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Psychological Aspects in Terms of Physical, Mental and Sociological Factors During Worldwide Corona Crisis

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Abstract

Severe acute respiratory syndrome is a viral disease affecting the respiratory system , cause by coronavirus. The epidemic lasted for 2 years till May 2004 when WHO declared China to be free of new cases but stated that " biosafety concerns remain" In 2019, SARS-CoV-2 was discovered which later in the present have caused the COVID-19 pandemic. The first case was identified in Wuhan, china and which later on turned to worldwide pandemic. The WHO had declared the outbreak a public health emergency of international concern on 30 January 2020 and a PANDEMIC on 11th march 2020.

Considering the present scenario , there are certain articles over the internet and journals which have given us a bunch of ideas over different aspects of life. Some of the long list of concepts are physical health, mental health, local market uprising, and concept of work from home .

Based on gathered information via discussions, surveys and personal interactions with thousands of individuals, differing in age, by gender and by occupations, these hereby key points and key lessons have come to light and here is a brief description in terms of physical health , mental health, effect of lockdown on local market and effect of work from home on people.

Keywords: *Pandemic, Corona, Psychological, Mental, Social, Physical.*

Introduction

Severe acute respiratory syndrome is a viral disease affecting the respiratory system , cause by coronavirus. SARS-CoV-1 being the first identified strain , causing the SARS outbreak in Foshan, Guangdong, China on 16th November 2002. The epidemic lasted for 2 years till May 2004 when WHO declared China to be free of new cases but stated that " biosafety concerns remain". In 2017, a group of scientists from China found traces of the virus in horseshoe bats in Yunnan. In 2019, SARS-CoV-2 was discovered which later in the present have caused the COVID-19 pandemic. It is called Covid-19 due to its beginning occurring in 2019 year. Its also called human coronavirus 19 or HCoV-19.The first case was identified in Wuhan, china and which later on turned to worldwide pandemic. The WHO had declared the outbreak a public health emergency of international concern on 30 January 2020 and a PANDEMIC on 11th march 2020.

Considering the present scenario , there are certain articles over the internet and journals which have given us a bunch of ideas over different aspects of life. Some of the long list of concepts are physical health, mental health, local market uprising, and concept of work from home .

"Millions of viruses, including coronaviruses, are circulating in wildlife and most of these have the potential to infect humans"[1]. " A need for undertaking joint surveillance by human health and animal health has also been expressed for early detection of zoonotic infections."[2]

As for India's situation regarding the Covid pandemic, the first case was reported on 30th January, 2020. " Indian government has some of legal acts in aspect of disease and disaster management, such as The

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Epidemic Disease Act of India , 1987 [3] and The Disaster Management Act, 2005 [4]", allowing the legalities to apply pandemic control measures and providing a platform for the medical health care workers to inform regarding the positive cases and required management of cases and control of the spread of disease.

"Special efforts were undertaken in India to protect and treat elder citizens. As per age distribution of those positive of covid-19, 14 percent of 45 years olds, approximately 35 percent of aged between 45 and 60 years, approximately 51 percent of more than 60 years olds, 42 percent of 60-75 years old and approximately 9 percent of above 75 years category and ones with comorbidities hold a percentage of 78 percent. "[5]

"Indian council of Medical Research (ICMR) and a wide network of around 65 Virus Research and Diagnostic Laboratories (VDRLs) have been a part of testing team. India, not having many laboratories for diagnosis during the start of pandemic. As from May 10, 2020 onwards, India had tested 1,609,037 samples for SARS-CoV-2 VIRUS, at a rate of approx.. 1200 tests per million people. Out of this, 62,937 tested positive.[6]. At the start of May 2020, reverse transcription-polymerase chain reaction (RT-PCR) test facility were available in 360 government and 112 private facilities across the country.[6], with testing of around 90,000 per day."

Materials and Methods

Based on clear and informative discussions among groups of people, divided on basis on gender, age and social status, key aspects were identified and purposeful lessons and ideas were gathered and correlated with each other in order to collectively suggest solutions different problems such as mental issues including depression, latent personality diseases, and other psychological factors that played crucial role in life. All these were highlighted after concerned group of people stayed at home in quarantine for about 3 months after lockdown had been initiated.

Some of the population were unaware about the spread of disease and they were concerned for the risk of staying at home for longer than they anticipated, children were among this population mostly and the issues parents had to face to keep their kids indoors. Such problems among the rest were addressed and certain health issues came to light and final solutions were suggested based of their needs.

Regarding physical health, concerns grew among middle aged people and mostly males who worked from home , mainly regarding weight gain and more chances of getting chronic diseases such as hypertension and diabetes.

Local market were surveyed and the benefit of lockdown were seen for such retailers since mostly shopping for medical purposes and groceries were done through local market rather than global markets. Basic interaction and other methods of approach to the considered group of people have helped in gathering useful and considerate material for discussion and concludes on basis of physical, mental and social factors happening around people during the time of crisis and how to be positive in this difficult time, on individual level and as a community.

Study Design:

Based on gathered information via discussions, surveys and personal interactions with thousands of individuals, differing in age, by gender and by occupations, these key points and key lessons have come to light and here is a brief description in terms of physical health , mental health, effect of lockdown on local market and effect of work from home on people.

Health

Physical health

Amid lockdown being declared at different times all over the world, people have been sitting at home and being unable to go out , have meant , to not be productive at home , which is wrong ideology. Being at home should ideally mean being at peace and the best possible way to rejuvenate from all the darkness that gets suppressed within us while we are out in the world.

Diet and exercise are two key ingredients for a healthy life. As per current guidelines, most common symptoms seen in a Covid positive patient are sore throat, cough and fever, which are also typical of flu or common cold. So at an individual level, taking care of our health and keeping a good immunity would help rule out diagnosis of Covid from other differential diagnosis. Working out by running , jogging, skipping or basic stretching exercises and yoga are some of proven effective ways to keep a healthy body and meditation as a whole works like magic when it comes to peace . Chronic stress can leave a drastic impact on health and

lower the immunity, making a person vulnerable to the disease and its speed of healing. At the end of the day we have to focus on soulful healing and it can be achieved with clear mind and a good sleep.

Mental Health

We have all been so affected by the current status of the world and for many reasons, whether it is from being at home for longer than we hope for or just with everything being good but still some of us feeling down. First of all we have to address the fact that it is normal to feel sad even if everything is good for you. Mental health and depression are underrated and have to be highlighted now. As many of us are in a similar situation, everyone is unique individual with different problems in life, no problem is small or big and even though we all know we will feel good in the future and it is about the present situation in which we feel bad.

"Depression—also called "clinical depression" or a "depressive disorder"—is a mood disorder that causes distressing symptoms that affect how you feel, think, and handle daily activities, such as sleeping, eating, or working. To be diagnosed with depression, symptoms must be present most of the day, nearly every day for at least 2 weeks. Feelings of hopelessness, worthlessness, and helplessness are the three basic criteria for experiencing depression."[7]

"One shall be able to identify these symptoms and consult for help , either with a close friend or to a professional. One can refer to National Institute of Mental Health NIMH Help for Mental Illnesses webpage at www.nimh.nih.gov/findhelp. Another Federal health agency, the Substance

Abuse and Mental Health Services Administration (SAMHSA), maintains an online Behavioral Health Treatment Services Locator at https://findtreatment.samhsa.gov/. You can also check online for mental health professionals; contact your community health center, local mental health association, or insurance plan to find a mental health professional. Hospital doctors can help in an emergency."[7]

Effect of lockdown on local market

With global market loss, there has been beneficial growth of income for local markets due to easy access to necessary items and less human contact to local societies. Crisis has hit the world economy in a highly fragile situation, persisting after the Great Financial Crisis & the Great Recession. High, persistent and partly rising inequality along with current account imbalances are some of the drawbacks to current situation along with weak recovery (secular stagnation), weak investment and low capital stock growth , low productivity growth. [8]

With the fear in mind that there could be a 2nd wave of the pandemic coming ahead and with permanent disruption of supply chains, lasting effects on supply side (capital stock, human capital) , an amendment or a seclusion of solutions have to be applied in order to be prepared for upcoming future.[9]

Pharmacies and grocery shops have been the most at gains during this lockdown for almost everyone have been compulsorily asked to keep a mask on at any time of the day that they are out. One drawback to this is shortage. Shortage of masks, gloves and sanitary products have been seen and people had found alternatives to that in forms of cloth masks, diluted disinfectant solutions and as such products made at home. One lesson we could learn are the talents of modifications at times of crisis by people in order to keep oneself and their families safe.

Effect of work from home on people

Work from home brought both drawbacks and benefits to individuals and the society. Drawbacks are procrastination, poor reports and performances by employees, success decline in some ways and benefits are ease of mind and no possible chance of burnout and no late hours of work.

Elaborating each point, due to no senior supervisor keeping an eye on employees and no 9 to 5 work hours rule applied, people have procrastinated over work and projects have been delayed leading to loss at company level. No second opinion and checks on work by another colleague, work quality had decreased and it can be solved at an individual level only for who is presenting with poor work can only solve their working efficiency.

New ideas are brought by inspiration but a mind block would lead to either no new ideas or poor ideas. Employees could be motivated by their seniors and regular call meetings may help as well.

Although being at home has relaxed minds and ease at home has helped in certain ways to bring about the best of potential in some people.

We have to understand that work is as important as keeping a successful carrier but it is not everything you seek for happiness and taking a break once in a whole is crucial for basic health.

Work from home have helped in keeping family dynamite alive and spending quality time with family helps in more productive work outcomes.

Discussion

As there is no proper and confirming news as to when the pandemic would come to an end, all we can understand is that we are all involved in this together and maintaining a balance between our daily life and world economy along with keeping a sane mind and healthy body is in our hands only[10].

With diseases that are deadly, pollution, corruption, disasters, and a lot more bad things happening that could not be stopped, all but hope can be in the mind for a better tomorrow. With recent and current affairs, one must understand the value of humanity and understanding and in my opinion, as a review to all the news , a lesson of kindness can be learnt and being kind to each other can solve even the biggest of world problems.

As a citizen of the world, it is our soulful responsibility to teach our newer generation better ways to live life and to learn from them the innocence that they hold as children. Healthy lifestyle is the best way prevent any disease and as it is said " prevention is better than cure" [11,12,13].

Mental health , which cannot be covered in just a paragraph, holds one of the biggest problem in the world and it is a crucial lesson to be learnt that we all have some anxiety in our mind, with less or more , but it is important to accept that it is present in all of us and addressing our anxieties will have to reach to the bottom and get the reason for why would you have anxiety in the first place then only a solution can be framed. Remember, accepting you have a problem solves half of the problem. Having someone to talk to and also loving yourself can help deal with the biggest of issues. Self-awareness and self-dependence are two important concepts to be introduces in someone's mind, helping in such a way that any problem in the future can also be dealt with without it growing inside , well enough, to cause mental issues.

Another concept that we have talked about in the article is "working from home" [14] and in conclusion all that is needed to know is that whether work is done from an office or at home , the important thing is doing work and projects without any distraction and procrastination. Also it is a positive way since "burn out" is avoided which imposes a much bigger problem for those who have to go for long hours of work out of their comfort zone, home.

The last concept addressed is "local market rise" which is talked about in terms of economic growth and it should be supported even in the future. Helping small scale industries to grow will not only make a country developed and self-dependent but local talents are also highlighted and appreciated. for example, as India is an agricultural country, people with different capabilities and new ideas lie in every corner and as long as international brands are appreciated, not only will it cost more money to purchasers but the hidden talents will forever be hidden. It is very important and national brand and national workers are supported and allowed to bring out their potential, whether in terms of clothing, food and agricultural products, science and technology or discoveries and inventions.

Conclusion

In conclusion, we saw certain numbers of cases growing by day in the world and nationwide and with the current status , we also observed how the government and first line health care workers have been working on toes to keep us safe.

We also discussed the factors that are affecting our day to day life and how we can overcome our problems. Maybe not all the problems are discussed and not all the solutions are highlighted but the gist of the article is every problem can have a solution if its seen clearly. Psychology is a vast term and till date, we have only scratched the surface and it is obvious we have a long way to go. Whether this pandemic will end soon or later, we have to keep going on with our life, our work and our children and new generation will come but it is our moral responsibility that the new generation survives and then this responsibility is passed on so in simple words, be kind to one another. Be human.

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Combined Effect of Neural Tissue Mobilization and Deep Friction Massage in Piriformis Syndrome: A Research Protocol

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Abstract

Background:- Piriformis syndrome is the musculoskeletal condition which is characterized by pain along with tingling and numbness which results because of compression of piriformis muscle. Various treatment approaches are present for treating the piriformis syndrome but neural tissue mobilisation is manual therapy similar to joint mobilisation. Deep friction massage is used to breakdown adhesion and release of scar tissues. This study protocol has been created to find out the combined effect of neural tissue mobilization and deep friction massage in piriformis syndrome.

Methods:-- On the basis of inclusion and exclusion criteria 30 participants between 20-60 years of age having piriformis syndrome will be select for study and are classified into two groups. Group A (n-15) for Neural Tissue Mobilisation, Group B (n-15) for Neural Tissue Mobilisation and Deep Friction Massage. Outcome measure are Oswestry Disability Index, Numerical Pain Rating Scale and Range of motion of hip joint. Follow up of the participants are done after every 2 days and treatment duration is of 10 days.

Discussion:- Effect of intervention will be analyzed by Numerical Pain Rating Scale, Oswestry Disability Index and Range of Motion of Hip joint. The result of this study will significantly provide the affirmation of Neural Tissue Mobilisation with Deep Friction Massage in Piriformis Syndrome.

Keywords:- *Neural Tissue Mobilisation, Deep Friction Massage, Piriformis Syndrome, Physical Therapy*

Introduction

Piriformis syndrome is the musculoskeletal condition which is painful resembles to sciatica secondary to entrapment in sciatic nerve at greater sciatic notch in Piriformis muscle[1]. Wallet sciatica, Hip Socket Neuropathy, Pseudo Sciatica are the terms referred for Piriformis syndrome[2].

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Incidence of Piriformis syndrome in patients with pain at the low back is 5-36% more common in women than men in the ratio of (6:1)[3]. In sedentary individual the prevalence of Piriformis muscle tightness was found to be 79.5%[4]. The characteristics clinical features are pain in the low back, hip region and posterior aspect of thigh which radiates to leg and side of foot with course of sciatic nerve, along with this patient also experience numbness, burning sensation, painful bowel movements, difficulty in walking and performing functional activities[5].

Modalities used in treatment are cold therapy, hot therapy, ultrasound therapy adjunct with stretching of Piriformis muscle. Conventional therapy approaches are massage therapy, muscle energy technique, Neural Tissue Mobilization, myofascial release technique, positional release technique[3]. Neural Tissue Mobilization therapy helps to decrease pain and inflammation thereby improve

axoplasmic blood flow, intra neural mobility, mechano sensitivity and intraneural blood flow[6]. Neural Tissue Mobilization exercises are derived from neurodynamic tests such as straight leg raise test , slump test which is effective in decrease pain symptoms[7]. Deep Friction Massage helps to break down the adhesions in the muscle and release scar tissue[5]. It also helps to maintain the mobility in soft tissue structures[3].

Aim

The study aims to find out the combined effect of Neural Tissue Mobilization and Deep Friction Massage for pain relief and improving flexibility of hip joint.

Methodology

Study setting

The trail will be carried out in musculoskeletal OPD of Ravi Nair Physiotherapy College, sawangi meghe (wardha) after approval from Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences Deemed to be University.

Study design and sample size

The design of the study is randomized control trial for individual diagnosed with piriformis syndrome. The number of participants enrolled in this study is (n-30)n-30. The participants will be randomized into 1:1 manner in Neural Tissue Mobilisation (Group A) and Neural Tissue Mobilisation with Deep Friction Massage (Group B) Before inclusion, the participants will be explained about the approaches and objectives of the study, and consent formed in written signed by the participants. The procedure of the study is shown in fig 1 (as recommended by standard protocol items: recommendation for intervention trials).

Figure no:-1 Schedule of enrollment, interventions and assesements.

	Study Period				
	Enrollment	**Allocation**	**Post Allocation**		**Follow up test**
TIME POINT	-t1	0	Intervention (t1)	Post Test (t2)	Tx
Enrollment					
Eligibility Screen	X				
Informed Consent	X				
Allocation		X			

***Cont.*. Figure no:-1 Schedule of enrollment, interventions and assesements.**

INTERVENTIONS					
Neural Tissue Mobilisation			X		
Neural Tissue Mobilisation with Deep Friction Massage			X		
Assessments Baseline Variables:- Positive Piriformis test, Tenderness over low back, low back pain,	X		X	X	
Outcome Variables:- NPRS, ODI, Range of motion of hip joint	X			X	X

Participants

The inclusion criteria for the participants are as under

1) Patient willing to participate

2) Both male and female between the age group of (20-60) years with diagnosed case of piriformis syndrome.

3) Tenderness over low back, buttocks and hip joint.

4) Low back pain radiating to posterior thigh, calf and foot.

5) Limitation in range of motion of hip joint.

6) Piriformis test positive

The exclusion criteria for the participants are as under

1) Patient not willing to participate.

2) Age below 20 and above 60 years.

3) Lumbo-Sacral disc lession and spinal stenosis

4) History of any spinal surgery

5) Spondylolisthesis

6) Degenerative spine and hip joint.

Participants Timeline

As the duration of the study is 6 months and the intervention duration is 10 days. Assessment of the patient is done on 1st days and intervention will be started. The evaluation (NPRS, Oswestry Disability Index, Range of motion of hip joint) will be performed at baseline and before their last session.

Recruitment

The orthopaedic and health care practitioners working under DMIMSU are invited to refer the prospective patients to our inpatient department. The patient who are already diagnosed with piriformis syndrome will be assessed systematically for the eligibility in the study as per the inclusion and exclusion criteria. Patient concerned will be cumulated before

the allocation and after elaboration the purpose, nature, procedure of the intervention.

Implementation

Randomization will be supervised by the research coordinator and principal investigators.

Study procedure

The participants will be categorized into two groups

Group A (Neural Tissue Mobilisation) – The participants in this group will undergo neural Tissue mobilisation daily for 8 day**s,** it will performed by the physiotherapist in the IPD.

Group B (Neural Tissue Mobilisation with Deep Friction Massage) – The participants in this group will undergo Neural Tissue Mobilisation and Deep Friction Massage for 40 minutes daily for 8 days.

Intervention:

Group A: Intervention will be Neural Tissue Mobilisation alone.

Group B: Intervention will be Neural Tissue Mobilization with Deep Friction Massage.

Procedure of Neural Tissue Mobilization

Neural Tissue Mobilization technique is applied 12 to 15 minutes with 30 seconds hold and 1 minutes relaxation phase. The leg is raised beyond 40 degree for induction of dural motion in sciatic nerve and sciatic nerve will stretched, the straight leg raise will provide traction on sciatic nerve and lumbosacral nerve root. The symptoms starts developing from piriformis muscle and in the distribution of sciatic nerve of affected lower extremity because of neural tension. Hip adduction was also done with adjunct to straight leg raise to develop more traction in the sciatic nerve.

Procedure of Deep Friction Massage

Deep Friction Massage is given for 10 to 15 minutes.

Frictions are classified into:-

Circular and Transverse

Circular Frictions: Finger tips are used for circular friction. Affected area is properly palpated by palpation technique so that tip of finger is properly placed over the affected area. With the finger tips pressure is applied in a circular direction initially slowly and then gradually increasing the pressure for 3 minutes then release the pressure and relax for one minute and repeat the procedure.

Transverse Frictions: Index finger and middle finger finger tips are used for transverse friction massage. Palpate the affected structure and provide frictions with finger tips over the affected area on the longitudinal axis by keeping the body alignment parallel to the movement. Finger tips are move in forward and backward direction over the affected area in order to produce proper separation of fibers, this procedure will last for 5 minutes and then relax and repeat the same technique..

Outcome measures:

1) The Oswestry Disability Index is an most important role which is use to measure patients permanent functional disability and this tool is also used for low back pain and disorders of spine the questionnaire gives us information as how your back pain affect activities of daily livin[8].

2) The Numerical Pain Rating Scale is one of the simplest and most used scale in clinical practice for measuring the intensity of pain, this is the 10 point scale where 0 represents no pain and 10 represent the worst[9].

3) The range of motion of hip joint is assessed through the universal goniometer.

Data Collection and Management

Data Collection:-

The assessment data will be properly collected from pre-established spreadsheet with the baseline characteristics variables. Testing data will be put into a secure REDCap database. The non electronic data such as consent forms, hardcopies of the assessment forms will be stored in the study setting.

Data Management:-

Data collection and proper documentation will be done under the guidance of principal investigator. The study documentation will be evaluated for accuracy.

At the end of the study the Excel spreadsheet will be released and analysis done by statistician. To prevent missing data checklists are used.

Statistical analysis plan:-

Data analysis will be done by using descriptive and interferences statistics using Chisquare test and student unpaired t test. Software used in the analysis will be SPSS 24.0 version. Graphpad prism 7.0 version and $p<0.005$ is considered as the level of significance. ($P>0.005$).

Discussion

Piriformis Syndrome it the musculoskeletal condition which is characterized by pain in the buttock and entrapment in the sciatic nerve. The aim of the research is to combined the effect of Neural Tissue Mobilisation and Neural Tissue Mobilisation with Deep Friction Massage in piriformis syndrome patients. Neural Tissue Mobilisation (NTM) is the therapeutic treatment showed significant improvement in pain relief, strength, range of motion. Deep Friction Massage (DFM) helps to break down the adhesion in the muscle and scar tissue and to maintain the mobility of soft tissue structures. In conclusion, this research protocol combines two manual technique in piriformis syndrome. The result of the current study will improve quality of life of patient and make functional independent. Outcome measures use in the study are NPRS, Oswestry Disability Index, Range of Motion of hip joint. These scales help to assess the intensity of pain, activities of daily living and range of motion of hip joint.

Result

After completion of the study it will provide the best treatment regime out of individual NTM or NTM with DFM of piriformis syndrome and result of both the study will help to determine the effect of both the treatments. Data will be completely analyzed after completion of the study and will be submitted in form of research paper.

Consent:

Consent form will be obtain from the patient and one of the relative on a printed form with signatures by principal investigator and giving proof of confidentiality.

Confidentiality:

Participants and the relative will be informed about the study program and personal information will be taken by principal investigator only, in consent form the confidentiality statement will be mentioned. Signatures of both investigator and patient with two witness will be taken and for the purpose of the study if needed to disclose some information complete assurance and consent will be taken from the patient.

Author's contribution

DJ suggested the design of the study. VA and DJ lead the creation and design of the study. VA wrote the manuscript of the article. VA and DJ read and approved the final manuscript for publication.

Declaration Of Interests:

The authors declares no conflicting interest.

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Police Brutality and Custodial Torture in Technological Era: Need for Anti-Torture Law in India - A Critical Analysis

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Abstract

Police audacity and brutality is not an uncommon phenomenon. It has caught the attention of the public and the judiciary and eventually a catena of directions was promulgated by the National Human Rights Commissions (NHRC), High Courts as well as the Supreme Court of India right from the ancient era to technological era. Yet, the menace and inhumane attitude of the police officials have not declined; instead, it is increasing day by day and one step ahead, Tamil Nadu has seen the worst of its effect that has resulted in the death of an innocent father and his son. This incident has grabbed the attention of not only the State of Tamil Nadu but also the entire Nation. In this situation, the authors intend to analyse the power of the police to manhandle the public, the liabilities and responsibilities of the police in case of death caused by such brutality, and third-degree methods used by the police. This article ponders on the question Does the police have the power to torture the public for any reasons whatsoever? If so, what are the limitations placed on the powers of the police while harassing the public? and so on… the authors aspire to analyse the duties of the police while making the arrest, the duties of the magistrates while ordering for judicial custody, and the authors also intend to suggest the remedial measures to curb the menace of lockup deaths.

Aim and Scope: To know the powers of the police to torture the public under the guise of investigation, to know the judicial approach to curtail the menace of torture by the police, and its implications and to know the feasibilities of technologies to be implemented during the investigating process.

Method: For the purpose of the research, secondary data such as books, articles, news papers, case laws, and legal provisions of Indian Laws are collected and analysed.

Conclusion: In spite of the Constitutional and statutory provisions including International Conventions, decision and directions promulgated by the Apex Court of India and the NHRC, there is a constant increase in the custodial torture, custodial death and custodial ill-treatment which is a disturbing factor that causes deep anxiety in the society. The Prevention of Anti-Torture Act is the need of the hour.

Keywords: *Judiciary, Legal Sanctity, Lockup Deaths, Police Brutality, Powers of Police, Rights of Public, Role of Technology.*

Introduction

Technology has changed the entire world. Everything is possible and nothing is impossible in the technological era. Number of technologies are adopted for doing, changing and accomplishing the tasks in various sectors in the world. Yet, the menaces of police atrocities, brutalities and deaths in lockups are not coming down. According to '*India: Annual Report on Torture, 2019' published by the National Campaign against Torture* (2020), the National Human Rights Commission (NHRC), India has recorded a sum of

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1,723 cases of loss of lives in judicial as well as in police custody throughout India. The Report dictates that the above number of cases includes 1,606 deaths in judicial custody and 117 in police custody, which discloses the death of an average of five persons in custody per day.

In addition, an analysis of the Human Rights Cases Statistics shows that a total of 735 custodial death cases have been registered from January – 1st July 2020 [1]. Among these, 42 cases relate to Police custody while the remaining 693 cases pertain to judicial custody. After careful analysis of the above monthly wise report, it comes to be known that roughly 4 persons per day have died either under police custody or under judicial custody (from January – June 30, 2020). Approximately Rs.2,77,551 *(Rupees Two Lakhs Seventy-Seven Thousand Five Hundred and Fifty-Five Only)* has been awarded as monetary relief per death. Hence, a sum of Rs. 1,36,00,000/- *(Rupees One Crore thirty-Six Lakhs only)* has been awarded for 49 deaths in judicial custody. To the shock and surprise of the public, none of the police officials were convicted [2]. Therefore, it is essential to analyse the lacuna in the existing criminal jurisprudence, and stringent measures are the need of the hour to control the menace of police audacity and atrocities.

Objectives of the Research

The research aims:

- To know the powers of the police to torture the public under the guise of investigation
- To know the relevant laws and its limitations on the powers of the police
- To know the judicial approach to curtail the menace of torture by the police, and its implications
- To know the feasibilities of technologies to be implemented during the investigating process.

Research Question

1. Whether the police have the unfettered rights to torture the detainees or accused? If so, to what extent?
2. Whether the laws in India allow the police officials to use third-degree methods in the name of investigation?
3. What are the judicial mechanisms to check the menace of torture by the police?
4. What are the possibilities of implementation of the technologies during the investigating process?

Methodology

This research is out and out based on non empirical method. For the purpose of the research, secondary data such as books, articles, news papers, case laws, and legal provisions of Indian Laws are collected and analysed.

Methods of Torture by Police

The Report 2019[3] highlights 15 top methods of torture and deaths in police custody. It states three significant situations under which the suspects are tortured, namely i) to punish them ii) to gather information and iii) to extract confession. Besides extracting crucial information by force from the suspects or their relatives, the report further emphasizes that the police use common methods of torture such as slapping, kicking with boots, beating with sticks, pulling hair etc[4]. The police use other brutal techniques like, hammering iron nails in the body, applying roller on legs and burning, *falanga*, stretching legs apart in the opposite side, hitting in private parts, stabbing with the screwdriver, electric shock, pouring petrol in private parts, applying chilli powder in private parts, beating while being hand-cuffed, pricking needle in the body. Other inhuman harassment methods are branding with a hot iron rod, beating after stripping, urinating in mouth, inserting a hard blunt object into the anus, beating after hanging upside down with hands and legs tied, forcing to perform oral sex, pressing fingernails with pliers, deprivation of food and water, beating with iron rods after the victim is suspended between two tables with both hands and legs tied, forcing to do *murga* pose or stress position and kicking in the belly of the pregnant women[5].

Here the questions are i) On what authority do the policemen indulge in the above mentioned brutality, inhumane and degrading activities? ii) Do the policemen have rights to torture the detainees even without maintaining the minimum amount of human decorum or humanenes? iii) Are the detainees under the servitude of the policemen? iv) Are the policemen above the law

of the land? With these questions in mind, the authors intend to discuss the legal provisions of the various enactments including the international conventions regarding the brutal torture by the policemen.

LEGAL SANCTITY OF TORTURE BY POLICE: VIEWS OF INTERNATIONAL CONVENTION/ COVENANTS ABOUT TORTURE

1. Clause 39 of the Magna Carta, 1215 states, "No freeman is to be taken or imprisoned or disseised of his free tenement or his liberties or free customs, or outlawed or exiled or in any way ruined, nor will we go against such a man or send against him save by lawful judgment of his peers or by the law of the land".

2. Universal Declaration of Human Rights (UDHR), 1948 and International Covenant on Civil and Political Rights (ICCPR), 1966, insist, "No one shall be subjected to torture or to cruel or inhuman or degrading treatment or punishment [6]".

CONSTITUTION OF INDIA AND THE TORTURE

Article 21 of the Indian Constitution states, "No person shall be deprived of his life or personal liberty except according to procedure established by law" while Article 22 (1) emphasizes, "No person who is arrested shall be denied the right to consult, and to be defended by, a legal practitioner of his choice." The expression "life or personal liberty" in Article 21 has been held "to include within itself a guarantee against torture and assault by the State or its functionaries [7]" and Article 22 mandates that the arrested person shall not be denied the right to consult the lawyers of his choice.

CODE OF CRIMINAL PROCEDURE, 1973 (AS AMENDED) ABOUT THE TORTURE

i) Section 41-D of Code of Criminal Procedure 1973 emphasizes, "When any person is arrested and interrogated by the police, he shall be entitled to meet an advocate of his choice during interrogation".

ii) Section 46 (2) states that while making the arrest, if such person forcibly resists the endeavour to arrest him, or attempts to evade the arrest, such police officer or other person **may use all the means necessary to effect the arrest**. Still, nothing in Section 46, gives a right to cause the death of a person who is not accused of an offence punishable with death or with imprisonment for life [8]. Further, the arrested person shall not be subjected to more restraint than is necessary to prevent his escape [9]. When any person is arrested, he shall be examined by the medical officer in the service of Central or State Governments [10] and the medical officer so examining the arrested person shall prepare the record of examination mentioning therein any injuries or marks of violence upon the person arrested and the approximate time when such injuries or marks may have been inflicted [11]. According to Section 55-A of Cr.P.C[12], it shall be the duty of the person having the custody of the accused to take reasonable care of the health and safety of the accused, and every police officer may interpose for the purpose of preventing, and shall, to the best of his ability, prevent the commission of any cognizable offence.

Above all, under Section 176 (1-A), it is emphasized that **where any person dies or disappears while such person is in the custody of police or in any other custody authorized by the magistrate or the Court,** in addition to the inquiry or investigation held by the police, **an inquiry shall be held by the judicial magistrate or metropolitan magistrate,** as the case may be, within whose local jurisdiction the offence has been committed[13]. Despite the mandatory nature of this provision, its compliance is rare. With respect to this provision, in a petition filed before the Supreme Court of India, it has been categorically mentioned that since its enactment, the section has been left untouched and has not been implemented properly, thereby leading to rising custodial deaths.

The Supreme Court of India accepted to hear the plea for mandatory judicial probe in cases of custodial death and rape [14]. Though there is a mandatory provision for judicial probe under the above Section 176 (1-A), the Judicial probe in Sathankulam incident *(Tuticorin District of Tamil Nadu State)* was held only after the intervention of the Madurai Bench of the Madras High Court [15].

POLICE STANDING ORDER AND TORTURE

Police Standing Order (PSO) [16] under the heading *"Ill-Treatment by the police"* states, "directly an accused person is placed under arrest, the investigating police

officer shall, as the first step in the police investigation, ask him whether he has any complaint, to make of ill-treatment by the police and shall enter in the case diary the question and answer. If an allegation of ill-treatment is made, the investigating officer shall there and then examine the prisoner's body, if the prisoner consents, to see if there are any marks of ill-treatment and shall record the result of his examination. If the prisoner refuses to allow his body to be examined, the refusal and the reasons therefore shall be recorded. If the investigating officer finds that there is reason to believe the allegation of ill-treatment, he shall at once so far suspend the investigation on which the prisoner has been arrested and to forward the prisoner with his complaint, the record of corporal examination, any other evidence available, and, if possible the police officer implicated by the prisoner's complaint to the nearest magistrate having jurisdiction to enquire into the case*".*

A bare reading of the above provisions under various enactments in India including the International covenants makes it crystal clear that ill-treatment, cruelty, torture, inhumane or degrading treatments are strictly prohibited and the persons arrested have to be treated with utmost care, caution and dignity. Instead, there are past and current reports that the police forces allegedly employed such practices in a routine manner, which resulted in custodial deaths either in the police station or in judicial custody.

COURTS' VIEW ON TORTURE AND THE CUSTODIAL VIOLENCE

The Apex Court of India in *Radul Sah Vs State of Bihar [17],* emphatically observes, "It is necessary to educate ourselves into accepting that, respect for the rights of the individual is the true bastion of democracy. Therefore, the State must repair the damage done by its officers to the petitioner's rights. It may have recourse against those officers". In *Smt. Nilabati Behera Vs State of Orissa [18],* the Supreme Court of India while observing that custodial death is perhaps one of the worst crimes in a civilized society governed by rule of law [19], held, "There is a great responsibility on the police or prison authorities to ensure that the citizens in their custody are not deprived of their right to life. The court further, held that the precious rights guaranteed by Article 21 of the Constitution of India cannot be denied to convicts, (under trials or other prisoners) in custody except according to the procedure established by law. Further, the court went on to state that the concept of sovereign immunity being inapplicable and alien to the concept of fundamental rights wrongdoer is accountable and the State is responsible, if the rights are violated [20]."

Dr J.S Anand, J in his concurring judgment[21], mandates, "It is an obligation of the State to ensure that there is no infringement of the indefeasible rights of a citizen to life, except in accordance with the law, while the citizen is in its custody". Moreover, in *Devinder Singh & Others Vs State of Punjab through CBI [22],* the Court held that "Act of beating or confining a person illegally is outside the purview of duties".

In *D. K. Basu v. State of West Bengal [23],* again the Supreme Court of India held as under

"Custodial violence, including torture and death in the lock-ups strikes a blow at the Rule of Law, which demands that **the powers of the executive should not only be derived from law but also that the same should be limited by law.** Custodial violence is a matter of concern. It is aggravated by the fact that it is committed by persons who are supposed to be the protectors of the citizens. **It is committed under the shield of uniform and authority in the four walls of a police station or lock-up, the victim being totally helpless.** The protection of an individual from torture and abuse by the police and other law enforcing officers is a matter of deep concern in a free society".

Further, the Court in the above case has held, "**If the functionaries of the Government become law-breakers, it is bound to breed contempt for law and would encourage lawlessness** and every man would have the tendency to become a law unto himself thereby leading to anarchy[24]". **No civilized nation can permit that to happen, the Court asserts [25].**

The Supreme Court further wonders whether *a citizen sheds off his fundamental right to life, the moment a policeman arrests him and it also questions if the right to life of a citizen be put in abeyance on his arrest.* The answers to the above questions were negative [26].

The Court further expresses its anguish, "police are, no doubt, under a legal duty and have legitimate

right to arrest a criminal and to interrogate him during the investigation of an offence but it must be remembered that the law does not permit the use of third-degree methods or torture of accused in custody during interrogation and investigation with a view to solving the crime. The end cannot justify the means. The interrogation and investigation into a crime should be in true sense, purposeful to make the investigation effective. By torturing a person and using third-degree methods, the police would be accomplishing behind the closed doors what the demands of our legal order forbid. No society can permit it [27]. "

The Top Court of India in the above case reiterates, "Death in custody is not generally shown in the records of the lock-up and every effort is made by the police to dispose of the body or to make out a case that the arrested person died after he was released from custody. Any complaint against such torture or death is generally not given any attention by the police officers because of ties of brotherhood. No first information report at the instance of the victim or his kith and kin is generally entertained and even the higher police officers turn a blind eye to such complaints".

Even where a *formalis* prosecution is set in motion by the victim or by their relatives, no direct evidence is available to prove the charges since all the tortures are happening away from the public look. Moreover, the eyewitnesses are either policemen or the co-prisoners who are disinclined to come out as a prosecution witness due to fear of retribution by the police officials. It is also very difficult to get evidence against policemen since they are in charge of the police station. Consequently, the erring policemen are liable to be acquitted for want of evidence. Therefore, the torture in police custody receives encouragement and builds up in the minds of the police that no one is against the police and none can harm the police. Eventually, the ruthlessness of the police is encouraged and the society suffers. "It is no part of an official to commit an offence, and never can be [28]."

REQUIREMENT OF SANCTION UNDER SECTION 197 OF CR.P.C TO PROSECUTE THE POLICE OFFICIALS FOR CUSTODIAL DEATH OR TORTURE

The Supreme Court of India in *Devinder Singh & Others Vs State of Punjab through CBI [29]* answered that ordinarily the shield of sanction under Section 197 of Cr.P.C is not required for offences which have no connection with the official duties. "A public servant not entitled to indulge in criminal activities and to this extent, the sanction has to be construed narrowly and in a restricted manner". Moreover, in some cases, the Court observed that "The protection of sanction is an assurance to an honest and sincere officer to perform his public duty honestly and to the best of his ability. However, the performance of an official duty under the colour of public authority cannot be camouflaged to commit a crime [30],[31],[32]." Interestingly, in *Devinder Singh & Others Vs State of Punjab through CBI [33],* the Supreme Court *upheld the argument put forth by the CBI that "*in cases of criminal activities, fake encounters, **custodial death due to torture etc.,** sanction to prosecute is not at all required as fake encounters, torture in custody and other criminal acts complained of do not form part of their official duties".

Conclusion and Suggestions

In spite of the Constitutional and statutory provisions including International Conventions, decision and directions promulgated by the Apex Court of India and the NHRC, there is a constant increase in the custodial torture, custodial death and custodial ill-treatment which is a disturbing factor that causes deep anxiety in the society. In these circumstances, the authors wish to recollect the order passed by the Tamil Nadu Government whereby the school teachers and parents are prevented from inflicting corporal punishment to their children assuming that such punishments are in contravention of Human Rights as well as the fundamental rights guaranteed by the Constitution. The author assumes that the duo, *teachers and parents* are, whenever there is a need (not in all cases) to mend the behaviour of their children with good faith and to mould them to make their wards suitable to their society are, as a means of correctional measures giving lineal corporal punishment during the early childhood and not after that. There is a proverb that *"as the twig is bent, so is the tree inclined"*. In such a situation like this, the authority with which the law enforcing agencies are brutally assaulting the people who are mostly from the underprivileged and weaker sections of the society is questionable. Remembering

the ongoing incidents in the State of Tamil Nadu, especially during the COVID-19 pandemic situation, the authors with a hardened heart, high trust and the only objective of protecting human lives from the hands of the law enforcing agencies particularly the policemen, the authors highly recommend The Prevention of Anti-Torture Act with a comprehensive analysis taking into consideration the 113th, 152nd and 273rd Reports of Law Commission of India and the UN Convention Against Torture and Other Cruel, Inhuman or degrading Treatment or Punishment. The authors further appeals to adopt the support of the technologies in this regard. Therefore, the authors to avoid any such human loss in future (in the technological era), suggest the following measures:

1. In all the police stations, *at least* two psychiatrists and one advocate must be appointed.

2. Proper and periodical training must be given to the police officials to inculcate right perception of the laws and their latest developments to secure human lives and values.

3. The presence of *at least* one advocate and one psychiatrist during the interrogations or investigation must be made mandatory in the station to prevent the third-degree methods. Advocates may be appointed either on a temporary or *Adhoc* basis in this regard.

4. The CCTV cameras must, if not installed, be installed forthwith in all the police stations and throughout the enquiry, investigation and till the accused produced before the court, the CCTV should be in operation. Since, the electronic evidences are, after the adoption the Information Technology Act, 2000, admissible before the court (*See* Evidence Act, 1872 Section 3); whenever necessary the CCTV footages must be produced before the court.

5. Penal provisions may be incorporated in I.P.C, 1860 for failure to have advocates and psychiatrists and for switching off the CCTV during the interrogations or investigations. If the CCTV is switched off or not working during the course of investigation *(i.e, right from the accused brought to the station and till he produced before the court),* the Court shall presume that the police has caused the torture or assaulted the accused in the police station.

6. Complaint against police officials in this regard should be sent through registered post with acknowledgement due to Chief Judicial Magistrate (CJM) in case of custodial violence that does not amount to death or Session Judge in case of custodial violence that amounts to death. They in turn, shall forward the complaint to the nearest Law College Principal for investigation who along with not exceeding two Professors/Associate Professors or with Assistant Professors shall conduct an enquiry and submit the report to the CJM or the Sessions Judge, for necessary action either for departmental action or for criminal proceedings or both as the case may be. The entire enquiry must be completed within one month. Based on the report, CJM or Session Judge as the case may be, may request the concerned Public Prosecutor to initiate criminal proceeding against such erring law enforcing agencies.

7. A copy of the report shall be forwarded to the concerned Superintendent of Police (SP) through the Principal District Judge (PDJ) to initiate action on the delinquent. Failing which, the PDJ shall request the concerned Public Prosecutor to intimate the matter to the D.I.G of police, who in turn, without any unnecessary delay should take action against such erring police officials including the SP.

8. Public awareness must be created about the rights of the public and the limited rights of the police.

9. Awareness must be created about Sections 330 and 331 of I.P.C, and Section 330 of I.P.C must be suitably amended to make the offence as non-bailable and triable by the Court of Session, considering the influential position of the law enforcing agencies.

10. Section 327 of IPC has to be suitably amended to include “money value” and the offence must be tried by the Court of Session, considering the influential position of the law enforcing agencies, and the erring law enforcing agencies have to be booked under this Section also.

11. A new Section 154A shall be incorporated in Code of Criminal Procedure 1973 as recommended by the Law Commission of India in its 152nd report and its recommendation clause 154A (2) may be modified in consonance with the suggestion mentioned in suggestion no.6.

12. Section 154 (3) of Cr.P.C may suitably be amended to provide disciplinary action for dereliction of duty, against the erring officer in charge of the police station, and Section 166A of I.P.C may also be amended to provide punishment up to three years and to make the offence 'non bailable'.

13. As recommended by the Law Commission of India in its 152nd Report, explanation to Section 197 shall be inserted in Code of Criminal Procedure, 1973 forthwith.

14. As recommended by the Law Commission of India in its 152nd Report, a provision *namely* "Section 114-B" shall be incorporated in the Indian Evidence Act 1872 forthwith.

15. Section 3 of The Prevention of Torture Bill 2017 must be revisited to include 'confession' and 'to the person himself', since it is mentioned in the Bill from any person *(See also The Prevention of Torture Bill 2010)* and a modified Bill must be introduced fixing fine amount 'not less than Rs. one lakh' (Section 4 of Bill, 2017) without undue delay in the Parliament, providing stringent and immediate measures.

"Fiat Justitia Ruat Caelum" – Let Justice be done though the heaven Falls

Future Scope

This paper focus on the brutal incidents that happened in the State of Tamil Nadu and the suggestions mentioned are having a wider scope for research in future also. Till the meance is considerably reduced, this paper this topic is researchable and debatable one.

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Research article

Study of Socio-Demographic Profile of Thoraco-Abdominal Injuries in Fatal Road Traffic Accidents in Mangalore City

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Abstract

Background: Victims in road traffic accidents sustain large varieties of thoracic and abdominal injuries. This post mortem study of profile of thoraco-abdominal injuries, type, pattern and nature of external and internal injuries involved will be an attempt to highlight the trends in this region. This study is aimed to study socio-demographic profile of victims died due to fatal thoraco-abdominal injuries in road traffic accidents in Mangalore city. **Material and Methods:** Present study was a record based retrospective autopsy study carried autopsies that had thoraco-abdominal injuries sustained in fatal road traffic accidents. **Results:** 247 cases sustained thoraco-abdominal injuries in RTA were studied. The most common age group was 20 – 29 (21.8%) years followed by 30 -39 years (20.2%). Male victims (85.02%) were more compared to females (14.98%) and male: female ratio was 5.6: 1. Most cases were happened on city roads (38.5%) & pedestrians comprise the greatest number of victims involving 46% cases. 69% cases had thoracic trauma with signs of external injuries while external abdominal injuries were present on 54% cases. 199 (81%) cases sustained internal injuries to thorax. 55% cases were associated with external injuries of thorax and 26% cases are without external injuries. Most common injuries were fractures of ribs (60 %), injuries to lungs (38%) & injuries to heart (23%). There were 136 (55%) cases of internal abdominal injuries, most common organ injured was liver (38%), followed by spleen (17%) & abdominal wall (14%). **Conclusion:** The present study was undertaken to focus light upon socio-demographic profile of thoraco-abdominal injuries in fatal road traffic accidents. Maximum numbers of the victims in the present study were males (85 %) in the age group of 20-39 years (42%), reported in city roads (39%), during summer season. Maximum numbers of victims were pedestrians (46%).

Keywords: *socio-demographic profile, fatal thoraco-abdominal injuries, road traffic accidents, road safety*

Introduction

"The fear of death is the most unjustified of all fears, for there's no risk of accident for someone who's dead" – Albert Einstein

Accidents are counter parts of civilized world. Accident is an unfortunate incident that happens suddenly and unintentionally, which normally results in damage or injury. Injuries from RTA is expected to be the third most common cause of universal disability and the second most common cause of disability in the developing world. [1]

The pattern of road traffic injuries in fact varies significantly across the countries. The risk factors

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include speeding, alcohol-impaired driving, and not using protective measures like helmet or seat belt. [2]

The incidence of accidental deaths has shown an increasing trend during the period 2003 - 2013 with an increase of 54.3% in the year 2013 as compared to 2003. The population growth during the period 2003-2013 was 15.0% whereas the increase in the rate of accidental deaths during the same period was 25.5% [(3)]. The number of vehicles in the country has increased by 78.0%, and the quantum of 'Road Traffic Accidents' has increased by 5.1% during the period of 2009 to 2013. [3]

Victims in road traffic accidents sustain large varieties of thoracic and abdominal injuries. This is due to the tremendous increase in the number of vehicles, high speed technology along with other contributing factors like congestion and poor condition of roads, intoxicating influence of alcoholor drugs, inexperienced drivers without proper driving license, ignorance or intentional violation of traffic rules etc

This post mortem study of profile of thoraco-abdominal injuries, type, pattern and nature of external and internal injuries involved will be an attempt to highlight the trends in this region. This study is aimed to study socio-demographic profile of victims died due to fatal thoraco-abdominal injuries in road traffic accidents in Mangalore city.

Material and Methods

Present study was a record based retrospective autopsy study carried out in the Department of Forensic Medicine of K S Hegde Medical Academy (a constituent unit of NITTE University) and Wenlock District Hospital, Mangalore. Study period was during January 2009 and December 2013. Study approval was taken from institutional ethical committee.

Inclusion Criteria

· All cases that had thoraco-abdominal injuries sustained in fatal road traffic accidents.

Exclusion Criteria

· The cases which are not road traffic accidents.

· Dismantled and dismembered bodies of which the abdominal organs are missing.

· Cases with no specific history.

· Decomposed bodies in which organs are liquefied.

All the data obtained was kept anonymous to protect the identity and confidentiality medico legal information. The data required for the study such as age, sex, information furnished by the police in the inquest papers (form 146 (i) & (ii)), autopsy findings, investigation reports if any and cause of death was collected retrospectively. The data collected was documented in proforma & entered in Microsoft excel sheet. Data was analysed with the help of SPSS software version 16.0. And results were derived in form of percentages.

Results

A total of 4271 cases were brought to the mortuary during study period. Among 4271 cases 859 cases were due to road traffic accidents, out of them 346 cases were from Mangalore taluka, & 247 cases sustained thoraco-abdominal injuries. The most common age group was 20 – 29 (22%) years followed by 30 -39 years (20%). The age group between 20 to 49 years contributes 147 cases (60%). Male victims (85%) were more compared to females (15%) and male: female ratio was 5.6: 1.

Table 1 - Age & gender wise distribution

	Total number of victims	Percentage
Age Group		
0 – 9	8	3%
10-19	18	7%
20 – 29	54	22%
30 – 39	50	20%
40 – 49	43	17%
50 – 59	36	15%
60 – 69	22	9%
70 – 79	13	5%
>80	3	1%
Gender		
Male	210	85%
Female	37	15%

In present study, most cases were happened on city

roads (39%) followed by on rural roads (36%) and on highways (25%). Among all the road users, pedestrians comprise the greatest number of victims involving 46% cases followed by two-wheeler rider (33%), pillion rider (11%), light vehicle occupant (9%), Heavy vehicle occupant (1%).

Table 2 – General characteristics

General characteristics	Total number of victims	Percentage
Place of incident		
City roads	95	38%
Rural roads	90	36%
Highway	62	25%
Road user category		
Pedestrian	113	46%
Two-wheeler rider	82	33%
Pillion rider	28	11%
Light vehicle occupant	21	9%
Heavy vehicle occupant	3	1%

Out of 247 cases, 68.8% cases had thoracic trauma with signs of external injuries such as abrasions (61%), contusion (11%) and laceration (10%). External abdominal injuries were present on 54% cases, 49% cases had abrasion followed by contusion in 9% cases and laceration in 6% cases.

Table 3- External injuries

External injuries	Total number of victims	Percentage
Thorax		
Abrasion	150	61%
Contusion	26	11%
Laceration	25	10%
Abdomen		
Abrasion	121	49%
Contusion	22	9%
Laceration	16	6%

Among 247 cases, 199 (80.6%) cases sustained internal injuries to thorax. 55.1% cases were associated with external injuries of thorax and 25.5% cases are without external injuries. Most common injuries were fractures of ribs (60 %), injuries to lungs (38%) & injuries to heart (23%). There were 136 (55%) cases of internal abdominal injuries, most common organ injured was liver (38%), followed by spleen (17%) & abdominal wall (14%).

Table 4- Internal injuries

Internal Injuries	Total number of victims	Percentage
Thorax		
Fracture of ribs	147	60%
Injuries to lung	92	37%
Injuries to heart	56	23%
Injuries to sternum	27	11%
Injuries to pericardium	26	11%
Crush injury	16	6%
Injuries to pleurae	11	4%
Injuries to vessels	8	3%
Injuries to thoracic vertebrae	8	3%
Injuries to spinal cord	4	2%
Abdomen		0%
Injuries to liver	93	38%
Injuries to spleen	41	17%
Injuries to wall	34	14%
Injuries to peritoneum	25	10%
Injuries to small intestine	11	4%
Injuries to kidney, diaphragm, large intestine, omentum, pelvis, stomach, bladder, lumbar vertebrae	47	19%

In present study, most cases (13%) were observed in the month of May followed by January (11%) and November (10%). Maximum number of cases observed in summer season i.e., March, April, and May (32%).

Table 5 - Month Wise Distribution

Month	Total number of victims	Percentage
January	28	11%
February	19	8%
March	22	9%
April	24	10%
May	33	13%
June	22	9%
July	9	4%
August	12	5%
September	16	6%
October	20	8%
November	25	10%
December	17	7%

Discussion

Among all accidental deaths, road traffic injuries assert 1.2 million lives every year and form the main cause of deaths from unnatural causes[1]. It is estimated that by the year 2020, 8.4million people will die every year of injuries due to road traffic accidents[2].

The bony thoracic cage contains vital centers of circulation and respiration. Trauma to chest challenges the integrity and even viability of vital organs. Complications of thoracic injuries include pneumothorax, haemothorax, interstitial emphysema, arterial air embolism, cardiac tamponade. The main complication of abdominal injuries is internal haemorrhage & shock, peritonitis and paralytic ileus.[4]

Maximum numbers of the victims in the present study were males (85 %) in the age group of 20-39 years (42%). The fact that males are usually the earning members of the families and were exposed to traffic makes them more vulnerable to the road traffic accidents. The subjects in the age group of 20-39 years lead more active life and are at the peak of their creativity having the tendency to take risk, thereby subjecting themselves to the dangers of accidents and injuries. Similar findings were also reported by, NB Kumar et al,[2] M R Lamb et al[5] and Meera Th et al[6].

In present study, maximum number of cases reported in city roads (39%) followed by rural roads (36%) and highways (25%). The commonest time of incident of road traffic accidents was 6 am to 12 pm (38%). The raise in population and vehicles in the city leading to increased congestion on the roads can be directly associated to the number of road traffic accidents. A study by Yogesh G done in Bangalore has shown that city roads were commonest place of RTA and 6 am to 12 pm as commonest time of incident, which was comparable to our study.[7] Maximum number of victims in present study were pedestrians (46%) followed by two-wheeler riders (33%). A demographic and topographic analysis of road traffic accidents done by B R Sharma et al[8] and Nuwadutta Subedi et al[9] noted similar results.

In thoraco-abdominal region, internal injuries may occur without any external injuries. In this study 81% of cases sustained internal injuries to thorax, in that 55% of cases were associated with external injuries and 26% of cases are without external injuries. The commonest injury of thoracic region was fracture of the ribs (60%) because anatomically rib cage is the one which takes more impact during accident. and amongst the thoracic organ's lungs were the most commonly affected thoracic organ (37%) followed by heart (22%). 55% cases showed external abdominal injuries and 55% cases showed internal abdominal injuries. The most common organ injured in this region is liver (38%), followed by spleen in 17% cases. Liver is commonly injured due to the anatomical position and it occupies the major area of upper part of abdomen. Similar findings were observed in study of Nuwadatta Subedi et al.[9], Pradeep K Saralaya et al[10], A Hanamantha et al[11].

A similar study on road traffic accidents showed that pedestrians were the most common victims in road traffic accidents followed by motor cyclist and light motor vehicle occupants. The most common organs involved in road traffic accidents were liver followed by Spleen and kidneys.[5]

According to this study, maximum number of cases observed in the month of May (13%) followed by January (11%) and November (10%). A statistical analysis of accidents by National Crime Records Bureau also reported that maximum number of cases occurring in May followed by January.[3] In the present study it was observed that during the summer season i.e., March, April and May, the number of cases is more compared to other seasons. This can be attributed to the completion of road repair works and asphalting of roads, damaged by the monsoon of previous year, usually draw to a close by summer. This may lead vehicle to move faster in summer in the newly laid roads, and may result in increased road traffic accidents compared to other seasons.

The alarming increase in morbidity and mortality owing to road traffic accidents over the past few decades is a matter of great concern. The risk stratification and the study of nature of the vehicles involved in RTA can help the authorities in propagating safety measures and better health care availability on roads.

Conclusion

The present study was undertaken to focus light upon socio-demographic profile of thoraco-abdominal injuries in fatal road traffic accidents. Maximum numbers of the victims in the present study were males (85 %) in the age group of 20-39 years (42%), reported in city roads (39%), during summer season. Maximum number of victims were pedestrians (46%). Strict execution of traffic rules, separate pavements or foot path for pedestrians & safety precautions while driving and travelling are very important to prevent mortality & morbidity related to thoraco-abdominal injuries in road traffic accidents.

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Research Article

Study of Mortality Due to Thoraco-Abdominal Injuries in Road Traffic Accidents in Mangalore City

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Abstract

Background: After the strict implementation of helmet rule the number of head injuries due to road traffic accidents has subsequently decreased, but injuries sustained to thoracic and abdominal regions are still on the rise. This post mortem study of profile of thoraco-abdominal injuries, type, pattern and nature of external and internal injuries involved will be an attempt to highlight the trends of mortality due to thoraco-abdominal injuries associated with road accidents in Mangalore city. **Material and Methods:** Present study was autopsy record based retrospective study conducted deaths associated with thoracic-abdominal injuries that have definite history of road traffic accidents. **Results:** From 247 deaths of RTA victims who sustained thoraco-abdominal injuries, most common age group was 20 – 29 (21.8%) years, male: female ratio was 5.6: 1. 21 % of cases were spot dead or dead on arrival to hospital, 60 % of cases died within 24 hours. 68.8% cases had thoracic trauma with signs of external injuries such as abrasions (60.7%), contusion (10.5%) and laceration (10.2%). External abdominal injuries were present on 54.2% cases, 48.9% cases had abrasion followed by contusion in 8.9% cases and laceration in 6.5% cases. 199 (80.6%) cases sustained internal injuries to thorax. Fractures of ribs were present on 59.5% cases. Injuries to lungs sustained in 37.2% cases and to heart in 22.6% cases. 136 (55.1%) cases of internal abdominal injuries, most common organ injured was liver (38%), followed by spleen (17%) & abdominal wall (14%). Among the 247 cases, cause of death in 47% cases was head injury followed by thoraco-abdominal injury (20%), head injury and thoracic injury (8%), only thoracic injury (7%), abdominal injury (6%). **Conclusion:** The present study maximum numbers of the victims were males, from age group of 20-39 years, reported on city roads, pedestrians, noted in summer season and cause of death was head injury followed by thoraco-abdominal injuries.

Keywords: *mortality, fatal thoraco-abdominal injuries, road traffic accidents, road safety*

Introduction

Injuries from road traffic accidents (RTA) is expected to be the third most common cause of universal disability and the second most common cause of disability in the developing world.[1] Mortality and Morbidity from road traffic accidents has greater importance in developing countries.

The number of vehicles in the country has increased by 78.0%, and the quantum of 'Road Traffic Accidents' has increased by 5.1% during the period of 2009 to 2013.[2]The population growth during the period 2003-2013 was 15.0% whereas the increase in the rate of accidental deaths during the same period was 25.5%.[2]In 2013, there was a decline of 0.9% in deaths due to causes attributable to nature and an increase of 1.5% in deaths

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due to un-natural causes as compared to 2012. 'Road Traffic Accidents' cases in the country have marginally increased by 0.7% during 2013 compared to 2012. Their proportion in total deaths due to un-natural causes has slightly increased from 35.2% in 2012 to 36.4% in 2013.[2]

The thoracic and abdominal cavities contain most of the vital organs; hence, injury to these organs leads to fatal outcome. Trauma to these cavities can be produced in various means such as road traffic accidents, railway accidents, fall from height and blunt weapons. Among this, road traffic accidents contribute maximum numbers. After the strict implementation of helmet rule the number of head injuries due to road traffic accidents has subsequently decreased, but injuries sustained to thoracic and abdominal regions are still on the rise.[3] Because of their size and anatomical position, they are major regions of trauma in road traffic accidents.

This post mortem study of profile of thoraco-abdominal injuries, type, pattern and nature of external and internal injuries involved will be an attempt to highlight the trends of mortality due to thoraco-abdominal injuries associated with road accidents in Mangalore city.

Material and Methods

Present study was autopsy record based retrospective study conducted in Department of Forensic Medicine & Toxicology of K S Hegde Medical Academy (a constituent unit of NITTE University) and Wenlock District Hospital, Mangalore. Ethical society approval was taken for present study.

Inclusion Criteria

· All cases of deaths associated with thoracic-abdominal injuries that have definite history of road traffic accidents.

Exclusion Criteria

· Cases with no specific history.

· Dismantled and dismembered bodies of which the abdominal organs are missing

· Decomposed bodies in which organs are liquefied.

Autopsies conducted during January 2009 and December 2013 was considered for this study. All the data obtained will be made anonymous to protect the identity and confidentiality medico legal information. Details of diseased person such as age, sex, information furnished by the police in the inquest papers (form 146 (i) & (ii)), autopsy findings ,investigation reports if any and cause of death were noted in proforma. The data was analysed with help of descriptive statistics.

Results

During study period 247 deaths of road traffic accident victims who sustained thoraco-abdominal injuries were noted. The most common age group was 20 – 29 (21.8%) years followed by 30 -39 years (20.2%). The age group between 20 to 49 years contributes 147 cases (59.5%).

Table 1 - Age wise distribution

Age Group	Total number of victim	Percentage
0 – 9	8	3%
10-19	18	7%
20 – 29	54	22%
30 – 39	50	20%
40 – 49	43	17%
50 – 59	36	15%
60 – 69	22	9%
70 – 79	13	5%
>80	3	1%

Male victims (85.02%) were more compared to females (14.98%) and male: female ratio was 5.6: 1.

Table 2- Gender wise distribution

Year	Male	Female
2009 (n=51)	42 (82%)	9 (18%)
2010 (n=39)	31 (79%)	8 (21%)
2011 (n=60)	53 (88%)	7 (12%)
2012 (n=56)	50 (89%)	6 (11%)
2013 (n=41)	34 (83%)	7 (17%)
Total (n=247)	210 (85%)	37 (15%)

In present study, most cases (13.3%) were observed in the month of May followed by January (11.3%) and November (10.1%). Maximum number of cases observed in summer season i.e., March, April, and May (31.9%). 21 % of cases were spot dead or dead on arrival to hospital, 60 % of cases died within 24 hours and 14% of cases survived more than 24 hours and less than 10 days. Only 5 % of cases survived more than 10 days.

Table 3- Duration of Survival

Duration Of Survival	Total number of victim	Percentage
Immediately - < 1 hour	51	21%
> 1 hour - < 24 hours	149	60%
1 day – 10 days	34	14%
> 10 days	13	5%

In present study, most cases were happened on city roads (38.5%) followed by on rural roads (36.4%) and on highways (25.1%). Among all the road users, pedestrians comprise the greatest number of victims involving 45.8% cases followed by two-wheeler rider (33.2%), pillion rider (11.3%), light vehicle occupant (8.5%), Heavy vehicle occupant (1.2%). Out of 247 cases, 68.8% cases had thoracic trauma with signs of external injuries such as abrasions (60.7%), contusion (10.5%) and laceration (10.2%). External abdominal injuries were present on 54.2% cases, 48.9% cases had abrasion followed by contusion in 8.9% cases and laceration in 6.5% cases.

Table 4- External injuries

External injuries	Thorax Total number of victim	Abdomen Total number of victim
Abrasion	150	121
Contusion	26	22
Laceration	25	16

Among 247 cases, 199 (81%) cases sustained internal injuries to thorax. Most common injuries were fractures of ribs (60%), followed by injuries to lungs (37%) cases and injuries to heart (23%). Crush injuries were observed in 6% of cases. There were 136 (55%) cases of internal abdominal injuries, most common organ injured was liver (38%), followed by spleen (17%) & abdominal wall (14%).

Table 5- Internal injuries

Thorax	Total number of victim (%)	Abdomen	Total number of victim (%)
Fracture of ribs	147 (60%)	Injuries to liver	93 (38%)
Injuries to lung	92 (37%)	Injuries to spleen	41 (17%)
Injuries to heart	56 (23%)	Injuries to wall	34 (14%)
Injuries to sternum	27 (11%)	Injuries to peritoneum	25 (10%)
Injuries to pericardium	26 (11%)	Injuries to small intestine	11 (4%)
Crush injury	16 (6%)	Injuries to kidney, diaphragm, large intestine, omentum, pelvis, stomach, bladder, lumbar vertebrae	47 (19%)
Injuries to pleurae	11 (60%)		
Injuries to vessels	8 (60%)		
Injuries to thoracic vertebrae	8		
Injuries to spinal cord	4		

Among the 247 cases, cause of death in 47% cases was head injury followed by thoraco-abdominal injury (20%), head injury and thoracic injury (8%), only thoracic injury (7%), abdominal injury (6%).

Table 6- Cause of Death

Cause Of Death	Total number of victim	Percentage
Head injury	117	47%
Thoraco-abdominal injury	50	20%
Head+thoracic injury	20	8%
Thoracic injury	17	7%
Abdominal injury	15	6%
Head + thoracic + abdominal injury	15	6%
Head+abdominal injury	13	5%

Discussion

The rapid and unplanned urbanization has resulted in an unprecedented revolution in the growth of motor vehicles globally. The alarming increase in morbidity and mortality owing to road traffic accidents over the past few decades is a matter of great concern.

The frequency of road traffic accidents in India is the highest in the world. Numerically, road traffic accidents accounts for the great majority of accidental deaths worldwide and the purpose of forensic examination in traffic accidents is to determine the nature and manner of causation of injuries on a pedestrian, or the occupant or rider of a vehicle[4].

The bony thoracic cage contains vital organs of circulation and respiration and trauma to these organs challenges the integrity and viability of entire organisms.[5] Injuries to abdomen are also significant as it contains

numerous vital organs like lungs, liver, spleen, kidneys, stomach, intestines and urinary bladder. Injuries to these organs are important as isolated injuries to liver, spleen, intestine can be saved if timely surgical aid is provided to them.[6]

In present 4 years forensic study, we noted that maximum numbers of the victims were males (85 %), from age group of 20-39 years (42%), reported on city roads (39%), pedestrians (46%), noted in summer season (32%) and cause of death was head injury (47%) followed by thoraco-abdominal injuries (20%). Similar results were noted in other Indian studies.[7-10]

In a study conducted in Bangalore, most of the victims were male and liver and spleen were the most common organs injured in road traffic accidents. Maximum number of accidents took place between 9am to 12 noon and 6pm to 9 pm, four-wheeler occupants met more accidents followed by truck, bus and jeep occupants.[7]

In the autopsy study conducted by Nuwadatta Subedi et al[8] in 122 cases of road traffic accidents, male (80%) victims were more compared to female (20%). The mean age of the victims was 30.76 years. In road user category pedestrians were involved in more (30.3%) cases followed by two wheelers (28.8%) cases. In external injuries abrasion were more followed by contusion and laceration. In this study they found that the most common organs injured in road traffic accidents were liver (57.5%) and spleen (37.5%).

A study conducted at rural medical college, Loni, maximum numbers of victims of thoraco-abdominal injuries were males (82.6 %) as compared to females (17.34 %) and most vulnerable age group were of 21–30 years in both fatal and non-fatal accidents, commonest cause for thoraco-abdominal injuries (84.9%) were road traffic accidents, 32.25% died on the spot, 23.64% died within 12 hours, 6.45% of victims survived for 1 to 7 days and only 3.22% were survived for more than a week after sustaining a injuries. The commonest injury of thoracic region were fracture of ribs and lung was the commonly affected organ. In abdominal injuries, liver was the most commonly affected followed by spleen and then kidney. The survival period is less than one hour in most of the cases.[9]

A prospective study of trends of fatal road traffic accidents in central India, during the period of January 2011 to September 2012 noted that young adult males of the age group of 21-40 years were common victims (52.71%) and peak incidence occurring between 6pm to 12 am. Male to female ratio was 4.6:1. Two-wheeler users were the most common victims followed by pedestrians. There were 24 cases of light vehicle occupant and 20 cases of heavy vehicle occupant. Most common cause of death was head injury followed by injury to vital organs, haemorrhage and shock, thoracic injury, spine injury and abdominal injury. The survival period is less than 24 hours in majority of cases (76.77%).[3]

In a study to emphasize the pattern of thoraco-abdominal injuries sustained by the victims of fatal road traffic accidents in Jaipur, Rajasthan, male predominance (81%) was observed. Road traffic accidents were the major cause of fatality due to blunt thoraco- abdominal trauma (74.50%). External thoracic injuries were more common than internal thoracic injuries whereas, in abdominal region, internal injuries were more common than external injuries. Fracture of thoracic cage was the most common thoracic injury (65%).[10]

The thoraco-abdominal injuries due to road traffic accidents require multidisciplinary approach of management. The lack of awareness and knowledge of traffic rules are also the reasons for the increasing number of Road traffic accidents This post mortem study of profile of thoraco-abdominal injuries, type-pattern and nature of external and internal injuries involved will be an attempt to highlight the trends in this region.

Conclusion

The present study maximum numbers of the victims were males, from age group of 20-39 years, reported on city roads, pedestrians, noted in summer season and cause of death was head injury followed by thoraco-abdominal injuries. Properly maintained wide roads can prevent accidents to a greater extend with strict execution of traffic rules & measures to keep the stray animals away from the roads. Trauma centers with well trained staff and facility should be established in every city can help to reduce mortality & morbidity due to road traffic accidents.

Conflict of Interest: None to declare

Source of Funding: Nil

Ethical Clearance: Taken

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Research article

Pattern of Thoraco-Abdominal Injuries in Fatal Road Traffic Accidents in Mangalore City

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Abstract

Background: After the strict implementation of helmet rule the number of head injuries due to road traffic accidents has subsequently decreased, but injuries sustained to thoracic and abdominal regions are still on the rise. Because of their size and anatomical position, they are major regions of trauma in road traffic accidents. This post mortem study of profile of thoraco-abdominal injuries, type, pattern and nature of external and internal injuries involved, was an attempt to highlight the trends & pattern of thoraco-abdominal injuries in fatal road traffic accidents in Mangalore city. **Material and Methods:** Present study was a retrospective, autopsy-record based study of thoraco-abdominal injuries sustained in fatal road traffic accidents. Details of deceased individuals such as age, sex, information furnished by the police in the inquest papers (form 146 (i) & (ii)), autopsy findings, investigation reports if any and cause of death were noted in proforma. **Results:** During study period among 4271 cases 859 cases (20%) were due to road traffic accidents. Out of these 859 cases of road traffic accidents 247 cases (29%) sustained thoraco-abdominal injuries. 38.5% cases happened on city roads & pedestrians were most common victims (45.8%). Most common injuries were fractures of ribs (60%), followed by injuries to lungs (37%) cases and injuries to heart (23%). Crush injuries were observed in 6% of cases. There were 136 (55%) cases of internal abdominal injuries, most common organ injured was liver (38%), followed by spleen (17%) & abdominal wall (14%). Among the 247 cases, most common cause of death was head injury (47%) followed by thoraco- abdominal injury (20%), head injury and thoracic injury (8%), only thoracic injury (7%), abdominal injury (6%). **Conclusion:** Most fatal accidents were reported on city roads, in summer season and cause of death was head injury followed by thoraco-abdominal injuries. Ribs, lungs and Liver were the organs most commonly injured.

Keywords: *fatal thoraco-abdominal injuries, road traffic accidents, road safety, Mangalore city accidents*

Introduction

Road traffic accident (RTA) is an unintended collision of one motor vehicle with another, a stationary object or person, resulting in injuries, death or loss of property. Mortality and Morbidity from road traffic accidents has greater importance in developing countries. Injuries from RTA is expected to be the third most common cause of universal disability and the second most common cause of disability in the developing world.[1]

The pattern of road traffic injuries in fact varies significantly across the countries. The risk factors include speeding, alcohol-impaired driving, and not using protective measures like helmet or seat belt.[2]A total of 4,00,517 accidental deaths were reported in the country during 2013 (5,535 more than such deaths reported in

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2012) showing an increase of 1.4% as compared to 2012. A total of 3,77,758 (94.3%) deaths were due to un-natural causes and the rest of 5.7% deaths (22,759) were due to causes attributable to nature, out of total 4,00,517 accidental deaths during the year 2013. Out of 4,00,517 accidental deaths Karnataka has a share of 5.6% cases.[3]

After the strict implementation of helmet rule the number of head injuries due to road traffic accidents has subsequently decreased, but injuries sustained to thoracic and abdominal regions are still on the rise. Because of their size and anatomical position, they are major regions of trauma in road traffic accidents. The thoraco-abdominal injuries due to road traffic accidents require multidisciplinary approach of management. This post mortem study of profile of thoraco-abdominal injuries, type, pattern and nature of external and internal injuries involved was an attempt to highlight the trends & pattern of thoraco-abdominal injuries in fatal road traffic accidents in Mangalore city

Material and Methods

Present study was a retrospective, autopsy-record based study of thoraco-abdominal injuries sustained in fatal road traffic accidents. Study was conducted in department of forensic medicine of K S Hegde Medical Academy (a constituent unit of NITTE University) and Wenlock District Hospital, Mangalore & autopsies conducted during January 2009 and December 2013 were considered for study. Research society & ethical committee approval was taken. All the data obtained was kept anonymous to protect the identity and confidentiality medico legal information.

Inclusion Criteria

· Autopsies of thoracic-abdominal injuries sustained in fatal road traffic accidents.

Exclusion Criteria

· Cases with no specific history.

· Dismantled and dismembered bodies, decomposed bodies.

Necessary details of deceased individuals such as age, sex, information furnished by the police in the inquest papers (form 146 (i) & (ii)), autopsy findings, investigation reports if any and cause of death were noted in proforma.

The data collected will be documented in proforma. The data will be analysed and results will be derived with the help of SPSS software version 16.0.

Results

During study period, 4271 cases were brought to the mortuary of Wenlock hospital and K. S. Hegde medical college, Mangalore for autopsy. Among 4271 cases 859 cases (20%) were due to road traffic accidents. Out of these 859 cases of road traffic accidents 247 cases (29%) sustained thoraco-abdominal injuries. 38.5% cases happened on city roads followed by 36.4% cases on rural roads and 25.1% cases were on highways. Among all the road users, pedestrians were most common victims (45.8%) cases followed by two-wheeler riders (33.2%), pillion riders (11.3%), light vehicle occupants (8.5%) & heavy vehicle occupant (1.2%).

Table- 1 General characteristics

General characteristics	Total number of victim	Percentage
Place of incident		
City roads	95	38.5%
Rural roads	90	36.4%
Highway	62	25.1%
Road user category		
Pedestrian	113	45.8%
Two-wheeler rider	82	33.2%
Pillion rider	28	11.3%
Light vehicle occupant	21	8.5%
Heavy vehicle occupant	3	1.2%

21 % of cases were spot dead- or dead-on arrival to hospital, 60 % of cases died within 24 hours and 14% of cases survived more than 24 hours and less than 10 days. Only 5 % of cases survived more than 10 days.

Table 2- Duration of Survival

Duration of Survival	Total number of victims	Percentage
Immediately - < 1 hour	51	21%
> 1 hour - < 24 hours	149	60%
1 day – 10 days	34	14%
> 10 days	13	5%

In 69% cases thoracic trauma with signs of external injuries such as abrasions (61%), contusion (11%) and laceration (10%) were noted. External abdominal injuries were present on 54% cases, 49% cases had abrasion followed by contusion in 9% cases and laceration in 7% cases.

Table 3- External injuries

External injuries	Thorax(n=247)	Abdomen(n=247)
Total number of victims	169 (69%)	134 (54%)
Abrasion	150 (61%)	121 (49%)
Contusion	26 (11%)	22 (9%)
Laceration	25 (10%)	16 (7%)

Among 247 cases, 199 (81%) cases sustained internal injuries to thorax. Most common injuries were fractures of ribs (60%), followed by injuries to lungs (37%) cases and injuries to heart (23%). Crush injuries were observed in 6% of cases. There were 136 (55%) cases of internal abdominal injuries, most common organ injured was liver (38%), followed by spleen (17%) & abdominal wall (14%).

Table 4- Internal injuries

Thorax	Total number of victim (%)	Abdomen	Total number of victim (%)
Fracture of ribs	147 (60%)	Injuries to liver	93 (38%)
Injuries to lung	92 (37%)	Injuries to spleen	41 (17%)
Injuries to heart	56 (23%)	Injuries to wall	34 (14%)
Injuries to sternum	27 (11%)	Injuries to peritoneum	25 (10%)
Injuries to pericardium	26 (11%)	Injuries to small intestine	11 (4%)
Crush injury	16 (6%)	Injuries to kidney, diaphragm, large intestine, omentum, pelvis, stomach, bladder, lumbar vertebrae	47 (19%)
Injuries to pleurae	11 (60%)		
Injuries to vessels	8 (60%)		
Injuries to thoracic vertebrae	8		
Injuries to spinal cord	4		

Among the 247 cases, most common cause of death was head injury (47%) followed by thoraco- abdominal injury (20%), head injury and thoracic injury (8%), only thoracic injury (7%), abdominal injury (6%).

Table 5- Cause of Death

Cause of Death	Total number of victims	Percentage
Head injury	117	47%
Thoraco-abdominal injury	50	20%
Head+thoracic injury	20	8%
Thoracic injury	17	7%
Abdominal injury	15	6%
Head + thoracic + abdominal injury	15	6%
Head+abdominal injury	13	5%

Discussion

The thoracic and abdominal cavities contain most of the vital organs; hence, injury to these organs leads to fatal outcome. Trauma to these cavities can be produced in various means such as road traffic accidents, railway accidents, fall from height and blunt weapons. Among this, road traffic accidents contribute maximum numbers. The injuries in thoraco–abdominal area is classified as penetrating or open injuries and non-penetrating or closed injuries. Complication of thoracic injuries includes pneumothorax, hemothorax, interstitial emphysema, arterial air embolism, cardiac tamponade. The main complication of abdominal injuries is internal hemorrhage& shock, peritonitis and paralytic ileus.[4]

In present study, we noted that most fatal accidents were reported on city roads and cause of death was head injury followed by thoraco-abdominal injuries. Ribs, lungs and Liver were the organs most commonly injured. Similar findings were noted in other Indian studies.[5-9]

A study conducted in Imphal noted that foremost cause of blunt thoraco-abdominal trauma (86.40%) was vehicular accident followed by assault by blunt weapon (8%). There were no associated external injuries to the thoraco-abdominal region in 12.80% of cases. Most number of victims died at the spot (47.2%), (12%) cases died within 1 hour, (10.4%) victims survived less than 2 hours and only (1.6%) victims survived up to more than 1 week. The commonest cause of death was haemorrhagic shock combined with head injury in (48.8%) cases followed by haemorrhagic shock alone in (44%) of the cases. There were 87.20% of the cases showed associated external injuries on the thoraco-abdominal region while the remaining 12.80% did not show any external injuries.[5]

A cross sectional hospital-based study in Maharashtra revealed that, 61.3% cases were due to vehicle-to-vehicle collision. Two wheelers (25.87%) constitute more accidents followed by four wheelers and truck. Minor injuries were 44.7%, fractures 23.4% whereas 12.8% had multiple injuries. 9.2% were referred to higher centre. 61.7% of all RTAs cases were treated, cured & discharged. 23.5% had disability at the time of discharge & 5.60% mortality was noted.[6]

A study conducted in Guwahati noted incidence of fatal two-wheeler accidents is 16.59%. Rib fractures were seen in 33.3% of riders and 17.64% of pillion riders. Laceration to the liver and spleen constituted 19% each in riders while it was 14.7% and 17.6% respectively in pillion riders. Laceration of the heart was seen in 7.14% riders and 4.76% pillion riders. [8]

A study conducted in Allahabad revealed that maximum fatalities 83.05% were recorded on highways and only 6 cases occurred in lanes. Pedestrians accounted for 35.79% of total fatalities followed by two wheelers 30.53% of cases. The majority of fatal RTA were due to heavy vehicles 58.52% of cases followed by light vehicles 21.58%. The heavy vehicles were mostly responsible for causing a large number of visceral injuries. Brain was found to be injured in a majority of cases followed by liver and lungs. The victims mostly died of head injuries alone followed by thoraco-abdominal injuries in combination with head injuries. Maximum number of cases (40.21%) died within 24 hours followed by spot death 32.42%.[9]

The present record based retrospective research study was aimed at understanding the profile of thoraco-abdominal injuries caused in road traffic accidents in and around Mangalore. This study can help the authorities in propagating safety measures and better health care availability on roads to reduce mortality and morbidity of thoraco-abdominal injuries associated with road traffic accidents.

Conclusion

The present study was undertaken to focus light upon the pattern of fatal thoraco-abdominal injuries in relation to various factors in Mangalore. Most fatal accidents were reported on city roads and cause of death was head injury followed by thoraco-abdominal injuries. Ribs, lungs and Liver were the organs most commonly injured.

Conflict of Interest: None to declare

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An Ayurvedic Approach for Treatment of Fissure in Ano: A Case Report of 32 Year Old Patient

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Abstract

Anal Fissure is one of the major causes for pain at anal region. In this case report we are presenting case of 32 year old male patient came to OPD of Shalya tantra with the chief complains of pain during defection since 20days, itching at perianal region since 15 days and bleeding per rectum since 13 days. Intervention- In this case report patient is treated by using Go ghrita for the management of acute fissure in ano. Go ghrita was used for local application along with sitz bath.The effectiveness of Go ghrita was assessed by pain bleeding itching and healing. The patient has found effective relief from pain, bleeding, itching at perianal region using Go ghrita which has anti-inflammatory activity, antiulcer activity. The outcome of this management is that *Go* ghrita *is effective in the management of Fissure in ano* . Patients who take all follow-up after treatment will have less chance of symptom reoccurrence.

Keywords - *Fissure in ano, Go Ghrita,Ayurveda, antiulcer activity*

Introduction

In the age of alimentation, there's a shift within the habit of taking food and its timings in addition as within the manner that has become sedentary. Both of these causes turn out disturbance within the system that ends up in several diseases of digestive system like piles, fissure, fistula, prolapsed etc.

For pain at anal region fissure in ano is one among most common causes[(1).] The fissure-in-ano is categorized into two type on the basis of clinical symptoms & durations of the disease; viz. Acute and Chronic [(2).] The two primary signs of this disorder are, bleeding and pain; pain is often unbearable [(3)]. In long-standing instances, sentinel tag and haemorrhoids can be associated with this. Pruritus ani can be another symptom of this disorder [(4)]. In males anal fissure typically occurs in the midline posterior- 90 percent and 10 percent much less frequently. Subsequently, female fissures on the anterior midline are somewhat more common than before [(5).]

Sources on Parikartika are available from all Bruhatrayi and corresponding writers of A*yurveda.*. The factors responsible for the causation of *Parikartika* can be found in various ayurvedic texts such as *Vamana – VirecanaVypada, Basti Karma vypada and Upadrava* of *Atisara, Grahani, Arsa,Udāvta.*· In this regard, *Acharya Sushruta* stated the aetiopathogenesis of disease that if a anyone is impaired, with *Mrudukoshta (mild digestive power), Mandagni (poor appetite) in* these circumstances, more intake of food has the quality of *Rukshna(dry),Ushna(hot),Lavana(salty)*etc[(6).] Diet which is having such quality will vitiate Vata & Kapha & leads to Parikartika . Parikartika also has symptoms such as pain in the, penis, anus, neck of the urinary bladder and umbilical region with flatus cessation[(7).]In

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Kashyap Samhita, in the chapter *Garbhini Chikitsa*, we receive *doshik* classification, aetiology, symptomatology and treatment of *Parikartika*[8] .*Acharya* Charaka further mentioned fissure in ano as a Vataj Atisara complication[9]. In ayurveda Go Ghrita is used for the treatment of vrana. The latin name of Go Ghrita is *Butyrum departum.*

Case Report

In this case report we are presenting case of 32 year old male patient came to OPD of Shalya tantra with the chief complains of pain during defection since 20days, itching at perianal region since 15 days and bleeding per rectum since 13 days. The patient was suffering from constipation since 6 months. Patient has no history of any systematic disorder.

History of present illness

The patient was apparently normal 20 days before followed by he developed pain at perianal region, itching and bleeding per rectum. For that he has taken treatment to a modern medicine practitioner but he did not get relief. So for the Ayurvedic treatment he came to our hospital.

1. Nadi (pulse) = 82/min.

2. Mala (stool) = Malavashmbha, Sarakta.

3. Mutra (urine) = 3times/day.

4. Jihwa (tounge) = Saam.

5. Agni = Mandagni.

6. Shabda (speech) = prakrut (Normal).

7. Sparsha (skin) = Anushnasheeta.

8. Druka (eyes) = Prakrut

9. Akruti = krusha.

10. Bala = Aavar.

11. Raktachaapa (B.P) = 130/80 mm/Hg

Investigation: Hb-12.2 gm%. TLC: White blood count (WBC) 7000/mm3.Kidney function test was normal. Liver function test was normal. Human immunodeficiency virus-l (HIV-l) testing was negative .and Hepatitis B surface antigen, non reactive.

Per rectal examination –Per rectum examination reveals fissure in ano at 6'o clock.

Spasm-Grade 1

Tenderness- Grade 2

No active bleeding present

Intervention-In this case report patient is treated by using Go ghrita for the management of acute fissure in ano Go ghrita was used for local application along with sitz bath. The effectiveness of

Go ghrita was assessed by pain, bleeding, itching and healing. Assessment was done on 0^{th} day , 15^{th} da,.$30^{th\ day}$ follow up was taken on 45^{th} day.

Observation & Results

After the study Significant reduction was observed in all subjective and objective parameters (Table no.1, Table no.2)

Table no.1 Assessment of SubjectiveParameter

Sr. No.	Parameter				Follow up
		Day 0	Day 15	Day 30	Day 45
1	Pain(0-4)	3	1	1	0
2	Bleeding(0-1)	1	0	0	0
3	Itching(0-3)	2	1	0	0

Table no.2 Assessment of Objective Parameter

Sr. No.	Parameter				Follow up
		Day 0	Day 15	Day 30	Day 45
1	Parikartika Healing(0-3)	2	1	0	0

Figure1 Fissure in ano 1st day

Figure 2 Fissure in ano 30th day

Discussion

As per *Ayurveda Go Ghrita* is Rakshoghna, Balya, Vranahara, Vishahara, Rasayana, Ayuvardhaka, Balakaraka,Agnivardhaka, Jivaniya, Varnya, Prasadana,. *Rasapanchak* of *Go Ghrita* is Rasa- *Madhura,Guna-Shita, Guru, Rasayana, Virya-Sheeta,Vipaka-Madhura,Karma – Tridoshahara.* It is known for *Vranaropak* properties. Fissure in ano is longitudinal tear in the anoderm distal of anal canal. In contemporary sciences surgical treatment available for fissure in ano are sphincterotomy, lord's dilatation, fissurectomy, but these surgical procedure having adverse effects such as bleeding, infection, incontinence. [10] Ghee is a complex lipid of glycerides (majorly triglycerides), free fatty acids, phospholipids, sterols, sterol esters, fat soluble vitamins, carbonyls, hydrocarbons, carotenoids, (only in ghee derived from cow milk). [11] Go-Ghrita has a soothing property and form a thin-film layer over them and that allows early epithelization of wound. [12] To sort out these problem we need treatment which is easily applicable and non surgical.

Conclusion

The patient has found effective relief from pain, bleeding, itching at perianal region using. Ghrita does not contains water, and as per ayurvedic literature it has long shelf-life, and having healing properties which improves with age. The outcome of this management is that *Go* Ghrita is effective in the management of Fissure in ano*(*Figure1, Figure2). Patients who take all follow-up after treatment will have less chance of symptom reoccurrence.

Conflict of Interest: None

Funding: NIL

Ethical Clearance- Taken from Institutional ethical committee

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Review Paper

Ozone Therapy - A Boon to Dental Sciences

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Abstract

Ozone therapy has successfully being used in the medical field for the treatment of various diseases for more than a decade. The versatility of ozone therapy, its unique properties like biocompatibility, antimicrobial, anti-inflammatory, immunostimulant, non-invasive nature, atraumatic, relative absence of side effects or adverse reactions, and better patient compliance were responsible for its widespread dental and medical use. Ozone (O_3) is used in dentistry as gas, ozonated water and as ozonated oils. This review of literature is an attempt to summarize its therapeutic potential in dentistry.

Key words: *ozone; ozone therapy; dental application; oxidative agent***.**

Introduction

Ozone (O_3) is a natural colourless gas made up of three oxygen atoms, a highly water soluble inorganic molecule with a pungent odor at room temperature. The word ozone originated from the Greek word ozein, which mean odor and was first discovered by German chemist Christian Friedrich Schonbein in 1840. He is considered as the "father of ozone therapy". Edward. A. Fisch first introduced ozone therapy in dentistry in the year 1930's, to aid in disinfection and wound healing during dental surgeries. Ozone therapy can be defined as a versatile bio-oxidative therapy in which oxygen/ozone is administered via gas or dissolved in water or oil base to obtain therapeutic benefits.[1,2] It is an excellent antimicrobial agent that induces oxidative stress, killing almost 99% of bacteria, fungi and virus when exposed for 10sec. Ozone has also proven itself as a powerful oxidizer agent for sterilization of medical instruments.[3,4,5] Currently, the role of ozone in dentistry mostly relays on its antimicrobial properties and treatment of jaw diseases.[6,7]

Despite the various benefits, ozone toxicity and clinical utility depend on its concentration, time and the site of administration. One of the major contraindications of ozone therapy is its lung inhalation causing increased airways resistance without changing the pulmonary compliance or elastic characteristics.[3,5] Intravenous injections of ozone/oxygen gas should not be practiced due to the possible risk of air embolism.[8] This article is based on information retrieved through valid textbooks, peer reviews, journals and medline/pubmed search.

Ozone Generation

Medical grade ozone generator for therapeutic use was first developed by German physicians named Joachim Hansler and Hans Wolff. Currently Corona Discharge method is used to generate controlled production of high ozone concentration where medical grade oxygen is made to flow through a high voltage tubes that contains a mixture of pure oxygen and pure ozone in the ratio of 0.05% to 5% of O_3 and 95% to 99.95% of O_2. In dentistry, two mostly used ozone units are the heal ozone [9] and ozotop.[10]

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Modes Of Ozone Administration

Ozone (O_3) is used in dentistry in three basic forms: gaseous, ozonated water and as ozonated oils. The route of Ozone administration is topical or locoregional. The ozone in gaseous form is applied through a silicon cup which gets adapted over the tooth, and is exposed for a minimum of 10 secs. The ozone in the silicone cup is collected again and reconverted to oxygen through a reducing agent by the apparatus.[11] Ozonated oils plant extracts - sunflower oil, olive oil and ground nut, are used externally in the form of thick gel containing ozonides. Irrigation is utilized for stomatitis, herpetic lesions and periodontal infections. Insufflation is used for decay, periodontal infections and endodontic treatment.[12-13]

Mechanisms of Action

The following properties of ozone therapy are employed:[7,11]

1) As disinfectant with sterilizing effect on bacteria, virus, fungi and protozoa due to oxidative damages. There is evidence that ozone directly inactivates bacterial toxins.

2) As a weak cytokine inducer that stimutates anti-inflammatory and immune responses.

3) Its hemostatic effect due to generation of H2O2 that causes irreversible platelet aggregation by activating phospholipase C, phospholipase A2, cyclo oxygenases and lipooxygenases, and thromboxane synthetase allowing increase in intracellular Ca2+ and release of prostaglandins E2 and F2a and Thromoxane A2.

4) Accelerates wound healing, improves oxygen supply due to stimulation of production of free radical scavengers like glutathione peroxidase, catalase, dismutase etc.

5) Biosynthesis and bioenergetics (activation of the metabolism of carbohydrates, proteins, lipids)

Clinical Applications of Ozone in Dentistry

With all the evidence of different actions and lack of toxicity, ozone has developed into a new non- invasive tool for treating myraids of problems related to dentistry.

Ozone Uses in Oral Medicine

Soft tissue lesions like herpes labialis[14], aphthae, removable denture ulcers, cuts, cheilitis, candidiasis, cysts and traumatic wounds can be treated with either ozonated water or oils. Ozonated oil applied on herpes labialis and mandibular osteomyelitis demonstrated faster healing times than conventional protocols.[8] Another study demonstrated that odontoblastic cells exhibited inflammatory responses against bacterial lipopolysaccharides (LPS). Ozonated water improved LPS-induced inflammatory responses.[15] Some studies have demonstrated that exposure for 60 sec kills 99.9% cariogenic bacteria such as Actinomyces naeslundii, Streptococcus mutans and Lactobacillus casei[16], in addition to degradation of salivary proteins. Thus 10-30 sec is recommended for killing a significant number of cariogenic bacteria.

Ozone Uses in Oral Surgery

Ozone therapy was found to accelerate wound healing[17] by facilitating oxygen release and blood suppy due to vasodilatation, following tooth extraction or implant dentistry.[18] Ozone therapy is found to be beneficial for the treatment of the refractory osteomyelitis and in cases of bisphosnate related osteonecrosis of jaw[19] as adjunct to treatment with antibiotic, surgery and hyperbaric oxygen.

Ozone Uses in Implantology

The use of ozone in implantology helps in bone regeneration. The socket is prepared conventionally and the ozone is bubbled into the prepared socket for about 40 sec. This is followed by placement of implant into the socket. This prevents infection and enhances bone regeneration. Studies have reported similar promising effects in cases of peri-implantitis.[20]

Ozone Uses in Periodontics

In a study by Nagayoshi et al.[21] dental plaque samples were treated with 4 mL of Ozone water for 10 sec and was observed that gram +ve and gram –ve oral microorganisms and Candida albicans was significantly reduced thereby reflecting its potential action in controlling micro-organisms in dental plaques. Also there is reduction in the plaque index, gingival index and bleeding index by using ozone irrigation as compared to

chlorhexidine.[22,23] Irrigation of the root surface of avulsed teeth for 2 min showed effective mechanical cleansing and decontamination of root surface without a negative effect on periodontal ligament cell proliferation.[24]

Ozone Uses in Prosthodontics

Gaseous O_3 was proved to be clinically useful for disinfection of dentures and other removable denture alloys without compromising their physical properties like reflectance, surface roughness and weight.[23,25] The exposure of dentures to ozonated water together with ultrasonication has antimicrobial activity against C. albicans.[23,26] Bacterial reduction was recorded in the ozone treated groups when compared with conventional and surgical methods to cure periimplantitis.[27]

Ozone Uses in Endodontics

The potential use of ozone gas, ozonated water and ozonized oil in endodontic therapy has been repeatedly reported in the literatures.[28,29] Intra canal gaseous ozone at a flow rate of 0.5–1 l/min with net volume of 5 gm/ml for 2–3min showed encouraging results against pathogenic microbes in the root canal. Ozonated water can be used as an intracanal irrigating solution and in cases with infected necrotic pulpal tissues enhancing tissue regeneration and healing process. Ozonated oils is found to be more efficient in canal sterilization than conventional irrigation by sodium hypochlorite (NaOCl), and sodium peroxide combination. Also when a root canal was disinfected by ozone water with sonification, the antimicrobial efficacy was comparable to 2.5% NaOCl.[30] The aqueous form of Ozone was found to be less cytotoxic than gaseous Ozone.[31] Ozone therapy also increases the scope of nonsurgical management of periapical lesions.

Ozone Uses in Orthodontics

Conventional orthodontic treatment requires the use of brackets bonded to the enamel surface of teeth for optimal tooth movement. Diffuse opacity white spot lesion develop at enamel bracket interface, due to microleakage or poor oral hygiene, causing demineralization of enamel. So, proper enamel prophylaxis is required in orthodontics, prior to bracket placement. Ghobashy et al.[32] evalauted the effects of ozonized olive oil gel in reducing enamel demineralization around orthodontic brackets. The use of ozonized olive oil gel along with standard prophylaxis showed significantly less decalcification of teeth among orthodontics patients. Pretreatment of enamel with prophylactic ozone enhances shear bond strength of orthodontic brackets bonded with total or self- etch adhesive systems.[33]

Ozone Uses in Pedodontics

The application of Ozone therapy helps to attain a positive rapport when dealing with a child patient to a great extent. The treatment with ozone is very fast, atraumatic, effective, painless and ensures better compliance and acceptability, making it an ideal choice for pediatric patients. Dahnhardt et al.[34] found 93% of total reduction of anxiety in treating carious teeth with ozone in anxious children. Ozonated water is also recommended before replantation of avulsed teeth.

Management of Dental Caries

The application of Ozone therapy in the treatment of dental caries is extensively studied and many studies have proved its effectiveness in the treatment of pit and fissure caries, root caries and interproximal caries. It acts by oxidizing the pyruvic acid produced by cariogenic bacteria to acetate and carbon dioxide.[35] The treatment provides an alternative therapy to conventional drilling and filling for non cavitated deciduous carious lesion. Ozone treatment either alone or combined with a re-mineralizing solution was found to be effective for remineralization of initial fissure caries lesions.[36,37] Randomized double blind standardized clinical studies are however required to establish Ozone therapy as the stand-alone treatment of dental caries. Application of ozone for 40-60 sec also reduces dentinal hypersensitivity of teeth, instantly.[30]

Ozone and Dental Unit Water Lines (Duwl)

Kohno *et al*., published their results that sbowed acidic electrolyzed water could be applied as an appropriate measure against bacterial contamination of the DUWL.[38] In model dental unit water lines, ozone achieved a 57% reduction in biofilm and a 65% reduction in viable bacteria in spite of being used in a very low dose and with a short duration of application.[39]

Conclusion

The use of ozone has opened new vistas in treatment modalities. Now, with ozone treatment the fear of injections and drills has become the thing of past. Treating patients with ozone reduces the treatment time with a great deal of difference, eliminates the bacterial count more effectively, and moreover, it is completely painless, so increase the patients' acceptability and compliance. Ozone, definitely, seems to be a promising treatment modality for various dental problems in future. On the other hand we have to keep in mind that presently ozone is an adjunct to other conventional treatment modalities and should be used in combination until more research shows its benefits in independent usage.

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Hyperammonaemia in Absence of Deranged Liver Enzymes with Valproate: A Case Report

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Abstract

Valproate is used for various neurological and psychiatric conditions. It has various adverse effects, most of which are mild and transient, however, hyperammonaemia in the absence of abnormal liver function is an uncommon and life-threatening side effect. The exact mechanism for this is unknown, but several mechanisms have been described for the same. We present a case of 11-year-old male patient belonging to a broken family. Initially he was diagnosed as a case of ADHD with conduct disorder. However due to non-response to medications, over time, a EEG was done and was found to have generalised epileptiform discharges, He was maintaining well on Valproate, but had an acute exacerbation of symptoms on being off medications for 2 weeks and grandmother restarting Valproate in high dose. Response to the treatment provided to the patient was rewarding.

Key words: *Hyperammonaemia, Sodium Valproate, ADHD with conduct disorder, Epileptiform discharges.*

Introduction

Valproate is branched chain fatty acid that is used in the treatment of various psychiatric and neurological disorders like epilepsy, Bipolar affective disorder, and Schizoaffective disorder. Although, most of the adverse effects associated with Valproate are mild and transient, an uncommon and serious complication of Valproate therapy is hyperammonaemia [1,2]. The exact incidence of this serious adverse effect is not certain, however, reported incidence of asymptomatic hyperammonaemia in children is around 20% and symptomatic was 5% [3]. Patients receiving other anti-epileptic drugs (AEDs) along with Valproate are at an increased risk of developing hyperammonaemia [3]. Hyperammonaemia can present in both ways, with or without hepatic dysfunction in both children as well as adult population [4]. This can occur even at therapeutic levels of Sodium Valproate [4].

Here, we report a case of a male child who developed an acute onset of hyperammonaemia without hepatic enzyme derangement.

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Case

Mr. NT is an 11-year-old male child from a broken family, parents separated immediately after birth and patient was brought up by maternal grandmother. The child has no access to the mother as mother remarried and stays in another state and biological father passed away due to unknown illness. He first attended Psychiatry clinic in 2015 with symptoms of agitation, restlessness, poor attention span, being “always on the go”, breaking things and physical aggression towards grandmother and classmates. He was first diagnosed as a case of ADHD with conduct disorder and was started on Tab. Atomoxetine 10mg per day along Tab. Risperidone 1mg per day. The dose was gradually increased to 20mg/day and 2mg/day, respectively. Patient’s inattention improved, but physical aggression and restlessness did not improve. An EEG was done to rule out co-morbid seizure disorder, as patient’s younger half-brother had a

history of seizure disorder and was under treatment. EEG was suggestive of generalised epileptiform discharges. He was then started on Tab. Sodium Valproate 300mg per day and dose was gradually increased but the patient did not respond and EEG showed persistent generalised epileptiform discharges. He was then started on Tab. Oxcarbazepine 300mg per day and dose was gradually increased to 600mg/day in divided doses along with Tab. Valproate (600mg per day in divided doses). Subsequent EEGs demonstrated no seizure activity. Patient demonstrated significant improvement in behaviour and started attending school regularly and there were no further complaints from his teachers and peers. He was maintaining on Tab. Sodium Valproate 600 mg in divided doses for 3 years, Tab. Oxcarbazepine 600mg in divided doses for 1.5 years, Tab. Atomoxetine 20mg OD for5 years and Tab. Risperidone 2 mg HS for 5 years. The child was maintaining well on these medications.

The grandmother skipped medications for about 2 weeks due to financial issues in February 2020. After obtaining medications again from a local pharmacy using old prescription, she restarted the patient on the same dosage about 6 days prior to the presentation in the OPD. Once the medication was restarted, the patient developed symptoms of reduced appetite, hyperactivity and aggressive behaviour in the form of biting and breaking things. He had become increasingly violent and was brought to the OPD with these symptoms for 3 days. The grandmother was asked to admit the patient as an exacerbation in psychopathological symptoms was suspected, but as the grandmother was not willing for admission, Tab. Clobazam 10mg in divided doses was added for the patient. Two days later, the grandmother reported to the emergency room with the patient complaining of an exacerbation in his symptoms of hyperactivity, impulsivity and violence directed towards self and others. The patient further complained of a generalised continuous headache, and reduction in appetite. On examination, he was found to have flapping tremors, hyperactivity, inability to concentrate, marked aggression, irritable mood and labile affect. He was admitted in the IPD under Psychiatry department and all the medications being given to the patient were withheld for 1 day. He was subjected to complete blood count (CBC), liver function tests (LFTs), kidney function tests (KFTs), Sr. electrolytes, Plasma Ammonia andSr. Valproate. His blood work was within normal limits for CBC, LFT, KFT, Sr. electrolytes.However, his plasma ammonia was found to be elevated at 124 µg/dL (Normal range at <77 µg/dL) and Sr. Valproate levels marginally elevated at 121 µg/mL (Normal range 50-100 µg/mL). Patient was restarted on all the medications except Valproate after obtaining the lab results along with lactulose. He was symptom free after 4 days of hospitalisation and was discharged in medically stable condition and normal plasma ammonia levels (65µg/dL). EEG was done before discharge and did not show any epileptiform activity. He was also restarted on all other medications other than Tab. Valproate. On his follow up after a month, patient reported no symptoms of irritability, aggression and hyperactivity.

Discussion

The common symptoms of hyperammonaemia can vary from irritability, aggression, flapping tremors to drowsiness, coma and paradoxical seizures [3].

The patient was suspected to have an exacerbation in his previous symptoms of psychopathology, i.e. hyperactivity, aggressive and violent behaviour. Hence, the addition of Tab. Clobazam was tried on the previous visit. However, when there was a worsening in symptoms, all the medications were withheld as raised ammonia levels secondary toValproate was suspected. Valproate induced hyperammonaemia is more common in children [3] and many reasons have been cited for the development of hyperammonaemia in patients undergoing Valproate therapy which include, but are not limited to, urea cycle enzyme deficiencies [5], underlying liver disease [6], concomitant AED usage [1], high initial dose of Valproate [7], chronic usage of Valproate [8] and high catabolic states like pregnancy, infection or trauma [1]. In our patient, initial high dose of Valproate was found to the risk factor.

When compared to patients of hyperammonaemia secondary to liver disease, patients of valproate induced hyperammonaemia have normal liver function tests, which suggests pathology other than liver cell dysfunction [9]. Our patient had normal liver function along with normal proteins and bilirubin levels. The patient was, however, not tested for any underlying urea cycle enzyme abnormality.

The mechanism of hyperammonaemia is not completely understood and it is believed several mechanisms are involved [1].

Decline in Carbamoyl phosphate synthetase 1 (CPS 1) activity: This enzyme is crucial for urea cycle as it is the first enzyme of ammonia cycle. Generation of Propionate, which is metabolite of Valproate, decreases the hepatic concentration of N- Acetylglutamate (NAG). NAG under normal circumstances, acts as an activator of CPS 1 [10].

· Depletion of hepatic carnitine levels: Metabolism of Valproate occurs through β fatty acid oxidation which requires carnitine [11]. Carnitine helps in generation of Adenosine triphosphate (ATP) from fatty acids. Since there is depletion of carnitine, Acetyl Coenzyme A (Acetyl Co-A) production is reduced owing to reduced β oxidation of fatty acids. This reduction Acetyl Co-A ultimately leads to disruption in urea cycle causing an accumulation of ammonia [10,11].

· Increment in mitochondrial glutamine transport: With an increase in mitochondrial glutamine transport, there is an increased uptake of glutamine and release of ammonia by the kidneys [10].

Hyperammonaemia and serum valproate levels have no direct correlation [12,13], however, they may have a synergistic effect [14]. It is also necessary to emphasize here that a previous tolerance to Valproate does not preclude the occurrence of Hyperammonaemia [3].

Hyperammonaemia is a serious and life-threatening adverse effect of Valproate therapy which needs a high suspicion to diagnose as it can occur in the absence of hepatic enzyme derangement. It has a varied clinical presentation ranging from tremors, lethargy, irritability, drowsiness, and coma, however, in psychiatric setting, it can present as an exacerbation in previous psychopathological symptoms and withdrawal of Valproate leads to promising results which is consistent with a reduction in ammonia levels and symptomatic improvement in the patient.

Ethical Clearance: Ethical clearance for publication of the case report was taken from the Institutional Ethics Committee, Datta Meghe Institute of Medical Sciences (Deemed to be University). Patient's consent was also sought.

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Original Article

Assessment of Anxiety during COVID-19 Pandemic among General Population of India

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Abstract

Introduction -The coronavirus COVID-19 illness makes a very unpredictable future the main psychological effect is elevated rates of anxiety, because of pandemic and the lockdown.

Aim -The aimed of the study was to Assess the level of anxiety during COVID-19 among general population in India.

Methodology -A Snowball sampling technique was used to collect the samples from different state and union territory of India. Total 829 participants respond and enrolled themselves in the study. Participants in the study belong to 27 states or union territories of India and majority of them are from Delhi.

Result-Most of the Participant in the study was female (54%), graduate (51%), age between 18-28 (60%) and Hindu (89.3%). Half of the participant were having minimal anxiety (57.1%) nearly one fourth of the participant were having Mild anxiety (28.5%) and least number of participants were having Moderate anxiety (11.0%) and Severe anxiety (3.5%).There was no significant association of Anxiety scores with Socio-demographic variable except place of stay, suffering from any health problem and During lockdown period time spent in watching news related to corona (COVID-19) on mobile, laptop, television etc.

Conclusion-The study concluded the outbreak of coronavirus disease 2019 (COVID-19) Cause anxiety among the people. Understanding the risk to yourself and people you care about can make an outbreak less adverse effect on mental and physical health of the people

Keywords- *Anxiety, COVID-19, General population of India*

Introduction

A public health emergency of worldwide concern-novel coronavirus disease (COVID-19) outbroke as a global health threat. Coronaviruses named due to the outer fringe of covering proteins resembling crown are a family of enveloped RNA viruses.[1] The new disease caused by the coronavirus; it was named as Novel coronavirus. In February, the World Health Organization gave the name COVID-19. The CO stands for corona, VI is for virus. And D means disease and the19 is stand for 2019, the year the disease first appeared in China.[2]

The war between Virus and Human started when Corona virus outbreak came to light on December 31, 2019 when Wuhan Municipal Health Commission, China informed the World Health Organisation about group of cases of pneumonia in Wuhan City because of unexplained cause. [3]Subsequently the disease spread like Firewood in China, and to the rest of the world.

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The WHO has declared the disease as a pandemic.[4] The World Health Organization has announced COVID-19 as the sixth public health emergency of international concern.[5]

Novel Corona Virus Disease (COVID-19) has soon spread all over the world, infecting people throughout the worldwide.[6] This phenomenon has led to an immense public reaction; the media has been continuously reporting about corona virus to keep all informed about the pandemic situation. People many focuses on how much active case is there in their states, how many recovery and numbers of death because of COVID-19 which effect their mental health and make them worried.[7]

On 24 March 2020, the Government of India declared a nationwide lockdown for 21 days for safety of the people and limiting movement of the entire 1.3 billion population of India as a preventive measure against the COVID-19 pandemic in India, followed by enforcement of a series of rule and regulations in the country's specially the COVID-19 affected regions to prevent the spread of the disease.[8]The country divided into red, orange and green coronavirus colour zones depending on the number of positive cases in the proposed extended lockdown period.[9]

COVID-19 prevention and control measures can be difficult to implement in humanitarian crises or places with low capacity.[10] Indeed COVID-19 has impacted the mental health of the entire community in one or another manner. This has created an unprecedented mental health challenge in the face of limited specialist resources.[11]

All these things are creating a lot of concern for people leading to heightened levels of anxiety. As COVID19 is a new disease and is having the most upsetting effects globally, its emergence and spread, causes confusion, anxiety and fear among the general public. Anxiety is a normal response to any uncertainty and things that may harm us and our close one. Mainly the vulnerable group may get more anxious.[12]

Anxiety, when above its normal level, weakens the immune system and as a result the risk of the virus infection increases. In addition, people's anxious reactions trigger public disruptive behaviours as people rush to stores, health centres, and pharmacies and health supply become scarce and the country health care service provision is affected.[13]

People worry about how to deal with his virus and manage their own and family members health both here and abroad. People may also have a lot of concerns about their life and finances, their ability to take part in important community and social events and hobbies, and other important parts of their lives. People who already experience a lot of anxiety and don't know how to deal with it may find their anxiety worsening. [14]

When anxiety affects a larger population, leading to exhaustion of resources. It also can lead to limitations in daily activities, avoidance behaviour causing limited socialization, increase anger, feel helpless etc Because of anxiety, people adopt various unwanted lifestyle dietary modifications and negative defence mechanism under the influence of rumours.

Materials and Methods

This was a cross-sectional, observational study carried out in general population of India during 8th May to 19th May 2020 at 4 PM IST. A Snowball sampling technique was used to collect the samples from different state and union territory of India. Total 829 participants respond and enrolled themselves in the study. An online questionnaire was developed by using google forms the link of the questionnaire was sent through emails, WhatsApp and other social media. The participants were encouraged to roll out the survey to as many people as possible. Thus, the link was forwarded to people a part from the first point of contact and soon once the Form is submitted, they give the informed consent. After they accepted to take the survey, they filled up the demographic details. Then a set of several questions related to Anxiety, which the participants have to answer all the questions no answer was skip. Participants with access to the internet and age should be 18 years and more, able to understand English and willing to participate were included.

The online self-reported questionnaire was validated by 5 Experts. The online self-reported questionnaire contained the following two sections related to demographic variable and Anxiety during the pandemic of the novel coronavirus done by GAD-7 Scale.

Description of Tool

Socio-Demographic Variable-The socio-demographic variables included which State belongs to in India, age, gender, marital status, Educational status, occupation, religion, number of members in the family, family Income, place of stay, occupation, are you suffering from any health problem and During lockdown period time spent in watching news related to coronavirus (COVID-19).

Generalized Anxiety Disorder 7 Scale- Anxiety related to novel coronavirus infection done by standardised tool General anxiety disorder scale a had 7 items that were rated on a 4-point Likert scale ranging from Not at all, Several days, More than half the days and nearly every day.

Result

An online survey was conducted to assess level of anxiety experienced in the community during the pandemic among Indian population. A total of 829 participants were filled the form. The study included only those participants who understood English and had access to the internet and age 18years and above, any gender and Unwilling and not submitted the google form were excluded.

Statistical analysis was done by SPSS 20 Version and Descriptive analysis was done in terms of mean and standard deviation with range for continuous variables and for ordinal and nominal variables frequency with percentage was used. Mean and standard deviation have been used to estimate the results of the study. All analyses were done with the help of SPSS Version 20.

The participants belong to 27 states or union territories of India. Majority of the participant belongs to Delhi (25.9%) followed by Haryana (23.6%), Uttarakhand (11.1%).Most of representative were belongs to the age 18-28 years (60%), Female (54%), Single (60%), educational qualification graduate (51%), 4-6 members in the family (66%), Family income 30,000-60,000 (30%), students (37%), suffering from no health problems (93.6%) and during lock down period time spent in watching news related to corona (COVID-19) on mobile, laptop, television etc is mostly 1-3 hours (81.7%) .

The level of anxiety among general population of India it shows that half of the participant were having minimal anxiety (57.1%) during COVID-19 Pandemic. Nearly one fourth of the participant were having Mild anxiety (28.5%) and least number of participants were having Moderate anxiety (11.0%) and Severe anxiety (3.5%). The Mean and Standard Deviation of anxiety score was 4.68±4.53 shows in Table 1.

Table 1- Frequency, percentage, Mean and SD in terms of level of Anxiety

Level of Anxiety	Range	f (%)	Mean ± SD
Minimal Anxiety	0-4	473(57.1%)	
Mild Anxiety	5-9	236 (28.5%)	
Moderate Anxiety	10-14	91(11.0%)	4.68±4.53
Severe Anxiety	Greater than 15	29 (3.5%)	

Further, Association of level of Anxiety with Socio-demographic variable was tested by using one-way ANOVA and Independent t-test among general population. There was no significant association of Anxiety scores with Socio-demographic variable except place of stay (t=7.48, p=0.00), suffering from any health problem(t=6.38, p=0.00) and During lockdown period time spent in watching news related to corona (COVID-19) on mobile, laptop, television etc (F=3.38, p=0.03) at 0.05 level of significance

Post Hoc showing the significant difference among period time spent in watching news related to corona (COVID-19) on mobile, laptop, television etc. It shows that the association was due to mean difference (0.69) in the time (1-3 hours vs 6 hours) i.e p=0.01 was significant at 0.05 level of significance shows in Table 2.Hence it concludes that those who spent in watching news related to corona (COVID-19) on mobile, laptop, television etc more they are having higher anxiety level.

Table 2- Post Hoc showing the significant mean difference in association of Anxiety scores among general population

N=829

Socio Demographic variable	Category	Mean Difference	Standard Error	P value
During lockdown period time spent in watching news related to corona (COVID-19) on mobile, laptop, television etc	1-3 hours vs 4-6 hours	0.39	0.47	0.39
	1-3 hours vs 6 hours	1.76	0.69	0.01*
	4-6 hours vs 6 hours	1.36	0.80	0.09

^{NS} -Not significant (p>0.05) *- Significant (p < 0.05)

Discussion

The present study aimed to assess the level of anxiety during COVID-19 among general Population of India. This survey was started during the 3rd locked in India when the cases were arising in number day by day and People were facing anxiety related to fear of having the diseases, social distancing, isolation and doing work from home.

In present study most of the participant were Hindu (89.3%) and graduate (51%) this finding consists with the study conducted by Kaustav Chakraborty and Moumita Chatterjee (2020) where they found that most of participant were Hindu (96.8%) and graduate (56.2%).[15]

The data showed that most of the participant was graduate and above and half of them were female (54%) and male (46%) these finding similar with the study result conducted by Roy D, Tripathy S(2020)[13] where they found that highest qualification of more than 90% of the population was graduation and among the participants, 51.2 % were females and 48.6% were males and same as the study finding conducted by Moghanibashi-Mansourieh (2020) where most of the participant where female (65.8% and male (34.2%).[16]

In addition, in the present study most of the participants were single (60.3%) and student (37%) and belongs to the age 18-28 (60%) Same finding showed by the study conducted by Gao J, Zheng P (2020) where most (47.9%) were aged 21–30 years. Many participants (62.2%) was having college education, more than half of them were married. [17]

These may affect mental health adversely. Media influences the mental well-being and add to the level of anxiety. The swine flu pandemic of 2009–2010, which resulted in high mortality world-wide also caught global media attention and evoked anxiety among the public significantly. [18]

In addition, During lockdown period time spent in watching news related to corona (COVID-19) on mobile, laptop, television etc more found significant difference in association between (1-3 hours) vs (6 hours) are more prone to develop anxiety this finding is similar with the finding (Huang Y, Zhao N, 2020) where people who spent too much time thinking about the out- break (≥ 3 hours) were likely to develop anxiety symptoms. The

more people followed coronavirus news, the more the level and severity of their anxiety symptoms increased. The research findings show that people who most follow COVID-19 news experience more anxiety and as WHO the media and the press should not only focus on the negative aspects of producing and publishing news. [19]

The anxiety was not having any different between gender during COVID-19 outbreak these finding is contrast with the finding of the study done by Guo et al., 2016; Gao et al. (2020)[20] where women were more likely to have anxiety than men. And also, there is no major difference in anxiety according to the age this finding also contrasts with the finding (Huang Y, Zhao N, 2020) where Younger participants (< 35 years) were more likely to develop anxiety and depressive symptoms during COVID-19 outbreak than older participants (≥ 35 years). [21]

In present study most of the participant having (57.1%) minimal anxiety and severe anxiety (3.5%) this finding similar with the finding (Ahmed MZ, Ahmed O, 2020) where the participant having mild anxiety (10.1%) and severe anxiety (12.9%). [22]A study in China showed that most of the participants experienced moderate to severe anxiety symptoms, and it complies with the findings of the present study. In this research half of the participant having minimal anxiety 473(57.1%) followed by mild anxiety 236 (28.5%), Moderate anxiety 91(11.0%) and sever anxiety 29 (3.5%). [23]

The study is limited to the people who had Internet to access to fill the form and the ability to Understand and read English. This represents the educated population of the country, so it should not be generalized to the whole population. The anxiety in uneducated people and dose who are not able to access internet may be differ from the findings of our study and we were unable to assess an individual's psychological conditions before the outbreak of COVID-19 among general population.

Conclusion

The outbreak of coronavirus disease 2019 (COVID-19) Cause anxiety among the people. Understanding the risk to yourself and people you care about can make an outbreak less adverse effect on mental and physical health of the people This survey was an attempt to asses anxiety during pandemic among Indian population. These findings implicated the government also need pay more attention to mental health problems, especially on anxiety among general population.

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A Conceptual Study on Prevention & Management of Covid -19 Pandemic with Special Reference to *Janapadodwamsa*

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Abstract

Introduction: Ayurveda being one of the oldest surviving Medical Tradition, has a key role to play in this crucial situation.Covid-19 it is not a simple infectious disease it is a pandemic disease caused by Coronavirus which reflects in human but stay as a host in animals. These viruses contain seven different strains; of which SARS-COV-2 is responsible for Covid-19. Its spread through droplets via air due to sneezing, cough, talking and direct or indirect contact with an infected patient such as handshake, touch surfaces contact by an infected person. This virus causes the severe acute respiratory syndrome. Its incubation period varies between 2 to 14 days, the disease can transmit from symptoms less person to person. **Material & Methods:** Covid-19research information was collected from the world health organization, books, research papers, journals. Ayurveda literature from reference books, internet, and opinions of experts utilize as a review. **Observations and Results:** COVID-19 can be provisionally understood from the Ayurvedic perspective as *vātakapha* dominant *sannipātajvara* of *āgantu* origin with *pittānubandha*. The asymptomatic, presymptomatic, mild, moderate, severe and critical stages of COVID-19 with varying clinical presentations have been analysed on the basis of *nidāna*, *doṣa*, *dūṣya*, *nidānapañcaka* and *ṣaṭkriyākāla.* **Conclusion**: It will be good to take preventive measures which boost our immunity in these times. Ayurveda extensive knowledge base on preventive care by which each individual can achieve by uplifting and maintain his or her immunity. In Ayurveda, Immunity comes under the heading of *Vyadhiksamatwa*. In Ayurveda, many single drugs or compound formulation were mentioned as *Rasayana* to boost up Immunity (*Bala* or *Vyadhiksamatwa*).

Keywords*: Vyadhiksamatwa, Janapadodwamsa,* pandemic diseas, Coronavirus.

Introduction

The present Pandemic of COVID-19 has brought the so-called human activity to a standstill, and has forced the entire world to seek very seriously for its cause, mechanisms of pathogenesis and its solution.[1] Ayurveda being one of the oldest surviving Medical Tradition, has a key role to play in this crucial situation.Covid-19 it is not a simple infectious disease it is a pandemic disease caused by Coronavirus which reflects in human but stay as a host in animals. These viruses contain seven different strains; of which SARS-COV-2 is responsible for Covid-19.[2] Its spread through droplets via air due to sneezing, cough, talking and direct or indirect contact with an infected patient such as handshake, touch surfaces contact by an infected person. This virus causes the severe acute respiratory syndrome. Its incubation period varies between 2 to 14 days, the disease can transmit from symptoms less person, and its vaccine trial is going on all of this situation makes disease fatal. As COVID-19 is proving to the world, tiny viruses are powerful biological agents, capable of rapidly effecting

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massive damage and fatality[3]. And yet, they're so simple: a virus is merely comprised of pieces of genetic material—either RNA or DNA—wrapped in a protein envelope, which is known as a "capsid." Yet this tiny organism carries with it a mortality rate (death rate). The present work uses the model of *Janapadodhwamsa* of *Acharya Charaka* and *Maraka* of *Acharya Sushrutha* along with the model of *Masurika* of *Acharya Madhava* to explain the Pathogenesis of COVID –19 with modern correlation. The study identifies it as Nija Jwara and *Oupasargika Roga*, proposes a Diagnostic Protocol based on [4]Acharya *Charaka's Jwara* model, identifies factors affecting its severity or complicating its course, along with their modern correlation and proposes management guide lines for different stages and various subsets of patients; while describing the general contraindication of drugs, their potential interaction with concomitant modern medicines, criteria for selection etc[5]. The study also identifies important preventive principles along with medications with aim to restore balance between man and nature.

Material & Methods

Covid-19research information was collected from the world health organization, books, research papers, journals. Ayurveda literature from reference books, internet, and opinions of experts utilize as a review

Observations and Results

The Causative factors

The causative factor of *Janapadodhwansa*[6]*:*-

· *Adharma*; means persons are following path those are not beneficial to humanity as well as animals.

· *Pradhnyaparadha*; it means a person doing the action by his intellect, past restraint memory and present memory is deranged,its reason for increasing disease-causing elements called Doshas.

· *Opasarga*; *Acharya Sushruta* mentioned *opasarga* elements are, direct contact of an infected person, sexual intercourse, inhalation, sharing same diet-cloths and placed. These reasons are responsible for skin disease, fever, *Shosha* (tuberculosis), ophthalmic conjunctivitis and contagious disease.

· The organism of other species those are not seeing by open eyes that enter inside the human body and cause *Janapadodhwansa*.

· Another reason is war due to the large population affected and killed.

· Due to the disorder of seasonal weather.

Causes of Infectious disease

Acharya Sushruta mentioned *Bhoot* Grahapida under the eading *Amanussopasarga Pratisedham* chapter it means an infectious disease caused by non-human being or invisible matter.[7] It is innumerable. Causes are external injuries, impurity, uncleanness, violence, coitus, demand worship and breaking the rules.[8]

Patho-physiology

Most patients with COVID-19 predominantly have a respiratory tract infection associated with SARS-CoV-2 infection. However, in a small proportion of cases, they can progress to a more severe and systemic disease characterized by the Acute Respiratory Distress Syndrome (ARDS), sepsis and septic shock, multiorgan failure, including acute kidney injury and cardiac injury. Autopsy findings in China and European countries showed endothelial damage of pulmonary vasculature, microvascular thrombosis and hemorrhage linked to extensive alveolar and interstitial inflammation that ultimately result in COVID-19 vasculopathy, pulmonary intravascular coagulopathy, hypercoagulability, ventilation perfusion mismatch, and refractory ARDS. Hypoxemia, secondary to ARDS may also activate the coagulation cascade.[9]

Disturbance of air*/Air Currents*: Power Sector (Thermal)/Industrial pollution/Military activity related Pollution/Automobile and Aviation sector related Pollution.

Disturbance to Ecology of water bodies: Pollution of water bodies/Sand mining from river beds/Unregulated damming etc./Contamination of Ocean beds.

Disturbance to Land Ecology*:* Rapid Urbanization and huge constructional activity with non-eco-friendly materials/Over exploitation and destruction of mountains, forests & ecosystems. Destruction of other life forms like Animals/Plants/Birds and displacement

from their natural habitats/Usage of Non–Biodegradable toxic materials like plastics pesticides etc.[10]

Activities of this kind vitiate and disturb the balancing forces of nature and gives rise to abnormal air currents, Cyclones, Floods, non-seasonal rains, extreme weather conditions etc. Destruction of Amazon wild forests or Destruction of Australian forests due to wild fire, Extreme weathers, non-seasonal rains experienced around Europe, Australia, India etc. can be taken as *Aristas* (warnings).

The vitiation of air currents, seasons, seasonal irregularities and disturbance of land and water ecology simultaneously induces leads to vitiation of *doshas* (Cytokines) in humans causing mass susceptibility to the same illness, while also affecting the *oshadhis* (food yielding plants), thus reducing the nutritive value and leading to under nourishment. When this combines with the causative factors causing agni mandya (sluggish metabolism) it leads to infectious diseases like COVID-19, probably more in future if corrective actions are not taken. The predominant pulmonary involvement of covid-19 can be explained by the commonality of *pranavaha srotas* vitiating factors with that of *vata*, (*Kshaya*-weight loss, *vega sandharan*-constipation, *rukshata*-low fat high fibre diet intake, *vyayamat kshudhitasya*-inappropriate excessive exercise.[11]

It is the site of *Kaphadosha*, Due to accumulation of *Kapha* causes *Rasavaha*, *swedavaha*, *ambuvaha*, *pranvahasrotasdhusti* (inflammation). Srotavahrodha results in an obstruction of *Vayu*, and *Pitta Dosha* results in a Fever, Cough, Difficulty in breathing, Pneumonia to severe respiratory failure. It's all depends on *Vyadhishamatva* (Immunity).[12]

Clinical Features:-

In case you're still unsure the three most important symptoms of coronavirus infection are:

- Dry cough
- Fever
- Difficulty Breathing

Other common symptoms are:

- Nasal congestion
- Phlegm accumulation
- Sore throat (pharyngitis)
- Fatigue
- Muscular aches (myalgias)
- Diarrhea
- Headache
- Joint pains

Principles of Ayurvedic Prevention and Management of Coronavirus:-

For any condition affecting *pranavaha srotas* (respiratory channels) one should apply the treatment protocols recommended for *Shvasaroga Chikitsa*—Breathlessness, cough, and related disorders.[13]

- To reduce the aggravated and accumulated Vata and Kapha *doshas*.
- To purify *rasavaha srotas* (blood plasma)
- To purify and unblock *pranavaha srotas*
- Secondarily to optimize *agni*.

Treatment

- *Sarve Abhyanga* (full body external friction-type oil massage) with *Dhavantaram* taila, *Dashmooladi* taila, or *karpooradi* taila with added lavana (crushed rock salt).
- *Ruksha sweda* (dry heat), *nadi sweda* (steam administered locally through tubes).
- Pinda sweda (boluses of hot herbalized rice balls) applied to chest and back.
- *Vamana* (physician-assisted gentle vomiting) using *Madanaphala* decoction + *yastimadhu* + vacha = salt
- Alternating Anuvasana and *Niruha basti* (oil-based and decoction-based enemas)

Preventive

Your best line of defense is to choose a healthy lifestyle. Following general good-health guidelines is the

single best step you can take toward naturally keeping your immune system strong and healthy. Every part of your body, including your immune system, functions better when protected from environmental assaults and bolstered by healthy-living strategies such as these:[14]

- Try to minimize stress
- Eat a diet high in grain, fruits and vegetables.
- Exercise regularly.
- Maintain a healthy weight.
- If you drink alcohol, drink only in moderation.
- Get adequate sleep.
- Observe meticulous hygiene, such as washing your hands frequently
- Don't smoke.

Conclusion

Ayurveda has the ability to deals with any disease. In the case of Covid-19Ayurveda has preventive as well as curative aspect. Preventive aspect is *Dincharya*, *Rasayana* and Panchakarma therapies. The therapeutic element contains *Rasayana*, *Panchakarama*, medicinal formulation, mantra therapies and spiritual therapies. The present need for Ayurveda researcher in clinical trials on the patient of Covid-19. Ayurveda cures disease and strengthens physical as well as mental levels. The present proposal is based on classical understanding of Ayurveda and it needs to be emphasized that as of date there are no known curative medicines for this virus. Hence this approach has to be applied pragmatically, taking into consideration various aspects, like a proper evaluation of the individual patient based on Ayurvedic parameters, with due advice and inputs from the colleagues from Modern medicine, proper discussion in a integrative scenario of possible drug to drug interaction and limitations of therapy one own experience with usage of particular drugs, with proper preventive contact and respiratory precautions in place, and results being documented systematically particularly when dealing with sick patients and critical conditions. But we strongly feel that Covid 19 presents a very valuable opportunity for Ayurveda to join hands with Modern Medicine and enrich itself and Science in general for betterment of Mankind.

Conflicts of Interest- No

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The Use of Information Gained under RTI Act- 2005 in Forensics and Toxicology- An Indian Perspective

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Abstract

The role of forensic reports are to prove without doubt in the court the cause of death, injury nature of death or accident but courts till date do not fully rely on forensic reports as evidence. A study conducted in 2011 by Supreme Court and High Courts, DNA has played an important role in only 47 cases. Out of these, 23.4% decisions were given by Delhi High Court alone. Furthermore, DNA evidence had been used in merely 4.7% murder cases and 2.3% rape and murder. The Committee on Reforms of Criminal Justice System also signifies that the present stage of applicability of forensic science in crime scene investigation is somewhat low in our country, with only 5-6% of the registered crime cases being referred to the Forensic Science Laboratories (FSLs) and Finger Print Bureau put together.

Article 20(3) of the Indian constitution states that "No person accused of any offence shall be compelled to be a witness against himself but the honourable Supreme Court held otherwise. In the case of The State of Bombay vs Kathi Kalu Oghad And Others (AIR 1961), the Supreme Court held that, compelling any person to give any sort of forensic evidence does not violate Article 20(3) of the constitution. The same is given in Section 73 of the Indian Evidence act, which states that any person can be asked to give the fingerprint or DNA examination even to the accused. Similarly, Section 53 of the criminal procedure code states that a person accused of any crime can be asked to undergo a medical examination if the officers feel that the examination can provide some evidence to the crime. Further Section 164A of the procedure allows the medical examiner to examine the victim of the rape case within twenty-four-hours of the crime.

Keywords:- *Right to Information, Legal knowledge, Forensic, Toxicology , use of information in Forensic and Toxicology.*

Introduction

The Right to Information Act, 2005 was passed by the parliament on 15th June 2005 and the basic objective of enacting this act was:-

"An Act to provide for setting out the practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, the constitution of a Central Information Commission and State Information Commissions and for matters connected therewith or incidental thereto by the parliament of India."

It is important to understand the views of Supreme Court with respect to the constitutional right of every citizen to seek information from the public authorities. In the case of Union of India vs. Association for Democratic Reforms and Another wherein the Honourable Apex Court referring to its earlier judgment delivered in Dinesh Trivedi M.P. and Others vs. Union of India and Others, reiterated the fact that the right to get information in democracy is recognised all throughout and it is natural right flowing from the concept of democracy. At this stage, we would refer to Article 19(1) and (2) of the International Covenant on Civil and Political Rights which is as under:

(1) Everyone shall have the right to hold opinions without interference.

(2) Everyone shall have the right to freedom of expression; this right shall include freedom to seek, receive and impart information and ideas of all kinds, regardless of frontiers, either orally, in writing or in print, in the form of art, or through any other media of his choice.

The RTI since 2005 has been a huge pillar in promoting huge transparency of various departments and helped unveil many scams and foul play by various government departments be it the Adarsh scam, 2G scam, or the Indian red cross scam amongst others. The right to information act of 2005 has given a way to human right activists to hold public departments accountable. These are just a few examples where the RTI has played a key role.

Constitutional validity of forensic reports and limited use by courts

Forensic science is defined as "The application of science to those criminal and civil laws that are enforced by the police agencies in a criminal justice system." The Indian Evidence Act 1872 defines a forensic report as a "belief" tendered by expert. An expert may be defined as a person who, by practice and observation, has become experienced in any science or in his area of expertise. He is one who has devoted time and knowledge to a special branch of learning, and is thus especially skilled in that field wherein he is called to give his expert judgment.

Article 20(3) of the Indian constitution states that "No person accused of any offence shall be compelled to be a witness against himself but the honourable Supreme court held otherwise. In the case of The State of Bombay vs Kathi Kalu Oghad And Others, the Supreme Court held that, compelling any person to give any sort of forensic evidence does not violate Article 20(3) of the constitution. The same is given in Section 73 of the Indian Evidence act, which states that any person can be asked to give the fingerprint or DNA examination even to the accused. Similarly, Section 53 of the criminal procedure code states that a person accused of any crime can be asked to undergo a medical examination if the officers feel that the examination can provide some evidence to the crime. Further Section 164A of the procedure allows the medical examiner to examine the victim of the rape case within twenty-four-hours of the crime.

The role of forensic reports is to prove without doubt in the court the cause of death, injury nature of death or accident but courts till date do not fully rely on forensic reports as evidence. A study conducted in 2011 by Supreme Court and High Courts, DNA has played an important role in only 47 cases. Out of these, 23.4% decisions were given by Delhi High Court alone. Furthermore, DNA evidence had been used in merely 4.7% murder cases and 2.3% rape and murder. The Committee on Reforms of Criminal Justice System also signifies that the present stage of applicability of forensic science in crime scene investigation is somewhat low in our country, with only 5-6% of the registered crime cases being referred to the Forensic Science Laboratories (FSLs) and Finger Print Bureau put together.[1]

Why is RTI applicable on forensic reports

Forensic reports and the forensic department come under the ambit of Section 4 of the RTI act where under

sub clause (a)of the section maintain all its records duly catalogued and indexed in a manner and the form which facilitates the RTI and to ensure that all records that are appropriate to be computerized within a reasonable time and subject to availability of resources, computerized and connected through a network all over the country on different systems so that access to such records is facilitated.

Forensic reports are kept under the ambit of the right to information because as one sees it from the above statistics, the courts do not rely on forensic reports. The primary reason of this is the unprofessional conduct of physical evidence, including improper collection, preservation, non-collection of clue evidence, non-maintenances of chain of custody, as well as negligent and delayed dispatch of physical evidence for scientific analysis.

Further Courts don't rely on the process followed like sending an accused for medico-legal examination, non-lifting of fingerprints by the Investigating Officer or when bloodstained mortal object had been sent for chemical examination without covering the same by a

wrapper immediately after the seizure. [2]

The delayed inspection of biological, serological and viscera exhibit in the cases of poisoning puts a big question mark on the legitimacy of evidence. The decomposition of such exhibits can generate alcohol in the exhibits, on long standing duration and may also not permit the detection of poison and conclusive serological results; likewise, in cases of drunkenness, the blood alcohol or urine alcohol negative samples may test positive for the presence of alcohol due to self-generation of alcohol on the putrefaction of samples and the huge backlog of cases delay in investigation and administration of justice. [3]

Yet the main objective of the RTI act is to promote accountability and transparency among public departments. The forensic departments also need to take accountability of the backlog of cases and the procedure of testing that takes place. As the job to determine the actual cause of death injury or the nature of death or injury is a job that needs high precision and skill, that may prove vital for investigation and evidence.

RTI making forensic labs accountable

In 2010 The FSL in Maharashtra had a huge backlog on then, to which an official of the forensic laboratory had said "Over the past year, we have been receiving around 15 RTI applications per month, the applications included policemen investigating the case and other concerned parties." The official admitted to the fact that several cases had been pending they were short staffed with lack of chemicals for testing and the laboratory had been attending to high priority cases[4]. Just because of a RTI the FSL had to admit to the delays and lot of cases being pending with the laboratory.

In 2011 the FSL in Delhi would have compromised criminal investigation as over a dozen of scientists were not qualified. They were hired after submitting false documents and false information. At least 15 senior scientific officers that were hired and half of them were appointed in the key facility. The Delhi high court issued notices to the recruiting authority i.e. UPSC after the appointments were questioned and exposed these appointments through a series of RTI in front of the High court.[5]

A RTI reply in the year 2013 revealed that there were a large number of cases that is cases of forensic reports, DNA tests and ballistic tests were pending in the FSL at Rohini in New Delhi , The reply states that the number of cases that were pending with the laboratory at the start of 2013 was 8991, the report also cites shortage of staff as a reason for the backlog where out of total sanctioned posts of 337 at FSL, 194 seats were lying vacant, with 5,443 cases pending with the chemistry department, 2038 case pending with the documentation department, 862 cases with the DNA department, 143 cases pending with the cyber unit and 46 cases with the physics department. As a result, a lower court had directed the Secretary, Home affairs of Delhi government to set up at least 12 forensic labs in the state to cope up with the stress.[6]

Similarly, a RTI reveal that the FSL in Maharashtra examined a total of 31,442 cases. This figure went up to 36,431 cases in 2014 to drastically fall to 26,514 cases being examined by the state laboratory in 2015. As a result of this, while 2013 saw 10,553 cases being carried forward to the next year and 9,013 such cases being forwarded in 2014, the year 2015 saw a drastic rise in the number of cases carried forward to the next year to 24,557. The officials claimed that they had a problem in hiring people in the time period and many of its employees moved to high paying jobs. [7]

In the year 2014-15 questions were being raised on the sanctity of tests conducted by one of the forensic labs after a dispute between the scientists and the CFSIL in Hyderabad led to samples of internal organs of 12 bodies allegedly thrown away. A second set of Viscera from the bodies were tested 16 months later after the home ministry finally decided to get involved in the matter. 15 letters had already been sent before the home ministry decided to jump in as revealed by a RTI, after the Supreme court's judgement that directed the forensic labs to "ensure that the viscera is examined immediately and report is sent to the investigating agencies/courts post haste". The ministry had finally set up a committee on 17th April 2015 to test the samples again. [8]

As of April 2019, 30,125 police cases were pending with 8 forensic laboratories and 2 mini labs. In a reply to an RTI by the Directorate of Forensic Sciences Laboratories has revealed that even though the numbers have slightly reduced in the later half of the year but

still there is a lack of Manpower in these kinds of jobs with only 733 posts filled out of 1500 posts that were sanctioned.[9]

The Right to information is not only making the forensic laboratories accountable for the huge number of cases that are pending with the labs but also in a way highlights the low infrastructure and the shortage of workforce in these laboratories that the government can eventually take action on.

Limitations to the use of Right to information in Forensic cases

One needs to keep in mind that there have been various instances where information has been denied under Section 8(1) which states that notwithstanding anything in this Act, there shall be no obligation to give information under certain circumstances.

In a case that is in the case of Mrs. Vijay Bala v. Central Bureau of Investigation, Central Forensic Science Lab, where the appellant had sought a lot of information from the CFSIL pertaining to a case. The public information officer denied the information under section 8(1) (h). The CIC observed that the reports in question were submitted to the CBI were being used for investigation. Revealing such information under an RTI would indeed compromise the process of investigation. The appellant could instead ask the CBI for such reports and it was up to the discretion of the CBI to provide or not to provide such information.

In yet another case that is Gouranga Paik vs Central Forensic Science the appellant had asked for the following details under the Right to information act

(1) The authority who did the test and signed the report

(2) The date and time when the tests were done

(3) Date and time when samples were provided for the test

(4) Education qualification of the person who conducted the tests

(5) Number of tests done by the C.F.S.L out of those tests how many of them were proved wrong and later stood corrected. The particulars of the appellant authority.

The PIO rejected the RTI stating the reason that the appellant had asked questions from a document held by them for forensic examination the request was rejected under Section 8(1) (g)& (h) and appealed in front of the central information commission.

The CIC held that the CFSIL that the documents in question are under the obligation to the forward authority and are not bound to reply to the RTI, revealing such vital information would lead to compromising the trial, investigation and the source of information

An important case where the power of RTI was limited was in the case of Pritam Kumar vs Forensic Science Laboratory. In this case the appellant was a director of a company and he alleged the other two directors used to forge his signature. The appellant had filed a case under section 420,468,471,120(B) and by the order of the Hon'ble Tis Hazari Court, New Delhi, his forged signature and original signature was sent to the Forensic science laboratory.

He alleged the Forensic science laboratory of corruption and delay. He appealed in the Central information and asked for the following:

(1) He claimed that even a normal person could tell the difference between the original signature and the forged one.

(2) The opinion of an expert under the RTI filed

(3) A Committee and a CBI enquiry to be set up probing the corruption and the procedure of investigation

The CIC dismissed the appeal stating that the commission cannot assume the role of a forensic expert and a public information officer cannot be burdened to create more information than he has just for the sake of the appellant. He can cross examine the information provided in the court but cannot use an RTI to cross examine the information provided.

These are huge limitations on the power of RTI the fact that you cannot crossly examine the information by Filing an RTI and the fact that what is under the ambit of section 8 (1) is not clear in such cases.

Conclusion

The RTI Act of 2005 is a powerful tool given to Citizens of India. Yet RTI on Forensics, doesn't have much role in ensuring the accountability of forensic labs Given that RTIs have revealed that the Forensic Labs have an acute shortage of manpower and Infrastructure bur what next did it solve the problem, did the efficiency of these labs increase? The question remains in one's mind when one looks at the number of cases pending with forensic labs that is 30 As of April 2019, 30,125 police cases were pending with 8 forensic laboratories and 2 mini labs. The power of RTI in the field of health is limited with no way to cross examine the information. The scope of information that one gets is also limited and the ambit of what falls under Section 8(1) in such cases is not clearly defined.

The fact that Courts too don't rely on the process of Forensic Collected and analysed tells us something about the credibility of these forensic labs and reports. In many cases the evidence that would have been crucial evidence in the case is not admitted by the court of law because the chain of custody was broken and yet reports of forensic labs in a case is considered as conclusive evidence in many cases. There is no doubt in my mind that a forensic lab, report plays an important role in determining the cause of death or accident and the nature of death But with so many cases pending and so many procedural issues that forensic evidence that can help the court reach a decision is rejected makes on think. The RTI Act of 2005 can only point out flaws and prove inefficiency of the public authority. The action that corrects these flaws revealed by an RTI on the Government. Government needs to act on these on an urgent basis because as the phrase goes justice delayed is Justice denied

Ethical Clearance- Taken from Ph.D guide of Amity Law School, Noida, Uttar Pradesh

Conflict of Interest - NIL

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Original Research Article

Profile and Pattern of Women Seeking Medical Termination of Pregnancy in a Tertiary care Centre in National Capital Region

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Abstract

Background: Induced abortion whether safe or unsafe, legal or illegal is a reproductive health service that is part of the lives of women, couples and communities all over the country. When faced with unintended pregnancies, especially in contexts in which women lack access to effective family planning, induced abortion is an important part of women's reproductive health care. In this backdrop the present study was undertaken with the objective to study socio-demographic profile, methods, various indications in Medical Termination of Pregnancy cases performed in our institution.

Method: This study was conducted in the Department of Forensic Medicine in collaboration with Department of Obstetrics and Gynaecology, at SMSR, GautambudhNagar, India. Information was obtained from the patient seeking abortion services, patient's case record file in the hospital, Medical Termination of Pregnancy (MTP) register and direct interview with concerned consultant in charge in the form of pre-designed questionnaire..

Conclusion: The most common reason for MTP was unwanted pregnancy due to non use of contraceptives by the women of reproductive age group. We recommend comprehensive contraceptive counseling can help prevent unintended pregnancies that lead to abortions and should include information on when fertility returns after a birth or abortion. Also dedicating a special cell to focus on a woman's unmet needs of contraception would go a long way in resolution of unintended pregnancies and abortions.

Key words: *induced, unintended, legal, reproductive*

Introduction

Women's reproductive' rights in general, and abortion in particular, have been the subject of intense debate globally. Even in countries where the law permits abortions women battle stigma, bias lack of awareness and information, all of which result in restriction of access to safe abortion.

Almost fifty years after abortion was legalized in India, a majority of women continue to lack access to safe abortion care. Unsafe abortion is the third leading cause of maternal mortality in India. Every day women die in India due to unsafe abortion related causes and many more suffer from morbidities such as pelvic inflammatory disease and infertility, which are related to unsafe abortion practices – ranging from home remedies

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to inserting sharp foreign objects into the cervix.

Women's rights often take a backseat, even when they remain at a higher risk of death and morbidities. In the context of a high burden of pregnancy related deaths, according to NFHS-4 data, 133 for every 100,000 cases in India, a progressive MTP act that support woman's choice is the need of the hour. The current Act in existence is conditional and does not allow women to exercise their reproductive rights.

In this light this study was adopted to study in depth the detailed profile of MTP cases presenting to our Hospital

Aims & Objectives

The objective was to study current scenario of MTP services existing in the hospital and the socio-demographic profile of patients presenting in the Department to procure abortion services.

• To study the profile and pattern of Medical Termination of Pregnancy cases performed in our institution over a period of one year.

• To observe the methods used for Medical Termination of Pregnancy in the institution.

• To identify indications for Medical Termination of Pregnancy performed in 1st trimester and mid-trimester of pregnancy.

Material & Method

The present study was conducted for a period of one year from January 2019 to January 2020 in the Department of Forensic Medicine in collaboration with Department of Obstetrics and Gynaecology in School of Medical Sciences & Research, Sharda University, a tertiary care institution of Greater Noida(U.P.).

Women who came to Department of Obstetrics and Gynecology either admitted through Family Planning Outpatient Department (OPD) or came to emergency department willing for MTP on valid grounds were included in this study after taking informed consent. Information was obtained from the patient, MTP register, patient's case record file in the hospital and direct interview with concerned consultant in charge in the form of pre-designed pre-validated questionnaire .The questionnaire focused on seeking in-depth knowledge of MTP with respect to socio-demographic profile of women seeking abortion, indications, method, outcome on maternalmortality and morbidity, post abortion care and counseling.

Inclusion criteria: All women presenting to the Obstetrics &Gynaecology Department of SMS&R for termination of pregnancy based on valid MTP grounds would be eligible for inclusion.

Exclusion criteria:

• Period of gestation more than 20 weeks(confirmed by LMP, clinical examination or ultrasonography reports at the time of presentation)

• Not given consent

Observation & Results

About 180 women who underwent Medical Termination of Pregnancy were studied. The data collected is summarized in the tables below.

Table1: Demographic profile of women seeking MTP (n =180)

Age(years)	Number of cases (180)	% cases
Less than 15	Nil	0
15-19	5	2.7
20-24	45	25
25-29	66	36
30-34	44	24
35-39	16	9
40-44	4	2.2

Cont... **Table1: Demographic profile of women seeking MTP (n =180)**

45 &more	Nil	0
Area		
Rural	22	12
Urban	168	93
Religion		
Hindu	159	88
Muslim	20	11
Sikh	Nil	0
Christian	1	0.5
Marital status		
Married	176	98
Unmarried	4	2
Educational status		
Uneducated	21	12
Primary	26	14
Middle	28	16
High school	61	34
Intermediate	39	22
Graduate	5	2.7
Post graduate	Nil	0
No. of children		
0	19	11
1	76	42
2	33	18
3	35	19
4	13	7
>5	4	2
Pregnancy weeks		
Upto 12 weeks	169	94
12-20 weeks	11	6

Table 2: Grounds for MTP (n =180)

Indications of MTP	Number of cases	% cases
Therapeutic	2	1
Humanitarian	Nil	0
Social (Failure of contraceptive device or other methods)	171	95
Eugenic	7	4

Table 3: Duration from the last termination of Pregnancy (n= 7)

Duration (in months)	Therapeutic No.	Eugenic No.	Social (Contraceptive Failure) No.	Humanitarian No.
<6 (n= 2)	-	-	2	-
6-12 (n=3)	1	-	2	-
>12 (n=2)	-	1	1	-

Table 4: Method used for MTP (n =180)

Methods used for Termination	Number of cases	% cases
Medical abortion (by prostaglandins)	114	63
Surgical abortion (Suction& Evacuation)	66	37

Table 5: Accepted Family Planning method after MTP (n =180)

Accepted Family planning method after MTP	Number of cases	% cases
Sterilization	7	4
Copper-t	25	14
OCP	Nil	0
Condom	121	67
Not any method	20	11
Unclassified	7	4

Discussion

A total of 180 women admitted for termination of pregnancy were studied. Findings revealed highest rate of abortion 36% in the age group of 25-29 years age groups and were mostly married (98%) followed by unmarried (2%). Abortion seeking women were mostly from Hindu religion and urban background. 94% of the patients presented within 12 weeks of gestation for termination followed by 6% between 13 to 20 weeks of gestation. Majority (46%) were multipara having 2-4 offspring with mean followed by primipara 11%. Unplanned pregnancydue to contraceptive failure along with social and financial constraints was the main reason for termination. In 63%the pregnancy was terminated by medical method (by prostaglandins).

Dhillon *et al*. in their study in 13 states of India found that three-fifths of the induced abortion seekers were between 25 and 34 years. [2] In contrast, the study conducted by Shivakumar and Vishvanath, 2011, showed that majority of the abortions was among the women of 20–29 years of age**.** [1]

In this study 34 % woman have attained secondary education. In contrast, Shivakumar and Vishvanath noted majority (57.3%) uneducated and (42.7%) educated women. [1] Agarwal and Salhan observed that majority of women (70.7%) were educated and (34.8%) were uneducated**.** [3] This difference reflects the urgent need about the awareness of the safe abortion practices.

In the present study, around 71% women had undergone one abortion and 22% women had 2 or more abortions. In contrast to our findings, Akinola *et al*., 2010 observed that only 6.3% of the patients had not experienced prior abortion and 75.5% patients reported only 1 abortion. [5]

The majority of abortions around 94% were performed in the first trimester and 6% in the second trimester. None of the cases were reported during the third trimester in our study. Agarwal and Salhan, 2008 also noted that majority of abortion cases (89.4%) were within 12 weeks of gestation and beyond 12 weeks only (10.6%) cases. In the study by Shivakumar and Vishvanath, 2011 had the majority of abortions (84.7%) during 5–12 weeks of pregnancy followed by 13–20 weeks (15.3%)**.** [3]

Among various reasons given by patients for undergoing an abortion, the most common problem was found to be contraceptive failure that accounted about 94%. InShivakumar and Vishvanath study, 2011, the most common reason behind abortion was unplanned pregnancy (30.7%) followed by contraceptive failure (29.3%).[3] Bahadur *et al*., 2008 cited termination of unplanned pregnancy (32.8%) as the most common reason, inadequate income in 24.6%, contraceptive failure in 22.3% and family complete in 20.3% women**.** [4] Bhattacharya *et al*., 2010 observed birth spacing as the main reason (59.8%) and female fetus was also reason for termination in 6.8% cases**.**[6]

Conclusion

The Medical Termination of Pregnancy Act, 1971 recognized the importance of safe, affordable, accessible abortion services to women who need to terminate pregnancy under certain specified conditions. However, women's right and access to abortion which the MTP Act enabled somehow became entangled in the fight against gender based sex-selection.

The widespread lack of awareness of the legal status of abortion services and women's rights under the existing law as well as of the facilities that offer abortion services calls for socially and culturally appropriate information, education and communication (IEC) campaigns.

In past many years several Writ Petitions have been filed before the Supreme Court and various High Courts seeking permission for aborting pregnancies at gestational age beyond the present permissible limit on the grounds of foetal abnormalities or pregnancies due to sexual violence faced by women. To keep up with the imperative need in recent times in wake of changing discourse on sexual and reproductive rights paired with the advancement of medical technology for safe abortion, there is a scope for increasing upper gestational limit for terminating pregnancies especially for vulnerable women and for pregnancies with substantial foetal anomalies detected late in pregnancy. Considering the need and demand for increased gestational limit under certain specified conditions and to ensure safety and well-being of women, Government of India has proposed to amend the said Act in early 2020,however it still continues to be long awaited for it to see the light of the day.

It is important that abortion services be seen not in isolation, but as part of a constellation of care tailored to the sexual and reproductive health needs of the modern day women.

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Comparative Study of Magnesium Sulphate added to Bupivacaine and Ropivacaine Local Infiltration of Wound for Postoperative Analgesia in Patients Undergoing Upper Abdominal Surgery: A Randomized Double Blind Study

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Abstract

Aim: To compare subcutaneous infiltration of Inj Bupivacaine with inj MgSO4and Inj Ropivacaine with InjMgSO4 for postoperative analgesia undergoing upper abdominal surgery. **Goal**: To determine duration of analgesia, doses of rescue analgesia, VAS at different time intervals.

Background: Post-operative pain is most neglected entity. We should use availableresources in optimummanner for improving pain managementstrategies.

Methods: A Prospective, double-blinded, randomized studyof 50 patients undergoing upper abdominal surgery divided into group B and R. Infiltration was done in the subcutaneous plane by surgeon before skin closure. Group B: Inj. Bupivacaine 50mg (10ml of 0.5% Inj . Bupivacaine) with Inj.MgSO4 500mg (1ml of Inj 50% MgSO4) diluted with NS 9 ml to make total volume of 20 ml whereas, Group R: Inj Ropivacaine 50 mg (6.7 ml of 0.75% Inj. Ropivacaine) with Inj MgSO4 500 mg diluted with NS (12.3 ml) making a total volume of 20 ml. In postoperative period, parameters noted were vitals and Visual Analogue Score (VAS) for 24 hours.

Results: In group R, moderate pain was seen in 40%of the patients at T12 whereas in group B 48% had moderate pain. Total no. of rescue analgesic doses required in Group R is 2.1 +/- 0.5 and in Group B is 2.6 +/- 0.5 in 24 hrs and has significant differencestatistically.

Conclusion: Group R had better VAS scores at T24 and reduced no.of rescue analgesic doses.

Keywords*: Local Infiltration, Ropivacaine, Bupivacaine, Magnesium Sulphate, Postoperative pain*

Introduction

Management of acute post-operative pain has received keen attention in recent years with considerable concurrent advancement in the field[1,2]. Severe abdominal pain after upper abdominal surgeries if treated inadequately, can cause atelectasis due to shallow breathing, retention of secretions and lack of cooperation in physiotherapy. This leads to increased stay in the hospital.

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Oral analgesics and Opioids are used for postoperative analgesia but they are not devoid of side effects. Wound infiltration with local anaesthetics are gaining popularity as there is minimal systemic absorption of the locally infiltrated drugs and thereby reducing any systemic side-effects as compared to oral analgesics and Opioids and has better patient comfort with minimum intervention. Local anaesthetics like Ropivacaine and Bupivacaine are being used as local infiltration for postoperative analgesia. Many adjuvants are added to prolong their effect. As Magnesium sulphate has minimal side effects we designed this prospective observational study to evaluate postoperative pain relief in patients undergoing upper abdominal surgery.

Aims & Objectives of Study

To compare addition of Inj Magnesium sulphate to Inj Bupivacaine and Inj Ropivacaine for local subcutaneous wound infiltration for postoperative analgesia undergoing upper abdominal surgery. Objectives of the study were to compare duration of analgesia, VAS at different time intervals and any postoperativecomplications

Material and Methodology

Institutional ethical committee permission was taken for the study to be conducted at Dhiraj General Hospital, S.B.K.S.M.I.R.C. in Department of Anaesthesiology. CTRI no. CTRI/2019/04/018833 registered on 30/04/2019.

We studied 50 patients of ASA I, II and III, allocated in 2 groups (n=25 in each group). The study was observational in nature. All the patients participating in the study were explained clearly about the purpose and nature of the study in the language they can understand. They were included in the study only after obtaining a written informed consent. All the basal parameters were noted at the time of presentation of the patient.

Patients were allocated in to two groups as **GroupR**:(n=25) were infiltrated with Inj. Ropivacaine 50 mg plus Inj. Magnesium sulphate 500 mg diluted with NS to make 20 ml and **Group B**:(n=25) were infiltrated with Inj. Bupivacaine 50 mg plus Inj. Magnesium Sulphate 500 mg diluted with NS to make 20 ml.

Inclusion Criteria: age >18 years <60 years, ASAI,II and III. Patientswho signed informed consent, Mallampatti class I and II , upper abdominal surgeries e.g. Open Cholecystectomy, Laparotomies, abdominal mass, etc.

Exclusion Criteria: Patients who refused for the study, ASA IV & V, Mallampatti class III and IV, knownallergyofanyofthedrugs, Patientnotplanned for extubation after the surgery.

Preoperative Examination

Thorough history,general physical examination and systemic examinations were done. Respiratory rate, pulse rate, SpO2 and blood pressure will be recorded preoperatively. Assessment of airway was done as per Mallampatti grading. Routine blood investigations, CXR, ECG were done. All patients were explained the visual analogue pain scale (VAS) during the preanesthetic evaluation3. (Figure1)

Pre-operative Preparation

Patients were kept nil by mouth for at least 6 hrs. prior to the surgery. The patients were explained about the procedure of general anaesthesia and a written informed consent was obtained. In OT Multipara monitors were applied and baseline respiratory rate, pulse rate, NIBP, SpO2 and ECG were recorded. 18 G intravenous line was secured and I.V. fluids were started. Patients were premedicated with Inj.Ondensetron 0.08mg/kg, Inj. Glycopyrrolate 0.004 mg/kg, Inj midazolam 0.05mg/kg IV, Inj Fentanyl 2 mcg/Kg IV. Patient were induced with Propofol 2 mg/Kg I.V. till the loss of eyelash reflex. Followed by Inj. Succinyl choline I.V. 2mg/kg. IPPR was given with 100% oxygenation on bag and mask with attached circuit and were intubated with appropriately sized cuffed endotracheal tube by direct laryngoscopy. After checking the equal bilateral airentry, tube was fixed. Anaesthesia was maintained with oxygen and nitrous oxide (50%-50%), isoflurane and Inj. Atracurium 0.5mg/Kg body weight as initial dose and 0.1 mg/kg as and when required.

15 min before the completion of surgical procedure, incision site was infiltrated with study group drug which was made by the allotted consultant anaesthetist in all sterile conditions and handed to the scrubbed surgeon. Patients were blinded for which group they were into, second blinding was at the level of the

investigator who will be collecting all the data and analysing. Neuromuscular blockade was reversed with Inj. Neostigmine 0.05 mg/kg and Inj. Glycopyrrolate 0.008mg/kg intravenously. Once all recovery criteria were fulfilled and after oropharyngeal suction, trachea was extubated. Patients were monitored in the postoperative recovery room for 24 hours. All the patients were observed for VASat 15 min, 1 hour, 2 hr,4 hr,6 hr, 8 hr, 16hr and 24 hr after extubation.Patients were given rescue analgesia whenever the VAS > 5 with Inj. Diclofenac 75 mg IV and total no. of doses were calculated. Patients were released from the study after 24hrs.

Results

Demographically both the groups were comparable and have no statistical difference. There was no statistical difference in SpO2, Heart rate, Systolic and diastolic blood pressure with pvalue>0.05 and both the groups were comparable. Table1 shows all the parameters with their P value. It was observed that at time 0, 68 % of the patients in Group R had no pain and 28% of the patients had mild pain and 4% had moderate pain whereas in Group B 48% of the patients had no pain,52% had mild pain andnone of the patients had moderate pain. Both the groups were comparable at time 0 and no statistical difference was significant. (Figure 2)

It was observed at 6 hrs postoperatively that only 24% of the patients were pain free, 40%had mild pain and 36% of the patients had moderate pain in Group R whereas in group B, it was 8%, 48 % and 44% respectively. Both the groups were comparable and no significant difference was present (p value >0.05). Figure3)

At 12 hrs postoperatively no patient was pain free, 60% had mild pain and40% of the patients had moderate pain in Group R whereas in group B, it was 0%, 52% and 48% respectively. Both the groups were comparable and no significant difference was present (p value>0.05) (Figure4) It was observed at 24 hrs postoperatively 60% patients in Group R had mild pain and 40% had moderate pain whereas in groupB, 52% of the patients had mild pain and 48% had moderate pain. Both the groups were comparable and no significant difference was present (p value >0.05). 48% of the patients in Group B requiring analgesia in 24 hrs whereas only 40% IN Group R. (Figure 5) Total no. of doses required in postoperative period of 24 hrs in group B is2.6+/-0.5andinGroupRis2.1+/-0.5whichisstatisticallysignificantwithpvalueof0.030 (<0.05)

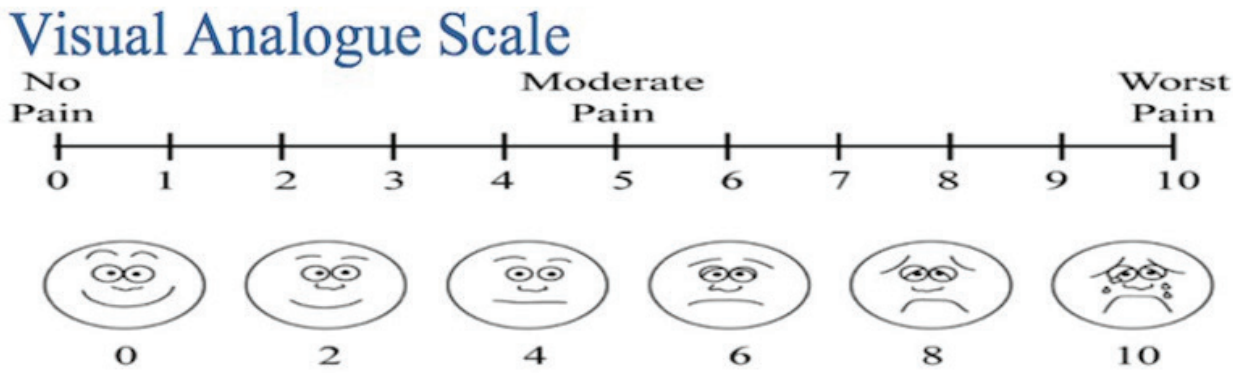

Figure 1: Visual Analogue scale

Table 1 :Table showing Demographic data and parameters of patients in both groups

Parameter	Group B	Group R	P value
Age (years) (Mean±SD)	39.67 ± 17.61	37.3 ± 17.5	NS
Gender (Male/Female)	14/11	14/11	-
ASA physical status (I/II/III)	14/8/3	14/8/3	-
Pain at 0 hr (no pain/mild/moderate)	12/13/0	17/07/1	0.266
Pain at 6hr (no pain/mild/moderate)	2/12/11	6/10/9	0.303
Pain at 12 hr (no pain/mild/moderate)	0/13/12	0/15/10	0.568
Pain 24 hr (no pain/mild/moderate)	0/13/12	0/15/10	0.568
Doses of rescue analgesia in 24 hrs	2.6 +/- 0.5	2.1 +/- 0.5	0.03*
Patients receiving Rescue analgesia	12	10	0.3
Spo2	100	100	NA
Heart Rate (HR)	82.10 ±11.259	76.20± 6.494	0.821
Systolic Blood Pressure (SBP)	129 ± 6.815	128.20 ±14.711	0.691
Diastolic Blood Pressure) DBP	80.60 ± 5.582	77 ± 12.832	0.606
*indicates: significant			

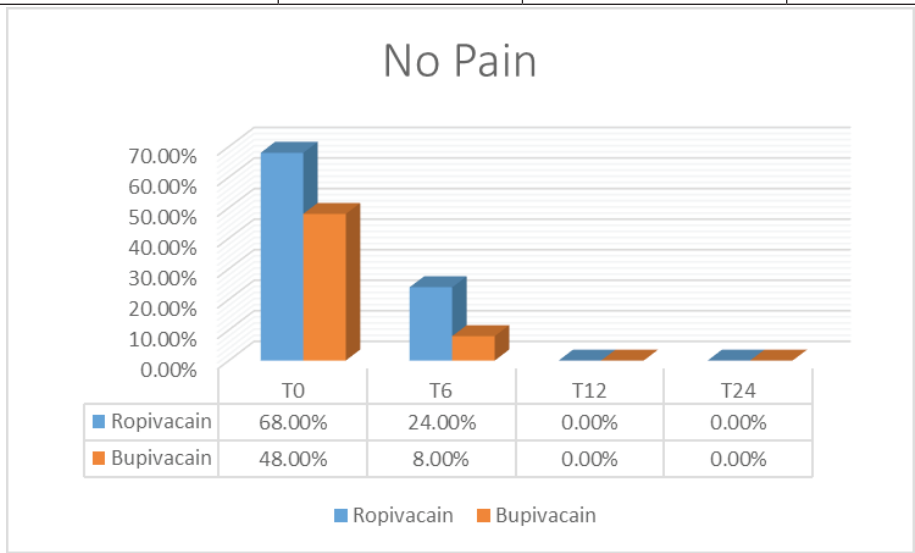

Figure2 : : Graph showing percentage of patients without pain at alltimes in both thegroups

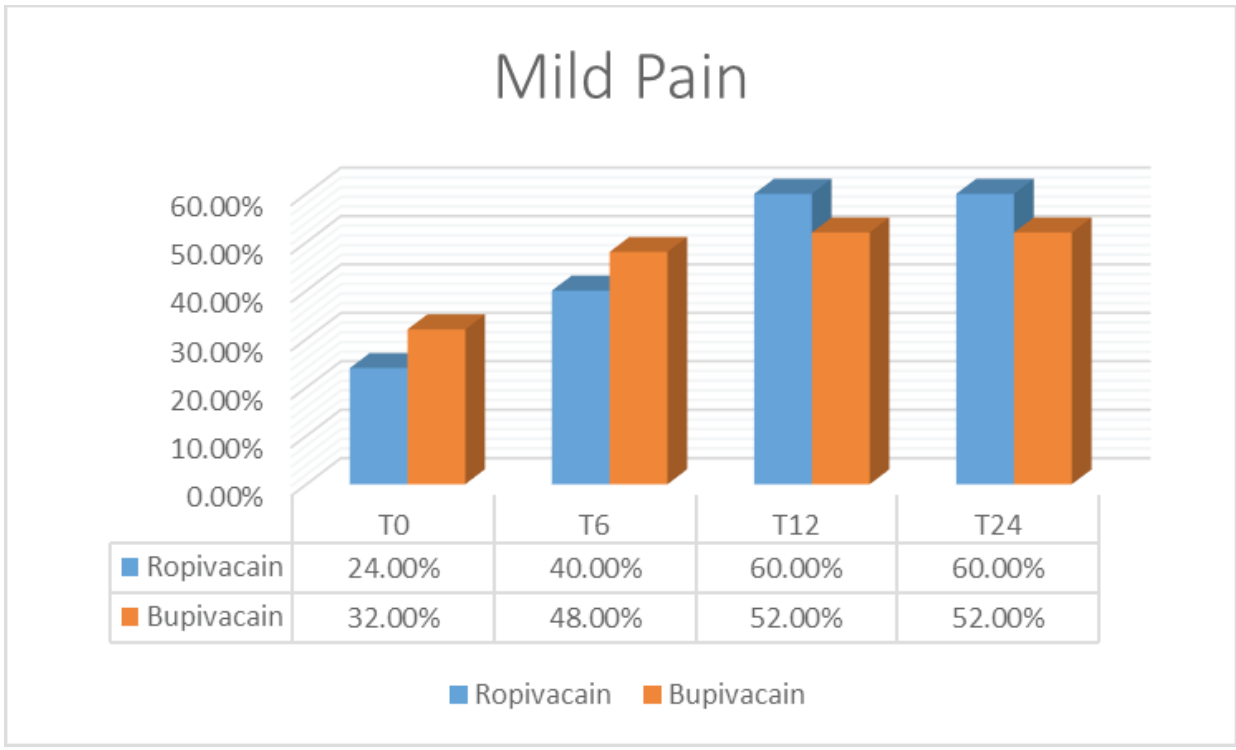

Figure3: Graph showing percentage of patients with mild pain at alltimes in both the groups

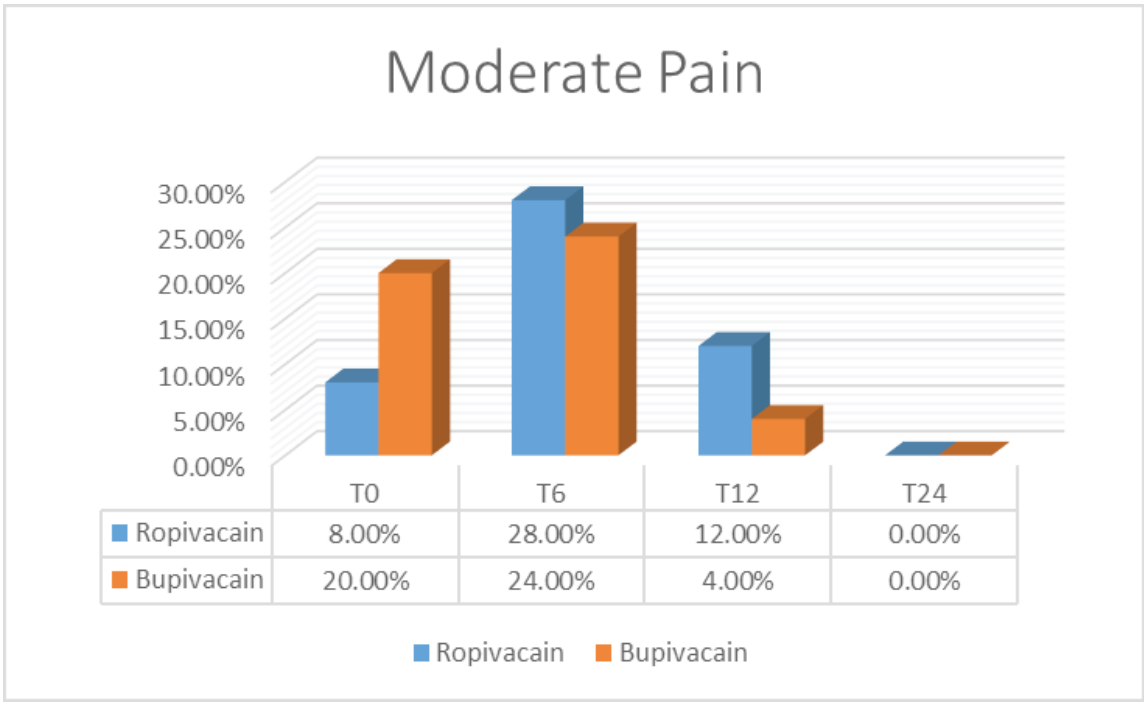

Figure 4 : : Graph showing percentage of patients with moderate pain at alltimes in both the groups

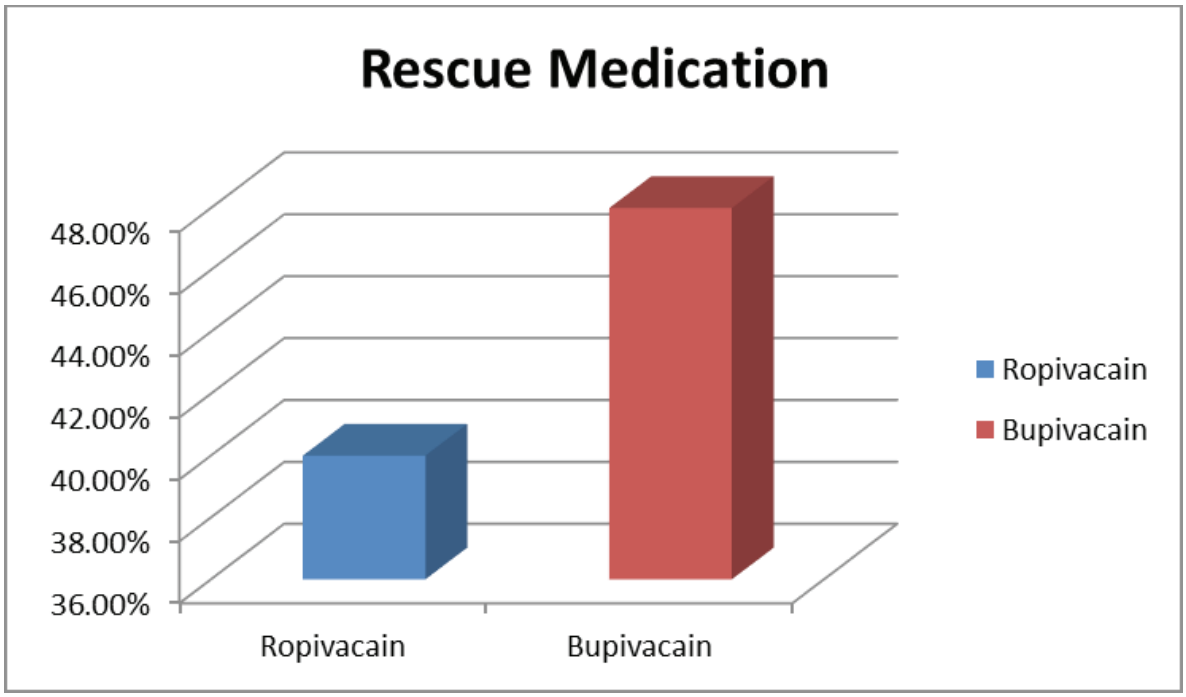

Figure 5 Graph showing number of patients receiving analgesics in two groups.

Discussion

Pain is one of the most important factor to be taken care of in the perioperative period. The results of our study showed that infiltration of local area with ropivacaine and magnesium sulphate provided better postoperative pain control in first 6hrs with 32% patients with no pain and 68% with mild pain. Even at 24 hrs postoperatively, 60% of the patients in Ropivacaine had mild pain whereas 48% patients in Group B had moderate pain. This is in contradiction to the study by Hazarika et al who found wound infiltration with bupivacaine and magnesium sulphate compared to ropivacaine and magnesium sulphate provided longer duration of postoperative analgesia in patients undergoing lumbarlaminectomy4.

Local wound infiltration was an effective method for postoperative analgesia since it was efficacious and side effects were minimal. Regional analgesia in the form of spinal and epidural instillation of medications had shown to have delayed neurological recovery5.Kundra S et al who studied efficacy of Magnesium Sulphate as an adjunct to Ropivacaine in localinfiltration for postoperative pain following Lower Segment Caesarean Section. Sixty parturient undergoing caesarean delivery were randomized to either group A or B in a double blinded manner. After uterine and muscle closure but before skin closure, Group A was administered local subcutaneous wound infiltration of Injection (Inj) ropivacaine 0.75% 150milligram (mg) or 20 millilitres (ml) whereas, group B patients were given a local subcutaneous wound infiltration of Inj magnesium sulphate 750 mg (1.5 ml of Inj 50% Magnesium sulphate) added to Inj ropivacaine 0.75% (18.5 ml) making a total volume of 20 ml. In postoperative period, Heart rate (HR), Mean Arterial Pressure (MAP), Visual Analogue Score (VAS),supplemental analgesic consumption and timing of each subsequent analgesic was noted for the initial 24 hours.6Kundra had not compared the two different local anaesthetics.Thus in this study we compared the two local anaesthetics i.e. Ropivacaine and Bupivacaine with addition of Magnesium Sulphate. In this study dose of Magnesium sulphate was 750 mg but we found similar results with a lower dose of 500 mg and thus further studies can be executed to decide the optimal dose for postoperativeanalgesia.

Magnesium sulphate, a known physiological antagonist of NMDA receptor is in its inactive state. NMDA receptors are not only present in the central nervous system but also in the peripheral tissues such as the skin and the muscles. It has got no analgesic property of its own, but its NMDA receptor antagonistic property abolishes the central mechanism of pain transmission,

modulation, sensitization and even modulates pain transmission from the peripheral tissues. This unique property of magnesium sulphate promoted its use in our study patients in combination with local anaesthetic. The dose of magnesium sulphate was selected as per Donadi et al7.In addition to their central location, recent studies identified NMDA receptors peripherally in the skin and muscles, and found that they play a role in sensory transmission of noxioussignals8.

Though we had few limitations assample size was small, ASA grade IV and V were not included with no severe underlying disease, therefore the results of the present study should not be generalized to all the patients.

Conclusion

LocalInfiltrationisabetteralternativeforpostoperativ eanalgesiawithnoside-effects.Lesser number of rescue analgesia was required in Group R. Though Ropivacaine group had better VAS scores, we should still recruit more patients with higher ASA grades for better results. More studies are needed in future to testify the role of magnesium for local infiltration in postoperative pain management strategies.

Ethical Clearance: Obtained from Sumandeep Vidyapeeth Institutional Ethical Committee

Source of Funding : Self

Conflicts of Interest : Nil

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Effectiveness of Warm Water Foot Bath Therapy on Quality of Sleep among Elderly

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Abstract

Background: Insomnia is a subjective complaint of inadequate night-time sleep. It is the most prevalent sleep disorder identified by older people.[1]

Depression, anxiety, and reduced cognitive function have been linked with severe lack of sleep. Poor sleep quality in the elderly reduces the quality of life, but sadly the most elder people do not report sleep problems unless expressly requested. Warm water foot bath provides a better sleep at night, as it calm downs and relaxes the body and mind. This works by increasing the body temperature slightly and after 15 minutes this starts to drop gradually. This may indirectly encourage sleep. Gradual decrease in body temperature makes us feel drowsy and so we feel better prepared for sleep. A warm water foot bath also diverts some blood from the head to lower parts of the body, decreases brain activity and mimics the state of pre-sleep.[2] We wanted to determine the effectiveness of warm water foot bath therapy on quality of sleep among elderly.

Methods: In this study the 60 older adults are selected based on inclusion criteria and were assigned to experimental and control group alternatively. The experimental group composed of 30 elder people and control group contained 30 elder people. The researcher developed rapport with older people receiving warm footbath and explained the benefits of intervention. The day we began the data collection, the demographic data and pretest took from elder people of experimental group and control group. On the next day, warm footbath was given by the researcher with duration of 15 – 20 minutes for five consecutive days in older people of experimental group. On the other hand, routine work is maintained in control group. Posttest was done on sixth day for both experimental and control group to assess the sleep quality among elderly by using Groningen Sleep Quality Scale.[4]

Results: Findings shows that 25 sample (83.33 %) had undisturbed sleep at night, 5 (16.67 %) had disturbed sleep at night using warm water footbath therapy and none of them had poor sleep at night. The minimum score was 2 and the maximum score was 7 and the mean score was 4.23 ± 1.30 with a mean percentage of 30.2 %. There significant association between qualities of sleeps and age group.

Conclusions: The significant difference between preexisting sleeping quality and effectiveness footbath interpreting effective quality of sleep using warm water footbath therapy among elderly. Mean value of preexisting sleeping quality is 8.93 and effectiveness of footbath therapy is 4.23 and standard deviation value of preexisting sleeping quality is 0.828 and effectiveness of footbath is 1.305. The calculated t-value is 14.75 and p-value is 0.000. Hence it is statistically interpreted that the effectiveness of footbath therapy among elderly was effective.

Key Words: *Warm Water Foot Bath, Quality of Sleep, Elderly People*

Background

To every human being sleep is the basic need; it is a universal biological process for all people. A sleep is considered as a state of unconsciousness. A sleep has come to be considered as an altered state of consciousness in which the individuals reaction and perception to 1 the environment are suppressed.[5] A sleeping disorder is depicted as an abstract complaint of depicted nighttime rest. It is the most well-known rest disorder articulated with the guide of matured individuals. Although alterations in sleep patterns are endemic amongst this population, sleep problems are hardly ever assessed in a traditional patient evaluation. Furthermore, sufferers frequently fail to mention signs to their caregiver, and when sleep is assessed, it is normally in the form of a single question. As a result, insomnia is often unrecognized and untreated.[6]

Discussion:

Sleep is the fundamental human need; it is universal biological process which is common among all humans. A warm-water foot bath is a local application of moist heat. It is non-invasive and simple procedure to implement at home. The results offer empirical support that a warm-water footbath relieves elderly people's exhaustion and insomnia issues. It can be a non-pharmaceutical solution to help people tackle tiredness and sleep problems.[4]

The total number of study population is 60, maximum sample are belongs to 61-70 yrs. of age group. the maximum study sample are male by their gender. The study Findings shows that 25 sample (83.33 %) had undisturbed sleep at night, 5 (16.67 %) had disturbed sleep at night using warm water footbath therapy and none of them had poor sleep at night. The minimum score was 2 and the maximum score was 7 and the mean score was 4.23 ± 1.30 with a mean percentage of 30.2%

The following certain studies are supported to present study studies, A study was conducted by AlleheSeyyedrasooli, Leila valizadeh, Vahidvzamanzadeh, khadijeh Nasiri, Hossein Kaiantri on the effect of Foot bath therapy to check sleep quality 46 yrs old men were selected to for the study. Then 46 were divided into two groups. One group had foot bath therapy and another did not have foot bath therapy .It continue for 6 weeks the researcher we Pittsburgh sleep quality index scale before and after foot bath therapy, a sleep quality were improved , those who taken foot bath therapy.[9]

A study was conducted by Kim HJ, LEE, and SOHNG.KV with titled, the effect of foot bath therapy on sleep among the elder adult in nursing home. The objective was check effect of foot bathing therapy using different water temperature on quality of sleep in elder receiving in nursing home. participated divided in three group. Experimental and control group .It perform for 30 min daily for four weeks. Water at 40^0c used for experimental, water at 36.5 ^{0} used for placebo and control group did not receiving intervention .amount of sleep and sleep efficiency was different for experimental group specially people having poor sleep. Long effect of therapy decrease in third week of therapy.[10]

The present study done by Liao WC, Wang L, kuo CP, Lo C, chin MJ, Ting H with titled, effect of warm water foot bath before bedtime on body temperature and sleep in older adult with good and poor sleep. This study examined the effect of warm footbath on body temperature and sleep in older adults with good and poor sleep. Result found that footbath therapy before sleep significantly increased and retained foot temperature in both good and poor sleep.[11]

Conclusion

The study concluded that present study the warm water foot bath therapy brought out improvement in the sleep quality among elderly people of experimental group, and there is the significant association with age of elderly adult and effects of warm water foot bath .

Ethical Clearance- Taken from IEC committee with reference no.**DMIMS(DU)/Sept-2019/8488**

Source of Funding- Self/

Conflict of Interest – Nil.

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Assess the Effectiveness of Information Booklet on the Knowledge of Wet Nursing among Women

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Abstract

Background: Breast milk is an ideal nutrition for infants. Breast milk contain a perfect combination of vitamins, protein and fat which is required for the optimum growth of the baby. Wet-nursing is defined as the act of providing breast milk someone else›s child. **Objectives of the Study:** To assess the pretest knowledge regarding wet nursing among women. To assess the post test knowledge regarding wet nursing among women.To assess the effectiveness of information booklet on the knowledge of women regarding wet nursing. **Materials and Methods:** 100 samples were selected by purposive sampling technique.pre experimental research design one group pretest and post without control group design was used. **Result:** In pre-test majority of women 58% of them had good knowledge regarding wet nursing among women. mean percentage of knowledge score was (38.20 %) and in post test majority of antenatal mothers (40% of them had excellent level of knowledge score regarding wet nursing among women.Mean percentage of knowledge score was (67%). Education and area of residence found to be associated with knowledge of wet nursing none of the other variable were found significantly related with the knowledge of women.**, Conclusion-**All the women aware about wet nursing to prevent complication during postnatal periods,.

Keywords: *wet nursing, breast milk, women, antibodies*

Introduction

Breast milk is an ideal nutrition for infants. Breast milk contains aperfect combination of vitamins protins and fat which is required for the optimum growth of the baby. Breast milk also has an incredible amount of antibodies which helps a baby battle viruses and bacteria. This also reduces the chance of developing baby Asthama and various allergies.Only breastfeeding for the first 6 months also decreases the risk of ear infections, respiratory infections and bouts of diarrhea for the infant. There are various cases in which the baby is deprived of breast milk, including mothers who are afraid of breastfeeding in public, mothers with active tuberculosis, mothers who are undergoing cancer treatment, mothers who are HIV positive, etc.[2]

A program undertaken in Turkey under the World Health Organization's "Promoting Breastfeeding and Babyfriendly Hospitals Programme," there was a key message that "babies should start breastfeeding right after birth and receive only breast milk for the first 6 months. Babies someti, forall these efforts, for several reasons relating to babies and mothers3For such a case,the World Health Organization recommends the baby might be given breast milk from another woman. The American Academy of Pediatrics also focuses on a statement that breast milk from donors can be used in children who can not be breastfeed by their own mothers4.Wet-nursing or cross-nursing is defined as the act of providing breast milk someone else's child. A wet nurse may have a healthy breast milk supply by providing breast milk for her own child, or she may

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stimulate a supply of breast milk for another woman's child.5

Wet breastfeeding is considered healthier in contrast to other types of infant feeding such as baby formulas andfeeding bottles as an alternative for mother's breast milk and was a common practice prior to these developments. The key goal of wet nursing is to ensure that breast milk is delivered to an child, which is vital for lifelong promotion of health and development.6Breastfeeding increases the susceptibility of children to lethal exposures. Infant and Young ChildFeeding in Emergency program puts special focus on breastfeeding and avoiding the excessive use of feeding materials. Global Infant Feeding Policy is based on values of fairness, security and human rights7

Recently there have been sporadic media reports in developed countries that said breastfeeding the baby by someones other than the babys own biological motheris getting more 'popular' day by day. In April 2007 the topic of wet nursing was also raised on television on The Today show and the syndicated Dr.phil although the sharing of breastfeeding was practiced through the 20th century as it has been for millennium, it was generally done for covered activity rather than openly. As such, there are no prevalence data for comparisons to see if this practice is, indeed, growing in popularity.7

Objectives of the Study

1. To assess the pretest knowledge regarding wet nursing among women

2. To assess the post test knowledge regarding wet nursing among women

3.To assess the effectiveness of of information booklet on the knowledge of women regarding wet nursing..

Materials and Methods

Sources of data: data collected from the women rural areas of wardha

Inclusion Criteria

All women who are/were pregnant or breastfeeding and have children

Exclusion Criteria

- Mother who are not willing to participate in the study.
- Women who already were the part of same research before.

Research approach : Quntitative approch

Research Design: Evaluative research design

Setting : study conducted in selected area in village.

Sampling technique: purposive sampling technique.

Sample size: total sample size were 100 women from urban rural area

Description of The Tool

It has two sections-

Part I – it consists of demographic variables of the wet nursing women tto be participated in the study (age, education, occupation, income, family status, residence, religion, current status of woman)

Part II – consist of knowledge items regarding wet nursing

Independent variable: Information booklet on wet nursing.

Dependent variable: The dependent variable in this study is knowledge of women.

MAJOR FINDING OF THE STUDYAND DISCUSSION

Distribution of pregnant women with regards to to demographic variables.

- 6% of the mothers were in the age group of 16-22 yrs, 29% were in the age group of 23-30 yrs., 23% were in the age group of of 31-39 years and 42% were of more than 39 years.
- 82% of women were Hindus, 16% were Buddhist and 2% of them were belonging to other religion.
- 64% of the women were from nuclear family,

31% were from joint families, 2% were from extended families and 3% of them were from blended family.

• 98% of the women were from rural areas and 2% of them were from slum area.

• 62% of women were educated up to primary standard, 26% of them were undergraduate, 4% of them were postgraduate and 8% of them were illiterate.

• 24% of the women were farm worker, 10% of them were doing private service, 1% of them were doing government service and 65% of them were homemaker.

• 3% of the women were newly married, 4% of them were pregnant, 60% of them were lactating, 27% of them were menopausal and 6% of them had no status.

• 43% of the women were having monthly family income of 10000-30000 Rs, 39% of them had between 40000-70000 Rs, 14% of them had between 80000-100000 Rs and 4% of them had monthly family income of more than one lakh Rs.

Table no. 1 Assessment with level of pre test knowledge

n=100

Level of pre test knowledge	Score Range	Level of pretest knowledge score	
		No of women	Percentage
Poor	1-5	42	42
Good	6-10	58	58
Excellent	11-15	0	0
Minimum score		2	
Maximum score		9	
Mean knowledge score		5.73 ± 1.58	
Mean % Knowledge Score		38.20 ± 10.54	

The above table shows that 42% of the mothers were having poor level of knowledge score and 58% of them had good level of knowledge score.

Minimum knowledge score in pretest was 2 and maximum knowledge score in pre-test was 9.

Mean knowledge score in pre test was 5.73 ± 1.58 and mean percentage of knowledge score in pre test was 38.20 ± 10.54.

Table 2. Assessment with level of post-test knowledge

n=100

Level of post-test knowledge	Score range	Level ofPost-test Knowledge score	
		No of women	Percentage
Poor	1-5	1	1
Good	6-10	59	59
Excellent	11-15	40	40
Minimum score		5	
Maximum score		13	
Mean knowledge score		10.05 ± 1.53	
Mean % Knowledge Score		67 ± 10.26	

The above table shows that 1% of the mothers were having poor level of knowledge score, 59% had good and 40% of them had excellent level of knowledge score. Minimum knowledge score in post test was 5 and maximum knowledge score in post-test was 13. Mean knowledge score in post test was 10.05 ± 1.53 and mean percentage of knowledge score in post test was 67 ± 10.26.

EVALUATION OF EFFECTIVENESS OF INFORMATION BOOKLET ON THE KNOWLEDGE OF WOMEN REGARDING WET NURSING

This section deals with the effectiveness of information booklet on the knowledge of women regarding wet nursing. The hypothesis is tested statistically with distribution of post test and post test mean standard deviation and mean percentage knowledge score. The level of knowledge during the he post-test and post test are compared to prove the effectiveness of information booklet. Significance of difference at 5% level of significance is tested with student's paired 't' ttest and tabulated 't' value is compared with calculated 't' value.Also the calculated 'p' values are compared with acceptable 'p'value i.e.0.05

Table 3: Significance of difference between knowledge scorein post and post-test of women

n=100

Overall	mean	SD	Mean diffrence	t-value	p-value
Pre test	5.73	1.58	4.32±1.73	24.91	0.0001 S,p<0.05
Post test	10.05	1.53			

This table shows the comparison of pre-test and post-test knowledge scores of women regarding wet nursing. Mean, standard deviation and mean difference values are compared and student's paired' test is applied at 5% level of significance. The tabulated value for n=100-1 i.e. 99 degree of freedom was 1.98. The calculated 't' value i.e. 34.91 are much higher than the tabulated value at 5% level of significance for overall knowledge score of mothers which is statiscally acceptable level of significance. Hence it is statistically interpreted that the Information Booklet on knowledge regarding wet nursing among women was effective. Thus the H1 is accepted.

Discussion

One of the study shows that, 650 infants were examined for breastfeeding patterns and role of some factor on exclusive breastfeeding at tthe Zanjan city, Iran.92%of of infants were breastfeed during the first year of life. 82% were exclusively breastfed in the first five days of life but declined to 44% at first month. Breastfeeding is the major determinants of infant's health and survival The present study findings showed that the information booklet was highly effective in improving knowledge of women. Present study conducted in rural areas of Maharashtra and subject was selected through purposive sampling technique. The tool for data collection was structural knowledge questionnaire. The pre test conducted on the first day followed by information booklet and post test conducted on seventh day. One of the study conducted in India.

Nursing Implication

The finding of the study has implication in nursing service, nursing,education,nursing administration, nursing research and nursing practice.

Nursing Administration:Study results may be used by nursing management to establish wet nursing education strategies and plans. The study will help the nursing administrator plan and organize continuing education for nurses , nursing students regarding wet nursing, so they can educate other women and their relatives through information booklets.

Nursing Education: The nursing student gains insight into wet nursing. This will help the students

develop their awareness about wet nursing. Findings will allow the nursing faculty to offer greater priority to the preparation and organization of wet nursing health education programs that will enhance student awareness about wet nursing

Nursing research The nursing research ‘s key aim is to strengthen the expertise of staff nurses by incorporating evidencebased practice.The studyprovides the basic data for carrying out other research studies. The research would be a catalyst for the prospective investigators to perform largescale related studies For research scholars the analysis will be a guide.

Recommendations

It is recommended that the following studies be performed based on the research findings. The following recommendations are offered for the further studies:

- A study can be replicated on larger sample size.
- Comparative study can be conducted in urban and rural areas.
- A study can be conducted to assess the knowledge and attitude of women towards the wet nursing.
- A community-based research can be conducted on the same topic.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: The study was approved by the Ethics committee of the institute.

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Effect of Grey Zone Samples Testing by Enzyme Immunoassay and Its Impact on Enhancing Blood Safety: Experience from Tertiary Care Hospital based Blood Bank in Central Gujarat

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Abstract

Background : Enzyme linked immunosorbent assay is a standard protocol adopted by majority of Blood Banks for screening of blood units for transfusion transmissible infections. The grey zone testing of samples increases sensitivity of ELISA tests which in turn enhances the safe blood transfusion practices. The present study was thus designed to know the effect of repeat testing of Grey zone Blood Donor samples for improving the sensitivity of ELISA Screening methods in blood banks.

Methods:- All Blood Donor Samples which were 10% below the cut- off value were marked as Grey zone samples and were repeated for ELISA screening . Interpretation of Repeat tests was done as per NACO and WHO guidelines and the samples which recorded their one or both O.D value above cut- off were labelled as reactive and samples which recorded one or both O.D value in grey zone were marked as indeterminate.

Results and Conclusion: In present study, a total of 10,425 Blood Donors were screened for TTI' s and out of 10,425 (15) Blood donor samples were found in grey zone. The results of repeat testing showed that 9 (60%) Blood Donor samples were found to be confirm reactive for TTI. Grey zone sample testing should be done routinely as a measure to enhance sensitivity of ELISA testing of Blood Donors in developing countries.

Key words:- *ELISA, Grey Zone, Transfusion transmitted infection,*

Introduction

Blood transfusion is a life saving procedure for the management of clinical and surgical patients . The use of Blood and components should always be done judiciously , weighing their benefits against the risks involved since Blood transfusion is associated with the risk of disease transmission. The collected units should be tested with highly sensitive and specific testing kits before transfusion. The important diseases transmitted through blood are HIV (Human Immunodeficiency virus), HBV (Hepatitis B virus), HCV (Hepatitis C virus), Malarial parasites and Syphilis. (1)

Following Blood transfusion , there is a risk of transfusion- transmitted infectious diseases, despite the use of current advanced screening technologies. The risk of Transfusion Transmitted Infection is estimated to be 1 in 6,77,000 units of HIV , 1 in 1,03,000 for HCV, and 1 in 63000 for HBV.(2) Since transfusion of blood and components involves entry of maximum viral load particles to be transmitted so sensitive and specific donor screening strategies are needed for safe blood transfusion to the patients. (3)

The various screening methods currently used for diagnosis of Transfusion Transmitted Infections are either Immunoassays e.g Enzyme linked Immunosorbent assay (ELISA) or Chemiluminiscence Assays (CLIA) and Molecular techniques like Nucleic Acid Testing (NAT).(4) ELISA is the most commonly performed screening test at blood banks and tertiary care centers testing a large number of samples a day. It is sensitive, specific and cost -effective.

As per Drugs and cosmetics act, 1940 and rule , 1945, the testing of Blood and components for viral markers of HIV, HBV, HCV, are mandatory in blood bank. Screening of all blood donors in India is done by complying with NACO and WHO guidelines. According to these guidelines samples with optical density (O.D) Values above or equal to cut-off are considered as reactive samples and those below the cut-off value as non reactive. Samples with optical density 10% below the cut off are marked as grey zone samples. Testing of grey zone samples enhances the sensitivity of screening of blood by ELISA and CLIA techniques as they identify seroconverters early in the course of disease and thus contributes to provide safe blood for use. [6]

Since there is very less data to know usefulness of grey zone testing in screening of transfusion transmitted infections . The present study thus aims to know the utility of grey zone calculation of Blood Donor Saamples and its role in increasing the sensitivity of ELISA technology used for blood donor screening at our blood bank.

Material and Methods

The present study was a prospective analytical study carried out in Department of Transfusion Medicine, SBKSMIRC, from January 2017- December 2029. All Blood Donors were explained about Blood Donation Process and an informed consent was obtained from blood donors that their blood samples will be screened for Transfusion transmitted infections like; HIV, HBV, HCV, Syphilis and Malarial Parasite.

In our blood bank, Immunobased assays , (ELISA) Enzyme Linked Immunosorbent assay (Lisaquant 4000 and Lisawash 4000) was used for screening of all blood samples strictly following manufacturers guidelines. HIV screening was performed by fourth generation Elisa kits (Qualisa) ,for HBV 3rd generation Elisa kits (Qualisa) were used , for HCV 3rd generation Elisa kits (Qualisa) were used . Validation and Quality control of ELISA was done by preparing Levy – Jenning charts and using west gard rules by running 30 borderline positive controls as per guidelines and procedures in National AIDS control organization (NACO) Quality Manual.[6]

The test run samples with OD value more than cut-off were considered reactive units . All reactive units were discarded and reactive donors were notified as per departmental standard operating procedures. Grey zone is defined as values 10 % below the cut off margin .Grey zone values are calculated with each day of run and samples falling in Grey zone area are removed from inventory to be discarded as per departmental standard operating procedures. . All initial grey zone samples are tested in duplicate and those samples showing one or both values above cut off were labelled as reactive units and those donors are notified and recalled for counselling and further management. The Grey zone samples which were showing one or both OD value again as grey zone on duplicate testing was marked as indeterminate. Indeterminate donors were documented as nonreactive and informed to visit Blood Bank for repeat testing after 6 months.

Results and Discussion

Among the 10,425 blood donors donating the blood during 3 years of study i.e. from January 2017 to December 2019 , 50 (0.47%) donors were reactive for HBsAg , HCV, and HIV in the TTI Screening tests and 15(0.0015%) donors samples were Grey Zone positive. Of total 50 TTI Positive Blood Donors , 25(50%) were positive for HBsAg , 8 (16%) were positive for HIV , 6 (12%) were positive for HCV and 10 (20%) were positive for VDRL and one positive (2%) for Malaria.

Donors having their TTI result in the Grey zone were 15 , of which HBsAg was 07 (46.6%) ,HCV 05 (33.33%), HIV 2 (13.33%), VDRL 1 (6.66%). **Table 1.**

On repeat testing of 15 Grey Zone samples, confirm reactive HBsAg samples were 4 and indeterminate was 1. For HIV Confirm reactive samples were 2 , For HCV Confirm reactive sample were 2 and indeterminate were 3, and for VDRL Confirm reactive samples were 1. **Table 2**

Table no.1: Shows number of TTI positive and grey zone samples in first run on Blood Donors , (n= 10,425)

Test	Sero reactive samples in first run (%)	Grey zone samples in first run. (%)
HIV	08 (16%)	02(13.33%)
HBV	25(50%)	07(46.6%)
HCV	06 (12%)	05(33.33%)
VDRL	10 (20%)	01(6.66%)
Malarial Parasite	01(2%)	00

Table no. 2: Shows interpretation of Grey zone samples on repeat testing.

TTI Marker	Sample in Grey zone .	Repeat non reactive	Repeat Seroreactive	Repeat Grey zone (Indererminate)
HIV	02	--	02	--
HBV	07	02	04	01
HCV	05	--	02	03
VDRL	01	--	01	--
Malarial parasite	00	--	--	--
Total	15	02	09	04

There are very few research studies conducted on reporting significance of Grey zone positive samples in increasing sensitivity of immunobased assays like ELISA and CLIA.(3) (4) The present study reported 0.14% (15/10,425) samples in Grey zone .

NAT testing though improves blood safety by decreasing the window period of infection but it is not routinely used in many blood banks because of economic reasons. The tests based on immunological assays like ELISA /CLIA testing remains one of the economical methods for screening of donor blood samples. To enhance the sensitivity of ELISA / CLIA as screening assay repeat testing of samples with O.D value 10 % below cut off should be practiced.

In one study by Pereire etal it was reported that there can be possibility of reporting precarious result values around the cut off zone in TTI screening , used for calculating the reactive samples. (7) The study thus concluded that samples in Grey zone were not always confirmatory source of test methods for notifying the donor about his TTI positive status, as chances , for these tests as confirmed positive is low. This study when compared with other Indian studies in analyzing the impact of Grey zone on TTI ,it was observed in one study conducted by Anitha et al. that 144 Blood Donors (n=21,908) were in Grey Zone. Repeat testing when done on all these Grey zone samples , 35(24.30%) samples were found to be reactive for TTI and 18 (12.5%) were in grey zone.

In our study out of 15 samples which were initially in grey zone 9 samples were reported confirm reactive and four samples were in repeat grey zone/Indeterminate.

One study conducted in Turkey reported 70% false positivity on repeat testing of grey zone samples[8]

In present study , 0.14% samples were reported in Grey Zone and 60% of Grey zone samples reported ,were repeat reactive for either of viral markers.

In similar studies Grey Zone repeat testing was 4.6-75%. (9,10,11) .

Repeat reactivity in grey zone testing is astounding condition and thus the more stringent screening technologies and donor selection methods is the need of hour in developing countries.

The only limitation in present study was the nonavailability of performing the confirmatory assays for Reactive and Indeterminate units and lack of follow up to look for seroconversion of infection for those donors whose samples were in Grey zone of ELISA .

Conclusion

Repeat testing of Grey zone samples will help in improving the safety of Blood transfusion in developing countries where more sensitive supplementary assay such as NAT technology is very expensive.

Ethical approval: Taken from ethics committee of sumandeep Vidyapeeth university.

Conflict of Interest : Nil

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Case Report

Multifaceted Physiotherapeutic approach in Snapping Hip Syndrome: A Case Report

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Abstract

Introduction: Snapping hip syndrome or "coxa saltans," sometimes called as dancer's hip, is seen in ballet dancers. It is a condition caused by the movement of tendon or a muscle over a bony eminence in the hip in which snapping sound is heard or snapping sensation is felt when a person walks, runs, swing leg around or get up from a chair. Snapping Hip Syndrome is just associated with the snapping sound and no such discomfort or pain is felt. Patients chief complaints were Low back pain radiating down her right leg and sometimes a click sound heard in her right hip. Experts estimate that 5 to 10 percent of the population has snapping hips with no symptoms or pain, and no treatment is necessary. On the other half some individuals has irritating pain which requires treatment or surgery. Diagnosis of External Snapping Hip Syndrome was confirmed when detail evaluation of patient was done using Special test - SLUMPS and Straight Leg Raise (SLR) test, Faber's test and Ober's test and Harris Hip Score was also noted.

Conclusion: This case report provides a comprehensive rehabilitation plan that helped relieve pain, spasm, increase strength and range of motion. Physiotherapy intervention includes stretching of the tighten muscle and purposeful strengthening to decrease pain and disability of patient. Physiotherapy treatment is found to be best to resolve patient's chief complaints. Improving the disability and Social skill improvement was checked by using Lower Extremity Functional Scale (LEFS) and Quality of Life (QOL).

Key words: *Snapping hip syndrome, Coxa saltans, Snapping sound, Snapping sensation, Dancers hip, Rehabilitation.*

Introduction

The snapping sensation is not always associated with pain but is audible. It is just associated with the snapping sound and no such discomfort or pain is felt, the condition can usually be ignored and is sometimes considered to be without any of the pathologic importance[1]. Tendons snapping over bony bumps (prominences) are the most common cause of snapping hip syndrome. The iliotibial band travels from the pelvis to the knee snaps over the greater trochanter of hip, leading to irritation of the trochanteric bursa (reduces friction between the greater trochanter and iliotibial band)[1].

The second most common cause of the iliopsoas tendon is inflammation where it attaches to the hip. The other causes may include loose pieces of the bone or the cartilage within the hip joint and a hamstring tendon snapping over the ischial tuberosity. Experts estimate that 5 to 10 percent of the population has snapping hips with no symptoms or pain, and no treatment is necessary. For a small number of people, hip snapping is not only annoying but can also accompany by pain and requires treatment.

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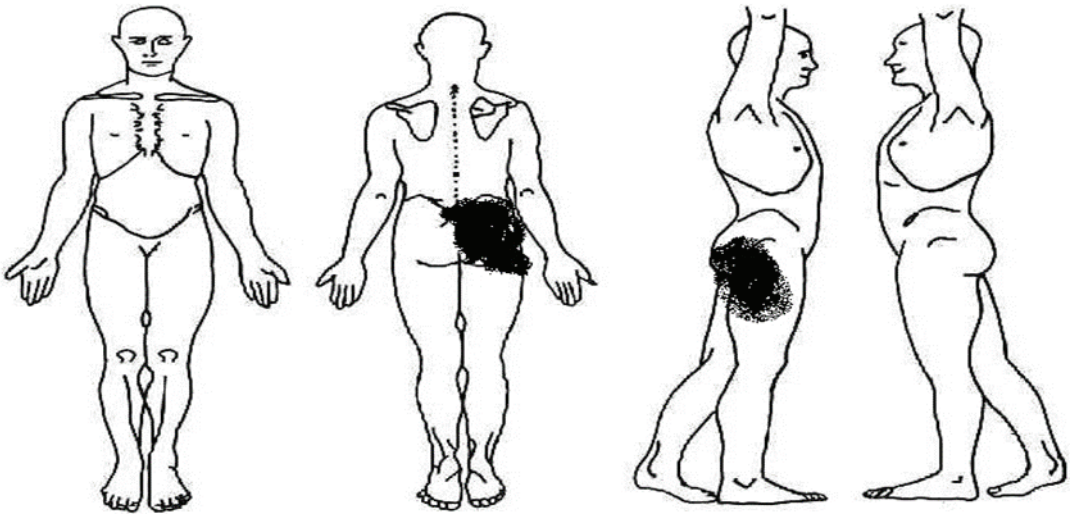
Several case reports suggest surgery for the patients with tenacious symptoms of external snapping hip, the surgery which releases symptoms is indeterminate. Iliotibial band (ITB)-modifying techniques include alteration in the shape of the lateral thigh and overload of the contralateral abduction mechanism. There is an advancement in the endoscopic technique which reduces the tension of the Iliotibial band complex by releasing the gluteus maximus tendon at the femoral insertion (GMT)[2].

PATIENT INFORMATION:

A 24 year old female complains difficulties in day-to-day tasks. She has history of pain since a year ago when she started experiencing low back pain which gradually started radiating to her right leg. Sometimes the patient also feels a click or pop sound at her hip which is observed more on extreme walking or long standing. On 12th September 2020 patient's parents brought her at Acharya Vinobha Bhave Rural Hospital (AVBRH), DMIMS (DU) Sawangi Meghe, Wardha, Maharashtra for further treatment. Patient was diagnosed with Snapping Hip Syndrome for which she was given medications and referred to physiotherapy department (Table 3).

Picture indicating pain area for patient.



Clinical Findings:

Patient was examined in standing position where lumbar movements, gait analysis and posture were observed. On assessment, pain was noted 7 on VAS, Tightness present in Glutei muscle, piriformis, IT Band. Special test - SLUMPS and SLR test was- negative while, Faber's test[3] and Ober's test[4] - were positive. Harris Hip Score[5] was noted as 75.8 (fair)[6]

Medical Treatment:

Rest, NSAIDS, COX2 inhibitors, Corticosteroids.

Therapeutic Management:

Goals

Short Term Goal is to Ease pain and muscle spasm

Long Term Goal is to Reduce weakness, restore movement and Avoid Recurrence, Back to Sports.

Management

Hot Pack given to ease the pain. Stretching of Quadriceps, Hamstring, Piriformis, Iliotibial Tract. Strengthening done by following exercises: Straight Leg Rise, Prone Straight Leg Rise, Side Straight Leg Rise,

Bridging, Squatting, Superman/ Quadruped, Lunges. Active movement for unaffected limbs to maintain their strength

Home Programme: Patient was asked to continue all exercises, gait training exercises ,Rest, Medications, Hot Pack .

TABLE NO. 1: MUSCLE STRENGTH

	Pre		Post	
Muscles	**Right**	**Left**	**Right**	**Left**
Hip				
Flexors	Poor	Good	Good	Good
Extensors	Poor	Good	Good	Good
Abductors	Poor	Good	Good	Good
Adductors	Poor	Good	Good	Good
Knee				
Flexors	Poor	Good	Good	Good
Extensors	Poor	Good	Good	Good
Ankle				
Plantar Flexors	Good	Good	Good	Good
Dorsi Flexors	Good	Good	Good	Good
Inversion	Good	Good	Good	Good
Eversion	Good	Good	Good	Good

TABLE NO. 2: RANGE OF MOTION

JOINT	**Pre**		**Post**	
	Active	**Passive**	**Active**	**Passive**
Hip				
Flexion	0-90°	0-100°	0-100°	0-110°
0Extension	0-40°	0-50°	0-50°	0-55°
Abduction	0-15°	0-20°	0-20°	0-25°
Adduction	0-10°	0-10°	0-10°	0-15°
Internal Rotation	0-15°	0-20°	0-20°	0-25°
External Rotation	0-10°	0-10°	0-10°	0-15°

TABLE NO. 2: RANGE OF MOTION

Knee				
Flexion	0-120	0-120	0-120	0-120
Extension	0-10	0-10	0-10	0-10
Ankle				
Plantar Flexion	0-45°	0-45°	0-45°	0-45°
Dorsi Flexion	0-15°	0-15°	0-15°	0-15°
Inversion	0-30°	0-30°	0-30°	0-30°
Eversion	0-20°	0-20°	0-20°	0-20°

TABLE 3: TIMELINE

Date	Event(s)	Diagnosis & Treatment	Home Program
1st Sept 2020	Initiation of pain		Nil
12th Sept 2020	Orthopaedic consultation	Snapping Hip syndrome Physiotherapy	Rest , Medications
14th Sept2020	Physiotherapeutic Assessment- Observation Inspection Examination Special test	Posture , Gait , Lumbar movements, VAS , LEFS ,Muscle Strength , Rom, SLR, SLUMPS, Faber's test, Ober's test.	Rest , Hot pack
15th Sept 2020 Onwards	Medical Management	Rest, NSAIDS, COX2 inhibitors, Corticosteroids.	Follow up done by patient as per orthopaedician
15th Sept-30th Sept 2020	Physiotherapeutic Intervention Week 1 Week 2	Week 1: Stretching of Quadriceps, Hamstring, Piriformis, and Iliotibial Tract. Strengthening done by following exercises. Week 2: Straight Leg Rise, Prone Straight Leg Rise, Side Straight Leg Rise, Bridging, Squatting, Superman/ Quadruped, Lunges.	Follow up Done by the patient as per therapist's guidelines.

Diagnostic Assessment:

Pain was noted 7 on VAS scale. Aggravating factors - Prolong walking and long standing. Reliving factors- Rest. Nature And behaviour of pain was Dull aching in low back and right leg. Tightness present in Glutei muscle, piriformis, IT Band. Special test - SLUMPS and SLR test was- negative while, Faber's test and Ober's test- were positive table 1 and 2. According to observation and assessment patient diagnosed as External Snapping Hip Syndrome. Medical Management: Rest, NSAIDS, COX2 inhibitors, Corticosteroids.

Discusssion

In this case, patient represented with low back pain which gradually started radiating to her right lower leg. After visiting to Orthopaedic, patient was diagnosed with snapping hip syndrome where she was given the medication and referred to Physiotherapist. A successful plan of care was made following a clinical evaluation. Goals for rehabilitation started from mild exercises to stretching and strengthening. All the exercises were performed three times a day and 10 sets; hot pack were applied around patient's hip to ease the pain caused discomfort during the session[7]. Precautions to be taken by patients were – stop sitting on low-floor, sitting cross legs, stop stair climbing. There by patient has benefited with the protocol. Home program exercises were continued including Hip range of motion exercises, isometrics exercises and eccentric strengthening exercises.

Conclusion

This case report provides a comprehensive rehabilitation plan that helped relieve pain , spasm , increase strength and range of motion. Physiotherapy intervention included stretching the tighten muscle and purposeful strengthening to decrease pain and disability of patient. Physiotherapy treatment is found to be best to resolve patient's chief complaints. Improving disability and Social skill improvement was checked by using Lower Extremity Functional Scale (LEFS)[8] and QOL[9].

Patient Informed Consent:

Proper patient consent was taken from patient for writing this case report.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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Xerostomia – An Unknown Oral Manifestation in AAA Syndrome

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Abstract

Background: The triple A syndrome (AAAS), also termed Allgrove syndrome (AS), is a very rare chromosome recessive disorder that was 1st identified in 1978 by Jeremy Allgrove and colleagues in 2 unrelated siblings. In most cases, there is no associated family history. The syndrome comprises of **A**chalasia, **A**ddisonianism and **A**lacrimia. It has an estimated prevalence of 1 per 1,000,000 individuals. About 200 cases have been reported worldwide as of date, showing a great variability in severity as well as in clinical manifestations. Consistent oral findings could be noticed such as high arched palate, oral pigmentation, fissured or atrophic tongue, and xerostomia

Case report: We report the case of a 25-year-old girl who presented with Triple A Syndrome with all characteristic clinical features.

Conclusion: The aim of this case report is to highlight the dental involvement in Allgrove syndrome. In addition, we aim to emphasize the need for the oral physician to accurately diagnose the condition, manage the oral manifestations, and to collaborate with other dental specialists to provide optimum dental care to these patients.

Keywords*: AAA Syndrome, Allgrove's syndrome, Dental, Oral Manifestation, Xerostomia,*

Introduction

Allgrove's syndrome is also popularly termed as AAA syndrome. This was first described by Jeremy Allgrove and his co-workers in 1978. It was considered as a rare chromosome recessive disorder[1]. Literature review reveals approximately 200 cases of AAA syndrome. Although the exact prevalence is unknown, it is estimated that 1 in 1,000,000 individuals suffer from this condition[2]. "Achalasia", "Adrenal Insufficiency" and "Alacrimia" are the major features of this syndrome[1]. Over-time more features of this syndrome were seen to be caused by dysfunction of autonomic nervous system. Gazarian et al. reported autonomic neuropathy in 4 children and suggested referring to the Allgrove's syndrome as "4A syndrome"[3,1]. Recently, studies have identified a mutation in a candidate gene on chromosome 12q13 in AAA patients[4].

AAA syndrome is normally seen during 1st decade of life with alacrimia and achalasia as the earliest manifestation[1]. Adrenal insufficiency and other related features are usually seen in adulthood. Mutation in the 3A's gene on chromosome 12q13 is known to cause the clinical manifestations of this syndrome. This gene is expressed in large quantities in the gastrointestinal system, renal system, adrenal and pituitary gland, and cerebellum[5]. A tryptophan aspartate repeat protein called ALADIN – which is a product of 3A's gene – is

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widely distributed in a large number of organ systems which explains the extensive systems affected[6].

The orofacial region is also widely affected in AAA syndrome – a long dysmorphic phase, elongated philtrum, thin upper lip, and prominent facial features. Intraorally, patients can present with a high arched palate with or without cleft, malocclusion (predominantly cross-bite)[7]. Tongue in these patients can have various presentations such as fissuring, atrophy, poorly developed papillae and occasionally enlarged. Adrenal insufficiency or addisonianism contributes to hyperpigmentation of the perioral region and oral mucosa[2].

Case

A 25-year-old female patient, complained of pain in the right lower back tooth region for the past 20 days and burning sensation in the mouth past 3 months. Patient also gave history of pain which was gradual in onset, continuous in nature, and mild in intensity, and aggravated on chewing food. Pain was radiating to the head and neck and subsided on its own after one hour. No history of nocturnal and postural changes was reported. Patient also complained of burning sensation (VAS 5) in the past 3 months leading to difficulty in eating food leading to loss of appetite. Difficulty in opening the mouth widely. Patient gave a history of difficulty in swallowing food (Fig 1). No h/o burning while micturition. Patient gives a history of dryness of mouth in the last 3 months. Difficulty in speech for long duration. Weight loss from the past 1 year. On asking about the medical history the patient informed she was diagnosed with case of Allgrove's disease at age of 3 yr. On which on asking, patient informed about sudden pigmentation of skin, recurrent respiratory infection, and absence of tears in childhood for which she was advised with artificial tears. Patient's mother informed the delivery was normal. Patient was informed she attained menarche at 14yr of age and had an irregular menstrual cycle. Also gives the history of joint pain from past few years. Patient parents were healthy with no history of similar condition in both maternal and paternal families. Parent's marriage was 2nd degree of consanguinity. Patients may have 2 sibling, and both are healthy. Patient is on Tab Wysolone 5mg (1-0-1/2), Tab Floricot 0.1mg (0-0-1), Tab Calspect 0-1-0, Tab Tayo 60k (one tablet in month). Patients brush once daily and consume a mixed diet with no deleterious habits. On general physical examination, patient walked in unassisted, well- oriented to time, place, and person. Appeared comfortable, Built: ectomorphic, Height: 4'9", Weight: 30 kg, Temperature: afebrile, Vital signs-Pulse: 94 beats/ min, B.P: 140/ 100 mm Hg, Respiratory rate: 24 cycles/ min. Pallor, Icterus, Clubbing, Cyanosis, Lymphadenopathy, Edema were absent. Examination revealed wasting of the thenar and hypothenar muscle (Fig 2). Facial appearance revealed dysmorphic facial features characterized by prominent ears, bulging eyes, and dysarthria with nasal speech (Fig 3). On extra oral examination, Lymph nodes: non-tender, non-palpable. TMJ examination: Bilaterally symmetrical, well- coordinated movements were noted with normal mouth opening. No clicking was noted. Mouth opening-30mm. On intra-oral examination of soft tissue, there was evidence of whitish yellow scrapable plaque on the dorsal and right lateral border of the tongue with multiple deep grooves measuring 1-2cm were seen on the anterior 2/3rd surface of the dorsal surface of the tongue (Fig 4). Tongue appeared erythematous, shrunken with dryness in the oral cavity. Moreover, evidence of crackling in the corner of the mouth bilaterally which were erythematous in appearance and tender on palpation (Fig 5). On salivary gland examination, there was reduced salivary flow without any glandular swelling. On hard tissue examination, Generalized demineralization of the tooth (Fig 6). Dental caries in relation to 11, 12, 16, 17, 31, 34, 35, 36, 37, 41, and 47. Tender on percussion 47, Calculus ++, Stains +. For investigation, the orthopantomogram (Fig 7) and cytological smear was taken from the dorsal and lateral aspect of tongue. Taking all intraoral findings and investigations in consideration, the provisional diagnosis of Oral candidiasis in relation to the lateral border of the tongue, Angular Cheilitis bilaterally, Xerostomia, Apical periodontitis in relation to 47, Dental caries in relation to 11, 12, 16, 17, 31, 34, 35, 36, 37 and 41.

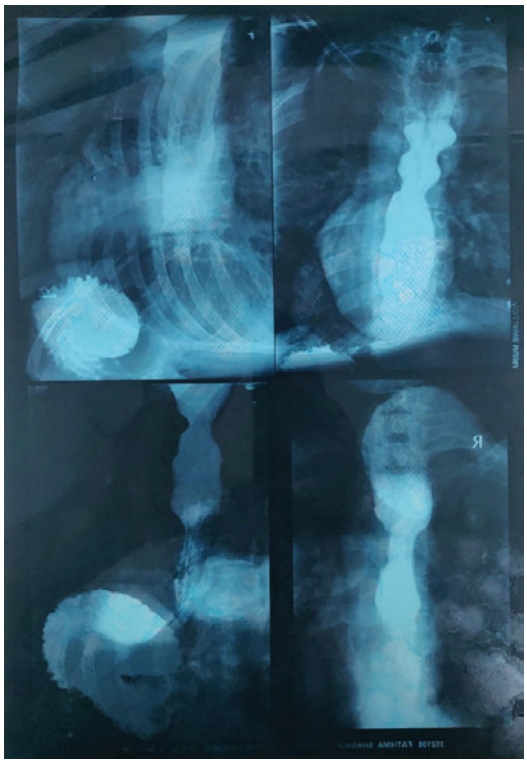

Figure 1: Oesophagography with Barium Swallow diagnostic of achalasia cardia

Figure 2: Wasting of Thenar and Hypothenar Muscle

Figure 3: Dysmorphic Facial Features Characterised by Hypertelorism, Prominent Ears, Prognathism

Figure 4: Dry and Fissured Tongue with scrapable white candida lesion on dorsal and lateral aspect

Figure 5: Angular cheilitis

Figure 6: Intra oral examination showing multiple carious tooth and erosion on palatal aspect of upper anterior

Figure 7: Orthopantomogram showing multiple carious and restored teeth

Discussion

Our patient presented with the characteristic features of the original 3A's of Allgrove's syndrome mainly (Achalasia, adrenal insufficiency, and alacrimia). She also had very pertinent dysmorphic facial features as discussed in the literature such as malar hypoplasia and mandible prognathism[8]. Previous reports by other authors describe the presence of carious lesions, premature exfoliation of teeth, periodontitis and xerostomia[9]. Studies have shown that salivary gland hypofunction (resulting in xerostomia) can influence the oral microflora thereby increasing the chances of developing opportunistic infection specially candidiasis. Xerostomia further enhances the patient's caries

developing risk[10].

Xerostomia represents the cardinal symptom in multiple secretory dysfunctions. There have been reports of an association between xerostomia and angular cheilitis. We believe xerostomia should be considered as one of the cardinal features of AAA syndrome as hyposalivation results in all other oral manifestations such as increased incidence of carious lesions, oral candidiasis and angular cheilitis. These oral findings may help in early diagnosis.

Apart from systemic management of patient, it is imperative that oral changes or oral condition should be managed to improve the quality of life of patients. Patient was prescribed with topical application of candid mouth paint thrice daily on the dorsal and lateral aspect of the tongue for 2 weeks and for xerostomia Limcee was prescribed to keep sublingually and the patient was advised to take frequent sips of water and increase vitamin C in diet. Taking the patients complaint in consideration, patient was referred to the department of endodontics for restoration followed by the department of periodontics for oral prophylaxis. These patients should be followed up periodically and provided with adequate dental care.

Conclusion

This case report highlights the importance of recognizing xerostomia as an important clinical finding in Allgrove's syndrome. Considering the majority of the oral complications are related to reduced salivary flow multidisciplinary approach with appropriate and timely dental care will improve the quality of life of the patient.

Ethical Clearance: Taken from ethics committee of Manipal College of Dental Sciences, Mangalore

Conflict of Interest: Nil

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Biomedical Waste Management in India-A Review

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Abstract

Biomedical waste is any kind of waste either solid or liquid containing infectious, potentially infectious materials of medical, laboratory or research origin from activities such as diagnosis, prevention & treatment of diseases. Bio-medical waste has a higher potential of infection and injury to the healthcare worker, patient and the surrounding community. It consists of human anatomical waste, animal waste, microbiology & biotechnology waste, waste sharps, discarded medicines & cytotoxic drugs, soiled waste, liquid waste, incineration ash & chemical wastes. Common generators of biomedical waste include hospitals, clinics, medical & veterinary colleges, blood banks, mortuaries, autopsy centers, biotechnology institutions, research laboratories, home health care & funeral homes. Hazardous chemicals & radioactive waste though non-infectious require proper disposal. World Health Organization states that 10% of hospital waste are infectious & 5% are non-infectious but hazardous waste. World Health Organisation has classified medical waste into 8 categories which include general, pathological, radioactive, chemical, infectious, sharps, pharmaceuticals & pressurized wastes. In India, Biomedical waste (Management & Handling) Rules 1998 along with further amendments regulate biomedical waste management. It consists of 6 schedules which includes Category of Biomedical waste, Colour coding & type of container, Label for Biomedical waste containers or bags which should be non-washable & prominently visible, Label for transport of Biomedical waste containers or bags, Standard for treatment & disposal, Schedule for waste treatment facilities like Incinerator, Autoclave, Microwave System. Operating Standards like combustion efficiency & Emission Standards are defined. The present review article focused on basic issues as definition, categories, problems relating to biomedical waste and procedure of handling and disposal method of Biomedical Waste Management.

Keywords: *Biomedical waste, Health care, Colour-coding, Segregation*

Hospital is one of the complex institutions which is frequented by people from every walk of life in the society without any distinction between age, sex, race and religion. This is over and above the normal inhabitants of hospital i.e patients and staff. All of them produce waste which is increasing in its amount and type due to advances in scientific knowledge and is creating its impact. [1]Hospitals produce waste, which is increasing over the years in its amount and type. The hospital waste, in addition to the risk for patients and personnel who handle them also poses a threat to public health and environment. Biomedical waste (BMW) is any waste produced during the diagnosis, treatment, or immunization of human or animal research activities pertaining thereto or in the production or testing of biological or in health camps. It follows the cradle to grave approach which is characterization, quantification, segregation, storage, transport, and treatment of BMW. The basic principle of good BMW practice is based on the concept of 3Rs, namely, reduce, recycle, and reuse.[2]Due to the increase in the procedures that are carried out at the various health care setups, excessive amounts of waste have been generated at the centers of care. India approximately generates 2

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kg/bed/ day and this biomedical waste encompasses wastes like anatomical waste, cytotoxic wastes, sharps, which when inadequately segregated could cause different kinds of deadly infectious diseases like Human immunodeficiency virus(HIV) hepatitis C and B infections, etc, and also cause disruptions in the environment, and adverse impact on ecological balance.

Biomedical waste (BMW) is a major issue of concern in modern times.[3,4] As per WHO 15 -25% of the waste generated in the hospital is dangerous and hazardous to health as it poses a risk to health of individual .As per estimates 32% of new Hepatitis B infection, 40 % of Hepatitis C infections and 5 % of new HIV infections occur every year due to contaminated sharps and syringes.[5] Health care waste consists of solid, liquid and gaseous waste contaminated with organic and inorganic substance including pathogenic radionuclide generated from in vitro analysis of body microorganisms. Hospital waste possesses serious tissues and fluid. WHO (1999) reported that, about 85% of health hazard to the health workers, public and air hospital waste is non-hazardous, 10% infective and 5% flora on the area not infective but hazardous.[6] The Government of India (notification, 1998) specifies that Hospital Waste Management is a part of hospital hygiene and maintenance activities. This involves management of range of activities, which are mainly engineering functions, such as collection, transportation, operation or treatment of processing systems, and disposal of wastes.[7]

Classification of Bio-Medical Waste according to World Health Organization

The World Health Organization (WHO) has classified medical waste into eight categories: ´

1. General Waste
2. Pathological
3. Radioactive
4. Chemical
5. Infectious to potentially infectious waste
6. Sharps
7. Pharmaceuticals
8. Pressurized containers

Major Sources of bio-medical waste.

· Govt. hospitals/private hospitals/nursing homes/ dispensaries.

· Primary health centers.

· Medical colleges and research centers/ paramedic services.

· Veterinary colleges and animal research centers.

· laboratories and research centres

· mortuary and autopsy centres

· animal research and testing laboratories

· blood banks and collection services

· nursing homes for the elderly

· Biotechnology institutions.

· Production units.

Minor Sources of bio-medical waste.

• Physicians/ dentists' clinics

• Animal houses/slaughter houses.

• Blood donation camps.

• Vaccination centers.

• Acupuncturists/psychiatric clinics/cosmetic piercing.

• Funeral services.

• Institutions for disabled persons

Need of biomedical waste management in hospitals

The reasons due to which there is great need of management of hospitals waste such as:

1. Injuries from sharps leading to infection to all categories of hospital personnel and waste handler

2. Nosocomial infections in patients from poor infection control practices and poor waste management.

3. Risk of infection outside hospital for waste handlers and scavengers and at time general public living in the vicinity of hospitals.

4. Risk associated with hazardous chemicals, drugs to persons handling wastes at all levels.

5. Disposable being repacked and sold by unscrupulous elements without even being washed.

6. Drugs which have been disposed of, being repacked and sold off to unsuspecting buyers.

7. Risk of air, water and soil pollution directly due to waste, or due to defective incineration emissions and ash.[8]

Benefits of Biomedical Waste Management

1. Cleaner and healthier surroundings.

2. Reduction in the incidence of hospital acquired and general infections.

3. Reduction in the cost of infection control within the hospital.

4. Reduction in the possibility of disease and death due to reuse and repackaging of infectious disposables.

5. Low incidence of community and occupational health hazards.

6. Reduction in the cost of waste management and generation of revenue through appropriate treatment and disposal of waste.

7. Improved image of the healthcare establishment and increase the quality of life.

Biomedical Waste Management Process

Mismanagement of hospital waste implies a combination of improper handling of waste during generation, collection, storage, transport and treatment. Improper handling comprises several unsafe actions, such as handling without personal protective equipment (PPE), poor storage (e.g. high temperature conditions combined with prolonged storage times before treatment), manual transport for longer distances, use of uncovered containers instead of closed plastic bags, etc. Other examples include exposure times beyond acceptable limits, lack of worker and equipment decontamination procedures, etc., all of which affect hospital workers in different ways. There is a big network of Health Care Institutions in India. The hospital waste like body parts, organs, tissues, blood and body fluids along with soiled linen, cotton, bandage and plaster casts from infected and contaminated areas are very essential to be properly collected, segregated, stored, transported, treated and disposed of in safe manner to prevent nosocomial or hospital acquired infection.

Six steps of Bio medical waste Management

1. Waste collection

2. Segregation

3. Transportation and storage

4. Treatment & Disposal

5. Transport to final disposal site

6. Final disposal

Safeguarding the health care workforce against occupational health risks arising from hospital-waste management calls for effective infectious waste control measures. In addition to protecting workers health, such control measures protect public health and the environment from the hazards posed by hospital waste. Proper management ensures that infectious waste is handled in accordance with established and acceptable procedures from the time of generation through treatment of the waste and its ultimate disposal.

Salient Features of Biomedical Waste Rules 2016

1. The scope of the rules have been expanded to include various health camps such as vaccination camps, blood donation camps, and surgical camps[9]

2. Duties of the occupier of HCFs have been revised. Occupier is the person having administrative control over the HCF that is generating BMW[10]

a. Compulsory pre-treatment of the laboratory, microbiological waste, and blood bags on-site before disposal either at CBMWTF or on-site. The method of sterilization/disinfection should be in accordance with National AIDS Control Organization (NACO) or WHO

b. The use of chlorinated plastic bags, gloves, blood bags, etc. should be gradually stopped and this phasing out should be within 2 years from the date of notification of these rules

c. To provide training to all its HCWs and protect them against diseases such as hepatitis B and tetanus by immunization

d. Liquid waste to be separated at source by pretreatment before mixing with other liquid waste

e. To set up a barcode system for BMW containing that is to be sent out of the premises for treatment and disposal

f. All major accidents including accidents caused by fire hazards, blasts, during handling of BMW, and remedial action taken by the prescribed authority should be reported

g. The existing incinerator should be upgraded/ modified to achieve the new standard within 2 years from the date of this notification

h. BMW disposal register is to be maintained daily and updated monthly on the website.

3. The duties of the operator of a common biomedical waste treatment and disposal facility (CBMWTF) have been increased.[9] They should assist in training of HCW from where the waste is being collected. Furthermore, there should be barcoding and global positioning system established for handling of BMW within 1 year. Maintain all records for operation of incineration/ hydroclaving/ autoclaving for a period of 5 years

4. The segregation, packaging, transportation, and storage of BMW have been improved. Biomedical waste has been classified into four categories based on color code-type of waste and treatment options. In addition, untreated human anatomical waste, animal anatomical waste, soiled waste, and biotechnology waste should not be stored beyond a period of 48 h. In case, there is a need to store beyond 48 h, the occupier should take all appropriate measures to ensure that the waste does not adversely affect human health and the environment.[9]

5. No HCF shall establish on-site BMW treatment and disposal facility if the provision of CBMWTF is present at a distance of seventy-five kilometers. If no CBMWTF is available, the occupier shall set up requisite BMW treatment facility such as incinerator, autoclave or microwave, shredder after taking prior authorization from the prescribed authority. After confirming treatment of plastics and glassware by autoclaving or microwaving followed by mutilation/shredding, these recyclables should be given to authorized recyclers

6. Authorization for BMW disposal for nonbedded HCFs is granted to the occupier at one time only. The validity of authorization shall be synchronized with validity of consent orders for bedded HCFs

7. Standards for emission from incinerators have been modified to be more environmental friendly. These are permissible limit for SPM-50 mg/nm3 ; residence time in secondary chamber of incinerator – two seconds; standard for dioxin and furans – 0.1 ng TEQ/Nm3

8. Ministry of Environment, Forest, and Climate change will monitor the implementation of rules yearly. The responsibility of each state to check for compliance will be done by setting up district-level committee under the chairpersonship of District Collector or District Magistrate or Additional District Magistrate. In addition, every 6 months, this committee shall submit its report to the State Pollution Control Board.

Table- Colour coding of BMW

Category	Types of Waste	Colour and type of Container
Yellow	Human Anatomical waste Animal Anatomical waste Soiled waste Discarded or expired medicine Laboratory waste Chemical waste Chemical liquid waste	Yellow colour non chlorinated plastic bags having thickness equal to more than 50microns or containers.
Red	Contaminated waste (Recyclable)	Red colour non chlorinated plastic bags having thickness equal to more than 50microns or containers.
White	Waste sharps including metals	White colour translucent, puncture proof, leak proof, temper proof containers.
Blue	Glassware Metallic body implants	Cardboard boxes with blue coloured marking or blue coloured puncture proof, temper proof containers.

Suggestions

The following solutions to remove obstacles to make progress in hospital waste management are recommended:

1. Calculate and monitor economical benefits from waste minimization in hospital.

2. More accurate monitoring and controlling of hospital waste separation process by the ministry of health and medical education and environmental protection agency.

3. Materials management in a way that the 3 primary criteria of using less, reusing and recycling to be considered.

4. Review reasonably related laws to facilitate the process of reducing the danger of hospital waste and removing their problems.

5. Review the laws in the process of the hospital waste separation at source and related definitions to prevent from confusion of officials responsible for hospital waste separation.

6. Participation of private sector's specialists in the process of hospital waste management.

7. Domestic and foreign investment to import modern technology in the country by public and private sector.

8. Meetings with managers and officials for a closer relationship and familiarity with available scientific and practical solutions.

9. Helping of the University to improve the implementation of the plans and suggests new scientific recommendations.

10. Advertising extensively by the public media for more awareness of the public.

11. Using new technology capable of reducing waste in different parts of the hospital, especially areas with potential of production of hazardous wastes.

Conclusion

Training programs need to focus on empowering the healthcare professionals on biomedical waste

management with broad scope and practical knowledge in all aspects. Training the staff with checklists and regular inspections can bring about accountability in the staff. Improper Biomedical waste management leads to environmental pollution, multiplication of vectors like insects, rodents & worms leading to transmission of diseases like typhoid, cholera, plague, hepatitis & AIDS. Recycling of disposable syringes, needles, intravenous sets, and glass bottles without proper sterilization leads to hepatitis, tetanus, HIV & viral diseases. Benefits of biomedical waste management include healthy surroundings, reduction in hospital acquired infections & cost of infection control, reduction in reuse of infectious disposables & prevention of occupational health hazards. Awareness about hazards of biomedical waste & its proper disposal is required for a safe & healthy future. All health care professionals regardless of their designation, experience and qualification , designation must be included in these interventions, so that it can avoid cross infections among the professionals and patients in the health care sector.

Conflict of Interest- Nil

Ethical Clearance- Nil

Acknowledgment- Nil

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Utility of the Cell Block Preparation as an Additive Tool to the Conventional Cytological Smears

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Abstract

Background: For the detection of malignancy in the serous fluids of the body, cytological examination of the fluid is very important. It is also of prime importance and seems difficult to differentiate the malignant cells from reactive mesothelial cells during fluid cytology. The cell block preparation is a useful and additive diagnostic tool when cellular features in cytology are inconclusive or suspicious. The aim of the study was to assess the utilization of the cell technique as an additional tool along with the conventional smears that we are using in routine practice.

Methods: 200 body (effusion) fluids were examined by the conventional cytological smears as well as by the cell block technique.

Conclusion: The diagnostic yield increases when we use the cell block preparation method along with the conventional smears because the architectural patterns and cell morphology are very well preserved in cell block as compared to the smears.

Keywords: *Conventional smear, Cell block, body (effusion) fluids, Fluid Cytology.*

Introduction

Cytological examination of serous body fluids causes detection of cancer cells and it also gives us the details about inflammation and various infections[1]. The most important reason for cytological examination of the effusion fluid is to determine if it contains malignant cells or not [2]. It is difficult to differentiate a malignant cell from reactive mesothelial cell in day to day practice. The sensitivity of cytological diagnosis of effusion is low, which is attributed to benign morphology of the cells and changes that have occurred during processing.[1]

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Cell block technique is the unique and one of the oldest techniques for preparation of the material for microscopy.[1] We can diagnose the cytological abnormalities like reactive mesothelial cells in the fluid or adenocarcinoma by using the cell block technique.[1] We can obtain increased cellularity as well as better morphological details because there is good conservation of architectural features in the cell block. [1] Many sections can be prepared from the block for further study like immunohistochemistry and this is the major advantage of cell block method.[1] The cell block method was described in the literature as early as 1900 and it is one of the traditional methods used for processing cytological material. 10% alcohol-formalin is used for fixation. The principle of the fixative is cross linkage of the proteins present in the effusion material and formation of gel that can be used for cell block formation. We can evaluate the usefulness of the cell block method in the diagnosis of serous fluids by present study.

Aims and Objectives

The present study done with the view to:

· To establish utility and accuracy of cell block method

· To study the cytological findings of serous fluids by conventional smears and compare it with the cell block preparation.

Material and Method

The present study included 200 cases of effusion fluids, out of which 117 were pleural, 82 were peritoneal and 1 was pericardial from a tertiary care hospital, Vadodara. The patient presented with complains of ascites, pleural effusions or pericardial effusions were included in the study. The patients were subjected to fluid analysis, by both conventional smears as well as cell block technique. [5] The clinical features of the patient and the laboratory reports of the patient were obtained. The received sample was separated into two parts for different processes. Half of the fluid was put into the centrifuge, the supernatant fluid was discarded, and smears were prepared; stained with Hematoxylin & Eosin and MGG. PAP stain was used only if there is a need. The other half of the sample was centrifuged at 1500 rpm, we discarded the supernatant and the sediment obtained after centrifugation was fixed in 10% formal alcohol for 24 hrs [1]. The sample obtained by this method is further processed as a normal histology sample. The sections were cut, prepared and stained with H&E for microscopy. Some of the sections were stained with the special stains like PAS and Mucicarmine wherever necessary. The slides prepared from the block were examined for cellularity, arrangement, nuclear and cytoplasmic features. The comparison of conventional smear and cell block technique was done.

Results and Discussion

We examined 200 effusion fluid samples, in which 117 were pleural fluids, 82 were peritoneal fluids and 1 was pericardial fluid. Out of 200 cases, there were 110 (55%) males and 90 (45%) were females. We also classified the cases according to the age of the patient and 98 (49%) cases of the total were noted in age group of 41-60 years and only 1 (0.5%) case was noted in age group 0-10 years. The cases of pleural effusion were more common amongst the males i.e. 77 (38.5%) whereas only 40(20%) cases were noted among the female. Our study showed more cases of inflammation i.e.70 (35%) and only 07 (3.5%) cases of malignancy. Age group of 41-50 years was having the highest number of cases and the age group of 0-10 years was having the lowest number of cases (Table 1). In case of pleural fluid, 4 smears were diagnosed as suspicious on cytology and the same cases turned out to be malignant by cell block technique (Table 2).

In case of ascitic (peritoneal) fluid, the higher numbers of cases were inflammatory cases i.e. 69 (34.5%) and 13 (7.5%) were malignant (Table-3). Age group of 51-60 years was having the highest number of cases and the age group of 71-80 years was having the lowest number of cases (Table-1). In case of ascitic fluid, 6 (3%) smears were diagnosed as suspicious on cytology and the same cases turned out to be malignant by cell block technique (Table-3).

In the pericardial effusion case, there was an only inflammatory change and it was in the age group of 41-50 years. It was having mixed inflammatory cells.

Table 1 Sample distribution according to age and sex for the effusion fluids

Age Group→ (In years)		0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	71-80 years
Pleural Fluid	Male	01	03	14	16	17	10	11	05
	Female		02	05	06	10	07	08	02
Peritoneal Fluid	Male		03	03	05	10	08	04	
	Female		01	02	06	12	23	02	03
Pericardial Fluid	Male								
	Female					01			
Total (200)		01	09	24	33	50	48	25	10

Table 2 Comparison of Pleural fluid cases diagnosed by smear and cell block technique with sex wise distribution

Diagnosis			Diagnosis by smear	Diagnosis by cell block technique
Inflammatory Cases	Male	71(35.5%)	106	106 (no cellularity cases are included)
	Female	35(17.5%)		
Malignancy Cases	Male	04(2%)	07	07
	Female	03(1.5%)		
Suspicious Cases	Male	03(1.5%)	04	04 (Diagnosed as malignant)
	Female	01(0.5%)		
Total cases	Male	78(39%)	117	117
	Female	39(19.5%)		

Table 3 Comparison of Ascitic fluid cases diagnosed by smear and cell block technique with sex wise distribution

Diagnosis			Diagnosis by smear	Diagnosis by cell block technique
Inflammatory Cases	Male	28 (14%)	69	69 (no cellularity cases are included)
	Female	41 (20.5%)		
Malignancy Cases	Male	02 (1%)	07	07
	Female	05 (2.5%)		
Suspicious Cases	Male	02 (1%)	06	06 (Diagnosed as malignant)
	Female	04 (2%)		
Total cases	Male	32 (16%)	82	82
	Female	50 (25%)		

The cell block technique was described by Mandlebaum in 1900 for studying the exudates [4]. The fixative used for fixation of the smears is 10% alcohol-formalin and the formalin present in the fixative solution causes cross-linkage of the protein material present in the fluid and forms a gel which can be used for further processing [5].

In our study of 200 cases, maximum numbers of cases were inflammatory 177 (88.5%) while malignancy was detected in 27 (11.5%) cases. Pleural effusion cases were the commonest among all the cases followed by peritoneal effusion cases and pericardial effusion cases. We have compared our study results with, Archana et al. [1], Meenu et al. [3] and Bhavana et al. [8]. Because of high prevalence of tuberculosis in the region of the study, there is predominance of pleural fluids in present study (Table 4).

Table 4: Various studies showing distribution of the cases

Name of the study	Pleural fluid	Ascitic fluid	Pericardial fluid	Total
Bhavana et. al. 8	139 (61.78%)	84 (37.34%)	2 (0.88%)	225 (100%)
Foot et al. 6	1301 (64.12%)	700 (34.5%)	28 (1.4%)	2029 (100%)
Van de Molengraft 7	171 (67.32%)	83 (32.68%)	-	254 (100%)
Present study	117 (58.5%)	82 (41%)	1 (0.5%)	200 (100%)

Lymphocytes were predominantly seen in 62 (31.3%) cases. 7 (3.5%) and 40 (33.3%) cases were noted showing low cellularity in various other studies like Bhavana et. al. [8] and Meenu et. al. [3] respectively (Table 5). Inflammatory smears prepared from pleural effusion were mainly showing leucocytes. Our study has more numbers of pleural effusion cases showing chronic inflammation with lymphocytes predominant and very less/ no mesothelial cells.

Mesothelial cells show a characteristic feature that is gaps or window formation due to microvilli that separates the cells [9]. Bedrossian described that in benign mesothelial cells, the microvilli are evenly distributed around the cells and they are slender; whereas in adenocarcinoma, the microvilli if present, are concentrated at the poles and are short [10]. In present study, predominantly mesothelial cells were present in 21% (42 cases) of inflammatory effusion cases. Generally the malignant cells may have molding of the nucleus, irregular nuclear membranes and prominent nucleoli with no gaps or windows.

The cell block technique can be used in the diagnosis of early stages of adenocarcinomas because there may be very less malignant features in the conventional smears but we can identify the features of malignancy by cell block and it is possible to stain and confirm the malignancy in the same sample with various special stains [11].

In the cell block, the carcinomatous cells have variable sizes, nucleus showing pleomorphism and overlapping, hyperchromatic nucleoli, intracytoplasmic vacuoles and occasional multinucleated cells. The malignant cells may form tubular structures with central lumina or show gland like arrangement. The nuclei of the individual malignant cells show abnormal mitoses, granularity of the chromatin and prominent nucleoli [1]. There are many advantages of Cell block technique as they can be utilized for immunohistochemistry. Eight to ten sections can be cut and used for evaluation of a large number of antigens. We can store the cell blocks very easily as compared to the conventional smears.

In the study done by Archana et al [1], out of 150 cases, only 29 cases were diagnosed as malignant by conventional cytological smears but 39 cases were found to be malignant by cell block technique and in our study, 27 malignancies were detected by cell block technique, but routine conventional smear could diagnose only 12 malignancies (Table- 6). Thus we can increase the diagnostic yield by using the cell block technique along with the conventional smears. Only 5 cell blocks in our study showed no cellularity. Technical errors such as degenerated samples or inadequate sampling can produce lack of cellularity in cell blocks.

Table 5: Various studies showing comparison of cellular component present in inflammatory smears

Cases	Bhavana8et al	Meenu3et al	Present study
Smears with Neutrophil Predominant	43 (21.7%)	26 (21.7%)	38 (21.5%)
Smears with Lymphocyte Predominant	62 (31.3%)	16 (13.3%)	71 (40.1%)
Smears with Low cellularity	7 (3.5%)	40 (33.3%)	8 (4.5%)
Smears with Mixed cellularity	40 (20.2%)	24 (20.0%)	33 (18.6%)
Smears with Mesothelial cells (Predominantly)	46 (23.2%)	6 (5.0%)	27 (15.3%)
Total	198 (100%)	120 (100%)	177 (100%)

Table 6: Various studies showing comparison of Diagnostic yield by smear and cell block technique

	Bhavana8et al	Archana1 et. al.	Present study
Cases of Effusion	225	150	200
Diagnosed as Inflammatory	183	77	177
Diagnosed as Suspicious on smear	15	10	10
Cell block showing no cellularity	7	34	5
Diagnosed as malignant on smear	12	29	14
Diagnosed as malignant by cell block technique	27	39	24

Conclusion

We can conclude that there is increased diagnostic yield with the use of cell block technique because the architecture can be preserved and the same technique can be used in some cases where there is confusion between the malignancy and reactive / inflammatory changes. For the accurate diagnosis, IHC can also be performed on the tissue from the cell block.

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Compare the Accuracy of IOL Power Calculations Using Ultrasound Biometry and Partial Coherence Laser Interferometry based Optical Biometry

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Abstract

Aims: To compare the accuracy of IOL power calculations using ultrasound biometry and partial coherence laser interferometry based optical biometry

Material and Methods: All cases of cataract with no other pathology involved presenting to, ophthalmology department at RNT Medical College and Hospital, Udaipur from august 2018 to December 2019.The patients are divided into two group 1st group IOL power calculated by optical biometer and 2nd group IOL power calculated by ultrasound biometry.

Results: Significant p-value (0.01) was observed between Group 1 and Group 2 among pre operative and post-operative VS patients.

Conclusion: optical biometry with the AL scan (Nidek) proved to be slightly more accurate than ultrasound biometry for IOL power calculation.

Keywords: *AL Scan, Ultrasound biometry, IOL*

Introduction

The refractive power of the human eye depends on thepower of the cornea and the lens, the position of the lensand the length of the eye.

Accurate assessment of thesevariables is essential in achieving optimal postoperativerefractive results. If these biometric measurements andcalculations are inaccurate, the patients may be left witha significant refractive error.

Studies conducted byOlsen T[1]showed that imprecision in measurements ofanterior chamber depth (ACD), axial length and cornealpower contribute to 42, 36 and 22%, respectively, of theerror in predicted refraction after implantation of an intraocularlens (IOL).

Biometry is the method of applying mathematics to biology. The term was originally used by Whewell initially in the 1800s for calculating life expectancy. The refractive power of the eye primarily depends upon the cornea, the lens, ocular media, and the axial length of the eye.

When planning for cataract surgery, in order to achieve the desired post-operative refraction, the required power of the intraocular lens implant can be calculated if the corneal refractive power, media type, and axial length are known.[2]

In the late 1990s an alternative to ultrasound biometry was introduced, IOL master (OPTICAL BIOMETRY) was based on the principal of partial coherence interferometry[3]. Ultrasound measurement has always

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had two major limitations. First ultrasound measures the anatomical axial length of the eye from the anterior pole to the posterior pole not the optical axial length along the visual axis. Second problem with ultrasound biometry is that it measures to the front of the retina the internal limiting membrane, but the photoreceptors used for sight are at the back of retina near Bruch's membrane. With the introduction of optical biometry these two problem were addressed. For optical biometry patients fixes on the target light as a result the device actually measures the distance from the corneal vertex to the fovea rather than the anatomical length. Second the optical signal measures to the Bruch's membrane the back of the retina rather than the anterior limiting membrane. The device measures the true distance.

However, there is one limitation to the technology to obtain the measurements a fairly good optical pathway is required. In an eye with grade 4 nuclear sclerotic cataract or a white cataract optical biometry cannot get the reading[4].

Ultrasound axial length measurement have been the gold standard for many years with the introduction of optical biometry, used not only for axial length but anterior chamber depth pachymetry and lens and retinal thickness measurement[5].

This study is being done to compare the clinical accuracy and post - operative refraction between ultrasound and optical biometry.

Materials and Methods

Study subjects:

All cases of cataract with no other pathology involved presenting to, ophthalmology department at RNT Medical College and Hospital, Udaipur from august 2018 to December 2019 was included in study

Exclusion criteria:

1. Patients not willing to participate in the study
2. Ocular pathology involved along with cataract.
3. Patients with mature senile cataract (MSC) andhypermature senile cataract (HMSC)
4. Patients with posterior polar cataract
5. Cataract surgery with intraoperative complication

Study area: Ophthalmology Department in RNT Medical College and Hospital,Udaipur.

Study period: One yearprospectivestudy from August 2018 to December 2019

Methods of collection of data:

Study design: HospitalbasedProspectivestudy.

Sample size: 200

Group 1 – optical biometry

Group 2 - A scan ultrasound

Data collection:

Medical records and case sheets was referred whenever necessary to collect addition information. Patient wasundergo keratometry andA- scan and values was documented IOL power was calculated using SRK T formula. Optical biometry values was measured and values was documented IOL power was calculated using SRK T formula.

Study Analysis

This was a prospective analysis performed on patients undergoing phacoemulsification cataract surgery with IOL implantation. The patients are divided into two group 1stgroup IOL power calculated by optical biometer, keratometric value in optical biometrygroup was measured by manual keratometry with Bausch & Lomb (B&L) Keratometer and axial length byoptical biometryand 2nd group IOL power calculated by ultrasound biometry used for measuring axial length (AL) and K_1 K_2 measured by B&L keratometer. The postoperative natural visual acuity and refractive error was carried out after 6 weeks of cataract surgery. Spherical equivalent obtained in both cases are studied excluding surgical induced astigmatism. The data was statistically compiled and analysedappropriate statistical tests was applied

Intervention and technique

Patients was asked to sit on the chair and look straight then under local anesthesia containing proparacaine

hydrochloride 0.5 percent with a- scan probe, center of cornea was touched to calculate the axial length. Average of thee readings was taken. K1 and k2 values are calculated by keratometry. In optical biometry the patients was asked to sit in front of the instrument and asked to look straight and the readings was taken.

Statistical Analysis

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. Descriptive statistics included computation of percentages, means and standard deviations. The data were checked for normality before statistical analysis using Shapiro–Wilk test. Quantitatively, parameter data were analyzed using t-test while nonparametric data were analyzed using Wilcoxon signed-rank test and Mann–Whitney U-test. Chi-square test and fisher exact test were used for qualitative data whenever two or more than two groups were used to compare. Level of significance was set at $P \leq 0.05$.

Observations

Mean age was 65.91 years in Optical biometry (group 1) and 63.23 years inA-Scan Ultrasound, (group 2)

Group1- Optical biometry were 52 (52%) female and male patients were 48(48%) whereas from Group 2- A-Scan Ultrasound male patients were 37(37%) and female patients were 63(63%).

Left eye patients from Group1- Optical biometry were 46 (46%) and right eye patients were 54(54%) whereas from Group 2-A-Scan Ultrasound LE patients were 41(41%) and RE patients were 59(59%).

24% and 22% sample patients taken under the study were from visual standards 6/60 respectively from Group 1 and Group2 respectively.

Group 1 and Group 2 were further categorized on the basis of NS2, NS3 and NS4 in which maximum patient found with NS 3 (41.9%) grade in both group.

Table 1: Comparison of AL and IOL readings in both the groups

		Mean	Std. Dev	P value
AL	Group 1	22.79	1.08	0.15
	Group 2	23.09	1.04	
IOL	Group 1	21.16	2.95	0.65
	Group 2	20.95	3.53	

Patients categorized on the basis of AL mean was 22.79, 23.09 and standard deviation was 1.08, 1.04 respectively for Group 1- Optical biometry and Group 2-A-Scan Ultrasound respectively. Insignificant calculated p-value (0.15) was observed.

Patients categorized on the basis of IOL mean was 21.16, 20.95 and standard deviation was 2.95, 3.53 respectively for Group 1- Optical biometry and Group 2-A-Scan Ultrasound respectively. Insignificant calculated p-value (0.65) was observed

Table 2: Comparison V.A. Unaided after surgery in both group

			V.A. Unaided					Total
			6/12	6/18	6/24	6/6	6/9	
Groups	Group 1	N	16	2	0	35	47	100
		%	16.0%	2.0%	0.0%	35.0%	47.0%	100.0%
	Group 2	N	31	6	1	26	36	100
		%	31.0%	6.0%	1.0%	26.0%	36.0%	100.0%
Total		N	47	8	1	61	83	200
		%	23.5%	4.0%	0.5%	30.5%	41.5%	100.0%

P value=0.01 (S)

Significant p-value (0.01) was observed between Group 1 and Group 2 among VS patients. Maximum patients were having 6/9 VA, 47% from Group 1 and 36 % were from Group 2.

Table 3: Spherical Refractive error(D)

Groups	Mean	Std. Deviation	P value
Group 1	0.09	0.45	0.74
Group 2	0.11	0.604	

Group 1 have 0.09 Spherical Refractive error(D) and group 2 have 0.11 Spherical Refractive error(D) which showed statistically non-significant result.

Table 4:Spherical RE(D) in Group 1 and 2

		N	Mean	Std. Deviation	P value
Group 1	No correction	52	0.00	0.00	0.02 (S)
	correction	48	0.19	0.64	
Group 2	No correction	47	0.00	0.00	0.02 (S)
	correction	53	0.22	0.81	

Among group 1, no correction have not any Spherical Refractive error(D) and correction have 0.19 Spherical Refractive error(D) which showed statistically significant result.

Among group 2, no correction have not any Spherical Refractive error(D) and correction have 0.22 Spherical Refractive error(D) which showed statistically significant result.

Table 5: Comparison of postoperative visual acuity after spherical correction in both the groups

		6/12	6/18	6/24	6/6	6/9	Total
Group 1	No.	3	0	0	74	23	100
	%	3%	0.0%	0.0%	74%	23%	100%
Group 2	No.	3	0	0	66	31	100
	%	3%	0.0%	0.0%	66%	31%	100%
Total	N	6	0	0	140	54	200
	%	3%	0.0%	0.0%	70%	27%	100%

P value =0.56

Significant p-value (0.01) was observed between Group 1 and Group 2 among VS patients. Maximum patients were having 6/6 VA, 74% from Group 1 and 66 % were from Group 2.

Discussion

In our study it is found maximum number of patient having cataract at age of more than 60 years. It is similar to study done in south India by Singh S et al[6] in 2019, they reported that the prevalence of cataract in population more than equal to 60 years in south India in both urban and rural population

In our study it is found that prevalence of cataract in female is more than male. It is similar to study done by PK Nirmalan et al[7] found that prevalence of cataract significantly lower in male.

SimilarlyVashistP et al[8] concluded that prevalence of cataract increases with age and was higher in women than in men.

Optical biometry offers many distinct advantages compared to US-guided biometry. It is a non-contact approach with likely accuracy and reproducibility in the context of non-severe pathology. When limitations such as dense media opacity, high axial myopia, and/or poor fixation prevent use of optical biometry, US-guided biometry becomes a useful alternative method, as it can be used in cases with significant media opacity[9,10]

In our study we have compared optical biometry (non contact) and A scan(contact method) to assists the better visual outcome in patients with cataract of various grades. This study compares the refractive outcome post surgery between the ultrasound A scan and the optical biometry.

Similarly, there were no statistically significant differences in AL measurements and IOL power calculation between the two groups.

We know from previous studies that optical biometry measurements are most reliable[11].

The results from this study help to determine that in such cases, optical biometry (IOL Master in particular) may still be utilized or, at the very least, clinically relevant when making IOL selections for a specific refractive target.

Even in cases where high-quality data is available, our results and literature also suggest that there is still room for improvement in order to achieve more precise postoperative refractive results. It is therefore still important to any discussion of cataract surgery that there may be a role for spectacles after surgery despite the array of treatments, technologies and formulae.

A recent study in 2017 reported 49% of patients were still spectacle-dependent for distance after cataract extraction with IOL implant using the most advanced technologies to target emmetropia. In addition, there was a strong correlation between the use of spectacles post-operatively and surgeons' advice to obtain them for optimal refractive outcome.[12]

Precise and accurate biometric data is a fundamental requirement for successful refractive outcomes with cataract surgery. However, pathology often highlights technological related limitations where data of lower

quality is the best available. Newer modalities including those utilizing Swept source-based technologies are being integrated to overcome the technological limitations of prior generation biometry.[13]

In our study we found that Optical biometry done in group 1 showed better visual outcome postoperatively than compared with ultrasound A scan in group 2.This is comparable to Kiss B et al[14] study, 6 (95.7% using PCI technique) and Rose LT et al[15] study(100% in PCI group and 71.42% in US group).

Sang woo moon et al[16] in their study showed the difference is not statistically significant intraocular lens calculation done by AL scan were nearly same in predicting postoperative optical refraction compare to those of applanation ultrasound.

Raymond S et al[17] in their prospective double-blind randomised clinical study demonstrated no clinical advantage of PCI technology over conventional aplanation US for IOL power calculation.

Also, Moeini et al[18] in their study show that was no significant difference in IOL power calculation.

Other authors demonstrate that results with optical biometry are more precise and have more predictable refractive outcome than the conventional ultrasound biometry[19,20]

It is generally accepted that the optical biometry offers superior reproducibility of axial length measurement in comparison with applanation ultrasound biometry.

In generally is agreed that the accurate biometry is the most important factor in achieving a successful refractive outcome after IOL implantation.

However, optical biometry is limited by its inability to measure AXL in dense ocular media, nonoptimal fixation as in cases of age-related macular degeneration and in patients with mobility problems in which ultrasound biometry still has a role.

Currently optical biometry is the most widely used technique for biometry. If measurement using ultrasound biometry is done correctly, results of both methods correspond significantly and the methods are mutually replaceable

Our results showed that optical biometry with the AL SCAN (NIDEK CO. LTD) proved to be slightly more accurate than ultrasound biometry for IOL power calculation. We did not evaluated for different types of cataract.

Conclusion

In conclusion, the optical biometer is quick and easy to use and provides a noncontact technique with no risk of infection or corneal abrasion and most accepted by patients. It allows accurate AXL measurement and determination of IOL power for cataract surgery.

Our results showed that optical biometry with the AL scan (Nidek) proved to be slightly more accurate than ultrasound biometry for IOL power calculation.

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Effectiveness of Nurse Led Intervention Regarding Management of Hypertension on Awareness, Treatment Compliance, Life Style and Quality of Life among Hypertensive Adults: A Systematic Review

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Abstract

Introduction: Hypertension is one of the most common conditions affecting the health worldwide and is also the leading cause of death around the globe. There is a persistent need for effective nurse led intervention for interrupting the further spreading roots of the hypertension in the community. There is also a great need for creating awareness, ensuring the compliance with the treatment, modification of lifestyle and improving the quality of life for the sufferers of this condition.

Methods: The study design was systematic review and it includes studies from databases of PUBMED, MEDLINE, Cochrane library, SCOPUS, Science direct, and Google scholar (2008-2019). The included studies were focusing on the management of hypertension among hypertensive adults in terms of awareness, treatment compliance, life style and quality of life.

Result: The data was grouped and analyzed in terms of meta-analysis. Total 20 relevant articles were undertaken. Abstracted information is related to study design, population characteristics, intervention and outcomes.

Conclusion: The nurse led intervention in the management of hypertension is an effective strategy in improving the health outcomes in terms of creating awareness, ensuring the compliance with the treatment, modification of life style and improving the quality of life among hypertensive adults.

Key words: *Hypertension, awareness, treatment compliance, life style, quality of life.*

Introduction

Hypertension is a worldwide risk factor affecting the health and the cause of cardiovascular diseases. There is strikingly increase in the prevalence of hypertension from 23% to 42% which was age and gender standardized according to 2011 population census of India.[1]About one third of urban adult and near to one fourth rural adults in India are hypertensive who have least awareness and no knowledge about status of their hypertension. There is need to provide multicomponent interventions for managing hypertension among Indians as current rates of awareness, treatment and control of hypertension are very low for those who are on treatment.[2]

After the diagnosis of hypertension is made, the main issue in the management is the patients' compliance with the treatment. Even after having adequate knowledge about the condition,poor drug compliance is mainly the result of forgetfulness and interruptions in the daily routines.[3]There are many other socio-demographic factors which can significantly influence the rate of

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adherence such as level of education, marital status and duration of disease.[4]

Life style modifications can help to improve the quality of life by changing the eating habits, weight control, exercising daily, abstaining from alcohol and smoking cessation and then can play a vital and effective role in management of hypertension. The rate of practicing the life style modifications among the hypertensive patients are on the lower side which indicates greater need for advice and support for controlling their blood pressure.[5]Only knowledge and attitude are not enough to manage the hypertension. It was reported that even after giving interventions to improve knowledge and attitude and awareness, participants adherence to the practices was still poor.[6]

Materials and Methods

Research design: Systematic Review

Inclusion criteria: It includes the studies related to development and evaluation of nurse led intervention for management of hypertension in terms of awareness, treatment compliance, life style and quality of life among hypertensive adults.

Exclusion criteria: The study excludes the studies those did not discuss about the nurse led intervention for management of hypertension in terms of awareness, treatment compliance, life style and quality of life.

This review article was written after doing a systematic review of several studies, journals and articles on web. Ethical issues have been addressed as there is no direct involvement of human subjects in this study. The referred articles and studies have been properly cited in the reference section.

Data Analysis: Relevant articles on the topics of development and evaluation of nurse led intervention for management of hypertension in terms of awareness, treatment compliance, life style and quality of life by search of significant articles PubMed/Medline, Science direct, SCOPUS, CINAHL, Elsevier and Google scholar.

Results

A systematic review was done and total of 128 studies were selected for review, out of which 20 were found to be appropriate. Data was divided into three sections:

Literature related to awareness of hypertension among hypertensive adults:

Out of five reviews, only two (40%) have shown that the hypertensive patients had relatively high awareness related to their disease condition but their uncontrolled hypertension was mainly related to barriers associated with treatment, average knowledge about condition and poor selfcare practices.[7,10]Other three cross-sectional studies(60%) concluded that the prevalence of hypertension is high but the awareness about hypertension, knowledge about status of their condition and adherence to treatment plan was poor.[8,9,11]

A cross-sectional study was conducted with aim to assess awareness, treatment, and control of hypertension among the rural population of Bengal. The content included predesigned interview and blood pressure monitoring. Random sampling technique was used to select a sample of 651 participants. The findings revealed that about half (48.2%) of the participants were aware of hypertension; 47.1% were taking treatment and only 15 (18.7%) among them had controlled BP which was 8.8%.The study concluded that awareness, treatment, and control rates of hypertension among participants were low.[9]

A cross-sectional, descriptive study was conducted in Saudi Arabia with aim to assess awareness and knowledge and its self-care practices among hypertensive patients. Total 211 patients were included in study who were interviewed using semi-structured, pretested questionnaire and blood pressure monitoring was also done. The level of awareness about hypertension was high in majority of cases (72.6%), knowledge level was average (54.7%)and most of the participants(74.4%) had below average level of self-care practices. Although there was an awareness and adequate knowledge on hypertension among hypertensive patients, but the findings indicated an uncontrolled blood pressure (BP) and poor self-care practices among the studied population.[10]

A cross-sectional study was conducted to assess the correlation between health literacy and health related quality of life in patients with hypertension. The sample consisted of 400 patients with hypertension. The data

was collected by using a demographic questionnaire, Short Form Health Survey(SF-36), and Health Literacy for Iranian Adults (HELIA) Inventory. The mean and standard deviation scores of health literacy and health-related quality of life were, respectively, 68.66 (±13.56) and 52.94 (±15.20). There was a significant positive correlation between health literacy and health-related quality of life ($p<0.01$, $r=0.30$). Based on the findings, it was concluded that patients' health and quality of life should be promoted by the nursing officials and policymakers by using appropriate interventions to creating awareness and improving knowledge of patients about management of hypertension.[11]

Literature related to treatment compliance of hypertension:

Most of the studies (80%) have revealed that the patients receiving antihypertensive therapy have poor compliance and adherence to management of hypertension. Patients taking treatment for hypertension show compliance at the beginning of the treatment but afterwards the compliance rate decreases due to many contributing factors. Most commonly reported reason for non-adherence to treatment or skipping of the dose is forgetfulness and otherfactors like discontinuation of medication when feeling well, smokers and alcoholics are likely to be non-compliant, as well as illiteracy and male gender is also one of the factors.[12,13]

A cross-sectional study involving 221 hypertensive patients was conducted in the Bamenda Health District to evaluate Knowledge of Hypertension and Compliance with Therapy Among Hypertensive Patients. Validated questionnaires were used to assess knowledge of hypertension using a 15-item scale, while compliance was assessed using the 8-item Morisky Medication Adherence Scale. The results showed, 14.0%, 53.4%, and 32.6% of participants had adequate, average, and poor knowledge of hypertension, respectively. The antihypertensive compliance rate was 43.9% which was low. Again, the reasons for non-compliance were forgetfulness, lack of motivation due to the incurable nature of the disease and lack of symptoms of the disease. The study concluded that knowledge positively affected the compliance, and good compliance was associated with good blood pressure control. There is a need to educate the people about treatment properly and warn them about potential complications.[14]

A study was carried out using a prospective design as quantitative method and in-depth interview was used as qualitative design to assess the adherence to hypertension medication in North of Vietnam among the subjects aged from 35-64 years who were selected randomly. Quantitative part included data on medication use and adherence whereas qualitative data includes understanding the factors affecting the level of adherence. Among 315 patients only 49.8% were adherent to treatment. The medication adherence was higher in females than the males but the difference was not statistically significant. As the age increases patients are more likely to be compliant as the result of information provided to them about the complications. [15]

A cross-sectional study was conducted among 488 Chinese hypertensive patients who were admitted in a tertiary hospital in Xi'an, China. Data were collected regarding socio-demographic factors and hypertension-related clinical characteristics to determine the treatment adherence. The adherence to treatment was assessed using the previously validated instrument: therapeutic adherence scale for hypertensive patients. A total of 27.46% of patients were compliant with their antihypertensive treatments. Three factors were identified to be independently associated with antihypertensive treatment adherence: gender ($P = .034$), residence ($P = .029$), duration of high blood pressure ($P < .001$).Treatment adherence among hypertensive patients in China was poor. It was thus concluded that more attention and effective strategies should be designed to address factors affecting treatment adherence. Education about hypertension knowledge should be strengthened for patients. Moreover, the importance of lifestyle modification during hypertension treatment is often neglected by patients, therefore, there is an urgent need to educate hypertensive patients about the adherence to lifestyle modifications.[16]

Literature related to life style and quality of life:

A systemic review was conducted to find effects of lifestyle modificationson the management of hypertension. Medline, Academia, Google Scholar, Embase, PubMed, PMC, Cochrane Library, American College o Physicians (ACP), American Association

of Clinical Endocrinologist (AACE) and International Journal of Hypertension (IJH) were searched for studies on hypertension and lifestyle modifications. Out of 51,063 retrieved studies 19 studies were included. The review helped to reveal that life style modifications help to make significant changes in the blood pressure. These modifications include weight control, dietary modifications (DASH diet), low sodium diet, abstinence from alcohol and exercising daily. These multilevel lifestyle modifications are effective in controlling the blood pressure among hypertensive candidates.[17]

A study was conducted in tertiary care hospital, Erode with aim to assess the quality of life in the hypertensive patients and to evaluate the functional capacity, physical aspects, pain, general health, vitality, social aspects, emotional aspects and mental health of the patient. A total of 300 participants were selected for the study period of 6 months. Short form 36 item (SF-36) health survey questionnaire was used to assess the quality of life in hypertensive patients. The study concluded that the mental health (Avg. score 25.8) was the mostly affected component in hypertensive patients followed by emotional aspects and vitality. There is need for proper treatment and awareness about hypertension for the patient's mental and emotional well-being.[18]

Dietary modification practices are also one of the factors which helps to manage the blood pressure but in two studies it is found that dietary modifications are low in practice among hypertensive adults. They used to take processed foods, not aware about salt levels in their food, added salt in food on table and do not consider salt as causative factor of hypertension.[19,20]

Discussion

A study conducted by **Karmakar N. et al.(2018)** found that awareness and control rates of hypertension were low. Another study conducted by **Naimi AJ. Et al. (2017)** had shown that there is a significant positive relationship between health-related education and improved quality of life. A study on adherence rate to treatment by **Akoko BM. Et al. (2017)** had found that compliance rates for antihypertensives are low and the major reasons for non-compliance were forgetfulness, lack of motivation and lack of symptoms of disease. Another study by **Organi et al. (2020)** found that life style modifications helps to manage blood pressure but their practice rate is low among hypertensive clients. [9,11,14,17]

Conclusion

The nurse led intervention can effectively help to control the spread of hypertension by creating awareness, adherence to treatment, life-style modifications and quality of life among hypertensive patients. Several studies in review have concluded that there is an emergent need to manage hypertension and extensive efforts by health care workers is required. Thus, it is recommended to carry out more extensive research in this area so that improved nursing interventions can help to curb the spread of hypertension.

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Endurance and Respiratory Training, an Approach to Delay the Symptomatic Progression in Multiple Sclerosis - A Case Report

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Abstract

Multiple sclerosis (MS) is an autoimmune disease which is inflammatory in nature and shows gliosis and demyelination which lead to acute and chronic symptoms impairing the quality of life due to disability. Presence of immune-mediated response in Central Nervous System leads to the above presentation. MS is the most common condition of CNS which causes disability. Treatment of Multiple Sclerosis consists of treating the exacerbations along with slowing the disease process and including symptomatic therapies. The most invalidating symptom in people with MS is Fatigue. This case reports shows the effectiveness of endurance and respiratory training towards the delaying the progression of symptoms in MS.

Key*words*: *Multiple Sclerosis, Endurance Training, Respiratory Training, Neurological Condition*

Background

Multiple sclerosis (MS) is an autoimmune disease which is inflammatory in nature and shows gliosis and demyelination which lead to acute and chronic symptoms impairing the quality of life due to disability [(1)]. Presence of immune-mediated response in CNS leads to the above presentation. MS is the most common condition of CNS which causes disability [(2)]. Treatment of Multiple Sclerosis consists of treating the exacerbations along with slowing the disease process and including symptomatic therapies [(3)]. The most invalidating symptom in people with MS is Fatigue. A number of direct and indirect mechanisms in the disease process and inactivity are responsible for fatigue. Physiotherapy can lead to physiological and psychological changes that may be responsible in reducing fatigue [(4)]. Exercise Training has shown plasticity related changes in skeleton muscles and its oxidative capacity in individuals with moderate to severe MS. [(5)].

This is a case of 35years old male present with the complaints of progressive weakness which worsened the ambulatory status with the passage of time along with bladder involvement and dysarthria. A peculiar feature like Uthoff's sign where symptoms worsen in the presence of heat was also observed.

Case

A 35-year-old male patient came to physiotherapy OPD with the following complaints: **1)** Weakness in both legs (started 1 year back and kept on increasing), **2)** Difficulty walking independently (1 year back patient had independent walking but walking became assisted with the further increase in weakness), **3)** Retention of urine (since 3 months) and **4)** Disturbed sleep and appetite (since almost a year when the symptoms started arriving). The patient had a history of urine retention for which catheterization was done and also for more than a year patient experienced the low-grade rise of

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temperature with severe episodes of giddiness where giddiness exaggerated on looking upwards and on exposure to bright light. This caused difficulty in working and increased lapse of a number of working days. The patient was able to ambulate independently till one year of the onset of the symptoms but later on, weakness progressed leading to slipping of chappals and even further progressed to ambulation requiring assistance which later on lead to major support in ambulation.

Patient was on Beta Interferon 1a and Glatiramer (Copaxone). Also the sympotomatic medicines where advised during the occurrence of the symptom (Antipyretics, Analgesics). Disease modifying drugs were started 10months back i.e. 2 months after the onset. Dosages were manipulated as per the requirements during subsequent follow ups with the local neurologist under whose consultation the patient was. Dietary advices were given in order to maintain the optimal nutritional status.

On Observation it was found that patient's general condition was fair but nutritional status was poor and postural changes present were: Excessive lateral rotation, Clawing of hand (Due to over-reactive Flexor Policis Longus), Tongue fasciculations, Marked wasting of upper and lower limbs, Dry atrophic skin and Prominent clavicle (b/l).

On Palpation grade II tenderness was present over calf regions bilaterally whereas swelling, warmth and pseudo hypertrophy were all absent.

On examination, the following findings were noted: Higher Mental Functions were normal, Cranial Nerves were intact. There was a presence of Dysarthria**.** All the superficial and deep sensations were normal bilaterally. In a reflex examination, it was noted that superficial reflexes like corneal, conjunctival and abdominal reflexes were normal and plantar reflex showed flexor withdrawal bilaterally. In deep tendon reflexes, it was observed that Rt Biceps jerk was 2++ and Lt was 2+, Triceps jerk and Brachioradialis Jerk (Bilaterally) were 2+, Knee jerk (Bilaterally) was 3+ and Ankle jerk (Bilaterally) was 5+. During tone assessment, it was noted that UL tone was normal and LL tone was mildly spastic (Grade 1 and 1+ on MAS) but spasticity exaggerated during stress, anxiety, fatigue and exposure to an overheated environment. The tightness was absent. PROM was full and AROM was compromised due to weakness. On Manual Muscle Testing, Shoulder, Elbow and Wrist had grade V whereas MMT for various Hand muscles ranged from fair to Good to Normal where Extensor Digitorum, FDM, ADM, Opponens Digiti Minimi, Dorsal Interossei and grip muscles were fair; FBP, APL, Opponens Policis, Palmar Interossei, ABP and Palmaris Longus were good and lastly, FPL was found to be normal.

During coordination examination it was seen that finger to nose and Finger to finger à movements were sluggish but coordinated, slight past pointing due to weakness. Whereas Heel to shin sluggish because of dysmetria and weakness. Equilibrium test was not possible to assess as the patient was unable to stand independently

Barthel Score was 35; Berg Balance Score was 46 which showed a high risk of fall and Fatigue Severity Scale (FSS) showed an average score of 5.3.

During investigations, MRI (Gadolinium based contrast was injected for the radiographic scanning) showed the presence of plaques in several areas of CNS. CSF analysis was done 1 year back during the diagnosis process. Lumbar puncture was done and CSF analysis showed clear and colorless appearance. Cell count was normal and total protein levels are normal with the increase in Immunoglobulin component. Increased IgG in CSF and plaques in MRI confirmed the radiological aspect of diagnosis. Quantiferone test for Tuberculosis was done and was negative. Eye examination was was done to rule out metabolic disorders and sarcoidosis. Routine hemogram was done along with renal and liver function tests, electrolyte levels, sedimentation, B12, Vit D, Thyroid Function test and anti HIV, HbsAg were all done

Diagnostic Challenge: As the nature of the disease is progressive, it became difficult to maintain the patient's motivation to undergo regular physiotherapy in order to keep a check on the severity of the condition. Also because symptoms worsened on exposure to the hot environment, management of visits during OPD timings was also difficult.

Differential Diagnosis included Neuromyelitis Optica (NMO) and Neuromyelitis Optical Spectrum

Disorders (NMOSD), Idiopathic Inflammatory Non-Demyelinating Diseases, SLE,Sarcoidosis, Acute Disseminated Encephalomyelitis (ADEM), Tuberculosis, Transverse Myelitis and HIV.

Diagnosis of MS was made based on the criteria which mentioned two or more attacks, atleast 1 month apart, lasting at least 24 hours (known as DIT), and the second is presence of two or more neurological findings showing the involvement of more than one region in the CNS (known as DIS)

Rehabilitation

Patient and family education was the most important aspect of rehabilitation as the condition was progressive in nature which was leading to worsening of the patient's overall condition. Spasticity was looked after by the combination of stretching, positioning and hydrotherapy/ cryotherapy. Strengthening program was incorporated in such a way that it had proper rest intervals so as to control fatigue. Also, fatigue management techniques were introduced i.e. Energy Effectiveness Strategies. Grip strengthening and functional training were carried out. LL strengthening was carried out using the FITT (Frequency, Intensity, Type and Time) principle and also using electrical stimulation for toe extensors and dorsiflexors. Balance training was introduced initially placing the patient with full support and wide BOS which was then reduced to normal BOS but support was not removed completely due to the progressive weakness. Pain in calf was managed through hydrotherapy and positioning limbs in an optimal position so as to reduce the occurrence of tightness. Exercises to improve Trunk and pelvic stability were given. Pelvic floor rehab (exercises and electrical stimulation) was carried out to reduce retention. Frenkel's Exercises were given for UL. Transfer training and Gait training with assistive devices were given. Treadmill training with the unweighing harness was included in the treatment. Breathing Exercises, Thoracic Expansion Exercises and Pranayama were practiced as a home session. The speech therapist was involved in the management of dysarthria. Counseling and Yoga were advised to prevent the occurrence of psychosocial issues. Also at last appropriate home exercise program was suggested considering patients fatigue intensity and ergonomic changes in the home environment were carried out in the presence of an occupational therapist. Gait assistive devices were kept handy so that when the patient's symptoms worsened up during stress episodes they were used and also family members made sure to discontinue it once symptoms touched the baseline.

Follow up was taken first at a span of 1 month, then 3rd month and at 6th month. After that patient discontinued attending OPD due to shifting in location. It was observed in all consecutive follow-ups that despite the progressing nature of the disease, patient's functional status and fatigue tolerance were the same suggesting the sustainability of the physical condition and delayed disease process.

Outcome Measure	Base Line	1 month later	3 months later	6 months later
Barthel Index	35	31	34	34
Berg Balance Score	46	46	38	48
Fatigue Severity Scale	5.3	5.2	5.7	5.5

Discussion

Limitations in muscular strength and endurance including the respiratory muscles are the factors affecting functional capacity and their exercise performance. Respiratory muscles weakness can cause low cough performance resulting in aspiration or acute ventilator failure. This commonly leads to death of people with MS. Respiratory function and efficacy of coughing may get improved by training respiratory muscles. [6]. Negligence towards the importance of respiratory muscles has been found while the whole focus is pointed

towards whole body exercises for reducing fatigue. Exercise performance and other working muscles get compromised due to limitations of inspiratory muscle function. As endurance is the ability to sustain a specific activity for a prolonged period of time, it shares a direct relationship with fatigue and delaying the disease progression. It is therefore required for a patient to undergo moderate to intense aerobic activity twice a week for 30minutes along with strengthening of major muscle groups twice a week for achieving important benefits [7]. A wide range of rehabilitation is required for a patient with MS which demonstrates the difficulty in developing a personal rehabilitation protocol by the team. [8]. Also QOL gets markedly affected due to fatigue which is one of the debilitating symptoms and it needs to be treated by including aerobic and resistance exercises along with Yoga and Ti Chi which in turn improves balance, coordination and depression [9].

Conclusion

This case report concludes that with the appropriate inclusion of aerobic exercises aiming to improve the endurance maintained the symptoms from worsening up. Progressive worsening of the neurological symptoms affecting the QOL should be immediately considered as MS is progressive in nature and appropriate rehabilitation helps in delaying the disease progression. Also, a wide area of symptoms should be well addressed while designing a rehab protocol.

Limitations:

As the disease is progressive in nature, the patient's wellbeing will be for limited time duration. Also, stress being an inevitable part of life will lead to worsening of the symptoms.

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Ethical Clearance: N/A

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Efficacy of Stretching vs Muscle Energy Technique in Postoperative Elbow Stiffness: A Research Protocol

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Abstract

Background: The elbow, intermediate joint of the upper limb, frequently undergoes pathological events and is especially prone to stiffness. Patients lose his capacity to conduct the compensatory movements which may result in intolerance. Fracture and dislocations are commonly seen in the individuals. Careful evaluation is must before treatment. Physical therapy plays an important role in recovering functional activities.

Methods/Design: The study will be designed as a Randomized Control Trial. Total 20 participants will be selected as per inclusion and exclusion criteria from AVBRH and RNPC OPD, Sawangi (Meghe). Duration of the study will be 6 months. 20 subjects will be grouped into two out of which 1 group of participants will receive Stretching (static stretching) and group 2 will receive Muscle energy technique (Post isometric relaxation). Visual Analogue Scale for Pain and Assessment of Range of Motion using Universal Goniometer will be the outcome measures for the patient.

Result: Outcome measures of the patient using muscle energy technique can have more effectiveness as compared to stretching. It may help to regain muscle strength and the patient will be able to perform the compensatory movement in adjacent joints.

Conclusion: Will be published after the results are analyzed..

Keywords: *Postoperative elbow stiffness, Post isometric Relaxation, Restoration of ROM.*

Introduction

Postoperative elbow stiffness, a frequently disabling complication which poses serious challenges for its management. Elbow stiffness is poorly tolerated in adjacent joints due to lack of compensatory motions. Bending arc of 30° to 130° is sufficient to perform most activities of daily living. Some patients complain of functional weakness and ask for help if they cannot stretch post 40^0 or more than 120^0. Fracture-dislocation accounted for 38% of cases, 20% dislocation and 30% isolated fractures[1].

Posttraumatic elbow stiffness is poorly tolerated due to lack of compensatory motion. Supination and Pronation are reduced too. Elbow Stiffness impairs hand work, since this is highly dependent on the forearm's elbow extension and bending and rotation. A 50% in elbow movement would reduce the position of the upper extremity by nearly 80%[2].

Elbow stiffness is associated with extreme morbidity. Whether intrinsic (intra-articular) or extrinsic (extra-articular) is classified. Patients who have established risk factors for the development of heterotopic ossification (HO) formation and who undergo serious elbow trauma. Prophylaxis, either by indomethacin or by radiation

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therapy. Early HO excision proved safe and successful. Elbow's range of motion (ROM) assessment carefully is important. From experience and physical examination, it is important to determine whether the patient has a physically compromised elbow ROM that has been proven to be stable and successful. Through history and physical examination, it is important to assess if the patient has an elbow ROM functionally[3].

The stiff elbow is challenging to treat hence postoperative management and rehabilitation program is required which aims to regain elbow motion, restore muscle strength and reincorporate the daily activities of living[4]

The target will never be accomplished without intense physiotherapy. Muscle will be elongated manually through low force and appropriate hold duration. (Usually 30 sec) which diminishes muscle spindle performance resulting in reduced stretch reflex activity (Osama)

Muscle Energy Technique (post isometric relaxation) - is the result of reducing muscle tone within a single or group of muscles following a short duration of submaximal contraction of the same muscle. It operates on the principle of autogenic inhibition[5]. Stretching (Static) has the following purposes: It increases muscle strength and minimizes the risk of injury. Muscle Energy Technique (Post isometric relaxation) has following purposes: A) It helps to lengthen the muscle through its method of contraction and relaxation. B) It affects subsequent tone reduction experienced by the muscle or muscle group[6].

Elbow stiffness is a common problem seen in postoperative patients with traumatic and non-traumatic injury of the elbow. Pain and reduced performance may interfere with day to day activities. So, a structural physiotherapy intervention is necessary to reduce the pain and improve functionality of the elbow.

Aim and Objective

Aim of the study is to improve the flexibility of the elbow. Objective is to evaluate the effectiveness of regular stretching vs post isometric relaxation (PIR) in muscle energy technique on postoperative elbow stiffness on the parameter of pain and range of motion.

Material/ Methodology

Study Design

This study will be conducted in Musculoskeletal OPD of Ravi Nair Physiotherapy College, DMIMS(DU), Sawangi(Meghe), Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University, (DMIMS, DU) Sawangi(Meghe), Wardha. Before inclusion, all the participants will be informed regarding the aim and procedure of research. Those participants who will meet the inclusion criteria must give the written informed consent. This study will be conducted with a population from 10 to 30 years which will be divided equally into two groups with simple random sampling with envelope method where each group consists of 10 subjects. Group A will be given static stretching and Group B will be given muscle energy technique (PIR).

Inclusion Criteria: -1-month old Postoperative cases of elbow stiffness, pain on palpation, structural deformity and muscle hyperactivity were taken for the study.

Exclusion Criteria: - The subjects to have been excluded from this research are subjects not willing to participate, subjects with compartment syndrome, fresh skin graft and hypermobility.

Outcome Measures:

VAS (Visual Analogue Scale)

Goniometer

Procedure:

Group A (Static Stretching): Each stretch is performed 10 repetition withhold duration of 30 sec and rest period 15 sec between each stretch.

Group B (Muscle energy technique-Post isometric relaxation): The procedure of muscle energy technique depends on the autogenic inhibition in post facilitation stretch. This technique is given to biceps brachii, brachialis and brachioradialis which is contracted during submaximal degree of effort for 5 to 10 secs and resistance is applied over the patient's effort as asked to relax. Hold the stretch for 10 sec. Then ask the patient to inhale during the procedure. This complete

procedure is repeated for 6 to 7 times.

Expected Result

Regular stretching has been proved to be beneficial in improving post-operative elbow flexibility. VAS and Goniometer as reliable and valid tools to measure pain and ROM. Post isometric relaxation and regular stretching would have positive impact equally or superseding on post-operative elbow flexibility in terms of managing pain and rom.

Discussion

The study protocol is to propel with postoperative elbow stiffness has been selected to restore the functional range of motion. It would seem to be that stretching alone is not enough but as an adjunct to stiffness it is helpful. The present study demonstrates significant improvement in restoring Range of Motion in group A treated with static stretching compared to group B treated with muscle energy technique-Post isometric relaxation.

Trauma to the elbow joint is the major cause for the elbow stiffness. prolonged pain leads to involuntary or voluntary muscle guarding during motion. And capsule into contractures and structures around it. Which impair its functional activities in children's and adults. Muscle Energy Technique is a type of manual therapy that uses the muscle's own energy to relax the muscles through autogenic or reciprocal inhibition and lengthen the muscle in the type of gentle isometric contractions. The technique involves voluntary contraction of the muscle against the force of therapists in a regulated direction. The effect decreases pain, tone, and helps to restore weak muscle strength and joint mobility.[7]

Physiotherapy reduces the adverse effects of immobilization and helps patients recover to the functional stage of pre-fracture. An essential component of physiotherapy in the management of elbow stiffness is therapeutic exercise, particularly stretching exercises. The main role of stretching exercise is to enhance the capacity of connective tissue through elastic and plastic deformation properties to respond to tensile loading.[8]

Conclusion

Will be published after the results are analyzed.

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Self Medication in Dentistry

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Abstract

Background: During mid 1980's World Health Organization gave approval for some medical drugs from prescription to reduce the burden of the medical professionals. Self-medication is one form of medication which is practiced worldwide by many people with or without medical knowledge. In dentistry the most common reason for taking the self-medication is phobia towards the dental procedures.

Aim and Objective: This study was conducted to assess the knowledge about self-medication practice among common people and create awareness on its ill effects.

Materials and Methods: A cross-sectional study was conducted among general population of age 18years and above. As the study was conducted during the pandemic time, a web-based survey was conducted with a set of 11 self-structured questions which was framed in English language and vetted with expert`s opinion. The questions were circulated through links to the participants through various social media platforms.

Result: The 50 participants were grouped based on occupational status. chi-square test was performed and it was seen that most of the questions were statistically significant ($p < 0.05$). High significance was seen when the participants were asked the commonest reason and drug that they would prefer for self-Medication ($p < 0.00001$).

Conclusion: The common reason for using of self-medication in dental issue is pain, lack of time and phobia. This has to be improved by conducting awareness programs and the patients have to overcome their fear and volunteer themselves to treat the cause.

Keywords*: self-medication, professional, phobia, dental problems, General population.*

Introduction

Self-medication is one form of medication which is practiced worldwide by many people with or without medical knowledge. It can be commonly defined as using the medication without prescription or medical professional guidance [1]. During mid 1980's World Health Organization gave approval for some medical drugs from prescription to over the counter to turn down the over burden of the medical professionals [2].Due to this Pandemic situation, People refuse to go to hospitals and clinics and prefer self-medication for treating pain, swelling and increase I temperature.

In dentistry the most common reason for taking the self-medication is phobia towards the dental procedures [3]. There are various dental problems that may start simple but may end up with major issues. Self-medication may even make the situation worsen. Using of medication from over the counter is safe and shows effectiveness in common, but over using of it without proper knowledge on drug dosage, side effects, and interaction may lead to severe problems [4]. Self-medication has it both pros and cons. It becomes benefit

when it is properly used with understanding on usage of medication [5]. In developing countries like India most of the people refuse to take medical professional`s advice or concern in taking the medication, instead they get medicines from the retail medical, from self-experience or from the neighbors who are with the same symptoms [6]. This study was conducted to assess the knowledge about self-medication practice among common people and create awareness on its ill effects.

Material and Method

A Cross sectional study was conducted among the general population with 50 participants. People who were willing to participate in the study, irrespective of gender variation and age above 18years were included in the study. As the study was conducted during the pandemic time, a web-based survey was conducted with a set of 11 self-structured questions which was framed in English language and vetted with expert`s opinion. The questions were circulated through links to the participants through various social media platforms.

The Questionnaire began with the informed consent along with their demographic details followed by the questions regarding self-medication in dentistry. The format of the questionnaire was provided with multiple choices with four options. These were done to prevent bias, and each participant was allowed to submit the response only once. The collected data were subjected to statistical analysis.

Result

The study was conducted with 50 participants of general population and they were grouped under occupational status. We subjected the data to evaluate the chi-square value based on their occupation. On analysis of occupation wise distribution, it was observed 40% were professionals (doctors, engineers, lawyers), 30% were students (engineering, medicine, allied health sciences, arts and sciences), 10% were self-employed (business) and 8% belong to unemployed category and 12% were others. Questions asked in the survey were mentioned in (table 1).

On observing (table 2), most of the questions showed significant values. ($p < 0.05$). High significance was seen when the participants were asked the commonest reason and drug that they would prefer for self-Medication ($p < 0.00001$). Meanwhile, the results also showed non-significant values when asked side-effects of taking self-Medication ($p > 0.05$).

TABLE 1: Question and options:

1. When was your last visit to dentist? a)3months b)6months c)Year d)Never
2. What was the reason for the visit? a)Emergency b)Pain c)Dental procedure d)Regular check up
3. Have you taken any medication on your own? a)Occasionally b)When I have pain c)Every time d)Not at all
4. When was the last time you took self-medication? a)Less than a month b)More than 3 months c)One year d)Never
5. Whom do you prefer to get an advice for taking self-medication? a)From family & friends b)From pharmacy c)From media d)Self-thought
6. What is the reason for not visiting dentist? a)Phobia of dental procedure b)Dental visit is expensive c)Lack of time d)Self-medication is the best

Cont... **TABLE 1: Question and options:**

7. What kind of self-medication do you use? a)Medicine from pharmacy b)Traditional home medicine c)Previous prescription d)From media
8. What is the commonest reason for taking self-medication? a)Pain b)Tooth mobility c)Swelling or Redness in gums d)Burning sensation
9. What are the side effects of self-medication? a)Organ damage b)Worsening the existing problem c)Skin rashes d)Addiction
10. What is the commonest drug you take? a)Paracetamol b)Amoxicillin c)Dolo d)Others

Table 1 showing the questions and options given to the participants for assessing their knowledge about self-medication.

TABLE 2: RESPONSE OF PARTICIPANTS ON SELF-MEDICATION:

QUESTIONS	CHI –SQAURE	p Value
1	21.52	0.00008
2	8.08	0.04439
3	18	0.00044
4	4.24	**0.23669**
5	19.28	0.00024
6	13.84	0.00313
7	18	0.00044
8	34.64	0.00001
9	6.64	**0.0843**
10	45.6	**0.00001**

It is seen from table 2 that most of them are statistically significant ($p < 0.05$) and the non-significant values were highlighted.

Discussion

Self-medication is a common practice in economically deprived communities and in developing countries [7]. Self-medication is inappropriate if used with poor knowledge about the dosage, duration, side effects and interaction of frequently used drugs [4]. This survey was carried out to access the perception of self-medication among general population related to oral problems.

In the present study, 6.66% of students and 40% of professional responded that they would visit the dentist once a year showing their concern and positive attitude about oral health. It was accordance the study conducted by Arun K. Simon et al., where 63% of them reported their dental visit less than a year.[8]

In a study done by Anjan Giri et al., showed that 74% of participants were taking self-Medication for tooth ache and mobility respectively.[9] Also in a study by Swetha Gandhi et al., majority of the participants reported that they take self-Medication for Odontalgia (57.69%) followed by tooth mobility (19.33) [10]. This was similar to our study were 73.33% of students have

said that they would take self-medicine for pain followed by 25% of unemployed participants who would take for mobility of the tooth.

Symptomatic treatment always reduces the pain but never the cause. People usually prefer to go to dentist only when the self-medication is not working and the situation becomes worsen. This was seen in our study where 60% of students and 40% of professionals and self-employed participants stated that pain and dental procedure followed by regular check-up were major reason for their dental visit in our study.

Regarding the source of advice, 46.66% of students preferred pharmacist and 50% of professionals preferred family and friends for taking self-Medication. On comparing the result between students and professionals revealed that students would rather get an advice from people having medical knowledge than family and friends. This was similar to the study done by Arun K Simon et al., and Vedrana et al., were 62.5% and 63.8% of participants stated that the principal instructor for self-medication was the pharmacist (62.5%) [8,11].

Among the total subjects, 60% of professional and self-employed confessed that lack of time was the major reason for not visiting a dentist which might be because of their work pressure. This was accordance to the study by Subhashini et al., where 39.49% had reported that they had no time for visiting a dentist [12]. Also, in the present study, 20% of self-employed participants revealed that they had anxiety and phobia for dentists and their practice which was similar to the study by Qaiser Ali Baig et al., who showed 52% of their participants preferred self-medication as they had dental phobia.[2]

When the participants were asked about the most preferred self-medicated drug`s name, 66.66% of the students and 55% of professionals responded that they would take paracetamol as their first choice of drug followed by DOLO, 15% said by the professionals in our study, which was similarly Henry James et al., study who reported that 81.3% used paracetamol [13]. Whereas in another study done by Mohammed Bilal et al., 52% of their participants preferred amoxicillin as their first choice of drug [14].

The limitation of the study is that, the results cannot be generalized as the study population was small and as the survey is circulated through online platforms, the accuracy of the answers and their truthfulness is not known.

Conclusion

The results are at the alarming rate of usage of self-medication with less knowledge on the side effects of it. The common reason for using self-medication in dental problem is phobia, pain, lack of time and also, many says the treatment is expensive. Now a days there is awareness among the people on side effects but it still has to be improved by conducting more health education programs to educate the people to overcome their fear and anxiety and volunteer themselves to treat the source without thoughtlessly following the medications from old prescription.

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Awareness of Oral Health Care among Pregnant Women During Pregnancy

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Abstract

Background: Pregnancy is a unique phase of women's life where it is associated with physiological changes. Poor oral hygiene maintenance and inadequate care for oral health have an impact and noticeable postnatal effects on the infants. There is only a minimal knowledge among pregnant women regarding oral health care during pregnancy.

Materials and Methods: A cross sectional study was conducted among 74 pregnant women. As the study was conducted during the pandemic time, a web-based questionnaire in Google forms was framed. The questionnaire was sent through various social media platforms and answers were collected and sent for statistical analysis.

Results: The responses were statistically analysed by SPSS software Version 18.0. Majority of the participants lacked knowledge about the facts that pregnancy increases existing dental problems and self-medications without physician's prescription during pregnancy are not advisable.

Conclusion: Increased hormonal secretion and fetal growth induce several systemic, as well as local physiologic and physical changes in a pregnant woman. During this period pregnant women should always take good care of her oral and systemic health. This not only improves the mother's overall health but also prevents associated problems in the fetus. Adequate knowledge by creating awareness about oral health and oral hygiene maintenance will prevent pregnant women from dental complications.

Keywords: *Pregnancy, Oral Health Care, Awareness, Dentist, Self-Medication.*

Introduction

The storm of hormones which is induced during pregnancy causes changes in the mother's body and the oral cavity. An increase in the secretion of the female sex hormones, oestrogen by 10 folds and progesterone by 30-fold, is important for the normal progression of a pregnancy [1]. Elevated levels cause an increased capillary permeability, predispose the pregnant women to gingivitis and gingival hyperplasia [2].

The immense population of pregnant women have reported about the oral health issues. The oral changes which are seen in pregnancy include gingivitis, gingival hyperplasia, pyogenic granuloma and salivary changes. This also includes facial pigmentation (melasma or chloasma). Studies have shown that there is an association between the periodontal disease with pre-term birth and low birth weight infants. Good maternal oral hygiene is beneficial to both the mother and infants [3]. Many pregnant women think that these issues are normal

during pregnancy and vanish by itself after parturition. So, they fail to look up for an expert dental care and treatment as they are frightened that dental treatment during pregnancy may affect their infants. In most of the prenatal clinics the pregnant women are referred to Dental professionals only if they have a major dental complaint which has to be treated immediately.

Aim and Objective

The Aim and objective of the study is to assess the knowledge and create awareness among the pregnant women regarding oral health care during pregnancy.

Materials and Method

A cross-sectional study was conducted to access the knowledge of pregnant women about oral health care among. The sample size was calculated to be 74. The participants who were pregnant and willing to participate in the study were included.

Since the study was conducted during COVID -19 pandemic lockdown period, it was difficult to collect live samples from the subjects. A web-based questionnaire comprising of 10 close ended questions including the demographic details were prepared in Google forms. The link was circulated on various social media platforms, with a note explaining the purpose of the study that comes under the consent and the eligibility criteria of the participants.

Results

The study was conducted with the total of 74 females who were pregnant. The responses were collected among the population group under the study and evaluated for statistical analysis by SPSS software Version 18.0.

Table 1 depicted that 83.78% of pregnant women have said that emergency treatment should not be done during pregnancy. Table 1 depicted that 83.78% of pregnant women have said that emergency treatment should not be done during pregnancy and 66.21% have feel that it not safe to undergo scaling during pregnancy. Most of the pregnant women in our study did not believe that pregnancy will worsen the existing dental problem. On the other hand, 89.18% of women were aware that 2nd trimester is the safe period during pregnancy to take few dental treatments and 63.51% of participants have even said that it is safe to undergo basic dental procedures during pregnancy. Additionally, 70.3% of participants have thought that regular check-up during pregnancy is necessary. The chi-square value and p-value showed in table 2 revealed that most of the questions showed significant values ($p<0.05$).

Table 1: Questions and Responses Distribution

Q	Question	Yes (Response) (%)	No (Response) (%)
1.	Is regular oral health check-up needed during pregnancy	70.3%	29.7%
2.	Will it be safe to undergo basic dental procedures during pregnancy?	63.51%	36.48%
3.	Can emergency dental procedures (root canal, extraction) be done during pregnancy?	16.21%	83.78%
4.	Is tooth cleaning safe during pregnancy?	33.78%	66.21%
5.	Can removal of teeth be done during pregnancy?	24.32%	75.67%

Cont... **Table 1: Questions and Responses Distribution**

6.	Is it safe to take dental treatments during 2nd trimester (4th to 6th month) of pregnancy?	89.18%	10.81%
7.	According to you, will taking x rays during pregnancy affect the brain of fetus?	81.08%	18.92%
8.	Have you noticed any changes in your gums during pregnancy?	79.72%	20.27%
9.	Is it advisable to take medicines without the doctor's opinion during pregnancy?	13.51%	86.48%
10.	Will pregnancy increase existing dental problems?	36.48%	63.51%

Table 2: INDIVIDUAL RESPONSES REPORT:

Question No.	Chi-square Value	p-value	Significance
Q1	12.162	.00049*	SIGNIFICANT
Q2	5.405	.02007*	SIGNIFICANT
Q3	33.784	< .00001*	SIGNIFICANT
Q4	7.784	.000527*	SIGNIFICANT
Q5	19.514	< .00001*	SIGNIFICANT
Q6	45.459	< .00001*	SIGNIFICANT
Q7	28.595	< .00001*	SIGNIFICANT
Q8	26.162	< .00001*	SIGNIFICANT
Q9	39.405	< .00001*	SIGNIFICANT
Q10	5.405	.02007*	SIGNIFICANT

***p<.05 significant**

Discussion

It has been said that the mother "loses a tooth for every baby". There is no medical literature to support this statement. The loss of a tooth by a pregnant woman most likely reflects a continuation of her current state of dental health. Oral health maintenance is important during pregnancy. People think that pregnant women should never go to a dental check-up irrespective of the trimester and the dental issue due to fear and myth. This

results in self-medication and worsening the present problem.[2] In an earlier study done by Rafi A. Togoo.,[4] 41.03% of pregnant women did not visit the dentist for regular dental check-up during pregnancy due to fear. This was in contrast to our study where 70.3% of pregnant women showed a positive attitude towards it.

During pregnancy it is not advisable to undergo long dental treatment procedures such as root canal treatment or extractions. But there are few elective procedures which can be recommended to pregnant women according to their trimester. 63.51% of participants were aware about this in our study. Whereas, in an earlier study conducted by Hans M et al.,[5] only 30.66% of pregnant women believed that basic dental procedures were safe.

Scaling can be done during pregnancy period. It is a minimally invasive procedure and poses no harm to the featus. In study conducted by Muralidharan C et al.,[6] 58.8% of pregnant women had their teeth cleaned during pregnancy. But, in our study, only 33.78% of pregnant women believed that scaling was safe during pregnancy.

There is a myth saying that pregnant women should not undergo any dental treatment during pregnancy irrespective of the tooth complaint and trimester. This was shown in the study done by Sajjan P et al,.[7] where most pregnant women felt that dental procedures are not advisable during pregnancy. On the other hand, 89.18% of pregnant women were aware that second trimester is considered to be the safe period during pregnancy for few dental treatments. The estimated fetal dose for one dental visit is 0.01 mrad. Dosage of less than 5 rad is shown to not cause any developmental disturbances. To be precautionary, it is better to avoid taking x-rays during pregnancy. But if deemed necessary, appropriate safety measures must be taken.[8] In our study 81.08% of pregnant women said that X rays should be avoided during pregnancy.

Many problems related to gums such as swelling, bleeding and bad breath during pregnancy are due to hormonal changes. In a study by Penmetsa GS et al.,[9] majority of pregnant women believed that gingival bleeding was common during pregnancy and any gingival changes during pregnancy will be resolved on its own after child`s birth. In another study by Rafi A. Togoo et al.,[4] 28.68% of pregnant women had developed gingival swelling, 27.49% women had bleeding gums during pregnancy. In our study 79.72% of pregnant women have noticed gum changes during pregnancy. This was in contrast to a study by Abiola et al.,[10] where 79% of women did not believe that pregnancy can lead to gum problems.

Not all medicines are safe during pregnancy. The FDA has categorized the potential teratogenic risk of medications by an A, B, C, D, X system. However, some drugs like paracetamol and vitamin supplements which belong to category A are considered to be safe drugs. In a study by Noha M. Zaki.,[11] 59.2% of pregnant women believe that all medications harm the featus and 78.9% pregnant women refrain themselves from taking medicines even in case of fever due to fear. Whereas, in our study 86.48% of pregnant women believe that it is not advisable to take medications during pregnancy without physician's opinion.

It is shown that only 36.48% of participants had the knowledge that pregnancy increases existing dental problems if oral hygiene is not properly maintained. Overall, in the present study, there is an increased positive response where pregnant women have a good level of awareness and knowledge about the oral health, dental treatments and medications that should be taken care of during pregnancy period.

There are few recommended dental procedures which can be provided to the patients during pregnancy.

For First Trimester (*conception to 14th week*): The greater risk of susceptibility to stress and teratogens occurs during this time and 50% to 75% of all spontaneous abortions occur during this period. It is not recommended that the procedures may be done at this time.

1. Patients should be assessed for their current dental health
2. Inform them of the changes that they should expect during their pregnancies.
3. Strict oral hygiene instructions to be given.
4. Limit dental treatment to periodontal prophylaxis and emergency treatments only
5. Avoid routine radiographs. Use selectively and

when needed. [2,12]

For Second Trimester (*14th to 28th week*): Some elective and emergent dentoalveolar procedures are more safely accomplished during the second trimester [2].

1. Oral hygiene instruction and plaque control.
2. Scaling, polishing, and curettage may be performed if necessary.
3. Control of active oral diseases, if any.
4. Elective dental care is safe.
5. Avoid routine radiographs. Use selectively and when needed. [1,2,12]

For Third Trimester (29th week until childbirth): Short dental appointments should be scheduled with appropriate positioning while in the chair to prevent supine hypotension. It is safe to perform routine dental treatment in the early part of the third trimester, but from the middle of the third trimester routine dental treatment should be avoided. [2]

1. Oral hygiene instruction, and plaque control.
2. Scaling, polishing, and curettage may be performed if necessary.
3. Avoid elective dental care during the second half of the third trimester.
4. Avoid routine radiographs. Use selectively and when needed. [1,2]

Conclusion

The changes that occur during pregnancy are the result of increasing maternal and fetal requirements for the growth of the fetus and the preparation of the mother for delivery. Increased hormonal secretion and fetal growth induce several systemic, as well as local physiologic and physical changes in a pregnant woman [13]. During this period pregnant women should always take good care of her oral and systemic health. This not only improves the mother's overall health but also prevents associated problems in the featus.[14] Through dental health camps and media, pregnant women should be given awareness about the regular dental check-ups and safe period for dental treatment during each trimester.

Ethical Clearence: Taken from Institutional Ethical Committee.

Source of Funding: Self

Conflict of Interest: Nil

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Clinical Profile Management of Liver Abscess with Percutaneous Pigtail Cathater

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Abstract

Background: Management of liver abscess has advanced in this era with many patients wanting its non-surgical treatment. Percutaneous drainage with Ultrasound guidance is one such minimal invasive procedure for management of abscess with its own limitation and advantages. We have undertaken this study at our institute to study clinical profile and management of liver abscess.

Methodology: All the patient enrolled after taking detailed history and Ultrasound was done. After giving consent an ultrasound guided pigtail catheter was insertion was done under local anesthesia. Post procedure all the parameters were observed and antibiotics was given and depending upon drainage of pus patients were followed.

Results: Total 30 patient of liquefied and partially liquefied liver abscess were enrolled in this study. Under local anesthesia ultrasound guided pigtail catheters of various sizes (14 F) were introduced in these patients using the Seldinger technique. There were 25 male and 5 female patients in this study.Most common affected age group was 31-40. Abdominal pain with fever was common presenting features. All the patients recovered in this study.

Conclusion: Ultrasound guided percutaneouspigtail catheter drainage ofliver abscesses is cost effective and minimal invasive procedure for liquefied and partially liquefied abscesses.

Keywords: *liver abscess, pig tail, needle aspiration*

Introduction

Liver abscess is pus filled cavitary lesion in liver caused by organism entering from enter hepatic system through biliary system and infecting hepatic cell and multiplying in liver.[1] Most of the abscess are pyogenic or amoebic or of mixed type. Rarely fungal abscess occur. It occurs in age group of 20-40 years with male predominance.[1] Around 60% of abscesses are solitary or can be multiple; mostly occur in 7th segment of right lobe of liverwhich may be attributed to flow of blood from superior mesenteric vein and portal vein to right lobe[1].Dull abdominal pain with or without fever is most common clinical presentation1.

Liver abscesses, both amoebic and pyogenic, still be a crucial reason for morbidity and mortality in tropical countries.[1] However, recent advances in interventional radiology, medical aid, newer antibiotic therapy, increased availability of ultrasound and computerized tomography scanning of the abdomen has proven to improve patient outcome by leading to early diagnosis and treatment of patients with liver abscess.[2] Percutaneous drainage of liver abscess has been one such important advancement in the management of pyogenic liver abscesses. Percutaneous treatment (needle aspiration or

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catheter drainage) is now a customary in management for liver abscesses.[3] Surgical management has very limited indications in this era.[4]Though needle aspiration avoids problems associated to catheter placement ,its care and long-term hospital, it has lower success rate than catheter drainage.[5,6] Another problem associated with needle aspiration is requirement of repeated needle insertions for multiple abscesses and for partially liquefied ones (average number of insertions per patient :1 - 5) over a short period of time within 5 to 14 days which might be painful and unpleasant for the patients and hence might not be acceptable to them.[7]Hence percutaneous pigtail catheter drainage which avoids these problems is now used as the first line tool in the treatment of liver abscesses.[8] The advantage of catheter drainage is that it provides a continuous output of pus and hence the issue of incomplete and repeated evacuations are not encountered.[9]

Methodology

This prospective study was conducted at Dhiraj Hospital Sumandeep Vidyapeeth Vadodara enrolling 30 patients with liquefied liver abscess. All the patients were enrolled after taking detailed history and Ultrasound was done.Patients having rupture liver abscess into peritoneum or pleura, having deranged coagulation profile were excluded.After confirming diagnosis,a written and informed consent is taken.

Patient is prepared for procedure and Inj Glycopylorate is given before procedure. After locating the point of insertion with an ultrasound, local anesthesia is given, skin incision is placed with a stab knife and under guidance pigtail catheter insertionusing Seldinger technique[9]is done and checked to placement in centre of cavity. Post procedure sterile dressing is done and all the vital parameters are observed and antibiotics are given.

Daily drain output was measured and charted. When there is no output in drain re-ultrasound is done to check for any remaining pus in cavity and if no pus is found pigtail is removed and patient is discharged. Follow up Ultrasound is done at 3 and 6months. and depending upon drainage of pus patients was followed. (FIGURE 1)

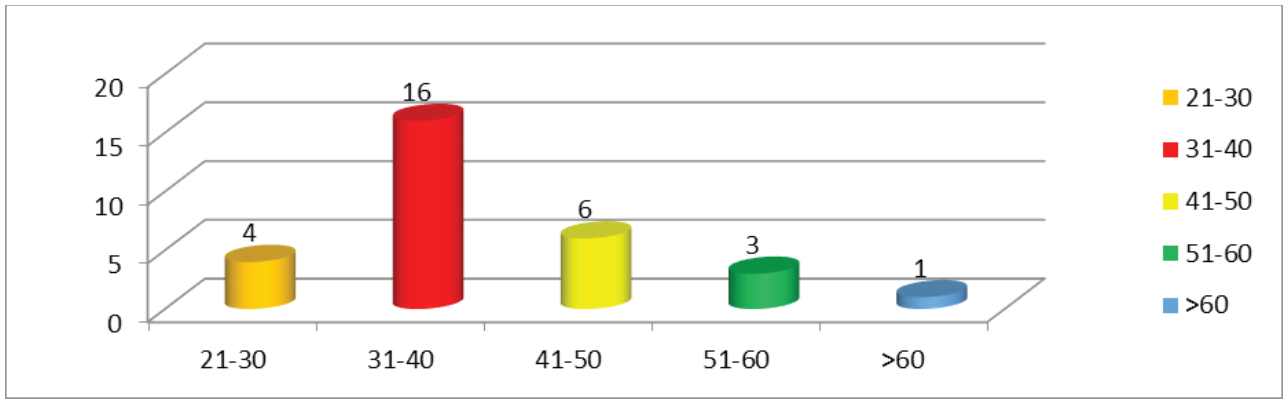
Figure 1: Pigtail in-situ.

Result and Discussion

1. Age Distribution:

Most of the patient in present study belonged to age group 31-40 which is working population as described in Graph 1.

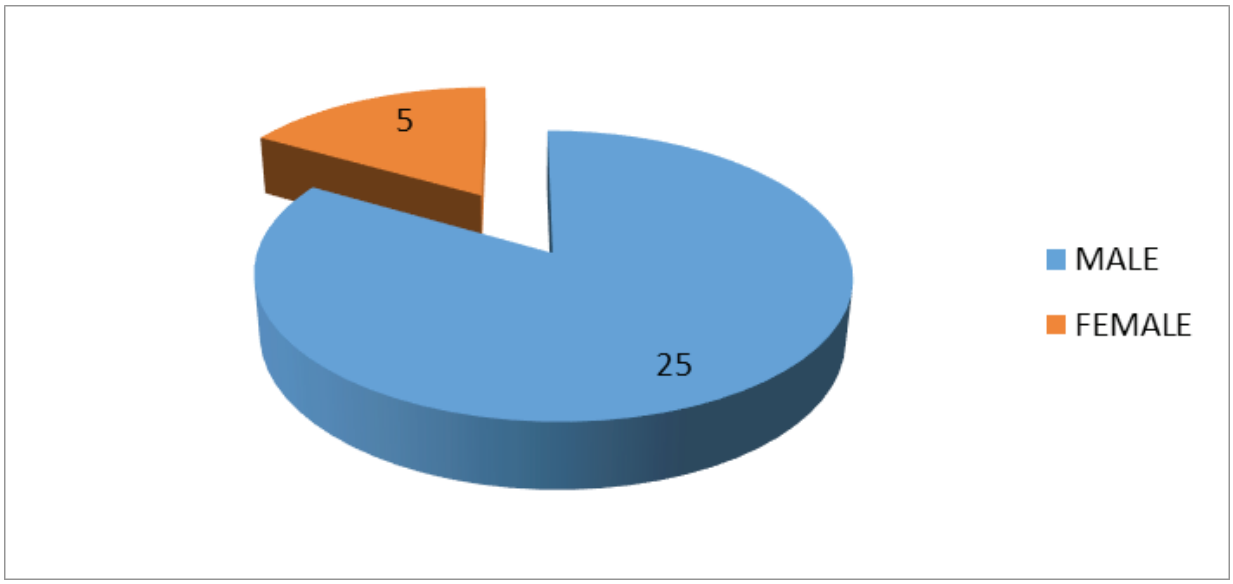
GRAPH-1AGE DISTRIBUTION:



2. SEX DISTRIBUTION:

There were 25 male and 5 female patients in present study. (Graph 2)

GRAPH 2: SEX DISTRIBUTION:



3. CLINICAL PRESENTATION:

Abdominal pain dull aching in nature in right hypochondriac region and epigastric region is most common presentation in 29 patient which was associated with fever in 28patients.Few patients presented with vomiting, diarrhea and jaundice. (Table 1)

Table 1: Clinical Presentation

Clinical Presentation	No Of Patients
Abdominal Pain	29
Fever	27
Vomiting	12
Diarrhea	11
Jaundice	8

4. Ultr4asound Findings:

Right lobe of liver is mainly involved in 23 patients while left lobe was involved in 7 patients that might be attributed to flow of blood from superior mesenteric vein and portal vein to right lobe[1]. 17 abscesses were completely liquefied while13 where partially liquefied of those with due time at hospital liquefied completely and patient were discharged. Most of the abscess in present study was > 5 cm in size.(Table 2, Table 3)

Table 2: Ultrasound Findings: Lobe and liquefaction

USG FINDINGS	LIQUEFIED	PARTIALLY LIQUEFIED
RIGHT LOBE	13	10
LEFT LOBE	4	3

Table 3: ultrasound Findings: Size of abscess

Size of abscess	No of patients
<5	2
5-8	10
8-12	12
>12	6

5. Pus Drainage:

In most of the patient amount of pus drained on first day was around 200- 300 cc and drainage was early in liquefied abscess compared to partially liquefied. In most of the patient drain was removed on day 6 or 7 after repeat ultrasound while in few cases with partially liquefied abscess it took around 11-12 days. Pus was send for culture and sensitivity and on gross examination in all the patients it was anchovy sausage appearance.

On follow up there was no recurrence. There were no complications of bleeding or diaphragm puncture during procedure.

Treatment of abscess of liver has evolved with decrease in mortality and morbidities. Also there is availability of newer antibiotics. At present liver abscess are being managed by Percutaneous drainage or surgical exploration either open or laparoscopic.[10] Percutaneous drainage is being performed in more patient compared to surgical procedure which may be due to advantage of low cost, non invasive and low morbidity associated with it[11].In present study also such advantage is noted. In few studies there were low success rates that may be due to early withdrawal of catheter or thick partially liquefied pus[12].These can be overcome by repeat ultrasound and use of antibiotics to liquefy abscess.

Though percutaneous drainage in present as well as few other studies has shown advantages advance study with more sample size may be helpful in deciding best method to treat liver abscess.

Conclusion

Ultrasound guided percutaneous pigtail catheter drainage ofliver abscesses is cost effective and minimal invasive procedure for liquefied and partially liquefied abscesses.

Ethical Clearance- Taken from SVIEC committee: SVIEC /ON/MEDI/BNPG19/020110

Source of Funding- Self

Conflict of Interest - Nil

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Dissection of Learning Opportunities and Obstacles While Learning Through Video Conferencing Platform (VCP) - During Covid 19 Lockdown

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Abstract

Background- COVID 19 pandemic has influenced our lives in all the aspects, even the younger generations are also not left untouched, specially their studies. Professional courses like nursing has integrated theory with practical learning and due to the lock down situation both aspects of learning - theory as well as the clinical learning are suffering. Though the teaching institutes have come up with the solutions like online lectures, using the video conferencing platform (VCP). This has expanded the horizon of traditional classroom. Though it is widely practiced in developed countries but in India it is still a new trend of learning. Purpose - of this study was to assess learner's satisfaction level while learning through video conferencing platform and various opportunities and obstacles learner's faced were explored. **Methods** – nursing students were the participants and online self-reporting method was adopted for the data collection. Self-developed questionnaire was completed by 138 participants and questionnaire had 36 items to respond on Video Conferencing Platform related to learner's satisfaction, also opportunities & obstacles encountered while learning through VCP.

Results – Majority (63%) of the participants were somewhat satisfied, learning through VCP and 26.1% were highly satisfied. Observation of the opportunities were found to be better learning outcome – brainstorming stimulation, self-motivated learning, able to ask and clear doubts and lecture was reported to be captivating. Another opportunity reported was comfort while learning which included the learning in personal space, no compulsion of physical presence at teaching institute and freedom to choose which lecture to attend. Obstacles reported were related to the classroom environment where the chances avoiding questions and fooling the instructor was reported by majority participants, technical challenges like slow internet disturbed the lecture and made it difficult to articulate lecture and also missed part of lecture. Other obstacles reported were decreased social interaction, participants found learning through VCP to be tiresome as compared to traditional classroom, connectivity issues disturbance create due to the surroundings. Association between the course of study with satisfaction level, opportunities and obstacles were found to be significant, whereas the association between the time spend on VCP was found to be significant only with obstacles.

Keywords*: video conferencing platform, learning, nursing students, opportunities and obstacle*

Introduction

COVID 19 has tremendously influenced the world in all aspects and education system has not been left untouched. Corona virus pandemic has significantly disrupted various sectors in India, since March 2020 the educational institutions are closed as precautionary and preventive measures. As per present situation there is an uncertainty when will these educational institutions will reopen. According to UNESCO Institute for Statistics data around 320,713,810 learners are affected in India due to COVID 19 lock down situation.[1]

Undoubtedly, this is crucial time for education sector when the entrance exams and university exams are held due to the pandemic, sessions are delayed and struggle to impart knowledge has been a challenge.

Multiple measure has been adopted by the educational institutions and their examining bodies like : Online sessions for teaching , revise the plan of academic calendar and postponing the examination. However, the challenges related to theory could be managed with video conferencing or online teaching. but the and inability to complete the academic requirements like practical or clinical duties.

Developed countries have already been using the web based / online teaching methods but India has adopted the teaching through video conferencing recently. This movement from traditional to internet based education requires engaging the students in their learning space which is compatible to their abilities and surroundings. In addition the teachers also need to innovate about the effective medium for learning, creating and sharing the ideas among learners. This is more feasible when video communication technologies are used and most common method is Video conferencing.

In higher education Video conferencing is the most commonly accessed via internet and it immediately facilitates learner's self directed learning[2,3]

Video Conferencing : video conferencing technology is a communication medium through which the learner or user is able to share visual and audio in real time. With this users transfer or exchange the ideas and thoughts using the presentations or text or slides through the Video Conferencing Platform (VCP).[4] However, this is dependent on one variable that is the speed of the internet and computer.

According to Campbell (2006) video conferencing has opened new opportunities for both the parties i.e, teachers as well as learners[5]. Majorly, teachers use this method for inculcating problem solving and competency among learners [6]

Benefits : Application of theory to practice is effectively learnt through video conferencing because it promote students' participation and problem solving[7]. Additionally this method allows learner to record the session which can be later accessed and reviewed whenever needed, this is not possible in traditional teaching method. The geographical limitation is overcome by this video conferencing method and helps learners to develop cultural competency as they are from different backgrounds[8].

Challenges: video conferencing limits the response and interaction to one person, only one person is allowed to speak at a time and this may cause lack of interest among rest all learners[9]. Another challenge is lack of infrastructure from learner side like lack of microphones or slow internet speed. Moreover the learners behave the same manner as they are in traditional classroom[10]. In addition, it was reported that video conferencing has no effect on the learners' outcome because they faced difficulty to use the equipment and they were not feeling free to express themselves during the session[11].

Literature on effectiveness of video conferencing in education have reported that environmental as well as individual factors influence learning experience and outcome of learning. Environmental factors like hardware or the desktop/laptop etc, and individual factors were attitude and knowledge[12].

The current limitation of COVID 19 has revolutionised the movement of higher education towards comprehensive learning experience through internet and most common platform adopted is video conferencing[13-15].

In view of above the study was conducted to explore the students' perspective related to teaching through video conferencing, in India. Though it is in practice in many countries but it's a new initiative in India. With video conferencing providing different educational environments, still there is lack of notable research to demonstrate the use of this method in nursing education.

Materials and Methods

In this study, during COVID 19 Lockdown - learning through video conferencing platform was assessed among nursing students. As, it was not feasible to reach to the students physically due to lock down situation, therefore, it was decided to collect data using the internet. However, the ethical permission was taken from the institutional ethical committee.

Measure: The tool used for data collection had two sections:

· Demographics details – age, gender, Institution , University, Course

· Video conference Platform related data: - Platform used, Version used, Number of hours spent, Device used, Previous experience.

· Opinionnaire related to learning through Video Conferencing platform. –

It was further subdivide into two opportunities and obstacles:

Opportunities related to :

i. Learning outcome

ii. Skill development

iii. Comfort

iv. Classroom environment

Obstacles related to :

v. Learning outcome

vi. Technical challenges

vii. Comfort

viii. Classroom environment

Objectives of the study were:

· Assess the satisfaction level of learner

· Assess the obstacles and opportunities experienced by the learner

· Find out association between satisfaction level of learner and demographic variables

RESULTS & DISCUSSION

FREQUENCY DISTRIBUTION OF DEMOGRAPHIC VARIABLES

Participants in the study were 138 and self-reporting method was adopted, among the 138 participants 102 (73.9%) were female, and 36 males (26.1%). All the **participants were B.Sc. Nursing** students and attended online classes through **Zoom video conferencing platform** 128 (92.8%) other platforms used were webinar and skype and majority participants 120 (87%) used free version only.

Duration spent on Video Conferencing platform majorly between 03-05 hours daily (56.5%) whereas 22.5% participants spent 05-08hours daily.

Device used by majority of the participants was mobile phone (92.8%) rest 07.2% used laptop. Participants (82.6%) had no previous experience of learning through video conferencing platform, on other hand thirteen percent had learn through video conferencing platform. An opinion reported was participants (43.5%) were not sure about the VCP to be safe for use, and 21% reported it to be safe, rest 35.5% did not find VCP to be safe for use.

TABLE 1: FREQUENCY DISTRIBUTION OF DEMOGRAPHIC VARIABLES

N=138

Variable	Frequency (f)	Percentage (%)
Age	Mean±SD(range)= 20.36±1.44 (18-24)	
Gender		
Female	102	73.9
Male	36	26.1

Course		
B.Sc. Nursing 1st year	33	23.9
B.Sc. Nursing 2nd year	37	26.8
B.Sc. Nursing 3rd year	30	21.7
B.Sc. Nursing 4th year	38	27.5
Video Conferencing platform		
Zoom	128	92.8
Webinar	6	4.3
Skype	4	2.9
Version		
Free version	120	87
Provided by institute	14	10.1
Premium version	03	2.2
Paid version	01	0.7
Time Spend on Video Conferencing platform		
Daily 1-2hours	29	21
Daily 3-5hours	78	56.5
Daily 5-8hours	31	22.5
Device Used		
Mobile phone	128	92.8
Laptop	10	07.2
Have you ever learnt earlier through Video Conferencing Platform (VCP)?		
Yes	18	13
No	114	82.6
Not Sure	06	4.3
In your opinion is it safe to use Video Conferencing Platform (VCP)?		
Yes	29	21
No	49	35.5
Not Sure	60	43.5

Satisfaction Level Of Learner

Satisfaction level of learner was assessed through Likert scale and it was found that majority participants (63%) were somewhat satisfied with learning through VCP. Score range for the satisfaction level was 10-50 with mean of 31.86 and Standard Deviation ±6.30. Hence, the inference was drawn that learning through the video conferencing platform was somewhat satisfactory and met the needs of learner. Also, 26.1% of the participants were highly satisfied while learning through VCP.

TABLE 2: SATISFACTION LEVEL OF LEARNER

Satisfaction level (score ranges= 10-50)	Frequency (f)	Percentage (%)	Mean	SD (Range)
Dissatisfied (10-22)	15	10.9	31.86	±6.30 (18-48)
Somewhat satisfied (23-36)	87	63		
Highly satisfied (37-50)	36	26.1		

OPPORTUNITIES & OBSTACLES EXPERIENCED

Opportunities and obstacles encountered by the learners were further divided into subsection

i. Learning outcome

ii. Skill development/ technical challenges

iii. Comfort

iv. Classroom environment

The **opportunities** reported related to the learning outcome were:

· Participants found that VCP instigated **brainstorming** among them (50%) indulged them into **self-motivated learning** (55.1%), and allowed then to **learn adequately** (42.8%), also **clarified the doubts of learner** (63.8%). Moreover participants found the **lecture to be captivating** (48.6%). However a contrasting opinion reported was that 34.8% disagreed that it is **better than a traditional classroom** method.

· Under **Skill development** majority participants agreed that VCP **improved time management skill** (39.9%)among them and **i**mproved skill of using e learning methods (56.5%)

· **Comfort** was reported in terms of **VCP being user friendly** (49.3%), allowed **learning in personal space** (58.7%). Also, **VCP provided freedom** to choose which lecture to attend (40.6%) and **no compulsion of physical presence** at the teaching institute (50.7%).

· Related to Classroom environment, participants (34.8%) reported that **chances of avoiding the question** or fooling the instructor was possible through VCP and **opportunity to disturb** classmates was easier (34.1%).

The **obstacles reported** related to the learning outcome were:

· Under **learning outcome** the obstacle reported was **Lecture to be monotonous** y (41.3%) and **difficulty to articulate** lecture mixed response was reported(agreed - 37%)(neutral – 36.2%).

· **Technical challenges** reported were **slow internet** (62.3%) and **connectivity of other** students created disturbance(40.6%).

· Obstacles related to comfort reported were **VCP being tiresome** (31.2%) and **lack of interaction between friends**(47.8%), i**nteraction with family and leisure time** also decreased (37.7% and 36.2% respectively).

· Obstacles related to classroom environment reported were **difficulty to concentrate** (41.3%) due to the **disturbances from surrounding** at learner's place (55.1%) and also from others' surroundings (50%). One of the **major obstacle** reported was **missing the part of the lecture** due to the connectivity/ environmental disturbance/ other students speaking (53.6%)

Observation of the mean of the opportunities clearly depict that comfort was the most rated opportunity with mean of 13.09 and overall the mean of all the opportunities was found to be 50.36. Among obstacles classroom environment was found to have highest mean 11.11 and overall obstacles were found to have mean of 29.44.

TABLE 3: MEANS OF OPPORTUNITIES & OBSTACLES PROVIDED BY VCP

Opportunities (Score range)	Mean ±SD (Range)	Obstacles (Score range)	Mean ±SD (Range)
Learning outcome (Score ranges=6- 30)	19.71 ±4.25 (11-30)	Learning outcome related (score ranges =2-10)	5.12 ±1.61 (2-8)
Skill development (Score ranges =2-10)	7.03 ±1.88 (2-10)	Technical challenges (score ranges =2-10)	3.85 ± 1.64 (2-10)
Comfort (Score ranges = 4-20)	13.09 ±2.07 (5-18)	Comfort (score ranges =4-20)	9.35 ± 3.20 (4-16)
Class room environment (score ranges =2-10)	5.68 ±2.13 (2-10)	Classroom environment (score ranges =5-25)	11.11 ±3.55 (5-21)
Overall Opportunities (score range = 14-70)	50.36 ±6.59 (29-66)	Overall Obstacles (Score Range=13-65)	29.44 ±8.14 (13-52)

Hence the inference was drawn that the better learning outcome and comfort were the opportunities while learning through Video Conferencing Platform and obstacle was related classroom environment.

ASSOCIATION OF SELECTED DEMOGRAPHIC VARIABLE WITH

LEVEL OF SATISFACTION , OPPORTUNITIES & OBSTACLES

Association of satisfaction level, opportunities and obstacles with year of study was calculated & it was found these variables were associated as the calculated values (for satisfaction level -13.59, opportunities - 17.34 and obstacles -10.99) were found to be significant.

TABLE 4

ASSOCIATION OF SELECTED DEMOGRAPHIC VARIABLE WITH LEVEL OF SATISFACTION , OPPORTUNITIES & OBSTACLES						
Variables → ↓	Satisfaction level		Opportunities		Obstacles	
B.Sc. Nursing	Mean Rank	Chi Square (df) P value	Mean Rank	Chi Square (df) P value	Mean Rank	Chi Square (df) P value

Cont... **Table 4**

1st year 2nd year 3rd year 4th year	70.45 88.57 57.07 59.92	13.59 (3) 0.004 significant	73.33 90.01 57.30 55.83	17.34 (3) 0.001 significant	52.20 82.99 66.57 73.71	10.99 (3) 0.01 significant
Time Spend on VCP Daily	Mean Rank	Chi Square (df) P value	Mean Rank	Chi Square (df) P value	Mean Rank	Chi Square (df) P value
1-2hours 3-5hours 5-8hours	61.88 73.16 67.42	1.79 (2) 0.4	60.07 72.90 60.07	2.18 (2) 0.3	76.83 74.84 49.21	10.37 (2) 0.006 significant

However, association of time spent on video conferencing platform was found to be significant only with obstacles (10.37)

Conclusion

The study revealed the different aspects in terms of opportunities and obstacles faced by the learners while learning through the Video Conferencing Platform. As VCP is new method adopted by the teaching institutes due to COVID 19 lockdown situation. Study brought out the fact that VCP brings better learning outcomes with learning at learner's comfort, whereas disturbance of surroundings and internet speed to be most reported obstacle. This study would act as base for the future learning process through virtual classroom which would be based on video conferencing platform.

Ethical Clearance was obtained from the institution's research & ethical committee

Source of Funding -Self

Conflict of Interest - Nil

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Quality of Life Among Chronic Liver Disease Patients: An Exploratory Cross Sectional Survey

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Abstract

Background: QOL is the general well-being of individuals and societies, outlining negative and positive features of life. It observes life satisfaction, including everything from physical health, family, education, employment, wealth, religious beliefs, finance and the environment.[1] Quality of life has an important status in patient management suffering from chronic liver disease.

Objectives: The present study was done to assess the quality of life among patients suffering from Chronic Liver Diseases.

Material and Methods: A descriptive explorative cross-sectional research design was used. Data was collected from 165 patients using purposive sampling technique. The quality of life Scale (QOLS) was used to assess the quality of life among chronic liver diseases patient admitted at Institute of Liver and Biliary Sciences. MELD and CTP score was used to assess the mortality and survival rate among chronic liver diseases patient. The study participants and their care givers were informed that the study is harmless; all the gathered data was treated confidentially and used for research purpose only.

Results: The results of the study revealed that 0.6 percent of the patients have poor, in 35.2 percent of the people have moderate whereas 64.2 percent of the people have good quality of life. In terms of physical wellbeing of patient 11.52 percent of the people have poor, 73.93 percent have moderate while only 14.55 percent have good quality of life. In relation with social wellbeing of the patient 0.61 percent of the people have poor, 27.88 percent have moderate while only 17.51 percent have good quality of life. As per emotional wellbeing of the patient 3.03 percent of the people have poor, 87.88 percent have moderate while only 9.09 percent have good quality of life. In terms of functional wellbeing of patient 3.03 percent of the people have poor, 60 percent have moderate while only 36.97 percent have good quality of life. The quality of life in terms of cost showed that 11.52 percent of the patient had poor, 73.93 percent had moderate, and 14.55 percent of the patient has good quality of life. The quality of life in terms of limitation due to signs and symptoms showed that 1.21 percent of the patient have poor, 81.82 percent have moderate whereas 16.97 percent of the patient had good quality of life. The weak positive correlation (r=0.48) was found between MELD and CTP score.

Conclusion: The study concluded that only 35.2 percent of the patient have moderate quality of life, more than half (64.2%) of the patient have good quality of life. So, the effective steps need to be taken to improve the quality of life among patients with Chronic Liver Diseases.

Key words: *Quality of life, Chronic Liver disease*

Introduction

Quality of life (QOL) was first used in world war II to describe the effect of material welfare on individuals' lives. QOL is the general well-being of individuals and societies, outlining negative and positive features of life. It observes life satisfaction, including everything from physical health, family, education, employment, wealth,

religious beliefs, finance and the environment.[1] QOL has a wide range of contexts, including the fields of international development, healthcare, politics and employment. It is important not to mix up the concept of QOL with a more recent growing area of health related QOL (HRQOL).[2] Quality of life should not be confused with the concept of standard of living, which is based primarily on income. Health-related QOL focuses on the effects of a disease or health conditions on the daily functioning of individuals with special attention for their physical and mental health[3].

Recently quality of life (QOL) has become the principal goal of medical care because of the increasing emphasis on the patients as focal point of health care, patients functioning preservation and wellbeing hence forth measurement of patients quality is receiving attention in medical research.[4] Quality of life (QOL) is a subjective multidimensional concept which includes functional status, emotional and social wellbeing as well as general health.[5] Quality of life has an important status in patient management suffering from chronic liver disease.[6]

Recently health related quality of life has gained importance as an outcome measure in clinical and epidemiological studies and has become a key component in the evaluation of therapeutic interventions in hepatology.[7]

Chronic means of long duration" Liver means "the large gland situated in the right upper area of abdominal cavity" which chief function is bile secretion, maintain blood composition and regulate metabolic process.[8] Whereas disease means "any departure from normal functions of liver" so chronic liver disease is long duration illness of large gland in which liver function is disturbed. In many patients, long term heavy drinking leads to chronic liver disease, liver failure and even death.[9] Globally cirrhosis/chronic liver disease caused by many entities, is responsible for major mortality and morbidity. Chronic liver disease is one of the common conditions for which individual are hospitalized.[5] Symptoms may be extremely variable in advanced disease, whereas compensated patients may be asymptomatic for years or decades. However, the impact of the disease on patients' functioning and well-being may be enormous. Patients may experience anxiety because of chronic viral infection, or emotional problems associated with alcoholism. Complications (ascites, encephalopathy, hemorrhage) and/or extrahepatic manifestations of advanced disease may affect QOL, not to mention therapeutic interventions, such as therapy with fast-acting loop diuretics, limiting outdoor activities, or interferon treatment, causing malaise and illness awareness.[10]

However, the research was taken up with a rationale to describe and assess the QOL of liver disease patients in India, in order to have baseline literature & empirical evidence to further have development of intervention to enhance the QOL of liver disease patients in India.

Methodology

Study Design

Descriptive exploratory cross section research design was used to assess the quality of life among Chronic Liver Diseases Patient.

Research Setting and Population

The study was conducted at selected Institute of Liver and Biliary Sciences, New Delhi. Data was collected from the various department like IPD and OPD. The patient who were present at the time of data collection and who were ready to participate were included in the study. The patients who were hemodynamically unstable and with hepatic encephalopathy were excluded from the study.

Sample Size

Sample size was not determined as all the patient visiting to the hospital were taken for the study. 165 patients were selected using purposive sampling technique from selected Institute of Liver and Biliary Sciences.

Data Collection Tool and Technique

It comprised of three sections:

Section A: It consists of sociodemographic variables which include age, gender, chronicity & occupation.

Section B: Two standardized scale i.e. MELD (Model of end stage liver diseases) and CTP (Child Turcotte- Pugh) score was used. MELD score was

checked to assess the mortality whereas CTP score was used to assess the survival rate among chronic liver diseases patients. The online calculators were used to calculate the score. The parameters of the MELD score were creatinine, bilirubin level, INR and sodium level of the patient. The score of 1 to 9 indicates poor prognosis, 10 to 19 indicates 6 percent mortality, 20-29 indicates 20 percent mortality, 20 -29 indicates 20 percent mortality, 30 to 39 indicates 52.6 percent mortality, the score of 40 indicates 71.3 percent mortality.

The next score is CTP score. The components of CTP score were encephalopathy, ascites, bilirubin level and PT or INR. The total score is 15. The score between 5 to 6 indicates 100 percent survival rate, 7 to 9 indicates 81 percent survival rate, 10 to 15 indicates 45 percent survival rate.

Section B: The structured questionnaire was used to assess the quality of life among chronic liver disease patient. The tool was validated by five experts from the field of the Nursing, and Hepatology. The tool has seven components which includes physical, social/ family, emotional, functional, spiritual wellbeing of the patient, and also cost and limitations due to signs and symptoms. The score of 0 to 50 indicates poor, 51 -100 indicates moderate, and score of 101 to 150 indicates good quality of life. In physical wellbeing the total score is 18. The score from 0 to 9 indicates poor, 10 to 14 indicate moderate, whereas 15 to 18 indicates good quality of life. The total score of emotional domains is 15. Where score between 0 to 7 indicates poor quality of life, score of 8 to 12 indicates moderate quality of life and score of 13 to 15 indicates good quality of life. Next domain of the scale is functional wellbeing. The total score is 36. The score of 0 to 17 indicates poor, 18 to 27 indicates moderate and 28 to 36 indicates good quality of life. The total score of the domain cost is 18. The score from 0 to 9 indicates poor, 10 to 14 indicate moderate, whereas 15 to 18 indicates good quality of life. The last domain of the scale is limitation due to signs and symptoms. The last component of the tool is limitations due to sign and symptoms. The total score is 30. The score of 0 to 14 indicates poor quality of life. The score of 15 to 22 indicates average quality of life and 23 to 30 indicates good quality of life.

Data Collection Procedure

Data was collected using interview techniques. Tool was developed in three sections. One dealt with the identification data & demographic variable like Age, Gender, Occupation and Chronicity of disease. The second one is MELD and CTP score. The third part was structured three-point scale where participants would opt for the best suitable life experience & activities. Official ethical clearance & permission was obtained from the institute ethical committee. Verbal informed consent was taken prior to data collection from study participants.

Data Analysis

Table 1: Frequency and Percentage Distribution of the Socio Demographic Variables

N=165

Sociodemographic Variables	Frequency (%)
Age in years	47.15±10.86
Gender	
· Male · Female	137 (83) 28 (17)
Chronicity	

Cont... **Table 1: Frequency and Percentage Distribution of the Socio Demographic Variables**

N=165

· <6 months	53 (32)
· 1-3 years	75(46)
· 3-5 years	37(22)
Occupation	
· Salaried	60 (36)
· Self employed	73 (44)
· Housewife/Retired	32 (20)

Table 1 illustrates that frequency and percentage of the sociodemographic variables like age, gender, chronicity and occupation. The mean age group of the patients was 47.15±10.86. The majority (83%) of the patient were male, whereas only 17 percent of the patient were female. In terms of the chronicity of the diseases, the duration of the illness was less than six months in 32 percent of the patients, 1-3 years in 46 percent of the patients while 3 to 5 years in 22 precent of the patients. Further in relation to occupation, 36 percent of the patients were salaried, 44 percent of the patients were self-employed and rest 20 percent of the patients were housewife or retired.

Table 2. Overall perception of quality of life among chronic liver diseases patient

N=165

Quality of life	f (%)
Poor	01(0.6)
Moderate	58(35.2)
Good	106 (64.2)

Table 2 is showing the overall perception of quality of life among chronic liver disease patient. It can be observed in the table that only (0.6%) of the patients were have poor, 35.2 percent of the patient have moderate, more than half (64.2%) of the patient were have good quality of life.

Table 3. Perception of quality of life in various domains among Chronic liver diseases patient

N=165

Various aspects of life	**Poor f (%)**	**Moderate f (%)**	**Good f (%)**
Physical Well being	19 (11)	122(74)	24 (15)
Social well being	01 (01)	46(28)	118(71)
Emotional well being	05 (03)	145 (87)	15 (10)
Functional well being	05(03)	99 (60)	61(37)
Spiritual well being	03(02)	148 (90)	14 (08)
Cost	13 (11)	113 (74)	39(15)
Limitation due to signs and symptoms	02 (01)	135 (82)	28(17)

Table 3 is showing the frequency and percentage of quality of life in terms of various aspects of life. In terms of physical wellbeing only 15 percent of the patient were having good quality of life, 74 percent were having moderate quality of life, whereas 11 percent of the patients were having poor quality of life.

Further in relations with social wellbeing more than half (71%) of the patient were having good quality of life, 28 percent were having moderate quality while only 1 percent of the patient were having poor quality of life. In view of emotional wellbeing 10 percent patients were having good, 87 precent were having moderate and 3 percent of patients have poor quality of life.

The quality of life in terms of functional wellbeing of the patient revealed that 37 percent of the patients were having good, 60 precent of the patient having moderate whereas only 3 percent of the patients have poor quality of life. Next in terms of spiritual wellbeing, 2 percent of the patient have poor, 90 percent of the patient have moderate and only 8 percent of the patient have good quality of life.

In context to the cost, it was found that only 15 percent of the patient have good, more than half (74%) have moderate and 11 percent of the patients have poor quality of life. Further the quality of life in relation with limitation due to sign and symptoms showed that 17 percent of the patient had good, 82 precent of the patients had moderate and 1 percent of the patient had poor quality of life.

Table 4. Frequency and percentage of the MELD and CTP score of the patient

N= 165

MELD Score	**f (%)**
Poor Prognosis	15 (10)
6 % mortality	74 (45)
20% mortality	53(32)
52.6 % mortality	18 (11)
71.3% mortality	05 (03)
CTP Score	
100 % (5-6)	17 (10)
81% (7-9)	110 (67)
45% (10-15)	38 (23)

Table 4 represents the frequency and percentage of the MELD score among chronic liver diseases patients. MELD score was used to predict the mortality among chronic liver diseases patients. It can be observed from the table that 10 percent of the patients were having the poor prognosis, 45 precent of the patients have 6 % mortality, 32 precent of the patients were having the 20% mortality, 11 precent of the patients have 52.6 % mortality and 3 precent of the patients have the 71.3% mortality. CTP score is used to predict the survival rate among chronic liver diseases patient. The above table showed that 10 percent of the patients have 100 percent survival rate, 67 precent of the patient have 81 precent survival rate while 23 percent of the patient have 45 precent survival rate.

Table 5. Correlation between MELD and CTP

Scores	MELD Score (Mean ±SD)	CTP Score (Mean ±SD)	R
	19.92±8.4	8.29±1.73	0.48

P<0.05

Table 5 depicts the corelation between MELD and CTP score. Pearson corelation was computed to see the corelation between MELD and CTP score. Correlation between CTP & MELD was assessed and weak positive correlation of 0.48 was found. Hence it can be concluded that the MELD Score can predict the prognosis or survival rate exactly.

Association of sociodemographic variables with quality of life among chronic liver disease patient was also computed using Chi Square. But there was no significant association found between the sociodemographic variables and quality of life among chronic liver disease patients.

Discussion

The chronic liver diseases are the serious illness which affects the quality of life of the patients. So, assessment of quality life among chronic liver diseases patient is important. Thus, the present study aimed at assessing the quality of life of chronic liver disease patients.

The present study showed that more than half of the patients have average quality of life.

The mean age of the patients was 47.15±10.86. The majority of the patients were male. The study findings were supported by the study conducted to assess the quality of life among chronic liver disease patient revealed that majority of the patients were male.[11]

In the present study the MELD score was used to assess the mortality among chronic liver diseases patient which showed that 32 percent of the patient has 20 percent mortality rate, 45 percent patients have 6 percent mortality rate. Similar study conducted to assess the quality of life among chronic diseases used MELD score to predict the mortality. The findings of MELD Score revealed that the majority (93.2%) of the patient were at low risk, 5.3 percent were at moderate risk whereas only 1.5 percent of the patient were at high risk of mortality.

The present study findings revealed a positive corelation with MELD and CTP score which concluded that MELD Score can predict the prognosis or survival rate exactly.

A study conducted to assess the health-related quality of life among chronic diseases patient showed that the overall CLDQ score was also low in patients below 45 years old ($p < 0.05$).[11]

The limitation of the study was sample size and data was collected from only one institute.[11]

Conclusion

The study concluded that only 35.2 percent of the patient have moderate quality of life, more than half (64.2%) of the patient have good quality of life. So, the effective steps need to be taken to improve the quality of life among patients with Chronic Liver Diseases.

Ethical Clearance: Ethical clearance taken from institutional ethical committee.

Conflict of Interest: Nil

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Attitude of Physiotherapists Towards Dealing Subjects with Mental Illness

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Abstract

Introduction: The concept of mental health receives negative opinions and are stigmatized in public despite evidence of importance of mental health for social and human capital. It is important that there should be a multidisciplinary approach when it comes to rehabilitation of patients with Mental Illness than concentrating only in the psychiatry aspect. Patients with Mental Illness can also suffer from physical health related co-morbidities in which physiotherapy can play a role in improving the lifestyle. **Objective:** The aim of the study was to assess the attitude of physiotherapists towards subjects with mental illness. **Methods:** A non-experimental study of convenient samples was carried out among 100 physiotherapists. Data collection was performed using the questionnaire MICA-4(Mental Illness Clinicians Attitude Scale). The questionnaire includes 16 items and has a 6- point Likert-type response format. Each item has its own definite score. A higher score depicts negative attitude and a lesser score shows a positive attitude. **Results:** The data was analysed using SPSS statistics version 23. The results revealed that 73% of the subjects had neutral attitude towards dealing individuals with any form of mental illness. **Conclusion:** The neutral attitude recorded is indicative of lack of exposure of physiotherapists in the field of psychiatry. Training at an early stage can be beneficial for future therapists to deal in a better way with mental illness patient having physical health problems. It is important to promote this area of specialisation both for clinical and research studies in order to implement strategies to avoid stigmatisation.

Keywords: *Attitude, Mental illness, Mental disorder, Mental Illness Clinician Attitude scale, Physiotherapist.*

Introduction

Brain disorders which are neurological, psychiatric, and developmental are a prime cause of mortality and disability worldwide and are responsible for a high proportion of the cause of disease in developing countries[1]. Brain disorders are predicted to increase in the forthcoming decades as an outcome of large-scale demographic and epidemiological shifts. According to a survey, by 2020 depression is projected to be the second and followed by stroke the leading cause of disability-adjusted life years (DALYs) lost worldwide .

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The social stigma associated with some of the disorders like epilepsy, schizophrenia, and mental retardation often acts as a barrier to people with these disorders from seeking and getting medical help . It also results in the loss of social, educational, and job opportunities to affected individuals and their family members .

According to WHO, a broad range of problems with different symptoms falls under the category of mental disorders. They are popularly characterized by some combination of abnormal thoughts, emotions, behaviour and relationships with others. Schizophrenia, depression, intellectual disabilities and disorders due to drug abuse are few of the common examples. Most of these disorders can be successfully treated.

According to American Psychiatry Association, Mental illness is a health condition involving changes

in emotions, thinking or behaviour or a combination of these and it is associated with distress and/or problems with functioning in social, work or family activities.

In a statement WHO revealed that 1 in 7 people worldwide are suffering from mental illness and 7.5% of the Indian population suffers from some form of mental illness. A study revealed that 35% of the population from their samples had family history of psychiatric disorders[2] People with serious mental health issues generally have poor physical health[3]. Majority of these physical health problems broadly falls under the scope of musculoskeletal, cardio-thoracic, nerve impairment etc.

The concept of mental health receives negative opinions and are stigmatized in public perception despite evidence of importance of mental health for social and human capital. When it comes to physical health consciousness and awareness are high among people comparatively to Mental Health but there is no true physical health without a healthy mind[4].

Most people with mental illness have poor physical health and high rates of co-morbidities[5] and in these cases, physiotherapy is capable of promoting functional movement which aims to optimize wellbeing. Physiotherapy plays a major role in restoring bodily movement which is affected by pain, age, any injury, stress, and other pathological and even environmental factors[6].

Several studies exist related to other health professional's attitude towards mental illness but only few literatures have investigated the views of physiotherapists in providing services to mental illness patients. One of the studies revealed the limitations faced by the physiotherapists in dealing subjects with severe persistent mental illness[7]. Yildrim et al. in their study found that the physiotherapy students showed moderate positive beliefs towards mental illness.

Therefore, the aim of our study was to assess the attitude of physiotherapists when it comes to dealing subjects suffering from any form of mental disorder.

Methods

This non-experimental study of observational type was conducted among the physiotherapist based on the inclusion criteria. The sampling was done using convenient sampling method. The entire sample consisted of academic and clinical physiotherapists(n=100). The therapists were explained the aim of the study and informed consent was received. The data were collected between December 2019 to February 2020 through an online survey. Undergraduate physiotherapy students were excluded from this study. The following questionnaire was used for the data collection. Demographic data was also collected.

STATISTICAL INSTRUMENT: The Mental Illness Clinician's Attitude scale (MICA) version4 was developed by Gabbidon J, Clement S et al in 2013 and the internal consistency of the MICA v4 was assessed using Cronbach's □ with an acceptable value of □ > 0.7 but not exceeding 0.9(Streiner and Norman, 1995) which is indicative of good internal consistency. It is a self-administered scale containing 16 questions, used to assess the attitude of health and social care professionals towards psychiatry and psychiatric patients through a set of validated questions[8]. An individual's MICA overall score is calculated by adding up the scores for each item. Items 1, 2, 4, 5, 6, 7, 8, 13, 14 and 15 are scored as follows: strongly agree = 6, agree = 5, somewhat agree = 4, somewhat disagree = 3, disagree = 2, and strongly disagree = 1. Other items 3, 9, 10, 11, 12, and 16 are scored as follows: strongly agree = 1, agree = 2, somewhat agree = 3, somewhat disagree = 4, disagree = 5, and strongly disagree = 6. The scores for each item are added to produce a overall score. A more negative (stigmatizing) attitude is reflected by a higher total score. There is no cut-off point mentioned in the scale as it is difficult to claim that there is a level above which attitudes are negative. It is recommended that the mean and standard deviation are to be used as it is a continuous scale. The results were tabulated with the help of SPSS software version 23.

Results

Out of 100 participants, 44(44%) were males and 56(56%) were females. On the basis of years of experience 32 participants had more than 5 years of experience and 68 participants had less than 5 years of experience.

The mean along with the standard deviation of the Mental Illness Clinician Scale questionnaire was 48.96

± 9.64 with the range of total score being from 27-71. As shown in Table.1 the attitude of physiotherapists was found to be 15% positive,73% neutral and 12% negative according to our study. The MODE (value that appears most often in a set of data values) value of the responses for each question is depicted in Figure.2 which helps in assessing the inclination of response towards each question which explains whether the majority of the Physiotherapist participating in this study agreed or disagreed to the given scenario in the questionnaire. The values 1 to 6 shown in Figure.1 depicts the choices picked by physiotherapists who took part in our study from the MICA-4 questionnaire which ranges from Strongly agree (1), Agree (2), Somewhat Agree (3), Somewhat Disagree (4), Disagree (5), Strongly disagree (6) respectively.

Table no.1:

Attitude shown by Physiotherapists

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	NEGATIVE	12	12.0	12.0	12.0
	NEUTRAL	73	73.0	73.0	85.0
	POSITIVE	15	15.0	15.0	100.0
	Total	100	100.0	100.0	

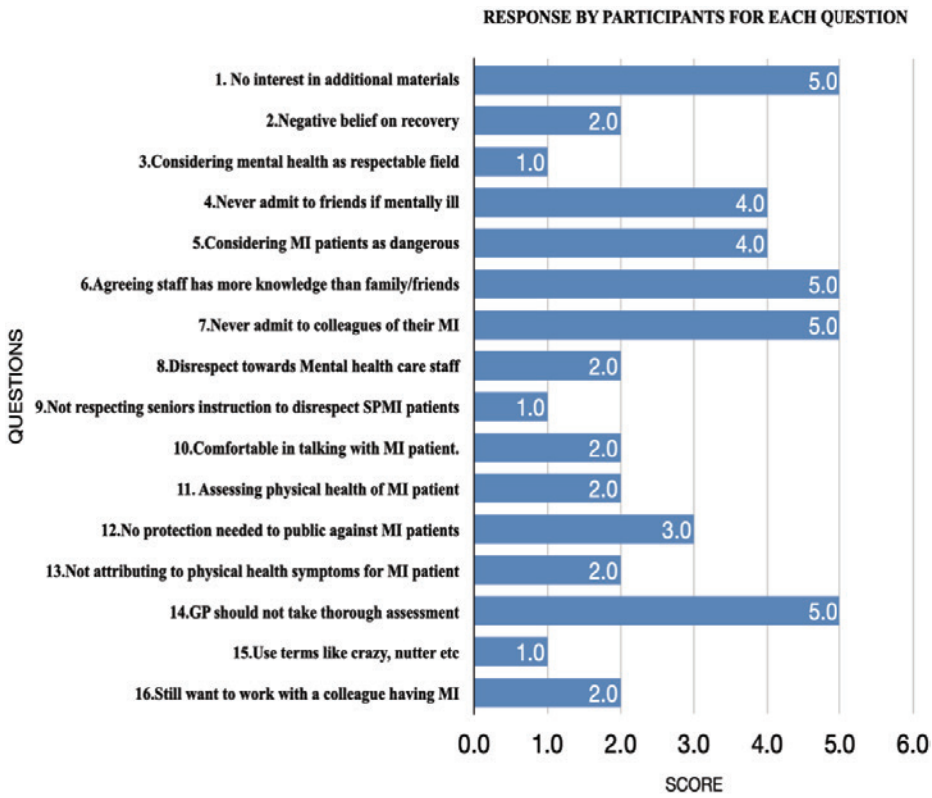

Figure no.1: Inclination of responses by the physiotherapists

Discussion

The results showed that the lack of exposure to the field of psychiatry by the Physiotherapists has resulted in majority of this population having neutral attitude. Reports from the study done by Yildrim et al. conveys that Psychiatry is a relatively new field of profession encountered by physiotherapists all over the world. In this study, the attitude of the Physiotherapists was assessed by using the Mental Illness Clinician Scale-4 questionnaire. There is a consistency between the current study and a study done by Robson and colleagues Robson et al in which the attitudes of mental health nurses towards physical care of people with severe mental illness was assessed using the physical health attitude scale for mental health nurses which concluded an overall positive attitude towards people with severe mental illness[9]. Similarly, Richa & Naddaf studied the perception of psychiatry and mental disease among Lebanese non-psychiatric doctors and revealed that there was a negative attitude among doctors and residents towards mental illness but positive attitudes towards psychiatric treatments, psychiatry, and psychiatrists[10].

Yildrim et al. in their study stated that the physiotherapy students showed moderate positive beliefs towards mental illness which was different from our study which showed neutral attitude among the physiotherapists. This concludes that training students at an earlier level during their course time for knowledge and confidence to treat patients with Mental illness will be more effective. Eleanor et al. in their study using an open ended questionnaire concluded that 93% of their participants agreed that physiotherapists should play a significant role in managing physical health problems of people with SPMI (Severe and Persistent Mental Illness) and comparing this to the neutral attitude present within the therapists in our study , with exposure and in depth knowledge about psychiatry it will help therapists to play their role effectively in the same and this would bring a change in their attitude positively. As the evidence about the attitude of physiotherapists are very minimal, this study can be used as an initial basis for further research in this direction in order to gain deeper knowledge about the limitations faced by the therapists when treating patients with physical illness along with co-morbidities involving their mental health. This study would be beneficial to explain patients and their care takers that a multidisciplinary approach is needed for patients with mental illness in which physiotherapists can play a vital role in treating the physical symptoms.

Conclusion

One of the greatest drawbacks in our society is the prevalence of stigma which contributes in making our society crippled that prevents people with mental illness to live a normal social and occupational life and thus from receiving an equal right of medical health care as other non-psychiatric patients. The neutral attitude in our study is indicative of lack of exposure of physiotherapists in the field of psychiatry. Training at an early stage can be beneficial for future therapists to deal in a better way with mental illness patient having physical health problems. It is important to promote this area of specialization in both clinical and research aspects in order to implement strategies that avoid stigmatisation.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance : Taken from Institutional Ethical Committee.

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Prevalence of the Risk of Obstructive Sleep Apnea in Relation to Obese and Overweight Collegiate Students

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Abstract

INTRODUCTION: Obstructive sleep apnea is described as monotonous collapse and reopening of upper airway during sleep which affect the ventilation. This Obstructive sleep apnea is common among obese and overweight individuals. **OBJECTIVE:** The objective of the study was to determine prevalence of the risk of obstructive sleep apnea in relation to obese and overweight collegiate students.

METHODOLOGY: It is a non- experimental study with sample size of 100 which include obese and overweight. 50 subjects with obese and 50 subjects with overweight are included and assessed for risk of sleep apnea. **OUTCOME MEASURES:** Berlin Questionnaire **RESULTS:** Percentage of low risk was about 56, whereas the high risk Obstructive sleep apnea was 44% among overweight. Percentage of high risk was 52 and low risk 48. **CONCLUSION:** This study concluded that the prevalence of Obstructive sleep apnea had a higher risk among obese when compare to the overweight individuals.

Keywords: *Obese, Overweight, Obstructive sleep apnea, Berlin questionnaire, Prevalence, collegiate students.*

Introduction

According to World Health Organization (WHO), Obstructive sleep apnea (OSA) is clinically considered by often intervals of breathing especially during sleep which is associated by loud snoring. Obstructive sleep apnea leads to impairment of ventilation and may leads to intermittent hypoxemia and hypercapnia which is mainly due to monotonous collapse and reopening of upper airways in sleep.[1] Clinically obesity and its associated factors have a dual relationship. Initially obesity as risk criteria for certain disease and act as an outcome of genetic, behavioral and socioeconomic factors. Obesity plays an underlying cause for cardiovascular diseases, diabetes mellitus, kidney diseases, arthritis and musculoskeletal problems.[2] The Etiopathogenesis of Obstructive sleep apnea were upper airways muscle dilation during sleep, ventilatory control instability, sleep state stability. It is also evident that genetic determinants pre disposes to Obstructive sleep apnea which include factors such as skeletal structure obesity epidemiology of fat distribution and respiratory control.[3] The Berlin Questionnaire was used to assess obstructive sleep apnea. The sensitivity for the Berlin questionnaire was 77.0% (95% CI: 71.0-82.0%) and the specificity was 97%. The Prevalence of Obstructive sleep apnea syndrome is estimated to 3.6% which was confirmed by population - based survey done in North India. Prevalence of Obstructive sleep apnea 14.7% the above study.[4] Now comparing the prevalence rates of OSA and Obstructive sleep apnea syndrome in Males were 13.4% and 4 % whereas females were 15.6% and 1.5% respectively. The main risk factor for incidence for OSA is Obesity since OSA and Obesity are interrelated

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in complex manner8.Adult population in south Indian countries with overweight (BMI 25 – 29.9 kg/m2) and obese is (BMI > =30 kg/m2).[5]

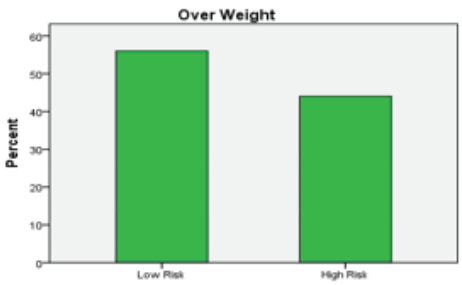
Methods

100 subjects were included. Selected subject were completely explained about the study and Berlin questionnaire was distributed. Ethical clearance approved by SRM scientific committee .

Results and Discussion

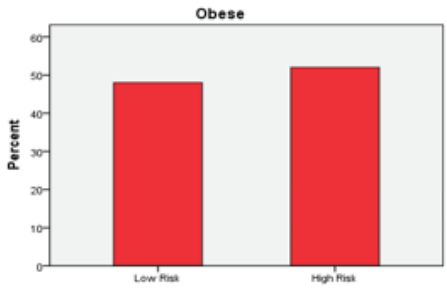
According to table 1 low risk Obstructive sleep apnea was more among overweight collegiate students that is, the cumulative percentage of low risk was about 56, whereas the high risk Obstructive sleep apnea was less (44%) among overweight. This is shown in graph 1. As per the table 2 the low risk Obstructive sleep apnea less in obese individuals but high risk sleep apnea was comparatively more among the same group. The cumulative percentage of high risk was 52 and low risk 48. This is shown in graph 2. According to the World Health Organization guideline the prevalence of overweight among college students was 24.3% and prevalence of obesity was 8.6%. In this study total sample of 100 was categorize into 50 as overweight and 50 obese. The prevalence of Obstructive sleep apnea was calculated under two terms such as high risk and low risk. The frequency of high risk for Obstructive sleep apnea among overweight was 44% and obese was about 56%. While concentrating the low risk for Obstructive sleep apnea among obese individual was 48% and it was 52 % among overweight individual. Even though the exact mechanism that lies behind the relationship between Obstructive sleep apnea and excess Body Mass Index is idiopathic. Due to the excessive fat deposit in the airway especially the upper region leads to airway collapse during sleep by constricting the airway and reducing the muscle activity around that area. This kind of apnea usually leads to obesity that is mainly due to sleepiness in the daytime and often these individual arouse for breathing and make able to overcome the unobstructed air flow. However it is usually noted that inadequate sleep pattern may leads to increased energy demand and food intake.

GRAPH I: OVERWEIGHT



The above graph shows that low risk of Obstructive sleep apnea is more common (56%) among overweight collegiate students

GRAPH II: OBESE



The above graph shows that high risk of Obstructive sleep apnea is more common (52%) among Obese collegiate students

GRAPH I: OVERWEIGHT **GRAPH II: OBESE**

Conclusion

This study concluded that the prevalence of Obstructive sleep apnea had higher risk among obese when compare to the overweight individuals.

Conflict of Interest: NIL

Financial Support: Self

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Influence of Index Plus and Index Minus Foot on Static and Dynamic Balance among Collegiate Girls

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Abstract

Background: Index plus foot is defined as the first metatarsus being lengthier than the second whereas the Index minus foot is a condition where the second metatarsus is extended than the first metatarsal**.** The prevalence of index minus foot is higher among females, a study on balance with index minus and index plus foot on normal individual females are lacking. **Objective**: The objective of the study is to determine the influence of index plus and index minus foot among collegiate girls **Methodology:** It is a non- experimental study with sample size of 100 are taken and tested for both static and dynamic balance. **Outcome Measures:** Flamingo balance test and Star Excursion Balance Test. **Results:** The mean and standard deviation of flamingo balance test for index plus group is 9.24+2.77423 and the index minus is 15.04+1.6655. The mean value of star excursion balance test for both groups resulted with less noticeable difference. **Conclusion**: The study concludes that there is a difference in static balance, whereas there is no differences in dynamic balance among collegiate girls.

Keywords: *Index plus and minus foot, static and dynamic balance, flamingo balance test, star excursion balance test.*

Introduction

The metatarsals in the feet are off varying length. Usually the first metatarsal is longer than the second metatarsal which is said to be Index plus foot. In some cases the second metatarsal is longer than the other toes that is Index minus foot. The prevalence of Morton's toe worldwide ranges from 20-30%. It is also noted that the prevalence is more in females compared to men. Clinically there are certain tools to measure the length of the metatarsal.[1]One among them is the Maestro and Barroco techniques.[1] It is done by obtaining a radiograph of the foot drawing lines in the radiograph accordingly. The other method of determining the metatarsal length easily at place is Palpation Based Test.[1] Balance is stated as maintaining equilibrium in which centre of mass adopts according to the individual base of support irrespective to the external and internal factors.[2] One of the required terminology in routine life is balance which can be both static and dynamic.[3] Within the provided optimal base of support balance is the desire of moving body in terms of both physiological and mechanical situation.[4] The static balance is measured using Flamingo Balance Test (FBT). It is one of the cost effective method.[5] The Star Excursion Balance Test is a method used to measure the dynamic postural control of an individual.[5] Since the body's total weight is finally transferred to the foot, even a slight modification in the alignment may alter the line of gravity to fall in the provided base of support.[6] As balance is more consider in daily activities it is necessary to understand the mechanism of balance in index minus and index plus foot.

Material and Methods

The length of the metatarsal is measured by palpation based test. In this method the participant was asked to stand in a piece of graph. One line that is in order to the great toe is drawn and the next line is drawn in horizontal manner in respect to the second toe. A line is drawn horizontally from the above lines at a distance of 2 cm. By using sliding Vernier caliper the length was measured from metatarsophalangeal joint to the new line drawn. If the difference between two lines are greater than zero, it is called index plus and lesser than zero it is called index minus foot.

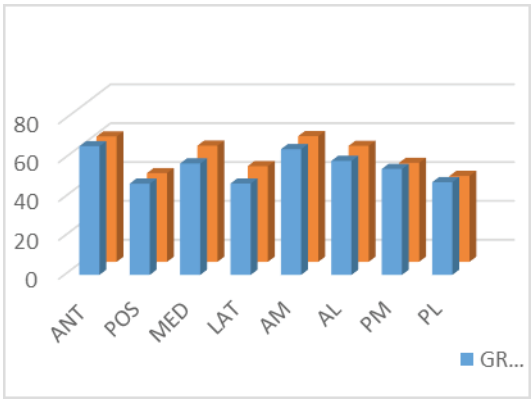
The static balance is measured by Flamingo Balance test and the dynamic balance is measured by SEBT.

Results and Discussion

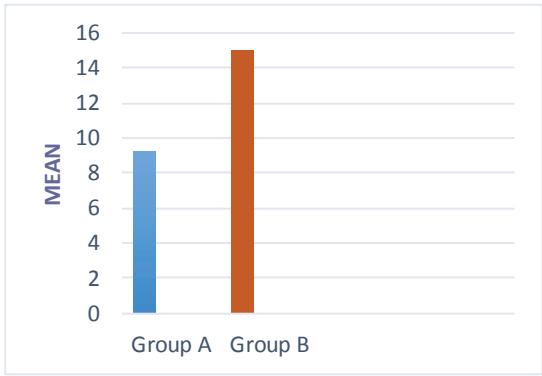
The flamingo balance test performed by the index plus group exhibited a total mean and standard deviation of 9.24+2.77423(Graph 1) whereas the index minus group shown a mean of 15.04+1.6655(Graph 2). The mean value of star excursion balance test for both groups resulted with less noticeable difference. As the weight falls into the calcaneus, it eventually spreads to the forefoot. The majority of the load is been received by the first metatarsal. The great toe is said to play a predominant role in static balance, this is taken into consideration that individual with index plus toe is more supreme in single leg static balance. Where in index minus foot the body load transmitted from the calcaneus should be received slightly more by the second metatarsal compared to the above state. This results is supported by **Agopyan et al suggesting** that there might be a difference in unilateral static balance with index minus toe. Considering the dynamic balance among the groups **In Hyouk Hyong. et.al** showed that there is no significant difference in dynamic balance among different foot shape in his study. It is stated that for an individual to maintain a dynamic balance the muscles play a vital role than the bones. This is in accordance with **Bannister. R** that enough muscle power is required for maintaining dynamic mobility. Thus this study on the basis of human biomechanics shows that static balance varies among the index plus and index minus foot whereas the dynamic balance do not have any significant difference.

Conclusion

On account of all the above, the study concludes that there is a difference on static balance among index minus and index plus foot. Regarding the dynamic balance there is no much difference among the groups.



Graph 1 Flamingo Balance Test.



Graph 2 Star Excursion Balance Test.

Conflict of Interest: Nil

Financial Support: Self

Ethical Clearance: Ethical clearance is obtained by the Institutional ethical committee.

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Study of Sperm Dna Fragmentation Index in Patients from Rural Areas of Wardha Region

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Abstract

Background: Sperm DNA fragmentation test is an advanced test for proper sperm function with respect to semen analysis, as semen analysis is used for motility and morphology assessment, it cannot explain sperm at molecular level and thus SDF test has been introduced to evaluate male fertility. The integrity of genetic material in sperm is crucial for successful fertilization & normal embryo development as this will lead to proper transmission of genetic information. Patients having varicocele, recurrent failure of ART tecniques and improper lifestyle (radiation, smoking, alcohol, BMI, ageing, etc) are considered for sperm DNA fragmentation test. SDF test is useful in selecting patients for the ART procedures i.e. IVF/ICSI/IUI and highest pregnancy and live birth outcomes.

Objectives:

· To predict male infertility with normal semen parameters & its diagnostic values.

· To investigate the clinical factors related to sperm DNA fragmentation in male with infertility.

· To investigate the rate of sperm DNA fragmentation in male related to improper fertilization, embryo development and recurrent pregnancy loss.

Methods: The participants (n=40) with male fertility problems will be included in randomised control trial. All 40 participants will be randomly selected to evaluate their sperm DNA integrity from their respective semen samples using sperm chromatin dispersion test.

Discussion: Efficiency of the Sperm DNA Fragmentation test is assessed by performing sperm chromatin dispersion test on respective processed semen sample using Halosperm G2 test kit.

Keywords: *Sperm DNA fragmentation (SDF), DNA fragmentation index (DFI), oxidative stress, genetic abnormalities, hyperthermia, gonadotoxins and endocrine abnormalities.*

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Introduction

9% of couples are diagnosed with infertility out of which 20% is due to male factor.(1) Especially in males, infertility factor has become quite common. most of infertility cases are due to damage to sperm

DNA (i.e. sperm DNA fragmentation). At an age, as small quantity of sperm can achieve pregnancy, hence it becomes important to select healthy sperms for ICSI/ IVF. However Semen analysis is used for morphology, motility and sperm concerntration assessment, it cannot evaluate the sperm at molecular level and therefore SDF test is nowadays used to evaluate male infertility. SDF(sperm DNA fragmentation) term is used to indicate abnormal genetic material in sperm which can lead to male infertility. The integrity of genetic material in sperm is important for successful fertilization and normal embryo development, as proper DNA integrity will lead to proper transmission of genetic information.

DNA combination protein blocks

Chromatin

23 chromosomes in sperm head are made up of chromatin that consist of DNA and proteins. Thus intact chromatin is important for proper pregnancy outcomes.

Research has been done on new testing methods on sperm abnormalities in the past 30 years and sperm DNA damage has come to be an area of interest.

It has been observed that Sperm DNA Fragmentation is highly recognized as important cause of male infertility. The correlation between sperm damaged DNA and failed reproductive outcome led to introduce SPERM DNA FRAGMENTATION or Sperm DNA Integrity tests for the evaluation of male infertility. Sperm DNA fragmentation test is an advanced and valuable test for proper sperm function with respect to semen analysis and this test is utilized during evaluation of male fertility. The integrity of genetic material in sperm is crucial for successful fertilization, normal embryo development and proper reproductive outcome as good DNA integrity will lead to proper transmission of genetic information. SDF term is used to indicate abnormal genetic material in sperm which can lead to male infertility.

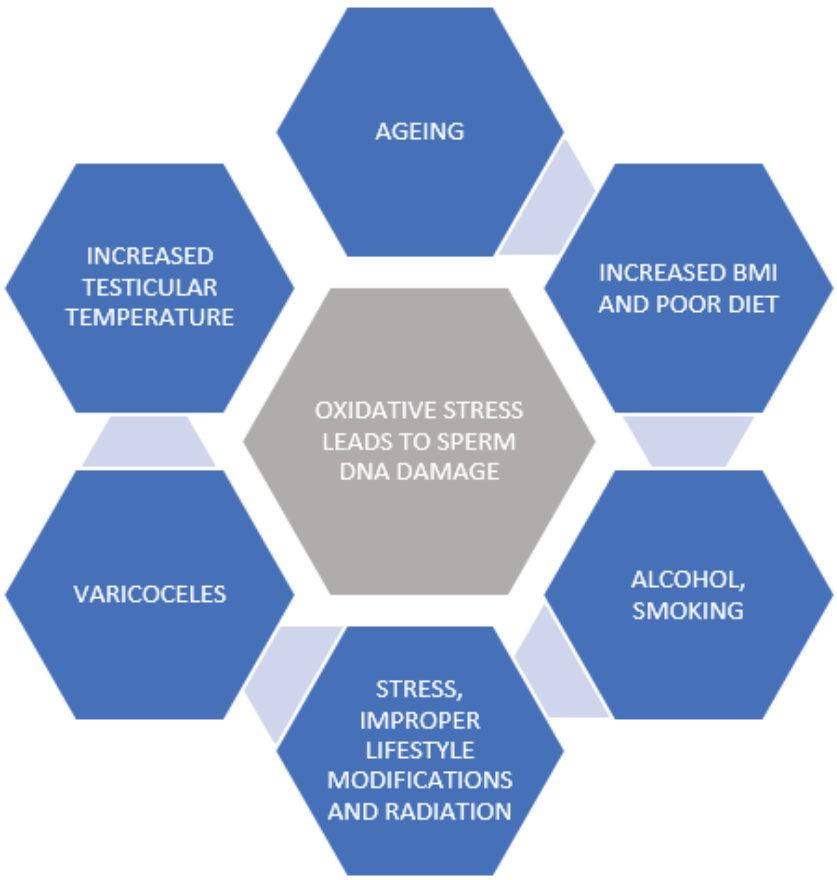

Fig: Factors associated with Sperm DNA Damage

Patients having varicocele, unexplained infertility, recurrent pregnancy loss, recurrent failure of assisted reproductive techniques (ART) and improper lifestyle/ environmental exposures are considered for sperm DNA fragmentation test. SDF test is useful in selecting patients for the ART procedures i.e. IVF/ICSI/IUI and highest pregnancy and live birth outcomes. [2] As Sperm DNA Fragmentation (SDF) test is considered as an important tool for measuring the quality of sperm on basis of its DNA fragmentation rate values. [4] Hence this method is more conventional and significant than the parameters analysed from semen analysis. Semen analysis is used for motility and morphology assessment, it cannot explain sperm at molecular level and thus SDF test has been introduced other than semen analysis to evaluate male fertility. High SDF rate largely affects the embryo quality, improper fertilization, miscarriages and failed IUI/IVF/ICSI.

High SDF rates is found in male with both normal and abnormal semen parameters, improper lifestyle modifications, smoking, radiation, oxidative stress, etc. "The percentage of cells having fragmentated DNA is represented by the **DNA FRAGMENTATION INDEX (DFI).**" DFI is recommended as element for prediction of fertility in males.

The Sperm DNA damage include defects in spermatogenesis (i.e. genetic abnormalities) and oxidative stress, hyperthermia, gonadotoxins and endocrine abnormalities).

Various tecniques used to measure sperm DNA integrity include:

· the sperm chromatin structure assay **(SCSA)**

· the terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end labelling **(TUNEL**) assay

· the single cell gel electrophoresis (Comet) assay & the sperm chromatin dispersion **(SCD)** test.

However, rate of sperm DNA fragmentation varies due to methods used for testing SDF and population. There are few studies which showed the correlation between clinical factors and sperm DNA damage.[3]

Clinical Factors/reasons leading to high sperm DNA damage are:

· lifestyle modifications (i.e. radiatons, heat, airborne pollutants, chemicals and sexually transmitted infections)

· biological factors (i.e. increased body mass index, diabetes and male age)

· Infection (i.e. inflammation and male genital tract infection)

· Oxidative stress

· Alcohol exposure (lead to alteration of endocrine system/ toxic effect to testis/genetic defect)

· Smoking (cause poor semen quality and genetic damage to sperms)

· Increased testicular temperature, varicocele

Clinical significance of sperm DNA damage for pregnancy:

It includes assesment of couples struggling with infertility and counselling strategies for patients with high SDF. This include

· Natural conception

· Intrauterine insemination (IUI)

· In vitro fertilization (IVF)

· Intracytoplasmic sperm injection (ICSI)

Treatments

· Damage due to free radicals: change in lifestyle and diet designed to reduce oxidative stress that will simultaneously reduce the levels of DNA fragmentation.

· Antibiotics if have existing infection

· Say no to drugs, smoking and tobacco

· Improve lifestyle modifications i.e. less exposure to radiation, pollution, etc

· Varicocele surgery

· Less BMI and good diet i.e. fresh foods, particularly those containing antioxidants and vitamin C & E

· TESA: Testicular aspiration of sperm (DNA damage occurs at the post-testicular level, hence testicular sperm may have a better DNA integrity than ejaculated sperm)

· ICSI rather than IVF

Background/Rationale

1) There was study[4] related to association of sperm DNA fragmentation with lifestyle factors and semen parameters of Saudi men. For this they selected total 94 couples for investigation with study parameters included were male age, body mass index, smoking, semen values, %sperm DNA fragmentation, fertilization rate and pregnancy outcome. In this they grouped semen samples on basis of % sperm DNA fragmentation into <15%, 15-30% and >30% i.e. low, moderate and high % spermDNA fragmentation respectively.

Results observed were, there was no difference in the ICSI outcome in low and moderate SDF. However in high SDF no patient achieved pregnancy. In this 53.19% Saudi men has low DFI, 32.98% moderate and 13.83% high DFI. It also showed that semen volume, sperm morphology and fertilization rate did not show any correlation with DNA fragmentation. But sperm concerntration and motility has negative correlation with DFI category. Also,

· BMI- +ve correlation (moderate DFI)

· SMOKING- +ve correlation (low DFI)

· AGE- +ve correlation (moderate and high DFI)

The study says 14% of Saudi men had high DFI.

2) There was study[5] to compare the result of TUNEL assay(i.e. tecnique used for SDF) applied to semen samples from males with proven fertile factor (n=47) and patient from infertile male population (n=66) to get discriminating threshold value. Results observed were that patients with male factor have high mean level of DNA fragmentation then proven fertile men.[5] The calculated threshold value for TUNEL assay to distinguish between both was 20% i.e. high for both positive value 92.8% and negative value 95.5%. (specificity-89.4%, confidence interval(CI)-95% and sensitivity-96.9%). This study demonstrated that SDF measured by TUNEL assay is highly valuable indicator of male infertility.[5]

Objectives:

· To predict male infertility with normal and abnormal both semen parameters & its diagnostic values.

· To investigate the clinical factors related to sperm DNA fragmentation (SDF) in male with infertility.

· To investigate the rate of sperm DNA fragmentation in male related to improper fertilization, embryo development and recurrent pregnancy loss.

· To investigate the relationship between Sperm DNA fragmentation index (DFI) and normal and abnormal both semen parameters and pregnancy rate of In Vitro Fertilization (IVF) and Intracytoplasmic sperm injection (ICSI).

· Prediction of outcome by evaluating increased SDFI (mean 95% confidence interval) and allowing with post infertility treatments.

Methods

Study Design:

POPULATION: male partner attending wardha test tube baby centre (AVBRH).

INTERVENTION: performing Sperm Chromatin Dispersion Test to evaluate Sperm DNA Fragmentation Index in male partner registered for SDF test with specific clinical parameters.

COMPARISON: Sperm DNA Fragmentation Index (DFI) with different clinical factors will be compared among patients.

OUTCOME: prediction of male infertility will be done by evaluating primary outcome i.e. increased sperm DNA fragmentation index (mean difference with 95% confidence interval) of patients and allowing male partner with post infertility treatments and success pregnancy rate.

Aim:

"To Study Dna Fragmentation Index In Patients Of Rural Area From Vidharbha Region With Different Clinical Parameters."

Methodology

Study Setting:

The trial will be carried out at Wardha Test Tube Baby Centre, AVBRH, Sawangi (Meghe), Wardha, after approval of Institutional Ethics Committee (IEC) of Datta Meghe Institute Of Medical Sciences (DU)

Study Design & Sample Size:

The design of the study is a randomized controlled trial of a male attending Wardha Test Tube Baby Centre, AVBRH, Sawangi (meghe), wardha.The number of participants enrolled in this experimental study is 40 (n = 40). Eight Groups are made considering 5 males in each group. Only single group of five people will be allowed for SDF test on particular day. The selected participants will be randomly allowed for collection of semen samples (well labelled with name, date & time of collection). Before inclusion, the participants will be explained about the objectives and approaches of the study, and written patient consent forms will be signed by them. The study procedure is shown in Figure 1

Fig 2: Patients Schedule, Interventions & Assesments.

Patient no./name	Male age (yrs ± SD)	Initial concerntration of sample (M×106/ml ± SD)	Initial motility (M% ± SD)	DFI (M% ±SD)
1.				
2.				
3.				
4.				
5.				

(test to be performed for 40 patients in group of 5)

M= mean, SD= Standard Deviation, DFI= DNA Fragmentation Index (by SCD test).

Participants

The inclusion criteria for the participants are as under:

1. Infertile male partner with poor semen quality.

2. Patients addicted to alcohol, tobacco and smoking.

3. Patient from rural area of wardha region with different lifestyle parameters (i.e. radiation, heat, air borne pollutants, etc)

4. Patient with IVF implantation failure.

Patient with IVF blastocyst formation failure.

Variables: This clearly defines all outcomes, exposures, potential confounders & effect modifiers. This will provide diagnostic criteria, if applicable.

Data sources/ measurement: For each variable of interest will provide sources of data & details of method of measurement.

The exclusion criteria for the participants are as under:

1. Patient not giving consent for treatment.

2. Patients having infections like HIV, HbsAG etc.

3. The cases with female infertility were excluded.

Sample Size: 40 infertile male

Statistical methods: Describe all statistical methods, including those used to control for confounding. Describe any methods used to examine subgroups and interactions.

Methodology

This study will be done in wardha test tube baby centre AVBRH (SAWANGI) WARDHA. Relevant data on the demographics and treatment history as well as the indications will be recorded. Counselling of all participants for research work will be done. The routine protocol in our set up will be followed and history of patient will be taken.(6)

Patients will be asked for medical history, physical examinations, conventional semen analyses, and blood tests including assessments of luteinizing hormone, follicle-stimulating hormone, and testosterone and for smoking and alcohol drinking status.(6)

Hormonal Analysis

LH, FSH and testosterone level will be analysed at Central pathology lab of AVBRH, Sawangi Meghe, Wardha.

Physical Examination

A general physical examination will be done. Patient will also be examined including examination of the penis i.e.

1) location of urethral meatus

2) Presence of both the vas deferens and epididymis

3) Palpation of the testes and their size

4) Presence of a varicocele (Varicoceles were diagnosed during scrotal examinations with the patient in a standing position)

Semen Collection and Analysis

Initially Patient will be asked to undergo for semen analysis. Patients were asked to have at least five days of abstinence before semen analysis. Semen sample will be collected at AVBRH IVF laboratory in semen collection room in a sterile plastic container by masturbation (acc. To WHO 2010). Sample is allowed to liquefy for 15 minutes prior to examination. Sample having less than 5 million/ml will be excluded, as sperm chromatin dispersion test require concerntration of 5-10 million/ml.

Sperm chromatin dispersion test

After analyzing semen sample, adequate sample will undergo sperm chromatin dispersion test Halosperm G2 kit. All the result from the test will be analysed and recorded to check DNA FRAGMENTATION INDEX % in patients.

Scope:

· Currently used sperm selection methods have been found to effectively eliminate sperm with damaged DNA, as a consequence of which the risk of using a genetically incompetent sperm for medically assisted reproductive technologies seems to be low and will increase the pregnancy outcome rate.

Limitations:

Ø One of the factor for assessing male infertility in my study is FSH, LH level measured/ testosterone conc. measured/varicocele daignosis and because cost many of the patient may not be willing for investigation.

Expected Outcomes/Results:

Participants: Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give reasons for non-participation at each stage. Consider use of a flow diagram.

Descriptive data: Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Indicate number of participants with missing data for each variable of interest.

Outcome data: Report numbers of outcome events or summary measures.

Main results: Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included. Report category boundaries when continuous variables were categorized. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.

Other analyses: Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses.

Discussion:

Efficiency of the Sperm DNA Fragmentation test is assessed by performing sperm chromatin dispersion test on respective semen sample (processed semen sample used)

using Halosperm G2 test kit (Halotech DNA madrid spain). Fragmentation in DNA of sperms are evaluated through no/small halos i.e. Sperm with DNA fragmentation and medium/large halos i.e. sperm free from DNA fragmentation.

Key results: Summarise key results with reference to study objectives.

Limitations: Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.

Interpretation: Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

Generalisability: Discuss the generalisability (external validity) of the study results.

1. Ethical clearance :
2. Source of funding: Self
3. Conflict of interest: Nil

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Estimation of Chronological Age from Advanced Glycation End Products Level in Vitreous Fluid

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Abstract

Background: Chronological age estimation is an important process for corpse identification. Advanced glycation end products (AGEs) are accumulated during lifetime and have been used for forensic identification. The AGEs in vitreous humor still lack of information for age estimation. *Objective:* To investigate correlation between the AGEs level contained in vitreous humor and chronological age in postmortem cases. *Methods:* The vitreous humor samples were collected from the postmortem cases (n=142). The AGEs levels were determined by spectrofluorometer and presented in microgram of quinidine hemisulfate equivalent (µg QE/mL) and nanogram of pentosidine equivalent (ng PE/mL). Stepwise linear regression was used to generate the equation. *Results:* The QE and PE showed mild positive correlation (r = 0.350 and 0.195; p <0.001, respectively) while the PE/QE ratio showed moderate negative correlation with age (r = -0.561, p <0.001). The predicted equation showed error value 12 years and gave accurate prediction in range 40-59 years old. *Conclusion:* The AGEs contained in the vitreous humor correlated with chronological age in postmortem cases. The other fluorescence molecules might be interfere during the AGEs measurement then the specific methods should be developed for measuring the AGEs level to decrease the error value.

Key words: *Age estimation, advanced glycation end products, vitreous humor, pentosidine*

Introduction

The formation of advanced glycation end products (AGEs) is a part of normal process in human body. The AGEs ,group of heterogeneous compounds, occurred from reducing sugars that reacted with free amino groups of proteins, lipids, or nucleic acids[1]. The AGEs ,normally pentosidine, ε*N*-carboxymethyllysine, ε*N*-carboxyethyl-lysine and methylglyoxal derivatives are found in the human body[2]. The AGEs play an important role in normal aging process and age-related diseases. Accumulation of the AGEs in tissues including serum, teeth, cartilage and skin are progressive during the lifetime[3]. The AGEs levels have been associated with aging process and correlated with age in volunteers, but AGEs levels in postmortem case is limited[4, 5].

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Vitreous humor is a gelatinous substance and filled in the eyeball where is a close space and is undisturbed by external environments, decomposition process and bacterial contamination[6]. Water, minerals and collagen are major components in the vitreous[7, 8]. The AGEs are found in the vitreous and associated with age in living person[9, 10]. Moreover, the vitreous humor is an alternative material for forensic identification. It was applied in postmortem diagnosis for example postmortem identification, death from hypothermia, sudden infant death, brain damage assessment and intoxication by bleach, but no apply in age estimation[11]. In addition, the process of vitreous humor collection is simple and rapid method.

High performance liquid chromatography (HPLC) coupled with fluorescence detector and enzyme-linked immunosorbent assay (ELISA) are suitable methods for the AGEs level analysis[12]. The HPLC is an expensive instrument and cannot provided in

laboratory. Spectrofluorometer is a basic instrument for determination of fluorescence substances level. An emission and excitation wavelengths are more specific to each substance properties and the pentosidine level was applied by fluorescence spectrometer[13].

In forensic medicine, age estimation is an essential process for person identification both living individuals and postmortem. There are many methods for the age estimation including radiological examination of skeleton and dentine, anthropological method, telomere shortening and chemical substances especially aspartic acid and AGE which are popular chemical substance for age estimation[14]. The AGEs level contained in teeth were highly correlated with chronological age[5]. However, the teeth samples were difficult for sample preparation. Using of the AGEs level contained vitreous humor might be simple and rapid method for age estimation. The aim of the study were to investigate correlation between the AGEs level contained in vitreous humor and chronological age in postmortem cases by spectrofluorometer.

Materials and Methods

One hundred and fourth two male who died within 24 hours were included in this study. All participants aged between 12 to 88 years old. The vitreous humor samples were collected during autopsy at Department of Forensics Medicines, Faculty of Medicine, Chiang Mai University during September 2019 to January 2020. The subjects who had decomposition and not known about gender, age, and race were excluded. The study was approved by the Research Ethics Committee Faculty of Medicine, Chiang Mai University (FOR 2562 06508).

Approximately, three milliliter of vitreous fluid was collected at eyeball position and the sample was added in sodium fluoride tube. The samples were stored at -80 °C before experimentation. The 0.5 milliliter of vitreous fluid sample was hydrolyzed with one milliliter of 6M hydrochloric acid at 110 °C for 20 hours. The hydrolyzed sample was centrifuged at 12,000 RPM for 10 minutes. Then, ten microliter of the hydrolyzed sample was diluted with 190 microliter of double distilled water.

The AGEs concentration was measured with spectrofluorometer[15]. Quinidine hemisulfate and pentosidine were used as standard substance. The excitation and emission wavelengths was 350/440 nm for quinidine hemisulfate and 328/378 nm for pentosidine, respectively. The results were expressed in microgram of quinidine hemisulfate equivalent per milliliter (μg QE/mL) and nanogram of pentosidine equivalent (ng PE/mL).

The efficiency of the age prediction models were assessed by self-consistency test and bias and inaccuracy value were calculated following equiation:

•Bias = Σ (predicting age – chronological age)/n

•Inaccuracy = Σ|predicting age – chronological age|/n

Data were expressed as mean ± standard deviation (S.D). The correlation between the AGEs levels and chronological age were analyzed by Pearson's correlation. The age prediction models were generated by multiple linear regression. The estimated age and actual age were compared with paired *t*-Test analysis. Independent t-Test was used to compare AGEs levels in cardiovascular disease group and non-cardiovascular diseases group. A *p*-value less than 0.05 ($p<0.05$) was considered significant.

Reults

In this study 142 males who age ranged from 12 to 88 years old were included. We classified the participants into seven groups and the results are showed in Table 1. Cause of death composed of trauma 84 cases (59.2%), sudden unexpected death 41 cases (28.9%), hanging 11 cases (7.7%), and miscellaneous 6 cases (4.2%). The underlying diseases of the participants were obtained from medical record. Hypertension, diabetic mellitus and renal disease found 18 (12.7%), 7 (4.9%), and 4 (2.8%) cases, respectively. The scatter plots between age and the QE, PE, and PE/QE ratio are shown in Figure 1. The value of QE, PE, and PE/QE ratio in these diseases showed similarly levels with the healthy participants or other diseases.

Correlation between the quinidine hemisulfate, pentosidine equivalent and chronological age

The Pearson's correlation was used for determining a correlation between the AGEs concentrations and chronological ages. The correlation between the QE and

PE showed mild positive correlation (r=0.35 and 0.195, respectively). While the PE/QE ratio showed negative correlation (r=-0.561). The correlation of chronological age and the AGEs levels are shown in Figure 1.

Age estimation model

The age estimation equation was generated by linear regression following stepwise method. The formula for age estimation is shown as follow:

Estimated age = 143.404 – (1040.781* PE/QE) + (24.277*PE) – (1.927*QE) (1)

When PE is pentosidine equivalent, QE is quinidine hemisulfate equivalent, and PE/QE is the ratio of pentosidine and quinidine hemisulfate equivalent. The R value of the linear regression equation was 0.607 (R^2 = 0.368, p <0.001). The beta coefficients of QE, PE, and PE/QE ratio in the equation presented -0.975, 1.076, and -0.889 respectively. The standard error of the estimation (SE) showed 15.89.

The self-consistency test was used for testing the efficacy and accuracy of the estimated equation. Bias value of the equation showed 0.028 years and inaccuracy demonstrated 12 years. The estimated age were compared with chronological age in each age range and the results are shown in Table 1. A different value (ΔA) between estimated age and chronological age was plotted with the chronological age. The ΔA in age range 40-59 years old revealed lower inaccuracy value than the other ranges and the graph is shown in Figure 2. The estimated ages were similar with the chronological ages in range 40-59 year old but others ranges were different.

Effect of cardiovascular diseases on the AGEs levels

The cause of death was diagnosed by a forensic pathologist. Coronary artery disease, myocardial infarction, and intracerebral hemorrhage were grouped in cardiovascular disease. For, pneumonia, sepsis, pulmonary embolism, and others were grouped in non- cardiovascular disease. The AGEs level in the cardiovascular disease group was similar level when compared with the level of non- cardiovascular diseases and the results are demonstrated in Table 2.

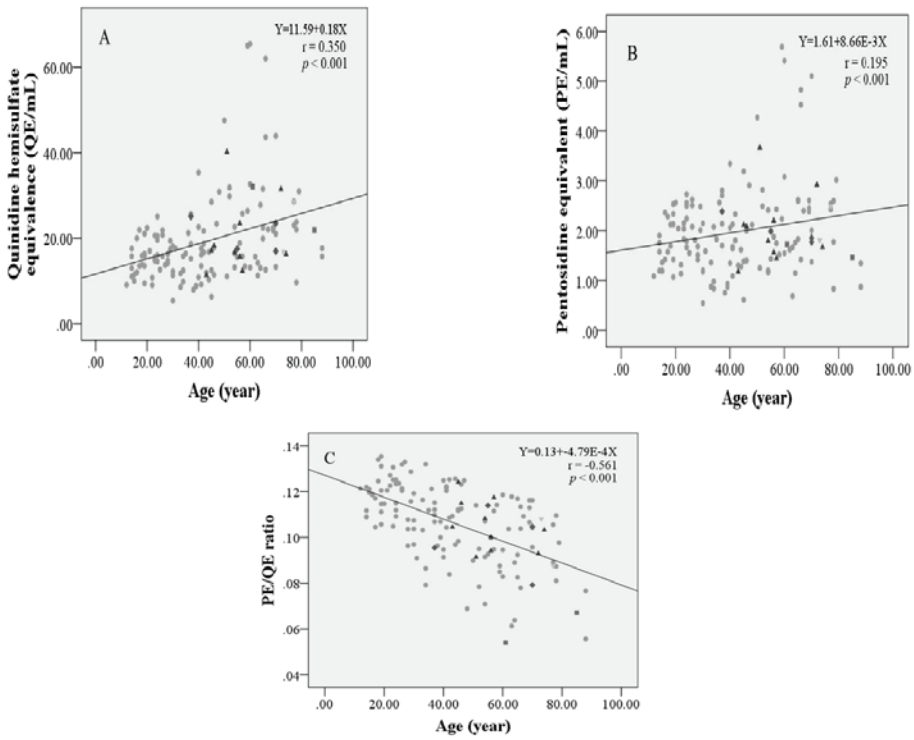

Figure 1. Distribution of the AGEs values in each chronological ages and diseases when p = hypertension, u = Diabetic mellitus q=, renal disease, n = hypertension + diabetic mellitus +renal disease and ˜ = healthy participants. Correlation of the QE, the PE and PE/QE ratio with chronological age are shown in Figure A, B and C, respectively.

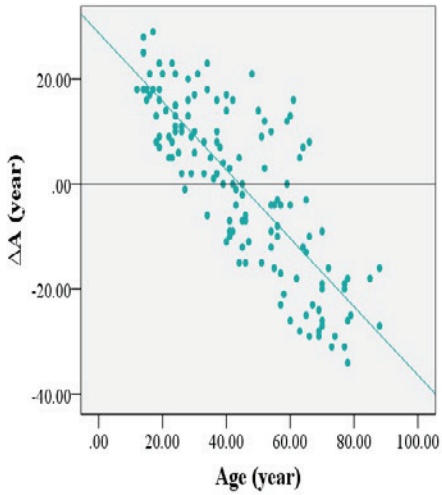

Figure 2. Relative of bias (the difference value of estimated age and chronological age (rA)) and chronological age.

Table 1. Comparison between estimated age and chronological age

Actual age	Number (n)	Mean of actual age (year)	Predicted age range	p-value
12-19	19	16.5	24-48	<0.001
20-29	23	24.8	21-49	<0.001
30-39	19	34.8	26-61	<0.001
40-48	23	43.2	24-64	0.270
50-59	22	54.6	33-66	0.091
60-69	18	64.5	32-75	<0.001
70-88	18	76.3	43-76	<0.001

$p < 0.05$ significant different between actual age and predicted age

Table 2. Comparison of the AGEs levels in cardiovascular diseases and non-cardiovascular diseases

Factors	Cardiovascular diseases (N=24)	Non- cardiovascular diseases (N=17)	p-value
Age	59.6±12.7	57.4±19.1	0.672
Quinidine hemisulfate (μg QE/mL)	23.1±11.7	23.3±13.5	0.948
Pentosidine (ng PE/mL)	2.2±1.0	2.2±1.3	0.888
PE/QE ratio (*10-2)	9.9±2.0	9.5±1.9	0.627

$p < 0.05$ significant different the AGEs level between the cardiovascular disease group and non- cardiovascular diseases group.

Discussion

The AGE formation is a normal process and develops in tissue during aging. The AGEs levels were determined in postmortem for age estimation. Estrogen, female hormone, reduce the AGEs level[16] and gender might effect on pentosidine accumulation in vitreous humor[10].Then only male participants were included in this study to decrease gender effect.

The QE and PE level presented mild positive correlation with chronological age. Both the AGEs and pentosidine in vitreous significant increased depending on age[17]. The PE/QE ratio showed moderate negative correlation with the chronological age. The correlation of the QE and PE level in vitreous were lower than teeth, cartilage, and bone samples[5,18]. The turnover rate of protein might be effect to the AGEs and pentosidine level in tissues. High turnover rate tissue including blood and vitreous humor has a shorter half-life of the AGEs than the low turnover rate tissue[19].

In age ranged 40-59 year old were highly accurate than the others range. The contaminated AGEs in food can absorb through circulation leading to high AGEs levels in blood or accumulate in other tissue[20]. Teenager's lifestyle like to consume about coffee, cocoa, cake, bread and grilled meat leading to highly the AGEs level in blood and might be disposed to the vitreous[21, 22]. Aging is the progressive accumulation of the AGEs from dietary, lifestyle, drug and unknown underlying disease [9, 23]. The AGEs damage to an organism overtime leading to disturbed function on the cellular, tissue and organs. In elderly age, the AGEs level normally higher level than the teenager leading to highly error estimated age.

High level of the AGEs presents in diabetes mellitus and cardiovascular diseases[1, 24]. The cardiovascular disease was only observed with forensic pathologist, but diabetes mellitus could not diagnostic with routine laboratory. The AGEs level in cardiovascular diseases did not different compared with the healthy participants. As same as, the QE, PE and PE/QE ratio in cardiovascular diseases group did not different compared with non-cardiovascular diseases which was discordance with the previous study[25]. In this study showed that cardiovascular diseases did not effect to the QE, PE and PE/QE ratio and might be effect on the age estimation process. However, the fluorescence spectrometry method might be affected by others fluorescence molecules. Then, the specific methods for the AGEs determination should be developed to give the highly predict equation for age estimation.

Conclusion

The PE/QE ratio of vitreous humor in postmortem cases showed moderately correlation with chronological age and suitable for age estimation. The predicted equation presented error value about 12 years and gave accurate range in 40-59 year old. The protein turnover rate and external factors including dietary intake, drugs, and unknown underlying disease might disturb on the AGEs. In the future, teeth, bone, and cartilage samples

should be determined the AGEs for more accuracy in postmortem age estimation. The specific methods should be developed for measuring the AGEs level to decrease the error value.

Conflicts of Interest: The authors declare that they do not have any conflict of interest.

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Study to Assess the Effectiveness of Structured Teaching Programme on Hospice and Palliative Care among Nursing Students

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Abstract

Context: Patient and family who are confronting the issues of life threatening diseases achieves personal gratification through palliative care.

Aim: The primary goal of the investigation was to determine the adequacy of "structured teaching programme" on hospice and palliative care amidst nursing students.

Setting: Quasi experimental study was conducted in Kular College of Nursing, Kishangarh.

Methods and Material: "Non-probability-convenience sampling technique was utilized to select sample. 60 subjects were selected for the study. Self structured knowledge questionnaire was used for data collection.

Results: The outcomes indicated that pre test mean score" was 16.86 & standard deviation was 3.1 while "post-test mean score" was 23.46 & standard deviation was 4.14.

Conclusion: "Structured Teaching programme" was efficacious in enhancing knowledge of nursing students.

Keywords: *Effectiveness, structured teaching programme/STP, hospice, palliative care, nursing students*

Introduction

"According to Worldwide Palliative Care Alliance (WBPC)" despite more than 100 million individuals over the world profited by hospice and palliative care, yearly under 8% of those in need access it[2]. Despite the fact that India positions at the base of the nature of the passing list in general score, it is assessed that in India around 7 million individuals are determined to have malignant growth consistently, around 80% of all disease is analyzed in the serious stage when treatment is less compelling and palliative care become significant. Consistently in excess of 60 patients die in India from hazardous diseases and in pain. In India the overall number who needs palliative care is probably going to be 6 million individuals, however under 1% has access to it.[3,4,]

For the life threatening diseases, sympathetic "hospice and palliative care" is provided to the patients. Moreover, "Palliative care" which is a part of "hospice care" can be served as a distinctive field in clinical practice while treating the patient. "Hospice and palliative care" fulfills patients bodily, sentimental, religious demands as well. For alleviating manifestations of patients sickness while receiving treatment "palliative and hospice care" is beneficial.[5]

Nurses assume a significant part in care of fundamentally sick and passing on clients. The absence of information regarding "palliative care" is man snag for "nurses" & other medical experts.[6]

Main goal is to refine the "palliative care" in every setting and upgrade the relatives experience watching the demise process of their friends and family. Nurses have the option to provide viable palliative concern once they can recognize the manifestations and necessities of the patient. Team of "Palliative care" aims to reduce the suffering by alleviating the symptoms & emphasis

on "psychosocial concern". They additionally try to organize care & interaction is enhanced among experts and within patient and family. Setting to setting team organization vary.

Considering the abovementioned, the current investigation was attempted to determine the adequacy of "structured teaching programme" about hospice and "palliative care" as far as information among student nurses. The goal of the investigation included assessment of the adequacy of "structured teaching programme" as far as information on hospice and palliative care among student nurses.

Materials and Methods

"Quasi experimental" study was conducted in Kular College of Nursing, Kishangarh. One group pre-test and post-test design chosen for the study. Participants were chosen utilizing "purposive sampling technique". The participants were students of B.Sc.(N) 2nd year. Informed consent was obtained from participants. Prior to the data collection, the necessary permission was obtained from the concerned authorities. Questionnaire was distributed to the participants for pre-test, in which the questionnaire consists of two parts, Part A and Part B. Part A was designed to investigate the demographic data, while part B was designed to measure the knowledge level regarding hospice and palliative care. After pre-test, "structured teaching programme" was implemented upon same group. "Post-test" was carried out following 7 days to assess knowledge level among participants. Approval from the ethical and research committee of Kular College of Nursing was taken before starting the study. Descriptive and inferential statistics were used.

Results

Results had indicated that most of the students who were in the age bunch 19 (63.3%) and followed by the age range over 20 year (23.3%) and least of them were in the age range of 18 (13.3%). Regarding habitat of the respondents rural frequency 25(83.3%) and urban 5(16.6%). Regarding religion of the respondents Sikh were 58(96.6%), Hindu were 2(3.3%) and no respondent were from Christian religion.

Table 1: Pre-test and Post-test results

Sr No.	Group	"Level of knowledge"					
		"Adequate knowledge"		"Moderate knowledge"		"Inadequate knowledge"	
		Frequency (f)	Percentage (%)	"Frequency" (f)	"Percentage" (%)	"Frequency" (f)	"Percentage" (%)
1.	Pre-test	-	-	12	20%	48	80%
2.	Post-test	2	3.33%	56	93.3%	2	3.33%

Table 1 depicts that in pre-test 20% had adequate knowledge and 80% had inadequate knowledge on Hospice and Palliative care according to post-test results majority of students i.e. 93.3% had adequate knowledge after structured teaching programme on hospice and palliative care, 3.33% had inadequate knowledge and 3.33% had inadequate knowledge.

Table 2: Distribution of "Mean and Standard Deviation" of pre and post-test results

Variables	Mean	SD	t test value	Table value	Inference
Pre- test	16.86	3.1	15.6		Significant
Post-test	23.46	4.14			

Table 2 shows that during "pre test mean score" was 16.86 & standard deviation was 3.1 whereas "post-test mean score" was 23.46 & standard deviation was 4.14. There is considerable connection between "structure teaching programme" and information score.

Table 3 "Association between the post-test knowledge scores with their socio demographic variables".

Sr. No.	Demographic variables	Level of Knowledge			df	Table Value	Chi square	p-value
		Adequate	Moderate	Inadequate				
1.	Age in years				2	5.99	6.787S	0.1475
	18	1	6	1				
	19	0	37	1				
	>20	1	13	0				
2.	Habitat				1	3.84	3.42NS	0.180
	Rural	1	48	1				
	Urban	1	8	1				
3.	Religion				1	3.84	28.96S	0.00
	Sikh	1	56	1				
	Hindu	1	0	1				

Table 3 presents that relationship within post-test information scores & "socio-demographic" variables, particularly, age, and religion found significant, as calculated value is greater than tabulated value at the p level of significance < 0.05. It means knowledge scores were associated with age and religion. And the association between post-knowledge scores and socio demographic variables such as habitat found non-significant, as calculated value is less than tabulated value at the p level of significance <0.05.

Discussion

The current investigation uncovered that there was a considerable variation among pre and post test scores of knowledge. The outcomes are upheld by the results of the investigations Shany Thomas (2018)[7] on knowledge with respect to "palliative care" among staff nurses. Majority of subjects (94%) had adequate knowledge and only 6% subjects had moderate knowledge regarding palliative care.

An experiment was directed by Rajaragupathy S. et al, to evaluate the knowledge of palliative care among undergraduate students of medication, nursing, pharmaceutics & physiotherapy. The investigation included absolute of 200 students. Findings uncovered that essential information about palliative care was insufficient among the undergraduate students of medical care. These results are consistent with the present study as 77 (96.25%) nurses had inadequate knowledge, while only 3 (3.75%) had adequate knowledge regarding palliative care.[8]

Conclusion

The "structured teaching programme" was discovered to be compelling in expanding information among student nurses. Participants gained significant knowledge after the implementation of teaching programme. There ought to be workshops of students nurses and in-service training for the nurses so they can be fully informed regarding the changing trends which thus improve the patient result. Palliative care is rising consideration in India, so nurses should be prepared and instructed on the different parts of palliative care and idea should be joined in educational program so that student nurses can pick up information as per evolving patterns.

Ethical Clearance: Taken from ethical committee of Kular College of Nursing, Khanna, Ludhiana, Punjab

Conflict of Interest: Nil

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Knowledge Regarding Computer Ergonomics and Incidence of Musculoskeletal Disorders among Desktop Users

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Abstract

The use of computers has become universal. Computer technology plays an integral role in our personal, professional and educational lives and neglecting the principles of ergonomics during computer utility raises the risk of health problems. Musculoskeletal disorders (MSDs), Visual symptoms and Insomnia are the common health problems among desktop users. Aims and Objectives: To assess the knowledge regarding computer ergonomics and incidence of musculoskeletal disorders Methodology: In this cross-sectional research study 130 desktop users were recruited as study participants. The tool used i.e. selected variables regarding study participants characteristics and Structured Knowledge questionnaire (35 items) Results: More than 2/3rd of study participants (90%) were reported about discomfort related to eye strain, head and neck, wrist and hand, elbow, upper back, lower back, hips and thighs and knees. Less than half of the study participants were at average level of knowledge related to computer ergonomics and There was significant association of selected variables (discomfort and severity of hand and wrist discomfort) was found with the Knowledge of computer ergonomics. Conclusions: Findings of this study concluded that there was majority of study participants reported discomfort related to the eye strain, head and neck, wrist and hand, elbow, upper back, lower back, hips and thighs and knees and severity of hand and wrist discomfort which showed that these organs get influence more by desktop based on body configuration.

Keywords: *Knowledge, Computer ergonomics, Musculoskeletal Disorders, Desktop users*

Introduction

Ergonomics (human factors) is the study of humans in relation to their work environment and work in surroundings. It is a broad science involving application of psychological as well as physiological principles.[1] The use of computers has become universal. Computer technology plays an integral role in our personal, professional and educational lives[2]. Neglecting the principles of ergonomics during computer utility raises the risk of health problems. Musculoskeletal disorders (MSDs), Visual symptoms and Insomnia are the common health problems amongst computer users[3]. Extended period of static sitting postures causes decreased circulation, stiffness and pain in the joints. Prolonged duration of continuous work increases the risk of cumulative trauma disorders, which may result in long-term disability[4]. A little knowledge of the principles of ergonomics of work station setup and exercises can prevent a lot of discomfort and maximize productivity[5]. (Knowledge of Computer Ergonomics among Computer Science Engineering and Information Technology Students in Karnataka, India. There is a need for more awareness regarding musculoskeletal disorders and why it's happening. Nowadays people have obesity, eye problem which has made them to uses spectacles at early age, disfigured body, some patches in wrist because not using mouse pad and swelling in legs due to prolonged sitting. These problems mainly arise when one doesn't

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sit regularly for work but when they sit they won't left it incomplete, finishing the work without considering their health and body aches, which makes have poor health[6]. discomfort arises from lack of knowledge about ergonomics get influence more by desktop based on body configuration[7]. Awareness among this generation need to be given with the cause of poor ergonomic postures This research was undertaken to assess the knowledge of computer ergonomics and incidence of musculoskeletal disorders among desktop users.

Materials and Methods

Assessment of Knowledge regarding computer ergonomics was done through questionnaire survey method from 130 desktop users. Questionnaire was prepared and circulated among computer users through google form. The data collected was handled confidentially. Research tool consists of two sections i.e. Section A related to Demographic profile consists of age, gender, qualification, discomfort related to eye strain, head and neck, shoulders, elbow, wrist, upper back, lower back, hips and thighs and knees, Dominant Hand, Frequency of Wrist and hand discomfort, severity of wrist and hand discomfort, previous knowledge, Hours of working on computer and years of working on computer and Section B related to Structured Knowledge questionnaire consists, was used to assess the knowledge regarding computer ergonomics among desktop users. The tool was developed on the basis of previous research evidences, based on extensive review of literature and opinion of experts and guides. It consists of 35 items and scoring was done as very good (28-35), good (23-27), average (18-22) and below average (0-17). The tool is valid and reliable to assess the knowledge regarding computer ergonomics.

Data Analysis

Descriptive statistics: Frequency, percentage distribution was used to describe selected variables

Inferential statistics: Independent 't' test and ANOVA test was used to find out the association of knowledge regarding computer ergonomics with selected variables

Results

Most of the study participants were female (76.2%) and most of the study participants in age group less than 30 years of age (75.4%) and more than half of the study participants were post graduated (55.4%). More than 2/3rd of study participants (90%) were reported about discomfort related to eye strain, head and neck, wrist and hand, elbow, upper back, lower back, hips and thighs and knees. Majority of the study participants (99.2%) dominant hand was right. Less than half of the study participants were neither having hand and wrist discomfort nor severity of hand and wrist discomfort. Less than half of study participants were at average level of knowledge regarding computer ergonomics. ANOVA and 't' test value for association of Knowledge score regarding computer ergonomics among desktop users with selected variables. The findings revealed that computed 'F/t' value between knowledge and age (F= 0.34, p=0.79), gender (t= 1.51, p= 0.13), qualification (F= 1.33, p=0.26), dominant hand (F= 0.69, p=0.48), Frequency of wrist and hand discomfort (F= 1.31, p=0.26), previous knowledge (t= 0.21, p= 0.83), hours of working on computer (t= 1.01, p= 0.31) and years of using computer (F= 0.45, p=0.67were found to be statistically non-significant except discomfort (t= 2.04, p= 0.04) and severity of wrist and hand discomfort (F= 2.167, p=0.03).

Table 1: Level of Knowledge regarding computer ergonomics among desktop users

N=130

Level of Knowledge	Actual Range of Score	f (%)
Very Good	28-35	4(3.1)
Good	23-27	35 (26.9)
Average	18-22	50 (38.5)
Below Average	0-17	41(31.5)

Discussion

In this study most of the study participants were female and most of the study participants belongs to age group of less than 30 years. These findings were supported by a cross sectional study conducted by Mohamed Sherif Sirajudeen et al (2018) where they found more than half of the participants (54.8%) were in 22-24 years of age group and more than half of the participants (57.5%) were females. In present study majority of study participants (90%) were reported about discomfort related to eye strain, head and neck, wrist and hand, elbow, upper back, lower back, hips and thighs and knees. These research findings were similar to previous research findings, conducted by A.K. Sharma et al (2006)[8] where majority of subjects (93%) had one or more than one computer related health problem

Conclusion

Based on our research findings, discomfort related to the eye strain, head and neck, wrist and hand, elbow, upper back, lower back, hips and thighs and knees and severity of hand and wrist discomfort are associated with the knowledge regarding computer ergonomics which emphasize the importance of computer ergonomics to decrease the related musculoskeletal disorders. There is a need to plan an appropriate learning programme to make the computer users more aware and make them understand about the computer ergonomics

Conflict of Interest: NIL

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Ethical Approval: Research ethics committee of Maharishi Markandeshwar Deemed to be University Mullana, Ambala (MMDU/IEC/1752)

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Clinical Profile of Pediatric Patients with Acyanotic Congenital Heart Disease in Dr. Soetomo General Hospital Surabaya

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Abstract

Congenital Heart Diseases (CHD) are classified as Acyanotic or Cyanotic CHD. Prevalence of CHD according to various studies is 8-10 per 1000 live births worldwide, 10% of cases became infant mortality in developing countries. This research aims to study the characteristics and clinical presentations in children with acyanotic CHD in Dr. Soetomo General Hospital. A retrospective observational study from children with acyanotic type of CHD from medical record outpatient clinic in the Division of Neonatology and Pediatric Ward at Dr. Soetomo General Hospital from June to December 2016. Age, sex, heart defect, comorbid and nutritional status were taken from the medical record. The most dominant clinical profile was female with a mean age of 4 years. Atrial Septal Defect was the most common type of left-to-right shunt, followed by Ventricular Septal Defect, and Patent Ductus Arteriosus. Pulmonary Stenosis was the most common type of obstructive lesion, followed by Aortic Stenosis. Complications of acyanotic CHD were dominated by recurrent respiratory tract infections followed by growth and developmental disorder, and heart failure. The most common nutritional status was moderate malnutrition.

Key Words: *Acyanotic, CHD, ASD, VSD, clinical profile.*

Introduction

Congenital Heart Disease (CHD) is a disease marked with abnormalities of the heart structure or cardiac circulation function that is present at birth.[1] CHD is the most common congenital abnormality, almost 1/3 of cases of congenital abnormalities are cases of congenital heart disease.[2] The prevalence of CHD worldwide ranges from 6-10 per 1.000 births. The distribution depends on the demographics. Currently, of the 220 million population of Indonesia, it is calculated that there are 6.600.000 newborn babies and 48.800 of them are diagnosed with CHD.[3]

CHD is classified into 2 groups, namely acyanotic and cyanotic congenital heart disease.[4] Cyanotic CHD is more complex and is characterized by cyanosis due to a right-to-left shunt so that blood from systemic veins that contain low oxygen will return to the systemic circulation. This acyanotic CHD has no symptoms or signs of cyanosis, but there was a left-to-right shunt or ventricular outlet obstruction. [3,4] The number of patients with cyanotic CHD is much greater than that of cyanotic by 3-4 times, but cyanotic CHD causes higher morbidity and mortality than acyanotic.[3]

The incidence of CHD is reported to be around 8-10 babies out of 1.000 live births and 30% of them have developed symptoms in the first weeks of life. If not detected early and not treated properly, 50% of deaths will occur in the first month of life. In developed countries, almost all types of CHD have been detected in infancy even at the age of less than 1 month, whereas in developing countries many are only detected after the child is older, so that in some severe types of CHD many children may have died before being diagnosed. In certain types of CHD, early recognition and diagnosis are needed so that the necessary treatment and surgery can be given immediately. To improve services in Indonesia, apart from the provision of funds and an adequate pediatric cardiology service center, it is also necessary to have early detection capabilities for CHD and knowledge of optimal referral times by general practitioners who are first dealing with patients.

Materials And Methods

This study uses a descriptive design. The research data were taken from all medical records of patients with Congenital Heart Disease in the Division of Neonatology and Pediatric Ward of Dr. Soetomo General Hospital Surabaya from January 1, 2016, to December 31, 2016. Incomplete medical records were excluded from this study. The sampling method is a total sampling. The data were entered into the Statistical Software Program for Social Science (SPSS) and analyzed using the chi-square and t-test.

Results and Discussion

From 355 children with acyanotic CHD, 158 incomplete medical records were excluded. The remaining 198 children were predominantly female with 127 female subjects (64.1%) and the mean age was 4.0 years old (SD 3.2). The characteristics of the research subjects are described in table 1.

Table 1 Level of Knowledge regarding computer ergonomics among desktop users N=130

Level of Knowledge	Actual Range of Score	f (%)
Very Good	28-35	4(3.1)
Good	23-27	35 (26.9)
Average	18-22	50 (38.5)
Below Average	0-17	41(31.5)

Table 1: Subjects characteristics

Characteristics	Case n (%)
Age	
· 1 year old	33 (16.7)
· 2 years old	36 (18.2)
· 3 years old	31 (15.7)
· 4 years old	31 (15.7)
· 5 years old	25 (12.6)
· 6 years old	18 (9.1)
· 7 years old	11 (5.6)
· 8 years old	1 (0.5)
· 9 years old	3 (1.5)
· 10 years old	1 (0.5)
· 12 years old	1 (0.5)
· 13 years old	1 (0.5)
· 14 years old	1 (0.5)
· 15 years old	1 (0.5)
· 17 years old	2 (1)
· 26 years old	2 (1)
Sex	
· Female	127 (64.1)
· Male	71 (35.9)
Nutritional Status	
· Good nutritional status	94 (47.5)

Cont... **Table 1: Subjects characteristics**

· Moderate malnutrition	95 (48)
· Severe malnutrition	9 (4.5)
Heart defect	
· Atrial Septal Defect	97 (49)
· Ventricular Septal Defect	72 (36.4)
· Patent Ductus Arteriosus	22 (11.1)
· Pulmonary Stenosis	4 (2)
· Aortic Stenosis	3 (1.5)
Complication	
· Recurrent respiratory tract infections	68 (34.5)
· Growth and developmental disorder	48 (24.3)
· Heart failure	37 (18.7)
· Asymptomatic	45 (22.5)

Atrial Septal Defect (ASD) was the most common type of left to right shunt, seen in 97 cases (49.0%). ASDs are associated with Mendelian inheritance, aneuploidy, transcription errors, mutations, and maternal exposures. Besides, ASDs have been associated with familial genetic disorders and conduction defects. Transcription factors important during the atrial septation include GATA4, NKzX2-5, and TBX5. More recent epidemiologic data suggest that ASDs occur in 1.6 per 1.000 live births.[5] Congenital heart disease is diagnosed more commonly in patients in developed countries who have higher incomes.[6] This explains ASD as the most common type of left-to-right shunt. Ventricular Septal Defect (VSD) seen in 72 cases (36.4%) was the next most common cause of left-to-right shunt. VSD develops when there is a developmental abnormality or an interruption of the interventricular septum formation during the complex embryologic heart morphogenesis. VSDs are frequently isolated; however, they can occur in association with other congenital heart defects such as atrial septal defects, patent ductus arteriosus, right aortic arch, and pulmonic stenosis. Several genetic factors have been identified to cause VSD including chromosomal, a single gene, and polygenic inheritance.[7,8] Pinto *et al*.[9] stated that isolated VSD accounts for 37% of all congenital heart disease in children. The incidence of isolated VSD is about 0.3% of newborns. Because as many as 90% may eventually close spontaneously; the incidence is significantly lower in adults. VSDs have no gender predilection. The percentage of each type is presented in the pathophysiology section. This is in accordance with the findings in this study. The third most common cause of left-to-right shunt is Patent Ductus Arteriosus (PDA) 22 cases (11.1%). Iwashima *et al*.[10], stated that in at least 10% of other congenital heart disorders, a patent ductus may be present. This statement supports the findings of this study.

Pulmonary Stenosis (PS) was the most common type of obstructive lesion seen in 4 cases (2%), followed by 3 cases Aortic Stenosis (AS) (1.5%). Samánek *et al.*[11] dan Stephensen *et al.*[12] stated that pulmonic stenosis accounts for 7%-10% of congenital heart diseases. There is a slight female predominance, and 2% of familial occurrences are without a genetic cause.[13] The differences in the findings of this study could be due to different research methods. Differences could also be caused by differences in the number and characteristics of the study samples in which the research conducted by Samánek *et al*.[11] has subjects of 779 children and were examined at least four times during infancy and at the age of three and four years. A study by Hoffman *et al.*[14] concluded that aortic stenosis accounts for approximately 3–6% of congenital heart defects. This is consistent with the findings in this study. The differences that arise could be caused by differences in the number of samples where Hoffman *et al*.[14] have as many as 19.044 children as research subjects.

Complications of acyanotic CHD were dominated by recurrent respiratory tract infections with 68 cases (34.5%). Some kind of CHD which leads to increased

pulmonary flow (e.g. VSD, PDA) or CHD with desaturation (e.g. TOF, DORV, truncus arteriosus) carries a higher risk of recurrent respiratory tract infections and increased frequency of hospitalizations.[15] This supports recurrent respiratory tract infections as the most common complication of CHD. The second most common complication is growth and developmental disorder with as much as 48 cases (24.3%). A study by Mehrizi and Drash[16] stated that patients with ASD or PS were more likely to develop growth retardation. This is in contrast to the findings in this study where cumulatively, 51% of patients experienced ASD or PS. This difference could be because the research by Mehrizi and Drash[16] was carried out in 1962 where perhaps advanced nutrition feeding was still inadequate. Heart failure is the third most common complication in CHD with 37 cases (18.7%). Heart failure is more common in adult CHD.[17] In a Dutch registry of 10,808 CHD patients followed for 21 years (median), the incidence of HF-admissions was 1.2 per 1.000 patient and the median age at first HF-admission was 46.7 years.[18] The most common CHD induced heart failure route is purely acquired and mechanical with no genetic element. This includes incomplete or palliative correction of a lesion leading to a chronic state of hemodynamic stress and subsequent heart failure.[19] The other route is congenital malformation and cardiomyopathy and reactivation of fetal genes that are in mutated pathways in the setting of CHD.[19] This supports the findings in this study which states that heart failure is not the most common complication of CHD.

The most common nutritional status was moderate malnutrition in 95 cases (48%). Malnutrition occurs among children with congenital heart disease, irrespective of the nature of the cardiac defect, and the presence or absence of cyanosis.[20] Children with congenital heart disease (CHD) are more prone to malnutrition for several reasons including decreased energy intake, increased energy requirements, or both.[21] This study and other studies show that patients with CHD are more prone to malnutrition.

The limitation of this study is that a large number of samples were excluded due to incomplete data on patient characteristics. Further research is needed to determine the main cause of a particular defect can be the most common type of left-to-right shunt or obstructive lesion.

Conclusion and Acknowledgement

Atrial Septal Defect (ASD) was the most common type of left-to-right shunt, followed by Ventricular Septal Defect (VSD), and Patent Ductus Arteriosus (PDA). Pulmonary Stenosis (PS) was the most common type of obstructive lesion, followed by Aortic Stenosis (AS). Complications of acyanotic CHD were dominated by recurrent respiratory tract infections followed by growth and developmental disorder, and heart failure. The most common nutritional status was moderate malnutrition.

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NSAID-Induced Adverse Drug Reaction: Mechanism and Management

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs to reduce pain or swelling. The use of these drugs in high doses or long-term can cause side effects or hypersensitivity problems, also known as Adverse Drug Reaction (ADR). A literature review was carried out using the PubMed database by inserting the keywords 'NSAID', 'adverse drug reaction', and 'hypersensitivity'. All studies related to NSAIDs and their adverse drug reactions were included in this review, while genetic or pharmacogenomics studies and NSAIDs' effectiveness were excluded. The results showed that gastrointestinal (GI) problems such as duodenal ulcers or erosive gastritis are the most common diclofenac effects (2.05%). Cardiovascular issues, such as acute myocardial infarction, were mostly caused by rofecoxib (2.12%). Hypersensitivity, both respiratory and skin, is commonly caused by ibuprofen with prevalence 50% and 67%, respectively. The most frequent kidney problem related to NSAIDs use is acute kidney injury. In comparison, the common hypersensitivity problems are asthma, urticaria, and angioedema. Adverse drug reactions can be prevented or treated by lowering the dose, reducing the duration of treatment, adding companion drugs, or changing the type of NSAID. In conclusion, it can be seen that ibuprofen severely caused kidney problems and hypersensitivity. On the other hand, diclofenac caused digestive issues, and rofecoxib caused cardiovascular problems.

Keywords: *NSAIDs, ADR, side effects, hypersensitivity*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) work by inhibiting the Cyclooxygenase (COX) enzymes [(1)]. COX-1 is responsible for platelet aggregation, renal artery vasodilation, and gastric mucosal protection beneficial to the body, while COX-2 is an enzyme that will increase during the inflammatory process[(2)].

Adverse Drug Reactions (ADR) can arise due to the body's response to a harmful and undesirable drug. Adverse Drug Reaction occurs at doses usually used in humans to prevent, diagnose, and treat disease or modify physiological functions [(3)]. ADRs are commonly categorized into two types, which are type A and type B, based on their respective mechanism. Type A ADR is the most common (85-90%) and predictable, resulting from high doses or long-term use. Meanwhile, Type B ADR occurs as much as 10-15% in ADR cases and is an idiosyncratic reaction [(4)].

The extensive use of NSAIDs causes many problems, including gastrointestinal diseases (ranging from

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dyspepsia to ulcers), kidney, cardiovascular disorders, and hypersensitivity problems [1, 2, 5]. The prevalence of ADR due to NSAIDs is 22%, with a considerable risk of gastrointestinal diseases 1-5%, cardiovascular 7.2%, renal toxicities 1-5%, and hypersensitivity 0.6-5.7% [6-9].

The literature search was carried out to update data about the NSAID class of drugs that commonly cause hospitals' problems globally. The mechanism of side effects, prevalence, and treatment or prevention of ADR due to NSAIDs are written in this review.

Methods

The literature search was performed using the PubMed database with the keywords 'NSAID', 'adverse drug reaction', 'hypersensitivity', and 'mechanism of action'. A total of 1008 studies were obtained in the initial search. Review articles, non-human studies, non-English studies, unrelated studies (genetic or pharmacogenomics), studies with no information related to the drugs used, and studies assessing the efficacy of NSAIDs with other anti-inflammatory drugs were excluded. Only 24 studies focusing on NSAIDs and the adverse drug reactions met the criteria and were included in the review.

Results and Discussion

General Classification of Adverse Drugs Reactions (ADRs)

Adverse Drug Reaction (ADR) is an unwanted and potentially dangerous response that may arise after consuming a drug or a combination of medications in regular doses [10]. The classification of ADR reactions is very complicated. In general, there are approximately five types of responses. ADR type A reaction is the most common reaction; this reaction is caused by a dose and tends to be predictable, which arises due to the pharmacological response. Type B ADR reaction is a rare but unpredictable reaction. Hypersensitivity reactions are included in type B because they are idiosyncratic and depend on each person. The other types of reactions (C, D, and E) are not caused by mechanisms but more likely to be clinical manifestations. Type C reaction is closely related to dose and time of administration. In contrast, type D reaction is commonly associated with the delayed response, so it is difficult to be diagnosed. Type E reaction is a reaction due to the cessation of drug use. However, type A and type B reactions are the most commonly acknowledged in the clinical setting and have been used for a long time [10, 11]. This review is focused on type A and B reactions.

Table 1. Article Review Results

No	Sites of Reaction	Types of Reaction	Name of drugs	Prevalence (%)	Ref
1.	Gastrointestinal	Peptic ulceration and unspecified gastrointestinal bleeding site	Diclofenac Loxoprofen Celecoxib Mefenamic acid	2.05 1.72 1.66 1.51	(12)
		Colorectal cancer	Aspirin	0.88	(13)
		Stomach cancer	Aspirin	0.14	
		Pancreas cancer	Aspirin	0.14	
		Small bowel injury	Celecoxib	N/A	(14)
		Gastrointestinal bleeding	Etoricoxib	N/A	(15)
		Small bowel lesion (erythema, erosions, ulcers, edema)	Diclofenac	N/A	
		Esophagitis and gastric/duodenal ulcers	Aspirin	N/A	

Cont... **Table 1. Article Review Results**

2.	Cardiovascular	Stroke	Ibuprofen Rofecoxib Celecoxib Diclofenac	3.36 1.14 0.87 0.58	(8, 16, 17)
		Myocardial infarction	Lumiracoxib Rofecoxib Celecoxib Diclofenac	2 0.89 0.86 0.54	
		Cardiovascular death	Rofecoxib Celecoxib	0.86 0.85	
		Acute Myocardial Infarction (AMI)	Ketorolac Indomethacin Diclofenac Rofecoxib Etoricoxib	2.31 1.66 1.33 1.33 1.32	
		Cardiovascular death	Etoricoxib Diclofenac	4.07 3.98	
3.	Renal Injury	Acute Kidney Injury (AKI)	Ibuprofen Naproxen Diclofenac 25 mg with acetaminophen Ketorolac Combination of ibuprofen and naproxen Combination of ibuprofen and ketorolac Rofecoxib	67 11 7.7 7 7 7 N/A	(18-22)

Cont... **Table 1. Article Review Results**

4.	Hypersensitivity (Respiratory)	NSAID-exacerbated respiratory disease (NERD)	Ibuprofen Acetaminophen Mefenamic acid Diclofenac Aspirin Naproxen	60 59 40 20 20 20	(23-27)
		Asthma	Aspirin Mefenamic acid Diclofenac Ibuprofen	50 20 20 10	
		Nasal rhinorrhea, wheezing	Ibuprofen	N/A	
		Nasal Polyps	Aspirin	9.7	
		Rhinitis	Aspirin	8.7	
	Hypersensitivity (Skin)	Urticaria	Ibuprofen Aspirin Metamizole	50 15 10	(28-33)
		Angioedema	Ibuprofen Aspirin Metamizole	37 20 10	
		Maculopapular eruption	Celecoxib	N/A	
		Exanthema	Ibuprofen	4.6	
		NSAID-exacerbated cutaneous disease (NECD)	Ibuprofen Diclofenac Acetylsalicylic acid Ketoprofen	24 15 14 8	

Note: Prevalence is expressed in percentage (%) and sorted from highest to smallest, which causes ADR

N/A: Not available

Type A Adverse Drugs Reactions and Its Clinical Manifestations

Type A was a reaction caused by most ADRs (85-90%). Usually, this type was predictable and could arise due to high doses or long-term use [(4)]. Type A reactions can be divided into three sites of reactions: gastrointestinal, cardiovascular, and renal injury due to reactions that arise due to the pharmacological effects of the drugs[(4, 5)].

In 2003, a study showed that NSAIDs such as naproxen and indomethacin could cause small intestinal injury [(33)]. In 2007, COX-2-specific NSAIDs such as celecoxib and rofecoxib were considered to increase the incidence of myocardial infarction and heart failure [(34)]. Aspirin, ibuprofen, ketorolac can cause interstitial nephritis, nephrotic syndrome, and papillary necrosis; the effects on the kidneys are generally caused by dosage, type of drug, and duration of use [(35)].

a. Gastrointestinal toxicities

According to Lai [(12)], problems related to gastrointestinal problems occurred due to the use of diclofenac (2.05%), celecoxib (1.66%), loxoprofen

(1.72%) (in Japan and Korea), and mefenamic acid (1.51%). In the use of aspirin, 1.4% could cause several types of gastrointestinal cancer during the treatment period. Common cancers were colorectal (0.88%), stomach (0.14%) and pancreas (0.14%). Meanwhile, the use of nonselective NSAIDs of 0.61% could cause colorectal (0.36%), pancreas (0.09%) and gastric (0.05%) cancers. On the other hand, COX-2 selective NSAIDs of 0.56% could develop several types of gastrointestinal cancer, especially colorectal (0.33%), pancreas (0.07%), stomach (0.04%), and oesophageal (0.04%) cancers [13].

The well-known mechanism regarding NSAIDs causing gastrointestinal toxicity was suppressing COX enzyme activity, where non-selective NSAIDs could induce injury to the gastric mucosa due to topical erosive effects combined with systemic effects characterized by the depletion of prostaglandins synthesized by COX-1 [5, 36].

b. Cardiovascular Disorder

The occurrence of cardiovascular-related NSAIDs included heart failure, myocardial infarction, ischemic stroke, and cardiac death. Of all patients who received treatment with NSAIDs, 7.2% experienced cardiovascular events during the treatment period. The use of ketorolac was also identified as increasing cases of acute myocardial infarction (AMI), followed by indomethacin, etoricoxib, rofecoxib, diclofenac [8, 16].

The increasing cardiovascular risk was demonstrated by COX-2 selective NSAID. Rofecoxib was often associated with the incidence of myocardial infarction, ibuprofen, and diclofenac for the risk of stroke, and etoricoxib and diclofenac for the risk of death from cardiovascular (myocardial infarction, arrhythmia, pulmonary embolism, and stroke) [17, 37].

COX-2 selective NSAIDs inhibited vascular prostacyclin synthesis (PGI2), where PGI2 functioned as an anti-platelet aggregation with vasodilator properties. PGI2 was a protective mediator for the cardiovascular system, which worked through IP receptors, and was expressed in various cell types. The increased risk was caused by a decrease in the formation of PGI2. However, this risk could be reduced by inhibiting COX-1 since COX-1 had an activity in the formation of thromboxane A2 (TXA2), which caused vasoconstriction[5].

The combination of these two effects could lead to a "pro-thrombotic state" with a significant risk of developing myocardial infarction or stroke. However, except for naproxen, none of the non-selective NSAIDs (apart from aspirin) could affect COX-1 platelets in a significant way needed for the inhibitory effect of platelets [5, 38]. In terms of cardiovascular safety, naproxen, and ibuprofen at the lowest effective dose were the best first choice with the lowest risk [8, 39-41].

c. Renal toxicity

In the event of kidney toxicity caused by NSAIDs, drugs with a high level of toxicity were ibuprofen (67%), naproxen (11%), ketorolac (7%), the combination of ibuprofen and naproxen (7%), and ibuprofen and ketorolac (7%). The combination of diclofenac with acetaminophen was also known to induce Acute Kidney Injury (AKI) occurrence, especially for patients with accompanying diseases such as liver cirrhosis or hypertension. COX-2 selective NSAID, rofecoxib, also showed an increased risk for AKI, although according to studies, selective NSAIDs showed a smaller risk compared to nonselective [18-20].

All NSAIDs could change kidney function by inhibiting COX-1 (which regulated renal hemodynamics and glomerular filtration) and/or COX-2 (which mediated the excretion of salt and water) expressed in the kidney [18, 42]. Although rare, NSAIDs could cause nephropathy due to high doses of some drugs. It was found that indomethacin was associated with more cases of kidney failure [37, 43].

Type B Adverse Drugs Reactions and Its Clinical Manifestations

Type B occurred as much as 10-15% in ADR cases. The reaction was unpredictable, was not dose-dependent, and only appeared in certain patients. Type B reactions were divided into several classifications of causes: excessive sensitivity to drug toxicity, idiosyncratic drug reactions, and hypersensitivity reactions, both nonimmunological and immunological [4].

Nonimmunological immune responses to NSAIDs (cross-reactive) were indicated by hypersensitivity reactions to NSAID-exacerbated respiratory disease (NERD), NSAID-exacerbated cutaneous disease

(NECD), and NSAID-induced urticarial/angioedema (NIUA). For immunological responses (non-cross-reactive), hypersensitivity reactions that might arise were single-NSAID-induced urticaria/angioedema or anaphylaxis (SNIUAA) and single-NSAID-induced delayed HS reaction (SNIDR) (24–48 hours after drug entry) [44].

In 2004, it was found that the leading cause of NSAID hypersensitivity was caused by the use of aspirin or nonselective NSAIDs. In contrast, skin reactions are commonly urticaria or angioedema with the similar use of drugs [45].

a. Respiratory Disease

NSAID-exacerbated respiratory disease (NERD) events were generally caused by ibuprofen (60%); mefenamic acid (40%); diclofenac (20%); acetylsalicylic acid (20%); and naproxen (20%). In Europe, the highest causes were acetylsalicylic acid (82%) and pyrazolones (9%), while in America, the highest causes were acetylsalicylic acid (80%) and ibuprofen (41%). Other studies showed that ibuprofen accounted for 63.8% and was followed by acetaminophen (59%). NSAID-exacerbated respiratory disease (NERD) was present in the form of respiratory symptoms such as asthma, rhinitis, nasal polyps, mild chest tightness, and nasal congestion [23, 24].

The suppression mechanism of COX-1 production influenced the reduction of prostaglandin E2 (PGE2) biosynthesis from arachidonic acid. The induction of COX-2 isoenzymes did not compensate for the lack of this anti-inflammatory mediator. The inflammatory process was more striking when COX inhibitors were used, and the respiratory tract's inflammation would persist even if the drug were no longer used [46-50].

b. Skin Eruption

Skin-related cases, both angioedema and urticaria were mostly induced by ibuprofen (43.5%), aspirin (17.5%), metamizole (10%). In another study sequencing from the most to the least frequently induced, there was ibuprofen (24%); diclofenac (15%); acetylsalicylic acid (14%); ketoprofen (8%). On the other hand, the reaction with a combination of two different NSAIDs showed smaller results. NSAID-exacerbated cutaneous disease (NECD) was indicated by the emergence of urticaria and angioedema (41.4%) and urticaria (28.5%), which could occur in a matter of hours. Skin lesions were commonly found on the face (eyes and lips). On the other hand, the delayed reaction would occur more than 24 hours after exposure. It would develop into specific skin diseases (exanthema, fixed drug eruption, toxic epidermal necrolysis, or Stevens-Johnsons syndrome) [9, 27, 28].

The NECD induction mechanism was the same as NERD because both of them resulted from hypersensitivity reactions. The use of COX-2 selective NSAIDs (celecoxib and etoricoxib) was recommended for patients with this hypersensitivity. Hypersensitivity events were rarely found when using COX-2 selective NSAIDs, but prior testing was needed to ensure tolerability [28, 51, 52].

Management of NSAIDs' Adverse Drugs Reaction in Clinical Settings

It is known that an adverse drug reaction (ADR) is an unexpected reaction from a treatment. Physicians or pharmacists commonly recommend changing the drugs with a similar group or the same mechanism or actions. However, it cannot be applied in general because not all medicines can replace each other. Each drug has its levels of efficacy, toxicity, and side effects [53, 54]. In the case of ADR, because of NSAIDs, there are recommended actions that could be applied to prevent or treated ADRs (Table 2).

Table 2. Management of NSAID Induced Adverse Drug Reaction

No	Type of Reaction	Reaction	Management	Ref
1.	Type A	Gastrointestinal symptoms (nausea, vomiting, abdominal pain)	Oral or intravenous H2 blockers or antiemetics or PPI	(55, 56)
		Small bowel injury	Conversion from nonselective NSAIDs to selective like celecoxib	(57)
		Acute Myocardial Infarction	Combination of naproxen and ibuprofen in low effective dose	(41)
		Acute kidney injury	Lowering dose	(58)
2.	Type B	AERD	Low salicylate diet	(59)
		Nasal symptoms, such as congestion	Topical nasal decongestants or corticosteroids, oral or nasal antihistamines	(55)
		Bronchospasm	short-acting bronchodilator	
		Urticaria/ angioedema	Addition of antihistamines and leukotriene antagonists	(60)

AERD: Aspirin Exacerbated Respiratory Disease

In type A reaction, it is recommended to reduce the dose or withhold the drug administration until the response disappears [61, 62]. In terms of gastrointestinal symptoms (nausea, vomiting, and abdominal pain), it is suggested to use H2 Blocker or antiemetics to prevent adverse reactions. Guidelines recommend various strategies to avoid problems in those at risk for NSAID-associated GI complications [55, 61]. Patients without gastrointestinal risk factors could use nonselective NSAIDs. On the other hand, patients with gastrointestinal risk could only receive nonselective NSAIDs with PPI. For patients with high gastrointestinal risk factors that needed NSAIDs, including those who had had previous bleeding ulcers or with concomitant anticoagulant therapy, COX-2 plus PPI inhibitors provided the most promising gastrointestinal protection[39, 56].

A recent study found that the use of histamine type-2 receptor antagonists (H2RAs) could prevent GI adverse events in NSAID users. It was recently proved during clinical trials, the use of ibuprofen/famotidine combination could reduce the risk of ulcers by 50% compared with ibuprofen alone [63].

It is recommended to use specific NSAIDs in cardiovascular and kidney disorders such as naproxen and ibuprofen in low doses [37, 41]. Some studies showed that cardiovascular risk has a strong association with cyclooxygenase (COX)-2 selectivity. The low COX-2

selectivity of naproxen results in a lower cardiovascular risk than other NSAIDs [64, 65].

For type B reaction, the identified management was specific for Aspirin Exacerbated Respiratory Disease (AERD), nasal symptoms, bronchospasm, and urticaria. To prevent AERD, patients should limit salicylate use, and it should be closely monitored [59]. To avoid respiratory disorder, especially in the nasal symptoms, topical nasal decongestants or corticosteroids, oral or nasal antihistamines should be administered. A study conducted by Braido [66] showed that inhaled steroids and antihistamine are the primary tools in allergies, rhinitis, and asthma, which are more safe and effective drugs are. A short-acting bronchodilator is recommended to prevent bronchospasm for high-risk patients when they use NSAID [57].

Some studies showed that patients could experience urticaria only after the intake of NSAID. However, if the patients have to use NSAIDs, short-term antihistamines with or without steroids should be administered to prevent exacerbation or control acute urticaria [60, 67]. On the other hand, a recent study revealed that the use of antileukotrienes is more effective in primary cold urticaria delayed pressure urticaria [68].

Conclusion

ADR risks due to the use of NSAIDs were mostly caused by ibuprofen with side effects of kidney problems and hypersensitivity to both breathing and skin, followed by diclofenac with gastrointestinal issues, and rofecoxib, which was not even recommended for patients with cardiovascular problems because it could increase the severity of the disease. Adverse drug reactions could be overcome or prevented by lowering the dose, reducing the duration of treatment, or changing the type of NSAID used.

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To Assess the Effectiveness of Planned Teaching on Knowledge Regarding First Aid Measures for Selected Minor Injuries among Anganwadi Workers

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Abstract

Background: First aid is the temporary and immediate care given to the person who is injured or suddenly become ill. First aid can save a victim's life especially if the victim is bleeding heavily and has stopped breathing it also prevents additional medical emergencies that can arise from an injury or illness. Anganwadi worker have an important role to play in child protection. Their role can only be properly carried out, if teachers are equipped with the relevant skills. Appropriate in servicers training in children protection for teacher is of key importance is both emphasizing child protecting issues and in promoting these skills in teachers. **Method**: Pre-experimental one group pre-test and post –test research design is used .Non-probability convenience sampling is used . Sample size are 100 Anganwadi Workers. Tools are Structured questionnaire for assessing the knowledge. **Results:** 8% of the Anganwadi workers in pretest had average level of knowledge score, 51% in pre test had good, 38% in pre test and 13% in post test had very good and 3% in pre test and 87% in post test had excellent level of knowledge score. the planned teaching programme on overall knowledge regarding first aid measures for selected minor injury among Anganwadi workers in Anganwadi centre was effective **Conclusion**: Planned teaching significantly brought out their improvement in the knowledge regarding first aid measure for selected minor injuries among Anganwadi workers. Analysis of the data shows that there was significant difference between pre test and post test knowledge.

Keywords: *First aid measures, Minor injuries, Anganwadi workers.*

Introduction

Now a day injuries are very common and it can occur at any point of time in our day to day life. Among them, injuries in school children have more possibilities of minor injury. The most frequent causes of school related injuries requiring hospitalization such as falls and sports activities. Playground equipment related injuries occur on school playground during school hours and these require adequate supervision.[1]

First Aid essentially uses simple techniques involving a basic knowledge, good practice of the principles of life preservation. It does not require sophisticated equipment or a great knowledge of medical matters. It is often as important to know what not to do as it is to know what should be done. The objectives of First Aid is to offer assistance to anyone injured or ill before expert help from a doctor or nurse is available, or before an ambulance arrives[2]

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International federation of Red Cross and Red Cross crescent societies consists of 97 million volunteer and member the international federation is the world largest humanization networks .everywhere one of its 178 members, red cross and red crescent societies is engaged in first aid programs either training first aider to save life in disaster of all kind of training the general public in basic life saving techniques and providing first aid services during sport and mass events[3]

Anganwadi worker have an important role to play in child protection. Their role can only be properly carried out, if teachers are equipped with the relevant skills. Appropriate in servicers training in children protection for teacher is of key importance is both emphasizing child protecting issues and in promoting these skills in teacher[4]

Assessment of knowledge of Anganwadi teachers regarding first aid measure will help the nursing personnel to plan and organized health education training programmes to enhance teacher's awareness. As Anganwadi teacher play pivotal role in taking care of children during emergency .the current information will definitely enhance the teacher knowledge and they can utilize it throughout their life time.

Objectives:

1. To assess the existing knowledge of Anganwadi workers regarding First aid measures.

2. To evaluate the effectiveness of planned teaching regarding selected first aid measure.

3. To associate the post test knowledge score with the selected demographic variable.

Assumption:

Anganwadi worker may have some knowledge regarding first aid measure for selected minor injuries in children

Hypothesis:

H_0: There will be no significant .knowledge regarding first aid measures for minor injuries among Aanganwadi worker with selected demographic variable

H_1: There will be significant knowledge regarding first aid measures for minor injuries among Anganwadi worker with selected demographic variable

Material and Method: This study was based on Quasi experimental one group pre-test and post – test research design**.** The population was Anganwadi Workers . In this study, 100 Anganwadi Workers are included**.**

Inclusion Criteria

· Anganwadi worker is present at the time of the study.

· Anganwadi worker who are willing to participate in the study.

Exclusion Critaria:

· Anganwadi worker who are not participated in such studies.

Anganwadi worker who are not present at the time of data collection

A structure questionnaire consists of two section i.e. Section I and section II.

Section I-

It is consisting of demographic characteristics regarding Anganwadi worker i.e. age, marital status, monthly income, religion, residential area , education.

Section II-

It is consisting of 25 multiple choice questions to assess the knowledge regarding first aid measure for selected minor injury in Anganwadi workers.

Results

· Distribution of subjects according to their age in years reveals that 12% were from in the age group of 20-30 years, 55% sample s from age group of 30-40 years, 27% samples from age group of 40-50 year and 6% sample from age group of 50 year above.

· Distribution of samples according to marital status shows that majority 70% of samples were married ,18% sample were unmarried, remaining sample were widow.

· Distribution of sample according to their educational qualification shows that 26% sample educated up to high school 50% samples where HSC pass 40% are holding in education and 10% having degree in education.

Distribution of sample according to their Monthly income 34% of the Anganwadi worker had income between Rs.2000-4000And 66% had income between 4000-6000Rs. Respectively.

· Distribution of samples according to their religion shows that the majority 70% were hindu,2%of sample were muslim,24%sample were Buddhist and 4%were other.

· Distribution of the sample according to residential area show that majority 60% of sample belonged to urban area and 34% sample belonged to rural area.

· Difference between pre test and post score knowledge regarding first aid measure for selected minor injuries showed that there was significant difference between pretest knowledge score and post test knowledge score of Anganwadi worker regarding first aid measure for selected minor injuries as t- value was22.04 and p- value 0.001 where $p<0.05$.Thus result shows that the planned teaching was effected in enhancing the knowledge of first aid measure for selected minor injuries among Anganwadi workers.

· In association of knowledge score in relation to demographic variable the result showed that there was no significant difference of knowledge scores in relation to the demographic variable such as age, marital status, education, residential area, monthly income. From the above finding it was concluded that no demographic variable had relation with pre test and post knowledge.

Discussion

Martin A Carrie, Red cross and U.S. Department of Education, was conducted a training program on first aid among teacher, Anganwadi worker and school student. Training program included instruction on how to handle unconscious victims and how to administer the proper bandage and splints etc[5].

Finding were also supported by A.J et al (2004) who had conducted a study to assess the knowledge of first aid practices among 654 Parents in USA, the study result showed that mean age (SD) was 38.5(13.8),56%were fememal,56% had at least a high school education. None of them answered all questions correctly with roughly half being answered all question correctly with roughly half being familiar with 60% of the question and it was concluded that knowledge was unaffected by age, gender and education[6].

Angela et al (2005) conducted a study by collecting data from 506 individuals to examine the self-perceived first aid abilities after Northridge earthquake. It is suggested that first aid training should be directed towards targets segments of population that they are not likely to have had instruction in these basic skills[7]

A study on parent and caregivers, theoretical and practical knowledge of first aid in case of accident and minor injuries in children in polish. They asked a group of 93 parents and tutors (11 men and 82 women) to complete a questionnaire containing 20 questions. The results revealed that, a great extent of self satisfaction in the questioned groups of parents, most of the (64.5%) think they know first aid rules , although only 35 people (37.6%) were ever trained in the field. Most parent (71%) have already trained minor injuries and dealt with minor accidents, which took place while they take care of child[8]

Nursing Implication:-

Nursing Practice

Nurses working in the clinical practice can teach the first aid measure to the patient of various common injuries. Community health nurse can teach people regarding various common injuries and first aid management.

Nursing Education

Injuries are the very common among all age groups. If the person has knowledge about the first aid he can minimize the morbidity and also prevent further consequences. First aid can be easily learned and mastered by practice. More theoretical and practical aspect should be included in the curriculum.

Nursing research

Almost everybody had come across with different kind of injuries in life. There is great need for research in the area of prevention of disability with effective first aid measures. This study had highlighted the knowledge of first aid among the Anganwadi workers and provided knowledge on first aid measure for selected minor injuries.

Recommendation

On the basis of the finding of the study, it is recommended that the following studies can be conducted.

1. A similar study may be conducted on a larger population for generalization of findings.

2. Studies may be conducted to evaluate the effectiveness of information booklet versus other method of teaching on first aid measure for selected minor injuries.

3. A study may be conducted to assess the existing knowledge and practice of Anganwadi workers in relation to applying the first aid measure

Conclusion

Planned teaching significantly brought out their improvement in the knowledge regarding first aid measure for selected minor injuries among Anganwadi workers. Analysis of the data shows that there was significant difference between pre test and post test knowledge.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: The study was approved by the Ethics committee of the institute.

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A Study to Assess the Effectiveness of Self Instructional Module on Knowledge Regarding Management of Selected Minor Ailments of Preschool Children among Anganwadi Workers

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Abstract

Background: Child healthy development is an investment in the growth and prosperity of the country. As an opportunity for the future, the value of maintaining child safety can not be overlooked. Most countries' advances have yielded nothing because this dimension was over-looked. An active and successful population cannot develop from unhealthy children chronically affected by recurrent minor ailments.**Objectives:**1. To assess the knowledge regarding management of selected minor ailments of preschool children among Anganwadi Workers.2. To assess the effectiveness of self instructional module on knowledge regarding management of selected minor ailments of preschool children among Anganwadi Workers.3. To associate the post test knowledge score with selected demographic variables. **Materials and Method:** This research was based on the design of Pre experimental one-group pre-test post-test study. This research examined 100 anganwadi workers. This research has attempted at various centers in Anganwadi. The sampling technique used in this study was a sampling technique intended for non probability. The data were obtained using structured questionnaire**. Results:** In the post-test 1(1 percent) of the women had very good knowledge and only 99(99 percent) of them had excellent knowledge score. The minimum score in the posttest was 20 and the highest score was 25, the mean score for the posttest was 24.05±0.892 with an average percentage score of 96.2 percent. **Conclusion**: The results of this study indicate that the post-test workers at Anganwadi had excellent knowledge. The post-test score was significantly higher at 0.05 than the pre-test score. Thus it was hypothesized that SIM as a teaching tool was found to be effective in addressing selected minor ailments.

Keywords: *Self-instructional module, information, management of minor illnesses, staff at Anganwadi*

Introduction

Kids in preschool are vulnerable to different mild illnesses. About 3/4th children are considered unhealthy and are surviving due to poor health status with impairment of physical and intellectual functions. Early detection and prediction of these minor ailments can prevent disability and impairment. This tends to reduce morbidity and mortality, due in particular to severe dehydration caused by diarrhoea and other minor ailment complications[1]

The word anganwadi in Hindi means "courtyard or refuge." It was inaugurated in 1975 mostly by Indian government as part of the Integrated Child Development Services program to fight child hunger and malnutrition. A typical anganwadi center also provides basic health care in Indian villages. It is a part of the public health-care system in India. Specific activities in health care include consultation and contraception delivery, nutritional awareness and supplements, and pre-school activities[2]

The anganwadi staff are local and voluntary staff who are provided three months of training to prevent and cure such diseases, providing supplementary nutrition, immunization, and providing pre-school mothers with health education. Former Indian prime minister Smt.

At the World Health Assembly Indira Gandhi stressed that programs must continue where people is or where problems arise.The child is a National Development and Productivity investment. We can not disregard the value of preserving child health as a potential investment. As anganwadi children form an important and significant population group, they are the future citizens and assets for their families and nation[5]

For non-formal education the Children of age group 3 to 4 years go to anganwadi. This age group symbolizes around 24.9 per cent of the children's population. This is a physical through and evolving time. Early disease detection is performed within a regulated population group[5]

Children spend much of the day in the anganwadi, and are vulnerable to different health issues. The specific acute and chronic conditions that can be experienced during the anganwadi period are fever, common cold and cough, diarrhoea and aspiration to foreign bodies[6]

Around 10.5 million children in the world's developed countries die each year before their fifth birthday. A significant proportion of deaths occur in the first year. Acute respiratory tract infections, diarrhoea, fever , malaria and common cold and cough are responsible for nearly 70 percent of these deaths. Any of those five disorders triggers at least three out of four cases of childhood illness.

Because of deprivation, inadequate access or insufficient information about the danger from sickness, a substantial number of sick children do not obtain access to the health facility7.

Minor conditions are one of the most important factors for increasing patient attendance at the outpatient pediatric clinic and overloading the clinic 's job. Minor ailments such as diarrhoea , dysentery, common cold, cough , fever, worm infestation and scabies can be treated by auxiliaries as per the institution's standing orders and do not require a person specially trained[8]

Minor ailments account for 60-70 percent of clinic visit. A review of what and where services can be done for minor ailments is required not through future research but by using health resources. Someone between the doctors and nurse can take active part in care of minor ailments.Good education and promotion of health is needed to minimize the demand for treatment of minor ailments. Common cold and flu, fever, cough and sore throat, head lice, indigestion, diarrhoea and constipation are just a few examples of minor ailments. Cold and cough, flu that children sometimes get is not particularly serious and can be managed[8]

Good education and promotion of health is needed to minimize the demand for treatment of minor ailments. Common cold and flu, fever, cough and sore throat, head lice, indigestion, diarrhoea and constipation are just a few examples of minor ailments. Cold and cough, flu that children sometimes get is not particularly serious and can be managed[8] Since anganwadi workers are an influential member of the community, they build a bridge between children , parents and also those participating in anganwadi children's health care.

Objectives:

1. To assess the knowledge regarding management of selected minor ailments of preschool children among Anganwadi Workers.

2. To assess the effectiveness of self-instructional module on knowledge regarding management of selected minor ailments of preschool children among Anganwadi Workers.

3. To associate the post test knowledge score with selected demographic variables.

Assumptions: The study assumes that anganwadi staff have little awareness about preschool children's minor ailments.

Hypothesis:

H_0: There will be no significant difference between pre-test and post-test knowledge score regarding management of selected minor ailments of preschool children among Anganwadi workers.

H_1: There will be significant difference between pre-test and post-test knowledge score regarding management of selected minor ailments of preschool children among Anganwadi workers.

Material And Method

This study was determined by the design of Pre experimental one-group pre-test post-test research. 100Anganwadi workers are included in this study. Criteria for inclusion: 1. Workers from Anganwadi happy to join. 2. Anganwadi personnel who can read or write Marathi Exclusion Criteria1) Anganwadi employees who attend the minor pre-school children's training program. Resources development: a structured questionnaire was used. Questionnaire method used to test awareness of pre-school children's specified mild illnesses management. This is consisting of two sections. Section I consisted of demographic features concerning Anganwadi workers.

The coefficient of reliability was calculated. It is assumed that the Questionnaires are accurate because the co-efficient is greater than 0.8. The questionnaire 's reliability co-efficient was 0.86, which was more than 0.8. The test was then considered valid.

The data was obtained over an average 20-day span. Samples were selected by purposive non-probability sampling, which was applicable during the study. The authority persons had obtained permission prior to collecting the data. And prior to starting the study, the informed consent of much of the participants was taken. Data is collected using questionnaires.

Results

Distribution by age of Anganwadi workers shows that 11 (11%) in 25-36 years, 15 (15%) in 31-35, 10 (10%) in 36-40 years, 16 (16%) in 41-45, 22 (22%) in 46-50 years, 15 (15%) in 51-55 years and 11 (11%) in 56-60 years. Distribution of anganwadi employees according to their survey education reveals that main is 31% (31%), secondary is 36% (36%) and graduation is 33% (33%). Their faith distribution shows that Hindu was 16 (16%), Muslim was 19 (19%), Buddhist was 20 (20%), Christians was 19 (19%) and others 26 (26%).

Distribution by residency shows that 40 (40%) came from urban areas and 60 (60%) were from rural areas. Distribution on the bases of their past experience shows that 100 (100%) had little experience of the management of identified minor illnesses among anganwadi employees and pre-school babies. The p-value measured in this analysis was 0.946, 0.125, 0.811 and 0.161 respectively for age, employment, ethnicity and location, which was more than the p-value (0.05), thereby demonstrating that there was little correlation between intelligence score and demographic variables.

Table no.1: Assessment of pretest knowledge regarding management of selected minor ailment of pre-school children among anganwadi worker. n = 100

Level of knowledge score	Score range	Percentage score	Pre Test	
			Frequency	Percentage
Poor	0-5	0-20%	2	2%
Average	6-10	21-40%	37	37%
Good	11-15	41-60%	61	61%
Very good	16-20	61-80%	0	0%
Excellent	21-25	81-100%	0	0%
Minimum score	5			
Maximum score	15			
Mean score	10.93± 2.257			
Mean %	43.72%			

Pre-test results show that 2(2%) of anganwadi workers had poor knowledge score, 37(37%) of them had average

knowledge score, but also 61(61%) had good knowledge score. The minimum score was 5 and the best total was 15, the total score was 10,93 ± 2,257 for a 43,72 percent overall score.

Table no. 2: Assessment of posttest knowledge score regarding management of selected minor ailment of pre-school children among anganwadi worker

n = 100

Level of knowledge score	Score range	Percentage score	Post Test	
			Frequency	Percentage
Poor	0-5	0-20%	0	0%
average	6-10	21-40%	0	0%
good	11-15	41-60%	0	0%
Very good	16-20	61-80%	1	1%
excellent	21-25	81-100%	99	99%
Minimum score	20			
Maximum score	25			
Mean score	24.05 ±0.892			
Mean %	96.2%			

Post-test confirms that 1(1%) of them had fairly better awareness score and 99 (99%) had excellent knowledge score. The minimum score was 20 and the highest score was 25, the average score was 24.05± 0.892 and the average score was 96.2%.

TABLE NO. 3. Percentage wise distribution of effectiveness of self-instructional module on knowledge regarding management of selected minor ailment of pre-school children among anganwadi worker

n=100

Tests	Mean	SD	't'-value	Degree of freedom	p-value	Significant
Pre Test	10.93	2.257	55.039	99	0.000	S, p<0.05
Post Test	24.03	0.892				

Analysis of pre-test post-test assessments reveals that there is a significant discrepancy between pre-test and post-test performance scores understanding successful self-instruction module on awareness of pre-school child management among anganwadi personnel. The mean pre-test value is 10.93 and the post-test value is 24.03 and the normal pre-test variance is 2.257 and the post-test value is 0.892. T-value estimated is 55.039, and p-value is 0.000. It is therefore statistically interpreted that the self-instruction module on knowing about the management of selected minor ailment among anganwadi pre-school children has been successful.

Thus, H_1 is accepted and H_0 is rejected.

Discussion

A comparative study was conducted to assess rural and urban anganwadi workers' awareness and attitude regarding selected minor conditions of pre-school children in the Ooty region. The evaluation was carried using concise comparative study design with the survey of 25 rural anganwadi workers and 25 urban anganwadi participants to test the awareness and attitude of rural and urban anganwadi workers in the Ooty region on identified minor conditions of pre-school children. The results indicate that the awareness and attitudes of rural and urban anganwadi staff members varied significantly[9]

A cross sectional research was carried out to determine the information of anganwadi workers in the district of Hooghly, West Bengal. The study was conducted by interviewing anganwadi workers and observing their own skills in the job. The period of study in the Hooghly district ICDS project was 1989-1999. Knowledge of anganwadi personnel was tested on various ICDS-related components.Results showed that fever could be identified by 11.8 per cent anganwadi staff, 91.2 per cent knew how to prepare oral rehydration solution and 74.9 per cent knew when to discard the solution. While anganwadi workers' knowledge about major activity was mostly adequate, they did not have adequate knowledge of some of the minor ailments such as fever. Therefore their expertise and skills need to be strengthened in many areas[10]

In Palakode, Dharmapuri district, Tamilnadu, a concise cross-sectional survey method was used to evaluate the anganwadi staff 's comprehension and attitude concerning care for children under five. Data were collected from eighty anganwadi employees using convenient sampling technique which was not likely to be used.The findings showed that 60.15% of anganwadi workers had good awareness and 89% of anganwadi workers had a positive attitude towards deworming children every six months, 70% of anganwadi workers had a positive attitude towards diarrhoeal disease,In the growth chart, 78 percent of workers had a positive attitude to the history of disease, immunization and diet, while 49 percent of workers had a negative attitude to the growth chart that it is useful[11]

A concise, comparative research was conducted to develop the awareness of caregivers in pre-school children about fever control. A convenience survey of 86 caretakers was performed at the study. We gathered data from 50 caretakers that can provide follow-up. The results were obtained using an open-ended questionnaire created by an investigator. Tests showed that caretaker awareness of the intensity of fever at which treatment was delivered was not drastically increased (chi-squared analysis,.012; df, 1; $p = 0.91$). Enhanced efficiency of the antipyretic medicine dose (chi-squared study, 13.13; df, 6; $p = 0.04$)

An ICDS programme, Gorakhpur, undertook a cross-sectional and longitudinal follow-up analysis to investigate the function of anganwadi staff in disability detection and prevention in children under the age of six. Results showed that among 1545 infants, anganwadi staff reported impairment in 118 cases in 126 topics checked by pediatricians[13]

Those studies support the current study.

A number of studies in this area have been checked with direct or indirect results among Anganwadi Employees on minor conditions of pre-school children[14-17]

Recommendations:

• A related analysis can be carried out for the generalization of the findings on a larger population.

• Research can be carried out to determine the efficacy of the information booklet over other teaching module approaches for distributing the knowledge to the population

•A analysis can be carried out to determine the current expertise and activities of anganwadi staff in the treatment of acute and chronic illnesses

• In hospitals, a structured learning system can be used to allow all nurses to engage in improving awareness of the management of minor ailments.

Conclusion

The outcome of this analysis indicates that 100 percent of anganwadi staff have outstanding post-test knowledge. To determine the efficacy of the 't'

self-instruction module test was introduced and t was calculated, the post-test value was substantially higher at 0.05 than the pre-test score. Therefore it was inferred that SIM was found to be successful as a teaching technique on the management of minor ailments.

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Ethical Clearance: The study was approved by the Ethics committee of the institute.

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Potential Stem Cells of Oral Origin- A review on Induced Pluripotent Stem Cell Technology

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Abstract

Mesenchymal stem cells are isolated from multiple tissues, such as bone marrow, peripheral blood, umbilical cord blood, adult connective tissue, dental tissues, placenta, and amniotic membrane. Oral derived mesenchymal stem cells (OMSC's) are multipotent cells, though differentiates into odontoblast, cementoblast, osteoblast, chondrocyte, adipocyte, melanocyte, endothelial cell hepatocyte, myoblast and neural cell but shows limited differentiating into only these specific types of cells or mesenchymal origin cells. Induced pluripotent stem cells (iPSC's) are the potential stem cells induced through reprogramming such somatic cells genetically by presenting with distinct or causative factors. Growth factors and signalling molecules have the capacity to induce cellular proliferation and cellular differentiation. Addition of transcription factors to a population of differentiated cells does not promise reprogramming of the potential stem cells. Furthermore, ethical issues related with the development of embryonic stem cells (ESC's) do not apply to iPSCs, which offer a non-controversial strategy to create patient-specific stem cell lines. Hence the present review article was aimed to briefly describe numerous oral mesenchymal stem cells (OMSC's) and role of Induced potential pluripotent stem cells (iPSCs) in the regenerative medicine and dentistry with a focus on various iPSCs reprogramming techniques in the current scenario.

Keywords: *Mesenchymal Stem Cells, Nuclear Reprogramming, Osteogenic Potential, Retroviruses, Somatic Cells, Transcription Factors.*

Introduction

Stem cells are the "precursor or predecessor" clonogenic cells potential for both self-regeneration and multi-lineage differentiation [1]. The term "stem cell" was presented for the first time by German biologist Haeckel in 1868 [2]. Later Wilson established the term "stem cell" [3]. In 1909 at congress of hematologic society in Berlin, Russian histologist, Alexander Maksimov, hypothesized the scientific existence of hematopoietic stem cells [4]. Based on their source two main types of stem cells namely embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells) were identified. Embryonic stem cells were collected from early stage embryo (4-5days old) and are capable of evolving more than 200 cell types [5]. Stem cells are also classified on their abilities to differentiate as totipotent, pluripotent, or multipotent. Multipotent stem cells are adult stem cells which only generate specific lineages of cells. Pluripotent stem cells are those cells that can give rise to every single cell of an organism except its extra-embryonic tissues like placenta [6, 7].

An indistinguishable recent significant development has been the generation of direct conversion of somatic cells through introduction of four major genes namely

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Oct-4, Sox2, c-Myc and Klf4 to induced pluripotent stem (iPS) cells. iPS cells are the pluripotent stem cells induced through reprogramming somatic cells genetically by presenting with distinct or causative factors. Addition of transcription factors to a population of differentiated cells does not promise reprogramming of the potential stem cells [8]. The low efficiency of reprogramming in vitro proposes that additional rare events are necessary to generate iPSCs. iPS cells have been shown to be similar to ES cells in morphology, proliferation, histo and morphodifferentiation capacity, genetic and epigenetic conditions [9].

Methodology

A structured literature search for articles written in the English language in PubMed, MEDLINE, Embase, Google Scholar and Web of Science databases from 1980 to till date was retrieved by using MeSH terms "Potential stem cells", "stem cells in dentistry", "oral stem cells", "induced potential stem cells", "pluripotent oral stem cells", "mesenchymal stem cells" and "pulpal stem cells".

ORAL MESENCHYMAL STEM CELLS (OMSCs):

Mesenchymal stem (stromal) cells (MSCs) also called adult or somatic stem cells or postnatal stem cells harvested directly from adults. They are multipotent cells, only differentiating into limited types of cells or mesenchymal origin have been identified by Alexander Friedenstein as a population of nonhematopoietic cells in various tissues, including umbilical cord, bone marrow, adipose tissue, oral and perioral tissues and have been broadly studied for their safe and effective clinical applications [10, 11]. MSCs draw attention worldwide for their multi lineage differentiation potential into tissue developing cells having the ability to aid repair processes in damaged sites, but more prominently for their paracrine mediated regenerative effects with potential to modulate the host immune responses by production of anti-inflammatory or immunosuppressive factors [12]. Serum components in MSC cultures are crucial for effective cell expansion and efforts to substitute commercially available bovine or calf serum with cGMP alternatives such as autologous or allogeneic human sera or human platelet lysate (HPL), have faced practical difficulties in achieving large-scale expansion of MSCs, and also in achieving the laboratory requirements of these cell culture lines [13].

Teeth are non-invasive natural source of dental stem cells, which are simple, suitable and inexpensive to collect, hold potential for a range of therapeutic applications. In the field of dental research, various oral MSC sources have been recommended as highly favourable for tissue engineering (TE) applications in the orofacial region. Numerous research and number of case reports have been conducted to test the regenerative potential of oral MSCs [14]. Dental pulp stem cells (DPSCs) and alveolar bone marrow stem cells (aBMMSCs) were identified as highly potential and have been extensively studied over the decades where as other dental stem cells such as stem cell from exfoliated deciduous teeth (SHEDs), Stem cells from apical papilla (SCAPs), Periodontal ligament stem cells (PDLSCs), inflammatory periapical progenitor cells (iPAPCs) and salivary gland stem cells (SGSCs) were also identified but not extensively studied or tested for its clinical implications. Oral MSCs are used in various applications, including periodontal and peri-implant therapies, cleft lip and palate management and revitalization of immature permanent teeth with necrotic pulp [15, 16].

DENTAL PULP STEM CELL (DPSC's):

A potential Oral source of MSCs with great significance in regenerative dentistry is Dental Pulp Stem Cell (DPSCs). These are Mesenchymal type of stem cells presenting inside dental pulp. DPSCs have bone and cartilage forming potential in vitro and can differentiate into dentin, in vivo and also differentiate into dentin pulp- like complex. In recent times, immature dental pulp stem cells were identified which is a pluripotent sub-population of DPSC generated using dental pulp organ culture. These DPSC's cells also have been studied extensively in-vitro for its Adipogenic, chondrogenic, neurogenic, angiogenic and myogenic lineage differentiation potential. Various in-vivo studies also proposed their ability to take apart in formation of functional dentin/pulp complexes and other tissues including bone, cementum, blood vessels and neural tissues [17, 18].

STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH (SHED's)

SHED was first discovered by Songtao Shi in 2003. Miura et al in his study confirmed the ability of SHEDs to differentiate into diverse cell types to a better extent than DPSCs including osteoblast-like, odontoblast-like cells, adipocytes, and neural cells [19]. Abbas et al in a study explored the possible neural crest origin of SHED. The cells appear to play a vital role in the formation of mineralized tissue, which can be used to enhance orofacial bone regeneration [20]. Banking of SHED cells is a major added advantage over other adult stem cells. SHED banking is more economical when compared to cord blood. Isolation technique of these cells is simple, painless and do not possess any risk of immune reaction or tissue rejection being an autologous transplant, hence immunosuppressive therapy is not required and are not subjected to same ethical concerns as embryonic stem cells. SHED may also be beneficial for close relatives of the donor such as grandparents, parents and siblings [21].

PERIODONTAL LIGAMENT STEM CELLS (PDLSCs):

Seo et al in his postnatal study observed the presence of multi potent stem cells in the human periodontal ligament (PDLSCs). When transplanted into rodents, PDLSCs had the ability to produce a cementum or periodontal ligament-like structure and contributed to periodontal tissue repair. These cells can also be isolated from cryopreserved periodontal ligaments while retaining their stem cell potential including strain generation, cementum or periodontal-ligament-like tissue regeneration, expression of MSC surface markers, multipotential differentiation [22]. Trubiani et al suggested that PDLSCs had regenerative potential when planted onto three dimensional biocompatible scaffolds, thus encouraging their use in graft biomaterials for bone tissue engineering in regenerative dentistry [23], whereas Li et al have reported cementum and periodontal ligament-like tissue formation when PDLSCs are seeded on bioengineered dentin [24].

STEM CELLS FROM APICAL PAPILLA (SCAPS):

In an experimental study autologous SCAP and PDLSCs were loaded onto hydroxyapatite/tricalcium phosphate and gelfoam scaffolds and implanted into sockets in the lower jaw of mini pig model. The study observed the formation of a bioroot encircled with the periodontal ligament tissue and in a natural relationship with the surrounding bone [25].

POTENTIAL ADULT STEM CELL (ASCs) VERSUS INDUCED PLURIPOTENT STEM CELLS (IPSs):

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are pluripotent stem cells. iPS cells are the pluripotent stem cells induced through reprogramming somatic cells genetically by acquainting with defined factors. Over the past decade extensive studies on iPS cells are focused on optimization methods, clinical use of these cells effectively and the mechanisms of iPS cell reprogramming technology. Since this initial discovery, researchers have rapidly enhanced the techniques to generate iPSCs, creating a powerful new way to "de-differentiate" cells whose developmental outcomes had been previously assumed to be determined [26]. iPS technology is considered to be one of the most substantial discovery in regenerative dentistry due to its significant role in regeneration of the damaged dental tissues and to regain the tooth morphology and functions [27].

Numerous methods have been followed or experimented over the years for preparation of Pluripotent Stem Cells especially of Oral origin. One such technique to achieve an effective iPSC's is nuclear reprogramming that implicates experimentally inducing a stable change in the nucleus of a mature cell that can then be maintained and replicated as the cell divides through mitosis. These changes are most commonly associated with the reacquisition of a pluripotent state, thereby providing the cell with developmental potential [28]. The strategy has historically been carried out using techniques such as somatic cell nuclear transfer (SCNT), altered nuclear transfer (ANT) and methods to fuse somatic cells with ESCs [29, 30]. In this method of nuclear programming mature somatic cells from an adult and the genes that encode critical transcription factor proteins, which themselves control the function of other genes vital for early phases in embryonic development was introduced. In 2006 it was described in a study that only four transcription factors (Oct4, Sox2, Klf4, and c-Myc) were required to reprogram mouse fibroblasts [31]. In

2007, two dissimilar research groups reached a new breakthrough by deriving iPSCs from human cells, using either the original four genes or a different combination containing Oct4, Sox2, Nanog, and Lin28 [32].

Another method of delivering the desired transcription factor is by targeting the DNA that encodes the production of such factors by integration into the genome of the somatic cells by using retroviral vectors. Retrovirus, an RNA virus that replicate in the host cell and incorporates into the host's genome, allowing the virus to replicate as part of the host cell's DNA from its RNA by using reverse transcriptase enzyme [33]. However, the forced expression of these genes cannot be controlled fully, leading to unpredictable effects. Lentivirus, a simple type of integrated virus can increase the efficiency of reprogramming. Decreasing the drawbacks of viral integration and maximizing reprogramming efficiency, researchers are exploring numerous approaches to reprogram cells in the absence of integrating viral vectors or to use possibly more effective integrative approaches [34].

APPLICATIONS OF INDUCED PLURIPOTENT STEM CELLS:

According to Yan et al DPSCs, SHED, and SCAP, could be induced to generate iPS cells by introducing 3–4 factors with higher efficiency than human fibroblasts [35]. Oda et al suggested programming of OMSCs derived from third molar can be used efficiently [36]. Induced Pluripotent stem cells from DPSCs, SHEDs, SCAPs, PDLSCs are potent resources of Dental pulp, gingival fibroblasts, oral mucosal fibroblasts and periodontal ligament. Mita et al in 2015 reported that intranasal administration of SHED in a mouse model of Alzheimer's disease (AD) could result in substantially improved cognitive function [37]. Similarly Oral Mesenchymal stem cells (OMSCs) derived from iPS programmed cell derivative has successfully been used to repair spinal cord injury and to restore feeling and movement in paralyzed human patients [38]. They can also be used to treat neuronal degenerative disorders such as Parkinson's disease, cerebral palsy, Alzheimer's disease, and other such disorders. A recent experiment has demonstrated the proof of principle in this regard as iPSCs derived from a patient with amyotrophic lateral sclerosis (ALS) were directed to differentiate into motor neurons, which are the cells that are destroyed in the disease [39]. Adipocytes can be used to treat various spine and orthopedic conditions, Crohn's disease, cardiovascular diseases and may also be useful in plastic surgery. Chondrocytes and osteoblasts have been used to grow intact teeth in animals. Ishikawa et al experimentally showed that factors secreted from dental pulp stem cells also showed multifaceted benefits for treating experimental rheumatoid arthritis in animal models [40].

Conclusion

One of the major limitations of the stem cell or progenitor cells is their differentiation potential is comparatively less than the embryonic stem cells. These induced potential dental or oral stem cells (iPSC's) could be of vital tool in cellular therapy and the subsequent development of techniques for use in regenerative dentistry and diseases with degenerative tendency. In addition, the reprogrammed cells could be focused to produce the cell types that are destroyed by the questionable or unknown originated disease. iPSCs generated from patients identified with a specific genetically-inherited disease can be used to prototypical present disease pathology. Thus a substantial evidence based research accompanied by numerous experimental efforts on development of reprogramming techniques under invivo as well as invitro is required to advance the dental regenerative therapeutics by using Induced potential pluripotent stem cells.

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Effectiveness of Gyro Based Handglove for Improving Activities of Daily Living among the Individuals Suffering with Hand Tremors

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Abstract

Objectives: Tremor patients can face many difficulties and challenges in performing their daily life activities. The impact of tremor on patients' lives has various dimensions, captured by questionnaires for tremor severity, disability, handicap, and quality of life. Quality of life is substantially decreased mainly for mental domains, indicating a major impact of psychosocial aspects. The purpose of this study is to reduce hand tremors with the help of gyro based hand glove to improve the activities of daily living of the patient.

Methodology: 24 subjects (M:16; F:8) suffering from hand tremors were selected on the basis of inclusion criteria. Name, age and gender of the individuals participating the research was documented. ADL and IADL score of all the individuals was calculated both pre application of the glove and post application of glove. The scale used for assessing the score of ADL and IADL was 'Katz index of independence in activities of daily living scale' and 'Lawton-Brody instrumental assisted activities of daily living scale' respectively.

Result: From this study we get to know that there is increase in the score of ADL by 17.8%. that there is increase in the score of ADL by 38.63%.

Conclusion: On the basis of the result of the study, it was concluded that subjects suffering with hand tremors had significant increase in the score of the activities of daily living as well as the score of their Instrumental activities of daily living.

Key Words: *Gyro, tremors, Quality of life.*

Introduction

Tremor is one of the most common involuntary movement disorders seen in clinical practice.[1] It is defined as an involuntary, approximately rhythmic, and roughly sinusoidal movement of one or more body parts.[1] It is differentiated from other involuntary movement disorders, such as chorea, athetosis, ballism, tics, and myoclonus, by its repetitive, stereotyped movements of a regular amplitude and frequency.[1] Tremor may be confused with rhythmic myoclonus (incorrectly termed cortical tremor), which is typically characterised by brief muscle twitches, confined to one limb or to adjacent body regions, associated with spike-wave complexes on the electroencephalogram (EEG) or spinal lesions.[1]

CLASSIFICATION OF TREMORS:[1,2,3]

Type of tremor and Definition

1. Rest tremor - Tremor that occurs in a body part that is not voluntarily activated and is completed supported against gravity.

2. Action tremor - Any tremor that is produced by voluntary contraction of muscle, including postural, isometric, and kinetic tremor. The last includes intention tremor.

3. Postural tremor - Tremor that is present while voluntarily maintaining a position against gravity.

4. Kinetic tremor - Tremor that occurs during

any voluntary movement. It may include visually or non-visually guided movements. Tremor during target directed movement is called intention tremor.

5. Isometric tremor - Tremor that occurs as a result of muscle contraction against a rigid stationary object.

6. Task specific tremor - Kinetic tremor that may appear or become exacerbated during specific activities.

Essential tremor (ET) is the most common tremor disorder [4,5,6,7] Prior studies of ET patients have demonstrated that tremor impacts on daily function and quality of life.[8,9,10] Embarrassment is a potentially important contributor to lower quality of life in patients with outwardly visible movement disorders, including ET. A recent study reported embarrassment to be very common among patients with ET, with 18.9% of a population sample and 58.2% of a clinical sample of ET patients reporting embarrassment from tremor.[11] 4.6% of individuals 65 years or older are thought to have ET.[12] Parkinson's disease (PD) affects 0.43–1.90% of individuals 60 years or older.[13] with the majority experiencing rest or postural tremor.[14,15] While rest tremor is typically associated with PD, most PD patients also have a postural or re-emergent tremor, which is the most disabling form of PD-related tremor.[15,16] The prevalence of dystonic tremor is unknown.[17] Although hand tremor has been reported in up to 70% of patients in a series of patients with dystonia of various types.[18] There are many other causes of tremor besides ET, PD, and dystonia, which can affect not only hands but also other body parts.[19] Treatment of tremor with oral medication often provides insufficient relief, especially when the tremor is severe, and is frequently associated with systemic side effects.[20,21]

"Gyroscope", symmetrical mass, usually a wheel, mounted so that it can spin about an axis in any direction. When spinning, the gyroscope has special properties. Many spinning objects exhibit some of these properties; the rotation of the earth about its axis gives it the properties of a huge gyroscope. Once a gyroscope starts to spin, it will resist changes in the orientation of its spin axis. For example, a spinning top resists toppling over, thus keeping its spin axis vertical. If a torque or twisting force, is applied to the spin axis, the axis will not turn in the direction of the torque, but will instead move in a direction perpendicular to it. This motion is called precession. The wobbling motion of a spinning top is a simple example of precession. The torque that causes the wobbling is the weight of the top acting about its tapering point. The modern gyroscope was developed in the first half of the 19th cent. by the French physicist Jean B. L. Foucault, and its first notable use was in a visual demonstration of the earth's rotation. In the second half of the 19th cent., with the invention of the electrically driven rotor, its uses multiplied. It became possible to rotate the gyroscope's wheel at desired speeds without interfering with the precession. Large gyroscopes are used in ship stabilizers to counteract rolling. The gyroscope is the nucleus of most automatic steering systems, such as those used in airplanes, missiles, and torpedoes. It is also used in the gyrocompass, a directional instrument used on ships. Unaffected by magnetic variations, its spinning axis, when brought in line with the north-south axis of the earth, provides an accurate line of reference for navigation.

In the present study we have tried to check the effect of the gloves made using the mechanics of gyroscope on the activities of daily living and soon the quality of life of the patients suffering with hand tremors.

Methadology

An approval for the study was obtained from the Protocol committee and the Institutional Ethical Committee of KIMSDU. Individuals were approached and those fulfilling the inclusion criteria were selected. Total 24 (M:16; F:8) individuals were selected. The procedure was explained and written informed consent was taken from those willing to participate.

Name, age and gender of the individuals participating the research was documented. ADL and IADL score of all the individuals was calculated both pre application of the glove and post application of glove. The scale used for assessing the score of ADL and IADL was 'Katz index of independence in activities of daily living scale' and 'Lawton-Brody instrumental assisted activities of daily living scale' respectively.

Data was documented and statistical analysis was done.

DATA PRESENTATION, ANALYSIS AND INTERPRETATION:

1. Gender distribution:

Table no.1 : Gender distribution table

GENDER	FREQUENCY	PERCENTAGE
MALE	16	66.67
FEMALE	8	33.33
TOTAL	24	100

Interpretation: from the above table and graph we get to know that, from total 24 individuals participating in the research 16 are male and 8 are female. We also get to know that out of 100% of individuals participating , 66.67% are male and 33.33 % are female.

2. Comparison of A.D.L scores on Pre and Post Application of gloves: 17.8

Table no. 2: Comparison of ADL scores on Pre and Post Application of gloves

GROUP	MEAN	SD	W	P VALUE	RESULT
PRE APPLICATION	2.083	0.7173	-300	<0.0001	Significant
POST APPLICATION	5.792	0.4149		<0.0001	

Interpretation: The above table shows the comparison between the ADL scores of individuals pre application and post application of the gloves. Comparison between this two variables was calculated by the Wilcoxon matched-pairs signed- rank test which found out to be -300 with the p-value of <0.0001 which is a significant value. We also get to know that there is increase in the score of ADL by 17.8%

3. Comparison of I.A.D.L scores on Pre and Post Application of gloves: 38.63

Table no. 3: Comparison of ADL scores on Pre and Post Application of gloves

GROUP	MEAN	SD	W	P VALUE	RESULT
PRE APPLICATION	5.5	7.625	-300	<0.0001	Significant
POST APPLICATION	0.5108	0.4945		<0.0001	

Interpretation: The above table shows the comparison between the IADL scores of individuals pre application and post application of the gloves. Comparison between this two variables was calculated by the Wilcoxon matched-pairs signed- rank test whish found out to be -300 with the p-value of <0.0001 which is a significant value. We also get to know that there is increase in the score of ADL by 38.63%**.**

Resullt

In this study 24 (16:M; 8:F) individuals were participated who had the problems of hand tremors. Tremor is one of the most common involuntary movement disorders seen in clinical practice.[1] It is defined as an involuntary, approximately rhythmic, and roughly sinusoidal movement of one or more body parts.[1] It is differentiated from other involuntary movement disorders, such as chorea, athetosis, ballism, tics, and myoclonus, by its repetitive, stereotyped movements of a regular amplitude and frequency.[1]

ADL and IADL score of all the individuals was calculated before the application of glove. The scale used for assessing the score of ADL and IADL was 'Katz index of independence in activities of daily living scale' and 'Lawton-Brody instrumental assisted activities of daily living scale' respectively.

Katz index of independence in activities of daily living scale have the following component for which the individuals were assessed, they are- bathing, toileting, transferring, continence, feeding, dressing. Individuals are told to perform the following activities and then there score is calculated by the standard scoring from the scale. Then the same score was calculated again after the application of the gloves. Comparison between this two variables (ADL score before the application and after the application of the gloves) was calculated by the Wilcoxon matched-pairs signed- rank test which found out to be -300 with the p-value of <0.0001 which is a significant value. We also get to know that there is increase in the score of ADL by 17.8%.

Lawton-Brody instrumental assisted activities of daily living scale have the following component for which the individuals were assessed, they are- ability to use telephone, laundry, shopping, mode of transportation, food preparation, responsibility of their own medications, ability to handle finances, housekeeping. Individuals are told to perform the following activities and then there score is calculated by the standard scoring from the scale. Then the same score was calculated again after the application of the gloves. Comparison between this two variables (IADL score before the application and after the application of the gloves) was calculated by the Wilcoxon matched-pairs signed- rank test which found out to be -300 with the p-value of <0.0001 which is a significant value. We also get to know that there is increase in the score of ADL by 38.63%

With the application of the glove there was increase in the confidence if the individuals in performing all the activities if daily living which over all also have the effect on the quality of life of the individuals.

Conclusion

On the basis of the result of the study, it was concluded that subjects suffering with hand tremors had significant increase in the score of the activities of daily living as well as the score of there Instrumental activities of daily living.

Conflicts of Interest: There were no conflicts of interest in this study

Ethical Clearance: Ethical clearance was taken from institutional committee of Krishna institute of medical sciences.

Source of Funding: Self

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Assess the Effectiveness of Planned Teaching on Knowledge Regarding the Ill Effects of Wearing High Heels among Adolescence Girls

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Abstract

Introduction: Fashion is like a circle; after some time it keeps changing and gets to the same position. High-heels are frequently worn by many women and form an important part of female gender identity, with high heels, the center with gravity shifts against a person wearing high heels and the buttocks and spine are out of balance.Long use of High Heels tends to increase buttock size. Aims: Assess the effectiveness of planned teaching on knowledge regarding the ill effects of wearing high heels among adolescence girls. Materials and methods : Present study is Pre experimental one group pretest ,post test design was used study has been conducted among 60 adolescence girls in R.S. Mundle Dharampeth, Arts and Commerce college, Nagpur. Structured questionnaires given to assess the existing knowledge and the planned teaching were given after the pre test and assess the post test knowledge to see the effectiveness of planned teaching. Results: The levels of knowledge were seen into 4 categories, poor, average, good and excellent of antenatal mothers, frequency and percentage wise distribution of antenatal mothers according to post test level of knowledge regarding ill effects of wearing high heels. The Mean score of knowledge in post test was 16.10 ± 0.70 and of excellent knowledge score in post-test was 94.70 ± 4.15.Conclusion- Present study identify the existing knowledge of adolescence girls structure teaching It was found that adolescence girls having a good level of knowledge regarding ill effects of wearing high heels in pre test and which were significantly more improvement after attending structured teaching and they have been more gained knowledge about the ill effects of high heels with scientific rationale. So planned teaching was effective in enhancing the knowledge of ill effects of wearing high heels.

Keywords: *High heels, adverse effects, adolescence girls.*

Introduction

Adolescence hood is a traditional stage of physical and psychological human development. Adolescence is exposed to vast number of images and ideas of what they are "supposed" to look and act like. Health is the level of functional or metabolic effectiveness of women's lives; this is the general condition of the mind and body of a person, which usually means that he is free of sickness, injury or pain. Many women around the world wear high heels to improve their professional and physical image, but their harmful effect has outstripped the pain many people bear to suffer when wearing such high shoes. The damaged ankle, leg, and back that await women who frequently wear high heels is not enough to dissuade most of them. Even when, after an hour of walking, they feel the effect A recent study at the University of North Carolina at Charlotte found that, although wearing high heels initially strengthens women's ankle muscles, it eventually leads to instability and weakening of those same muscles. Regularly wearing a high heel can lead to permanent changes in posture in adolescents and young adolescence s, as well as spine and leg malposition. Almost two-thirds of the usual high heel wearers report back pain in lumber, compared to less than 20 percent in the general population.[1]

Materials and Methods

A pre-experimental pre-test, post-test research design was used in this interventional study

The study was conducted during September 2019 and the setting was selected in the R. S. Mundle Dharmpeth Arts and Commerce College Nagpur, after getting ethical permission (Ref. no: DMIMS(DU)/ IEC/ Sep 2019/8479 on dated 27 /9/2019 , by using non-probability convenience sampling technique, 60 adolescence were Selected based on the calculation.

Statistical Analysis

The demographic data, collected in pre-test stage, analysis was done in terms of frequency and percentage. The paired t-test was used to compare pre and post test knowledge scores. Chi-square test was applied to find out the association between selected variable with practice score and post-test knowledge score. For statistical analysis SPSS version 16.0 was used.

Method of data collection were used for this study in

Section A: Demographical data about adolescence girls **including** e.g. Age (in years) Height in cm, Education, Economical status, Religion, Marital Status etc.

Section B: Structured knowledge questionnaire on ill effects of wearing high heels.

Major Findings of The Study

In the Table 1 shows that majority of college going adolescence girls According to age (yrs), for the 17-18 age group 35.0 percent of girls belonged, for the 19-20 age group 48.3 percent belonged. 16.7 per cent and more belong to the 21-23 age groups.

According to their education, 8.3% of the girls were educated up to B.A, 45.0% were educated up to B.com, 30.0% were educated up to BBA and only 16.7% of them were educated up to BCCA According to the religion, 100% of the adolescence girls were belongs to Hindu religion.

According to their marital status, 100% of the adolescence girls were unmarried. According to their economic status, 100% of the adolescence girls were belongs to middle-socio economic status. According to their height (cm), 21.7% of the adolescence girls had ≤ 150 cm height, 61.5% of the adolescence girls had 151 – 160 cm height, 16.5% of the adolescence girls had160 – 170 cm height.

According to Table 2, the findings showed that 78.33 % had good level of knowledge regarding ill effects of wearing high heels where as in post test scores 100% college going students had excellent knowledge regarding ill effects of wearing high heels. This table 2 shows a comparison of girls ' pretest and post-test awareness scores regarding ill effects of wearing high heels. Furthermore, standard deviation is compared with mean difference values and student's the combined "t" test is performed at a sense point of 5 percent. For girls overall knowledge score, the calculated "t" value, i.e. 32.80, is much higher.

Association with demographical variables finding showed that there was no significant association of post test knowledge score regarding ill effects of wearing high heels among the adolescence girls with their selected demographic variables .e.g. Age (in years), education Religion, Economical status and marital status and height of the girls.

Table 1: Percentage of wise distribution of girls according to their demographics.

n=60

Demographic Variables	No. of girls	Percentage (%)
Age(yrs)		
17-18 yrs	21	35.0

Cont... **Table 1: Percentage of wise distribution of girls according to their demographics.**

19-20 yrs	29	48.3
21-23 yrs	10	16.7
Education		
BA	5	8.3
B.Com	27	45.0
BBA	18	30.0
BCCA	10	16.7
Religion		
Hindu	60	100
Muslim	0	0
Christian	0	0
Other	**0**	**0**
Marital Status		
Unmarried	60	100
Married	0	0
Married Single	0	0
Single	0	0
Economic Status		
Low	**0**	**0**
Middle	60	100
Higher Middle	0	0
Higher	0	0

Height in cm		
≤150 cm	13	21.7
151-160 cm	37	61.7
161-170 cm	10	16.7

Table 2 : Significance of disparity between girls ' pre -and post-test awareness score n=60

Overall	Mean	SD	Mean Difference	t-value	p-value
Pre Test	9.60	1.31	6.50±1.53	32.80	0.0001 S,p<0.05
Post Test	16.10	0.70			

Discussion

The present study pre test finding show that the study participant i.e. adolescent girls had inadequate knowledge regarding ill effects of high heels. During administration the proposed teaching programme pre -test and post-test data found that the post-test score was higher than the pre-test score.

The following study supported to the present study the research was conducted by Hap Sari V, Yang S, to identify the alteration in the walking by wearing high heels. According to the study findings it was concluded that there was no significant effect of different height of shoe heel (3-5 cm) on the dynamic equilibrium of different height of shoe heel. These effect should be considered when clinician manage young and middle age females and should give care to the dynamic balance In order to identify early disturbances caused by this habit and to help prevent complications for these subjects with regard to the balance of disturbances[2].

The study supported that Mechanical factors relate to pain in knee osteoarthritis.

Maly, Monica R; Costigan, Patrick A; Olney, Sandra J, et al 2008-07-01, Pain experienced by people with knee osteoarthritis is linked to psychosocial factors and articular tissue damage and/or the pathway of pain itself. Mechanical variables were hypothesized to cause this perception of pain; but mechanics in this community were not established as a source of pain. This research aimed to establish whether mechanics might describe variation in pain intensity Mechanical measures included weight-bearing varus-valgus alignment, body mass index and iso kinetic quadriceps torque. Gait analysis captured the range of adduction-abduction angle, range of flexion-extension angle and external knee adduction moment during level walking. Pain intensity was significantly related to the dynamic range of flexion-extension during gait and body mass index. A total of 29% of the variance in pain intensity was explained by mechanical variables. The range of flexion-extension explained 18% of variance in pain intensity. Body mass index added 11% to the model. The knee adduction moment was unrelated to pain intensity. The findings support that mechanical factors are related to knee osteoarthritis pain. Because limitations in flexion-extension range of motion and body size are modifiable factors, future research could examine whether interventions targeting these mechanics would facilitate pain management.3

This study supported to present study conducted by Saumya raju and R. Babu et al aimed to conduct A quasi experimental, one group pre-test and post- test

design, was adopted for the study and it was conducted in The Oxford College of Engineering, Bommanahalli, Bangalore. Purposive sampling technique was used for selecting 40 Engineering Students. The result Shows that pre test mean knowledge score obtained by the subject was 7.4250. After administering structured teaching programme post test mean knowledge score increased to 31.5750 and found to be significant at the level of P<0.05, which evidenced that the developed structured teaching programme has increased knowledge regarding side effects of using high heeled foot wears among adolescence girls in selected colleges .Conclusion is the structured teaching programme was effective in improving the knowledge level of the adolescence girls on side effects of using high heeled foot wears.[4].

According to max burnish, Heather May Morgan , Jean Barnish *et al ,* The study gave evidence that heel high is harmful to health and documented initially all review articles of any kind that either assessed the psychosexual benefits or the negative impacts of high heels on musculoskeletal health. Many women around the world wear high heels to improve their professional and physical image, but their harmful effect has outstripped the pain many people bear to suffer when wearing such high shoes. A study of done to examine the gait of regular heel wear compared to non wears in the year 2012, in this study recruiting 10 non wear us and regular heel wear (40+hrs per week) clear gait and biomechanical deference become evident .[5]

The study was conducted by Matthew R Titchenal,Jessica L Asay, Julien Favre, Thomas p Andriachhi, constance R *et al*. In this study alteres walking biomechanics have been suggested to play a role in knee osteoarthritiies development and progressive Altered walking biomechanics have been suggested to play a role in knee osteoarthritis (OA) development and progression.1,2 Knee OA is roughly two times more common in women compared to men, with incidence increasing substantially in females over age 50.3 Because women and men were observed to have similar knee biomechanics during barefoot walking,4 gender differences in footwear, specifically high heeled shoes, have been implicated as a possible factor for the higher incidence of OA in women. The result was Subjects reported variable experience and usage frequency of wearing heels greater than 5 cm with mean of 10.41 6.44 years (range: [3.75,30.00]) of experience and a mean usage of 2.10 2.55 hours/week (range: [0.06,9.00]). No significant correlations were observed between usage frequency or experience level and any of the gait variables wearing high heels.[6]

Recommandations:

Based on the findings of the study, the following studies are recommended:

- A comparative investigation to assess knowledge of parents regarding ill effects of wearing high heels from urban area could be conducted.
- A review may be performed to determine the efficacy of a related issue instructional module.
- A comparative study to assess adolescent girls ' knowledge and practices in relation to high heels

Conclusion

Based on the above cited findings, it was concluded that the teaching designed has been successful in improving awareness ill effects of wearing high heels. Prepared by Researcher in the form of organized teaching has helped adolescence girls develop their awareness. We can reduce the rate of ill effect of wearing high heels by encouraging them to not wear the high heels on regular basis.

Conflict of Interest- Nil

Financial Support - Self

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Socio-Epidemiological Variables and Medicolegal Considerations in Freshwater Drowning Deaths: A Decadal Autopsy Study (2006-2015)

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Abstract

Drowning is significant public health problem commonly affecting the most productive age groups. The decadal retrospective study presents detailed analysis of socio-epidemiological characteristics of 120 victims of drowning death and stresses upon prevention of such deaths. It also undertakes medicolegal analysis of drowning deaths with regard to identity, manner and time since death.

Drowning deaths comprised nearly 30% of violent asphyxial deaths, following hanging (50.37%). Overall, the most affected ages were third and fourth decade of life accounting for 46.7% cases. Young adults in their second decade of life were commonest victims of accidental drowning. Suicidal deaths were more frequent among females. Canal water was the most common source of fatal drowning in 62% cases, followed by ponds, river and drains.

Significantly 28.3% victims of drowning were unidentified and in about 2/3rd of these cases time since death exceeded 3 days. Among 82 victims with known manner of death, accidental deaths accounted for 79.3% cases; suicides for 18.3% cases and 2.4% were homicidal cases. Minor injuries were observed in 16.6% cases, none contributing to the cause of death.

Key words: *drowning, submersion, accidental, canals, time since death*

Introduction

Drowning is classified under 'violence and injury' for Global health statistics by WHO. Drowning is significant public health problem and commonly affects the most productive age groups. Often ignored but these deaths are preventable. Epidemiological studies of individual characteristics, variables and patterns of drowning helps to formulate effective preventive strategies and to identify the vulnerable groups.

As per World Congress on Drowning 2002 *"Drowning is the process of experiencing respiratory impairment from submersion/immersion in liquid"*. The latest WHO Global Health Estimates indicate that over 3,20,000 people lost their lives to drowning in 2016. Nearly 60% of these deaths occurred among those aged under 30 years, and is third leading cause of death worldwide for children aged 5-14 years.[1]

As per NCRB report on Accidental deaths and suicides in India in 2018, out of 23,690 accidental deaths due to deliberate or negligent conduct on the part of human beings, drowning constituted a share of 7.5%. Intentional drowning deaths comprised 4.9% share among total suicides (n=6579) in India in 2018. Homicidal drowning is rare and involves infants and children.[2]

In forensic context, drowning causes asphyxial death and diagnosis primarily relies on meticulous autopsy examination, macro-pathological findings, individual characteristics and due consideration to the circumstances. Water bodies are also used as means to dispose of dead body after homicidal killing (post-mortem submersion). Advanced putrefaction, difficult or no identification, mutilation by aquatic animals, altered injuries and drifting of body away from the place of incidence further complicates the difficulty encountered in diagnosing these deaths.

In this retrospective study based on 120 cases of fresh water drowning the socio-epidemiological variables are studied and authors tried to highlight important medicolegal considerations.

Materials and Methods

All cases of alleged drowning autopsied at Government Medical College, Amritsar during a period of ten years from 01.01.2006 to 31.12.2015, where the final cause of death was either affirmative or consistent with drowning, are considered for the study. Four cases of post-mortem submersion were not included in the study.

Police inquest papers and autopsy reports were available for perusal in all cases. The subject's individual profile, cause and manner of death, time since death as certified by the forensic pathologist, and circumstantial evidences were examined thoroughly.

Results

During the period 2006 to 2015 a total of 10,217 autopsies were conducted at the institute. The violent asphyxial deaths viz. hanging, strangulation, traumatic asphyxia, drowning, suffocation, smothering etc. accounted for 3.98% (n= 407) cases. The highest reported cases were that of hanging (n=205) comprising more than half of cases of violent asphyxial deaths.

Deaths attributed to drowning (120 cases) comprised nearly 30% of violent asphyxial deaths and 1.17% of total autopsies. All drowning deaths were either spot deaths or dead at arrival or directly brought by police for autopsy. No case of delayed death from drowning was observed.

The present study found male preponderance (n=100) with 83.33% cases **(Table 1)**.The commonest age group affected was 31 to 40 years, followed by 21 to 30 years. Children below 10 years and elders above 50 years of age comprised 9.2% and 8.3% respectively **(Table 2)**.

A substantial number of drowning deaths (n=34), comprising 28.3% were unidentified/unclaimed **(Table 1)**. Out of 86 identified cases 76.7% (n=66) were from rural area and nearly half of the victims (52.3%, n=45) were married.

Canal water was the most common source of fatal drowning (n=74), accounting for nearly 62% deaths, followed by ponds 15.8% (n=19), river 10.8% (n=13) and drain **(Table 3)**. 19 bodies recovered from canal waters, 5 from ponds and 4 from drains remained unidentified, having a share of nearly 25% from each source. About half of bodies recovered from river waters (n=6/13) remained unclaimed.

As regards manner of death in drowning cases (n=120), 26.7% (n=38) deaths were of undetermined intent **(Table 4)**. Among 82 victims with known manner of death, accidental deaths accounted for 79.3% (65 deaths); suicides for 18.3% (15 deaths) and 2.4% (2 deaths) were homicidal **(Table 5)**. Accidental drowning was common among males causing 81.2 % deaths as compared to 69.2% deaths in females. Males in second decade of their life were most common victims (n=18) of accidental drowning, comprising 22% of total cases **(Table 5)**. Marriage was not a variable for unintentional drowning deaths; however, most of suicide victims (intentional drowning) were married. 30% of females committed suicide as compared to 16% males **(Table 5)**.

Two cases were of homicidal intent as verified from police inquest. Both were children below 10 years. A 4 year male child who was kidnapped, was found drowned in a canal; the other one was 1.5 year male child drowned in a drain during assault on his family.

A substantial number of the bodies were submitted for autopsy within 24 hours of death (41.5%). However, there were significant number of deaths (n=41, 34%) in which time since death exceeded beyond 3 days; most of them (n=22) were unidentified **(Table 6)**.

Table 1: Sex-wise distribution of cases

	Identified	Unidentified	Total
Males	73	27	100 (83.33%)
Female	13	7	20 (16.67%)
Total	86	34	120

Table 2: Age distribution of victims of drowning

Age group (years)	Male	Female	Total	Percentage of total
<10	8	3	11	9.17%
11-20	19	2	21	17.5%
21-30	20	4	24	20%
31-40	26	6	32	26.67%
41-50	19	3	22	18.33%
>50	8	2	10	8.33
Total	100	20	120	100%

Table 3: Distribution of cases as per source of drowning

Source of drowning	Cases	Percentage
Canal waters	74	61.67%
Ponds	19	15.84%
Drains	13	10.83%
River	10	8.33%
Gutter	4	3.33%
Total	120	100%

Table 4: Manner of death in drowning cases

Manner	Cases	Percentage
Accident	65	54.2%
Suicide	15	12.5%
Homicide	2	1.6%
Indeterminate	38	31.7%
Total	120	100%

Table 5: Age, sex and marital status of accidental and suicidal drowning cases (n=80)

	Manner	Accidental (n=65)				Suicide (n=15)				Total
	Gender	Male (n=56)		Female (n=9)		Male (n=11)		Female (n=4)		
	Marital status	U	M	U	M	U	M	U	M	
Age groups	<10 years#	5	0	2	0	0	0	0	0	7
	11-20 years	15	3	2	0	1	0	0	0	21
	21-30 years	7	6	1	1	0	2	1	1	19
	31-40 years	1	9	0	2	0	3	0	2	17
	41-50 years	0	9	0	0	0	3	0	0	12
	>50 years	0	1	0	1	0	2	0	0	4
	Total	28	28	5	4	1	10	1	3	80

**U=unmarried, M=married # 2 cases of homicidal drowning in children below 10 years age group noted*

Table 6: Distribution of cases as per time since death

Time since death	Identified cases	Unidentified cases	Total	Percentage
<1 day	43	7	50	41.67%
1-3 days	24	5	29	24.17%
3-7 days	18	19	37	30.83%
>7 days	4	3	4	3.33%
Total	86	34	120	100%

Article Type: Original Research Article

Discussion

Socio-epidemiology:

Indian economy is primarily agriculture-based and Punjab region is considered as breadbasket of India. The fertile land is fed by many perennial rivers and has extensively developed irrigation canal system. Males are engaged in agricultural activities viz. irrigation, fishing or swimming etc. and are more exposed to water resources. The present study reports slightly increased male preponderance of victims (83.3%) as reported by various authors (70% to 76%) in studies from different regions of India - Kumar N[3] (76.16%), Kumar AGV[4](70.45%), Tokbi S[5](75.31%) and Kumar A[6](74.73%).

The commonest affected age group was 31 to 40 years, followed by 21 to 30 years; together accounting for 46.7% of cases. Phad LG[7] (50.56%), Kumar A[6] (55.3%), Kumar N[3] (54.8%) reports a similar combined incidence during third and fourth decades of life. The probable reason for this could be attributed to careless adventurous nature of young adults during recreational activities near water bodies; and familial or financial problems during fourth decade of life.

Out of 86 known cases, 76.7 % (n=66) were from rural areas, this finding correlates with the studies of Delmonte[8] and Murkey[9]. Phad LG[7] reports higher incidence among urban dwellers (57.38%). The findings of present study are along expected lines owing to abundant water bodies and prevalent agriculture related activities in adjoining rural areas of the city.

Water source of drowning :

Canal water was the most common source of drowning deaths accounting for nearly 62% of deaths, followed by ponds, rivers and drains. In developed nations and urban dwellings, significant numbers of drowning deaths in bathtub or swimming pool are reported, but no such source has been found in present study.

In India, most common water bodies for drowning fatalities have been reported as canals by Kumar A[6], ponds by Sugatha M[10], lakes by Chidanand C[11] & Phad LG[7] and rivers by Kanchan T[12] & Kumar N[3], depending upon geographical landscape of area of study. Wells by Rao[13] & Phad LG[7] and ditches by Sugatha M[10] have been reported as other important sources. The present study reports canal waters as commonest source of drowning fatalities (62%), which is in agreement with the study from Haryana by Kumar A[6] (55.37%).

About half of bodies recovered from river and one-fourth from canal waters, ponds or drains

were unidentified. Rivers and canals are sources of running water and drift the body away from the site of occurrence, more so during the rainy season, making it difficult to establish the identity. Besides the body may be recovered late in running water, by the time when decomposition changes had already set in. Mutilation by aquatic fauna is also cause of concern.

Cause, manner and time since death:

The common external morpho-pathological findings characteristic of drowning include copious fine leathery froth from nostrils, cyanosis and skin changes. Internal findings include froth in trachea, voluminous lungs with overlapping anterior borders or rib impressions, blood-stained froth on dissection of lungs and mud/water in trachea/bronchi, stomach and small intestines. But none of them are conclusive. The autopsy diagnosis of drowning is not solely based upon macro-pathological findings; police investigations, histopathology, biochemistry, diatom test etc. need to be considered. Despite all these investigations it may still remain diagnoses of exclusion.

The present study considered only those cases where the cause of death was attributed to drowning by the forensic pathologist. Associated mechanical injuries were observed in form of minor abrasions or bruises or small lacerations in 16.6% (n=20) cases; none contributing to the cause of death. No significant pathological disease was detected at autopsy to contribute towards death. However the possibility of diseases like seizures, cardiac channelopathies etc. precipitating the unintentional drowning cannot be ruled out.

The pattern of accidental and suicidal drowning deaths as reported by different authors, support the findings of present study. Kumar N[3] reports 45.2% accidental deaths, 16.4% suicidal deaths and 35.7% of indeterminate intent. Mukherjee AA[14] mentions 26 cases (37.14%) of accidental, 19 cases (27.14%) of suicidal and 25 cases (35.72%) of indeterminate intent. Nayak GH[15] et al found 13 cases (56.52%) of accidental drowning and 10 (43.47%) suicidal cases. Kumar A[6] reported 55.91% accidental and 44.08% suicidal fatalities. However, Kumar AGV[6] reports more of suicides 50%, 27% accidental and 23% indeterminate drowning deaths.

In agreement to present study, Rao[13] reports young adults in second decade of life as commonest victims of accidental drowning and suicidal deaths more frequent among females as compared to males. The author also reports that the frequency of accidental and suicidal deaths equals in third decade of life, irrespective of the gender. The trend shown by present study is suggestive of preponderance of accidental deaths in both sexes in all age groups except in elders above 50 years of age, where suicides exceed unintentional deaths. The reasons for suicidal drowning in elderly population could be negligence or chronic ailments. The authors didn't find mention about time since death in most of studies.

Conclusion

The study concludes that drowning is potentially lethal and unintentional drowning is the leading cause of these fatalities. Male adolescents and young adults in second decade of life especially in rural areas are common victims. This group constitutes important preventable part of unintentional drowning. They can be educated and preventive strategies can be developed viz. compulsory swimming in schools, adult supervision when adventuring near water bodies, use of fencing, signage and markings etc.

Canal waters remain the leading source of drowning fatalities in Punjab region. Canal safety campaigns to raise awareness among inhabitants living close to the canals, and community participation may help.

A significant number of drowning deaths remain unidentified and commonly recovered from waters after several days. There are diagnostic challenges associated with these deaths, on account of decomposition changes or altered morpho-pathological findings, effects of extraneous factors; lack of circumstantial evidences and failure to identify. Such cases warrant meticulous autopsy to establish cause of death.

Conflict of Interest: None

Ethical Clearance: Not required as the study is based on retrospective data.

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Prevalence of Microscopic Calcium Oxalate crystalluria in Biochemically Normal Urine Samples

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Abstract

Background: Calcium oxalate is the major cause of human kidney stones in all over the globe. These are colorless, refractile, and envelope-shaped. Sometimes dumbbell-shaped or peanut-like forms are seen. They are soluble in dilute Acids. Consumption of some types of foods like tomatoes, spinach, cabbage, asparagus, etc. causes upsurge in its number. A large number are seen in ethylene glycol poisoning.

Method : This present research was aimed at investigating the prevalence of calcium oxalate crystalluria in biochemically normal urine samples came to Clinical pathology section of Central Diagnostic Laboratory, Dhiraj General Hospital for the period of two months from 1st December 2019 to 31st January 2020. The urine samples were centrifuged at 3000 rpm for 15 min. The supernatant were decanted and the sediments were viewed under microscope using high power objective lens with 40x magnification. Total 400 urine samples with normal urine protein and normal urine sugar were analyzed microscopically using an Olympus microscope 4000x. Results showed that calcium oxalate crystals had the prevalence rate of 13.26 %.

Conclusion: The study showed that this much of prevalence of COC was associated with food intake and the social life style.

Keywords: *Calcium oxalate crystalluria, Microscopy, Prevalence, Biochemical.*

Introduction

Calcium oxalate crystal is a chemical compound that forms an envelope shaped crystal, known in plants as raphides; a major constituent of human kidney stones.[1] Majority of the renal stones are the calcium stones, usually in form of calcium oxalate. This oxalate is a naturally occurring substance found in routine diet, includs some fruit , vegetable, nuts and chocolates. Human liver also produces oxalate, dietary factors, high doses of Vitamin D and several metabolic disorders can increase the amount of calcium oxalate in the urine.[2] Calcium oxalate crystals (COC) are the most frequently observe crystals in urine, and 75 % of renal calculi have calcium oxalate crystals as component. Calcium oxalate crystal can form at any pH, and have various microscopic morphologies. It is estimated that about half of the oxalate in urine comes from ascorbic acid (Vitamin C) which is a precursor to calcium oxalate. COC are also associated with ethylene glycol ingestion; another oxalate precursor is calcium carbonate (CaCO3), the chief element in egg shells.[3]The most common metabolic disorder that causes kidney stone in children is hypercalciuria, which causes extra calcium to collect in urine. Other more rare metabolic conditions involve breaking down of oxalate, a substance made in the body found in some foods.[4] These condition include hyperoxaluria, too much oxalate in urine oxalis characterized by deposits of oxalate and calcium in the body tissues.

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Calcium oxalate is found in rhubarb leaves in large quantity and in species of oxalis, Areacaceae taro, kiwifruits, tea leaves, agaves, alocasia and spinach in varying amount while insoluble calcium oxalate crystals are found in plant stem, root and leaves. Vegetable like okra, parsley, leeks, Swiss chard, collard and quinoa are among the most oxalate-dense vegetables.[5] Calcium oxalate is known to be a major constituent of human kidney stone. Even a small dose of calcium oxalate is enough to cause intense sensations of burning in the mouth and throat, swelling, and choking that could last for up to two weeks, and in greater doses it can cause severe digestive upset, breathing difficulties, coma or even death.[6] Urinalysis was the first laboratory test performed in medicine and has been used for quite a few years. Urinalysis continues to be a powerful tool in obtaining crucial information for diagnostic purposes in medicine.[7] Urine analysis is a test that evaluate a sample of urine, it is use to detect and assess a wide range of disorder, such as UTI, renal disease and diabetes. It involves a number of tests to detect and measure various compound that passes through the urine.[8] For example bilirubin pigment in bile may indicate liver diseases. Hemoglobin; iron-containing pigment in red blood cells may indicate injury of the urinary tract, anemia or infection. Cells from the lining of the urinary tract (epithelial cells), crystals may indicate metabolic disease.[9]

These are colorless, refractile, and envelope-shaped. Sometimes dumbbell-shaped or peanut-like forms are seen. [10]

Dihydrates may appear at pH 6 or in neutral urine. Their classic form is that of a small, colorless octahedron that resembles an envelope. Dumbbell shapes and ovoid forms may occur. Longer forms occur in calcium oxalate monohydrate. COCs are insoluble in acetic acid. Oxalate crystals in large numbers may reflect severe chronic kidney disease or ethylene glycol or methoxyflurane toxicity. Oxaluria has come into eminence as a reflection of the increased absorption of oxalates from food following small bowel disease and resection, notably for Crohn's disease. Oxaluria may also be present in genetically susceptible persons following large doses of ascorbic acid. [11]

This present study was carried out in order to determine the prevalence of microscopic calcium oxalate crystals in the urine with normal biochemical parameters at Dhiraj General Hospital.

Material and Method

This was retrospective cross sectional study, conducted to get the prevalence of calcium oxalate crystalluria in biochemically normal urine samples came to Clinical pathology section of Central Diagnostic Laboratory, Dhiraj General Hospital (Sumandeep University) for the period of two months from 1st December 2019 to 31st January 2020.

The material used were Microscope, centrifuge, urine sample bottles, glass slides, dropping pipette, ethanol, water bath, etc. All glass wares were sterilized prior to use and after use.

Urine has a long rich history as a source for measuring health, well-being and remains an important tool for clinical diagnosis. The clinical information obtained from a urine specimen is influenced by the collection method, timing and handling. A vast assortment of collection and transport containers for urine specimens are available. Determining which urine collection method should be used depending on the type of laboratory test ordered. Randomly selected specimens were used for this research since it is the easiest to obtain and it is readily available.

10 mL of the urine sample collected were centrifuged at 3000 rpm for 15 min. The supernatant were decanted and the sediments were viewed under microscope using highest power objective lens 400x. The number of calcium oxalate crystals and other microscopic parameters were reported .[12]

Photographic shots were made to portrayed. Raw data obtained were expressed as mean ± SD and %. Significance differences between means were compared using software. Statically significance was achieved at p value of ≤ 0.05.

Results And Discussion

Urine is sometimes supersaturated with calcium oxalate crystals (COC), which means that it contains oxalate crystals that formed spontaneously. COC exist

in monohydrate and dehydrate forms which can be differentiated by the shape of their respective crystals. The shape of COC is octahedral, and they can grow at any pH naturally in normal urine. Calcium oxalate monohydrate crystals vary in shape such as prisms, dumbbells, spindles, ovals or picket fences.[6] In this present study, the shapes of these crystals are mainly octahedral (Figure 1 and 2 (10x and 40x). COC is known to be major components of human kidney stones. [1]

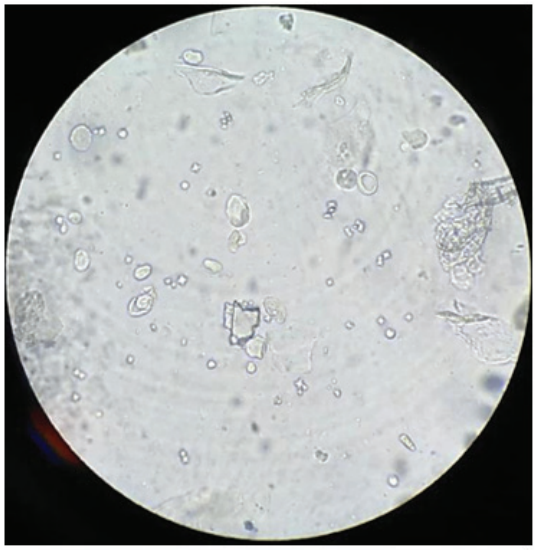

FIGURE:1: COC seen under low power. 10x

FIGURE:2: COC seen under high power. 40x

The presence of these crystals in urine were seen in 53 samples out of 400 cases studied. The distribution of these crystals in urine samples investigated was age dependent as adult patients had the highest percentage prevalence rate. It is possible that the social and food habits of the students must have contributed greatly to this anomaly. In their diet history, majority of them were vegetarian by their diet (89.9%).

In this study, the distribution of octahedral shape COC in urine may leads to blockage of the urinary tracts. This can lead to pain; most commonly beginning in the flank, groin or lower back. This pain is often known as renal colic and typically comes in waves lasting 20-60min. [13] Crystals found in urine may indicate an abnormality, however they may also be present in normal urine as an artifact .[14] There are few relatively health condition that require strict oxalate restriction. These conditions include absorptive hypercalciuria type II, enteric hypercalcuria, and primary hyperoxaluria, Dietary oxalates are usually restricted to 50 mg per day under these circumstances.[15] The frequency of these crystals and other microscopic parameters in the urine samples of these students calls for urgent medical attention. For example, pus cells in urine signifies that the body is fighting an infection in the lower or upper urinary tract, or it contains dead skin cells, bacteria or white blood cells. Pyuria for instance, mostly exist as result of urinary tract infection .[16]

Apart from COC; Triple phosphate, Urate, Billirubin crystals were also found while studying the data. The presence of intact red blood cell (RBC) in urine usually signifies source of blood loss in the lower part of the urinary tract such as urethra, bladder and ureter because gross haematuria may be related to trauma of the bladder, urethra, tumors or hemorrhage.[17] Patients with this in their urine may likely experience this disease if not checked properly. Epithelial cells in the urine can deliver some information of diagnostic use based on the type and the numbers of the cells.[2,17] Transition epithelial cells which lines the urethra and bladder are also commonly seen under microscopy.

Conclusion

This study showed that most of the urine samples collected randomly from patients with normal urine sugar and normal urine protein, has significant level of COC. However, after this study, it is recommended that persons with high level of COC in their urine should reduce the rate of consumption of oxalate rich foods such as spinach and they should take much fluid (water) each

day as this will help to dissolve the crystals in urines.

Conflicts of Interest: The author has no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: There were no ethical issues involved as the study was analytical and did not violate any ethical principles. Also this article has used only urine samples received in Laboratory and does not comprise any interventional studies on animal or human.

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Exfoliative Cellular Study of Pathological Body Fluids – 1 year Retrospective Study

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Abstract

Background: Body fluids are straightforwardly gained by tapping. For fluid routine and microscopic examination, laboratory receives pleural, peritoneal, CSF, Urine and Synovial fluids. The cytological evaluation and biochemical study of these received fluids can be fruitful as assistance to the diagnosis. Present study is an attempt to analyze all the samples referred for cytological evaluation.

Method: The study is cross-sectional descriptive, analytical and retrospective kind of study carried out in Central Laboratory of Dhiraj Hospital, over a period of one year from June2019 to May2020. Fluids such as peritoneal, pleural, CSF, synovial, etc. were received were examined under light microscope and found pathologies are classified in malignant and non-malignant entities. A total of 506 body fluids were received in the one year period. Majority of the cases were Peritoneal fluid comprised of 242 (47.8 %) followed by pleural fluid with 162 cases (32.0 %). Majority cases were categorized under Non-malignant group. Slight male dominance was observed with the mean age of 46.2 years.

Conclusion: Thorough analysis and appropriate clinical correlation will aid in improving patient treatment and care. **C**ytology of the fluid is a rapid and simple diagnostic technique for diagnosis of pathological conditions in malignant and non-malignant cases.

Keywords: *Body fluids, Cytology, Peritoneal, Pleural, CSF*

Introduction

Fluid cytology routine and microscopic test evaluation is found simple, swift, cost effective and quite patient-centric investigation done in regular. Allocating both as a therapeutic as well as diagnostic intervention, tapping of these cavity fluids helps in enhanced understanding of the underlying pathological process. Peritoneal, pleural, CSF, Synovial, BAL and pericardial fluids comprise the major chunk of body fluids.[1] Generally, all the body cavities are lined by single layered epithelial cells and have nominal free fluid in them, with main function of lubrication and protection of underlying visceral organs. If the balance between fluid formation and its loss or removal leads to effusion of the fluid. This is defined as Starling's law.[2] Various pathological processes include acute / chronic infectious, inflammatory and neoplastic etiology. Presence or lack of atypia or frank malignant cells is a absolute guide to the disease progression. The overall cytological assessment with its clinical, physical and radiological examination is an aid to the provisional diagnosis. By these means pathological progression can be checked whereas response of disease to therapy can be evaluated with reasonable accuracy.

Our study aims to do analysis the different exfoliated body fluids received in Cental Laboratory of Dhiraj

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Material and Method

The study is cross-sectional descriptive, analytical and retrospective kind of study carried out in Central Laboratory of Dhiraj General Hospital (Pathology Unit), of a tertiary care hospital over a period of one year from June 2019 to May 2020. Various body fluids such as peritoneal, pleural, cerebrospinal fluid (CSF), Synovial, BAL and pericardial fluids were received in our laboratory were centrifuged at 2000 rpm for 20 minutes, supernatants were thrown out and thin and thick smears were prepared and stained with sedimented cellular button. Two slides (thin and thick each) were air dried and stained with the Field's stain. Individual cell morphologies were studied under the light microscope for their cellular details and classified in malignant and non-malignant groups by the expert pathologist. For the total cell count, improved Neubaur's chamber was used. Patient's history and clinical findings were collected from cytological requisition forms. All the data was then summarized and analyzed further.

Result

Total of 506 body fluids received in the duration of one year. Preponderance of the patients was in the 4th decade with the mean age of 46.2 years. Observed age range varied from 15 to 75 years. 261 cases (51.5 %) out of 506 were male which the majority is. And females founded of 245 cases (48.4%). We received pleural, peritoneal , CSF, sputum, pericardial, BAL and synovial fluids. All the fluids were classified on the basis of malignant and non-malignant cellular details. Cases in which no opinion was possible due to paucity in cellularity, inadequacy of sample and stored / degenerative changes were also noted. Amongst these total fluids examined, peritoneal fluid (ascitic fluid) comprised of maximum cases with 242 (47.8 %) followed by pleural fluid with 162 cases (32.0 %). The next most frequently came across fluid was CSF with 68 (13.4 %) cases. Sputum, Synovial, BAL and pericardial fluids were total comprised of 6.71 % (34 cases).

On evaluation, malignant cases were 43 (8.49, of which 28 were encountered in peritoneal fluid whereas 15 were found in pleural fluids. We also received 16 cases of sputum of which 13 were non-malignant whereas no opinion was possible in a 3 cases. Of the 5 pericardial fluids we received 4 cases which were non-malignant while no opinion was possible in single case. Also 5 synovial fluids and 8 BAL fluid were there. [Table 1,2].

Table 1: Distribution of cases according to diagnosis

Sr. No	Type of fluid	Total	%
1	Peritoneal	242	47.8
2	Pleural	162	32.0
3	CSF	68	13.4
4	BAL	8	1.58
5	Sputum	16	3.16
6	Pericardial	5	0.98
7	Synovial	5	0.98

Table 2: Distribution of cases according to diagnosis

Sr. No	Type of fluid	Total	Malignant	Non malignant	No opinion possible
1	Peritoneal	242	28	214	00
2	Pleural	162	15	147	00
3	CSF	68	00	60	08
4	BAL	8	00	07	01
5	Sputum	16	00	13	03
6	Pericardial	5	00	05	00
7	Synovial	5	00	05	00

Of the 68 cases, opinion was not possible in 08 (1.58 %) in CSF. Of the 60 non-malignant diagnoses of CSF 43 showed normal findings, rest of the cases showed neutrophil cell infiltration indicating bacterial meningitis, cryptococcal meningitis and viral meningitis. We received 05 cases of synovial fluids, none of which were malignant. In both the genders, the most common fluid to be tapped was pleural followed by peritoneal. On further evaluation, demographic characteristics and gross appearances of fluids is revealed below. [Table 3,4]

Table 3: Demographic characteristics of various fluids

Sr. No	Type of fluid	Total (total 506)	Mean age (years)	Male (total 261)	Female (total 245)
1	Peritoneal	242	42.5	125	117
2	Pleural	162	41.2	80	82
3	CSF	68	46.0	38	30
4	BAL	8	53.2	3	5
5	Sputum	16	58.5	8	8
6	Pericardial	5	49.2	3	2
7	Synovial	5	38.6	4	1

Table 4: Gross characteristics of various fluids

Sr. No	Type of fluid	Total (total 506)	Clear	Turbid	Hemorrhagic
1	Peritoneal	242	47	32	163
2	Pleural	162	21	105	36
3	CSF	68	50	0	18
4	BAL	8	0	8	0
5	Sputum	16	0	15	0
6	Pericardial	5	0	3	2
7	Synovial	5	0	4	1

Transudates comprised of 283 (55.9%) cases among all fluids. Peritoneal fluid had the highest frequency of transudate, followed by Pleural fluid, CSF, and synovial fluid. Out of 506 cases, 223 (44.1%) cases had exudative effusion. Majority of them were pleural fluid with followed by peritoneal fluid, CSF, BAL, sputum, and synovial fluid.

Discussion

The pioneers of effusion cytological examination were Lucke and Kiebs, whose concept was revealed in 1867.[1] They are accredited with the narrative of malignant cells/ atypical cells in the peritoneal fluid. In 1882, malignancy in pleural effusion was first labeled by Quincke. For CSF, when lumber puncture was first introduced in 1891, its examination gained momentum.[1] Over the past many years various pathologies have come across in the innumerable literatures, which have mentioned the potential etiologies for different effusions. Owed to all these facts it is authoritarian that exact diagnosis of the underlying disease is known. Pathological fluid aspiration is a simple, cost-effective, swift and patient friendly technique. Over and above cytological microscopy, biochemical and microbiological analysis of these fluids are also important. In our study, males and females had almost equal incidence with males 208 (50.24 %) cases being slightly more than female 206 (49.75%) cases. Majority of the cases were in forth decade. Present study was in concordance with the studies done earlier by Shulbha et. al., Pradhan et. al., Joshi et. al., and Wasim et. al. [3,4,5] The peritoneal fluid was the most frequently encountered with 242 (47.8 %) followed by pleural fluid with 162 cases (32.0 %). A total of 43 (8.49 %) cases were malignant and 463 (91.50%) cases were non-malignant in our study. Our conclusions were in concordance with other studies' observations. [3,4,5] Transudates cases were maximum in our study with 55.9% whereas exudates constituted of 223 44.1%. Kumavat PV et. al. stated similar findings.[1] We encountered 0.72% cases of cryotococcal meningitis. India ink preparation was applied for cryptococci identification. In India, cryptococcal meningitis is the most common opportunistic CNS infection.[6,7] In our study, majority of the malignancies were found in the peritoneal fluid with maximum cases being adenocarcinoma. In one study done by Wong JW et. al., amongst all the fluids, pleural fluid was found to have the highest positivity for atypical/malignant cells.[8] However Jha R et. al., found among all fluids, adenocarcinoma (AC) as the most common finding.[9, 10] Overall, current study was in agreement with other previously conducted studies. Hemorrhagic fluid raises strong suspicion of malignancy as was discovered in our study with majority of malignancies presenting as haemorrhagic effusions. And the presence of malignant cells in the effusion deteriorates the prognosis.

Conclusion

For all the treating physicians, cytological assessment of pathological cavity fluid is a definite aid to the. This is a simple and safe investigation method which aids in understanding disease progression and pathology. Additionally, tumor markers studies can be done from fluids, which can help in accurate diagnosis and improve patient's morbidity and mortality.

Conflicts of Interest: All authors have no conflicts of interest to declare.

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Ethical Clearance: The ethical clearance was taken for the present study from the institute ethical committee.

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A Retrospective Study on Seroprevalence of Transfusion Transmitted Infections in Qs at Dhiraj Hospital, Vadodara

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Abstract

Background: Transfusion of blood and its components help in saving lives but it can also be a life-threatening hazard. Prevention of transmission of infectious diseases through blood transfusion in developing countries is difficult, since the resources available are limited.

Methods and Material: A retrospective study was undertaken to determine the seroprevalence of TTI among the blood donors who donated blood to Dhiraj Hospital Blood Bank between January 2015 and December 2019.The TTI reports of the donors was obtained from the blood donor data records.

Each blood unit was tested by ELISA method for HIV, Hepatitis B and Hepatitis C virus. Syphilis was tested by Rapid Plasma Reagin (RPR) card test. Malaria was tested by antigen rapid diagnostic test.

As is the practice in the Blood Bank, all positive samples were subject to repeat test for confirmation, before the seropositive blood unit is discarded

The information extracted from the Dhiraj Hospital blood bank database also included donor Id, Age, Sex, Residential address, donation type, donation frequency.

Results: The data analysed in the study consisted of a total 20,711 blood donations, of which 2728 (13.1%) were voluntary and 18,288 (86.9%) were replacement donation.

The seroprevalence rate of HIV was 0.1, that of HBV was 1.4, HCV 0.1, syphilis was 0.5 and Malaria was 0.3 among all the blood donors. The TTIs were more frequently found in replacement donors in comparison to volunteer donors.

Conclusions:

Ø The prevalence of TTI is higher in replacement blood donors than voluntary blood donors, hence collection of blood from replacement donors should be at least reduced, if not totally eliminated.

Ø Stringent criteria need to be used for selection of blood donors.

Ø Potential Voluntary blood donors need to be identified and motivated to donate blood regularly.

Key-words: *Blood donor, Seroprevalence, Transfusion transmitted infections.*

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Introduction

Transfusion of blood and its components is an important intervention in current medical practice. However, such interventions not only carry a potential

risk of immune-hypersensitivity reaction, but also a potential risk of transmission of TTI like Human Immunodeficiency Virus 1 and 2, Hepatitis B Virus, Hepatitis C Virus and syphilis.

It is estimated by some reports that the risk of transfusion transmitted infection in developing countries is about 1%[1].

As per the WHO, it is recommended that all blood donors and units should be compulsorily tested for HIV 1 and 2, HCV, HBV and Syphilis infections in order to limit the risk of transmission of TTI [2].

The incidence and prevalence of TTI has significantly decreased in countries which have adopted the WHO recommendations and also introduced newer and more sensitive screening test.

However, in the lower income countries, the risk of TTI continues to remain high [3].

The practice of excessive reliance on rapid testing kits for screening blood donors for TTI has largely contributed to the increased risk of transmission of TTI in these countries. This practice of reliance on rapid testing kits has been even questioned by an International QC survey [4].

Moreover, the low sensitivity of these rapid kits to detect the TTI during the window period or in a low-level carrier state also adds to the problem.

The risk factors for TTI in blood donors, are the high prevalence of TTI in general population, sourcing of blood largely from replacement donors, inadequate screening facilities and excessive reliance on rapid testing kits rather than EIA or NAT for screening.

There is an added risk of septic transfusion due to bacterial contamination during the procedure of blood collection and processing, if proper aseptic precautions are not taken [5].

The above situation highlights the need for utilizing the epidemiological data available on TTI and utilize it for risk assessment and selection of blood donor for collection of blood.

Many countries have studied the seroprevalence of TTI among their blood donors. This study was undertaken to define the prevalence of the Hepatitis B Virus surface antigen (HBsAg), anti-HCV, anti-HIV, and anti-TP among donor population of Dhiraj Hospital Blood Bank.

Materials and Methods

The study was undertaken to define the seroprevalence rates of the various TTI in the blood donor of Dhiraj Hospital Blood bank during the 5 years period from January 2015 and December 2019.

Study area:

The data utilized in the study was extracted from the Blood Bank of Dhiraj Hospital database.

The data pertained to results of serological Tests for HIV, HBV, HCV and Syphilis that were performed in the TTI Laboratory of the Dhiraj Hospital Blood bank in accordance with the national testing policy and guidelines

Study design:

The retrospective study analyzed the results of TTI of the blood donor who donated between January 2015 and December 2019, obtained from blood bank data records to find out seroprevalence of TTI in the blood donors

The information extracted from the Dhiraj Hospital blood bank database also included donor Id, Age, Sex, Residential address, donation type, donation frequency

The results of HBV, HCV, HIV, Syphilis tests as well as the overall incidence of TTI were expressed as seropositivity rates.

Study population:

The study data included all blood donors who donated blood to the Dhiraj Blood Bank between January 2015 and December 2019 which included both voluntary and replacement donors. Donors were selected for blood donation as per the pre-set criteria of Dhiraj Blood Bank.

Statistical Analysis

The data retrieved from Dhiraj Hospital Blood Bank Database was presented as number of seropositive samples per year and utilized to calculate the prevalence

rate of various TTI like anti-HIV, HBsAg, anti-HCV and anti-TP expressed as percentages.

Study selection Criteria:

Inclusion criteria:

1) All volunteer and replacement donors.

2) All donors fit into pre-set criteria of Dhiraj Blood Bank.

Exclusion criteria:

1) Deferred patients

Results

Chart below shows 2.2% of donors found positive for TTI infection between January 2015 to December 2019.

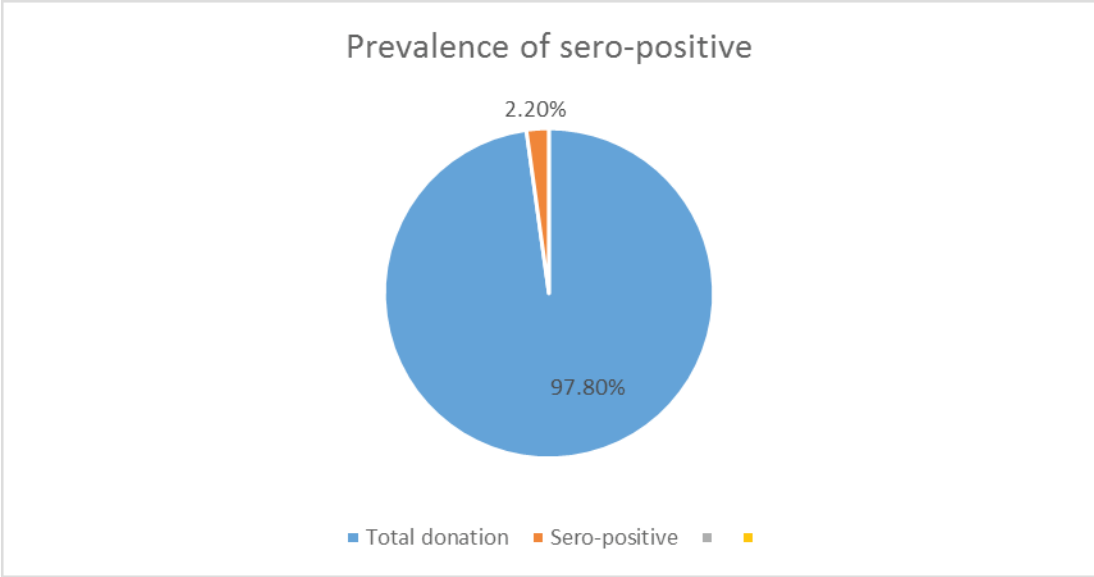

Table 1: Socio-Demographic characteristics of blood donors who donated blood to the Dhiraj blood bank from January 2015 to December 2019.

Characteristic		Nos.	Percentage
Sex	Male	20184	97.4%
	Female	527	2.6%
Age	18-30	6172	29.8%
	30-40	7766	37.5%
	40-50	5012	24.2%
	50-60	1761	8.5%
Donor type	Volunteer	2728	13.1%
	Replacement	18288	86.9%
Blood Type	O	9361	45.2%
	B	5509	26.6%
	A	4577	22.1%
	AB	1264	6.1%
Rh Type	Rh Positive	18432	89.01%
	Rh Negative	2279	10.99%
Total Donors		20711	

Socio-demographic characteristics of the donors:

The TTI data of the 20,711 blood units/blood donors whose blood was collected by the Dhiraj Hospital between January 2015 and December 2019 were analysed and the demographic features of blood donors is presented above in the table 1.

Majority of the blood donors were males (97.4%), while only 2.6 % were females. The age of the blood donors ranged from 18 to 60 years. The highest number of blood donors belonged to the age group of 30 to 40 years.

Majority 86.9 % of the blood donors were replacement donors, while the number of voluntary blood donors was lower.

The blood group of the blood donors studied was type O in 45.2%, type B in 26.6%, type A in 22.1% and type AB in 6.1%. Most of the blood donors i.e.89.01% were Rh Positive and only 10.99% were Rh negative.

Table 2: Number and rate of Transfusion Transmitted Infections (TTI) among the donors by year (January 2015 - Dec 2019)

Year	Donations	HIV +ve	HBsAg +ve	HCV +ve	VDRL +ve	MP +ve	Total (%)
2015	*4037*	*4*	*61*	*3*	*32*	0	100(2.4%)
2016	4913	12	67	0	19	3	101(2.0%)
2017	4878	7	56	3	46	0	112(2.2%)
2018	3384	6	57	5	3	4	75(2.2%)
2019	3500	3	64	14	6	1	88(2.5%)
Total	20711	32(0.1%)	305(1.4%)	25(0.1%)	106(0.5%)	7(0.03%)	476(2.2%)

Seroprevalence of TTI among the donors:

The Table 2 presents the results of TTI tests of a total of 20,711 blood donors. The seropositivity rates for each of the TTIs were HBV 1.4 %, HCV 0.1 %, HIV 0.1 %, Syphilis 0.5 % and Malarial Parasite 0.03%.

The HBV was observed to be the most prevalent TTI all through the five-year study period. Of the total blood units tested, about 2.2 % of all the donors had at least one TTI, while 0.1% had multiple infections.

In our 5 year study, the incidence of TTI was the highest in 2019(2.5%) and lowest in 2016(2.0%). Our study data also shows that the incidence of TTI is higher in male donors than in female donors. Further our study also noted that the incidence of TTI is more in Replacement donors as compared to Voluntary donors.

Discussion

India has over the years, managed to reduce the TTI in blood donors significantly with the introduction of modern screening tests and strict enforcement of proper guidelines and protocols. However, the prevalence of transfusion transmitted viruses among blood donors continues.

Transfusion transmitted infections (TTIs) are a matter great concern with regards to the safety of the blood transfused to recipient patients.

The risk of transmitting a TTI through blood transfusion has declined over the years in the developed world. However, in the developing countries each blood unit still has a 1 % risk of transmitting a TTI

A voluntary donor is one who is self-motivated to donate blood regularly without any inducement., while a replacement donor donates blood only when a near or dear one is in need of blood.

Thus, replacement donors (RD) should be motivated and proper guidance and knowledge of blood donation should be given to them to promote more voluntary blood donation.

On comparing our study with other similar studies, it has found that seroprevalence of HBV is highest among all the TTIs.

The seroprevalence of HCV as reported by Indian studies varies from a high of 1.09 % (Gupta et al,2004) to a low of 0.2% (Diwan R et al 2012)

Sexually transmitted infections are more in developing countries compared to developed countries and constitute a major public health issue.

A reliable source of safe blood for a blood bank is a Voluntary blood donor, but the voluntary blood donation in India is less than desired, probably due to the lack of information and the myths associated with it.

In order to increase voluntary blood donation, it is necessary/essential to create awareness about it among the local community/population. This would help in minimizing the risk of TTI.

The risk of TTI is higher with blood procured from replacement/professional donor masquerading as replacement donor. Therefore, it is best to avoid taking blood from replacement donors or accept it only in emergency situations

Blood transfusion should not cause any harm to recipients, therefore blood unit should be fully screened for infection like HIV, HBV, HCV etc and also checked for bacterial contamination.

Unsafe blood remains a major threat to the recipient. The risk of infection is relatively higher in HB than with the other TTIs, although its prevalence has reduced after introduction of hepatitis B surface antigen (HbsAg) testing and after its inclusion as a routine screening test.

The rapid testing kits utilized for screening of blood for TTI are unable to detect the presence of virus during the "Window Period"

Many countries now use kits for detect antibodies directed against HBV core antigen or HBV NAT testing to detect chronic HBV carrier state with a low viral load. The Nucleic Acid

Nuclei Acid Amplification Techniques NAAT is currently recommended for the early detection of the TTI viruses as it targets the Viral RNA /DNA regions/ genome. This also reduces the Window Period.

Conclusion

The increase in the prevalence of seropositivity for HCV and HBsAg in both replacement and voluntary donors is a cause of worry. A large number of replacement donors have been found to have HIV, HBV, HCV, Malaria and Syphilis infections.

Hence, it is recommended that stringent criteria be used for selection of blood donors and also standardization of the testing methods used for the screening of the blood unit to ensure blood safety.

The practice of testing the blood units using Nucleic Acid amplification techniques has helped the western countries to decrease the risk of transmission of these infections and therefore needs to be widely adopted.

Moreover, it is also important to promote voluntary blood donation activity by identifying and motivating potential voluntary blood donors from within the local community.

Ethical Clearance- Taken from institutional ethical committee.

Source of Funding- Self funded.

Conflict of Interest – Nil.

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In Silico Prediction of Potential Compounds of Nigella sativa as Aromatase Agonists and Ability to Cross the Blood-Brain Barrier for treatment of Alzheimer's Disease

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Abstract

Background: Alzheimer's disease (AD), is a chronic neurodegenerative disease which condition is characterized by a decline in memory, thinking skills, and the ability to perform basic activities of daily living. Study showed that postmenopausal women with AD had a lower aromatase activity (p450 aromatase) in the brain compared to menopausal women without AD. To improve cognitive function in AD patients, Nigella sativa (NS) was found to have a protective effect on memory, and cognitive function. This study therefore, aimed to investigate in silico prediction of potential compounds of Nigella sativa as aromatase agonists and ability to cross the Blood-Brain Barrier (BBB) for treatment of Alzheimer's Disease. **Methods:** The data sets used in this study were collected from databases KnapSack Kanaya, PubChem, and PASS server Way2Drug. HitPick and Autodock Vina was performed. Additionally, the systematic analysis involved ADMET, LMMD, and SWISS ADME. **Results and Discussions:** In silico test results revealed the most promising constituents in 36 active NS compounds that may have potential to reduce the severity of the AD, owing to its anti-inflammatory, cytochrome p450 stimulants, free radical scavengers, antioxidants, and immunomodulators. The ability of NS to cross the BBB was proven by admetSAR LMMD with an analysis average value of 0.91 (from the highest value of 1). Further, NS can trigger cytochrome P450 aromatase activity via Quercetin 3-(6'' ''-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside which has a better binding affinity value than its control (androstenedione). NS through oleic acid compounds may bind to peroxisome proliferator-activated receptor gamma (PPRAG), similar to Rosiglitazone which may affect transcription and activation regulation of PPARG. **Conclusion:** To be concluded, Nigella sativa could be used as a potential medicinal plant for the treatment of Alzheimer's disease.

Keywords: *In silico, Nigella sativa, Aromatase agonists, Blood-Brain Barrier, Alzheimer's Disease*

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Introduction

Alzheimer's disease (AD), is a chronic neurodegenerative disease which condition is characterized by a decline in memory, thinking skills, and the ability to perform basic activities of daily living. AD had been also identified as the most common cause of dementia in later life[1-3]. The increased risk of

developing severe symptoms of AD was also associated with many factors including brain estrogen deficiency in females with AD. A previous study showed that postmenopausal women with AD had a lower aromatase activity (p450 aromatase) in the brain compared to menopausal women without AD[4].

Many studies have been searching for ways to cure AD, but none of the drugs can stop the progression of disease[5]. Currently, the treatment strategy of AD may reduce some physical symptoms and controlling behavioral symptoms that impact daily life. Interestingly, a study found that 25% of the treatment regimens today were related to natural products. The increased use of herbal products is due to their affordable and accessible choice, fewer side effects in contrast to synthetic drugs, and a more effective selection in patients with chronic diseases[6]. The usage of natural products increases when prescribed medicines were ineffective in the treatment of chronic diseases. The identification of potential drugs to effectively inhibit the progression of AD is crucial, thus many natural plants including *Nigella sativa* (NS) are also being proposed for its phytopharmaceuticals in the treatment of AD[7-9].

With respect to improve cognitive function in AD patients, Nigella sativa (NS) was found to have a protective effect on memory, and cognitive function. NS has a powerful constituent of antioxidants and protects against damage induced by the oxidative processes and the abilities to binding to protein targets. Thus, it may prevent neuronal cell death and improve cognitive function[8,10-12]. NS, as a herbal medicine has been widely recognized for its potential biological activities, such as a diuretic, antihypertensive, antidiabetic, anticancer, immunomodulatory, antimicrobial, anthelmintic, analgesic, a spasmolytic, bronchodilator, anti-inflammatory, antitussive, gastroprotective, hepatoprotective, reduce LDL, renal protective and antioxidants[13-20]. In addition, the essential oil of NS seeds contains various active ingredients, such as thymoquinone (TQ) (30–48%), thymol, thymohydroquinone (THQ), dithymoquinone, p-cymene (7–15%), carvacrol (6–12%), sesquiterpene longifolene (1 –8%), 4-terpineol (2–7%), t-anethol (1–4%), and α – pinene[7], nigellimine-N- oxide, nigellicine, nigellidine and alpha-hederin[21]. Further, the antioxidant and anti-inflammatory activities of NS play a crucial role to help prevent oxidative damage in cells, especially in the brain area[10].

Due to its natural plants, a safety, and efficient along with favorable phytopharmaceuticals properties, NS has been suggested to be a potential for further study in the treatment of AD. This study therefore, aimed to investigate in silico prediction of potential compounds of Nigella sativa as aromatase agonists and ability to cross the Blood-Brain Barrier (BBB) for treatment of Alzheimer's Disease.

Materials and Methods

The data sets used in this study were collected from databases KnapSack Kanaya and PubChem. KnapSack Kanaya revealed the potential biological activities of NS compounds. Meanwhile, the PubChem database was used for identifying the SMILE (simplified molecular-input line-entry system) structure of these compounds. The SMILE structure was employed for tracing and exploration of NS compounds using the PASS server Way2Drug. Basically, the PASS server Way2Drug identified the probability activator (Pa), and probability inhibitor (Pi). Pa value describes the potential properties of NS for AD treatment, while the Pi value represents the potential inhibitors of the NS compounds. Pa value > 0.7 means that the input compounds have a high efficacy of similarity with the compounds that have been proven in the database as AD treatment. However, if the predictive value between 0.3-0.7, then the compound will computationally be known to play a vital role in the treatment of AD, yet the similarity to compounds that have been proven in certain activities, could be less.

HitPick was used for predicting NS compounds-protein target interactions. The higher the value of prediction precision (maximum 100%), and similarity (highest is 1), the higher the probability of these compounds can interact. After that, String-db was used to identify multi protein interaction. In addition, to evaluate the binding affinities/docking scores between ligands (NS compounds) and Aromatase (PDB ID 3EQM), Autodock Vina was performed, using PyRx 9.5 program. Docking results was evaluated based on the binding affinity value. The more negative the value, the stronger the interaction between ligands and protein. PyMol 2.3 program 1 was used to visualize docking results, while the LigPlot 2.1 program was used

to see amino acid interactions. The docking grid box was as follows: Center X: 87,914 Y: 54,097 Z: 44,738 Dimension Armstrong: X: 4,542 Y: 15,916 Z: 32,648.

Additionally, the systematic analysis involved Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET), Laboratory of Molecular Modeling and Design (LMMD), and SWISS ADME. These are tools for predicting and analyzing drug pharmacokinetics. The higher the value, the better the ability to penetrate, and the highest value of this analysis is 1[22].

Results and Discussion

In silico test results revealed the most promising constituents in 36 active NS compounds that may have potential to reduce the severity of the AD, owing to its anti-inflammatory, cytochrome p450 stimulants, free radical scavengers, antioxidants, and immunomodulators. Tabel 1 showed the results of database tracing using KnapSack, there were 36 active compounds in NS. Further exploration was carried out using the Pass server Way2 Drug to determine the potential of NS compounds as AD therapy.

Table 1: Probability active components in *Nigella sativa* (NS)

Metabolites	Anti-inflammation	Cytochromes p450 stimulants	Cytochromes p450 inhibitor	Free radical scavengers	Antioxidants	Immunostimulants
Thymol	0.564	0.396		0.348	0.314	
Carvacrol	0.673	0.396		0.295	0.313	0.225
alpha-Thujene	0.807	0.35				
alpha-Pinene	0.476	0.442		0.160		
beta-Pinene	0.601	0.308				
Myrcene	0.287	0.325		0.38	0.469	0.622
Lauric acid	0.515	0.305		0.315	0.229	0.504
Oleic acid	0.614	0.273		0.36	0.283	0.549
Anisaldehyde		0.755	0.273	0.35	0.233	0.284
Apiol	0.469	0.174		0.459	0.358	0.167
Estragole	0.447	0.389	0.259	0.486	0.337	0.205
Myristicin	0.38		0.19	0.552	0.359	0.206
(+)-R-Citronellol	0.53	0.26		0.365	0.588	0.585
p-Cymene	0.643	0.517	0.213	0.365	0.144	0.173
(+)-Fenchone		0.369				0.184
alpha-Phellandrene	0.395	0.447				
gamma-Terpinene	0.463	0.441		0.163		0.237
Longifolene	0.57	0.256		0.157	0.208	0.225
(Z.Z.Z)-Octadeca-9.12.15-trienoic acid	0.812	0.255		0.281	0.363	0.504
Thymoquinone	0.6	0.48		0.238	0.229	0.242
Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.751			0.977	0.926	0.677
Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.753			0.997	0.928	0.639

Metabolites	Anti-inflammation	Cytochromes p450 stimulants	Cytochromes p450 inhibitor	Free radical scavengers	Antioxidants	Immunostimulants
Quercetin 3-(6’’’’-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	0.737			0.997	0.889	0.482
Salfredin B11	0.519	0.357		0.485	0.422	
Fuzitine						
Nigellicine	0.426			0.174		
Nigellidine	0.698	0.307		0.218	0.159	
Nigellimine	0.318	0.395	0.278	0.219		
4-Terpineol	0.603	0.312		0	0.151	
4(10)-Thujene	0.853	0.25	0.173	0		
Nigeglanine	0.48	0.218		0		
Nonane	0.424	0.517		0.266	0.170	0.372
Carvone	0.469	0.322		0.15	0.193	
alpha-Longipinene	0.377	0.3		0.18	0.164	
Dihydrocarvone	0.476	0.35			0.172	
Nigellidine 4-O-sulfite					0.165	
Mean	0.554	0.360	0.231	0.321	0.351	0.373
SD	0.147	0.115	0.041	0.265	0.235	0.179

This study found that the highest mean of probability active components in NS, Pa value of anti-inflammatory activities = 0.554. There were several compounds that had Pa > 0.7, which were investigated as potential compounds based on in silico and laboratory tests, known as anisaldehyde (Pa = 0.755) for Cytochrome p450 Stimulant. Anti-inflammatory activity was found in 6 compounds with the highest Pa of 0.853 in compound 4(10)-Thujene. Free radical scavengers and anti-oxidant activities were found in 3 compounds, namely Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside Quercetin 3-(6’’ ‘’-feruloylglucosyl)-(1->2)-galactosyl-(1->2) -glucoside and Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside. The highest Pa of 0.997 for free radical scavenger activity was found in Quercetin 3-(6’’ ‘’-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside and Quercetin 3-glucosyl-(1->2)-galactosyl-(1-> 2)-glucoside. The highest Pa of 0.928 for anti-oxidants was found in Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside.

Hitpick analysis was performed to determine the target protein of the active NS compounds. The input compounds included in the Hitpick program were 36 active NS compounds. After data processing, 10 protein targets were obtained, namely Fatty Acid Binding Protein 7 (FABP7), FABP4, FABP3, Peripheral myelin protein 2 (PMP2), Ganglionic Long-Term Potentiation (GLTP), Free fatty acid receptor1 (FFAR1), FABP5, FABP1, GC and albumin (ALB). These target proteins were protein targeted by oleic acid. The selection of protein targets was based on similarity and target precision, namely 1.0 and 100%, indicating that the target based on the HitPick database has a high confidence score. Several proteins act as biomarkers in Alzheimer’s such as: FABP7, FABP4, FABP3. Other protein targets were associated with PMP2, GLTP, FABP5 and FABP1 neurogenesis and neurogenesis. Meanwhile, other proteins were considered as proteins that were widely found in the body such as ALB, so they were not specific.

In addition, using the String-db program, protein interaction analysis determined the interactions of the target protein. From the Hitpick results, eight proteins were entered into the String-db program and were then analyzed. The result obtained was FABP5 interaction

which activated VEGFA protein which was known as a neuroprotective cytokine that induced neurogenesis and angiogenesis. VEGFA protein had a positive effect on reducing Alzheimer's symptoms, as well as depressive symptoms in adults, increasing VEGF protein in the hippocampal region and neurogenesis[23]. The String-db data also showed that VEGFA activated FABP4, which was one of the biomarkers in frontotemporal dementia[24].

Furthermore, the analysis of the interaction of oleic acid with peroxisome proliferator-activated receptor gamma (PPARG) was a ligand that activates transcription factors with their biological activities regulating glucose metabolism, fat and suppression of inflammatory gene expression[25]. Oleic acid was predicted to have interactions with PPARG. The interactions of compounds and proteins were predicted using the STITCH-DB program.

This study showed that oleic acid could bind to PPARG and the interaction that occurred was transcriptional regulation. Administration of PPRAG agonists had been shown to improve AD pathology in AD experimental animals. Likewise, the administration of Rosiglitazone - a PPARG agonist - demonstrated a significant improvement in memory and cognition in AD patients. Rosiglitazone was previously given to treat type 2 diabetes mellitus (DM) as well as thiazolidinedione (TZD) which was also a PPARG agonist[25-26]. Rosiglitazone could influence transcription and activation regulation of PPARG. From the results of the compound and protein interaction analysis, it was possible that NS could be used as a potential medicinal plant for AD drugs with the PPARG target protein.

The aromatase enzyme was an important enzyme in the process of steroidogenesis, a family of cytochrome P450, and was encoded by the CYP19 gene on chromosome 15[27-28]. P450arom, also known as estrogen synthase, was a terminal enzyme complex from the steroidogenesis pathway that catalyzes the conversion of C19 androgens (androstenedione and testosterone) to C18 estrogens (estrone and E2). This enzyme complex consists of cytochrome P450 aromatase and flavoprotein NADPH-cytochrome. Aromatase was involved in various brain functions such as synaptic plasticity, neurogenesis, neuroprotection and regulation of sexual and emotional behavior in mice, was also involved in the pathophysiology of AD and autism spectrum disorder (ASD) in humans[29]. To determine the role of the active NS compound in triggering the activity of cytochrome P450 aromatase, an affinity bond comparison was carried out with the control 4-ANDROSTENE-3-17-DIONE which had bound to aromatase. Of the 37 docking ligands, there was one ligand that had a better value than the control, namely Quercetin 3-(6'' ''-feruloylglucosyl) - (1->2) -galactosyl-(1->2)-glucoside.

Table 2: admetSAR analysis using Laboratory of Molecular Modeling and Design (LMMD) to predict Nigella sativa (NS) permeability to cross the blood-brain barrier (BBB)

Metabolites	BBB Penetration Score	
Thymol	0.9381	BBB+
Carvacrol	0.9381	BBB+
alpha-Thujene	0.9550	BBB+
alpha-Pinene	0.8959	BBB+
beta-Pinene	0.9229	BBB+
Myrcene	0.9478	BBB+
Lauric acid	0.9488	BBB+
Oleic acid	0.9539	BBB+
Anisaldehyde	0.9073	BBB+
Apiol	0.9399	BBB+
Estragol	0.9383	BBB+

Cont... **Table 2: admetSAR analysis using Laboratory of Molecular Modeling and Design (LMMD) to predict Nigella sativa (NS) permeability to cross the blood-brain barrier (BBB)**

Metabolites	BBB Penetration Score	
Myristicin	0.9504	BBB+
(+)-R-Citronellol	0.9409	BBB+
p-Cymene	0.9677	BBB+
(+)-Fenchone	0.9849	BBB+
alpha-Phellandrene	0.9049	BBB+
gamma-Terpinene	0.9431	BBB+
Longifolene	0.9687	BBB+
(Z,Z,Z)-Octadeca-9,12,15-trienoic acid	0.9314	BBB+
Thymoquinone	0.8138	BBB+
Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.9144	BBB+
Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	0.7248	BBB-
Quercetin 3-(6’’’’-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	0.9251	BBB+
Salfredin B11	0.8952	BBB+
Fuzitine	0.7793	BBB-
Nigellicine	0.6906	BBB-
Nigellidine	0.8590	BBB+
Nigellimine	0.9868	BBB+
4-Terpineol	0.9104	BBB+
4(10)-Thujene	0.9554	BBB+
Nigeglanine	0.9890	BBB+
Nonane	0.9821	BBB+
Carvone	0.8689	BBB+
alpha-Longipinene	0.9400	BBB+
Dihydrocarvone	0.9256	BBB+
Nigellidine 4-O-sulfite	0.7214	BBB-

The blood–brain barrier (BBB) was a term used to describe the unique properties of the microvascular central nervous system (CNS), which was a semipermeable, highly selective boundary and separated circulating blood from the brain from deep extracellular fluid (CNS)[30]. This system allowed the passage of water, some gases, and fat-soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids which are essential for nerve function. BBB LMMD was an analysis to predict whether these compounds could penetrate the BBB. Additionally, 36 secondary metabolites of NS were analyzed using the SAR LMMD admet with the highest value of the analysis being 1. The higher the value (close to 1), the better its ability to penetrate BBB[31]. From this analysis in Table 2, it was found 10 metabolites with an analysis number > 0.95 and 3 metabolites that were unable to cross the BBB, namely Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside, Fuzitine, and Nigellidine 4-O-sulfite. The average value of the analysis was 0.91. However,

the ability of NS and TQ to cross the BBB still needs to be studied further[8]. Thymoquinone and THQ in the form of glycosidic, binding to aglycone which then allowed it to cross the BBB[32]. Thymoquinones could cross the BBB and had neuromodulator activities[33].

Conclusion and Acknowledgement

This study showed 36 active NS compounds, and the most potential properties were anti-inflammatory. Other biological activities of NS compounds were Cytochrome p450 Stimulants, free radical scavengers, anti-oxidants, and immunomodulators. Further, NS had an ability to cross the BBB and there was one ligand of Quercetin 3-(6'' '-eruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside which showed a better value than its control (androstenedione). Quercetin could trigger cytochrome P450 aromatase activity. Oleic acid could bind to PRAG similar to Rosiglitazone which then affected transcription regulation and activation of PPARG. In conclusion, Nigella sativa could be used as a potential medicinal plant for the treatment of Alzheimer's disease. This study would not have been possible without the support of Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya and Department of Obstetrics and Gynecology, Faculty of Medicine, University of Muhammadiyah Malang. I am especially grateful to all of those with whom I have had the pleasure to work during the study.

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Glycated Haemoglobin- Recent Developments and Review on Non-Glycemic Variables

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Abstract

Glycated hemoglobin (HbA1c) is the current tool for monitoring glycemic control once a diagnosis of diabetes is established. Its role in the diagnosis of diabetes has only recently come to attention. In the past, many international organizations have discussed the role of HbA1c in the diagnosis of diabetes and rejected this application as appropriately DCCT-aligned assays were not used or available globally. Considering the high biological variability, the dynamics of glucose, as well as the limitations of blood glucose monitoring technology, at that time, the possibility of obtaining an integrated average glycemia value by the measurement of a single biomarker elicited immense interest and provided a powerful tool in both diabetes research and clinical management. HbA1c testing was soon facilitated by the development of a new analytical methodology that was suitable for use in clinical laboratories. However, a consensus statement in 2007 on assays used to report HbA1c has now further strengthened the case for a change in the diagnosis of diabetes. Using HbA1c as a screening or diagnostic tool has some logistical advantages over traditional glucose testing (either oral glucose tolerance test [OGTT] or fasting plasma glucose [FPG]). Patients can present for a relatively quick test in a non-fasted state at any point of the day, allowing more scope for opportunistic screening. HbA1c assay readings are less prone to recent influences of physical or emotional stress and provide an indication of longer term glycemic control spanning the last 2–3 months. Owing to such logistical advantages there are calls for HbA1c to become the preferred diagnostic tool over glucose tests. Performing the HbA1c test regularly allows the assessment of glycemic control and verification of the efficacy of medication treatment and of education for self-care. It is estimated that 33% to 49% of people with DM2 cannot achieve adequate goals for glucose, blood pressure, or lipid profile control and only 14% reach normal parameters in these measurements.

Keywords- *Diabetes Mellitus, Glycated hemoglobin, Hyperglycemia, Blood Glucose.*

Background

Diabetes mellitus refers to a group of metabolic disorder that shares the phenotype of hyperglycemia characterized by insulin resistance initially, impaired insulin secretion, insulin deficiency, increase glucose production and decreased glucose utilization and the complications arising from this disease is the major cause of death worldwide. The cells of the body cannot metabolize carbohydrate due to relative or complete lack of insulin and body breaks its own protein, fat, glycogen resulting in hyperglycemia.[1-3]

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Glycated Hemoglobin

Glycated hemoglobin (HbA1c) was first described by Rahbar *et al*. in 1969.[4] Hemoglobin (Hb) is a tetramer

formed of two alpha and two beta globin chains. On exposure to high levels of blood glucose, hemoglobin gets non-enzymatically glycated at different sites in the molecule.[5] Glycation is the non-enzymatic addition of sugar residue to the amino groups of proteins. Glycated Hb also known as HbA1c , is used as a guideline to check the status of patients glycemic status for preceding 3 months or it represents value for glucose preceding 8 to 12 weeks and provides criteria for assessing glucose control, Glycated Hb values are free of day to day glucose fluctuations and unaffected by recent exercise or food ingestion.[6,7]

HbA1c of 6.5% is recommended as the cut point for the diagnosis of diabetes.[8] Human Adult Hb consists of HbA (97% of the total, it is made up of 4 polypeptide chains, 2α and 2β), HbA_2 (2.5% , made up of 2α and 2δ) and HbF (0.5%, and made up of 2α and 2γ). Several minor hemoglobin's are identified in chromatographic analysis of HbA_1, namely, HbA_{1a}, HbA_{1b} and $HbA_{1c,}$ which are referred as HbA_1, fast hemoglobins, glycohemoglobins, or glycated hemoglobins .[6,9]

HbA1c is formed by combination of aldehyde group of glucose and hexoses non-enzymatically which binds with amino terminal of the β-chain of Hb, to form an unstable Schiff base (aldimine, pre-HbA1c) before undergoing an Amadori rearrangement to form a more stable ketoamine, HbA1c and this process is known as glycation of Hb occurs in life span of 120 days. So this characteristic of Hb biomarker is used to monitoring the average blood glucose levels.[9,4] So the measurement of HbA1c is used for checking blood sugar control in pre-diabetic patients and monitoring sugar levels in patients with elevated HbA1c.[10]

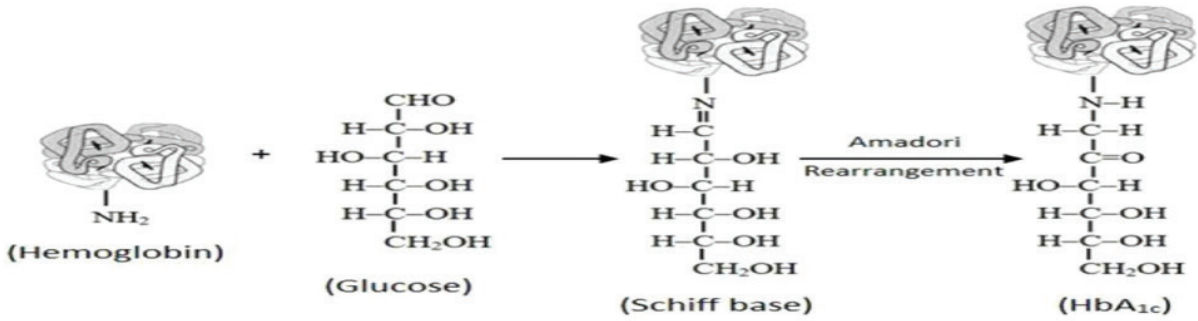

Figure 1. Formation of HbA1c[9]

Non-glycemic Variables affecting HbA1c

Non glycemic variables affecting HbA1c are age, ethnicity, gender, erythrocyte turnover, anaemia, pregnancy, Haemoglobin variants, thyroid disease, liver disease, HIV and Kidney diseases. It has long been recognized that hemoglobin variants interfere with HbA1c synthesis and measurement, and this interference depends on the nature of the congenital disorder afflicting hemoglobin synthesis and the analytical method that is used to measure HbA1c. [11] Thalassemia traits, HbS, HbC, HbE and HbF are among the most abundant hemoglobin-related interferences.[12]Additionally, other posttranslational modifications of hemoglobin such as carbamylation by uremic toxins in end-stage renal disease may significantly interfere with some HbA1c assays.[13] It should be noted that the majority of interferences have been mitigated by improvements of analytical methodologies, and the remaining interferences have been depicted and rigorously scrutinized.[14]

Age was found to be associated with a gradual increase of HbA1c levels in non diabetic individuals independently of sex and level of glycemia, indicating that age-specific reference intervals/clinical cut-off points may improve the clinical accuracy of this test in both the diagnosis and management of diabetes.[15] There are ethnic differences in HbA1c values even when glycemia levels are the same; a recent meta-analysis revealed that Caucasians have slightly lower HbA1c values in comparison to persons of other ethnic groups .[16] While the clinical relevance of this finding needs

to be further investigated, the understanding of the molecular mechanisms behind this observed between-race variability in HbA1c may improve its clinical applicability. Nonglycemic factors affecting HbA1c levels include erythropoiesis, hemoglobin synthesis and conditions influencing red blood cell survival. Deficiency anemias generally elicit falsely increased HbA1c levels due to the increased levels of aged erythrocytes that are found in patients with this disease, whereas falsely decreased HbA1c levels can be observed in hemolytic anemias of any cause.[17] Nonhematological conditions influencing HbA1c values include pregnancy, chronic renal failure and certain medications.[11] Variability in the normal erythrocyte lifespan is another significant confounder of HbA1c accuracy. Malka et al[18] recently proposed a mechanistic mathematical model integrating hemoglobin glycation and red blood cell kinetics that provided a personalized insight into average glucose levels and reduced the occurrence of diagnostic errors due to a misinterpretation of average glycemia (as reflected by HbA1c) by more than 50%. The applicability and clinical utility of the proposed model have yet to be determined.

The glycation extent of haemoglobin (glycated haemoglobin A1c) is an indicator of plasma glucose levels over the ~100 days prior to a venipuncture, and the laboratory assays for its quantification are steadily changing.[19,20] The remainder of glycoproteins are collectively termed fructosamines [21], and most of these are glycated albumin. Investigations into both HbA1c and fructosamine values are appropriate for clinical use. [22,23]The difference between the measured HbA1c value and the HbA1c value predicted from fructosamine values is termed the glycation gap. It has a broad distribution in patients with nephropathy (from −3.0% to 5.5%) .[24] Glycation gap values oscillating within >−0.5 and 0.5 was considered metabolically unfit. [25,26]Glycation gap evaluations currently comply with the reference change value criteria for glycaemic control, as documented by their stability in the follow-up of patients with type 2 diabetes.[27] To date, the glycation gap ranges in healthy controls are unknown. It has been suggested that glycation gap is not associated with chronic kidney disease in nondiabetic individuals .[28]

GLYCATED HB IN THE DIAGNOSIS OF DIABETES

Diabetes mellitus is a chronic non communicable disease characterized by hyperglycaemia and associated with long-term complications. DM management requires an accurate evaluation of glycaemic control. The traditional blood glucose estimations give results that are influenced by biological variability, variability due to pre-analytical factors (such as blood collection method and blood sex hormones in glucose homeostasis as well as to different attitudes and behaviours related to diabetes care.

In 1985 WHO report mentioned the utility of HbA1c in diabetes. By 2010 ADA and major expert committee and association across the globe recommended HbA1c for the diagnosis of type 2 diabetes mellitus.[4] In 2010 ADA-organized international expert committee and recommended adoption of HbA1c for the diagnosis of diabetes at a cut off 6.5%[9] clinical significance of HbA1c in T1DM was given by the Diabetes Control and Complications Trial (DCCT) and significance on T2DM was given by United Kingdom Prospective Diabetes Study. Measurement of HbA1C and blood glucose levels are used in routine management of patients with T1DM and T2DM. [10]

HbA1c measurement is recommended for checking blood sugar control in pre-diabetic and monitoring blood sugar control in patients with elevated level.[10]

Table 1: Factors that influence HbA1c and its measurement[10]

1.Erythropoiesis Increased HbA1c: Vitamin B12 deficiency, decreased erythropoiesis, Iron. Decreased HbA1c: Administration of erythropoietin, iron, vitamin B12, reticulocytosis and chronic liver disease.
2. Altered Haemoglobin Chemical or Genetic alterations in haemoglobin: Haemoglobinopathies, HbF, methaemoglobin, may increase or decrease HbA1c

Cont... **Table 1: Factors that influence HbA1c and its measurement[10]**

3.Glycation Increased HbA1c: Alcoholism, chronic renal failure, decreased intraerythrocyte pH. Decreased HbA1c: Aspirin, Vitamin C and E, certain intra-erythrocyte pH. Variable HbA1c: Genetic determinants.
4.Erythrocyte destruction Increased HbA1c: Splenectomy, increased erythrocyte life span, . Decreased HbA1c: Haemoglobinopathies, splenomegaly, rheumatoid arthritis or drug such as antiretrovirals, ribavirin and dapsone, decreased RBCs life span
5.Assays Increased HbA1c: Hyperbilirubinaemia, carbamylated haemoglobin, alcoholism, large doses of aspirin, chronic opiate use. Variable HbA1c: Haemoglobinopathies. Decreased HbA1c : Hypertriglyceridaemia.

Pathophysiology of Hyperglycemia

1. Non-enzymatic Glycation of proteins, mainly haemoglobin, low density lipoproteins, collagen and tubulin in pheripheral nerves leads to accumulation of advanced glycosylated end products causing injury by stimulating pro-inflammatory factors (complement and cytokines).

2. Polyol pathway: There is accumulation of sorbitol and fructose in the glucose metabolism by increased intracellular aldose reductase which leads to cell proliferation, changes in vascular permeability and capillary structure by stimulation of protein kinase C and transforming growth factor beta.

3. Abnormal microvascular blood flow impairs nutrient and oxygen supply. Microvascular occlusion due to vasoconstrictors (endothelins and thrombogenesis) and leads to endothelial damage.

4. Other factors formation of Reactive oxygen species and stimulation of growth factor TGF-β and VEGF. These growth factors are released from ischaemic tissues and causes proliferation of endothelial cells.[29]

METHODS OF MEASURING HbA1c[30]

Method of Testing	**Procedure**	**Advantage**	**Disadvantage**
Chromatography based HPLC assay	Assay uses an HPLC instrument and ion exchange or affinity column to separate HbA1c molecules from another hemoglobin molecules.	HbA1c overestimation leads to aggressive glucose management, resulting in more frequent hypoglycaemic episodes.	a. Altering the normal process of glycation of HbA to A1C. b. Causing an abnormal peak on chromatography, making estimation of A1C unreliable. c. Making the red blood cell more prone to hemolysis, thereby decreasing the time for glycosylation to occur and producing a falsely low A1C result.

Cont... **METHODS OF MEASURING HbA1c[30]**

Boronate affinity	Glucose binds to m-aminophenylboronic acid	Minimal interference from hemoglobinopathies, HbF, and carbamylated Hb	Measures not only glycation of N-terminal valine on β chain, but also β chains glycated at other sites and glycated α chains
Antibody based immunoassay	Uses a specific antibody (usually monoclonal) to the glucose and the first 5 to 10 amino acids of the β-chain. This antibody is latex coated. The agglutinator reacts with the antibody to give a scattering of light and an increase in absorbance. From this the amount of HbA1c is calculated, and the total hemoglobin can be determined by measuring at or near the Soret absorption band of hemoglobin (410 - 420nm) or by Drabkins method (oxidation and conversion to cyanmethemoglobin) at about 540nm, or using the alkali hematin assay.	Reduces the scattering of light and the absorbance.	a. Time required to complete the analysis. b. Technical skills required for handling. c. High price of reagents.
Enzyme based enzymatic assay	Lysed blood samples are subjected to proteolytic digestion. Glycated valines are released and serve as substrate for fructosyl valine oxidase. The produced hydrogen peroxide is measured using a horseradish peroxidase-catalyzed reaction with a chromogen	a. Enzymatic assay proved to be a robust and reliable method for HbA1c measurement suitable for routine practice in clinical chemistry laboratories. b. The assay is designed to report %HbA1c values directly without need for a separate measurement of total hemoglobin and is not adversely affected by interferences from common hemoglobin variants in samples.	A disadvantage of the enzymatic method is its relatively high cost.

Conclusion

Globally, the incidence and prevalence of type 1 and type 2 diabetes mellitus (DM) has increased significantly over the past two decades and is expected to continue to increase in the future. Diabetes is associated with several chronic complications that lead to increased morbidity and mortality. HbA1c is an accurate and easy-to-manage test with onsite results availability. It can be an effective tool for diagnosing and prognosis of diabetes, especially in low- and middle-income countries and in hard-to reach populations. Hyperglycemia is a key biochemical feature of diabetes that should be rigorously controlled and maintained in a range as close to normal as possible to mitigate the risk of diabetic complications. Both the level of and exposure to hyperglycemia, as well as glycemic variability, contribute to the pathogenesis of diabetic complications, with different patterns of disease pathogenesis in patients with type 1 or type 2 diabetes. Despite its analytical and biological limitations, HbA1c

remains the key biomarker of long-term glycemic control. However, it has become apparent in recent years that other glycated proteins, 1,5-AG, and integrated measures from direct glucose testing by SMBG/CGMS may provide valuable data complementary to HbA1c, particularly in circumstances when the HbA1c results may be unreliable or insufficient to assess the risk of adverse outcomes Long-term associations of these alternative biomarkers of glycemia with the risk of diabetic complications need to be investigated to provide clinically relevant cut-off values and validate their utility in diverse populations of patients with diabetes. Better understanding of the limitations of HbA1c may improve prediction and diagnostic accuracy, much needed in order to identify those individuals who require fast track into lifestyle modification and/or other therapeutic programs currently, used as a complementary test to FPG or OGTT remains likely to be the approach that is most beneficial.

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Role of Omega 3 Fatty acids, Vitamin D, Vitamin B12, Vitamin B6 and Folate in Mental wellbeing- A Short review of Literature

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Abstract

Neuropsychiatric conditions have been linked to around 14 percent of the global disease burden, mainly due to the chronically debilitating nature of depression and other widespread mental disorders, alcohol and drug use disorders, and psychosis. The science and lay press have confirmed that vitamin D is an important factor that can have major health benefits in the prevention and treatment of many chronic diseases. Most people have inadequate vitamin D levels in this country. This is also relevant for people with depression and other mental illnesses as well. Inadequate food consumption, lifestyle or other factors are a major causes of Vitamin D deficiency. Successful diagnosis and treatment of insufficient levels of vitamin D in people with depression and other mental illnesses could be a simple and cost-effective therapy that could enhance the long-term health outcomes and quality of life of patients. It is now well known that omega-3 fatty acids are important for physical health, and there is growing evidence that omega-3 fatty acids may also be important for mental health. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the two major omega-3 fatty acids in fish oil, have essential biological roles within the CNS. DHA is a major structural component of neuronal membranes, and modifying the neuronal membrane fatty acid composition contributes to functional changes in the behaviour of phospholipid membrane-embedded receptors and other proteins. EPA has major physiological roles that can influence the behaviour of neurons. For innumerable aspects of brain functioning, adequate nutrition is needed. A modifiable risk factor for depression may be the poor quality of diet. The goal was to evaluate and synthesize the existing understanding of the role of nutrition in mental well-being. The risk of depression is increased by low omega-3 fatty acid status. Both fish oil and folic acid supplements have been successfully used to treat depression. The response to antidepressants is decreased by folate deficiency. Deficiencies in folate, vitamin B12, Vitamin D, Omega 3 fatty acids, Vitamin B6 tend to be more prevalent in depressed individuals than in nondepressed individuals. In this short review of Literature we have focused on role of Omega 3 Fatty acids, Vitamin D, Vitamin B12, Vitamin B6 and Folate in Mental wellbeing.

Keywords : *Mental health, Vitamin B12, Vitamin D, Omega 3 Fatty acids, Folate, Vitamin B6*

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Introduction

Mental well-being is a core component of optimal health, and is a status that individuals can manage stress from daily living and make positive achievements pursuing public interest and contribution to the community. [1]

The World Health Organization (WHO) estimates that several hundred million people in the world suffer from a mental or behavioural disorder at the beginning of the new millennium. In addition, mental illnesses are the second leading cause of disability worldwide.[2] Mental disorders, which are the same as psychiatric disorders, are clusters of syndromes which disturb an individual's cognition, emotion regulation or behaviour. Common mental disorders include bipolar disorders (manic disorder, depression, and manic-depression), dementia, schizophrenia, and panic disorder. [3] Depression, the most common mental disorder, is one of the major global public health problems and projected to become the second leading cause of burden of disease by 2030.[4,5] Maintaining individual's mental health is important to improve personal life values, to reduce medical cost and other social expenses to deal with mental disorders, and to enhance national competitiveness. Several factors affecting the development of mental disorders include genetic factors, stress, diet, physical inactivity, drugs, and other environmental factors.[6-8]Among these factors, dietary factors may aggravate or ameliorate symptoms and the progression of the disorders although those are not major etiologies. Nutritional deficiencies, resulting from insufficient intake or absorption of nutrients critical to human health, are now a recognized risk factor for psychiatric disorders.[9] Nutritional factors having beneficial effect on mental health are polyunsaturated fatty acids (PUFAs), especially omega-3 FAs, phospholipids, cholesterol, niacin, folate, vitamin B6, vitamin B12, and vitamin D.[10-13]

Omega 3 fatty acids and mental health

Omega-3 fatty acids play a critical role in the development and function of the central nervous system. Emerging research is establishing an association between omega-3 fatty acids (alpha-linolenic, eicosapentaenoic, docosahexaenoic) and major depressive disorder. Omega-3 PUFAs play fundamental roles in the development, functioning and aging of the brain. In humans, dietary deficiencies of omega-3 PUFAs, such as docosahexaenoic acid and eicosapentaenoic acid, have been associated with an increased risk of various mental disorders. Omega-3 fatty acids are long-chain, polyunsaturated fatty acids (PUFA) of plant and marine origin. Because these essential fatty acids cannot be synthesized by the human body, they must be derived from dietary sources. Flaxseed, hemp, canola and walnut oils are all generally rich sources of the parent omega-3, alpha linolenic acid (ALA). Dietary ALA can be metabolized in the liver to the longer-chain omega-3 eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). This conversion is limited in human beings, it is estimated that only 5–15% of ALA is ultimately converted to DHA .[14] Aging, illness and stress, as well as excessive amounts of omega-6 rich oils (corn, safflower, sunflower, cottonseed) can all compromise conversion.[15] They have long been investigated for their cardio protective and anti-inflammatory roles, which has lead to their increased use as dietary supplements. [16]A new application for omega-3 fatty acids has emerged recently, the treatment of certain forms of mental illness. Such a use is biologically plausible given that omega-3 fatty acids, in particular DHA, are abundant in the brain and are involved in, or modulate, the mechanism by which brain neurons communicate .[17] The role of omega 3 fatty acids in improving cognitive functions has been extensively studied in a variety of populations ranging from infants to the elderly, and from healthy individuals to patients with psychiatric, neurodegenerative or neurodevelopment disorders. For example, omega 3 fatty acids supplementation has been shown to improve depressive symptoms and verbal fluency in elderly with mild cognitive impairments. They have been shown to alter the functioning of neural systems utilising dopamine and serotonin, both of which are thought to play an important role in mental illness and are major targets of psychoactive medications. [16,17]Furthermore, animal models of mental illness have suggested that omega-3 fatty acids can affect brain processes such as those that control mood and anxiety.[18-20]

Vitamin D and mental Health

Vitamin D deficiency is commonly defined as levels of 25-hydroxyvitamin D (25OHD) less than 25 nmol/L, insufficiency as between 25 and 50 nmol/L and sufficiency as greater than 50 nmol/L. Its RDA is 400 IU or 10 mg, it binds to the receptor of target cells and regulate through gene expression.[21] Poor diet, lack of sun exposure, decreased synthesis of vitamin D and decreased renal hydroxylation of 25(OH) D due to old age are the main cause of Vitamin D deficiency. [22]

Nearly 30 percent to 50 percent of people are estimated to have deficiency of vitamin D, and insufficiency and vitamin D deficiency are recognized as global health issues in the world. [23] Scientific findings about the role of vitamin D in healthy people and patients with numerous diseases increasingly accumulate in medical literature. [24]The fact that, taking into account recommended optimal values, about a third or more of adult population in societies with higher life standard had a deficiency of vitamin D, raises additional, widespread concern. [25,26] Vitamin D3 (cholecalciferol) is taken in the diet (fortified dairy products and fish oils) or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation. It is transported in the blood by the vitamin D binding protein (DBP) to the liver. In the liver it is hydroxylated, resulting in the formation of 25 hydroxyvitamin D3 (25(OH) D3). This is then transported to the kidney by DBP. In the proximal renal tubule 25(OH)D3 is hydroxylated, resulting in the hormonally active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), which is responsible for most if not all of the biologic actions of vitamin D.[27] Vitamin D is crucial for several key physiological processes, including brain development, DNA repair, and regulation of many genes. Evidence indicates that prenatal and early postnatal vitamin D deficiency increases autism risk, probably through multiple effects, including impaired brain development and increased de-novo mutations.[28] Vitamin D receptors (VDR) were found in neurons and glial cells in brain areas responsible for the development of depression and suggesting a role of vitamin D for development of some mental disorders.[29,30] Vitamin D helps protect against oxidative stress, which is a key cause of DNA damage, and also aids in the repair of DNA damage once it occurs [31,32]

Vitamin B12 and Mental Health

Vitamin B12 plays a crucial role in cell reproduction, normal erythropoiesis, nucleoprotein and myelin synthesis, normal growth, DNA synthesis, and one carbon metabolism.[33] Normal daily requirement is about 1 µg.[34] Vitamin B12 helps in synthesis of methionine from homocysteine and conversion of methylmalonylcoA to succinylcoA. Methionine is converted to SAM which donates its methyl group to myelin, membrane phospholipids and various neurotransmitters and free THF is liberated from N5 methyl THF which is used in synthesis of purine, pyrimidine and nucleic acid.[35]An elevated level of Hcy as a neurotoxin was also shown to affect the redox signalling pathways in neurons through the generation of reactive oxygen species (ROS) and a decrease in endogenous antioxidants. If patterns of DNA methylation in redox-related genes can modulate cognitive impairment caused by vitamin B12 deficiency and hyperhomocysteinaemia is therefore of interest, low levels of vitamin B12 can cause serious cognitive dysfunction.[36] Psychiatric symptoms attributable to vitamin B_{12} deficiency have been described for decades. The earlier studies are for the most part in accord with more recent ones, despite being diagnostically less precise in psychological and hematologic terms. These symptoms tend to fall into many clinically distinct categories: slow cerebration; confusion; memory changes; delirium, with or without hallucinations and/or delusions; depression; acute psychotic states; and more rarely) reversible manic and schizophreniform states.[37]

Folate and Mental Health

Folate deficiency and insufficiency are common among patients with mood disorders and correlate with illness severity.[38] Folate deficiency may specifically affect central monoamine metabolism and aggravate depressive disorders.[39] There must be two reasons of folate deficiency, the biologically active form of folate, L-methylfolate, may act as a trimonoamine modulator and enhance the synthesis of three monoamines including dopamine, norepinephrine, and serotonin, and this has been reported to be involved in the efficacy of antidepressant. Patients with a methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism produce a less active form of the enzyme. The TT genotype is associated with major depression and bipolar disorder.[40] Dietary folate must be converted to L-methylfolate for use in the brain. Other potential explanation may be due to malnutrition or poor nutrition intake.

Vitamin B6 and Mental Health

Vitamin B6 deficiency has been associated with neuropsychiatric conditions, including seizures, migraines, chronic pain and depression. In the control of mental function and mood, vitamin B6, containing three chemically distinct compounds, pyridoxal, pyridoxamine, and pyridoxine, is involved. Vitamin

B6 is also an important re-methylation cofactor for homocysteine, and deficiency is associated with an increase in levels of homocysteine in the blood. Homocysteine is a cerebrovascular disease risk factor and may also have a direct toxic impact on central nervous system neurons. [41]

Conclusion

Nutrition deficiency leads to serious mental disorders, as body require adequate amount of nutrition for its proper functioning. Poor nutrition status in mental disorders leads to folate deficiency which appears to predominate in melancholic depression; anorexia and weight loss are common features of this type. In these settings, supplementation with folate or its derivative, methylfolate, may be found to play an important role in effective antidepressant treatment. Both folate and vitamin B12 deficiency may cause similar neurological depression, dementia, and a demyelinating myelopathy. Deficiency of omega 3 fatty acids, vitamin D, vitamin B12, folate, vitamin B6 are commonly seen in mental disorders, so adequate amount of nutrition and supplements should be given to reverse the cause of damage.

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Ethical Clearance- Not Required

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Clinical Indicators for Quality Improvements-A Mini Review of Literature

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Abstract

The quality of health care is on the agenda in most health care systems. Much of this interest in quality of care has developed in response to recent dramatic transformations of health care systems, accompanied by new organizational structures and reimbursement strategies that may affect quality of care. Assessing the quality of care has become increasingly important to providers, regulators, and purchasers of care. Indicators for performance and outcome measurement allow the quality of care and services to be measured. This assessment can be done by creating quality indicators that describe the performance that should occur for a particular type of patient or the related health outcomes, and then evaluating whether patients' care is consistent with the indicators based on evidence-based standards of care. Quality of care can be defined as 'the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge', and can be divided into different dimensions according to the aspects of care being assessed. This paper will focus define clinical indicators in a standard way for a global audience, and to review a few classifications of clinical indicators that may be useful to those who wish to measure quality of care and describe the performance of health care and related outcomes.

Keywords- *Indicators, Quality, Healthcare*

Introduction

Introduction to indicators

Indicators provide a quantitative basis for clinicians, organizations, and planners aiming to achieve improvement in care and the processes by which patient care is provided. Indicator measurement and monitoring serve many purposes. They make it possible to: document the quality of care; make comparisons (benchmarking) over time between places (e.g. hospitals); make judgments and set priorities (e.g. choosing a hospital or surgery, or organizing medical care); support accountability, regulation, and accreditation; support quality improvement; and support patient choice of providers. The use of indicators enables professionals and organizations to monitor and evaluate what happens to patients as a consequence of how well professionals and organizational systems function to provide for the needs of patients. Indicators are, however, not a direct measure of quality. Because quality is multidimensional, understanding quality requires many different measures.

Indicators have been defined in several different ways:

1. Indicators measures a particular health care process or outcome.[1]

2. Indicator are used as quantitative measures that can be used to monitor and evaluate the quality of important governance, management, clinical, and support functions that affect patient outcomes.[2]

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3. Indicators are used as measurement tools, screens that are used as guides to monitor, evaluate, and improve the quality of patient care, clinical support services, and organizational function that affect patient outcomes.[3]

Following are the key characteristics of an ideal indicator:

(i) Indicator is based on agreed definitions, and described exhaustively and exclusively

(ii) Indicator is highly or optimally specific and sensitive, i.e. it detects few false positives and false negatives

(iii) Indicator is valid and reliable

(iv) Indicator discriminates well

(v) Indicator relates to clearly identifiable events for the user (e.g. if meant for clinical providers, it is relevant to clinical practice)

(vi) Indicator permits useful comparisons

(vii) Indicator is evidence-based. [4]

Purpose of the use of indicator

The use of indicators enables professionals and organisations to monitor and evaluate what happens to patients as a consequence of how well professionals and organisational systems function to answer the needs of patients. However, indicators are not a direct measure of quality. As quality is multidimensional, understanding quality requires many different measures. Indicators provide a quantitative basis for clinicians, organisations and planners aiming at achieving improvements in care and the processes by which care is provided.

Indicator measuring and monitoring serve many purposes making it possible to:

· Document the quality of care

· Make comparisons and benchmarking over time between places (e.g. units, hospitals)

· Make judgments and set priorities (e.g. choosing a hospital or surgery or organising medical care)

· Support accountability, regulation, and accreditation

· Support quality improvement − Support patients' choice of providers.[5,6]

Quality Characteristics of Indicators [7]

Quality characteristics of the indicators		
1	Relevancy	Relevant to important aspects (effectiveness, safety, and efficiency) and dimensions (professional, organisational, and patient oriented) of quality of care.
2	Validity	Strong correlation with the current quality of care - Valid on the basis of good scientific proof and experience
3	Reliability	Low inter and intra observer variation - Available and reliable date sources - Statistically reliable, i.e. reported as an average or median with confidence intervals and valid for comparison, i.e. corrected for case mix and sociodemographic variables.
4	Feasibility	Easily available - Applicable to quality improvement; i.e. easy to build in improvement initiatives - Sensitive to improvement in time - Useful to base decisions on (caregivers, patients, regulating agencies) - Applying to those who should use them

Criteria used to evaluate potential quality indicators

Face validity: An adequate quality indicator must have sound clinical or empirical rationale for its use. It should measure an important aspect of quality that is subject to provider or health care system control.

Precision: An adequate quality indicator should have relatively large variation among providers or areas that is not due to random variation or patient characteristics. This criterion measures the impact of chance on apparent provider or community health system performance.

Minimum bias: The indicator should not be affected by systematic differences in patient case mix, including disease severity and comorbidity. In cases where such systematic differences exist, an adequate risk-adjustment system should be possible using available data.

Construct validity: The indicator should be related to other indicators or measures intended to measure the same or related aspects of quality. For example, improved performance on measures of inpatient care (such as adherence to specific evidence-based treatment guidelines) ought to be associated with reduced patient complication rates.

Fosters real quality improvement: The indicator should be robust to possible provider manipulation of the system. In other words, the indicator should be insulated from perverse incentives for providers to improve their reported performance by avoiding difficult or complex cases, or by other responses that do not improve quality of care.

Application: The indicator should have been used in the past or have high potential for working well with other indicators. Sometimes looking at groups of indicators together is likely to provide a more complete picture of quality.[8]

Types of Indicators

(i) Structural indicators

'Structure' refers to health system characteristics that affect the system's ability to meet the health care needs of individual patients or a community. Structural indicators describe the type and amount of resources used by a health system or organization to deliver programs and services, and they relate to the presence or number of staff, clients, money, beds, supplies, and buildings. The assessment of structure is a judgement on whether care is being provided under conditions that are either conducive or inimical to the provision of good care.[9,10]

Examples of structural indicators

- Proportion of specialists to other doctors
- Access to specific technologies (e.g. MRI scan)

· Access of specific units (e.g. stroke units)

· Clinical guidelines revised every 2nd year

· Physiotherapists assigned to specific units

(ii) Process indicators

Process indicators assess what the provider did for the patient and how well it was done. Processes are a series of inter-related activities undertaken to achieve objectives. Process indicators measure the activities and tasks in patient episodes of care. Some authors include the patient's activities in seeking care and carrying it out in their definition of the health care process. Others limit this term to care that health care providers are giving. It may be argued that providers are not accountable for the patient's activities and these, therefore, do not constitute part of the quality of care, but rather fall into the realm of patient characteristics and behaviour that influence patients' health outcomes.

Example of Process indicators

· Proportion of patients with diabetes given regular foot care

· Proportion of patients with myocardial infarction who received thrombolysis

· Proportion of patients assessed by a doctor within 24 hours of referral

· Proportion of patients treated according to clinical guidelines

(iii) Outcome indicators

Outcomes are states of health or events that follow care and that may be affected by health care. An ideal outcome indicator would capture the effect of care processes on the health and wellbeing of patients and populations. Outcomes can be expressed as the five Ds

The Five Ds:

(i) Death: a bad outcome if untimely

(ii) Disease: symptoms, physical signs, and laboratory abnormalities

(iii) Discomfort: symptoms such as pain, nausea, or dyspnea

(iv) Disability: impaired ability connected to usual activities at home, work, or in recreation

(v) Dissatisfaction: emotional reactions to disease and its care, such as sadness and anger.

Example of Outcome indicators

Intermediate

· HbA1c results for diabetics

· Lipid profile results for patients with hyperlipidaemia

· Blood pressure results for hypertensive patients

End result (should be specified for diseases)

· Mortality

· Morbidity

· Functional status

· Health status measurement

· Work status

· Quality of life Patient satisfaction

Research methods on the application of indicators

Acceptability

The acceptability of the data collected using a measure will depend upon the extent to which the findings are acceptable to both those being assessed and those undertaking the assessment. For example, the iterated consensus rating procedure consults lay professionals as to the acceptability of indicators. Campbell and colleagues conducted a quality assessment in 60 general practices in England but only used quality indicators rated acceptable and valid by the nurses and doctors working in the practices.[11]

Feasibility

Information about the quality of services is often driven by data availability rather than by epidemiological and clinical considerations.[12] Quality measurement cannot be achieved without accurate and consistent information systems.[13,14] Current administrative data, both at the macro (health authority

or "large organisation") and micro (individual medical records) levels, are constrained by inconsistent and often unreliable data.[15]Medical records are a poor vehicle for collecting data on preventive care and the recording of symptoms.[16] In addition, aspects of care being assessed by quality indicators must relate to enough patients to make comparing data feasible

Reliability

Indicators should be used to compare organisations/ practitioners with similar organisations/practitioners, or confounding factors such as socioeconomic and demographic factors, as well as factors outside the control of practitioners, should be taken into account (that is, compare like with like or risk/case mix adjust). This is because the environment in which an organisation operates affects the care provided.

Examples include admission rates or surgery rates. Indicators must also have explicit exclusion and inclusion criteria for applying the indicator to patients-for example, age ranges co-morbidities, case mix, and clinical diagnoses. The inter-rater reliability of an indicator can also be tested when applying indicators

Sensitivity to change

Quality measures must be capable of detecting changes in quality of care in order to discriminate between and within subjects.[17] This is an important and often forgotten dimension of Lawrence's definition of a quality indicator.

Conclusion

This review article shows that the indicators are instruments to evaluate and improve the quality of care. Clinical indicators measure the extent to which set targets are achieved. They are expressed as numbers, rates, or averages that can provide a basis for clinicians, organizations, and planners aiming to achieve improvement in care and the processes by which patient care is provided. They can be measures of structure, process, and outcome, either as generic measures relevant for all diseases, or disease-specific measures that describe the quality of patient care related to a specific diagnosis. For the healthy population, indicators can also be important with regard to prevention, quality of life, and satisfaction with health care.

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Leukotrienes and Inflammation –A Review

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Abstract

Leukotrienes, together with the prostaglandins and other related compounds, are derived from 20 carbon (eicosa) fatty acids that contain double bonds (enoic). Hence this group of substances is called the eicosanoids. The name leukotriene derives from the original discovery of these substances in white blood cells (polymorphonuclear leucocytes) and the fact that they all have in common 4 double bonds (hence the 4 subscript), 3 of which are in a conjugated triene structure. Leukotrienes do not exist preformed in cells. They are formed from the breakdown of arachidonic acid, a polyunsaturated 20 carbon fatty acid. In its esterified form, arachidonic acid is bound to the phospholipids of the cell membranes. Both immunological and non-immunological stimuli can release arachidonic acid from membrane phospholipids by activating phospholipase A_2. The glucocorticosteroid drugs can inhibit phospholipase A_2 and thereby decrease the production of all the leukotrienes and hence leukotriene-mediated responses. Generally, inflammation leads to vasodilation, vascular hyperpermeability, increased blood flow and recruitment of leukocytes to inflamed sites. These events cause enhanced production of cytokines, chemokines, chemical mediators and lipid mediators such as LTs and prostaglandins. Acute inflammation occurs over a short time (seconds, minutes and hours). In contrast, chronic inflammation is a long-lasting inflammatory and immune response that occurs over months to years and results in diverse diseases including asthma, allergies, atherosclerosis, arthritis, obesity, cancer and other age-related diseases such as AMD. In this review article we aimed to highlight the evidence that implicates LTs in physiological function and also in disease processes.

Keywords – *Leukotrienes, Inflammation, Cardiovascular Disease, Asthma, Rheumatoid Arthritis.*

Introduction

The name "leukotriene" is referring to the cellular source (leukocytes are one of the major sources) as well as the conjugated triene that characterizes their structure. Lipid mediators, which denote bioactive mediators derived from lipids, play roles in immune regulation, self-defense, and the maintenance of homeostasis in living systems. They include prostaglandins (PGs) and leukotrienes (LTs), lysophospholipids (including sphingosine 1-phosphate), and endocannabinoids. Leukotrienes, together with prostaglandins, thromboxanes, and lipoxins, are the major constituents of a group of biologicallyactive oxygenated fatty acids known as eicosanoids [1] and constituent family of lipid mediators with potent biological activities.[2] Because myeloid cells contain substantial amounts of esterified arachidonic acid (AA)[3] and constitutively express all of the enzymes necessary to hydrolyze it and metabolize it via the5- lipoxygenase (5-LO) pathway, they are capable of generating large quantities of products termed leukotrienes (LTs) within seconds to minutes after encountering an activating stimulus. The systemic name of arachidonic acid is 5,8,11,14- eicosatetraenoic acid, symbolized as C20:4, indicating a total of 20 carbons (twenty in greek is eicosi) and presence of four

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doubal bonds at the indicated positions.[4] LTs, which are derived from arachidonic acid (5Z,8Z,11Z,14Z-eicosatetraenoic acid; AA) through two steps catalyzed by 5-lipoxygenase (5-LO), are inflammatory mediators that function in normal host defense and play roles in inflammatory diseases.[5,6] Leukotrienes (LTs) are short-lived lipid mediators, which act in an autocrine and paracrine manner. They can be subdivided into two groups: the first group is represented by leukotriene B4 (LTB4) alone. The second group is constituted by the cysteinyl leukotrienes (cys-LTs), namely leukotriene C4 (LTC4), leukotriene D4 (LTD4) and leukotriene E4 (LTE4).[7,8]

Inflammation is the response of living tissue to damage. The inflammatory process is the reaction of blood vessel, which brings about an accumulation of fluid and white blood cell in the extravascular tissue.1 The acute inflammatory response has functions of destroying and eliminating the components of exudate. The damaged tissue can be broken down and partially liquefied, and the debris removed from the site of damage.[9]1 Inflammation is caused by release of chemicals from tissues and migrating cells after injury. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, more recently, platelet activating factor (PAF) and interleukin-1 and other various mediators.[10]

DISEASES THAT HAVE ROLE OF LEUKOTRIENE

Allergic diseases: Asthma, Allergic rhinitis, Rhino sinusitis, Atopic dermatitis, Urticaria, Allergic fungal sinusitis.

Fibrotic diseases: Airway remodeling in asthma, Bronchiolitis obliterans after lung transplantation, Idiopathic pulmonary fibrosis, Scleroderma, Asbestosis.

Other pulmonary syndromes: Acute lung injury or adult respiratory distress syndrome, Viral bronchiolitis, Obstructive sleep apnea, Chronic obstructive pulmonary disease, Cystic fibrosis and other forms of bronchiectasis, Bronchopulmonary dysplasia.

Other local inflammatory diseases: Arthritis (including osteoarthritis and gout), Glomerulonephritis, Interstitial cystitis, Psoriasis, Inflammatory bowel disease.

Systemic inflammatory diseases: Rheumatoid arthritis, Vasculitides (systemic lupus erythematosus, Churg–Strauss syndrome, Henoch–Schonlein purpura), Transplant rejection.

Cancer: Solid tumors (including melanoma, mesothelioma, and pancreatic, lung, esophageal, prostate, and colon cancers), Leukemias, Lymphomas, etc.

Cardiovascular disease: Atherosclerosis, Aortic aneurysm, Sickle cell crisis, Ischemia–reperfusion injury, Pulmonary arterial hypertension, Sepsis.[11,12]

Biosynthesis of Leukotrienes

In response to a hormonal or other stimulus a specific phospholipase-A2 present in most types of cells attacks membrane phospholipids releasing arachidonic acid. Phospholipase-A2 is specific for the carbon-2 position of the phospholipids, to which arachidonic acid is attached. After the release of arachonic acid is released into the cytosol, it can follow on of the 2 pathways. One is cyclooxygenease pathway (Produces prostaglandins and thromboxanes), and other is Lipooxygenease pathway (Produces Leukotrienes).

Lipooxygenease Pathway For Synthesis of Leukotrienes.

Lipooygenease catalyzes the addition of a single oxygen molecule in the arachidonic acid to form Hydroperoxyeicosatetraenoic acids (HPETEs). Three Lipooxygenases are present in human cells-5-Lipooxygenase, 12-Lipooxygenase and 15-Lipooxygenase. They operate in same manner but insert the oxygen at different places in the arachidionic acid chains. Different cells contains different lipooxygenease, like 5-Lipooxygenase is rich in Neutrophil and leukocytes. Platelets are rich in 12-lipooxygenase. Eosinophil leukocytes are rich in 12-lipooxygenase. Only 5-lipooxygenase forms leukotrienes and they are present in leukocytes and converts arachidonic acid to 5-hydroxyperoxyeicosatetraenoic acid. This is converted to leukotriene-A4, the precursor of other leukotrienes.[13]

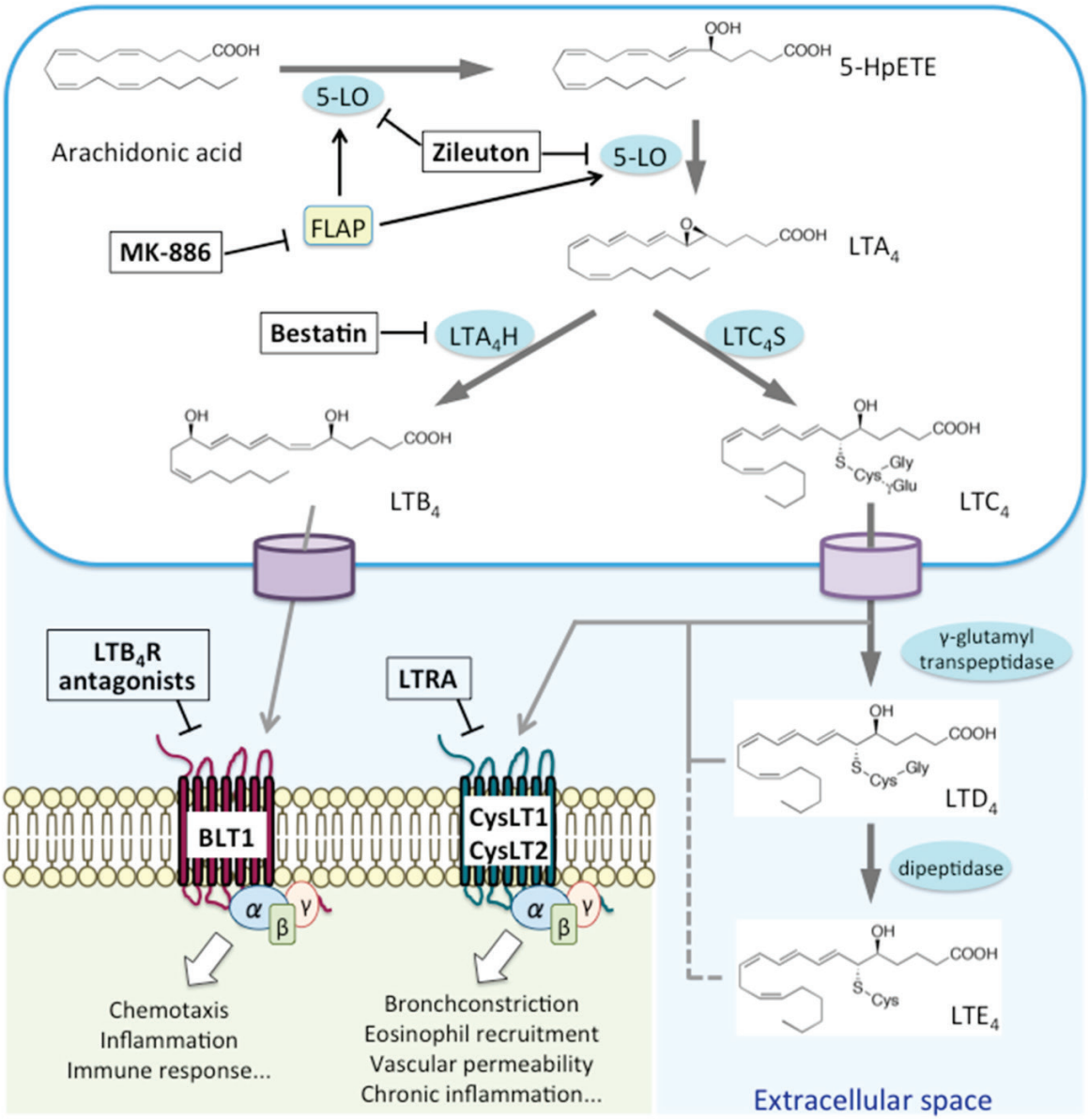

Figure 1- Receptors and Biosynthetic pathway of Leukotrienes[14]

Source of Figure - Jo-Watanabe A, Okuno T, Yokomizo T. The role of leukotrienes as potential therapeutic targets in allergic disorders. International journal of molecular sciences. 2019 Jan;20(14):3580.

Mechanism

Leukotrienes exert their effects in an autocrine or paracrine manner by binding receptors. These receptors are G protein-coupled (GPCR) receptors that activate a G protein once it is bound. Either the Gq protein, which leads to increases in intracellular calcium, is activated by leukotriene receptors, or the Gi protein, which leads to decreases in intracellular cAMP. A cascade of kinase reactions is then signalled by each of these G proteins, leading to changes in both transcriptional activity and cellular motility. There are both typical and distinctive effects of the various forms of leukotriene. In general, LTB and cysteinyl leukotrienes exert various effects across different receptor binding groups.LTB binds to receptors 1 and 2 (BLT1 and BLT2) of B leukotriene, respectively. Most importantly, LTB functions as a potent chemotactic neutrophil receptor. This activity highlights the inflammatory propellant aspect of leukotrienes, as the main LTB products are also neutrophils. Cysteinyl leukotrienes bind to cysteinyl leukotriene receptors type 1 and type 2 (cysLT1 and cysLT2), respectively). Airway

shifts, including bronchoconstriction, airway edema, and mucus secretion, are mainly regulated by cysLT1. On the other side, cysLT2, as it evokes increases in vascular permeability and tissue fibrosis but has no effect on the airways, is primarily an inflammatory stimulator. It is worth noting that increases in vascular permeability induced by leukotriene are 3 to 4 times more potent than histamine.[15]

Sites of Leukotrienes Biosynthesis

The cellular distribution of the enzymes regulating each stage of the biosynthetic pathway determines the locations in which the leukotrienes are synthesised. The distribution of 5-lipoxygenase is restricted to a specific number of myeloid cells: neutrophils, eosinophils, monocytes, macrophages, mast cells, basophils, and B lymphocytes. The distribution of 5-lipoxygenase is limited to a specific number of myeloid cells: neutrophils, eosinophils, monocytes, macrophages, mast cells, basophiles, and B lymphocytes. Most of these cells, with the exception of human monocytes and macrophages, contain large amounts of either LTB4 or LTC4, but not both. All other cells known as leukotriene secretors have been shown to almost exclusively release either LTB4 or C4.Neutrophils have been shown to synthesise significant quantities of LTB4, possessing 5-LO and LTA4-hydrolase, whereas eosinophils and mast cells preferentially synthesise LTC4, according to the presence within these cells of 5-LO and LTC4-synthase.[16,17]

Leukotrienes Receptors

The LTC4, LTD4, and LTE4 cysteinyl leukotrienes are lipid inflammation mediators that function through two G protein-coupled receptors (GPCRs), type 1 cysteinyl leukotriene receptor (CysLT1R) and type 2 (CysLT2R)1. Although LTD4 is CysLT1R2's preferred endogenous ligand, CysLT2R responds to LTC4 and LTD43 similarly. CysLTRs have bronchoconstrictive and pro-inflammatory effects and are also considered to play a role in asthma, allergic rhinitis, cardiovascular disease and cancer.Several selective CysLT1R antagonists have been approved as antiasthmatic drugs, such as zafirlukast, pranlukast, and montelukast, but a significant fraction of patients do not respond to this therapy. In physiology and pathology, the various expression profiles, tissue distribution, and endogenous ligand sensitivity for CysLTRs, their heterodimerization and cross-regulation, as well as the prevalence of asthma-associated polymorphisms in CysLT2R suggest different functions for each receptor subtype. On the basis of an animal asthma model induced by LTC4, it was proposed that CysLT2R-selective or dual antagonists can improve treatment of cases of severe asthma.[18]

Cysteinyl leukotrienes

Bioconversion of LTC4 into LTD4 and LTE4 does not seem to be a catabolic inactivation because for most biological activities, LTD4 is at least as potent as LTC4, and LTE4 seems to be only marginally less potent. Infusion of radio-labeled LTC4 and LTE4 in ordinary subjects results in rapid bloodstream disappearance along with identification of fractional quantities of LTE4 in the urine within the first 2 hours.[19] Later on, considerable quantities of omega- and betaoxidized LTE4 metabolites are found in urine.[20] A multiple increase in urinary LTE4 excretion reported in patients with severe liver dysfunction indicates that the liver could be the source of CysLT catabolism.[21]

Leukotriene B4

No urinary metabolites of LTB4 have been identified so far, unlike what has been observed for Cys-LTs. In purified polymorphonuclear leukocyte preparations, LTB4 undergoes rapid metabolism to deliver 20-hydroxy LTB4 and 20-carboxy LTB4 .[22] This conversion is catalysed by a particular enzyme called cytochrome P-450, but occurs primarily after the release of intact LTB4 and reuptake by adjacent cells.[23] In view of the decreased biological activity of the 20-carboxy metabolite, omega-oxidation may be a mechanism for the local inactivation of LTB4. On the other hand, in monocytes, eosinophils, and macrophages, this metabolism is not observed, and insufficient evidence exists that it can occur in vivo.

Role of Leukotrienes in Inflammation

Leukotrienes and Cardiovascular Disease

The potential participation of LTs in the production of damage caused by myocardial infarction has been of great concern in recent years. An increased risk of stroke and myocardial infarction (MI) is associated with the genetic variants within the 5-LO pathway; in addition,

the development of CysLTs increases in ischaemia-reperfusion injury in both patients and animal models. LTs are difficult to measure reliably in the blood because of their rapid metabolism and excretion, while elevated plasma concentrations of these mediators have been recorded after acute MI. They affect coronary vascular resistance, infarct size, pulmonary vascular resistance, bronchial tone, and renal vascular resistance directly or indirectly; in addition, they are main inflammatory regulators and thus potential targets to influence healing after MII.[24]

Leukotrienes and Asthma

LTs have been assessed in asthmatic patients' exhaled breath condensate (EBC), sputum, BAL fluid and urine. In patients with asthma, sputum CysLT concentrations are elevated, indicating asthma severity . In patients with asthma, particularly those with nocturnal asthma, LT concentrations are increased in the BAL fluid. Measurement of LTs is likely to indicate pulmonary synthesis of LTs in BAL fluid, sputum and EBC. To test the systemic synthesis of CysLTs, urinary measurement of LTE4, the most abundant CysLT excreted in the urine, is used as circulating concentrations of LTs are normally undetectable. No or only minor variations between healthy and atopic asthmatic subjects in urinary LTE4 concentrations have generally been documented under basal conditions. Urinary LTE4 excretion is elevated in atopic asthmatics after allergen challenge, in aspirin-sensitive asthmatics in nocturnal asthma.[25]

Rheumatoid arthritis and Leukotrienes

Rheumatoid arthritis (RA) is among the most prevalent autoimmune diseases (1-3% worldwide). RA is a prototypic inflammatory disease, characterized by a changed homeostasis state in which immunological stimulation and unwanted inflammation take precedence. The disordered inflammation has painful and deteriorating immediate effects while causing accumulated tissue damage that could contribute to symmetric polyarthritis leading to lifelong discomfort, impairment and shorter life expectancy.[26] LTs are allowed to play an increasingly significant role in the pathophysiology of Inflammatory disorders, particularly those events that occur in this includes activation of leukocytes and control by LTB4 of proinflammatory cytokines and suggests that this The receptor-ligand pair leads to the inflamed joint's recruitment of leukocytes. Leukocytes, immunocomplex, and rheumatoid factor are linked to RA synovium, At the stage of LTB4, and LTs, LTB4 in particular, RA bone remodelling was also involved. Local blood flow is regulated by Proinflammatory PGs and LTB4, Vascular dilation and changes in the permeability required at the Place for adhesion, diapedesis and recruitment of leukocytes. To date, drugs that inhibit 5-LOX have been approved for Diseases of the bones. Provided that LTB4 is also a strong inducer of Migration by neutrophils. In synovium, this effect may have wider functional significance.[27]

Allergic rhinitis and Leukotrienes

CysLTs are synthesised by mast cells and basophils during the early phase of antigen response and by eosinophils and macrophages during the late phase via the 5-lipoxygenase metabolism of arachidonic acid. In patients with allergic rhinitis, the levels of cysLT in nasal secretions are elevated after short-term allergen instillation and in the allergy season. By interacting with receptors, particularly the cysLT1 receptor, on target cells, these lipid mediators function locally and systemically. Evidence obtained from topical application of cysLTs in the nose and from the effects of LTRAs suggests that nasal mucous secretion, congestion, and inflammation are caused by cysLTs.By improving immune responses and the formation, adhesion, migration, and survival of inflammatory cells such as eosinophils, CysLTs promote allergic inflammation. They also increase the generation of a number of other proinflammatory mediators, such as cytokines, which in turn increase the development of cysLTs and receptors. CysLTs fulfil the requirements for relevant allergic rhinitis mediators through their various effects on the structural components of disease that are immune, inflammatory, and local. LTRAs provide a valuable approach to the treatment of this severe and widespread condition by blocking the cysLT1 receptor responsible for most of these symptoms.[28]

Conclusion

Leukotrienes are inflammatory chemicals, they released from body after coming into contact with an allergen. Leukotrienes cause airway muscles to contract and excess mucus and fluid to be created. By binding to unique G-protein-coupled receptors, they exert their biologic effects. In this review article we

aimed to highlight the evidence that implicates LTs in physiological function and also in disease processes.

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Recent Updates in Female Infertility: A Short Review of Literature

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Abstract

Infertility is defined as the inability to conceive after 1 year of unprotected intercourse. It has been estimated that 93% of healthy couples practicing unprotected intercourse should except to conceive within 1 year. Females presenting after this time should therefore be regarded as possibly infertile and should be evaluated. Although infertility is considered by some as primarily a woman's problem, men often contribute to and are also affected. Infertility problem often arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. Human infertility is a complex problem, which has numerous consequences depending on the society and cultural background, age, hormone problems, environmental problem, gender, lifestyle, sexual history, of the people it affects. Infertility is a global public health concern this is partly due to its complexity in etiology as well as difficulty in preventing, diagnosing and treating it.

Keyword: *Female infertility, Testosterone, Endometriosis, Prolactin, PCOD*

Introduction

Parenthood is considered one of the most important life achievements in the Indian society.[1] It is widely accepted that human existence reaches completeness through a child and fulfils the individual's need for reproduction.[2] Infertility disrupts the basic social structure (Family-building) and there by the domestic and social economic wellbeing of a couple is not achieved. Although good documentation of the prevalence of infertility is lacking, it is generally believed that more than 70 million couples suffer from infertility worldwide. [2,3] In India the prevalence of primary infertility was 12.6 percent.[4] Conception normally is achieved within (12) months in (80-85) of couples who use no contraceptive measures.

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Females presenting after this time should therefore be regarded as possibly infertile and should be evaluated. Data available over the past (20) years reveal that in approximately (30%) of cases, pathology found in the women alone, and in another (20%) both the man and woman are abnormal. [5-6] The women reproductive years begin when she starts her menstrual cycle during puberty (about the age 13) years, and the ability to have a child usually ends around the age (45) years, although it is potentially possible for a women to be pregnant until her periods end with menopause (about the age 51) years. [7] Born girl already carries in her body about (400000) immature eggs (oocytes). These are stored in her ovaries in tiny fluid-filled sacs called follicles. Once she enters her reproductive years, she starts having monthly one egg (or, less commonly, more than one), which may join with a male motile sperm cell during fertilization and being a pregnant. [8] The development and release of the egg depend largely on a delicate balance of hormones (chemicals that signal the body organs to do a particular task). Some of these hormones are produced in the ovaries, others from the two glands in the brain, the

hypothalamus and the pituitary. [9] Primary infertility is a term used to describe a couple that has never been able to conceive after a minimum of one year of attempting to do so through unprotected intercourse. Causes of primary infertility include a wide range of physical as well as emotional factors. [10]

The principal causes are:

1. Ovulatory or hormonal abnormality: Failure of ovulation is the single most common cause of infertility in females. The normal ovarian cycle is so complex that even small deviations may disrupt the cycle and prevent ovulation. [11] Ovulatory disorders are most often caused by abnormality in one of the controlling hormone. However problems can also arise if the ovaries themselves are resistant or non responsive to normal levels of hormones. In addition, absent, damaged or diseased ovaries will prevent ovulation [12]. The principal symptoms associated with ovulatory disorders are: Amenorrhea, Oligomenorrhoea, Irregular menstrual cycle, Obesity, Excessive weight loss Galactorrhoea, Hirtism and Acne.[13]

2. Anatomical disorders: Disorders of the female sex organs are much more common than those of the male. This is especially true of infection and inflammatory conditions. [14]

3. Chromosomal disorders: Infertility can arise when there are abnormal chromosomes or abnormal numbers. Chromosomal aneuploidy refers to an alteration in chromosomal number from the normal diploid chromosomal complement in somatic cells or haploid complement in gametes. Chromosomal aneuploidy is the leading cause of pregnancy loss and developmental disabilities in humans. [15]

4. Unexplained infertility (Idiopathic): This is a diagnosis of exclusion.

5. Pelvic inflammatory disease (PID): Pelvic inflammatory disease (PID) is an infection of the female reproductive organs. It is a common illness. PID occurs when bacteria move from the vagina and cervix upward into the uterus, ovaries, or fallopian tubes. The bacteria can lead to an abscess in a fallopian tube or ovary. Long-term problems can occur if PID is not treated promptly.

PID can lead to serious, long-term problems: one in 10 women with PID becomes infertile. PID can cause scarring of the fallopian tubes. This scarring can block the tubes and prevent an egg from being fertilized.

Ectopic pregnancy—Scarring from PID also can prevent a fertilized egg from moving into the uterus. Instead, it can begin to grow in the fallopian tube. The tube may rupture (break) and cause life-threatening bleeding into the abdomen and pelvis. Emergency surgery may be needed if the ectopic pregnancy is not diagnosed early.

Chronic pelvic pain—PID may lead to long-lasting pelvic pain.

6. Endometriosis: Endometriosis is a disorder in which tissue similar to the tissue that forms the lining of your uterus grows outside of your uterine cavity. The lining of your uterus is called the endometrium.

7. Polycystic ovarian syndrome: Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. It is characterised by a combination of hyperandrogenism (either clinical or biochemical), chronic oligo/anovulation, and polycystic ovaries. It is frequently associated with insulin resistance and obesity. PCOS receives considerable attention because of its high prevalence and possible reproductive, metabolic, and cardiovascular consequences. It is the most common cause of anovulatory infertility. Ovulation induction with an aromatase inhibitor or anti-oestrogen is the first-line medical treatment. The aim of ovulation induction is monofollicular growth to avoid multiple pregnancy. The second-line treatments include gonadotrophins and laparoscopic ovarian drilling. The role and benefit of metformin in ovulation induction is uncertain. Woman with PCOS undergoing IVF are at significant risk of ovarian hyperstimulation syndrome. Women with PCOS are also at an increased risk of developing gestational diabetes, pregnancy-induced hypertension, and pre-eclampsia.

8. Advanced age, Environmental and occupational exposure to chemicals, Congenital abnormalities, Hormonal imbalance. [16,17]

The impact of the reproductive system disorders:

Female infertility may be clue to solve ovulation

problems, anatomical problems, such as fallopian tube damage or endometriosis.[18] In womb, congenital disorders, muscle tissue gland, endometriosis, polyps and adhesions can also reduce fertility.[19]The reasons for male infertility include unusual characteristics of semen, reproductive infection and disorder, erectile dysfunction or ejaculation disorder. After testing and treating each etiologic factor, couples may look for assisted reproductive technology. [18]

Endometriosis

Endometriosis is a benign estrogen-dependent inflammatory disease which develops due to the ectopic endometrial implants. Endometriosis affects more than 10%-15% of women who are of childbearing age. [18] The symptoms of endometriosis vary. Some women experience mild symptoms, but others can have moderate to severe symptoms. The severity of your pain doesn't indicate the degree or stage of the condition. You may have a mild form of the disease yet experience agonizing pain. It's also possible to have a severe form and have very little discomfort. Pelvic pain is the most common symptom of endometriosis. You may also have the following symptoms: painful periods, pain in the lower abdomen before and during menstruation, cramps one or two weeks around menstruation, heavy menstrual bleeding or bleeding between periods, infertility, pain following sexual intercourse, discomfort with bowel movements, lower back pain that may occur at any time during your menstrual cycle. The relationship between endometriosis and infertility has been mentioned in many resources. However, a cause and effect relationship is still controversial. The pelvic anatomy is deformed and fertility is decreased through mechanical adhesion, such as pelvic adhesion. These adhesions cause damage to ovule release or selection, decrease sperm motility, and causing disruption in myometrium contraction, such as fertilization disorder and embryo transfer. Infertile women are more likely to develop the disease. However, so much debate has been raised on the impact of diseases on fertility. Anti-genetic growth factors, inflammatory cytokines and normal genes are among the endometriosis-related etiologic factors of infertility.[20]

The impact of Genital Infections and Diseases

Untreated sexually transmitted infections (STIs) are among the factors that cause damage to the reproductive system of men and women. Chlamydia and gonorrhea are most common STI which lead to infertility in men and women. This disease has no symptom. Thus, it cannot be diagnosed and treated. Chlamydia causes problems for men and women. If chlamydia is left untreated, the woman will be also at risk of pelvic inflammatory disease (PID). PID is a serious condition where the upper part of the female reproductive system and its supporting structures become inflamed.[21] The studies show that pre-pregnancy diagnosis of Chlamydia trachomatis reduces the abortion caused by PID in 4 weeks. Valid evidence also shows that women with PID are at risk of ectopic pregnancy, fallopian tube infertility and chronic pelvic pain.[22] A relationship was observed between infertility and previous Chlamydia infection in women.[23] There is so much evidence indicating the relationship between trachomatis infection and any changes in semen quality. Trachoma can affect the sperm function. In vitro experiments show that C. trachomatis Tyrosine phosphorylation affects sperm proteins, causes sperm premature death and develops an apoptosis-like reaction in sperms which increase sperm surface fragmentation levels.[24]

The Impact of Age

Studies show that the risk of infertility increases with growing age.[18] Young people's knowledge of infertility is hopeful and leads to the maintenance and improvement of pregnancy health.[23]Evidence shows that age may play an important role in fertility. Pregnancy before 30 for women and before 35 for men has more chance of success. Men and women should know each other's ages that they could come to an agreement for a successful pregnancy.[25]

The Impact of Hormonal Disorders

There are many hormonal disorders that cause infertility. Hypothyroidism, hyperprolactinemia (high male hormone levels) and luteal phase defect (low progesterone) are a few examples of these disorders. Hormonal disorders are a major cause of infertility in women. The inability of women at ovulation and regulation of hormone levels leads to too high or too low production of hormones. These hormonal disorders are characterized with symptoms such as irregular menstrual cycles, excessive bleeding, or very little bleeding, pelvic and abdominal cramps, absence of menstruation or long

menstruation and excessive weight loss or weight gain. The following factors may cause hormonal disorders: gland problems such as thyroid gland, pituitary gland and hypothalamus gland problems. These preliminary glands are responsible for the production of sex hormones. Birth control pills, stress and some diseases such as hypothyroidism affect these glands. If any of these glands encounter any problem, disorder can prevent from the full process of ovulation, and thereby, pregnancy will become difficult. In addition, some treatments can cause hormonal disorders. Targeted cancer therapies can cause anatomical and hormonal changes which negatively affect the breast cancer patient's sexual potential. There are large differences in the evidence-based interdisciplinary treatment and management of breast cancer young patients who are treated and are fertile now and there are concerns about pregnancy after cancer treatment. [26]

PCOD

Polycystic ovary syndrome (PCOS) affects 5%-20% of women of reproductive age worldwide and is characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology.[27] The 2003 Rotterdam criteria are currently the internationally accepted criteria by which PCOS is diagnosed. Patients are diagnosed with PCOS when two out of three criteria are satisfied: oligo- ovulation or anovulation, clinical and/or the presence of polycystic ovaries(PCO) and exclusion of other etiologies(congenital adrenal hyperplasia and androgen secreting tumors).[28] There also exists the androgen excess and PCOS society definition which recommends that clinical or biochemical hyperandrogenism should be essential for diagnosis, but also ovulatory dysfunction is required in the form of either oligo-anovulation or PCO.[29] The pathophysiology of PCOS is multifactorial, and it is believed that a genetic predisposition exists that is exacerbated by excess adiposity. It is thought that the pathophysiology of PCOS involves the interaction between abnormal ovarian morphology, due to excess androgen production by the PCOhyperinsulinemia, and elevated luteinizing hormone (LH) levels. [30]According to the world health organization(WHO), PCOS is the commonest cause of an ovulatory infertility. Upto 70% of women with PCOS have insulin resistance meaning that their cells can't use insulin properly. When cells can't use insulin properly, the body demand for insulin increases. The pancreas makes more insulin to compensate extra insulin triggers ovaries to produce more male hormones. [31]Women with PCOS often have increased levels of inflammation in their body. Being overweight can also contribute to inflammation. Studies have linked excess inflammation to higher androgen levels.[32]

COMPLICATIONS

Many studies have been performed comparing pregnancy outcome in women with PCOS vs controls.

1. MULTIPLE PREGNANCIES: Multiple pregnancies are the most important cause of the increased perinatal morbidity observed following fertility treatments, with special regard to women with PCOS affected by an ovulatory infertility. Most of the risk of pregnancy complications is due to preterm delivery rates of multiple births.[33]

2. MISCARRIAGE: It is still debated whether women with PCOS have an increased risk of miscarriage compared to women without fertility disorder.[34]

3.PREGNANCY INDUCED HYPERTENSION AND PREECLAMPSIA:

All three meta-analysis reported 3-4 times increased risk of pregnancy induced hypertension in women with PCOS. Women with PCOS also represent 3-4 fold increased risk of developing pre-eclampsia during pregnancy.[35]

The Impact of Smoking and Alcohol Consumption

Meta-analyzes have shown that 40% of infertile men are smokers.[18] Chemicals (such as nicotine, cyanide and carbon monoxide) in cigarettes cause a rapid destruction of ovules. Unfortunately, ovules cannot be replaced if destroyed. It means that female smokers experience menopause one to four years sooner. The number and quality of sperm decrease in male smokers; sperm mobility is reduced and the number of sperms with abnormal appearance increases. Smoking may make the sperm unable to fertilize eggs.[36] Female smokers do not get pregnant like non-smoking women. Infertility rate in male and female smokers is almost double the infertility rate in non-smoking women. The reproductive risk increases with the daily number of cigarettes. Even

fertility treatments such as in vitro fertilization (IVF) may not be able to completely eliminate the effects of smoking on fertility. During IVF, female smokers need more drugs to stimulate ovulation and have fewer ovules. Female smokers have 30% lower chance of pregnancy through IVF method compared to non-smoking women. [26] Alcohol consumption in men reduces sperm count; and motility and number of normal morphology sperm in them.[18]

The Impact of Immune Responses

Immunological mechanism plays an important role in sexual problems, such as recurrent miscarriage (RM) (three or more RSA), infertility and implantation failure. This shows that successful pregnancy depends on the maternal immune response to the growing semi-allogeneic fetus.[37] Embryo implantation is affected by local and systemic immune responses including immunoglobulins, cytokines, and hormonal and endometrial factors. Contribution of these factors to the success of implantation and pregnancy is critical. Natural killer cells play an important role in female sexual function. This cells are correlated with inductive failures, NK cell cytotoxicity induced abortion or infertility and gene expression. [38]

The Impact of Mobile Phone

Use Mobile device emits radio frequency electromagnetic waves that can reduce sperm quality and disrupt the normal function of the body. There are many real mechanisms emphasizing the impact of mobile phones on sexual ability of men. All these mechanisms rely on the impact of mobile phones on biological system, such as thermal and non-thermal effects. Since testicles are a shallow organ, it may absorb the radiant energy more than other organs. Oxidative stress (OS) developed in testicles because cell phones cause free radicals in sperm. OS is a major cause of infertility in men. [39]

The Impact of Sexual Violence

The literature shows that the history of sexual violence is associated with infertility. The psychological trauma caused by sexual violence leads to ovulation infertility or sexual dysfunction. Infertile women had experienced sexual violence three times in their lives compared to fertile women. Although the relationship between sexual violence and fallopian tube infertility could be easily explained, this relationship with any infertility other than fallopian tube infertility is vague and confusing. This may be due to the fact that fallopian tube pathology was diagnosed using human chorionic gonadotropin (HSG) against laparoscopy as a gold standard method. HSG is an unreliable test for tube openness with an intensity of 62% and sensitivity of 83%. Therefore, non-tubal infertility group includes an unknown number of undetectable tubal infertility.[40]

The Impact of Anxiety

Although the impact of anxiety on male infertility has not been clearly proved, the incidence of such problems in the lives of men may cause anxiety. Once faced with their infertility, men may have excessive stress, including the anxiety related to power, masculinity, and sexual adequacy. The anxiety may decrease the intimacy between partners and avoid the sexual behaviour. [41]

The Impact of Obesity

According to the researchers, obese women, especially women with abdominal fat hardly become pregnant and have low chance of infertility treatment. In women with a body mass index (BMI) >25, compared with BMI <25, the pregnancy rate is lower (respectively, 10.5% vs. 253%). The role of obesity in pregnancy focuses on the physiology of fat body and metabolic disorders. [36] Articles show that nearly 10% of sub-fertile or infertile women have been diagnosed with diminished reduced ovarian (DOR). [43] Polycystic ovary is one of the common reasons of ovulation disorder in women of childbearing age. [44] Some studies have shown that usually 30%-70% of women with PCOS are obese. Overweight and obesity in women with PCOS exacerbates the severity of androgen and disorders metabolic profile.[45]

The impact of Diseases That Can Cause Infertility

Systemic lupus erythematosus (SLE) is a multiple automatic system that affects fertility in women and men. Genital tract in women and men may be affected by cytotoxic treatment due to the disease activity. Fertility disordermay be due to the problems which prevent from successful fertilization and thus lead to infertility, avoid

the effective implantation in the uterine wall and cause problems in maintaining pregnancy after implantation and complications during childbirth. [46] Varicocele is a pathological enlargement of the pampiniform venous plexus in the scrotum that affects testicular function and can lead to infertility. However, the pathophysiological mechanism that leads to varicocele infertility is not fully understood. The following five mechanisms seem to contribute to the impact of varicocele on testicular function, reduced blood flow leading to hypoxia, thermal stress, oxidative stress, hormonal imbalance and detoxification.[47]

The Impact of Nutrition

Although no definitive relationship was discovered, selection of supplements or food groups is crucial before and during pregnancy attempt to improve fertility in women and men.[48] A high saturated fat diet is associated with reduced sperm quality. Changes in diet over the last decades may partly explain the high frequency of abnormal sperm count. Decrease in saturated fat intake in general and reproductive health benefit.[49] Lower sperm concentration and total sperm count are correlated in men with saturated fat diet.[18]

Unknown Factors

There are numerous factors affecting infertility and nature has multiple roles in this fact. The special impact of some of these factors on fertility is still uncertain and unclear. The relationship of these factors with fertility in humans remains unclear and further research is required in this regard.[25]

HORMONES IN INFERTILITY

ROLE OF HCG IN REPRODUCTION

The glycoprotein hormone family is believed to share similar structural features and the structure of hCG has been elucidated.[50,51] The a- and h-subunits each have remarkably similar folds. When the heterodimer is formed, the single loop of one subunit lies adjacent to the double loops of the other subunit. The h-subunit of hCG has six disulphide bonds and the a-subunit has five. The crystal structure revealed that the previously defined disulphide bonds could not all be made.[52,53] These disulphide bonds appear to play an integral role in maintaining the heterodimeric structure. These structural features, together with the observation that the individual subunits are inactive, have implied that the quaternary structure provided by the assembly of the a- and h-subunits is important for hCG function. However, work with genetically fused a- and h-subunits have shown that the heterodimeric configuration is more important for assembly and secretion than for biological activity.[54-56]hCG is heavily glycosylated with the carbohydrate content accounting for 30% of its molecular weight. There are two asparagine linked carbohydrate chains on each subunit and the carboxyl terminus of hCG has four O-linked glycosylation sites. The O-linked oligosaccharides extend the life of the hormone in the circulation. If the unique C-terminal extension of hCG bearing these oligosaccharides is added to FSH, then the in vivo half life of the hormone is increased.[57] The Asn-linked carbohydrates may also be of importance in the folding and correct disulphide bond arrangement of hCG.[58] The glycosylation status of the free a-subunit and hCG varies throughout pregnancy, with hCGa becoming more highly branched and both hCGa and hCG more fucosylated as gestation progresses.[59] These carbohydrates prevent association of the a- and h-subunits, and the free a-subunits are linked to prolactin secretion.[60,61]

FSH

FSH is synthesized and secreted by gonadotropins in the anterior pituitary gland; it is a glycoprotein that regulates the development, growth, pubertal maturation and reproductive processes of the human body. In females, it initiates follicular growth, specifically affecting granulose cells. With the concomitant rise in inhibin B (a complex protein that down regulates FSH synthesis and inhibits its secretion). FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. [62]

LH

LH is produced also by the anterior pituitary gland; it is a glycoprotein and essential for reproduction. In females, at the time of menstruation, LH initiates follicular growth, specifically affecting granulose cells. [63]

Testosterone

Testosterone is a steroid hormone from the androgen group. It is the principal male sex hormone, produced by testes in men and by thecal cells of the ovaries and placenta in women. Testosterone is also synthesized by zona reticulais of the adrenal cortex in both sexes. In general, Testosterone has both anabolic and virilizing effects.[64,65]

Prolactin (PRL)

Prolactin is mainly synthesized in the pituitary gland and involved in many different biological functions including behavior, immunology, endocrinology, metabolism and reproduction. [66] More than (300) different biological functions have been attributed to PRL, the major ones being induction of differentiation and growth in mammary epithelia and stimulation of milk protein secretion[67] PRL is secreted mainly by lactotrope cells, breast deciduas and immune system. [68] Hyperprolactinemia is one of the most common endocrine disorders of the hypothalamic pituitary axis. It is more commonly diagnosed in women than in men and if it persists, it usually causes infertility, amenorrhea, galactorrhea, oligomenorrhea, hyperandrogenism, hirsutism, acne, regular menses; but with anovulatory cycles.[69]

Conclusion

Infertility is a difficult process to overcome, but dealing with infertility is instrumental to pursuing a positive family building journey in the future. Social stigma due to infertility is seen in many cultures throughout the world in varying forms, when women cannot conceive the blame is put on them, even when 50% of infertility issues come from man. The effect of infertility can lead to social shaming from internal and social norms surrounding pregnancy, which affects women around the world. Many infertile women tend to cope with immense stress and social stigma behind their condition, which can lead to considerable mental distress. The long term stress involved in attempting to conceive a child and lead to emotional distress that may manifest as mental disease. Present review concludes that treatment and self-care significantly improves the chances conception which includes:

Hormone treatments, fertility drugs, timed intercourse, medications like estrogen, anti-diabetic medications and sex hormone suppression improves the chances of conception

- Eat balanced and nutritious diet with plenty of fresh fruits and vegetables.
- Treating or preventing existing diseases such as diabetes and hypothyroidism.
- Regular physical examinations help to detect early signs of infections or abnormalities.
- Do not delay on parenthood

Ethical Clearance- Nil

Source of Funding- Nil

Conflict of Interest- Nil

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Methicillin Resistant *Staphylococcus Aureus* Carrier Status among Indian Healthcare Workers: A Systematic Review and Meta-Analysis

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Abstract

Infection with Methicillin Resistant *Staphylococcus aureus* (MRSA) is a threat to quality care in the hospital. Asymptomatic colonization of MRSA escalates the burden of infection. The rate of MRSA colonization among healthcare workers (HCW) is not homogenous across the globe. Even though there are individual study reports on MRSA colonization, no pooled data is available in India. Therefore, it is important to evaluate the problem of MRSA colonization to develop a policy on preventive measures. We performed a systematic review and meta-analysis of MRSA carrier status among Indian HCWs using five databases (Scopus, PubMed-Medline, IndMed, CINAHL and Google-Scholar) from the articles published from 2008 to 2017 (10 years). STATA 13.0 with metaprop package in STATA was used to find the rate of colonization. Among 2,349 HCWs, the pooled prevalence of MRSA colonization in throat, nose, axilla, palm, fingertips and web-spaces was nine percent (CI 6% - 13%; p=0.001, I^2 91.68%). Further, the forest plot of MRSA colonization in nasal cavity alone was performed, and the prevalence of nasal colonization of MRSA among 1,251 Indian HCWs was found to be 11% (CI 5-17%, p=0.001, I^2 93.3%). The colonization rate is not very high nor negligible. Therefore, the hospital administrator along with the hospital infection control committee needs to formulate a policy on periodic screening and decolonization of HCWs in high-risk areas.

***Keywords**: MRSA, colonization, nasal carriage, healthcare workers, systematic review*

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is a strain of antibiotic resistant *Staphylococcus aureus*. It is a Gram-positive bacteria, resistant to β-lactam antibiotics (Penicillin derivatives such as Methicillin, Oxacillin, etc.) and difficult to treat[1]. MRSA has become a threat to healthcare facilities in many countries, including India. The proportion of MRSA ranges from 20% to 80% around the globe, and this high proportion requires second-line antibiotics in prophylaxis[2]. Infection with MRSA attributes to increased risk of mortality, readmissions, excessive utilization of healthcare resources and thereby added healthcare costs[2]. MRSA can be community acquired or hospital acquired. Though community acquired MRSA exhibits susceptibility to many antibiotics, hospital associated MRSA has amplified resistant pattern to a good number of generally used non-beta lactum antibiotics[2].

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MRSA contributes to 40-60% of total hospital associated infections (HAI)[3]. However, the prevalence of MRSA infection is not homogenous in India[4]. MRSA is seen in healthy individuals as a carrier. The common locations of harboring MRSA as carrier are nose, armpit, groin, throat, palm and finger tips[5-6]. The primary colonized site in man is anterior nares[7]. Asymptomatic carrier status of MRSA endangers public health as the diagnosis of MRSA is challenging. The impact of carrier status or mere infection with MRSA is difficult to treat and increases the duration of treatment[2]. The healthcare workers serve as a reservoir, vector or victim of MRSA transmission[8].

A meta-analysis published in 2008 with 127 studies, which involved screening of 33,318 healthcare workers for MRSA carrier status around the world showed a prevalence of 4.6%[8]. There are no meta-analyses or systematic reviews published in the recent years on screening of MRSA colonization among healthcare workers. However, there are many studies reporting varied rates of MRSA carrier status ranging from 0 to 74%[9-10]. Yet, there is no systematic review available in India to estimate the burden of MRSA carrier status. Therefore, this systematic review and meta-analysis is performed with the aim to identify the prevalence of nasal carriage of MRSA among Indian healthcare workers.

Method

Inclusion criteria

We included studies conducted among healthcare workers in Indian hospitals. Healthcare workers comprised of doctors, nurses, technician and various therapists or students of any health disciplines who are involved in direct patient care activities. The articles involving both the gender and all age group of healthcare workers working in public or private hospitals were reviewed. We have included the articles published in English language only. Observational studies such as cross-sectional, descriptive, cohort, case-control or prevalence surveys were included. Case reports, case series, reviews and conference reports were excluded. The study period was for last 10 years (articles published from January 2008 to December 2017). The studies in which the sample was obtained from nose, and Methicillin resistance confirmed with Cefoxitin susceptibility testing according to guidelines of Clinical and Laboratory Standards Institute (CLSI) were included[11].

Search strategy

The major electronic data bases were systematically searched. They are Scopus, PubMed-Medline, IndMed, CINAHL and Google Scholar. The keywords used were: methicillin resistant *Staphylococcus aureus*, healthcare worker, nasal colonization, observational study and geographical area such as India. Appropriate search strategies were built as per the requirement of each database.

Quality assessment

The quality of studies was assessed by using the Joanna Briggs Institute Checklist for Systematic Reviews and Research Syntheses. The checklist consists of three sections: 1) the study details (authors details, publication related items and aim and objectives); 2) the study methods (study design, study duration, sample characteristics, dependent variable, outcome measured, ethical considerations, method of diagnostic test, method of data analysis); 3) the study results (prevalence or incidence, authors comments or limitation of the study). Two reviewers independently responded to each of the items mentioned in the checklist with a 'yes' or 'no'. Each 'yes' carried a score of one and 'no' carried a score of zero, with the total possible maximum score of 10. Section 2 and 3 were considered for quality assessment. The studies with the score of six or more than six were considered for review and meta-analysis.

Data extraction

The data were extracted using checklist prepared on the basis of the 'Meta-analysis of Observational Studies in Epidemiology (MOOSE)' guidelines[12] and 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)' guidelines[13]. The data extracted from each study were: the last name of the first author, name of the journal, year of publication of the study, aims and objectives, setting of the study, number of healthcare workers recruited in the study, types of healthcare worker, methodology of the study, source of sample obtained, method of testing the presence of MRSA and prevalence of MRSA carrier status. Two

reviewers have independently extracted the data. The third reviewer helped in resolving the inconstancies between the reviewers.

Statistical Analysis

The meta-analysis was performed by using STATA 13.0 version. Forest plots were built with the help of metaprop package in STATA. This review has included cross-sectional studies only. As cross-sectional studies are observational, a considerable amount of heterogeneity is expected. Hence, a random effects model was adopted instead of fixed effect model. The pooled prevalence of MRSA carrier status with 95% confidence interval along with I^2 statistic that helps in quantifying the heterogeneity of the studies was reported.

Results

From the five data bases, 333 articles were identified using appropriate search terms (Figure 1). Microsoft excel was used to remove the duplicates. After the title and abstract screen, 18 articles were considered for full text review and 315 were excluded. This review also included the article which has used CLSI with Cefoxitin (30 µg) disc diffusion for testing MRSA. Among 18 studies, seven studies were excluded. We were unable to get the percentage of MRSA colonization in one study[14] and another study reported different sample size at different time[15]. Five studies were excluded as Oxacillin disc diffusion method was employed for identifying MRSA[16-20]. Finally, 11 studies were included to find the prevalence of MRSA colonization among HCWs of Indian hospitals (Table 1).

Table 1: Characteristics of studies included in the systematic review and meta-analysis

Author (year) setting	Study title	Sample size	Screening location	MRSA Carrier status (%)
Shinde (2016) Belagavi (Karnataka)21	Screening for Methicillin Resistant Staphylococcus aureus (MRSA) colonization in healthcare workers working in critical care areas at a tertiary care hospital, Karnataka, India	63	Nasal, palm	6.4
Nambirajan, (2016) Tamilnadu22	Alarming carrier status of Methicillin Resistant Staphylococcus aureus (MRSA) among adolescent learners- prevalence and antibiotic susceptibility of the organism	619	Nasal, palm, Index finger and Dorsum	8.9
Malini (2012) Bengaluru23	Methicillin Resistant Staphylococcus aureus carriage among the healthcare workers in a tertiary care hospital	150	Nasal, throat, palms and web spaces	10
Agarwal, (2015) Uttar Pradesh24	Nasal carriage of Methicillin- and Mupirocin-resistant S. aureus among healthcare workers in a tertiary care hospital	200	Nasal	14
Satpathi, (2015) West Bengal25	Nasal carriage of Staphylococcus aureus and the quantum of their Methicillin Resistance amongst the healthcare workers in a peripheral tertiary care centre of Eastern India	183	Nasal	6.6
Radhakrishna, (2016) Karnataka26	Nasal carriage of staphylococcus aureus with special emphasis on Methicillin Resistant Staphylococcus aureus among students of a South Indian medical college - prevalence and antibiogram pattern	148	Nasal	6.1

Cont... **Table 1: Characteristics of studies included in the systematic review and meta-analysis**

Author (year) setting	Study title	Sample size	Screening location	MRSA Carrier status (%)
Radhakrishna, (2013) Mangalore, Karnataka27	Prevalence of Methicillin Resistant Staphylococcus aureus carriage amongst healthcare workers of critical care units in Kasturba Medical College Hospital, Mangalore, India	200	Nasal	2.5
Verma, (2017) Bhopal28	Utility of chromogenic medium for early detection of nasal carriage of Methicillin Resistant Staphylococcus aureus (MRSA) in healthcare professionals	120	Nasal	21.7
Visalachy, (2016) Chennai, Tamilnadu29	Carriage of multidrug resistant bacteria on frequently contacted surfaces and hands of healthcare workers	157	Hands	1.3
Hema, (2017) Bengaluru, Karnataka10	Prevalence of nasal carriers of Methicillin Resistant Staphylococcus aureus among dental students: An in vivo study	400	Nasal	18.5
Renushri, (2014) Karnataka30	Screening for Methicillin Resistant Staphylococcus aureus carriers among individuals exposed and not exposed to the hospital environment and their antimicrobial sensitivity pattern	119	Nasal, throat, Palm	11.8

NA: not available MRSA: Methicillin Resistant *Staphylococcus aureus* MSSA: Methicillin Sensitive *Staphylococcus aureus*

The total population included in the meta-analysis of MRSA colonization was 2,359. The population consisted of doctors, nurses, nursing orderlies, auxiliary nurses, health science students such as medical students, nursing students, dental students, technicians and housekeeping staff (Table 1). Most of the articles were published in recent years (2 articles published in 2017, 4 articles in 2016, 2 articles in 2015 and 1 each in 2014, 2013 and 2012). The key features of included studies are given in Table 1.

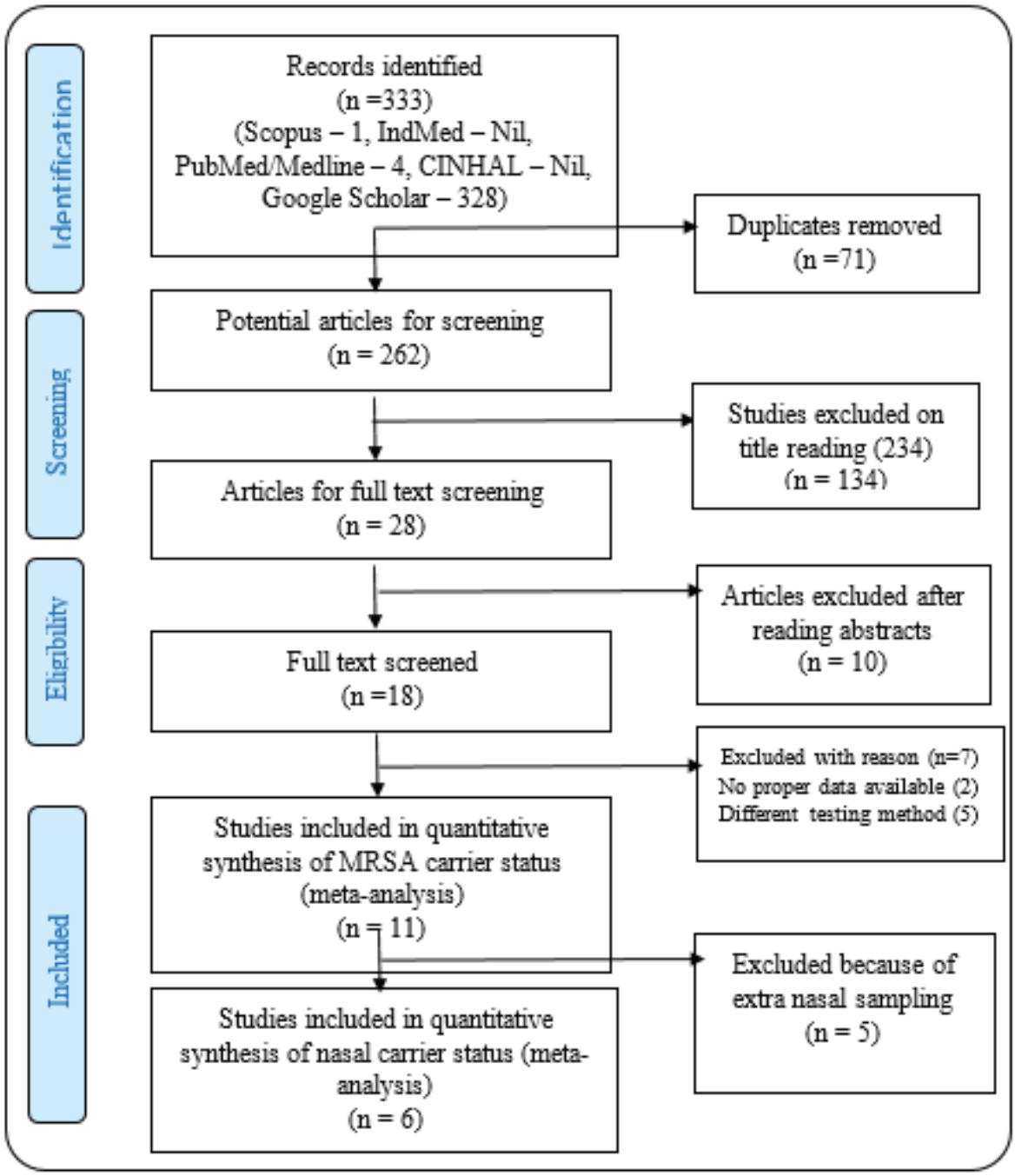

Figure 1: PRISMA Chart Detailing Study Selection for the Review

The forest plot (figure 2) on MRSA colonization among Indian healthcare workers shows the overall pooled prevalence of MRSA colonization with 95% confidence interval was nine percent (6%, 13%; p=0.001, I^2 91.68%).

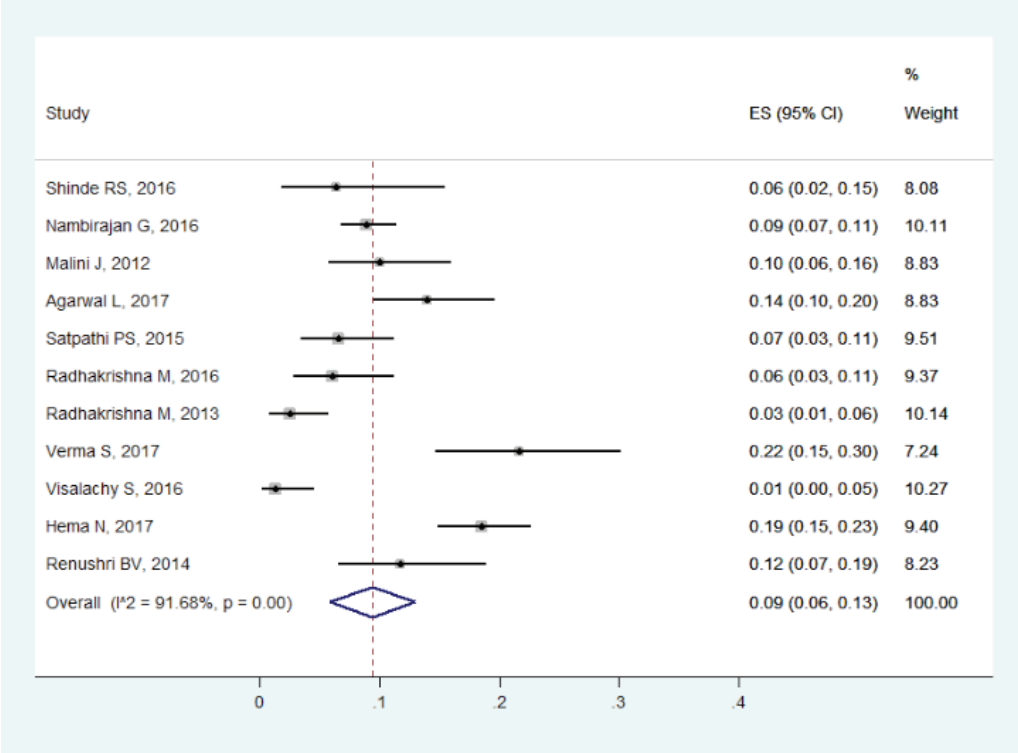

Figure 2: Forest plot of prevalence of MRSA carriage among Indian healthcare workers

From the 11 studies included in the meta-analysis, six studies have investigated nasal carrier status and rest five studies have multiple sampling from palm, finger tips, web spaces, axilla, etc. Therefore, six studies[10,24-28] were further analyzed for estimating the prevalence of nasal carrier status of MRSA. A total of 1,251 healthcare workers were included for the meta-analysis (Table 1). The prevalence of MRSA colonization in nasal cavity alone is given in figure 3. The pooled prevalence of nasal carrier is 11% (5%, 17%; p=0.001, I^2 93.3%).

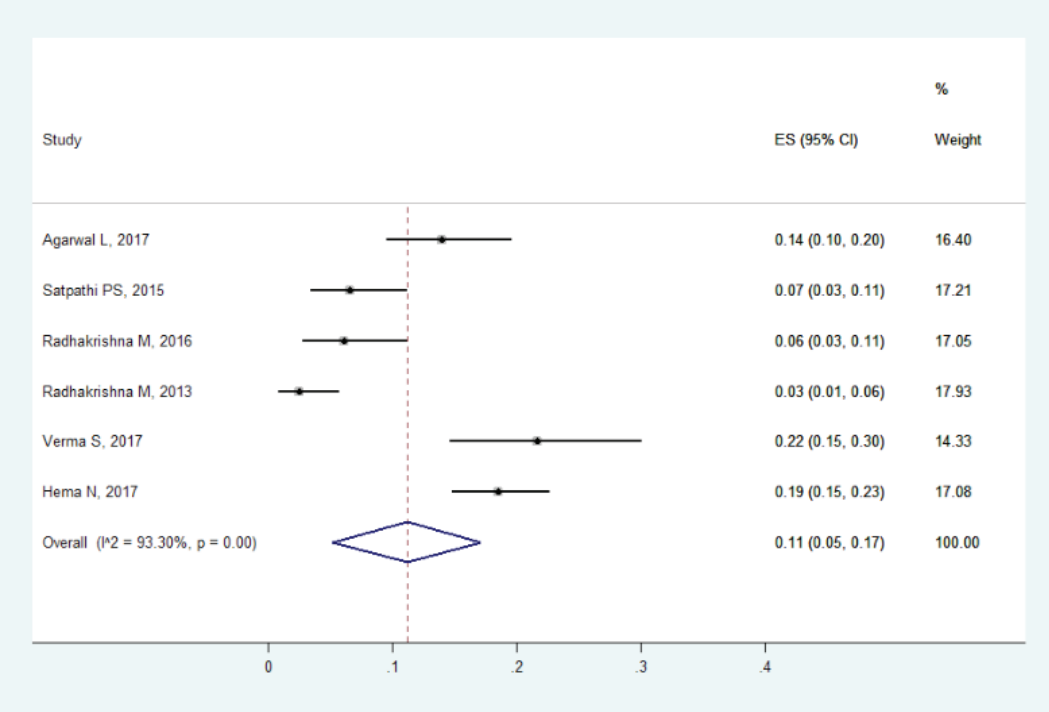

Figure 3: Forest plot of prevalence of nasal carriage status of MRSA among Indian healthcare workers.

Discussion

HCWs can spread infection to patients, as they are closely associated with them in the hospital. Doctors and nurses regularly encounter their patients in a hospital setting during patient care activities. The hands of HCWs are the common transportation for the hospital-associated organisms to cross contaminate between the patients and the hospital atmosphere[31]. Therefore, testing the carrier status and decolonizing the healthcare workers becomes essential to reduce MRSA infection in the hospital. Frequent screening with six months to one-year gap helps in reducing carrier status among HCWs[32].

A meta-analysis empowers greater precision by summarizing the results of all the significant studies conducted in different areas in a specified time. The meta-analysis presented here includes 11 published studies regarding MRSA colonization among healthcare workers from different hospitals of India. We observed more number of studies published in recent years (2016, 2017). However, MRSA can be screened at different locations such as nose, armpit, groin, throat, web spaces of hand and palm and fingertips, most of the studies have included nasal screening. It is also evident that nasal colonization of MRSA is more common than other location in human body[20,30].

But, nasal colonization of MRSA is not homogenous globally[33-37]. In the present meta-analysis, the prevalence of MRSA is nine percent among HCWs. A meta-analysis from Iran shows 32.8% (95% CI: 26.0-40.4) MRSA[39]. In Egypt, a review revealed that the prevalence of nasal carriage of *Staphylococcus aureus* among Egyptian HCWs was 22.9%, in which 58.8% was MRSA[36]. In Ethiopia, 28.8% HCWs were colonized with *Staphylococcus aureus* and 44% of them was MRSA[36]. In China, the prevalence of *Staphylococcus aureus* was 21.6%, in which 4.7% was MRSA[32]. 8.9% HCWs were nasal carriers in Ireland non-acute care health facilities[39]. MRSA carriage among HCWs in Europe and the United States was 1.8%[40]. A meta-analysis reported the average MRSA prevalence among HCWs was 4.6% globally[8]. This different rate among the countries may be due to sampling and sampling technique, sample size, the location of sampling, test performed to detect MRSA, and compliance to infection control policies in hospitals[34,41].

Healthcare workers have higher rate (11.8%) of MRSA carrier status compared to people not exposed (4%) to hospitals[30]. A study has noticed that the postgraduates in a hospital have higher rate of MRSA carriage than that of interns[10] indicating longer the exposure to hospital, higher the rate of colonization. MRSA is detected in the hospital environment such as stethoscopes, aprons, neck ties and frequently touched surfaces like computer monitors, tap and door handles[42,43] and have the ability to survive on abiotic surfaces up to 12 days[44].

Mupirocin is the most frequently used topical antibiotic of choice to combat MRSA colonization among HCWs. It can be used to treat isolated cases as well as during institutional outbreaks[45]. The use of nasal mupirocin is advisable twice daily for five days for decolonization.

MRSA is a global burden and one of the leading cause of hospital-associated infection. Screening every patients who visit hospital may not be feasible in India as we have a highly dense population. At the same time, the high risk of HCWs transmitting the infection to their patients cannot be ignored. Therefore, periodic screening of HCWs is recommended and there should be a mandatory policy on screening of HCWs. The other measure that can bring about reduction in transmission of infection from HCW to patient is by proper hand washing[2].

Noncompliance to hand hygiene, lack of knowledge on infection control measures and its importance, antibiotic abuse, under resourced hospital facilities are the causes of increased MRSA carrier status among HCWs[38]. Few healthcare workers have sufficient knowledge, and they also claim that they are practicing infection control measures during patient care. However, their actual practice of infection control was low[46]. Hence, newer method of hand hygiene monitoring system need to be implemented for the better compliance.

Infection control is considered as one of the quality indicators of a hospital. Most of the hospitals in India have formulated Hospital Infection Control Committee (HICC) striving hard to bring down Hospital Associated Infections (HAI). Screening of HCWs during recruitment is not a routine practice in many of the Indian hospitals. However, HCWs are screened during outbreaks or when

needed. A well formulated hospital policy may address these problem.

This systematic review has few limitations. The representation of the studies were not available from entire geographical regions in India. Hence, the generalizability of the calculated rate must be used cautiously. This review has included published articles only. The reviewed articles have included varied subjects such as doctors, nurses, technicians, students and housekeeping staff. Therefore, additional studies are necessary to find the prevalence rate in each categories of healthcare workers to formulate the policy on periodic screening and decolonization to reduce MRSA burden in the country.

Conclusion

Exposure to infection is an inherent risk of contracting varied infections among healthcare workers. It is an immense challenge as HCWs have frequent contact with patients and may transmit MRSA more frequently. Therefore, screening and decolonizing the healthcare workers becomes significant in reducing MRSA infection. No systematic review on MRSA colonization among healthcare workers was available. This study helps in understanding the burden of MRSA colonization and may be used in policy development on periodic screening and decolonization of healthcare workers. Perhaps, further systematic reviews and meta-analysis are needed among different cadres of healthcare workers to recognize the problem and implement preventive measures.

Ethical Clearance - Permission was obtained from institutional research committee.

Source of Funding- Self

Conflict of Interest - Nil

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Effect of Early Pre-Ambulatory Training on Functional Mobility Skill and Quality of Life In Low Paraplegia: A Research Protocol

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Abstract

Background: Paraplegia defined as near total dysfunction of the entire or part of the trunk and both lower limbs, caused by lesions of the thoracic or lumbar spinal cord or cauda equina. 43% of SCI results in paraplegia for thoracic, lumbar, or sacral lesion. The purpose of physical therapy management is to make the patient relaxed and to achieve pre-ambulatory preparations, pre-ambulatory activities and ambulation activities.

Methods/Design: The study will be designed as experimental study. Total 60 participants will be selected as per inclusion and exclusion criteria from AVBRH, Sawangi Meghe for the study. The duration of study will 6 months with intervention. Physiotherapy intervention includes respiratory care, skin protection, range of motions and early reinforcement, early mobility activity, bed mobility skills, transfer activities and wheelchair skills. ASIA Impairment Scale, Functional Independent Measure, Wheelchair Skill Test, WHOQOL-BREF will be major outcome measure for the patient.

Discussion: The study protocol details, in people with SCI the intervention strategy selected is largely based on the amount of preserved motor function. Independence in functional skills in patients with complete motor SCI (ASIA A and B) is largely achieved through compensatory mechanisms and interventions are developed accordingly. Expected outcome from the study will be the effects of early pre-ambulatory training on functional mobility skills and quality of life in low paraplegia.

Key Words: *Spinal cord injury, paraplegia, pre-ambulatory training, quality of life, rehabilitation*

Introduction

Spinal cord injury is a moderately low – rate, high – cost injury that outcomes in huge change in a person's life.[1] Traumatic and non-traumatic injuries are two specific etiological categories of spinal cord injury. Trauma is the most common cause of injury in adult recovery settings. Injury occurs from damage caused by traumatic situations such as road traffic accidents(40.4%), falls(27.9%), crime (15%), and athletics (8.0%)[2].

In adult populations non-traumatic injury generally results from illness or pathological effect. Tetraplegia and paraplegia are two specific functional categories of spinal cord injury. Paraplegia defined as to complete paralysis of the entire or part of the trunk and both lower limbs, caused by lesions of the thoracic or lumbar spinal cord or cauda equina. [1]

Approximately 1.5 million people live with SCI in India. Every year approximate 20,000 new cases of SCI

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are combined and 60-70% of them are illiterate, poor.[3] 43% of SCI results in paraplegia for thoracic, lumbar, or sacral lesion[2]. The ability to strengthen results is with combinatorial strategies, and the effects on functional outcomes that support the recovery and quality of life. Life expectancy for people without a SCI has improved over the years but is still lower than that. Age at the onset and level and extent of neurological injury are variables that affect life expectancy. Patients with incomplete neurological SCI have a longer life expectancy than those with complete injuries, and patients with more serious injuries often have a higher life expectancy. Individuals with SCI may benefit from recent neurotechnology advances designed to enhance function and quality of life. [1]

The neurological conditions are spinal shock, sensory and motor impairment, autonomic dysreflexia, spastic hypertonia, postural hypotension, coronary heart disease, impaired temperature control, pulmonary impairments, bowel and bladder dysfunction, sexual abnormality. Secondary medical complications are developed in 82% patients by rehabilitation. After one year of injury, pressure ulcers (15%), pneumonia (4%), and deep vein thrombosis (2.5%) are the most common secondary complications. [1]

The summary purpose of physical therapy management is to make the patient relaxed and to gain functional freedom necessary for daily life, jobs and recreation.[4] [5] During this early stage of recovery the primary focus areas are the evaluation of sensory and motor control, respiratory function, skin integrity, PROM, and early mobility ability performance. Early pre-ambulatory training includes strengthening, isokinetic strength testing exercises, resistance training program, endurance training, bed mobility skills, sitting stability, transitions, mat-to-wheelchair transfers, locomotive training. [1]

Improvements in functional damage, the capacity to carry out tasks that are significant to the individual and a return to engaging in regular, preferred social roles can be achieved through therapies based on compensatory approaches, restorative approaches or a mixture of the two. In people with SCI the intervention strategy selected is largely based on the amount of preserved motor function. Independence in functional skills in patients with complete motor SCI (ASIA A and B) is largely achieved through compensatory mechanisms and interventions are developed accordingly.

Aim & Objective

To study the effect of early pre ambulatory training on functional mobility and quality of life in low paraplegia. The objectives are to achieve pre-ambulatory preparation, pre-ambulatory activities and ambulation activities.

Methodology

Study setting

The study shall be incorporated at ICU setup of Acharya Vinoba Bhava Rural Hospital Sawangi Meghe, Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences, Sawangi Meghe , Wardha

Study design and sample size

The number of participants enrolled in this experimental study is 60. Clarify to the participants before inclusion about the study's goals and methods. The patients will be screened and random allocation shall be done; written consent form will be obtained from all participants' family.

Participants

Inclusion Criteria: Subjects of both genders from the age of 18 to 65 years, neurological level of injury from T12 or below, motor complete and incomplete injury, medically stable patients, sufficient upper extremity and range of motion.

Exclusion Criteria: Spinal instability, deep vein thrombosis, heterotopic ossification, cognitive deficits, contractures, osteoporosis and skeletal fracture, pain, skin injury, upper extremity soft tissue injuries like contusion, sprain and strain.

Participant timeline

The study duration is of 6 months with intervention. Simple random sampling (envelop) method will be done. Before the initial examination, the patient must be stable enough to undergo analysis. Assessment will be done on 1st day of visit then in last of intervention. In patients,

the treatment process varied from 8 weeks to 12 weeks, respectively. The evaluation (FIM and WHOQOL-BREF) will be performed initial to the last session.

Recruitment

Regular visit to neuro ICU, neurosurgery wards will be done and contact will maintain with doctors, record maintaining office for cases that will enrolled in hospital so that can be taken for the study. The patient who are already undergoing rehabilitation in our in-patient department (IPD) and diagnosed with low paraplegia will be systematically assessed for the eligibility in the study as per the inclusion and exclusion criteria.

Implementation & Blinding: Randomization will be supervised by the research coordinator and principal investigators. Tester(s) will be blinded.

Physiotherapy interventions:

Management of physical therapy in an acute recovery period: In physical therapy assessment examination of skin integrity, sensory and motor function, respiratory function, passive range of motion and performance of early mobility activity will be done. Physical therapy intervention will be respiratory care, skin protection, range of motions and early strengthening, recent flexibility interventions, education.

In active rehabilitation, physical therapy strategies include strengthening, cardiovascular/ Endurance, bed mobility activities, rolling, progression supine to/ from sitting, sitting stability, transitions, mat-to-wheelchair transitions, locomotive exercise, locomotive training for individuals with motor full SCI and locomotive training for individuals with partial SCI. Wheelchair Skills are forward and backward propulsion, turning, ascending and descending inclines, guess and continue to maintain wheelie, propulsion on uneven terrain.

Study procedure

The physiotherapy program focused mainly not just on patient recovery but also on improving their quality of life. In patients, the treatment process varied from 8 weeks to 12 weeks, respectively.

Treatment plan will be followed in some key steps:

Pre-ambulatory preparation, Pre-ambulatory activities, Ambulatory activities. (Time duration will vary according to patient's condition).

Pre-ambulatory preparation will be: Postural hypotension and cardiovascular impairments. In postural hypotension: When an erect or vertical position is assumed there is a decrease in blood pressure that is called orthostatic hypotension. It is due to a lack of sympathetic regulation of the vasoconstriction. The problem is compounded by lack of muscle tone, causing pooling of the peripheral venous and splanchnic beds. The vital signs should be closely monitored. Using compression stocking and an abdominal binder should reduce these symptoms further. In cardiovascular impairments: Below the level of lesion this causes bradycardia, and peripheral vasculature distension. Due to the disturbed equilibrium from sympathetic to parasympathetic device, and lack of active muscle contraction and extended bedtime. Cardiovascular preparation provides major safety advantages for SCI sufferers. A variety of studies have shown that aerobic fitness may be improved by strength training. The most popular form of aerobic training is upper limbs - based movements such as arm ergometry, wheelchair movements and swimming. Another form of endurance training is in people with SCI with adequate walking ability locomotive training on an TM with or without BWS.

Pre-ambulatory activities will be as follow: Rolling, Progression supine to/from sitting, sitting stability. To facilitate functional mobility independence bed mobility skills are required. It involves rolling, transition from sitting at the bedside to / from the supine and lower limb management. Independence is also required for dressing, placement in bed and screening of the skin. Basically, two methods of progression "walking" on bent elbows and going straight up, from supine. Independent sitting stability, in both the low sitting and the high sitting, is an important ability for several various basic activities like transitions, dressing and mobility in the wheelchair. Number of surfaces should undergo balance treatments. Surfaces are: rigid mat, bed, thick mattress, soft sofa and so on. Balance interventions should also be practiced while sitting in the patient's wheelchair.

Ambulatory activities will be as follow: transitions, mat-to-wheelchair transfers, and locomotive training

and wheelchair skills. In transitions, the sitting-pivot has three components: Preparatory step; away from the transferring surface trunk is flexed forward and laterally, lift phase; buttocks are lifted off the seating surface as the trunk rotates and end of the descent phase when the buttocks are on the other sitting surface. Locomotive training will be: Locomotive Training for Individuals with Motor full SCI and locomotive Training for Individuals with partial SCI. Basic wheelchair mobility skills will be included.

OUTCOME

Primary outcome measures:

The primary outcome measures will be ASIA Impairment Scale and Functional Independent Measure. ASIA standards should be used to assess the level of lesions and preserved motor function. In sensory examination key points in each of the dermatomes is tested bilaterally and in motor examination key muscle functions of the myotomes are tested bilaterally. Motor and sensory examination of the standards can be reliable if carried out by professional examiners. For classification of injury ASIA standards are validated. [6] The Measurement of Functional Independence (FIM) is an 18-item measure of physical, psychological and social dimensions functionality. The FIM uses an individual's level of assistance to rate the functional status from complete independence to complete assistance.

Secondary outcomes measures:

The secondary outcome measures will be the Wheelchair Skill Test. To evaluate a manual wheelchair user's skill performance WST 4.1 is a reliable tool. The wheelchair circuit is a appropriate and valid instrument for assessing the manual functionality of wheelchairs in subjects with SCI. The main objective of this review is to evaluate record, examine and critically assess the performance-based wheelchair skills assessments currently available in international literature for manual wheelchair users, particularly those with spinal cord injury. [7] The WHOQOL-BREF will be used to determine the quality of life (QoL) of paraplegic people and their self-esteem. It contains 26 items, assessing the following specific domains: physical and psychological health, social relations, and environment. It has been shown that WHOQOL-BREF exhibits strong discriminating validity, material validity and reliability for test-retest.

DATA COLLECTION AND MANAGEMENT

Data collection

The data of assessment will be collected from the pre-established spread sheet with variable baseline characteristics. In a secure REDCap database testing data will be stored. The non-electronic data, such as hard copies of assessment forms, signed consent forms, etc. will be stored securely in the study setting.

Data management

Data collection and documentation will take place in the context of principal investigators guidance. For accuracy will being evaluated thoroughly by the study documentation. To prevent missing data due to inappropriate staff procedures checklists are used.

Statistical Analysis Plan

Data analysis will be undertaken utilising qualitative and interpretation statistical data through using Chi-square test and the student's unpaired t test. The device used for interpretation will be SPSS24. 0 version, Graph pad prism version 7.0 and $p<0.005$ are considered to be of relevance ($p>0.005$).

Bias

Measures will be taken to prevent this from happening attrition bias by giving reminder calls prior to each intervention and by providing travel assistance to those who need it. So, we expect a low percentage of dropouts.

Discussion

The study protocol details, in people with SCI the intervention strategy selected is largely based on the amount of preserved motor function. As the average life expectancy of people with SCI has increased due to improvements in medicine, the HRQL of persons with SCI should be examined more thoroughly because quality of life (QoL) provides a good prediction of survival 15 years after injury .As the average life expectancy of people with SCI has increased due to improvements in medicine, the HRQL of persons with SCI should be examined

more thoroughly because quality of life (QoL) provides a good prediction of survival 15 years after injury. The study protocol details, in people with SCI the intervention strategy selected is largely based on the amount of preserved motor function. Because of advances in medicine, the average life expectancy of people with SCI has improved, the HRQL of people with SCI should be studied more closely because quality of life (QoL) offers a strong predictor of survival 15 years after injury.[8] The individuals and their families seems to have a substantial impact from the sudden onset of spinal cord injury.[3]

Exercise and gait training in persons with paraplegia and its potential impact on mass and strength assets. The connection between changes in the muscle properties and factor of gait of paraplegic people using orthotics are investigated by the authors. For developing countries with environmental barriers, these results are significant and relevant. Reinforcement of the upper extremity will be used in the recovery of paraplegic patients qualified for orthotics ambulation.[9]

Expected outcome from the study will be the effects of early pre-ambulatory training on functional mobility skills and quality of life in low paraplegia. Rehabilitation measures may be compensatory or recovery-based depending on the patient's appearance. The physiotherapist, recovery team and patient will use these projected outcomes to set goals and expectations. [1]

Ethical Approval and Dissemination:

Ethical approval will be taken from institutional ethical committee. The DMIMS who will fund for research and the subjects who will participate in the study can access the main findings of the research. Data held safely for the enrolled subjects a minimum of five years. After completion of data collection, statistical analysis a completion report will be formed and after review by institutional research cell will be send for publication.

Patient consent

Principal Investigators will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality.

Confidentiality

The research plan will be explained to the participants and personal details will be taken from one of his / her relative and the principal investigator. The consent form must contain a statement of confidentiality and the principal investigator, patient and 2 witnesses' signatures. If needed to reveal any details for the analysis, the patient's consent will be taken with full confidentiality guarantee.

Declaration of interest

The authors declare no conflicting interest.

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Assess the Knowledge and Attitude Regarding Respiratory Problems among Petrol Pump Workers

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Abstract

Introduction: Occupational health is the promotion and maintenance of the highest degree of physical, mental and social well-being of employees in all professions through the avoidance of health departures, the management of risks and the adaptation of work to people and their jobs. As the petrol pump workers work constantly for more than 8 hours per day for 6 days a week and thus being constantly exposed to petrol/diesel fumes, are at a high risk of developing respiratory problems.

Objectives:1) To assess the knowledge of petrol pump workers regarding respiratoryproblems. 2) To assess the attitude towards respiratory problems among petrol pumpworkers. 3)To find out the association between knowledge score with selected demographic variables 4)To find out the association between attitude towards respiratory problems with selected demographicvariables.

Hypothesis:H_1-there will be a significant association between petrol pump workers and selected demographic variables. H_2- there will be no significant association between knowledge of respiratory problems and selected demographicvariables.

Methods and Materials:A descriptive study was conducted in Wardha district to assess the knowledge of petrol pump workers regarding respiratoryproblems. Total 100 petrol pump workers were selected by probability simple random sampling technique.Modified structured questionnaire and Likert scale was used to assess knowledge and attitude of petrol pump workers respectively.Descriptive statistics like frequencies and percentages were used for representing the analyzed data.

Result: Findings shows that 3% of the petrol pump workers were having poor level of knowledge score, 13% had average, 49% had good and 35% of them had excellent level of knowledge score.87% of petrol pump workers had positive attitude. There was positive correlation was established between knowledge score and attitude score of petrol pump workers(r=0.283, p=0.0001)

Conclusion: Findings suggest that petrol pump workers were aware about the respiratory problems due to occupational hazards and somewhat their attitude was positive towards their health.

***Key words**: knowledge, attitude, respiratory problems.*

Introduction

Occupational health is the promotion and maintenance of the highest degree of physical, mental and social well-being of employees in all professions through the avoidance of health departures, the management of risks and the adaptation of work to people and their jobs.(1)

Health is very important for ruling the life, changes in the health will occurs with the changing environment. The environment of the workers where they are working will affect on their health. There are several types of environment which exists in surrounding, in that mostly the physical environment which play an important role. Air, noise, heat and radiation are the main source of environmental pollution.Air pollution is a major

problem for developing countries, Which has dangerous consequences for human health and the environment.[2]

The number of petrol pumps is increasing due to increased number of vehicles automobiles. Petrol pump workers exposed to organic and inorganic component present in petrol. Volatile aromatic hydrocarbons are present in the atmosphere of service station.[3]Continues exposure of petrol pump workers to petrol and diesel vapor affect on lung function abnormality. The severity of respiratory impairment will depend on duration of exposure.[4] Petrol pump workers those are working more than 5 years are vulnerable to develop lung diseases.[5]

According to one study exposure to petrol vapors and fumes, hydrocarbons leads to decreased the pulmonary functions forced expiratory volume (FEV1, FEV6, FEV1/FEV6), and peak expiratory flow rate (PEFR) relative to their age-matched controls**.**[6]

This study shows that persons working in petrol pumps have respiratory abnormalities in the form of restrictive lung impairment. This impairment increases as the duration of exposure at work place increases. [7]

In petrol and diesel fumes the petroleum content of benzene is absorbed into the human body either through the respiratory tract or through epidermal touch.This can cause an irritant effect on the bronchial epithelium, affect the cilia and clara cells of the lungs and release proteolytic enzymes from macrophages at peak expiratory flow rate (PEFR).[8]

Workers working in retail outlets and the petrol industry will face health problems depending on their working conditions in exploration, refinery, terminal, transportation and dispensing to vehicles and containers. So to avoid health problems safety measures should be taken while working. Petroleum products are very inflammable and harmful to human being, environment and earth also. Because oil products are highly inflammable and can cause accidents. Petrol content is benzene and in nature it is carcinogenic. Petroleum inhalation causes skin problems, vomiting and, occasionally, euphoria.[9]

There is increases frequency of cell abnormalities in petrol pump workers those are exposed to gasoline fumes.Continuous exposure to gasoline fumes can cause the disease cancer. Peak expiratory flow rate (PEFR) of exposed petrol pump workers are decreased as compare to other persons and it will depend on duration of exposure.[7]

Lung impairment can occur in petrol pump workers and it increases as the duration of exposure at workplace. Spirometry should be done periodically to assess lung function measurement of petrol pump workers.[10]

Relatively unexplored among workers who feel fuel is the health effects of occupational exposure to petrol and air pollution from vehicle sources.Diesel and gasoline exhaust particles are readily inhaled and cover a large surface area where organic matter can quickly adsorbed.[11]

They can stay airborne for longer periods of time and can stick in large numbers and deeper into the lungs than large particles. Benzene occurs naturally in crude oil and is a gas component. The concentration of benzene in motor vehicles in India is about 3%. Petrol pump staff exposed to petrol fumes exhibit numbers of clinical signs and symptoms that may be due to exposure of benzene. [10] This may reduce the working of the central nervous system (CNS) and trigger organ damage. If even small amounts of hydrocarbons reach the bloodstream.[12]

The sensitivity of gas pump staff to the fumes has a number of effects on respiratory functions. Relatively unexplored among workers who feel fuel is the health effects of occupational exposure to petrol and air pollution from vehicle sources.Description of the respiratory problems among petrol pump staff and such a research that is missing in this geographical region had to be carried out. For the susceptible categories, the Wardha air quality index (AQI) and the PM 2.5 air pollution index is 117 unhealthy. Every day petrol pump workers who exposed to petrol fumes are suffering from memory loss and reduced muscle function.

Hypothesis.

H_1-there will be a significant association between petrol pump workers and selected demographic variables.

H_2- there will be no significant association between knowledge of respiratory problems and selected demographic variables.

Material and Methods

A descriptive study was conducted in Wardha district to assess the knowledge of petrol pump workers regarding respiratoryproblems. Duration of study was one year.Approval had been taken from Institutional Ethics Committee (under protocol number DMIMS (DU)/IEC/ SEPT-2019/8491).The participants were petrol pump workers. Total 100 petrol pump workers participated in this study. Probability simple random technique was used to select sample. Modified structured questionnaire and Likert scale was used to assess knowledge and attitude of petrol pump workers respectively. Question was divided in three sections. Section one includes demographic variable such as age ,education, working hours/day, years of experience in petrol pump, any substance abuse including habit of smoking. Section two includes knowledge based 20 multiple choice questions to assess the knowledge regarding respiratory problems among the petrol pump workers. One mark is given for right answer and zero mark is given for wrong answer.Section three consists of ten items on attitude scale. Scoring was given 1 to strongly disagree, 2 to disagree, 3 to neutral, 4 to agree and 5 to strongly agree. The data was collected from 22th December 2019 to 29th December 2019. The investigators visited to the respective petrol pumps in advance and obtained the necessary permission from the owners of petrol pumps in Wardha district.20minutes were allotted to solve the questions. Studywas conducted in accordance with the ethical guidelines prescribed by central ethics committee on human research.The collected data was entered and analysed in MS Excel. Descriptive statistics like frequencies and percentages were used for representing the analysed data.

Results

Table 1: Percentage wise distribution of petrol pump workers according to their demographic characteristics

N=100

Demographic variables	No. of Petrol Pump Workers	Percentage(%)
Age(yrs)		
20-30 yrs	45	45
31-40 yrs	32	32
41-50 yrs	15	15
>50 yrs	8	8
Educational Level		
Primary	4	4
Secondary	39	39
Higher Secondary	49	49
Graduation	6	6
PG and above	2	2
Working Hours		
8 hrs	35	35
12 hrs	51	51
24 hrs	14	14

Cont... **Table 1: Percentage wise distribution of petrol pump workers according to their demographic characteristics**

N=100

Years of experience in petrol pump		
1-5 yrs	38	38
6-10 yrs	23	23
11-15 yrs	27	27
16-20 yrs	8	8
History of substance abuse		
Yes	0	0
No	100	100

The above table shows the demographic variables of petrol pump workers.

- 45% of the petrol pump workers were in the age group of 20-30 years, 32% were 8% of them were more than 50 years of age.
- 4% of the petrol pump workers were educated upto primary standard, 39% of them were educated up-to secondary, 49% of them were educated upto higher secondary, 6% of them were graduate and 2% of them were PGs and above.
- 35% of the petrol pump workers were having 8 working hours, 51% of them had 12 hours and 14% of them had 24 working hours.
- 38% of the petrol pump workers had working experience of 1-5 years, 23% had 6-10 years, 27% had 11-15 years, 8% had 16-20 years and 4% of them had working experience of more than 20yrs
- All (100%) of the petrol pump workers had no history of substance abuse

Table 2: Assessment with level of knowledge score

N=100

Level of knowledge	**Score Range**	**Level of Knowledge Score**	
		No of petrol pump workers	**Percentage**
Poor	1-5	3	3
Average	6-10	13	13
Good	11-15	49	49
Excellent	16-20	35	35
Minimum score		3	
Maximum score		19	
Mean knowledge score		13.64 ± 3.41	
Mean % Knowledge Score		68.20 ± 17.09	

The above table shows that 3% of the petrol pump workers were having poor level of knowledge score, 13% had average, 49% had good and 35% of them had excellent level of knowledge score Minimum score for knowledge was 3 and maximum score for knowledge was 19. Mean knowledge score was 13.64± 3.41 and mean percentage of knowledge score was 68.20 ± 17.0

Table 3: Assessment with level of attitude score n=100

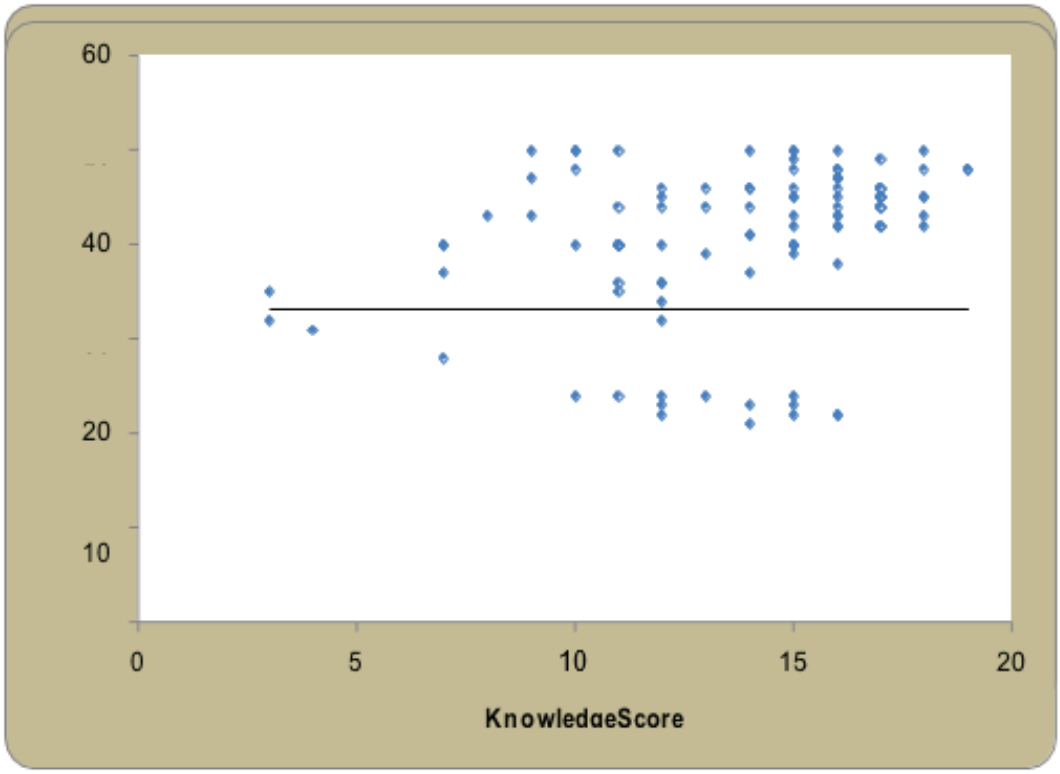
Level of attitude	Score Range	Level of Attitude Score	
		No of petrol pump workers	Percentage
Positive Attitude	>50%	87	87
Negative Attitude	≤50%	13	13
Minimum score		21	
Maximum score		50	
Mean attitude score		40.55 ± 8.25	
Mean % Attitude Score		81.10 ± 16.50	

The above table shows that all(100%) of the petrol pump workers were having positive attitude.Minimum attitude score was 21 and maximum attitude score was 50.Mean attitude score was 40.55 ± 8.25 and mean percentage of attitude score was 81.10 ± 16.50.

Table 4: Correlation between knowledge score and attitude score of petrol pump workers n=100

Overall	Mean	SD	Correlation 'r'	p-value
Knowledge Score	13.64	3.41	0.283	0.004 S,p<0.05
Attitude Score	40.55	8.25		

This table shows the association between knowledge score and attitude score of adults. Knowledge score and attitude score are compared and Pearson's Correlation Coefficient is applied at 5% level of significance. The tabulated value for n=100-2 i.e.98 degrees of freedom was 1.98. The 'p' value i.e. 0.0001 are much less than 5% level of significance. Hence it is statistically interpreted that the significant positive correlation was established between knowledge score and attitude score of petrol pump workers(r=0.283, p=0.0001)



Graph 1: Correlation between knowledge score and attitude score of petrol pump workers

Discussion

The present descriptive study focuses on assessing the knowledge and attitude regarding respiratory problems among petrol pump workers in Wardha district. Sampling technique was non probability.The population of the study consists of petrol pump workers of age above 18 year working in petrol pumps of Wardha district. The sample size included 100 petrol pump workers. As tool structured questionnaire used to assess the baseline information, knowledge and attitude of the workers respectively.

The study result are discussed under the following headlines –

1. Baselines variables of petrol pump workers
2. Knowledge score of petrol pump worker
3. Attitude score of petrol pump worker
4. Association of knowledge and attitude score and selected demographic variable

Baseline variable of petrol pump workers

The subject in the study consist of 100 petrol pump workers workings in 10 different petrol pumps in Wardha district. Workers selected for the study were in the age group above 18 year. Findings revealed that majority in (45%) of worker were in age group of 20-30 years. This findings showed that more young population working in petrol pump.

Similarly finding were showed by another study were the majority (65.2%) of the workers were within the age of 18-27 years1. In the contrast to the present study findings, another study conducted on knowledge of petrol pump workers revealed that (75.6%) of the workers age ranged between 30-49 years and only 7.3% of workers fall between the age group of 22 to 29 years. (13)

With regards to education, majority of workers (49%) had high school education, workers with the primary education had (4%) and post graduation (2%) are very less which revealed that petrol pump workers had basic education and none of them were illiterate.

Similarly, another study conducted knowledge of workers revealed that (49.39%) had intermitted education i.e. high school education. 2.53% worker had primary education none of them are illiterate.[1]

In the case of present study majority of workers (51%) had up-to 12 hours duty per day. Out of them 14% workers had 8 hours duty. This is mainly because most of the worker had an option for selecting the working hours. A small portion of workers were found as part time worker and continuing education.

A similar study conducted to described that (75.5%) had a duty of ≤48 hours per week and

24.5% had a duty of more than 48 hours per week. (13)

Considering the year of experience, present study finding showed that 38% had experience of 1 to 5 year and only 4% had experience more than 20 years in the petrol pumps. This indicates that most of the workers were not considering their job in petrol pump as permanent job.

Similar study findings were showed by another study represent that the most of workers (69.5%) had experience <5 years and few had experience of more than > 15 year.

In the present study none of them having the history of smoking and also not having history of substance.

Association between knowledge and attitude score and selected baseline variables of petrol pump workers.

The present study findings revealed that there was significant association between knowledge and attitude of workers. The obtained P value($P< 0.05$) indicate that positive correlation was established between knowledge score and attitude score of petrol pump workers.

Age in year of petrol pump workers is statistically associated with the knowledge score. Workings hours of petrol pump workers is statistically associated with the attitude score.

Nursing Implications

Nursing services

The nursing services should be based on felt needs of the people the nurse should be responsible for preventive, promote, curative, and rehabilitative aspects of care. It should be helpful in bringing out awareness in workers regarding prevention and management of occupational health hazards due to their work environment and assist them in promoting good health and control of hazards. Most important is the preventives aspect of the care which is based achieved through the health education. The present study will hope the nurses for coordinating health care services to health careprofessionals.

Nursing administration

Nurse administrator should take great interest in formulating occupational health policies to protect high risk groups liable to suffer with health problems caused by particular occupation.Arrangement of in service education for nurses on occupational health hazards, especially due to petroleum products and prevention of further diseases.

Nursing Education

The awareness about respiratory problems among the respiratory problems should be emphasized in the curriculum .the knowledge of heath professional can be improved by conducting in services education in thecurriculum.

Recommendation

Keeping in view the research conclusions, the following guidelines made:

1. Similar study can be replicated in a large group to generalize thefindings.

2. A study on attitude and practice regarding use of personal protective equipments among workers can be carriedout.

3. A study can be conducted to assess the knowledge and attitude regarding respiratory problems among the petrol pumpworkers.

4. A study can be conducted on knowledge of workers regarding the hazards of their workenvironment.

5. Descriptive survey method design is used to assess the knowledge and attitude regarding respiratory problems among the petrol pumpworkers.

Limitations

The study was conducted only in Wardha district and sample size was also small.

Conclusion

Findings suggest that petrol pump workers were aware about the respiratory problems due to occupational hazards and somewhat their attitude was positive towards their health.

Conflict of Interest: There is no conflict of interest.

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Ethical Clearance: Taken from Datta Meghe Institute of Medical Sciences.

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A Cross Sectional Study "Impact of Corona Virus Pandemic on Nursing Education: A Paradigm Shifting or Sustaining the Roots?

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Abstract

Background:COVID 19 outbreak miens critical concern to global education system across the world. The closure of universities has left billions of students behind. Nursing educators and students relied on technology to continue learning online during the pandemic. However, online education is hampered by the lack of networks, power, infrastructure such as inaccessible and lack of digital skills.

Aim: The aim of the study was to evaluate the impact of the COVID 19 outbreak on nursing education.

Objectives:

1. To assess the perception regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications among nursing educators.

2. To find out the association between perception of nursing educators with their socio-demographic variables.

Material and Methods: Quantitative Cross sectional research design was adopted with Non probability technique(Convenient sampling) .Data were collected through a Google form consisting of self-structured Likert scale on perceptions of nursing educators. The Subdomains of the perception were impact of COVID-19 outbreak on Nursing education, Limitations of e-learning and essential modifications required.

Result: of the study revealed that the corona pandemic outbreak has adversely impacted nursing education and urgent need to implement effective e-learning teaching learning strategies of nursing educators to understand their perception and issues they are facing during e- classes to foster teaching-learning.The mean and SD of (7.9 + 3.9) shows negative impact of COVID pandemic on nursing education and are of limitations in E-learning methods with a mean and SD score of (6.2 + 2.4).

Conclusion: The process of imparting education has seen a sea change during the threat of the COVID pandemic. Besides, technology is affecting every side of society and changing it regularly. Challenges in the E-learning context shows the requirement for up-gradation in institutional technology infrastructure and teacher's capacity building training to improve technological expertise.

***Keywords**: COVID 19; Nursing Education; E-learning;Perception; Limitations; Educators*

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Introduction

The COVID -19 outbreak has threatened to aggravate educational results without proactive interventions. The virus epidemic worldwide has burdened learner's immediate learning need and health

costs too. In relation to the clinical setting the nursing education is precisely designed with minute details of theory and clinical teaching learning in terms of credits and define hours. Corona deadlock has moved the system to online platform. The efficacy of using online platform or e learning methods brought few challenges for nursing educators. The professional competence of nursing skills through e learning mode raised questions unanswered. Initially nursing educators also excited to adapt the change and to shift on virtual platform. Then they initiated to face lots of limitations and challenges as they progressed. This move has raised the questions over the teacher to show their capability to cope with technology.

E-learning involved learning delivery along with technology that provided a range of instructional plans and formats. E-learning often knew with added words such as 'internet-based learning,' 'online learning,' 'computer-assisted learning,' and 'web-based learning.'1 The faculty of nursing needs to have skills that are distinct from the requirements of e-learning, especially when it comes to pandemics. Review of literature do not support the studies representing impact on nursing education or None of article on education revealed the influence on perception of nursing educators towards corona pandemic. The present study was undertaken with a aim to find out the perception of educators towards COVID -19 outbreak as it is pivotal to identify how e-learning support nursing .education at time of pandemic and social distancing.

Research question

1. What is the perception regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications among nursing educators?

2. Is there any association between perception regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications among nursing educators with their socio-demographic variables?

Objectives of the study are

1) To assess the perception regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications among nursing educators.

2) To find out the association between perception of nursing educators with their socio-demographic variables.

Methodology

Research Design	**Cross sectional research design**
Setting	Nursing Institutions, across India
Ethical Permission	Manikaka Topiwala Institute of Nursing, Gujarat, India
Sampling techniques	Nonprobability technique (Convenient sampling)
Sample size	386 nursing faculties
Variables	1) Socio-demographic variables 2) Nursing educator's perceptions regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications.

Cont...

Explanation of the tools

Tool 1: Socio-demographic profile

It consists of personal information of nursing educators i.e. gender, age, qualification (in nursing), designation, type of working institution, teaching experience, previous experience of E-teaching and whether they have taken E-classes during lockdown.

Tool 2: Likert scale on Nursing educator's Perception regarding impact of corona pandemic on nursing education, limitations of E-learning & required modifications.

This likert scale about nursing educator's perceptions is consisted of 15 items which is further divided into 5 domains.

1) Domain of perception of nursing educators regarding impact of corona pandemic on nursing education (4 items)
   - Each item score ranges from 1 to 5, where 1 refers to strongly agree, 2 refers to agree, 3 refers to neutral, 4 refers to disagree and 5 refers to strongly disagree.
   - Total score ranges from 4 to 20.
   - Score 12 refers to overall neutral response.
   - Lesser the score, more is the adverse impact of corona pandemic on nursing education
2) Domain of perception of nursing educators regarding limitations of E-learning (3 items)
   - Each item score ranges from 1 to 5, where 1 refers to strongly agree, 2 refers to agree , 3 refers to neutral, 4 refers to disagree and 5 refers to strongly disagree.
   - Total score ranges from 3 to 15.
   - Score 9 refers to overall neutral response.
   - Lesser the score, more are the limitations of E learning.
3) Domain of nursing educator's perceptions towards required modifications (5 items)
   - It contains 5 items.
   - Each item score ranges from 1 to 5, where 1 refers to strongly agree, 2 refers to agree , 3 refers to neutral, 4 refers to disagree and 5 refers to strongly disagree.
   - Total score ranges from 5 to 25.
   - Score 15 refers to overall neutral response.
   - Lesser the score, more is the agreement of nursing educators towards required modifications mentioned in items.
4) Domain of nursing educator's perceptions towards importance of required modifications (2 items)
   - Each item score ranges from 1 to 5, where 1 refers to very important, 2 refers to important, 3 refers to moderately important, 4 refers of little importance and 5 refers to unimportant.
5) Domain of nursing educator's perceptions towards potential of required modifications (1 item)
   - Each item score ranges from 1 to 5, where 1 refers to definitely, 2 very probably , 3 refers to possibly, 4 refers probably not and 5 refers to very probably not.

Statistics	**Descriptive statistics: mean, median, SD, frequency distribution** **Inferential statistics: Association**

Results

Findings related to Socio demographic Data of nurse educators:

As per socio-demographic details, findings revealed that 73.1% nursing educators were females. 51% of the nursing educators belonged to (31-40) years of age group and 59.1% were post-graduates. Most of the nursing educators (46.6%) were working as clinical instructors/ Nursing tutors and 60.1% were serving under private institutions. A total of 39.6% nursing educators were

having teaching experience of less than 4 years and 50.3% were having previous E- teaching experience. Most of the nursing educators (73.3%) have taken E-classes during lockdown because of COVID-19 pandemic.

Findings Related to perceptions regarding impact of corona pandemic

The nursing educators showed fair agreement that COVD-19 pandemic has negative impact on nursing education with a mean and SD of (7.9 + 3.9). Data also showed that there are limitations in E-learning methods with a mean and SD score of (6.2 + 2.4) and findings of the study also suggested that nursing educators agreed with the mentioned modifications to improve the status of nursing education during these pandemic situations with a mean value of (9.6 + 3.6).

Figure 1 represents findings revealed degree of agreement shown by nursing educators regarding impact of corona pandemic on nursing education and limitations of E-learning. Most of the nursing educators strongly agreed and agreed that corona pandemic has very adversely impacted nursing education and teaching through E-learning methods has lot of limitations.

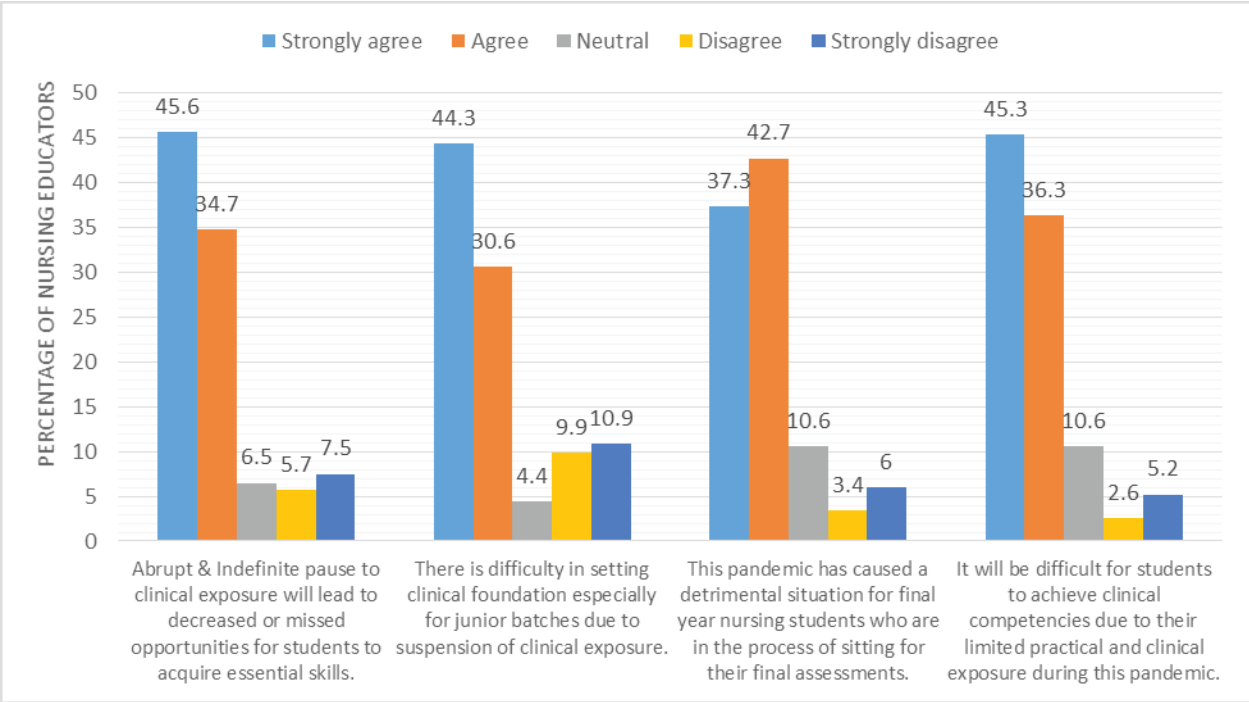

Figure 1 represents the Nursing educator's perceptions towards impact of corona pandemic on nursing education

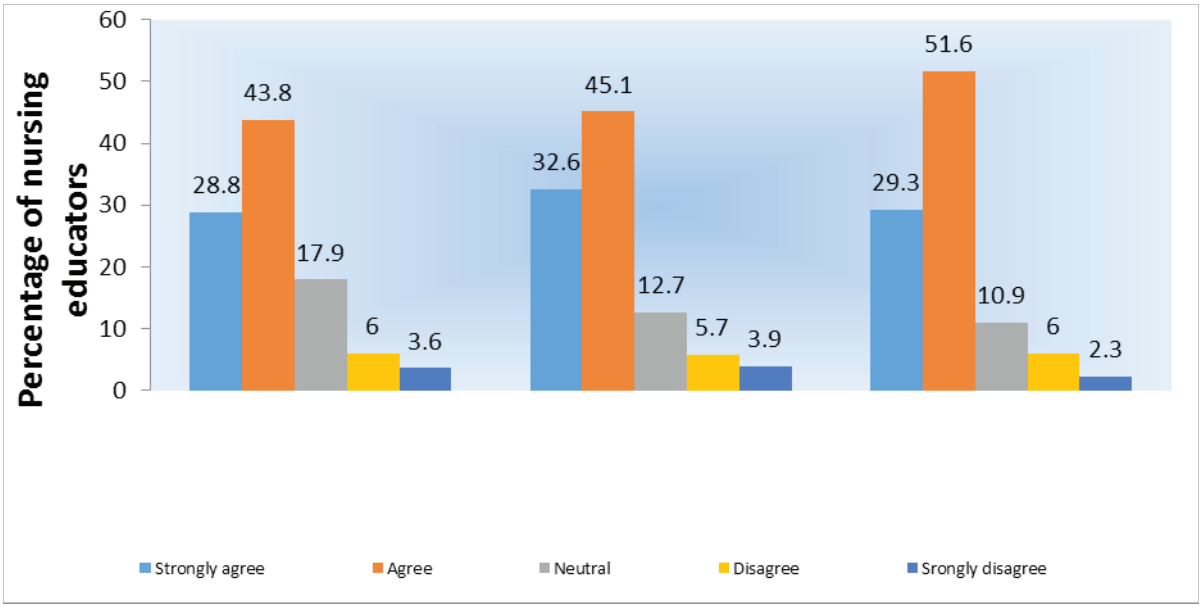

Figure 2 represents the Nursing educator's perceptions towards limitations of E –learning n =386

Figure 2 represents the findings revealed degree of agreement shown by nursing educators regarding impact of corona pandemic on nursing education and limitations of E-learning. Most of the nursing educators strongly agreed and agreed that corona pandemic has very adversely impacted nursing education and teaching through E-learning methods has lot of limitations.

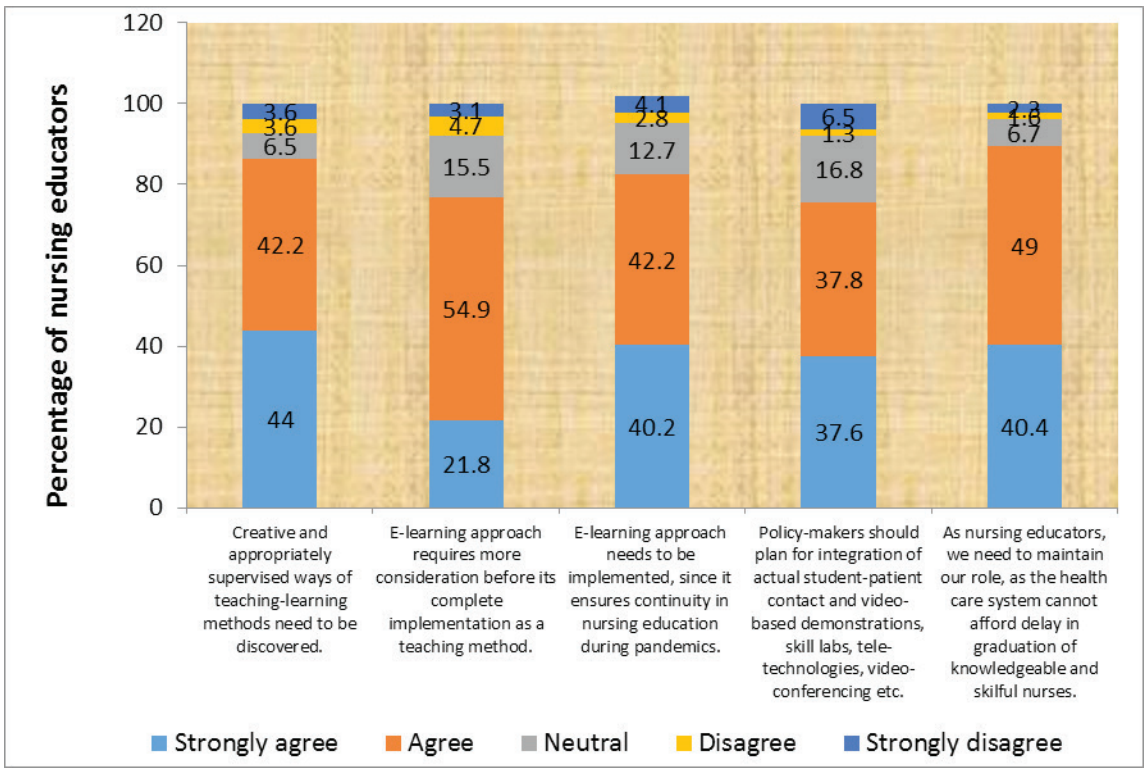

Figure 3 represents the Nursing educator's perceptions towards required modifications.

Figure 3 and 4 represents perceptions of nursing educators towards required modifications, its importance & potential in nursing education to deal with these pandemics. Findings revealed that most of the nursing educators agreed for the necessity of modifications and think that these modifications are important to carry out and have great potential to bring positive changes in nursing education.

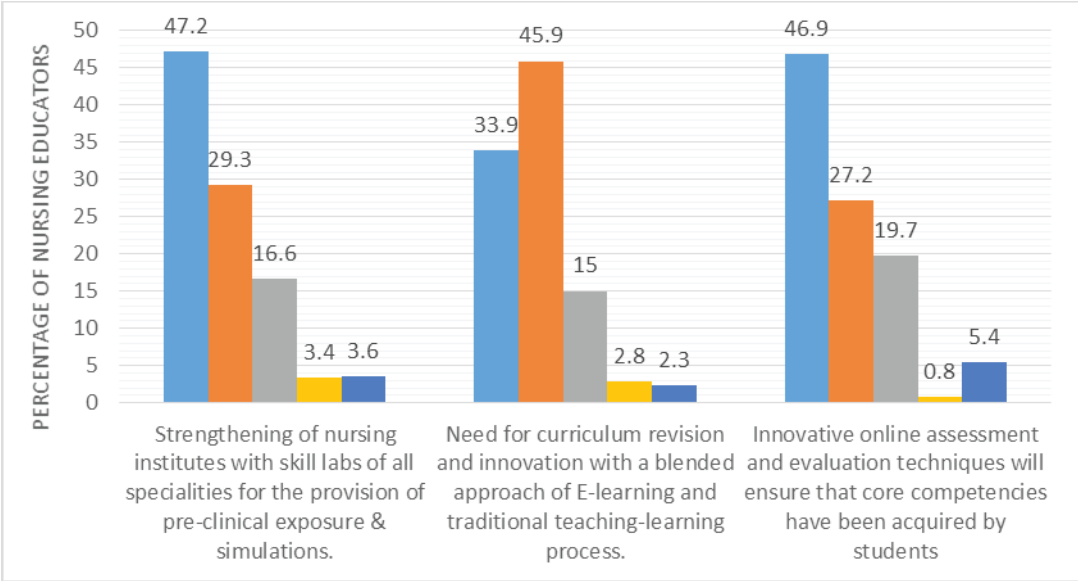

Figure 4 represents the Nursing educator's perceptions towards importance and potential of required modifications

Finding Related to Association

Table 1 and Table 2 represents the association of nursing educator's perceptions regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications with selected variables and data in Table 5a revealed that nursing educators who were females, working in government institutions, with previous experience of E-teaching and have taken E- classes during lock-down showed better agreement of adverse impact of corona pandemic on nursing education with a statistically significant p-value of 0.03, 0.003, 0.01, 0.0001 respectively. Nursing educator's agreement towards limitations of E-learning was not found to be significantly associated with any of the variables. Nursing educators who have taken E-classes during lockdown were having statistically significant better agreement towards mentioned modifications to improve nursing education during the pandemic situations.

Table 1 represents the association of nursing educator's perceptions regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications with selected variables. n= 386

Impact of corona pandemic on nursing education		Mean + SD	Mean diff (95% CI)	t-value	p-value
Gender	Female	7.7 + 3.8	-0.96 (-0.8 to -1.8)	2.14	0.03*
	Male	8.6 + 4.0			
Type of working Institution	Private	8.4 + 4.3	1.2 (2.0 to 0.42)	3.0	0.003*
	Government	7.2 + 3.0			
Previous experience of E-teaching	Yes	7.7 + 3.4	-0.93 (- 0.15 to -1.7)	2.3	0.01*
	No	8.3 + 4.2			
Taken E-classes during lockdown	Yes	7.3 + 3.4	-2.0 (-1.1 to – 2.8)	4.6	0.0001*
	No	9.4 + 4.7			
Limitations of E-learning					
Gender	Female	6.2 +2.6	0.38(0.9 to -0.16)	0.013	0.16
	Male	5.9 +1.8			
Type of working Institution	Private	6.3 + 2.6	0.44 (0.93 to -0.05)	1.75	0.07
	Government	5.8 + 2.0			

Cont... **Table 1 represents the association of nursing educator's perceptions regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications with selected variables. n= 386**

Previous experience of E-teaching	Yes	6.12 + 2.4	-0.07 (0.4 to -0.56)	0.3	0.76
	No	6.19 + 2.4			
Taken E-classes during lockdown	Yes	6.1 + 2.2	-0.10 (0.44 to -0.65)	0.37	0.7
	No	6.2 +2.7			
Required modifications					
Gender	Female	9.5 + 3.7	-0.54(0.27 to -1.3)	0.62	0.19
	Male	10.0 + 3.4			
Type of working Institution	Private	9.7 + 4.1	0.43(1.1 to -0.3)	1.16	0.27
	Government	9.3 + 2.7			
Previous experience of E-teaching	Yes	9.2 + 3.3	-0.66 (0.60 to -1.3)	1.8	0.07
	No	9.9 + 3.9			
Taken E-classes during lockdown	Yes	9.0 + 3.0	-2.2 (-1.4 to -3.0)	5.6	0.0001*
	No	11.2 + 4.4			
Independent t test* p-value<0.05					

Table 2 revealed that impact of corona pandemic on nursing education was significantly associated with age, designation and teaching experience with a p-value of 0.0001, 0.0001 and 0.0001 respectively. Data represents that nursing educators in age group of 21-30 years, working as professors and with teaching experience between 0 to 4 years were having better agreement of adverse impact of corona pandemic on nursing education. Limitations of E-learning was also found to be significantly associated with age, designation and teaching experience with a p-value of 0.001, 0.0001 and 0.0001 respectively. Nursing educators whose age group is 50 years and above, working as professors and with teaching experience between 0 to 4 years agreed the most, towards limited scope of E-learning. Requirement of modifications was found to be significantly associated with age, qualification, designation and teaching experience with a p-value of 0.0001, 0.007, 0.02 and 0.0001 respectively. Nursing educators whose age group is 50 years and above, done doctorate or pursuing doctorate, working as professors and with teaching experience between 0 to 4 years agreed the most, towards requirement of modifications in current teaching-learning methods to deal with these pandemics as far as nursing education is concerned.

Table 2 represents the association of nursing educator's perceptions regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications with selected variables. n= 386

Impact of corona pandemic on nursing education		Mean + SD	F-value	p-value
Age	21-30	7.2 + 3.2	8.63	0.0001*
	31-40	7.8 + 3,5		
	41-50	11.0 + 5.9		
	50 years and above	10.0 + 7.9		
Qualification	Graduate	8.4 + 4.4	1.01	0.3
	Post-Graduate	7.74 + 3.6		
	Doctorate (Including Pursuing Scholars)	7.78 + 4.0		

Cont... **Table 2 represents the association of nursing educator's perceptions regarding impact of corona pandemic on nursing education, limitations of E-learning and required modifications with selected variables. n= 386**

Designation	Clinical Instructor/ Nursing Tutor	7.77 + 3.5	6.54	0.0001*
	Lecturer	8.1 + 4.2		
	Professor	7.1 + 3.4		
	Principal/ Administrator	11.5 + 5.5		
Teaching Experience (In years)	0-4 years	6.9 + 2.5	11.71	0.0001*
	5-9 years	8.4 + 4.1		
	10-14 years	7.2 + 4.1		
	15 years and more	10.7 + 5.4		
Limitations of E-learning				
Age	21-30	6.0 + 2.2	5.66	0.001*
	31-40	6.1 + 2.2		
	41-50	7.7 + 3.7		
	50 years and above	4.6 + 2.6		
Qualification	Graduate	6.17 + 2.7	0.09	0.9
	Post-Graduate	6.18 + 2.1		
	Doctorate (Including Pursuing Scholars)	6.03 + 2.8		
Designation	Clinical Instructor/ Nursing Tutor	5.9 + 2.2	7.29	0.0001*
	Lecturer	6.3 + 2.5		
	Professor	5.8 + 2.2		
	Principal/ Administrator	8.5 + 3.0		
Teaching Experience (In years)	0-4 years	5.5 + 1.4	7.82	0.0001*
	5-9 years	6.48 + 2.6		
	10-14 years	6.40 + 3.1		
	15 years and more	7.2 + 2.8		
Required modifications				
Age	21-30	9.2 + 4.1	9.1	0.0001*
	31-40	9.5 + 2.7		
	41-50	12.9 + 4.7		
	50 years and above	7.8 + 2.0		
Qualification	Graduate	10.5 + 4.9	5.01	0.007*
	Post-Graduate	9.4 + 3.1		
	Doctorate (Including Pursuing Scholars)	8.8 + 2.4		
Designation	Clinical Instructor/ Nursing Tutor	9.9 + 3.9	3.32	0.02*
	Lecturer	9.8 + 3.7		
	Professor	8.5 + 2.6		
	Principal/ Administrator	10.0 + 2.9		
Teaching Experience (In years)	0-4 years	8.5 + 2.6	9.50	0.0001*
	5-9 years	10.6 + 4.0		
	10-14 years	9.1 + 4.5		
	15 years and more	10.6 + 3.0		
ANOVA * p-value<0.05				

E-classes during lockdown was significantly associated with qualification, designation, type of working institution, and teaching experience with a p-value of 0.0001, 0.0001,0.0001 and 0.0001 respectively. Data revealed that 92.1% of nursing educators with doctorate degree, 100% of nursing educators who are working as principal/administrator, 85% of educators serving in government institutions and 95.9% of educators already experienced with E-teaching have taken classes during lockdown period of COVID-19 pandemic.

Discussion

This study aimed to assess the impact of COVID-19 pandemic on nursing education and investigating the perception of nursing educators for e-learning and to inquire factors comprise nursing educators' efficacy and measures of quality of e-learning during the COVID-19 pandemic environment. The body of evidence supporting the urgent need to implement effective e-learning teaching strategies with several of collaborative activities such as knowing experiences of nursing educators to understand their perception and issues they are facing during e- classes to foster teaching, student's satisfaction, and achievement of educational outcomes.

Majority of study participants were young, having less than 5 years' experience. More than half of study participants were responded from private nursing institutions. Almost two by third nursing faculty were taking e-classes during pandemic. About the existing nursing curriculum, absolute practices of clinical competencies are among the prime concern for learners to achieve routine hospital practical experiences during clinical hours as it assures to prepare clinical efficient workforce [3]. 43.8% study participants reported to get it difficult to provide actual clinical experience to their students with e-learning as teaching method. Hence, it's required to keep the most practical and reliable skills through technology as such a simulation technique can useful for students to impart professional and factual clinical learning.

Even though all efforts are being taken by nursing institutions and raising the use of information technology in nursing education, the efficacy of utilizing e-teaching methods in clinical learning is imprecise.[4]

Half of the study participants (54.9) were in favor of strategic planning before its implementation as of teaching, learning method in nursing to get maximum results. Studies also supported the prime factors in preparing nursing curriculum program should be making learning simple, efficient and future of technology ready [2, 5]

Another notable concern of nursing educator's participant was clinical exposer of junior batches nursing students due to lockdown constraints and COVID-19 pandemic, clinical posting was suspended for an undecided time. In contrast with other similar studies junior batch nursing students, mainly in initial semesters, are in the early phase of psychomotor, clinical critical thinking skills. Hence, attending complete e-learning based clinical practices, the skills lab may not as useful.[6]

Nursing is a practice-led profession and leadership skills promote nursing students to improve the quality of care along with direct participation in clinical care.[7] Almost half of the study participants agreed to the statement that leadership quality, teamwork, and group discussion may be affected in the absence of face to face classroom teaching where students work together with a common goal.

Previous research work also reported that knowledge adherence and consistency was higher in traditional classroom teaching as compared to e-learning. [8-10] Furthermore Lathi *et al*. (2014)[11] did not find statistically significant differences among e-learning and traditional classroom learning groups concerning knowledge, skill, and satisfaction of nursing students. In our study, 45.3% of participants were accepting that this will be difficult for nursing students to achieve proficiency in clinical practices due to the termination of regular hospital visits in the wake of the COVID-19 pandemic.

Another observation during the study was found that 43.8% of participants were affirmation that nursing student's skills to make clinical diagnosis would suffer in the absence of regular clinical practices. Accordingly similar study, since its beginning, nursing is a purely practice-based discipline and clinical education is the basis of the nursing education program. Besides, the available literature, nursing students are spending almost 50% of their curriculum hours during their clinical practices.[10, 12] Lack of clinical competency can threaten

patient care and safety.[13]

Since the beginning of lockdown, nursing faculties are put up with the high expectation to use e- technologies with full expertise with little or no formal computer training. More than half of our study participants agreed to the requirement of advanced planning before implementing e-learning. Vary from many researchers' views by being either nursing clinical practitioners or educators the teacher's perception with use of information technology for education purposes likely influenced by their previous exposure.[14] High quality of technical support and facilities can boost opportunities for e-learning in nursing education . Here almost half of participants have had not been part of any e-learning teaching exposer before the pandemic. During the COVID -19 pandemic crisis, well-planned e-learning programmes for nursing students can support in maintaining the development of nursing students' skills and knowledge. It is suggested to support further shreds of evidence related to the perception of nursing educator about e-learning for enhancing nursing learning, improved clinical skills and heightened learner satisfaction among nursing students.

Conclusion

As technology advances and training pressures rise, e-learning is an economical, innovative and reputable platform for program directors to incorporate into their curricula.

The effectiveness of e-learning in nursing education remains unknown regarding how the learning can be transferred to change practice and affect patient outcomes. However different and well planned e-learning programmes can support the skills and educational development of nursing students. It is suggested to include training of nursing faculties for handling technical devices and including computer literacy in nursing curriculum

Recommendations

- Nursing academics in present and future will be left with, no choice but to prepare nursing students technology ready.
- The COVID pandemic imposed lockdown left with no choice for educators but to go with the use of technology in nursing teaching, learning. This created an immediate need to prepare a technologically competent workforce.
- Nursing faculty progress in technology fluency is crucial and needs to be continuing with a focus for nursing institutions and administrators to enhance student learning and retain faculty by providing training to handle technical aspects.
- Where most nursing institutions are already facing a shortage of teaching faculty. Mastering competency in e- technology and planning e-courses is a further burden, on already occupied nursing faculty and could push forward the departure of extremely qualified nursing faculties from nursing institutions, thus worsening the already existing nursing shortage
- Developing or developed in every country, the paucity of training is quoted as an important issue of shortage of nurses due to inadequate nursing institutions and faculty globally.
- COVID-19 Pandemic is a health emergency worldwide and that can outcome in severe disruption of health and social infrastructure. Nurses are playing a crucial part as members of the response team in the century's biggest health crisis. During such never seen situation keeping nurse's institution working and continuity in nursing education is the great service nursing faculties are doing by accepting the challenge of e-learning
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COVID-19 Lockdown & Online Education: Impact of World-Wide Pandemic on Nursing Education in India

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Abstract

Background : The technology has taken over all most all aspects of our life including our education system. The present pandemic COVID 19 has affected the education of nursing students. So many institutions have adopted online teaching so that students can learn and improve their self-confidence.

Objectives: The aim of this study is to assess the nursing student's attitude towards online education and factors affecting online education.

Methods: A total of 780 participants from different nursing institutions of Odisha completed a questionnaire-based survey on the impact of pandemic on education of nursing students. A self-structured questionnaire was used to collect data. Participants were selected by convenient sampling method. The demographic characteristics were represented by frequencies and percentage. Descriptive statistics for all categories calculated based on the responses.

Results: Among the participants (n=780), all most all participants 87.9% were females, most participants 79.4% of participants were between 18 to 28 years. Among all participants 56.8% are doing Basic Bsc. nursing, 10.1% are continuing Msc Nursing, 29% are in GNM course, and 4.1% are continuing post basic nursing. Maximum of 48.8% are in 1st year. All most all 98.5% of institution conducting online education. 39.3% of students have positive attitude and 28.4% of students have negative attitude towards online teaching. 72% students have poor internet connection, 52% students have lack of knowledge regarding using the software, 67% students feel audio was not clear, 56% students feel letters are small to read, 47% participants feel time is not sufficient, 68% participants feel there was interruption during class.

Conclusion: The online education has not developed overnight. This system of education adopted by many higher educations and in many institutions. Online teaching

Key words*: Impact, pandemic, nursing, online education, lockdown*

Introduction

When we look back 10 to 20 years from now, our life has transformed into a newer form. The technology has become a vital part of everyone's lives. All most all aspects of our day to day lives are influenced by technology[1]. The technology has taken over every field of our lives even in education system. The biggest change in education by technology is online education[2]. All we need one computer and internet connection. Now what is online education is? It teaches the students by using electronic technologies outside the traditional classroom teaching[3]. Every citizen of India has right to education, weather a child belongs to rich society or poor society or lives faraway. Online education made it easy to teach every child[4]. By online education every child can get education at anytime and anywhere.

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Due to the current pandemic COVID 19 situation and lockdown the education of children is also affected. But online education came as a savior for students during this pandemic[6]. Due to pandemic it became a common regular mode of teaching for many higher education institutions. To continue their education and to improve their confidence during lockdown every institution adopted the online education[7]. Now the biggest question is whether this online education is effective? Weather it is more effective than traditional classroom teaching, can online education replace the classroom teaching? The Pew Research Center conducted a research in 2010-11 year and the result shows, 89% of colleges offering online courses[8]. Another study conducted with students who got in to college admission in 2013 revealed that at least 32% has attended one online course[9]. The aim of present study is to assess the student's attitude towards online teaching and factors affecting the online educations.

Material & Methods

Research design and sampling

A cross sectional survey was used to collect data from study subjects by using self-structured questionnaire. In present study there are 780 participants from different nursing institutions of Odisha. The date was collected by using convenient sampling technique. Students from different institutions who agreed to participate in the study had included in the present study.

Methods of data collection

The present study was conducted from May 20th to 30th 2020. The data was collected during the lockdown period that is why it was not feasible for author to collect data by face to face interview, so we collected the data online. The participants were given an introduction of author, purpose of study, instructions to fill the answers, confidentiality of participants, consent from participants and self-structured questionnaire. The questionnaire was formed by using Google docs and was posted in What's App group and E-mail. The participants were requested to the entire questionnaire by selecting only one response. A self-structured questionnaire was formed based on student's attitude towards online classes and factors affecting online classes. The questionnaire has three parts which is based on demographic data, attitude towards online classes and factors affecting online classes. The demographic data contains age, gender, course and year of course. Thirteen questions based on attitude of students towards online class and seven questions related to factors affecting online classes. To measure the attitude five-point scales was adopted i.e. from strongly agree to strongly disagree. Each question has five options. For strongly agree response 5 points was given gradually the score decreases and for strongly disagree one score was allotted. If score is more than 50 it was considered as positive attitude, the score is 49-26 it was considered as neutral and scores less than 25 was considered as negative attitude. The collected data was coded and entered into excel sheet. Descriptive statistics were used to calculate demographic characteristics, attitude and factors.

Results

In present study there were total of 780 participants. Among them most of participants (87.9%) were females, most participants (79.4%) of participants were between 18 to 28 years. Among all participants 56.8% are doing Basic Bsc. nursing, 10.1% are continuing Msc Nursing, 29% are in GNM course, 4.1% are continuing post basic nursing. Maximum of 48.8% are in 1st year, 32.2% are in 2nd year, 17.2% are in 3rd year and only 6.8% students are in internship. All most all i.e. 98.5% of institution conducting online education. More than half of students 66.6% responded that all subjects covers in online classes. Only 58.3% students responded that demonstration shown through video.

Table – 1: Frequency and percentage of attitude of nursing students towards online classes calculated and results shows that only 9.2% of students have all required technology and knowledge how to access the online classes. Only 1.5% of students strongly agree that online teaching is easy to understand. More than half 51.3% of students want the topic to repeat in classroom again. Less than half 45.6% of students agree on online classes give more opportunity to know new learning materials. Only 30.8% of students agree that online classes are equal to the traditional classroom teaching in understanding the topics. 41.7% of students disagree that it is easy to understand online class compared to classroom teaching. Only 31.3% of students feel confident that you can take an exam based on topics covered online.

Table - 1: Frequency and percentage of attitude of nursing students towards online classes.

N=780

Sl no.	Questions	Strongly agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly disagree (%)
1.	Online classes give more opportunity to know new learning materials.	8	45.6	24.6	19.2	2.6
2.	Online classes are equal to the traditional classroom teaching in understanding the subjects	2.9	30.8	18.9	39.1	8.3
3.	Online classes increase my potential to learn new things compared to classroom teaching	2.7	32	25.4	34.6	5.3
4.	Online classes motivate to learn independently compared to classroom teaching	5.3	44.1	21.6	25.7	3.3
5.	Online classes encourage to ask questions and improve interaction with teacher compared to classroom teaching	6.2	41.7	22.5	24.3	5.3
6.	online classes improve information, communication technique skills compared to classroom teaching	5	37.3	23.7	29	5
7.	Attention towards class longer in online compared to classroom teaching	2.3	30.8	30.5	28.1	8.3
8.	I have all required technology and knowledge how to assess the online classes	9.2	50	25.7	13.9	1.2
9.	It is easy to understand online class compared to classroom teaching	1.5	21	28.7	41.7	7.1
10.	Do you have chance to ask doubts to your teacher after the class got over?	14.2	57.6	19.5	5.6	3.1
11.	Do you want topics taken online to be repeat in classroom	11.2	51.3	24.9	8.4	4.2
12.	Do you want online class to be continue after your college resume classroom teaching	2.4	32.6	36.1	24.6	4.3
13.	Do you feel confident that you can take an exam based on topics covered online?	6.7	31.3	38.8	16.7	6.5

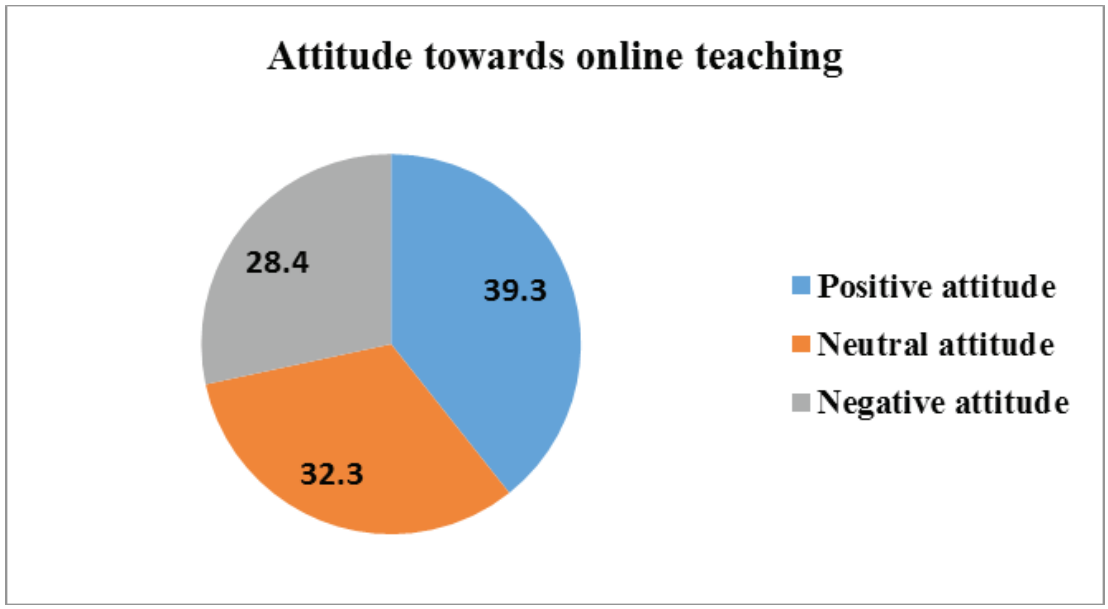

Figure – 1: level of attitude of nursing students towards online teaching shows only 39.3% of students have positive attitude and 28.4% of students have negative attitude towards online teaching.

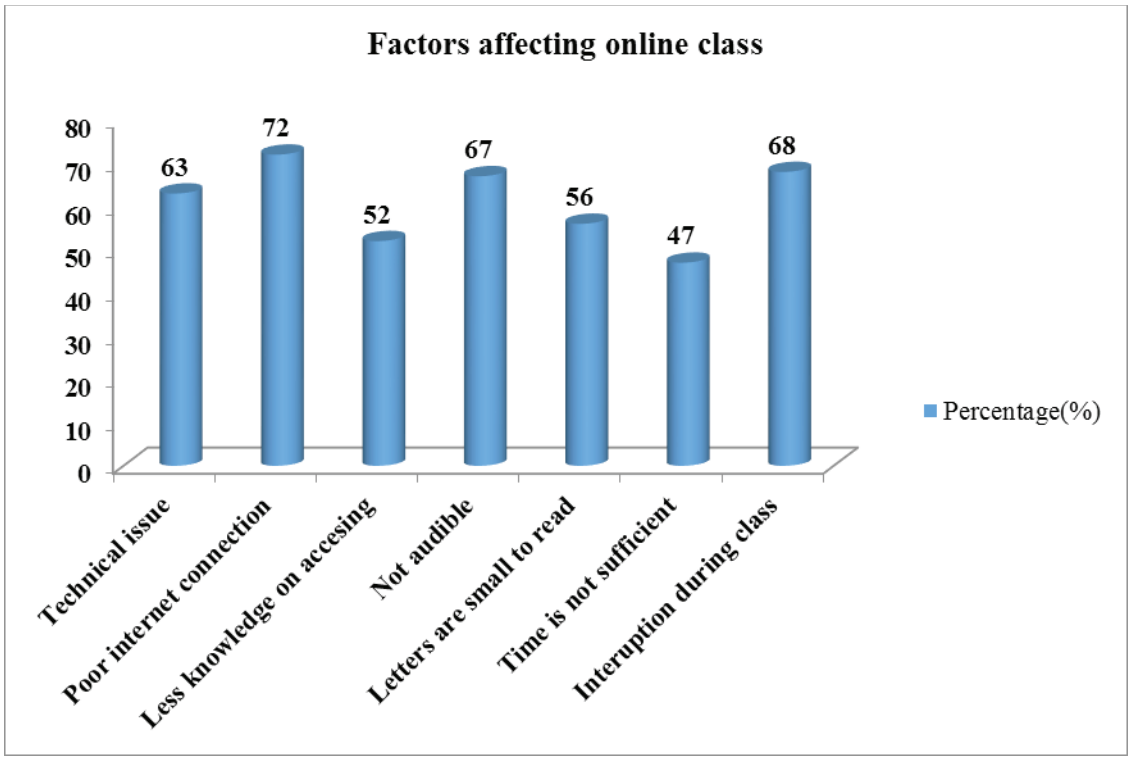

Figure 2: Factors affecting online teaching reveals the most 72% affected factor is poor internet connection and least 47% affected factor is time is not sufficient.

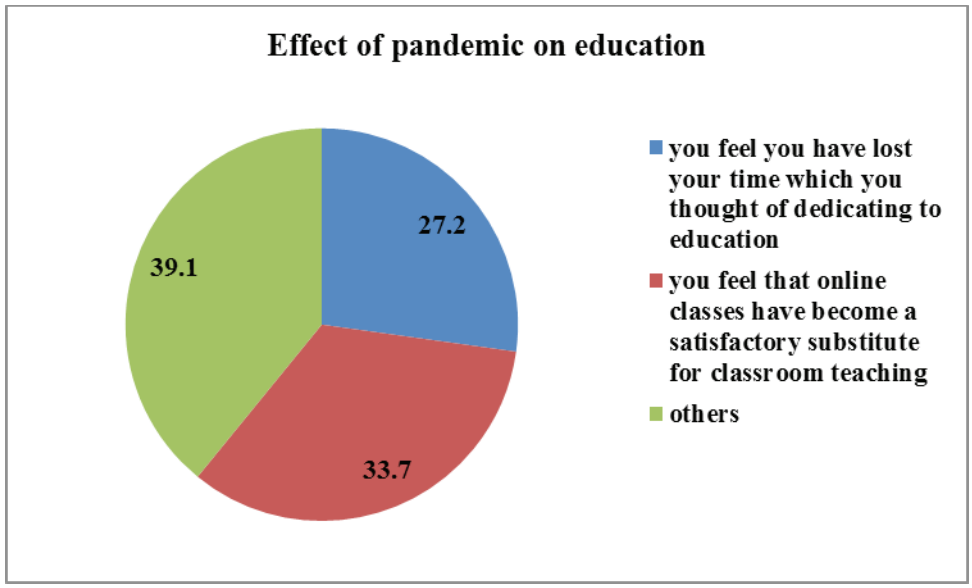

Figure – 3: The effect of this pandemic on your education shows 27.2% of students feel they have lost their time and only 33.7% of students feel online classes have substituted classroom teaching.

Discussion

This survey was conducted among nursing students. The maximum participants were female and most participants were between 18 to 28 years. 45.6% of students feel online classes give more opportunity to know new learning materials. 44.1% of students feel online classes motivate to learn independently compared to classroom teaching. 57.6% students get chance to ask doubts to teacher after the class got over. 24.6% students don't want online class to be continued after your college resumes classroom teaching. 37.3% of students feel online classes improves information, communication technique skills compared to classroom teaching. 32% of students feelonline classes increase their potential to learn new things compared to classroom teaching. More than half 51.3% of students want the topic to repeat in classroom again. 23.2% students are not confidence that they can secure good percentage after online class. Only half of students know the technology related to online class.39.3% of students have positive attitude, 28.4% of students have negative attitude and 32.3% of students have neutral attitude towards online teaching.The researcher found out many factors which are affecting the online teaching such as 63& students has technical issue, 72% students has poor internet connection, 52% students has lack of knowledge regarding using the software, 67% students feel audio was not clear, 56% students feel letters are small to read, 47% participants feel time is not sufficient, 68% participants feel there was interruption during class.

Zahoor Ahmad Lone conducted a study on impact of Online Education reveals that, in India thousands of students who enroll in various higher education courses which is taught by online. The concept and view towards online teaching has changed, people should know what can be the issue behind online education. Technology has changed our day to day life; even it influenced the educational sector also. He found out some factors can affect the online teaching such aslack of Infrastructure, lack of hardware and software facilities, lack of skilled manpower to teach students in rural areas, lack of computer based courses[10].

Lori Kupczynski, Marie-Anne Mundy, Don J. Jones conducted a study with 1438 students to assess the factors affecting online student success in graduation level, reveals that there were significant relationships between the presentation, feedback, caring and instructor rating[11].

Anna Sun and Xiufang Chen Rowan conducted a study on effective practice of online education. They found out effectiveness of online teaching depends on the content of course should be well designed, the teacher and learner should motivate to take well

organized online teaching, both teacher and students should well-prepared and support to each other, should create an online teaching community, should improve the technology and internet connection. After doing all those, it can be anticipated that this may strengthen the effectiveness of online teaching[12].

Neuhauser, C. conducted a research on effectiveness of Online and classroom teaching, the study divided students into two equal groups. The two groups were taught by the same instructor and used same materials. He found out there was no significant differences in given assignments and scores, though the average score of online groups was little higher. 96% students found the online teaching was as effective as face-to-face teaching. The result also shows there was no significant difference between learning styles and courses, learning preferences and grades of students of both groups. The result showed that both online and face to face teaching is equally effective[13].

There are many studies which show that both online teaching and traditional classroom teaching was equally effective. In present study researcher found many factors which can affect the online teaching such as poor internet connect, technical issue, audio, time and small letters. So, researcher recommends that if these problems can be fixed and a team of expert online community can be formed, university can strengthen the technical and network issue the online classes can be more effective.

Conclusion

The education system is now not only the face to face teaching but also using new technology to improve the student's skills. All most 3 billion students in India using the online learning or e-learning. The India Govt. also gives more effort in making digital India and online education so that all children from every corner can learn easily.

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Knowledge of Students Regarding COVID-19 Amid its Hasty Expansion

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Abstract

Background: The pandemic of COVID-19 has been spreading around the world very rapidly and has took a terrible shape. The one of the better ways to deal with this terrible pandemic is to have thorough knowledge about it and act consequently.

Objectives: The present study aim at assessing and comparing the knowledge of Students about COVID 19 and its hasty expansion.

Material and Methods: Descriptive cross-sectional research design was adopted for the study. Data using online structured knowledge questionnaire was collected from 316 participants which was selected using convenient sampling technique from selected University of Ambala. The data was collected from students of Nursing, Physiotherapy and IT department.

Results: The mean age of participants among Nursing was 21.75 + 1.77, 23.43 + 2.62 among physiotherapy whereas 23.22+ 2.28 among IT department. In relations to Gender it was found that most (76 %) of the participants were female and only 24 percent were male among Nursing, 66 percent's participants were female, and 34 percent were male among physiotherapy while 56 percent were female and 44 percent were male in the IT department. 47 percent participants from the Nursing, 40 percent participants from physiotherapy and 20 percent participants form IT were having good knowledge. The knowledge score among Nursing Participants ranged from 17 to 46, 15 to 47 among Physiotherapy whereas it was 17 to 47 among IT participants. The mean knowledge score of the Nursing participants was 33.84 + 5. 48, 32.91+6.67 among physiotherapy and it was 31.63+5.98 among IT. Repeated measure ANOVA was calculated which shows a statically significant difference (F=1.07, p=0.00) among three groups

Conclusion: The study concluded that continuing teaching experience is required to the students regarding preventive measures as well as change in the treatment regimen

Key words: *Knowledge, Understanding, University students, COVID 19,*

Introduction

COVID-19 is an infectious disease caused by virus diagnosed newly i.e. severe acute respiratory syndrome coronavirus. The virus was reported in Wuhan city of China.[1] Coronaviruses (CoV) are a broad family of viruses that are known to cause serious and sometimes fatal pulmonary diseases such as, Severe Acute Respiratory Syndrome (SARS-) and Middle East Respiratory Syndrome (MERS-).[2] As of now, globally 35,109,317 are the confirmed cases of COVID 19, including 1,035,341 deaths all over the world, whereas in India 6,623,815 confirmed cases of COVID-19 with 102,685 deaths.[3]

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The unique feature of the virus is high transmission rate and low pathogenicity. High infectious rate as well as mortality rate associated with COVID-19 till present and has a matter of great public health concern globally.[3]

The transmission mode of COVID 19 is still vague but, it is believed that the virus transmits through respiratory aerosol by coughing and sneezing of the infected person, or via close personal contact. Touching the objects that are contaminated with coronavirus may also spread the infection. The treatment of COVID 19 is the matter of a great concern because as of now there is no specific antiviral medicine or vaccine to treat or prevent the disease. Only symptomatic relief can be done. Treatment for symptomatic relief can be done. Possible vaccine and medications are still under trial.

The Medical Students are directly or indirectly related to such pandemics. So, they must have a higher level of knowledge and better attitude towards the disease. [4]

The student compliance is an essential component in the prevention and control of the COVID 19 which is directly related to their knowledge. Hence, we undertook a study to assess the knowledge among students of various department at Selected University of Mullana, Ambala, Haryana during very quickly rise of COVID 19 outbreak.

Methodology

Study Design

Descriptive cross section research design was used to assess and compare the knowledge of Students about COVID 19. The study was conducted in the month of April 2020 at selected University of Ambala.

Research Setting and Population

The study was conducted at selected University of Mullana, Ambala. The students of Nursing, Physiotherapy and IT departments who were present at the time of data collection and who were willing to participate in the study were included in the study.

Sample Size

Initially 582 students were enrolled for the study. But at the time of data collection only 317 students have participated in the study. Among 317 participants 136 students were form Nursing Departments, 81 from the Physiotherapy department and 101 students from the Non-Nursing Department.

Data Collection Tool and Technique

Section A:

The demographic variable includes age, gender, area of residence, previous knowledge and source of knowledge of COVID 19.

Section B:

41 Questions related to various aspects of COVID 19 like Place of Origin, Incubation period, Risk factors, Mode of transmission, Clinical signs and symptoms, Diagnostic tests and Precautions and management of Covid-19. Every question has four possible answers whereas only one was the correct answer. The correct answer was given one marks whereas zero for the wrong answer. The total score is 35. Maximum score was 35 and minimum score is 0.

Structured knowledge questionnaire was made based on the guidelines of WHO, ICMR, CDC and MOHFW to measure the knowledge. Tool was validated from the department of Physiotherapy, Nursing and Non-Nursing.

The questionnaire was administered with the help of Google Form.

Data Collection Procedure

Ethical approval was taken to conduct the study from Ethical Committee. Consent was taken from the participants. Data collection was done in the month of April 2020.

Data Analysis

Table 1: Frequency and Percentage Distribution of the Socio Demographic Variables

N=317

Sample Characteristics	Nursing F(%)	Physiotherapy F (%)	IT F (%)
Age (mean+ SD)	21.75 + 1.77	23.43 + 2.62	23.22+ 2.28
Gender			
Male	33 (24)	27 (34)	44 (44)
Female	103 (76)	53 (66)	57 (56)
Previous Knowledge			
Yes	336 (100)	80 (100)	101 (100)
No	00(0)	00 (00)	00 (00)
Source of Knowledge			
Television	40 (30)	10 (13)	23 (23)
Social media	48 (35)	30 (37)	22 (22)
Newspaper	32 (24)	20 (25)	36 (36)
Webinar	15 (11)	20 (25)	20 (19)
Current area of residence			
Punjab	47 (34)	18 (23)	28 (28)
Himachal	39 (29)	24 (30)	38 (37)
Haryana	30 (22)	26 (32)	30 (31)
Jammu Kashmir	20 (15)	12 (15)	05 (05)
Knowledge score (Mean+ SD)	33.84 + 5. 48	32.91+6.67	31.63+5.98
Good (35 to 51)	65 (47)	32 (40)	20 (20)
Average (18 to 34)	70 (52)	47 (58)	80 (79)
Below Average (1 to 17)	01 (01)	02 (02)	01 (01)

Table 1 represents the frequency percentage of sociodemographic variables. It can be observed in the table 1 that the mean age of participants among Nursing was 21.75 + 1.77, 23.43 + 2.62 among physiotherapy whereas 23.22+ 2.28 among IT department. In relations to Gender it was found that most (76 %) of the participants were female and

only 24 percent were male among Nursing, 66 percent's participants were female, and 34 percent were male among physiotherapy while 56 percent were female and 44 percent were male in the IT department. All of the participants among all the groups have previous knowledge regarding COVID 19.

Further regarding source of knowledge, 30 percent of the participants in Nursing, 13 percent in the Physiotherapy and 23 percent's in IT, Television is the source of knowledge. 35 percent of the participants from Nursing, 37 percent from the physiotherapy, and 22 percent from the IT department know about COVID 19 through Social Media. Further, 24 percent's participants among Nursing, 25 percent from Physiotherapy and 36 percent from IT department participants source of Knowledge is Newspaper. Webinar was the source of Knowledge among 11 percent Nursing participants, 25 percent Physiotherapy Participants and 19 percent among IT participants.

As per the Current area of Residence, 34 percent participants from Nursing, 23 participants from Physiotherapy and 28 percent from IT were residing in the Punjab. Further 29 percent participants from Nursing, 30 percent from Physiotherapy and 37 percent from the IT, were from Himachal. Next, 22 percent participants from Nursing, 32 percent from the Physiotherapy and 31 percent from IT were from Haryana. Furthermore, 15 percent participants from Nursing, 15 percent from Physiotherapy and 05 percent from the IT, were from Jammu and Kashmir.

Table 2: Frequency and percentage distribution of Level of Knowledge among Participants

N=31

Level of Knowledge	Nursing	Physiotherapy	IT
Good (35 to 51)	65 (47)	32 (40)	20 (20)
Average (18 to 34)	70 (52)	47 (58)	80 (79)
Below Average (0 to 17)	01 (01)	02 (02)	01 (01)

Table 2 showed the frequency and percentage distribution of Level of knowledge among participants. It can be interpreted from the table 2 that 47 percent participants from the Nursing, 40 percent participants from physiotherapy and 20 percent participants form IT were having good knowledge. Then 52 percent participants from the Nursing, 58 percent participants from physiotherapy and 79 percent participants form IT were having Average knowledge. Next 01 percent participants from the Nursing, 02 percent participants from physiotherapy and 01 percent participants form IT were having below Average knowledge.

Table 3 Range, Mean, Median and Standard Deviation of Knowledge Scores of students N=317

Group	Range	Mean± SD	Median
Nursing	17 to 46	33.84 + 5. 48	34
Physiotherapy	15 to 47	32.91+6.67	33
IT	17 to 47	31.63+5.98	31

Table 3 representing the Range, Mean, Median and standard deviation of knowledge score of students regarding COVID 19. The knowledge score among Nursing Participants ranged from 17 to 46, 15 to 47 among Physiotherapy whereas it was 17 to 47 among IT participants. The mean knowledge score of the Nursing participants was 33.84

+ 5. 48, 32.91+6.67 among physiotherapy and it was 31.63+5.98 among IT. The median of the knowledge of the participants of Nursing, Physiotherapy and IT was 34, 33 and 31.

Table 4. Repeated Measure ANOVA of Knowledge Score of the Participants

N=317

Area	Group 1 Mean± SD	Group 2 Mean± SD	Group 3 Mean± SD	F	Df	p
Introduction and Origin of Covid-19	9.1±2.21	9.3±1.88	8.89±2.24	2.54	1	0.00S
Epidemiology, incubation period and risk of Covid-19	5.13±1.36	5:01±1.43	4.98±4.41	3.22	1	0.04 S
Clinical Sign and Symptoms, Screening and diagnostic test	8.88± 2.22	8.31±2.51	7.76±2.23	9.92	1	0.00 S
Precaution and management of Covid-19	10.64± 2.18	10.26±2.38	9.80±2.13	3.94	1	0.02 S
Total	33.84 + 5. 48	32.91+6.67	31.63+5.98	1.07	1	0.00 S

Table 4 depicting the Repeated Measure ANOVA of Knowledge Score of the Participants. The total mean knowledge score of the Nursing students was 3384 + 5. 48, which was 32.91+6.67 among Physiotherapy students and 31.63+5.98 was on IT students. Repeated measure ANOVA was calculated which shows a statically significant difference (F=1.07, p=0.00) among three groups, so it can be concluded that there was a statically significant difference found among the knowledge of the Nursing Students.

Discussion

The present study aim was to assess and compare the knowledge of students regarding COVID 19. The results of the study revealed that 47 percent participants from the Nursing, 40 percent participants from physiotherapy and 20 percent participants form IT were having good knowledge. Then 52 percent participants from the Nursing, 58 percent participants from physiotherapy and 79 percent participants form IT were having Average knowledge. Next 01 percent participants from the Nursing, 02 percent participants from physiotherapy and 01 percent participants form IT were having below Average knowledge. Similarly, study conducted to assess the knowledge of Nursing students showed that the students had average knowledge regarding COVID 19.[5]

Similarly, another cross-sectional study was done to assess the knowledge of Nursing Students regarding COVID 19 showed that over all 75.58±3.21 participants showed good knowledge and awareness about COVID-19. Around 87.47% participants were aware about high risk age group for COVID-19. 83.54% participants were aware about concept of hand hygiene and 83.37% were well aware of PPE for suspected or confirmed COVID-19 cases.[6]

Limitations:

- The study was limited to only one university.
- The study was limited only to students of the Nursing.

Recommendations:

Nursing Practice

- There should be hands on experience for Nursing Students to deal with COVID 19 and it will help to reduce the fear among students.
- Continuing teaching for the students regarding preventive measures and changing treatment regimen needed to update the knowledge.

Future Research

- The study can be done between multiple colleges to compare the knowledge regarding COVID 19.
- Further research is needed to determine the preventive behaviour and risk perception of Nursing Students related to COVID 19.

Conclusion

The study concluded that continuing teaching experience is required to the students regarding preventive measures as well as change in the treatment regimen.

Ethical Clearance: Ethical clearance taken from the ethical committee.

Conflict of Interest: Nil

Source of Finding: Self

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Parenting Knowledge and Anxiety among Primiparus Couple in Odisha, India: A Cross Sectional Study

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Abstract

Background: Turning into the parent bring difficulties, challenges and series of changes such as emotional adjustment, couple relationship issues and trouble overseeing regular newborn child practices. Preparing for the progress to parenthood brings changes in life roles and finding a way to oversee such changes. New parents can get ready for parenthood by training of skills, child rearing tasks and assets that can help with turning into a parent.

Objective: To evaluate the level of knowledge of primigravida mother and father regarding parenthood. To determine the level of anxiety between couples. To find out the association between level of knowledge with selected demographic variable.

Methods: The present study is a cross sectional non experimental descriptive survey. The total samples for this study are 380 primi couples. A self-structured questionnaire was prepared to assess the level of knowledge of primiparus couples and standardize anxiety tool to assess anxiety between parents. Data was collected by interviewing the study samples. Pilot study was conducted with 30 samples from different settings to check the reliability & feasibility of the study.

Results: The finding of this study is (29%) of couple has good knowledge and (37%) couple has poor knowledge regarding preparedness of parenthood. Mean and SD of knowledge score of father and mother is 17.06±3.60 and 18.36±3.99 respectively. More than half i.e. 41.31% couple has mild to moderate anxiety and only 42.89% couple has minimum anxiety. There is no significant association between level of knowledge and age, occupation, level of education and monthly income of couple as it is less than P value 0.05.

Keyword*: primiparus, parenting, knowledge, anxiety,*

Introduction

Being a parent is an alternate encounter. Parents are world to their kids and they have a significant job in the improvement of their youngsters. It has been recommended that child rearing is the hardest activity any individual will ever have. Turning into a parent implies our lives are changing and we should get ready for that progression. Often newly parents are having anxiety and fear about childcare. Preparing couples for parenthood is start from the antenatal period.[1] The couples prepare themselves by physically, mentally and financially.[2]Turning into a parent is set apart by a progression of changes in the life of a mother or father. These progressions can be both brilliant and testing. The parenthood has a lot of challenges with changes in their daily routine, habits, and changes in their relationship, sleep deprivation, infant care tasks and life style [3,4]. After became parents a few couples feel less fulfilled in their relationship and there is increment in couple strife[5]. These early long periods of family arrangement are significant for the psychological well-being of baby and mental prosperity of parents. Dysfunctional child rearing practices and family struggle have the effect on passionate advancement of kid which can cause conduct issues from an exceptionally youthful age[6]. Studies have demonstrated that it is regularly distressing and brings

numerous changes in the family life style which is not easy to adopt for parents. Stress in parents during the early years of family formation has the negative impacts on children[7-8]. Most eager moms and fathers experience pregnancy, birth and early parenthood with no genuine mental medical problems and have a safe attachment with their infant during the postnatal period[9,10]. But many parents experience different degrees of tension and variable state of mind during the pregnancy. Primi mothers experience anxiety and fear about child birth and child care. Girija Kalayil Madhavan prabhakaran et al1 found that during third trimester there is 93%severe anxiety about child birth and care of newly born baby[11]. So, the couples should be educated regarding newborn care during antenatal period through different classes on child birth. The couple should be taught about emotional and physical aspects of pregnancy, early parenthood coping skills which will increase the knowledge and confidence of parents and reduce the anxiety[12]. Therefore, it is important to prepare and support couples for their new role being a parent and transition to parenthood[13]. The aim present study is to assess the knowledge of parents regarding rearing of child and provide information and advice about child care to new parents during the pregnancy to reduce their anxiety and to adjust with sudden changes in life style.

Materials & Methods

Research design

The research design was a cross-sectional descriptive survey. The research approach used for the present study was quantitative approach. In present study a self-administered questionnaire administered to all study participants followed by an interview administered perinatal anxiety screening scale (PASS)

Sampling

In present study sample were 380 married primiparus couples who were expecting their first child and data was collected from couples attending selected antenatal clinic in Bhubneswar, Odisha by using purposive sampling technique. The study subjects were selected from interested moms and fathers who were familiar with local language to get relevant data.

Inclusion and exclusion criteria

In present study only married couple who are going to be new parents of any ages and willing to participate were included. The parents who had a child already and who did not meet the criteria were excluded from the study.

Description of tool

The present study tool has three sections. Section A: This section contains socio demographic information like age, height, weight, education, occupation, monthly income, types of family, information on trimester and so on. Section B: This segment is made to assess the level of knowledge regarding preparedness of parenthood. 28 questionnaires are included in this section. Each question has four options with one correct option. For each correct response score is one and for incorrect response score is zero. The maximum score was 28. The score was categorized in 3 levels. Good score: 28-18 average: 17- 9 and Poor:8-1. Section C: this section contains the standardize anxiety tool i.e. perinatal anxiety screening scale (PASS) to assess the level of anxiety between couples. It contains 31 questions and it has score 0 – 3. The participants were interviewed and score as given according to their response. Score 0= not at all, score 1= sometimes, score 2=often and score 3= almost always. The score was categorized in three levels. Minimal anxiety (0-20), mild- moderate anxiety (21-41) and severe anxiety (42-93).

After collecting all data, the data was entered into the Statistical package for Social Sciences (SPSS) licensed to the institute was used for data analyses. Descriptive and inferential statistics were calculated to summaries the variables. $P<0.05$ was taken statistically significant.

Ethical consideration

Participation in the present study was voluntary and an informed written consent was taken from all the participants. After the study was approved by the research committee, permission was obtained by the significant authorities to collect the data.

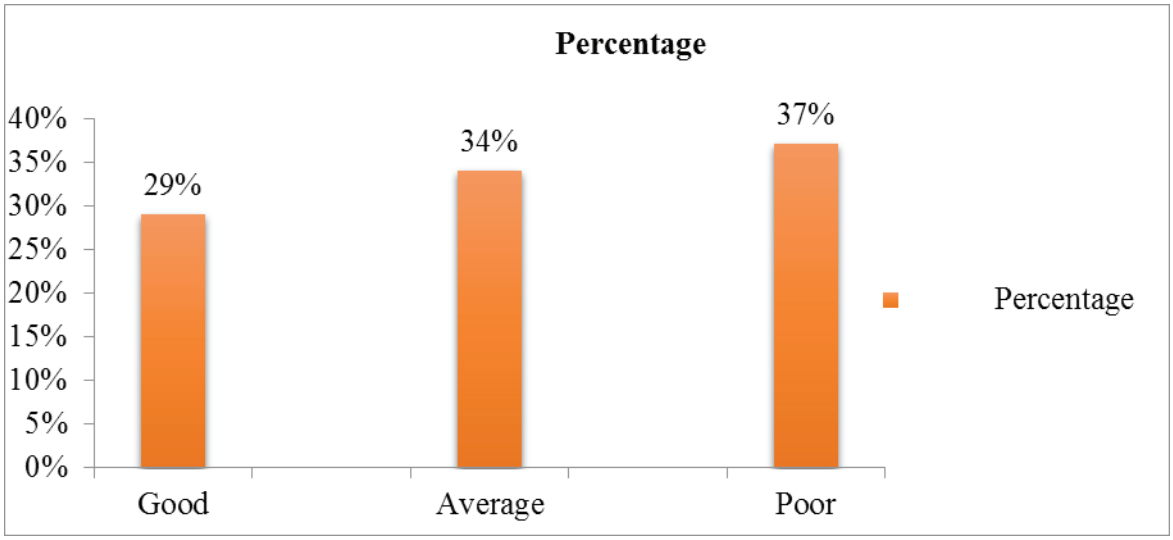
Results

Demographic characteristics

A total of 380 parents participated in the present study. Mean age of parents was 29.62 years with a standard deviation of 3.28. Majority of couples (70%) belongs to Hindu religion. Nuclear family (76%) was identified as most common type of family. (58%) of male were doing service and only (27%) women are working and (57%) female are housewife. (32%) couple was graduate and (28%) couple passed class 10th.

Figure-1: percentage of level of knowledge among primiparus couple.

N=380



Above figure depicts that only 29% of couple has good knowledge and 37% couple has poor knowledge.

Table-1: frequency and percentage of level of anxiety among primiparus couple.

N=380

Level of anxiety	Frequency	Percentage (%)
Minimal Anxiety	163	42.89
Mild-Moderate Anxiety	157	41.31
Severe anxiety	60	15.78

Table 1- shows only 15.78 % couple has severe anxiety and maximum 42.89% couple has minimum anxiety.

Table-2: Mean, mean%, standard deviation (SD), regarding knowledge assessment of primipara couple.

N=380

Mean	SD	Mean (%)
17.06 (Father)	3.60	60.92
18.36 (Mother)	3.99	65.57

Table- 3: Association between level of knowledge and selected demographic variable.

N=380

Variable	x 2	df	P value
Age	0.43	1	0.51
Occupation	0.47	1	0.49
Level of education	1.40	1	0.23
Monthly income	0.67	1	0.41

Above table depicts that there is no significant association between level of knowledge and age, occupation, level of education and monthly income of couple as it is less than P value 0.05.

Discussion

The present study assessed the knowledge and level of anxiety among the primiparus couple. A significant quality of this study is that the researcher assessed both the individual and couple, not like earlier research which had only centered around mothers only. The result shows maximum 37% couple has poor knowledge, 34% has average knowledge and 29% couple has good knowledge regarding preparedness of parenthood. Mean of father's knowledge is 17.06 with mean % of 60.92% and SD is 3.60. In case of primi mothers mean is 18.36, mean% is 65.57%, standard deviation is 3.99. Most of couple i.e. 41.31% has mild- moderate anxiety, 15.78% has severe anxiety and only 42.89% has minimal anxiety.

In 2016, Laura Vismara, Luca Rollè, Francesca Agostini et al. conducted a study on parenting anxiety, stress and depression among newly fathers and mothers during perinatal period in Italy. The study participants were 362 parents (181 couples) and their healthy 208 babies among those 55.8% boys and 44.2% girls. From the participants 70% were married couples and 30% were live-in relationship couples. This non-parametric test results of this study revealed that, the mothers (43.6%) were more likely to experience severe anxiety than their partners (26%) at p value <0.001[14].

In 2018, Korja R, Nolvi S, et al conducted a study at maternal welfare clinics in the South-Western Hospital in Finland. A total 3808 number of mothers and2623 number of fathers were participated in this study. Anxiety of both parents was evaluated at gestational weeks- 14, 24 and 34 by using the anxiety scale of Symptom Checklist 90 (SCL-90). In this study, among participants the mothers were having higher educational levels than fathers and also mothers reported more anxiety and depressive symptoms at each assessment point in comparison to fathers. Anxiety& depressive symptoms of mothers were inter-related at all assessment points during pregnancy ($r = .58$-$.62$, $p < .001$). Same as, paternal anxiety and depressive symptoms were inter-related at all assessment points ($r = .58$-$.59$, $p < .001$)[15]. A cross sectional study was conducted by Patel BB, Gurmeet P et al in 2016 among 384 pregnant women who were attending the antenatal clinic in a Tertiary Care Hospital of Pune, Maharashtra. The Study shows that about 58% mothers had sufficient information with respect to ANC. It was also revealed that all the variable age, education, occupation, parity, kind of family, and financial status (SES) had a significant association with awareness about ANC. 100% women were having a positive attitude toward ANC and 70%, women were adequately practicing, and variables such as education and SES had a significant association with practices about ANC[16].

Punyatoya Bej conducted a cross sectional study on Knowledge, attitude and practices among pregnant women about antenatal care, danger sign during pregnancy and adopting family planning method in 2018 with 54 pregnant women during antenatal care check. The result reveals that pregnant women have positive

attitude i.e. 93% about antenatal care and antenatal care practices was 62%. The knowledge regarding antenatal care was only 17%[17].

Conclusion

For recently moms, during pregnancy being nervousness and misery are genuinely normal. Ladies, when becomes pregnant gets a new experience and fathers too experience significant changes in their personal life, in family and in society. These transitions can cause pressure and tension, nearby positive feelings as well. The change to parenthood is a time of disequilibrium, with first-time parents encountering new desires and circumstances. Some first-time parents report that they are not prepared for the change to parenthood. Many parents get ready for the change to parenthood by getting information from their parents, social media and friends on pregnancy and how to care the infant. Sometimes they become misguided and less clear about the information. Although it has been observed that for most couples, these feelings are transient and gradually reduce after some time. It's a responsibility of all health care personnel to guide and counsel the new parents through antenatal classes.

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Assessment of Salivary Flow Rate and Carbon Monoxide Level among Smokers, Tobacco Chewers and Control Group

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Abstract

Aim: To assess salivary flow rate and carbon monoxide level among smokers, tobacco chewers and control group.

Methodology: The study has total of 45 subjects, which were divided into 3 groups and 15 subjects in each. Group A- smokers' group, Group B- chewers' group, Group C-control group. Unstimulated whole saliva was measured by performing modified Schirmer tear strip test(MST). Smokerlyzer used for measuring CO levels from exhaled breath.

Result: On analysing the data of SFR between smokers, chewer's and control group, there was statistically significant result at p value of 0.05 between all groups. On evaluating the co level in smoking group 53.3% presented with higher CO level and 60% chewers presented with lower CO level. In control group 100% were under no risk.

Conclusion: The salivary flow rate is reduced in tobacco users in either form. Since smoking group is at higher risk monitoring of carbon monoxide level can be used as the important tool in motivating the tobacco users to quit the habit.

Aim: To assess salivary flow rate and carbon monoxide level among smokers, tobacco chewers and control group.

Keywords: *Smoker's, Tobacco chewer's, Salivary flow rate, Xerostomia, Premalignancy, Carbonmonoxide level*

Introduction

Tobacco consumption is a problem which is seen universally and tobacco is consumed in various forms such as smoking, chewing, snuffing and dipping. Many life threatening medical complications and health issues are caused by using tobacco for a long time period [1]. Tobacco chewing habit and premalignant lesions such as oral sub mucous fibrosis are said to have strong relation [2]. Both systematic and localized ill effects are created by using tobacco [3]. Saliva is the first victim to encounter the ill effects caused by the toxic chemicals present in the tobacco [4, 5]. Measuring of salivary flow rate (SFR) is done on stimulates and unstimulated saliva.

SFR for unstimulated saliva is 0.3ml/ minute and stimulated SFR is 1.5 to 2.0 ml/minute [6]. Earlier in 18th century it was discovered that salivary gland activity

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is increased in smoking. But the observation was that only during the initial stages of smoking habit people had increased salivary gland activity. [7]. Long term use of tobacco causes alteration in the taste receptors function leading to decrease in salivary reflux. Main content of tobacco is nicotine which acts on cholinergic receptors of brain causing decrease in salivary secretion. [8]. This decrease in salivary flow leads to disorders such as dysgeusia, pain and burning mouth, dental caries and other oral infectious diseases. [9]. During smoking carbon monoxide (CO) enters the blood circulation from cigarette smoke and forms carboxyhaemoglobin (COHb).Elimination of CO occurs primarily through respiration and a strong correlation exists between CObreath and COHb[10, 11, 12] enabling it to be used as a important tool for assessing smoking status.

This study is conducted to assess salivary flow rate in different groups with many parameters along with assessment of carbon monoxide level in smokers and tobacco chewers.

Materials and Methodology

The study participants are the patients reported to department of oral medicine and radiology of a private dental college in Chennai. The subjects were explained about the purpose and procedure of this study and consent was obtained. The demographic details with history of smoking and chewing habit were also collected from the subjects. Ethical clearance was obtained from the institutional ethical committee.

Inclusion criteria include subjects of age above 18 years and who has a habit of smoking, chewing for at least 6 months and the subjects who do not have any systemic disease.

Exclusion criteria include subjects below 18 years of age, subjects with systemic diseases, salivary gland disease, the patients under radiotherapy and the patients who had quit their habit.

The study has total of 45 subjects, which were divided into 3 groups (A, B, and C) and 15 subjects in each.

Group A- subjects who have smoking habit only (smokers group),

Group B- subject who have chewing habit only (chewers group)

Group C- subject who are healthy and without smoking or chewing habit (control group).

Assessment of Salivary Flow Rate.

Unstimulated whole saliva was measured by performing modified Schirmer tear strip test(MST). Before performing the test, the participants were asked to sit upright in dental chair and relax. The subject were then asked to swallow the salivary secretion in the mouth and told not to swallow during the test. Also the subject was asked to elevate the tongue during the procedure and were retracted gently to avoid inappropriate wetting of test strips. With the help of the cotton plier the test strip is held vertically and the rounded end place in the floor of the mouth either side of lingual frenum. According to the length of the wetting, the reading were noted at 5 minutes. Inference: reading 1 to 5mm/ 5minutes was taken as severe, 6 to 10mm/ 5minutes as moderate, and above 10mm to 24mm/ 5minutes as mild, 25mm to 30mm/5mm as normal.

Assessment of Xerostomia: This was done using a questionnaire which is modified from Fox et al. (1987), and Pai et al. (2001), questionnaire [13, 14]. [Table.1]

TABLE- 1 Modified questionnaire of assessment of xerostomia.

1.	**Do you feel your mouth dry?**	**Mild xerostomia**
2.	Do you sip liquids to aid in swallowing dry food?	
3.	Do you feel thirsty very frequently?	Moderate xerostomia
4.	Do you have difficulties swallowing any food?	
5.	Does your mouth feels dry throughout the day?	Severe xerostomia
6.	Do you chew gums/hard candies/minutest daily to relieve oral dryness	

Based on the severity of the symptoms, subjects were classified as mild, moderate and severe xerostomia.

Assessment of Co Level:

CO levels were monitored by breath carbon monoxide monitor which is product of BEDFONT scientific Ltd. Approved by ISO13485 medical devices quality management. Smokerlyzer used for measuring CO levels from exhaled breath according to manufacturer direction. It was an immediate, non-invasive and well established used to clarify from smokers to non-smokers. The smokerlyzer were placed in the patients mouth. The patient is asked to exhale into the smokerlyzer for 15seconds without pausing, inhalation and in a uniform place. Two readings were recorded in 10 minutes time interval. The highest of these 2 CO levels and carboxyhaemoglobin percentage obtained were recorded. Inference 0-1(reading:01-06ppm) no risk,1.1-3(reading:07-10ppm) lower risk, >3(reading:11-30ppm) high risk.

Statistical Analysis

Data was analysed using the statistical package for social service (spss) software, using student t-test, chi-square test ANOVA. These were applied to assess between the group differences. P-value of less than 0.05 was considered as statistically significant. Significance level of 0.05 and confidence level of 95% was considered.

Results

The study included forty five subjects. On analysing the given data among smoker group the mean age was 37.8±16.88 (mean ± SD) having predominant male population (100%). In chewers group the mean age was 37.8±16.88 (mean ± SD) with 87% of male and 13% of female population. The mean age of control group was 36.13 ± 11.69 (mean ± SD) with 53.3% of males and 46.6% of the female population. In age distribution the P-value is 0.4784 which is not statistically significant at $p < 0.05$ but in gender distribution p-value is 0.13124 which is significant at $p<0.05$.[Table.2]

Table.2. Gender Distribution.

GENDER	smoking	Chewing	control	Row Totals
MALE	15 (12.00) [0.75]	13 (12.00) [0.08]	8 (12.00) [1.33]	36
FEMALE	0 (0.00) [0.00]	2 (2.20) [0.42]	7 (4.00) [4.00]	9
Column	15	15	15	45 (Grand Total)
The chi-square statistic is 8.6667. The p-value is .013124. The result is significant at p < .05.				

On analysing various parameters like frequency, duration and quantity of tobacco consumption between the smokers and chewers group, there was no significance in frequency and duration but a significance at $p<0.05$ in the quantity of tobacco consumption (1 - 5 packets/day) in smokers as 46.6% , and in chewers 87% was obtained.[Fig.1]

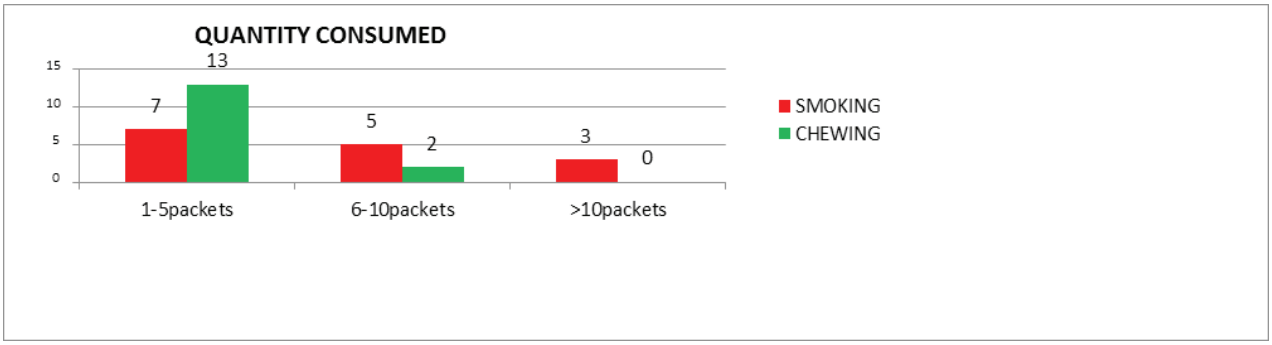

Figure.1- QUANTITY CONSUMED

On evaluating the oral mucosal lesions both the smokers and chewers group presented with 86.6% where as in control only 7%. The p value is 0.00001. Indicating significance at $p<0.05$ for oral lesions among smokers, chewers and control.[Fig.2.]

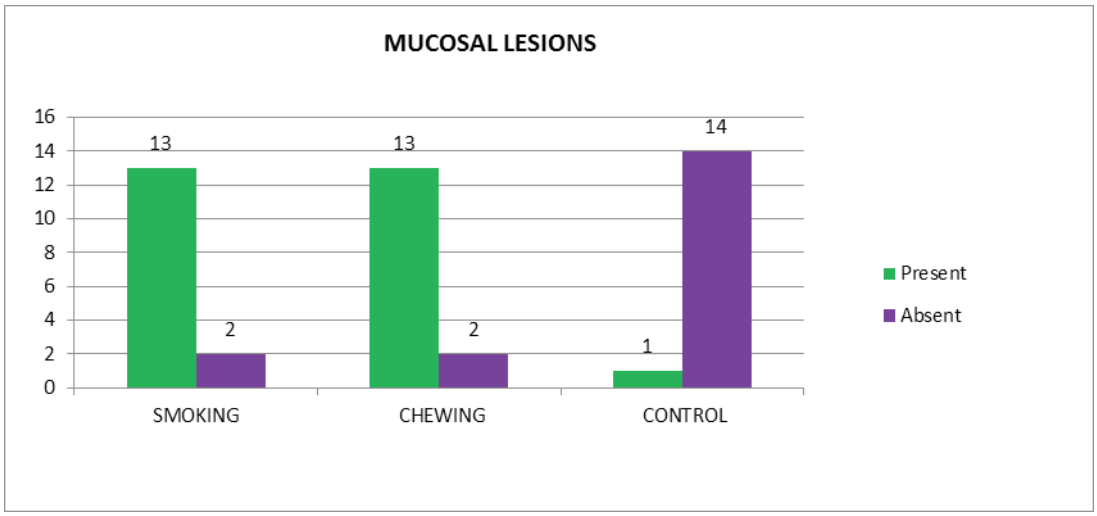

Figure.2-MUCOSAL LESIONS

Both smokers and chewer's presented with 20% of premalignant lesions where as none was in control group. The p value is 0.04908 which is significant at $p<0.05$.

On evaluating periodontal lesions among the 3 groups: 26.66% of smokers and 40% of chewers and 33.3% in control presented with the lesion with a P value of 0.7408, there was no significance at $p<0.05$.

Likewise on evaluating the dental caries there was no significance, 53.3% in smokers group, 80% in chewers group and 86.6% in control group presented with dental caries with a p value of (0.7408).

On analysing the data of SFR between smokers and control group using student t test the mean value was -3.600 with the p value of 0.003 [Table.3]. Likewise the mean value was -2.533 between chewers and control with p value of 0.012 [Table.4.]. There was statistically significant result at p value of 0.05 between smokers and control group, and chewers and control group.

Table.3. SFR between smokers and control group

Mean	SD	Std. Error Mean	95%ConfidenCe Interval of the Difference			Df	p-value
			Lower	Upper			
-3.600	3.961	1.023	-5.793	-1.407	-3.520	14	.003

Table.4. SFR between chewers and control group

Mean	SD	Std. Error Mean	95%ConfidenCe Interval of the Difference		T	Df	p-value
			Lower	Upper			
-2.533	3.420	.883	-4.427	-.639	-2.869	14	.012

Xerostomia:

On analysing the data for xerostomia between smokers and control the mean value was 0.267 with the p value of 0.484. Likewise the mean value between chewers group and control is 0.133 with the p value of 0.653. There was a statistically significant difference between smokers and control group. But no significance between chewers and control group.

CO Level:

On evaluating the co level the smoking group (53.3%)presented with higher CO level (high risk) In chewers (60%) presented with lower CO level(lower risk). The control group (100%) were at no risk. [fig.3.]

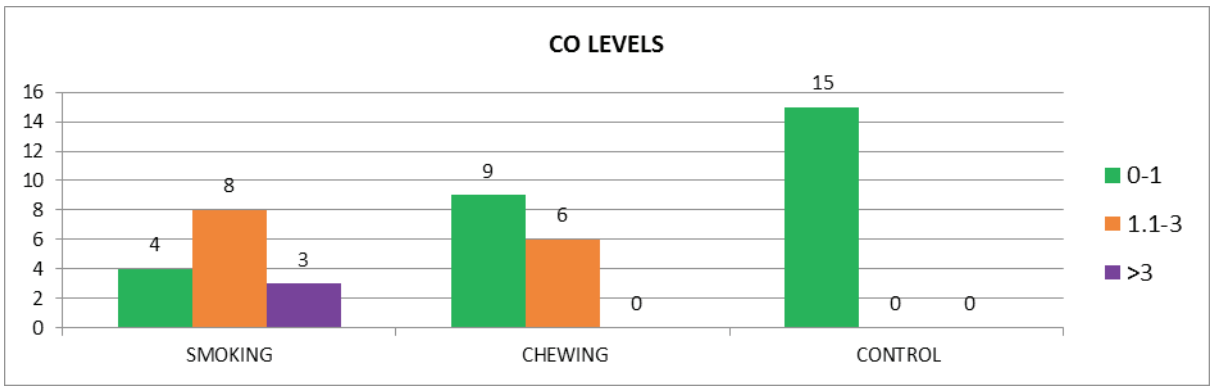

Figure.3-CO LEVEL

Discussion

In maintaining the oral environment saliva has a important role [15]. Taking this into account the alterations in salivary flow rate either qualitatively or quantitatively may lead to local adverse effects such as dental caries, oral mucositis, oral infections, chewing disorders, or extra oral adverse effects like dysphagia, halitosis, weight loss [16,17,18]. Measurement of salivary secretion can be done through various methods such as unstimulated whole saliva and stimulated saliva. Unstimulated whole saliva reflects basal salivary flow rate, it is present in our mouth for 14 hours and provides protection to the oral tissues while stimulated saliva is present during food intake and for 2 hours [19]. In our study, we have chosen to measure unstimulated saliva, as it is easy, non-invasive and comfortable procedure for the patients.

Tobacco both in smoked and smokeless form contains many toxic compositions which causes changes functionally and structurally in saliva [20].

In the present study SFR was reduced in smokers and chewrer's group when compared with the control. This finding is in accordance with the study conducted by Rooban et al[21](2006) and Alpana Kanwar et al[22] (2013) on long term effects of tobacco on resting whole

mouth salivary flow where there was a significant difference between two groups of smoked and smokeless tobacco. This might be due to effects of nicotine REF 8Other factors that influence salivary flow rate are gender and smoking status, women show lower salivary flow rate[23, 24, 25]. The relation between salivary secretion and smoking is controversial, as normal secretion and presence of hyposalivation have both been reported [26, 27, 28, 29, 30].

In the present study xerostomia was observed in smokers but not in chewers and control group. This is in accordance with the study conducted by Maryam Rod et al [5] in which smokers had reduced salivary flow rate and xerostomia.

Oral mucosal lesions were seen in both smoker's and chewer's at 86.6% where as only 7% in control. This shows that the habit of using tobacco has a higher chance of oral mucosal lesions. According to the study conducted by Sujatha et al(2012)[31] detectable mucosal changes were seen in 38.2% of patients who used smoked form of tobacco when compared with smokeless tobacco users no changes were seen in 43.3%. There was a significant correlation of occurrence of lesions with the duration of habit. In contrast in the present study frequency and duration did not have any significance and only quantity of tobacco consumed add a significant value for 5 packets per day in both smokers and chewers group. Dose response relationship is important because it gives a evidence for educating tobacco users about the ill effects of such habits and to reduce the quantity or completely stop such habits [31].

In a study conducted by C H-Lee(2003) et al [32] risk of premalignant lesions was seen in non-smokers who had the habit of chewing quid without tobacco when compared with those who used tobacco. Likewise in our study both the smokers and chewers presented with premalignant lesions and none was presented in control group.This shows that tobacco is the important risk factor in causing premalignant lesions.

In our study there was no significance for both the periodontal lesions and dental caries among the 3 groups but comparatively chewer's group had a slight predominance for both the conditions. This is in accordance with the study conducted by Offenbatcher et al (1985) [33] where using smokeless tobacco was a risk factor for the prevalence of gingival pathology and dental caries. In a study conducted by Sandberg et al (2011) [34] on assessing carbon monoxide level smokers group were at the higher risk group than non-smokers. Likewise in the present study smokers were at a high risk and this shows that breath carbon monoxide level assessing tool can be used to motivate the smoker's and help to quit the habit.

Conclusion

From the present study we can conclude that the salivary flow rate is reduced in tobacco users in either forms smoke and smokeless along with smoker's being at a higher risk for carbon monoxide level .It is also found that using tobacco makes oral mucosa vulnerable to many oral mucosal and dental diseases. Breath carbon monoxide level monitor can be used as an important tool in motivating the tobacco users to quit the habit as it is easy and non invasive.Since this is a preliminary study in which the sample size is small with multiple factors in future it can be carried on a larger sample.

Conflict of Interest: Nil.

Source of Funding: Nil

Ethical Clearance: Obtained.

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Assessment of Knowledge, Attitude and Practice of Forensic Odontology among Undergraduate Dental Students

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Abstract

Aim: The aim of the study is to assess the level of knowledge, awareness and practice of forensic odontology among undergraduate dental students.

Methodology: The data was collected through a questionnaire which contained 15 close-ended questions shared through online platform. And circulated amongst undergraduate dental students through online platform, all data were completely filled and statistical analysis was done.

Result: The results of this study show that Interns were the group with the most Knowledge about Forensic Odontology (p=0.0004). 75% of the Interns scored >90 and contrastingly, around 30% of the IV, III, II and I years scored >90.

Conclusion: This study divulges that there was adequate knowledge and awareness of forensic odontology amongst the Interns and very few of other group students (I, II, III and IVyear) have adequate knowledge and awareness regarding forensic odontology. Although the significance of maintenance of dental records are established, it has not been practiced among the students.

Keywords: *Forensic Odontology, Age estimation, Sex determination, Bite marks, Cheiloscopy, Ante-mortem records.*

Introduction

Forensic odontology relates science and knowledge to the discovery and investigation of crimes and the administration of justice. It has extended global consent as an authorizing field for using knowledge in legal measures [1]. Forensic odontology is a division of forensic science and comprises supporting in the documentation of dead persons and criminals [2]. The Federation Dentaire Internationale (FDI) describes forensic odontology as that division of dentistry which deals with the proper handling and examination of dental evidence and with the appropriate evaluation and presentation of dental discoveries in the interest of justice [3].

Forensic odontology is the recognition restraint based upon the revealing distinctive features occurs in each entity's dental tissues [4].Forensic Odontologists discover in recognizing strange human leftovers, victim's identification in mass disasters, defining the gender of skeletal remain, age assessment of both existing and deceased, evaluation of mishandling cases and identification of bite marks at crime acts [5]. Teeth have the capacity to tolerate disintegration and undergo intense modifications in temperature, due to which dental authentication assessment and estimation is between the most steady and reliable way of identification [6]. It can be harmonized by assessing characteristics of an unknown records (post-mortem dental records) with a known record (ante-mortem dental records).

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The importance of forensic odontology consists of documentation of human remains by dental records and handling the crime scene by the signs of bite marks or physical injuries [7]. The reason why the dental practitioners should have knowledge about the forensic science is the records given by dentists are exact source of identification. Recently natural disasters are increasingly seen in India, the bodies of the individual become unidentifiable, in this situation dentist play a significant role in identifying the individuals [8].

Dentist are the health-care specialists who regularly evaluate the head and neck of the patients and have a countless chance in detecting the signs of abuse and disregard [9]. With the growth in misfortunes, criminal cases, and mass tragedies or disasters, private dental practitioners may tolerate an essential role in resolving these types of circumstances [10]. The several tools used in identification of human remains are the personal stuffs like fragments of clothing, ornaments, fingerprints from blood group and dentition [11]. Keeping this as the background, the aim of this survey was to assess the knowledge, awareness and practice of forensic odontology among dental students in Chennai.

Materials and Methods

The aim of the study is to assess the level of forensic odontology among undergraduate dental students. The target population was 18 to 25 years old, college-going undergraduate students from Chennai. The questionnaire was prepared in English, focusing both on the demography of the subjects and to assess the knowledge, awareness and practice of the students. This questionnaire was converted into the online platform called Google forms, distributed to various college students. The demographic questions were to determine the age, sex and the year of study of the participants. Participants were informed about the study and were assured that their participation was purely voluntary. The required information was collected from various articles pertaining to the subject. The data was collected from the structured questionnaire which consists of 15 close-ended questions, which helps in the assessment of knowledge, awareness, attitude and practice of forensic odontology among dental students. On completion of the survey, their responses were collected and subjected to statistical analysis. A total of 102 students completed the survey with willingness and upon clarification of the purpose of the questionnaire prior to it.

\Table 1: Questionnaire:

1. Are you aware of Forensic Odontology as a branch in Dentistry?
2. What are the ways of identification of the suspect/deceased?
3. Are you aware of age of an individual can be determined by examining the teeth?
4. How do you think age of an individual is determined?
5. Are you aware that forensic odontology can determine the gender of an individual?
6. Do you think lip print patterns of human can be used for identification of an individual?
7. Do you consider bite marks pattern of human teeth as an important aid for assessing crime scene?
8. What is the source of your knowledge about forensic dentistry?
9. Do you maintain dental records in your college?
10. Do you know the significance of maintaining dental records?
11. Are you willing to take courses in Forensic Odontology?
12. Are you interested in pursuing Forensic Odontology as a profession?
13. Are you aware that you can testify as an expert witness in court to present forensic dental evidence?
14. How will you identify Physical/sexual/ psychological abuse in children and adults?
15. Do you know any Forensic Odontologists in India?

Statistical Analysis

The collected data were analyzed using SPSS version 16.0 (Ilanos, NY, USA).One-way ANOVA analysis was performed to identify the interrelationship between and among the study groups. Data were analyzed using the Chi-square test.

Descriptive statistics were used to recapitulate the responses to the questionnaire, with the results being presented as frequencies and percentages. Chi-square tests were used to compare the 5 groups, and the level of significance was set at $P \leq 0.05$. Correct responses or "Yes" were given a score of 1 and incorrect responses or "no" were given a score of 0; the scores were summed to obtain the overall scores.

To objectively assess the overall level of (a) knowledge and awareness; (b) attitude; and (c) practice, the responses were classified into four levels: ≤ 50 ("poor"), 50-70 ("moderate"), 70- 90 ("good"), and ≥ 90 ("very good"), which were assessed by group using Chi-Square test.

Results

A total of 102 undergraduate students took this questionnaire survey. On analyzing the demographic data, the mean age is 21.5. Of the 102 people attending this study, 74 of it were Female and 28 were Male (shown in *Table 2*). Maximum number of participants for this study has been deduced to be Interns (48%) followed by final years (26.5%) and the remaining by first, second and third years. The Chi-square test was used and the *p* value < .05.

Table 2: Demographic details:

Variable	Description	Frequency	
		N	%
Age	18-20 years	27	26.5
	21-25 years	75	73.5
Gender	Female	74	72.6
	Male	28	27.4
Year of study	I year	4	3.9
	II year	6	5.9
	III year	16	15.7
	IV year	27	26.5
	Internship	49	48

A total of the 15 questions were presented and the data collected were used to evaluate the following components.

1**. Knowledge and awareness**: regarding the significance of forensic odontology, identification of an individual dental records, dental age estimation, bite marks, and acting as a court witness.

2. **Attitude**: Interest in Forensic odontology as a career.

3. **Practice**: regarding maintenance of dental records and attitude towards maintenance of records.

For'knowledge questions', 96% of the participants were aware of forensic odontology and 85% of them knew that it can be used in the identification of an individual's age and gender, which can be achieved by the use of lip prints, bite marks and teeth.

regarding the question the ways to identify an individual, 71.6 % of participants were aware that it's a combination of DNA, fingerprinting, lip prints and bite marks.

Over 77% of participants were aware about the signs of different types of abuses. Regarding the source of knowledge of forensic odontology, 38% replied as lectures, 38% through internet and 19% through books. Forensic Odontologists in India were known by 59.8% of the participants.

The findings of 'attitude questions' show that more than 75 % of the participants were interested in taking a course and pursuing forensic odontology as a career.

For the questions related to 'practice', 81% of the participants maintained dental records in their colleges and 76.5 % knew that they can testify as an expert witness to present forensic evidence.

FIGURE 1-SCORE ANALYSIS OF PRACTICE

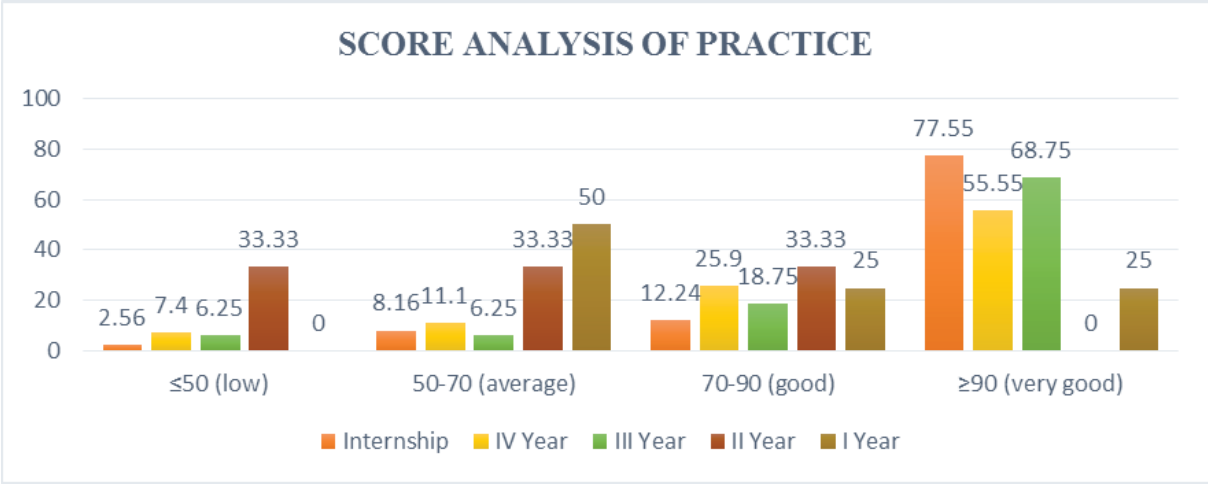
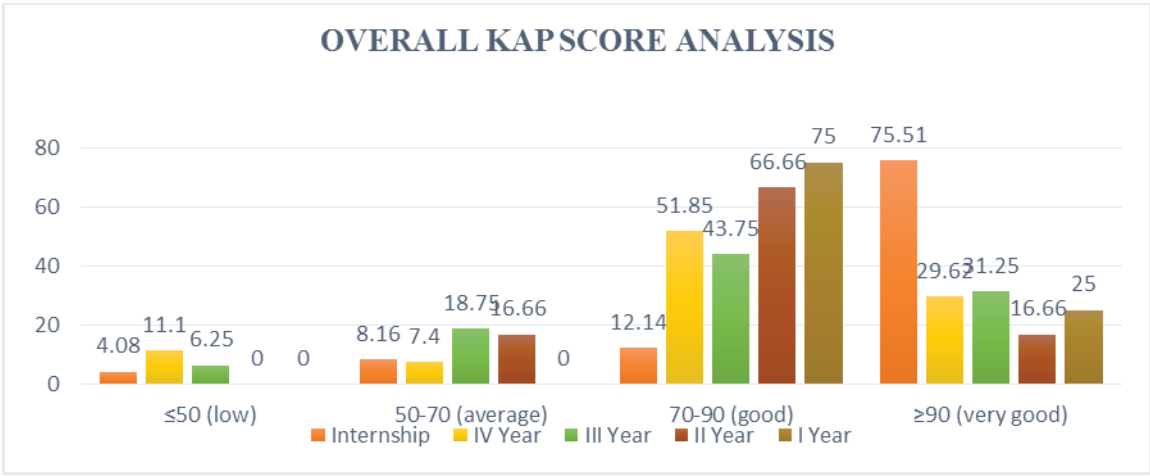


FIGURE 2-OVERALL KAP SCORE ANALYSIS



Discussion

Forensic odontology is a significant part of forensic medicine and is one of the most important step in the identification of an individual in the case of mass disasters, crime suspects like child abuse cases, assault or homicide cases. Teeth are considered to be 'the most reliable and consistent way of identification' as it is tolerable to physical and environmental insults. There are various methods to positively identify an individual, such as, bite marks, DNA analysis, Cheiloscopy, Rugoscopy, Facial reconstruction, Denture identification, tongue prints, etc. As this specialty is constantly evolving, one can only wonder what the future holds. This study helps the assessment of knowledge and in pin-pointing the area that needs improvement and resolving it.

About 96% of the participants in the present study were aware of forensic odontology as a branch of dentistry and we have obtained statistically significant *p* value. These results were similar to the study conducted by Rahman et al (2017)[12] in which 96.1% of the participants were aware of it. This shows that most of the undergraduate students of dentistry are having awareness of forensic odontology. In our study 71.6% of the participants agreed that combination of fingerprint analysis, lip prints and bite marks as the ways of identifying the suspect or deceased. Likewise, the results of the studies conducted by Hannah et al (96%)[13] and Abdul et al (72%) [14] have revealed that forensic odontology can aid in identification of individuals during mass disasters.

On analyzing the age determination, 94.1% of the participants were aware of it, which was similar to the study conducted by Duraimurugan et al (2017)[15], in which 81.5% of the study participants knew that forensic odontology can be used in determination of the age of an individual. A study conducted by Hashim et al[16] in 2020 revealed that only 28.3% of the participants were aware that gender determination can be achieved by forensic odontology methods. In contrary to that, there was a statistical significance in the present study, 92.1% of participants revealed that they are aware of it. This shows the better knowledge in this regard.

Regarding the awareness of lip prints in the identification of an individual, 85.3% of the present study participants, and bite marks being an important tool in assessing a crime scene there was awareness of 89.2% of the study participants, likewise, were aware of it. 77.5% of the participants in a study conducted by Rahman et al[12] in 2017 and 42% of the participants in the study conducted by Naik et al[17] in 2018 were aware of the use of lip prints in forensic odontology. Similar to the results of our study, 90.2% of the Rahman et al[12] study participants were aware of that bite marks are an essential tool in assessing a crime scene but on contrary, only 57% of the participants in the Naik et al[17] study was aware of it.

In the present study 81.4% of the participants have revealed that they maintain dental records in their colleges. The results were similar to the study conducted by Abdul et al[14] in 2019, which revealed that 87.5% maintain dental records. Furthermore, 86.3% of the study participants agreed that they are aware of the significance of the record maintenance. Similar results were obtained from the studies by Naik et al[17] (2018) which revealed 92% of its participants and the study by Naram et al (2019)[18] which revealed that 61% of the participants in the study were aware of the significance of record maintenance and on contrary to the results obtained by the study by Hannah et al[13] which revealed only 19% of its participants were aware.

79.4% of the present study participants expressed interest in pursuing courses in forensic odontology and these results were higher than the results obtained by Sujatha et al (2018)[19] which is 48.8% but similar to the results given by Hannah et al (2017)[13] which is 83%. In the present study, 76.5% of the participants agreed they would take up forensic odontology as a profession where as a study conducted by Abdul et al[14] in 2019 revealed contradictory results (21.3%).

As the job entails, forensic odontologists are to testify as an expert witness in court. This was known to 76.5% of the study participants and the *p* value obtained was 0.03 which is statistically significant. The studies that revealed similar results were by Abdul et al[14] and Naik et al[17] which are 80% and 76% of its study participants. When asked about the signs to identify abuse in children and adults, 77.5% of our study participants agreed that it was a combination of physical injuries, scars and behavior changes. The results of the study conducted by Rudraswamy et al (2017)[20] revealed that 48% of its

study participants answered that physical/sexual abuse can be identified solely by behavior changes and the results of the study by Naik et al[17] revealed that 98% of its participants identify abuse solely on physical injuries.

Regarding the awareness of any forensic odontologists in India, 59.8% of the present study participant were aware. The results of the study conducted by Sahini et al (2016)[9] in assessing the knowledge of dental practitioners on Forensic odontology revealed that only 41% of its study participants were aware of it.

With respect to the Knowledge score, Attitude score and Practice score, the Interns have topped consistently. 75% of the Interns have scored "very good" score in the overall KAP score, followed by 30% of the IV and III years. This is due to the late introduction of Forensic odontology in the BDS curriculum and diminished awareness of the opportunities this unique and fascinating subject has to offer.

Conclusion

This study divulges that there was adequate knowledge and awareness of forensic odontology amongst the Interns and very few of other group students (I, II, III and IV) have adequate knowledge, awareness and practice regarding forensic odontology. Although the significance of maintenance of dental records are established, it has not been practiced widely among the students. It can be enhanced by prompting forensic dentistry as subject in dental curriculum at both under-graduate and post-graduate levels and by conducting periodic CDE Programs, conferences and workshops and career counselling should be arranged to ensure opportunities in forensic odontology as a profession.

Conflict of Interest – Nil

Source of Funding – Self

Ethical Clearance- Obtained From Instituitional Ethical Clearance Committee

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Assessment of Knowledge, Attitude, and Practice of Recent Diagnostic Aids for Oral Cancer among Dental Students

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Abstract

Aim: The aim of the present study was to assess the knowledge, attitude and practice of recent diagnostic aids for oral cancer among dental students.

Materials and Methods: This study was conducted among 100 students using a 15-item self-administered questionnaire during COVID-19 pandemic period involving both undergraduates and postgraduate dental students.It was formatted on google forms and shared through social media. The data collected was analysed statistically.

Results: A total of 100 dental studentsparticipated in the survey. The total knowledge, attitude, and practice about recent diagnostic aids for oral cancer (based on summing all the positive responses -- for all the relevant questions -- and calculating the percentage) was 90% for post-graduates (highest), 88% for interns group, 84% for final years and 80% for third years (least) respectively.The results of this study show that postgraduates had good knowledge about recent diagnostic aids for oral cancer when compared to other groups.

Conclusion: These findings concerning dental students suggests that they have sufficient knowledge in the recent diagnostic methods of oral canceras they are the ones who have higher chances of diagnosing oral cancer at earlier stages as they routinely examine oral cavity.

***Key Words**: Attitude, diagnostic aids, knowledge, oral cancer, practice, questionnaire.*

Introduction

Oral cancer is defined as the growth or proliferation of malignant cells in the oral cavity. Sites which are majorly affectedincludes the lips, tongue, lining of the cheeks, floor of the mouth, hard and soft palates, gums, and teeth. [1]Causes majorly includesusage of tobacco. In addition to smoking,smokeless tobacco has also been strongly associated with the development of oral cancer.[2].Nearly 40% of head and neck malignancies are said to be squamous cell carcinomas arising in the oral cavity.3The oral cancer can be found at an early stage by means of visual and tactile examinations, and dentists play a key role as a health professionals in early detection of this disease [4].Oral cancer screening tests includes both visual and clinical examination whereas others prefer various specific or non-specific oral cancer diagnostic tools such as optical coherence tomography, Positron emission tomography, DNA ploidy tumor markers, and Polymerase chain reaction (PCR).[5] Most of the oral cancer cases is not diagnosed until an advanced stage, which has been one of the major reasons for minimally improved survival rate over the years[6].Treatment of oral cancer at an earlier stage is less complicated and has higher survival rates comparatively the cost of treatment for a stage IV oral cancer patient is more than three-times the cost of treatment for a stage I patient according to a study conducted in Greece,[7] and a similar result was obtained in a study conducted in Italy.[8,9]The current trends in diagnosis focuses in the areas such as molecular biology and advanced diagnostic aids which will transform our traditional way of approaches to another era.[10,11]Many studies have been done in the past regarding the factors behind the diagnostic delay of oral cancer but early detection still remains constant over

recent days.

Materials and Methods

This study was designed to measure the knowledge, attitude and practice of recent diagnostic aids for oral cancer among dental students. The questionnaire consisted of 15 questions; they are mentioned below (*Table 1*). The questions were converted into google forms and shared to the participants via social network. The data collected was analysed statistically.

Table 1: **Questionnaire**

1. Have u came across patients with oral cancer?
2. Do you practice complete oral cavity examination besides palpating lymphnodes routinely on patients?
3. Have you taken biopsy in patients with suspicious lesions?
4. Which stain is used to assess the status of margins around oral cancer during resection?
5.What method does PCR relies on?
6) Which biomarker is used in detecting oral cancer in molecular biology?
7) The tools of Nano diagnostics used in cancer diagnosis include?
8) Optical coherence tomography (OCT) is based on the scanning images of a?
9)VELSCOPE SYSTEM used in oral cancer diagnosis decreases?
10) Cancer proteomics encompasses the identification and quantitative analysis of?
11) Chemiluminescence illumination imaging device emits light at wavelength of?
12) DNA image cytometry measures ploidy status by measuring?
13)Which is the novel way to provide molecular biomarkers for oral cancer detection?
14) Do you think saliva is a used to measure specific examining proteomic/genomic targets?
15)Classification of brush biopsy?

Statistical Analysis

On statistical evaluation using SPSS version16.0 (Ilanos, NY, USA) it was observed all the 100 samples were valid for the study with Cronbach's alpha reliability score of 0.837 (significantscore**).Descriptive statistics were used to summarize the responses to the questionnaire, with the results being presented as frequencies and percentages.** One way ANOVA analysis was performed to identify the interrelationship between and among the study groups. The analysis shows F value of 23.6445 with p value of <0.0001(significant at p<0.05) suggesting significant relationship**.Chi-square tests were used to compare between 4 groups, and the level of significance was set at $P \leq 0.05$. The analysis focused on the correct responses in the groups to the 15 questions (10 were related to knowledge, 2 were related to attitude and 2 were related to practice regarding recent advances in oral cancer diagnosis).**

Results

Of 100 participants, from age group of 20 years to 30 years participated in this study on analysis of the given data the mean age of study population was observed as 23.36 ± 1.834 (mean ± S.D) comprising of 33 (33%) male and 67 (67%) of female participants. No significant differences were observed between age and gender wise distribution among the study participants

On analysis of the given data it was observed majority of the study participants 52% pursue internship

program (52 out of 100) followed by 26% (26) postgraduates, 11% (11 out of 100) final year students and 11% (11 out of 100) 3rd year students (*Table 2*). Chi-square test analysis to correlate interrelationship between the year wise distribution of the study participants showed chi-square statistic of 44.88 with p values <0.0001. The result is significant at p < .05.

Table 2:Year wise distribution:

YEAR	N	FREQUENCY
THIRD YEAR	11	11%
FINAL YEAR	11	11%
INTERNS	52	52%
POST GRADUATES	26	26%
TOTAL	100	

Around 46% dental students do not came across patients with oral cancer.On evaluating the students whether they do complete oral cavity examination besides palpating lymph nodes. 34% interns responded that they do only if it is needed and not in all patients.23%interns, 20 %postgraduates responded with yes.This signifies that early detection of oral cancer is possible can be done if each student suggests biopsy for suspicious patients. In our study when asked about the chemiluminescence illumination 41% students are aware about the wavelength used.

when questioned the students about the stain used to assess the status of margin of tissue at the time of resection.44% dental students selected the correct answer 'toluidine blue and lugols iodineby this analysis we can conclude that interns and postgraduates have fair knowledge about stain used in oral cancer diagnosis 'The chi-square statistic is 17.8623. The *p*-value is .036805.

The result is significant at $p < .05$.On evaluating the students whether they know the method which pcr relies on . 42% of interns and 22% of postgraduates selectedthermal cycling and tissue imaging and 44% of dental students identified the biomarker used in molecular biology for detecting oral cancerwhich is TSG p53, p15and BAX,BCL-2.41%dental students answered correctly that chemiluminescence illumination uses wavelength of 430nm,540nm,and 580nm,followed by 39.4% students identified VELscope system diagnosis decreases nicotinamide adenine dinucleotide and flavin adenine dinucleotide and 37% students stated optical coherence tomography is based on swept-source OCT system. This signifies that undergraduates and postgraduates have fair knowledge on pcr, biomarkers and all other recent diagnostic methods used in dentistry.

The overall level of (a) knowledge (b) attitude, and (c) practice. Regarding the overall knowledge, attitude, and practice percentage score, 75% of the 52 intern respondents were categorized as "very good" and 17.30% were "good." The "good" and "very good" together constituted 92.30%. Among the 11 fourth (4th) year respondents, only 45.45% were "very good" and 36.36% were "good". The "good" and "very good" together constituted 81.81%. Among the 11 third (3rd) 36.36% were "good" and 27.27% were "very good". Among the postgraduates 76.62% were "very good" and 15.38% were "good" together constituting about 92%. There was a significant between-group difference in the total percentage score (x^2=31.4641) (P<.0001). This signifies that interns and postgraduates have good knowledge on recent diagnostic aids used in oral cancer when compared to third years and final year dental students.(Table 3)

Table 3: Overall percentage scores

OVERALL	Internship	postgraduates	Final Year	Third Year	X2	P
≤50 (low)	1 (1.92)	1(3.84)	1 (9.09	1(9.09)	31.4641	<0.0001*
50-70 (average)	3 (5.76)	1 (3.84)	1 (9.09)	3 (27.27)		
70-90 (good)	9 (17.30)	4 (15.38)	4 (36.36)	4 (36.36)		
≥90 (very good)	39 (75)	20 (76.62)	5 (45.45)	3 (27.27)		
Total	52 (100.0)	26 (100.0)	11 (100.0)	11 (100.0)		

Discussion

The present study indicated that only 49.5% of the dental students referred patients with suspicious lesions to a specialist's clinic for further evaluation. This is notably troublesome as some of the dental students do not wish to undertake invasive procedures due to lack of clinical knowledge and exposure to such cases.

In the current study when asked about classification of oral brush biopsy 31%identified the correct option and this suggests that dental students are quite aware of brush biopsy. In two recent articles on oral cancer diagnostic aids, Patton et al. (2008)[12]and Lingen et al. [13] concluded that based upon published studies, oral brush biopsy with computer-assisted analysis has been demonstrated to be valuable for detecting dysplasia and cancer when evaluating "clinically suspicious" lesions. Both groups suggested that the accuracy of the oral brush biopsy for testing"minimally suspicious" lesions has not yet been established

In our present study 44% of them knows how DNA image cytometry measures the ploidy status which is bynuclear DNA.Recent research by Vijayavel T et al (2018) has described the probable use of DNA ploidy analysis to predict the character of premalignant lesions in oral cavity. And they use nuclear DNA to measure it. DNA ploidy analysispotentially aid in directing the management of the lesion, and probably suggest more aggressive treatment.[14]

In present study 38% dental students stated that they practice complete oral cavity examination besides palpating lymph nodes routinely on patients and among them only 49.5% dental students accepted that they take biopsy in patients with suspicious lesions. Similarly, in a study conducted by Alonge et al (2003) A mere 37% of the dentists declare palpating the lymph nodes during the complete oral cavity examination.[15]

Similarly, in Brazil , a study conducted by Leão JC et al (2005),36% of the dentists reported that they did not palpate the lymph nodes. The authors also stated that oral cancer examinations performed seemed to depend on a number of factors, the most pertinent of which were time and the comfort level and training of the dentist [16]. To detect oral cancer routinely at an early stage and to counsel the patients in prevention of the disease is a continuous challenge for the dental profession.

50% of dental students accepted that saliva is the novel way to provide molecular biomarkers for oral cancer detection.Cancerproteomics encompasses the identification and quantitative analysis of differently expressed protein.45.5% students had knowledge about this question,when compared to other years majority of the interns answered correct. This indicates some level of certainty among dental students about their knowledge on recent diagnostic aids of oral cancer

Conclusion

Dentist and dental students plays a major role in prevention and detection of oral cancer. Dental students

have high chances of diagnosis of the oral cancer as they routinely examine oral cavity. To make this possible, the dental students must be aware of diagnostic methods of oral cancer. Though post-graduates are likely to be trained to be effective in this field, graduate students should have a technical, scientific, humanistic and ethical knowledge aimed at prevention of prevalent oral diseases. Dental students should at least have a basic knowledge on the prevention and early diagnosis of oral cancer. Early diagnostic techniques are very useful in differentiating normal mucosa from dysplastic or cancerous lesions. These techniques can facilitate diagnosis in real time, and they are non-invasive. These methods also helps to improve their capability to detect and manage potentially malignant oral lesions. By our study we concluded that dental students have a good knowledge about diagnostic aids used in detection of oral cancer.

Conflict of Interest-Nil

Source of Funding-Self

Ethical Clearance- Obtained From Institutional Ethical Clearance Committee

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The Effect *Allium sativum* (Garlic Extract) as Prebiotic Substance on the Activity of Probiotic Bacteria *Lactobacillus acidophilus* Against Some Locally Isolates of Pathogenic Bacteria

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Abstract

The aim of this study was to improve the growth and inhibitory activity of the selected *Lactobacillus* isolate. A total of twenty samples of dairy products (fresh yogurt) were collected. Seven isolates of *Lactobacillus* species . were obtained, and identified as three *Lactobacillus fermentum*, three *Lactobacillus acidophilus* and one of *Lactobacillus brevis*. The growth of *lactobacillus acidophilus* was counted , also inhibitory activity of *Lactobacillus acidophilus* against some pathogenic bacteria was tested) for two different incubation periods before the addition of Allium sativum(garlic extract) as prebiotic substances. three different concentrations of it were used by addition of it to

De Man, Rogosa and Sharpe agar (MRS) medium as prebiotic substances , and two incubation periods (24, 48) hour. The effect of *Allium sativum* on the growth of probiotic bacteria was evaluated, measurement of *Lactobacillus acidophilus* growth curve was done after and before adding garlic extract. For analyzing inhibitory activity of *Lactobacillus acidophilus* with the *Allium* ,results showed that *Lactobacillus acidophilus* propagated in MRS fortified with 20% and 30% v/v garlic extract with incubation period 24 hr by giving the highest inhibition zone.

It was concluded that *Lactobacillus.acidophilus* isolate can grow well and exhibit good effect against Gram-negative bacteria in a medium containing garlic extract (that is added as a substrate supporting probiotic growth and activity). also it was clear that filtrate of *Lactobacillus .acidophilus* isolate that propagating in spesific broth fortified with 20% garlic extract at 48 hr incubation period produce antibacterial effect better than 10% , it was concluded that prebiotic treatment enhance the growth of *Lactobacillus acidophilus* especially at 24 hr. this may due to fructooligosaccharide content in garlic , also when propagated in MRS without prebiotic treatment, and which may due to highly decrease in pH which cause inhibition the growth of *Lactobacillus acidophillus*.

Key words : *Prebiotic , probiotic, garlic extract, Lactobacillus, (MRS) medium*

Introduction

Recently a great attention was paid to use microorganisms or their metabolites in the industry, food safety and treatment of some diseases. Bacteria are the first type of microorganisms used in this approach[(1)] Among the bacterial group is the *Lactobacillus* spp. which have a great role as probiotics, due to their presence in mucous membrane of intestine and digestive tract of human as normal microflora. Also it is safe used in food industry and their ability to produce inhibited materials such as organic acids, H_2O_2 , CO_2 , amino acid, di-acetyl, acetaldehyde and bacteriocins[(2).]

Beneficial effects of feeding an exogenous probiotic may be enhanced and extended by simultaneous administration of a prebiotic , that is non- digestible food ingredient improving the human health [(3).]

Many types of prebiotic were found naturally as prebiotic carbohydrate found naturally in fruits, vegetables and grains as well as other classes like dietary fiber and oligosaccharides. All those materials enhance growth of probiotics in several ways.

These synergistic effect of probiotic and prebiotic contributes to health, one example of prebiotic is disaccharides (lactose) which is primary carbohydrate of mammalian milk, and it is hydrolyzed into two monosaccharides (glucose and galactose) by the intestinal brush border enzyme lactase [4]. Lactose that is not digested by small intestine becomes a substrate for fermentation by bacteria in the colon by β- galactosidase enzyme, producing of short chain fatty acids that restrict the growth and activity of less beneficial species [5].

Dairy by-products are the main sources of the disaccharide lactose, which is a by-product of cheese industry. It contains diverse biologically components like lactose as a carbohydrate and other components of health benefits [6].

For such reason, this study was designed to:-

Isolation and identification of lactic acid bacteria from local sources as probiotic. . Using garlic extract as prebiotic available substances evaluation their effect in enhancing properties of the probiotic bacterial growth and/or activity. Determining the minimum inhibitory concentrations of concentrated filtrate of *Lactobacillus* bacteria before and after addition of garlic extract.

Methods

Isolation and identification of *Lactobacillus* spp

Yogurt samples (20) of dairy food product were purchased from Baghdad market for isolation of *Lactobacillus* spp, Lactic acid bacteria were isolated from the samples by captivating a portion of 1 ml of sample and added to sterile test tube with 10 ml MRS broth, incubated at 37 ºC for 24 hr, under anaerobic conditions. Serial dilutions prepared and 0.1 ml from last dilution was streaked on the surface of MRS agar containing 1% $CaCO_3$ in petri dishes, incubated for 24 hr at 37 ºC. The suspected LAB isolates were identified by microscopic examination (Gram stain) to examine cells shape, Gram reaction, grouping and non-spore forming phenomena. Then biochemical test in which catalase**,** oxidase**,** gelatinase tests were **,** also acid manufacture and clot creation test done then production of ammonia from arginine tested. Finally to confirm the diagnosis the species of *lactobacillus* bacteria, carbohydrate fermentation test used to consult lactic acid bacteria species, It was done by using sterilized MRS broth after substituting glucose and meat extract by 1% of each of the autoclaved sugars (lactose, fructose, raffinose, maltose, mannitol, sucrose) ,also filtered sugars are used for carbohydrate fermentation test such sugars are (arabinose, xylose, galactose), then adding 0.004% of chlorophenol red reagent as an indicator, the pH was adjusted to 6.5 [7].

Also gelatin medium agar was used to detected gelatin liquification in tubes, by inculcating 1% of LAB isolates, and incubating at 37 ºC for 48 hr. After that it was put into the refrigerator at 4 ºC for 30 min. This test was performed to demonstrate the ability of isolates to hydrolyze gelatine [8]. acid production and clot formation test

Done in which tubes containing 10 ml of litmus milk medium were inoculated by 1% of the suspected bacterial culture and then incubated at 37 ºC for 48 hr. to detect color change, crude production and pH decrease as positive result, Growth at 45 ºC and 15 ºC Tubes containing 10 ml MRS broth were inoculated with 1% of lactic acid bacterial culture then incubate at 15 ºC and 45 ºC for 24 hr. After incubation, growth was observed in the tubes and compared with control.

Growth Curve Measurement of *Lactobacillus acidophilus* isolate grow in MRS

To check the effect of garlic extract as prebiotic, on the growth of *Lactobacillus acidophilus* , measurement of *Lactobacillus acidophilus* growth curve [9] were done before then after the addition of garlic extract to MRS medium.

Two hundred ml of MRS broth was inoculated with 2 ml culture of *Lactobacillus acidophilus*, incubated on shaker incubator under anaerobic condition of 180 rpm at 37 ºC, optical density read in spectrophotometer at wave length of 600 nm at the time of inoculation and then each 2 hr for 24 hr**.** The producers were reiterated via MRS broth with prebiotic substances (garlic extract) in three different concentration (10,20,30% v/v) inoculated with

probiotic bacteria *Lactobacillus acidophilus*, to find the best one for enhancing activity on probiotic bacteria *Lactobacillus acidophilus.*

Inhibitory Activity of *Lactobacillus acidophilus* Isolates against pathogenic test bacteria

Determining Inhibitory Effect of *Lactobacillus acidophilus* Isolate that chosed randomly from other species against pathogenic bacteria *Salmonella typhimurium* and *Pseudomonas aeruginosa***)** by using well diffusion method as following[10].

First, before adding prebiotic, inoculating MRS broth with of *Lactobacillus acidophilus* isolate in a test tube, the tube was incubated at 37 ºC for two different incubation periods (24and 48 hr). The culture was centrifuged at 6000 rpm for 10 min this to get the supernatant which contain the filtrate of the grown bacteria in medium. After that, it was filtered through Millipore filter 0.22µm unit. Wells diffusion method was donw to detect the inhibitory effect of *Lactobacillus acidophilus* against test pathogenic bacteria by making wells on nutrient agar surface, filling them with the filtrates of *lactobacillus acidophilus*. After incubation the diameter of inhibition zones around wells were measured and compared with control.

Secondly, it was determining inhibitory effect of *Lactobacillus acidophilus* propagating in MRS broth that mixed with garlic extract as prebiotic. by inoculating 1% of previously activated bacteria *(lactobacillus acidophilus* isolate) into the MRS broth enriched with garlic extract at different concentrations (10,20, 30 v/v), then adjust pH to 6, and incubate at 37°C for (24 and 48) hr. Then centrifuged at 6000 rpm for 10 min, cell free supernatant was taken and filtered throughout millipore filte (0.22µm).Well diffusion method was applied and inhibition zone diameter was measured as following, each pathogenic bacteria was streaked on surface of nutrient agar plate then wells were made by the aid of a crok borer, the wells was filled with the filtrate of *Lactobacillus acidophilus* treated with garlic extract , then incubated at 37ºC for 24hr,the inhibition zone diameter was measured.

Results and Discussions

Isolation and identification of *Lactobacillus* species:

Seven isolates of *Lactobacillus* were isolated from a total of 20 yogurt samples, when grown on MRS agar, suspected *Lactobacillus* isolates produced colonies surrounded by clear zones. Colonies were white to pale in color, round, soft, mucoid, convex and having smooth edges. the gram staining demonstrated that suspected *Lactobacillus* isolates were gram +ve, short or long bacilli, grouped in long and short chain containing (3-8) cells but sometimes were single, non-spore former and non-motile. Biochemical tests were done suspected *Lactobacillus* isolates yield clot when grown in litmus milk medium leading to reduction the pH from 6.5- 4.5. Furthermore, all suspected isolates provided negative results for the catalase test so no bubbles were detected after addition of hydrogen peroxide to the colonies. The isolates also give negative results for both oxidase and gelatinase tests. In addition, they were not capable to crop ammonia from arginine-supplemented medium when the color of medium waited unchanged (orange) after addition of Nessler reagent, but not all species. Besides that, all isolates were not capable to grow on nutrient agar. Some isolate were able to grow at 45ºC, while other able to grow at 15º C.

The seven isolates of *Lactobacillus* species, were different in their capability to ferment the carbohydrate sources used.

It was shown that the *Lactobacillus* isolates which fermented all sugars but unsuccessful to ferment only mannitol were recognized as *Lactobacillus fermentum*. Isolates that fermented all sugars with the exception of maltose, mannitol and lactose were classified as *Lactobacillus brevis*. Isolates that unable to ferment both xylose and manitol but ferment other sugars were *Lactobacillus acidophilus.*

According to the above results, three isolates were identified as *Lactobacillus. acidophilus,* three isolates *Lb. fermentum* and one for *Lb. brevis*

Lactobacillus acidophilus isolates chosen to detect its effect on pathogenic bacteria before and after adding prebiotic material randomly.

Growth Curve Measurement of *Lactobacillus acidophilus* isolate in MRS broth before addition of

garlic extract

Growth characterization of the selected *lactobacillus acidophilus* was done to determine the extent of their growth phase when proliferated in MRS broth alone, comparing with their propagation in MRS broth containing garlic extract as prebiotic substance.

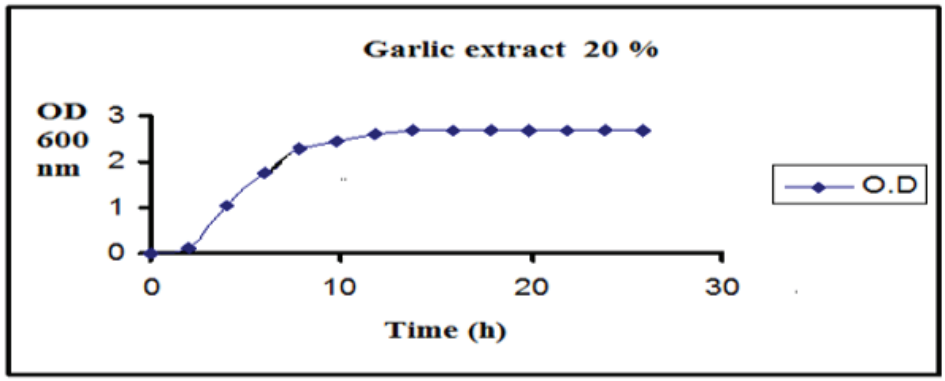
When a bacterium inoculated into a fresh MRS broth culture medium, it exhibits a characteristic of growth curve [9].

It clear that lag phase took about 2 hr. During this phase no upturn in cell number was detected which may be associated to preparation of cells for synthesis of DNA, inducible enzymes looked for cell division and reproduction. This phase existed followed by the log phase when the number of cells was bigger. A logarithmic growth remarked during the first 8 hr, then growth rate become at a slower speed for 24 hr. at which the isolate arrived the stationary stage [11].

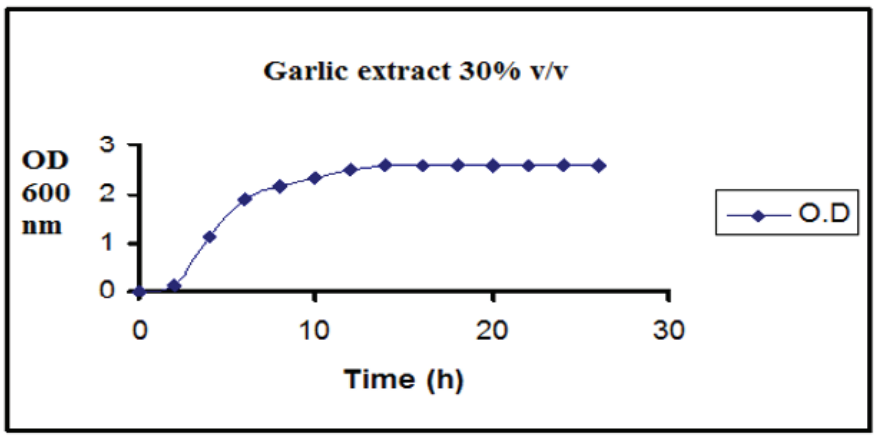
Growth Curve Measurement of *Lactobacillus acidophilus* isolate Propagated in MRS Broth Fortified with garlic extract as Prebiotic Substances

Testing the improving effect of garlic extract on probiotic growth was observed. The growth curve of *Lactobacillus acidophilus* isolate propagated in MRS broth containing 10% garlic extract were examined

As soon as, *Lactobacillus acidophilus* was propagated in MRS broth containing 20% and 30% (v/v) of garlic extract The lag phase also take about 2 hr and result showed that there was increase in the growth of *lactobacillus acidophilus* with garlic extract as prebiotic substance that mention in figure (1) and (2),noticing that there is no difference in growth curve between 20%and 30 %.



Figure(1). Growth Curve of *Lactobacillus. acidophilus* isolate in MRS medium fortified with garlic extract 20% v/v



Figure(2). Growth Curve of *Lactobacillus acidophilus* isolate in MRS medium fortified with garlic extract 30%

In general , in comparing growth of probiotic bacteria *lactobacillus acidophilus* before and after the addition of garlic extract, it was found that there is increase in growth curve of *lactobacillus acidophilus* with 20% and 30% of garlic extract. That is mean the enhancement growth of probiotic bacteria by adding garlic extract to the MRS medium, may be due to composition of garlic and its active compound that found in it ,such as the fructooligosaccharide (FOS) that found in garlic, it has effect on the growth of *Lactobacillus. acidophilus* bacteria when it added to the media it chang in growth behavior [12, 13].

Inhibitory Activity of *Lactobacillus acidophilus* isolates against pathogenic test bacteria

Well diffusion method used to evalute the inhibition activity of selected *Lactobacillus acidophilus*. Filtrates bacteria was applied in this experiment after propagation in MRS broth at two incubation periods (24, 48 hr) against the tested pathogenic bacteria. This done by making wells on nutrient agar plates that have been cultured by the tested bacteria , then the wells were filled with *Lactobacillus. acidophilus* filtrate . It was found that *Lactobacillus acidophilus* isolate give highest inhibitory activity when grown in liquid medium for all incubation periods used, when maximum inhibition zone diameters reached 14mm, This due to the ability of MRS broth to exhibit wide spectrum inhibitory effect against Gram-positive and Gram-negative bacteria as *Lactobacillus* sp. Propagated on it [14].

Table (1) and figure (2) displays the inhibitory effect of *Lactobacillus acidophilus* filtrate, as well as, It was establish that 24hr period of incubation showed best inhibitory effect against test bacteria when inhibition zone diameter reached to 14 and 15.5 mm for *Salmonella typhimurim* and *Pseudomonas aeruginosa* respectively, but after 48 hr the inhibition zone for *Salmonella typhimurim* still the same while it decrease to 13 mm for *Pseudomonas aeruginosa*

Table (1): Inhibitory Activity of Unconcentrated Filtrate of *Lactobacillus acidophils* Isolate Against Test Bacteria after Propagating in MRS Broth for 24 hr and 48hr.

Inhibition Zone Diameter(mm)		
Pathogenic bacteria isolates	**Incubation Periods(hr)**	
	24	48
S. typhimuriuum	14	14
Pseudomonas aeruginosa	15.5	13

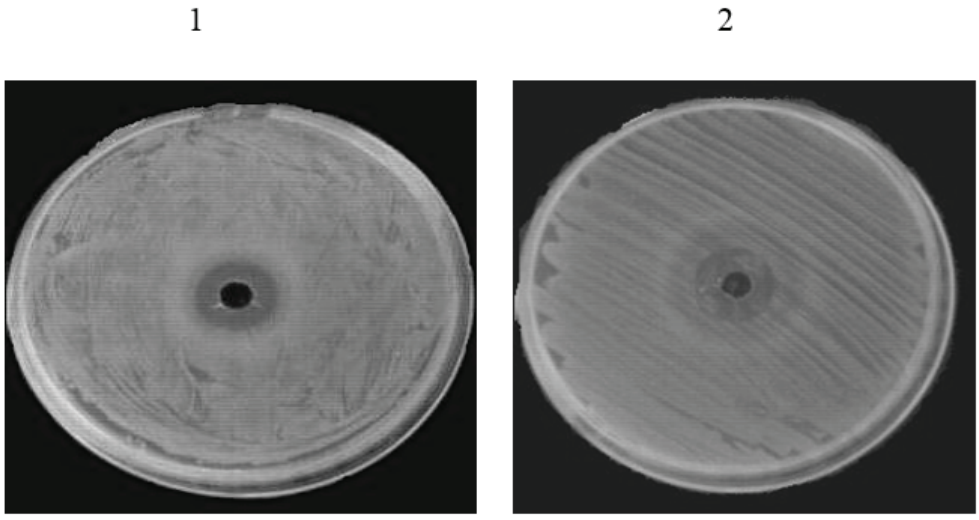


Figure (2)Inhibitory Activity of *Lactobacillus .acidophilus*Isolate against Test Bacteria after Propagating in MRS Broth for 24 hr, 1: for *Salmonella typhimurium*, 2: for *Pseudomonas aeruginosa* .

These results in line with Al-Jebory (2005)([15]) who found that increasing incubation period to 48hr lead to unablty for increase the inhibitory activity instead less activity. While attained results was disagreement with those obtained by Al-Dulemy (2005)([16]) who found that the inhibitory effect increased after 48 hr. The reason for such result may be that the inhibitory materials (plantaracin) are secreted outside the cells after increasing the incubation time causing decrease in the inhibitory activity of *Lactobacillus* against the pathogenic isolate.

Inhibitory Activity of *lactobacillus acidophilus* Propagated in MRS Medium after addition of *Allium sativum* (garlic extract)

Three concentrations of garlic extract (10, 20, 30% v/v) and two incubation periods (24, 48 hr) were used for assess the inhibitory activity of *Lactobacillus. acidophilus* isolate against the pathogenic bacterium.

Results in table (2) and figure (3) showed that, 10% garlic extract have not raised *Lactobacillus acidophilus* activity at all incubation periods when inhibition zone diameter range between 14-15.5 mm, against pathogenic bacteria, but it was clear increasing in inhibition zone diameter when garlic extract concentration raise to 20% and 30%, so cause increase in inhibitory activity as with 20% of garlic extract at 24hr incubation period when inhibition zone diameter increased to a range between 18-19 mm against pathogenic bacteria. This range of inhibition zone diameter was still at the same rang when incubation period is 48hr. The same increases were recorded at 30% of garlic extract at 24hr and 48hr when inhibition zone ranged between 18 -19 mm against pathogenic test bacteria.

Table (2). Inhibition Zone Produced By *Lactobacillus acidophilus* Isolate Propagating in MRS Broth Fortified with Different garlic extract Concentration against Test Bacteria

Conc. (%v/v)	Incubation period(hr)	Salmonella typhimurium	Pseudomonas aeruginosa
10	24	14	15.5
	48	14	15.5
20	24	19	18
	48	19.5	18
30	24	19	18
	48	18	18

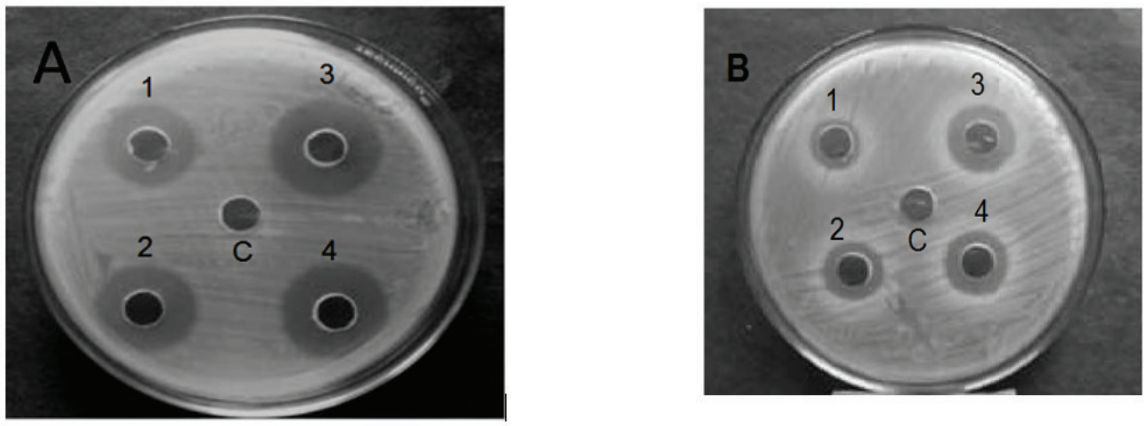


Figure (3). Inhibitory Activity of *Lactobacillus acidophilus* Filtrate against Test Bacteria after Propagating in MRS Broth Fortified with garlic extract for 24 hr. C: control, 1: *Lb. acidophilus* in MRS media alone, 2: *Lb. acidophilus* in MRS media with garlic extract 10% v/v, 3: *Lb. acidophilus* in MRS media with garlic extract 20% v/v, 4: *Lb. acidophilus* in MRS media with garlic extract 30% v/v, A: *Salmonella typhimurium* as pathogenic bacteria, B: *Pseudomonas aeruginosa* as pathogenic bacteria.

Almost similar results were obtained by Kontula *et al*., (1999)([17]) who found that LAB may utilize lactose, and the probiotic action of the strains could be enhanced..

It was concluded that *Lactobacillus.acidophilus* isolate can grow well and exhibit good effect against Gram-negative bacteria in a medium containing garlic extract (that is added as a substrate supporting probiotic growth and activity). From above results it was clear that filtrate of *Lactobacillus .acidophilus* isolate that propagating in MRS broth fortified with 20% garlic extract at 48 hr incubation period produce antibacterial effect better than 10% but as same effect as in 30% against pathogenic bacteria.

The compromise of scientists in the European Project ENDO (DGXII AIRII-CT94-1095) was to spot the uniquely well accepted prebiotic action of beta(2-1)fructans (fructooligosaccharides and inulin [(18).] which may increase the growth of probiotic bacteria *lactobacillus acidophilus* so act as prebiotic material that increase activity against pathogenic bacteria as mention above.

It is clear that the fructooligosaccharide (FOS) content in garlic have the best effect on the growth of *Lactobacillus. acidophilus* bacteria, when the FOS content in garlic was about 3.34% (b/b). This is consistent with the study of Altuntas and Korukluoglu **,** which reported that FOS is favored and fermented by *Lactobacillus* and *Bifidobacterium*. FOS and galactooligosaccharides are a composite of oligosaccharides (medium-chain carbohydrates) [(12).] FOS mixtures are found in fruits and vegetables, including onion tubers, such as garlic (1-2%) [(13)]

This is consistent with [(19)] **,** who informed that the addition of foods containing prebiotics such as oligosaccharides, the composition of Lactobacillus spp., will be increased by 81%. Supplementation with prebiotics at 0.1-0.2% can enhance the growth and activity of beneficial bacteria [(20),(21).]

From above results it was concluded that prebiotic treatment enhance the growth of *Lactobacillus acidophilus* especially at 24 hr. this may due to fructooligosaccharide (FOS) content in garlic have the best effect on the growth of Lactobacillus. acidophilus bacteria , when the FOS content in garlic was about 3.34% (b/b). but less effect was observed after 48 hr incubation period f, and also when propagated in MRS without prebiotic treatment, and which may due to highly decrease in pH (less than 3) and this may cause inhibition the growth of *Lactobacillus acidophillus* cells and hydrolysis of its cells.

Despite that, several healthy important components are found in garlic extract, but it was rarely used due to many causes. Condensed (dried) garlic extract is easier to use due to its potential in various field such as prebiotic. However not much attention was conveyed to use some materials as prebiotics in Iraq

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Conflict of Interest: Nil

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Knowledge and Attitudes amongst Medical Students on Health Research and Scientific Publications

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Abstract

Introduction: Most of the medical colleges, in general, have been suffering from lack of health research and scientific publications among the students in particulars and faculties in general. Health research and scientific publications are important medical students' activity for healthy and prosperous medical education. Objective: This study assesses the level of knowledge and attitudes regarding health research and scientific publications among medical students. Methodology: It is a cross-sectional descriptive study conducted among 100 medical students and were randomly picked up at Assam Medical College and Hospital in the year 2019. A prefix pretested proforma was used to determine the knowledge and attitudes of the students. Informed consent was taken. The data thus obtained was analyzed using MS Excel. Results: A total of 25(25%) students feel confident in interpreting and writing a research paper, while 39(39%) says they can do it with seniors' support.

In comparison, 20(20%) says no it saying their incapabilities of doing it. Majority 70(70%) students say that they have not participated in a research project (apart from mandatory academic projects) and 90(90%) says that they were never written any scientific paper. A good number of 80(80%) students feel that lack of a mentor is the cause of no publication while 60(60%) students believe that undergraduate students can plan and conduct a research project and write a scientific paper. Lack of facilities for doing research is accepted by 50(50%) of students to be another cause of no research and publication while 40(40%) receive lack of funding to be the root cause of no study. Conclusion: With the limited resources of this study, our findings have highlighted a low to moderate level of knowledge of undergraduate medical students in research methodology principles and the critical impact of research methodology workshops. These findings can be utilized for future health research planning to improve the situation in India's field of medical education.

Keywords*: Medical education; research methodology; skill.*

Introduction

Currently, it is widely acknowledged that research becomes critical for the development of countries and innovations; and outcomes derived from primary and applied research tremendously benefit the community.[1, 2]

Furthermore, shifting research paradigm considers physicians as crucial creators of science through clinical and translational researches. In this regard, medical students who play critical roles in helping the progress of science as potential physicians are encouraged to contribute to research projects to promote their independent training and skills.[3] In other words, health research training is an essential part of medical

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education. There is growing evidence on the importance of the involvement of students in research.[4]

This is more emphasized in developing countries where financial and human resources are limited. There is a critical need for research as a tool to make efficient decisions to prevent unnecessary waste. In this regard, it is essential to remember that there is some evidence that students with extracurricular research programs and experiences are more likely to become future scientists or physician investigators. [4-7]

The development of strong research skills and the provision of medical care are inextricably linked. That's why the research experience is stressed during the undergraduate medical education program. Therefore, this study has suggested research methodology as integrated research into the core curriculum, as well as offering several programs for students to become involved throughout their years of study.

This study has aimed to assess undergraduate medical students' knowledge in principles of research methodology and its contributing factors.

Material and Methods

It was a cross-sectional descriptive study. The sample size was 100 and randomly picked up from the undergraduate student community in their basic science stage at Medical College at Assam Medical College, Dibrugarh, Assam in the year 2019. To determine knowledge about principles of research, participants filled a pre-text and pretested questionnaire. Informed consent was taken. The data thus obtained was analyzed using MS Excel. Ethical clearance was taken from the ethics committee (human).

Result

One hundred students were responded to the questionnaire; therefore, the response rate of 100% was observed. 60(60%) of participants were female, and 40(40%) were male, as shown in **Fig. 1**.

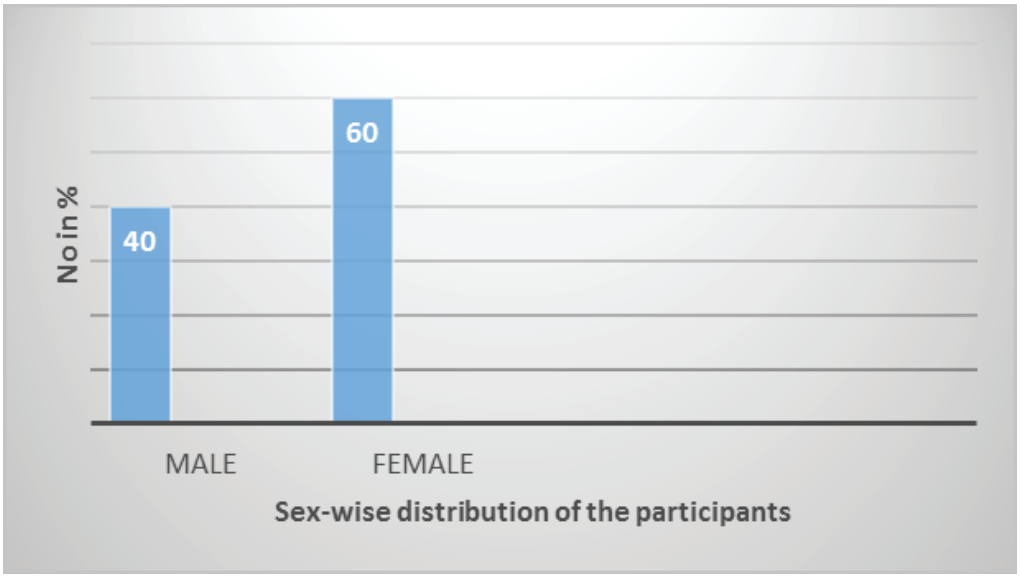

Fig. 1 Sex distribution of the study participants

Table 1 summarizes knowledge of the students in different aspects of research and publications. We observed that 25(25%) students feel confident in interpreting and writing a research paper, while 39(39%) says they can do it with seniors' support. In comparison, 20(20%) says no it saying their incapabilities of doing it. Majority 70(70%) students say that they have not participated in a research project (apart from mandatory academic projects) and 90(90%) says that they were never written any scientific paper. A good number of 80(80%) students feel that lack of a mentor is the cause of no publication while 60(60%) students believe that undergraduate students can plan and conduct a research project and write a scientific paper. Lack of facilities for

doing research is accepted by 50(50%) of students to be another cause of no research and publication while 40(40%) accept lack of funding to be the root cause of no study.

Table 1 Principles of research and publication knowledge of participants

Statement	No. of students (%)			
	Yes	No	Yes with support of seniors	Don't know
Do you feel confident in interpreting and writing a research paper?	**25**	**20**	**39**	**16**
Have you ever participated in a research project (apart from mandatory academic projects)?	10	70	10	10
Have you ever written a scientific paper?	1	99	0	0
Lack of mentor is the cause of no publication or lack of research participations?	80	15	0	5
Do you think UG can plan and conduct a research project and write a scientific paper?	60	10	25	5
Do you think that lack of facility is the cause of no publication or lack of research participations?	50	40	0	10
Lack of fund is the root cause of no scientific research or lack of publication?	40	30	0	30
Lack of knowledge of research methodology is the root cause of no publication or lack of research participations?	70	20	0	10
No future benefit or privileges from Govt. of doing research and scientific publication is the cause of all?	30	40	0	30
Lack of knowledge of seniors in research and publication is the cause of demotivation, leading to all these problems?	40	30	0	30

Lack of knowledge of research methodology is the root cause of no research, and 70(70%) students have considered its subsequent publication. Interestingly 40(40%) students say that it has no future benefit or privileges for research and scientific publication.

Discussion

Our study shows undergraduate medical students have low-to-moderate knowledge about principles of research and publications. In line with this finding, in a survey by Windish et al. on understanding medical residents of biostatistics and interpretation of results, the mean correct answer was 41.4%, indicating low-to-moderate knowledge of the residents these issues.[8] Similar findings were observed among physicians practising in an academic medical center[9], medical students, first-year Croatian medical students[10], Pakistani medical students[11], and medical students in South-East Europe.[12]

Because some medical curriculum chapters are related to health and epidemiology basics, it was assumed that passing these courses would enormously improve students' knowledge on principles of research methodology. However, our findings indicate that the medical curriculum is exclusively meaningful in understanding research and publication. Yet, other team works, motivation from seniors, source of fund, benefits of such works regarding research achievements, etc. may also contribute to the knowledge of students' in principles of research and publications.

In interpreting the results, one should consider our study's limitations: First, the study was cross-sectional. Prospective studies would have been beneficial in overcoming this limitation. Second, this study was performed over the students of a single medical college and did not evaluate the same in other institutions, improving students' knowledge of research principles and publications. Furthermore, similar academic and research potentials of these students have not been evaluated over the understanding of analysis principles.

Conclusion

Apart from the limitations, our findings highlight the low-to-moderate level of knowledge of undergraduate medical students in research and publications and the critical role of seniors' guidance and others in improving students' knowledge. Hence, we suggest further multicenter research on students studying in different institutions and students at different medical education stages, which could provide useful information for education and research policymakers.

Conflict of Interest: No conflict of interest associated with this work.

Ethical Issues: Ethical clearance taken from the institutional ethics committee.

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Prevalence of Hypertension in Patients with Coronary Artery Diseases with and without Type-II Diabetes Mellitus

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Abstract

Background: - Hypertension is one of the major public health issue and also have an important area of research. Its high prevalence and being a major risk factor for cardiovascular diseases and other complications. Hypertension accounts for the majority of hypertension in individuals with diabetes, particularly those with Type-2 diabetes.

Objectives. To study association of hypertension with diabetes and also find out the prevalence of hypertension in type-II diabetes, CAD patients with or without type-II diabetes mellitus.

Materials & Methods: - It is a type of case control study among the rural population of kamothe, where A total of 120 subjects were included in the study among which 90 were in study subjects and 30 were in control groups and among the study subjects (i.e.-90) 30 were in CAD with diabetes, 30 were in CAD and 30 in Diabetes mellitus respectively. BMI was calculated by height and weight and BP was recorded. Blood sugar were measured by enzymatic method on EM-200 analyzer

Results:-The mean level of BMI in the group –II that is in the coronary artery disease with diabetes is 24.22 ± 1.0, in only coronary artery diseases was 20.65 ± 2.0 and in only diabetes subjects was 24.39±1.0 of the study subjects in the comparison of control subjects i.e. 23.17 ± 1.78 and we have also found the statistical significance (P-0.001) in all the study groups. In the aspects of Blood pressure distribution, we have distributed it into different stage of hypertension and found that more number of patients from group –III i.e. only CAD and found 83% diastolic BP in pre hypertension stages and 60% were hypertensive and in only DM, there were 56% SBP and 60% DBP in pre-hypertensive.

Conclusion: - Periodic evaluation of patient with pre hypertension and hypertension by applying life style modifications and may favor the good outcome and also prompt the treatment of hypertension and hyperglycemia.

Keyword: - *Diabetes mellitus, Coronary artery diseases, Hypertension &Body mass Index.*

Introduction

Type 2 diabetes mellitus (T2DM) is a class of chronic metabolic syndrome and has a gradual increasing prevalence in the world. It is one of the fast epidemic in the some of the developing countries like India and this trend of diabetic added more burden towards healthcare providers.(1) India is now considered as diabetic capital of the world. Interestingly every fifth diabetic in the world is an Indian. Cardiovascular disease is the major cause of morbidity and mortality among diabetic patients, accounting for 75% of hospitalizations and 70-80% of deaths. (2) Atherosclerotic coronary artery disease (CAD) and other forms of cardiovascular disease (CVD) are the major causes of morbidity and mortality in type II DM (T2DM). (3) As per the International Diabetes Federation

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(IDF) Diabetes Atlas 2011, it is expected that there is an addition in existing number of cases for diabetes and rises from 366 million (2011) to 525 million by 2030 if preventive measure and awareness program would not bring into health care system.(4) The estimated worldwide prevalence of diabetes for all age groups individual will rise by 4.4 % in 2030. (5) Type-2 diabetes mellitus (T2DM) is the predominant form of diabetes worldwide, accounting for 90% of cases globally.(6) Sex, age, and ethnic background are important factors in determining the risk of developing T2DM. (7)

As per the Joint National Committee-7 (JNC7), Hypertension (HTN) is defined as abnormally high arterial blood pressure (systolic BP level of ≥140mmHg and/or diastolic BP level ≥ 90mmHg) whereas the normal range considered for blood pressure is a systolic BP < 120mmHg and diastolic BP < 80mmHg. (8,9) Hypertension is a very common in the patients of diabetes and it well established interrelation that strongly linked with the predisposition of atherosclerotic plaque in arterial wall. The prevalence of coexisting hypertension and diabetes appears to be increasing in industrialized nations because populations are aging and both hypertension and non-insulin dependent diabetes mellitus incidence increases with age.(9&10)

Hypertension is also have major contribution to diabetic complication like retinopathy, nephropathy etc. and having 35-75 % linked between diabetic cardiovascular and renal complications (11) For all these reasons hypertension and diabetes should be recognized and treated early and aggressively. Chen *et al.* (12) stated that hypertension to account for 30% of deaths in diabetes patients and for 25% of cardiovascular events in diabetes patients. In contrast, when hypertension and diabetes mellitus were regarded as independent, the population-attributable risk from diabetes mellitus was only 7% for all-cause mortality and 9% for any major atherosclerotic cardiovascular event.

So looking into above aspect, we have plan to study association of hypertension with diabetes and also find out the prevalence of Hypertension in type-II diabetes, clinically confirmed CAD and CAD with type-II diabetes mellitus.

Materials and Methods

The present study was a case control study in the patients of coronary artery diseases with or without type-II diabetes mellitus, who visited into OPD department of Medicine at MGM Hospital and study were done with the collaboration of Biochemistry department and cardiology department after getting the approval from Institutional Ethics Committee. (Approval No: 2015/SC/96) The patients were

Included in the study according to inclusion criteria. A total 120 subjects of either sex having (age group of 25-70 years) were enrolled in this study and were categorized into following four groups:

Group I have 30 Healthy Controls

Group II have 30 patients with type-II diabetes Mellitus with Coronary Artery Disease

Group III have 30 patients with coronary artery diseases.

Group IV have 30 patients with type-2 diabetes Mellitus

Further participants of all the four group were categorized as in normal, pre-hypertension and hypertension on the basis of their blood pressure.

Patients with sexually transmitted disease, rheumatoid arthritis, sepsis, asthma, malignancy, renal disease, liver disease, chronic illness, malnutrition pregnant women and were excluded from the present study. General Information about detailed history was noted. Anthropometric (height, weight and calculated body mass index) and vital sign (blood pressure) were recorded from each and every subjects. There were two series of samples were collected. In the first series - About 2 ml of fasting blood sample (FBS) was collected from each subject then the subjects were instructed to take breakfast or meals. After two hours of meal approx. 2 ml of post prandial blood sample (PP2BS) was collected in fluoride vacationer. The samples were collected with all the aseptic precautions than the blood sample was centrifuged at 3000 rpm for 10 minutes to get plasma for biochemical investigations.

Methodology

ü Height (m) and weight (Kg) of each subject were measured.

ü Body mass Index (BMI) in kg/m^2 was calculated by formula height (m2)/weight (Kg).

ü Systolic and diastolic Blood pressure of the each subject were recorded by using Sphygmomanometer.

ü Estimation of Fasting blood sugar and post-prandial blood glucose were done by GOD-POD enzymatic method.

Statistical analysis: - Data of the present study were analyzed by 'SPSS, Version 21' and presented in the form of mean ± S.D. for categorical data, values were presented in the form of frequency or percentages (%). A *'p'* value of ≤ *0.05* was considered as significant.

Results

Table-1: Gender wise distribution in control and study subjects.

Gender	Frequency (%)			
	Control	Study Subjects		
	Group-I (n=30)	Group-II (n=30)	Group-III (n=30)	Group-IV (n=30)
Male	67% (20)	47% (14)	70% (21)	67% (20)
Female	33% (10)	53% (16)	30% (09)	33% (10)

Table-2: Comparison of Blood Pressure, BMI and Blood sugar in control and study subjects

Parameter	Control	Study Subjects			Test of Significance (P- Value)
	Group-I (n=30)	Group-II (n=30)	Group-III (n=30)	Group-IV (n=30)	
BMI (m/kg2)	23.17 ± 1.78	24.22 ± 1.0	20.65 ± 2.0	24.39±1.0	0.001**
Systolic BP (mm/Hg)	119.77 ± 5.25	140.2 ± 5.38	137.90 ± 28.91	140.2 ± 5.0	0.0001**
Diastolic BP (mm/Hg)	77.1 ± 4.25	86.7 ± 4.28	83.60 ± 3.49	86.0 ± 4.0	0.0001***
FBS (mg/dl)	91.61 ± 9.6	140.66 ± 41.83	96.69 ± 9.42	140.66 ± 41.83	0.001**
PP2BS (mg/dl)	129.35 ± 10.6	257.8 ± 56.27	139.48 ± 11.11	257.0 ± 56.0	0.0001***

*** Highly significant, ** significant # not significant

Table-3: Blood pressure distribution (Systolic and diastolic) among study subjects:

Stages of hypertension	Study subjects					
	Group-II (n=30)		Group-III (n=30)		Group-IV (n=30)	
	SBP	DBP	SBP	DBP	SBP	DBP
Normal	0	4 (13%)	3 (33%)	5 (16%)	0	1 (3%)
Pre-hypertension	13 (43%)	15 (50%)	9 (30%)	25 (83%)	17 (56%)	18 (60%)
hypertension	17 (56%)	14 (46%)	18 (60%)	0	13 (43%)	11 (36%)

Discussion

With rising population figures, like any other developing country India is undergoing demographic and epidemiological transition and with changing dietary habits, the number of non-communicable case are rising as nutrition plays a key role in the prevention and causation these diseases.

(13) This case control study is a community based study to find out the prevalence of prehypertension and hypertension in urban areas of kamothe population. In the present study, A total of 120 subjects were included in the study among which 90 were in study subjects and 30 were in control groups and among the study subjects (i.e.-90) 30 were in CAD with diabetes, 30 were in CAD and 30 in Diabetes mellitus respectively. In our study, we have done the various anthropometric measurement, vital sign and routine biochemical parameter. In the gender wise distribution in control groups and various study groups and we found that a majority number male subjects are getting affect from coronary artery diseases i.e. 70% and majority number of female are having CAD as well as diabetes (53%). In the aspects of anthropometric measurement we have calculated Body mass index (BMI) by World health organisation formula (WHO)-2005, that is BMI is calculated by weight (Kg) upon height (m^2) and we found the mean level of BMI in the group –II that is in the coronary artery disease with diabetes is 24.22 ± 1.0, in only coronary artery diseases is 20.65 ± 2.0 and in only diabetes subjects is 24.39±1.0 of the study subjects in the comparison of control subjects i.e. 23.17 ± 1.78 and we have also found the statistical significance (P-0.001) in all the study groups.

For the biochemical measurement we have measured the concentration of Fasting and pot prandial plasma glucose and found the mean levels of FBS in study subjects i.e. group-II- 140.66 ± 41.83, group-III-96.69 ± 9.42 and in group –IV- 140.66 ± 41.83 in the comparison with control (91.61 ± 9.6) and found statistically significant. (P-0.001). for the post prandial sugar (PP2BS), there were highly increasing mean level of PP2BS in group-II is 257.8 ± 56.27, group-III is 139.48 ± 11.11and in group IV is 257.0 ± 56.0 as compared with healthy control (129.35 ± 10.6). Which was found highly significant (P-0.0001). For the blood pressure (systolic as well as diastolic) there were increased mean levels of systolic in study subjects that is group-II is 140.2 ± 5.38group-III is 137.90 ± 28.91 and in group IV is 140.2 ± 5.0 as compared with healthy control (119.77 ± 5.25). and for diastolic the mean levels are group-II is 86.7 ± 4.28 group-III is 83.60 ± 3.49and in group IV is 86.0 ± 4.0 as compared with healthy control (77.1 ± 4.25) and Test of significance for blood pressure was also found statistical significant (P-0.001) shown in table-2. In the aspects of Blood pressure distribution, we

have distributed it into different stage of hypertension and found that more number of patients from group – III i.e. only CAD and found 83% diastolic BP in pre hypertension stages and 60% were hypertensive and in only DM, there were 56% SBP and 60% DBP in pre-hypertensive.

A majority of hypertension patient accounts for type-II diabetes and it constitute about more than 90%, who has dual diagnosis for diabetes and hypertension. Our study is also have similar with the Priya *et al and* Ramachandran *et al.* (14) Observed similar pattern few aspects that is 38% study subjects were hypertensive. Nephropathy was present in 88 (44%) patients. In that, 50 (56.18%) patients had hypertension

Venugopal K et al -2014 [15] has also done the similar study on 250 diabetic patients with various complication and found Prevalence of hypertension noted in 64 (25.6%) patients. BP was normal in 55 (22%), 131 (52.4%) patients were pre-hypertensive. Macro vascular complications noted in 120 (48%) and micro vascular complications noted in 60 (24%) patients.

Conclusion

The findings of the present study clearly showing an increasing prevalence of hypertension in patients with coronary artery disease. When the patients of coronary artery disease with diabetes were compared with those without diabetes, more number of former group had hypertension compared than the other group.

Periodic evaluation of patient with pre hypertension and hypertension by applying life style modifications and may favor the good outcome and also prompt the treatment of hypertension and hyperglycemia.

Conflict of Interest: - Nil.

Source of Funding: - Self

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Predisposing Risk Factors Associated with Acute Myocardial Infarction (AMI): A Review

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Abstract

Among non-communicable diseases, cardiovascular diseases are major contributors to the resulting morbidity and mortality all over the world and India is no exception. Being one of the densely populated country and categorized as developing country, the proportion of subjects who are being afflicted with this disease has been progressively increasing. One of the leading causes for cardiovascular complications is myocardial infarction. The incidence of Acute Myocardial infarction has been observed across all the populations and from early adulthood to old age. Over the years there has been change in life style and dietary habits which is further contributing to increased prevalence. A number of risk factors have been found to be causative factors for development of the disease. Which include, genetic, environmental, life style modifications etc. The risk factors can be categorized in modifiable and non-modifiable risk factors. Those factors which can be controlled are categorized as modifiable and by changing them the risk level can be decreased whereas non modifiable risk factors cannot be changed and by knowing them one can assess the amount of risk involved. The present review is focused on various risk factors which are known to cause cardiovascular diseases and the role of each risk factor in the development of Acute Myocardial Infarction.

Keyword: *Acute myocardial infarction, Modifiable risk factor and Non-modifiable risk factor.*

Introduction

Acute myocardial infarction (AMI) is one of the leading causes of deaths in developed and developing countries. In event of irreversible damage of heart muscle, a complete absence or deficient of oxygen supply will lead to impairment of cardiac function making the person prone to arrhythmias. All this ultimately leads myocardial necrosis that's we call it Heart attack. [(1-2).] Most commonly it occurs due to a sudden decrease or stoppage of coronary blood flow as clogging (cholesterol pilling up in the inner wall) and narrowing of coronary artery leads to necrosis of heart muscle. As per the function of cardiac muscle is concerned like any other tissue, which is depending upon the demand or workload. The blood supply or oxygen must be ensured if there is any imbalance between supply and demand ratios, especially when there is rapid heart rate under these condition as there is higher demand and the demand is not fulfilled than there is occurrence of damage of cardiac muscle will lead to drop in blood pressure and ultimately leading myocardial damage. Chest pain is the hallmark symptoms of acute myocardial infarction, which may start from center or left side of the chest and may radiate into left arm, neck or back and remains for few minutes [(3-4)]. Globally the incidence of acute myocardial infarction varies greatly. Approximately 6, 50,000 and 1, 80,000 acute myocardial infarction patients are reported from USA and UK respectively every year [(5)]. More than 3 million people with STEMIs and 4 million with NSTEMIs episodes are reported every year globally.[(6)] However with the comparison of different population of other developing countries, it is reported that Indians

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have four time higher AMI due to various predisposing factors (mainly genetic and lifestyle factors) and leading to one or other metabolic dysfunction in them. The incidence rate of myocardial infarction in India is 64.37 per 1000 people and the mortality rate of MI in India is 31.7% whereas in other countries its 30 %[7-8].

Risk Factors:

Many risk factors have been documented and the prevention of those would immensely help to reduce the burden of diseases. But this requires an increase in the knowledge and awareness of type of risk factors existing among various geographical populations. As per the INTERHEART study, there are various predisposing risk factors for acute myocardial infarction which are generally categorized into modifiable (treatable) and non-modifiable (untreatable) risk factors. Which are described hereunder; [9]

Modifiable risk factors: Among the various modifiable risk factor such as habits, smoking, imbalance in dietary intake, sedentary lifestyle etc. other modifiable risk factor confined each individual which are impaired glucose tolerance, hypertension, diabetes mellitus, changes in blood lipid, overweight and obese condition.[10]

A. Physical activity or Sedentariness:

Physical activity is a key determinant for energy balance and weight control. Enhancement of vasodilatation and vasomotor activity is endothelial function, if this function is improved it will reflect on these functions also. Physical activity improves this function. Apart from this physical activity has many positive implications such as contributing in glycemic control, improving blood pressure and regulating blood lipid profile and improving insulin sensitivity [11, 12&13]. Approximately 20-30% reduction is the risk of coronary heart disease may be contributed by physical activity [14]. Many studies have reported people with sedentary life styles with multiple cardiac risk factors have more likely to develop AMI than others. A number of worker reported, there is no common effect of different physical activity. Effect of one physical activity may differ from other physical activity in the theory of development of risk of cardiovascular disease It has also been reported and it is difficult to pinpoint the effect of one type of physical activity from another but there will be definitely a cumulative positive effect. When we look at various physical activity, somewhere someone is involved in various domestic that may not provide much protection against cardiovascular diseases. But regular walking, climbing of steps and cycling are known to provide protection against myocardial infarction. [15, 16, & 17]. **Many workers have carried out studies to explore the relationship between type of physical activity. Some workers have reported the effects of low physical activity with relation to AMI. [18]One group of workers reported that less physical activity is an indicator of consequent risk of AMI whereas other suggested beneficial effect of light indoor, activity to reduced risk of AMI (AMI**D'Avanzo et al-1993 &Gong et al.-2013)[19, 15]

B. Smoking:

Among the many habits, one of the well-established habit proven to a definitive major risk factor for development and progression of cardiovascular diseases and myocardial infarction is smoking and tobacco chewing. The mechanisms behind increase in the risk of AMI by Cigarette smoking are such as **(a) The damaging effects of cigarette smoking is due to the presence of** Nicotine and production of carbon monoxide and these components cause arteries to lose their distensibility leading to plaque development. (b) In cigarette smokers there are high levels of non-esterified fatty acids and also increase in serum LDL and triacylglycerols and decreased HDL cholesterol leading to increased risk. In addition to, smokers there is high production of free radicals which oxidize LDL cholesterol triggering monocyte recruitment and T-cells facilitating more uptake of damaged LDL through receptors and finally leading to formation foam cells which form in intima and leading to atherosclerotic plaque. These events evoke inflammatory response (higher serum C-reactive protein levels)[19, 20].(c) In cigarette smokers there is a short supply oxygen from oxyhemoglobin to myocardium as there is more production of carboxyhemoglobin as this type of hemoglobin does not allow release of oxygen by hemoglobin. (d) One of the toxic products of cigarette smoking and tobacco is nicotine. Nicotine has many detrimental effects as it activates sympathetic nervous system(SNS) leading to arterial vasoconstriction and that in turn increases the heart rate and systolic blood

pressure[21, 22]. Other effect of the toxins of tobacco smoke are decreasing cardio protective HDL cholesterol levels and increasing the atherosclerotic LDL cholesterol levels [21]. The extent of risk may be different in males and females. Males are reported to have more risk ranging from two to six fold risk to develop myocardial infarction compared to females who are exposed to cigarette smoking. If we compared with smokers and non-smokers are associated with three fold increase for unstable angina. In the literature, there are many reports regarding the congestive heart diseases and years or duration of the smoking, number of cigarette smoked and the age of starting of smoking. [23].

C. Alcohol Consumption:

Many occasional alcohol consumers have been reported to be associated with hugely higher risk of myocardial infarction in the successive hour of its consumption. However, regular alcohol consumers with light to moderate consumption of alcohol known to be associated with a decreased risk of development of coronary artery disease, angina pectoris and myocardial infarction [24, 25].Heavy alcohol consumption which is episodic (called as binge drinking) is known to promote the progression of atherosclerosis in the carotid arteries and the binge drinking most likely to trigger an embolic stroke and MI [26-28]. Two studies, one from Finland and the second one from Austria have reported a strong association between binge drinking with fatal myocardial infarction.

Kauhanen*et.al.-1997*[28] reported that the risk of death from fatal MI was more than six times greater in men who consumed six or more beers per session than in those who drank less than three beers. In another study, McElduff and Dobson-1997[30] revealed an odds risk ratio of 2.62 for a major coronary event in men who consumed nine or more drinks daily for 1 or 2 days per week compared with those men who consumed the same amount of alcohol spread over the entire week. [27,29] Martin McKee et al-1998 [31] reviewed potentially four main possible mechanisms of Binge drinking pattern, which are reported to be the causes of cardiovascular deaths. These are:

(1) Effect on lipids: Binge drinking increases the risk of coronary artery disease. Regular, moderate alcohol consumption is believed to exert its cardio protective effect by increasing the anti-atherogenic high density lipoproteins (HDL). Though there is an evidence that chronic alcohol consumption suppresses levels of the atherogenic lipoprotein (a), but once the alcoholics stop drinking it is shown that this lipoproteins rises substantially.

(2) Effect on clotting: increasing chances of the risk of thrombosis. Regarding binge drinking there is no certain pattern of its effect on clotting system. Alcohol known to inhibit platelet responses to a range of physiological factors leading to reduction in platelet aggregation but in heavy drinkers there may be thrombocytopenia and other effects may lead to myocardial infarction whereas moderate regular drinking with meals have been reported to carry cardio protective effects.

(3) Effect on the myocardium or its conducting system leading to a greater risk of arrhythmias. Excessive indulgence of alcohol drinking occasionally will lead to histological changes in myocardium and also to a reduction in threshold for ventricular fibrillation.

(4) Effect on blood pressure: causing either an acute increase or sustained hypertension. Alcohol has an acute presser effect, binge drinking pattern reported to lead to significantly higher systolic but not diastolic blood pressures [30].Augustin *et al*.-2004 [32] and Biyik et al.-2007 [32]reported that regular alcohol intake during meals was inversely related to the risk of AMI, whereas alcohol intake during and outside mealtimes or only outside mealtimes was not related to the risk. This might be related to the metabolic effects of alcohol consumption as alcohol induces an increase in the HDL-C level, reductions in low-density lipoprotein and lipoprotein (Lpa) levels and a decrease in insulin resistance[31,33]. Some workers reported that 35% of acute myocardial infarcted patients show hypoglycemic and hypoinsulinaemic effect when alcohol (especially wine) ingested with food [33]

A large controversy has been generated regarding beneficial and detrimental effects of alcohol consumption and risk of AMI. One group of workers [34] reported that alcohol consumption can increase HDL cholesterol, apoprtoein-a1 and Adiponectin and reduction in fibrinogen levels and among these HDL cholesterol proved to be main protective factor which could explain that a 50% causal relationship attributed to increased

HDL cholesterol and decreased risk of acute myocardial infarction. But some of the other workers believed based on low HDL cholesterol under these conditions and attributed only 16% causal relationship between HDL-cholesterol levels and the risk of acute myocardial infarction [35, 36]

Some more workers who believe in the beneficial effects alcohol consuming give reasons for this beneficial effects such as increase in the levels prostacyclin in blood vessel walls leading to improvement in the functions of vascular endothelial cells not only that there is an increase in the sensitivity of insulin and resistance to thrombosis. Moreover, long-term regular alcohol consumption is known to improve heart rate variability (HRV) and thus diminished risk for onset of MI. But many other workers did not find beneficial effects of alcohol consumption. These workers reported that alcohol consumption would lead to increase in LDL cholesterol, triacylglycerols, heart rate and blood pressure increases the risk of atherosclerosis, atrial fibrillation and anoxia resulting in the damaging of cardiac muscle cells and cardiovascular system and also another effect is production of an inhibitor of fibrinolytic enzyme. [38,39] Some of the worker found that there is no association between consumption of alcohol and morbidity but heavy drinking on day to day basis might lead to increase serum triacylglycerol leading to increase in blood pressure, cardiomyopathy and stroke development.[40].

Table-1: Effects of alcohol consumption on clinical outcomes, atherosclerosis progression and blood coagulation parameters.32

Events	Binge drinking large Regular light-to-moderate	Binge drinking large Regular light-to-moderate
Morbidity and mortality	Increase	Decrease
Major coronary event	Increase	Decrease
Atherosclerosis progression	Increase	Decrease
Fibrinolysis	Decrease	Increase
Platelet activity	Increase	Decrease
Vascular tone	Increase	Decrease

D. Hypertension (Blood pressure):

One of the risk factors which has a direct correlation with an increased probability of developing coronary artery diseases and substantially documented in various populations across the globe.[41] Blood pressure (systolic and diastolic) increases the risk for MI, hence hypertension increases and there will be greater risk for the development of disease. Hypertension can be a considered as major risk factor for causing atherosclerosis in coronary artery, and that can leads to heart attack or myocardial infarction. As age advances hypertension becomes even worse to heart and many studies reported that 70% of heart diseases are due to this condition. [42]. **In the prospective Ttrialists groups study it is concluded that, rise in blood pressure by 20/10 mmHg can double the risk of ischemic heart disease**

and stroke over the range of 115/75 mmHg to 185/115 mmHg in those individuals who are between the age group of 40-90 years Lewington et al. [41]. It is one of the predisposing condition to develop atherosclerosis. It is a synergistic mechanism where inflammation and oxidative stress is observed in arterial wall, of course there is every possibility that the effect of hypertension on the onset of coronary artery diseases may be influence or modulated by hereditary and environmental factor [43] In the older patients an association between the development of acute myocardial infarction and hypertension has been reported by **Sadeghi R et al. [44]** It is further reported that, for the development of coronary artery diseases, increase in the blood pressure is one of the frequently found risk factor. Ciruzzi et al. [45] showed that hypertension is also frequently found independent risk factor for acute myocardial infarction in argentine population and suggested that the control of hypertension can be done with strict compliance of appropriate medications and adopting life style modifications facilitating the reduction in the risk of myocardial infarction. [45]

E. Dyslipidemia (Hypercholesterimia)

There is a close association between the levels of various lipid parameters and development of myocardial infarction. Except a few most of the other lipids are increased, this condition is known as dyslipidemia, which is a predisposing risk factors for acute myocardial infarction. Lipids increased levels have a negative impact on health are: Various serum lipid parameter some of atherogenic and other are anti-atherogenic. In the group of atherogenic lipids, Triglyceride, low density lipoprotein cholesterol, very low density lipoprotein and Apo protein B where as anti-atherogenic are high density lipoprotein cholesterol and Apo protein A present in it. [46, 47].

The principle cause of injury to the artery and vascular smooth muscle cells (SMCs) is mainly due to the increased levels of triglyceride and low density lipoprotein cholesterol. The known consequences are: If there is an accumulation of LDL-cholesterol in vascular endothelial, leukocyte will start to accumulation and attachment to the endothelium leading to further accumulation of lipids finally resulting into formation of foam cells and second effect is due to oxidative damage to the LDL-cholesterol. Deregulation of receptor uptake of LDL become unregulated and that will lead more uptake of LDL cholesterol lead to formation of atherosclerosis. [23, 48]. Apart from the above some other independent predictors of cardiovascular death in very high risk patients are: High levels of vonWillebrand factor, D-dimer, ADP induced platelet aggregation, triglycerides, end-diastolic volume, end-diastolic dimension, and ventricular septal thickness etc. **(Table-2).** These indicators endorse a close relationship between lipid metabolic and hemostatic disturbances between endothelial dysfunction and intra-cardiac hemodynamic worsening in the patients. [49] High density lipoprotein-cholesterol is known to be anti-atherogenic is prevents or inhibits pro-inflammatory effects of oxidized LDL. The other effect is to permotes various factor such as platelet activation factor, acetyl hydrolase and different antioxidant enzymes like catalase, myeloperoxidase and paraoxonase. [48, 50]. One group of workers reported that dyslipidemia condition in younger age group patients with acute myocardial infarction. These workers also categorized that increased cholesterol and LDL as potential risk factor in the development of acute myocardial infarction and also decreased HDL-c levels contributed to it. A relationship between dyslipidemia and coronary artery diseases has been established. An increased in concentration of total cholesterol, triglyceride, LDL-cholesterol and decreased levels of HDL-cholesterol and also reestablished of major risk factor in development of atherosclerotic coronary artery diseases. Correction of dyslipidemia have every possibility to remove the risk of myocardial

Lipid related cardiac abnormalities	Cardiovascular abnormalities	Metabolic abnormalities
ü Various serum lipid parameters levels abnormalities including: LDL-Cholesterol, Triglycerides, HDL-Cholesterol	ü Atherosclerosis ü Endothelial dysfunction ü Vascular inflammation ü Myocardial Infarction ü Coronary Heart Diseases (CHD) ü Peripheral artery diseases (PVD)	ü Nephrotic syndrome ü Acute coronary syndrome (ACS) ü Recurrent ischemic syndrome ü Thyroid disorder ü Chronic renal failure ü Growth Hormone Disorder ü Niemann-Picks Diseases type-c

infarction by Framingham study [52]

Table-2:[49]Showing important lipid abnormalities and metabolic disorders related atherogenesis.

F. Overweight/Obesity and BMI:

In recent times overweight/obesity has become a pandemic especially in younger population due to life styles adopted. Obesity is one of the many risk factors for development of metabolic syndrome and it is directly attributed to increase in the incidence of myocardial infarction. Zhu et al. [53] in a meta-analysis of previous studies and suggested that overweight and obesity are associated with higher risk of AMI**.** Overweight is one of the major contributor for the development of obesity. In the individual with obesity, a levels of free radicals is a key precursor for development of metabolic syndrome. [54] It is also reported that obesity has an association of pro-inflammatory effects of certain cytokines as an increase in the production of interleukin with the increase adipocyte mass. Subsequently stimulate production of C-reactive protein in the liver and both play a role in endothelial dysfunction by decreasing nitric oxide (NO), leading to vasoconstriction and increasing vascular resistance. [55] This association remained significant even after adjusting for other risk factors (non-HDL, HDL, smoking and hypertension). Before the onset of clinical manifestation in adolescent and young adult obesity found to the progression of atherosclerosis. **(56).** Schargrodsky et al.-1994[57] and its co-worker observed that overweight is one of the independent risk factor for the development of myocardial infarction. Yusuf et al.-2004 [58] shown the relationship between increase in the risk of acute myocardial infarction to abdominal obesity in the different age group and in the both gender.

G. Type 2 diabetes mellitus (T2DM):

Diabetes mellitus is another potential risk factor, and its predominant form especially type 2 diabetes mellitus (T2DM). There is a distinctive association between type 2 diabetes and cardiovascular diseases. Diabetes mellitus is due to decreased secretion of insulin or decreased effect of insulin. There are many factor behind the diabetes mellitus, they can be either genetic or environmental being the part of metabolic syndrome. Patients of diabetes mellitus have disturbance in the glucose metabolism leading to increase in its production. It has been clearly established a strong link between diabetes mellitus and increased mortality and morbidity of cardiovascular diseases compared with non-diabetic subjects the rate of mortality is very high in patients with diabetes mellitus. Patients with diabetes bear greater risk of atherosclerotic vascular disease in the heart as well as in other vascularized areas. Coronary artery disease accounts for more than 80% of all deaths and 75% of all hospitalizations in diabetic. **(59-61)**

Diabetes mellitus escalations the chances for the risk of MI because it upturns the rate of atherosclerotic progression and having an adverse effects on lipid profile, which facilitates the formation of atherosclerotic plaque. **(62)** Some workers reported diabetes as one of the major individual risk factor in the causal of heart diseases. In myocardial infarction case fatality, diabetes can be considered as an important risk factor as the available

data suggests that myocardial infarction is more fatal in diabetic subjects compared to non-diabetic individuals. (63)

G. Stress:

An element stress is present in every individual and many times it gives impetus to carry out a task but chronic high levels of stress will definitely lead to disturbances in homeostatic aspects as it affects the status hormones and ultimately many metabolic process and including free radical generation. Many studies have clearly delineated a relationship between constant chronic stress, social isolation and anxiety to the increased risk of heart attack and stroke. It is a common observation preceding that there are various reasons like affected person have intense grief, which may be due to death of close relatives leading to onset of myocardial infarction. The relation between acute attacks of myocardial infarction followed by stressful emotional behaviour. This results hemodynamic changes leading to the stress of coronary artery causing the rupture of atherosclerotic plaque and thrombosis.(64)

Non-modifiable risk factors:

Many of the risk factors which cannot be changed or controlled and bound to be happen. Theses factor will not be much use in the part of its management.

A. Age:

There are many studies which reported the incidence of myocardial infarction in different age groups but majority of these studies indicated a relationship between advancing age with an increased mortality in acute myocardial infarction. There are no definitive answers to explain the mechanism behind the significant mortality rate in the older people though some inference can be drawn linking to the processing of ageing along with the metabolic changes which take place like hardening of blood vessels etc. It is often reported that 80% of heart diseases related mortality occur in people with 65 years or above. The age range of occurring this event in most of the males is between 50-65 years whereas in females it is 10 years later especially after the onset of menopause. This aspect has many serious implications in many countries as there is an increasing trend in the number of geriatric populations in many countries. (67)

A. Gender:

It is well established that more men suffer from heart attacks compared to corresponding age group of females. Men tend to get heart attack at a younger age compared to females. The incidence of heart attack in female increases after onset of menopause even then the post-menopausal incidence rate in females is lower than that of males. While going through the data of deaths due to heart diseases one can certainly come to know that males are more prone than females (66). Sex hormones play very important role to provide protective mechanism against CAD during premenopausal years in women. Women before menopause are protected against CAD, because of the impact of sex hormones function. (68)

B. Family history

Among the different non modifiable risk factors family history is one of the most important independent risk factor for AMI. If a first degree relative is associated with AMI then there is a double risk to be afflicted with this disease. Comparison is drown from the number of cohort study and observed that person who have first degree of relative with cardiovascular diseases have higher risk of afflicting with the diseases compared to those person who do not affected with first degree of relative. (69) In case of family history of acute myocardial infarction is one of the crucial risk factor while assessing various modifiable or non-modifiable risk factors. A of strong evidence suggested that the family history have strong association. (70, 71)

Genetic factors:

Heredity is a crucial aspect to predict the likely event to happen in the family with the history of a coronary event at a younger age. Coronary artery disease and myocardial infarction are the repeatedly causes for death across many families. In many families every second myocardial infarction is lethal and hits the patients unexpectedly without previous signs or symptoms. A comparative analysis of molecular genetic testing had demonstrated that: In first aspect, the genetic study it has been observed that the myocardial infarction locus is unique and it does not overlap with other risk factor. Second aspect is lipoprotein a, type-2 diabetes mellitus and serum lipid and hypertension comes under strong

CVD risk factor have very strong relationship with genetic and target the candidate genes for myocardial infarction pathogenesis. (65)

Other predisposing risk factor for acute myocardial infarction (AMI): are as follows

A. Socio-economic status: - Social strata determines how a person adopts life style and social status is directly determined by economic status of that individual or family. Generally the higher the person in social status the existence better health indicators. Persons in higher echelons of society are within the reach of power, prestige and access to resources. There are many structural mechanism which are responsible for creating differential social positions of any individual such as governance, education system, labor market structures and the reach to various welfare policies by the way of causing an impact on behavioural, economical, emotional, day to day lifestyle and various metabolic aspect.(72). There is an established relationship between development of diseases and social status. Cardiovascular diseases has no exception to it. Better social status, reachability to nutritious diet and better working condition will improve to overall increasing the various cardiovascular diseases. Working into the aspects, there are some reports, which have made the recommendation. These recommendation discussed in WHO member states and passes a resolution called Health welfare policy in 2009 with three recommendation that are: (i) Day to day living condition enhancement (ii) Equal sharing of supremacy or resources. (iii) Observation of health discriminations. (73)

B. Consanguineous marriage: - In many regions of various countries there is a practice of consanguineous marriage practices. It is well known that marriages between cousins will lead not only to many hereditary diseases but also other diseases. One of such disease is cardiovascular disease. The frequency of consanguineous marriage is common in the third world countries which are 50.3%, 54.3%, 28.9%, 38.6%, and 25% in Jordan, Kuwait, Egypt, Iran, and Lebanon, respectively and in many regions of Asia including India there is a practice of this system from so many past years. Genetic studies lead to recognition of new complex diseases causing variants, and focus on the association between polymorphism and risk of coronary artery disease. (68)

C. Gout: - Gout is due to the accumulation of uric acid in the disturbance in metabolism of purine. In the patients of gouty arthritis there will be inflammatory response. One of the case of initiation and progression in the atherosclerosis. Toxin can infer the presence of inflammatory components in gout also increases the risk for acute myocardial infarction (AMI). Which in turn leads to a series of event and finally promoting a pro-thrombotic environment for acute coronary events such as angina or MI (74)

D. Periodontal Diseases: -One of the risk factor which is found to be associated with cardiovascular diseases is the presence of a periodontal diseases. Periodontal diseases are group of inflammatory disease where in the principle etiological agents, bacteria and their byproducts. (75). It is well established a strong link between dental disease, especially periodontal disease and atherosclerosis leading to increase in the risk of occurrence of CHD. (76, 77)

Conclusion

In this review, we have reviewed various risk factors, which have been found to be causative factors for development of acute myocardial infarction. Among the various risk factors, there are modifiable risk factor such as imbalance in dietary intake, habits and sedentary lifestyle. Sedentary life style is one of the significant risk factors and many workers found that the leading active life decreases the risk of AMI. Another modifiable risk factor is smoking, compared with non-smokers, smokers are associated with three fold increase in unstable angina. The risk combined by smoker is also associated with number of cigarettes he or she smoked. Alcohol is also one of the risk factor however many researchers have reported beneficial effects of limited intake of alcohol . We have also come across reports suggesting a connection between hypertension, dyslipidemia and acute myocardial infarction. One of the most important aspects is atherosclerosis, which has been directly associated with the risk CAD. Stress is one of the risk factor for the association with AMI. We have also included non-modifiable risk factors such as gender, family history, genetics etc. reported to have an association with AMI. Finally we have also reviewed various predisposing factors which act as precursor

for the development of CAD. Some of such are socio economic status, consanguineous marriage, gout and periodontal diseases.

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Efficacy of Innovative Table for Traction, Myofascial Release Along with Medicated Steam (Swedan) in Non-Specific Low Back Pain Patients: A Research Protocol

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Abstract

Background: Low back pain (LBP) is a common condition associated with work absenteeism, disability and large health care costs with an annual prevalence range from 22% to 65%.Chronic low back pain is the condition where the back pain lasts for more than three months. Lumbar traction is the process of applying a stretching force to the lumbar vertebrae through body weight, weights, and/or pulleys to distract individual joints of the lumbar spine. Myofascial release (MFR) is a form of manual medicine which involves the application of a low load, long duration stretch to the myofascial complex, intended to restore optimal length, decrease pain, and improve function. Medicated steam therapy is a method of giving medicated steam over the affected muscular area in order to provide local effect of pain relief. All the three methods will be applied together to see the effect.

Methods/Design: The study will be designed as a pilot study. A total of 30 samples will be taken with nonspecific low back pain. NPRS and Modified Oswestry Disability index was taken before treatment. Intervention including traction, myofascial release therapy and medicated steam will be given. . Two sessions will be given once a week for 3 weeks and outcome will be recorded.

Discussion: Various studies have been conducted over the effect of lumbar traction over low back pain. Traction distracts the muscles and the joint helps in relieving the pain. One of the therapeutic options is traction, which can be combined with other physical therapy modalities. Combining effect of traction with hot fermentation has been effective study have shown. It has been claimed that traction therapy results in lessening the muscle spasm

Key words: *Traction table, Low back pain, Steam therapy, Myofascial release.*

Introduction

Low back pain (LBP) is a common condition associated with work absenteeism, disability and large health care costs1 with an annual prevalence range from 22% to 65%. Such epidemiological data translate into significant negative economic and social effects. Low back pain (LBP) is a common condition associated with work absenteeism, disability and large health care costs1 with an annual prevalence range from 22% to 65%2. Such epidemiological data translate into significant negative economic and social effects. So, Management of low back pain and avoiding recurrence is important. Lower back pain is many times ignored and this led to chronic changes in musculature as well as the body mechanics. A proper posture and proper biomechanics of body should be present to lead a healthier life. All the possible measures should be taken to avoid as well

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as treat low back pain. Chronic low back pain is the condition where the back pain lasts for more than three months. Chronic back pain reduces the efficacy of work and major consequences are faced because of that by the person as well as the society. lumbar extension traction had a significant and stable effect on the intervertebral movements at most levels[1]

Various Physiotherapy interventions are used to reduce low back pain. Traction and myofascial release have shown significant effect on reducing pain and relaxing muscle. Lumbar traction is the process of applying a stretching force to the lumbar vertebrae through body weight, weights, and/or pulleys to distract individual joints of the lumbar spine. Which distracts spinal segments and provides relaxation which helps in reducing pain. Lumbar traction also stretches the muscles which surrounds the area providing a relaxation effect. In cases like prolapsed intervertebral disc the lumbar traction has been found effective. Lumbar traction reduces the compressive forces on the disc and hence provides the disc the time to heal. The supposed mechanical effects of traction are vertebral separation and widening of the intervertebral foramen[2]. Traction acts as a mechanical intervention therapy. Which distracts the muscles and the joint helps in relieving the pain[2]

Myofascial release (MFR) is a form of manual medicine which involves the application of a low load, long duration stretch to the myofascial complex, intended to restore optimal length, decrease pain, and improve function[3]. Myofascial practitioners believe that by restoring the length and health of restricted connective tissue, pressure can be relieved on pain sensitive structures such as nerves and blood vessels. Direct technique MFR is thought to work directly on restricted fascia; practitioners use knuckles or elbow or other tools to slowly sink into the fascia, and the pressure applied is a few kilograms of force to contact the restricted fascia, apply tension, or stretch the fascia. Indirect MFR involves application of gentle stretch- the pressure applied is a few grams of force, and the hands tend to follow the direction of fascial restriction, hold the stretch, and allow the fascia to 'unwind' itself. The rationale for these techniques can be traced to various studies that investigated plastic, viscoelastic, and piezoelectric properties of connective tissue[4]. When overused tightness develops in connecting tissue, it is unclear if the tightness is due to collagen fibre, fibroblast or ground substance. Myofascial release therapy helps in reducing the tightness[3]. Trigger points are small, tender points in the muscle or connective tissue that can cause pain attributed to distant sites. Trigger points develop in cases of prolonge back pain and they are also treated by myofascial relase as well as by traction.[5].

Instrument assisted soft tissue mobilisation is the technique which uses a tool to mobilise the soft tissue, to remove the adhesions. It reduces the force required by the therapist to palpate the tissue through hands and provides the same amount of tension release in fascia.

Dashmuladhi is of medical importance in Ayurveda. It is a pain relieving medication extracted from the roots of 10 different herbs. Medicated steam therapy is a method of giving medicated steam over the affected muscular area in order to provide local effect of pain relief. The process which relieves stiffness, heaviness, coldness of the body and produces sweat is called Swedana**.** The sedative effect produced relieves the pain in the area. This may be because of the increased blood supply which produces removal of waste products hence getting good nourishment. Heat is also a counter-irritant i.e. the thermal stimulus may affect the pain sensation as ex-plained in pain gate theory of Melzack and Wall. This explains the effect of heat applied on the nerves. Vāta is the responsible factor for inducing pain in the body and the properties of Vāta and Swedana are exactly the opposite. Hence, the heat can cause reduction in pain by reducing the swelling, removing the coldness at the site and causing perspiration. Traction, Instrument assisted soft tissue mobilisation and steam therapy all have significant effect of nonspecific low back pain. Steam therapy is generally used by Ayurvedic doctors with herbs in order to reduce pain .Treating a single patient with all the three techniques is time consuming. If all the three therapies are combined, the time required to treat the case will be reduced and the outcome will change. Even the manpower required of treating the patient will be reduced. This study was conducted in order to see the combined effect of all the three modalities.

Aim & Objective

1) To find out the combined effect of traction,

instrument assisted soft tissue mobilisation and medicated steam therapy (Swedan).

2) To find out a dose response relationship of the newly developed traction device.

Methodology

Study Setting: The trial will be carried out in the Out Patients Department of Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University.

Study Design and Sample Size:

The design of study is a pilot study. The participants number, enrolled in the experimental study will be 30 (n = 30).

Participants

Inclusion Criteria:-

1. Chronic Low Back Pain
2. Both genders
3. PIVD
4. Age 20-40

Exclusion Criteria: -

1. Individual with acute low back pain
2. Individuals with Cognitive impairments
3. Individuals with sensory deficit
4. Individuals with recent fractures.
5. Individuals with osteoporosis

PROCEDURE:

Participant Timeline:

Study duration is of 6 months and intervention duration is 3 weeks so participant will be enrolled during first 5 months of study so 2 week intervention will be completed successfully. Assessment will be done on 1st day of visit then in midway (1st week) and end (2nd week) of intervention.

Implementation:

Research coordinator and principal investigator will supervise randomization. Participants will be asked to manually select from the envelope, sealed group allocation for the recruitment into either group.

Blinding:

Tester(s) will be blinded to assign the subjects to the group. To ensure binding, subjects will be mandated not to reveal any details of their treatment to the tester

Study Procedure:

After the ethical approval an informed consent will be taken from the patients. Proper procedure will be explained to them. A total of 30 samples will be taken with nonspecific low back pain. NPRS and Modified Oswestry Disability index was taken before treatment .Comfortable clothing will be provided to the patient as the part which need to be treated should be exposed. Patient will be made to lie supine on the traction table. 1/3rd of the patient's weight will be used to apply traction. Patient will be positioned in such a way that the painful segment will be placed on the roller. Traction belt will be applied and the machine will be started. At the same time medicated steam will be given to the patient. Traction will be given for 15 mins while steam will be given for 10 mins. Two sessions will be given once a week for 3 weeks and outcome will be recorded.

Outcome will be recorded in between the study and after completion of 3 weeks.

Outcome Measures:

Pain: Outcome measure will be by Numeric Pain Rating Scale[6]. NPRS is the clinical tool used to assess pain. The maximum score is 10. Where Zero is no pain and 10 is as bad as it could be.

Disability Index: *Modified Oswestry Low Back Pain Disability* will be used. Total 10 components are present with maximum score of 5 in each section. Disability is calculated in percentage[7].

DATA COLLECTION AND MANAGEMENT:

Data collection:

Information about study given at time of recruitment

(elaborating the purpose, nature, procedure, benefits and after effects of the intervention) with all baseline tests and assessment will be repeated on 2 more occasions.

2nd – midway of intervention (10th day), 3rd- end of intervention (3rd week)

Data management:

Principal investigators will supervise documentation & data collection. For precision and accuracy brief evaluation of the study documentation will be done. After completion of study, Excel spreadsheet will be released to an allocation blinded statistician for conducting the analysis, following which unblinding of the groups will be done.

Discussion

Various studies have been conducted over the effect of lumbar traction over low back pain. Traction acts as a mechanical intervention therapy. Which distracts the muscles and the joint helps in relieving the pain. One of the therapeutic options is traction, which can be combined with other physical therapy modalities[2]. Combining effect of traction with hot fermentation has been effective study have shown**.** It has been claimed that traction therapy results in lessening the muscle spasm[8]

Myofascial release therapy is used to release tension in the fascia as well as muscles. Studies have suggested that when IASTM was applied for 4 weeks in 30 patients with chronic lumbar pain, the pain decreased significantly. However, the literature on the efficacy of lumbar traction as a part of conservative treatment is conflicting [9].Sufficient joint ROM is needed for optimal musculoskeletal function and insufficient flexibility can make one become vulnerable to overuse syndrome and acute injuries (Hreljac et al., 2000; Sainz de Baranda and Ayala, 2010). Therefore, having sufficient ROM is important for improving exercise performance, in addition to the rehabilitation or prevention of sports injury. Myofascial release especially instrument assisted soft tissue mobilisation reduces the muscle tension hence helping in restoring the range of motion.

The Cochrane Database of Systemic Review has indicated that traction may make little or no difference in pain intensity, functional status, and global improvement or return to work when compared to placebo, sham traction, or no treatment. To date, the use of traction as treatment for non-specific LBP is not supported by the best available evidence[10].

Ethical Approval and Dissemination:

Ethical approval will be taken from institutional ethical committee. The DMIMS who will fund for research and the subjects who will participate in the study can access the main findings of the research. Data held safely for the enrolled subjects a minimum of five years. After completion of data collection, statistical analysis a completion report will be formed and after review by institutional research cell will be send for publication.

Patient consent:

Principal Investigators will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality.

Confidentiality:

The research plan will be explained to the participants and personal details will be taken from one of his / her relative and the principal investigator. The consent form must contain a statement of confidentiality and the principal investigator, patient and 2 witnesses' signatures. If needed to reveal any details for the analysis, the patient's consent will be taken with full confidentiality guarantee.

Declaration of interest:

The authors declare no conflicting interest.

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A Case Report of 57 Year Old Male Having Post Fracture Stiffness of Right Shoulder with Supraspinatous Tear Case Report

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Abstract

A case of lateral clavicle fracture associated with frozen shoulder is reported, which had reduced the abduction and lateral rotation due to frozen shoulder due to pain patient's fear of being unable to do any shoulder impact operation. MRI ruled out supraspinatous tear. Medical examination of the frozen shoulder confirms the diagnosis. He was successfully treated with the combination of physical therapy treatment with the TENS, IFT, ultrasound and shoulder girdle mobilization electrotherapy modality. Scope of motion comment per goniometer at 2, 4, 6, 8 weeks shoulder at. All the ranges are increased significantly**.**

Key Word-*: Frozen shoulder, lateral fracture of clavicle, combine physical therapy, electrotherapeutic modality, shoulder mobilization.*

Introduction

Shoulder stiffness is the main complication after fracture shoulder immobilization and diabetics contribute to frozen shoulder which is the most common complication in diabetic mellitus patients. In the initial phase of adhesive capsulitis, patients may typically perform all of the everyday living operation despite pain, at which time patients can also suffer from sleeping disorder. Pain typically reduces during the second phase but minimal movement and patient is unable to perform everyday exercise. There are three stages of first phase adhesive capsulitis 2-9 months, second phase 4-12 months and third phase 5-26 months [1]. Physiotherapeutic therapy is improving the frozen shoulder patient by almost 90 percent. Treatment with physiotherapy consists of physical therapy, behavioral exercises. The aim is pain relief, and movement normalization. Ultrasound therapy induces vasodilation that eliminates metabolitis from affected tissues and relieves muscle spasm. Transcutaneous stimulation of electric nerves is efficient in relieving pain. Therapeutic exercises include mobilization of soft tissue using cyriax process, peripheral mobilization to boost controlled ROM. In stage 2 frozen shoulder joint mobilization i.e. glenohumeral joint passive ROM mobilization along with active assisted ROM, strengthening and stretching exercises.

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Patient information-

The patient is 57 year old male with right hand dominance and his body mass index 29kg/m^2. He was the case of fracture clavicle of chief pain complaint on the right shoulder, he was diagnosed with fracture lateral 1/3 clavicle and was treated with conservative sling for 2 months due to immobilization for a long period of time, patients suffering from shoulder stiffness due to diabetic

patients, which may contribute to frozen shoulder. There was no previous surgical experience, and with the same reason no experience of hospitalization, there is history of diabetics and hypertension. Patient was initially able to perform elbow, wrist and finger ROM normally after 2 months removal of shoulder sling patient experiences extreme pain around the shoulder when shifting pain to interrupt the sleep of the patient and Pain is mostly at night due to pain patient's fear of not having to do both elbow and wrist movement.

Clinical findings-

Musculoskeletal assessment was perform with drop arm test, apply's scratch test, Hawkins and NEER impingement tests, range of shoulder joint measured by goniometer after 2 week flexion 80 degree, extension 20 degree, abduction 60 degree, medial rotation 20 degree and lateral rotation 15 degree.

There is positive apply's test , Hawkins- kennedy test, NEER impingement test.

Diagnostic assessment-

Patient with x-ray prior to conservative management and shows lateral 1/3 clavicle fracture after removal of x-ray sling patient again showing fracture becoming united but patient complaint with severe pain. MRI patient after it shows degenerative changes in the acromioclavicular joint causing impingement over the supra spinatus tendon causing fiber thinning and mild edema suggesting partial tearing of the thickness.

Therapeutic intervention-

Intervention type is physiotherapeutic exercises, self care

Therapeutic ultrasound, transcutaneous electrical nerve stimulation, interferential therapy, Russian current is most effective, eight-week low-level laser therapy[2] can be more efficient than exercising alone in a four-week period, and work for up to two months.

Plan of care decide as per week-

1. First 1-2 week- therapeutic ultrasound in continuous mode, interferential therapy in clover leaf pattern causes deep tissue effect which reduce pain and synchronized muscle tissue modality given to the patient 3 time day each modality for 7-8 minute. In second week isometric exercise start shoulder isometric and grade 1 mobilization for 5 minute 4 times a day.

2. 3-4 week- low level laser therapy[2] and Russian current start for 8 minute also interferential therapy given for 8 minute for 4 times a day along with grade 1 mobilization for 10 repetition finger ladder upto the pain free movement for 5-6 repetition and 3 times a day effective in the reduction of pain, increase the shoulder abduction range in adhesive capsulitis[3].

3. 5-6 week- patient treated through shoulder mobilization grade 1-2 along with therapeutic ultrasound and TENS[4]. Different exercises like codman's and finger ladder and mechanotherapy by shoulder wheel exercise. Shoulder isometrics active assisted, pectoral muscle stretches along with mobilization each isometrics hold for 10 sec. and for 20 repetition each 3-4 times a day .

4. 7-8 week- therapeutic ultrasound and low level laser therapy for 3 times a day each for 7-8 minute. Shoulder isometrics 10 sec hold and times a day, shoulder wheel 20 repetition and 4 times a day. Finger ladder 10 repetition each exercise 4 times a day it gives effective result to achieve normal range of motion and pain relief[6]. Pectoral muscle stretch, scapular rotation, shoulder flexion, extension, rotation supination-pronation, medial and lateral rotation for 10 times and home exercise program[1].

Follow up/ outcome- Goniometer measurement

Shoulder movement	First day	2 week	4 week	6 week	8 week
Flexion	60	80	110	134	165
Extension	10	20	25	35	47
Abduction	30	60	80	140	165
Internal rotation	10	20	40	55	72
External rotation	5	15	25	37	50

Patient follow up for treatment 2 month on regular basis.

Discussion-

Mobilization of soft tissue along with home exercise supports patients suffering from frozen shoulder[7]. I n this case of frozen shoulder patient had been able to return his previous work and ADL after getting combine regime for 8 weeks. TENS, and medical ultrasound, IFT, have been shown to help relieve pain[3]. Exercise and home software help to boost strength and endurance and to render flexible joints[8]. It's time for a cycle to take time and there are several challenges to recover the full range of frozen shoulder.

This case shows remarkable improvement in the treatment of frozen shoulder combining electrotherapeutic modality with manual therapy, mobilization and home exercise[9]. Best evidence and patient effort in physiotherapy and exercise at home provide successful results[1].

Conclusion

Most of the evidence shows that after 45 years of illness, females are more likely to develop frozen shoulder than males. Pain usually presents symptoms, mainly abduction and external rotation limiting movement, tenderness, pain limiting the activity. Physiotherapy play important role in treatment of patients suffering from frozen shoulder. The treatment of primarily aims at relieving pain and increasing motion range[10]. Only manual therapy alone, does not give the satisfactory result along with electrotherapeutic modality with proper dose work on the patient.

Ethics and Dissemination: The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review

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Conflict of Interest: Nil

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Familiarity and Desire to Perceive Medical Education Courses among School Students in India- A Pilot Study

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Abstract

The number of dentist serving the rural areas is relatively low and the practice of seeking general physicians for dental treatments can still be evident in areas where dental services are not accessible. But it is unclear whether students from suburban regions prefer to pursue dentistry as their first choice of preference. Hence, the present study was aimed to find the interest in dentistry among the aspiring school students, to evaluate if the existing exams remain a challenge, from choosing a medical course, and also to assess if the emergence of COVID-19 has actually increased the popularity of traditional medicines. A survey questionnaire with 16 questions was shared through online media to 200 school students both in urban and suburban regions after validation for this pilot study. After consent to participate, the responses collected were tabulated and statistically analyzed. It was observed that only 64% (urban) and 73% (suburban) were aware of dentistry with a slight increase in student's choice for traditional medicine courses over allopathic medicine (40% urban, 48% suburban) post pandemic**.** Within the limitations of this study it is evident that school students are less aware of various dentistry courses, and access to training for the exams can motivate the number of students seeking admission to dentistry courses.

Keywords: *Dentistry, Medical education, Psychology of school students, Traditional Medical courses.*

Introduction

Traditional medicine practices are very popular in India and the rural population still practice home remedies based on these traditional therapies. Minor ailments are treated by home remedies on advice from elderly people at home in rural areas of India. Although India has been home to origin of these ancient medicines [1], allopathic remains the most sought medical treatment among Indian population [2]. Owing to this demand, the number of aspirants seeking admission to medical universities has increased multifold. This increase in student's number has led the governing authorities to standardize the procedure of admitting the students to medical universities through common entrance exams based on common curriculum [3]. The number of dentist's patient's ratio in India appears to be satisfactory [4] and the number of dental institutions also offers treatment at nominal cost. But the number of dentist serving the rural areas is low and the practice of seeking general physicians for dental treatments can still be evident in areas where dental services are not accessible. Despite all these, the number of students seeking admission to dental colleges is not satisfactory [5]. This can be due to the fact the awareness about dental education courses are lower students choose dentistry if they fail to secure medical admissions.

This COVID 19 emergence appears to have certain impact on the mental attitude of the school students aspiring to be physicians. The fact that traditional medicine in the form of diluted and boiled solutions, commonly called as "**kashayams**" in Ayurveda and siddha has been effectively used in the treatment of COVID-19 has increased the fame of these ancient traditional medicines [6]. Patient who consulted

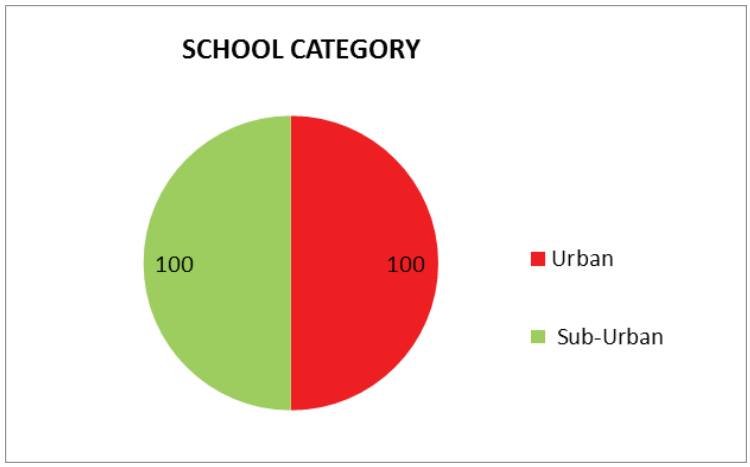
physicians often for their illness, have reduced the frequency of visits during this pandemic and have tried these traditional therapies at home after teleconsultation and there has been a good patient satisfaction in this regard [7]. Hence the interest in pursuing these medicine courses also appears to be increased. However the definite number of students actually interested to continue these traditional medicine courses was not clearly evident. With this scientific background a study aimed to find the interest in dentistry among the aspiring school students, to evaluate if the existing exams remain a challenge, from choosing a medical course, and also to assess if the emergence of COVID-19 has actually increased the popularity of traditional medicines.

Materials and Methods

The study was approved by Institutions Review Board and a survey questionnaire was prepared and shared through online media to the school students both in urban and suburban regions. The study was designed by the principal investigator and two field investigators were involved in this study. The investigator designed it as a pilot study as many students could not be contacted during this pandemic crisis. Educational institutions of the participants were contacted and explained about the study. The students of each institution were explained about the study by the institutions over phone and after their willingness to participate in the study were obtained, their contact details were collected. The questionnaire was then shared to the participants through online resources and the responses were obtained. The survey consisted of 16 questions with one open ended question (**Figure 1**). Based on the validity of the questionnaire 200 samples were included for this pilot study. The responses collected were tabulated and statistically evaluated by using SPSS software ver20.0 (IBM Corp., Armonk, N.Y., USA).

Result

Nonprobability, convenient sampling technique was employed that yielded information from 200 school students consisting of 100 participants each from urban and suburban regions (**Graph 1**) grouped as 125 boys and 75 girls by gender (**Graph 2**). On statistical evaluation it was observed all the 200 Samples were valid for the study with Cronbach's alpha reliability score being **0.88447 (Highly Significant score**). Category wise analysis showed 180 out of 200 (90%) participants belong to class 10th followed by 5.5% (11 out of 200) class 11th and 4.5% (9 out of 200) class 12th students (**Graph 3**). Chi-square test performed for category-wise distribution showed Chi^2 value of 48.02. The **p-value is <.00001**. The Result is significant at $p < .05$.Kruskal-wallis test calculator was performed to evaluate the interrelationship within and among the groups followed by Comparative T-test was performed to estimate the level of significance between the study populations based on the questionnaire responses. It was observed that between the urban and suburban population interrelation within the group was found to be statistically significant at p<.05 [Urban (H) = 30.0031 at p<.00001; Suburban (H) = 27.3765 at p<.00001]. Similarly relationship among the study groups was also found to be statistically significant at p<.05 [H= 29.977 at p<.00001].



GRAPH 1: GRAPH SHOWING THE DISTRIBUTION OF STUDY POPULATION AMONG SCHOOL CATEGORY

GRAPH 2: GRAPH SHOWING THE GENDER WISE DISTRIBUTION OF STUDY POPULATION

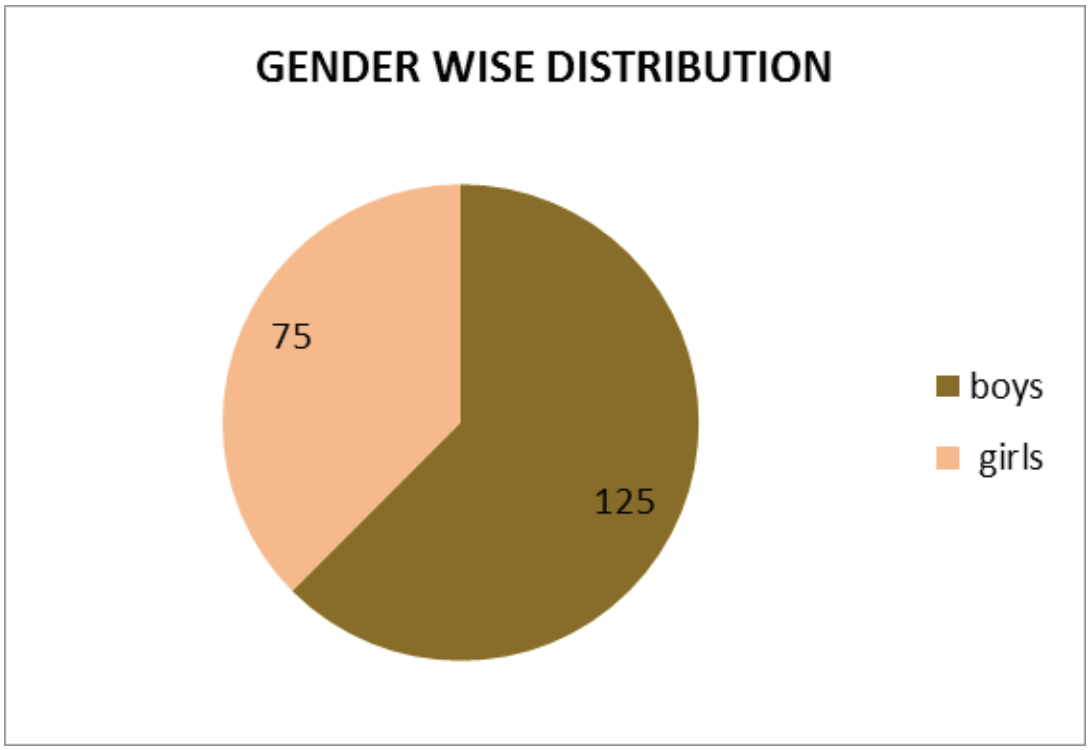
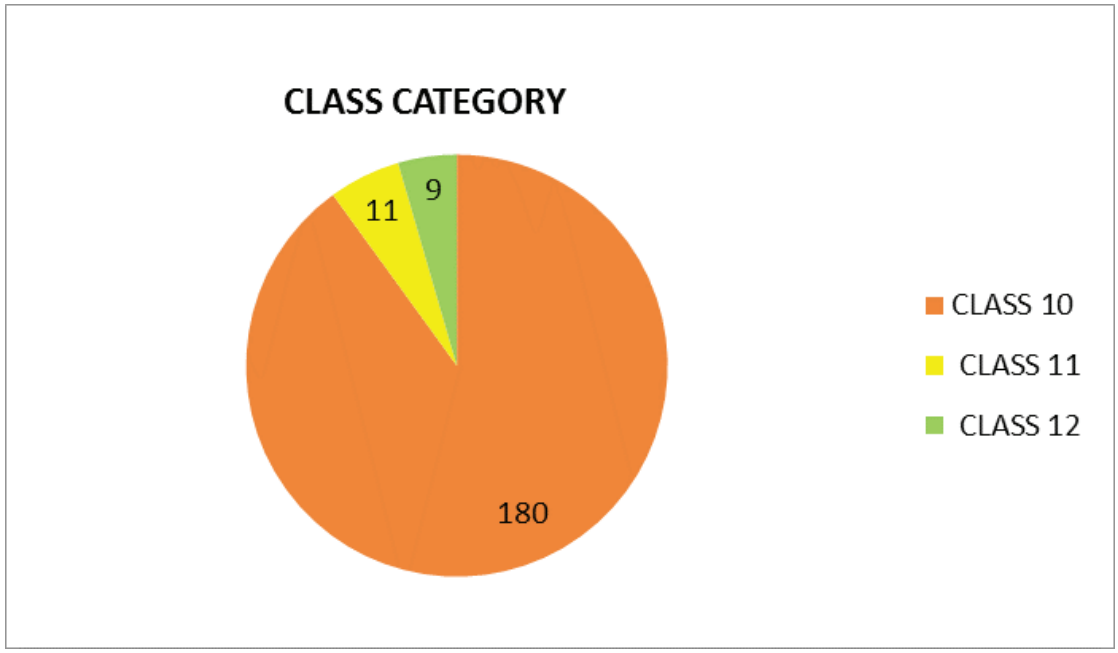


FIGURE 1: IMAGE SHOWING THE SURVEY QUESTIONNAIRE USED TO EVALAUTE THE STUDY



GRAPH 3: GRAPH SHOWING THE CLASS CATEGORY DISTRIBUTION OF STUDY POPULATION

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School name: **Class:** **Gender: M / F**

Urban school/ Sub urban school **10^{th}/11^{th} /12^{th}**

1) Which group will you take in your higher secondary to join in a dental course?

a) Allied health sciences b) Biology c) Commerce d) Computer science

2) Which branch in health science is related to oral health?

a) MBBS b) BHMS c) BAMS d) Dentistry

3) Are you aware that dentistry is one of the health science courses like MBBS?

a) Yes b) NO c) Not sure

4) Are you aware that qualifying NEET is required to join Dentistry?

a) Yes b) NO c) Not sure

5) How many times have you visited dentist in your past?

a) Once b) Twice c) Thrice d) Never

6) If yes, what is the reason for your visit?

a) Routine dental visit b) tooth ache c) bleeding gums d) cosmetic reasons

7) Do you know that dentistry's course duration is same as MBBS?

a) Yes b) No c) Not sure

8) Are you aware that dentistry is the highest paid profession in western countries?

a) Yes b) No c) Not sure

9) Are you aware of the integrated course of Dentistry with Master's in European countries?

a) Yes b) No c) Not sure

10) Do you know that the basics of MBBS & Dentistry are the same?

a) Yes b) No c) Not sure

11) Do you know that there are different diploma courses like Dental mechanics & Dental technician?

a) Yes b) No c) Not sure

12) Are you aware of Dental nurse?

a) Yes b) No c) Not sure

13) Would you intend to join dentistry after schooling?

a) Yes b) No c) Not sure

14) Are you attending any Entrance coaching classes (NEET)?

a) Yes b) No

15) A) Do you know that there are different specialities in dentistry?

a) Yes b) No

B) Could you name a few specialisations?

16) What is the source of information for you about dentistry?

FIGURE 1: IMAGE SHOWING THE SURVEY QUESTIONNAIRE USED TO EVALAUTE THE STUDY

Discussion

The approach of the school students and their desire for choosing a particular career are of great importance for strategy creators around the world. The students have a number of career choices and it becomes difficult for them to choose a particular career [8]. Most commonly the students depend on parent's guidance in choosing their higher education. Decision process involves a number of factors that includes personal interest, peer pressure, self-motivation, financial reasons, and better quality of life. Dental career is unique profession, influenced by several factors such as gender, parental support, family background, parent's socio-economic status, prestige, income and faculty role models, controllable life style versus non controllable life style, local market forces, committed relationship, and influence of a faculty advisor and the perceptions of employment availability [9]. According to the Indian education board, students who are interested to pursue higher education would be choosing biology and physical sciences as their secondary level course. Among the class 10 students, 65% preferred to choose the biology stream in the urban area and 46% preferred in the suburban area similar to studies performed by Rajeev et al [10], Bhat et al [11], Aggarwal et al [12] among Indian students and also by Dastjerdi MV et al [13] among Iranian students. This preference indicates that pursuing higher studies in medicine courses is preferred by students among both the population group could be attributed by several factors such as personal choices, student's motivation, information from social medias, peers, parental influences, support, family background, parent's socio-economic status and the perceptions of employment availability. Another reason for choosing this biology stream could be due to the limited choices available. After secondary level, the students are left with the choice of choosing biology stream, technical stream or commerce stream with few vocational subjects. However the new education policy has proposed to offer a wide choice of subjects to choose, which could influence this outcome in the future.

In the present study it was observed that 64% (urban) and 73% (suburban) of participant groups respectively are aware of dentistry as a branch in health sciences which deals with oral health. But the student's motivations for choosing dentistry as a career may differ amongst students. It also appears that though the students are conscious that dentistry is a health science course like medicine, they lack the awareness that common entrance exam is necessary to secure an admission to dentistry courses (51% urban, 53% suburban). In a study performed by Hawley et al [14], Aggarwal et al [12], in India, it appears that the increase of dentists per population in urban areas and the expected decrease in financial gains from working in rural areas may discourage students from choosing dentistry as a career.

There was a mixed response when asked about the duration of courses. Most of the students (64%) lack the knowledge about various courses offered in dentistry. However 30% (40% urban, 20% suburban) of the participants obtained information about dentistry through social media, thus signifying the importance of various social media platform in creating awareness about the course. This is contrast to the studies conducted by Karibe et al [15], Marino et al [16], Jover et al [17], and Tangade et al [18] who reported that parents were very influential among Asian students.45% of the suburban students were unaware that dentistry is the highest paid profession in western countries in contrast to the urban students (54%) whereas most of the study participants in both urban and suburban group were unaware of several other dental diploma courses and are not sure about the role of dental nurse.In the study by Karibe et al [15] 35.5% of dental students in Canada and 44.2% in Thailand preferred working abroad; reasons obtained were participation in a specific postgraduate program at an overseas university or hospital, working as a researcher in an overseas laboratory, working at a clinic or managing their own clinic after obtaining an overseas dental license, and international volunteer activity.

Owing to the current pandemic, there is a shift in the student's preference to continue their higher studies. There appears to be a slight increase in student's choice for traditional medicine courses over allopathic medicine (40% urban, 48% suburban). When asked about the choice of choosing dentistry over medicine, most of them were unsure about the choice of the course (54%). As behavioral change is a difficult area to measure in non-practicing medical students; very few studies were deemed to adequately assess behavioral change. It was also observed that 83% of urban and 99% of suburban population were not physically attending any entrance

exam coaching classes due to the current pandemic. However the global trend of online learning has reduced this challenge of non-accessibility to these training programs [19, 20]. The survey did not analyze the student's access to online coaching classes that were offered during this period. It was noted that communication challenges was predominantly faced by both the study groups rather than financial or other issues. Efforts to improve the awareness and accessibility of entrance examination coaching programs in suburban region could improve the attitude towards the importance of health care services in suburban areas [21]. This could influence the number of students seeking admissions to medical professional courses from suburban areas and indirectly positively influence the overall health care infrastructure in those areas.

Conclusion

Based on the results of this pilot study, it appears that more awareness about the various courses in dentistry is required to motivate students to choose dentistry as their career. More access and training to perform in the admission exams could help in increasing the number of students from suburban population to choose dental courses and the emergence of online learning trend is set to change this scenario.

Conflict of Interest: Nil

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Ethical Clearance: Not Applicable

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Study of the Motives of Hanging Cases Brought for Medico Legal Autopsy in a Tertiary Care Medical College & Hospital of Assam

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Abstract

Death is inevitable for every living being however sudden demise of a human being which is unexpected due to unnatural causes is an immense loss of valuable human resources causing irreparable loss for the family and society at large. There are various modes of committing suicide but death by hanging is quite common among the masses. The present study was conducted at Tezpur Medical College & Hospital, Tezpur, Assam to find out the various motives of hanging cases in medico legal autopsies during the period 1st January 2019 to 31st December 2019.

Key Words: *hanging, socio demographic, autopsy, motive*

Introduction

One, who succeeds in completing the act of suicide, has nothing to bother about and it is only the survivors who are the bear the consequence. Society must identify the potential cases of suicide prone and help and treat them in time irrespective of the method they apply to commit it. Analysis of changing social order is relevant to the understanding of his rising phenomenon of alienation. The joint family being a large group has multiplicity to relationship within a group and individual in times of stress, emotional or socio-economic, could rely upon support from any of the members of such a family.[7] The change over from joint to single and then to nuclear family system has increased the emotional dependence of spouses on each other and therefore a sense of mutual partnership understanding and co-operation has become the sign-quo-non of a happy and adjusted family life. Any misunderstanding between the spouses, or a slight disregard of one by the other or a little deviation from the norms of family life, creates a sense of isolation and self-estrangement.[6] The changing values of social norms, including the sex behavior also add to this problem.

Emile Durkheim[3] 1912 had revolutionized the society's outlook on the problem of suicide. In his classic work "Le Suicide" Durkheim[3] had emphasized the social aspects of this highly individualized phenomenon. He said that "suicide rate varies inversely with degree of integration within social group of which the individual forms a part."

Durkheim [3] attempted to analyze the social forces that had their impact on the suicidal individuals. The emphasis was on the point that, the determining factor in the case of suicide was social and not psychological.

He classified the suicide into three categories – (A) Egoistic suicide – resulting from lack of integration and involvement of the individual with the society. (B) Altruistic suicide – due to over integration of the individual with society. The customs the individual's life is being rigidly controlled by the regulations and the customs of the society; he belongs and therefore has too little choice or will on his own. (C) Anomic suicide

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– here individual's adjustment or support to society is suddenly disrupted as during moorings into the economic depressions.

For Durkheim [3] suicide was entirely a social problem. He has proved in his work that wherever social integration was more powerful and abiding, suicide was less than in societies where social disintegration was pronounced.

Cavan [2] 1928 in 'Suicide' accepted that social disorganization must be of critical significance in the etiology of suicide in western society. Cavan[2] found three major factors leading to suicide (I) fixity of idea (II) lack of objectivity (III) aggressive (resentment, anger, hate) /depressive emotions.

Meninger [5] in his book "Man against himself" 1938 has written that a self destroyer suffers from (I) the wish to die (II) the desire to kill (III) the desire to be killed (IV) unidentified wish.

Stengel E [9] 1963 in his classical work "Suicide and social Isolation" told that social isolation seems to be one known common denominator running through all the factors which contribute to suicidal acts, but this does not mean that it variably leads to such act. They happen as the result of a constellation of conditions, one of which is crisis in the individual's life situation. It may be brought about by disease, social i.e. they are more liable than others to react with suicidal acts under conditions of stress. Stengel [9] also stressed that suicide proneness could be based on certain personality factors that are part of individual's innate endowment.

Material and Methods

The study has been carried out in the Department of Forensic Medicine, TMCH, Tezpur from 1st January 2019 to 31st December 2019 by examination of the inquest report and dead body challan, personal interview of accompanying police, attendants of deceased, in case of hospitalized patients from hospital records and findings recorded in autopsy report of the various medico legal autopsies during that period. A total of 278 autopsies were carried out during 1st January 2019 to 31st December 2019 out of which 45 cases were death due to hanging.

Observation and Results

A total of 278 medico legal autopsies were performed in the Department of Forensic Medicine, Tezpur Medical College & Hospital, Tezpur during the period from 1st January 2019 to 31st December 2019. Out of these, 45 cases were death due to hanging, constituting16.18 % of the total autopsies performed.

Deaths due to hanging (Table – 1):

Total Number of autopsies	Number of deaths due to hanging	Percentage%
278	45	16.18

From Table 1 it is seen that out of the 278 autopsies conducted during the year 2019, 45 cases were deaths due to hanging (16.18%).

Sex distribution of death due to hanging during this period is as shown below (Table –2):

Sex of the victim	Number	Percentage %
Male	29	64.44
Female	16	35.56
Total	45	100

From Table -2 it is evident that male victims outnumbered the female victims.

Age and sex-wise distribution of deaths (Table -3):

Age group in years	Male		Female		Total	
	No.	%	No.	%	No.	%
0-9	0	0	1	2.22	1	2.22
10-19	4	8.89	4	8.89	8	17.78
20-29	7	15.56	5	11.11	13	26.67
30-39	6	13.33	2	4.44	8	17.77
40-49	7	15.56	2	4.44	9	20
50-59	3	6.66	1	2.22	4	8.88
60 & above	2	4.44	1	2.22	3	6.66
Total	29	64.44	16	35.56	45	100

From Table -3 it is evident that in the age group of 20 to 29 years deaths due to suicidal hanging were most common.

Table-4: Mental status of the victims

Mental status	Male		Female		Total	
	No.	%	No.	%	No.	%
Normal	14	31.12	10	22.22	24	53.34
Unstable	3	6.67	2	4.44	5	11.11
Depressed	10	22.22	3	6.67	13	28.89
Unknown	2	4.44	1	2.22	3	6.66
Total	29	64.44	16	35.56	45	100

From Table -4 it is found that most of the victims (53.34%) had normal mental status.

Table-5: Seasonal variation of the cases

Month	No. of cases	Percentage %
Summer (May, Jun., July, Aug.)	20	44.45
Spring (March, April.)	5	11.11
Winter (Nov., Dec., Jan., Feb.)	15	33.33
Autumn (Sept., Oct.)	5	11.11
Total	45	100

From Table -5 it is seen that most of the victims (44.45%) committed suicide by hanging during the summer months.

Table-6: Motive behind suicides

Motive behind the suicides	Male		Female		Total	
	No.	%	No.	%	No.	%
Poverty	5	11.11	2	4.44	7	15.55
Unemployment	3	6.66	0	0	3	6.66
Failure in love	2	4.44	3	6.66	5	11.10
Failure in examination	4	8.89	2	4.44	6	13.33
Family quarrel & unhappiness	7	15.56	5	11.11	12	26.67
Chronic disease	1	2.22	1	2.22	2	4.44
Illegitimate pregnancy	0	0	1	2.22	1	2.22
Failure in business	1	2.22	0	0	1	2.22
Loss of service	3	6.66	1	2.22	4	8.88
Not known	3	6.66	1	2.22	4	8.88
Total	29	64.44	16	35.56	45	100

From Table -6 it is depicted that majority of the victims committed suicide by hanging due family quarrels and unhappiness (26.67%) followed by poverty (15.55%).

Discussion

I. The present study shows that out of the 278 number of autopsies conducted at TMCH, Tezpur during the study period 45 cases were deaths due suicidal hanging which comprises 16.86% out of the total autopsies which varies with Singh Amandeep [10] who found a low incidence rate of 1.26% and AP Patel et al [8] who found an incidence rate of 4.65%.

II. This study shows male preponderance, 29 male cases followed by 16 female cases which is similar with Singh Amandeep et al [10] and AP Patel et al[8].

III. In the age group of 20 to 29 years deaths due to suicidal hanging were most common is similar with study by Patel AP et al [8] and variance with Azmak D etal [1] who describes most of the victims were in the 30 to 39 years age group.

IV. Most of the victims (53.34%) had normal mental status followed by persons with depression (28.89%)

V. Most of the victims (44.45%) committed suicide by hanging during the summer months.

VI. Majority of the victims committed suicide by hanging due family quarrels and unhappiness (26.67%) followed by poverty (15.55%).

Conclusion

Douglas [4] in his "Social meaning of Suicide" 1967 defined suicide as self-murder of self-slaughter and treats suicide as an important clinical and sociological problem. He described that "Suicide is caused by suicidogenic currents which are in turn caused by lack (or decrease) in equilibrium (or integration) between two sets of opposing forces or by one of the sets changing its strength." He also states that "in a given state of society a certain number of persons must put an end to their own life. This is the general question as to who shall commit the crime depends, of course, upon special laws, which, however, in their total action must obey the large social law to which they are all subordinate. It is a belief that suicide has been rising steadily because of the moral control or repression by society of the deviant tendencies of individuals is decreasing.

Ethical Clearance: Taken

Conflict of Interest: None declared

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Perception about Dental Myths among General Public

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Abstract

Introduction: Myths are part of everyone's life. Considering the outcomes of beliefs, one should be aware about the myths about health care as it may result in outrageous complication. Certain culture and cultural beliefs have a greater impact in health and sickness which represents different ideas shared by different people in India.

Aim: This study was aimed to assess the knowledge about dental myths among general public, in Tamil Nadu.

Methodology: A cross sectional study was conducted using self-administered questionnaire, through Google forms, among 200 people from the age group of 17-54 years in Tamil Nadu, India. Statistics was done and tabulated.

Results: Most of the participants were not having adequate awareness and knowledge about dental myths.

Conclusion: Thus, awareness and knowledge about dental myths among general public has to be improved by community oral health programs.

Keywords: *Dental myth, Awareness, Brushing, Oral health programs.*

Introduction

Oral health and hygiene are an important component for maintaining healthy life style among people of all age groups. Recent reports have shown oral diseases have been increasing in greater manner especially in well-developed countries[1]. Due to the limited resources and poor health infrastructure available, many countries fail to cope up with the demand for oral care[2].

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India being one of the largest countries with huge population consists of different kind of people from different cultures, ethnic, geographical and religious diversity which had strong impact on myths about health[3].

The word myth was derived from, Greek word "Mythos" meaning the stories evolved from group of inhabitants that creates impact on obtaining general and dental treatment even during sickness[4].

Myths are part of everyone's life. Considering the outcomes of beliefs, one should be aware of the myths about health care as it may result in outrageous complication[3]. Certain culture and cultural beliefs which represents different ideas shared by different

people always has a greater effect on their overall health including oral health and hygiene[2].

Usually a dental myth arises from traditional belief or from untrained or unqualified dental professionals called quacks. Over a period of time these ideas have created a strong impact on people's mind which had further led to a threat for them in accepting modern treatment that are available in these recent days[5].

Even in modern dentistry, some people still believe in false dental notations. These dental notations may end in severe disability. Hence it is necessary to break all those dental myths at early stage[6].

This study is designed to appraise the perception of dental myths among general public and its impact on public attitude towards the modern treatments available.

Methodolgy

This study was designed to measure the knowledge about Dental myth among general public. A cross sectional study was conducted among randomly selected people from age group of 17-54 years in Tamil Nadu, India. A total of 200 individual participated, in this study. The participants were informed about the study and assured that their participation was purely voluntary. It was conducted in Tamil Nadu, India in October 2020 during COVID 19 pandemic lockdown period. The self-administered structured questionnaire consisting of 15 questions in English, which included demographic details, socio economic status and substantive questions exploring the respondents about dental myths among general public was shared with participants. The questionnaire had a combination of selected response to the question and close ended questions (Yes/No). It took about 5-10 minutes to complete the questionnaires for the participants. The questions were converted into Google forms and shared to the participants via social network. The purpose and aim of the study were explained with requesting for participation, to the participants. After collecting responses, the data was analyzed by subjecting it to statistical analysis.

Results

A total of 200 participants, from age group of 17 years to 54 years participated in this study. The mean age was 29.41 $\pm$ 8.60. Among them, 59.5% were females and 40.5% were males. Majority of the participants were recorded from Chennai, in Tamil Nadu. With regard to socio-economic status, the majority of the participants were recorded in the lower middle-class category, of yearly income less than Rs.75,000.

In this present study, about 58.5% of populations believe that all dental treatments are painful (Fig-1). Around 56.5% of participants don't believe in a myth, that there is no need to visit a dentist unless they develop a dental pain exists (Fig -2). 51.5% of the participants of the current study believed that a tooth once treated doesn't require any other further treatment (Fig -3). Around 59.5% of population believed that brushing leads to shaking of the teeth (Fig -4). 57.5% participants consider that home remedies are far better when compared to various modern dental treatments (Fig-5).

In regarding replacement of the natural teeth, around 63.5%, of the participants stated that replacing missing teeth with artificial teeth may cause problems to adjacent natural teeth. About 62.5% participants think that gingival bleeding is a serious disease. When questioned on effects of flossing on gums around 72.5% of participants considered that flossing will harm the gums (Fig-6). Majority of the participants uses toothpaste for brushing their teeth. 40% of the people use neem stick, tobacco, charcoal etc. to clean their teeth.

Table 1: Distribution of study participants based on knowledge of Dental myth.

S.No	QUESTION	Option	Frequency	Percent
1	Do you think all dental treatments are painful?	Yes	103	51.5
		No	83	41.5
		May be	14	7
2	Do you think that, Extraction of teeth in upper jaw can cause vision loss?	Yes	42	21
		No	99	49.5
		May be	59	29.5
3	Do you feel cleaning of teeth cause shaking of teeth?	Yes	77	38.5
		No	81	40.5
		May be	42	21
4	Do you think that home remedies are better for dental problems?	Yes	64	32
		No	85	42.5
		May be	51	25.5
5	Do you think systemic disease has major impact on oral health?	Yes	79	39.5
		No	70	35
		May be	51	25.5
6	Have you ever thought that gingival bleeding is a serious disease?	Yes	69	34.5
		No	75	37.5
		May be	56	28
7	According to you, which among the following is best used for cleaning the teeth?	Tooth paste	120	60
		Charcoal	37	18.5
		Neem stick	37	18.5
		Tobacco	6	3

Figure -1 Distribution on belief of "All dental treatments are painful"

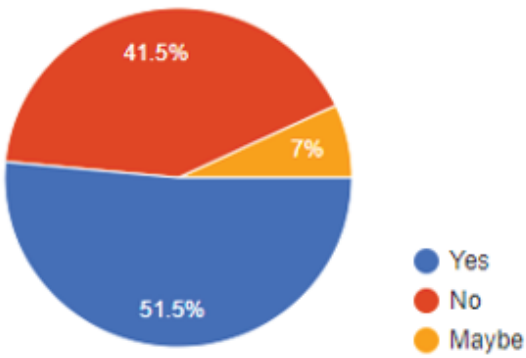


Figure -2 Distribution of visiting a dentist without pain.

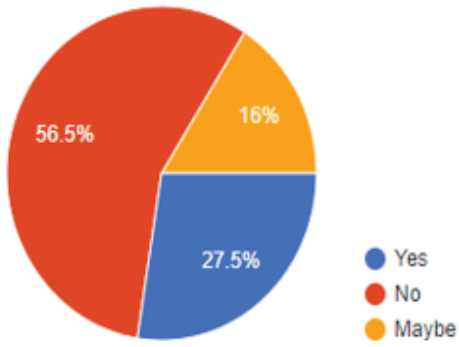


Figure -3 Distribution on belief that treated teeth doesn't require further treatment.

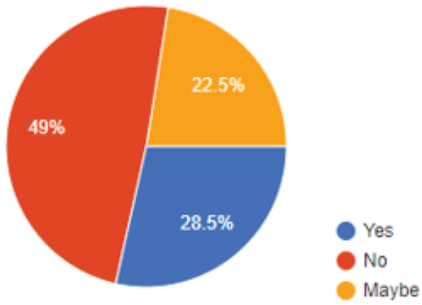


Figure -4 Distribution on belief that Scaling causes mobility of teeth.

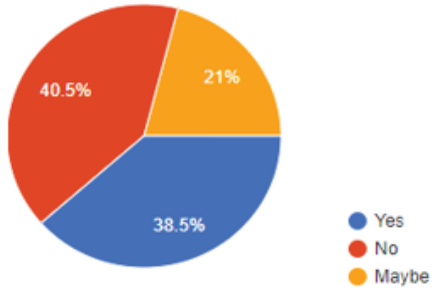


Figure -5 Home remedies are better than dental treatments

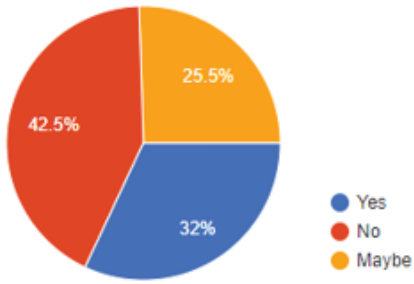
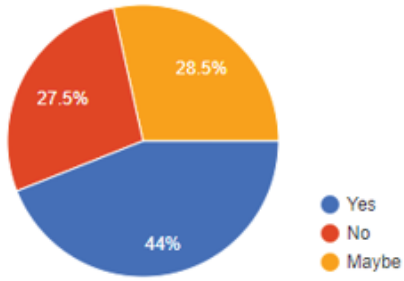


Figure – 6 Distribution on belief that flossing harms gums.



Discussion

Majority of the participants think that, there is no need to care for milk tooth in children. This result is in accordance with the study done by Sabrina et al (2018), which shows that the myth about the primary tooth is still prevailing among people, which should be eradicated[7].

Most of the participants responded that all the dental treatment to be painful, which is in negative correlation with the study done by khan et al (2012)[8].

Most of the participants responded that the extraction of upper teeth does not cause vision loss, which is positively correlating with the study done by Harshada et al (2020). Knowledge in this study about the extraction of teeth was higher, but still it has to be improved[9].

Majority of the participants think that they don't need to visit a dentist unless they develop a pain, which is in accordance with the study done by Sharma et al (2015)[10].

Most of the participants think that removal of wisdom teeth, can affect the wisdom. The results are similar, with the study done by Yadhavi et al (2015), which shows that people still believe in myth about removal of wisdom teeth[11].

The greater number of the participants believes that once teeth treated does not require any further more treatments, which had a positive correlation with the study done by Vignesh et al (2012). This shows that people still believe in myths about frequency of dental treatments[12].

When questioned about whether scaling causes mobility of the teeth, majority of the population in this study believed this myth; this is positively correlating with the study done by Gambhir RS et al (2015), which thereby infers us to provide more awareness and education to population for better oral health and

hygiene of an individual[13].

Most of the participants believe that home remedies are much better than dental treatments available in these modern days which is similar to the study done by Sakshi Joshi et al (2020)[14].

Regarding the fact that systemic disease has major impact on oral health majority of the population had good knowledge in this aspect. Urban population seems to have better knowledge on relation between systemic disease and oral health when compared to rural population who require more educational programs to bring awareness among them.

Most of the participants think that only ageing leads to mobility of the tooth and they are not aware of the other factors that causes mobility which is in negative correlation with the study done by Teweri et al (2014)[1].

The greater part of the participants use tooth paste for cleaning, whereas still some people use charcoal, neem stick to clean their teeth in this study conducted in Tamil Nadu which has negative correlation in accordance with the study done by Sumit et al (2014) in Sangrur district of Punjab[15].

Therefore, Myths are deep rooted among peoples, so it is essential to make availability of good education to them by community oral health programs, campaign etc., to get rid of these myths and misconceptions prevailing among general public.

Conclusion

Though many recent advances are developing in treatment modalities in various fields of dentistry, there is a huge difference prevailing among general public regarding the dental myths and false beliefs in dental treatments. Hence this should be eradicated by conducting more oral health awareness programs, campaigns and researches to improve the awareness among the people for their better oral hygiene.

Ethical Clearance: Institutional ethical clearance obtained

Source of Funding: Self

Conflicts of Intrest: Nil

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Awareness and Attitude about Social, Ethical and Legal issues Related to Assisted Reproductive Techniques (A.R.T) amongst Medical Students and Interns in a Tertiary Care Hospital at Chennai

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Abstract

Infertility is an issue on the rise worldwide. Infertility treatment globally has undergone a gradual revolution from the traditional treatment to the use of assisted reproductive technology (ART). ART is presently the most sought after technology that successfully treats millions of infertile couples over the world. However, the utilization of ART still differs between various nations owing to varying socioeconomic, cultural and religious reasons. The sudden increase of this technology has introduced numerous social, ethical, and legal challenges. This paper assesses the awareness level and attitude amongst medical students and interns in Chennai regarding some of the most vital and tricky topics in the field of ART and outlines the ethical, legal and social challenges they bring in. This questionnaire-based study involved 427 participants who encompassed medical students and interns from tertiary care hospital at Chennai. The responses were collected, tabulated and statistically analysed. About 2/3rd of the participants were females. 94% of the respondents declared that they are aware of ART (401), 63% answered that all the information was obtained from the medical college. Over 2/3rd of the study population was aware of one of the ART methods. 70.8% (302) amongst the participants felt that ART solved all problems of infertility.86.7% of the respondents (370) knew that Spousal agreement to opt for A.R.T is a must. 90.6% (387) of the study population were of the opinion that revealing the identity of the sperm donor would cause psychological, social and attitudinal issues among the recipients & the spouse. 29.6% of the participants felt that donation of the surplus embryos for research purposes was the best option. The gaps in knowledge about newer methods and procedures regarding ART were identified. Medical students must be agents of socio-medical information and this makes them good ambassadors for healthy living and fertility awareness. The introduction of government run ART centres and reduction in the cost of the services will go a long way in encouraging more persons to use the services.

Keywords: *Assisted reproductive technique (ART), fertility, surrogacy, awareness, ovarian reserve.*

Introduction

Infertility is a common problem affecting one in six couples. The World Health Organisation (WHO) defines infertility as a disease of the reproductive system characterized by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse**.** [1,2] There are two types of infertility namely primary and secondary. The male infertility is accountable for 20–30% of infertility cases, while 20–35% is due to female infertility and the rest is due to combined problems. Increase in cases of infertility could be because of a few factors such as delayed childbearing, alterations in semen quality due to habits such as tobacco and alcohol, obesity and

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changes in sexual behaviour. In recent times there has been an increase in malignant diseases which require high-dose chemotherapy or radiotherapy as treatment options. Many young patients receiving these treatments are at risk of developing reproductive failure, a number of fertility preservation options ranging from embryo cryopreservation to ovarian tissue cryopreservation are now available which can be done prior to chemotherapy or radiotherapy. [3,4,5]

Assisted reproductive technology (ART) is used to treat infertility. It includes fertility treatments that handle both a woman's egg and a man's sperm. It works by removing eggs from a woman's body. The eggs are then mixed with sperm to make embryos. The embryos are then put back in the woman's body. In vitro fertilization (IVF) is the most common and effective type of ART. ART has successfully treated millions of infertile couples the world over. However, the explosion of this technology has introduced a myriad of new social, ethical, and legal challenges. [6,7]

This study was undertaken to assess the awareness level and attitude amongst medical students and interns in a tertiary hospital at Chennai towards such impending issues related to Assisted reproductive technology (ART).

Methodology

A cross sectional questionnaire based study was undertaken after obtaining Institutional Ethical Clearance at Saveetha Medical College and Hospitals, Chennai. Sample of 427 volunteers (consent obtained) inclusive of medical students and interns were chosen randomly. Confidentiality regarding the participant response for the questions was ensured and participation was voluntary. A pretested, semi-structured questionnaire was administered. The questionnaire had details about demographic data like age, gender and religion, followed by few questions to assess knowledge and attitude of ethical , social and legal aspects of ART. Score was allotted to each question based on the response. This study was carried out for 6 months from April 2020 to September 2020. Statistical analysis was done using Microsoft excel and SPSS statistical package version 24.

Observation and Results

The study comprised of 427 participants. The participants from 1st year to internship were 56, 102, 85, 94 and 90 respectively. [Fig 1] This study had a female preponderance with 65% participation (278 females) with F: M ratio being 1.87:1. The girls reported higher mean scores in knowledge (7.37±1.01). 85% of the study population were Hindus (363), 10% were Muslims (43) and the rest were Christians(21). Ninety four percentage of the respondents declared that they are aware of ART (401), although 13% (55) personally knew infertile people that have used or are using ART. Amongst the population that was aware (n=401), when asked about the information source regarding ART, 63% answered that all the information was obtained from the medical college, 30% from the social media,books, journals and TV, 4 % from family members, 3 % from scientific meetings. [Fig 2]

Based on the questionnaire, the following observations were made.

Around 90% of the total students were aware of surrogacy, over 3/4th of them were familiar with donor egg procedures, IVF, and intra-uterine insemination (IUI). More than 2/3rd of the study population knew about intracytoplasmic sperm injection (ICSI) and more than half of them were aware of cryopreservation of oocytes, embryos and blastocysts. while a significant lack of knowledge was observed regarding procedures like ovarian tissue cryopreservation and vitrification. Over two third of the respondents (69.78 %)were comfortable to use one of the techniques in future if required. [Table 1]

93.4% (399) of the study population of this study agreed that Hippocratic oath formulated the moral ground of clinical practice. 34.9% of the participants felt that it was unethical for male doctors to refuse a patient in the absence of female attendant. 70.8% (302)amongst the participants felt that ART solved all problems of infertility.90.6% (387) of the population in this study believed that cost of A.R.T does not encourage its use in certain economic classes and 80.2% of this study population felt that infertile couples of lower socio economic background preferred alternative treatment like herbs (Siddha/Ayurveda) to modern/scientific A.R.T. Approximately half of the participants (52.8%) agreed that religious beliefs did not go against A.R.T. Almost 1/3rd of participants belonging to the study

(30.4%) felt that babies born out of ART are always physically or mentally deformed/challenged.

75.1% (321) of the population of this study knew that artificial insemination could not be used for gender selection.More than 3/4th of the study participants (86.7% , 370) knew that Spousal agreement to opt for A.R.T is a must. 73.5% (314) of this study population were aware that donation of gametes (sperm or oocyte) without knowledge of spouse to another infertile couple is illegal. 90.6% (387) of the study population were of the opinion that revealing the identity of the sperm donor would cause psychological, social and attitudinal issues among the recipients & the spouse. Hence 82.9% (354) participants mentioned the need for the couple to undergo psychological screening or counselling prior to gamete donation. However, 63.2% (270) of the participants were of the opinion that socio-psychological issues in A.R.T could lead to an increased misunderstanding, leading to divorce among the couples who have received it. 57.5% (245) participants thought that it was good to disclose the identity of biological parents to the child at an appropriate age.

36.8% participants made an observation that unmarried or widowed women could not avail artificial insemination and 20.8% of the population of this study were aware that it was not legal for a lesbian couple to opt for ART.

64.6% (276) of the population of this study stated that artificial insemination does not amount to consummation of marriage and 33.72% (144) of them were aware that donor could not be held guilty of adultery in India.

On the questions related to surrogacy , 85.3% (364) of the study population knew that both husband and wife were required to consent for surrogacy and also agreed that it was an ethical prerequisite that oocyte donors were to participate voluntarily and without coercion or undue influence .81.1% (346) of the participants agreed that surrogacy was a good option for infertile couples, 3.8% disagree, 15.1% were not aware about it. 69.5% (297) of the population in this study had the knowledge that payments were to be made to surrogate mothers for their services.

While 46.2 % (197) of the respondents were neutral , 38.7 % (165)of them agreed on the prerequisite of racial resemblance between the surrogate mother and rearing mother.

29.6 % of the study population felt that it was better to donate the surplus embryos for research purposes where as 10.8% preferred to discard/ thaw as against 21.8% of them who preferred to store them indefinitely. However 37.8% of them wanted to donate the embryos to other infertile couples.

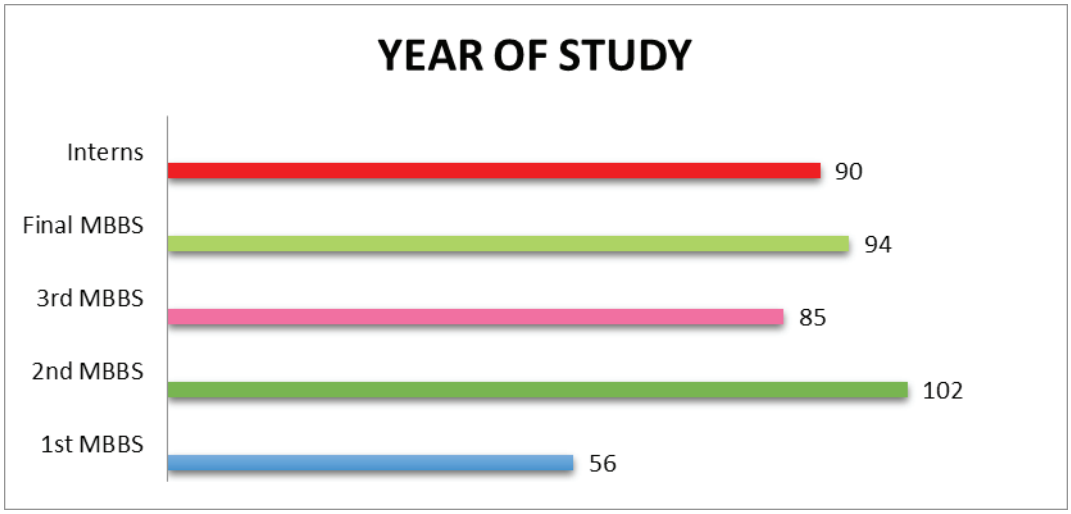

Fig 1: Year of Study of respondents

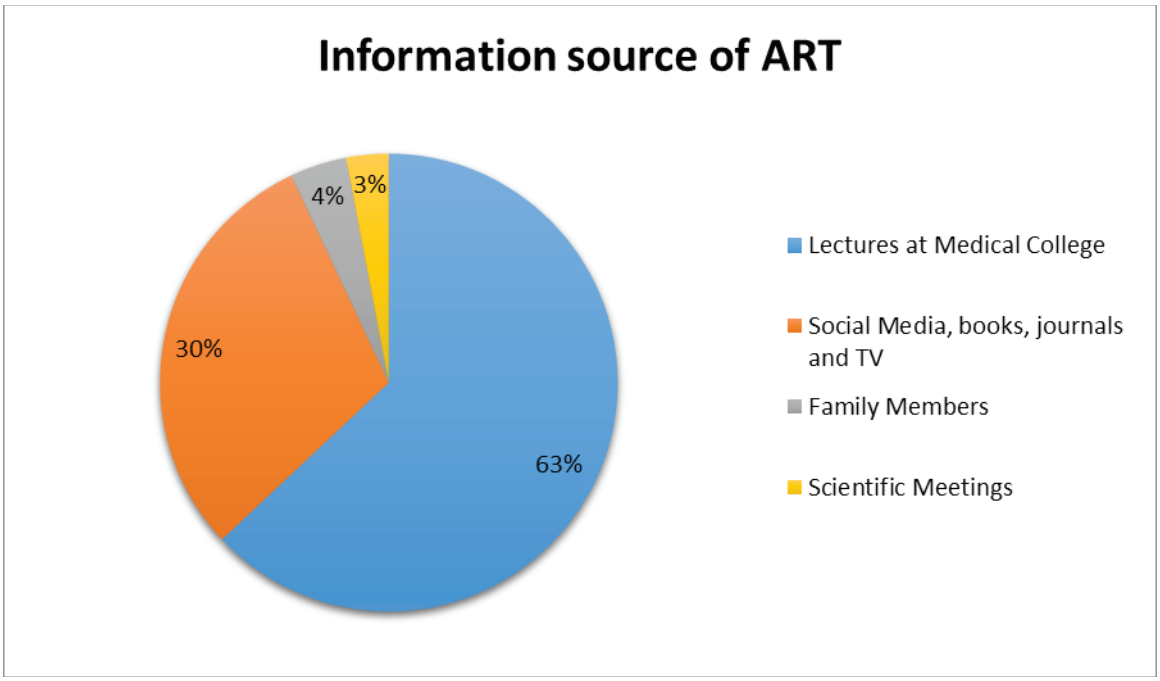

Figure 2: Information Source of ART amongst study population

Table 1 : Awareness regarding methods of Assisted reproduction technologies

Question	Yes	No
Are you familiar with the following methods: (n=427)		
Intra-uterine insemination	329 (77.1)	98 (22.9)
In vitro fertilization	340 (79.6)	87 (20.4)
Intracytoplasmic sperm injection	298 (69.7)	129 (30.3)
Oocyte, embryo, blastocyst cryopreservation	237 (55.5)	190 (44.5)
Ovarian tissue cryopreservation	106 (24.9)	321 (75.1)
Vitrification	36(8.5)	391 (91.5)
Surrogacy	381 (89.23)	46(10.77)
Donor egg	369 (86.4)	58 (12.6)

Discussion

Knowledge plays a major role in decision making in health care management system. Ninety four percentage of the participants declared that they are aware of ART. This is slightly higher than studies done by other authors in African countries. The study showed that awareness of ART was more among female respondents as compared to male respondents. Irrespective of the gender, there was an association between knowledge about infertility and the possibility of using ART. [6,8]

Inequitable access to healthcare is an ongoing debate for over a decade in the developing countries. Equitable access to IVF remains a critical issue in India where only a small fraction of the population have access to higher end health insurance leaving a larger population uncovered towards expensive procedures like IVF. Hence this would lead to lesser utility by the couple belonging to lower socioeconomic groups which resonated with the thoughts of the respondents of this study. According to few similar studies from other nations (Brazil South Africa and Nigeria), ART is predominantly private sector driven, expensive and remains unaffordable for those in need. [9-11] This is not in accordance with the findings from developed nations (European countries) where a specific number of cycles of IVF treatment are reimbursed by their governments. [12]

About the observation by the respondents that babies born out of IVF would be susceptible to birth defects has been documented by many authors. This is due to the transfer of multiple embryos to the uterus to ascertain that at least one or two survive and develop until birth. [11,13]

The present study revealed that the participants had good perception of ART techniques and their related ethical and legal issues. This finding corresponds with the study conducted in a developed country in the year 2012. [14]

Studies from developed countries have shown that the viability of the frozen embryos reduces with longer storage time.[15] Certain religious views against destruction of the embryos also made a larger difference. 37.8% of our study population wanted to donate the embryos to infertile couple which was in accordance with a study done by Bello et al. [16]

Conclusion

Medical students are being taught about the importance, legal, social, ethical dilemmas of ART in their medical curriculum. The availability of internet and other resources have made the information regarding ART accessible at a click away. In this study, we found that the medical students had reasonable understanding of the utility of ART. Being the future health care providers, these findings could have implications for the development and implementation of government run infertility-related programmes in the near future that would help to overcome the inherent challenges undermining the full utilization of ART services in developing nation like India. Tailored public health education program about the availability of such services is necessary.

Conflict of Interest : Nil

Ethical Clearance: Obtained (SMC/IEC/2020/03/285)

Source of Funding: Self

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Awareness about Consumer Protection Act amongst Patients Visiting a Private Medical College and Hospital at Chennai: A Descriptive Study

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Abstract

Objectives: To assess the knowledge regarding Consumer Protection Act amongst patients visiting a tertiary care hospital at Chennai.

Methodology: A cross-sectional descriptive questionnaire based survey was carried out on 550 patients visiting a private medical college and hospital at Chennai and the data was collected. The interview consisting of closed ended questions on CPA. Statistical analysis was done using appropriate statistical tools in SPSS software.

Observation and Results: Young male respondents were found to have more awareness in comparison with females. Education and monthly income were found to have positive association with the level of awareness.62% of the study participants have heard about CPA. In cases of medical negligence, 78% participants would visit police station rather than a consumer redressal forum.80% of the participants were unaware of the location of consumer court. Approximately half of the respondents (45%) felt that medical services by doctors were liable for punishment under CPA in case of negligence.

Conclusion: Knowledge of what may influence patients is of utmost importance in cases of meeting their expectations and demands. Although the Consumer protection Act empowers patients to demand quality care, still complaints against medical professionals for errors, unfair access and poor satisfaction in service rendered remains. Hence awareness of patients rights and legislations would pave a path towards improvement in the quality of medical service rendered , thereby providing satisfaction to both patients and health care provider.

Keywords: *Consumer protection act, Patient, Knowledge, Awareness, Medical, Service.*

Introduction

India has a mixed medical practice with the dominant private medical practice alongside a tiered public health system. Studies on patients' rights in India give a limited picture of the situation. Many studies in the past on patient-physician communication have identified gaps between practice and guidelines, and this trait is attributed to the existing social and legal contexts of the physician-patient interaction in India. Physicians in India have always held disproportionate power over their patients, and classical paternalism in physicians' behavior is the rule rather than an exception. Gender has been defined as a decisive factor in physicians' dominance in their relations and communication with patients. Certain times there are a deep mutual distrust between them which hamper standardisation of the quality of care and affects patients' rights as a whole. [1]

More than three decades of working in the Consumer Protection Act, 1986 necessitated a paradigm shift in the post globalization and digitization phase of India. The Consumer Protection Act, 2019, passed and received the presidential the assent on 9 August 2019, repealing the Consumer Protection Act, 1986, to strengthen consumer rights and dispensing consumer justice. It envisages a

robust grievance redressal mechanism in the context of e-commerce and e-governance. The slew of legal measures entails the inclusion of e-commerce, Central Consumer Protection Authority (CCPA), alternative dispute resolution (ADR), suo moto action against unfair trade practices, pecuniary jurisdiction, amplifying of grounds of complaints, penalties to deceptive advertisements and product safety and liability.[2]

This study was undertaken to assess the awareness about Consumer Protection Act amongst the patients visiting a private medical college and hospital at Chennai. We aimed to analyse the gaps that existed and also to ensure that patients are informed about their rights, thereby ascertaining healthy doctor patient relationship.

Methodology

Questionnaire based study was undertaken in the out/in patient departments of Saveetha Medical College, Chennai from April to September 2020. A total of 550 patients were interviewed with the help of semi structured and pre validated questionnaire. Confidentiality was maintained and also participation was made voluntary. Voluntary subjects who are above 18 years of age were included .People who could not comprehend the questions in spite of assistance were excluded from the study. A Part of the questionnaire involved the sociodemographic details and other part involved the questions related to consumer protection act 2019. After collection of data, it was entered in Microsoft excel 2010 and analyzed using SPSS software version 24. Descriptive statistics were obtained and percentage distribution of responses to questions were calculated. Each positive response was scored as 1 and negative as 0. The individual scores were summed up to yield a total score. The participant's total score was a simple sum of responses. Categorization of scores was done at three levels—low (0–5), medium (6-10) and high (11–15).

Observations and Results

Males were predominant in this study with the Male to Female ratio being 1.35:1. **[Figure 1]** The knowledge of the males was found to be higher than females in this study. Among the study participants, 61% had low knowledge scores, 26% had a medium score and 13% had a high score.Among 550 subjects, 64.19 % were belonging to age group of 18 to 29 years. Followed by 17.81% between 30 to 39 years, 9.28% from 40 to 49 years, 6.90 % from 50 to 59 years whereas least number of subjects 1.82% from age group of 60 and above**. [Table 1]**

According to modified Kuppuswamy classification scale 2020, most of the participants belonged to upper lower class (46%) , followed by lower middle class (22%) with education qualification of 10^{th} standard (43%) and graduates comprised of 25 % of the study population.

62% of the respondents had heard about the Consumer Protection Act (CPA). The major source of awareness of CPA was media contributing a majority of 164, which is 48 % and the remaining acquired knowledge through friends or peer (22%), professional meetings (19%) and internet (11%) **[Figure 2]** 38% of the study population stated that CPA was applicable to medical services, while 54% of them were not aware. Approximately half of the respondents (45%) felt that doctors irrespective of providing free treatment or paid services were liable for punishment under CPA. Approximately 4/5th of them were not aware of the location of consumer court. Only 22% of the study population was aware of the procedure to file a complaint in the consumer court and the rest would want to approach the police station for the same.8% of them had filed a case and the consumer forum was able to redress to the grievances of only 6% of them. To the other questions on time period to file the complaint, only 13% of them were aware and 26% of them knew about the party that could sue a physician in case of negligence while treating a minor. **[Table 2]**

Figure 1:Gender wise distribution of study subjects

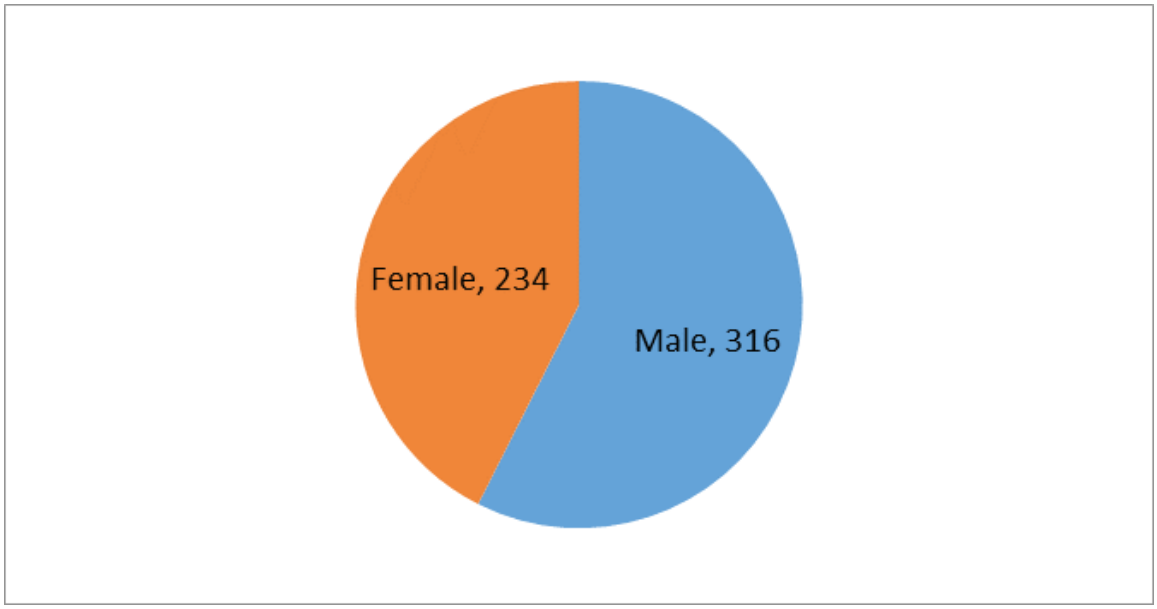

Figure 2: Source of Awareness of CPA amongst study subjects

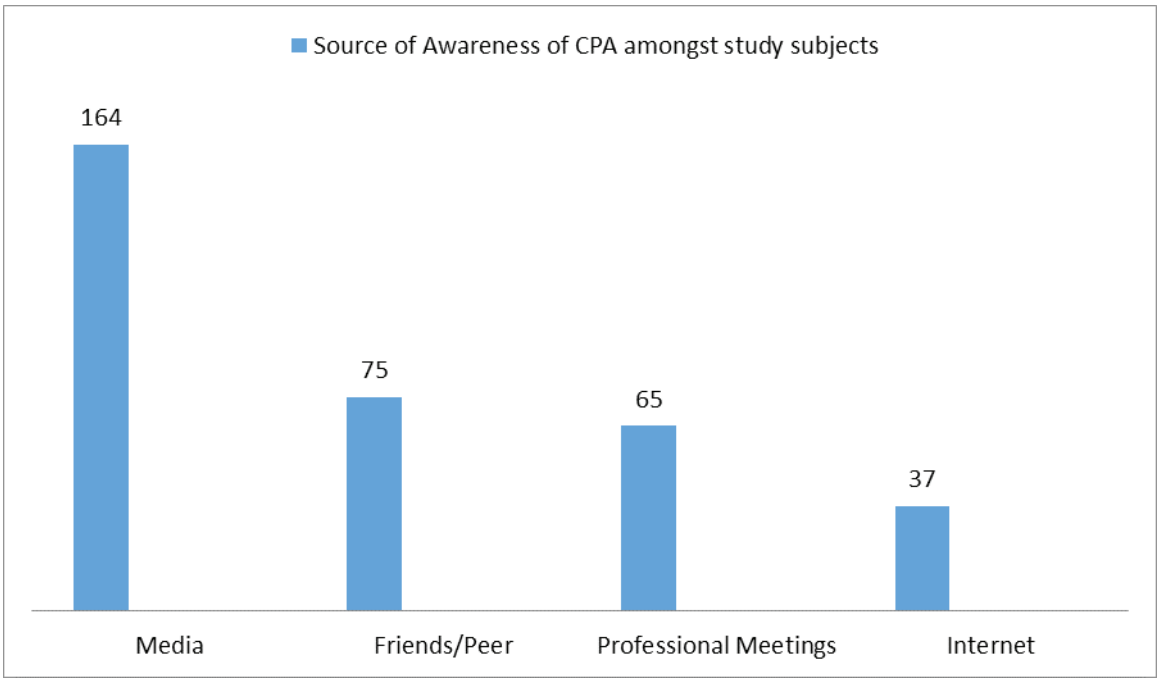

Table 1 : Distribution of study subjects according to age groups

Age groups (yrs)	No. Of subjects	Percentage
18-29	353	64.19
30-39	98	17.81
40-49	51	9.28
50-59	38	6.90
60 and above	10	1.82
Total	550	100

Table 2: Response of subjects to various questions regarding COPRA :

SL no	Question regarding CPA	Yes (%)	No (%)	No answer (%)
1	Heard about Consumer Protection Act (CPA)	62	38	-
2	CPA is only applicable for goods (or commodities) that are purchased	18	32	50
3	CPA is applicable to medical services	38	08	54
4	Healthcare is excluded under CPA	25	48	27
5	Doctors who take fee for the services they provide are liable under CPA	45	29	26
6	Do you have to pay any fees to file complain in consumer court	15	22	63
7	Consent should be regularly taken from patient before procedure	38	48	14
8	Can you file (or pleed) a complaint without the presence of a lawyer	30	18	52
9	Aware of location of Consumer court	20	79	1
10	Consumer court gives speedy redressal than civil court	36	4	60
11	Are you aware of the procedure to lodge a complaint as per CPA	22	78	-
12 (a)	Have you filed a case in the consumer forum	12	78	12
12 (b)	If yes, is the consumer forum able to redress to your grievances	10	2	
13	Are you aware of the monetary compensation under CPA	8	78	14
14	Compliant can be filed in a consumer court where the complainant either resides or works	10	80	10
15	Specific provision of product liability exists as per CPA	9	89	2

Discussion

More than 50% of the study population had low knowledge scores regarding CPA .This might be due to majority belonging to lower socioeconomic strata with educational qualification upto 10th standard. This also attributes to the deficiency in the nation's educational system which lacks information on CPA, both in formal and informal ways. Also with increase in knowledge, awareness levels also increased. Men had higher knowledge in comparison to women in the present study. This could be due to the fact that males leading more social life, comparatively devote more time toward their acquiring knowledge through newspapers and other social media platforms than females. These findings are in accordance to some other study reports.[3,4,5,6] This finding was contradictory with the results of study done at Ghaziabad. [7]

In the present study, less than 10% participants had the knowledge about the maximum Compensation under CPA which can be claimed by the patients.This is in accordance with a few other authors recording similar findings . [7]

CPA has been planned to be customer friendly with no court fee payment allowing the complainants to plead their own case, and the fast track justice with decision taken within 3-6 months. However, Majority of the participants were not aware of these aspects and would report to the police station instead of approaching lawyer or to consumer court. This shows that even after 3 decades of its enactment, CPA has not gained its due importance among the general population. These findings were similar to few other studies. [7,8]

In this study, 38 % of the respondents were aware regarding informed consent for general anaesthesia, surgical operation and major diagnostic procedures. Few participants had appropriate knowledge about the exclusion of healthcare under CPA, non liability of physician giving free treatment and that complaint against the deficiency of such free treatment service cannot sustain generally, time frame to file a case under CPA. Less than half of them were aware that paid medical services to patients is included under the purview of the Consumer Protection Act, 2019.These are in accordance to a few other studies.[9,10]

Conclusion

The present study makes an alarming observation regarding understanding of CPA by the patients. Even though approximately 2/3rd of the respondents had heard about consumer protection act, the knowledge regarding the same was low.

Hence there is a need to raise awareness among public regarding consumer protection act to ensure delivery of quality medical service to them. This would set the tone to curtail unfair medical practices in future.

Conflict of Interest : Nil

Ethical Clearance: Obtained (SMC/IEC/2020/03/292)

Source of Funding: Self

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Knowledge and Attitude Towards Euthanasia and Advance Directives amongst Medical Students in a Private Medical College and Hospital at a Metropolitan City of India

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Abstract

Background - Decision making about 'End of life' is value-laden with respect to the context of culture and ethics. Advanced and improved medical technologies and well qualified health care professionals are always trying to sustain life , sometimes even when there is no hope of recovery .This has led to a need for an end of life decision making legislation more than ever.

Euthanasia (Painless or mercy killing) and advance directives are less popular concepts in Indian context as against the many of the nations worldwide.

Objective- To study the knowledge and attitude of medical students toward euthanasia and advance directives.

Methodology - 400 medical students were chosen randomly and a cross-sectional descriptive questionnaire based study was conducted administering a pre-tested, semi structured questionnaire to assess their knowledge and attitude regarding euthanasia and advance directives in a private medical college and hospital at Chennai. The data collected was statistically analysed using appropriate statistical tools in SPSS software.

Results- Female preponderance, 246 (61.5%) was noted in the study with female to male ratio being 1.6:1. 76% of them had heard about Euthanasia and 29% of them were aware of the concept of advance directives. 61% of them wanted active euthanasia to be legalised. 54% of them believed that this concept may easily be misused and that was the reason against opting for euthanasia. 34% of them were aware that the mental health act (MHCA) , 2017 promoted advance directive in India.Although none of them had helped any patient write an advance directive, 77% of them felt that health care providers should always anticipate discussion about end-of-life issues with the patient.

Conclusion- This study concludes that majority of the medical students in teaching medical colleges and hospital had moderate to high level of knowledge of euthanasia and moderate to poor level of knowledge regarding advance directives.

Keywords: *Euthanasia, Advance directives, Medical students, Awareness, Knowledge, Attitude.*

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Introduction

'Euthanasia' or 'good death' in Greek is painless mercy killing of patient suffering from a incurable / irreversible and painful diseases. Physician assisted killing , by the act of commission is called active euthanasia. According to a study in Netherlands 54% of all the practising physician have performed active

euthanasia. [1] In India, active euthanasia is not legalised, because it is not considered as an noble death. Passive euthanasia is a death of the patient brought by the act of omission.Aruna Shanbaugh case was one of the main reasons in sparking legalisation of passive euthanasia in India under strict guidelines and reviewing of the Article 21 as 'right to die is a component of right to dignity'. Although euthanasia is considered to be practical by many, there are some who prefer the word unethical and against the law of nature. [2,3,4]

Advance Directives is a living will which gives durable power of attorney to a surrogate decision-maker, remaining in effect during the incompetency of the person making it. USA was the first country to introduce advance directives.According to a survey conducted in Filipino patients and their families a favourable attitude towards advance directives has been observed. However by large people still are hesitant across the world about advance directives as it can be easily misused [5.6]

There are surplus research about living healthy lives and successful aging. Nevertheless, there is a paucity of information on end of life (EOL) issues including euthanasia and advance directives amongst the health care providers in many developing countries including India . Hence it necessitates further studies amongst the future forerunners in health care regarding their attitude towards EOL issues.

Methodology

This cross sectional study was undertaken after obtaining Institutional Ethical Clearance at Saveetha Medical College and Hospitals,Chennai. Randomly chosen 400 medical students who volunteered to be a part of this study were included(consent obtained). Anonymity and confidentiality of respondents for the questions was ensured. A pretested, semi-structured questionnaire was administered. The questionnaire had details about demographic data like age, gender and religion, followed by few questions to assess knowledge and attitude. Score was allotted to each question based on the response. Subjects who secured 50% or more were considered as having adequate knowledge, and those with less than 50% were considered as having inadequate knowledge towards EOL questions. This study was carried out for 6 months from April 2020 to September 2020. Statistical analysis was done using Microsoft excel and SPSS statistical package version 24.

Observation and Results

A total of 400 study population constituted in this study with female and male subjects 246 (61.5%) and 154 (38.5%) respectively and a female to male ratio being 1.6:1. The girls reported higher mean scores in knowledge (7.48±1.29) and attitude (8.38±0.92). The study consisted of 98, 112, 99 and 91 students belonging to 1st, 2nd, 3rd and Final year MBBS. **[Figure 1]** Amongst the study population , 76% of them had heard about Euthanasia and 29% of them were aware of the concept of advance directives. 37% of them said that passive euthanasia was legal in India . 72 % of them believed that Euthanasia was ethical ,75% were happy about legalising passive euthanasia by the Indian Government and 61% of them wanted active euthanasia to be legalised. 88% of the respondents were against using euthanasia for covering medical lapse**.[Table 1]** 44% of them felt that the main reason to support euthanasia was due to the right of an individual to die with dignity**.[Figure 2]** However 54% of them believed that this concept may easily be misused and that was the reason against opting for euthanasia.76% of them would prefer euthanasia in a criminal with an incurable painful disease than letting the patient suffer for their guilt.Family members were the preferred ones to take decision regarding Euthanasia according to 63% of them the respondents followed by the treating doctors (18%), Court of law(9%) , Lawyer appointed by patient(6%), Religious or spiritual leaders(4%). 39% amongst them felt Doctors from Surgical specialties would support Euthanasia closely followed by those from medical specialties (34%), Obstetrics and Gynecology (25%) and Pediatrics (2%).

With respect to Advance directives, 24% of the study population felt it to be reasonable to exist, 57% of them felt the surrogate decision makers might take advantage of the power to make money whereas over half of them (52%) also felt that surrogate decision makers (loved ones) in case of euthanasia would make the same decision as that of the patient. 34% of them were aware that the mental health act (MHCA),2017 promoted advance directive in India. Although none of them had helped any patient write an advance directives, 77% of them felt that health care providers should always anticipate

discussion about end-of-life issues with the patient .23 % of them felt Advance directives were very useful. In cases where patients can no longer communicate and have no written ADs, as the doctor in charge 94 % of them were willing to communicate to the close relatives of the patient in order to determine the patient's wishes and about 54% of them would be an active participant in decision making if EOL situation was seen in their own family. **.[Table 2]** According to the study population , the main criterias' to decide on the right moment to discuss end-of-life issues with the patient was mostly during request to do so by the patient (56%), Spiritual availability of the patient (42%) and rarely at the time disposal of the professionals (2%). This was largely governed by the efficiency of health professionals to discuss ADs with good rapport with the patient (60%) , communication skills (36%) ,and self-awareness of the health professional towards it (4%). According to the respondents, in cases where the patient could not communicate anymore , the relative was expected to communicate to the physician the patient's wishes and allow the physician to take the decision independently (28%),take decision jointly (49%), communicate his/her own wish and make the decision jointly with the doctor (18%) , not be involved at all (5%).

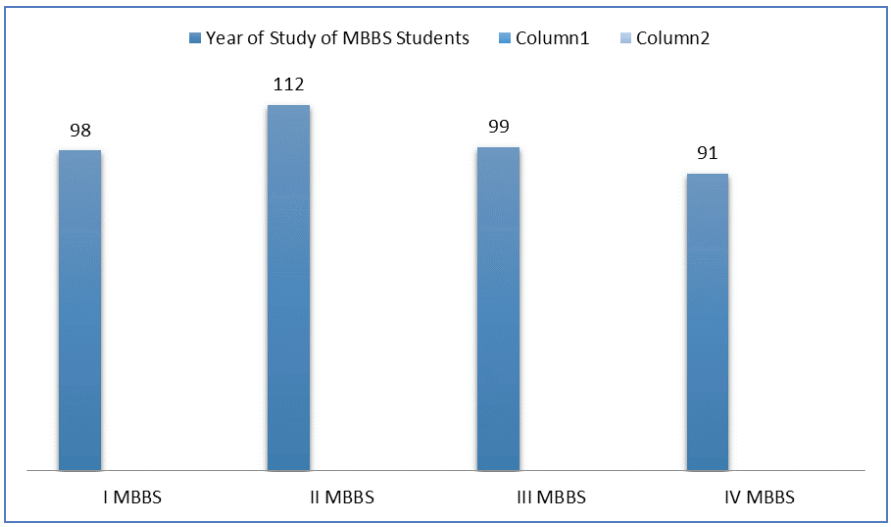

Fig 1 :Year of Study of MBBS Students

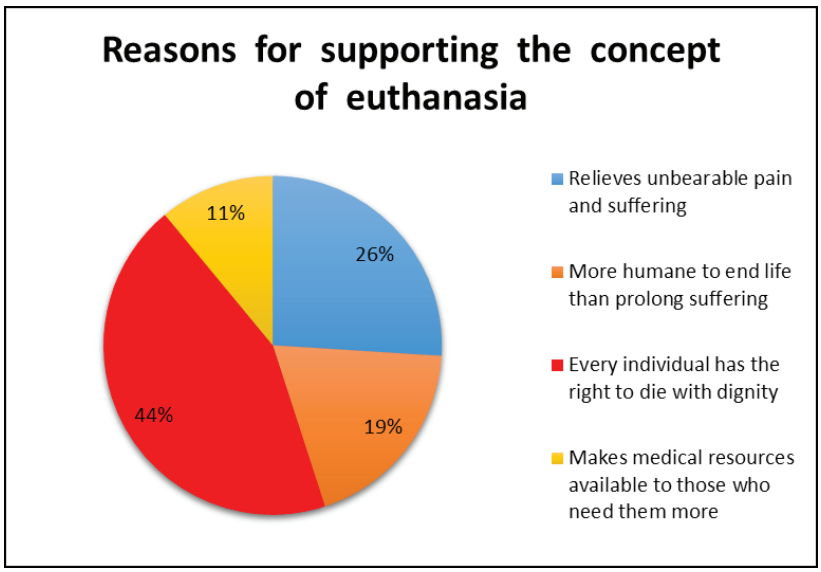

Fig 2 :Reasons in support of Euthanasia

Table 1: Questions related to Euthanasia

Question	Yes	No
1)Euthanasia is the painless killing of a patient who is suffering from an incurable and painful disease. Do you think euthanasia is ethical?	72	28
2) Is euthanasia is legal in India?	37	63
3)Do you think active euthanasia should be legalised in?	61	39
4)Do you think using euthanasia for covering medical lapse is correct?	12	88
5)Aruna Shanbaug case sparked the legalisation of passive euthanasia in India .Do you think the measures taken by Indian government is correct?	75	25
6) When a person has a disease that cannot be cured and is living in severe pain, do you think doctors should be allowed by law to assist the patient to commit suicide if the patient requests it?	38	62

Table 2: Questions related to Advance directives.

Question	Yes	No
1)Do you think advance directive is a living will that gives the surrogate decision maker the durable power to make the decision due to the incompetence of the person to do it?	29	71
2)Do you think concept advance directive is the reasonable thing to exist?	24	76
3)Do you think surrogate decision makers would take advantage of the power (decision making) to make money?	57	48
4)Do you think surrogate decision makers (loved ones) in case of euthanasia will make the same decision as that of the patient?	52	48
5)Does mental health act (MHCA) , 2017 promote advance directive in India ?	34	68
6)Do you think that we should always anticipate with the patient the discussion about end-of-life issues	77	23
7)Did you already help a patient to write his ADs?	0	100
8)Do you think that ADs are useful?	23	77
9)If your relative can no longer communicate and has no written ADs, the doctor has the duty to speak to the close relatives of the patient in order to determine the patient's wishes: a)Are you satisfied with this role? b)Would you take a more active role in the decisions that affect your family? c)Would you prefer not to be involved at all in decisions about your relative?	 94 54 69	 6 46 31

Discussion

The study was conducted on 400 medical student volunteers. Almost three fourth of them were aware about Euthanasia and believed it was ethical as well as being happy about legalising passive euthanasia by the Indian Government. Similar findings were observed in a survey conducted on Swedish medical students [7]. Over one third of the students supported euthanasia mainly because of belief in right to die with dignity just as entitled by Article 21 as discussed in Aruna Shanbaug case [4] .Over half of the students (54%) were hesitant about the concept of legalising euthanasia as they believed it could be easily misused for personal benefits similar to those performed during Nazi regime. [8]

With respect to advance directives, only 29% of students had basic idea about advance directives . Although 52% of students supported the advance directives and thought that loved one will make exactly same decision as that of the patient , almost equal number of students (57%) were convinced that they could be easily misused similar to observations by other authors [6,9].

However, the students were convinced about the importance of doctors especially regarding communication to the patients during euthanasia as well as in cases of advance directives.

Limitation

The limitations of the study comprise only a single questionnaire administration was done with limited questions related to hypothetical situations. Factors influencing students' responses such as personality, value system, and emotional state were not assessed. Further studies with more volunteers are required to attain statistical significance.

Conclusion

This study concludes that majority of the medical students are aware of euthanasia and a very few are aware about advance directives. Many of the medical students support euthanasia practice and recent changes in law that involved euthanasia. Most students expressed concerns of abuse if euthanasia and advance directives were legalised and practiced. Future studies are required to explore precisely the factors that influence medical students' attitudes toward euthanasia and advance directives and many awareness programs are required to be held for the same.

Conflict of Interest : Nil

Ethical Clearance: Obtained (SMC/IEC/2020/03/286)

Source of Funding: Self

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Knowledge and Attitude Towards Antibiotic Prescription among Dental Students

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Abstract

Introduction: Antibiotics play a crucial role in dental practice for both prophylactic and therapeutic purposes. Excessive consumption of antibiotics or using them unreasonably not only cause emergence of resistant bacterial strains and hostile drug reactions but also create an economic liability on the overall health system of a country.

Aim: The present study was aimed to evaluate the knowledge and attitude towards usage, antibiotic prescription and various concerns associated with antibiotics among dental students.

Methodology: A cross sectional study was conducted using self-administrated questionnaire, through Google forms using various social media platforms such as whatsapp, facebook among 202 dental students of third year, final year, interns and postgraduates across dental colleges in Tamil Nadu, India during COVID-19 pandemic lockdown period. Statistics was done and tabulated.

Results: Our study results show that the majority of dental students are aware of antibiotic prescription, uses of antibiotic and antibiotic resistance.

Conclusion: Though the knowledge and attitude about antibiotic prescription is high among dental students, there are still few areas where they lack adequate knowledge; these deficits can be rectified by encouraging them to attend cde programs, conferences related to antibiotic prescription.

Keywords: *Antibiotics, Adverse drug reaction, Dental students, Prescription, Irrational practices.*

Introduction

Resistance to antibiotic is an evolving and rapidly threatening serious concern leading to difficulty in treating infections caused by microorganism's particularly bacterial species. Excessive consumption of antibiotics or using them unreasonably not only cause emergence of resistant bacterial strains and hostile drug reactions but also create an economic liability on the overall health system of a country [1].

Self-medication, sharing medication with other people, compliance of the patient and keeping part of the course for another occasion are the various patient related factors [2].

Among all these factors, the irrational use of antibiotics has contributed to the bacterial resistance with substantial clinical and economic impact. Recommending a particular antibiotic medication is a difficult task that requires theoretical and clinical knowledge combined with practical skills. Antibiotics play a pivotal role in dental practice for both prophylactic and therapeutic purposes[3].

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The clinical effectiveness and efficacy of antibiotics depends on several influences such as the fear of losing a patient's trust, lack of accurate information on indications of antibiotic usage and forced usage of antibiotics due to pressure from patients and families and partially on their precise usage, on patients, physicians and merchants [4].

It is high time to create awareness, find and bridge the gap about different dimensions of antibiotic uses including antimicrobial stewardship and inculcate a practice of rational uses of drugs in general and antibiotics in particulars among budding medical and dental practitioners [5].

Hence the present study was aimed to evaluate the knowledge and attitude towards usage, antibiotic prescription and various concerns associated with antibiotics among dental students.

Methodology

This study was designed to evaluate the knowledge and attitude towards usage, antibiotic prescription and various concerns associated with antibiotics. A cross sectional study was conducted among dental students of third year, final year, interns and postgraduates across dental colleges in Tamil Nadu, India during COVID-19 pandemic lockdown period. A total of 202 individual participated, in this study. The participants were informed about the study and assured that their participation was purely voluntary.

The self-administrated structured questionnaire consisting of 15 questions, in English, which is simple and easily understanding questions which helps the respondents. The questionnaire had a combination of selected response to the questions and closed ended questions (Yes/No/options-based). It took 5-10 minutes to complete the questionnaires for participants. The questions were converted into google forms and shared to participants social media platforms such as whatsapp, facebook. The purpose and aim of the study were explained with requesting for participation, to the participants.

Statistical Analysis

On statistical evaluation using SPSS version20.0 (Ilanos, NY, USA) it was observed all the 202 samples were valid for the study with Cronbach's alpha reliability score of 0.8947.

Results

On analysis of the given data it was observed majority of the study participants 57.42% pursue internship program followed by 15.8% final year students, 3rd year students and 11.38% postgraduates.

In the present study on analysing the knowledge about antibiotic prescription 47.5% of participants choose amoxicillin-clavulanic acid as their choice of drug (fig: 1).Around 87% of students are aware that antibiotics are effective against bacterial infections (fig: 2). 74.5% are unaware that ceftriaxone is a beta-lactam antibiotic. About 75% of the study participants prescribe antibiotics over a period of 5 days as recommended (fig: 3).On analysing the attitude towards prescription of antibiotics 87.5% students prescribe antibiotics for acute pulpitis. 87% of students prescribe antibiotics before any extraction procedure and 77.5% of students prescribe antibiotics for pain. 90% of the study participants believe antibiotic resistance as a public health issue (fig: 4) and recommended addressing this issue is important. In the present study the overall Knowledge and Attitude score was found to be good among postgraduates and interns, where as it was least among final years and third years respectively.

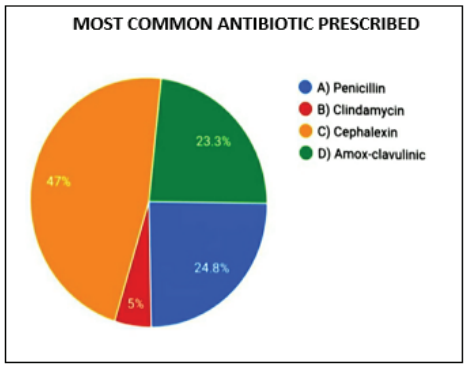


Figure 1

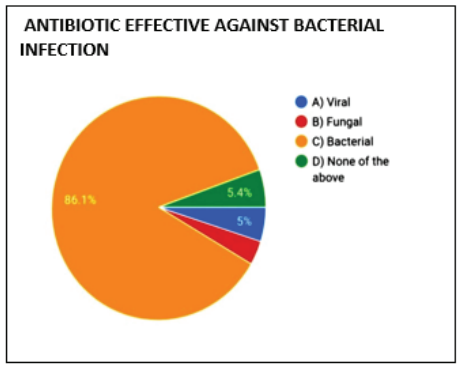
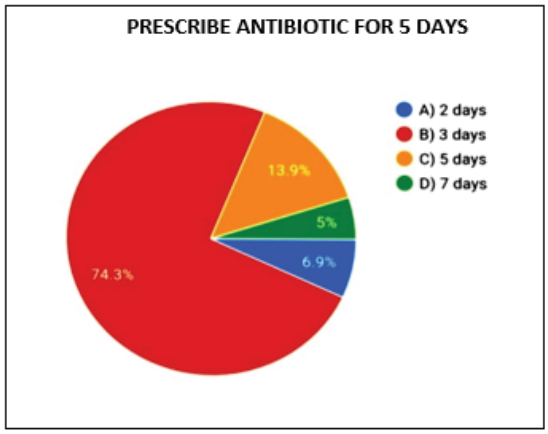


Figure 2



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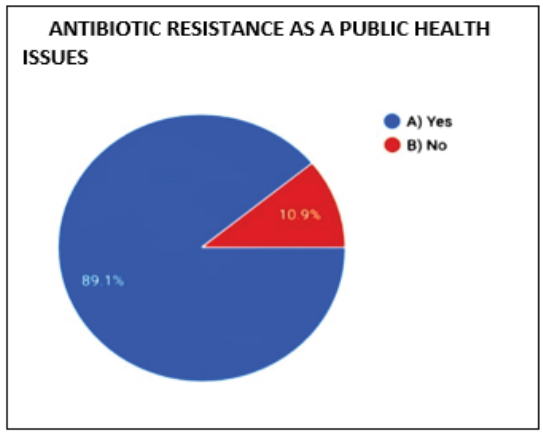

Figure 4

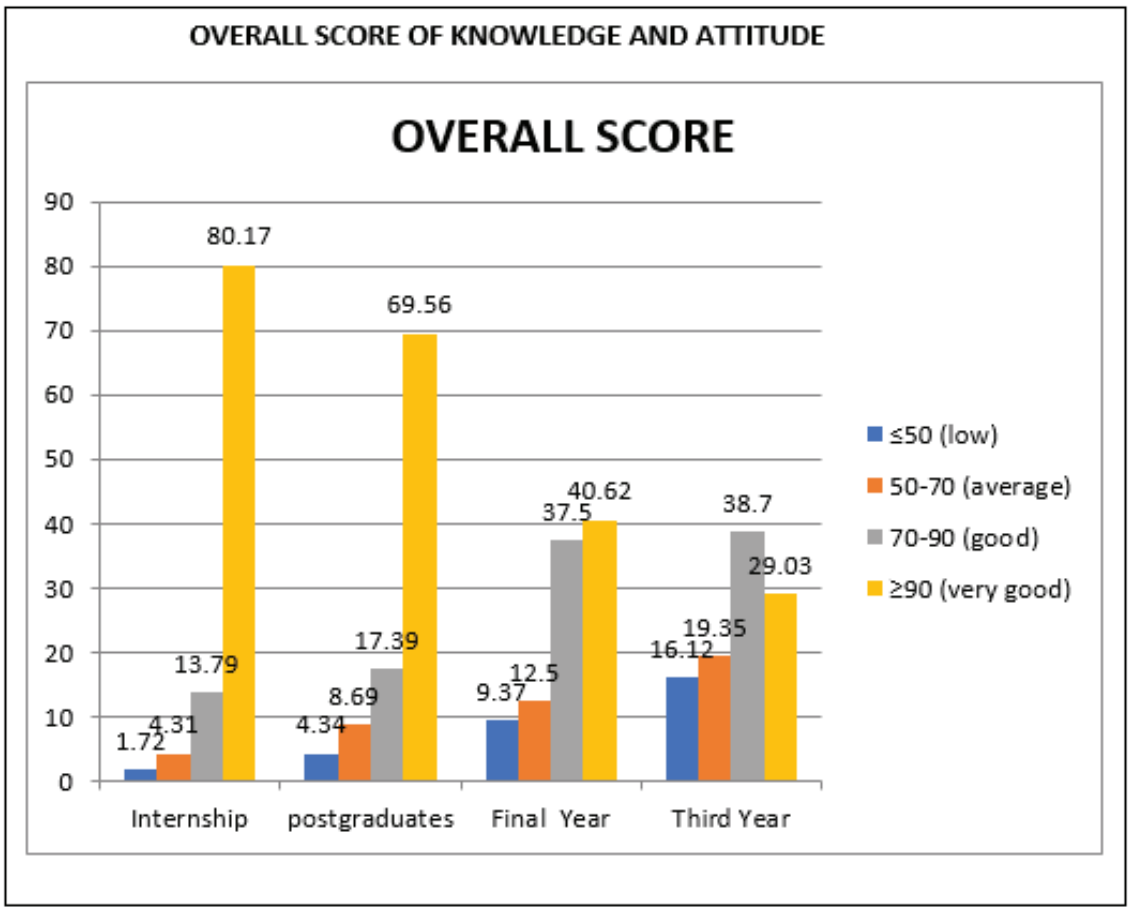

Figure 5

Discussion

Misconception in prescribing medicine is very common, particularly with new practitioners. The fundamental issue which adds to this irrational prescription is that in the clinical and therapeutic undergraduate studies, students are not satisfactorily trained and wherever it is taught, the learning provided is just theoretical [4]. Majority of our respondents had knowledge about the antibiotic resistance pertaining to ability of the drug to resist the effect of drugs. This was in contrast to Dyar et al [5] and Haque et al [6] who found that medical student's knowledge on antibiotics and antibiotic resistance was limited.

In the present study on analyzing the knowledge about antibiotic prescription, 47.5% of participants choose amoxicillin-clavulanic acid as their choice of drug. This is similar to the study by Tancawan et al [7,8] who showed amoxicillin-clavulanic acid was well-tolerated drug of choice with a safety profile consistent with the known pharmacologic effects and effective agent.

Around 87% of students accepted antibiotic as effective mode of treatment against bacterial infections. 74.5% of students are unaware that ceftriaxone is a beta-lactam antibiotic.

About 75% of the study participants prescribe antibiotics over a period of 5 days as recommended. Pouwels et al in 2019 [9] in his study concluded with the similar results thus stating that considerable reductions in antibiotic exposure and limiting the usage can be accomplished by bringing the duration of antibiotic prescriptions with guidelines.

On analyzing the attitude towards prescription of antibiotics 87.5% students prescribe antibiotics for acute pulpitis, 87% of students prescribe antibiotics before any extraction procedure and 77.5% of students prescribe antibiotics in patients complaining of tooth pain. This is similar to the study by Dar-Odeh NS [10] et al who demonstrated antibiotics are prescribed by dentists for treatment as well as prevention of infection. The Indications for systemic antibiotic use in dentistry are limited, since most dental and periodontal diseases are best managed by operative intervention and oral hygiene measures.

90% of the study participants believe antibiotic resistance as a public health issue and recommended addressing this issue is important. Similar observation was made by Sharma et al [11] where Self-medication and excessive choice of antibiotics holds key to development of antibiotic resistance. Large number of participants in our study had practiced self-medication with antibiotics; that is similar to a study done by Shubha R et al [12]. Majority of our participants had advised their friends and relatives to take antibiotics for which they are not qualified and not supposed to. However, the writing provides evidence of inadequate prescribing practices by dentists, due to a number of factors ranging from inadequate knowledge to social factors. There should be a mandated guide line that who should prescribe antibiotics and who shouldn't and it should be enforced strictly at the national level.

The total knowledge and attitude **towards antibiotic prescription among dental students** based on summing all the positive responses was 93% (highest) for interns group, 86% for post-graduates, 78% for final years and 69% for third years (least) respectively. Our study results were similar to the study by Nyquist et al [13] among 503 interns and senior physicians who showed 98% of physicians in France were aware of the resistance. Study done by Huang et al [14] that showed 83% of the Chinese medical students considered antibiotic resistance as a serious national issue. The study was contrast to various studies that shows an excessive use of antibiotics especially among senior medical students, reflecting a deficiency of antibiotics usage instruction in their curriculum.

According to the study conducted by Guzman-Alvarez et al [14] only 30% of dental students followed the WHO guidelines for prescribing, and the remaining did not follow them as most of them were not aware of such guidelines which in contrast to the present study where 75% of study participants were aware of WHO guidelines.

Conclusion

This survey was able to assess the knowledge and attitude of dental students toward antibiotic prescription. Under the limitations of the present study, it can be concluded that most dental students are aware of antibiotic resistance. However, there is no concurrence

among participants regarding important aspects of prescribing antibiotics such as dose, frequency and duration. Awareness need to be done through educational interventions. Students need to be updated on the current scenario and keep up with the latest emerging trends. Though the knowledge and attitude about antibiotic prescription is high among dental students, there are still few areas where they lack adequate knowledge; these deficits can be rectified by encouraging them to attend cde programs, conferences related to antibiotic prescription.

Conflicts of Intrest: Nil

Source of Funding: Self

Ethical Clearance: taken from institutional ethical clearance

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Factors Affecting Natural Illnesses during the Covid-19 Pandemic- Autopsy Findings in a Series of Cases

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Abstract

The Covid 19 pandemic brought about many changes. People were stressed, also they didn't get treatment on time. Because of this there was worsening of pre-existing disease. There was also increase in stress related diseases like myocardial ischemia. Lockdown and sudden change of plans also affected the treatment of cases. Some cases could also be due to undiagnosed Covid infection, since there is no test that is 100% sensitive and specific to detection of the virus[1] especially during the post-mortem period. Here we are discussing some selected cases which we have come during the period and the autopsy findings thereof. This article highlights the importance of negating fear and increase awareness. It also conveys the significance of verbal autopsy as a tool to help certify death.

Key words: *Covid 19, Natural death, verbal autopsy*

Introduction

The Covid 19 pandemic has affected all areas of life. It has made people think differently. Even the methods used in autopsy has changed. Many guidelines for handling bodies and conducting autopsy were issued [2-8]. While some countries did not permit autopsy without full facilities and safety protocols, some countries allowed performing minimal autopsy and using less invasive methods. Many have started to use ancillary methods like virtual autopsy and verbal autopsy. The pandemic has also shown the harassment people have to face due to fear of unknown factors. Most doctors are confused as to the legal guidelines in certifying cause of death. An overall tendency of patients to avoid hospital visit is seen. Unannounced lockdown caused disruption of treatment plans of patients travelling to a better centre for treatment. Increasing suspicion of Covid warriors to be Covid carriers is also seen. Here we are discussing a series of cases of brought dead to the hospital with natural causes during the Covid-19 pandemic.

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Case Presentation

Case 1

A 34yr old male with a ten day history of hepatitis with sub hepatic failure and hepatic encephalopathy was investigated and treated for the same. He had worsening of symptoms and was brought dead to the hospital. RT PCR test for Covid 19 was negative. The doctor was not sure if its correct to issue cause of death certificate if by chance it was a false negative report.

Case 2.

A 46year old male diagnosed with Unstable angina, old inferior wall MI, Triple vessel disease and type 2 diabetes was treated by bypass surgery and saphenous vein graft. He also had other investigations including whole body CT scan was done which showed mild hepatomegaly, renal microliths, trace pleural effusion, trace pericardial effusion with calcific thrombus in left

ventricular apex. He was on regular follow up. He had chest pain and collapse and was brought dead to the hospital. RT PCR for Covid 19 was negative. However the doctor was not sure if it is correct to issue cause of death certificate. Hence cause of death certificate was not issued.

Case 3

An 80 year old male was sitting on a chair at home. He had chest discomfort and collapsed and died. The case was brought dead to the hospital. He also tested negative for SARS Covid 19. The case was also intimated to the police not because the doctor had suspicion but because of the lack of clarity as to issuing death certificate in the context of Covid-19 pandemic

Case 4

In the initial phase of the pandemic sudden lockdown was implemented. A 58year old male with moderate left ventricular hypertrophy, calcific aortic valve with stenosis, grade 2 mitral regurgitation(rheumatic heart disease) was referred from another bordering country to the hospital for surgical management . Incidentally he reached hospital for treatment on 23rd march when lockdown was initiated. He had to undergo quarantine and waited for the appointment. Unfortunately, his condition worsened during the time and he succumbed to the illness.

Case 5

The pandemic caused fear in people to take hospital treatment. Those who were suffering from mild symptoms waited for a better day to go to the doctor. A 37yr, male had acidity like features along with chest discomfort. He took symptomatic home remedies including antacids. He was found collapsed on the floor the next morning. There was family history of sudden cardiac death. Covid test was negative

Case 6

There were illnesses in relation to stress. Long hours of work, uncertainties all affected the people especially those having work from home in the IT sector. A 29 yr, male had chest pain and sudden collapse and death. Covid test was negative.

Case 7

There is increasing suspicion of Covid 19 in the servers of society. Cleaners, police officers, doctors are considered to be carriers of the illness. A 38yr, Female working as a sweeper in the government sector was having fever and cough. She was tested thrice for covid-19antigen which was negative. Unfortunately she didn't get cured of the illness and died .

Case Findings

Case1.

Autopsy features were consistent with the history of illness . Hemorrhages were seen over the surfaces of internal organs

Case 2

Autopsy findings were consistent with the history. The scar of previous surgery was seen

Case 3

Autopsy showed recent myocardial infarction

Case 4

Autopsy showed findings consistent with the history with calcific aortic stenosis

Case 5

Autopsy showed pale , dull area in the myocardium consistent with recent myocardial infarction which was confirmed on histopathology.

Case 6

Autopsy showed complete occlusion of left anterior descending coronary artery with thrombus and myocardial infarction

Case 7

Autopsy showed pulmonary oedema. Histopathology showed foci of myocardial infarction and pulmonary oedema with autolysis.

Discussion

The first three cases describe the tendency to make non medicolegal cases as medicolegal cases due to lack

of clarity of doctors and general practitioners as to the process of certification during the Covid 19 pandemic. They need to be made aware that as per ICD [9], Covid can come in part 2 of the certificate if it is a comorbidity and it is not a crime to issue death certificate in a person dying of natural causes when the cause of death is obvious.

Unpredicted lock down and quarantine has many socioeconomic consequences[10]. It also restricted mobility of people and resulted in many problems consequent to the same.

People are increasingly afraid to visit hospital even in serious illnesses due to fear of contracting the viral infection. In many countries people are dying at home since they avoid going to hospital.[11]

There are increasing issues in relation to stress during the pandemic.[12] The increasing cortisol response to stress causes increase incidence of cardiac events as well.

The problems faced by the so called 'Covid warriors' when they are unwell are many. In addition to the stigma of possible Covid infection they also have to face the wrath of people which is uncalled for. Efforts are being undertaken all around the globe for reducing stigma against health care workers [13]and servers of society.

Verbal autopsy instrument is developed and is being validated by WHO.[14] It helps to diagnose conditions when facilities are less. Since it is a standardised tool it can be applied by during certification of death and also during medicolegal autopsy[15]. Since resources are scarce in many institutions, and also since safety protocols for Covid 19 require a higher standard, this can act as a complementary tool to minimal autopsy.

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Research Protocol

Combined Effect of Proprioceptive Neuromuscular Facilitation And Electrical Muscle Stimulation In Hemiplegic Stroke Patients To Enhance Upper Extremity Function: A Research Protocol

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Abstract

Background- : Stoke is characterized as rapid clinical signs of focal (or global) impairment of brain function, with symptoms that last longer than one day or lead to death, with no other apparent cause than vascular origin. Globally, Cerebrovascular(CVE)damage is the second-biggest root cause of death and the third-largest cause of disability. This is 1 in 19 deaths. Stroke is a significant source of injury in the long term and is more disabled than fatal. This protocol has been created that describes the design of experimental study to evaluate the combined effect of Neuromuscular Electrical Stimulation(NMES) with Proprioceptive Neuromuscular Facilitation(PNF) to enhance the upper- extremity functions in hemiplegic stroke patients.

Methods-The participants (n=26) will be recruited in the study suffering from stroke and meeting the inclusion criteria. Two groups will be formed such that patients in group A will be treated with conventional therapy and PNF group B will be treated with PNF and NMES in alongwith conventional therapy. The protocol will cover 6 weeks of treatment. Regular assessment at 0,3rdand 6th week will be carried out. In the rehabilitation period, we will evaluate upper extremity function, activities of daily living(ADLs), mental status examination at regular interval during the rehab.Our outcome measures will be- Glasgow coma scale (GCS), Mini-Mental Scale Examination (MMSE), Modified Ashworth Scale (MAS), Modified BarthelIndex(MBI) and Action Research Arm Test(ARAT).

Discussion-Efficacy of the intervention will be evaluated by analyzing the upper extremity function using Action Research Arm Test (ARAT) and level of disability by using Modified Barthel Index Scale. The result of the study will significantly provide affirmation on using combination therapy of PNF with NMES in stroke patients.

Keywords- *Stroke, Proprioceptive Neuromuscular Facilitation, Neuromuscular Electrical Stimulation, Conventional Therapy, Physiotherapy.*

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Introduction

The WHO defined stroke as: 'quickly developing signs of focal (or global) disturbance of brain function, with symptoms lasting more than 24 hours or longer, or leading to death, with no apparent cause other than vascular origin"[(1)]

Cerebrovascular injury (CVE) is Globally, 2ndlargest reason of death and the third-largest cause of disability

as per the stroke fact sheet.CVE is the Number 4 cause of death in America, killing more than 140,000 people a year (about 142,000). This is 1 in 19 deaths. Stroke is a significant source of injury in the long term and is more disabled than fatal[2].

In India, stroke is the single number one cause of death and disability. The median range of strokes adjusted prevalence rate, in rural areas 84-262/100,000 and in urban areas 334-424/100,000. The incidence rate is 119–145/100,000 based on recent population-based studies[3]

Clinically, a stroke presents with number of deficits such as motor, sensory, perceptual, mental and language impairments. Most prominent feature is motor weakness or hemiparesis on side of body opposite to side of lesion leading to disability[4].

Hemiplegia is a palsy affecting single side of the body. It is frequently referred to as either right or left hemiplegia, depending on which side of the body is involved. As many as "9 out of 10 patients suffer some degree of paralysis immediately after a stroke, according to the National Stroke Association"[5].

Spasticity is a motor disorder characterized by an change in 'muscle tone' based on velocity with exaggerated deep tendon reflexes, as one feature of upper motor neuron syndrome"[6].Disabling spasticity after stroke seems more common in the upper limb versus the lower limb[7]and influencing overall role of the upper extremity contributing to reliance on others.

Proprioceptive Neuromuscular Facilitation (PNF) is one of the key therapeutic strategies aimed at improving the essential functional features required for hemiplegic patients such as muscle tone, strength and flexibility[8]. PNF is a therapeutic exercise strategy, which functionally combines generated diagonal motion patterns, uses Neuromuscular Facilitation approach is used to elicit motor responses and improve control and function of the neuromuscular system[9].

Electrical Muscle Stimulation (EMS) is a modality of electrotherapy which helps to maintain muscle strength and tone. Neuromuscular electrical stimulation (NMES) is widely used as a therapy to promote motor recovery, minimize pain and spasticity and improve poor muscle recovery. There is growing evidence that NMES has a positive effect on the regeneration of upper limb motor control in stroke patients[10].

Limb physiotherapy for the hemiplegic limbs involves passive, active-assisted and active range of motion progressing to strength training. This can be an important therapy for the spasticity and avoidance of limb contractures as per AHCPR (1995) is recommendation[11].

Aim-

This study aims to evaluate the extent to which the combination of PNF and conventional therapy to PNF with NMES in combination with conventional therapy, reinforces the upper extremity and functional recovery in stroke patients.

Methodology-

Study setting-

The study will be carried out in Neuro-Physiotherapy OPD of Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha after getting approval from Institutional Ethical Committee of Datta Meghe Institute Of medical sciences, Deemed to be university.

Study design and sample size-

The design of the study is experimental study enrolling 26 participants. The participants enrolled in this study will be randomized in 1:1 manner into PNF and Conventional Therapy intervention group (Group A) and rehabilitation program of PNF with NMES in combination plus conventional therapy (Group B), for 6 weeks each. Before inclusion, the participants will be explained about objectives and approaches of the study, and written informed consent forms will be signed by them. The study schedule of enrollment, intervention and assessment of study(as recommended by standard protocol items: a recommendation for intervention trials (spirit),2013) [12]is illustrated in fig 1.

	STUDY PERIOD						
	Enrolment	**Allocation**	**Post- allocation**				**Follow-up test**
TIMEPOINT	**$-t_1$**		**Intervention (t_1-t_6) t_1 t_3 t_6**			**Post-test T_7**	**t_x**
ENROLLMENT:							
Eligibility screen	X					X	
Informed consent	X						
Allocation		X					
INTERVENTIONS:							
[PNF +Conventional] [PNF+NMES]							
			X	X	X		
			X	X	X		
ASSESSMENTS:							
Baseline Variables- MAS, GCS,MMSE	X						
Outcome Variables: MBI, ARAT		X		X	X	X	X

Fig. 1 schedule of enrollment, assessment and intervention.

PARTICIPANTS-

The inclusion criteria of participants are under-

1. Patient willing to participate
2. Age 30-70 years old
3. Both male and female patients
4. Glasgow Coma Scale(GCS) score more than 10
5. Mini Mental Scale Examination Score(MMSE) more than 18
6. On Brunstrom grading patient scoring 2,3 or 4.
7. On MAS patient scoring 1+,2,or 3

Exclusion criteria for participants are under-

1. Not willing to participate
2. Demyelinating diseases
3. Traumatic head injury
4. Psychological or psychiatric disorder
5. Myopathies, Peripheral Nerve Injury

6. Tumours

7. Recent fractures

8. Visual, hearing deficits

9. GCS score less than 10

10. MMSE score less than 18, On Brunstrom grading patient score 1,5 or 6

11. On MAS patient scoring 4, 5.

Particpant Timeline-

As study duration is of 6 months and intervention duration is 6 weeks so participant will be enrolled mostly during first 4 months of study so 6 week intervention will be completed successfully. Assessment will be done on 1st day of visit then in 3rd week and last on 6th week of intervention. Participant will have to visit 5 days a week for 6 weeks for treatment.

Recruitment-

The Neurologists and health care practitioners working under DMIMSU are invited to refer the prospective patients to our In-patient department (IPD) and Out-patient department (OPD). The patients who are already undergoing rehabilitation in our IPD and are diagnosed with stroke will be systematically assessed for the eligibility in the study as per inclusion and exclusion criteria. After enrollment in the study participants will be randomized in one of the group A or B and accordingly will undergo the rehabilitation program for 6 weeks with intermediate assessments. Informed patient consent will be taken before allocation and after explaining the purpose of the study, procedure, prospective benefits and after effects of intervention.

Implementation

Randomization will be supervised by the research coordinator and principal investigators. Participants will be asked to handpick a sealed group allocation for the recruitment into either group from the envelope.

Blinding

Tester(s) will be blinded to assign the subjects to the group. To ensure blinding, subjects will be mandated not to reveal any details of their treatment to the tester.

Study Procedure

The participants will be categorized into two groups-

Intervention for Group A- It includes PNF approach with conventional therapy, in PNF approach diagonal patterns of upper extremity will be used for enhancing the upper extremity function. Two diagonal patterns that are D1 & D2 flexion-extension pattern will be incorporated in rehabilitation program that involves overall movement of upper- extremity in flexion, extension, abduction, adduction & rotation and involve maximum joints of the upper-extremity. Duration and frequency of treatment, each pattern should be passively practiced then progress to active-assisted and with increased manual resistance or therabands use. Every pattern is practiced five times a week for 6 weeks for 10 repetitions. In conventional therapy Traditional therapy will include strength training, coordination, manual dexterity exercises, effective task practice where possible, and stretching / weight-bearing of the involved arm, & training of daily living activities (ADLs) using the less-affected hand for 6 weeks 5 days a week[13].

Intervention for Group B- It includes PNF in combination with NMES i.e., when patients will perform PNF pattern electrodes of NMES will be placed at desired points of upper- extremity such that there will movement by patient effort and NMES will produce contraction of muscles simultaneously thus enhancing the function of extremity. Daily this combination will be used for patients to find the desired results. EMS will be incorporated with PNF patterns with proper rest time for period of 6 weeks[14] for 5 days a week on regular basis.

NMES Parameters to be implemented;

Stimulus pulse: Symmetric Biphasic

Amplitude: 0-60mA

Pulse width: 300μsec

Frequency: 25 to 50 Hz

Duty cycle: 10 sec off 10 sec on

Outcomes-

Primary outcome measures-

1. **Modified Ashworth Scale (MAS)-** It is used

in patients with core conditions like stroke, spinal cord injury, etc to assess spasticity. It has 6 grades ranging from 0,1,1+,2,3,4 which helps to determine spasticity at different ranges of motions and thus helps to make treatment program. Scale validity and reliability is high, and is typically used to calculate spasticity[15].

2. **Modified Barthel Index (MBI) -** It is used for assessing the activities of daily living. It is composed of 10 questions that link the degree of independence to daily living activities. Collin et, al; proposed amendment in 1988, in which each domain was scored in one-point increments, ranging from 0 to 2 or 3 for each activity with a maximum score of 20 suggesting functional independence[16]. It is highly reliable and has a good correlation with other disability measures [17].

3. **Action research arm test (ARAT)-** It is a scale of 19 items that assesses the patient's function in 4 aspects (Fine and gross movement of the arm and hand). Every item's presentation is graded on 4 point ordinal scale ranging from 3,2,1,0, It helps to make treatment programs according to performance level of the patient. It is highly reliable and valid for measuring function of upper extremity with good measuring properties [18].

Secondary outcome measures-

1) Glasgow Coma Scale (GCS) is used to assess consciousness of patient and it is a baseline parameter to be assessed. It measures 3 function that is eye opening, verbal response and motor response and total score values between 3 and 15 as least score in each function is 1[19].

2) Mini-mental scale examination (MMSE) is used to assess the cognitive status of the patient that includes attention, memory, orientation and visuospatial proficiency of the patient. Patient is assessed at different levels and is given scores, the least score is 0 and maximum is 30 so on the basis of score achieved patient's cognitive level is assessed.

Data Collection and Management

Data collection

The evaluation data will be obtained from a pre-established spreadsheet with variable baseline characteristics. Research data will be placed in a secure database. Non-electronic records, such as hard copies of assessment forms, signed informed consent, etc., will be stored safely in the study setting.

Data management

Data collection and reporting will be carried out under the supervision of the principal investigators. The research reports must be carefully checked for accuracy. The Excel spreadsheet will be published at the conclusion of the study and given to the statistician for the required analysis. Checklist can be used to avoid lost data due to incorrect staff procedures.

Statistical Analysis Plan

Data analysis will be undertaken utilizing qualitative and interpretation statistical data through using Chi-square test and the student's unpaired t test. The device used for interpretation will be SPSS24. 0version, Graph pad prism version 7.0 and $p<0.005$ are considered to be of relevance ($p>0.005$).

BIAS

Measures will be taken to prevent this from happening attrition bias by giving reminder calls prior to each intervention and by providing travel assistance to those who need it. So, we expect a low percentage of dropouts.

Discussion

Stoke is characterized as rapid clinical signs of focal (or global) impairment of brain function, with symptoms that last longer than one day or lead to death, with no other apparent cause than vascular origin[1]. This study aims to evaluate the extent to which the combination of PNF and conventional therapy to PNF with NMES in combination with conventional therapy, reinforces the upper extremity and functional recovery in stroke patients. Proprioceptive Neuromuscular Facilitation (PNF) is one of the key therapeutic strategies aimed at improving the essential functional features required for hemiplegic patients such as muscle tone, strength and flexibility[8]. As per the study by Chaturvedi et al, PNF exercises are very effective in generating voluntary contraction and improving the functional activities in stroke patients[20]. Neuromuscular electrical stimulation (NMES) is a Electrotherapeutic modality used widely

as a therapy to promote motor recovery, minimize pain and spasticity and improve poor muscle recovery[10]. As per the study by Sheffler et al, NMES activate the paralyzed muscle in precise sequence and magnitude so as to directly accomplish functional task. It also has therapeutic effect of motor-learning thus improving the functional recovery[21]. In conclusion, this research seeks to explore the combined effect of PNF and NMES in hemiplegic stroke patients. The result of the study will help patients for faster recovery and improve their quality of life. Major Outcome measures of the study are MAS, Modified Barthel Index and ARAT scales. These 3 major scales will help to assess spasticity, activities of daily living and ability to do activities with upper extremity.

Result

Successful completion of this study will provide evidence on the best treatment strategy out of individual PNF or PNF combine with NMES for Stroke patients to improve their upper extremity function and result of this study will lead us to better understanding on both treatments. Once the study result is complete data will be analyzed using paired t-test and will be submitted in form of research paper.

Ethical Approval And Dissemination

Ethical approval will be taken from institutional ethical committee. The DMIMS who will fund for research and the subjects who will be participating in the study can access the main findings of the research. Data held safely for the enrolled subjects a minimum of five years. After completion of data collection, statistical analysis a completion report will be formed and after review by institutional research cell will be send for publication.

Patient Consent

Principal Investigator will obtain the informed consent from the patient and one of the relatives on a printed form with signature and given the proof of confidentiality.

Confidentiality

The study program will be elaborated to the participant and one of his/her relative, and principal investigator will take personal information as a part of procedure. The consent form will include the confidentiality statement and signatures of the principal investigator, patient and 2 witnesses. If required to disclose some information for the study, consent will be obtained from the patient with complete assurance of his/her confidentiality.

Author's contribution

DJ suggested the design of the study. PD and DJ led to the creation and design of the study. PD wrote the manuscript of this article. PD and DJ read and approved the final manuscript for publication.

Declaration of interest

The author declares no conflicting interest.

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Original Article

Sexual Dimorphism in Superadded Teeth - A Cross Sectional Study Conducted among Students of South Indian States

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Abstract

The present cross sectional study was conducted on students of south Indian states of SSIMSRC, Davangere. A study casts of 200 was taken (104 males and 96 females) in the age group of {18-25} years. The Mesio-distal and Buccolingual diameters of first and second permanent molars of both maxillary and mandible were measured on the study casts, using sliding verneir callipers.

The buccolingual and mesiodistal dimensions measured, in both maxillary and mandibular molars, showed higher values in males compared to that of females. Amongst the parameters studied, the buccolingual dimensions of maxillary molars showed highest sexual dimorphism with an accuracy of 82.1% and the mesiodistal dimension exhibited least sexual dimorphism. In present study showed that the maxillary molars exhibited highest percentage of dimorphism, compared to the dimensions of mandibular molars, the existence of statistically significant sexual dimorphism in maxillary molars especially the first maxillary molars.

Keywords: *Sexual dimorphism; Buccolingual; Mesiodistal; Molars.*

Introduction

Identification of dead is an essential part of post-mortem examination, for various reasons that include ethical, humanitarian need to know which individual has died, to allow legal investigations and to facilitate police enquiries into overtly criminal or suspicious deaths, as the identity of deceased person is vital factor in initiating investigations. In case of decomposed corpses and human remains many features may be partially or wholly lost, but more information can be obtained than from a skeleton[1]. The most commonly used techniques for sex determination are based on the assessment of the morphological characteristics of the pelvis and skull[2]. However, it is not uncommon to recover the pelvis and the skull in a fragmentary state in forensic settings. In this case, teeth can be used as an additional tool for sex determination. Their durability in the case of fire, trauma and bacterial decomposition makes them invaluable for identification[3]. Teeth play a very important role in identification as the dental characteristics are unique to each individual; moreover the teeth are not destroyed in extreme conditions as they can withstand temperatures upto 11000 celcius[4]. Teeth are unique as individual as fingerprints. Even when there is a lack of antemortem records, evaluation of the dentition is a worthwhile aid

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for investigations to provide information regarding the age, sex, socioeconomic grouping, race, food habits and cultural heritage from which we can identify the person[5]. Present study was carried out to know the Sexual dimorphism among maxillary and mandibular superadded teeth's (Molars).

Materials and Methods

A cross sectional study consisting of about 200 South Indian subjects divided into 106 males and 94 females between the age groups of 18-25 years among the students of SSIMS&RC, Davangere was conducted. Informed written consent from the subjects is obtained prior to taking the collection of specimen. Impressions of both maxillary and mandibular arches are made with irreversible hydrocolloid (alginate) material and cast poured immediately in type II dental stone to minimize dimensional change. After taking the alginate impressions of both maxillary and mandibular arches it would be molded into plaster models. Using a sliding vernier caliper, the mesio-distal and bucco-lingual diameters of first and second molar teeth will be determined from the plaster models of both maxillary and mandibular arches obtained from the subjects, by indirect anthropometric measurement. [6]

MD [MESIODISTAL] diameter of the crown: This measurement is the greatest mesiodistal dimension between the contact points of teeth on either side of jaw (FIGURE 1).

BL [BUCCOLINGUAL] diameter of the crown: This measurement is the greatest distance between buccal and lingual surfaces of the crown, taken at right angles to the plane in which the mesiodistal diameter is taken (FIGURE 2).

The measurements, Mesiodistal and Buccolingual dimensions of both maxillary and mandibular, first and second molars on both right and left side were taken using Sliding Vernier callipers and tabulated. Data was entered in master chart in terms of numbers and percentages. Results are presented as mean, standard deviation, percentage of sexual dimorphism for each first and second molar tooth. Unpaired 'T' test was used to compare the means between males and females. P value of 0.05 or less was considered as statistically significant. The mean values of BL and MD dimensions of males and females were subjected to the formula to calculate sexual dimorphism. [7]

$$\text{Sexual dimorphism} = \frac{Xm - 1}{Xf} \times 100$$

Where Xm = mean values for males and Xf = mean values for females.

Results and Observation

In the present cross sectional study about 200 South Indian subjects studied of which 106 males and 94 females between the age groups of 18-25 years, the parameters, the Mesio-distal and Bucco-lingual diameters of both maxillary and mandibular molars were higher in males compared to females with $p<0.001$. Among the molars, the left maxillary first molar (M1) was found to exhibit the greatest sexual dimorphism (82.1%) in terms of buccolingual dimensions while the least dimorphic value was that for right mandibular first molar (M1) (8.8%) in terms of mesiodistal dimensions. Among the maxillary molars, the left maxillary first molar (M1) was found to exhibit the greatest sexual dimorphism (82.1%) in terms of buccolingual dimensions while the least dimorphic value was that for left maxillary second molar(M2) (13.1%) in terms of mesiodistal dimensions. Among the mandibular molars, the right mandibular first molar (M1) was found to exhibit the greatest sexual dimorphism (75.5%) in terms of buccolingual dimensions while the least dimorphic value was that the same teeth (8.8 %) in terms of buccolingual dimensions. Comparing the linear measurements, the buccolingual dimensions of maxillary first molars were found to exhibit greater sexual dimorphism than mesiodistal dimensions of the same teeth (Table 1).

ABBREVIATION	FULL FORM
BL	Bucco-lingual
LTMD	Left mesio-distal
LTBL	Left Bucco-lingual
MD	Mesio-distal
M1	First molar
M2	Second molar
RTMD	Right mesio-distal
RTBL	Right bucco-lingual

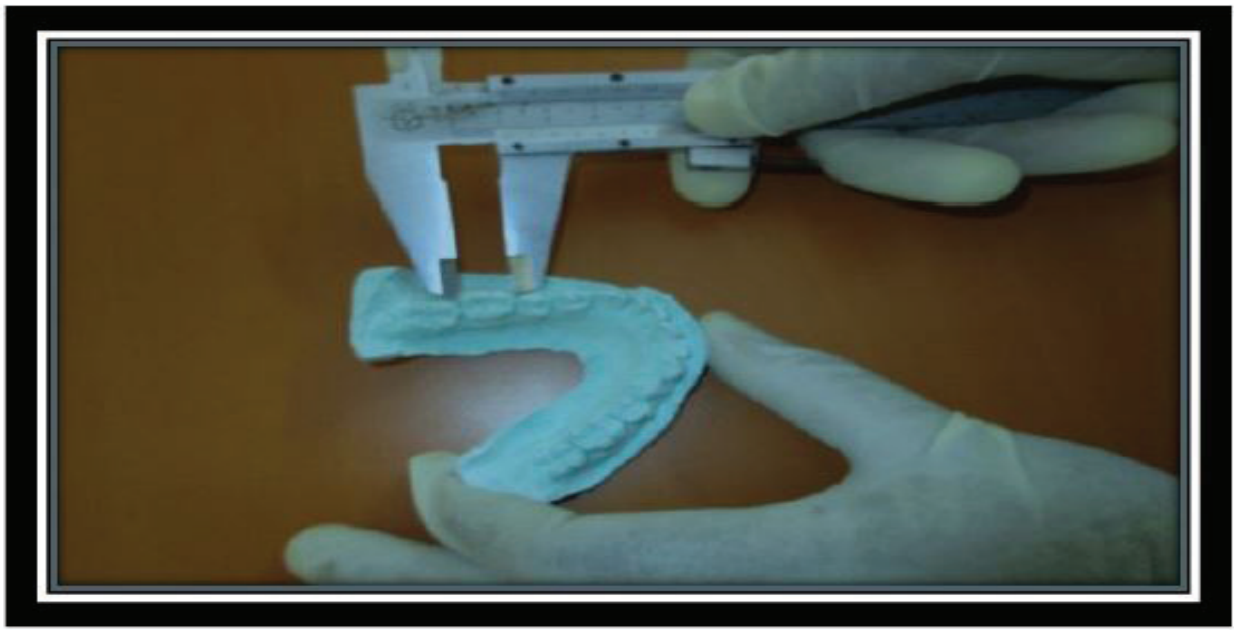

FIG 1: MESIODISTAL MEASUREMENT [MD]

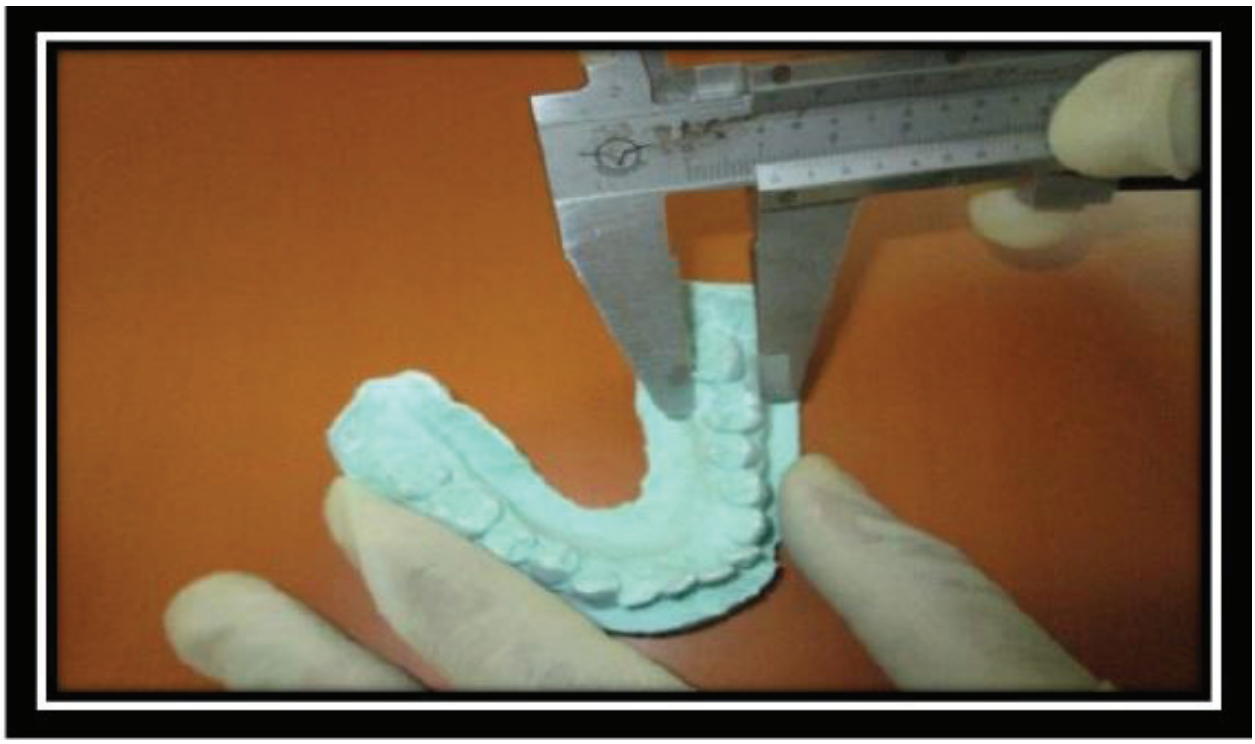

FIG 2: BUCCOLINGUAL MEASUREMENT [BL]

TABLE NO 1: COMPARISON OF THE MEAN VALUES OF DIFFERENT PARAMETERS IN MALES AND FEMALES USING UNPAIRED T-TEST.

ToothType			Males		Females		Diff (M - F)	% Dimorphism	Rank
			Mean	SD	Mean	SD			
UPPER M1	RT MD	UPM1RTMD	0.80	0.08	0.66	0.08	0.14	17.2	8
	RT BL	UPM1RTBL	4.92	4.56	0.90	0.95	4.02	81.7	15
UPPER M1	LT MD	UPM1LTMD	0.80	0.08	0.69	0.07	0.11	14.2	6
	LT BL	UPM1LTBL	4.92	4.56	0.88	0.95	4.04	82.1	16
UPPER M2	RT MD	UPM2RTMD	0.75	0.07	0.63	0.07	0.12	15.4	7
	RT BL	UPM2RTBL	3.36	4.10	0.88	0.96	2.48	73.8	13
UPPER M2	LT MD	UPM2LYMD	0.74	0.07	0.65	0.07	0.10	13.1	5
	LT BL	UPM2LTBL	2.31	3.38	0.89	0.95	1.42	61.4	12
LOWER M1	RT MD	LOWM1RTMD	0.83	0.07	0.76	0.06	0.07	8.8	1
	RT BL	LOWM1RTBL	7.16	4.24	1.74	2.70	5.42	75.7	14
LOWER M1	LT MD	LOWM1LTMD	0.81	0.08	0.74	0.08	0.07	9.2	2
	LT BL	LOWM1LTBL	5.18	4.57	2.11	3.18	3.07	59.3	10
LOWER M2	RT MD	LOWM2RTMD	0.84	0.90	0.75	0.97	0.09	10.9	3
	RT BL	LOWM2RTBL	3.58	4.24	1.39	2.26	2.19	61.1	11
LOWER M2	LT MD	LOWM2LTMD	0.77	0.07	0.67	0.08	0.10	13.0	4
	LT BL	LOWM2LTBL	3.01	3.90	1.37	2.27	1.64	54.4	9

** Using Unpaired t test, $p<0.001$, HS

Discussion

The present study established the significant impact of sex factor on the morphometry of both maxillary and mandibular first(M1), second(M2) molars. Buccolingual and mesiodistal diameters of right and left maxillary and mandibular first, second molars in males and females were measured on study casts. The comparison of mean values of parameters measured between males and females showed highly statistically significant differences with $p<0.001$ and these results are in agreement with the studies done by Perzigian AJ [8], Ghose LJ *et al*. [9], Stroud JL *et al.* [10], A Study conducted by Hattab FN et al. [11] on 198 Jordanians (86 males and 112 females), showed the BL width of 1st maxillary and mandibular molars was observed and significant sexual dimorphism was only observed in the maxillary molar which is in agreement to present study. A Study conducted by Rai B *et al.*[12], and Ghodosi A *et al.*[13], in which they have observed that the males had larger teeth than females in all the dimensions as our studies are in agreement with that, A study conducted by Joseph PA et al.,[14] showed that the odontometric sex assessment in Indians found that the extent of sexual dimorphism in Indians of all tooth variables being statistically larger in Indian males, which are in agreement with the present study, with accuracy rate of odontometric sex assessment is 72%, and the present study showed 82.1% accuracy. Acharya AB and Mainali S[15] in their study on Nepalese subjects found that MD dimensions had recognizable greater accuracy in sex identification than BL measurements contrast to that in the present study BL dimensions had more accuracy than MD dimensions. Sonika V et al. [16] in their study on Haryana population found that the greater accuracy of dimorphism was shown by maxillary molars than mandibular molars which is in agreement with the present study. The present study showed that the left buccolingual dimensions of maxillary first molar are greater than its counterparts. Similar results were obtained by study done by Lund H and Mörnstad H[17], Zarringhalam M[18], who found that dimensions of all permanent teeth were greater on the left than the right in upper jaw.

Conclusion

Forensic Odontology is an emerging field in India. It relies a lot on inexpensive and easy means of identification of persons from fragmented jaws and dental remains. The present cross sectional study was conducted on study casts of 200 was taken (104 males and 96 females) in the age group of 18-25years. the Mesio-distal and Bucco-lingual diameters of both maxillary and mandibular molars were higher in males compared to females with $p<0.001$ by using unpaired t test. The maxillary molars showed

highest sexual dimorphism when compared to the mandibular molars. Bucco-lingual dimensions of maxillary molars showed highest sexual dimorphism of 82.1%, compared to the mandibular molars. It was concluded from the study that the metric analysis of teeth can be of immense help in determining the sex of an individual, the results also showed that bucco-lingual diameters of first maxillary molars exhibited significant sexual dimorphism among the students of South Indian states and thus can be used as an additional tool in sex determination, even in absence of antemortem data, genetic and medicolegal purposes.

Conflict of Interest: None.

Source of Funding: Self.

Ethical Clearance: Ethical clearance was obtained from the Institutional Ethical Committee.

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Bacteriological Profile of Health Care Associated Infection and Antibiotic Resistance Pattern of Isolates at Picu in a Tertiary Care Hospital

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Abstract

Background: Healthcare Associated Infections (HAIs) are a major cause of morbidity and mortality in Pediatric intensive care units (PICUs). The aim was to determine bacteriological profile of HAI and to establish an antibiogram of isolates in our PICU.

Materials and Method: This was a retrospective study conducted at the Department of Pediatrics (Pediatric Intensive Care Unit) of AIIMS, Jodhpur. Data was collected over 2 years from January 2017 to December 2018. Among the114 culture positive isolates, 50% (57/114) were gram negative and 22.8% (26/114) were gram positive organisms. Culture positivity for fungal growth was 14 % (17/114) and rest 12.2% (14/114) were contaminants. The most common isolate in gram negative organism was Acinetobacter baumannii and in gram positive organism most common isolates were Enterococcus. Most commonly detected HAI types were ventilator associated pneumonia (VAP) - 34% (20/59). All gram negative organisms were resistant to carbepenems except Pseudomonas (100% sensitivity). Among gram positive organisms, methicillin resistant staphylococcus aureus (MRSA) was 100% sensitive to gentamicin, amikacin, vancomycin and linezolid.

Conclusion: The retrospective study showed that both gram positive and negative bacteria as well as fungal organisms are responsible for HAIs. Most of the strains were multi drug resistant.

Key words: *VAP- ventilator associated pneumonia, CRBSI- catheter related blood stream infection, CAUTI - catheter associated urinary tract infection, PICU - Pediatric intensive care units*

Introduction

Healthcare Associated Infections (HAIs) are a major cause of morbidity and mortality in Pediatric intensive care units (PICUs).Centers for Disease Control and Prevention –(CDC) defines a HAI as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or toxin(s). There must be no evidence that the infection was present or incubating at the time of admission. [(1)]This is due to the increased use of medical instruments such as mechanical ventilators, blood and urine catheters, invasive monitoring devices and increased resistance of the microorganisms isolated from Intensive care unit (ICU) patients to most commonly used antibiotics, which in turn is a result of overt use of broad-spectrum antibacterial agents.[(2)]

HAIs are common among children in ICUs and have been reported to occur in 9% to 21% of critically ill children.[(3)] The most common HAIs in ICUs include bacteremia particularly CLABSI (central line associated blood stream infection), ventilator associated pneumonia (VAP), and Catheter associated Urinary Tract Infections (CAUTIs), the former being the leading cause of death in ICU patients. (2) Catheter-related blood stream infections (CR-BSIs) (defined as the growth of the same

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pathogen from catheter tip and peripheral blood culture), which represent up to 30% of cases, and primary blood stream infections (BSIs), accounting for around 35% of cases, are the most common types of BSI in ICUs. [4]Ventilator-Associated-Pneumonia (VAP) is 2nd most commonHAI. CDC defines Ventilator-associated pneumonia (Pneumonia in persons who had a device to assist or control respiration continuously through a tracheotomy or by endotracheal intubation within the 48-hour period before the onset of infection.[1]According to the literature, pediatric VAP rates vary, with incidence reported up to 32% and rates up to 27.1 per 1000 ventilator days.[5] UTI is the third most common HAI after CLABSI and VAP. As per NHSN (National Health Safety Network) the incidence of catheter- associated UTI is 5 per 1000 catheter days.[6]

In the developed countries, gram positive organism such as CONS (Coagulase negative staphylococcus) is the most frequent pathogen isolated in patients with blood stream infections whereas gram negative organisms such as Pseudomonas and Escherichia Coli are common causative organisms for VAP and UTI. [7] In our country, gram negative organisms such as Klebsiella, Acinetobacter, Pseudomonas and E. coli are the commonest organism isolates in children with various types of HAI.[8].

The infections caused by multidrug resistant (MDR) organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics.[9]Therefore, this retrospective study was undertaken to determine bacterial flora of various HAIs and their antibiotic resistance pattern in our PICU.

Material and methods: This retrospective study was conducted at PICU at AIIMS, Jodhpur after the ethical clearance from institute`s ethical committee. Data was collected from medical record of the patient admitted between Jan 2017 to December 2018 retrospectively. Information regarding demography, presence of infection at admission, use of devices, type of devices, treatment details was collected. The study followed the Strobe guidelines. The inclusion criteria were children from one month to 18 years of age who were admitted in PICU. Blood sample, BAL and urine sample were taken after 48 hour of admission in PICU. Samples taken before 48 hour of admission were excluded. Three common HAIs (CLABSI/CRBSI, VAP, and CAUTI) were diagnosed according to the CDC definitions.[1]

1. A bloodstream infection was defined as CLABSI when it was associated with a central intravascular line. Diagnosis includes laboratory-confirmed bloodstream infection.

Laboratory-confirmed bloodstream infection was defined when either of the following criteria was observed:

a) A recognized pathogen was cultured from one or more blood cultures of a patient, and this pathogen was not related to an infection at another site.

b) A patient had signs or symptoms of infection not related to an infection at another site, and a common skin contaminant (such as coagulase-negative Staphylococcus) was cultured from two or more blood cultures drawn on separate occasions. [1]

2. CDC defines VAP (Pneumonia in persons who had a device to assist or control respiration continuously through a tracheostomy or by endotracheal tube within the 48-hour period before the onset of infection. Briefly pneumonia was defined when all of the below were satisfied.

a) There was new or progressive and persistent infiltrate, or consolidation, or cavitation (for all ages), or pneumatoceles (for infants ≤1 year of age) on a chest radiograph.

b) A patient had one of the following signs or symptoms: fever (temperature ≥38 °C), leucopenia (<4000 WBC/mm^3 and leukocytosis>12000WBC/mm^3

c) There were at least two of the following signs or symptoms: (i) new onset of purulent sputum or a change in property of the sputum, increased respiratory secretions and increased suctioning requirement (ii) new onset or worsening cough, dyspnea, or tachypnea, (iii) rales or bronchial breath sounds, or (iv) worsening gas exchange.(1)

3. CAUTI was briefly defined when a patient with a urinary catheter (UC) had one or more of the following signs or symptoms, with no other recognized infection: fever (temperature ≥38 °C), urinary urgency, frequency,

dysuria, or suprapubic tenderness, and this patient had a positive urine culture ($\geq 10^5$ colony-forming units per ml) with no more than two pathogens isolated. (1)

4. Bronchoalveolar lavage (BAL), non bronchoscopic BAL and aspiration of tracheal secretions was performed if required according to the patient's condition using an aseptic technique.

5. We used a closed drainage system for urinary catheterization. A urine sample was aseptically obtained from the sampling port of the UC or by bladder catheterization if required.

6. The incidence of HAI per 100 patients was calculated by dividing the number of patients with infections by the total number of patients and then multiplying the result by 100.

7. Antibiotic susceptibility of each pathogen was recorded for the purpose to create an antibiogram.

Statistical Analysis

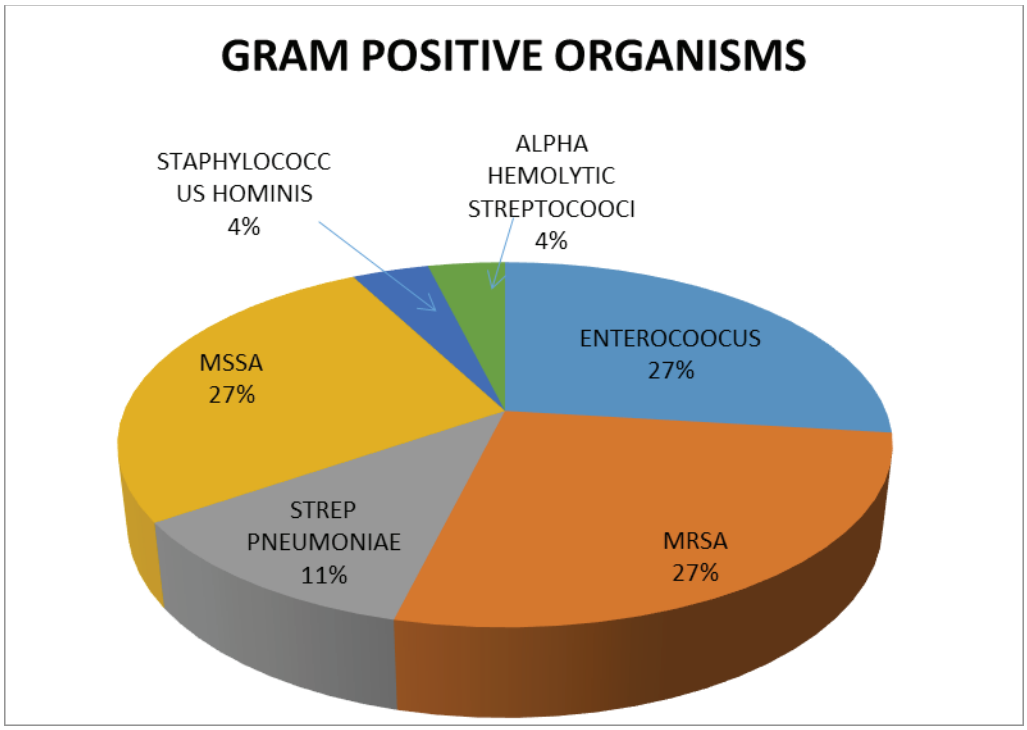
Data was analyses using SPSS software version 20.

Results and Discussion

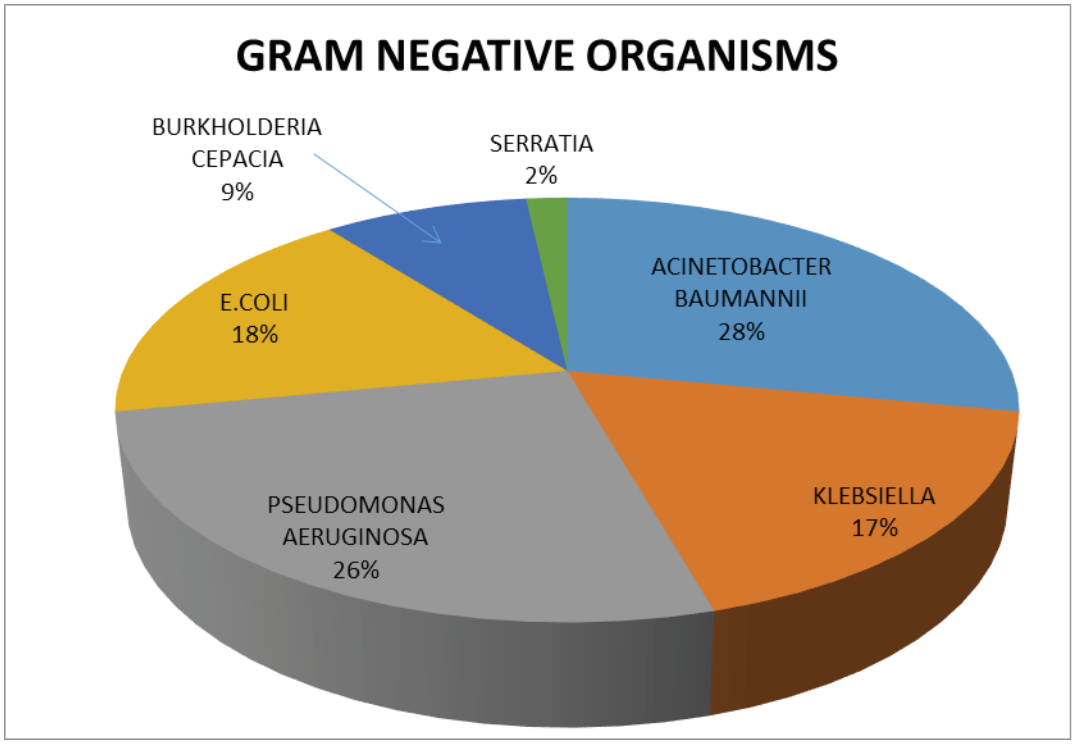
A total of 255 patients were admitted during the study period. Out of these, 155 (60%) were male children.610 samples were from different sites were analyzed during the study period.Samples were collected blood from peripheral line as well as central line, BAL, mid-stream urine sample, central line tip, peritoneal fluid, pleural fluid and wound swab whenever indicated. Total number of positive cultures obtained in the conducted study was 114 (18.6%). Among the culture positive isolates, 57 (50%) were gram negative and 26 (22.8%) were gram positive organisms.Culture positivity for fungal growth was 17 (14 %) and rests were contaminants. Fig 1 and 2 are showed species distribution according to culture. In the two years period of study, 59 HAI were detected from 255 patients. Based on the retrospective data, annual HAI rate for each year from 2017 to 2018 were 19.4% and 26% respectively. The overall HAI rate was 23.2%.

The most common isolate was Acinetobacterbaumannii (14.4%, n-16) followed by Pseudomonas (13.2%, n- 15), Klebsiella (8.7%, n-10) and E. coli (8.7%, n-10). Among Gram positive organisms Enterococcus(6.1%,n-7),*Methicillin-resistant*staphylococcus aureus (MRSA) (6.1%,n-7) and *Methicillin*-sensitive staphylococcus aureus (MSSA) (6.1%,n-7) were common. Culture positivity for fungal growth was 14 % (17). Candida Albicans was the most common fungus responsible for HAI.

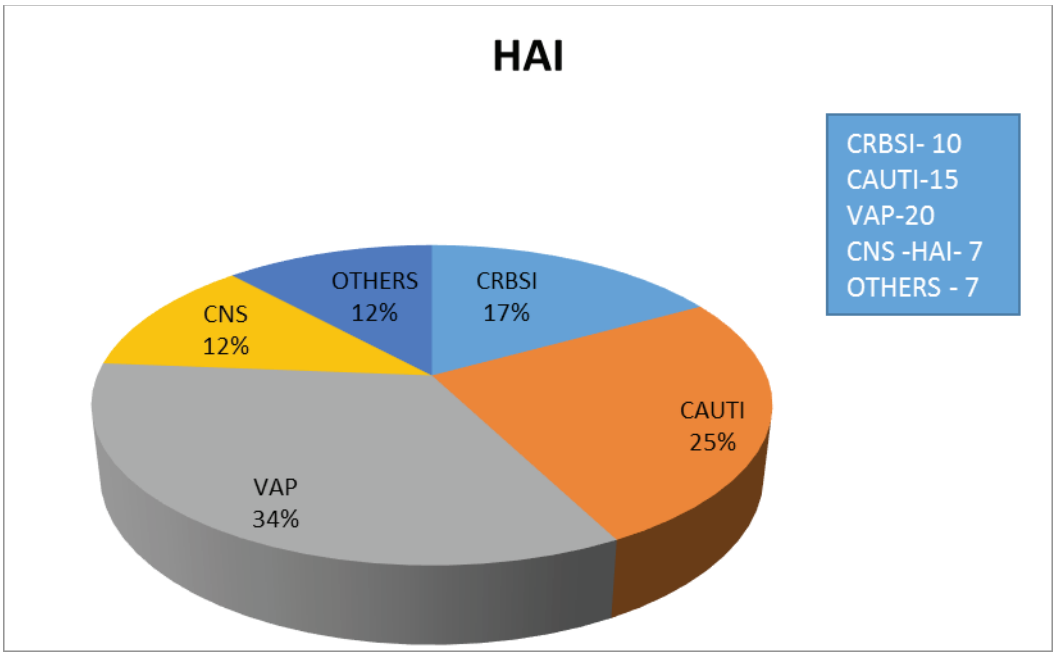
Regardless of years of observation the three most commonly detected HAI types were VAP (34%), CAUTI (25%), and CRBSI (17%). Most common organisms responsible for VAP were Pseudomonas Aeruginosa 11% (7/59) and Acinetobacterbaumannii 11% (7/59). CAUTI was most commonly caused by E. coli 5% (3/59) and Candida Albicans and tropicalis 10%(6/59). CRBSI/CLABSI was most commonly caused by Burkholderiacepacia(n=2), Klebsiella(n=2),Acinetobacterbaumannii (n=1) and E. coli(n=1). Species distribution in health care associated infections is shown in table no 1.



Graph 1: Gram positive organism distribution according to culture



Graph 2: Gram negative organisms distribution according to culture



Graph 3: Health care associated infection distribution

Table 1: Species distribution in health care associated infections

Organism	HAI –BC CRBSI (n)	CAUTI(n)	VAP(n)	HAI-CSF(n)	Others (n)	Total (n)
AcinetobacterBaumannii	1	0	7	2	1	11
Klebsiella	2	1	1	0	0	4
Pseudomonas Aeruginosa	0	0	7	1	1	9
E. Coli	1	3	0	0	2	6
BurkholderiaCepacia	2	0	1	0	0	3
Serratia	0	0	1	0	0	1
Enterocoocus	0	2	0	0	0	2
Methicillin resistant staphylococcus aureus	1	0	2	0	0	3
Strep. Pneumoniae	0	0	0	0	0	0
Methicillin sensitive staphylococcus aureus	0	0	0	1	1	2
Staphylococcus Hominis	0	0	0	0	0	0
Alpha HemolyticStreptocooci	0	0	0	0	0	0
Fungal	2	6	0	1	2	11
Mixed And Commensal	1	3	1	2	0	7
Total	10	15	20	7	7	59

Table 2: Antibiotic sensitivity pattern of gram negative organisms

Antibiotics	Acinetobacter Baumannii(%)	Klebsiella (%)	Pseudomonas Aeruginosa(%)	E.Coli(%)	Burkholderia Cepacia(%)	Serratia(%)
Ceftazidime	15	0	83.3	0	50	ND
Ceftriaxone	0	50	ND	0	ND	0
Amikacin	0	50	85.7	25	0	ND
Meropenam	12.5	50	100	50	50	ND
Piptaz	8.3	55.5	81.8	50	75	0
Imepenem	0	20	100	50	50	100
Colistin	100	100	100	100	ND	ND
Tigecycline	100	100	ND	100	ND	ND

Table 3: Antibiotic sensitivity pattern of gram positive organism

Antibiotics	Enterococcus (%)	MRSA (%)	MSSA (%)	Strep. Pneumoniae (%)	Staph. Hemolyticus (%)	Alpha Hemolytic Streptococci (%)
Linezolid	100	100	100	100	100	100
Vancomycin	100	100	100	ND	100	100
Teicoplanin	ND	100	100	ND	100	100
Clindamycin	ND	0	100	100	ND	ND
Gentamycin	50	100	100	ND	ND	100
Amikacin	ND	100	ND	ND	ND	ND
Cotrimoxazole	0	37.5	16	66	ND	ND
Erthyromycin	ND	0	50	100	ND	ND
Ciprofloxacin	ND	0	ND	ND	ND	0
Penicillin	ND	0	ND	100	ND	ND

Antibiotic sensitivity patterns

We attempted to correlate the organisms isolated with the antibiotic sensitivity pattern so as to formulate antibiotic protocols. Most of the gram negative organisms were resistant to multiple groups of antibiotic including Carbapenem. Acinetobacter baumannii and Klebsiella were sensitive to colistin and tigecycline only, however pseudomonas was sensitive to Carbapenems. Among gram positive organisms, MRSA was sensitive to gentamicin, amikacin, vancomycin and linezolid. Out of 17, antifungal sensitivity was done only for 4 isolates. In which Candida was sensitive to caspofungin and amphotericin B and resistance to fluconazole and Voriconazole. Antibiotic sensitivity pattern of gram positive and gram negative organisms are summarized in table 2and 3.

In the present study culture positivity rate was seen in (114/610) 18.6% samples and HAI was diagnosed in 59 (23.2%),whereas in other similar report prevalence of HAI varies from 6.6 – 37%. [8-12] In the current study, the incidence of infections due to gram negative organisms was 50% while 22.8% isolates were gram positive organisms. It is in accordance with other study. [8, 10-12] In present study, Acinetobacter baumannii was isolated in 14% of samples and Pseudomonas in 13.2%. It is in accordance with the study conducted by Kepenekli et al. However Deep et al.,Atici et al and Armouret et al. reported Klebsiella as the most common organism in 33.3%, 19.4% and 25% of the HAIs respectively. Acinetobacter species is emerging as an important nosocomial pathogen in PICU environment.This high prevalence observed in our study may be related to non-compliance with the recommendations for mastery the hospital environment, lack in hands hygiene and misuse of antibiotics.Difference in the incidence in different institutes is because of difference in set up and the type of antibiotic policy used in these hospitals.

In present study, fungal infection was reported 14.9%. It is in rising trend because of the increasing use of broad spectrum antibiotics, longer duration of hospital stay, use of central line catheter, urinary catheter and peritoneal catheter, mechanical ventilation, total parenteral nutrition, immune-compromised conditionsand multifocal colonization. We should have high index of suspicion and advocate early use of anti-fungal in high risk patients to prevent catastrophic events.

In present study, VAP is most common HAI because suction procedures and mechanical ventilationthe infectious particles lodged within thetracheal tubes can get dislodged into distalairways causing VAP. The risk of VAP increases if ET/TT is colonized. Prior respiratory disorders may put thepatients at a higher risk as there is a greaterchance of airway colonization by gramnegative bacilli in these patients andcompounding factors like impairment ofmucosal clearance and loss of mucosalintegrity also exists.The incidence of VAP increased with the durationof ventilation.Our study corroborated this fact as proven by Salata, *et al.*[13] and Schaberg, *et al.*[14]

In this study, mortality rate was higher in patients who did not develop HAI and it is contradictory finding compared with study done by Gupta A. et. al.[15]This could be explained by the fact that most of the patients who died in our PICU were referred from other hospitals and had already received antibiotics for more than 3 daysin other centers and hence their cultures were not positive andfew were post-operative patients with surgical complications.

In this study, another important finding was antimicrobial resistance which was very high and this may cause a serious therapeutic challenge to the management of common infections. Most of the GNB were multi drug resistant with a very high resistance to betalactam antibiotics (100% resistance to amoxyclav, 50 to 85% to ceftazidime). Carbepenem resistancewas detected in 88.5% isolates of Acinetobacter spp., 50% of Klebsiella spp. and 50% of E.coli spp. All gram negative organisms had developed resistance tocarbepenem except Pseudomonas (100% sensitivity). Klebsiella and Acinetobacter spp. were 100% sensitive to colistin and tigecycline. These findings correlated with findings of Kepenekli E. et. al.[10]

Among the gram positive organisms maximum resistance was seen with penicillin and erythromycin. MRSA displayed markedly high level of drug resistance to most commonly used antibiotics like penicillin, erythromycin, cotrimoxazole, ciprofloxacin and clindamycin.

Enterococcus, MRSA, MSSA, Streptococcus pneumoniae and Staphylococcus hominis were sensitive to vancomycin, linezolid and teicoplanin. These findings were in accordance to the study by Jamshidi M. et. al.[2] This higher degree of antibiotic resistanceis probably because of indiscriminate use of antibiotics in our city and patientsreaching our hospital after receiving broad spectrum antibiotics at various other hospitals on many occasions.

Conclusion

So it is concluded that HAI is an important cause of morbidity and mortality. The causative agents of HAI are acquired in hospitals. This retrospective study showed both gram positive and negative bacteria as well as fungal organisms are responsible for HAIs, but most commonly the gram negative organisms. Most of the strains are multi drug resistant. To bring down the incidence of HAI, judicious use of antibiotics according to the prevalent resistance pattern is essential. Moreover, there is a need for strict aseptic precautions on the part of health care workers.

Limitations

1. Since it is retrospective study, so the results may not be truly representative.

2. Antibiotic sensitivity pattern of all fungal pathogens were not doneat our institute and so resistance pattern of allfungal organisms to various anti-fungal could not be studied.

Conflict of Interest: Nil

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Type of Study- Original Article

A Comprehensive Study of Deaths from Corrosive Poisoning

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Abstaract

Paracelsus, the father of toxicology, once wrote:"Everything is poison, there is poison in everything. Only the dose makes a thing not a poison." A corrosive is a substance which has surface-destructive effect on contact. Corrosive poisoning results from ingestion, topical exposure or inhalation of compounds, that causes tissue injury by chemical reaction. An average home contains a dozen different cleaning products. These products are used in many industries. These are responsible for a large number of accidental and intentional poisoning. This study "A Comprehensive Study Of Deaths from Corrosive Poisoning" is taken up Over 47 corrosive poisoning fatalities autopsied at the Department of Medicine and Department of Forensic Medicine, Osmania General Hospital, Hyderabad to make an attempt to ascertain the frequency of poisoning fatalities and to suggest suitable remedial measures to reduce the morbidity and mortality due to corrosive poisoning. Incidence in males (66.91%) is more than in females (33.09%).The most frequently involved victims belong to the age groups 21 to 30 years, than 11 to 20 years and 31 to 40 years. In majority of the cases, the poisoning was suicidal (93.62%) and then they were accidental (6.38%).

Key Words- *corrosive poison, manner of death, type of poison.*

Introduction

Poisoning is the one of the commonest method for suicide in developing countries like India(1). A **corrosive substance** on contavt damages another surface or substance. A corrosive agent is a substance that causes both functional and histological damage on contact with body surfaces. Although there are many ways to categorize corrosive agents, they are most typically classified as acids and alkalis. They may come in solid or liquid forms with different viscosities and concentrations of solution.In the past there were many incidences of accidental poisoning but later their number went down after adopting mandatory warning labels on the containers, increased education and product regulation (especially of acids)have decreased morbidity and mortality from caustic exposures in both adults and children. But in underdeveloped countries exposure to caustics remains a significant problem [2,3,4,5].

The term corrosive poisons are alkaline or acidic agents.It is a common belief that alkalis bite the oesophagus and licks the stomach due to dilution by its acidic contents and acids spare the oesophagus and damage the stomach. Corrosive poisoning results from ingestion, topical exposure or inhalation of compounds, that causes tissue injury by chemical reaction. These products are used in many industries and house as a cleaning products. Which may leads accidental and intentional poisoning. These compounds are available easily and popular for suicidal and homicidal purpose. Most people use these compounds for suicidal purpose. Death rate is also high in these cases. The out-come depends also on the dose taken, availability of hospital care. Some studies show that about 80% of corrosive poisoning occurs in children < 5 yrs. Adult exposure has

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more mortality & morbidity due to significant volume of exposure & possible combined ingestion caustic substances are chemical when ingested causes injury to the gastrointestinal mucosa. Which are also known as corrosive poisons i.e., strong acids. They coagulate the tissue proteins, extract the tissue fluids. if the person survives then there is ulcer formation, sloughing of the necrosed tissue and scar formation with contractures.

Mineral acids and alkalis have direct local action, but may have indirect action, when ingested and absorbed in the system like circulatory and respiratory systems. Organic corrosive chemicals causes direct local as well as remote action as they are absorbed and reach to the different systems. At present, there are no official or even suggested standards limiting the toxicity of corrosive usage. In our study, we made an effort to go into the social and economic factors, sex, race and occupational factors to throw some light and to suggest certain parameters for early detection and treatment. We also studied various incidences referring to fatal dose, fatal period and the most important clinical sign and chemical examination.

Aims and Objectives of the Study

The incidence of poison deaths from corrosive substances constitutes a miniscule percentage of deaths out of total deaths from various poisons. Even though corrosive substances are available easily as an out-of-the shelf article compared to other poisonous substances particularly as household and toilet cleaners of limited usage, they can cause a chemical significant violet death. As such their use is limited to suicidal poisoning and at no occasion they are used as homicidal poisons but for rare incidents of acid throwing. Therefore corrosive substances are used as an ingested poison for committing suicide where classical findings of corrosion of tissues are observed during autopsy.

Now-a-days they have changed their application as a tool for vitriolage, particularly in young generation to settle scores in frustrated love affairs. Hence the incidence of homicides from topical corrosion has not been an uncommon feature. Apart from this, because of its dubious consistency particularly with oils, rare occasions of accidental poisoning are also seen. Accidental chemical injury also does occur in industrial accidents.

Truly speaking, corrosives are the only substances which has changed its mode of administration with the progress of civilization particularly urbanisation and industrialisation and in near future corrosives and corrosive sublimates may become main liners in the overall incidence of unnatural mortality from poisons.

This study "A Comprehensive Study Of Deaths from Corrosive Poisoning" is taken up to make an attempt to ascertain the frequency of poisoning fatalities and to suggest suitable remedial measures to reduce the morbidity and mortality due to corrosive poisoning.

Objectives

The objectives of the present study are to determine:

1. The frequency of fatal cases of acute corrosive poisoning in relation to age and sex.

2. The frequency of fatal cases of corrosive poisoning with regard to their marital status.

3. The frequency of fatal cases of corrosive poisoning according to their socio-economic status.

4. The various corrosive substances responsible for death in the order of their frequency.

5. The survival period of the person in cases of particular corrosive poisoning.

6. The causes of death.

7. The manner of death.

8. Suggestions to prevent the incidence and to reduce mortality and morbidity in the present scenario.

Materials and Methods

A study of 701 cases of various poisoning, of which 47 corrosive poisoning fatalities autopsied at the Department of Medicine and Department of Forensic Medicine, Osmania General Hospital, Hyderabad has been made.

The records maintained at the Department of Medicine and the Department of Forensic Medicine, Osmania General Hospital, includes copies of the following:

1. Hospital case sheet extracts.

2. Police requisition and inquest reports.

3. Reports of clarification of the circumstances surrounding the death.

4. The post-mortem reports.

5. Forensic Science laboratory reports of the chemical analysis of viscera of the deceased.

Information was also extracted from the relatives, attenders and eyewitnesses (in some cases), regarding the mode of poisoning and the type of poison used.

The presence or absence of a poison in the viscera sent for analysis, to the Forensic Science Laboratory was taken as the criteria to consider a case as a "Poison Fatality". This was because in some cases, if the chemical analysis report was received as negative, it was not considered for the study as the precise cause of death was not identified and at best a calculated guess would have been made.

An attempt has been made to ascertain the manner of death, taking into consideration the history given in the inquest report, the age of the deceased, the nature of the poison responsible for death and any other available information. The history given by the police should only be taken as a hint. The autopsy findings should always prevail upon the history furnished in case of any inconsistencies between the two.

Observations and Discussion

In this study, 47 cases of corrosive poisoning fatalities were studied in detail.

AGE: Analysing the age group of the 47 cases of corrosive poison fatalities, it was noted that 14.89 % deaths were between the ages of 11 to 20 years. 29.79 % were between the ages of 21 to 30 years. 25.53 % were between 31 to 40 years. 17.02 % were between 41 to 50 years. 8.51 % was between 51 to 60 years. 4.25% were between 61 to 70 years.

The predominance of this age group is due to the fact that the age group 21 to 50 years is the most active period of life. People have a tendency to take more risks in pursuit of success, and frustration and depression are more common in personal as well as in professional life.

SEX: Sex distribution in this study shows male preponderance. There were 469 males and 232 females in a total number of 701 cases of poisoning. Out of 47 Corrosive poisonings,28 male and 19 female fatalities were noted.

The male preponderance is due to the fact that all over the world males are seen as sole breadwinners and when they fail in doing something, whether professionally or personally; there is a sense of shame, which drives people to suicide. Also as men take more risks and tend to lead a more care-free life. The females lead a less active life and also stay indoors with an overall less amount of exposure to the various poisonous substances.

MARITAL STATUS: On studying the marital status of the persons in this study, it is observed that 37 people were married including 22 males and 15females. And 10 persons were unmarried including 4males and 6 females.

This data suggests that the rate of poisoning fatalities is more among the married population compared to the unmarried population. This may be due to the fact that the married people have more responsibilities, duties and financial burden due to the dependants and consequently get more frustrated and are liable to take their own lives. Also, as a majority of adults are married, the rate of poisoning also reflects this trend. Unmarried people on the other hand are more carefree and "happy go lucky" which explains the lower number of fatal cases.

PERIOD OF SURVIVAL: Incorrosive poisoning, it is observed that 26 (55.32%) victims succumbed within 24 hours of consumption of the poison. Thirteen persons (27.66%) died in between 24-48 hours after consumption of the poison and 4 persons (8.51%) survived in between 48-72 hrs. Three persons (6.38%) survived 72-100 hours and a single person (2.13%) survived more than 100 hrs. but later died of complications. Obviously, all these persons received some sort of treatment at a hospital. This indicates the degree of toxicity of these compounds as well as the large quantity of the substance consumed. In some cases, the treatment could not have reached its logical conclusion of saving the patient's life due to financial problems of the family and sometimes due to careless and recklessness of the patients' attendants in understanding the gravity of the situation and taking proper precautions and care of the patient and his needs.

MANNER OF POISONING: In this study, the manner of death was found to be suicidal in a large number of cases (93.62%), followed by accidental (6.38%). There were no homicidal and unknown manners of poisoning. The high percentage of suicides by corrosive poisoning is due to known toxicity of these agents and also their easy availability and low cost. They are usually found in most Indian homes for floor and toilet cleaning.

Suicide is a subject of great sociological and psychiatric importance with many unexamined and unresolved problems. In our country, the study of this particular subject is rendered difficult by the lack of statistics about the number of attempts and successful suicides. It is always interesting to consider the reasons. Corrosive compound are routinely used in many Indian homes and can be easily mistaken for something else and consumed with disastrous consequences.

TYPES OF CORROSIVE POISONING: In this study deaths due to sulphuric acid were 43 (91.49 %) out of which 25 male, 18 female, Carbolic acid - 2 deaths (4.25 %), 1 male, 1 female, Nitric acid 1 death-male (2.13%), and Hydrochloric acid 1 death- male (2.13%).

Conclusion

The incidence of cases of corrosive poisoning is on the increase. Corrosive Poisoning cases that came to Mortuary of Osmania General Hospital are from Sulphuric acid, Nitric acid, Hydrochloric acid and Carbolic acid. They are easily available and most effective, kills the person in a very short period. Therefore, the study of poisoning by corrosives is thought necessary and an attempt is made here.

All the findings are critically discussed and the following are noted:

1. The incidence of corrosive poisons was found on the increase.

2. Incidence in males (66.91%) is more than in females (33.09%).

3. The most frequently involved victims belong to the age groups 21 to 30 years, than 11 to 20 years and 31 to 40 years.

4. In majority of the cases, the poisoning was suicidal (93.62%) and then they were accidental (6.38%)

5. Signs and symptoms:

a. Vomiting,

b. sluggish reaction of pupil to light,

c. loss ofconsciousness,

d. Respiratory disturbances and death.

6. Fatal dose - could not be ascertained

7. Treatment:

a. demulcents,

b. I.V. fluids,

c. Inj. Pantocid,

d. Anti-biotics,

e. Oxygen inhalation,

f. Injections of atropine and adrenaline and

g. ventilation support.

8. Post-mortem appearance: signs asphyxia, corroded areas of skin mucous membranes were brownish black, teeth chalky white stomach mucosa converted into soft sponge black massinflammation of upper respiratory tract, corrosion of oesophagus , perforation of stomach and chemical peritonitis were observed in sulphuric acid poisoning. In case ofnitric acid poisoning yellowish corroded areas of skin, teeth and mucous membranes were observed.Distinct odour of phenol in mouth, nostrils and gastric contents, brownish corroded areas, gastric mucosa soft, cerebral and pulmonary oedema were found in carbolic acid poisoning.

9. Chemical analysis: revealed presence of corrosive substances.

Conflict of Interest – Nil

Source of Funding – Self

Ethical Clearance- Instititional Ethical Committee Clearance Taken.

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Predicting 10-year Cardiovascular Risk Using WHO/ISH Risk Prediction Chart among Rural Population in Haryana, India

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Abstract

Background: CVD are leading cause of premature Deaths worldwide. Coronary vascular disease (CVD) risk estimation tools are a simple means of early identifying those at high risk in a community. It seems to be a cost-effective strategy for CVD prevention in developing countries. The WHO /ISH risk prediction charts estimates risk of people having CVD's for next ten years, who do not develop coronary heart disease or stroke. **Methods:** A total of 100 subjects between 40 to 70 years of age were included in this cross sectional study. Total 100 families were visited and subjects were selected randomly. CVD risk was calculated by asking information from the subjects regarding Age, gender, smoking status, measuring their blood pressure, history of diabetes. The chart classifies an individual into different risk of having CVD'S that categorized into low (<10%), moderate (10% to <20%), high (20% to <30%), and very high (>30%) risk groups. **Results:** Less than two-third (62%) of population has less than 10% CVD risk, followed by less than one-third (31%) of the population had CVD risk of between 10- to <20% for the next 10 years. Only 6 subjects had 20-<30% of risk followed by 1% CVD risk between 30-<40%.Age, More SBP, Abdominal obesity, smoking, diabetes mellitus and More BMI ($p<0.0001$) were significantly associated with higher CVD risk. **Conclusions:** WHO/ISH Chart should be used by peripheral health workers for early identification of people with a high risk for CVD and provide timely services in reducing the complications and premature deaths related to CVD in India.

Keywords: *Cardiovascular diseases, WHO/ISH chart, Risk prediction*

Introduction

Cardiovascular diseases (CVDs) are classified as diseases of heart and blood vessels. Globally CVDs are the number one cause of death. Most of the people every year die due to CVDs as compared to other causes. In 2016, CVDs responsible for 17.9 million deaths, that makes 31% of global deaths. Out of these deaths, Heart attack and stroke both are the single most cause responsible for 85% deaths. Low and middle income countries are mostly affected by CVD's as maximum of deaths take place in these countries.[1] Most CVDs can be prevented by addressing and creating awareness among community regarding ill effects of behavioral risk factors like tobacco use, unhealthy diet, overweight and obesity, sedentary lifestyle and lack of physical activity. Practical and feasible approaches are needed to prevent people from getting heart attacks and strokes. Early identification and screening of risk factors among individuals in Health and wellness centers and primary health centers would be a potentially benefit step for primary prevention efforts that critically contribute in reducing the burden of CVD in India.

There are various simple CVD tools which help in early identification of population at high risk. The tool should be reliable and cost-effective so that it can be used in low resource setting areas and in developing countries. The WHO /ISH risk prediction chart helps in estimating the risk of developing atherosclerotic disease, stroke and other heart diseases for the next 10 years among people who are free from these disease but having some risk

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factors. Very few studies have been conducted in India to estimate the cardiovascular risk of community using this chart. This study was undertaken to estimate 10 year cardiovascular risk among rural population using WHO/ ISH risk prediction charts.

Aim

The objective of the study was to estimate 10 year risk of cardiovascular diseases among rural population in Haryana by using WHO/ISH risk prediction charts.

Definition of variables

Smokers: All current smokers and those who quit smoking less than 1 year.

Current drinker: Those who consumed alcohol in the year preceding the Survey but ≤5 (for women ≤4) standard drinks on any occasion.

Insufficient physical activity: Insufficient physical activity can be defined as less than 150 minutes moderate activity per week, or less than 60 minutes of vigorous activity per week.

Examples of moderate intensity physical activity includes walking briskly, gardening, dancing, swimming, bicycling, volleyball, scrubbing floors, carrying water from river or well, manual grinding or pounding of cereals, manual washing of clothes.

Vigorous intensity activity includes jogging, running, high impact aerobic exercise, rowing, lifting heavy loads, digging or construction work, football and cycling uphill.

Abdominal obesity: Waist circumference >90 cm in males and > 80 cm in females.

Method

A cross sectional study was conducted in the village Adhoya of Ambala district. Written Informed consent from the study participants was obtained prior to the collection of data.. Total 100 families were included under the study. Eligible respondent was selected from each of the households. More than 40 years of adults were included in the study. In case a household had more than one eligible respondent, one respondent was selected randomly. After three times visit houses found locked were excluded from the study. Clients with coronary heart disease and stroke were excluded from the study. The study participants were interviewed with the help of a structured questionnaire. The tool was divided into three sections that included demographic variables, behavioral risk factors, and physical measurements. The behavioral risk factors were smokers, current drinkers, and insufficient physical activity. Physical measurements included waist circumference, weight, Height, BMI and blood pressure measurement using the standardized techniques.

WHO/ISH CVD risk prediction chart

In present study SEAR (D) risk estimation chart without cholesterol was used to estimate the total 10-year risk of all subjects. Risk was calculated by asking information from subjects regarding Age, gender, current smoker or not, checking mean systolic BP, history of increased blood sugar. The chart categories an individual into different groups according to risk that includes low (<10%), moderate (10% to <20%), high (20% to <30%), and very high (>30%) risk.

SPSS software package version 19.0 was used for entering and analyzing of data. To find the association between the risk factors (alcohol use, physical inactivity, and abdominal obesity, smoking, BMI) and cardiovascular risk Chi-square test was performe.

Results

Table 1: Frequency and percentage distribution in terms of sample characteristics

N=100

Variables	f (%)
1. Age in years	
40-49	54 (54)
50-59	20 (20)
60-69	26(26)
2. Gender	
Male	25 (25)
Female	75 (75)
3. Level of Education	
Non-Formal education	33 (33)
Primary School completed	19 (19)
Middle school completed	8 (8)
High school completed	19 (19)
Degree/diploma	21 (21)
4. Socio-economic status*	
Upper class	20 (20)
Upper middle class	21 (21)
Middle class	23 (23)
Lower middle class	19 (19)
Lower class	17 (17)
5. Religion	
Hindu	57 (57)
Muslim	12 (12)
Sikh	29 (29)
Christian	02 (02)
6. Systolic BP (mm of Hg)	
Less than 120	29 (29)
120-129	47 (47)
130-139	17 (17)
140-160	07 (07)
7. Smoking	
Yes	10 (10)
No	90 (90)
8. Diabetes Mellitus	
Yes	12 (12)
No	88 (88)
9. Alcohol Use	
Never	82 (82)
Current Drinker	18 (18)
10. BMI (Kg/m2)	

Cont... **Table 1: Frequency and percentage distribution in terms of sample characteristics**

N=100

Underweight	07 (07)
Normal	42 (42)
Overweight	31 (31)
Obese	20 (20)
11. Diet	
Vegetarian	76 (76)
Non-vegetarian	24 (24)
12. Physical Activity	
Yes	31 (31)
No	69 (69)
13. Abdominal Obesity	
Yes	48 (48)
No	52 (52)

*Modified B.G Prasad scale

Table 1 shows a total of 100 subjects between 40 to 70 years of age were included in the study. More than half (54%) of the subjects were in the age group of 40-49 years. Majority of the subjects (75%) were females. 33% of subjects had no formal education followed by 21% subjects with Degree/diploma (21%).

Less than one fourth (23%) of subjects belonged to the middle class followed by upper middle class (21%). More than half (57%) of the subjects were Hindu. Less than half (47%) of the subjects had SBP in between were 120-129 mm of Hg. Only 17% of respondents had Systolic Bp between 130-139 mm of Hg followed by 7% between 140-160 mm of Hg.

10% of males were current smokers and 18% were current drinkers. On the other hand all the female respondents were lifetime abstainers. Most of the subjects (88%) were non-Diabetic. Only12 % of subjects had Diabetes and was on regular treatment. In terms of Body Mass Index (BMI) Less than half (42%) had normal BMI, less than one third (31%) of subjects were overweight and 20% of subjects were obese. Majority (76%) of subjects was vegetarian and one-fourth (24%) were non- vegetarian. More than two-third (67%) of subjects were engaged in some kind of physical activities. Less than half (48%) of the population has abdominal obesity.

Figure 1 shows Less than Two-third (62%) of subjects has less than 10% CVD risk, followed by less than one-third (31%) of the subjects has CVD risk of between 10- to <20% for the next 10 years. Only 6 subjects had 20-<30% of risk followed by 1% CVD risk between 30-<40%.

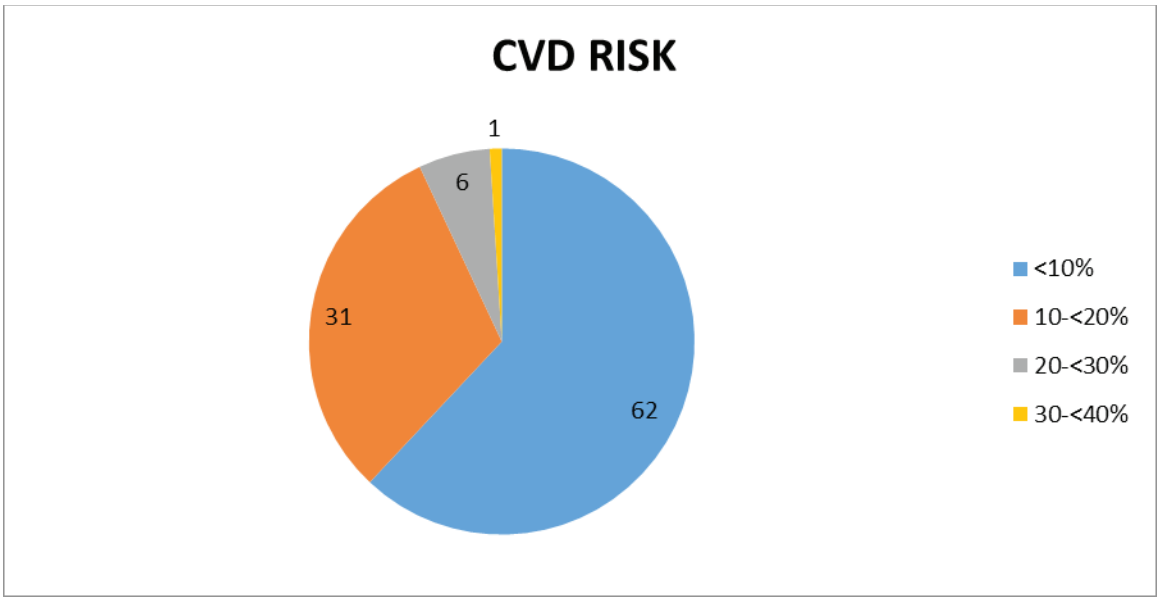

Figure 1: Pie chart showing CVD risk among study participants

Table 2: Chi-square value showing association of CVD with selected variables

N=100

Variable	CVD Risk		p
1.Age in Years	<10% n=62	>10% n-38	
40-49	48	06	<0.0001
50-59	12	08	
60-69	02	24	
2.Gender			
Male	13	12	0.23
Female	49	26	
3.Socio-economic status			
Upper class	12	08	0.50
Upper middle class	15	06	
Middle class	11	12	
Lower middle class	12	07	
Lower class	12	05	
4.Systolic BP (mm of Hg)			
Less than 120	25	04	< 0.0001
120-129	29	18	
130-139	07	10	
140-160	01	06	
5.Smoking			
Yes	01	09	<0.0001
No	61	29	
6. Diabetes Mellitus			
Yes	1	11	<0.0001
No	61	27	
7.Alcohol Use			

Cont... **Table 2: Chi-square value showing association of CVD with selected variables**

N=100

Never	57	25	<0.0001
Current Drinker	05	13	
8.BMI (Kg/m2)			
Underweight	05	02	<0.0001
Normal	33	09	
Overweight	20	11	
Obese	04	16	
9.Diet			
Vegetarian	59	17	1.0
Non-vegetarian	03	21	
10.Physical Activity			
Yes	08	23	<0.0001
No	54	15	
11. Abdominal Obesity			
Yes	21	27	<0.0001
No	41	11	

Table 2 shows that the CVD risk was found to be significantly associated with Age, SBP, smoking, diabetes mellitus, alcohol consumption, BMI, Physical activity, Abdominal obesity (p=<0.0001).

Discussion

With the increase in the burden of Non-communicable diseases, CVD being a leading cause of premature death. Prevention of CVD is an essential component in reducing overall mortality related to NCDs. Detecting risk factors contributing towards NCDs at the earliest along with predicting next 10 years of CVD risk helps in preventing any unexpected outcome in the future. In present study, less than one-third (31%) of the subjects has moderate risk for next 10 years. A study conducted in Salem (Premanandh K, Shankar R (2018)) revealed that most of subjects had low CVD risk.[4] A study was conducted in Malaysia (2013) with a cross-sectional design. Total 196 subjects (aged 40-70 years) were selected who was attending outpatient clinic, USM hospital. Results of study showed that majority of patients showed <10% CVD risk.[5] A study conducted in three countries of Asia (Cambodia, Malaysia and Mongolia) on adults of age 40-64 years using data from national STEPS survey. It was found that majority of people were on developing CVD risk of <10% in next 10 years. In Mongolia (89.6%) to 94.4% in Malaysia and 97% in Cambodia.[6]

In the present study there was significant relationship was seen between age and risk of CVD. The results was similar with the findings of Dhungana RR et al which was conducted to assess the Risk of CVD among adults in Kathmandu, Nepal.[7] The results was also consistent with the results of a study carried out in rural areas of north India by Bansal P et al (2016).[8]

The present study identified high blood sugar and high systolic Blood pressure as strong foretell of CVD risk. This result was similar to the findings of a study carried out in rural Nepal that concluded Increase blood pressure and presence of diabetes mellitus are the strong risk factors for the development of CVD.[7] A study done in 2013 by Norhayati et al identified diabetes and hypertension as a major factor in contributing towards cardiovascular diseases.[5] The results was also similar with the results of a study carried out in rural areas of north India by Bansal P et al (2016).[8]

Study result showed abdominal obesity had a higher cardiovascular risk compared with normal individuals, and was statistically significant (p=0.0001). Waist circumference (WC) used as a measure of central

obesity. It is simple to measure with very few articles and considered as more practical. In present study Alcohol consumption was also a strong risk factor associated with CVD. A cross-sectional study in Salem (Premanandh K, Shankar R (2018)) also showed that abdominal obesity and alcohol consumption were significantly associated with higher CVD risk.[4] A study with community based approach and cross-sectional design was carried out in rural areas of Mysuru concluded that High BMI, abdominal obesity, smoking and alcohol is significantly related to Increasing the risk of CVD.[9]

Conclusion and Recommendation

WHO/ISH CVD risk charts which are simple and colour coded chart can be used as a tool for early screening of CVD's among high risk group especially in low-socio-economic countries by the peripheral health workers. This can further helps the Governments in planning and implementation of strategies and policies among identified high risk population which can prevent further catastrophe of cardiovascular diseases.

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Conflict of interest: No conflict of interest

Ethical Clearance: Taken from Faculty of Nursing, MM (Deemed to be) University, Mullana, Ambala.

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Observational Study to Compare Transdermal Nitroglycerin and Placebo Patch in Enhancing the Post-Operative analgesic Effect of Intrathecal Clonidine with Bupivacaine in Abdominal Hysterectomies

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Abstract

Background: Hyperbaric Bupivacaine 0.5% is the most commonly used for spinal anaesthesia.To improve the quality & duration of analgesia many adjuvants have been used.Intrathecal clonidine binds with postsynaptic alpha-2 receptors at the dorsal horn of the spinal cord,resulting in anti-nociceptive action.Transdermal nitroglycerin (tNTG) has been found to be converting nitroglycerin to Nitric Oxide(NO),which results in pain modulation in the central and peripheral nervous system by cyclic Guanosine Monophosphate(cGMP) through which the post-operative analgesia effect enhances.

Method: Sixty patients Of ASA I/II aged between 20to60 years posted for hysterectomy under spinal anaesthesia were selected.Patients were randomly allotted into 2 equal groups.

Group A received:Hyperbaric bupivacaine 0.5% (17.5 mg)+clonidine(50 mcg) + placebo patch

Group B received:hyperbaric bupivacaine0.5% (17.5 mg) + clonidine (50 mcg) + nitroglycerin (tntg) patch(nitroglycerin 25 mg/patch)

We have observed onset and duration of sensory.and motor block,two segment regression time, duration of analgesia, hemodynamic changes and complication if any.

Results: There was no statistically significant difference in demographic data and onset and duration of motor and sensory block. Duration of analgesia was significantly prolonged in clonidine with tNTG group as compared to clonidine group with placebo group.

Conclusion: Transdermal Nitroglycerin patch with intrathecal clonidine prolonged duration of analgesia without any changes in onset and duration of sensory and motor block.

Keywords: *Bupivacaine, Clonidine, Spinal Anaesthesia, Transdermal Nitroglycerin (tNTG) patch*

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Introduction

Pain is always a conscious and subjective perception that cannot be satisfactorily defined objectively[1]. Morgan & Mikhail Said "Pain.is an unpleasant emotional and sensory experience.associated with actual.tissue damage. Acute postoperative pain management is a key aspect of postoperative care, as acute pain, regardless of its site,

can adversely affect nearly all organ function, and so affects the postoperative mortality and morbidity"[1]. The aim of an anaesthesiologist is to render the patient pain free, during a surgical procedure and provided effective pain relief in initial postoperative period[1].

The subarachnoid blockade is the common form of central neuraxial blockade performed for abdominal hysterectomy. The disadvantage of spinal anaesthesia using hyperbaric bupivacaine alone is relatively shorter duration of action[2]. Postoperative analgesia can be provide by adding adjuncts like α_2 receptor agonist[2] (clonidine, dexmedetomidine), opioids[3] (fentanyl, morphine,etc.), benzodiazepines[4,5] (midazolam), ketamine[6] etc to intrathecal local anaesthetic drugs.

Clonidine is frequently used drug as an.adjuvant in spinal anaesthesia to improve the quality.and duration of post-operative analgesia[2]. Clonidine is α2 adrenergic receptor agonist and it have been the focus of interest due to analgesic, perioperative sympatholytic and sedative properties[2]. Its analgesic effect is due to binding with. postsynaptic α2 receptors (G-protein coupled inhibitory receptors) in the dorsal horn of the.spinal cord[2].

Transdermal nitroglycerin.helps in enhancing the post-operative analgesia effect of intrathecal.clonidine[7], fentanyl[8] and neostigmine[9] by release of nitric oxide(NO).

Nitroglycerine is short acting nitrates group of drug. Nitroglycerin converted to Nitric Oxide(NO). This NO increases the concentration of intracellular cyclic Guanosine Monophosphate (cGMP). NO-cGMP.. cascade in endothelial cell has been found to mediate Ach-induced vasodilatation[10] as well as Ach or morphine induced anti-nociception[11], which produces pain.modulation in the peripheral and central.nervous system .

Nitroglycerine is readily absorbed from the skin[12]. A transdermal patch in which drug is incorporated into a polymer bounded to adhesive plaster and the drug is held in a reservoir between an occlusive backing film and a rate controlling micropore membrane[12]. The micropore membrane is such that rate of delivery of

drug to skin surface is less than the slowest rate of absorption from skin, so providing steady delivery for 24 hrs.[12]

Method

This was an observational study, conducted at the Anaesthesia Department, S.B.K.S. Medical Institute. and Research Centre, Piparia, Vadodara. in 2014-2016. After obtaining approval from the ethical committee, we conducted a study on 60 patients of American Society of Anaesthesiologists (ASA) grade I and II aged between of 20-60 years, who were admitted for abdominal hysterectomy under spinal anaesthesia. The patients were randomly.divided by chit.method into two equal groups.

Total Sample Size: 60 patients; Group - A (30 patients) & Group – B (30 patients)

Group A: 0.5% hyperbaric bupivacaine (17.5 mg) + clonidine(50 mcg)+ placebo patch

Group B: 0.5% hyperbaric bupivacaine (17.5 mg) + clonidine (50 mcg)+ nitroglycerin (tntg) patch(nitroglycerin 25 mg per patch)

Patients with medical complications like anemia, heart disease, severe hypovolemiac shock, septicemia, hypertension, coagulation disorders or on anticoagulant therapy, local. infection at the site of puncture for spinal anaesthesia and patient's refusal were exclude from the study. Pre anaesthetic checkup and basic investigations were done. Written, informed consent was obtained. On the day of surgery, the patients were brought to the pre anaesthetic room and base line vital parameters (pulse rate, blood pressure, respiratory rate, SpO2 and temperature) were recorded.

IV line was secured in each patient and preloading was started with Ringer lactate (RL) 10ml/kg/hr 30 minutes before surgery. Transdermal Nitroglycerin (tNTG) patch or placebo patch were applied over the right side of chest region as per group.

Patients were premedicated with Inj. Glycopyrolate 0.004mg/kg, Inj. Ondensetron 0.08mg/kg and Inj. Ranitidine 1mg/kg intravenously.

Standard monitors were applied including.ECG (Electrocardiogram) leads, NIBP (Noninvasive Blood Pressure) cuff, oxygen saturation.probe and baseline parameters like Pulse rate (PR), Systolic.and Diastolic

Blood Pressure (SBP and DBP) and SpO2.were recorded.

Under all aseptic & anti-septic.precautions lumbar.. puncture was performed at L_2-L_3interspace using a 25G Quincke's type spinal needle with patient in left lateral position. Study drug was injected into.the subarachnoid space after free flow of CSF at the rate of 1ml given in 3 seconds.

Onset of sensory and motor block as well as duration of sensory and motor block, two segment regression time and duration of analgesia were noted.

The onset of sensory block was measured from the time of injection till T10 sensory block was achieved which was determined bilaterally using pin prick test and cold test using.spirit.

Duration of block was measured from time of onset till the sensory blockade was regressed to L1 dermatome.

Motor blockade was tested by Bromage scale[13].

Bromage scale:

· Bromage 0: the patient is able to move the hip, knee and ankle.

· Bromage 1: the patient is unable to move the hip, but is able to move the knee and ankle.

· Bromage 2: the patient is unable to move the hip and knee, but is able to move the ankle.

· Bromage 3: the patient is unable to move the hip, knee and ankle.

Duration of motor block assessment was done from the time of onset (Bromage scale ≥1) till normal motor function returned.

All patients of.both groups were monitored.for Heart rate (HR), blood pressure (SBP&DBP), Oxygen Saturation (SpO2) at 0,3, 5, 10,15,30,45,60 minutes and then half hourly till the surgery was completed and then for every 15 min for next one hour.If the systolic arterial pressure (SAP) decreased more than 30% below the pre- anaesthetic value, was considered to be significant hypotension and Inj.Mephenteramine 6 mg in increments was given intravenously.

Significant Bradycardia (HR <60 beats min–1) was treated with Inj.Atropine sulphate 0.6 mg intravenously.

Post-operative.pain was.assessed with **VAS (Visual Analogue Scale).**[13]

Duration of analgesia was assessed from onset of sensory block upto 1st rescue analgesic requirement (VAS score≥4)

For visual analogue score, the patient was taught.on 10cm scale telling that 0 (zero) represents 'no pain' and 10 represent 'worst pain'.

0	1	2	3	4	5	6	7	8	9	10
No	**Mild Pain**			**Moderate Pain**			**Severe Pain**			
	Good coughing on physiotherapy & no assistance in ambulation			Poor cough Require moderate assistance to ambulate			Unable to cough Require full assistance to ambulate			

When the VAS ≥4, rescue analgesic (Inj. Diclofenac 75mg I.M.) was given.

Side effects like nausea, vomiting, bradycardia, hypotension, rigors were noted intra and postoperatively and treated accordingly if any. Patients were also observed for delayed side effects like headache & backache for 3days.

Observation & Results

Study was carried out on 60 patients.belonging to ASA I & II undergoing Abdominal.hysterectomy at S.B.K.S M.I. & R.C., Dhiraj Hospital, Piparia, Vadodara.

The distribution of patients with respect to demographic data and ASA Grading was statistically not significant in both the groups.

Table-01: Mean onset and duration of sensory and motor Block

	GROUP A	GROUP B	P value
	MEAN± SD (Minutes)	MEAN± SD (Minutes)	
Onset of.sensory block	7.93±0.78	8.06±0.82	0.531
Duration.of sensory block	137.83±7.03	138.66±6.81	0.644
Onset.of motor block	6.13±1.40	6.03±1.37	0.774
Duration.of motor block	176.83±7.12	177.83±8.37	0.620

The difference between the mean onset and duration of sensory block and mean onset and duration of motor block in both groups was statistically not significant. (p value > 0.05) (Table: 1)

Table-02: Pre operative, intra operative and post operative mean pulse rate and mean oxygen saturation

	Pulse rate			Oxygen saturation (Spo2)		
Time Interval (min)	Group A	Group B	P value	Group A	Group B	P value
	Mean± SD	Mean± SD		Mean± SD	Mean± SD	
Pre op	104.66±10.5	102.06±7.65	0.27	99.1±0.84	98.96±0.92	0.54
0 min	88.13±5.79	88.33±5.40	0.89	99.93±0.25	99.76±0.56	0.13
3 min	80.46±4.09	80.13±4.66	0.77	99.7±0.74	99.9±0.30	0.17
5 min	74.13±2.28	74.53±3.52	0.60	99.96±0.18	99.76±0.56	0.19
10 min	70.06±2.80	71.53±4.89	0.15	99.83±0.53	99.76±0.56	0.77
15 min	65.6±1.92	65.33±2.18	0.61	99.8±0.55	99.83±0.46	0.81

Cont... **Table-02: Pre operative, intra operative and post operative mean pulse rate and mean oxygen saturation**

30 min	68.13±3.96	67.8±2.12	0.68	99.86±0.43	99.76±0.56	0.75
45 min	63.53±3.47	63.26±2.43	0.73	99.8±0.66	99.9±0.30	0.45
60 min	62.53±1.88	62.26±2.08	0.60	99.86±0.34	99.8±0.61	0.60
90 min	61.75±2.23	61.5±2.22	0.69	99.79±0.50	99.79±0.50	1.00
120 min	60.33±0.81	60±1.63	0.67	99.66±0.51	99.5±0.57	0.65
Post op-0 min	63.86±3.10	64.33±2.46	0.51	99.86±0.43	99.83±0.46	0.79
Post-op 15 min	70.26±2.21	69.73±4.60	0.56	99.86±0.43	99.83±0.46	0.79
Post op 30 min	73.76±2.92	74.13±3.23	0.64	99.86±0.43	99.9±0.30	0.67
Post op 60 min	81.53±3.01	81.33±3.29	0.80	99.73±0.73	99.73±0.73	1.00

There was statistically no significant difference in pulse rate and Oxygen saturation (Spo_2) between the two groups (p value > 0.05) at any interval of time during the pre operative, intra-operative and post operative period. (Table: 2)

Table-03: Pre operative, intra operative and post operative mean Systolic blood pressure & mean diastolic blood pressure (in mm of hg)

	Systolic BP			**Diastolic BP**		
Time Interval (min)	**Group A**	**Group B**	**P value**	**Group A**	**Group B**	**P value**
	Mean± SD	**Mean± SD**		**Mean± SD**	**Mean± SD**	
Preop	137.13±4.25	137.93±4.40	0.48	87.73±2.76	87.66±2.88	0.92
0 min	123.46±5.19	114.4±4.56	0.0001	82.73±2.19	74.33±3.28	0.0001
3 min	105.46±4.51	98.06±3.03	0.0001	73.8±2.89	68.4±1.92	0.0001
5 min	101.2±1.78	98.66±2.53	0.0001	70.4±1.77	64±2.40	0.0001
10 min	92.93±2.77	89.46±3.14	0.0001	65.13±2.01	59.4±1.40	0.0001
15 min	107.46±4.32	103.93±3.42	0.0009	60.93±1.36	57.8±2.48	0.0001
30 min	103.26±6.67	100.26±4.54	0.046	63.86±4.13	60.93±1.36	0.0005
45 min	106.93±4.57	101.6±4.37	0.0001	62.33±3.64	60.06±3.084	0.011

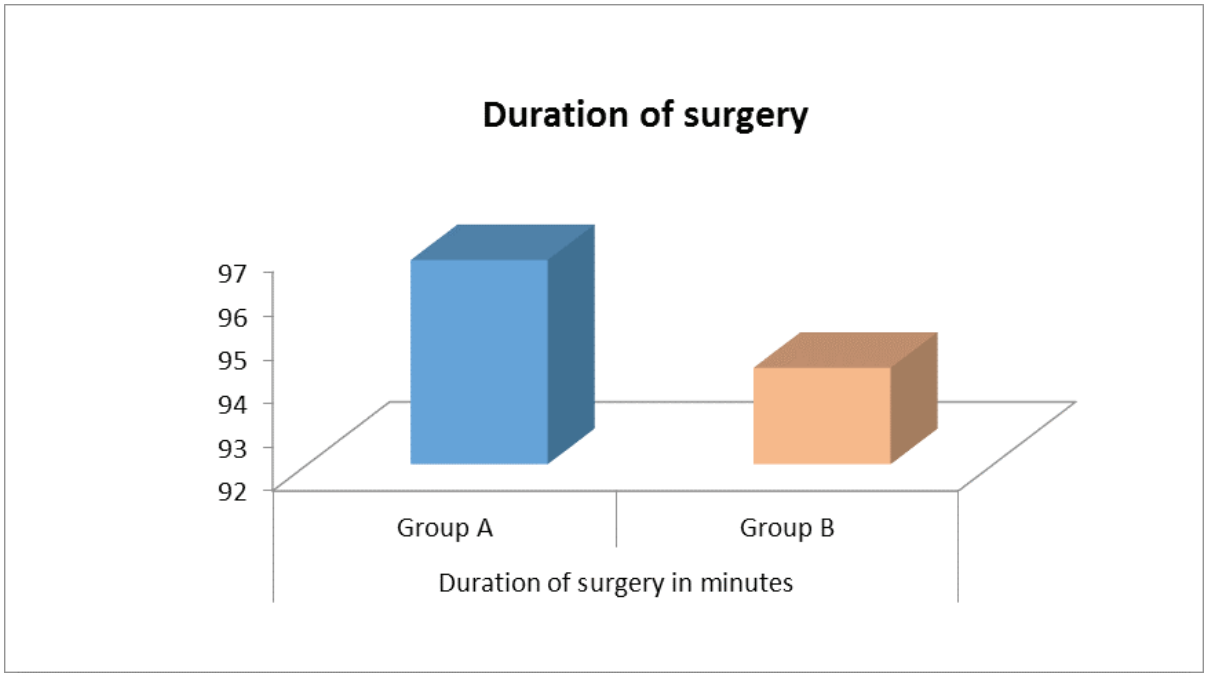
Cont... **Table-03: Pre operative, intra operative and post operative mean Systolic blood pressure & mean diastolic blood pressure (in mm of hg)**

60 min	103.6±4.59	100.53±3.78	0.0060	60.2±3.20	58.66±2.05	0.048
90 min	103.83±4.56	99.91±3.65	0.0019	63.08±3.82	60.91±2.70	0.02
120 min	102.66±5.75	102.5±1.91	0.95	63.66±3.20	63.5±2.51	0.93
0 min	103.73±4.41	103.06±4.77	0.48	65.33±2.53	65.06±1.46	0.61
Post op 15 min	104.86±5.52	104.06±2.8	0.48	70.6±2.17	70.26±2.14	0.54
Post op 30 min	107.36±5.38	108.93±4.77	0.23	73.2±3.46	73.66±3.15	0.59
Post op 60 min	113.9±6.95	112.93±4.97	0.52	75.13±2.81	75.33±3.25	0.79

There was no statistically significant difference in pre-operative SBP and DBP between the two groups (p value > 0.05). (Table: 3)

Intra-operatively, SBP and DBP were lower in Group B compared to Group A which was statistically significant (p value < 0.05). At 120 min there was no statistically significant difference in SBP between the two groups (p value > 0.05). (Table: 3)

In post operative 1 hour period, there was no statistically significant difference in SBP and DBP between the two groups (p value >0.05). (Table: 3)

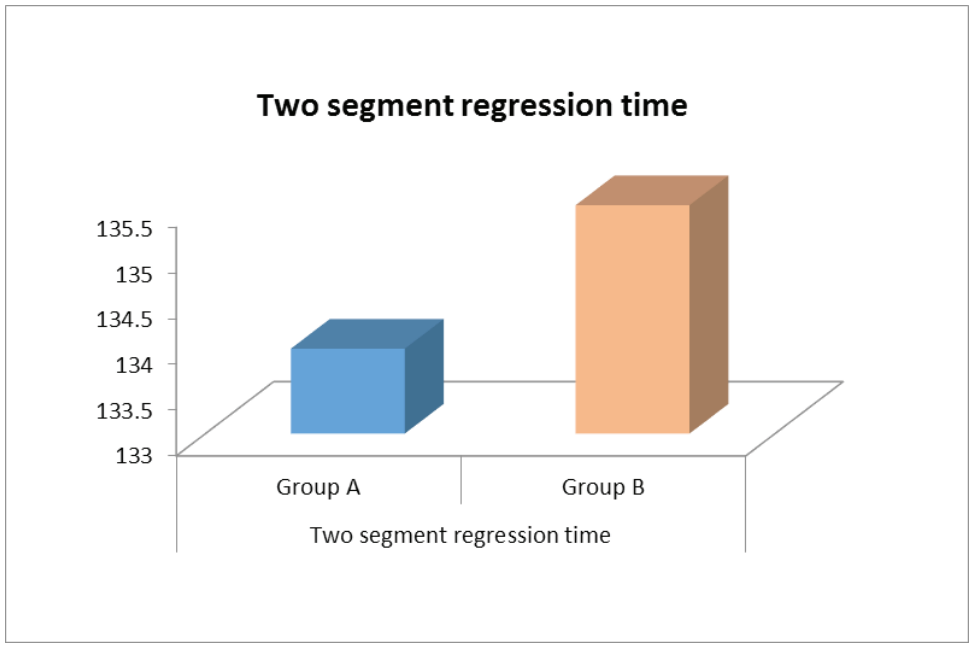


Graph 1: Mean duration of surgery

The mean duration of surgery was 96.66 ± 18.60 minutes in group A and 94.2 ±

17.07 minutes in group B, which was comparable and statistically not significant

(p value > 0.05).



Graph 2: Mean Two Segment Regression (in min.) in both groups

The mean two segment regression was 133.93 ± 8.28 minutes in group A & 135.5

± 8.13 minutes in group B which was comparable and statistically not significant

(p value > 0.05).

Table -4. Mean duration of analgesia (Time of 1St rescue analgesia)

Time of 1St rescue analgesia	Group A	Group B	P value
	Mean± SD	Mean± SD	
Time Interval (Min.)	281.66±25.16	397.33±30.73	0.0001

The mean duration of Analgesia (Time of 1St Rescue Analgesia) was 281.66666 ± 25.16611 minutes in group A and 397.3333 ± 30.7305 minutes in group B which was tatistically very significant (p value <0.05).(Table:4)

Table 5: Complications or Side Effects

Complications Or Side Effects	Group A	Group B
Bradycardia	00 (0.0%)	03 (10%)
Hypotension	16(53.33%)	20(66.66%)
PONV	00 (0.0%)	00 (0.0%)
P/O Headache	00 (0.0%)	00 (0.0%)
P/O Backache	00 (0.0%)	00 (0.0%)
Allergic Reaction	00 (0.0%)	00 (0.0%)

In our study, we observed 10% patients in Group B showed bradycardia and

66.6 % patient showed hypotension,while in Group A 53.3% patient showed hypotension and none of patient showed bradycardia.

Discussion

Spinal anaesthesia is the most commonly used regional anaesthesia technique. One disadvantage is that with spinal anaesthesia, there is an early need of analgesia for the postoperative pain[12].

There is a need for an adjuvant which increases the duration of analgesia without increasing the duration of motor blockade, prolonging postoperative analgesia, reducing postoperative analgesic requirements, without significant side effects and facilitating early ambulation with reduction in hospital stay of the patient[13].

In our study, we had added 50 mcg of clonidine to hyperbaric bupivacaine in both groups. Out of this, Group A received placebo patch and Group B received transdermal Nitroglycerine (tNTG) patch of 25 mg before spinal anaesthesia.

Racle et al (1987) [17] in their study used 150 mcg of clonidine interathecally with isobaric bupivacine for hip surgery. He observed prolongation in motor block and two segment regression, but showed more episode of hypotension in clonidine group. The aim of combining. different analgesic drugs is to.obtain synergistic or additive action which allows the use.of a smaller dose of each agent, hence improving the safety profile and reducing related side effects[15].

NO is a molecule with different effects; although it is essential in nociception, it is also an important messenger in tonic cholinergic pain inhibition[15]. NO-cyclic guanosine monophosphate (cGMP) cascade is involved in acetylcholine or morphine-induced peripheral antinociception.[15] The activation of descending pain pathway has been shown to involve the participation of NO through a mechanism of action that is likely to include activation of second messengers such as cGMP[17] . Wide-dynamic-range neurons in the. superficial dorsal horn and high-threshold cells in the superficial or deep layers show.reduced response after exposure to (cGMP)[17]. Pre-emptive usage of 25 mg transdermal nitroglycerine patch (NO donor) in patient undergoing surgery under spinal anaesthesia resulted in significant prolongation of postoperative analgesia and decreased postoperative analgesic consumption.

Khandawal M et al[7], Ahemed F et al[18] in their studies observed that onset and duration of sensory and motor block was statistically not-significant. This result is similar to our study.

Ahmed F et al[18] demonstrated no clinical significant difference in the hemodynamic parameters like heart rate, systolic blood pressure and diastolic blood pressure and oxygen saturation (SpO_2) which is similar to our study.

Khandelwal M et al[7] observed two segment regression in placebo and NTG patch was 136 ± 19 and 139 ± 21 minutes respectively which was statistically not significant. This results are comparable to our study.

Lauretti GR et al [9] in their study they observed time of first rescue analgesia was longer in nitroglycerine patch group as compared to control group this difference was statistically significant. We also observed that.the time of first rescue analgesic dose in the Group B (397. 33± 30.73 min.) was longer as compared to Group A (281.66± 25.16 min.) , which was statistically highly significant, this is due to the synergistic effect of nitroglycerine patch in groupB.

Khandelwal M et al [7] in their study, observed 36% patients showed hypotension and 3% patients showed bradycardia in clonidine group and 40% patients showed hypotension and none of patients had bradycardia in nitroglycerin patch with clonidine group, without any major complications.

In our study, 10% patients showed bradycardia and 66.6% patient showed hypotension in Group B, in Group A 53.3% patient showed hypotension and none of patient had bradycardia. Clonidine leads to vasodilation, decreased in blood pressure, heart rate and cardiac output[19]. Nitroglycerin also leads vasodilation and decreased in blood pressure[20]. There for when used together chances of hypotension are more.

Conclusion

We conclude that addition of transdermal Nitroglycerin (tNTG) patch (25 mg) with 50 mcg intrathecal Clonidine in 0.5 % hyperbaric bupivacaine

in spinal anaesthesia leads to prolonged duration of analgesia (Time of 1st rescuer analgesia), without any changes in onset and duration of sensory and motor blockade.

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Development of Simulated Educational Joint Mobilization Module Using Leap Motion Controller

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Abstract

Background: Joint mobilization is a complicated task in terms of learning as well as teaching and is characterized by great inter subject variability. With reference to the acquisition of skills such as joint mobilisation, only a few studies have investigated the effectiveness of the provision of real-time visual feedback on a computer screen in terms of students' ability to establish optimal force and demonstrate adequate performance. There is lack of training in theory and practical skills in mobilization given to the Physiotherapy students. Thus, an effort has been made in to practice it virtually using Leap Motion Controller.

Method: Total 138 participants will be recruited as per the inclusion and exclusion criteria. A brief explanation about the study will be given. Participants will be given exposure to the software and modality being used along with demonstration. Followed by, a questionnaire to evaluate the effective learning using the System Usability Scale.

Conclusion: The results from the study will significantly provide efficacy of the simulative device and evidence on the use of LMC for learning joint mobilization using the System Usability Scale.

Key words: *Biomechanics, Glenohumeral Joint, Mobilization, Shoulder Joint, Simulation*

Introduction

In healthcare professions, innovative methods and modalities are actively pursued to improve teaching and learning, and followed by patient care and outcomes[(1)]. Digital education could include a range of approaches focused on learning tools, ideas, material, objectives, teaching methods and delivery environment. Studies on the use of digital technologies in healthcare education have generally shown its advantages over traditional learning in terms of improved diagnostic reasoning skills, interpersonal and professional skills, long-term knowledge retention, problem-solving skills, self-direct / life-long learning skills, higher-order thinking skills, self-perception and trust[(2)]. Virtual Reality (VR) is a groundbreaking advanced system of technology that allows users to share a ' virtual world'. Potential advantages for VR in medical education are massive and recent advances in technology and improved accessibility has made it the exciting and evolving sector today. Their use in undergraduate training as a complement to conventional seminars, lessons and self-directed learning continues to be less well studied; however, there is evidence that VR has been shown to improve the retention of information and study drive. VR headsets produce 360-degree immersive images that can shift as the person moves around the world in real time[(3)].

Therapeutic touch has been used in human beings to soothe aches and pains. Manual therapy techniques are skilled hand movements intended to improve tissue extensibility; increase range of motion (ROM); induce relaxation; mobilize or manipulate soft tissue and joints; modulate pain; and reduce soft tissue swelling, inflammation, or restriction. The primary techniques included in manual therapy are mobilization and

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manipulation of joints and associated soft tissues[4]. Rationales for the reasons why manual therapies may work have been investigated and include reducing muscle inhibition, correcting joint displacement, adjusting joint subluxations, restoring bony alignment, reducing nuclear protrusion[5], and placebo effect[6,7]. Manual therapy is indicated for pain and loss of motion that occurs secondary to neuromusculoskeletal dysfunction. The patient may have pain with motion or rest, pain in the midst of available ROM or at the end of available ROM, or pain or stiffness caused by postural changes[4].

Joint mobilization is a complicated task to learn and to teach and is characterized by great inter subject variability. Joint mobilization, a form of passive movement with the application of force to the joint in a specific direction, is a common therapeutic technique used by orthopedic physical therapists to reestablish the arthrokinematics, osteokinematics, and function of a joint[8]. Skill acquisition usually involves the use of different modes of feedback to enhance motor learning. The traditional methods of learning joint mobilization include instructor demonstrations and students practicing on each other. Information, including the perceived resistance and movement by instructors, is conveyed to the student while the technique demonstrated on the student. The nature of feedback given to the subject is one of the empirical factors affecting a skill-acquisition process and its outcome[9].

The last decade has seen the proliferation of simulation in health care education. This trend has reached physical therapy education and is redefining experiences for physical therapy learners[10]. Physiotherapy education aims to prepare students to practice independently by developing complex clinical skills such as patient assessment, clinical reasoning, technical, communication and therapeutic skills[11].

Within clinical practice, simulation can support skill development, continued competency, and be part of a risk management approach to revisit a breakdown of care, evaluate a near miss event, or rehearse new workflow procedures. Simulations must be thoughtfully designed, executed, and followed with effective, structured debriefing to achieve the positive learning that will translate to improved clinical practice[10].

In the field of manual therapy with reference to the acquisition of skills such as joint mobilisation, only a few studies have investigated the effectiveness of the provision of real-time visual feedback on a computer screen in terms of students' ability to establish optimal force and demonstrate adequate performance[11]. There is lack of training in theory and practical skills in mobilization given to the physiotherapy students[9]. Thus, an effort is being made to learn and practice join mobilization using leap motion controller. Leap Motion's leap motion controller provides a means to capture and track delicate hand and finger movements, while manipulating a virtual environment that involves hand-arm coordination as part of virtual tasks[12].

The simulated joint to be used in the study will be the glenohumeral joint. The glenohumeral joint is a multiaxial, ball and socket type of synovial joint. The joint has 3 axes and 3 degrees of freedom involving flexion, extension, adduction, abduction, internal rotation and external rotation. With the contraction of the rotator cuff muscles, the humeral head is pushed or translate anteriorly, posteriorly, inferiorly, superiorly or in combination of these movements. The common pathologies occurring at shoulder joint are frozen shoulder, impingement syndrome, stiffness, shoulder pain, etc. for which the joint mobilization is frequently used treatment.

This study aims to evaluate the impact of leap motion controller(LMC) for training and practicing joint mobilization techniques at shoulder joint.

The objective of the study is to bear out the suitability of the system to be used as an aided method for learning mobilization of the shoulder joint. Aiming this, we'll investigate the following three aspects:

1. The capability of the system to assign different levels of mobilization grades of the shoulder joint; thus, to elucidate whether, despite the variable working loads, the training remained under controlled and moderate motion conditions according to the current clinical condition of the patient, to avoid any harmful movement, as a fundamental requirement in rehabilitation.

2. The effects of the variable difficulty training conditions on the kinesiologic participant's performance; then, to design a new predictive model of the participant's

progress, as a tool for the therapist for individualising the training intensity.

3. The evaluation and acceptance of the system by participants.

Materials and Method

Study Setting: This trial will be carried out in the HumEn research lab of Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University.

Study design: Quasi experimental study (one group design)

Sample size: The participants enrolled in this experimental study is 138 (n = 138). Before inclusion, all the participants will be explained about the purpose and procedures of the research.

Participants:

The inclusion criteria for the participants are as under:

1. Second to final year undergraduate students of physiotherapy.

2. Those who are willing to partake in the study and sign the informed consent form.

The exclusion criteria for the participants are as under:

1. Those with fracture of upper limb.

2. Those with any neurological pathology of the UL

3. Those having any psychological problems.

4. Those unwilling to participate.

Study duration: 6 months

Outcome measures:

Primary Outcomes:

1. SUS (System Usability Scale)

2. Student satisfaction with the approach used

Secondary Outcomes:

1. Time to teach the technique

2. Time to perform the technique

Instrumentation:

1. Leap motion controller

2. LED screen

Study procedure:

In collaboration with IIT, Nagpur, a software program will be developed depicting the virtual 3D anatomical model of the shoulder joint , which will be mobilized with Leap Motion Controller. It will be designed such that the participants can view the model in 360 degrees. Glides will be given virtually in real time to carry out mobilization. Different pathological conditions of shoulder will be demonstrated in the virtual environment and the subjects will be instructed to carry out mobilization under the supervision of PI. The direction of the glides will be marked by an arrow. The subjects will be trained by the PI to carry out mobilization via LMC. Further the use of haptic gloves can also be considered so that the subjects will have the actual feedback of how much pressure to apply while giving the glides.

After getting approval by the ethical committee , informed consent from the participants will be taken. Proper Information about the shoulder joint's osteokinematics and arthrokinematics will be given before training the participants. Further, the subjects will be trained to use the devices properly for which practical demonstration will be given.

The subjects are required to fill the questionnaire for feedback at baseline and after the completion of the program.

Mobilizations that are commonly used include posterior/anterior glides, superior/inferior glides. Posterior glide is used to increase flexion and internal rotation. Anterior glide is used to increase extension and external rotation. Inferior glide is used to increase abduction range ofshoulder joint. Grade I and grade II mobilization will be considered first[13].

Statistical analysis plan:

Analysis of data will be carried out using concise and inferensive statistics using unpaired t test students in chi square. The software used in the study will be the SPSS 24.0 version, the Graphpad prism 7.0 version and the degree of significance < 0.005 (p>0.005 m) is considered.

Ethics And Dissemination:

The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of inclusion and exclusion criteria, the patients are taken for review.

Observation And Expected Results:

The study's expected outcome will concentrate on evaluation of efficacy of the simulative device using the System Usability Scale to develop shoulder joint mobility. The results from the study will significantly provide evidence on the use of LMC for learning joint mobilization.

Discussion

This study protocol aims to assess the efficacy of the simulative device using the System Usability Scale to develop shoulder joint mobility. The purpose of this study will help to explain the process of creation of the simulative device designed to improve shoulder joint mobility. The study's expected outcome will concentrate on the evaluation of the usability of simulative device and the impact of LMC for training and practicing joint mobilization techniques at shoulder joint.

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Conflict of Interest: The authors declare no conflict of interest.

Funding Source: The research has not received any external funding.

Informed Consent: Written & Oral informed consent will be obtained from all individual participants included in the study. Additional informed consent will be obtained from all individual participants for whom identifying information is included in this manuscript.

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Perception of Nursing Students Regarding Clinical Learning Environment, Supervision (CLES)

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Abstract

Background: In nursing education, clinical placement is a vital part of nursing students' education. The clinical learning environment is considered as first place of professional practice for nurses and student's opinion contributes to its improvement. Theaim of the current study was to assess the perception of nursing students regarding clinical learning environment supervision in obstetric and gynecological department. **Method:** A quantitative research approach with descriptive survey research design was used to collect data from 222 nursing students using total enumerative sampling technique from September 2017 to April 2018. Clinical Learning Environment and supervision scale (CLES) was used to assess the perception of nursing students with their clinical learning environments. **Results:** The study results showed that the sub domain of supervisory relationship (90.62%) was having highest mean percentage and relationship between student, staff and teacher sub domain has lowest mean percentage (84.38%). The nursing students evaluated the sub domain of relationship between student, staff and teacher (22.07%) as 'need to improve' andintegration of theory and practice (67.57%) as 'good.'There is significant positive co-relation between all domains of clinical learning and supervision.

Conclusion: It was found that the student valued supervision, pedagogical and learning environment. Majority of nursing students experienced the content of supervisory relationship was the most important dimension in CLES. This means that nursing students were oriented tounit settings/ wards by clinical instructor/teacher, they felt that they receive individualized supervision and continuous feedback and suggestions of improvement from clinical instructors/teacher and there were frequent bed side rounds.

Key words: *Clinical Learning Environment & Supervision, clinical placement, obstetrics & gynecological nursing, nursing students.*

Introduction

The clinical learning environment is important in facilitating students to achieve their learning outcomes. Studies have shown that high quality of clinical learning environment and supervision has significant influence on the development of professional identity and competency[1] and clinical skills[2] among students. An effort to develop the quality experience within a supportive and pedagogically adjusted clinical learning environment is a concern for educational institutions[2]. Relevant studies revealed that quality of clinical learning environment usually depends on the quality of curriculum[3], supportive relationship between student and mentor, peer support and sense of belonging[4] among

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students. If students are welcomed, incorporated and appreciated in the clinical setting then the experience of clinical learning environment and supervision is considered as positive.[5]

The clinical learning environmentand supervision is an interactive forces' network within the clinical setting that influence the leaning outcomes. It comprises everything that surrounds students and affects their professional development in the clinical setting[4].When the students enter the unfamiliar area of the clinical setting, the atmosphere of this particular setting can have enduring influences on their learning experience[2]. The clinical learning environment can have a great influence in the development of the attitude, knowledge, skills, and problem solving ability of students who engage into this situation[3]. In addition, one to one supervision in the clinical setting has significantly increased students satisfaction. Although the basic premises for supervision include implementation, adequacy and effectiveness of supervision along with sufficient resources are dependent on the pedagogical premises of the work unit. Quality supervision is essential in which the individual learning needs and goals of the student are met[6].

In this context, development of decent learning environment depends on five distinctive constructs: the ward's pedagogical atmosphere includes teamwork and personnel's interest in students' learning needs; the supervisory relationship constructs reflect the sense of trust, student/mentor equality and continual feedback. The third construct is leadership style which represents the relationship between ward managers, staff and the students. The premises of nursing on the ward refers to the organization of the nursing care and the role of teacher in clinical practice reflects the teacher's ability to minimize the theory practice gap.[7]

Mostly studies have been done in the hospital setting to explore the attitude, satisfaction and perception of health care students regarding clinical learning environment[8]. Nursing is a respectable profession and the acceptability of male nurses in the hospital setting has increased over the years. A study showed that the male nurses' student expressed discomfort in the beginning, low acceptance and more role strain than the females especially while providing care to the mothers in the obstetrical and gynecological ward[9].It is beneficial to understand expectation of the students for their clinical experience to provide a positive CLE and proper supervision[6]. Hence, the investigators felt the need to assess the nursing students' perceptions with their clinical learning environment and supervision in a selected college of Nursing of Mullana, Ambala, Haryana.

Methods

A quantitative research approach was adopted witha descriptive survey design using the CLES (α=0.89) to collect data from nursing students**.** For the purpose of this study, required permission to use the CLES was obtained from its original developer through consecutive emails. CLES was developed and validated by Vikas Choudhary et al[8] which was designed toevaluate the nursing students' perceptions of clinical learning environment. The CLES scale has 53 itemsthat classified into three dimensions 1. Clinical learning environment 2. Role of clinical instructor/ teacher 3. Supervisory relationship. Nursing students' were asked to score their perception of each item on 3-point likert scale i.e. 3 (Always), 2 (sometime), 1(never). [5]

The ethical approval (IEC: 1307) for the study was obtained from the institutional ethical committee. Formal administrative approval was obtained from the Principal (M.M. College of Nursing, Mullana, Ambala, Haryana). Nursing students were explained the nature of the study and informed consent was obtained from the nursing students regarding their willingness to participate in the study.

Setting and sample

The study was conducted at M.M College of Nursing which has 1150 bedded parent hospital for clinical experience of nursing students. Study was conducted between September 2017 to April 2018. Totalenumeration sampling technique was used to select nursing students (N=222) Completed their posting in obstetrics and gynaecology nursing department during this time frame. They were assigned to clinical placements in the obstetrics and gynecological area and were posted into antenatal ward, labour room, postnatal ward, and postnatal intensive care unit, feeding rooms, high risk room, septic room, obstetrics and gynecological outpatient department (OPD), obstetric & gynecological

wards. The students spent 48 hours/week in the clinical area during their clinical placement and had teachers as their educational supervisor along with duty staff nurses. Data was collected after completion of their clinical posting in obstetrics and gynecology area via google form to get fair response and to maintain anonymity.

Results

Data were entered in Microsoft Excel 2007 and using SPSS (Armonk, NY:IBM Corp) version 20 for analysis. Majority (78.38%) of the nursing students were females **(FIGURE 1)**. Less than half (43.6%) of the nursing students were from B.Sc. Nursing 3rd year and only (2.25%) were from M.Sc. Nursing 1st and 2nd year. **(FIGURE 2)**

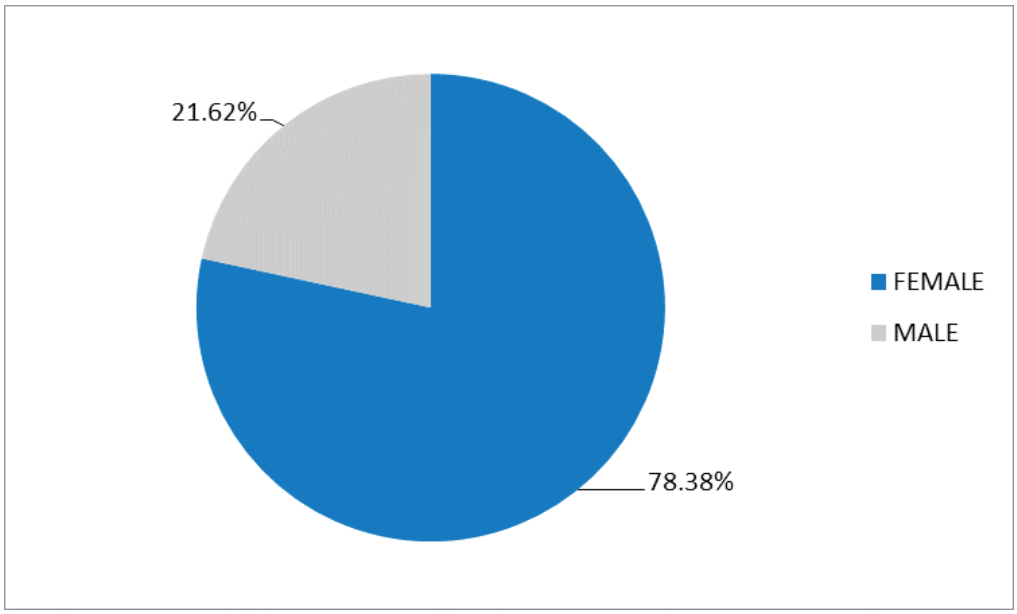

Figure 1: Pie diagram showing percentage distribution of students in terms of Gender.

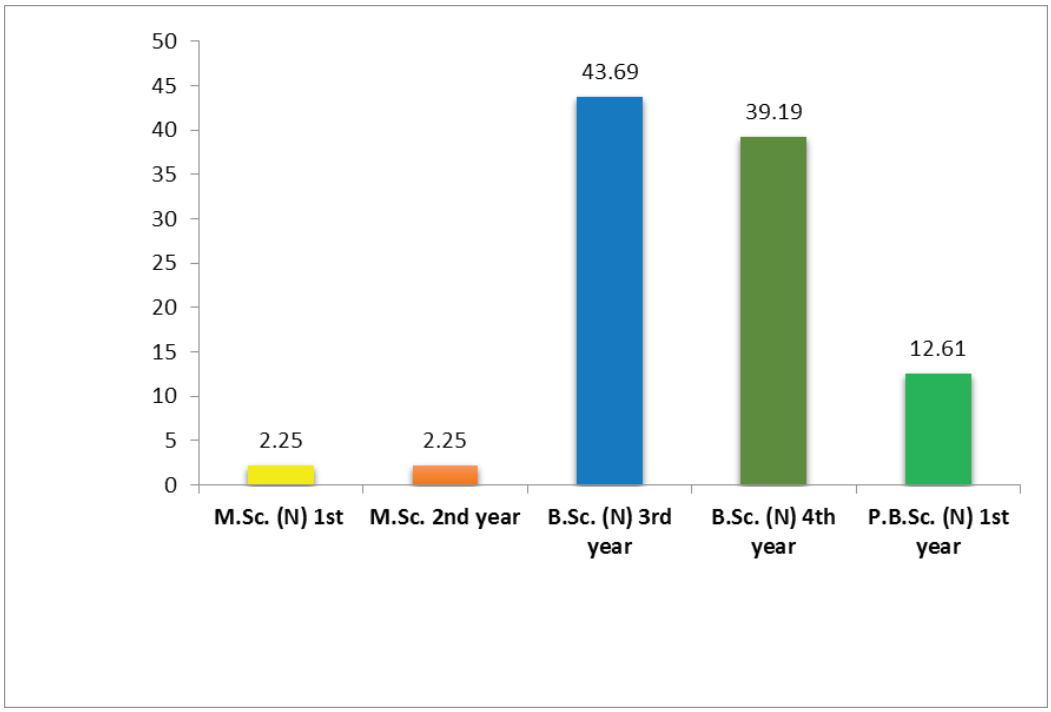

Table 1: Domain Wise Range, Mean, Standard Deviation, Mean Percentile and Rank of CLES

N=222

CLES Domains	Sub domains	Range	Mean + SD	Mean percentile	Rank
The learning environment	Pedagogical environment	16-27	23.95 + 2.54	88.62	V
	Leadership style	06-15	13.44 + 1.74	89.58	IV
	Nursing care in ward	07-21	18.49 + 2.34	88.05	VII
Role of clinical Instructor	Integration of theory and practice	17-30	27.62 + 2.79	92.05	I
	Cooperation between nursing staff and teacher	03-09	8.19 + 1.18	90.99	II
	Relationship between student, staff and teacher	03-09	7.59 + 1.40	84.38	VIII
Supervisory Relationship	Occurrence of supervision	10-17	14.99 + 2.04	88.15	VI
	Content of supervisory relationship	16-33	29.91 + 3.16	90.62	III

Table 1 illustrates domain wise mean, standard deviation, mean percentage and rank of clinical learning environment & supervision (CLES) in obstetrics & gynecological area. The sub-domain with highest mean percentage indicated lowest deficit area and lowest mean percentage indicated highest deficit area. Hence, rank was given according to the scores which depicts that nursing students were highly satisfied with the integration of theory and practice in their clinical learning environment and were least satisfied by the student, teacher and staff relationship in their clinical leaning environment and supervision.

Table 2: Frequency and Percentage Distribution of Nursing Students in terms of Level of CLES Scale

Domains	Sub domains	Need to Improve		Average		Good	
		F	%	f	%	F	%
Learning Environment	Pedagogical environment	07	3.15	110	49.55	105	47.30
	Leadership style	31	13.96	63	28.38	128	57.66
	Nursing care in ward	27	12.16	78	35.14	117	52.70

Cont... **Table 2: Frequency and Percentage Distribution of Nursing Students in terms of Level of CLES Scale**

Role of clinical Instructor	Integration of theory and practice	12	5.41	60	27.03	150	67.57
	Cooperation between nursing staff and teacher	16	7.21	86	38.74	120	54.05
	Relationship between student, staff and teacher	49	22.07	102	45.95	71	31.98
Supervisory Relationship	Occurrence of supervision	32	14.41	79	35.59	111	50.00
	Content of supervisory relationship	14	6.31	63	28.38	145	65.32

Highest percentage of nursing students evaluated all the domains of clinical learning environment and supervision as a 'good'. Less than one fourth (22.07%) of nursing students evaluated relationship between student, staff and teacher as ' need to improve' followed by 14.41% of nursing students evaluated occurrence of supervision as 'need to improve'. Nearly half (49.55%) of nursing students evaluated pedagogical environment as 'average' followed by 45.95% of nursing students evaluated relationship between student, staff and teacher as ' average'. More than two third (67.57%) of nursing students evaluated integration of theory and practice and 65.32% of nursing students evaluated content of supervisory relationship as 'good'. It inferred that nursing students described that relationship between student, staff and teacher as weakest sub- domain and integration of theory and practice as a strongest sub-domain. **(Table 2)**

Description of CLES subscale and it's domain

The mean value of the sum variables in the main sub domains that were measured on the likert scale varied between 2.53 to 2.76. The supervisory relationship (Mean=2.76, S.D. 0.03) and integration of theory and practice (Mean=2.76, S.D. 0.09) sub domain received highest evaluation. The lowest evaluation was in the sub domain of relationship among student, staff and clinical instructor (Mean= 2.53, SD=0.17). The leadership style, nursing care, integration of theory and practice, cooperation and relationship with students and supervisory relationship showed good reliability equals to or more than 70%) whereas reliabilities of occurrence of supervision and pedagogical environment were 0.47 and 0.69 respectively.

Table 3: Bivariate Correlation between Total Perception and CLES Domains (N= 222)

TOTAL PERCEPTION	PEDAGOGICAL ENVIRONMENT	LEADERSHIP STYLE	NURSING CARE IN WARD	ROLE OF TEACHER	SUPERVISIORY RELATIONSHIP
SPEARMAN CORR. COEFFICIENT	0.888**	0.927**	0.898**	0.523**	0.862**
p-VALUE	<0.001	<0.001	<0.001	<0.001	<0.001

** Correlation is significant at α= 0.01

Result of Spearman's Rho correlation coefficient was statistically significant between the overall perception and all of the five sub domain ($p<0.001$) . It inferred that overall nursing students perception was positively correlated with all the sub-domain of CLES scale. ($p<0.001$) **(Table 3)**

Table 4: Association of Gender and Clinical Learning Environment and Supervision among Nursing Students

N=222

CLES Domains	Sub domains	Male		Female		Independent sample t-test	
		Mean	SD	Mean	SD	't' value	p value
The learning environment	Pedagogical environment	23.9	2.06	23.9	2.67	0.88	0.03*
	Leadership style	13.5	1.44	13.4	1.82	0.56	0.11 NS
	Nursing care in ward	18.4	1.9	18.4	2.4	-0.04	0.15NS
Role of clinical Instructor	Integration of theory and practice	28.3	1.7	27.4	2.9	2.2	0.00*
	Cooperation between nursing staff and teacher	8.4	.68	8.1	1.27	1.9	0.01*
	Relationship between student, staff and teacher	7.8	1.09	7.5	1.4	1.3	0.00*
Supervisory Relationship	Occurrence of supervision	15.6	1.4	14.8	2.1	2.4	0.00*
	Content of supervisory relationship	30.3	2.18	29.7	3.3	1.0	0.01*

*** Association is significant at the 0.05 level (Independent t-Test)**

Table 4 exhibits association of gender with clinical learning environment and supervision among nursing students. For both genders, highest mean score was obtained for supervisory relationship and lowest mean score for relationship between student, staff and teacher. All sub-domains showed significant association with gender (male and female) except leadership style and nursing care in ward which inferred that clinical learning environment and supervision were independent on leadership style and nursing care in ward.

Table 5 :Association of Year of Study and Clinical Learning Environment and Supervision among Nursing Students

N=222

Groups	Mean	SD	F value	p value
B.SC 3RD YEAR	149.5	9.5	15.26	0.00*
B.SC 4TH YEAR	136.5	15.2		
P.B.SC. 1ST YEAR	148.2	8.30		
M.Sc. 1ST YEAR	152.8	5.2		
M.Sc. 2ND YEAR	142.8	9.8		

Table 5 reveals association of year of study and clinical learning environment and supervision among nursing students. The highest mean score were reported in M.Sc. (N) 1st year and lowest mean was reported for B.Sc. (N) 4th year. Analysis of variance confirmed that there was a significant mean score difference for all domains among different year of study.

Discussion

The clinical education is beneficial in preparing today novice nursing students to become tomorrow proficient health care providers.This study deals to assess nursing student's clinical learning environment and supervision in obstetric and gynecological nursing area.

The findings of the current study revealed that the total mean score of the CLES scale was 144.1 (89.27%), the participating nursing students have perceived their clinical learning environment in obstetrics and gynaecology nursing area as good.. Similar findings have been reported by Papastavrou E, et al.[10], who assessed the nursing student's experiences and satisfaction toward their clinical learning environment and found good level of satisfaction.

Also, The findings of the present study are similar to what was reported by Sundler, et al.[11], they examined nursing students' satisfaction with their clinical learning environment and found that, the total mean score of CLES was good which indicate good satisfaction and good experience with the clinical posting in obstetric and gynecological nursing.

The present study reveals that the pedagogical environment and role of clinical instructor/teacher was found the most important domain and sub domain during clinical placement and was evaluated as 'good'. the results strengthen the importance of a good pedagogical atmosphere by Warne et al[12]and a positive learning environment by Antohe et al.[13]

Present study shows positive co-relation between all domains of clinical learning and supervision Similar findings depicts by Dunn SV[14] that clinical learning environments requires positive relations among the dimensions of clinical learning. This is in line with the findings of previous studies[15,16] which showed that CLE is related to the quality patient care and patient contact. It is confirmed by O'Driscoll MF et al.[16] that a task oriented approach to nursing care is considered as a barrier to students' learning.

Domain wise analysis of current study showed a surprising score that is (92.05%) of nursing students experienced the integration of theory and practice as the

most important dimension in CLES. This means that nursing students were oriented to ward /unit settings by clinical instructor/teacher, they felt that they have capability of more critical reflection of nursing practice and in-depth understanding of essential content of nursing competence and received continuous feedback and suggestions of improvement from clinical instructors/ teacher. Levett-Jones et al.[17] approved that students need feedback and to maintain open communications with their faculty trainers. Research have shown that alert nurses who are in charge of designing practical placements leads to successful completion of leraning outcomes in exact appointed approach among students.

The present study exhibits association of gender with all domains of clinical learning environment and supervision among nursing students. It reveals the significant mean score difference between male and female nursing student's similar findings revealed by Suriya Khatoon et al.[18] showed significant mean score difference between male and female students except premises of nursing domain.

Limitations of the Study: One of the limitations of the current study was that the participants were selected from one nursing College in Haryana. Perception regarding clinical placement is assessed only in one clinical area.

Nursing students represent the future nursing workforce, thus nursing education is an important investment for the quality of the provided clinical nursing care. During a nursing programme, students are systematically prepared to reach the minimum standards of 'competencies' on knowledge, attitude and skill in order to be certified for their professional capability.

Extensive researches can be conducted to compare the experiences of student nurses with their clinical learning environment, studying in different nursing colleges. The present study recommends that interventional studies may be conducted in the areas of satisfaction as expressed by student nurses and qualitative research may be conducted on a similar topic. Seminars and workshops should be organized for the mentors/ supervisors to understand the importance of team work and create a healthy clinical learning environment so that adequate preparation will be made to achieve educational objectives.

Conclusions

Based on the findings of the study, it can be concluded that nursing students were highly satisfied with the integration of theory and practice in their clinical learning environment and were least satisfied by the student, teacher and staff relationship in their clinical leaning environment and supervision.Nursing student valued supervision, pedagogical and learning environment. Therefore, careful understanding of students' satisfaction of their clinical learning environments essential to design effective teaching and learning process to meet learning objectives and to meet quality standards of nursing education and practice.

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Conflict of Interest: None

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Evaluation of Halitosis and Sialometry in Emirati Diabetic Patients before and after Periodontal Treatment

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Abstract

Halitosis is an important social complaint which affects both healthy and periodontally diseased individuals. Oral malodor is mainly caused by a microbial degradation of both sulfur-containing and nonsulfur-containing amino acids into volatile, bad-smelling gases. Anaerobic gram-negative bacteria, the same species that have been linked to periodontal diseases, are especially involved in this process, explaining the link between oral malodor with periodontitis. The following study was done to investigate the parameters of halitosis and sialometry of Emirati diabetic patients in correlation with oral concentration of volatile sulfur compounds (VSC), salivary flow rate before and after non-surgical periodontal treatment (scaling & root planning) for patients having moderate Generalized and/localized chronic periodontitis.

Subjects and Methods: Eighty-one (81) Emirati subjects volunteers were recruited from the dental hospital, with average age 38-49 years. They have been divided into in three groups: Group I (negative control), Group II, and Group III and Group IIIa. Halitosis measurements for all volunteers had been recorded using Gas Chromatography. For group III samples were collected before periodontal treatment, and subsequently at two- & four-weeks following start of non-surgical periodontal treatment (Scaling & root planning).

Results: There were 54 patients participated in this study. Of the 54, 27 were control and the remaining were controlled DM. The mean and SD of all variables before and after the intervention showed that the values of all variables reducing after the intervention. All the difference observed was statistically significant ($P<0.001$). This study observed that the mean value of unstimulated salivary flow rate and stimulated salivary flow rate was less in the controlled DM group compared to control group, but for probing picker depth, CAL and average bone loss was more in controlled DM group compared to control group.

Conclusion: Based on the findings, it can be stated that diabetes is a major risk factor for periodontitis, and the risk of periodontal disease increases if glycemic control is poor; it was also proved beyond doubt that people with poorly controlled diabetes are at an increased risk of periodontal disease and loss of alveolar bone. Controlling diabetes by successfully improving glycemic control will reduce the risk and severity of periodontal diseases.

Keywords: *Periodontitis, glycemic control, diabetes, oral malodor, gram negative bacteria.*

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Introduction

Oral malodor is a serious psychological handicap which affects the quality of life and has negative effects on individuals community engagement and social life in many aspects. To the extent that it may shake the

self-esteem of certain personnel. It was found that the prevalence of halitosis in the common population fall in the range from 23% to 50%, respectively.[1,2]The causes of oral malodour may be systemic which represented only 10% while intraoral causes about 90%. The most common cause from various intraoral causes is the tongue coating and periodontal diseases. Mainly the bad breath is due to the release of volatile sulfur compounds (VSCs) in mouth air such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, which are produced by various types of anaerobic bacteria[1]. So, VSCs are considered as the most common factor contributing to breath malodor, and thereby provide a biomarker to analyse the breath quality.[1,2] It was found recently that VSCs in addition to causing oral malodor, may also contribute to the pathogenesis of periodontal disease. The following gram-negative bacteria including Porphyromonas gingivalis, Fusobacterium nucleatum and Treponema denticola which are key periodontopathic bacteria, are found to be associated with halitosis[2,3]. Gram negative anaerobic bacteria degrades the sulphur-containing amino acids cysteine and methionine and contributes to the production of VSCs like hydrogen sulphide (H2S), methyl mercaptan (CH3SH) and dimethyl sulphide ((CH3)2S), which are proven contributors to the oral malodor.[4,5] The Halimeter measurements are expected to cross 160 ppb, when the bad breath is expected upon.[5-8] It had been proved that long standing hyperglycemia was associated with periodontal disease which now considered as one of the six opathy of diabetes.[7-11] It is important to note that diabetes is a regional affliction as UAE, Saudi Arabia, Bahrain, Kuwait and Qatar all featuring in the top fifteen countries in terms of prevalence worldwide. It was found in 2015, that almost one in five people, of the UAE population between the ages of 20 and 79 have type 2 diabetes, according to the International Diabetes Federation (IDF). In 2015, there were over 1 million people living with diabetes in the UAE, placing the country 13th worldwide for age-adjusted comparative prevalence. However, an increasing demand for quality of life among UAE nationals necessitate to find a way to overcome periodontal disease and oral malodour usually accompanied type 2 diabetes by accurate identifying these problems, related factors and its control.

Aim of the Study

To investigate the parameters of halitosis and sialometry of Emirati diabetic patients in correlation with oral concentration of volatile sulfur compounds (VSC), salivary flow rate before and after non-surgical periodontal treatment (scaling & root planning) for patients having moderate Generalized and/localized chronic periodontitis.

Materials and Methods

Eighty-one (81) Emirati subjects volunteers were recruited from dental hospital,with average age 38-49 years. They have been divided into in three groups:

Group I (negative control)- 27 subjects with good systemic and oral health.

Group II- 27 controlled diabetic patients without periodontal disease.

Group III- 27 controlled diabetic patients with periodontal disease (before non-surgical periodontal treatment).

Group IIIa—27 controlled diabetic patients with periodontal disease of group III (after non-surgical periodontal treatment).

Halitosis measurements for all volunteers had been recorded using Gas Chromatography

For group III samples were collected before periodontal treatment, and subsequently at two & four weeks following start of non-surgical periodontal treatment (Scaling & root planning).

Study population

a) Inclusion criteria

· Emirati nationals who signed consent of participation in the study

· Controlled type II diabetic patients evaluated by HbA1c test. The test will be carried out using the HPLC technique (Gold standard). The lab carrying out the test will follow appropriate laid out approved protocols. The laboratory is accredited by the college of American Pathologists (CAP) and adheres to NGSP (National Glycohemoglobin Standardization Program)

level 1 reporting. Glycosylated haemoglobin test shows the average blood glucose level over a period of the past 3 months prior. Patients with a value between 6.2 - 8 % will be included in the study. Patients with values more than 8% will be excluded from the study.

- Duration of diabetes history more than 3 years.
- Free from any other systemic diseases
- Non pregnant or in menstruation period (female)
- No smoking or related habits

b) Exclusion criteria

- Any respiratory diseases
- Diabetic Patients with ketoacidosis assessed by

a) HbA1c more than 8% (to rule out uncontrolled diabetes)

b) Urine analysis for ketone bodies as done by Uristix ™

- Any drug which could affect halitosis
- Alcohol containing mouth rinses or drugs

Sample size calculation

For the calculation of sample size, we have taken the maximum value of VSC among people with no disease as 160ppb and for those with periodontal disease the maximum value as 230ppb. The Standard deviation was taken as 40% of the mean (92ppb). The power of the study is taken as 80% and the level of significance as 5%[15]. Thus the minimum number of patients required for each group is 27. Eighty-one (81) Emirati subjects volunteers.

Results

The present study aimed to determine the parameters of halitosis and sialometry of Emirati diabetic patients in correlation with oral concentration of volatile sulfur compounds (VSC) and salivary flow rate. Also,to determine BANA test before and after non-surgical periodontal treatment (scaling & root planning) for patients having moderate Generalized and/localized chronic periodontitis.

There were 54 patients participated in this study. Of the 54, 27 were control and the remaining were controlled DM. With regard to age, the minimum age observed was 24 and the maximum was 40. The mean age of males was 31.6 years with a standard deviation of 3.6 and for females the mean age was 31 years with a standard deviation 3.9 years. The age was divided into two groups. In the control group, more participants were in the age group of <=30 years whereas in the controlled DM, only 14% in the age group of <=30 years. The Chi-square test shows there is a statistically significant association between age group and the gender ($p<0.001$). The details of the distribution are given in table -1.

Table -1: Age distribution of the participants Vs. Group

Age	Group				Total
	Control		Controlled DM		
	No.	%	No.	%	
<=30	18	66.7	4	14.8	22
>30	9	33.3	23	85.2	32

The mean and SD of parameters such as HbA1C, H2S, CH3SH and CH32S are given in table -2. All the parameters except CH32S, the value observed was high in the control DM group compared to control group. The difference observed was statistically significant in HbA1C and H2S ($P<0.05$).

Table -2: Mean and SD of parameters before treatment

Parameter	Group				P
	Control		Controlled DM		
	Mean	SD	Mean	SD	
HbA1C	5.3	0.3	7.0	0.3	<0.001
H2S	138.7	20.4	154.9	26.6	<0.05
CH3SH	133.3	17.0	138.6	19.4	NS
CH32S	124.8	7.3	124.8	7.3	NS

The mean and SD of all variables before and after the intervention showed that the values of all variables reducing after the intervention. All the difference observed was statistically significant (P<0.001). The details are given in table -3

Table -3: Mean and SD of parameters before and after treatment

Parameter	Before/After						P
	Before			After			
	Mean	SD	No.	Mean	SD	No.	
HbA1C	7.0	0.3	27	6.7	0.4	27	<0.001
H2S	146.8	24.9	54	123.3	14.4	54	<0.001
CH3SH	135.9	18.3	54	121.0	10.9	54	<0.001
CH32S	124.8	7.3	54	114.2	8.4	54	<0.001

This study observed that the mean value of unstimulated SFR and stimulated SFR was less in the controlled DM group compared to control group, but for probing picker depth, CAL and average bone loss was more in controlled DM group compared to control group. All the difference observed was statistically significant. The details are given in table -4.

Table -4: Mean and SD of parameters in the controlled DM and control groups

Parameter	Group						P
	Controlled diabetic			Control			
	Mean	SD	No.	Mean	SD	No.	
Unstimulated SFR	0.19	0.10	27	0.26	0.06	27	<0.01
Stimulated SFR	0.35	0.11	27	0.41	0.09	27	<0.05
Probing pocket depth (PPD): mm	5.07	1.43	27	2.98	0.88	27	<0.001
CAL mm	2.07	1.43	27	0.50	0.65	27	<0.001
Average bone loss %	19.22	10.45	27	7.15	7.06	27	<0.001

Full mouth plaque score ranged from 0 to 3. In the controlled D M group, more than 50% with score more than or equal to 2 whereas in the control group, less than 30% with FMPS score more than or equal to 2.

Table - 5: Full mouth plaque score (FMPS) Vs. Group

Full mouth plaque score (FMPS)	Group				Total
	Controlled DM		Control		
	No.	%	No.	%	
0	3	11.1	5	18.5	8
1	7	25.9	14	51.9	21
2	10	37.0	6	22.2	16
3	7	25.9	2	7.4	9

To determine the relationship between HbA1C, CH3SH and H25, scatter diagram was drawn (Fig-1). and correlation coefficient was calculated. The correlation coefficient showed a very weak correlation between the variables. (Table 6).

Table -6: Correlation between HbA1C, CH3SH and H25 (Before)

Variables	Correlation coefficient
HbA1C and CH3SH	0.14
HbA1C and H25	0.38
CH3SH and H25	0.40

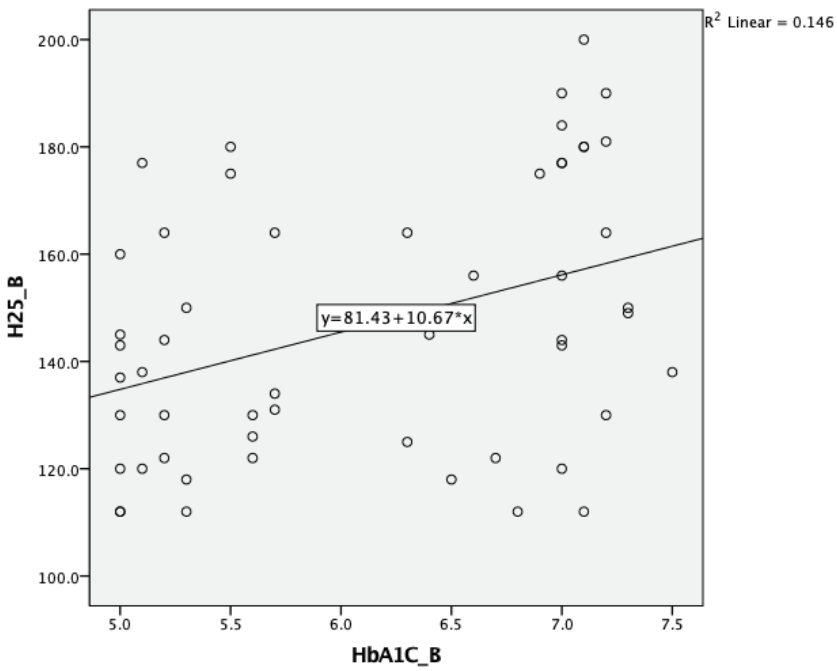

Fig-1A: scatter diagram for HbA1C and H25

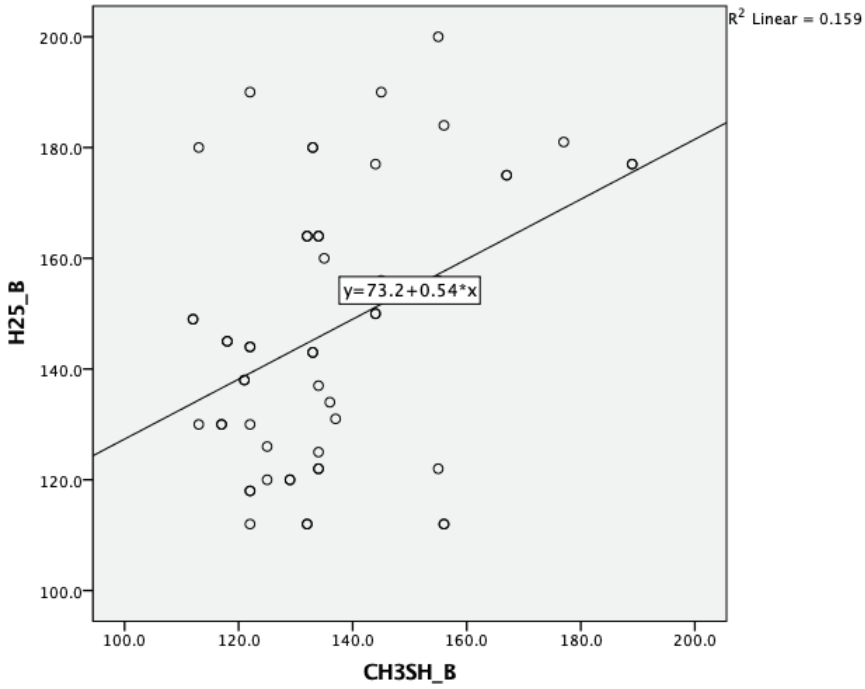

Fig-1B: scatter diagram for CH3SH and H2S

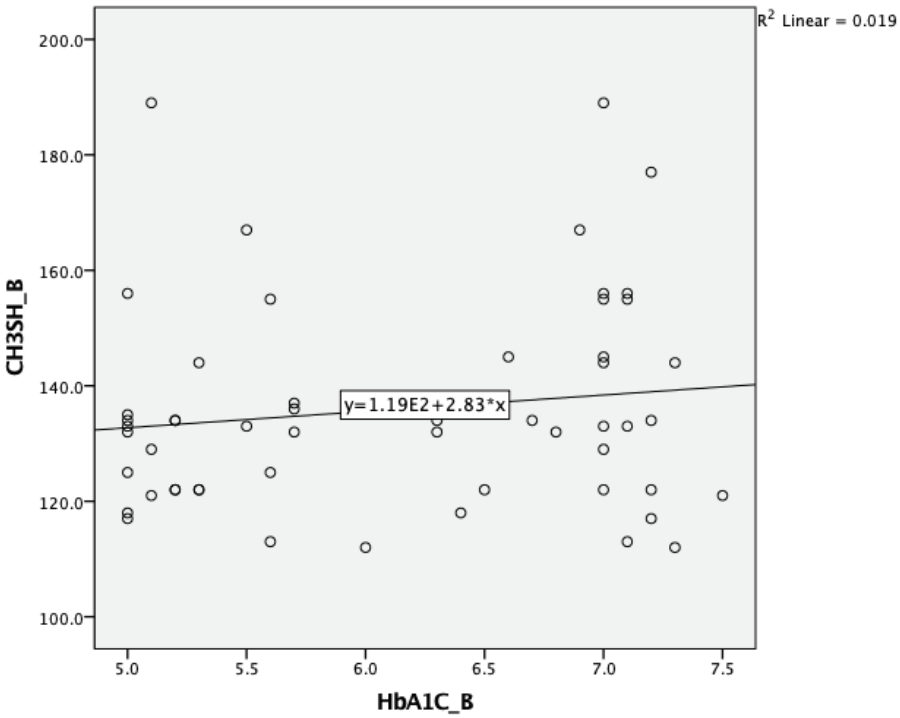

Fig-1C: scatter diagram for HbA1C and CH3SH

Discussion

The most common chronic inflammatory disease is chronic periodontitis which is characterised by destruction of the supporting structures of the teeth (the periodontal ligament and alveolar bone). The disease is highly prevalent and nearly 10-15% of the adults are affected according to the recent surveys. The disease will lead to a number of negative impacts on the normal quality of life which needs to be addressed. A number of studies were done to find the correlation between diabetes and periodontal disease, which shows a positive correlation between the two. This was also confirmed by the epidemiological data obtained from the research done earlier. Based on this it was concluded that diabetes is a major risk factor for periodontitis. A threefold increase in the susceptibility to periodontitis was seen in people with diabetes. Further studies established a clear relationship between degree of hyperglycaemia and extent of periodontitis. Even though the mechanisms that link these two conditions are not completely clear, it was found to involve aspects of immune functioning, cytokine biology and neutrophil activity. It was later proved beyond doubt that there exist a two-way relationship between diabetes and periodontitis, with diabetes leading to an increase in the risk for periodontitis, and inflammation of the periodontium negatively affecting the glycaemic control. As stated earlier, it is still unclear about the impact of periodontal inflammation on the glycemic control of diabetes and the involved mechanisms and processes. It was found that the inflammation of the periodontium may increase insulin resistance, thereby aggravating glycemic control.[12]

Based on this study, 85.2% patients in age group of above 30 years had controlled diabetes mellitus. This signifies the progress and control of diabetes mellitus among the population examined.

All the parameters except CH32S, the value observed was high in the control DM group compared to control group. The difference observed was statistically significant in HbA1C and H2S ($P<0.05$).

When the impact of periodontal disease on the probable changes in HbA_{1c} was assessed in non-diabetic individuals, it was found that those participants with the most severe periodontal disease at baseline demonstrated an greater increase in HbA_{1c} over the subjects compared with those with no periodontal diseases at baseline (change in HbA_{1c} $0.106 \pm 0.03\%$ vs $0.023 \pm 0.02\%$).

The mean and SD of all variables before and after the intervention showed that the values of all variables reducing after the intervention. All the difference observed was statistically significant ($P<0.001$). This shows that maintenance of proper oral hygiene combined with professional scaling and root planning brought down the probing depths significantly in controlled diabetes patients. The salivary secretions were low in patients with diabetes when compared to the controlled group which also emphasize the progression of periodontal diseases in the diseased group. Also, it was observed that the probing pocket depth, CAL and average bone loss was more in controlled DM group compared to control group. This shows that patient with diabetes are more prone to periodontal problems when compared to patients in the control group. It can be said based on the evidences available that improvement in metabolic control can be expected following successful management of periodontitis. As mentioned earlier the mechanisms by which this occurs are still unclear but may probably relate to the reduction in systemic inflammation following the periodontal management and resolution of periodontal inflammation. Larger randomised trials are to be performed to probe this further as these reductions in HbA_{1c} are associated with a reduced risk of diabetes complications.

This study observed that the mean value of unstimulated SFR and stimulated SFR was less in the controlled DM group compared to control group, but for probing picker depth, CAL and average bone loss was more in controlled DM group compared to control group. All the difference observed was statistically significant.

Full mouth plaque score ranged from 0 to 3. In the controlled D M group, more than 50% with score more than or equal to 2 whereas in the control group, less than 30% with FMPS score more than or equal to 2. This proves that along with decreased salivary flow, there is lesser amount of cleansing action in the oral cavity even in the presence of proper home care by the patients.

Conclusion

Based on the findings, it can be stated that diabetes is a major risk factor for periodontitis, and the risk of

periodontal disease increases if glycaemic control is poor; it was also proved beyond doubt that people with poorly controlled diabetes are at an increased risk of periodontal disease and loss of alveolar bone. Controlling diabetes by successfully improving glycaemic control will reduce the risk and severity of periodontal diseases. Furthermore, a lot of evidences suggests that reduction in the inflammatory status of the periodontium improves metabolic control, though large, multi-centre, randomised controlled trials are further needed validate these findings.

Conflict of Interest: Nil

Ethical Clearance: Obtained from the Research Ethical Committee

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Case Report: 25-year-old Female at Term Labour and Normal Delivery

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Abstract

Labor has been defined as that of the initiation of periodic painful contractions of progressive deletion as well as dilation of a cervix, followed by a decent part going to lead to the expulsion of its fetus & placenta from the uterus of mother. Post - natal treatment was its individualized treatment provided that meet the needs of the baby and mother after delivery/childbirth.

Case Report –Mrs. Nikita Matram 25-year-old female was admitted in AVBR Hospital with the complaint of pain in abdomen and increased the fetal movement on date 4/01/2020 and she delivered female baby on date 6/01/2020 and the baby weight was 2.8 kg at 1:40 am now her complaint was pain on suture side and weakness. But the patient has a previous history of abortion after marriage of four month two and half month of baby was abort. That time dilatation and curettage was done.

Discussion – Patient delivered female baby throughout the vaginal delivery and baby and mother was normal after delivery baby was kept with mother on same bed and initiate breastfeeding within one hour after delivery.

Conclusion – Case report conclude that after normal delivery and post-natal care mother and baby was normal.

Key words *– Full term normal delivery, post-natal care, newborn, delivery.*

Introduction

Giving birth, commonly defined of labor and birth, seems to be the conclusion of pregnancy once an or maybe more kids exit the mother via going through the womb or even the caesarean delivery and, throughout delivery, a baby is released through the womb. There are different forms of vaginal delivery. Supported vaginal and instrumental vaginal birth, accidental vaginal delivery, triggered vaginal birth, natural vaginal delivery.

Treatments that are consistent to protection. Given medical and scientific innovations through managing complex health problems, this same current setting with maternity services had also increased rates for healthy mothers and infants. There have been fears all around the community that evidence-based strategies and procedures across labour and childbirth are standard occurrence.[1] There is significant miss-use of advantageous techniques, over-use of detrimental or inefficient methods, or lack of desire and over consequences of insufficiently measured methods.[2] Enhance the efficiency with maternity services for both developed and emerging nations is an essential part with efforts to reduce maternal and infant mortality and morbidity.

Patient Case Report

Mrs. Nikita Matram 25-year-old female diagnosed with G2, A1, with 38 .2 wks. of gestational age with full term normal delivery was admitted in AVBR Hospital with the complaint of pain in abdomen and increased the fetal movement on date 4/01/2020 and she delivered female baby on date 6/01/2020 and the baby weight was 2.8 kg at 1:40 am now her complaint was pain on

suture side and weakness.. Before pregnancy the patient menstrual cycle was regular 28 days of cycle and duration was 3-4 days and Last menstrual period was 11/04/2019 and Expected date of delivery was 16/01/2020.

Mrs. Nikita Matram has a previous history of abortion after marriage of four month & two and half month of baby was aborted. That time dilatation and curettage was done. She has not used any contraception method & no any medical history like DM, Hypertension, TB, and Asthma. she lives in joint family in their family five member are living together including newborn and she was belonging from middle class family and in their houses all facilities are available like electricity, water supply from municipality etc. her monthly income was 25000/- per month. Mrs. Nikita and her family members were psychological stable. She maintains good interpersonal relationship with others. She is taking only vegetarian diet. She doesn't have any allergic reaction from any food and no any history of any bad habits like chewing tobacco, smoking etc.

Patient general examination was state of health was unhealthy, conscious, Body built thin, Posture erect, and hygiene was good. General parameter height was 154 cm, weight 45 kg. Vital sign is Temperature 98˚c, Pulse 84 b/m, Respiration – 20 b/m, BP – 120/80 mmHg. In breast some changes occur because of pregnancy enlargement of breast, nipple was large erectile and discharge was present of milk secretion. In abdomen linea nigra, striae gravidarum present. Lochia rubra was present.

Investigation -

· CBC

Hb 12.7 gm %

Total RBC 3.92 million /cu mm

Total WBC 13900 cu.mm

Total platelet counts 2.69 lacs/cu.mm

· LFT

SGPT 17

SGOT 31

Albumin 3.6 g/dl

· USG –

Fetal no. – Single

Lie – variable

Placenta – anterior, grade – II

Presentation – variable

Fetal movement – present

Liquor – adequate

Impression of USG – single intrauterine live fetus of average gestational age of 27 weeks 1 day and corresponding to weight of 999 GMS.

The drugs are used Inj. Metrogyl 100cc, I.V., TDS. Action – Metronidazole injection is also to prevent infection when used before, during, and after colorectal surgery. Metronidazole injection is in a class of medications called antibacterial. It works by killing bacteria and protozoa that cause infection, Inj. C-Tax 1 gm, I.V., BD., Action - C Tax 1gm Injection is an antibiotic medicine used to treat bacterial infections in your body. It is effective in infections of the brain, lungs, ear, urinary tract, skin and soft tissues, bones and joints, blood and heart. It is also used to prevent infections during surgery, Inj. Oxytocin 10 IU. Oxytocin is a uterine stimulant, prescribed for the initiation of uterine contractions and induction of labor in women as well as stimulation of contractions in cases where the uterus does not contract enough during labor.

If any complication occurs during normal delivery than patient refer for lower segment Cesarian section. Mother should take care of self and self-care may include Rest and ambulance - early ambulation after delivery, hospital stay, diet, perineal care, care of bladder, care of bowel, sleep, care of breast, rooming in, aseptic and antiseptics, immunization post-partum exercise, follow up, daily observation and care of newborn. After normal delivery mother and baby should come for check-up and follow up after discharge of six weeks, and explained the client about if any sign of infection and side effect of medication immediately informed to the doctors. Immunization of baby follow up is necessary.

Discussion

Present case reveals that the antenatal patient come in hospital with the complaint of pain in abdomen and increased the fetal movement on date 4/01/2020 and she delivered female baby on date 6/01/2020 and the baby weight was 2.8 kg at 1:40 am now her complaint was pain on suture side and weakness. After delivery mother and baby was normal Apgar score was ten and baby was kept with mother in same bed it will help to initiate breast feeding, thermoregulation, create bonding between mother and child, mother learn how to provide the care to baby etc. after that pain was manage by painkiller. Condition of baby and mother was good.

Most broadly, the phrase 'natural life' in scientific research and healthcare policy refers to conception without and with minimal surgical intervention. The 2007 systematic review by both the prenatal care Planning Group, Making Natural Childbirth a Fact, provided for such a uniform approach of hospital birth to improve trust for auditors or tracking practice patterns a subsequent description defined natural delivery or non-assisted vaginal delivery without intervention of labour; epidural, spinal and general anaesthesia or episiotomy. Unlike many other meanings, an interpretation with werkmeister has been restricted to a pregnancy and birth and it does not apply to birth outcomes like vertical presentation as well as intact perineum.[3]

This same delivering of a full-term baby originally referred with service only at gestational age of 37-42 weeks, even though defined by last menstruation cycle or through ultrasound dating as well as assessment. A Naegel rule is indeed a frequently was using formula besides predicting a due date focused mostly on date from the last menstruation cycle. Its legislation states a 28-day menstruation periods as well as a mid-stage ovulation. Ultrasound dating will be much more accurate, especially because once implemented early in the pregnancy and used to substantiate or adjust a due date focused on last menstruation cycle. About 11 per cent of singleton birth is pre-term and 10 per cent of all births are post-term. As a result, nearly 80 per cent of babies are born on even a full-term basis, but only 3-5 per cent of births arise mostly on expected delivery date.[4]

Healthcare offers patient care, encourages convenience, listens to emotional needs through a comprehensive wellbeing paradigm, and teaches nutrition or self-care. Even so, in today's popular health care setting, postnatal nurses are very often responsible to devices or for mothers and babies. It is critical that organizations develop trust and expertise in order to make a transition towards a humanizing birth feasible. This topic problem in such a major nursing review offers important resources to help caregivers encourage, endorse and defend regular births Nursing staff have a special and significant role to play in deciding the treatment procedures encountered by women. Unfortunately, nursing staff may well be functionally removed from important methods of data interchange as well as from making a contribution to a treatment plan. This can influence the ability of nursing to successfully encourage, help or secure regular births. Since medicalized conception presents a threat of iatrogenic damage to the both mothers and infants, nurses have a duty obligation to encourage natural delivery in order to improve patient health.[5]

Conclusion

After delivery mother and baby was normal. Apgar score was ten at birth. Condition of baby and mother was good after pain management pain was reduced. Vaginal delivery is a process of natural birth.

Ethical approval: Not applicable

Patient inform consent: While preparing case report and for publication patient's informed consent has been taken.

Conflict of Interest: The Author declares that there are no conflicts of interest.

Funding: Not applicable

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To Assess the Effectiveness of Information Booklet on Knowledge of Mothers Regarding Home Management of Common Illness among Children Aged 2 Months to 5 Years in the Group

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Abstract

Background: Care for children is often the responsibility of mothers. Mothers' awareness of child care therefore determines the type and standard of care had given to the children. Many studies had shown the level of education of mothers had a positive effect on their own experience and how they deal with health issues of children. **Objectives: 1**. To assess the pre-test knowledge of the mothers regarding home management of select common illness of children aged between 2 months and 5 years. **2**. To evaluate the information of booklet on knowledge regarding home management of selected common illness of children aged between 2 months and 5 years. **3.** To associate the post test knowledge score of mothers regarding home management of the common childhood illness among children aged between 2 months and 5 years with selected demographic variables. **Methodology:** One group pre-test and post-test design was used. Participants were consisted of 50 mothers of children aged between 2 months and 5 years. **Result:** Overall mean knowledge score in pre-test and post-test which reveals the post-test knowledge score was higher i.e. 18.16 with the SD of ± 1.68 when the compared to pre-test knowledge score which was 9.24 with the SD of ± 2.90. The statistical paired 't' test implies the difference in the pre-test and post-test knowledge score found to be 21.06 statistically significant at the level 0.05%.

Conclusion: It was statistically interpreted as had been accurate information booklet for the knowledge of mothers of home management for the chronic illness of children age between 2 months and 5 years.

Keywords *: Assess, Knowledge and Common childhood. illness*

Introduction

Children 0-4 years of age with the associated acute respiratory tract infection have a common mortality rate is 8.9 per 1,000. The prevalence of the infection was 32.8 and the prevalence of bacterial acute respiratory tract infection was 32.8. Health is both a right and a liability. Protecting children's developmental health is a long-term commitment to the country as a whole's growth and development. Children below the age of five make up 15 to 20 per cent of the Indian population. Their protection is a greatest investment for the economics of the country prosperity and political stability[1].

The most of the children died in each year with the viral infection and it could be save by low technology, cost effects of measures such as vaccines, antibiotic micronutrients, supplementation, improved family care and poor breastfeeding practice and oral rehydration therapy. In addition to providing to children with vaccines and antibiotics, moms could also educated on how to make simple changes to living conditions such as improving hygiene to enhance their children's health[2].

Every year more than 10 million children under the 5 years age group children died in developed countries.

Most of the death is preventable and is caused primarily by infectious etiologies such as diarrhea, food allergies, respiratory tract infection etc. Other factors leading to illnesses in this age group, aside from poverty, are inadequate living conditions, contaminated drinking water, bad hygiene and overcrowded[3].

Throughout India, diarrheal diseases are a major public health problem among children under the age of five years, with up to one-third of total pediatric admission due to diarrheal disease and up to 17% of all death throughout indoor pediatric patients associated with diarrhea[4].

In India, diarrheal disease is also a major public health problem among children under the age group of five years children up to one-third of total pediatric admission is due to diarrheal disease and up to 17% of all deaths in indoor pediatric patients are associated with diarrhea[5].

The mortality rate among the children under the age group of five years for acute respiratory tract infection is very high. This study calculated that in children under five years of age nearly 6.1 per cent of infant mortality per year. Statistically the raw mortality rate was 14.3 per 1,000 0-4-year-old babies, the average mortality rate for subsequent acute respiratory tract infection is 8.9/1000.

Viral infection were prevalent at 32.8, and bacteremia acute respiratory tract infection were 6.7 per 1,000 children deals with the acute respiratory tract infection[6].

Vomiting is common symptoms for children those who have always need for healthcare. Although vomiting can be the problem from the gastrointestinal tract itself. It can also signal more generalize systemic disorders. Vomiting in children is mostly normal and they can only treated by positive steps. Vomiting is common in children there are many possible causes, including viral infection and reflux. Many common illnesses such as colds and ear infection can also cause vomiting. Vomiting in young children can usually be treated at home. The healthcare provider usually won't prescribe medication to prevent vomiting unless symptoms are severe. That's because there is a greater risk of serious side effect when this type of medicine is used in young children. The main danger from vomiting is dehydration. To prevent the dehydration, you may be told to replace lost body fluids with oral rehydration solution[7].

Fever is one of the most common symptom for children. That is 30% of visits to the paediatric clinics are associated with fever. The caregivers will take medical help for their children. The present study was therefore designed the objectives to study the awareness of parents of under the age group of five years children about fever and its home management, awareness and to identify the co-relates of fever home management practices among rural parents[8].

Most of the children have food allergy (peanuts, milk, wheat, soy) in the first five years of child life. An allergen is an infectious disease marker. Previously, the American Academy of Pediatrics (AAP) recommended that introduction of certain highly allergenic foods be delayed in high risk children. However, the AAP now recognize that the early introduction of peanuts decreases the chance of developing peanuts allergy. Slowly introduce the allergy of foods after the addition of solid foods, which often occurs during a baby's first four to six months of the life[9].

Objectives

1. To assess the pre-test knowledge of the mothers regarding home management of selected common illness of children aged between 2 months and 5 years.

2. To evaluate the information of booklet on knowledge regarding home management of selected common illness of children aged between 2 months and 5 years.

3. To associate the post-test knowledge score of mothers regarding home management of the common childhood illness among children aged between 2 months and 5 years with selected demographic variables.

Assumption:

· The mothers may have some knowledge on management of common illness of among children aged between 2 months and 5 years.

· Mothers knowledge on management of common illness may vary with their demographic variables.

Hypothesis:

There will be significant association between among children aged between 2 months and 5 years. Sex, parents education, economic status, number of children in the family.

Material and Method

One group pre-test, post-test design was used. Participants were 50 mothers of children aged between 2 months and 5 years.

Criteria:

· Mothers of children age group between 2 months and 5 years.

· Mothers, who can understand Marathi, Hindi or English.

· Mothers who are willing to participate in this study.

Tools For Data Collection

Part A: Items on demographic variables are the mother's age, education of mother, family income, occupation of mother

Part b: for assessing the knowledge, questionnaire on home management for common illness among children aged between 2 months and 5 years

Result

Section A-

This section deals with the percentage wise distribution of the mothers according to their demographic variables.

· Distribution of the mothers according to their age reveals that 76% of mothers were between 20 and 30 years of age, 22% were between 31 to 40 years o age, 20% were between 41 and 50 years of age and 0% were over 50 years of age.

· Distribution of mothers according to their monthly income reveals that 34% mother's income were from 2000-4000 in rupees, 32% mother's income from 4001-6000 in rupees. 20% mothers were from 8001-10000 in rupees and 14% mothers were from 6001- 8000 in rupees.

· Distribution of mothers according to their occupation revels that 84% mothers were housewife. 10% mothers were farmer, 4% mothers were doing private job and 2% mothers were doing government job.

Section B-

Assessment of the mother's knowledge regarding home management of selected common illness among children aged group of 2 months and 5 years in selected rural area.

Table 1: Distribution of the subjects with regard to pretest knowledge of mothers regarding home management of the selected common illness among children aged between 2 months and 5 years.

n=50

Level of knowledge score	Score	Percentage score	Pre-Test	
			Frequency	Percentage
Poor	0-5	0-25%	6	12%
Average	6-10	26-50%	26	52%
Good	11-15	51-75%	18	36%
Very Good	16-20	76-100%	0	0
Mean ± SD			9.24±2.90	
Mean Percentage			46.2%	

Table 2: Distribution of subject with regard to posttest knowledge about home management of common illness among children aged between 2 months and 5 years

n=50

Level of knowledge score	Score	Score of percentage	Post -Test	
			frequency	percentage
Poor	1-5	0-25%	0	0%
Average	6-10	26-50%	0	0%
Good	11-15	51-75%	3	6%
Very Good	16-20	76-100%	47	94%
Mean ± SD			18.16±1.68	
Range			14-20=6	

Section C:

Analysis of effects of information booklet on knowledge about home management of the common illness among children aged between 2 months and 5 years

Table 3: Significance of difference between to pre test and post test knowledge of mothers regarding home management of the common illness among children aged between 2 months and 5 years

n=50

	Overall	Mean	SD	t-value	p-value
Knowledge of the score	Pre -Test	9.24	2.90	21.06	P<0.05
	Post -Test	18.16	1.68		

Discussion

Children are our future citizens, who can constitute a stronger of the nation. Growing age of the children in their tender age are at increased risk of becoming ill, specially children of under-five years. Common illness is (diarrhea, fever, vomiting, food allergy and acute respiratory tract infection) the cause of morbidity and mortality for under-five years children.

This five common illness occur more frequently than any other illness. Mother's being very crucial members in taking care of their children with this common illness in the family. So, it is essential that, they should have current knowledge about home management of common illness among children age group of 2 months and 5 years. The present study was taken up is an effort to assess the knowledge of mothers of the under the age of five years children through the one group pre-test and post-test method. Health education carried out through information booklet of the home management of common illness among children aged between 2 months and 5 years to mothers of under the age of five years children who lack of the knowledge about home management of common illness. A post-test conducted to find out the effects of information booklet about the home management of common illness among children aged between 2 months and 5 years. It was observed that the knowledge level of mothers of under-five children about home management of common illness among children aged between 2 months and 5 years was

increased in post pest.

Implications To Nursing:

Implication for nursing service

The content of information booklet module will help the nursing personnel in all area like hospital as well as community area and clinic for informing the mother's about home management of illness among children aged group of 2 months and 5 years. The findings will help the nursing personnel to estimate the effectiveness of information booklet module. The content of information booklet module will help the nursing personnel to known much more about different home management, causes, sing and symptoms, prevention and treatment of common illness among children aged between 2 months and 5 years. Which will help to explain the mothers among children age group between 2 months and 5 years while giving the health education.

Implication for nursing education

The nurse educator will be using the information booklet module to teach the students as well as peripheral level health workers and individuals to improve their knowledge, attitude towards the home management of the common illness among children age group between 2 months and 5 years. The institutes of nursing education should be playing an active role in conducting education programs, workshop and continuing education programmers to educate nursing personnel of hospital about the home management of common illness among children. The nurse educators can target the nurses and multipurpose health workers in the community areas to continuing nursing education programs along with training of trainer's programs can be organized to help then in imparting education of home management of common illness among children. it can be used as a training module for educating the individuals. The information booklet module can be used by the nursing students to educate the individuals regarding home management of common illness among children age group of 2 months and 5 years.

Implication for nursing research

Nursing research will help to know the nurse's role in developing knowledge of the people and developing the attitude related to home management of common illness. Research to conduct to provide the awareness about home management of common illnesses.

Implication for nursing administration

Nursing administration should take active part in policy making, developing, validating, approving protocols, procedures and standing orders concerning information booklet for mothers of 2 months to 5 years children. They should concentrate on proper selections, placement and effective utilization of the nurses in all areas, giving rooms for creativity, interest and ability in providing information booklet for the needed areas must provide opportunity for innovations, trial of emerging trends in the information booklet in fostering care. An ongoing education program on educative role of the nurses along with good supervision of nursing care service would motivate nurses to carry out the role in day to day nursing care. Efficient administrators can help in dissemination of research-based knowledge through organization of in-service education program. Nursing administration should promote necessary facilities and opportunities for nursing staff to equip themselves with the knowledge to deal with various aspects such as health practices which help for maintenances home management of common illness.

Conclusion

The researchers conducted the research on interventional topic. "To assess the effectiveness of information booklet on knowledge of mother's regarding home management of common illness among children aged between 2 months and 5 years." The researchers aimed to improve the level of knowledge of age between the 2 months to 5 years children mothers. We pre determined the certain objectives, to precede the study. Those objectives were adequate to reach into the findings. A particular time period has allocated for each step. Investigators had presented our hypothetical views about the study in its beginning. The study had done by separating the topic into 5 chapters. And finally, the research reached into.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: The study was approved by the Ethics committee of the institute.

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Evaluation of Lip Prints and Its Association with Sex Determination and Blood Group in Adults

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Abstract

Introduction: Cheiloscopy is the study of lip prints. Since the inception of the technique, the techniques is being used to evaluate the individual identity and its association with sex determination. As the blood stains may also be present at a crime scene, further studies have also been done to evaluate the association of common blood group pattern with lip prints.

Aims and Objectives: To study the patterns of lip print and its association with blood group and gender in adults.

Methods: In this prospective observational study, data were collected from 150 subjects (75 males and 75 females). All the subjects fulfilling the inclusion and exclusion criteria were in the age group of 18-30 years. In this study lip print pattern samples were collected by paper fold method and blood group samples were collected and both were analysed according to Tsuchihashi classification and Landsteiner's classification respectively.

Results: A The predominant lip print pattern found in males, females and both the gender together was Type 1- complete vertical in both upper and lower lips. Predominant blood group found in this study was B+ve. Most frequent lip print pattern found in all blood group types was Type 1- complete vertical.

Conclusions: Our study revealed an association between distribution of lip print pattern and gender. There was no correlation found in between blood group types and the cheiloscopic patterns. Further studies in different populations with large sample size are required to obtain a more definitive picture and statistical significance of this correlation.

Keywords: *Lip print; sex determination; blood group; Cheiloscopy.*

Introduction

Based on scientific principles individual identification is important task in forensic medicine. Traditional identification techniques involve evaluation of finger prints, dental pattern and DNA. In some situations, if the appropriate samples are not available, investigator may have to switch to other lesser known modalities of individual identification. Cheiloscopy is the Forensic investigation technique which includes study of lip prints. In 1961 first study of lip print was carried out by Tsuchihashi and Suzuki in Hungary and they established that human lips have individual and unique arrangement of lines on the red part. They conducted multiple studies over the next decade[1, 2, 3, 4, 5] to establish it further and came up with the classification[6] as following:

• Type I - Vertical grooves seen clearly

• Type I'- Same as type I but with partial length

• Type II – grooves with branching pattern

• Type III – Grooves are intersecting

• Type IV- Pattern of grooves is reticular

• Type V – Grooves not falling in above categories.

Other important evidence found at the crime scenes are blood stains. Landsteiner classified blood group system as the 'ABO' and ' Rhesus ' group. Despite blood grouping being an independent variable in determining the identity, its association with the lip prints may increase the efficacy of the technique.

The purpose of this study was to evaluate the pattern of lip prints and its possible association with blood group and sex determination in young adults in the state of Uttarakhand.

Material and Method:

The study was conducted in Department of Forensic Medicine, Himalayan Institute of Medical Sciences in year 2019 over three months. A total of 150 subjects were recruited (75 males and females each) from 18 to 30 years of age.

Inclusion criteria:

All subjects in the age group of 18-30 years, who gave consent to be recruited in the study after applying exclusion criteria.

Exclusion criteria:

1. Allergy to Lip stick
2. Any trauma or inflammation of the lips
3. Subjects undergoing orthodontic treatment
4. Congenital abnormality of lips

Materials used:

Red color lipstick, white A4 sheet, cellophane tape and magnifying lens.

Print recording:

Red lipstick was evenly applied on cleaned lips. Sample was collected on a clean white folded paper which was placed between the lips and the subjects were asked to press from centre to the corners. To make the impression permanent, the cellophane strip was stuck to the A4 sheet and visualized using magnifying lens. Blood sample was collected for blood group determination.

Statistical analysis:

All the data was compiled, classified and data was analysed using software SPSS version 11.

Results

Table 1: Pattern of Lip prints: Overall

Upper Lip					
		Frequency	**Percent**	**Valid percent**	**Cumulative**
	Complete vertical	108	72.0	72.0	72.0
	Incomplete vertical	13	8.7	8.7	80.7
	Bifurcation	14	9.3	9.3	90.0
	Intersection	6	4.0	4.0	94.0
	Reticular	9	6.0	6.0	100.0
	Total	150	100.0	100.0	
Lower lip					
		Frequency	**Percent**	**Valid percent**	**Cumulative**
	Complete vertical	88	58.7	58.7	58.7
	Incomplete vertical	7	4.7	4.7	63.3
	Bifurcation	33	22.0	22.0	85.3
	Intersection	8	5.3	5.3	90.7
	Reticular	14	9.3	9.3	100.0
	Total	150	100.0	100.0	

The most prominent upper lip print pattern found in present case study was complete vertical followed by bifurcation incomplete, vertical reticular and intersection. The most prominent lower lip print pattern was complete vertical followed by bifurcation, reticular, intersection and incomplete vertical.

Table 2: Pattern of Lip prints: Gender based

Upper Lip				
		Females	**Males**	**Total**
	Complete vertical	54	54	108
	Incomplete vertical	4	9	13
	Bifurcation	8	6	14
	Intersection	6	0	6
	Reticular	3	6	9
	Total	75	75	150
Lower lip				
		Females	**Males**	**Total**
	Complete vertical	48	40	88
	Incomplete vertical	5	2	7
	Bifurcation	15	18	33
	Intersection	5	3	8
	Reticular	2	12	14
	Total	75	75	150

Most common pattern of upper lip print was complete vertical in females followed by bifurcation, intersection, and incomplete vertical and reticular. Similarly, the most common upper lip print pattern was complete vertical followed by incomplete vertical, bifurcation and reticular for males. In females most frequent lower lip print pattern was complete vertical followed by bifurcation, incomplete vertical and intersection and reticular. In males most frequent lower lip print pattern was complete vertical followed by bifurcation, reticular, intersection and incomplete vertical.

Table 3: Chi square test (Relation of lip prints with gender)

Upper Lip						
	Value	**Df**	**Asymp. Sig. (2-sided)**	**Monte Carlo Sig. (2-sided)**		
				Sig.	**99% Confidence Interval**	
					Lower Bound	**Upper Bound**
Pearson Chi-Square	9.209a	4	0.056	0.053b	0.047	0.058
Likelihood Ratio	11.597	4	0.021	0.033b	0.028	0.038
Fisher's Exact Test	9.205			0.051b	0.045	0.057
Number of Valid Cases	150					
Expected count was less than 5 for 4 cells (40.0%). We expected a minimum count of 3.00.						

Lower Lip						
	Value	**Df**	**Asymp. Sig. (2-sided)**	**Monte Carlo Sig. (2-sided)**		
				Sig.	**99% Confidence Interval**	
					Lower Bound	**Upper Bound**
Pearson Chi-Square	9.929a	4	0.042	0.037b	0.032	0.041
Likelihood Ratio	10.760	4	0.029	0.042b	0.037	0.047
Fisher's Exact Test	10.024			0.036b	0.031	0.041
Number of Valid Cases	150					
Expected count was less than 5 for 4 cells (40.0%). We expected a minimum count of 3.50.						

In Fisher's Exact Test the P value is 0.051 which is more than 0.05. This shows that the parameters gender and lip print pattern of upper lip are statistically nonsignificant correlated. In Fisher's Exact Test the P value is 0.036 which is less than 0.05. This shows that the parameters gender and lip print pattern of lower lip are statistically significant correlated.

Table 4: Relation of blood group with lip prints

Upper Lip						
Blood Group	**complete vertical**	**Incomplete vertical**	**Bifurcation**	**Intersection**	**Reticular**	**Total**
A+ve	19	1	1	0	2	23
A-ve	2	0	0	0	0	2
B+ve	44	6	5	3	3	61
B-ve	2	1	0	0	0	3
AB+ve	13	1	3	0	1	18
AB-ve	1	0	0	1	0	2
O+ve	23	4	4	1	1	33
O-ve	4	0	1	1	2	8
Total	108	13	14	6	9	150
Lower Lip						
Blood Group	**complete vertical**	**Incomplete vertical**	**Bifurcation**	**Intersection**	**Reticular**	**Total**
A+ve	14	1	3	1	4	23
A-ve	2	0	0	0	0	2
B+ve	30	3	18	3	7	61
B-ve	2	0	1	0	0	3
AB+ve	14	1	3	0	0	18
AB-ve	2	0	0	0	0	2
O+ve	18	1	8	4	2	33
O-ve	6	1	0	0	1	8
Total	88	7	33	8	14	150

The most frequent lip print pattern of upper lip in respective blood group subjects: -

(1) O+ve- complete vertical followed by incomplete vertical, bifurcation, intersection and reticular.

(2) A+ve- complete vertical followed by reticular, incomplete vertical and bifurcation.

(3) A-ve- complete vertical.

(4) B+ve- complete vertical followed by incomplete vertical, bifurcation, intersection and reticular.

(5) B-ve- complete vertical and incomplete vertical.

(6) AB+ve- complete vertical followed by bifurcation, incomplete vertical and reticular.

(7) AB-ve- complete vertical and intersection.

(8) O-ve- complete vertical followed by reticular, bifurcation and intersection.

The most frequent lip print pattern of lower lip in respective blood group subjects:-

(1) O+ve- complete vertical followed by bifurcation, intersection, reticular and incomplete vertical.

(2) A+ve- complete vertical followed by reticular, bifurcation, intersection and incomplete vertical.

(3) A-ve- complete vertical.

(4) B+ve- complete vertical followed by bifurcation, reticular, incomplete and intersection.

(5) B-ve- complete vertical and bifurcation.

(6) AB+ve- complete vertical followed by bifurcation and incomplete vertical.

(7) AB-ve- complete vertical.

(8) O-ve- complete vertical followed by incomplete vertical and reticular.

Table 5: Chi square test (Relation of lip prints with blood group)

Upper Lip			
	Value	**Df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	28.620a	28	0.432
Likelihood Ratio	21.777	28	0.791
Linear-by-Linear Association	3.026	1	0.082
N of Valid Cases	150		
a. Expected count was less than 5 for 33 cells (82.5%). We expected a minimum count of 0.08.			
Lower Lip			
	Value	**Df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	20.239a	28	0.856
Likelihood Ratio	25.631	28	0.593
Linear-by-Linear Association	2.850	1	0.091
N of Valid Cases	150		
a. Expected count was less than 5 for 32 cells (80%). We expected a minimum count of 0.09.			

The P value was 0.432 which is more than 0.05. The two parameters blood group of subjects and lip print of upper lip are statistically non-significantly correlated. The P value is 0.856 which is more than 0.05. The two parameters blood group of subjects and lip print of lower lip are statistically non-significantly correlated.

Discussion

During investigation of several cases of disasters or death due to unnatural reasons, it becomes important to determine the identity of deceased or criminal. Though there are several more commonly used methods to compare the previously recorded database of the person with currently available anthropometric records, lip print records have also got its place in investigating cases. Though it's a useful technique, its practical use has remained uncommon. After the study on a large number of patients and coming up with a classification system pertaining to different types of lip prints from Suzuki and Tsuchihashi[6] there was a surge to validate their observation. Literature reported from different continents have equivocal results and our study is also an effort to observe the pattern in the state of Uttarakhand, India.

Type I – Complete Vertical pattern was found to be the most common one, in males, females and overall. There is enormous variation in this finding in literature reported before. Shilpa Patel et al[7] studied 100 subjects in the age group of 18-24 years and found Type 1- Complete vertical to be the most common pattern in males. Type II – Bifurcation type was the most common in females and overall. Nisha Kaul et al[8] conducted similar study in western part of Uttar Pradesh recruiting 200 subjects with the similar conclusion to our study mentioning Type 1- complete vertical to be the commonest lip pattern. Basheer S et al[9] studied a larger group of 800 subjects from North Kerala and concluded that type II - Bifurcation was predominant in males, Type - IV Reticular – in females and Type I - Complete vertical, in overall population. Verma et al[10] studied 208 random subjects and found type II - Bifurcation to be the commonest in males and females. Their findings were similar to other studies done by Sivapathasundram et al[11], Varghese et al[12] and Augustine et al[13].

Smudging of the lip prints lead to difficulty in ascertaining the pattern. Such problems have been encountered while data collection in past.[14] We did not have any such problems and all the prints could be studied properly. Thus, we found significant variation in the pattern of lip prints in the different geographical areas. Despite the variation in prevalence, it remains a useful technique for identification. In India, Aggarwal[15] mentioned about a case investigation and proved that lip prints are equally good as finger prints in identification of criminals.

The current study revealed that the most common blood group seen in both males and females was B+ve. Similar results were shown in studies conducted by Nisha Kaul et al[8] (western U.P) and Pradhuman Verma et al[10] (Rajasthan). Study conducted by Santosh Hunsagi et al[16] (Karnataka) and Basheer et al[9] (Kerala) showed blood group O to be the most common. The current study revealed that the most frequent lip print pattern in both upper and lower lips in all blood group types was Type I - complete vertical. Similar results were shown in study conducted by Nisha Kaul et al.[8] Similar study was conducted by Shilpa Patel et al[7] in which most frequent lip print pattern found in A+ve blood group subjects was Type II - bifurcation, in B+ve blood group subjects was Type 1 and in O+ve blood group subjects was Type II - bifurcation. Study conducted by Shaini Basheer et al[9] (Kerala) found that in all blood group types the upper lip pattern showed a predominance of Type II whereas lower lip pattern showed Type I. Study conducted by Pradhuman Verma et al[10] showed that in all blood group types Type II was predominant. Study conducted by Santosh Hunsagi[16] (Karnataka) showed that the most predominant lip print pattern in blood group A was Type III, in blood group B- Type II, in blood group AB - Type I and Type III and in Blood group O was Type II.

Conclusion

Our study revealed that there was an association between distribution of lip print pattern and gender. The most common lip print pattern seen in males, females and including both the gender overall was Type 1- 'complete vertical'. There was no correlation found between blood group types and cheiloscopic patterns. Most frequent lip print pattern found in all blood group types was Type 1- complete vertical. Though the method

of sample collection varied in all studies performed till now, our results were similar to previously reported literature. Further studies in different populations with larger sample size would be required to obtain a more definitive picture and statistical significance of this correlation.

Ethical Clearance: All ethical clearances were obtained from Institutional review board and ethics committee.

Source of Funding: Self

Conflict of Interest: Nil

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Estimation of Sexual Dimorphism by Mesiodistal Dimension of Permanent Maxillary Incisors and Canines

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Abstract

Introduction: Sexual dimorphism is the condition where whether the specific body part or the whole body of two sexes of the same species exhibit different characteristics except for the differences in primary sexual organs. Teeth are helpful in determining the sex, stature, race, age etc. same as bony tissues when identification is not possible from rest of the body. Most of the studies conducted earlier are on mandibular dental tissue and literature is sparse on evaluation of maxillary area. We decided to conduct the study on maxillary teeth.

Aims and Objectives: To find the correlation between mesiodistal dimension of permanent maxillary incisors and canines with the gender.

Methods: The study was conducted in Forensic Medicine and Toxicology department, Himalayan Institute of Medical Sciences, SRHU. The objective of the study was For the study sample of 100 individuals were taken out of which 50 were male and 50 female, all in the age group of 17-25 years of age. For measuring the dimension of teeth there comes mesiodistal, Buccolingual, labiolingual, crown heights, diagonal length etc. dimensions, but for our study we selected mesiodistal dimension. Subjects were taken according to the inclusion and exclusion criteria. The results were statistically analysed and compared with other studies.

Results: The study revealed about the definite association between mesiodistal dimensions of anterior maxillary permanent teeth and gender.

Conclusions: The results showed a positive correlation between the width and sexual dimorphism in maxillary canines, incisors and other teeth. Male teeth were greater in width as compared to female teeth in Indian population. More studies with larger number of subjects would be required to establish a proper correlation between maxillary dentition and gender.

Keywords: *Maxilla; sex determination; Teeth; Mesiodistal; permanent.*

Introduction

Sexual dimorphism is the condition where the specific body part or the whole body of two sexes of the same species exhibit different characteristics except for the differences in primary sexual organs. It is a pivotal component of individual identification in forensic medicine.[1] Krogman, conducted several studies regarding accuracy in determination of sex by skeletal remains and concluded that using entire skeleton, sex can be determined with 100% accuracy whereas long bones show 80% accuracy.[2] The cases where only dental tissues were recovered instead of whole skeleton like in cases of mass disaster like flood, earthquakes,

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hurricanes, fire etc. or mutilated body parts, bacterial decomposition, abrasion, high temperature etc., dental tissues may be found intact because of its high resistance against physical and chemical threats among other skeletal tissues. Tooth can be used for purpose of identification and helping the law.[3] Like the other bones of the body, teeth are helpful in determining the sex, stature, race and age when identification is not possible from rest of the body.[4] Garn et al[5] (1967), studied sexual dimorphism using dental tissues, and gave a formula for the determination of degree of sexual dimorphism between males and females as:

$$Sexual\ dimorphism = [\{Male\ mean - Female\ mean\}/\ Female\ mean] \times 100$$

Most of the studies were conducted on mandibular dental tissues earlier. In our study, we chose maxillary dental tissues over mandibular dental tissues for identification purpose due to sparse literature and data available.

The purpose of this study was to look for the correlation between mesiodistal dimension and sexual dimorphism in anterior maxillary teeth in adults and estimation of the percentage of sexual dimorphism in anterior maxillary teeth.

Material and Method:

It is an observational cross sectional study conducted in the Department of Forensic medicine and toxicology, Himalayan Institute of Medical Sciences, SRHU, Dehradun. Ethical clearance was obtained from the Institute's ethical committee prior to conduction of the study. A written informed consent was taken from the subjects after explaining them the procedure, risk and complications. In this study, the mesiodistal dimension of anterior permanent maxillary teeth was measured for determining of sex. 100 subjects (50 male and 50 females). were selected on the basis of the following inclusion and exclusion criteria.

Inclusion criteria-

1. Age of the subjects - above 18years
2. The canines of the subjects should be healthy and fully erupted.
3. There should be no gap in the teeth and no crowding should be present.

Exclusion criteria-

1. Ectopic location of tooth
2. Unhealthy gingiva or any infection
3. Incompletely erupted teeth
4. Various teeth
5. Restoration of canines and incisors
6. Endocrine abnormality affecting the eruption of teeth.

Materials used – Digital Vernier caliper, isopropyl alcohol, cotton.

Procedure- The vernier caliper was set on zero reading and was also checked for no or minimum instrumental error. Then the edges of the vernier caliper with which readings were to be taken were sterilized by using isopropyl alcohol. Procedure along with risks and benefits was described to the subject and the subject was told to widely open his/ her mouth so that all of his/her anterior maxillary dentition was clearly visible to examiner. The edges of the vernier caliper were opened and fixed approximating the edges of the teeth of which the dimensions were to be taken. The readings were noted down up to two decimal digits. The measurements were taken by single observer so as to minimize interobserver error. Readings were taken twice and an average was calculated for each tooth so as to reduce inter-observer error.

Mesiodistal dimension means the width of the tooth between the contact points with other teeth from medial to lateral side.[6]

Table-1 shows that the group statistics comparing the mean values, standard deviation and standard mean error between the two sexes. The mean values for each tooth was taken under observation for both sexes are as follows

The recorded data was put in Excel sheet for statistical analysis by the software SPSS.

Results

Table-1: Group statistics

	Gender	N	Mean	Std. Deviation	Std. Error Mean
Right Canine	M	50	7.5230	0.74836	0.10583
	F	50	6.9698	0.67353	0.09525
Right lateral Incisor	M	50	6.9400	0.85090	0.12033
	F	50	6.2266	0.75144	0.10627
Right central incisor	M	50	8.4510	0.75956	0.10742
	F	50	7.9198	0.56642	0.08010
Left central incisor	M	50	8.4728	0.68031	0.09621
	F	50	7.9604	0.63025	0.08913
Left lateral incisor	M	50	6.9088	0.79678	0.11268
	F	50	6.2284	0.72174	0.10207
Left canine	M	50	7.4874	0.71300	0.10083
	F	50	6.7718	0.74148	0.10486

For males the mean values for Right canine was 7.5230+_0.74836, Right lateral incisor - 6.9400+_0.85090, Right central incisor - 8.4510+_0.75956, Left central incisor - 8.4728+_0.68031, Left lateral incisor- 6.9088+_0.79678, Left canine- 7.4874+_0.71300 and for Females the mean values for Right canine - 6.9698+_0.67353, Right lateral incisor - 6.2266+_0.75144, Right central incisor- 7.9198+_0. 56642, Left central incisor- 7.9604+_0.63025, Left lateral incisor- 6.2284+_0.72174, Left canine- 6.7718+_0.74148.

On comparing these mean values for both males and females, the mean values of all teeth taken under consideration for males were greater in comparison to those of females. By using these mean values and putting them in the formula given by Garn et Al., we can estimate the percentage of sexual Dimorphism in the teeth taken under observation. After putting the data in the formula, the percentage of sexual dimorphism exhibited by each teeth were as follow:

Right canine – 7.93%, Right lateral incisor- - 11.45%, Right central incisor – 6.70%, Left central incisor – 6.4%, Left lateral incisor – 10.92%, Left canine – 10.56%. Among all teeth taken under observation, all teeth show significant amount of sexual dimorphism and the maximum values were of right lateral incisor followed by left lateral incisor then left canine and right canine then right central incisor then left central incisor.

Table 2 shows that when independent t-test was done, the sig (2-tailed) value came out to be 0.000 (i.e. $p<0.05$). Due to this result we can say that there is statistically significant difference in mean mesiodistal dimension of tooth in males and female

Table-2: Significance of data by t-test and Levene's test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Right Canine	Equal variances assumed	2.447	.121	3.885	98	.000	.55320	.14239	.27064	.83576
	Equal variances not assumed			3.885	96.932	.000	.55320	.14239	.27060	.83580
Right lateral Incisor	Equal variances assumed	.381	.538	4.444	98	.000	.71340	.16054	.39481	1.03199
	Equal variances not assumed			4.444	96.524	.000	.71340	.16054	.39475	1.03205
Right central incisor	Equal variances assumed	7.306	.008	3.964	98	.000	.53120	.13400	.26529	.79711
	Equal variances not assumed			3.964	90.625	.000	.53120	.13400	.26502	.79738

Cont... **Table-2: Significance of data by t-test and Levene's test**

Left central incisor	Equal variances assumed	.407	.525	3.907	98	.000	.51240	.13115	.25213	.77267
	Equal variances not assumed			3.907	97.433	.000	.51240	.13115	.25212	.77268
Left lateral incisor	Equal variances assumed	.964	.329	4.475	98	.000	.68040	.15204	.37869	.98211
	Equal variances not assumed			4.475	97.057	.000	.68040	.15204	.37865	.98215
Left canine	Equal variances assumed	.094	.760	4.919	98	.000	.71560	.14548	.42691	1.00429
	Equal variances not assumed			4.919	97.850	.000	.71560	.14548	.42690	1.00430

Table-3: Paired sample statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Right central incisor	8.1854	100	0.71806	0.07181
	Left central incisor	8.2166	100	0.70141	0.07014
Pair 2	Right lateral Incisor	6.5833	100	0.87542	0.08754
	Left lateral incisor	6.5686	100	0.83003	0.08300
Pair 3	Right Canine	7.2464	100	0.76092	0.07609
	Left canine	7.1296	100	0.80811	0.08081

Table-4: Paired sample tests

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Right central incisor - Left central incisor	-.03120	.55905	.05591	-.14213	.07973	-.558	99	.578
Pair 2	Right lateral Incisor - Left lateral incisor	.01470	.81920	.08192	-.14785	.17725	.179	99	.858
Pair 3	Right Canine - Left canine	.11680	.69669	.06967	-.02144	.25504	1.677	99	.097

Table 3 According to our hypothesis, there should be no difference in mean mesiodistal dimension of a pair of teeth in same person.

Table 4 On applying paired t-test, the sig (2- tailed) value were found much higher than 0.05. This shows that there is a significant difference between the dimensions of both teeth of a pair.

Discussion

The exact values of sexual dimorphism are calculated by the formula given by Garn et al. Other studies were also completed by using the same formula.

Rajbir Kaur khangura et al. in their study found that males show a greater mesiodistal dimension as compared to females. The canines in study of Zeinab Davoudmanesh et al showed a greater mean width in case of males as compared to females. Contradictory to this, the maxillary right canine in the study of Sharlene Sara Babu showed a greater mean width for females as compared to males.

In our study, all the teeth under observation showed a greater mean mesiodistal width for males and significant values of percentage of sexual dimorphism as for Right canine- 7.93%, right lateral incisor- 11.45%, right central incisor- 6.70%, left central incisor- 6.4%, left lateral incisor- 10.92%, left canine- 10.56%. Right lateral incisor leading the values followed by the left lateral incisor then the left canine. The canines in study of Gloria Staka also showed greater value of mean mesiodistal width with the percentage of sexual dimorphism of 3.71%.

Here, in our study the maximum sexual dimorphism is shown by the right lateral incisor but in the study done by Neelakshi Pandey and Mang Shin Ma maximum sexual dimorphism is exhibited by right maxillary canine. In our study, the mean value of right canine in males is greater than that of females and can be supported by the evidence that the mean values of mesiodistal dimensions of male maxillary incisors is greater than that of females as seen in the study conducted by Madhavi Yuwanati in 2012.

Conclusion

The results show a positive correlation between the width and sexual dimorphism in maxillary teeth, and not only canines and incisors, other teeth also exhibit sexual dimorphism. Male teeth are greater in width as compared to female teeth in Indian population. More studies are required to be done on dentition, not only incisors and canines' of maxilla only, but other teeth also are required to be studied. Mesiodistal dimension is not the only one parameter for sexual dimorphism, but other parameters are also there that are needed to be touched.

Ethical Clearance: All ethical clearances were obtained from Institutional review board and ethics committee.

Source of Funding: Self

Conflict of Interest: Nil

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Knowledge of Pregnant Women in Regards to Oral Health of the Expected Child

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Abstract

Background: Maternal oral health literacy, positive attitude and behavior in regards to infant's oral health dramatically reduce the chances of early childhood caries. The aim of the study is to assess oral health knowledge among pregnant women and to report their beliefs regarding dental care for their expected child. The study also evaluates the contribution of health care providers in regards to the oral health of the expected child.

Method: The cross-sectional study was conducted among 422 pregnant women, who attended an antenatal clinic at a tertiary health-care facility in Lucknow.

Closed-ended questionnaire was used to collect the data. The validity and reliability of questionnaire was tested. Frequency, distribution tables and descriptive statistics were calculated for all variables. Mann-Whitney U test and Kruskal Wallis H tests were applied to check for any significant difference between socio-demographic characteristics and general knowledge about oral health care as well as practices. Bi-variate and multivariate analysis was done.

Result : 40.8% pregnant women had poor knowledge, 56% had poor oral hygiene practices and 46.8% had poor knowledge regarding dental care of their expected child. Education was the only factor to be significantly associated with both variables- knowledge, and practice (p- value<0.01).

Conclusion and Recommendation: The present study highlights the need for improved communication and coordination between prenatal health care professionals, dentist, and pregnant women to facilitate optimal care of their child's oral health.

Keywords: *pregnant women, oral health care, early childhood caries, oral hygiene practice, education, referrals.*

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Introduction

Early childhood caries (ECC), a severe form of dental caries, has been defined as the presence of 1 or more decayed (non-cavitated or cavitated), missing (due to caries), or filled tooth surfaces in any primary tooth, in age less than 72 months, by the American Academy of Pediatric Dentistry (AAPD). [1]

Early Childhood Caries continues to be spread worldwide, with prevalence varying between countries and continents, despite all recommendations by the World Health Organization (WHO), for prevention of ECC. Dental caries affects nearly 30% to 50% of children in developed countries and upto 90% in developing and underdeveloped countries and other vulnerable populations. [2,6]

The occurrence of caries in a tender and budding age has a huge negative impact on the child's physical and mental development[7]. Oral malaise due to ECC, like experience of pain, trouble with eating, chewing, smiling and communication due to missing, discolored or decayed teeth, impedes a child's activities which eventually leads to loss of many potential school hours and playtime[7]. Besides, untreated tooth decay radically increases the risk of permanent tooth caries and poor overall health of the child due to poor nutrition.[7, 8]

Toddlers affected by caries may have to undergo a lot of psychological trauma due to fear and discomfort of getting treated. The idea of visiting a dentist, receiving injection and treatment in the oral cavity, is distressing for a juvenile, leading to sleeplessness and nitpickiness at meals with increased risk of domestic violence towards a child.[9] The distress and suffering, which an innocent juvenile undergoes due to the negligence on part of caregivers, need a call for attention for allcaregivers.

To manage ECC rationally, policies must pay attention on the right cause at the right time, thereby, preventing initiation of caries rather than in managing severity (10). WHO experts recommended that the critical period for preventive intervention of ECC is during the first 1000 days i.e. from pregnancy through to the child's second birthday.[11,12.] Dental caries could be prevented through coordinated efforts of mothers/ would-be mothers, the health care professionals i.e. the gynecologist, pediatrician, dentist, general practitioners and, other caregivers. Good oral health of the mother, good oral health knowledge and positive attitude about the infant's oral health, among mothers dramatically reduces the chances of ECC (13).

The purpose of the present study is to explore the oral health knowledge and self-care practice of pregnant women and their beliefs about the oral health care of the expected child. The study helps to assess the need for educating expecting mothers and motivating the health care professionals for oral health carepromotion.

Methods

This is a descriptive cross-sectional study. Pregnant women, regardless of financial status, social class or ethnicity, trimester, visiting the outpatient department of the anti-natal clinic (ANC) at the Department of Obstetrics & Gynecology, King Georges Medical University, Lucknow, India were systematically randomly selected for the study. Physically or mentally disabled females, females with some systemic health issues, those who refused to give written consent or those who could not read English or Hindi, were excluded from the study. Considering an estimate of 50% knowledge level (as the prevalence of knowledge of oral health in this community was unknown), with 5% precision of error, 95% confidence interval, and 80% power, the required sample-size was 384. To account for 10% anticipated non- responsiveness, this estimate was increased to a final sample size of 422. A review of past outpatient department attendance revealed that on an average "60" number of pregnant women attend ANC clinic at the Department of Obstetrics & Gynecology per day. A total of about 3600 (60 x 60= 3600) pregnant women are expected to visit ANC clinic during the 60 days of data collection. Hence, to enroll the desired sample size of 422 pregnant women, every 3600/422th i.e. every 9th pregnant womanwas enrolled for the study after assessing the inclusion and exclusion criteria. Ethical clearance was obtained from the institutional ethical committee (**90th ECM II B-IMR-S/P2).** The study has been conducted in full accordance with the World Medical Association Declaration ofHelinski.

The study participants were presented with a standardized questionnaire based on the WHO (**Table 1**)oral health questionnaire for adults as well as specific questions relating to oral health care of infants were added from similar studies.[14] A pilot study was conducted earlier on 20 expecting mothers to check the validity and reliability of the questionnaire. Editing was done until there was an acceptable clarity of the questionnaire. The questions were closed ended and in Hindi and English dialect. Questionnaires were completed, under the supervision of the principal investigator, by selecting the most relevant answer with

no interpersonal communications. The questionnaire was divided into 5 sections- section one, gathered information about the socio-demographic and socioeconomic background, section two consisted of seven questions assessing general oral health knowledge, section three had ten questions regarding the knowledge of expectant mothers about oral health care of the expected child and section four assessed oral health care practice of pregnant women. The fifth section assessed the attitude of pregnant women and health care professionals towards oralhealthoftheexpectedchild. Forassessingknowledgeandpracticeinsectionstwoand

three, correct answers were given a score of 1 whereas incorrect answers and 'I don't know` answers were given a score of zero. Later subjects were divided as per their scores. Participants who scored four or more were categorized in high knowledge group and rest in low knowledge group. For categorization, on basis of practice, those scoring five or higher were considered with adequate oral health care practice. In third section, those who scored six or more were classified as having good knowledge regarding oral health care of a child. Separate percentages were reported for the section-assessing attitude regarding of oral health care.

Statistical analyses were done using the Statistical Packages for Social Sciences (SPSS) software version 20.0. Frequency and descriptive statistics were calculated for all variables. Further, bi-variate and multivariate analysis(Table2) was performed. Chi-square test of association was performed. Ranks of scores were compared across various socio-demographic characteristics using the Mann Whitney U test (2 groups) and Kruskal Wallis (>2 groups) test.The p-value of less than 0.05 was considered to be statistically significant.

Ethics: The study has been approved by the InstitutionalEthical Committee: Reference number: 90th ECM II B IMR-S/P2.

Results

The data was collected for four hundred individuals.

The responses regarding oral health knowledge, knowledge about oral health care of the child and practice are presented in Figure 1.1, 1.2 respectively. Overall, out of 400 women, 40.8% had low knowledge about oral health, 46.8% percent of women had poor knowledge about the oral health care of the child and 56% of the women surveyed did not adequately followed good oral health care regimen.

Table 1 shows the response of prenatal care providers towards oral health care of the expected childChi-square test of association was performed. Education and occupation of women were found to be significantly associated with general knowledge about oral healthcare .Age andnumber of children had significant association with practice of oral healthcare. Age, education, number of children and period of gestation were all significantly associated with knowledge about oral health care of the child.

Initially, bi-variate analysis was done to check for any association between socio- demographic variables and knowledge about oral health care and practice Further, to study the effect of socio-demographic variables on the knowledge and practice of oral health care among pregnant women, multi variate analysis was performed. For general knowledge eaboutoral healthcare, occupation was significant in the multi variate model in **table 2**. As compared to women who were housewives, women who were employed had four times higher knowledge about oral health care of the child. For practice, both age and number of children were significant in the multivariate model. Womenagedbetween18 to25 years had five times highero dds of following good practice of oral healthcare than women of 35 years and above. Women having no child were four times more likely to follow good practice of oral health care as compared to women having 3 or more children. Lastly for knowledge about oral health care of the child, number of children and women's education were significant in the multivariate model . Women who completed college or university were almost eleven times more likely to have good knowledge for health care of the child as compared to women having no education. Similarly, women having no child were four times more likely to have better knowledge about oral health care of the child than women having 3 or more children.

Table 1: Attitude of respondents towards oral health care (as frequency percentage).

Attitude	Yes	No	I am Planning	
	Frequency (%)	Frequency (%)	Frequency (%)	
Did you visit any dentist during pregnancy? (n=400)	112(28%)	264(66%)	24(6%)	
If Yes, what was the reason of your visit? (n=112)	Dental Problem	Routine Check up	For advice regarding oral health care for the child	
	99(88%)	9(8%)	4(4%)	
If you visited the dentist, did the dentist give you advise regarding oral health care for the expected child? (n=112)	Oral Advice	Written & Oral Advice	Written Advice	Not given any advice
	26(23%)	0(0%)	0(0%)	86(77%)
Were you recommended to visit the dentist for oral health care advice for the child during your pregnancy? n=400	Gynecologist/ Pediatrician/ Any physician 5(1.25%)	Family/Friends 3(0.75%)	Self – Motivated 2(0.5%)	Not recommended 390(97.5%)

Table 2: Multivariate analysis

General Knowledge about oral health care		Practice		Knowledge about oral health care of the child	
Variables	**OR (95% CI)**	**Variables**	**OR (95% CI)**	**Variables**	**OR (95% CI)**
Education No Education College or Univ.	1.00 2.125(0.80-5.62)	Age 35 & above 18-25 yrs	1.00 5.42(2.34-12.54)*	Age 35 & above 18-25 yrs	1.00 0.47(0.17-1.33)
Occupation Housewives Employed	1.00 4.168(1.77-9.80)*	No. of children 3 or more No child	1.00 3.79(1.56-9.18)*	Education No Education College or Univ.	1.00 10.77(2.24-51.67)*
				No. of children 3 or more No child	1.00 3.82(1.03-14.19)*

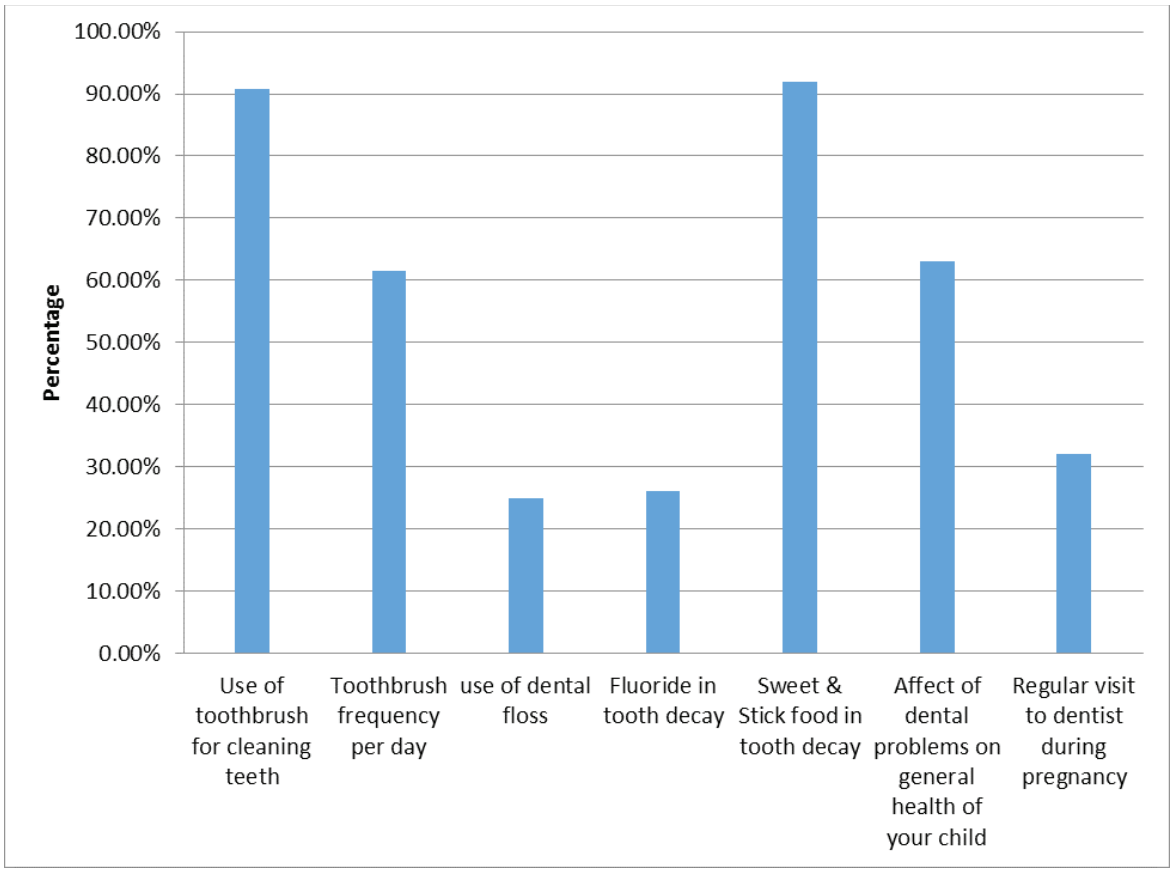

Figure 1.1. General Knowledge about oral health care

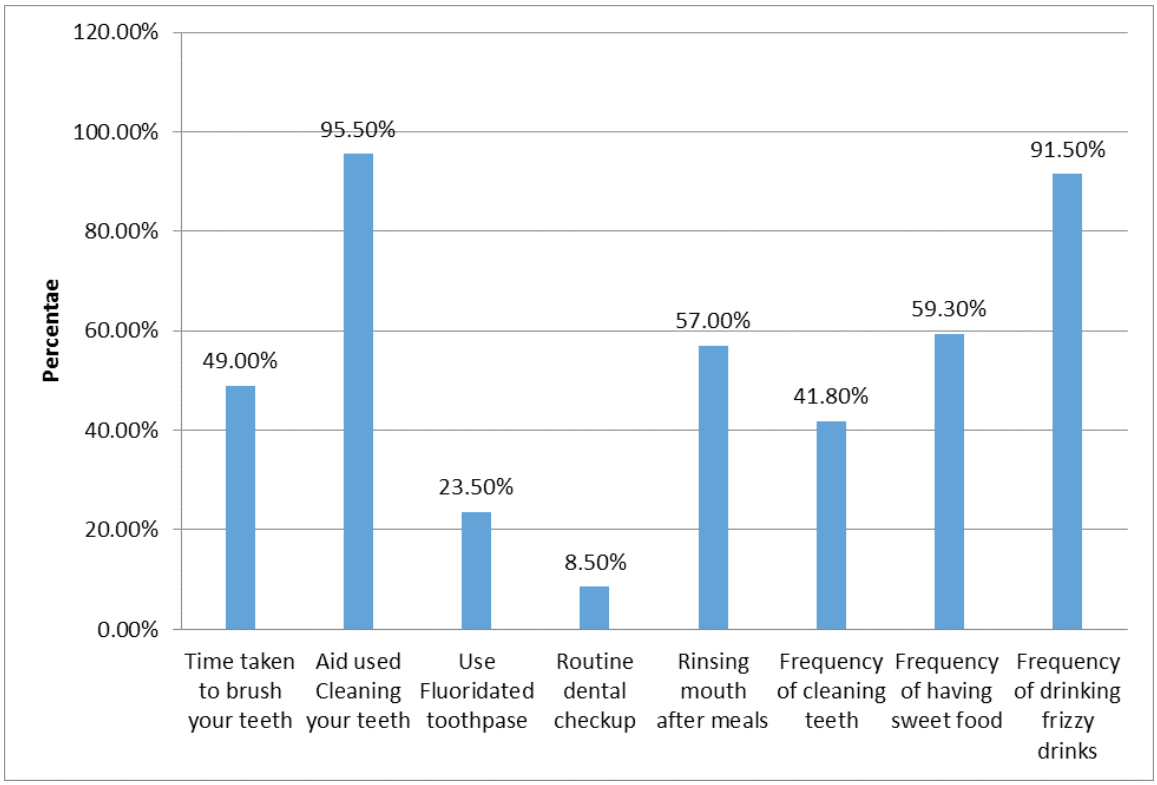

Figure 1.2. Distribution of practices

Discussion

To ascertain that future generations of children, have better oral health and improved quality of life worldwide, we need to consider strategies to promote preventive approach rather than the general curative approach. A systematic reviewbyLeongetalin2012doneontherisk factors during the first year of life for ECC highlights the need for early maternal intervention to reduce the likelihood of ECC.[15]

The impediment in reducing the prevalence of ECC is that many women are ignorant of the effects of their oral health behavior on themselves and their babies earlier to, during and after pregnancy. Moreover, many of those who are aware, lack the orientation to take appropriate actions.[15, 17]

The overall score of the present study revealed poor oral health knowledge and poor oral hygiene practices among pregnant mothers. Expectant mothers also showed deficient knowledge regarding infant oral health care in many aspects. Educational qualification of the pregnant women had a significant role in their oral health knowledge, oral hygiene practices and knowledge about the oral health care of the expected child. Earlier studies have also shown a direct influence of educational level on oral health knowledge and practices. [18]

There was good knowledge about the use of toothbrush in cleaning teeth and role of sweet and sticky food in tooth decay and limited knowledge of the role of fluoride and flossing in caries prevention, which well correlated with the results of earlier studies.[19, 22] A cohort study by Correia surveyed the future plans of pregnant women to provide dental care for their expected child and reported deficient knowledge.[22] The findings were similar to our study but they did not report the variability in knowledge in regards to education level and employment status.

Poor oral health of the mother due to inadequate oral hygiene practice inadvertently affects the overall health and oral health of the unborn child. In our study, 56% of female participants did not follow adequate oral health practices. Mothers need to know that if they have a higher level of mutans streptococci (MS), a microbial risk factor of ECC, their infants are at greater risk of acquiring MS than those mothers having low levels.[23, 24]

In our study positive correlation was observed between general knowledge about oral health and practices and knowledge about the oral health care of the child. This is in agreement with a study by Vann et al in 2010 on the impact of oral health literacy among female caregivers on outcomes in early childhood. He concludes that poor oral health literacy of the caregiver was associated with deleterious oral health behaviors, including nighttime bottle use and no daily brushing/ cleaning and this may have a multidimensional negative impact on oral health outcomes in infants and young children.[25]

As far as the best of our knowledge, this is the first study to evaluate the role of prenatal care providers in preventing ECC and promoting oral health. Out of 400 expectant mothers visiting the antenatal clinic, only 5 had been recommended for a dental visit by health care professional. Also, disharmony was seen between the prenatal providers and dentist; prenatal care providers fail to assess and refer the pregnant patient for oral health care training for herself and for the expected child. Moreover, it was seen that many dentists are providing dental care only by treating the patients and not by advising for proper oral health care protocol. Out of 112 patients who visited the dentist during pregnancy, 86 women were not given any guidelines regarding oral health care for the expected child. Laxity in the form of lack of communication and recommendations on part of the prenatal care providers is contributing to lack of awareness and motivation among pregnant women leading to a high prevalence of ECC.

Moreover, consideration of oral diseases is intended at the secondary and tertiary level of prevention, when the child has already acquired the disease, neglecting early prevention. Primary or early prevention is paramount to ECC management. Pregnant women, early mothers, primary caregivers, policy makers, and health care professionals, all should recognize the common risk factors of ECC and promote healthy oral healthpractices.[11]

There is increased approachability of women during pregnancy by health care professionals therefore maternal and infant oral health care messaging during this period is opportune. Brief counseling and strategies that move would-be mothers from inaction to action

should be adopted. Written guidelines in regards to oral health care for pregnant women and infant should be given, well explained and reinforced to the patient on every visit.

The results of the study are assessed within the context of study restraints. Limitation of the study was that convenience sampling was used which may have led to some bias, though the sample of pregnant women was almost equally distributed among the residential blocks in Lucknow. Moreover, the sample was not representative of health-care professionals in general because the data was collected at an antenatal clinic in a primary health care center. The use of a self-administered questionnaire might have introduced some bias as some of the participants might have under reported bad practices or over reported some good activities. Despite these limitations, it is still justified to consider the study results which, call for strategies for implementation of programs to motivate prenatal care providers to bring out awareness about the importance of oral health and theirimplications.

Conclusion and Recommendation

Our findings report poor and deficient knowledge among pregnant mothers regarding oral health care. Expectant mothers do not know all the factors that can influence caries in their children. There was a lack of initiative from health care professionals in providing referrals for dental visits during pregnancy. The present study highlights the need for improved communication and coordination between prenatal health care professionals, dentist, and pregnant women to facilitate optimal care of their child's oral health.

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Conflict of Interest: All authors declare that there is no conflict of interest.

Practice Relevance:

1. A preventive rather than curative approach will help decrease the oral health related ailments and also will be cost effective in the longer run.

2. A proper referral system from pre-natal health provider to a dentist if setup, will help bridge the gap of knowledge regarding oral health in pregnant females and a proper counselling will move them from inaction to action and thereby decreasing the suffering of the child.

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Incidence of Distant Metastasis in Head and Neck Cancer: A Hospital based Study

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Abstract

Aims and Objectives: To study the incidence of distant metastasis to bone and soft tissues from head and neck cancers and to assess the primary site of head and neck cancer, its clinical staging and its correlation with the site of distant metastasis.

Materials and Methods: Medical records of patients admitted for management of head and neck cancers in Medical and Radiation Oncology for a period of 6 years, were assessed for primary site of the tumour, staging, histopathological diagnosis and the site of distant metastasis.

Results: Out of 125 patient records evaluated, the incidence of distant metastasis was 15.2%. Majority of the patients (60%) presented at Stage III and IV of the disease. Thyroid and tongue malignancies showed the highest incidence of distant metastasis and this incidence was most common in the 5th and 6th decades of life. The most common sites of distant metastasis were to the bones (31.6%) and lungs (15.8%).

Conclusion: Cancers of the head and neck is typically loco-regional in nature, with metastasis to cervical nodes. Distant metastasis, though uncommon, may adversely impact the survival and quality of life of the patient. Understanding the risk factors and incidence for metastatic head and neck cancers may be useful in treatment planning and follow-up protocols for newly diagnosed patients.

Keywords: *Head and neck cancer, distant metastasis, incidence, staging, primary tumor*

Introduction

Cancer remains one of the main reasons of morbidity and mortality globally. A recent Lancet survey draws an alarming picture of increasing mortality (<5lakhs) due to cancer in India in 2010.[1] Among, 1 million new cases reported yearly in India, two hundred thousand affects the craniofacial region[2]. In the global scenario, the prevalence of cancer affecting the craniofacial region is six times more prevalent in India[2], owing to the extensive use of tobacco and its related products and varying lifestyle habits. Cancers of craniofacial region is considered predominantly a loco-regional disease, commonly metastasizing to the lymph nodes of the cervical region. Metastasis to distant sites usually occurs in later stages of the disease progression[3].

Distant metastasis is an intricate process of selection of a certain subpopulation of tumour cells for survival in an unfamiliar and hostile micro-environment.[3] These metastatic tumor cells possess the capacity to invade the basement membrane, extravasate and survive in the systemic circulation. Following which, they invade the microvascular walls and proliferate in a foreign distant organ. Numerous molecular adaptations involving numerous cellular processes such as adhesion,

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proteolysis, apoptosis and angiogenesis occur in order to begin the metastatic disease.[4,5]

The overall clinical frequency of distant metastases in craniofacial malignancies is relatively less in comparison to others, varying from 4.3% to 26% [6,7,8,9]. Leemans et al (1993) mentioned that in autopsy studies there was increased prevalence (40-57%) of metastases to distant areas associated with craniofacial cancer[6]. Lungs and bones are the commonest sites of metastasis from craniofacial cancer[10]. The other areas of metastatic deposits are in the liver, mediastinum, brain and skin although unexpected sites of spread such as cardiac metastasis from carcinoma of the tongue[11,12] and other primary tumours to the peritoneum, oesophagus and spleen, have also been noted. Sites of bone metastases have been found mostly in the vertebrae, skull and ribs[2,10]. This varying rate of metastasis could be influenced by the site of tumour, staging of neoplasm and the degree of involvement of regional nodes[2,3].

Distant metastasis adversely impacts the survival and standard of living in cancer patients. A thorough knowledge about the possible factors for metastatic craniofacial cancers is useful in planning treatment and formulating protocols for follow-up of recently diagnosed patients. The present study is aimed to study the incidence of distant metastasis to bone and soft tissues from head and neck cancers also with an objective to assess the site of primary cancer in craniofacial region, its clinical staging and its correlation with the site of distant metastasis.

Material and Methods

Archival data from the medical records of all patients with cancer in the craniofacial region admitted in the Departments of Medical and Radiation Oncology in the past 6 years were assessed. Patients with clinical and histological diagnosis of malignancy of known primaries of the craniofacial region were included in our study.

The primary site, staging of the tumour, histopathological diagnosis and the site of distant metastasis were recorded from the archival data.

Results

A total number of 125 patient records were assessed. Socio-demographic analysis revealed 44.8% (56) of the study group to be women and 55.2% (69) to be men, aged 10-80 years.

Of all head and neck cancers, thyroid malignancies showed the highest number (34.4%), followed by the maxillary bone and sinus (11.2%), tongue (8.8%), buccal mucosa (8.8%), lymph nodes (7.2%) and the alveolus (4.8%). (Table 1) Among thyroid cancers, papillary variant of thyroid cancer showed the highest percentage (27.2%), while in oral cancers, squamous cell carcinoma (SCC) recorded the highest percentage (well differentiated SCC=12%, whereas poorly differentiated SCC=15.2%). 75 out of 125 patients (60%) presented in advanced stage of the disease (combined Stage III and IV). All patients had been treated with either surgery or chemotherapy or radiotherapy or a combination.

The incidence of distant metastasis recorded in the 125 patient records was 15.2% (19 patients). These metastatic lesions were discovered either incidentally or due to new presenting complaints such as bone pain, cough, loss of appetite or lassitude. A thorough physical examination followed by an appropriate investigation such as a radionuclide scan, CT, MRI, chest x-ray, liver/ thorax ultrasonography was advised.

When comparing the metastatic pattern in relation to the primary site, we found that thyroid malignancies metastasized in 12 cases, tongue in 2, and 1 each was observed from the alveolus, intraosseous mandible, nasal cavity, lymph node and the palate.(Graph 1) Spread of metastasis was more in multiple areas of the same site than localization to a single lesion in a site. The sites of metastasis recorded were to the bones (6 cases, 31.6%), lungs (3 cases, 15.8%), liver (2 cases, 10.5%), to the mid-brain (1 case, 5.3%) and oesophagus (1 case, 5.3%). Lesions were also seen to be metastasizing in a combined manner to lungs and liver (4 cases, 21.1%), lung and bone (1 case, 5.3%), and lungs, liver and bone (1 case, 5.3%). (Table 2) When comparing sites of primaries with sites of metastasis, we found thyroid and tongue malignancies metastasizing to almost all the above mentioned-locations. (Table 3)

Comparing risk factors and its correlation between age of patients and metastasis, revealed that metastasis more common in the 50-60 years age group, followed by 40-50 year age group (Table 4). Only 24% patients'

records had a habit history recorded, out of which 33% patients had a combined habit of smoking cigarette/ beedi, chewing gutkha and consuming alcohol. But a proper correlation between the habit history and incidence of distant metastasis could not be arrived at.

Table 1: Primary site of tumour

Primary site	Frequency	Percentage
Lip	3	2.4
Buccal mucosa	11	8.8
Tongue	11	8.8
Alveolus (maxilla/mandible)	6	4.8
Maxilla and maxillary sinus	14	11.2
Mandible Intraosseous	2	1.6
Nose/nasal cavity	2	1.6
Thyroid	43	34.4
Cervical lymph node	3	2.4
Unknown origin	11	8.8
Nasopharyngeal/ tonsils/ arythenoids, larynx	9	7.2
Palate	2	1.6
Floor of the mouth	1	0.8

Table 2: Sites of Metastasis

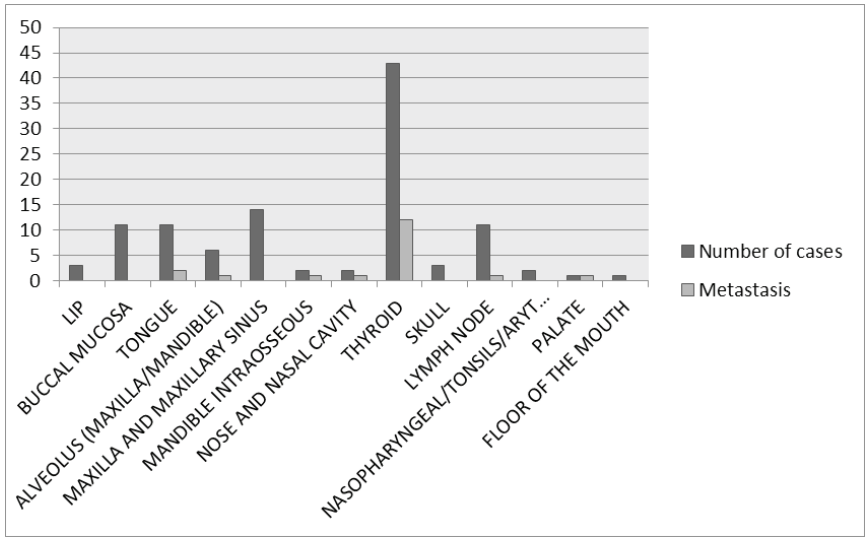
Sites	Frequency	Valid percent
Lung	3	15.8
Liver	2	10.5
Bone	6	31.6
Midbrain	1	5.3
Esophagus	1	5.3
Lung, Liver	4	21.1
Lung, Bone	1	5.3
Lung, Liver, Bone	1	5.3

Table 3: Correlation between primary site of tumor and site of metastasis

Site à Primary site â		Lung	Liver	Bone	Others	Lung, Liver	Lung, Bone	Lung, Liver, Bone
	Tongue	1	0	0	0	0	0	1
	Alveolus	0	0	1	0	0	0	0
	Mandible	0	1	0	0	0	0	0
	Nasal cavity	1	0	0	0	0	0	0
	Thyroid	1	1	4	1	4	1	0
	Lymph node	0	0	1	0	0	0	0
	Palate	0	0	0	1	0	0	0
Total		3	2	6	2	4	1	1

Table 4: Age and metastasis

Age		Frequency	Percent
	<20	5	4.0
	20 -30	6	4.8
	30 -40	13	10.4
	40 -50	31	24.8
	50 -60	34	27.2
	60 -70	26	20.8
	>=70	10	8.0
	Total	125	100.0



Graph 1: Primary site of tumour and metastasis

Discussion

Due to the rich lymphatic drainage in the orofacial region metastasis to the lymph nodes in the immediate vicinity is a common feature associated with head and neck cancers.

Recent reviews have suggested a genetic drive towards early metastatic diffusion to the lymph nodes in the neck[13]. According to the Seed soil theory of metastasis by Stephen Paget, the ''seeds'', or tumor cells grew in the microenvironment of certain organs, ''the soil.''[14,15] Few subpopulations of tumour cells acquire the capacity to invade the surrounding structures and basement membrane of the primary tumour and the local vasculature. Once in the systemic circulation, they must survive and invade the microvasculature of a distant organ. These processes require various molecular level interactions such as activation of receptor tyrosine kinases epidermal growth factor receptor (EGFR), signal transducers and activator of transcription 3 (Stat3) as well as nuclear factor-κB (NF-κB)[4].

Crile (1923) reported 1% prevalence of distant metastases in an autopsy series of craniofacial cancers[16,17]. Series based on clinical material have an incidence varying from 5.3% to 23.7%, whereas autopsy series report distant metastases in 17% to 57% of patients. The prevalence of distant metastasis in cancers of craniofacial region is low when compared to breast and lung cancer (3%-50%).[3] This variation may be due to several reasons like: differing in the assessment of parameters, such as tumour characteristics (primary tumour sites and staging of disease); various treatment modalities, the period of diagnosis of distant metastasis (clinical examination, during the course of disease or during autopsy); staging techniques and the length of follow-up.

Metastatic potential can be an inherited feature of many tumours or can present as an acquired characteristic in the process of tumorigenesis. Accordingly, it may present late onset of the disease or in certain cases, may be a presenting symptom. The prevalence before initial treatment could range from 5% to 17%.[3]

In this population of 125 patients, over a 6-year follow-up period, 15.2% (19) of the patients were found to have distant metastasis, none with an initial presentation of metastasis. These findings find correlation with studies by various studies. Probert and associates (1974) while assessing both clinical and autopsy cases among 749 patients found 12% distant metastasis and also noted that the large lesions were most likely to metastasize.[10] The study of Merino et al. (1977) stated that 10.9% of cancer patients had distant metastases, but the drawback of the study was that it did not take loco-regional status into account[8]. De Bree et al (2000) in his retrospective study among 101 cancer patients scheduled for surgery found that 17% had distant metastasis.[18] Various other authors have reported varying incidence of distant metastasis to be: 12%,2.82%,35%[19,20,21] respectively.

Though studies related to cancer in the craniofacial region have reported primaries in various oral and hypopharyngeal sites, we have not come across thyroid malignancies being included in any study with cancer associated with craniofacial region. Considering the high incidence of thyroid cancer among the Indians and the initial presenting complaint of dysphagia and swelling in the neck, we included various forms of thyroid cancers in our study for a more comprehensive view of cancers of the craniofacial region. In our study, we found an increased frequency of distant metastasis in thyroid cancers when compared to oral squamous cell cancers.

According to Kotwall et al, [22]the common sites of distant metastasis in descending order were: lungs (80%), mediastinal nodes (34%), liver and bone (31% each). Bree et al in their study reported metastasis to lung (12 %), bone metastases (4%) liver metastases (1%)[18]. Our study results were similar to those reported by Merino et al (1977)[8] where they found that the sites of metastasis from primaries originating in the nasopharynx were bone (54%), followed by lungs (23%). Study by Bhandari et al (2013) [2] also showed bone (skull, humerus, femur) to be the most common site of metastasis. However, in our study, bone metastasis was seen originating primarily from the thyroid and the alveolus. Also, vertebral metastasis was common in comparison to other bones.

Cancer research in the past were more focussed on primary tumor features, but in the recent the importance or the focus is more on tumor -specific factors and quality of life of cancer patients.Liao et al[22] in 2006 reported the age at highest risk of metastasis $\leq$ 40 years of age (in cases of tongue cancers) whereas in our study, we found

the age group of 50-60 years to be more commonly affected. Our study also showed metastasis more commonly in females than males. That could be due to the higher number of thyroid malignancies recorded in our study, and a female preponderance of thyroid malignancies. In our study higher rates of metastasis was found in thyroid malignancies. A univariate analysis for distant metastasis of thyroid cancer done by Machens et al revealed distant metastasis in 17 patients (12.69%) from follicular variant of thyroid carcinomas, 16 patients of papillary carcinoma of thyroid (5.6%) and in 40 patients (13.5%) of medullary carcinoma of thyroid.[24] The proposed hypothesis of distant metastasis in these cases is through direct accession of primary tumour cells to extra-thyroidal tissues and the rich blood vessels and an indirect pathway through dissemination via lymphatic channels.

Autopsy series by Kotwall et al.[22] found a correlation between the presence of distant metastasis and primary tumor site. Highest incidence of tumor metastasis in descending order was hypopharynx (63%), base of tongue (53%) and anterior tongue (50%). Papac et al[17] revealed metastasis to the tongue to be 2%. In our study, we found tongue to be the site of metastasis in 10.5% of cases. Our study also found that 60% of the patients who presented with advanced stage of the disease (Stage III and Stage IV) showed metastasis to various sites. This is in accordance with studies by Bhandari et al[2], who reported that 72.7% of cases with metastasis had presented initially with Stage III and IV.

Conclusion

Patients with advanced staging of the cancer have a higher incidence of distant metastasis. Thus such patients should be followed up with proper treatment protocols to evaluate any signs of distant metastasis. A detailed recording of patient demographics, lifestyle habits and tumour characteristics is a pre-requisite for further epidemiologic studies and formulation of a protocol of treatment and follow-up of head and neck cancer patients. Though numerous studies have been done to assess rates of metastasis from nasopharyngeal cancer, data on incidence of distant metastasis from thyroid cancers is lacking. We propose the inclusion of thyroid malignancies along with oral, nasopharyngeal and hypopharyngeal malignancies to study the incidence rates of distant metastasis from the head and neck region, for a more comprehensive data so as to plan appropriate treatment strategies. We also believe a thorough habit history and assessment of nodal status will give an insight into the correlation between development of cancer and distant metastasis to the risk factors.

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Conflict of Interest - Nil

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Surgical Management of Intestinal Obstruction

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Abstract

Background : Intestinal obstruction is one of the common abdominal emergencies and if not treated and delayed it may lead to bowel ischemia. Hence diagnosis and management of the patient with intestinal obstruction is one of the most important and challenging step to decrease its morbidity and mortality. This mortality due to acute intestinal obstruction can be decreased with better understanding of pathophysiology, improvement in diagnostic techniques, fluid and electrolyte correction, much potent antimicrobial hence our study focuses on early diagnosis of obstruction, skill full operative management, proper technique during surgery and intensive postoperative treatment and carries grateful results.

Aim and Objectives :

1. To study the various causes of intestinalobstruction.

2. To study the symptomatology of intestinalobstruction.

3. To study the various Modalities of treatment(surgical) and also the role of imaging studies in determining the etiology and intervention in intestinal obstruction.

Conclusion : Through our study we came to the conclusion that men are more commonly involved than females . Plain X-ray abdomen and ultrasonography were important for diagnosing out of which Plain X-ray erect abdomen was almost conclusive in all cases. Where diagnosis was inconclusive an ultrasonography was carried out. Amongst the causes, Postoperative adhesions are the common cause to produce intestinal obstruction. Intestinal obstruction with tuberculosis intestine are more likely to develop postoperative complications, proper anti-tubercular management is necessary to prevent mortality and morbidity. Early operations depending upon the etiology of obstruction are mandatory to avoid perforation, the development of peritonitis and systemic sepsis associated with multisystem organ failure. Through our study, Adhesiolysis followed by resection and anastomosis were the two procedures which were commonly performed. In Postoperative period, few patients had wound infection followed by would dehiscence, chest infection, septicemia and fecal fistula. Morbidity increased in old age patients and those whose presentation was late.

Keyword : *Intestinal obstruction, classification, causes, small bowel obstruction, large bowel obstruction*

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Introduction

Acute intestinal obstruction is one of common abdominal emergency and is associated with significant morbidity and mortality, especially if it progresses to bowel ischemia.

The diagnosis and management of the patient with intestinal obstruction is one of the more challenging

emergency that a general surgeon can come across.

Although the mortality due to acute intestinal obstruction is decreasing with better understanding of pathophysiology improvement in diagnostic techniques, fluid and electrolyte correction, much potent anti-microbials but still mortality ranges from 10-15% and more so in developing countries.

Early diagnosis of obstruction, skill full operative management, proper technique during surgery and intensive postoperative treatment carries grateful results.

Aim and Objectives

To study the various causes of intestinalobstruction.

Tostudythesymptomatologyofintestinalobstruction.

To study the various Modalities of treatment(surgical) and also the role of imaging studies in determining the etiology and intervention in intestinal obstruction.

Inclusion Criteria

- Age above 18 years
- Cases proven to have intestinal obstruction clinically and radiologically

Exclusion Criteria

- Age below 18 years.
- Patient with failed medical management attempt.

CLASSIFICATION OF INTESTINAL OBSTRUCTION

Intestinal obstruction may be classified into two types [1] :

1. Dynamic :Where peristalsis working against a mechanical obstruction.

A. Intraluminal

- Impaction
- Foreignbodies
- Bezoar
- Gallstones

B. Intramural

- Stricture
- Malignancy

C. Extramural

- Bands/adhesions
- Hernia
- Volvulus
- Intussusception

2. Adynamic:

This may occur in two forms:

- Aperistaltic e.g.: Paralytic ileus
- Peristalsis may be present in a non-propulsive form e.g.: Mesenteric vascular occlusion,Pseudo-obstruction.

Clinically the obstruction is classified [2] into:

 Acute obstruction – occurs in small bowel usually with sudden onset of colicky

central abdominal pain, distention, with early vomiting and constipation.

 Chronic obstruction – Usually seen in large bowel obstruction with lower abdominal colic and absolute constipation followed bydistention.

 Acute on chronic – There is short history of distention and vomiting against a background of pain andconstipation.

 Sub acute obstruction – Implies incompleteobstruction

Four cardinal feature of acute intestinal OBSTRUCTION are [2] :

1. Pain
2. Vomiting
3. Constipation
4. Abdominaldistention.

Associated with these cardinal features may be dehydration, oliguria, toxemia, hypovolemic shock, or respiratory distress. Fever may suggest strangulation or inflammatory pathology.

ON EXAMINATION

- **Type of abdominal distension**: Central in small bowel obstruction and upper abdominal in high-level obstruction. Distension will be more in the flanks in colonic obstruction.

- **Type of peristalsis**: Central step ladder type of peristalsis seen in distal small bowel obstruction and left to right over the umbilicus in the colonic obstruction.

- **Palpation**: Abdomen was to be examined for presence of any palpable mass, localized abdominal tenderness, rebound tenderness and mass - suggestive of strangulation. Generalized rigidity and tenderness suggests of peritonitis. During pain heaped up coils of intestine or prominent distended coils may be seen. Obliteration of liver dullness may be associated with perforation of intestine or primary perforation causing peritonitis andileus.

- **Per rectal examination:** It is one of the important examinations. Typically in intestinal obstruction, rectum.

  - Will be empty and ballooned (mechanicalobstruction).
  - Pelvic tumour may be palpable through rectalmucosa.
  - Apex of intussusception may be felt [3] .
  - Impacted faces or mass can befelt.
  - Bloody slime Passage of dark brown or black coloured stools may indicate possibility ofgangrene.

Difference between small and large bowel obstruction [4]

Sl no	Signs & symptoms	Small bowel obstruction	Large bowel obstruction
1	Duration	Short-in hours	Longer- in days
2	Vomiting	Early- bilious	Late- feculent
3	Pain	Early-periodical	Late- gradually increasing
4	Location of pain	Periumbilical	Peripheral, flanks
5	Features of shock	Early	Rare
6	Past history	H\o laparotomy	H/o bloody stool and Obstipation
7	Distention of abdomen	More in central	More in periphery
8	Palpation	Palpable coils of Intestine	Most of times palpable Growth
9	Dehydration	Extreme	Less marked

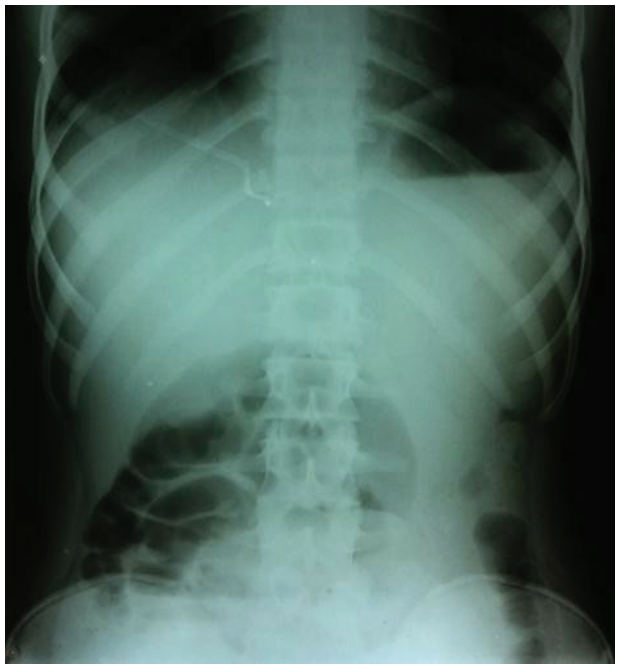
Following are the distinguishing features of obstruction at different levels

Levels of obstruction	Onset	Pain	Vomiting	Distension
High small bowel	Sudden	Around the umbilicus	Profuse	Absent/slight upper Abdominal
Low small bowel	Gradual	Severe colicky/dull	Less profuse	Moderate centraland Abdominal

INVESTIGATIONS

1. BLOOD INVESTIGATIONS
2. RADIOLOGICAL –

- PLAIN XRAY ABDOMENERECT



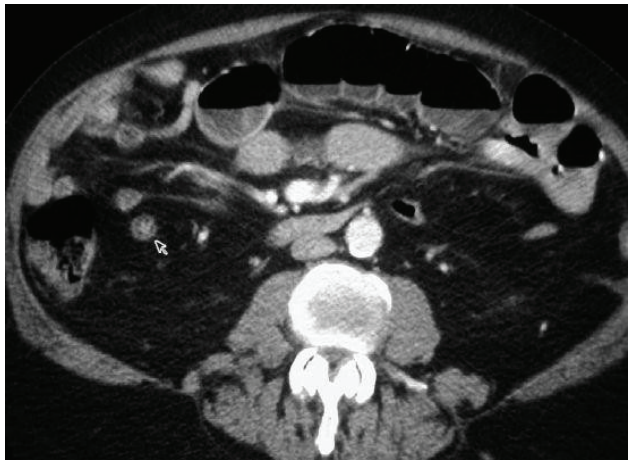
- Image 1: Plain X-ray erect abdomen showing multiple air fluid levels in a patient with intestinal obstruction due to an adhesions of small bowel.

- **Image 2: Plain X-ray erect abdomen showing large bowel obstruction secondary to volvulus.**

- ULTRASONOGRAPHY OF ABDOMEN AND PELVIS

- BARIUM MEAL

- CT SCAN



- **Image 2: CT abdomen showing multiple air fluid levels in a patient with intestinal obstruction.**

MANAGEMENT OF PATIENT

CONSERVATIVE TREATMENT / NON OPERATIVEMEASURE [5]

Indications for conservative management includes:

1. Doubts about mechanicalobstruction.
2. A relentingobstruction.
3. Multiple earlier obstructive episodes managedconservative.
4. Paralytic ileus of non-organicorigin.
5. Intestinal obstruction due to postoperativeadhesions.
6. Obstruction due to inflammatory abdominaldiseases.
7. Obstruction due to wormimpaction.

8. Obstruction secondary to fracture of thoracolumbarvertebra

IT INCLUDES

- GASTRIC DECOMPRESSION
- FLUID ELECTROLYTE IMBALANCE MANAGEMENT
- ANTIBIOTICS

NON OPERATIVE (NOT OPENING BOWEL) MANAGEMENT INCLUDES

1. Lysis of adhesions & bands.
2. Reduction of incarceratedhernia.
3. Derotation ofvolvulus
4. Manipulative reduction of intussusceptions

Contraindications to nonoperative management include suspected ischemia, large bowel obstruction, closed-loop obstruction, strangulated hernia, and perforation.

OPERATIVE MANAGEMENT [6] : The timing of surgical management or intervention is dependent on the clinical picture, early operation is indicatedin:

1. Obstructed and strangulatedhernia
2. Internal intestinalstrangulation
3. Acute obstruction

Pughe found a band was obstructing the ileum near the caecum. He divided the band and patientrecovered.

Thomas in 1886 described some methods of abdominal section for obstructed

bowel.

1. Colostomy through extra peritonealapproach.
2. Dissection of anterior abdominal wall, exploration of abdominal cavity, correction of any abnormality & thus relievingsymptoms.
3. Dissection of anterior abdominal wall, detection and excision of thelesion.
4. Section of abdominal wall in front & seizing the first portion of bowel & then suturing it to the previously made incision & incising the bowel so as to allow its contents to escape continuously permitting the original cause of obstruction run a chance of correction by naturalprocess.

OPERATIVE MANAGEMENT DEPENDS UPON THE CAUSE OF OBSTRUCTION [7]

A) ADHESIOLYSIS

B) SIGMOIDOPEXY

C) RESECTION AND ANASTAMOSIS

D) STRICTUROPLASTY

E) APPENDICECTOMY

F) MECKEL'S DIVERTICULECTOMY

G) CAECOPEXY

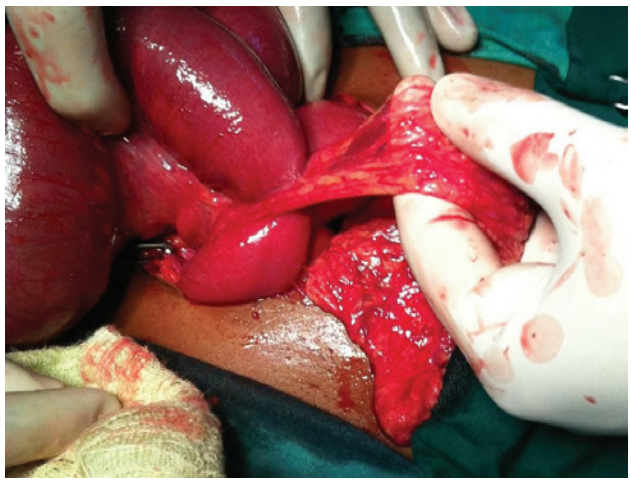


Image 3: Omental bands causing obstruction

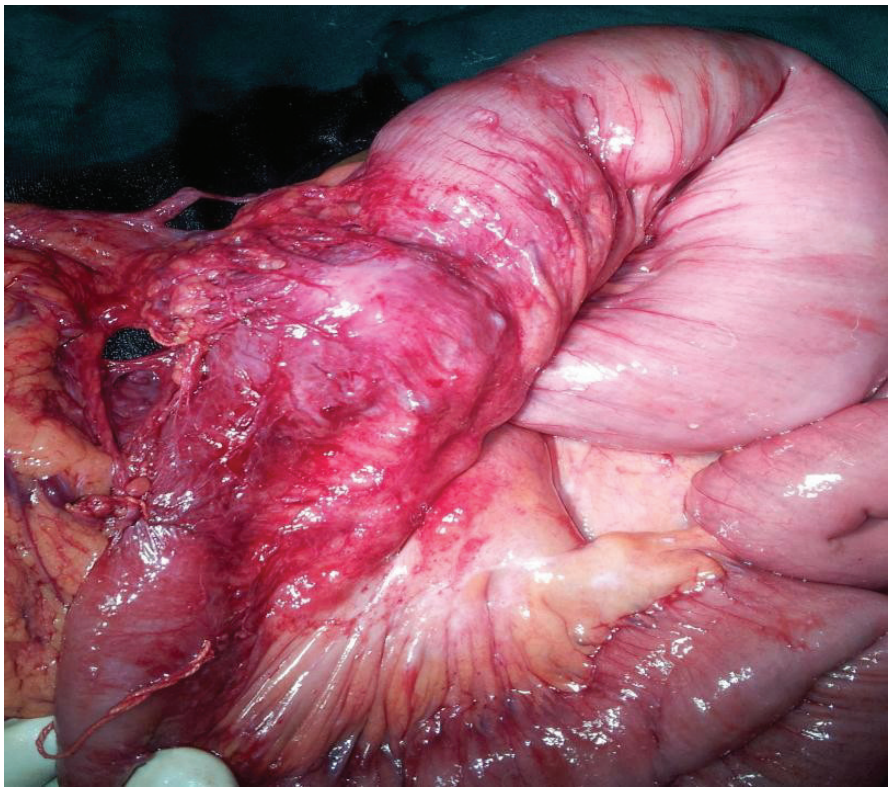


Image 4: TB stricture of ileum

Methods

Number of cases – **60**. After admission to Dhiraj Hospital, Vadodara . Investigations and operative procedures performed, were collected from the inpatients, were interpreted.

Results

- Intestinal obstruction is more common in the age group of 30-60 years .
- Mean age distribution was39.4years.
- Incidence in male (70%) was more as compared to female population(30%).
- Painabdomen–80%(24cases)Vomiting–83%(25cases)Distentionabdomen
- – 72% (22 cases) and Constipation – 60% (18 cases) were noted as many patients had coinciding symptoms.
- Commonest cause of acute intestinal obstruction as studied/noted at out institution was postoperative Adhesions and next is obstructed hernias followed with intestinal T.Bstrictures.
- Adhesiolysis & Resection/anastamosis was the most common procedure performed.
- Post operative complication are more in cases of T.B intestine as the etiology of intestinal obstruction.

Table-1 : Analyses of symptoms and signs:

Sl No	Symptoms & signs	No. of cases	Percentage
1	Pain abdomen	24	80
2	Vomiting	25	83
3	Tenderness	28	93
4	Distension	22	72
5	Constipation	18	60
6	Bowel sounds- increased	13	43
7	Bowel sounds- decreased	5	16
8	Bowel sounds- absent	2	7
9	Groin swelling	4	13
10	VP	5	16
11	Gaurding	14	46
12	Rigidity	4	13
13	Palpable mass	-	-
14	PR findings(significant)	1	3

- Levels of obstruction:

There were more of small bowel obstruction (67%) when compared to large bowel Obstruction(37%).

Table-2 : Level of Obstruction

Small Bowel	Large Bowel
40	20

Table 3 : Age Distribution

AGE	TOTAL CASES
21-30	6
31-40	25
41-50	20
51-60	4
61-70	3
>71	2

Sex incidence:

The occurrence of intestinal obstruction was common in male (70%) with comparison to female (30%). There were 42 male & 18 female with male to female ratio 2.2 : 1

Table-4 : Sex Incidence

Age	Male	Female
21-30	4	2
31-40	20	8
41-50	12	6
51-60	2	2
61-70	2	0
>71	2	0

Etiology of intestinalobstruction:

a. Small bowel obstruction 40 cases(67%)

TABLE-5.a

Cause	Case	Percentage
Adhesions & bands	18	30
Obstructed hernias	10	17
TB strictures	6	10
Small bowel volvulus	2	3
Intussusception	2	3
Meckel's diverticulum	2	3

b. Large bowel obstruction - 20 cases (37%)

TABLE-5.b

Cause	Case	Percentage
Neoplasm	12	17
Large bowel volvulus	4	7
T.B strictures	2	3
Intussusception	2	3

Interpretation and Conclusion

- More commonly found in males than in females.
- Intestinal obstruction still remains an important surgical emergency.
- Main complaint was pain in abdomen followed by vomiting, distension and constipation.
- Vigorous correction of fluid and electrolyte is very important.
- Plain X-ray abdomen and ultrasonography were important for diagnosing.
- Postoperative adhesions are the common cause to produce intestinal obstruction.
- Mechanical obstruction is not associated with any specific biochemical marker, which can help the surgeon for differentiate simple obstruction from ischaemia.
- Intestinal obstruction with tuberculosis intestine are times more likely to develop postoperative complications, proper anti-tubercular management is necessary to prevent mortality and morbidity.
- Early operations depending upon the etiology of obstruction are mandatory to avoid perforation, the development of peritonitis and systemic sepsis associated with multisystem organ failure.
- Plain X-ray erect abdomen was almost conclusive in all cases. Where diagnosis was inconclusive an ultrasonography was carried out.
- Intra operative findings varied from simple bands to malignancy.
- Adhesiolysis followed by resection and anastomosis were the two procedures which were commonly performed.
- Few patients had wound infection followed by would dehiscence, chest infection, septicemia and fecal fistula.
- Morbidity increased in old age patients and those whose presentation was late.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearence : taken from SUMANDEPP VIDYAPEETH INSTITUTIONAL ETHICS COMMITTEE**.**

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Nursing Management for Covid 19 Pandemics

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Abstract

Nurses represent the backbone of any health care system. Today in the fight against Covid-19, several nurses find themselves on the frontline. This report is a clear reminder of their important position and a wake-up call to ensure that they receive the help they need to keep the planet safe. [1] Nurses play a very important function, locally as well as internationally. They are on the front lines, day in and day out, taking care of these patients. "They are able to see what is working, as well as things that can be done differently to provide more effective and efficient care, and offer suggestions for improvement." 2020 is recognized by the World Health Organization as the Year of the Nurse and Midwife – On the function of nurses in the preparation and reaction to disasters. (World Health Organization) Globally, the way the world views nurses and health care professionals already changes. Because not everyone knows what is happening in a health care facility, the COVID-19 pandemic is high The COVID-19 pandemic has brought nurses to the forefront of people's minds and there is a daily capture of the media attention and public gratitude to health care professionals.[2] This short communication concludes with the key role of nurses for effective management during covid-19 pandemics.

Keywords*: COVID-19, pandemic, messenger, educator, advisor, protector,manager,care provider*

Introduction

Nurses proved to be the health warriors who took on the responsibility with their heart and soul in a very willing manner. The profession is critical to fighting the pandemic, and their calling has been "stepped up and gone beyond" by nurses in every country. We are working in the frontline and are overseeing patient screenings, placement as well the treatment of patients in the COVID region.[2] The World Health Organization has officially declared COVID-19 to be a pandemic and has called on "all countries to continue efforts that have been successful in reducing the number of cases and halting the spread of coronavirus infection." The prime purpose of Lockdown is to stay at home and maintain communal space.[3]The COVID-19 pandemic means that many of us are staying at home and doing less in terms of social interactions and exercise. This can have a negative effect on your physical and mental health.[4] In this COVID-19 pandemic, proclaiming "passion" For crisis preparation and emergency response, disaster preparedness and response, disaster management prevention, An epidemiology of disasters and more fields that are very far common. Nurses responsible for more than half of all health professionals worldwide, delivering essential resources across the health care system. They are displaying their humanity, bravery and courage around the world as they react to the COVID-19 pandemic and their importance has never been shown more clearly before. Every day we see the bravery, expertise and devotion of nurses and other healthcare staff the front lines of the COVID-19 pandemic, as they serve bravely.[5]

As messenger and educator role:

Live with you at home please. I recognize this might be really hard, but if you're not an important mitarbeiter who has to leave job, stay home. Avoid excursions to the grocery store, where possible. It's not time now to walk down Target's aisles, check all things, search for new home decor or have a brief chat with other customers. Get your things, and go home[5]. The illness causes respiratory disease (such as flu) with symptoms like cough, nausea, and trouble breathing in more extreme cases. We should protect ourselves by constantly washing our hands,

avoiding touching our face and eviting near touch with the unwell people (1 meter or 3 feet) [6]

As role of Manager:

The role of nurses in it's so important to reduce infectious diseases spread, because of one of the key we want things to do is reduce mortality and slow spreading the disease and improve the patient condition by providing quality care. Health Crisis response nurses are important as they are a critical connection between the client and the other health care member. During their whole shift, they are with their patients and are able to detect slight shifts in their patients by observation and analytical thinking that may mean that they decompense, or get worse, or get better.

Care provider:

In the present COVID 19 emergency medical situation, paramedics are playing a great role as a healthcare professional in rural areas also. They are providing advanced levels of care for emergencies and trauma. The majority of our rural paramedics are based in the field and close to the community. They are providing out-of-facility services and some referral services. The people they see are ill, very, very diseased. This virus affects everyone. Many of the patients they care for sickness and giving symptomatic treatment, according to patient condition needing intensive care unit, medication and intubation.

As role of Trainer:

Because nurses are the largest part of the health-care workforce, it is likely that nurses will take care of all victims and patients should a catastrophe occur. It's important they are qualified and have the expertise and action skills, be they on the front lines of the crisis or in a hospital, they care about others.

As role of protector: Personal Protective Equipment and Staff Safety

Most important role of the nurse is to protect themselves from contacting or transmitting COVID-19. Adequate personal protective equipment (PPE) should be provided to all health care workers, especially emergency nurses. Even when caring for patients who are critically ill, it is important to protect one's self and take the time to don the appropriate PPE, despite instincts to want to render aid immediately. Education and training around appropriate donning (putting on) and doffing (removing) of PPE is essential, as these are times when health care workers are at a high risk of accidental contamination. [7]

One of the main issues to do is to have enough PPE nice for use when caring. Health care staff are introducing other techniques To better protect yourself from threats, including changing their working wardrobe Before they reach their houses, they shower as they arrive at own house and several health related professionals are now isolating themselves from me in a separate room in their homes.

The critical role of nursing

Nurses are also the first and highest level primary care provider. They are important for extending healthcare access to remote communities. Research indicates that nurses are also instrumental in increasing efficiency in the health sector and patient satisfaction, and they are less costly to train and employ than other health care practitioners. Investing in the health care sector a triple health return, economic development and gender equality.

Throughout the latest emergency response situation at COVID 19, paramedics also play a significant role throughout remote areas as a healthcare professional. They provide advanced levels of emergency and trauma treatment. Most of our rural paramedics are in-field based and close to the community. We have facilities and other referral services out of facility.[8]

A crisis intervention plan:

Administrations in hospitals can help alleviate tension by proper coordination and sensible decision taking. "Triage is most important—it is the process of assessing if a patient requires ICU, non-ICU, or home care. This way, we can allocate the limited resources to people who need it the most," Given that senior doctors make the call, it takes away the burden from the juniors[9].

Pandemic COVID-19 poignantly underlined the vital The job of nurses and other health-care workers safeguarding the health of people and To save Long live. Long live. A new research, about the state of the Nursing Calls for a Global 2020 further involvement leadership

of schooling, working conditions of nurses to boost their medical-care commitments.[10]

As role of advisor:

The easiest thing to do is stay home sometimes hand washing, and holding surfaces frequently hit purify. Everybody needs to adapt a modern living way and probably feel frustrated. Keep in mind taking care of your mental state is critical. Some approaches to help combat tension include establishing a timetable and planning, taking regular changes, plan for busy, having adequate rest, use relaxation therapies, keeping hydrated and consuming ideal, nutritional diet. Inform yourself, but also update your knowledge. As our federal executive continues to say, remaining physically distant is vital but that doesn't mean you need to be socially distant.

Nursing staff play a vital role in providing health care services. These are the people who devote their lives to caring for mothers and children; offering lifesaving immunizations and health advice; caring for the elderly and generally meeting the basic health needs of daily life.[9] Schools are closed due to the COVID-19 outbreak for an uncertain period of time, parents face the daunting task of figuring out how to help their child's learning and more likely how to keep them entertained, while social distancing is either required or encouraged.[11]

Corona virus disease 2019 (COVID-19) can cause respiratory, physical, and mental dysfunction in patients. Hence, pulmonary rehabilitation is important for both admitted and discharged patients of COVID-19.[12]

In crises, nursing staff have more tasks to satisfy patients and their families; therefore, nurses must be well equipped with essential knowledge and skills in managing crises involving clinical treatment, decontamination, isolation, communication, triaging, psychological support, and palliative care if necessary.[13]

The latest study, made available World Health Organization (WHO) in collaboration Highlights of the The Foreign Nurses' Council (ICN) and Nursing. Now at World Health Day 2020 the achievements and issues faced by 28 million nurses worldwide, who responsible for over half of all health care persons worldwide.

Conclusion

Nurses are on the frontline, and they have a significant role in fighting COVID-19. Nurses are facing critical shortages of nursing staff, beds, and medical supplies. Nurses have also been life-saving at the center and people-care. Yet their worth was never greater this is more that it is. We deserve not only our respect but also concrete aid for securing we can contribute to their abilities in its best.

Ethical Clearance- Not required

Source of Funding- No funding

Conflict of Interest- Nil

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A Protocol on Effect of Cryotherapy and Myofascial release Technique in Calf Muscle Spasticity of Spastic Diplegic Cerebral Palsy Children

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Abstract

Background: Cerebral Palsy is been one of the developmental disorders affecting the infants brain. The most common form of Cerebral Paralysis being Spastic Cerebral Palsy affects children functionally and prevents their social functioning. Most of the physiotherapy interventions to treat spasticity are Cryotherapy, Myofascial Release technique. Cryotherapy is effective in reducing Spasticity and some suggest Myofascial Release Technique is also effective in reducing spasticity. **Aim:** To find out the effect of Cryotherapy and Myofascial Release Technique in calf muscle of Spastic Diplegic Cerebral Palsy Children. **Methods:** 30 Participants with Spastic Diplegia will be selected on the basis of inclusion as well as exclusion criteria. The patients will be evaluated using Modified Ashworth Scale and Modified Tardieu Scale for calf muscle prior to the treatment. The 30 participants will be divided into 3 groups. All the groups will receive Cryotherapy, MFR technique and combined effect of both the techniques separately. The patients will be again evaluated after the treatment by using MSA and MTS. **Conclusion:** This Study Concluded that there may be significant reduction in calf muscle spasticity in spastic diplegic cerebral palsy children by using combine effect of both Cryotherapy and Myofascial Release technique ore one of the method.

Keywords: *Cerebral palsy, Spastic Diplegia, Cryotherapy, Myofascial Release Technique, Modified Ashworth Scale, Modified Tardieu Scale.*

Introduction

Cerebral palsy (CP) identifies a category of chronic movement and posture developmental disorders that cause impairment of activity due to nonprogressive disorders in the development of the fetal or infant brain [(1)]. More than 15 million people worldwide have already been estimated to have cerebral palsy (CP). According to statistical reports of PSO, 1-2 cases per 1000 existing infants are reported with Cerebral Palsy. The incidence of Cerebral Palsy in various countries around the world though differs from 1.5 to 2.5 cases per thousand infants[(2)].

With about 70-80 percent of all diagnosed cases, the most common form of Cerebral Paralysis being Spastic Cerebral Palsy. It is the most leading cause to impairment in children, which prevents movement and thus suppresses the social functioning. Hence, that spasticity is one of the key challenges in the care of these children [(3)]. There are many approaches to treat spasticity, including treatment of medical, physical and surgical therapy. Important physiotherapy techniques for spasticity lowering include stretching, strengthening the antagonistic muscle, positioning, bracing and casting inhibitors, as well as exercises for weight bearing. Several physical therapies were widely used in combination with certain types of physical therapy, such as massage,

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myofascial release as well as **cryotherapy** [2].

Myofascial therapy is definable by "the facilitation of mechanical, neural and psycho physiological adaptive potential as interfaced by the myofascial system". Deep Myofascial Release is meant for reducing the barriers in the deepest parts in fascia. This will be obtained by trying to stretch the muscle flexible structures of a fascia, and increasing the viscosity of just the base tissue of a fascia. Myofascial release strategies is used in a broad range of conditions or diagnoses; pain, obstruction of movement, spasm, spasticity, neurological dysfunction, such as cerebral palsy, head and birth injury, cardiovascular problems, scoliosis[2].

Ice or cold therapy is a therapeutic procedure commonly used for treating various types of acute and chronic conditions. There is several tissue-based effects that is facilitated with cold therapy, including postinjury swelling and reduction of oedema, increased local circulation, decreased tissue-induced acute inflammation, reduced muscle spasm and pain suppression. Other result with cold therapy is a time-related spasticity reduction after administration of the ice for a while. Ice may be given to the body in various ways: indulging itself within cool water, using ice cubes or ice packs, or cooling sprays like ethyl chloride [4].

The Modified Ashworth Scale for subjects with neurological disorders is considered the main clinical indicator of muscle spasticity. The Modified Ashworth Scale is applicable to all muscles in the upper and lower body. The Modified Ashworth Scale indicates contradictory findings in terms of reliability and validity [2].

Tardieu scale is a spasticity measurement scale which takes into consideration passive movement resistance at slow along with fast speeds. Using Improved Tardieu Scale (Morris, 2002), the frequency of muscle response at accelerations and the degree with which the muscle resistance appears are shown in the spasticity calculation. R1 and R2 being determined by Modified Tardieu; R1 is degree of muscle reaction, and R2 is maximum PROM. At a very low velocity (V1) the full ROM angle (R2) is taken. The angle of muscle reaction (R1) is defined as the degree to which a catch either clonus is observed throughout a fast stretch (V3). R1 is deducted from R2, and it also implies the dynamic tone portion of the muscle. The Tardieu Scale is highly accurate and valid, varying from spasticity to contracture [2].

Aim: To find out the effect of cryotherapy and myofascial release technique in calf muscle spasticity of Spastic Diplegic Cerebral Palsy children.

Methodology

The study will be carried out in Ravi Nair Physiotherapy College and AVBRH, Sawangi (Meghe), Wardha after getting approval from Institutional Ethical Committee of Datta Meghe Institute of Medical Sciences (Deemed to be University). This Interventional study will be included 30 participants which will be selected randomly from Ravi Nair Physiotherapy College and AVBRH, Sawangi (Meghe), Wardha on the basis of the inclusion and exclusion criteria. The male and female Spastic diplegic CP children between the age group of 3-7 years with Modified Ashworth Scale 3 or less than 3 for both genders will be included. Those participants who have an orthopaedic operation before, given injection of botulinum toxin in the last 6 months, have endured serial casting during the last six months, taking oral or intrathecal myorelaxant drugs, with extreme limitations on the passive range of motion at the lower extremities, with certain cognitive and perceptional disabilities and contractures will be excluded. Before the Intervention the participants will be explained about the objectives and approaches of the study and a written informed consent forms will be signed by them. The participants will be divided into three groups i.e. 10 for group A receiving Cryotherapy, 10 for group B receiving Myofascial Release Technique and 10 for group C receiving combine effect of both Cryotherapy and Myofascial release technique (Fig./Table 1). The participants will be assessed pre and post treatment by using Modified Ashworth Scale (MAS) and Modified Tardieu Scale (MTS).

Procedure:

Selection of Patients visiting RNPC and AVBRH, Sawangi (Meghe), Wardha

30 subjects will randomly selected based on inclusion and exclusion criteria.

Assessment of Patients on Modified Ashworth Scale and Modified Tardieu Scale.

Patient will Receive Cryotherapy and Myofascial Release Technique.

Patients will be divided into three groups.

Group A (10 Patients) Group B (10 Patients) Group C (10 Patients)

(Cryotherapy) (Myofascial Release Technique) (Combine effect of both techniques)

Figure/Table 1. Showing the theme of conducting the current study.

DATA COLLECTION AND TREATMENT TOOLS:

A) Modified Ashworth Scale: The Modified Ashworth Scale for subjects with neurological disorders is considered the main clinical indicator of muscle spasticity. The Modified Ashworth Scale is applicable to all muscles in the upper and lower body. The Modified Ashworth Scale indicates contradictory findings in terms of reliability and validity.

The reliability of AS and MAS interrupters ranged from moderate to excellent, in accordance with ICC rankings. 0.54 to 0.87 AS ICC grades.

B) Modified Tardieu Scale: Tardieu scale is a spasticity measurement scale which takes into consideration passive movement resistance at slow along with fast speeds. Using Improved Tardieu Scale (Morris, 2002), the frequency of muscle response at accelerations and the degree with which the muscle resistance appears are shown in the spasticity calculation. R1 and R2 being determined by Modified Tardieu; R1 is degree of muscle reaction, and R2 is maximum PROM. At a very low velocity (V1) the full ROM angle (R2) is taken. The angle of muscle reaction (R1) is defined as the degree to which a catch either clonus is observed throughout a fast stretch (V3).R1 is deducted from R2, and it also implies the dynamic tone portion of the muscle. The Tardieu Scale is highly accurate and valid, varying from spasticity to contracture. Intrarater and interrater reliability of Modified Tardieu Scale is good to excellent.

C) MFR technique: Patients in prone position with a hold of 120 seconds, were given MFR for the calf muscle. Finger pads have been destined to fall into the middle part of calves to deliver the MFR. This would sustain the tissue softening for 120 seconds, and then extend laterally myofascial structures until the first fascial barrier. MFR will be given 15 minutes.

D) Cryotherapy: Cryotherapy to the calf muscle will be given with patient in prone position. The entire lower limb must be carefully and decently exposed and all children will be checked for the sensation of the skin to ensure that none of them has an impaired skin feel. Cold pack (Compress Reusable Cold Gel Pack) is applied to the skin of the treated region over a wet towel to prevent unnecessary local cooling (ice burning); dry toweling will keep this pack in place. Upon application, the skin underneath the cold pack is examined over one minute and check for adverse effects and sometimes irregular skin color changes. It would be repeated after 5 minutes, if the initial test did not reveal any adverse reactions. For 20 minutes, the cold pack will be applied apply, then remove and dry the skin.

After completing the treatment the immediate result will be seen and the participants will then be tested with Modified Ashworth Scale and Modified Tardieu Scale.

Statistical Analysis

The data will be collected and analyse utilizing inferential and descriptive statistics by using Chi-Square test and students 't' test (unpaired and paired) & software for analysis will be SPSS 22.0 version and Graph pad prism 6.0 version & level of significance will be considered as $P<0.05$.

Discussion

Cerebral palsy (CP) identifies a category of chronic movement and posture developmental disorders that cause impairment of activity due to nonprogressive disorders in the development of the fetal or infant brain [(1)].

Spasticity is defined as velocity dependent increased resistance to passive muscle stretch or as abnormal voluntary muscle activity associated with upper motor neurons paralysis [(8)]. The most common clinical type of CP observed was bilateral spastic CP [(9)]. Physiotherapy helps to facilitates developmental skills that are important to the daily life activities [(10)]. Physiotherapy treatment has a beneficial effect on the physical and functional status of children with CP[(11)].

This study protocol detail two physiotherapy intervention in patients with Spastic Diplegic Cerebral Palsy to reduce calf muscle spasticity. The Present study was conducted to see the effect of Cryotherapy and Myofascial release technique in calf muscle spasticity of Spastic Diplegic Cerebral Palsy Children.

The result showed that, all the treatment groups that is Cryotherapy and Myofascial release Technique alone and combined effect of Cryotherapy and Myofascial release technique show significant reduction in calf muscle spasticity. Result also shows that Combined effect of Cryotherapy and Myofascial release technique gives more effect in reducing calf muscle than Cryotherapy and Myofascial release technique alone.

Some Studies shows that and reported that Cryotherapy and wrapping procedures are combined to reduce lower limb spasticity in hemiplegic cerebral palsy children[12]. Cold therapy has been considered useful for spasticity reduction in upper motor neuron lesionand muscle retraining to promote muscle contraction[5].

Several research performed suggests that stretching is used alongside MFR in minimizing spasticity in spastic CP children rather than using stretching simply [6].

Sandra L, et al (2010) in their research, MFR was in some children with cerebral palsy benefits, including decreased spasticity, increased body symmetry range of motion. Myofascial release can be useful in reducing spasticity and improving the quality of life in cerebral palsy children [1].

For children with CP, a report showed the muscle tone measured with the Ashworth scale increases up to 4 years of age and then decreases up to 12 years of age [7].

Conclusion

This Study Concluded that there may be significant reduction in calf muscle spasticity in spastic diplegic cerebral palsy children by using combine effect of both Cryotherapy and Myofascial Release technique ore one of the method.

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Conflict of Interest: The author declares no conflict of interest.

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Successful Biological Treatment of Cutaneous wound Healing with Comparation of Aqueous Humour Fluid and Fat Graft; Cattle as Model

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Abstract

Aqueous humour fluid and fat graft effect on wound healing by containing growth factors. **Aim** of this study to evaluate the benfit effect of aqueous humour fluid and fat graft in wound healing in cattle. Twenty seven adult female local breed randomly divided into 3 equal groups. G1 have been treated by aqueous fluid, G2 treated with fat graft, while the last group (control group) leaft without treatment. **In conclusion** fat graft treated group appear better healing during study period compare with other groups.

Keyword*: aqueous humour fluid, fat graft, wound healing*

Introduction

Wound is known as degradation integrity of epithelial tissue, wound healing is a four stages complex biological process [(1)]. Skin wound healing is a cellular function mechanism involving the interaction of several cells, growth factors and cytokines, there are many techniques used to overcome wound healing limitation, new techniques that used for treatment wound involving scaffolds activation by bioactive molecule, growth factor and genetically modified cells [(2)]. Two of these techniques are fat graft with their adipose-derived stem cells [(3)], and use of aqueous humour fluid[(4)].

Fat grafting is a common procedure in plastic and reconstructive surgery because of it is low cost, ease of harvest, safety, and high content of stem cells and growth factors[(5)], also adipose tissue is available in large amount and easy to manipulate [(6)]. Fat grafting used in many purposes including lower limb ulcers [(7)], improve healing of burns [(8)], softening of surgical scars [(9)] and in wound healing [(5)]. Adipose tissue contain stem cells, secret anti-inflammatory mediator and growth factors [(10)], this stem cells contributing in wound healing by differentiated to various phenotypes wound healing cells such as keratinocytes, fibroblast and endothelial cells [(11)], also encourage neovascularization and tissue regeneration [(12)], this lead to suggest use fat in wound healing. Adipose derived stem cells differ from other mesenchymal stem cells by that it can be harvested with minimum donor site morbidity and without ex vivo culturing and expansion [(13)]. The other biological method used to overcome wound healing limitation is aqueous humour fluid, it is contain number of growth factors, cytokines capable of effecting fibroblast activity and wound healing[(14)]. Growth factors formed in the corneal cells in the eyes such as epidermal growth factor, transforming growth factor and keratinocyte growth factor then secreated them in the aqueous humour fluid and tear to maintain and renewal of normal tissue [(15)]. Composition of aqueous humour is approximately similler to cerebrospinal fluid and arterial plasma with high ascorbate, low level of plasma, protiens and blood cells [(16)]. Trasforming growth factor play an imprtant role in fibrosis during wound healing [(17)].

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Materials and Methods

The work was conducted on twenty seven local breed cattles, their body weights 550 ±25kg aged ±2 years. lived in the Animal field of the College of Veterinary Medicine/ University of Al-Qadisiyah under

standard feeding with free administration of clean water. All the procedures were accompanied with the ethics of the college committee. Animals divided randomly and equally into three groups, group 1 treated with aqueous humour fluid, group 2 treated with fat graft and group 3 leave without treatment as control group.

Fat graft (100 gm) was collected from the slaughter house of Al- Dewania/ Iraq immediately after slaughtering, immersed in 0.9% normal saline, at the same time under strict sterile conditions. Aqueous humorous fluid(AHF) (15 ML) was collected from the cow eyes after slaughtering and the two samples were preserved in ice box. and brought to the laboratory. The fat graft was washed ten times with normal saline, harvested with sharp knife and centrifuged with PBS 1% at 3000 rpm/ 5 minutes. The mid layer was collected and preserved under 4ºC until used. Then we make an inscion of about 1cm length in the thural area of animals in each group.G1 treated with aqueous humour fluid, 0.5ml of it locally on inscion was started directly and repeated for 7 consecutive days. G2 trated with 0.5ml of fat graft and continue for 7 consecutive days.

Samples were collected from all groups at 14 and 21 days for histo-pathological examination. The histo-pathological slides were stained with hematoxiline and eosin stains and examed under light microscope (olympus Incorporation, Japan).

Results

The histological study of G1 after 14 days post wound. There was an early stage of regeneratal layer and accumulation of inflammatory cells with fibroblast in the margin on newly formed epithelized tongue, however there was sheltered with a thin reepithelization formation of superficial crust and regenreation of epidermal layer as well as inflitration of inflammatory cells in the subepithelial area (fig.1). In 21 days post wound we reporting that slower healing wound with presence of granulation tissue below the epithelial layer also formation of new blood vessels (fig.2). In seconed group the cattle treated with fat graft,after 14 days post wound histololgic examination revealed that reepithelization regenrated epideramal layer and infiltration of inflammatory cells with formed epithelial tongue, also the wound show good regeneration of epithelial and subepithelial layers as well as infiltraion of inflammatory cells and fibroblast in the wound margin (fig.3). Like wise, in 21 days in wound area was noted that there was rapid healing of epidermis layer covered by a thin of scar tissue and formation of blood vessels due to proliferation of endothelial cell with presence of collagen (fig.4). the third untreated control group, the histological evaluation in day 14, there were afew accumulation of inflammatory cells in the sub epithelial area (fig.5). In day 21 we found that there was poor healing wound of both epidermal and subdermal layer with increase infiltration of inflammatory cell in the area (fig.6).

Fig:1-G1(treated by aqueous humors fluid in 14 days); Skin wound shows sheltered reepithelization;

formation of superficial crust (black arrow) and regeneration of epidermal layer (white arrow) as well as infiltration of inflammatory cells in the sub epithelial area. H&E, 100X.

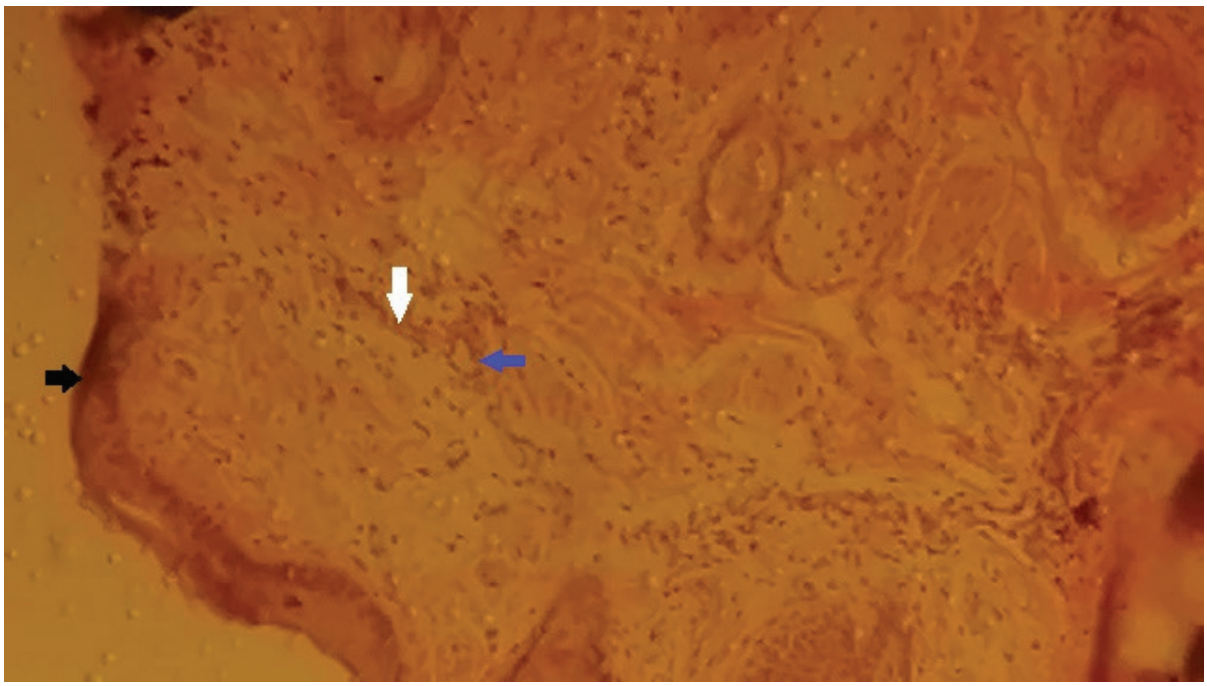

Fig:2-G1(treated with aqueous humors fluid in 21 days); Skin wound shows slower healing of epidermis layer (black arrow) and mild presence of granulation tissue as well as infiltration of few inflammatory cells (white arrows) in the sub dermal and newly formed blood vessels (blue arrow). H&E, 400X.

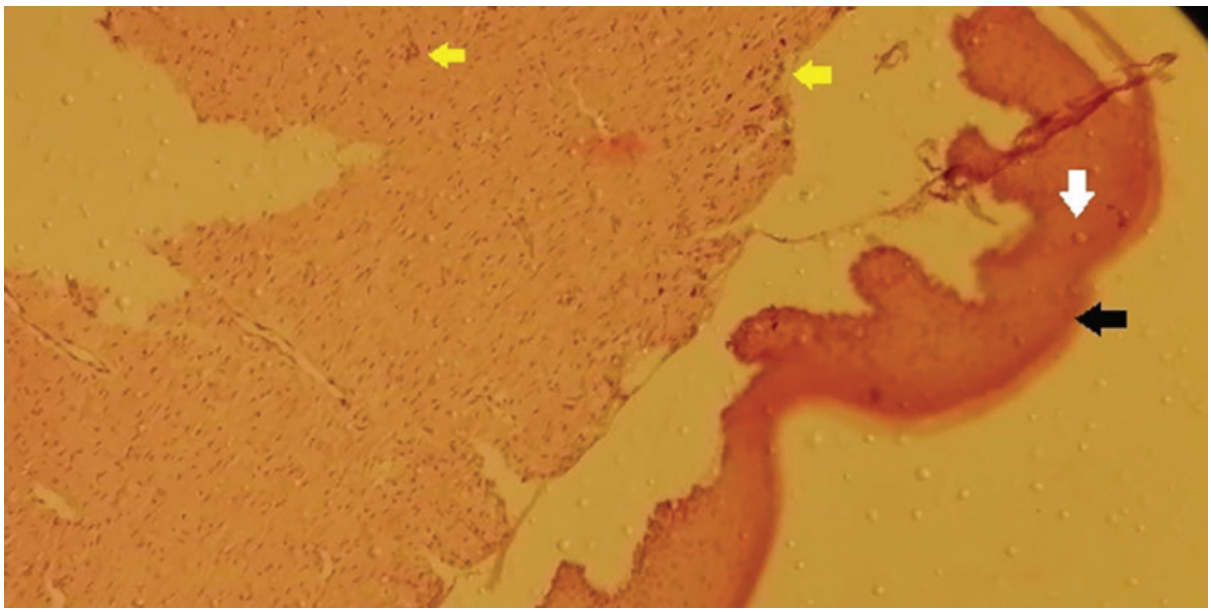

Fig:3-G2 (treated by fat graft in 14 days); Skin wound showed in the early stage good regeneration of epithelial (black arrow)and sub epithelial layers (white arrow) as well as infiltration of inflammatory cells in the sub epithelial area (yellow arrows). H&E, 100X.

Fig:-4-G2(treated with fat graft in 21 days); Skin wound shows rapid healing of epidermis layer (yellow arrows) covered by a thin layer of scar tissue (black arrow), and formation of blood vessels due to proliferation of endothelial cell (white arrows). H&E, 100X.

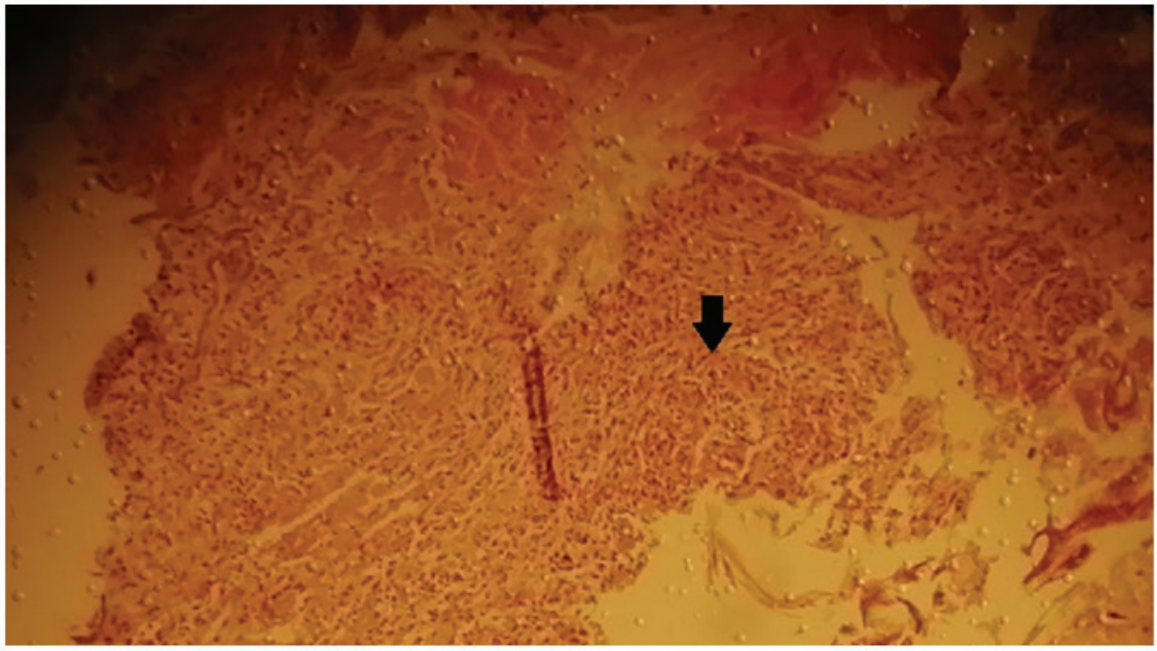

Fig:5-G3(control group without treated in 14 days); Skin wound shows a few accumulation of inflammatory cells in the sub epithelial area (black arrow). H&E, 100X.

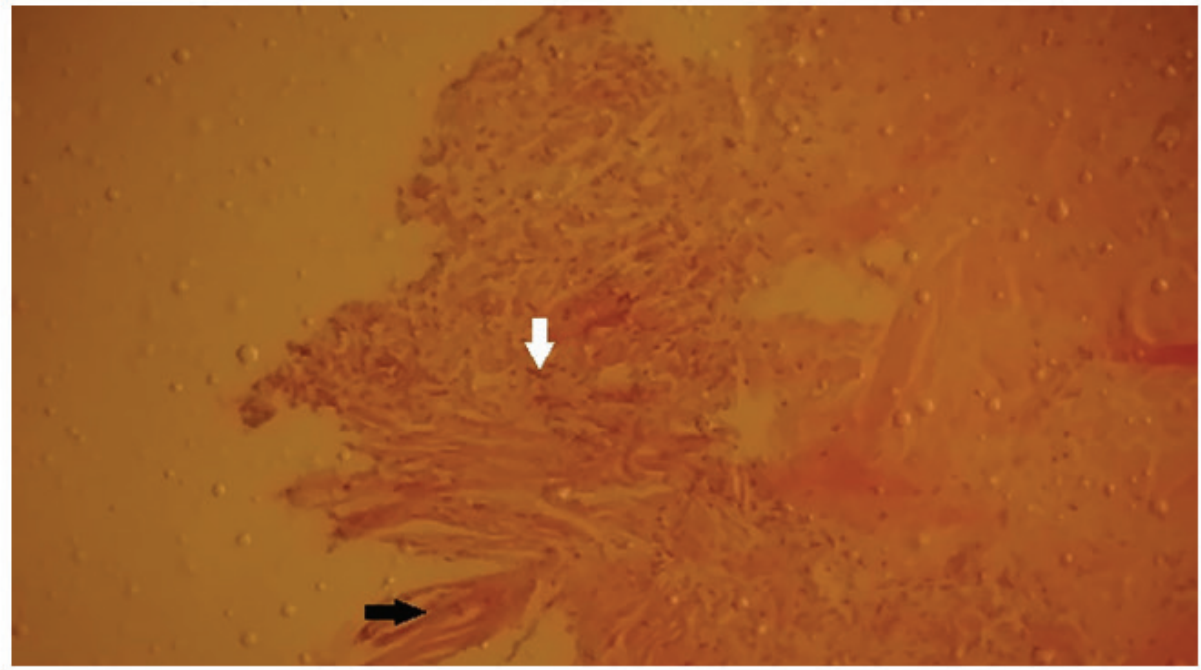

Fig:-6- G3(control group without treated in 21 days); Skin wound shows a poor healing process of both epidermal (black arrow) and sub dermal layer (white arrow). H&E, 40X.

Discussion

Fat graft first used by plastic surgery in lipotrophy of the face and many studies shown that it's have a good effect on wound healing. Fat graft when used in burns reported improvement in wound healing, scar suppleness and fibrosis [18]. Which agreed with our study. Also Rahul and Sharma use fat graft in wounds and the study show significant enhancing to epithelialization from margins, production of healthy granulation tissue [8]. On the other hand aqueous humour fluid accelerated wound healing by affecting on fibroblast active also decrease scar formation [19]. The study conclude that fat graft treated group show an accelerate process of wound healing in compassion with aqueous humors fluid treated group by increase formation of blood vessels and thin scar tissue, however the two groups show better healing than control group.

Conflict of Interest: None

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Ethical Clearance: Not required

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Sensitivity and Specificity of Several Malnutrition Screening Tools for Chronic Kidney Disease Patient: A Narrative Review

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Abstract

Introduction: Malnutrition remains a critical risk factor for morbidity and mortality among CKD patients. However, malnutrition is preventable and treatable. Thus, early malnutrition screening of CKD patients is crucial. The present paper is a narrative review of the several studies related the use, sensitivity and specificity of developed malnutrition screening tools for CKD patients.

Material and Method: Articles were searched from five electronic databases i.e., PubMed, PMC, Semantic-scholar, Science-direct, and Google scholar. Several keywords used for literature searching including "malnutrition screening tools CKD"; "malnutrition CKD"; "malnutrition screening CKD". Inclusion criteria of this narrative review was the subject of intervention should be an aged 1 – 64 years old with experimental randomized control trial (RCT) or non-RCT study design. The exclusion criteria for this literature study were research published before 1990, literature studies, meta-analysis, comments, short communication, and editorial letters. Data then narratively described.

Result and Conclusion: Twelve publication were narratively described in final analysis. Development of malnutrition screening tools for CKD patient had been done since 1992 and still counting until 2020. At least thirteen screening tools had been tested for its use, sensitivity and specificity. Among 13 tools we analyzed, *Simple Nutrition Screening Tool* (SNST) and *The Renal Nutrition Screening Tool* (R-NST) revealed the highest sensitivity and specificity value which suitable to be used in adult population, while in children, only *Pediatric Digital Scaled MAlnutrition Risk Screening Tool* (PeDiSMART) showed a good sensitivity and specificity. Further study should be focusing to look for sensitivity and specificity in other specific population to ensure the right use of malnutrition screening tool.

Keywords: *malnutrition screening, chronic kidney disease, sensitivity, specificity*

Introduction

Hospital malnutrition conditions are prone to occur in hospital patients and can have an impact on increasing patient morbidity, affecting the healing process of disease, reducing quality of life, extending the duration of hospitalization and increasing the burden of financing[1]. Chronic Kidney Disease (CKD) is a type of degenerative disease which is a kidney function disorder, is irreversible, and results in insurmountable physiological changes that lead to significant mortality and morbidity[2]. Chronic Kidney Disease in the world is currently experiencing an increase and becoming a serious health problem, the results of the 2010 Global Burden of Disease study, Chronic Kidney Disease was the 27th leading cause of death in the world in 1990 and increased to 18th in 2010[3]. In Indonesia, the prevalence of CKD in 2013 was reported to be 2% with the lowest prevalence of 1% and the highest of 4%[4].

The severity of CKD can be reduced with proper nutritional care. However, a study in one hospital

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in Indonesia showed that 54.2% of CKD patients undergoing routine hemodialysis had malnutrition or already in malnutrition status[5]. This number is high and needs to be intervened as early as possible because malnutrition in patients with CKD can affect the response to therapy and quality of life. CKD patients undergoing hemodialysis therapy have a greater risk of malnutrition due to the hemodialysis process itself; where in the hemodialysis process, it not only removes metabolic waste and harmful substances in the blood, but also often causes the loss of important nutrients such as amino acids, glucose, and water-soluble vitamins[6].

Studies show a high prevalence rate of malnutrition in both children and adults with chronic kidney disease. A study among Southern Nigerian patients showed a prevalence of malnutrition increased significantly across CKD stages 2 to 5 (p=0.020), higher in male patients which also marked by higher level of serum albumin and creatinine[7]. Malnutrition among CKD patients caused by interrelated abnormal pathophysiological factors such as low ghrelin which leads to decreased appetite, lower energy and nutrient intake, metabolic imbalances, increased catabolism, hormonal derangements, inflammation, and dialysis related abnormalities[8].

Nutritional screening is a first-line process of identifying patients who may be at risk of becoming malnourished. If based on the nutritional screening tools, patients are at risk, then patients should be undergoing a comprehensive nutrition care process by a dietitian to prevent malnutrition risk. However, malnutrition is rarely diagnosed because of low sensitivity and specificity of screening tool. This narrative review aims to narratively described the use of several nutritional screening tools in CKD patients as well as comparing each sensitivity and specificity.

Material & Method

Articles were searched from five electronic databases i.e., PubMed, PMC, Semantic-scholar, Science-direct, and Google scholar. An online search was conducted in August 2020 for all articles related to development, use, or evaluation of malnutrition screening tools among CKD patient published in 1992 – 2020. Several keywords used for literature searching including "malnutrition screening tools CKD"; "malnutrition CKD"; "malnutrition screening CKD".

This narrative review aims to answer research questions "how is the use, sensitivity and specificity of several malnutrition screening tools in CKD patients?". The sample in this literature study was CKD patients aged 1 – 64 years who participated in experimental or non-experimental research that measured the provision of various malnutrition screening tools in CKD patients. Inclusion criteria of this narrative review was the subject of intervention should be an aged 1 – 64 years old with experimental randomized control trial (RCT) or non-RCT study design. The exclusion criteria for this literature study were research published before 1990, literature studies, meta-analysis, comments, short communication, and editorial letters. Two reviewers assessed articles for inclusion and study quality and extracted data. Only studies published in English were included. Data were summarized narratively.

The scope of this narrative review is in line with the aim that is to describe the use of several malnutrition screening tools in different population all over the world, describing its sensitivity and specificity in capturing malnourished patient.

Findings

We retrieved 40 papers at first literature search, eighteen publications were excluded because it met exclusion criteria i.e., literature studies. Twelve publication were narratively described in final analysis.

First published malnutrition screening tools was in 1992 called Subjective Global Assessment (SGA), the use of SGA was tested among 59 chronic uraemic patients treated by haemodialysis (n = 36) or CAPD/ Continuous Ambulatory Peritoneal Dialysis (n = 23). SGA is now also widely used in clinical setting for adult patient. The SGA instrument diagnoses malnutrition based on two criteria, namely medical history and physical examination. The medical history is based on five questions, while the physical examination is based on one examination. The results of the six questions were then classified into good / normal nutrition, mild/ moderate malnutrition, and severe malnutrition[9].

This SGA assessment aims to find out the etiology of malnutrition, whether due to decreased food intake, malabsorption, maldigestion or increased demand. Physical examination assesses the loss of muscle mass

and fat as well as the presence of ascites and is useful for identifying changes in body composition due to the effects of malnutrition or the influence of the disease process[10]. However, this first SGA for CKD patients did not establish its validity but showed a good reliability and claimed to be inexpensive method which can be recommended for a more frequent assessment of nutritional status in dialysis patients[9]. Furthermore, SGA was validated by Cooper (2002)[11] among 76 dialysis patients and Gurreebun, et al. (2007)[12] among 141 hemodialysis patients using biochemical characteristics such as serum albumin and Total Body Nitrogen (TBN) somehow still showing that SGA were less sensitive to detect malnutrition; means that while patients were malnourished based on biochemical data but not detected as malnourished by SGA. A review of SGA tool for CKD patients suggests that SGA is a useful tool for nutritional screening, but its validity and reliability should be established first in the selected population to ensure clinicians in targeting malnourished patients[13].

The SGA then developed to another tool called PG-SGA (Patient-Generated Subjective Global Assessment) by Desbrow, et al[14]. PG-SGA Includes medical history (weight, intake, symptoms, functional capacity, metabolic demand) and physical examination; then also categories patients into SGA categories (A, B or C) as well as providing a numerical score for triaging[15]. His study with total 60 hemodialysis patients proven that PG-SGA has good internal consistency, also high sensitivity and specificity (83% and 92%). Recently, Kosters, et al[16] also tested validity of PG-SGA among 123 CKD patients which also revealed a good sensitivity (78%) and specificity (94%). To differ, Kosters, et al simplify PG-SGA form to be Patient-Generated Subjective Global Assessment Short Form (PG-SGA-SF) using only 4 indicators, those are nutritional impact symptoms, food intake, daily functioning and weight.

The Renal DETERMINE Nutrition Screening Tools was also one of early tools developed to screen malnutrition[17]. The Renal DETERMINE Nutrition Screening Tools is a self-administered which aims to educate patient and increase awareness of their malnutrition risk; but also, can be administered by the healthcare provider as well. DETERMINE stands for ***D****isease,* ***E****ating poorly,* ***T****ooth loss, swallowing difficulty, or gastric distress,* ***E****conomic hardship,* ***R****educed social contact,* ***M****ultiple medications,* ***I****nvoluntary weight loss or weight gain,* ***N****eeds assistance with self-care, and* ***E****lderly over 65*. The Renal DETERMINE Nutrition Screening Tools consists of 13 Yes/No questions. No cut off points determined and also for its sensitivity nor specificity. To our understanding, this tool is no longer used considering lack of data on its accuracy to detect malnutrition at early stage; even though this tool is quite simple and easy to administer.

In 2016, Xia, et al.[18] study developed and tested new tool called *The Renal Nutrition Screening Tool* (R-NST) among 122 acute kidney injury, chronic kidney disease, and dialysis. The new R-NST was developed by using some of the content of the MST (e.g., weight and appetite) and incorporating best practice dietetic content (e.g., using specific biochemical measures). The R-NST consists of 9 questions, which must be completed in 3 steps—steps 1 and 2 provide 2 separate screening pathways, and step 3 calculates the final score. Step 1 consists of 5 compulsory questions (1-5) including recent involuntary weight loss and any eating behavior change, and renal specific nutrition indicators (e.g., serum phosphate and potassium and episodes of peritonitis). Step 2 includes 4 questions (6-9) assessing any episodes of persistent gastrointestinal symptoms that may be indicative of increased risk of undernutrition, as well as 3 renal specific biochemical markers, namely albumin, C- reactive protein, and serum urea. Final step 3 is total score from step 1 and 2, using the cutoff point >3, patient screened as at risk of undernutrition and should be referred to dietitian[18]. This tool has not been validated in other population.

Study done by Susetyowati[19] has also been developed new tools specifically used for Indonesian population called *Simple Nutrition Screening Tool* (SNST). Even though it was not developed for CKD patient since the first place, SNST proved to be a better tool than the NRS 2002 in malnutrition hemodialysis (MHD) patients against either SGA (Sensitivity 94.3% vs. 82.9%; Specificity 60% vs. 58.6%). The study which included MHD patients concludes SNST as a valid screening tool that is valid, accurate, simple and can be quickly filled, east to be administered by any health-care provider. SNST consist of six yes/no questions including does patient look skinny; experienced weight loss (clothes feels loose and feeling involuntary weight loss);

experienced a decrease in food intake in the last 1 week; experienced fatigue and suffering from any disease that cause decrease food intake. Patient will be put as in malnutrition risk if total score >3[20]. This study also validated Nutrition Risk Screening Score (NRS-2002) in CKD patient which also prove that NRS-2002 have a good validity in predicting malnutrition risk compared to objective measures (BMI, MUAC, HGS and energy intake). In line with this study, Müller, et al.[21] also validate NRS-2002 in more CKD patients (489). His study explained that NRS-2002 also found to have good sensitivity to predict malnutrition in CKD patient. The NRS combines both a measure of current potential undernutrition and a measure of disease severity and has been validated in various patient groups. The risk for malnutrition was defined as an NRS-2002 score of >3 points.

Malnutrition Inflammation Score (MIS) is another screening tool for CKD patient that showed 87.09% of sensitivity and 45.45% of specificity after tested in 73 hemodialysis patients[22]. Compared to previously describe tools, MIS has lower specificity which could generates many false-positive results and should be considered more to be used in clinical setting. Another three malnutrition screening tools was *Dialysis Malnutrition Score* (DMS), *Nutritional Risk Index* (NRI), and *Malnutrition Universal Screening Tool* (MUST)[23,16]. DMS and NRI were validated among 226 hemodialysis patients aged >18 years. Compaing all of those three, only DMS showed a good both sensitivity and specificity (81,3% and 71,4%), while NRI and MUST only showed good specificity but low sensitivity. Low sensitivity might result in higher false-negative results and not suitable to detect malnutrition among CKD patients.

Chronic Kidney Disease can also occur in pediatric patient in which malnutrition causes morbidity and mortality. A validated tool for malnutrition screening in children is limited. One study was found to validate *Pediatric Digital Scaled MAlnutrition Risk Screening Tool* (*PeDiSMART*) which showed a good sensitivity (87%) and a specificity (75%)[24]. This study could be duplicated in another population to be used for pediatric population.

This narrative review could be beneficial to be used as a as a reference to determine the differences in the various detection tools for malnutrition in patients with CKD.

In conclusion, out of twelve published paper narratively reviewed, among adult CKD patients, Simple Nutrition Screening Tool (SNST) proved to have highest sensitivity and specificity, followed by The Renal Nutrition Screening Tool (R-NST); while in children, only Pediatric Digital Scaled MAlnutrition Risk Screening Tool (PeDiSMART) showed a good sensitivity and specificity. We suggest every hospital or any other healthcare undergone a validity, sensitivity and specificity test for targeted population because the results presented in this study might be difference if used in other specific population.

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Review Article

Neonatal Transport - Review Article

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Abstract

Various factors lead to neonatal mortality, in which transport of neonates play an important role in mortality and morbidity of neonates. In India neonatal transport has not developed well. Many sick neonates succumb during transport or become very serious during transport. Transport duration, type of transport, treatment given during transport , if pretransport treatment received, maintenance of hemodynamic stability, maintenance of temperature and blood sugars during transport is essential for reducing the mortality of the baby. Regionalizing neonatal care, will help in deciding the transport of neonates from one centre to an higher level of care. Hence a well-organized transport system should be in place for the transport of the sick neonates. This review article discusses about the neonatal transport and its implications.

Keywords: *Neonatal, Transport, Mortality, Morbidity, Sick.*

Introduction

In India we have a poor transport network with an unequal distribution of hospitals, which makes it difficult to transport a sick newborn to an equipped hospital.(1) The NMR of India is 29/1000 live birth ,which contributes to 56% of the under 5 mortality rate. (2) It was found that preterm birth complications, birth asphyxia, infections and malformations were the most common causes of mortality of newborns in India. (3) However various factors contribute to the neonatal morbidity, in which transport of the neonates to a higher center plays an important role. In utero transfer of the fetus and institutional delivery is the best, but due to unprecedented causes like preterm delivery and perinatal illness results in continuous needs to transfer the babies after delivery.(4) During transport of a newborn, his or her homeostasis is affected which can cause further worsening of the condition, especially when the quality of monitoring and treatment during transport cannot be ensured. Thus any complication occurring while shifting to higher center may result in greater morbidity and mortality .Most of the babies are transported by family people in a private vehicle without any treatment before or during transport. Many of these newborns thus transported are hypothermic, cyanosed with low blood sugar and 75% of such babies when transported in a bad condition will have serious clinical implications.(5)

Neonatal Transport

Neonatal transport may be defined as transferring a neonate from a lower level of nicu care to an higher level of care, which was not available in the previous set up.(6) Most sick neonates in India are transported by their parents or paramedical personnel either in private vehicles or poorly equipped ambulance.(7) An organized transport service provides almost the same level of monitoring and the quality of care during the transport that is available in the advanced care facility. (7)

Levels of NICU care (8)

Level 1- Neonatal resuscitation at birth, Postnatal care, Pretransport stabilization of sick newborn

Level 2- Level 1 + Care of babies less than 32 weeks and more than 1.5 kg due to immaturity or some temporary conditions. Provision of CPAP and Mechanical Ventilation. Pretransport stabilization of sick newborn

Level 3- Level 2 + Proper NICU care with medical and surgical consultation, Mechanical ventilation,

Diagnostic imaging availability

Level 4- Level 3 + Specialized surgical for repair of various congenital/ acquired conditions.

"**Indications for Transport** [8]

· Gestational age <32 weeks, birth weight < 1.5 Kg

· Respiratory distress requiring CPAP / FiO2 > 60%

· Respiratory failure due to hypoxia requiring invasive mechanical ventilation

· Persistent pulmonary arterial hypertension

· Congenital heart disease or cardiac arrhythmias

· Congenital anomalies and/or inborn errors of metabolism

· Hypoxic-ischemic encephalopathy

· Seizures

· Other conditions that may be indications for neonatology consultation and/or transfer

Severe hyperbilirubinemia that may require exchange transfusion

Infant of diabetic mother with hypoglycemia or other complications

Severe intrauterine growth restriction

Birth weight between 1,500 and 2,000 g and gestational age between 32 and 36 weeks

Procedures or therapies unavailable at referring hospital (ECHO, surgery, extracorporeal membrane oxygenation [ECMO], etc."

Contraindications for neonatal transport [9]

· Congenital anomalies incompatible with life.

· Immature, Nonviable infants (<400gm and < 23 weeks of gestational age).

· Unstable infants not responding to resuscitation and who can die during transport.

· Unfavorable transferring conditions in terms of staff, instruments , medical devices and materials, drugs and capability of the referral hospital.

Types of Transport [9]

1. Ground transport-

Patient can be transported in the ambulance in bad weather conditions. Transport can be interrupted and patient can be stabilized. Shaking of the babies during transport can cause problems and limited speed of transport can lead to prolonged duration of transport.

2. Air transport-

Helicopters can decrease the transport duration to 1/3rd.However the patient cabin is small, air conditions will affect the transport ,landing space is required and ear plugs have to be used in the neonates.

Airplanes can be used for long distance travels and they have a larger patient cabin. But apart from air conditions, landing in an airport is required with transfer to a hospital in an ground ambulance.

Transport Materials [9]

· Ambulance

· Multipara monitors

· Ventilators

· Oxygen supply

· Medications - IV fluids, Antibiotics, Inotropes, Surfactant, PGE1

· Intubation equipment's and Aspirator

· Incubator and Phototherapy unit

Transport team [8]

Transport team is made by doctors and nurses who are trained and experienced in neonatal critical care and know the needs of the neonate during transport. It includes general nurse, critical care nurse, neonatologist/ paediatrician, paramedic. These people should be trained for various situations during transport. Their skills should be assessed from time to time and they should be able to perform various procedures.

Pre transport stabilization [8]

Before referring the neonate, the patient has to be stabilized. Airway, Oxygenation of the baby should be maintained by Oxygen support, if needed intubating the baby before referral. Circulatory status and temperature should be maintained. IV lines should be secured appropriately or umbilical lines should be secured and feeding tube to be inserted before transport. Glucose levels to be maintained. First dose of antibiotics and other medications like anticonvulsants to be given. All primary investigations with its reports, obstetric and antenatal history and what treatment given in the present hospital along with the referral note should be attached in a separate file. The referred hospital should be informed prior to the referral about the patient details.

Medical Management during transport [8]

During transport vitals has to be monitored. It is difficult to assess air entry during transport, hence etCO2 has to be monitored. If further deterioration occurs during transport, vehicle can be stopped during ground transport and patient can be reassessed and procedure if needed can be done and patient can be stabilized and transport can be continued after that. If the patient is deteriorating further then it is better to return back to the referring hospital or get admitted on the way to the nearest hospital. During transport it is essential to communicate with the relatives of the patient about the patient's condition.

TOPS Score- [10]

TOPS score includes checking of temperature, oxygenation, perfusion and sugar on admission of the baby. It is a simple test which helps in prognostication of the babies on admission. High score in the TOPS score means increased risk of mortality during the course in stay in the hospital.

Arrival at the referred hospital [8]

When nearing the hospital the NICU should be informed telephonically about their arrival so that all equipment's and medications are kept ready for the patient. Relevant documents to be given at the arrival hospital. Parents have to be informed of the arrival at the hospital. Patient's condition during transport and in the previous hospital, handing over has to be given properly.

Medico legal issues associated with transport [7]

- In case of cardiac arrest, stop the vehicle and Cardiopulmonary resuscitation done according to the Neonatal Resuscitation Programme guidelines.
- If death occurs on the way, baby has to be first shifted to the higher health facility.
- Admit in the emergency room. Explain to parents and then the medical officer of the higher centre should issue the death certificate.
- It's the obligation of the transport team to make death certificate of baby.
- A consent form for transport of the baby from the parents has to be got before transportation of the baby.

Government programmes [11]

Under National Rural Health Mission (NRHM) the following programmes/schemes and awareness campaign is launched by the Government to reduce infant mortality rate:

Establishment of Sick New Born Care Units at District Hospitals, newborn stabilization Units at Community Health Centres (CHCs) and New Born Care corners at 24x7 Primary Health Centres (PHCs) to provide new born and child care services.

Navjaat Shishu Suraksha Karyakram (NSSK), a programme for training health care providers one essential newborn care.

Home Based New Born Care (HBNC) through ASHAs with series of home visits.

Janani Shishu Suraksha Karyakaram (JSSK) has been launched on 1st June, 2011, which entitles all pregnant women delivering in public health institutions to absolutely free and no expense delivery including Caesarean section. The initiative stipulates free drugs, diagnostics, blood and diet, besides free transport from home to institution, between facilities in case of a referral and drop back home. Similar entitlements have been put in place for all sick infants accessing public health institutions for treatment. However there are no government programmes for neonatal transport to higher

centre.

Discussion

In a study conducted by Verma et.al [10] it was found that 275 (70.51%) babies came in ambulance either government or private which was equipped with oxygen line rest came in unequipped own vehicles. Referral was done commonly for respiratory distress (42.56%), perinatal asphyxia (27.18%), prematurity (25.64%) and meconium stained liquor (15.90%). At admission hypothermia was present in 46.67% neonates, 39.23% were hypoxic, 21.28% were hypoglycemic and 14.61% had poor perfusion. Highest mortality 36/57 (63.16%) was found in poor perfusion group, whereas hypoglycemic babies had the least expiries 27/83 (32.53%) ($p<0.05$).

Sachan et.al [2] found that the diagnosis at admission was neonatal hyperbilirubinemia (38%), sepsis (34.7%), asphyxia (33.1%), prematurity (26.5%), , MAS (10%), and others (3%). Only 32.6% (80) of the patients used government ambulance facility for neonatal transport. Prior stabilization before and during transport will reduce morbidity and mortality.

Ghanghoriya et.al [12] found that 23.4% were hypothermic,5.6% were hypoglycemic ,75.8% had hypoxia and 40.48% neonates had delayed CRT. Statistically significant association was found between bad outcome and hypothermia, prolonged CRT, and hypoxemia (SPo2 < 90) at the time of admission. The most common cause for admission was respiratory distress (55.95%), birth asphyxia (46.03 %), LBW <1800 gms (19.44 %), meconium aspiration (17.85%), prematurity <32 weeks (16.26 %) and not accepting feeds well (13.09%) respectively. Neonates who were stabilized by treatment with i.v fluids and oxygen (77.77%) before and during transport had significantly lower deaths (18.87%) than the babies who were not managed properly during transport (32.14%). Increased duration of transport, more than 120 minutes had i had thrice the rate of neonatal death (22.59%) as compared to those who reached in 120 minutes. At the time of arrival 75.8% neonates had saturation <90, 72.2% neonates had respiratory distress, 40.48% neonates had prolonged capillary refill time, 23.4% neonates had hypothermia. "The survival of these neonates with deranged physiological status at the time of arrival was poor and 45.76% were expired among hypothermic newborns, 39.21% among abnormal CRT, 30.22% among newborns with respiratory distress, and 27.22% among newborns with low SPO2."

Aggarwal et al [13] it was found that Only 10.5% babies were transported in ambulances provided by hospitals. One third of the babies were referred without any referral note.

Regionalization of neonatal transport has to be done for a highly specialized neonatal intensive care unit. [14] A number of critical rare cases in neonates were reported by Taksande et al [15-18]. Gupta et al reviewed about Neonatal Gastric Perforations[19]. Neonatal hyperbilirubinemia and related article was also reported by Kurhade et al[20].

Conclusion:

It is found that the mortality rate was high in referred neonates. These deaths can be reduced by promoting tertiary care hospital deliveries, early identification of neonatal problems, prereferral stabilization, communicating to the referral hospital about the transported neonate, providing a good transport system for safe transport of babies in stable condition and giving a proper handover to the referred hospital and keep the parents informed about the status of the baby.

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Atrial Myxomas: A Case Report on Rare Benign Cardiac Tumour and its Histopathological Diagnosis

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Abstract

Cardiac tumours are a very rare entity of tumours which comprises of both malignant and benign group. The atrial myxomas are benign cardiac tumour which occurs most commonly. This case report illustrates a diagnosis of rare atrial myxoma through its characteristic signs, symptoms, most common site of occurrence and histopathological examination assisted by radiological examination.

The diagnosis is made by classical clinical presentation, histopathological examination along with 2D echocardiography to rule out the malignant activity. The clinical signs and symptoms vary with the location and size of tumour, which usually present as pulpy non-specific mass.

The histopathological study corresponded with classical pattern. Since the tumour may present as an emergency in the form of symptoms mimicking sudden cardiac death and other complications like embolization, prompt resection of tumour is the mainstay treatment associated with low mortality. However, the most common complication is recurrences hence the patient should be kept in regular follow up.

Keywords*: Atrial Myxoma, Histopathological examination, Benign tumour.*

Introduction

Primary cardiac tumours are rare. The incidence accounts for about 0.3% of patients with cardiac surgery. In cardiac tumours, the cardiac myxomas are most common accounting for about 50%.[1]Although the prevalence of cardiac tumours only with respect to autopsy is known which ranges from 0.001% to 0.3%, of more than 50% of benign cardiac tumours are myxomas. It has genetic origin in seven per cent of cases and rises as a component of a heritable disorder with some clinical manifestations. Over 72% of primary cardiac tumours are benign. In adults, the majority of benign lesions are myxomas.[2,3] Most common origin of cardiac myxomas is from the left atrium (about 75%) and less commonly the right atrium (18%), right ventricle accounts for (three per cent) and also very rarely from the left ventricle (three per cent). A small fraction of less than one per cent arises from the valves. Because of the non-specific symptoms, early diagnosis of cardiac myxomas can always be challenging.

Case History: A 42-year-old male patient was presented with complaints of shortness of breath associated with cough and pedal oedema for two months. His physical examination, on admission for cardiac surgery, revealed normal vital sign and neurological examination was normal. On gross examination, specimen was a reddish-brown jelly like pulp aggregating 16×2×1.5cm. A small whitish flap structure measured 1.5cm, as shown in Figure 1.

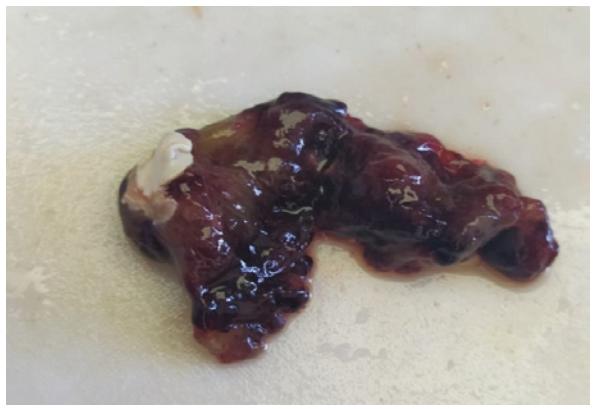

Figure 1: Reddish mass measuring 16×2×1.5cm

Figure 2: *2-D echocardiography revealed a left atrial tumour with no associated thrombus.*

[Figure3.jpg]

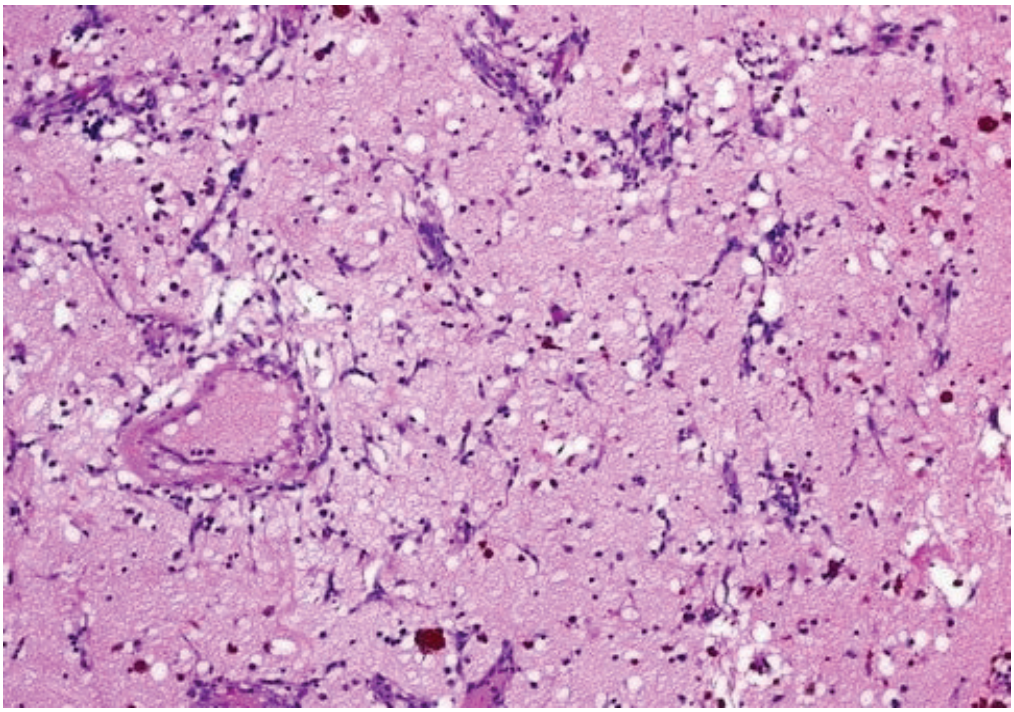

Figure 3: *The section shows stellate cells against abundant myxoidstroma. There is presence of hemosiderin laden macrophages along with delicate blood vessels.*

Discussion

With a rare incidence of 0.002% in general population, cardiac myxoma is the most common heart tumour, representing 50% of all cardiac tumours, and about 75% of them are in the left atrium.[5]

In our case as well, tumour arose from left atrium**.** Left atrial myxomas produce symptoms when they reach a weight of about 70 grams, right atrial myxomas to be symptomatic it can attain a weight of twice that of left atrial growth. Tumours size varies from 1 centimetre to 15 centimetres in diameter.[6]In our case, tumour size was 16 centimetres. The origin of myxoma is thought to originate from embryonic foregut, which is entrapped, therefore they arise from multipotentmesenchymal cells capable of both epithelial and neural differentiation.[7,8] Histologically it consists of mucopolysaccharidestroma containing tumour cells. Myxomas produce Vascular Endothelial Growth Factor (VEGF), which probably is responsible for angiogenesis and the early stages of tumour growth.[7,8]

The signs and symptoms depend upon the tumour size and involved heart chamber. Blood flow obstruction within the heart can produce heart murmurs and its associated clinical manifestations.[9]

Grossly, cardiac myxomas are polypoidal, round, or oval may be often pedunculated and soft gelatinous in consistency along with a smooth, villous, or friable surface.[10] Commonly observed symptoms and signs are dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea, pulmonary oedema, cough, haemoptysis, oedema, and fatigue.[11] On histopathological examination, tumour cells were shaped stellate, spindle, oval or round. The nucleus was shaped cylindrical or oval. There was no mitotic activity. The cytoplasm was stained pink. The pink or light blue staining myxoid material was filled with the tumour cells.[12]

Williams et al. (1970) demonstrated that myxoma cells produce a tumour stroma through its secretory function. They further concluded that stromal material is synthesized in the endoplasmic reticulum and Golgi zone which are known to be the site of acid mucopolysaccharide and glycoproteins production.[13,14,15]

In our case, classical histopathological features like background stroma of myxoid material and tumour cells in the form of stellate cells were observed.

For initial diagnostic evaluation, echocardiography is a simple, non-invasive, and largely available technique. With echocardiography, myocardium and cardiac chambers can be visualized which generally shows a presence of mass. In addition, echocardiography gives an indication of any other obstructive pathology which could be a source of emboli.[16]

Therefore, from the signs, symptoms, 2D echo, gross and histopathological examination, we concluded it to be a case of atrial myxoma.

Vaideeswa et al. (2012) analysed 20 patients of non-typical myxoma characterized by multi focal presentation, of whom four cases had recurrence, suggesting that young people with multiple lesions or family history are more likely to relapse. Such patients should always receive routine echocardiography examination throughout their life.[17]As risk factors for myxoma includes embolization including sudden cardiac death; prompt resection of tumour is advised once it has been initially diagnosed. The result of resection generally has a very good prognosis of under five per cent mortality rate.[18]

Conclusion

With the annual incidence of less than one per cent, atrial myxomas are one of the common benign cardiac tumours arising from most commonly the left atrium. The correct diagnoses of atrial myxomas by the aid of histopathological, radiological mean are necessary as it may be life threatening if misdiagnosed as malignancy or for that matter also benign and not followed up correctly.

Ethical Clearance: Taken from institutional ethical committee.

Sources of Funding: Self funded

Conflict of Interest: Nil

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A Rare Case Report of Giant Cervical Angiomatous Polyp

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Abstract

Cervical polyps which are giant are less commonly seen in day to day patient examination. It is called giant when it is of more than 4 cm. It can mimic cervical cancer also. This case which we are presenting here is about the cervical angiomatous giant polyp in a woman who was presented with pain in abdomen and increased flow of menses since 1 month. Tissue section was studied and diagnosed as giant cervical angiomatous type polyp which is seen very very less commonly.

***Key-Words**: Angiomatous polyp, giant, benign, dilated and congested vessels, highly vascular.*

Introduction

Polyp of cervix which is more than 4cm of big size is seen very less commonly. In females cervical polyp is seen many times but usually not more than 2 cm or between 2 to 4cm. They do not look like benign polyp.[1] We here discussing about giant angiomatous polyp of the cervix occurring in a multiparous woman present with pain in abdomen and increased flow of menses since 1 month. Histopathology of it came out to be angiomatous polyp (highly vascular) of cervix that is seen very very less commonly.

Case Report

A 30 year old woman visited to obstetric and gynaecology department of sumandeep vidyapeeth, waghodia, Vadodara with a complaint of pain in abdomen and increased flow of menses since 1 month. There is also increasing frequency of micturition. No associated bowel dysfunction. Patient was examined and pulse, blood pressure, temperature all were within normal limits. tender. Pain is increasing in nature during examination. There was no lymphadenopathy. Routine haematological and biochemical investigations were within normal limits. Because of pain and increased menses for diagnostic purpose D & C material was taken and a large single brownish black cervical polyp also dissected which was measured 5.3x3.2x2.3 cm.[Fig-1] On cut section, reddish homogenous areas were seen. [Fig-2] On microscopic examination, dilated endocervical glands and endocervical epithelial cells are seen. Angiomatous areas are seen with dilated congested thin walled capillaries. Focal areas show haemorrhage and areas of infarction. [Fig-3], [Fig-4]

[Fig-1]: Gross image of a brownish black giant cervical polyp

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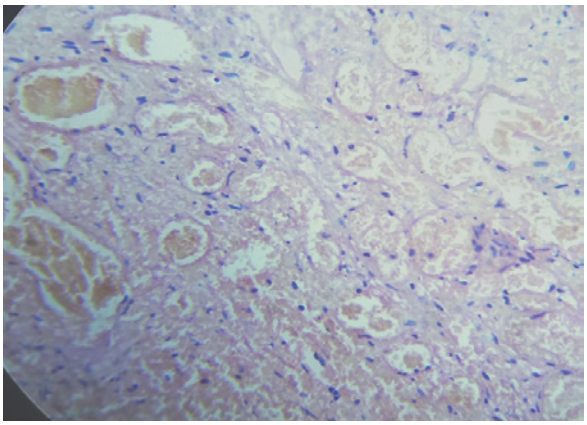
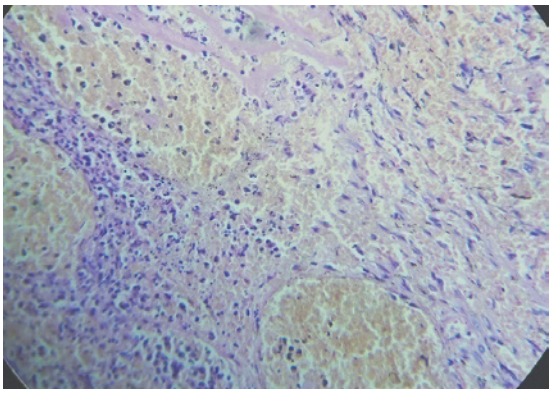
[Fig-2]: Gross image of a Polyp on cut section



[Fig-3]: Dilated congested blood vessels with focal hemorhage and necrosis (10X)



[Fig-4]: Dilated congested blood vessels with focal hemorhage and necrosis with few endocervical cells (40X)

Discussion

Polyps of cervix seen many times after adulthood with less frequently seen in cervical pathological cases. [2] Reported case was about giant polyp from endocervix. The histology of this case was reported as a giant angiomatous cervical polyp. Yadav *et al*. discussed about cervical polyp which was present most of the times at outer part of the cervix and benign.[2] It can happen due to inflammation from a long time. More than one delivery and foreign bodies also are the factors.[3] Khalil *et al*. also discussed about polyp of cervix which was giant. Tissue section was studied and it was finally concluded as glands of endocervix with fibrous and vascular tissue.[4] Benign to malignant transformation is very very less.[5] They can look like malignant clinically. Hence, examination and all tests should be properly done. Surgical approach or removal is main treatment and tissue section udy (histopathology) is final for confirmation.

Conclusion

Giant angiomatous cervical polyps are seen with very very less incidence. Only few cases are reported till date and all are benign. It can falsely present as prolapse or malignancy. So, proper examination and investigation is needed.

Ethical Clearance: Taken from institutional ethical committee.

Conflicts of Interest: There are no conflicts of interest.

Sources of Funding: Self funded

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Toxicological Assessment of Ethanolic Leaves Extract of *Kalanchoe pinnata* in Rats

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Abstract

Kalanchoe pinnata is a perennial plant that is widely used in the folklore treatment of kidney and gall stones and urinary insufficiency. The present study aims to evaluate the potential toxicity of the leaf extract of this plant upon acute and sub-acute (28 day) exposure in Wistar rats when administered orally. In acute oral toxicity(n=3) female Wistar rats were treated with a single dose of 2000mg/kg b.wt and observed for 14 days and sub-acute toxicity(n=6) male and female Wistar rats were treated with the dose of 500 and 1000mg/kg b.wt of leaf extract for 28 days consecutively. No mortality, morbidity or adverse clinical signs of toxicity were observed during the experimental period. No significant changes in the body weight was observed. Gross necropsy did not indicate any treatment-related pathological changes in any of the animals in the acute and sub-acute toxicity studies. Histopathology of the liver, heart and kidney did not show any remarkable lesions that could be related to the administration of the leaf extract. Ethanolic leaf extract of *Kalanchoe pinnata* is relatively safe with an LD_{50} value greater than 2000 mg/kg. From the sub-acute study, the no-observed adverse effects level (NOAEL) of the extract can be derived as 1000 mg/kg b.wt in male and female Wistar rats.

Keywords: *Kalanchoe pinnata, traditional medicine, safety, acute toxicity, sub-acute toxicity.*

Introduction

Kalanchoe pinnata (*K. pinnata*) is a medicinal plant of ethnomedical importance and used by the Asian folklore for various ailments.[1] The leaf extracts of this plant have been routinely used for ailments like microbial infections, kidney stones and wound healing. A number of active compounds, including flavonoids, glycosides, triterpenoids, steroids, bufadienolides and organic acids have been identified in *K. pinnata* that have been shown individually to possess variety of biological activities.[2-6]

Rajsekhar et al.[7] reviewed the pharmacological potentials of this plant which included wound-healing, antioxidant, anti-cancerous, anti-proliferative, antimicrobial, antiviral, anti-protozoal, anti-leishmanial, anthelmentic, insecticidal, anti-allergic, analgesic, antinociceptive, anti-edematogenic, anti-inflammatory, muscle-relaxant, antipyretic, anticonvulsant, antidepressant, sedative, antilithiatic, hepatoprotective, gastroprotective, antidiabetic, nephroprotective, haemoprotective, antihistamine, anti-hypertensive and immunosuppressant activities. Fernandes et al.[8] illustrated the presence of novel bioactive molecules in *K. pinnata* against bothropic venom. Zakharchenko et al.[9] explored the fungicidal potential of transgenic *K. pinnata* extract containing CecP1 and recommended their use as a candidate drug for treatment of wounds infected with *Candida albicans*.

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Despite the fact that medicinal plants such as *K. pinnata* have immense therapeutic value, the safety profiling needs to be performed using validated protocols so that it can be continuously utilized in folklore treatment.[10] To address this, a safety evaluation study of the ethanolic leaf extract of *K. pinnata* was conducted in Sprague-Dawley rats following the methodology described in the OECD guidelines for testing for acute (OECD 423) and repeated dose toxicity (OECD 407) as an initial screening for safety.

Materials and Methods

Plant material

Fresh leaves of *Kalanchoe pinnata* were collected from ITWWS (Irula Tribe Women's Welfare Society) Thandarai village, Chengalpattu, Tamil Nadu, India. The plant material was authenticated by Dr.P. Jayaraman, Director, Plant Anatomy Research Center, West Tambaram, Chennai. [PARC/3213]. The voucher specimen was deposited in the herbarium of PG and Research, Department of Plant biology and plant Biotechnology, Presidency collage, Chennai.

Extraction

The extraction was performed according to the method adopted by Azwanida.[11] Fresh leaves of *K. pinnata* were washed to remove any impurities present and shade-dried. The dried leaves were pulverized and the preparation was taken for extraction. The coarse particles were cold macerated with ethanol and kept in a shaker for seven days at 37 ºC and later filtered (using Whatman filter paper No.1 (11 µm pore size)) and dried on the hot plate at 40ºC. The crude extract was stored in refrigerator (2 -8ºC) until use.

Animals

Healthy young adult animals (8 to 12 weeks old) of Wistar strain were obtained from Sainath agencies, Hyderabad, India. The females were certified to be nulliparous and non-pregnant. The health status of the animals was provided by the vendor. All the animals were found to be health and active. The animals were acclimatized for a period of six days and housed individually in polypropylene cages with stainless steel top grill. Sterilized paddy husk bedding was provided to the animals. Standard laboratory diets (Rodent pellet) and purified drinking water was provided *ad libitum* throughout the experiment except when fasting was necessary. The animals were maintained under controlled environmental conditions at a temperature of 22°C (± 3°C) and relative humidity of 36-64% with 12 h light and 12 h dark cycle. Individual animals were identified by tail marking with unique numbers.

Acute oral toxicity

The evaluation of acute toxicity in a limit dose test was performed using the procedures described by the Organization for Economic Cooperation and Development (OECD 423). A limit test at one dose level (2000mg/kg body weight) was carried out with 6 female animals (3 animals per step). The ethanolic leaf extract of *K. pinnata* was administered as a single dose orally to overnight fasted rats. Food was withheld for a further 3 hours after dosing. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. Individual weights of animals were determined shortly before the test substance is administered and weekly thereafter. All test animals were subjected to gross necropsy. Gross pathological changes (if any) were recorded for each animal.[12]

Repeated dose toxicity

Following the acute toxicity study, the effect of 28-day repeated oral dosing of the extract in Wistar rats was evaluated following the OECD 407 Test Guideline. The method used provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on the nervous, immune and endocrine systems.

In the repeated dose oral toxicity study, 18 animals were randomly allocated to the control and treatment groups with 3 males and 3 females in each group. The animals in the treatment group were dosed with the ethanolic extract of *K.pinnata* at 500 and 1000mg/kg body weight daily 7 days each week for a period of 28 days. Animals in the vehicle control group received purified drinking water at 10ml/kg body weight. The extract/vehicle was administered in a single dose to the animals using a suitable intubation cannula.[13]

General clinical observations were made at least once daily. Mortality and morbidity observations were made twice daily. Body weight and feed measurements were made weekly. At the end of the 28-day treatment period, animals were euthanised. At gross necropsy, blood samples were collected from the retro-orbital sinus for haematology and biochemistry analyses.

Hematology and Biochemistry

Blood was collected from the retro-orbital sinus under mild anaesthesia using diethyl ether, into heparinized and nonheparinized tubes for hematology and biochemistry analyses respectively. Hematological parameters analyzed include Hematocrit, Clotting time, Hemoglobin concentration(Hb), Red blood cell count(RBC), White blood cell count(WBC), Differential count(DC) and Platelet count. Serum samples were analysed for the following Biochemical parameters: Sodium, Potassium, Glucose, Total cholesterol, Urea nitrogen, Creatinine, Total protein, Albumin, Total bilirubin, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

Histopathology

The animals were later euthanised by CO_2 asphyxiation. Gross necropsy (External and internal) was performed; Major organs (Liver, Heart and Kidney) were collected for histopathological evaluations.

Statistical analysis

Data obtained were subjected to paired Student's *t*-test to evaluate the difference between the treated and control groups. P value was set at 0.05. Values were represented as Mean ± SD.

Results

Acute oral toxicity

No mortality/morbidity was observed any of the animals treated with the ethanolic extract of *K. pinnata* at 2000mg/kg body weight during the 14-day observation period. All animals showed an increase in body weight relative to that recorded prior to treatment. Based on the observations, the median lethal dose (LD_{50}) of *K. pinnata* extract in female rats was estimated to be greater than 2000 mg/kg body weight.

Repeated dose toxicity

Repeated oral administration of the extract did not induce any adverse clinical signs of toxicity in any of the treated animals in both the sexes. No mortality or morbidity was observed during the course of the treatment period until termination. No significant changes in body weight or feed consumption were observed. Gross necropsy did not reveal any treatment-related pathological lesions. All treated animals gained appreciable body weight relative to that recorded prior to dosing (Table 1).

Table 1. Repeated dose toxicity study – Mean Weekly Body weight (in grams)

Treatment	Days					BWC
	0	7	14	21	28	
Control 10mL/kg b.wt	128.25 ± 2.12	134.3 ± 1.71	141.3 ± 2.08	146.65 ± 3.06	152.75 ± 3.70	24.5 ± 2.78
KPEE 500mg/kg b.wt	127.27 ± 3.40	133.73 ± 2.53	139.95 ± 2.52	145.62 ± 2.31	152.43 ± 2.91	25.1 ± 1.49
KPEE 1000mg/kg b.wt	125.73 ± 2.43	132.25 ± 2.27	138.87 ± 2.59	144.53 ± 2.12	152.75 ± 3.70	24.5 ± 2.78

Values are expressed as Mean ± S.D, N=6(both 3male&3female), KPEE – *Kalanchoe pinnata* Ethanolic Extract, BWC - Body Weight Change

Hematology and Biochemistry

The effects of sub acute oral administration of *K.Pinnata* extract on hematological and biochemical parameters are presented in Tables 2 and 3. No significant changes were observed in the levels of Hematocrit, Clotting time, Hemoglobin concentration(Hb), Red blood cell count(RBC), White blood cell count(WBC), Differential count(DC), Platelet count were observed with respect to the untreated control. No statistically significant changes were observed in the biochemical parameters assessed.

Table 2. Effect of ethanolic extract from *K. pinnata* leaves on Hematological parameters

Hematological Indices		Control 10mL/kg b.wt	KPEE Treated 500 mg/ kg b.wt	KPEE Treated 1000 mg/ kg b.wt
RBC(106 / **µL)**		8.22 ± 0.24	8.21 ± 0.48	7.88 ± 0.59
Hb(g/dL)		13.73 ± 0.38	13.82 ± 0.35	13.33 ± 0.29
HCT(%)		44.37 ± 0.86	43.92 ± 2.21	43.36 ± 1.37
WBC(103/**µL)**		11.3 ± 0.34	11.02 ± 0.35	10.68 ± 0.65
PLT(103/**µL)**		949.94 ± 21.91	944.7 ± 31.94	947.33 ± 16.14
CT(s)		133.01 ± 6.7	132.97 ± 8.64	136.92 ± 3.01
Differential Count	L (%)	74.35 ± 2.37	72.17 ± 2.21	73.18 ± 0.83
	M (%)	5.48 ± 0.32	5.46 ± 0.43	5.36 ± 0.28
	G (%)	22.54 ± 1.37	21.62 ± 2.27	20.33 ± 0.71
MCH(pg)		16.03 ± 0.84	15.95 ± 0.45	16.55 ± 0.59
MCHC(g/dL)		29.55 ± 1.48	30.19 ± 1.47	30.61 ± 0.69
MCV(fL)		54.31 ± 1.35	55.01 ± 2.19	55.71 ± 1.93

N=6(both 3male&3female), KPEE – *Kalanchoe pinnata* Ethanolic Extract. Values are expressed as Mean ± S.D; Hb, hemoglobin; RBC, red blood cell count; HCT, hematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; WBC, white blood cell count; L, lymphocyte percentage; M, monocyte percentage; G,granulocyte percentage; PLT, platelet count; CT, clotting time.

Table3. Effect of ethanolic extract from *K. pinnata* leaves on Biochemical parameters

Biochemical Indices	Control 10mL/kg b.wt	KPEE Treated 500mg/kg b.wt.	KPEE Treated 1000mg/kg b.wt.
Sodium(mEq/L)	145.41 ± 2.05	144.95 ± 2.35	146.84 ± 1.34
Potassium(mEq/L)	4.89 ± 0.29	5.17 ± 0.31	5.32 ± 0.44
Glucose(mg/dL)	106.32 ± 2.86	105.11 ± 1.82	105.01 ± 2.29
Cholesterol(mg/dL)	99.35 ± 2.19	97.54 ± 3.76	98.05 ± 3.88
Total Bilirubin(mg/dL)	0.37 ± 0.07	0.42 ± 0.04	0.34 ± 0.05
Total Protein(g/dL)	6.89 ± 0.18	6.89 ± 0.37	6.44 ± 0.47
Albumin(g/dL)	3.73 ± 0.32	3.97 ± 0.28	3.84 ± 0.36
Urea(mg/dL)	16.6 ± 0.53	16.39 ± 0.75	16.79 ± 0.29
Creatinine(mg/dL)	0.56 ± 0.05	0.63 ± 0.11	0.58 ± 0.09
AST(IU/L)	75.53 ± 2.52	75.41 ± 2.35	76.91 ± 1.09
ALT(IU/L)	19.2 ± 1.11	19.34 ± 1.13	20.5 ± 0.89

N=6(both 3male&3female), KPEE – *Kalanchoe pinnata* Ethanolic Extract Values are expressed as Mean ± S.D; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

Euthanasia and Gross necropsy:

The animals were euthanized by carbon dioxide asphyxiation and gross necropsy of the external and internal organs did not reveal any treatment-specific changes.

Histopathological Examination

Figures 1 represent the microscopic images of the stained tissue sections of liver, kidney and heart in the control group (A) and treatment groups (B, 500mg/kg b.wt and C & D, 1000mg/kg b.wt). No treatment-specific lesions, inflammation or pathological changes related to were observed in the organs of the animals from the treatment group compared to the untreated group. In general, microanatomy of liver, kidneys and heart did not present any treatment-related adverse toxicological effects in the animals that received 500mg/kg b.wt and 1000mg/kg b.wt.

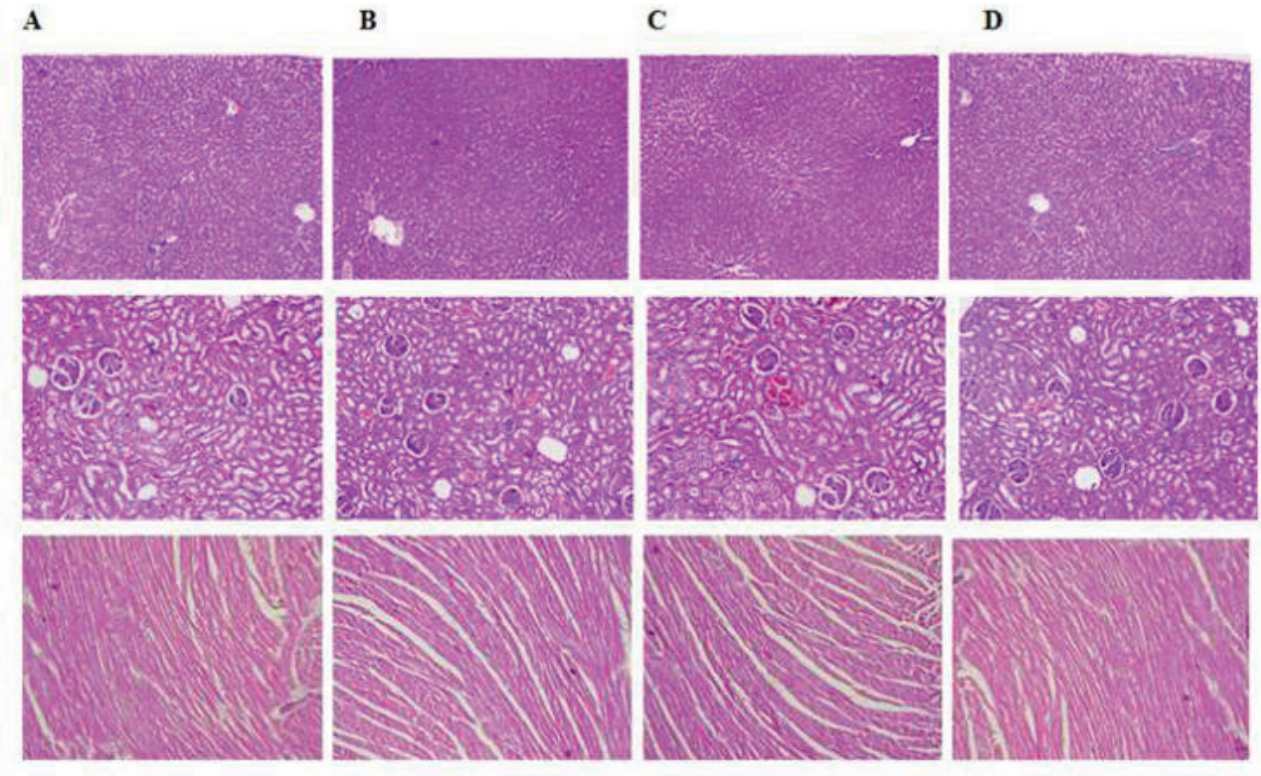

Figure 1: Hematoxylin and eosin stained cross sectional liver, kindney and heart views of control(A) and KPEE treated 500 mg/kg b.wt(B) and 1000 mg/kg b.wt (C and D) in sub-acute toxicity study in male and female rats (magnification, x40)

Discussion

Medicinal plants are an important source of substances which are claimed to induce anti-inflammatory and antioxidant effects. *K. pinnata* found in the temperate and tropical regions and largely used in Indian system of medicine(Folk, Siddha and Ayurvedic) for the treatment of kidney stones, gastric ulcers, pulmonary infections, rheumatoid arthritis.

Ozolua et al.[14] studied acute and sub-acute oral toxicity of the aqueous extract of *K. pinnata* in Sprague Dawley rats. No mortality was observed at the acute oral dose of 5 g (5000 mg) per kg body weight by the oral route. The medial lethal dose following intraperitoneal administration was 1.8 g (1800mg) per kg body weight. Sub acute treatment did not significantly alter animal weights, organ-to-body weight ratios, fluid intake, hematological indices and the levels of AST, ALP and albumin. ALT level was significantly reduced in the treated group. Total bilirubin and conjugated bilirubin levels remained unaffected.

Zakharchenko et al.[9] indicate that ethanol extracts have become increasingly popular form of *Kalanchoe* application in therapy. The therapeutic efficiency is attributed to the presence of lipophilic constituents such as the bufadionolides, polyphenols and flavonoids.

Nayak et al.[15] studied the wound healing potential of the ethanolic extract of *K. pinnata* leaf in rats and concluded that there was increased wound retraction and hydroxy proline content in the extract treated groups indicating a significant wound healing potential consistent with the traditional healing practice. Mathew et al.[6] reported the analgesic and anti-inflammatory potential of the ethanolic extract of the stem of *K. pinnata* in rat models, anti-depressant potential in mice, and anti-diabetic potential in alloxan-induced diabetic rats. In all these studies, the extract as such which served as the control did not exhibit any adverse effects in the treated animals, indicating its safety.

Nevertheless, an independent safety evaluation of the ethanolic extract has not been reports so far. The

present study attempted to evaluate the safety of the ethanolic extract of *K. pinnata* leaves during acute and sub-acute treatments in Wistar rats. The results obtained are similar to those obtained for the aqueous extracts performed by Ozolua et al.[15] in terms of the absence of mortality and the haematological and biochemical indices studied. In addition, histopathological evaluation of the major tissues (Liver, Heart and Kidney) did not indicate any remarkable lesions that could be correlated to the repeated dosing of the plant extract. The results therefore indicate the safety of the ethanolic extract of *K. pinnata* leaves complementing its use in traditional medicine.

Conclusion

Toxicological evaluation of the ethanolic extract obtained from leaves of *K. pinnata s*howed no signs or symptoms of acute toxicity. The median lethal dose (LD_{50}) value of the extract was derived to be higher than 2000 mg/kg b.wt indicating that the extract is non-toxic. Repeated dose toxicity study with the extract did not induce any adverse clinical signs of toxicity in the treated animals; in addition, hematology and biochemistry parameters remained unaffected in the treated animals. No histopathological changes (in the organs studied) related to treatment with the plant extract were recorded. The extract is therefore considered safe for repeated administration up to 1000mg/kg/day. In conclusion, the ethanolic extract of *K. pinnata* does not contain any toxic phytochemical constituents as evidenced by the lack of adverse clinical effects in acute and repeated dose toxicity studies in rats and can therefore be concluded as safe for oral consumption.

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Conflicts of Interest - The authors declare that no conflict of interest.

Ethical Clearance - This study has been approved by the Institutional Ethical Committee of GLR Laboratories Chennai.

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Analysis of Risk Management Cycle on Capitation Mechanism at First Level Healthcare Facilities in East Java

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Abstract

The implementation of risk management is an essential function in an organization to control risk. Based on the preliminary research that has been carried out, only 4.2% of FLHF in East Java implemented the complete risk management process, starting from establishing context to evaluating risks. Modifications of risk management cycle implementation are made by reviewing the opinion of Carroll (2001) and ISO 31000:2009. This research aims to analyze the influence between the stages of implementing modified risk management based at 95 FLHF in East Java. The results show that each stage of the capitation mechanism risk management has a strong significant influence on each other. The results provide recommendation that FLHF should improve the understanding and awareness of the risks and expected to be able to apply risk management cycle as a whole.

Keywords: *risk management cycle, capitation mechanism, first level healthcare facilities*

Introduction

The implementation of risk management is an essential function in an organization to control risk. This function will reduce the negative impact that may arise from various activities carried out by the organization. This function includes carrying out assessments and determining efficient ways to control risk.(1)(2)(3)(4)(5)(6) The implementation of risk management includes two major stages that are connected, namely the risk identification and analysis stage as well as the handling of such risks. Carrol (2001) describes the relationship between these two stages with the risk management process structure. The risk management process structure explains that failure in risk management can result in decreases control over losses that may occur.(3)

Risk is a possibility of an unwanted loss with a certain severity. The existence of a severity level associated with the risk allows analysis and prevention of the risk. The element of uncertainty in risk requires effective control measures in order to reduce the negative impacts that may occur. Therefore, a management concept is formed to control risk, known as risk management.(2)(7)(8) Based on ISO 31000:2009, the risk management process has five main parts, namely: (a) communication and consultation, (b) establish context; (c) risk assessment (identification, analysis, and evaluation) (d) risk management and (e) monitoring as well as review. Those standards state that every part is a systematic application of management policies, procedures, and evaluation. (6)

First Level Health Facilities (FLHF) according to the Minister of Health Regulation (Permenkes) No. 71 of 2013 (9) consists of community health centre (Puskesmas), primary clinic (pratama clinic), class D hospital, and private dentist practice. FLHF is an

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institution that acts as the earliest health service provider (PPK). As an organization engaged in the health sector, FLHF certainly cannot avoid risks. Therefore, FLHF urgently needs the implementation of risk management..

Since 2014, Indonesia has implemented the National Health Insurance (JKN) policy. FLHF, in collaboration with Healthcare and Social Security Agency (BPJS Kesehatan), will act as gatekeepers in the program and could face risks that need to be managed. The capitation mechanism is a payment method used in JKN, which is implemented in Indonesia by adjusting a separate policy. Among the various risks faced, it is deemed necessary to analyze health services in the FLHF from the capitation mechanism approach as a payment method in FLHF.

The number of FLHFs working with BPJS Kesehatan throughout Indonesia until July 2020 is more than 27.000 FLHFs. In East Java, the number of FLHF who has worked with BPJS Kesehatan amounted to around 2.400 FLHFs.(10) Total FLHF cooperated with BPJS Kesehatan in East Java reached 11,4% out of the total FLHF in Indonesia. As many as 38.9% consisted of Puskesmas, and the rest consisted of individual practice and Pratama clinics. Based on the preliminary research that has been carried out, out of the total number of FLHFs in East Java, only 4.2 % of FLHFs implemented the overall implementation of risk management, starting from establishing the context to evaluating risks.

Taking a statement from ISO 31000:2009(6) regarding the risk management process, this study then considers modifying the risk management process. Modifications are made by reviewing the opinion of Carroll (2001)(3) regarding the two stages in the process of implementing risk management and ISO 31000:2009 regarding the five main parts of risk management which are then adjusted to its application in FLHF. In conclusion, the risk management cycle in FLHF can be simplified into five components: (a) establish context; (b) risk identification; (c) risk analysis; (d) risk treatment, and (e) risk evaluation.

Establish context means determining the risk context of the capitation mechanism through socialization that affects the achievement of organizational goals. Risk identification is the study of potential sources of events and triggers that may cause harm to the organization due to the capitation mechanism. Risk analysis is the implementation of an investigation regarding possible causes, severity, urgency, and seriousness of losses related to the risks of the identified capitation mechanism. Risk treatment is the management of potential events that may result in organization losses due to the capitation mechanism. Risk evaluation can be interpreted as the implementation of activities to assess the management of events that may result in organization losses due to the capitation mechanism.

The modification results are considered to be more representative of the actual risk management cycle. Several essential points that make this modification different from existing concepts are found in the risk analysis and risk evaluation stages. In the risk analysis stage, there is a risk evaluation process in which risk is evaluated, so that the risk evaluation stage does not evaluate risk anymore. The risk evaluation stage is used to evaluate the overall risk management cycle. With the overall evaluation, it can be seen that the stages are still less than optimal in implementation. This research aims to analyze the influence between the stages of implementing modified risk management based on a combination of two statements.(3)(6) Modification of risk management implementation stages has been adjusted by its application in FLHF (Puskesmas and Pratama clinic) in collaboration with BPJS Kesehatan, especially in East Java Province.

Method

This research is an observational study with a cross-sectional approach using primary data from FLHF in East Java Province in collaboration with BPJS Kesehatan. The sampling method used was proportional stratified random sampling. The sample size after proportioning by type of FLHF has obtained as many as 54 community health centres and 28 primary clinics. Large sample proportionally distributed in districts or cities selected based on the number FLHF cooperation with BPJS Kesehatan. The results of the data collection obtained 95 FLHFs.

Research respondents were FLHF leaders and staff who were involved in the management of capitation funds and patient care for JKN participants. The minimum number of respondents for each FLHF was three people (according to FLHF

conditions) consisting of 1 (one) leader and 2 (two) staff who are the key persons in the organization. The questionnaire used in data collection is the risk management implementation questionnaire. The assessment is carried out on a scale ranging from 1 (never implementing) to 5 (always implementing). The establish context variable was represented by 17 statements, risk identification variable 23 statements, risk analysis variable 22 statements, and risk treatment and risk evaluation variable 24 statements. In this statement, the risk elements studied include financial, legal, social and performance aspects (performance, time, physical).

Results and Discussion

The implementation of capitation mechanism risk management in FLHF in this research consisted of five stages, namely establish context, risk identification, risk analysis, risk treatment, and risk evaluation. The results of research related to the implementation of capitation mechanism risk management are shown in Table 1.

Table 1. Distribution of Mean, Standard of Deviation, and Normality Test Score of Capitation Mechanism Risk Management Implementation at FLHF in 4 Districts and Cities in East Java 2019

No	The implementation of capitation mechanism risk management	n	Score					Annotation
			Mean	SD	Min	Max	p	
1	Establish Context	95	63,51	13,33	23	85	0,518	Normally distributed
2	Risk Identification	95	94,76	13,29	65	120	0,280	Normally distributed
3	Risk Analysis	95	87,64	14,12	57	115	0,797	Normally distributed
4	Risk Treatment	95	95,88	15,45	57	125	0,585	Normally distributed
5	Risk Evaluation	95	96,06	15,99	52	125	0,586	Normally distributed
Total score		95	437,85	67,33	297	570	0,630	Normally distributed
Factor analysis		95	0,001	1,00	-2,11	1,96	0,654	Normally distributed

Based on Table 1, it is known that all measurement data are normally distributed. The result of the factor analysis from the total score of all sub-variables is the score that will be used for the frequency analysis of the risk management implementation intensity. The scores obtained are then grouped to make it easier to identify the intensity of risk management implementation in FLHF. Four groups of implementation of risk management intensity were obtained, which are very low, low, high, and very high. The results of the division of the capitation mechanism risk management implementation intensity by FLHF type can be seen in Table 2.

Table 2. Frequency Distribution of Risk Management Implementation Intensity by Type of FLHF in 4 Districts and Cities in East Java 2019

No	FLHF Type	Very Low		Low		High		Very High		Total	
		n	%	n	%	n	%	n	%	n	%
1	Puskesmas	5	7,7%	24	36,9%	19	29,2%	17	26,2%	65	68,4%
2	Pratama clinics	7	23.3%	12	40,0%	6	20,0%	5	16,7%	30	31,6%
Total		12	12,6%	36	37,9%	25	26,3%	22	23,2%	95	100,0%

The analysis result shows that in general, FLHF has not fully implemented the capitation mechanism risk management correctly. This result can be concluded from the number of FLHF that have implemented risk management with low and very low intensity, namely 48 FLHF (50,5%). This amount is slightly greater than FLHF that have implemented risk management with high and very high intensity (49,5%).

Meanwhile, based on the type of FLHF, risk management implementation with high and very high intensity is proportionally greater in Puskesmas (55,4%) rather than in Pratama clinics (36,7%). Organizational leaders must be able to become "system thinkers" who demand in-depth analysis of security issues, replace punitive reactions to mistakes to be open and proactive in dealing with any risks. So it is hoped that there will be an opportunity to build a safer health organization.(11)

Furthermore, a continuation statistical analysis was carried out that observed the influence between stages of the capitation mechanism risk management implementation at FLHF in 4 districts and cities in East Java. The results of the statistical analysis can be seen in Table 3.

Tabel 3. Statitical Analysis of The Capitation Mechanism Risk Management Stages at FLHF in East Java 2019

No	Independent variables	Dependent variables	B	b	p	R2	Annotation
1	Establish Context	Risk Identification	0,667	0,669	0,001	0,45	Significant
2	Risk Identification	Risk Analysis	0,986	0,928	0,001	0,86	Significant
3	Risk Analysis	Risk Treatment	1,062	0,971	0,001	0,94	Significant
4	Risk Treatment	Risk Evaluation	1,013	0,979	0,001	0,96	Significant
5	Risk Evaluation	Establish Context	0,572	0,687	0,001	0,47	Significant

After the statistical analysis has been done, it was found that all stages of risk management cycle influence each other. This result is consistent with research by Malilay, et al. (2014) on the role of applied epidemiology methods in disaster management cycle. In said research, it is stated that there are three stages of the disaster management cycle, namely predisaster, disaster and post-disaster. The three stages are interconnected in their implementation. If one stage is not appropriately implemented, it will have an impact on the other stages. (12)

Other research by Evans, Dalkir, dan Bidian (2014) also stated the same thing in a different field, namely knowledge management cycle (KMC). KMC consists of 7 phases, namely identify, store, share, use, learn, improve, and create. The 7 phases are interrelated in their utilization and are grouped into the KMC model.(13) Research by Morita, Flynn, dan Ochiai (2011) on the strategic management cycle, also stated the same thing. The strategic management cycle consists of 4 stages, namely organizational visionary planning, formulating strategy, operations and practice, coordination and storing of practices. The success of the strategic management cycle is a function of how well each stage is carried out. An organization will survive and grow when management cycles are implemented effectively. (14)

Another research by Jorgensen (2007) regarding the sustainable environmental management system (EMS) stated a similar thing. Said research stated that to create

an efficient and dynamic system, it takes synergies from several areas. In addition, implementation in each of the six stages of consisting of environmental policy, planning, implementation and operation, checking, management review, and continual improvement is also crucial. Each stage of an EMS is interconnected, so compatibility between stages is essential.(15)

Based on the statistical analysis of the capitation mechanism risk management implementation cycle, the following results were obtained.

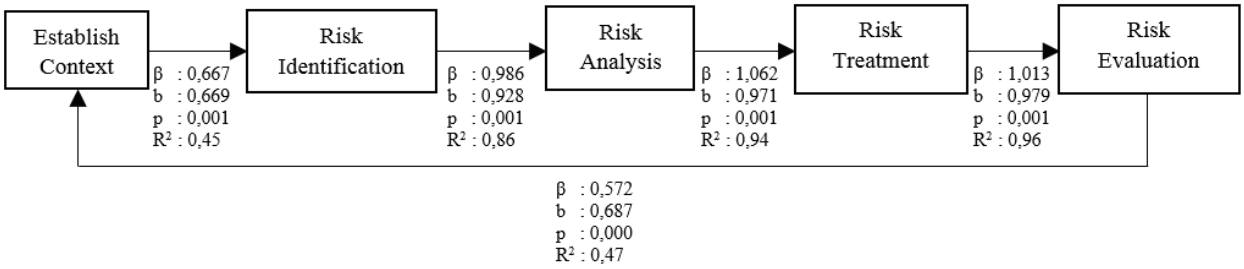

Figure 1. Statistical Analysis Results of Capitation Mechanism Risk Management Implementation Cycle

Figure 1 shows that each stage of the capitation mechanism risk management has a significant influence on each other. The strength of influence between stages of capitation mechanism risk management implementation is strong. This result is in line with research by Sendlhofer, et al. (2014)(16) related to the systematic implementation of clinical risk management in hospitals. The results indicate that clinical risk management plays a dominant role in enabling identification, analysis, and potential risks management. The application of clinical risk management into routines in hospital organizations can be challenging. Each stage in the clinical risk management application affects the quality of the risk management implementation cycle.

Based on the research, it can be concluded that various conditions will influence the risk management in the subsequent stages of implementation. This stage starts from establishing context to risk evaluating. Errors in the establishing context will affect every next step, up to the risk evaluation stage. The better the FLHF understanding and awareness of the risks faced, the better the implementation of identification, analysis, treatment and evaluation of these risks.

Conclusions and Suggestions

Conclusions

1. As many as 49.5 % of FLHF implemented risk management with low and very low intensity, more than those with high and very high intensity. FLHF have not fully implemented the capitation mechanism risk management correctly.

2. There is a strong influence at each stage of risk management implementation (establish context, risk identification, risk analysis, risk treatment, and risk evaluation). It is important to note that understanding and awareness of the risks faced will determine how the FLHF manages each risk.

Suggestions

1. The implementation of risk management will improve if the understanding and awareness of the risks faced are well enough so that the enforcement of the risk context is known in the FLHF. It is expected to be able to apply risk management as a whole.

2. Organizational leaders need to monitor each stage of the risk management implementation in detail (starting from establish context, identification, analysis, treatment to evaluation) because they are closely related to one another.

Conflict of Interest : Nil

Ethical Clearance : This research has been proved by Health Research Ethics Committee, Faculty of Nursing Universitas Airlangga

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Effectiveness of Cervical and Upper Shoulder Stabilization Exercises in Subjects With Rheumatoid Arthritis Patients Associated with Neck Pain

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Abstract

Background: Rheumatoid arthritis is an immunological mediated, chronic inflammatory multisystem disease which involves inflammation of the peripheral joint. Ra affects about 24.5 million people as of 2015 and the incidence rate is more dominated amongst female. Historically the involvement of the cervical spine in later stages of rheumatoid arthritis isn't uncommon. Specifically, C1 and C2 are involved as they are the only vertebrae without intervertebral disc and synovial lining and hence they are very susceptible to inflammation due to autoimmune reaction. The alteration in the range of motion is quite significant at the cervical spine. The biomechanics of the cervical spine and shoulder joint are interlinked with each other. The protocol of specific cervical and upper shoulder stabilization exercises with electrical modalities like hot-moist pack and transcutaneous electrical nerve stimulation and short wave diathermy therapy was conducted to find out the effectiveness of scheduled, designed physiotherapy protocol of specific stabilization exercises along with electrotherapy for cervical spine and upper shoulder stabilization exercises to see the improvement in cervical and shoulder range of motion and pain in patients with rheumatoid arthritis

Methodology: An observational study was conducted in 25 patients at Karad with a study duration of 6 months. The inclusion criteria were the patient with rheumatoid arthritis any age and gender willingly to participate. The outcome measures were universal goniometer for assessing cervical and shoulder range of motion, the visual analogue scale for pain assessment.

Conclusion: The study concluded that there was a significant reduction in the range of motion in both the cervical spine and shoulder joint. The visual analogue scale suggests there was significant pain present in the shoulder joint and cervical spine. Post-treatment assessment suggests the improvement in the range of motion and pain that leads to improvement in the quality of life.

Keywords: *Rheumatoid arthritis, cervical spine, shoulder joint, mobility, range of motion, pain, stabilization exercises, visual analogue scale, physiotherapy, electrotherapy.*

Introduction

Rheumatoid arthritis is systemic inflammatory disease clinically represents as polyarthritis involving small and large diarthrodial joints of the extremities usually in a symmetrical pattern along with the involvement of haematological, neurological and cardiovascular abnormalities. The disease prevalence worldwide is ranging from 0.3% - 0.8% and in India it ranges from 0.5% - 0.75% .

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Pathophysiology

As rheumatoid arthritis is an autoimmune disease the precise pathology is not known. It seems to

involve a complex interplay between genetic factors, environmental triggers and stochastic factors. The most important genetic susceptibility locus associated with RA is class 2 major histocompatibility complex MHC allele namely human leukocyte antigen-DR (HLA-DR) specifically HLA-DRI and HLA. The subtypes of HLA-DR4 contribute significantly to RA as they linked with RA risk encode a conserved sequence of five amino acid with position 70-74 with 'QKRAA' that surrounds the peptide-binding pocket of the antigen-presenting molecule called the ' shared-epitope' who binds and presents processed putative arthritic antigens to helper T-cell in some unknown altered way and /or shape the T-cell repertoire participating in broader self- reactive adaptive immune responses.

Environmental factors trigger antigenic activity in immunologically predisposed individuals. The role of HPA- axis and cytokine production, hormones, pregnancy and related foetal-maternal interaction have been implicated .the non-genetic factors triggers the persistent low-grade inflammation and quantitative and qualitative alteration in citrullination of self -mucosal proteins through the activation of innate immune with toll-like receptors. The process of Citrullation catalyst the autoimmune reaction as the Argenin converts into *citrulline presenting type-2* collagen fibres and vimentin with the help of an enzyme called *peptidylarginine deiminase* (PADI). And hence self-antigens gets formed. This self- antigen triggers the susceptible genes like HLA-DR4 and HLA-DR1 and hence activation of antigen-presenting cells occurs which also activates the CD4+ T –cells which produce autoantibodies namely *anticitrunillated protein antibody* (ACPA). The chemical mediators like cytokinesis i.e TNF-α, interferon- Gamma, IL-1 and IL-6 within the joint and leads to inflammatory response and hence activation of B- lymphocytes and macrophages along with endothelial cells. Activation of B-cells release Ig-M antibody against the Ig-G. This leads to a proliferation of synovial cells and pannus formation. This results in the swollen synovial membrane due to granulation and scar tissue. The pannus damages the articular cartilage and erodes bone and damage other soft tissue structures.

Involvement of the cervical spine and shoulder in RA:

In 1952, Kornblum and colleagues were the first to recognize the relationship between RA and cervical spine instability[1]. The involvement of the cervical spine is common and not so rare because of anatomy and biomechanics of axis and atlas vertebra. The occiput –C1 and articulation of C1 –C2 are the only joint in the spine without intervertebral disc[1] and this only consists of the synovial joint makes them more vulnerable in RA. Axis provide a platform for the skull to rest on it as it serves the purpose of the ring. The transverse ligament of C1 articulates with the posterior aspect of send via a synovial joint. Because of this structure, it allows axis and atlas increased mobility at the cervical spine. The rotation of the cervical spine is allowed due to articulation of the odontoid process with the atlas transverse ligament which results in the nearly 50% of cervical spine rotation. The integrity of transverse ligament decides the stability of Atlanto-axial structure. The alar ligament provides secondary stabilization. In RA, the transverse ligament is affected significantly and make it incompetent due to inflammation of the synovial articulation. if secondary stabilizes are involved then stability gets hampered. Synovial inflammation leads to erosion of the odontoid process. Axial skeleton involvement is a common feature of RA. It has a variable prevalence ranging from 25 to 80% (3), with the cervical spine being almost exclusively affected[2]. There are three types of cervical spine involvement in rheumatoid arthritis(RA). The commonest abnormality was erosions of the odontoid process (47%), followed by atlantoaxial dislocation and apophyseal joint involvement (24%) and only 5% of patients had abnormalities of spinous processes of vertebral bodies. The most common manifestation is AAS (49%), followed by superior migration of the odontoid (SMO, 38%) and sub axial subluxation (SAS, 10-20%)[2.] The cervical myelopathy is also predominant in some cases[6.] The neck pain was present in 68% in patients with rheumatoid arthritis as per the study conducted[4]. The condition of most patients with C1-C2 alignment abnormalities remained unchanged or became worse with time (i.e., the misalignment became fixed, subluxation increased, or AAI developed)[5.] The study was done to suggest that the degenerative changes in disc plates and osteophytes formation is also evident in the upper cervical region is also prominent in some age group studied[7].

The studies have found that patients with rheumatoid arthritis (RA) risk impaired shoulder function due to the inflammatory process. The knowledge of shoulder function in the early years of the disease is limited[8]. The aim was to compare shoulder function and activity limitation related to the shoulder-arm-hand in women with RA in early disease course compared to age-matched healthy women. Patients with rheumatoid arthritis (RA) risk impaired shoulder function due to the inflammatory process. The knowledge of shoulder function in the early years of the disease is limited[8]. The strength of the shoulder was found reduced up to 65% in patients. In a study at the time of the assessment, 53.4 % of the patients and 20.4 % in the reference group reported present shoulder symptoms. Thirty-three (32.0 %) patients had unilateral symptoms and 22 (21.4 %) bilateral symptoms and unilateral shoulder symptoms were more significant than the bilateral shoulder symptoms. The mean shoulder pain was significantly higher for the patient's dominant arm. Study indicates that patients with RA have reduced shoulder muscle strength and limited function already 1.5 years after disease onset, even if they do not complain of symptoms from the shoulder[8].

Over the past decade, numerous studies have shown an association between reduction in the strength and endurance capacity of the cervical muscles and neck pain. Studies of patients with neck pain symptoms have found decreased maximal isometric strength and isometric endurance of the cervical flexor muscles. The studies have that shown the effectiveness of cervical stabilization exercises in improving neck pain and cervical muscle performance in a randomized trial of patients with cervicogenic headache[9]. Stabilization exercises are much safer than the other kind of exercises and hence can be done in complicated conditions also.

Some guidelines for a chronic neck pain population, which state that programs should be multimodal to include active exercise and education. Exercising a minimum of 3 times a week for approximately 30 to 60 minutes at an intensity to induce strength gains and reduce pain and disability is recommended[9]. The combination of exercises + HMP+ TENS could be benefitting the patient.

Aim:

To study the effect of cervical and upper shoulder stabilization exercises in subjects with rheumatoid arthritis patients associated with neck pain

Objectives

To find the effectiveness of cervical and upper shoulder stabilization exercises on pain in subjects with rheumatoid arthritis patients associated with neck pain.

To find the effectiveness of cervical and upper shoulder stabilization exercises on cervical and shoulder range of motion in subjects with rheumatoid arthritis patients associated with neck pain.

To find out the effectiveness of cervical and upper shoulder stabilization exercises along with electrotherapy on quality of life.

Materials :

1)data collection sheet

2) Consent form

Methodology:

1) Type of study – Experimental
2) Study design - pre and post
3) Place of study- Karad
4) Sample size – 25
5) Sampling technique – convenient
6) Study duration – 3 months

Inclusion criteria :

Males/females suffering from rheumatoid arthritis.

Patient with neck pain associated with neck pain.

Exclusion criteria **:**

symptoms other than rheumatoid arthritis.

Data Collection :

The study was conducted in Krishna hospital and the around area. The 25 subjects were selected according to the inclusion and exclusion criteria. Individuals were

not willing to participate and individuals with symptoms other than rheumatoid arthritis were excluded. The written consent was taken from subjects and their family members those willing to participate. Institutional ethical committee approval was obtained before the beginning of the study.

The format of the treatment programme was explained to the patient before the treatment started. The red flags and yellow flags were checked. Exercises were given 3 days per week after giving all the postural ergonomics and all exercises were demonstrated properly before the treatment starts just to ensure that the training protocol was learned properly. The procedure of exercises was translated into the mother language of the patient and written copies of translation were given to the individual patient.

Procedure :

The programme will be started with the application of a combination of TENS and HMP followed by the sitting and walking with the neutral head position.

Electrical agents :- HMP + TENS

TYPE - burst

Frequency – 100 Hz

Duration - 15 min

Intensity – as comfortable

The exercises were started in supine position progressing to the sitting and standing. Cervical isometrics with a towel under the neck to all movement with the hold period of 10 seconds followed by the break of 15 seconds. 5 repetitions in a progressive manner were given.

1) Cervical exercises –

walking and sitting with a neutral head position

Supine and prone neutral head position

Supine and prone retraction

Supine and prone head lifts

Superman exercise

Isometrics of the neck in sitting and standing with towel and

Resistance band

Chin tuck and chin out exercises

2)Shoulder and scapular exercise –

scapular retraction and protractions

Prone T scapular stabilization

Prone Y scapular stabilization

Wall arm circles

Swiss ball exercises

Resistance band exercises

All exercise was done for 10 seconds hold with a break of 15 seconds. 5 repetition per each set was performed for 3 sets.

Statistical Analysis

Descriptive statistic such as mean, standard deviation and the percentage was used to present the data. The pre and post-treatment effectiveness was calculated by using paired “t-test”. T-test was done to check out if the data passed the normality test or not. P-value > 0.10 was considered significant. Age-wise association of altered mobility and pre and post effect at cervical spine and shoulder was assessed by ANOVA P test. “P” value less than 0.030 were considered as significant. Data analysis was performed by using Microsoft Excel and SPSS v16.0.

Discussion

Rheumatoid arthritis is the autoimmune disease that involves inflammation of peripheral joints. The studies have shown that cervical spine involvement is also a frequent feature of RA. The atlas and axis vertebrae are more prone to inflammation due to absence of intervertebral disc lead to joint erosion, altered mobility at the cervical joint. alongside with cervical spine involvement, shoulder involvement is also marked. The studies have been shown this. In this study, we’ve attempted to calculate the altered mobility of cervical and shoulder joint. Every movement at the cervical spine and shoulder joint is measured and treatment is given to

the patient to assess the effects on mobility of both joint. Values got recorded without any gender discrimination and the majority of the patient were females as RA is a female dominant disease.

25 samples were taken with the age ranging from 41 to 70 years. The mean age was 52.72 with SD 7.329. Data of cervical and shoulder ROM were collected before the treatment starts and the data was recorded as "pre-data values". Post-treatment assessment was done to find the effect of treatment on ROM.

In the dominant shoulder joint, the most affected movement found was the shoulder adduction with the mean ROM of 42.08° with 70.13% ROM available i.e 29.87% ROM was reduced which is quite significant followed by Internal Rotation with total 23.8% loss of movement. Reduction in a movement like External rotation, extension, abduction and flexion were found as follows 22.76%, 17.54%, 11.73%, 10.98%. After the treatment session, there was a significant improvement in range of motion in all movements. The most significant improvement was seen in adduction of 10.16° that 16.93% of improvement. The other movements like internal rotation, external rotation, abduction and extension were improved by 11.71%, 8.84% and 4.87%. The least improvement was seen in flexion ie 4.22% but flexion was one of the least hampered movement.

In Non-dominant hand, the movements were seen as little reduced as it is less used or less functional arm when compared to the dominant arm so it is expected to have different as compared to the dominant arm. The pattern of reduction in movements was the same. As in the dominant arm, the most affected movement was the abduction and the sequence of reduction of ROM was the same as in the dominant hand. The recovery pattern was quite similar as of dominant hand.

1. **Shoulder range of motion**

Table No: 1 : Dominant arm

dominant arm	**Average of available ROM**		**Normal - α**		**% of α**		**100-γ**		
	(α)°		**(β) °**		**(γ)**		**(δ) °**		
movement	pre	post	pre	post	pre	post	pre (**δ** 1)	post (**δ** 2)	**δ**1 - **δ**2
flexion	160.24	167.84	19.76	12.16	89.02%	93.24%	10.98%	6.76%	4.22%
extension	49.48	52.4	10.32	7.6	82.46%	87.33%	17.54%	12.67%	4.87%
abduction	141.24	149.56	18.76	10.44	88.27%	93.47%	11.73%	6.53%	5.20%
adduction	42.08	52.24	17.94	7.76	70.13%	87.06%	29.87%	12.94%	16.93%
internal rotation	53.32	61.52	16.68	8.48	76.17%	87.88%	23.83%	12.12%	11.71%
External rotation	69.52	77.48	20.48	12.52	77.24%	86.08%	22.76%	13.92%	8.84%

Table No : 2: Non-dominant arm.

non-dominant arm	Average of available ROM		Normal - α		% of α		100-γ		
	(α)°		(β) °		(γ)		(δ) °		
movement	pre	post	pre	post	pre	post	Pre (δ_1)	post (δ_2)	δ 1 - δ2
flexion	159.2	166.28	20.8	13.72	88.44%	92.37%	11.56%	7.63%	3.93%
extension	48.08	51.04	11.2	8.96	80.13%	85.66%	19.87%	14.34%	5.53%
abduction	139.04	147.88	20.96	12.12	86.90%	92.42%	13.10%	7.58%	5.52%
adduction	40.32	50.72	19.68	9.28	67.20%	84.53%	32.08%	15.47%	16.61%
internal rotation	52.2	60.36	17.8	9.64	74.57%	86.22%	25.43%	13.78%	11.65%
External rotation	69.48	76.44	20.52	13.56	77.70%	84.93%	22.30%	15.07%	7.23%

α = available range of motion in degrees.

β = difference between the normal and available range of motion i.e reduced range of motion in degrees.

γ = % of the available range of motion (to indicate how much % of ROM is available).

δ = % of reduced ROM.

$\delta_1 - \delta_2$ = percentage of improved ROM.

Table No : 3: Pre and post-treatment analysis of both shoulders

Shoulder joint	dominant		non-dominant	
movement	pre	post	pre	post
flexion	160.24	167.84	159.2	166.28
Extension	49.48	52.4	48.08	51.04
Abduction	141.24	149.56	139.04	147.88
Adduction	42.08	52.24	40.32	50.72
Internal rotation	53.32	61.52	52.2	60.36
External rotation	69.52	77.48	69.48	76.44

The study indicates that there was marked reduction in ROM at shoulder joint in both dominant and Non-dominant arm but the improvement was seen in all movements. The paired t-test was done for every movement between pre-treatment ROM and post-treatment ROM. we calculated the mean and SD. P-value has been found. 2 tailed P value was calculated and P>0.0001 was extremely significant. P-value >0.05 suggested that the data passed the normality test for all movements except extension. The following table shows the mean and SD for all movements before treatment and after treatment.

In the cervical spine, the most affected movement was right lateral flexion with mean available ROM of 40.64° that is 73.89% of available ROM and total reduction of 26.11%. The percentage reduction in movements in descending order as follows left lateral flexion, left rotation, right rotation, flexion and extension with 25.46%, 24.98%, 24.85%, 18.88%, 18.15%. The marked improvement was the scene in the ROM post-treatment right lateral flexion. The post-treatment mean ROM for right lateral flexion was 6.4° which is total 11.81% of total ROM. Other movements like left lateral flexion, right rotation left rotation improved significantly. The following chart shows all value for the cervical spine.

2. **Cervical range of motion**

Table no: 4: Cervical spine

Cervical spine	**Average of available ROM**		**Normal - α**		**% of β**		**100-γ**		
	(α)°		**(β) °**		**(γ)**		**(δ) °**		
movement	pre	post	pre	post	pre	post	pre (δ_2)	post (δ_2)	**δ** 1 - **δ** 2
flexion	68.96	74.96	16.04	10.04	81.12%	88.18%	18.88%	11.82%	7.06%
extension	65.48	70.6	14.52	9.4	81.85%	88.25%	18.15%	11.75%	6.30%
L .rotation	67.52	73.24	22.48	16.76	75.02%	81.37%	24.98%	18.63%	6.35%
Right rotation	67.64	74.96	22.36	15.04	75.15%	83.28%	24.85%	16.72%	8.13%
L. Lateral Flexion	41	46.68	14	8.32	74.54%	84.87%	25.46%	15.13%	10.33%
R. Lateral flexion	40.64	47.04	14.36	7.96	73.89%	85.70%	26.11%	14.30%	11.81%

α = available range of motion in degrees.

β = difference between the normal and available range of motion i.e reduced range of motion in degrees.

γ = % of the available range of motion (to indicate how much % of ROM is available).

δ = % of improved ROM.

$\delta_1 - \delta_2$ = percentage of improved ROM.

3. **Pain assessment for shoulder joint** :

The pain assessment was done by visual analogue scale (VAS). VAS scores at rest and on activity were taken for both shoulders. The average VAS score at rest for dominant shoulder and non-dominant shoulder was 1.64 and 1.72 respectively. The non-dominant shoulder has scored more VAS score than that of the dominant shoulder. The reason may be because pain relives on

movement and dominant arm is busy arm at most of the work. The pattern of pain follows the pattern of reduced movement. The greater the affected ROM, the greater the pain. Adduction was the most affected movement in both dominant and non-dominant arm hence the pain was more significant ie 3.56 for non-dominant arm and 3.52 for dominant arm followed by the internal rotation, external rotation, abduction, extension and flexion. The post-treatment effect was significant on both shoulders. Adduction was the most improved movement in terms of pain ie 1.72 and 1.80 for dominant and non-dominant shoulder followed by an external rotation, internal rotation, abduction, extension and flexion. Hence the overall improvement was seen in the shoulder joint.

Table No : 5: Visual analogue scale score for shoulder joints

Shoulder joint	VAS dominant			VAS non-dominant		
movement	pre (P1)	post (P2)	P1-P2	pre (Y1)	post (Y2)	Y1 -Y 2
at rest	1.64	0.76	0.88	1.72	0.84	0.88
flexion	2.32	1.24	1.08	2.36	1.28	1.08
extension	2.44	1.28	1.6	2.52	1.36	1.16
abduction	2.56	1.48	1.08	2.64	1.44	1.2
adduction	3.52	1.8	1.72	3.56	1.76	1.8
internal rotation	2.76	1.52	1.24	2.8	1.6	1.2
external rotation	2.6	1.48	1.12	2.68	1.4	1.28

4. **Pain assessment for the cervical spine:**

The VAS score was taken at rest and on activity for the neck. The pain wasn't significant at rest but it was significant for movements. The pain was more significant right lateral flexion that is 3.68 on visual analogue scale followed by a left rotation, right rotation, left lateral flexion, extension and flexion. The significant improvement was a scene in all movements but the marked improvement was seen in left rotation ie. 2.08 on VAS scale. cervical pain follows a significant pattern of movement. The most affected movement shows maximum pain score o VAS. There wasn't any significance between age and pain score on VAS. Age factor wasn't significant in the improvement of pain after treatment. The following chart shows the VAS score for the neck.

Table No: 6: Visual analogue scale for neck

movement	pre (P1)	Post (P2)	P1-P2
at rest	1.88	0.92	0.96
flexion	3	1.36	1.64
extension	3.2	1.44	1.76
left rotation	3.6	1.52	2.08
right rotation	3.48	1.6	1.88
left lateral flexion	3.4	1.72	1.68
right lateral flexion	3.68	1.64	2.04

To compare the pre and post-treatment effect the paired t-test was done. P-value >0.05 passed the normality test and p>0.001 considered extremely significant. All movements except the cervical extension and left lateral flexion were failed to pass the normality test. The overall difference was extremely significant. following table shows the mean and SD for each cervical movement for pre and post-treatment ROM data.

After the paired t-test. the age-wise comparison for shoulder movements and cervical spine was done. The patients were categorized under the group of 41-50, 51-60, 61-70. The no. of patients were different from each other.

Age group 41-50 represented 11 patients, group 51-60 had 10 patients and 3rd group of age 61-70 consist of 4 patients. The pre and post data were compared to each other with respect to the age group by ANOVA test. P-value was recorded by comparing SD and means. There wasn't any significant relationship between increasing age and hypomobility and post-treatment effects. They were unrelated to each other for both shoulder and cervical region.

The study suggests that there is a significant reduction in the range of motion at the shoulder joint and cervical spine. but the post-treatment analysis suggested that there is a marked improvement in range of motion. but there is no significant relationship between the age and reduced mobility and also the improvement shown by the patient.

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Changes in Intra Ocular Pressure after Cataract Surgery

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Abstract

Background: Rise in Intraocular Pressure (IOP) is the major contributing factor to the pathology of glaucoma. Along with cataract, it is one of the leading causes of preventable blindness all over the world. Cataract surgery can help reduce the IOP and our study helps confirm the same.

Methods: Our study included 150 eyes of 150 patients undergoing cataract surgery by phacoemulsification by a single surgeon. Complete ocular examination was performed on all the patients which included uncorrected distance visual acuity (UCVA) and best corrected visual acuity (BCVA) by Snellen's chart, anterior segment by slit-lamp, IOP by Goldmann's Applanation Tonometry (AT), gonioscopy by 4 mirror gonioscope, Anterior Chamber Depth (ACD) and Axial Length (AL) by A-scan. IOP measurements were taken between 9am and 10am.

Conclusion: Our study divided the patients into 3 groups based on IOP i.e., <15, 15-19, and 19-23 mmHg of IOP. All the 3 groups showed a lesser IOP post cataract surgery. There was a significant decrease in IOP when baseline was compared with post op week 1, week 4 and week 12. Therefore our study confirms that cataract surgery with PCIOL causes reduction in IOP which remains sustained for months.

Keywords: *Cataract, Phacoemulsification, Intra Ocular Pressure*

Introduction

Cataract and Glaucoma are the two leading causes of preventable blindness in the world. According to the World Health Organization (WHO), cataract is the leading cause of blindness all over the world, responsible for 47.8% of blindness and accounting for 17.7 million blind people. (1) (2) Up to 80% of bilateral blindness in India can be attributed to cataract, which is responsible for much of the social and economic burden in our country. (3) (4) Cataract and Glaucoma are known co-morbidities. Several physiological or sporadic factors or even daily activities can contribute to increase in Intra Ocular Pressure (IOP) and thereby the disease pathology. (5) (6) (7) (8) The progression of the disease can be prevented by two major factors –

1. Preventing major changes in the diurnal variation of IOP
2. Good setting of the target IOP (9)

The accuracy of IOP on measurement with an applanation tonometer (AT) can be affected by the Central Corneal Thickness (CCT). (10) (11)

A thorough examination before operating for cataract must also include the measurement of Anterior Chamber Depth (ACD) which plays a role in diagnosing the type of glaucoma.

According to several studies, post cataract surgery there is a significant reduction in IOP with or without intraocular lens implantation. Cataract removal causes long term control of IOP along with widening and deepening of anterior chamber angle. (12) (13)

The advent of modern phacoemulsification techniques has played a huge role in rehabilitating patients with loss of

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vision due to cataract. Cataract surgery is one of the most common and the most cost-effective methods and the use of phacoemulsification technique in the treatment of cataract is increasing at a rapid pace worldwide. [14] Modern day surgery produces rapid visual recovery with minimal post-operative side effects. One of the major post-operative complications is changes in IOP.

Several studies have reported the changes in IOP post phacoemulsification with intraocular lens implantation. [15] [16] According to some studies, the pressure changes are in the range of

+1.3mmHg to -2.5mmHg, and they are sharper and more sustained. [17] [18]

The aims of this study were to evaluate the effect of an uncomplicated phacoemulsification on IOP in normal population with cataract who are fit for the surgery.

Materials and Methods

This was an observational and prospective cohort study conducted in the Department of Ophthalmology in Dhiraj Hospital, a tertiary care hospital.

Our study enrolled 150 eyes of 150 patients undergoing cataract surgery by phacoemulsification by a single surgeon. All the patients of age more than 40 years with the angle grade 3 or 4 using Schaffer's grading criteria were included.

All the patients with history of medications like steroids or anti-glaucoma drugs, patients having undergone trabeculectomy surgery before/after enrolment and all the known cases of glaucoma were excluded.

All the participants gave informed consent before the study. The study was reviewed and approved by Institutional Ethics Committee and also adhered to the tenets of Declaration of Helsinki.

Complete ocular examination was performed on all the patients which included uncorrected distance visual acuity (UCVA) and best corrected visual acuity (BCVA) by Snellen's chart, anterior segment by slit-lamp, IOP by Goldmann's Applanation Tonometry (AT), gonioscopy by 4 mirror gonioscope, Anterior Chamber Depth (ACD) and Axial Length (AL) by A-scan. IOP measurements were taken between 9am and 10am.

Statistical Analysis

Mean and standard deviation were calculated and the student's unpaired 't' test was used for the comparison between two groups whereas repeated measured ANOVA was applied for comparison between more than two groups. When ANOVA was applied, Bonferroni Post Hoc multiple comparison was done to know the one to one relation.

Result and Discussion

Cataract surgery has evolved in a colossal way from Aphakia to Femtosecond Laser Assisted Cataract Surgery (FLACS), still phacoemulsification remains the most common technique of choice in the treatment of cataract. Very wide variations have been reported in IOP fluctuations post cataract surgery and a lot of research work has been conducted to establish relationship between the same. [19][20][21][22][23] [24] Our study was conducted to find out if uncomplicated phacoemulsification could be an alternative for IOP control.

Several mechanisms for the reduction of IOP after uneventful phacoemulsification have been proposed. These may be enhanced outflow facility by stretching of the trabecular meshwork, a direct effect of the IOL on the ciliary body or via- capsular bag contraction in reducing aqueous humor formation, ciliary body traction via the zonules preventing collapse of Schlemm's canal, widening of the anterior chamber angle and biochemical or blood-aqueous barrier permeability alterations. Some postulate increase in prostaglandin F2 levels post operatively leading to increased uveo-scleral outflow.

Out of 150 eyes operated in this study, 64 were right eyes and 86 were left eyes. The total mean age of 150 patients enrolled in this study was 60.26±10.86 out of which mean age of 80 (52.10%) male patients was 61.45±11.79 and mean age of 70 (47.90%) female patients was 58.90±9.51. There was no gender predilection in our study.

Mansberger et al. in 2012 found that in the cataract group, postoperative IOP was significantly lower than the preoperative IOP (19.8±3.2 mmHg vs. 23.9±3.2 mmHg; P=0.001) for at least 36 months. [25] The average decrease in postoperative IOP from preoperative IOP was 16.5%. A greater reduction in postoperative IOP occurred in the eyes with the highest preoperative IOP. In our study there was 11.4% decrease in IOP when compared post op week 12 with baseline IOP, which is in accordance with the above

study.

Shingleton et al. in 2006 compared the Non glaucomatous group (NG) (59 patients) at 3 years follow-up NG 1.7±3.1mmHg (P=0.0005). [15] At the final follow-up visit (mean near 5 y for all groups) the IOP was significantly decreased in NG 1.5±2.5mmHg (P<0.0001). In another study

by Issa et al. in 2005, IOP dropped by a mean of 2.55±1.78mm Hg post operatively. [26] Results from our study indicate a reduction of IOP by 1.69±2.313 from pre-operative IOP measurement which is in accordance to above studies.

Tong et al. in 1998 showed that the mean pre-operative IOP of the eyes operated on was 15.8 mm Hg ± 0.2 (±SE) (range 8 to 28 mm Hg). [27] One week postoperatively, the mean lOP dropped by 6.8% to 14.5 ± 0.2 mm Hg *(P* <.001) Six weeks postoperatively, mean lOP was down by 14.6% to 13.3±0.2 mm Hg *(P* <.001). Six to 8 months after surgery, it was down by 12.2% to 13.6±0.2 mm Hg *(P* <.001). In our study, mean IOP drop at post op week 1 was 14.22 ± 2.346 (down by 7%) and at 1 months was 13.70 ± 2.185 mmHg (down by 10.39%) and at 3 months was 13.60±2.210 which is in accordance to the above study. (Table No. 1)

In the present study we have divided patients into three groups based on IOP i.e., <15, 15-19, and 19-23 mmHg of IOP. At Baseline, 34.00% patients had less than 15mmHg of IOP compared to 66.67% at post op week 12. At Baseline, 59.33% patient had less than 15-19mmHG of IOP compared to 32.67% at post op week 12. At Baseline, 6.67% patient had less than 19-23mmHG of IOP compared to 0.67% at post op week 12. (Chart 2)

Intergroup comparison was also done to determine change in IOP among different post- operative follow ups. Significant change was noted in decrease in IOP when baseline was compared with post op week 1, week 4 and week 12 (p = 0.000). In that we found that there was no significant change in IOP levels between Baseline IOP & and Post op day 1. Similarly there was

no significant change in Post op -Week 4 & Week 12, whereas we found significant change in IOP from Baseline to Post op Week 1, Week 4, Week 12. (Table No. 2)

Transient IOP rise on post op day 1 can be attributed to either retained viscoelastic material, cortical matter or pigment dispersion from iris chafing. [15][27]

We also compared changes in IOP with different cataract grading and there is a steady decline in IOP with each cataract grading. However, our data propose no correlation between cataract grading and decrease in IOP. But there was higher decline in IOP in eyes with higher preoperative IOP which was in accordance of the above quoted study. [25]

We have also compared ACD of operated eyes. The mean pre-op Anterior chamber depth of eyes operated were 2.80±0.449 whereas post-op depth were 4.03±0.180 which was statistically significant (p=0.000). (Table No. 3) The mean increase in ACD of 150 eyes was 1.23±0.314. Issa et al. in 2005 demonstrated that the ACD increased by a mean (SD) of 1.10 (0.44) mm (p=0.00001). [26]

SHIN et al in 2010, compared intraocular pressure, axial length (AL) and anterior chamber depth (ACD) between patients with narrow occludable angles and open angles chosen for cataract surgery. [28] Mean pre op ACD was 3.08 which increased to 4.06±0.98 at week 4 and 4.07±0.98 at week 12. The mean increase in ACD 12 weeks postoperatively was statistically significantly greater in the occludable angle group than in the open-angle group (P<0.05). In our study we have excluded patients with occludable angles but the findings of open angle are in accordance with our study. We measured ACD at post op week 12 which was 4.03±0.180.

We compared Axial Lengths of operated eyes with baseline and post op IOP changes. (Chart No.

3) We found that most of the AL ranging from 21.00 to 24.99 showed significant change in IOP (p=0.000). Groups 5 and 6 were excluded due to less number of patients. Higher AL values could not be compared as there were no patients who were fulfilling our inclusion criterions. Lower AL were excluded because of narrow angles on gonioscopy as per exclusion criterion. We concluded that there is no significant lowering of IOP after intergroup comparison on the basis of AL.

Tuula et al in 2001 compared AL of operated eyes with changes in IOP and found no statistically significant correlations. [29] Not many studies have been conducted in this aspect.

Conclusion

Our study confirms Cataract surgery (phaco) with PCIOL causes reduction in IOP which remains sustained for months,

as the duration of study was 3 months only. Though anterior chamber angle anatomy, lens vault, lens thickness have been the technical limitation of our study are the strong predictors expected for the post-operative reduction in IOP; yet, elucidation of such factors has been suboptimal to date. The mechanism that leads to IOP reduction following cataract surgery in patients with open angles remains poorly understood, further research will likely allow a better understanding of these postoperative changes.

TABLE 1: MEAN INTRAOCULAR PRESSURE OF 150 OPERATED EYES

IOP	N	Mean	SD	p value
Baseline IOP	150	15.29	2.417	0.000
IOP at Post op Day 1	150	15.41	2.824	
IOP at Post op Week 1	150	14.22	2.346	
IOP at Post op Week 4	150	13.70	2.185	
IOP at Post op Week 12	150	13.60	2.210	

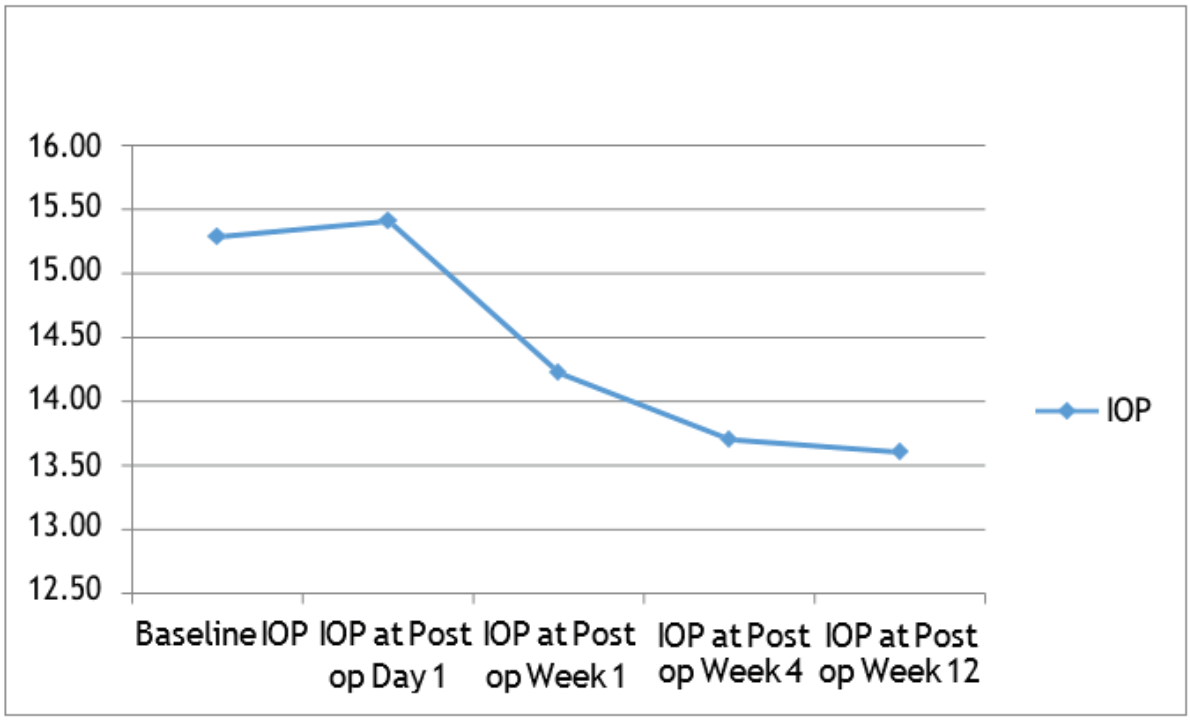


CHART 1: LINE CHART MEAN INTRAOCULAR PRESSURE OF 150 OPERATED EYES MEAN INTRAOCULAR PRESSURE OF 150 OPERATED EYES

The mean intraocular pressure was calculated at baseline (i.e., pre-operative), post op day 1, post op week 1, post op week 4 & post of week 12.

TABLE 2: AN INTERGROUP COMPARISION OF MEAN IOP FROM BASELINE TO POST OP WEEK 12

IOP		SE	p value	95% Confidence Interval for Difference	
				Lower Bound	Upper Bound
Baseline	Post op Day 1	0.170	1.000	-0.604	0.364
	Post op Week 1	0.143	0.000	0.660	1.473
	Post op Week 4	0.165	0.000	1.117	2.057
	Post Week 12	0.173	0.000	1.195	2.179
Post op Day 1	Baseline	0.170	1.000	-0.364	0.604
	Post op Week 1	0.126	0.000	0.829	1.545
	Post op Week 4	0.154	0.000	1.268	2.145
	Post op Week 12	0.175	0.000	1.308	2.305
Post op Week 1	Baseline	0.143	0.000	-1.473	-0.660
	Post op Day 1	0.126	0.000	-1.545	-0.829
	Post op Week 4	0.103	0.000	0.226	0.814
	Post op Week 12	0.112	0.000	0.300	0.940
Post op Week 4	Baseline	0.165	0.000	-2.057	-1.117
	Post op Day 1	0.154	0.000	-2.145	-1.268
	Post op Week 1	0.103	0.000	-0.814	-0.226
	Post op Week 12	0.073	1.000	-0.108	0.308
	Baseline	0.173	0.000	-2.179	-1.195
Post op Week 12	Post op Day 1	0.175	0.000	-2.305	-1.308
	Post op Week 1	0.112	0.000	-0.940	-0.300
	Post op Week 4	0.073	1.000	-0.308	0.108

On the basis of Bonferroni post hoc test intergroup comparison of changes in IOP from baseline to post-operative groups was performed.

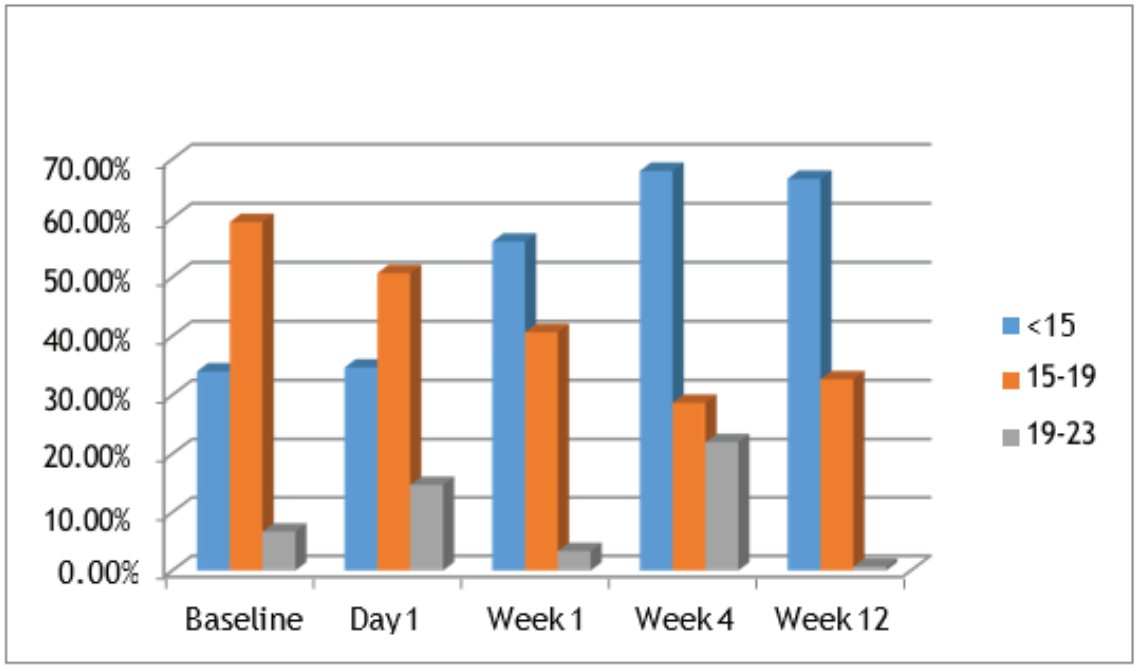


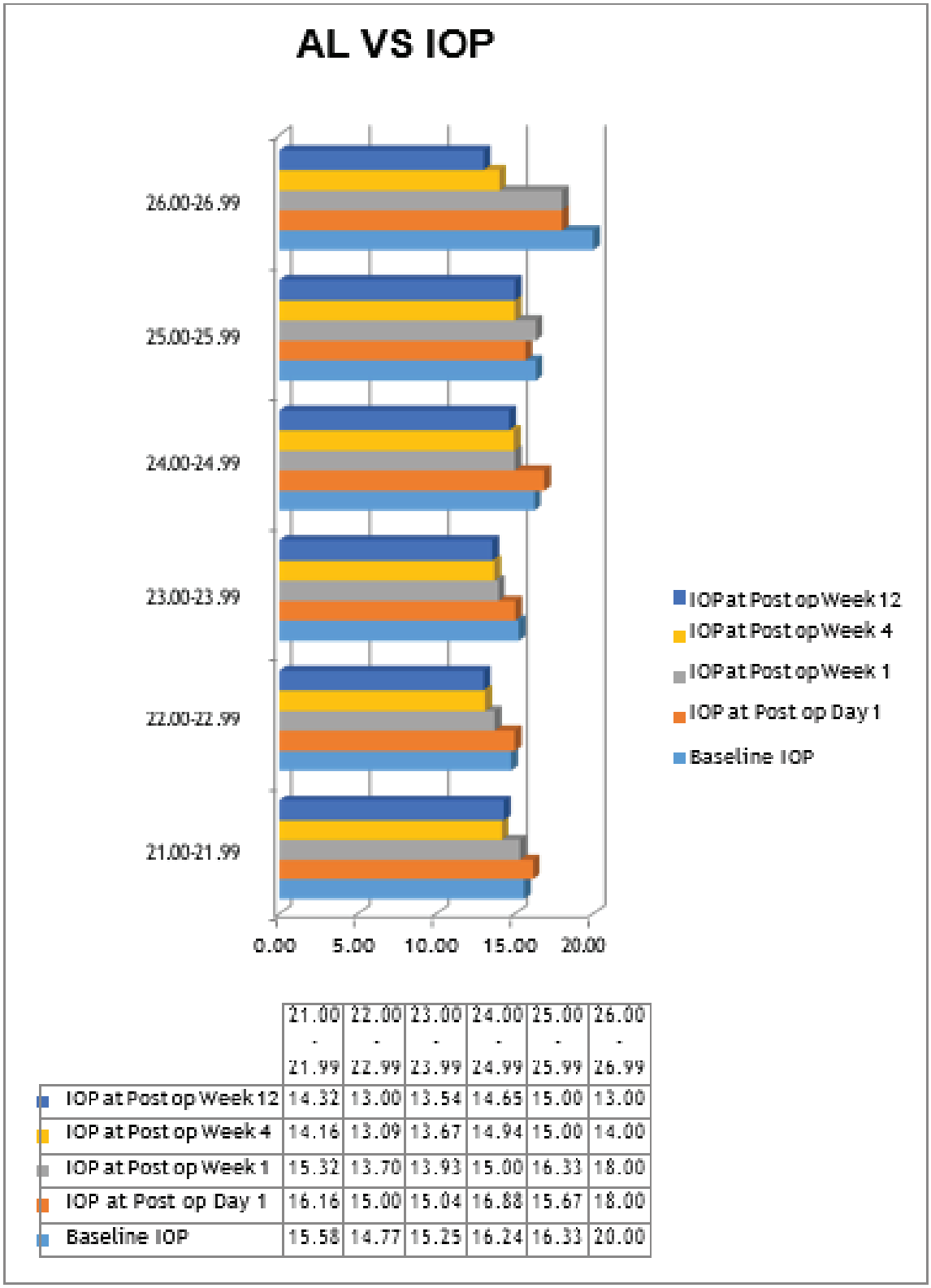
CHART 2: COMPARISION OF FREQUENCY DISTRIBUTION OF PATIENTS (N) AMONG 3 IOP GROUPS FROM BASELINE TO POST OP WEEK 12

TABLE 3: MEAN OF PRE-OP & POST OP ANTERIOR CHAMBER DEPTH OF 150 PATIENTS

ACD	Mean	SD	p value
Pre OP	2.80	0.449	0.000
Post OP	4.03	0.180	

In this study mean PRE-OP Anterior chamber depth of eye selected for cataract surgery is 2.80± 0.449 whereas POST-OP after cataract surgery was 4.03±0.180 which wasstatistically significant (p=0.00).

	21.00	22.00	23.00	24.00	25.00	26.00
	-	-	-	-	-	-
	21.99	22.99	23.99	24.99	25.99	26.99
IOP at Post op Week 12	14.32	13.00	13.54	14.65	15.00	13.00
IOP at Post op Week 4	14.16	13.09	13.67	14.94	15.00	14.00
IOP at Post op Week 1	15.32	13.70	13.93	15.00	16.33	18.00
IOP at Post op Day 1	16.16	15.00	15.04	16.88	15.67	18.00
Baseline IOP	15.58	14.77	15.25	16.24	16.33	20.00



	21.00 - 21.99	22.00 - 22.99	23.00 - 23.99	24.00 - 24.99	25.00 - 25.99	26.00 - 26.99
IOP at Post op Week 12	14.32	13.00	13.54	14.65	15.00	13.00
IOP at Post op Week 4	14.16	13.09	13.67	14.94	15.00	14.00
IOP at Post op Week 1	15.32	13.70	13.93	15.00	16.33	18.00
IOP at Post op Day 1	16.16	15.00	15.04	16.88	15.67	18.00
Baseline IOP	15.58	14.77	15.25	16.24	16.33	20.00

CHART 3: CHANGE IN IOP IN RELATION TO AXIAL LENGTH (AL)

Source of Funding - Self

Conflict of Interest - Nil

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Trends of Fatal Poisoning in Jhalawar Region of Rajasthan: A 5 - Year Retrospective Study

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Abstract

India is predominantly an agriculture based economy. Problem of poisoning is one of the important causes of mortality and morbidity in India and other agriculture based developing countries due to easy availability, low cost and no restriction on sale and supply of insecticidal compounds. The aim of this study is to find out the association of episodes of fatal poisoning with various factors like age, gender, area of residence, religion, occupation type of poison and manner of death. This retrospective study was carried out on total poisoning cases brought to mortuary of Jhalawar Medical College and associated group of hospitals, Jhalawar (Rajasthan) in 5 year duration from 1st January 2014 to 31st December 2018. In this study maximum cases of poisoning were of age group 21 - 30 year, incidence were more among males belonged to rural population who were involved in agricultural work. Majority of poisoning cases were due to Organophosphorus compounds.

Key words *:- Poisoning, Jhalawar, Fatal, Agrochemicals.*

Introduction

Every unnatural death represents a tragic waste of human life and resources whether accidental, suicidal or homicidal. Death due to poisoning is no exception. Poison has been one of the major cause of unnatural death since the beginning of civilization.[1] By definition poison is a substance (solid, liquid or gaseous), which if introduced in living body, or brought into contact with any part thereof , will produce ill-health or death, by its constitutional or local effects or both.[2] There is a substantial growth in the sector of industries, medicine and agriculture. So that a significant number of new compounds have appeared as new poisonous substance.

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The exact incidence of this problem is uncertain but as per WHO three million acute poisoning cases with 2,20,000 deaths occur annually worldwide particularly among agricultural workers. Of these, 90% of fatal poisoning occur in developing countries. This figure could be just the tip of the iceberg since most cases of poisoning actually go unreported, especially in the third world countries.[3] The exact incidence of poisoning in India is uncertain due to lack of data at central level as most cases are not reported, also mortality data is a poor indicator of incidental of poisoning. It has been estimated that 5-6 per cent per lakh population die due to poisoning every year[2]. Acute poisoning forms one of the most common cause of emergency hospital admissions. The poisoning is the second most common mode of suicide after hanging and the incidence of suicide by poisoning in the year 2012 were 29.1% of the total number of suicides.[4] Developing countries such as India & Sri Lanka have reported alarming rates of toxicity and death due to poisoning. In the US death due to poisoning are more than 775 per year.

In early 19th century arsenic poisoning was prime status in India, then the trends shifted to opium and

barbiturate poisoning. Presently organophosphorus poisoning is said to be commonest. The incidence of poisoning is increasing day by day because of its low cost , easy availability and insidious nature. Pattern of poisoning depends upon variety of factors and vary from case to case , region to region, availability and access to poisoning, popularity, socioeconomic status of individual, knowledge of local population about the poisonous properties of the agents , psychological factors, religious & cultural influences which changes over period of time[6]. Knowing the pattern of poisoning in a particular region helps not only in early diagnosis and treatment but also to take preventive measures. The epidemiology of this study will help in upgradation of the academics and autopsy surgeons will also take the help of this study in difficult or suspected cases. So this study is taken to analyse the pattern of poisoning among age , sex , religion , area and occupation etc .Such type of study has not been conducted in this region so far.

The aim of this study is to know the prevalence of fatal poisoning cases in study period of Jhalawar region of Rajasthan , which will act as useful planning tool for government authorities and planning bodies to plan and execute strategies towards prevention of poisoning incidences.

Material and Method

This study was conducted on all cases of poisoning brought for medico-legal autopsy in the Department of Forensic Medicine and Toxicology, Jhalawar Medical College, Jhalawar during the period January 2014 to December 2018 and collected their chemical analysis reports from regional forensic science laboratories. Total 1699 autopsies were conducted during the study period and out of these 335 were alleged poisoning deaths.

Inclusion Criteria - Cases of alleged history of fatal poisoning and those persons who declared dead on arrival in causality and suspected to be case of poisoning were sent for medico legal autopsies during the said study period .

Exclusion Criteria - Death due natural causes , undetermined causes , snake or insect bite poisoning and cases where datas were incomplete.

Collection of data - All selected cases were studied for data collection and the proforma was filled for each case , the datas were collected from sources such as inqeust reports, statement of relatives and police, autopsy reports and chemical analysis reports.

Table 1. Age and sex wise distribution of poisoning cases

Age(Yrs)	Male	Female	Total
0-10	1(0.29%)	2(0.59%)	3(0.88%)
11-20	23(6.86%)	33(9.75%)	56 (16.61%)
21-30	85(25.37%)	37(11.04%)	122(36.41%)
31-40	60(17.91%)	15(4.77%)	75 (22.68%)
41-50	31(9.15%)	11(3.27%)	42(12.42%)
51-60	18(5.37%)	4(1.19%)	22 (6.56%)
61-70	10(2.98%)	3(0.88)	13(3.86%)
71-80	1(0.29%)	0	1(0.29%)
>80	1(0.29%)	0	1(0.29%)
Total	230(68.65%)	105(31.35%)	335(100%)

Table 2. Religion wise distribution of deaths due to poisoning

Religion	No. of cases
Hindu	326(97.32%)
Muslim	07(2.09%)
Others	02(0.59%)
Total	335(100%)

Table 3: Area wise distribution of deaths due to poisoning

Area	Total
Rural	262(78.2%)
Urban	73(21.8%)
Total	335(100%)

Table 4: Occupation wise distribution

Occupation	No. of cases
Farmer/Agriculture worker	108(32.24%)
Laborer	84(25.07%)
Student	74(22.09%)
Housewife	42(12.54%)
Self Employed	27(8.06%)
Total	335(100%)

Table 5: Route of poison consumption

Route	No. of cases
Oral	211(62.99%)
Inhalation	123(36.72%)
Injection	01(0.29%)
Total	335(100%)

Table 6: Manner of poisoning and sex

Manner of poisoning	Male	Female	Total
Accidental	85(25.37%)	69(20.60%)	154(45.97%)
Suicidal	143(42.69%)	36(10.75%)	179(53.44%)
Homicidal	02 (0.59%)	0	02 (0.59%)
Total	230(68.65%)	105(31.35%)	335(100%)

Table 7: Type of poison and sex wise distribution

Type of poison	Male	Female	Total
OP*	96(28.65%)	40 (11.97%)	136(40.61%)
OL**	56(16.71%)	27(8.06%)	83(24.77%)
Celphos	48 (14.32%)	25 (7.46%)	73(21.79%)
Other	30(8.95%)	13 (3.88%)	43(12.83%)
Total	230(68.65%)	105(31.35%)	335(100%)

* Organophosphorus , ** Organochlorines

Observations

During the 5 year study period i.e. from year 2014 to 2018, a total number of 1699 autopsies were carried out at Jhalawar Medical College, Jhalawar, Rajasthan, out of which 335 cases (19.71 % of total) were poisoning cases.

In males, maximum number of poisoning occurred in age group of 21 to 30 years accounting to 25.37% and age group of 21-30 years accounting to 11.04% in females. The least affected age group was more than 70 years age group (Table-1).

In the present study maximum poisoning cases were seen among Hindu religion (97.32%) as compared to Muslim (2.09%).(Table-2)

The incidences of poisoning were more common in rural area (78.20%) as compared to urban (21.80%). (Table-3)

It is observed that the fatal cases of poisoning were seen more amongst the people engaged in agriculture works (32.24%) followed by labor class (25.07%) and students (22.1%).(Table-4)

In maximum number of poisoning cases, the route of poison administration was more by oral route accounting to 62.99% as compare to inhalation 36.72%.(Table-5)

According to the present study, the most common type of manner of poisoning was found to be suicidal (53.44%) then accidental (45.97%). (Table-6)

Organophosphorus was the most common poisonous substance consumed, found in 40.61% followed by organochlorines in 24.77% cases, Celphos in 21.79% cases and other poisons 12.83%. (Table -7)

Discussion

In the present study, 21-30 years of age group is showing maximum incidence of poisoning reports (36.41%) which is similar with other reasearcher's observation, [11, 13, 14, 16, 17, 18] which can be explained by the fact that the people of this young age group face stress of modern lifestyle, failure or less percentage in the exams, failure in love, scolding from parents or teachers, loss in business, family problems etc. When these problems and tensions become unbearable, ending one's life seems to be only solution for them.

In this study, males (68.51%) showed higher incidence of poisoning than the females (31.34%). On comparison with other authors, [7, 9, 13, 14, 16, 17, 18, 19] our study showed similarity. Though all the studies conducted in different parts of India, male predominance was a common and constant feature in all, which suggests that males are more active in various social activities and customs and hence they are vulnerable from stress and tension.

In this study rural population (78.20%) were more affected than urban population (21.80%) which is similar to other author's findings [5, 14, 16, 18, 19] This is because of demographic situation of the hospital and Jhalawar is rich in agricultural land farmers who are always at the risk of exposure of poisonus compounds but this finding is showing contradiction from the study of other.[17]

In the present study the highest poisoning cases were reported among agricultural workers (32.24%) and lowest number are seen in self-employed (8.06%). Agricultural workers solely depend on agricultural income for their livelihood. and due to some reasons (i.e. either lack of water and flood) if they are not able to generate the required income for their day to day living and commitments, they may get frustrated and result in suicide by their agricultural pesticides, insecticides or weed killer etc.[12,19] Lack of education and knowledge about uses of various pesticides / insecticides these workers may accidently expose to poisonous compounds.

In this study we observed that most of victims were from Hindu religion (97.32%) which can be explained by the fact that major population is Hindu in this Jhalawar region. In our study as well as most of other studies Hindus are more common than others religion.

In this study route of administration of poisoning was maximum (62.99%) by oral route, this is due to easy and convenient method of administration of poison, which is similar with most of the other studies.[8, 16, 19]

In the present study incidence of suicidal cases (53.44%) were highest followed by accidental (45.97%) and lowest homicides (0.59%). This may be because of reasons like economic crisis, examination failure, love failure , quarrels , unemployment and chronic illness. Our findings are consistent with a study of various authors. [8, 12, 14, 16, 17, 19]

In the present study maximum cases were of organophosphorus (40.61%) followed by organochlorines (24.77 %) and celphos (21.79%) . This could be explained easily that Jhalawar district is agricultural land, so most of farmers are more exposed to these agrochemical compounds intentionally or unintentionally. [12, 14, 17, 18, 19] The reason for more number of victims to choose organophosphorus were due to low cost , easy availability, no restriction on the sale and supply of these compounds. Poisoning by agrochemicals is practically inevitable because modern farming is unthinkable without the use of these compounds and especially for a developing countries like ours India as dependent on argriculture.

Conclusion

Present study indicates that younger age and males are as higher risk for suicide as well accidental poisoning. Poisoning is an important preventable public health problem especially in the developing countries. Major occupation in Jhalawar region of Rajasthan is farming with the majority of population living in rural areas so the farmers should be educated regarding the appropriate measures which need to be taken in cases of accidental exposure. Reducing deaths from self poisoning require preventive strategies include treating the problem leading to suicidal behaviors. The primary health centres should be upgraded and developed with better infrastructure to provide immediate effective treatment to poisoning specially to manage the cases of organphophorus poisoning in an emergency.

Conflict of Interest - NO

Source of Funding - Nil

Ethical Clearance - Taken from ethical committee of JMC Jhalawar.

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A-b Ridge Count and Its Correlation with Epilepsy

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Abstract

The study of ridges of skin is called dermatoglyphics. It is recognized as a valuable method for medicolegal and anthropological studies. The permanency of the finger patterns, the extreme variability from one individual to the other and easy analysis are some of the reasons for its wide applications in a variety of conditions. Dermatoglyphic patterns are determined by genetic factors. It has been proved that genetic factors are responsible for idiopathic epilepsy. Hence there is a scope for the study of relationship between epilepsy and dermatoglyphic patterns.

Objectives : • To study the A-b ridge count in generalized tonic clonic seizure patients.

• To compare this dermatoglyphic configuration of cases with the normal population.

Methods : The ink method was followed to take finger and palm prints. The palmar prints of 50 epileptic patients and 50 normal individuals of both sexes were collected for the study.

Conclusion: There is no significant association found between a-b ridge count and epilepsy patients when compared to normal.

Key Words: *Dermatoglyphics, a-b ridge count, Epilepsy.*

Introduction

Dermatoglyphics is often claimed to be associated with several diseases like congenital heart disease (Alter and Schllenberg 1970), Schizophrenia (Bramon et al 2005), cancer (Abbasi et al 2006), genetic disorders. (Mukharjee 2007) and diabetes (Kumbani 2007). Abnormality in the genetic configurations of parents is inherited by children and is reflected in the dermatoglyphic pattern.[4] Hence dermatoglyphic study proves to be a very useful, easily applicable, inexpensive, indispensable tool as an indicator in the diagnosis of hereditary diseases in patients. Epilepsy is a chronic brain disturbance of varied and complex origins, characterized by recurrent convulsive seizures, not necessarily presenting a defined external trigger factor. The etiology of the epilepsies allows a classification of syndrome features into two groups – idiopathic or cryptogenic epilepsy, which has isolated primary symptoms without apparent cause and is probably hereditary and finger print configurations are inherited with an embryonic origin common to nervous system. Their attractions indicate pleiotropic effects of the genotype responsible for enaphalographic irregularity and convulsive seizures[3]

Palmar Pattern Configuration : The palm has been divided into several anatomically defined areas for dermatoglyphic analysis in different individuals, such as thenar, hypothenar and interdigital areas. These areas approximate the sites of embryonic volar pad. Thenar and first interdigital area : Thenar and first interdigital areas are considered as one area labelled thenar/ first interdigital (Th/I1). In most of the cases there is no pattern in this area but the ridges follow a mild curve around the base of thumb. Patterns when present are most often loops; whorls are rarely encountered.

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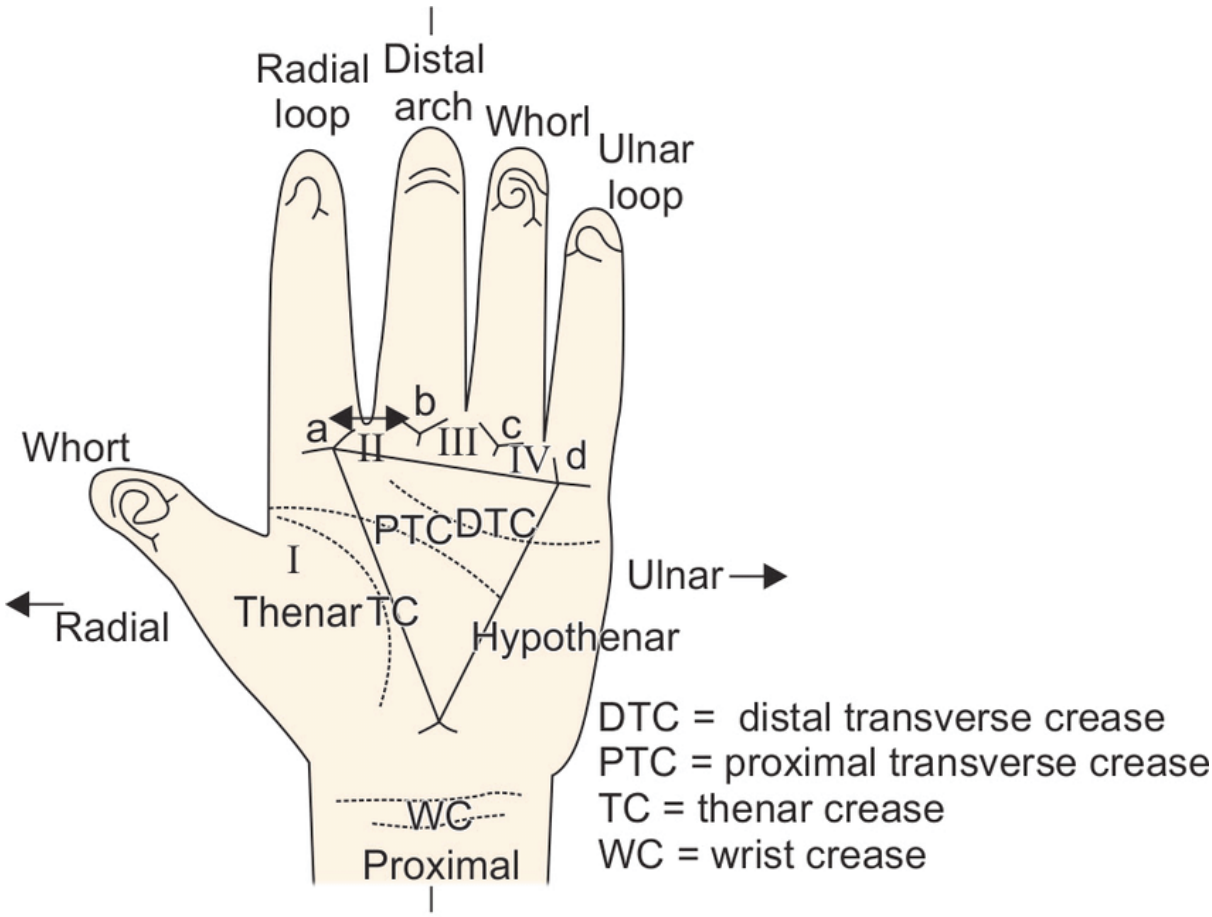
Second, third and fourth interdigital areas : The second, third and fourth interdigital areas are found in the distal palm in the region of heads of metacarpal bones. Each interdigital area is bordered laterally by digital triradii. Digital triradii are labelled a, b, c and d, starting from the triradius located at the base of digit II and moving toward the triradius associated with digit V. The second interdigital area I2 lies between triradii a and b, the third interdigital area I3 between triradii b and c and the fourth interdigital area I4 between triradii c and d. If a digital triradius is absent, the midpoint of the base of corresponding digit can be used to separate interdigital areas. Configurations of an interdigital region may be a true pattern (loop, whorl), a vestige or an open field. They are the most common patterns found in the distal palm.[1,2]

a-b ridge count : It is defined as the number of ridges intersected by a line drawn between 'a' triradius (at the base of index finger) and 'b' triradius (at the base of middle finger) of the palm in each hand.[1]



Method of collection of data : The palmar prints of consecutive patients diagnosed as Generalised tonic clonic seizures were collected for the study.

Inclusion criteria :

• Age between 15 to 60 years.

• Both male and female sex included.

Exclusion criteria :

• Patients associated with other psychiatric illnesses.

• Patients associated with mental retardation.

• Patients with history of trauma and other medical illnesses. Equal number of age and sex matched individuals who have no medical and psychiatric illness, with no past history or family history of seizures served

as controls.

Sample size : 50 cases and 50 controls.

METHODOLOGY : Standard INK method as described by Cummins and Midlo was adopted to take palm prints in present study. The hands of the subjects were cleaned with soap and water and dried. Subsequently the hands were wiped with spirit lightly to remove any greasy particles. Required amount of daub was placed on glass slab. It was uniformly spread by rubber roller to get a thin even ink film on glass slab.

The palm of the individual's right and left hand was inked with the help of rubber roller. The crystal bond paper was rolled forwards on to the wooden roller. The wrist of the palm was kept on the roller and it was rolled backwards by applying pressure on the zone of flexion creases at wrist, ulnar margin, flexion creases in central region of hand and over the knuckles. 40 Thus the print appeared on the crystal bond paper. Soon after the print was taken the sheets were coded with Name, Age and Sex for Cases and Controls, and then subjected to detailed analysis, ridge counting with magnifying lens and sharp needle.

Materials used : 1) Kores quick drying duplicating ink, 2) Rubber roller, 3) Wooden roller 4) Inking slab, 5) Cotton puff and spirit, 6) Scale, 7) Crystal bond paper, 8) Pencil, 9) Protracter,10)Magnifying lens, 11)Soap and towel.(Fig 1)

Fig 1: Materials used

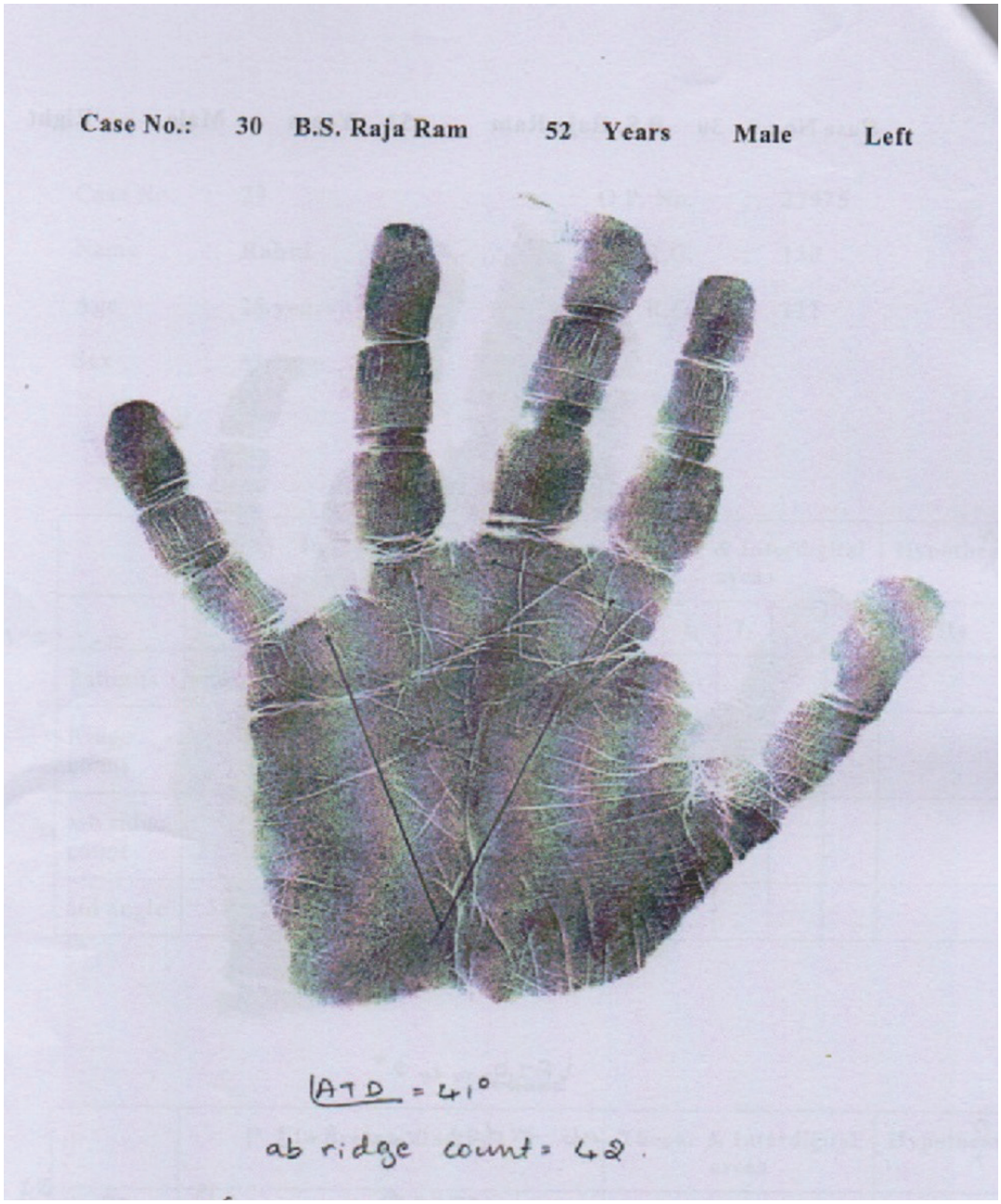


Fig 2: Number of ridges intersected by a line drawn between 'a' triradius (at the base of index finger) and 'b' triradius (at the base of middle finger) of the palm in each hand was measured.

Statistical Analysis

Descriptive statistics were presented as mean and standard deviation for continuous data and number and percentage for categorical data. Z-test / unpaired 't' test was used for comparing the means of two groups (cases Vs controls). Categorical data was anlaysed by chi-squire test and Fisher's exact test wherever necessary. P-value of 0.05 or less was set for statistical analysis.

Results

TABLE NO 1: STATISTICAL CALCULATION OF a-b RIDGE COUNT IN RIGHT AND LEFT HAND OF MALE EPILEPTICS AND CONTROLS

ab ridge count	Males					
	Cases (25)		Controls(22)		t	P
	Mean	SD	Mean	SD		
Right	35.3	4.4	34.0	6.2	0.84	0.41, NS
Left	35.3	7.2	33.2	6.0	1.08	0.29, NS

TABLE NO 2: STATISTICAL CALCULATION OF a-b RIDGE COUNT IN RIGHT AND LEFT HAND OF FEMALE EPILEPTICS AND CONTROLS

ab ridge count	Females					
	Cases (25)		Controls(28)		t	P
	Mean	SD	Mean	SD		
Right	33.8	5.1	35.3	7.3	0.86	0.39, NS
Left	34.2	5.8	34.3	6.2	0.06	0.95, NS

TABLE NO 3: STATISTICAL CALCULATION OF a-b RIDGE COUNT IN RIGHT AND LEFT HAND OF COMBINED SERIES OF MALE AND FEMALE EPILEPTICS AND CONTROLS

ab ridge count	Male+Females					
	Cases (50)		Controls(50)		t	P
	Mean	SD	Mean	SD		
Right	34.6	4.8	34.8	6.8	0.17	0.87, NS
Left	34.7	6.5	33.8	6.1	0.71	0.48, NS

The present study shows decrease mean value in right hands of cases and increase mean value in left hands of cases when compared to controls and difference found in a-b ridge count between epileptics and controls is statistically not significant.

Discussion

Nandlal, R.K. Sureka observed that the mean values of a bridge count for right and left hands in controls were 34.62 and 35.9 respectively, while in case of epilepsy the values were 36.88 and 39.04 respectively. In the present study the mean values of ab ridge count for right and left hands in controls are 34.8 and 33.8 respectively, while in case of epilepsy the values are 34.6 and 34.7 respectively.[17]

P.Ranganath that there was no significant difference in a-b ridge count between patients and controls in male or female.[19]

A study by Schaumann in 197 adult Caucasian males with epilepsy showed decreased a-b ridge count on both left and right palm[24]

Ana Tarca observed a surprising decrease of the distance between triradia "a" and "b" marking the limits of this compartment much under the average values recorded for Romanian populations.[20]

The present study shows mean a-b ridge count is lower in cases than controls on right hand and higher in cases than in controls on left hand. The difference found is statistically not significant.

Conclusion

The present study has been undertaken to find out the existence of any correlation between palmar dermatoglyphic parameter ab ridge count of patients having generalized tonic clonic seizures (GTCS) and normal individuals and the data obtained testify to a certain diagnostical and prognostical value of dermatoglyphical features. These results provide further evidence that dermatoglyphic abnormalities exist and that the a-b ridge count may not be a marker of disruption, probably environmental, that occurs when the brain may also be particularly vulnerable to such insult.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance : Taken from institution.

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Oral Health Status of 12-15 Years Old School Going Children in Lucknow City

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Abstract

Aim: To evaluate oral health status of 12-15 years old school going children in Lucknow.

Material and Method: The study was carried out during January 2019 to August 2019. Total 1200 school going children of age 12 to 15 years were taken in this study. Schools were selected by multistage random sampling technique. There were total 814 schools in the Lucknow, out of which 24 schools were taken in the study area in which 11 were government schools and remaining 13 were private schools. Closed-ended questionnaire consisting of demographic data, three questions of oral hygiene practices and four indices were used. Dental examination was done to assess the dental caries status, oral hygiene status, presence of malocclusion and gingival status. Data was analysed by using SPSS software version 21.

Results: Toothpaste and toothpowder were used as material by 73.2% and 25.3% of the subjects respectively. The maximum DMFT scores was seen among 12 years, then 15 years, then 14 years and least in 13 years. Maximum 13 years old students reported fair oral hygiene index. The comparison of study participants with regard to age of the student was not found to be significant.

Conclusion: To conclude, the results of the present study confirmed that there is increased prevalence of malocclusion among children in the 12 to 15 year age group. Among all the age group, oral hygiene status was observed to be fair.

Keywords: *Dental Caries, Malocclusion, OHI-S, Students*

Introduction

Oral health is an integral part of general health. Dental problems can be avoided if identified at an early stage. School age is regarded as the phase of childhood during which a child acquires the knowledge of the norms and values of a society and emerges as a contributing member to the community. At the age of 12 years permanent teeth (except 3rd molars) would have erupted and by 15 years age these teeth are exposed to the oral environment for almost 3 years. WHO has recommends both these ages as the index ages for oral health assessment.[1]

According to the World Health Organization (WHO) the prevalence of dental caries among school aged children is estimated to be as high as 90% in some countries. For children in particular, poor oral health can have negative impacts on quality of life and academic performance at school. Apart from causing chronic pain and discomfort, untreated dental caries can impact daily activities in terms of play, sleep, eating and school activity.[2] Untreated oral diseases in children frequently lead to serious general health problems, significant pain,

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interference with eating, and lost school time. Poor oral health in childhood often continues into adulthood, affecting economic productivity and quality-of-life.[3]

India, a developing country, faces many challenges in rendering oral health needs. As developing countries have limited resources allocated for oral health services, as for instance in India where less than seven percent of the gross national product is spent on health care, it is anticipated that self-reports can be utilized together with clinical indicators to assess the need for dental care.[4]

Considering the epidemiological triad for the causation of dental caries, there is a need to assess the host factors such as oral health knowledge, oral hygiene practices, dental visits, and eating habits of the children.[5] Some oral hygiene practices recommended for children include twice brushing with the appropriate dentifrice including fluoride containing pastes or gels. Chewing stick and sponge used by some rural homes also contain anti-bacterial substances which reduce activities of the bacteria in the mouth.[6]

Hence the aim of this study was to assess the oral health status of 12-15 years old school going children in Lucknow and to determine the prevalence of dental caries and malocclusion in the population which would help us in planning and implementing necessary preventive measures.

Material and Method

The present study was a descriptive cross sectional survey done to assess the oral health status of 12 to 15 years old school going children in Lucknow City. The study was carried out during January 2019 to August 2019. Total 1200 school going children of age 12 to 15 years were taken in this study. Schools were selected by multistage random sampling technique. Ethical permission was taken from the ethical committee of the college before the commencement of the study. There were total 814 schools in the Lucknow, out of which 25 schools were taken in the study area in which 12 were government schools and remaining 13 were private schools. Those Children who were willing to participate in the study; present on day of examination and whose proforma was completed were included in the study. Those children who were absent during the time of examination and children with any systemic diseases, on antibiotic therapy in the previous six months and who refused to participate was excluded from the study.

The sample size for the study was calculated based on the data collected from the pilot study. Expecting, at least 25% poor health status (effect size) in 12 to 15 years old school going children in Lucknow city, with considering 5.0% margin of error (Type I error), 80.0% power (Type II error) and 1:1 ratio, then minimum sample size required was 1200, evaluated using $n=Z^2pq/d^2$ formula.

Thus, minimum 290 school going children required for one age group and total 1160 or round up 1200 children required for four age group (12 to 15 yrs) to estimate 25% prevalence of poor oral health status. Data was collected from a cross-sectional survey, using a Survey Proforma which comprised of a Questionnaire and Clinical examination.

Closed-ended questionnaire consisting of demographic data, three questions of oral hygiene practices and four indices were used. Dental examination was done to assess the dental caries status, oral hygiene status, presence of malocclusion and gingival status. DMFT given by Henry T.Klein, Carrole E.Palmer and Knutson J.W in 1938 later modified by World Health Organization in year 1997, Gingival Index given by Sillness and Loe in 1967 and Oral hygiene index simplified given by John C. Greene and Jack R.Vermilion in 1964 was used to measure overall oral hygiene status.

An intra-oral examination was carried out by a single examiner to assess the Oral Health Status. Clinical examination (type III) including dental caries, gingival and oral hygiene status examination using WHO standard criteria was done. Individual consent will be taken from each student. The collected data was registered in a diagnostic chart for each child. Calibration of the examiner was assessed by kappa stastistic.

Statistical Analyses

All responses was tabulated using Microsoft – Excel 2010 Software for data analysis. Data was analysed by using SPSS software version 21.

Results

Out of 1200 students, 709 (59.1%) students were

female and 491 (40.9%) were male. Among 1200 students, maximum (30.5%) were of 13 years old followed by 14 years old (28.3%), 12 years old (25.9%) and least were 15 years old (15.3%).

Table 1 shows the distribution of students according to oral hygiene practices. 80.4% of the students clean their teeth with brush. More than 90% of the subjects clean their teeth in the morning. Toothpaste and toothpowder were used as material by 73.2% and 25.3% of the subjects respectively.

The mean DMFT among the subjects was compared using one way anova test. It was found to be significant. The maximum DMFT scores was seen among 12 years, then 15 years, then 14 years and least in 13 years. On pair wise comparison, significant differences were seen among 12 years and 13 years, 12 years and 14 years and 12 years and 15 years only (table 2).

The comparison of study participants with regard to age of the student and prevalence of malocclusion was done using chi square test. It was not found to be significant. Although maximum students of 14 years old reported high prevalence of malocclusion (table 3).

The comparison of study participants with regard to age of the student and oral hygiene index was done using chi square test. It was found to be significant. Maximum 13 years old students reported fair oral hygiene index (table 4).

The comparison of study participants with regard to age of the student and gingival index scores was done using chi square test. It was not found to be significant (table 5).

Table 1: Distribution of students according to oral hygiene practices

Variables		Frequency	Percent
Method of cleaning	With Brush	965	80.4
	With Finger	213	17.8
	Any Other	22	1.8
Time of brushing	After Every Meal	29	2.4
	At Night	47	3.9
	In The Morning	1124	93.6
Material used	Toothpaste	878	73.2
	Tooth Powder	304	25.3
	Both	18	1.5
	Total	1200	100.0

TABLE 2: Age wise comparison of mean DMFT score

Age in years	N	Mean	SD	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
12	311	1.80	1.936	1.58	2.02
13	366	1.29	1.378	1.15	1.43
14	339	1.33	1.362	1.18	1.47
15	184	1.42	1.397	1.22	1.63
F value		7.471			
p value		0.001*, Sig 12 years> 13,14,15 years			

Sig: Significant

TABLE 3: Comparison of study participants with regard to age of the student and prevalence of malocclusion

			Prevalence Of Malocclusion		Total
			Present	Absent	
AGE	12	N	200	111	311
		%	26.2%	25.4%	25.9%
	13	N	240	126	366
		%	31.5%	28.8%	30.5%
	14	N	207	132	339
		%	27.1%	30.2%	28.3%
	15	N	116	68	184
		%	15.2%	15.6%	15.3%
	Total	N	763	437	1200
		%	100%	100%	100%
Chi Square Test			1.151		
p value			0.648, NS		

NS: NON SIGNIFICANT

TABLE 4: Comparison of study participants with age of the student to oral hygiene index

			OHI-S			Total
			Good	Fair	Poor	
AGE	12	N	64	178	69	311
		%	22.9%	24.2%	37.5%	25.9%
	13	N	79	240	47	366
		%	28.3%	32.6%	25.5%	30.5%
	14	N	79	217	43	339
		%	28.3%	29.4%	23.4%	28.3%
	15	N	57	102	25	184
		%	20.4%	13.8%	13.6%	15.3%
	Total	N	279	737	184	1200
		%	100%	100%	100%	100%
Chi Square Test			22.403			
p value			0.001, sig			

sig: significant

Table 5: Distribution of study participants according to gingival index to age of the student

			Gingival Index				Total
			Normal Gingiva	Mild Inflammation	Moderate Inflammation	Severe Inflammation	
AGE	12	N	1	191	100	19	311
		%	.3%	61.4%	32.2%	6.1%	100.0%
	13	N	1	247	101	17	366
		%	.3%	67.5%	27.6%	4.6%	100.0%
	14	N	5	226	94	14	339
		%	1.5%	66.7%	27.7%	4.1%	100.0%
	15	N	1	128	48	7	184
		%	.5%	69.6%	26.1%	3.8%	100.0%
	Total	N	8	792	343	57	1200
		%	.7%	66.0%	28.6%	4.8%	100.0%
Chi Square Test			10.099				
p value			0.343, NS				

NS: NON SIGNIFICANT

Discussion

School provide a platform for the promotion of health and oral health not only for the students, but also for the staff, families and members of the community as a whole.[2] Hence, present study which was a descriptive cross-sectional survey, conducted with an aim to assess the oral health status of 12 to 15 years old school going children in Lucknow City, India and also to determine the prevalence of dental caries and malocclusion in the population which would help us in planning and implementing necessary preventive measures.

The present study was conducted among 1200 (709 males and 491 females) school going children of Lucknow City. A similar study was conducted by **Ndanu TA et al (2015)[6]** among 9-15 years old Ghanaian school children, in that the sample size was taken 1040 in which males were 465 and females were 575 and by **Fotedar Shailee et al (2013)[7]** , in that the sample size was taken 1011 (626 males and 385 females). It was found that number of male subjects (59.1%) were comparatively higher as compared to female subjects (40.9%) in all the age-groups. This difference in male to female ratio could be due to the reason that in rural areas still the women are not encouraged for school education.

In the present study, the age of children was taken 12 to 15 years as children in these age groups are more vulnerable to oral health problems. At age 12, when most of the permanent teeth were erupted, over 90% of school children have experienced dental caries. Accordingly, 15 year olds are used as a reference group to study the trend in and severity of caries.[8] A similar study was conducted by **Dubey et al (2015)[9]** in that, majority of school children were of 12 to 15 years old and also similar to study conducted by **Saima (2018)[10].**

In the present study, out of 1200 school going children 965 children (80.4%) were cleaning their teeth with brush which include 248 (79.7%) students of 12 years age group, 296 (80.9%) students of 13 years age group, 277 (81.7%) students of 14 years age group and 144 (78.3%) students of 15 years age group, 213 (17.8%) were using fingers to clean their teeth and rest 22 (1.8%) were using any other method to clean their teeth. Most of the children 1124 (93.6%) were brushing once a day which included 286 (91.9%) students of 12 years age group, 347 (94.8%) students of 13 years age group, 318 (93.8%) students of 14 years age group and 173 (94%) students of 15 years age group. This study is similar to the study done by **Richa G et al (2015)[1]** which showed more than 90% children brushed once daily whereas very few children were brushing their teeth twice daily and also by **Saima S (2018)[10]** which showed 91% children brushed once daily whereas 8% of children brushed twice daily and 1% brushed thrice daily. This finding is relatively lower compared to 96.6% morning brushing in **Ndanu TA et al (2014).[6]**

In the present study,12 years to 15 years old children had a mean DMFT of 1.80, 1.29, 1.33, 1.42 respectively. It was found that the mean DMFT was more in 12 years than 15 years than 14 years and than 13 years. This finding is in contrast with the study conducted by **Ndanu et al (2014) [6]** in that mean DMFT was 0.331 while in a study done by **Richa G et al (2015)[1]**, the mean DMFT was 1.26 in 15 year age group and 0.82 in 12 year age group and also by **Mohammed et al (2014)[11]** in that mean DMFT was 4.62, 4.29, and 5.50 respectively for 12 year, 13 year, 14 year old children. Mean DMFT was higher in the males (1.52) as compared to females (1.35). This is in contrast with the study conducted by **Shivananda GS et al (2017)[12]** in that the mean DMFT score was high in girls (0.95) as compared to boys (0.79) and also by **Mohammed et al (2014)[11]** which showed female children had a higher mean DMFT value (5.23) than male children (4.74).

In the present study, among the total population of 1200 students, 763 (63.6%) had presence of malocclusion. Rest all (437) were free from malocclusion. This may be due to premature exfoliation of deciduous teeth and pernicious habits during childhood. Tooth loss in early childhood has a direct influence on future development and establishment of occlusion. This value is relatively lower as compared to 83.3% presence of malocclusion in **Retna K et al (2016)[13],** 61.1% presence of malocclusion in **Punith S et al (2017)[14]** and 89.9% presence of malocclusion in **Shobha et al (2019).[15]** Maximum malocclusion was found in 13 years old (31.5%) than 14years (27.1%) than 12 years (26.2%) and than 15 years (15.2%). Maximum males (57.3%) reported high prevalence of malocclusion. This relation was found to be statistically significant (p value = 0.05).

Oral hygiene of 61.4% students were fair, 23.3% students were of good oral hygiene and rest all were of poor oral hygiene. Maximum fair oral hygiene was found in 13 years (32.6%) than 14 years (29.4%) than 12 years (24.2%) than 15 years (13.8%) with p value 0.001. Maximum poor oral hygiene was found in 12 years (37.5%) than 13 years (25.5%) than 14 years (23.4%) than 15 years (13.6%). A similar study was conducted by **Saima S et al (2018)[10]** in that, majority (65%) of the sample showed fair oral hygiene and by **Deepak PB et al (2010)[16]** in which majority of students had fair oral hygiene, followed by good oral hygiene status and 2% had poor hygiene status. This is in contrast with the study conducted by **Shivananda GS et al (2017) [7]** in that oral hygiene was good in about 81.7%, fair in 17.5% and 0.7% poor and also by **JR Sukhabogi et al (2014)[12]** which showed that oral hygiene status was good among 39.1% of the school children, fair and poor among 38.7% and 22.2% of the study participants respectively.

In the present study, out of 311, 12 year old children, 191 had mild gingivitis, 100 had moderate gingivitis and 19 had severe gingivitis. Out of 366, 13 year old children, 247 had mild gingivitis, 101 had moderate gingivitis and 17 had severe gingivitis. Out of 339, 14 year old children, 226 had mild gingivitis, 94 had moderate gingivitis and 14 had severe gingivitis. Out of 184, 15 year old children,128 had mild gingivitis, 48 had moderate gingivitis and 7 had severe gingivitis. The high prevalence of gingivitis in present study can be attributed to poor oral hygiene and the weak or absent of oral preventive programs that should be followed in schools. This study is similar to study done by **Abdullah GA et al (2006)[17]** which showed mild gingivitis had the highest percentage (59.3%) among the school children while moderate gingivitis was (30.3%) in 12 year old school children.

There were certain limitations in the present study. Since, it was a cross sectional study, it provides limited information. As the present study was a field survey therefore radiographs were not used in the diagnosis of dental caries. It was likely, therefore, that the prevalence of dental caries may have been under estimated.

Conclusion

To conclude, the results of the present study confirmed that there is increased prevalence of malocclusion among children in the 12 to 15 year age group. Among all the age group, oral hygiene status was observed to be fair. Presence of malocclusion was found 63.6%.The awareness regarding the oral health was very minimal among the study population. It may be due to ignorance, lack of knowledge or the lack of motivation. In this study, majority of children were suffering from mild gingivitis. Females were more affected than males. This study also showed that gingival problem also formed one of the major problems of 12 to 15 year old school children.

Conflict of Interest: Nil

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Nanotechnology: A New Strategy to Treat Dental Hypersensitivity

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Abstract

Dental Hypersensitivity is the most commonly encountered dental problems associated with short, sharp pain rising from exposed dentine in response to stimuli such as tactile, osmotic and chemical. Hypersensitivity may present on several teeth, in one area of the mouth, or on one specific tooth. DHS should be differentiated from another tooth sensitivity which may elicit from other clinical conditions such as dental caries, microleakage, cracked tooth or fractured restorations. Several treatment strategies such as desensitization of nerve endings, masking of dentin tubules, occlusion of dentin tubules, and iontophoresis, are prevalent in clinical practices. Stannous ions, most commonly used in toothpastes as stannous fluoride, have been demonstrated to occlude dentine tubules in vitro. SnF2 toothpaste formulations have been used for several decades and are widely accepted as an effective DH treatment. Recently advanced nanotechnology has taken a wide leap in improving the various measures in the treatment of hypersensitivity by its nano particles. Nano structures have superior hardness, elasticity and flexural strength leading to its better application. Biomimetic particles desensitizing effect of nanocrystals which is caused by the progressive closure of the tubular openings of the dentine with plugs. These advancements have progressed the growth in the treatment of Dental Hypersensitivity and have evoked a great amount of attention for improving disease prevention.

Keywords: *Dental Hypersensitivity, Nanotechnology, Stannous Fluoride, Biomimetic Particles.*

Introduction

Dentine hypersensitivity (DHS) is one of the most commonly encountered dental problems. It is characterized by short, sharp pain arising from exposed dentine in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other dental defects or pathology [1]. Dentinal hypersensitivity is defined as an exaggerated response to a stimulus that usually causes no response in a healthy tooth & also an exaggerated response to a non-harmful stimulus which evoke pain without causing pathologic alteration to the dentin-pulpo complex. A modification of this definition is usually recommended by the Canadian planning board on Dentinal hypersensitivity in 2003 which suggested that disease should be substituted for pathology. The definition provides the clinical description of the condition & identifies Dentinal hypersensitivity as a distinct clinical identity [2–4]. Others terms to explain DH are created by substituting the word dentinal, adding site descriptors, like cervical or root, and mixing this with either hypersensitivity or sensitivity.

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Hypersensitivity may present on several teeth, in one area of the mouth, or on one specific tooth [5,6]. DHS should be differentiated from another tooth sensitivity which may elicit from other clinical conditions such as dental caries, microleakage, cracked tooth or fractured restorations [3]. The clinical management of DHS has been a challenge for clinician, various treatment modalities are available, but the success of any sound treatment plan is depended on taking a detailed clinical and dietary history, differentially diagnose the condition from other dental pain conditions and identify and manage etiological and predisposing factors[6–9]. Despite the existence of those different terms, various authors prefer the term DH, commonly used and accepted for several decades, to explain a selected painful condition of teeth, which is distinct from others sorts of dentinal pain having different aetiologies.

Prevalence and epidemiology

Various studies highlighted the incidence of DHS in major population ranges between 10-30% of the general population and the age range differs from 20-50 years with the peak incidence present at the end of the third decade and reduces during the fourth and fifth decades of life [10–12]. Such variations were performed to the differences in populations, dietaries, and methods of investigations which are usually through patient questionnaires or clinical examinations. The higher incidence is observed in females than in males which may cause hormonal influence and dietary practices [13,14]. In contrary, one study showed no difference in prevalence of dentine hypersensitivity in either gender, suggesting overall that as many males as females are susceptible. The type of teeth most widely involved, canines and premolars of both the arches are the most affected teeth. It was also reported that buccal aspect of cervical area is the commonly affected site [14].

Pathogenesis

It has been reported in the literature that DH develops in two different phases: lesion localization and lesion initiation [15]. Lesion localization occurs by losing the protective covering over the dentin leading to exposure to external environment. It includes reduction of enamel via tooth wearing problems. Another cause for lesion localization is gingival recession which may flow from toothbrush abrasion, tooth preparation for crown, pocket reduction surgery ,excessive flossing or secondary to periodontal diseases [16]. As stated earlier, not all exposed dentine is sensitive. For DH to occur, the lesion localization has got to be initiated. It occurs after the protective covering of smear layer is removed, resulting to exposure and opening of dentinal tubules.

Mechanism of Dentin hypersensitivity

Three major mechanisms of dentinal sensitivity are proposed within the literature (Figure 1):

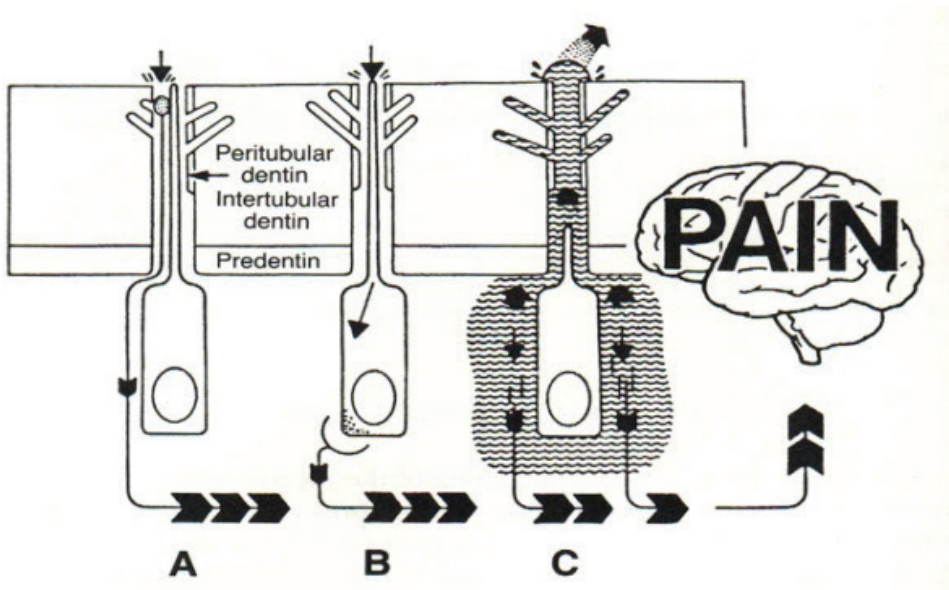

Figure 1. The schematic picture of the proposed theories [16]

- Direct innervation theory
- Odontoblast receptor
- Fluid movement/hydrodynamic theory

As per the direct innervation theory, nerve endings enters through the dentine and hold forth to the dentino-enamel junction [17]. Direct mechanical stimulation of these nerves leads to initiation an action potential. There is no evidence that outer dentin, which is typically the foremost sensitive part, is innervated. Developmental studies have reported that the plexus of Rashkow and intratubular nerves does not establish themselves until the tooth has erupted; whereas the newly erupted tooth is sensitive. Moreover, bradykinin does not induce pain when applied to dentine, and immersing with local anaesthetic solutions shows no signs of prevent pain, which does so when applied to skin.

Odontoblast receptor theory states that odontoblasts acts as receptors and relay the signal to a nerve terminal [18]. But maximum studies have shown that odontoblasts are matrix forming cells and hence they are not considered to be excitable cells, and no synapses are demonstrated between odontoblasts and nerve terminals [3].

Brannstrom (1964) has suggested that dentinal Hydrodynamic theory, proposed by Gysi and explained further by Branstrom[19] remains the most widely credited. Based on this theory, DH reduces with the depletion in fluid flowing within dentinal tubules [20]. Pashley (1986) suggested that DH might be reduced physiologically by the formation of intratubular crystals from dentinal fluids and saliva minerals [21]. Several treatment strategies such as desensitization of nerve endings, masking of dentin tubules, occlusion of dentin tubules, and iontophoresis, are prevalent in clinical practices.

Classification of Desensitizing Agents:

I. Mode of administration

At home desensitizing agents

In-office treatment

II. On the basis of mechanism of action

1. Nerve desensitization

- Potassium nitrate
- Protein precipitation
- Gluteraldehyde
- Silver nitrate
- Zinc chloride
- Strontium chloride hexahydrate

2. Plugging dentinal tubules

- Sodium fluoride
- Stannous fluoride
- Strontium chloride
- Potassium oxalate
- Calcium phosphate
- Calcium carbonate
- Bio active glasses (SiO_2–P_2O_5–CaO–Na_2O)

3. Dentine adhesive sealers

- Fluoride varnishes
- Oxalic acid and resin
- Glass ionomer cements
- Composites
- Dentin bonding agents

4. Lasers

- Neodymium:yttrium aluminum garnet (Nd-YAG) laser
- GaAlAs (galium-aluminium-arsenide laser)
- Erbium-YAG laser

5. Homeopathic medication

- Propolis

At-home therapy:

When the desensitizing tooth pastes were presented on the market for the first time were those which contained strontium salt and fluoride that had the property to occlude dentinal tubules, whereas those

which contained formaldehyde, destroy vital elements inside the tubules. A t the present moment most of the desensitizing toothpastes contain potassium salts such as potassium chloride, and potassium nitrate [22]. The studies have revealed that potassium salts move along the dentinal tubules by blocking the axonic action of the intra-dental nerve fibres which reduces the excitability of the tooth [4].

Mouthwashes and chewing gums:

Findings reported that mouthwashes which consisted of potassium nitrate and fluoride reduce DH. A few studies on the chewing gums containing potassium chloride has been reported fair but not much reliable. About 2-4 weeks after at- home therapies, the degree of DH would be reinvestigated. If the pain still existed, the patient should start the next phase of the therapy; in-office therapy [23].

In-office therapy:

Theoretically, in-office therapy of DH should cause prompt relief of the pain. Classification of various types of clinical desensitizing agents depend upon their mechanism of action and includes occluding dentinal tubules and disturbs the transmission of nerve impulses.

Management of Dentine Hypersensitivity focuses on two approaches: occluding dentine tubules or blocking impulse transmission in dentinal nerves. The former depends on ingredients that form hard deposits which partially occlude dentine tubules so external stimuli do not produce substantial shifts within fluid movement. Such agents include strontium or stannous salts, arginine plus insoluble calcium salts. Blocking of impulse transmission has been achieved by potassium ion; however, repeated administration appears to be required before symptomatic relief occur.

Short-term studies have always been positive for SnF2 toothpastes applied using the focused brushing technique, with many demonstrating clinical efficacy versus a control toothpaste.

According to a randomized clinical study conducted by Jonathan et al, DH was assessed using evaporative (Schiff scale) and tactile (Yeaple probe) stimuli. Participants applied toothpaste to two sensitive teeth by fingertip (60 s each); DH was re-assessed, prior to brushing. Test treatment participants brushed their sensitive teeth, with all participants then brushing all teeth for ≥60 s, twice daily for 3 days. DH was re assessed. Data were analysed by study and the pooled analysis indicated test treatment significantly reduced DH versus control, Studies indicated that single, fingertip application of a SnF2 toothpaste reduced DH and provides relief increased over 3 days. Stannous ions, most commonly used in toothpastes as stannous fluoride, have been demonstrated to occlude dentine tubules in vitro. SnF2 toothpaste formulations have been used for several decades and are widely accepted as an effective DH treatment [24].

Recent Advances

Nanotechnology (Nanohydroxyapatite Crystals): Recent Advancement in Treatment of Dentinal Hypersensitivity

Norio Taniguchi in 1974 First elucidated, "Nanotechnology mainly consists of the processing of separation, consolidation, and deformation of material by one atom or one molecule" [25].

Nanotechnology has achieved a phenomenal progress in the last several decades. These are those materials with parts less than 100 nm in at least one dimension, which included clusters of atoms, fibres those are less than 100 nm diameter, grains less than 100 nm in size, films less than 100 nm in thickness, composites and nanoholes. They have evoked a great amount of attention for improving disease prevention, treatment and diagnosis. The definition provided by the National Nanotechnology Initiative, nanotechnology proposed specific phenomena and manipulation of materials over the nanoscale [26]. Nanotechnology, it is the research and production of materials, devices and systems exhibiting physical, chemical, and biological properties which varies from those on a larger scale [25].

Freitas in 2000 proposed the term nano dentistry and stated that "new treatment opportunities, permanent hypersensitivity cure, complete orthodontic realignments during a single office visit, covalently bonded diamondized enamel and continuous oral health maintenance through the use of mechanical dentirobots" [25].

Properties of Nanoparticles:

1. Particles have significant surface, size and quantum effects.

2. They have special properties, including chemical, magnetic, optical and electro-optical properties, which are different from those of individual molecules or bulk species.

3. Nanostructured materials are the development of self-building an autonomous organization of components into various patterns without human intervention [12].

Fabrication Techniques:

In 'top - down' techniques, the micro-nano dimensions of the hydroxyapatite are obtained by milling larger particles of commercial synthetic hydroxyapatite. Only recently, the development of nanotechnologies has opened new opportunities in obtaining cheap HA micro-nanoparticles using the ''bottom up'' methods, in order to improve the biological responses of natural HA. The bottom-up fabrication technique has been summarized in Figure 2.

Various Nanostructures[25]

1. Nanopores

2. Nanotubes

3. Quantum dots

4. Nano shells

Gary and Leinfelde (2009) have summarized the types of nanoparticles in composite resins as below[26]

Type I: Subtype A: Nanometric particles disseminate as a single unit within the resin matrix.

Type I: Subtype B: Comprises of agglomerated cluster of nanoparticles.

Type II: Consists of cage like formation that is composed of 8 silicon atoms and 12 oxygen atoms.

Characteristics of nanofillers in dental composites make the nanocomposites superior to the traditional composites and blend with natural tooth structure far better.

1. Superior hardness

2. Superior flexural strength

3. Superior modulus of elasticity

4. Superior translucency, excellent colour density, high polish, esthetical appeal and polish retention

5. About 50% depletion in filling shrinkage

6. Good handling properties

Nano-resins are "utility in fielder" with ability to move over the walls of the cavity preparation, dampen the surface of dentine to assure good adaptation. These materials are often used as sealants and also as cement for thin staked porcelain veneers. Septodont's N'Durance- Dimer flow is a latest flowable composite, which features unique chemistry and nanotechnology formulation. They have less shrinkage with simple handling and is a very aesthetic material.

Nanotechnology in Dentistry:

Teeth Remineralization by Nanotechnology:

Hydroxyapatite Ca10(PO4)6(OH)6 building block of enamel, are the most constituent of dental tissues representing in enamel and dentin 95% - 97% wt. and 75 % respectively and responsible for mechanical behaviour of dental tissues. Hydroxyapatite (HA) is the most stable form of calcium phosphate. Enamel prismatic HA crystals consist of a weaving of prisms ranging from 3 to 5 μm in diameter. A single prism reveals a highly organized array of fastened needle like HA crystallites (approximately 30 nm thick, 60 nm wide and numerous mm long). Unlike bone, in enamel and dentine when HA is dissolved or abraded, it cannot spontaneously remineralize because enamel is bereft of deprived of regenerative cells and contrarily dentine apposition occurs only towards the pulp tissues [27].

Biogenic Hydroxyapatite V/S Biomimetic Synthetic:

Hydroxyapatite (Nanohydroxyapatite)

Biogenic carbonate hydroxyapatite (CHA) nanocrystals, constituents' mineral phase of calcified tissues like bone, dentin and enamel. They comprise of 4-8 wt.% of carbonate anions, which is approximately

25 nm wide, 60 nm in long and 2-5 nm thick. They exhibited a nonstoichiometric composition and have considerable low degree of crystallinity. Synthetic biomimetic CHA nanocrystals are similar to biogenic CHA nanocrystals. Biomimetic CHA nanocrystals are synthesized, comprising 4 ± 1 wt.% of carbonate ions, mostly 100 and 20 nm in size with a plate and acicular morphology respectively. They are stoichiometric in large amount of Ca/P molar ratio of about 1.6-1.7 [28]. Synthetic bioresorbable biomimetic hydroxyapatite nano and micro crystals provides good properties like bone filler biomaterial, such as bioactivity, osteoconductive, biocompatibility, direct bonding to bone, etc., exciting new applications of HA in the fields of bone tissue engineering and orthopaedic therapies.

Presently, synthetic CHA biomimetic nanocrystals proven to produce, In Vitro, re-mineralization of the altered enamel surfaces and closure of dentinal tubules, thus providing a prospective use in desensitizing dentifrices. Hefferren et al. have suggested that, increased

re-mineralization occurs frequently with apatite particles sizes <4 μm [29]. There is a potentially desensitizing effect of biomimetic CHA nanocrystals, is caused by the progressive closure of the tubular openings of the dentine with plugs within a few minutes until the regeneration of a mineralized layer has occurred within a few hours. Natural hypersensitive teeth have 8 times higher surface density of dentinal tubules and diameter with twice as large than non-sensitive teeth [14]. Reconstructive dental nanorobots, using native biological materials, could selectively and precisely occlude specific tubules within minutes, offering patients a quick and permanent cure [30]. Field Emission Scanning Electron Microscope (FE-SEM) observation of the nano-HAP-treated dentine surface showed that nano-HAP uniformly occluded the dentinal tubules with a dentinal plug and a protective layer on the surface of the dentine was also formed [31].

Challenges Faced by Nanodentistry:

• Accurate positioning and assembly of molecular scale part

• Economical nanorobot mass production technique

• Biocompatibility

• Simultaneous coordination of activities of large numbers of independent micron-scale robots

• Social issues of public acceptance, ethics, regulation and human safety

Conclusion

Dentinal hypersensitivity is a relatively common and significant dental problem which can be successfully managed by a very wide variety of procedures, agents and formulations applied locally either “in office” or “at home”. These hydroxyapatites are surface nanostructured and have higher surface area and consequently higher reactivity, allowing them to bind to enamel and dentine apatite producing a biomimetic coating on enamel, contrasting plaque formation and sealing dentine tubules and annulling hypersensitivity. Advancements in the management of dental hypersensitivity is slowly taking a new step into technological development.

Ethical Clearance- Ethical approval taken from Institutional Ethical Committee.

Source of Funding- None

Conflict of Interest - NIL

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Original Research Article

Correlation of Lipid Profile with Thyroid Stimulating Hormone in Pregnant Females

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Abstract

Introduction: Pregnancy is a state which requires more metabolic fuels. Changes in the hormonal levels in pregnancy alter lipid profile also in pregnant women. Thyroid hormone abnormality without any history of thyroid disease may lead to alteration of triglycerides, phospholipids, total cholesterol, and various lipoproteins levels in pregnancy which increases the risk for CVS disease.

Aim: The aim of the study is to find out the association among TSH and lipid profile in pregnant females.

Materials and Methods: The observational study was conducted at Biochemistry Department, SBKS Medical Institute & Research Center, Piparia, Vadodara, Gujarat, India. 100 antenatal cases from were enrolled after taking an informed consent. 50 pregnant women were in 2nd trimester and 50 pregnant women were in 3rd trimester. Blood samples were drawn to estimate lipid profile and TSH. Interpretation of data was done using Medcalc software.

Results: The mean level of TSH was 1.71(1.26) in 2nd trimester pregnant females and 2.68(0.72) in 3rd trimester pregnant females, difference between them was statistically significant. Significant negative correlation was found between TSH and total cholesterol in 3rd trimester pregnancy. There was a significant positive correlation was seen between TSH and triglyceride and between TSH and VLDL in 3rd trimester.

Conclusion: Significant increase in TSH level is found in 3rd trimester pregnant females compare to 2nd trimester pregnant females. Significant correlation is also found between TSH and lipid profile in 3rd trimester. Regular checkups of Thyroid hormones are required in later stages of pregnancy to prevent further complications.

Key Words: *Lipid Profile, TSH, Pregnancy.*

Introduction

Pregnancy is a state which requires more metabolic fuels. Changes in the hormonal levels in pregnancy alter lipid profile also in pregnant women[1]. Thyroid gland structure as well as function is significantly affected by pregnancy. For foetal neurological development and maternal health, thyroid hormone levels are very much important. Maternal thyroid hormones are utilised by foetus till 12 to 14 weeks of gestation, after that thyroid gland of the foetus starts working by their own. In a pregnant female, hypothyroidism leads to adverse impact on maternal health and health of the foetus. Low levels of thyroid hormones affect mental development of the child which leads to mental retardation and cognitive

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delay in early childhood[2]. Pregnancy complications of the untreated thyroid disease are high risk of miscarriage, placental abruption, hypertensive disorders, and growth retardation. However thyroid disease is the 2nd most common endocrine disorder in reproductive age group of the females[3].

Pregnancy is an exceptional physiological condition in which the maternal metabolic functions are changed to obtain enough amount of energy for proper growth and development of the foetus. Lipid metabolism derangements in pregnant female are fat accumulation, high tissue lipolysis and maternal hyperlipidaemia[4]. It is complex to conclude which level of lipid derangement is physiologic or pathologic in pregnancy. In early months of pregnancy, mild elevation of lipid profile is seen. Significant elevation of lipid profile is seen in 3rd trimester of pregnancy[5]. Both triglyceride (TG) and total cholesterol (TC) go to the placenta then metabolized and transported to the growing fetus. Lipids are necessary for the proper growth & development of the fetus. However, increased levels of cholesterol an d triglyceride in mothers are linked with preterm birth and preeclampsia[6].

Thyroid hormone abnormality without any history of thyroid disease may lead to alteration of triglycerides, phospholipids, total cholesterol, and various lipoproteins levels in pregnancy which is the risk factor for CVS disease. In the normal population, correlation was found between thyroid profile and lipid profile[7]. The indirect effect of TSH on lipid profile is through its effect on thyroid hormones and partially direct effect by extra-thyroidal effect of TSH on lipid profile. General pregnancy changes of lipid profile are non-harmful, and decrease to normal levels after delivery of a child[8,9]. Therefore, the aim of the present study is to find out the correlation among TSH and lipid profile in pregnant females.

Material and Methods

This observational study was done at Biochemistry Department, SBKS Medical Institute & Research Center, Piparia, Vadodara, Gujarat, India. 100 antenatal cases from January 2019 to June 2019 were taken for the study after taking an informed consent. Among them, 50 pregnant women were in 2nd trimester and 50 pregnant women were in 3rd trimester. All singleton pregnant women irrespective of parity and gravida were included. Pregnant women having chronic hypertension, Obstetric and Foetal abnormalities, Diabetes Mellitus, Renal Disorders and Thyroid Disorders were excluded.

Performa was filled by the investigator. Performa contained biodata – age, gender, clinical examination findings and clinical investigations. Study purpose was informed to the participants. Fasting blood was collected in plain vacutainer tubes in the 16th and 32nd week of gestation from pregnant women. Centrifugation done for 15 min.at 3000 r/pm. Serum was separated which was used for the estimation of lipid profile and TSH-Thyroid stimulating hormone.

Methodology

Serum TSH was estimated by sandwich chemiluminescence immunoassay. TSH was estimated on MAGLUMI fully auto chemiluminescence immunoassay analyzer. Serum Total cholesterol and Serum Triglyceride were done by Cholesterol oxidase peroxidase & Glycerophosphate oxidase (GPO) end point method respectively. HDL-C was estimated using HDL direct reagent based on modified polyvinyl sulfonic acid (PVS) and PEGME coupled classic precipitation principle method. Serum VLDL and LDL were calculated by Friedewalds formula. Lipid profile was performed on EM-200 fully auto chemistry analyzer.

Statistical Analysis

Data were presented as Mean and SD values. Comparisons between 2nd and 3rd trimester pregnancy were performed using the Independence student t- test. A p-value less than 0.05 ($p< 0.05$) was considered as statistical significant. For statistical analysis, Medcalc software was utilised.

Result

Results of biochemical parameters of 2nd and 3rd trimester pregnant females were given in Table 1 as mean and SD. The mean level of TSH was 1.71(1.26) in 2nd trimester pregnant females and 2.68(0.72) in 3rd trimester pregnant females, difference between them was statistically significant. Differences between lipid profile parameters in 2nd and 3rd trimester pregnant females were not significant.

Table 2 shows correlation of serum TSH with lipid profile among the 2nd trimester pregnant females. In 2nd trimester, significant correlation was not found between TSH and lipid profile. Table 3 shows correlation of serum TSH with lipid profile among the 3rd trimester pregnant females. Significant negative correlation was found between TSH and total cholesterol in 3rd trimester pregnancy. Significant positive correlation was there between TSH and triglyceride and also between TSH and VLDL in 3rd trimester pregnant females.

Table 1: Mean distribution of biochemical parameters in 2nd and 3rd trimester pregnancy

Values are expressed as means and SD.

Parameters	2nd trimester		3rd trimester		P value
	Mean	SD	Mean	SD	
Total cholesterol (mg/dl)	222	25.61	255	31.43	0.25
Triglyceride(mg/dl)	191	28.33	228	23.21	0.11
LDL Cholesterol(mg/dl)	136	12.71	165	20.11	0.09
HDL Cholesterol(mg/dl)	48	5.89	44	4.88	0.32
VLDL Cholesterol(mg/dl)	38	6.31	46	5.78	0.12
TSH(microIU/ml)	1.71	1.26	2.68	0.72	<0.01

$p < 0.05$ - significant, $p < 0.01$ - very significant, $p \geq 0.05$ - not significant TSH: Thyroid stimulating hormone

Table 2: Correlation of serum TSH with lipid profile among the 2nd trimester pregnant females

Parameters		Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Serum TSH	r value	- 0.025	-0.101	-0.097	0.091	-0.077
	P value	0.11	0.74	0.16	0.21	0.74

$p < 0.05$ - significant, $p < 0.01$ - very significant, $p \geq 0.05$ - not significant

TSH: Thyroid stimulating hormone

Table 3: Correlation of serum TSH with lipid profile among the 3rd trimester pregnant females

Parameters		Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Serum TSH	r value	- 0.233	0.089	-0.01	0.075	0.082
	P value	<0.01	<0.05	0.09	0.06	<0.05

$p < 0.05$ - significant, $p < 0.01$ - very significant, $p \geq 0.05$ - not significant

TSH: Thyroid stimulating hormone

Discussion

Thyroid profile estimation in pregnant women is of very important for maternal and fetal health. Physiologic changes linked with pregnancy need more availability of thyroid hormones by 40% to 100% to acheive the maternal and foetus requirements throughout the pregnancy[10]. The most seen derangement is the elevation of thyroxine-binding globulin (TBG) levels. This starts increasing early in the first trimester, reaches at plateau during middle of the gestation, and stays elevated until shortly after delivery. Increase in TBG is because of stimulation of its synthesis by increased maternal estrogen levels, because of decreased hepatic clearance of TBG due to estrogen-induced sialylation. Elevated TBG levels lead to increase in extra-thyroidal pool and because of that there is an increase in thyroid hormone synthesis in mother[11].

In our study, we found significant increase in TSH in 3rd trimester pregnant females than 2nd trimester pregnant females. Elevated maternal thyroid stimulating hormone (TSH) has been associated with an increased risk of preterm birth, placental abruption, fetal death, and impaired neurological development in the child[12]. Dhanwal DK had found out in the study that there is a high prevalence of hypothyroidism is seen in pregnancy[13]. One study demonstrated that in an iodine sufficient people, thyroid hormone levels were almost same in normal pregnancies and in non-pregnant females[14].

Our study demonstrated that, total cholesterol, triglyceride, LDL and VLDL levels were high in 3rd trimester pregnant females than 2nd trimester pregnant females. HDL levels were lower in 3rd trimester than 2nd trimester pregnant females. Study done by R. Pusukuru, et al showed in their study that all lipid profile parameters except HDL were elevated in both second and third trimester. HDL-Cholesterol was less in third trimester than second trimester. These findings are correlated with our study findings[15]. Link between early pregnancy triglyceridaemia in mother and risk of pregnancy induced hypertension, gestational diabetes,mellitus and preterm child deliveries was seen in study done by B Ghodke, et al[1].

In our study, in 2nd trimester, we didn't find any significant correlation between TSH and lipid profile. Significant negative correlation was found between TSH and total cholesterol in 3rd trimester pregnant females. There was a significant positive correlation seen between TSH and triglyceride and among TSH and VLDL in 3rd trimester pregnant females. Mehta V et al concluded that TSH levels were increased in third trimester than the second trimester. Also they found significant inverse correlations among TSH and cholesterol & TSH and VLDL in third trimester of the pregnant females[7]. This coincides with our findings. Some studies have shown that alteration in thyroid hormones can affect body weight and lipid profile. The influence of body weight on the interaction of thyroid hormones and lipid profile is not studied well[16]. In pregnancy, physiological weight gain of female can affect lipid profile and thyroid hormone levels in the body.

Conclusion

In our study, we found significant increase in TSH in 3rd trimester pregnant females than 2nd trimester pregnant females. Significant negative correlation was found between TSH and total cholesterol in 3rd trimester pregnant females. Significant positive correlation was seen among TSH and triglyceride and between TSH and VLDL in 3rd trimester pregnant females. More number of studies involving large population size are needed to ascertain these finding as our population size is small. Thyroid hormone abnormality can lead to pregnancy complications. Mother and fetus both are affected by thyroid dysfunction. Regular checkups of Thyroid hormones are required in later stages of pregnancy to prevent further complications.

Ethical Clearance: Ethical clearance was taken from Institutional ethical committee.

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Conflict of Interest: There is no conflict of interest.

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Thyroid Profile and Its Correlation with Serum Creatinine in Chronic Kidney Disease Patients in Tertiary Care Hospital

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Abstract

Background: Chronic kidney disease (CKD) is defined as abnormalities in kidney structure or function present for > 3 months and have other health implications. Thyroid hormones are important for electrolyte homeostasis and also for the growth and development of the kidney. Study shows that hypothyroidism is commonly seen in later stages of CKD. This study was conducted to investigate thyroid hormone profile and its correlation with creatinine in CKD patients.

Materials and Methods: The present case control study was conducted at Dhiraj General Hospital, Piparia, Vadodara, Gujarat, India in which 100 subjects were enrolled, out of which 50 were cases of CKD patients and 50 were controls. The age group for the study was 18 to 70 years. Blood samples were drawn to measure blood urea, serum creatinine, thyroid profile. Interpretation of data was done using Medcalc software.

Conclusion: Serum TSH levels were high in cases compared to controls but results were not significant. Significant negative correlation was found between free thyroid hormones and serum creatinine. Correlation of creatinine with T3, T4 and TSH were not significant. Subclinical hypothyroidism is found in our population with undialyzed CKD. More studies involving large population size are required to ascertain these findings.

***Key words**: CKD, Serum Creatinine, Thyroid Hormones.*

Introduction

Chronic kidney disease is defined as abnormalities in kidney structure or function that are present for > 3 months and have other health implications. The disease is classified on the basis of cause and category of glomerular filtration rate (G1 - G5) and albuminuria (A1 – A3) KDIGO Classification of CKD is used for the diagnosis of Chronic Kidney Disease (CKD)[1]. It is a serious disease to human being. The incidence and prevalence of CKD increase with aging. In many cases, CKD leads to end stage renal disease and eventually it leads to death. Therefore, accurate evaluation of renal function is important for healthy individuals and CKD patients[2].

The thyroid gland regulates the most of the body's physiological actions. The interactions between kidney function and thyroid hormones are wildly studied. Thyroid hormones are important for water and electrolyte homeostasis and also for the growth and development of the kidney[3]. Metabolism, degradation and excretion of thyroid hormones are regulated by kidney; renal dysfunction affects thyroid hormone production, its distribution, and excretion. Data suggest that predialysis patients with chronic kidney disease have an increased risk of hypothyroidism and subclinical hypothyroidism[4].

Hypothyroidism is commonly seen in later stages of CKD[5]. Metabolism, degradation and excretion of thyroid hormones depend on kidney function. CKD

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affects the hypothalamus pituitary thyroid axis. CKD will lead to complications like thyroid dysfunction, lipid abnormalities and cardiovascular diseases. Thyroid gland related complications in CKD patients include decrease in circulating thyroid hormone levels, distorted peripheral hormone metabolism, inadequate binding to carrier proteins, condensed tissue thyroid hormone content and altered iodine storage in the thyroid gland. Thus, thyroid hormone metabolism is very much affected in CKD[6]. This study was conducted to investigate thyroid hormone profile and its correlation with creatinine in CKD patients.

Materials and Methods

The present case control study was conducted at Dhiraj General hospital, Piparia, Vadodara, Gujarat, India in which 100 subjects were enrolled, out of which 50 were cases of CKD patients and 50 were controls. Study was conducted from July 2019 to Dec 2019.

Case Groups: - Undialyzed cases of Chronic Kidney Disease

Controls:-Age and Sex matched Normal healthy Individual in and around the Dhiraj General Hospital is termed as controls.

Undialyzed CKD patients who are willing to participate, both male and female with the age group of 18-70 years were included in the study. Patients with known history of thyroid disorder, acute renal failure, patients on drugs which affects thyroid hormone levels, pregnant females, and previous history of dialysis and also patients who are not willing to participate were excluded from the study.

Sample Collection & Processing: -

Data were collected by personal interview, with every individual. Performa was available for the filling of biodata like age and gender, clinical examination findings and investigations. Participants were informed of the study purpose. We had taken 50 Undialyzed CKD patients as cases and 50 healthy individuals as controls within the age group of 18-70 years attending the medical outpatient department and inpatient department of Dhiraj General Hospital. Written Informed consents were taken in their respected languages and fasting blood was collected in plain vacutainer tubes. Then it was centrifuged for 15 min. at 3000 r/min. Serum was separated which was used for the estimation of blood urea, serum creatinine, total triiodothyronine (T3), total thyroxine (T4), free triiodothyronine (FT3), free thyroxine (FT4) and Thyroid stimulating hormone (TSH).

Methodology

Serum Creatinine was estimated by enzymatic method. Blood urea was estimated by urease method. Urea and creatinine were performed on EM-200 fully auto chemistry analyzer. Serum T3 and T4 were estimated by competitive chemiluminescence immunoassay. Serum FT3 and FT4 were estimated by CLIA(Chemiluminescence immunoassay). Serum TSH was estimated by sandwich chemiluminescence immunoassay. Serum T3, T4, FT3, FT4 and TSH were estimated on MAGLUMI fully auto chemiluminescence immunoassay analyzer.

Statistical Analysis

Data were presented as Mean and SD values. Comparisons between cases and controls were performed using the Independence student t- test. A p-value less than 0.05 ($p< 0.05$) was considered as statistical significant. Medcalc software was used for statistical analysis.

Results and Discussion

The age and sex distribution in cases and controls were comparable, and difference between groups was not statistically significant. Results in cases and control groups were given in Table 1 as mean±SD. The mean level of T3 was 0.87±0.52 in cases and 1.74±1.97 in controls, difference between them was statistically significant. The mean level of T4 was 5.09±1.19 in cases and 6.11±1.28 in controls, difference between them was statistically not significant. The mean level of FT3 was lesser in cases (1.35 ± 0.22) than controls (2.53 ± 0.82); the difference between them was statistically significant. The mean level of FT4 in cases was 0.77 ± 0.31, and in controls, it was 1.49 ± 0.32, and the significant difference was found between both the groups. There was higher mean serum TSH in cases (2.85 ± 0.94) as compared to controls (1.39 ± 0.66), and the insignificant difference was found between them. Significantly higher mean blood urea and mean serum creatinine were found in CKD cases compared to controls.

Table 2 shows correlation of creatinine with thyroid profile parameters among cases. A significant negative correlation was found between serum creatinine and serum FT3 ($r = -0.771$, $P < 0.01$) and between serum creatinine and FT4 ($r = -0.592$, $P < 0.01$). There were no significant correlations found between creatinine and T3, T4 and TSH.

Table 1: Mean distribution of biochemical parameters in CKD cases and controls.

Values are expressed as means ±SD.

Parameters	Cases (n=50)	Controls (n = 50)	P value
Age (yrs)	34.30 ± 7.37	32.72 ± 9.71	0.81
Blood Urea (mg/dl)	75.58 ± 30.67	27.17 ± 9.43	<0.001
Serum Creatinine (mg/dl)	9.62 ± 5.26	0.83 ± 0.21	<0.001
T3 (ng/ml)	0.87±0.52	1.74±1.97	<0.001
T4 (microg/ml)	5.09±1.19	6.11±1.28	0.17
FT3(pg/ml)	1.35 ± 0.22	2.53 ± 0.82	<0.001
FT4(ng/dl)	0.77 ± 0.31	1.49 ± 0.32	<0.001
TSH(mIU/ml)	2.85 ± 0.94	1.39 ± 0.66	0.11

$p < 0.05$ - significant, $p < 0.001$ - very significant, $p \geq 0.05$ - not significant

T3- triiodothyronine, T4 – thyroxine, FT3: Free tri-iodothyronine, FT4: Free thyroxine, TSH: Thyroid stimulating hormone

Table 2: Correlation of serum creatinine with different variables and biochemical parameters among the cases.

Parameters		T3	T4	FT3	FT4	TSH
Serum creatinine	r value	0.228	0.012	-0.771	-0.592	0.188
	P value	0.071	0.88	<0.01	<0.01	0.323

$p < 0.05$ - significant, $p < 0.001$ - very significant, $p \geq 0.05$ - not significant

T3- triiodothyronine, T4 – thyroxine, FT3: Free tri-iodothyronine, FT4: Free thyroxine, TSH: Thyroid stimulating hormone

Abnormal thyroid hormone levels have been reported in association with chronic kidney disease patients. We have done a case–control study to correlate thyroid hormones status with serum creatinine between undialyzed CKD patients and healthy individuals. Studies showed that dialysis can affect the serum thyroid hormone levels in the CKD patients. Thyroid autoimmune diseases and subclinical primary hypothyroidism are highly seen in renal failure patients[4].

Possible ways by which CKD causes thyroid abnormalities include the following: 1.The blunting effect of uremia on TSH receptors in the hypothalamic–pituitary axis. 2. Declining glomerular filtration rate causing decreased clearance of cytokines such as tumor necrosis factor alpha and interleukin-1, which decrease the peripheral conversion of T4 to T3 as well as decreased clearance of iodine and other goitrogenic substances which causes hypothyroidis. The earliest thyroid hormone abnormality in CKD patients is decreased total T3 hormone level. This is due to chronic metabolic acidosis and chronic protein malnutrition which affect iodothyronine deiodination, protein binding of T3, decreasing the peripheral conversion of T4 to T3[7]. This explains low T3 levels in our study.

In our study, we found that serum levels of T3, FT3 and FT4 were low and serum creatinine and urea were high in cases as compared to controls, difference between them were statistically highly significant. TSH levels were insignificantly high in cases as compared to controls. There was significant inverse correlation found between the creatinine and serum FT3 and also between creatinine and FT4. Serum creatinine was positively correlated with T3, T4 and TSH, but correlation was not significant.

Our findings are coincides with study done by Okaka EI,et al which showed overt biochemical hypothyroidism and subclinical hypothyroidism were the predominant thyroid abnormalities found in nondialysis-dependent patients with CKD. Hypothyroidism was defined as the presence of elevated TSH and low T4 in a patient while SCH (subclinical hypothyroidism) was defined as the presence of elevated TSH values with normal T4 values[8]. In CKD patients who don't require chronic dialysis, subclinical hypothyroidism is more common. Michel chonchol et al showed this in their study[9]. Evaluation of total and free thyroid hormone levels in CKD patients was done by Mehta et al. They showed in their study that significant low levels of TT3, FT3, and FT4 were associated with increasing kidney damage[10]. Chandra A, et al observed in their study that the prevalence of hypothyroidism was more in patients with low GFR[11]. In one Study, a significant inverse correlation found between the renal parameters and FT3, FT4. There was no significant positive correlation found between the serum creatinine and TSH[12]. These findings are similar to our findings. TSH showed significant positive correlation with creatinine in overt hypothyroidism[13].

Cardiac dysfunctions seen in hypothyroidism include impaired systolic and diastolic function, impaired vasodilation at the endothelium, dyslipidemia and atherosclerosis. Some studies have shown new mechanisms for the increased mortality of CKD patients with hypothyroidism include increased vascular calcification and platelet reactivity leading to thromboembolic events[8]. So it is important to diagnose hypothyroidism in CKD patients for the prevention of further complications.

Conclusion

In our study, significant negative correlation was found between free thyroid hormones and creatinine. Correlation of creatinine with T3, T4 and TSH were not significant. Subclinical hypothyroidism is found in our population with undialyzed CKD. More studies involving large population size are required to ascertain these finding as our population size is small. Hypothyroidism can cause cardiovascular complications in CKD patients so it is important to screen CKD patients for thyroid gland dysfunction in order to prevent further cardiovascular complications.

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Association of BMI with Serum Homocysteine in Polycystic Ovarian Syndrome Women

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Abstract

Background: Women of reproductive age group are prone for Polycystic ovarian syndrome (PCOS). Recent studies have shown that hyperhomocysteinemia is a risk factor for cardiovascular diseases. Prevalence of cardiovascular disease and cardiovascular morbidities in women with PCOS are increased nowadays. The purpose of the study is to assess correlation of BMI with serum homocysteine in PCOS females.

Materials and Methods: This is done on 50 PCOS patients and 50 voluntary age matched healthy women with no menstrual abnormalities as controls. The age group for the cases and controls is 18-35 years. The study was conducted at Biochemistry Department, SBKS MI & RC, Piparia, Vadodara, Gujarat, India. Measurements of BMI and waist circumference were taken. Fasting blood samples were collected for estimation of FBS, Lipid profile and homocysteine. Medcalc software was used for all statistical analysis. A p-value less than 0.05 ($p < 0.05$) is considered as statistically significant.

Conclusion: Our study has shown significant higher levels of homocysteine in obese PCOS cases when compared with non-obese PCOS cases. Also, significant higher level of homocysteine and triglyceride are seen in PCOS cases compared to controls. Regular follow up for lipid profile and homocysteine is required for PCOS patients to prevent CVS complications.

Key Words: *BMI, Homocysteine, Lipid profile, PCOS.*

Introduction

Women of reproductive age group are prone for Polycystic ovarian syndrome (PCOS), an endocrine disease. Rotterdam criteria were used for PCOS diagnosis. According to that presence of any 2 of the following: oligomenorrhoea or amenorrhoea, hyperandrogenism (clinical and/or biochemical), and polycystic ovaries (≥12 follicles of the ovaries measuring 2–9 mm in diameter, or volume of ovary is 0.10 ml in at least one ovary) are the requirement for the diagnosis of PCOS[1,2]. Clinical features seen in PCOS are menstrual irregularities, hirsutism, obesity, infertility, anovulation, and acne. PCOS is also linked with obesity, insulin resistance, and metabolic syndrome leading to complications like cardiovascular diseases and diabetes mellitus[3].

Homocysteine (hcy), an amino acid, is an intermediary product of methionine metabolism. The only source of homocysteine is methionine which is an essential amino acid. Major causes of homocysteinemia include the imbalance in intake of folate, cobalamine, pridoxine and methionine or genetic variations[4]. Recent studies have shown that hyperhomocysteinemia is a risk factor for cardiovascular diseases. Homocysteine plays an important role in endothelial injury. Hyperhomocysteinemia leads to impairment of

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endothelial dependent vasoreactivity and due to it, endothelium thromboresistance is also decreased. And because of all these events, atherogenesis is developed in hyperhomocysteinemia patients[5]. If the homocysteine level in the blood is more than15 micromol/L, then it is considered as hyperhomocysteinemia[6]. Prevalence of cardiovascular disease and cardiovascular morbidities in women with PCOS are increased nowadays[7].

The reason for increased homocysteine in PCOS women is not well-established. Factors affecting serum homocysteine levels are age, gonadocorticoids and gonadal steroids levels, Body Mass Index (BMI), insulin resistance (IR) and chronic inflammatory conditions. Hyperinsulinemia inhibits hepatic cystathionine β-synthase activity which causes increase in serum homocysteine level[8]. The purpose of the study is to assess correlation of BMI with serum homocysteine in PCOS females.

Material and Methods

This is a case control study, which is carried out on 50 PCOS patients and 50 voluntary age matched healthy women with no menstrual abnormalities as controls. The age group for the cases and controls is 18-35 years. The study was done at Biochemistry Department, SBKS MI & RC, Piparia, Vadodara, Gujarat, India. Rotterdam criteria is used for the diagnosis of PCOS. Patients with diabetes mellitus, hypertension, dyslipidemia, renal and liver failure, thyroid disorders and other endocrine diseases and patients which are on treatment for PCOS were excluded from the study.

Informed consent was taken from all the participants in their respective languages. A performa was used to collect the data. Age, BMI, medical history, clinical examinations and investigations were included in performa.

Body weight and height were measured. Calculation of BMI was done as weight (kg) divided by height in meter square (m^2). Centralized obesity also known as waist circumference was measure at middle point between the lower margin of last palpable rib and highest point of iliac crest. FBS (Fasting blood sugar), serum homocysteine and lipid profile were measured in all participants from fasting blood samples. Serum Homocysteine level was measured by Enzymatic Method[9] in the Laboratory of Dhiraj hospital. Serum Total cholesterol was measured by Cholesterol oxidase peroxidase. Serum Triglyceride was estimated by Glycerophosphate oxidase (GPO) end point method. HDL cholesterol was measured by HDL direct reagent based on modified polyvinyl sulfonic acid (PVS) and PEGME coupled classic precipitation principal method. Serum VLDL and LDL were calculated by Friedewalds formula. Lipid profile was performed on EM-200 fully auto chemistry analyzer.

Statistics Analysis

Data was presented as Mean and SD Values. Test of significance was assessed by independent t-test. A p-value less than 0.05 ($p< 0.05$) is considered as statistically significant. Medcalc software was used for all statistical analysis.

Results

Table 1: Biochemical parameters in PCOS cases and controls. All the values are given as means ±SD.

Parameters	PCOS Cases	Controls	P Value
Waist (cm)	80.92 ± 8.47	77.09 ± 9.18	Non significant
BMI (kg/m2)	25.56 ± 4.73	23.64 ± 3.87	Non significant
FBS (mg/dl)	91.44 ± 8.21	86.12 ± 6.78	Non significant
Total Cholesterol (mg/dl)	181.54 ± 8.62	171.89 ± 5.22	Non significant

Cont... **Table 1: Biochemical parameters in PCOS cases and controls. All the values are given as means ±SD.**

Triglyceride (mg/dl)	125.35 ± 18.42	100.28 ± 13.60	<0.01
HDL cholesterol (mg/dl)	45.14 ± 11.72	56.22 ± 5.81	<0.01
LDL cholesterol (mg/dl)	110.24 ± 18.79	96.11 ± 20.97	Non significant
Homocysteine (micromol/L)	13.9 ± 3.29	9.91 ± 2.88	<0.003

$p < 0.05$ - significant, $p < 0.001$ - very significant, $p \geq 0.05$ - not significant

BMI – Body Mass Index, FBS- Fasting Blood Sugar, HDL – High Density Lipoprotein, LDL – Low Density Lipoprotein, PCOS – Polycystic Ovarian Syndrome

Table 2: Comparison of Homocysteine in obese and non-obese PCOS cases based on BMI

Subgroups	**PCOS cases (BMI ≥25) (n=34)**	**PCOS cases (BMI <25) (n=16)**	**P Value**
Homocysteine	11.19 ± 4.97	8.87 ± 3.71	<0.001

$p < 0.05$ - significant, $p < 0.001$ - very significant, $p \geq 0.05$ - not significant

Table 3: Comparison of Homocysteine in obese and non-obese PCOS cases based on Waist circumference

Subgroups	**PCOS cases (Waist ≥ 80) (n=32)**	**PCOS cases (Waist <80) (n=18)**	**P Value**
Homocysteine	9.79 ± 2.14	7.34 ± 2.87	<0.001

$p < 0.05$ - significant, $p < 0.001$ - very significant, $p \geq 0.05$ - not significant

Table 1 showed comparison of different biochemical parameters between PCOS cases and controls. Mean BMI, waist circumference, FBS, Lipid profile and homocysteine were increased in PCOS cases when compared to controls. Significant increase levels of triglyceride and homocysteine were found in PCOS cases compared to controls. HDL levels were significantly decreased in PCOS cases than the controls. In table2 and table 3, Homocysteine was compared between Obese and non-obese PCOS cases based on their BMI and Waist circumference respectively. There was a significant increase in homocysteine in obese PCOS cases compared to non-obese PCOS cases.

Discussion

Polycystic ovarian syndrome women are at considerable risk for the development of metabolic and CVS abnormalities apart from reproductive abnormalities. Reproductive age group women are more prone for the development of insulin resistance, type 2 diabetes mellitus, obesity, hypertension, dyslipidemia[10]. Hyperhomocysteinemia is considered a risk factor for

CVD. Increased homocysteine levels may be seen in nutritional deficiencies of folic acid, pyridoxine, and vitamin B_{12}. Decreased serum vitamin B_{12} level in PCOS patients is linked with insulin resistance and obesity[11].

In our study, waist circumference, BMI and FBS were higher in PCOS cases compared to controls. But difference among them was insignificant. Significant increase levels of triglyceride were found in PCOS cases compared to controls. HDL levels were significantly decreased in PCOS cases than the controls. These findings are similar to study done by Ahmed M. Mohamadin. They also found increase in homocysteine levels in PCOS cases compared to controls which also coincides with our finding of homocysteine[12]. Significant increase in mean BMI, Waist circumference and serum homocysteine are observed in PCOS women then the normal controls in study done by priyanka Maleedhu, et al[7]. While Annamaria Fulghesu, et al showed in their study that total cholesterol, HDL, LDL, triglycerides and homocysteine levels did not differ between PCOS cases and control groups[13]. One meta-analysis study showed that increase in homocysteine was found both in normal and obese women with PCOS. So according to this study, homocysteine concentration was not associated with body weight[11] which is contrast to our study. In our study, there was a significant increase in homocysteine in obese PCOS cases compared to non-obese PCOS cases. BMI and waist circumference affect homocysteine levels in PCOS women. Schachter M, et al concluded in their study that hyperinsulinaemia in PCOS women is correlated with increased serum homocysteine, regardless of BMI[14]. Suleiman RR, et al found significant high homocysteine level in PCOS patients compared to control. They also showed there was no significant difference in homocysteine levels between the BMI categories and marital status in PCOS cases and controls[3]. Our study coincides with study done by Priyanka Maleedhu et al which showed the increase in homocysteine was associated with increase in BMI and waist circumference in PCOS cases. While some studies didn't show any association between PCOS and homocysteine levels in PCOS women[15,16].

Foam cells and atherosclerotic plaques are formed when metabolite of homocysteine combines with LDL-cholesterol. Oxidation of reduced homocysteine forms free radicals which cause damage to the endothelial cells and because of this there is a marked platelet aggregation. Production of nitric oxide is impaired because of long-lasting exposure of endothelial cells to homocysteine. All these events are responsible for cardiovascular complications. Thus hyperhomocysteinemia has been linked to cardiovascular complications[17].

Conclusion

Our study has shown significant higher levels of homocysteine in obese PCOS cases when compared with non-obese PCOS cases. Also, significant higher level of homocysteine is seen in PCOS cases compared to controls. Dyslipidemia is also seen in PCOS cases compared to controls. Hyperhomocysteinemia and dyslipidemia are the risk factors for cardiovascular diseases. Further studies are required to find the correlations between lipid profile and homocysteine levels in PCOS patients so that further CVS complications are prevented in reproductive age women.

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Source of Funding: This study was self funded.

Conflict of Interest: There is no conflict of interest.

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Refugees in the COVID-19

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Abstract

COVID-19 brought about most unprecedented public health and socio-economic crisis in our lifetime. It affects us all, especially the vulnerable populations, which refugees and migrants often find themselves in.

COVID-19 could have even more dire consequences in refugees camps than in general populations. Refugees and migrants are potentially at increased risk of contracting diseases, including COVID-19, because they typically live in overcrowded conditions without access to basic sanitation. The ability to access health-care services in humanitarian settings is usually compromised and exacerbated by shortages of medicines and lack of health-care facilities. Moreover, refugees typically face administrative, financial, legal and language barriers to access the health system.

Forced migration is at record highs in 2020, with around 70.8 million people around the world being forcibly displaced. The risk of communicating the novel covid 19 increases in case of the refugees because they come from the diverse regions of the world which might be facing a higher risk to lives due to the pandemic. Therefore, the health professionals need to be more vigilant.

As the COVID-19 situation around the world changes, CDC (Centers for Disease Control and Prevention) is monitoring COVID-19 risk in each destination and making travel recommendations. Travel Health Notices inform travelers and clinicians about current health issues that impact travelers' health, like disease outbreaks, special events or gatherings, and natural disasters, in destinations around the world.

The sudden outbreak of the pandemic has posed a serious ultimatum to the world of providing proper shelter, asylum and healthcare facilities to the refugees. However, public health professionals, community organizations as World Health Organization (WHO) and UN High Commissioner for Refugees (UNHCR), resettlement agencies, and healthcare providers can assist refugees arriving in the United States by providing them with information they need to protect themselves from COVID-19.

Keywords: *Asylum, Covid-19, Exacerbated, Health Risks, Refugees, Ultimatum.*

Introduction

The novel corona virus pandemic poses unique challenges to forcibly displaced populations around the world. Months into the pandemic, countries are still scrambling to enact policies that mitigate the outbreak and minimize the strain on their health-care infrastructures and economies.

Article 1 of the 1951 Refugee Convention defines a refugee as a person who is outside his/her country of nationality or habitual residence; has a well-founded fear of persecution because of his/her race, religion, nationality, membership in a particular social group or political opinion; and is unable or unwilling to avail himself/herself of the protection of that country, or to return there, for fear of persecution. The United Nations High Commissioner for Refugees (UNHCR) continues to work with member states to provide guidance and assistance to those populations protected under their mandate. However, there is great concern regarding the ability to appropriately provide for displaced

populations, as they tend to be hosted in areas that lack access to health care and proper hygiene materials. The situation has been exacerbated by the temporary suspension of refugee resettlement across the globe.

The Corona-Virus Disease 2019 (COVID-19)[1] exposes these populations to a new threat, one that could prove to be more devastating than the events forcing them to flee their homelands; Refugees are vulnerable to COVID-19 as they live in conditions that disproportionately increase their risk of contagion. For example, Bangladesh has confirmed COVID-19 cases and hosts almost 1 million Rohingya refugees from Myanmar, with 600,000 concentrated in the Kutupalong-Balukhali Expansion Site (mean age, 21 years; standard deviation [SD], 18 years; 52% female)[2].

The World Health Organization anticipates that people who have both COVID-19 and other infectious diseases, such as tuberculosis and malaria, may have poorer treatment outcomes, especially if tuberculosis treatment is interrupted. This prediction is alarming, considering that tuberculosis and malaria are highly prevalent in refugee populations, as are non-communicable diseases, such as type 2 diabetes, known to increase susceptibility to severe COVID-19. This situation is compounded by language barriers that refugees face in host communities and their limited access to health care for obtaining health information, testing and treatment, which some may even avoid out of fears of being deported.

Although the refugees population is quite high but the rate of positive covid 19 patients among them is less as they escape testing due to the fear of being isolated in quarantines and separated from their families, or even killed to slow the pandemic.

The social stigma associated with COVID-19 may encourage illness concealment, delay early detection and treatment, increase distrust in health authorities, lower the likelihood of compliance and prolong recovery. News media reports from refugee-sending countries, such as Iraq, indicate that stigma is a major barrier to prevention and treatment.

Statistics:

Forced migration is at record highs in 2020, with around 70.8 million people around the world being forcibly displaced. About 41.3 million people are internally displaced within their country of origin, 25.9 million are forcibly displaced outside of their country of origin (identified as *refugees*), and 3.5 million are seeking asylum in another country (UNHCR, 2019). The population protected by the UNHCR has doubled since 2012 (UNHCR, 2019) and around 78 percent of refugees are displaced longer than 5 years (UNHCR, 2019)[3]. Nearly 80 percent of displaced populations are hosted in low- and middle-income countries (UNHCR, 2020b) and about 60 percent of refugees live in cities with Turkey hosting the largest refugee urban population (USA for UNHCR, n.d.). Around 25 percent of refugees live in refugee camps, the largest of which are hosted in Bangladesh, Uganda, Kenya, Jordan, Tanzania, and Ethiopia (USA for UNHCR, n.d.).

Each host country faces its own specific challenges in facing a global health crisis due to differences in size, housing infrastructure, and environment. As of March 31, 2020, 203 countries have been affected by coronavirus; 96 of these countries host refugee populations (UNHCR, 2020b)[4]. The majority of refugees reside in countries with health□care systems that were already overwhelmed before the outbreak of coronavirus (United Nations Human Rights Office of the High Commissioner, 2020). Many refugees live in makeshift shelters or reception centers that are overcrowded, with limited access to healthcare services and clean water and/or sanitation (United Nations Human Rights Office of the High Commissioner, 2020). Against this backdrop, on March 17, 2020, the UNHCR announced that resettlement departures for refugees would be suspended temporarily (UNHCR, 2020, para 4). This response was spurred by actions resettlement countries were beginning to take to restrict the entry of international travelers to limit exposure to coronavirus, and out of concern for the safety and health of refugees during the resettlement process[5].

The economic crisis caused by efforts to contain the pandemic is worsening the refugees' already precarious situation in informal labour markets. Unemployment, lack of governmental support compel the refugees to starve and lead a shabby life. The United Nations High Commissioner for Refugees reports that a recent survey in Jordan showed that only 35% of Syrian refugees said they had a secure job to return to after the lifting

of COVID-19 restrictions. This economic hardship might interact with past trauma exposure to prolong and exacerbate mental health conditions in refugee populations. Humanitarian agencies serving refugees emphasize the importance of global support for the receiving countries so they can continue their efforts of solidarity, medical care and economic support. Hence, we strongly endorse *The Lancet*'s guiding principle of public health networks leaving no one behind during the COVID-19 pandemic, recommending epidemiologic risk assessments and the timely deployment of outbreak response teams within refugee camps, promoting health education in a culturally sensitive manner and ensuring health care access without refoulment for refugees.

As resources are constrained, agencies and professionals serving refugees should consider giving priority to screening vulnerable subgroups (that is, those with chronic health conditions) to manage co morbidities more effectively, encourage patients to reduce high-risk behavior, provide treatment and reduce transmission rates. Overlooking mental health conditions, exacerbated by the socioeconomic hardship caused by this pandemic, will complicate refugees' integration and increase the uncertainty they endure. Governments, public health professionals and organizations should act now to prevent the spread of COVID-19 in refugees.

Newly Resettled Refugee Populations:

The recently resettled refugees in the United States may experience such living arrangements or working conditions that put them at greater risk of getting COVID-19. Some refugees also have limited access to health care, compared to the rest of the U.S. population.

Nearly 750,000 refugees resettled in the United States from 2008 to 2019, and thousands more have resettled since then. Refugees are eligible for U.S. government-funded resettlement help, including healthcare benefits such as short-term health insurance for up to 8 months after arrival, and a medical exam within 90 days of arrival[6].

The impact of COVID-19 on the lives of resettled refugees remains unknown, as COVID-19 is a new disease.

Guidance for Refugees upon Arrival in the United States:

Public health professionals, community organizations as WHO and UNHCR, resettlement agencies, and healthcare providers can assist refugees arriving in the United States by providing them with information they need to protect themselves from COVID-19.

· CDC's Welcome Booklet for Refugees icon provides important information to help refugees and their families stay healthy during the COVID-19 pandemic after arriving to the United States. Further they must be provided with the contact information of the heath care providers.

· CDC's After You Travel recommendations for inbound international travelers provide useful information for arriving refugees.

What Can Be Done for Refugee Health during the COVID-19 Pandemic:

· Share information and available resources about COVID-19 and work with resettlement agencies and other partners, such as employers, healthcare systems and education, faith-based, transportation, and housing organizations[7].

· Statistics pertaining to the socio-demographic an clinical characteristics of COVID-19 positive refugees.

· Identify and secure funding for services to support refugees communities during the pandemic.

· Educating refugees through health promotion audio and video materials.

· Disseminate information in the local languages and in accordance with the literacy levels of the refugees through various channels and other means of communication.

· Ensure contact tracing programs reaching refugee populations.

· Community health workers and patient navigators familiar with working with refugees should be integrated into contact tracing programs and trained

accordingly.

· Information pertaining to the services available to refugees during the pandemic.

· Collaboration with health systems and community partners to identify strategic locations within refugee communities to offer free or low-cost COVID-19 testing. Ensure either in-person or phone interpretation is available at testing sites.

· Provide background information for healthcare professionals and health systems to understand key demographic, cultural, and health characteristics of specific resettled refugee groups.

Why Refugees May Be at Increased Risk during COVID-19 Pandemic:

The arrival of the coronavirus posed an immediate threat to the refugees all over the world. Unlike other people, refugees cannot socially distance from others, as they have to live in a small area together. This makes them even more prone to the pandemic and reduces the chance of recovery[8].

· Generations residing in one house can make it hard to protect older family members, as well as those of any age who have certain underlying medical conditions, such as diabetes or asthma. This may also make it difficult to isolate those who are sick.

· Living in neighborhoods that are far from health care, grocery stores or pharmacies can make it harder to avail them.

· Refugees rely on public transportation which are limited in some areas, especially during the current pandemic.

· Non access to proper technologies and low literacy levels make the refugees ignorant of the required information

· Less integration into the community limits potential support and links to friends, religious leaders, or community organizations.

Work circumstances:

With the help of resettlement agencies, refugees often work in entry-level jobs, where the type of work, and workplace policies, can increase the risk of getting sick with COVID-19. Two factors may increase risk:

· **Being a critical worker:** The risk of infection may be greater for refugee workers in essential industries, such as meat-packing plants, grocery stores, and factories. These workers often are required to be at the job site despite outbreaks in their communities, and many can't afford to stay home.

· **Not having sick leave:** Refugees without paid sick leave may be more likely to keep working when they are sick or be exposed to someone who is sick with COVID-19.

Underlying medical conditions and lower access to care:

Some refugees have underlying medical conditions and face barriers to healthcare, which may put them at increased risk of COVID-19. These include:

· **Lack of health insurance:** Refugees can access healthcare in the early resettlement period, with the help of resettlement agencies. However, after their short-term health insurance expires, up to 50% of refugees may be uninsured[9].

· **Language barriers:** Language barriers can affect healthcare access at every stage, from making an appointment, to relaying health concerns, to filling a prescription, or to taking medicine as prescribed. Healthcare providers, clinics, or community groups can provide interpretation services to help fill these gaps.

· **Underlying medical and mental health conditions: R**efugees may have certain underlying medical conditions (for example, diabetes) that put them at increased risk of more severe illness from COVID-19, as well as mental health conditions (e.g., post-traumatic stress disorder and depression).

· **Stigma and perceived discrimination:** These issues may prevent refugees from seeking testing or care or being honest with their healthcare providers about their health needs.

Despite these barriers, refugees have the support of resettlement agencies, community groups, state refugee coordinator sexternal icon, and healthcare providers. State, local, and community response to COVID-19

should include working with these resource groups in their COVID-19 response efforts, who are trusted members of refugee communities.

United Nation Agency and WHO Agreement Regarding Refugees health Condition:

The World Health Organization (WHO) and the United Nations High Commissioner for Refugees (UNHCR), the UN Refugee Agency, have signed a new agreement to strengthen and advance public health services for the millions of forcibly displaced people around the world to support ongoing efforts to protect some 70 million displaced people from COVID-19 infection. Around 26 million are refugees, 80% of whom are sheltered in low and middle-income countries with weak health systems[10].

"The principle of solidarity and the goal of serving vulnerable people underpin the work of both our organizations," said Dr Tedros Adhanom Ghebreyesus, WHO Director-General. "We stand side by side in our commitment to protect the health of all people who have been forced to leave their homes and to ensure that they can obtain health services when and where they need them..."

Extensive collaborative efforts from WHO and the Government of Serbia have seen refugees and migrants provided COVID-19 protection equal to that of the host population in the spirit of universal health coverage.

"WHO is working with governments around the world to ensure supply chains remain open and lifesaving health services are reaching all communities," said Director-General Dr Tedros Adhanom Ghebreyesus. Health education materials in 7 languages were distributed to all migrant centres and NGOs that work with migrants in Serbia. Personal protective equipment (PPE), personal hygiene products and disinfectant were delivered to asylum and migrant reception centres throughout the country.

WHO has primary responsibility for promoting the health of refugees and migrants, with a current focus on prevention and responses during the COVID-19 pandemic. Refugees and migrants face the same health risks as host populations, but due to various barriers – geography, facilities, discrimination, language and costs – they may lack access to the health services required to control and treat illness. A recently published Lancet article warned of the increasing risks facing refugees and migrants, particularly those in camp settings where simple preventative measures like social distancing and self-isolation are harder to implement.

In countries that host a large number of refugees and migrants, WHO country offices have been working with ministries of health and other partners in their efforts to prevent and control COVID-19. Who is also collaborating with other UN agencies to provide interim technical guidance on scaling up outbreak readiness in humanitarian situations, including refugee camp and non-camp settings, Similar guidance has been released specifically for countries in the European and Eastern Mediterranean regions where refugee populations are large.

The WHO Eastern Mediterranean Regional Office (EMRO) has developed a reporting system to monitor the occurrence and trend of COVID-19 among displaced populations in camps and non-camps settings. The WHO Country Offices in Djibouti, Sudan, Lebanon, Syria and Yemen report rumours immediately and aggregate data every week. Also, to enhance interagency coordination for country support, WHO EMRO in collaboration with IOM, ESCWA and ILO, has established a Regional Taskforce on COVID-19 and Migration/Mobility.

Conclusion:

The pandemic has not only forced the general public to health related hardships but also the refugees. But it is also true in the US: although testing is ostensibly free, refugees and people of color are less likely to be referred for it. A key reason is the new "public charge rule," under which immigrants who have used (or seem likely to use) public benefits have their visa applications rejected.

There can be the difference between life and death not only for recipients, but also for anyone with whom they come in contact. And tests need not even be expensive: The Mass General Brigham Center for COVID-19 Innovation Direct-to-Consumer Working Group.

COVID-19 may disproportionately affect the poor

and marginalized, but that does not mean that everyone else is safe. On the contrary, the only way to address the pandemic effectively is to ensure that everyone – rich or poor, refugee or local – is protected. The health of each depends on the health of all.

Therefore, the local and national governments must incorporate refugees into pandemic-response plans, as the UNHCR and the World Health Organization have, by guaranteeing access to masks, hand sanitizer, testing, tracing, treatment, and ultimately, a vaccine. Portugal has exemplified adaptive leadership and pioneered such an inclusive approach, temporarily giving all migrants and asylum seekers with pending applications access to the health-care system.

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Original Article

Psychosocial Impact of Lockdown Due to Novel Corona Virus Covid 19 among Medical Undergraduate Students

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Abstract

Background: The outbreak of Novel Corona Virus Disease (COVID-19) which started in the late 2019, in Wuhan in Hubei province of China because of its high infectivity and fatality rate, has caused universal psychosocial impact on people Mass fear of COVID-19, termed as "Coronaphobia", has generated a plethora of psychiatric manifestations across the different strata of the society. So, this study has been undertaken to define psychosocial impact of COVID-19 on Medical graduants with an objective to assess the reasons for breaking the lockdown and the level of psychosocial impact of lockdown on a Medical graduants during the COVID-19 lockdown period. A cross sectional prospective study was done on 285 students of First to final year MBBS **V**alidated Psychosocial Impact Scale was used for data collection. The results showed the intensity of negative impact of lockdown on the students studying in medical college. 18.60% students showed normal response to the lockdown situation in this COVID 19 pandemic. 47.72% students showed borderline negative impact of lockdown. 29.12 students had mild negative impact, 4.56 % students had moderate negative impact and no student found to be in range of severe negative impact of lockdown.

Key words: *COVID 19, psychosocial impact, Coping, Medical students.*

Introduction

The outbreak of Novel Corona Virus Disease (COVID-19) which started in the late 2019, in Wuhan in Hubei province of China because of its high infectivity and fatality rate, has caused universal psychosocial impact on people by causing mass hysteria, economic burden and financial losses. Mass fear of COVID-19, termed as "Coronaphobia", has generated a plethora of psychiatric manifestations across the different strata of the society. (1)

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The 2019 Corona Virus Disease (COVID-19) outbreak has been declared an international public health emergency on January 30, 2020 by the World Health Organization (WHO) as the disease, first reported from China in December 2019, continues to surge through the continents affecting many countries from Europe, America and Asia severely and is still widening its burden of disease (2). A wide fragment of world's population currently is primarily restricted to their homes, owing to nationwide lockdowns and home-confinement strategies implemented in the majority of the COVID-19-hit countries after China to prevent further disease transmission (3,4). This unpredictable, fast spreading infectious disease has been causing universal awareness, anxiety and distress, all of which according to WHO are natural psychological responses to the randomly changing condition(5). Adverse psychosomatic outcomes among common people are nevertheless expected to increase significantly due to the pandemic

itself and also due to constant flow of readily available information and reinforced messaging obtained via online social networking services of almost all forms. As a consequence, rapidly expanding mass hysteria and panic regarding COVID-19 may beget enduring psychological problems in public from all the socioeconomic domains, which could potentially be even more detrimental in the long run than the virus itself [6]. Prior studies elucidated that mental well-being had been heavily affected in this kind of global pandemic [7, 8]. It creates a negative concept that can have an impact on one's mental and physical well-being. Psychological or psychosocial distress is the stress induced on our mind. It is related to both external and internal factors.[9] Therefore, it is imperative to determine the various possible ways in which COVID-19 pandemic will be impacting the world's mental health [10,11,12].

So, this study has been undertaken to define psychosocial impact of COVID-19 on Medical undergraduate students.

Aim

Assessment of the level of psychosocial impact of lockdown on the students of Medical profession during the COVID-19 pandemic lockdown period.

Objectives:

1. To assess the reasons for breaking the lockdown by Medical students.

2. To assess the level of psychosocial impact of lockdown on the students of Medical profession during the COVID-19.

Materials & Methods

Study Setting

The study was conducted in Wardha city of Maharashtra state which was an initial Green Zone for 50 days and later declared an Orange Zone. The students were from Jawaharlal Nehru Medical College residing at home.

Inclusion Criteria:

1. Students from First MBBS to Final MBBS

2. Students from Jawaharlal Nehru Medical college, DMIMS

3. Students who are staying at home in lockdown situations

4. Students who are willing for participation.

Exclusion Criteria:

1. Students from other constituent colleges of DMIMS i.e. Dental, Ayurvedic, Nursing, Physiotherapy

2. Students who are staying at hostels in college campus during lockdown period in COVID 19 pandemic.

3. Students who are not willing for participation.

Study design: Cross Sectional Prospective study. An Online survey with Google forms of questionnaire

Study population: College students from the disciplines of Medical sciences (From First To Final Year MBBS) in the age group of 18- 25 years both Male and Female included in the study.

Study duration: June-July 2020

Sample Size: 285

Tool for data Collection:

Psychosocial Impact Scale:

To evaluate the psychosocial impact of lockdown, the scale used is developed by Dr. Vaishali Tendolkar, Professor, Mental Health Nursing, Datta Meghe College of Nursing, Nagpur, and Maharashtra. (r=0.85).[13,14,15,16] The Part I caters to the demographic information of the students and includes, age, residence, course of study, year of study, types of family, number of family members, during lock down living with, etc. Part II of the tool evaluated the reasons for breaking the lock down and number of times the lock down was broken by the student in past 15 days. The Part III of the tool was a self-rating scale with 35 statements for which response was elicited on 5 point scale. The response was to be graded based on how often the student felt that way in lockdown days. Higher the response higher was the impact. Each item had minimum score of 0 and maximum score of 4. The responses ranged from minimum 0 to maximum 140. The score range of 0 – 25 was considered as normal response to the event of lock down. 26 – 45 score was

considered as borderline negative impact. Scores range from 46 – 75 was treated as mild negative impact, 76-105 was put into moderate negative impact category and scores range 106 – 140 were put into severe negative impact category.

Methods of data collection:

The students were contacted through mobile messaging services such as Watts App. The link of the Google form was shared with students. The participation in the survey was purely on voluntary basis. The investigators were accessible through phone for clearing their doubts.

Data Analysis:

The collected data was coded and analyzed on the basis of the objectives of the study. Descriptive statistics of mean, standard deviation, frequency percentage was used to describe the students as per their responses.

Limitations:

1. The study is limited to the students of Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha (M.S) in Central India.

Results

Part I - Demographic information of the students

The distribution of students according to their demographic characteristics reveals that the mean age of the students is 20.62+ 1.56 (18-26 years). Total number of students were 285, 42.80 % were male and 57.20% were female participants. All the students were from MBBS course. 34.03% participants were studying in first year, 20.35% in second year, 29.82% in third year and and 15.78% in final year. 16.49% students were from rural areas, 67.72% students lived in urban areas and 15.79% students residing in semi urban areas. Maximum students belonged to Hindu religion, 5.61% belonged to Muslim religion, 1.40% belonged to Christian religion, 0.35% belonged to Buddhist and 1.05% belonged to Jain religion. The students belonged to nuclear family was 74.04% and 25.96% belonged to joint Family. 24.21% students had 2 to 3 family members in their home. 45.96% students had 4 members in their family and 17.54% student had 5 members in their family and 12.28% students had more than 5 members in their family. 1.75% students revealed that they were living alone during this lockdown period, 1.40% students residing with friends, 94.74% students residing with parents and 2.11% residing with relatives.

Part II- Distribution of students according to practices during lockdown

Part II of the tool evaluated the reasons for breaking the lock down and number of times the lock down was broken by the student in past 15 days. The number of times students went out of their house and also mentioned the reasons for leaving their house during the period of lockdown in COVID 19 situation. 67.7% students have been out on roads during lockdown. 7% students went out for their some job, 46.8% students were out for medicines, 31% students out for grocery and vegetables, 27.5% students went out to meet their friends 2.9% students were out as they had to attend funeral, 10.2% students went out to attend family function, 24.9% students went out to complete academic assignments. 53% students went out to refresh themselves, 29.5% students went out to help neighbor/ relatives.

Part III- Distribution of students according to psychological impact of lockdown

It shows the responses of the participants describing their feeling and state of mind during the period of lockdown. 39.29% participants felt that Govt. has straddled their activities unnecessarily, 95.43% participants were happy for being with their family, 92.97% students worried about their educational loss, 46.32% students bored seeing same faces day and night. , 84.56% participant didn't like being instructed every now and then, 79.31% participants felt that they were expected to study round a clock. 65.62% felt that their privacy is challenged. 47.37% participants were feeling helpless as they have to be at home, 28.41% students were feeling feel they are being jailed for no reason, 90.17% participants were relieved that they are getting enough time to study, 65.26%, participants were feeling lonely as they can't meet their friends, 71.93% students felt social media contact with friends and relatives is not sufficient. 46.67% students regretted to be the part of this lockdown, 37.89% students didn't feel like eating, 75.09% felt that they have slowed down in their actions, 74.39% students feeling irritabilitility in this lockdown days, 52.98% participants having sleepless night, 51.58%

students worried about existence of human race. 31.93% students cried in these days, 50.17% felt that they have become touchy /sensitive because of being in one place, 48.08% felt hopeless about their future, 54.39% felt depressed when they read/view on social media about lockdown, 47.01% students have become suspicious these days, 53.34% felt that third world war has started, , 86.34% felt uncertainty about the duration of lockdown , 28.08,% students felt that they were victimized during lockdown, 90.17% participants felt sad when they heard about the death on news channels, 40.69% participant didn't think that they will be able to meet their friends with same warmth again, 60% students thought social distancing has distanced people from one another, 79.31% participants felt that lockdown shattered their dreams, 65.62% participants were worried about their economic loss, 47.37% participants feared that they may be jobless 28.41% students felt hopeless about their financial condition. 90.17% participants didn't like working these days.

Table 01: Distribution of students according to level of psycho-socio impact

Psycho-socio impact	Score Range	No of students	Percentage
Normal	0-25	53	18.60
Borderline	26-45	136	47.72
Mild Negative	46-75	83	29.12
Moderate Negative	76-105	13	4.56
Severe Negative	106-140	0	0
Total		285	100
Mean±SD		40.43 ± 17.50	
Mean% ± SD		28.88 ± 12.50	
Range		2-99	

The above table and the graph show the intensity of negative impact of lockdown on the students studying in medical college. 18.60% students showed normal response to the lockdown situation in this COVID 19 pandemic. 47.72% students showed borderline negative impact of lockdown. 29.12 students had mild negative impact, 4.56 % students had moderate negative impact and no student found to be in range of severe negative impact of lockdown.

Discussion

In early December 2019, an acute respiratory infectious disease resembling pneumonia of unknown cause was first reported on 31 December 2019 and on 30 January 2020 the outbreak was declared a Public Health Emergency of International concern causing increased mortality which was reported in Wuhan in Hubei Province in China. On 11 February 2020 the World Health Organization (WHO) announced a name for this novel virus infection COVID-19 has been responsible for the symptoms. The disease had a widespread all over the world with rapid rate affecting millions of people across the globe. Most of the countries are forced to take drastic steps of lockdown to curb the spread of the disease. In India too lockdown was forced as per the WHO guidelines forcing to shut down all establishments, Institutions and Commercial trade. These lead to varied problems in the society affecting the people of all strata due to either financial loss, job loss or academic loss due to fear of anxiety and apprehension. The education got halted due to lockdown. The students got stranded at their places and were forced to study through online mode to complete the academic session and undertake the examination. Students with no proper reading material

at home had extra burden of studying teaching material available through net with the available apparatus. Hence through structured questionnaire based study the psychological impact of lockdown in the Medical students was evaluated. It is found that the fear of the disease and lockdown situation has affected the mental status of Medical graduants where 47.72% showed borderline, 290. 12% of students showed Mild and 4.56% showed Moderate Negative impact of lockdown. None of the students showed severe negative impact. It is imperative to do the psychological counselling of the students so that they can cope up with the prevailing situation. Investigators didn't get the exact supporting studies for these results but in a study by P. Gonzalez revealed that moderate to extremely severe scores of anxiety, depression, and stress were reported by 21.34%, 34.19% and 28.14% of the students of Spanish university, respectively. A total of 50.43% of respondents presented moderate to severe impact of the outbreak.[17] In another study respondents from the age group of 21-40 years showed that hypochondriacal thoughts showed highest significance with depression (Z=-4.528, p=0.000) and stress (Z=-6.606, p=0.000) compared to other domains. Excessive fear of contamination & contracting COVID-19 showed highest significance with anxiety (Z=-6.481, p=0.000).[18]

Conclusion

Proper counseling and regular follow up of the students informing them to follow the guidelines issued by WHO, ICMR and the Government agencies from time to time will not only help to curb this disease but also will help all to cope up and live a normal healthy life.

Ethical Clearance- Ethical clearance is obtained from the Institutional Ethical Committee of JNMC, Sawangi (Meghe), Wardha. (Vide Reference no: DMIMS (DU)/IEC/2020-21/9032)

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Ajan Vruksha/ Khandu Chakka (*Ehretia Laevis Roxb)*. Plant Leaves as A Effective Healer in Chronic Varicose Vein Ulcer. – A Case Report

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Abstract

Introduction— Varicose vein ulcer is caused by abnormal function of veins due to chronic vein insufficiency, vein damage, ageing, obesity, congestive heart failure. Ehretia Laevis Roxb. plant is traditionally used for wounds[(1)] · Also its efficacy is tested in various wounds except chronic varicose ulcer.[(2)]

The main symptoms of the patient and the important clinical findings:- Patient having swelling, redness at right foot, yellowish pus discharge, blackness of skin and presence of ulcer since three years with severe pain and inability to perform his regular duties

The main diagnoses, therapeutics interventions, and outcomes:- Patient having chronic varicose vein ulcer at right leg since three years. Ehretia Laevis Roxb. leaves paste was prepared under all aseptic precaution and daily dressing of Ehretia Laevis Roxb. paste done on wound. Severe pain was reduced within 48 hrs after application of paste, which was significant finding. Wound was healed within 40 days without any complication and use of antibiotics and pain killer

Conclusion— Patient was very happy as he got immediate relief from pain and his wound was healed without any invasive procedures by application of Ehretia Laevis Roxb. leaves paste.

***Key Words**: - Chronic Varicose Vein Ulcer, Khandu Chakka, Ajan Vruksha, Ehretia Laevis Roxb.*

Introduction

In western word 10-20 % and in India 5% population is affected by varicose vein.[(3)] Varicose vein ulcer is caused by abnormal function of veins due to chronic vein insufficiency, vein damage, ageing, obesity, congestive heart failure. Ehretia Laevis Roxb. plant is traditionally used for wounds. Also its efficacy is tested in various wounds except chronic varicose ulcer. Plant has various chemical compounds useful in wound healing. This plant is named as Khandu Chakka and also known as Ajan Vruksha.[(4)] Ehretia Laevis Roxb. plant has anti microbial activity against various gram positive and gram negative organism. [(5-6).]

Patient Information:- Mr. Sridhar Khandar Age-65, Vadhona Bazar, Yavatmal(MS) was reported at MGACH & RC with leg ulcer with severe pain and insomnia due to chronic pain.

Clinical Findings:- Patient having chronic varicose vein ulcer at right leg since three years with severe pain and inability to perform his regular duties. Patient was reported at MGACH & RC with swelling, redness at right foot, yellowish pus discharge, blackness of skin and presence of ulcer. Patient has no history of Diabetes Mellitus, Hyper tension and no specific family history of major diseases. Patient was taking allopath treatment including pain killer and antibiotics since two and half years. Then he has taken homeopathy and ayurvedic

treatment. But he has not get relief for wound and pain. Due to chronic pain patient was suffering from insomnia and irritation.

Timeline: - Patient was suffering from ulcer with pain since three years

Diagnostic Assessment:- Patient was investigated for complete blood culture, bleeding time and clotting time, HIV,HBs Ag and Random Blood Sugar.

Wound is measured by following formula on every alternate day.

SAI – SACi /SAI x 100 = _____% reduction

(*SAI= surface area (LxW) on admission, SAC = surface area currently)

Sr. No	Name of investigations	Results
1	Hb%	14.1.gm%
2	MCV	100 Cub.Micron
3	MCH	30.6 Pico gm
	MCHC	30.6%
4	Total RBC count	4.61 millions/cu/mm
5	RDW	11.5 %
6	HCT	46.2%
7.	Total WBC count	9500/cu.mm
8	Monocytes	3%
9.	Granulocytes	74%
10.	Lymphocytes	21%
11	Eosinophils	2 %
12.	Basophils	0 %
13.	Total Platelets Counts	5.63 Lacs/cu.mm
14	Bleeding time	190 seconds
15	Clotting time	330 seconds
16	HIV	Non Reactive
17.	HBsAg	Non Reactive
18.	Random Blood Sugar	113mg%

Wound swab was sent for culture and sensitivity test. Klebsiella pneumonia gram-negative, Proteus mirabilis is a Gram-negative bacterium, Methicillin sensitive coagulase positive staphylococci organism were found in culture report. Klebsiella pneumonia gram-negative was sensitive to Amikacin, Ciprofloxacin, Ceftazidime, colistin, Co-trimaxazole, Gentamicin, tetracycline. Proteus mirabilis is a Gram-negative bacterium was sensitive to Amikacin, Ciprofloxacin, Colistin, Co-trimoxazole, gentamicin and resistant to Ceftazidime, tetracycline. Methicillin sensitive coagulase positive staphylococci was sensitive to Amikacin, Ciprofloxacin, Linezolid, Penicillin, Vancomycin and resistant to Ciprofloxacin, Erythromycin.

Therapeutic Intervention:- Ehretia Laevis Roxb. leaves paste was prepared under all aseptic precaution and daily dressing of Ehretia Laevis Roxb. paste done on wound.

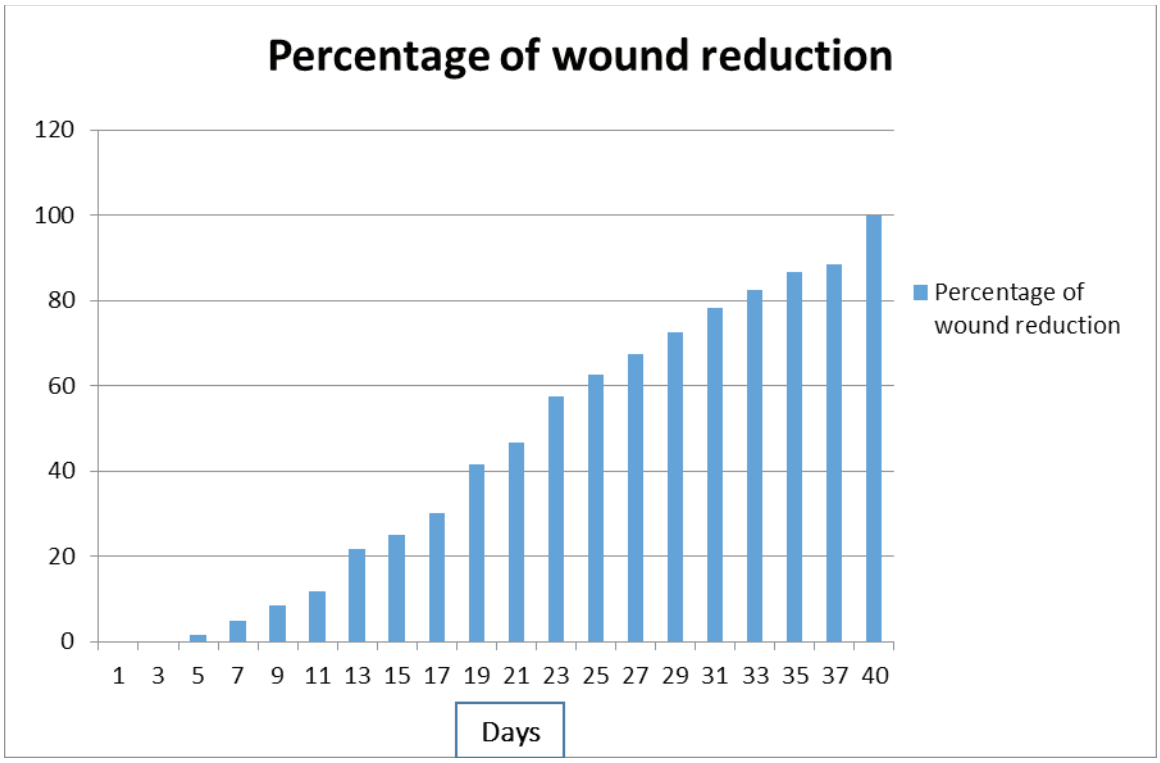
Follow-up and Outcomes: - Severe pain was reduced within 48 hrs after application of paste, which was significant finding. After two days there was no pain. Due to no pain his insomnia and irritation improved. Slough was removed after four days and granulation tissues were appearing from forth day. Wound was healed within 40 days without any complication and use of antibiotics.

Wound is measured by following formula on every alternate day by following formula

SAI – SACi /SAI x 100 = _____% reduction

(*SAI= surface area (LxW) on admission, SAC = surface area currently)

Sr. No.	Day	Percentage of wound reduction
1.	1	0%
2.	3	0%
3.	5	1.6%
4.	7	5%
5.	9	8.33%
6.	11	11.66%
7.	13	21.66 %
8.	15	25 %
9.	17	30 %
10.	19	41.66
11.	21	46.66
12.	23	57.5 %
13.	25	62.5 %
14.	27	67.5 %
15.	29	72.5 %
16.	31	78.33 %
17.	33	82.5 %
18.	35	86.66 %
19.	37	88.33 %
20.	40	100 %



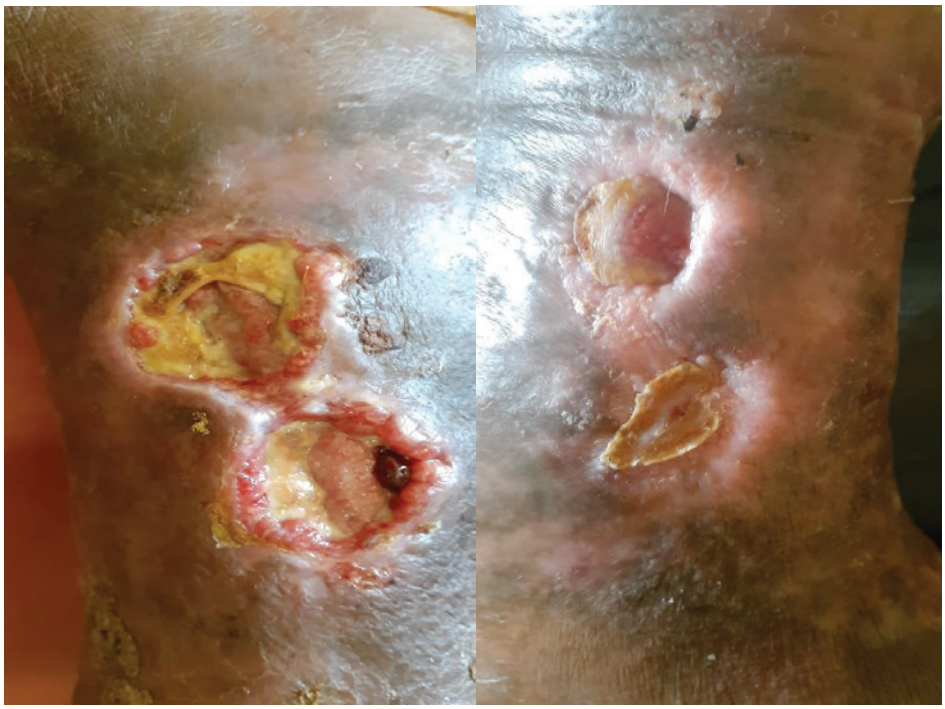


Figure:-1

(Chronic varicose vein ulce treatment)

Figure:-2

(Chronic varicose vein ulcer after before yreatment)

Discussion

There is swelling in varicose vein, causes skin changes and damage. When there is swelling, there is lack of blood supply and supply of required nutrition and oxygen for tissues. This hampers healing procedures. Due to continuous tension of blood in veins there is continuous chance of heavy bleeding of blood even by minor injuries, but it may control by Ehretia Laevis Roxb. laves paste as it has blood clotting properties.[7]

There is chance of blood coagulation. Up to three of individuals having varicose vein suffering from very pain full. blood clots. In about 25% patients suffering from blood clot landed into the deep vein thrombosis.[8] It is medical emergency and requires immediate interventions. The main risk of deep vein thrombosis is embolism in lungs and brain.

Chronic varicose veins result into stagnation of deoxygenated blood There is constant tension in tissues, there is leakage of fluids into the encompassing tissues, results in the swelling and results in chronic non healing venous ulcer. This results in delay in healing even by minor injuries. Reduction in swelling results in proper supply of nutrition to injured tissue. Application of Ehretia laevis Roxb. reduce swelling as it has anti inflammatory property and helps for proper supply of required nutrition. As swelling and pain is reduced by application of Ehretia laevis Roxb. leaves paste patient has got relief from insomnia and irritation within three days . As his stress was reduced he can continue his routine works.

Patient Perspective:- :- Patient was very happy as he got immediate relief from pain and his wound was healed without any invasive procedures, antibiotics and pain killer. This results in financial saving of needy farmer.

Conflict of Interest:- Nil

Source of Funding:- Self

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Internal Use of Ajan Vruksha/ Khandu Chakka *(Ehretia Laevis Roxb)*. Plant Leaves Powder in Shoulder Pain Management. – Case Report

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Abstract

Introduction— Shoulder pain and stiffness are common features among working women. It is affecting their day to day life style.

The main symptoms of the patient and the important clinical findings:- Patient was suffering from severe pain in right shoulder with restricted movement of right shoulder from 8 days. Patient was assessed by (SPADI) Shoulder Pain & Disability Index.

The main diagnoses, therapeutics interventions, and outcomes:- *Ehretia Laevis Roxb*. leaves powder capsules was made up of 250 mg. Patient was advised to take two capsules in morning and two capsules at night after food. Daily 1 gm powder was administered internally for 7 days

On the day zero pain score was 80%, on third day of treatment pain score was 54% and on seventh day pain score was 0. On the day zero disability score was 95%, on third day disability score was 55% and on seventh day score was 0.

Conclusion—. Patient cured from pain and shoulder disability by internal use of *Ehretia Laevis Roxb*. leaves powder within seven days.

***Key Words**: - Pain Management, Khandu Chakka, Ajan Vruksha, Ehretia Laevis Roxb. Shoulder Pain.*

Introduction

Shoulder pain and stiffness are common features among working women. It is affecting their day to day life style. Musculo skeleton pain is major burden on individual and health care system

The prevalence of shoulder pain in the United Kingdom population was 7% [(1).]

1069 subjects were participated in the study, 245 (22.9%) reported with shoulder pain in Rohtak District of Northern India. [(2)]

Shoulder pain causes inability to do daily routine work. Prevalence of shoulder pain is as 67 percent for whole life. The incidence of shoulder pain is between 0.9%-2.5% and prevalence rate is from 6.9-26% for point prevalence to 6.7-66.7% for lifetime prevalence.[(3)]

Shoulder pain may caused by bursitis & tendinitis, tear of tendon, arthritis, fracture, malignancy, infection, and neurotic problems.

Common treatment including avoiding overexertion or overdoing activities, involves rest. medications, surgeries.

Ricinus communis & *Commiphora Mukul, Boswellia serrata*, *Withania somnifera,Dashamool,*

Mahayograj Guggul, Vatavidhwansa, Yograj Guggul, Suvarna Bhasma commonly used for pain management in Ayurveda. [4]

Analgesic drugs act on the peripheral and central nervous systems. Painkillers include (NSAIDs) such as the salicylates, ibuprofen & COX-2 inhibitors, opioid drugs such as morphine and oxycodone, and acetaminophen.

Leaves and Bark of Ehretia Laevis Roxb. is being traditionally used for Pain Relief, wound healing and minor fracture.[5]

Ehretia Laevis Roxb. contains chemical compounds like Naphthoquinone derivative, Baurenol, ursolic acid, Rutin, Phytol, α and β amyrin, Betulin & Betulinic acid, Lupeol, β-sitosterol, Histidine, Hexadecanoic acid, Benzoic acid, Arachidonic acid show analgesic, antinociceptive and anti inflammatory activities.[6]

It has anti microbial properties [7-8] and its wound healing[9] property is also proven on scientific ground.

Patient Information:- Mrs. Archana Dilip Sawarkar Age-42, Sawangi(M) Wardha,(MS) India was reported at MGACH & RC Salod(Hi), Wardha(MS) with severe pain in right shoulder with stiffness.

Clinical Findings:- Patient was suffering from severe pain in right shoulder with restricted movement from 8 days. She has taken hot fomentation at her house but did not get any relief. There was no history of trauma, old injury, diabetes mellitus, and hyper tension.

Timeline: - Patient was suffering from severe pain in right shoulder from 8 days.

Diagnostic Assessment:- Patient was investigated for complete blood culture, Random Blood Sugar, Kidney Function Test, Liver Function Test, Lipid Profile, Serum Calcium, Rheumatic Arthritis. All tests were within normal limits.

Patient was assessed by (SPADI) Shoulder Pain & Disability Index [10]. on first, third and seventh day.

(SPADI) Shoulder Pain and Disability Index

Pain scale

0 = no pain to 10 = the worst pain imaginable

Table:- 1

	Day- 0	Day- 3rd	Day-7th
At its worst?	8	6	0
When lying on the involved side?	8	6	0
Reaching for something on a high shelf?	8	5	0
Touching the back of your neck?	8	5	0
Pushing with the involved arm?	8	5	0
Total Pain score	40/50X50= 80%	27/50X100= 54%	0%

Disability scale

0 = no difficulty to 10 = so difficult

Table:- 2

	Day-0	**Day-3rd**	**Day-7th**
0 = no difficulty and 10 = so difficult it requires help. Washing your hair?	8	5	0
Washing your back?	10	5	0
Putting on an undershirt or jumper?	10	5	0
Putting on a shirt that buttons down the front?	10	5	0
Putting on your pants?	9	5	0
Placing an object on a high shelf?	10	5	0
Carrying a heavy object of 10 pounds (4.5 kilograms)	10	7	0
Removing something from your back pocket?	9	7	0
Total Disability score	76/80X100= 95%	44/80X100= 55%	0%

Total SPADI Score

Table:- 3

	Day -0	**Day-3rd**	**Day-7th**
Total SPADI Score	89.23 %	54.61%	0 %

Therapeutic Intervention:- *Ehretia Laevis Roxb*. leaves were shed dried, powdered and filled in capsules in GMP And FDA approved Dattatrya Ayurved Rasashala Wardha(MS). Each capsules was of 250 mg. Patient was advised to take two capsules in morning and two capsules at night after food. Daily 1 gm powder was administered internally for 7 days.

Follow-up and Outcomes: - Patient's shoulder pain was assessed on the day of reporting, 3rd day and on seventh day by Shoulder Pain and Disability Index (SPADI). On day zero when treatment started SPADI was assessed. On the day zero pain score was 80%, on third day pain score was 54%. Within three days patient got significant pain relief. And on seventh day score was 0%. Patient got complete relief from pain.

On the day zero disability score was 95%, on third day disability score was 55%. Within three days patient got significant relief from disability. And on seventh day

disability score was 0%. Patient got complete relief from disability on seventh day along with complete relief from pain.

Patient cured from pain and shoulder disability by internal use of *Ehretia Laevis Roxb*. leaves powder within seven days.

Discussion

Ehretia Laevis Roxb. plant is a being used for various ailments traditionally. It is commonly used for pain relief, wound healing and minor fractures.

Velappan et al experimented methanolic extract (500 mg/kg) of the *Ehretia Laevis Roxb.* leaves in in mice,and found that it reduces paw volume by 56%, paw oedema to 60% [(11).]

N.Jyothirmai et al stated that the chloroform, methanolic and aqueous extract of Ehretia laevis reduces inflammation in animal model.[(12)]

Subodh et al shown anti arthritic activity of medicated oil of Ehretia Laevis Roxb. leaves by local application in human beings . [(13)]

Internal use of Ehretia Laevis Roxb. leaves powder is tested first time in this study for pain management in shoulder pain and assessed by(SPADI) Shoulder Pain & Disability Index And found very effective without any untoward side effects. Patient got near about 50% relief for his complaints within three days of administration and complete relief within seven days.

This study will pave the path for further study of internal use of *Ehretia Laveis Roxb*. in various ailment including pain management.

Also use of this herbal plant in pain management will minimise the side effects of modern medicine like Non-steroidal anti-inflammatory drugs.

Patient Perspective:- :- Patient was very happy as she got complete relief from pain and disability of her shoulder without using modern medicine and side effects of modern medicine was avoided.

Conflict of Interest:- Nil

Source of Funding:- Self

Consent of Patient:- Taken

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Evaluation of *Ehretia Laevis Roxb.* (Khandu Chakka/Ajan Vruksha) in the Wound Healing Adjudged by Histological Examination of the Tissue

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Abstract

Many herbal plants are mentioned in Ayurveda for wound healing. Folklore medicine for various ailments including wound healing is being practiced in India. *Ehretia Laevis Roxb*. contains many such chemical compounds useful for promotion of healing & repair. Tissue renewal goes hand in hand with inflammation influenced by multiple processes. *Ehretia Laevis Roxb*. called Khandu Chakka & Ajan Vruksha and traditionally being used for wound healing, body pain & minor fractures in the form of local application by folklore in the state of Maharashtra India. Objectives of the present study is to know the effect of *Ehretia Laevis Roxb*. in the wound healing on the histological parameters in animal model. Phenytoin ointment as a control was used. The present study has found put that leaf extract paste of *Ehretia Laevis Roxb.* has enhanced the process of wound healing ascertained on histological parameters as compared to topical phenytoin application. *Ehretia Laevis Roxb.* paste is found to be immensely useful at wound healing as compared to Phenytoin. *Ehretia Laevis Roxb.* is concluded to promote the healing significantly as observed by feature of granulation, collagenisation & re epithelisation of the wounds suggesting early healing by primary intension

***Key words**:- Ehretia Laevis Roxb., Phenytoin, Khandu Chakka, Ajan Vruksha, Wound healing , Histological study.*

Introduction

Many herbal plants are mentioned in Ayurveda for wound healing. Folklore medicine for various ailments including wound healing is being practiced in India. *Ehretia Laevis Roxb*. contains many such chemical compounds useful for promotion of healing & repair. These chemical compounds act as anti bacterial, anti fungal, anti viral, anti-inflammatory, anti parasite, analgesic, anti-oxidant, anti-apoptotic, clotting of blood plasma, improves immune responses, promotes collagen synthesis & regeneration of tissue. These properties are important for phenomenon of wound healing. Both of these processes can be mediated by external application of herbal extract of *Ehretia laevis Roxb*.[(1)]

Tissue renewal goes hand in hand with inflammation influenced by multiple processes. These processes includes the regeneration of cells by two underline processes such as wound healing by primary intension and wound healing by secondary intension.

Ehretia Laevis Roxb. called Khandu Chakka & Ajan Vruksha and traditionally being used for wound healing, body pain & minor fractures in the form of local application by folklore in the state of Maharashtra India. ([1])

Infections, old age, local tissue stress, metabolic disorder, diabetics, chemotherapy drugs, obesity, alcohol, habits such as chronic smoking, mal

nourishment influenced wound healing. Such situations require bearing of high expenditure on modern medicine drugs like expensive antibiotics.

There are not many studies in literature that has proven the role of *Ehretia Laevis Roxb*. in the wound healing, by histological evidences gathered by time graded biopsies collected from the site of wound after application of *Ehretia Laevis Roxb*. leaves paste.

Modern medicine uses Phenytoin for local application at enhancement of wound healing. [3]. Most of the studies reviewed for the present work & compared the results of Phenytoin with that of *Ehretia Laevis Roxb*.[3-9]

Study has been planned to evaluate & address their research gap involving histological evaluation.

Objectives of the present study is to know the effect of *Ehretia Laevis Roxb*. in the wound healing on the following parameters, that unable to judge the processes of wound healing secondary experimental burn into the Albino Rabbits.

· Quantification of granulation tissue after application of Ehretia Laevis Roxb. compared to Phenytoin paste application.

· Gradation of inflammatory infiltrates after application of paste of Ehretia Laevis Roxb. compared to Phenytoin.

· Quantification & pattern of collagen after application of paste of *Ehretia Laevis Roxb*. compared to Phenytoin.

Review of Literature

Drug Review:-

Ehretia laevis Roxb. known as Khandu Chakka & referred to Sant Dnyaneshwar Alandi Maharashtra, India too called as Ajan Vruksha[10], when he took Sanjivan Samadhi. Folklore believes this plant for its plenty of medicinal healing properties. [1]

Plant Description:- Following is the binomial nomenclature for the plant in the botany.

(Figure 1 & Figure 2)

Planta Tracheophyta Magnoliopsida Boraginales Boraginaceae

Ehretia Ehretia Laevis (Roxb)

Figure 1 **Figure 2**

Ehretia Laevis Roxb. **plant shows anti microbial activity on salivary microflora , B.subtilis , S. aureus, E. Coli. Pseudomonas aeruginosa[11-14]**

Materials and Methods

Study Material:

- Healthy 10 Rabbits were selected from DMIMS animal house. Then they were divided into two groups of 5 in each group.

- Leaves paste was prepared under all aseptic precautions. Leaves paste was applied on wound in first group and considered as Group A.

- Phenytoin ointment as a control was used in second group and considered as Group B.

- Animals were anesthetized before creating wound

- The skin shaved, disinfected with 70% alcohol and injected with 1 ml of Lignocaine HCl (2%, 100 mg/5 ml).

- Approval from institutional animal ethical committee was taken.

External wound protocols

· Physically external wound was made by burn injury of 2X2cm. The edges of the wound was sampled by forcep biopsy.

· Daily leaves paste was applied in Group A and Phenytoin ointment in group B. Biopsies for assessment of healing & repair were taken on 7^{th} and on 15^{th} day.

Assessment of biopsy from wound site was done by following histological features.

- Epithelialization
- Leucocytic infiltration
- Vascularity
- Fibroblastic cell proliferation
- Appearance of granulation tissue

Parameters to calculate healing score [15]

Histological Parameter

The following histological parameters were used to calculate healing score.

1. Quantity of granulation tissue (profound -1, moderate – 2, scanty-3, absent-4)

2. Inflammatory infiltrate(plenty -1, moderate – 2, a few- 3)

3. Collagen fiber orientation (vertical -1, mixed -2, horizontal-3)

4. Pattern of collagen (reticular -1, mixed-2, fascicle-3)

5. Quantity of early collagen (profound-1, moderate -2, minimal-3, absent-4)

6. Quantity of mature collagen (profound-1, moderate -2, minimal-3)

Histological assessments of wound were Semi Quantitative and Quantitative methods as described below.

· Semi-quantitative method:- Wound reepithelialisation,Migration of keratinocytes, Bridging of cells, keratinisation, Inflammatory cells: absence/ Presence: (mild/ moderate/ marked), Fibroblasts: absence/ presence(mild/ moderate/ marked),New vessels; absence/ presence(mild/ moderate/ marked),Collagen: absence/ presence(mild/ moderate/ marked)

· Quantitative method:- Polymorpho nuclear leucocytes/ tissue macrophages ratio, Percentage of reepithelialisation, Area of the granulation tissue

Results

The following observations were made for healing score & other histological parameters in conclusion of process of healing.

Table 1 Shows the Healing score of comparison between *Ehretia Laevis Roxb*. and Phenytoin for comparison over the time grade of 0, 7^{th}, 15^{th} day

Table:-1 Histological Healing Score

Day	Date of burn injury 0 day		Biopsy done on 7th day		Biopsy done on 15th day	
Parameters	**Group A Ehretia Laevis Roxb.**	**Group B Phenytoin**	**Group A Ehretia laevis Roxb.**	**Group B Phenytoin**	**Group A Ehretia laevis Roxb.**	**Group B Phenytoin**
Quantity of granulation tissue (profound -1, moderate – 2, scanty-3, absent-4)	4	4	2	3	4	4
Inflammatory infiltrate(plenty -1, moderate – 2, a few- 3)	3	3	2	3	3	3
Collagen fibre orientation (vertical -1, mixed -2, horizontal-3)	-	-	2	2	3	2
Pattern of collagen (reticular -1, mixed-2, fascicle-3)	-	-	2	2	2	2
Quantity of early collagen (profound-1, moderate -2, minimal-3, absent-4)	-	-	1	2	1	1
Quantity of mature collagen (profound-1, moderate -2, minimal-3)	-	-	2	3	1	2

Description of Table-1

It is seen from table one, the *Ehretia Laevis Roxb*. as compared to Phenytoin application works well for the synthesis of collagen fibres & its orientation. The quantity however of mature collagen was high on the 15th day by Phenytoin as compared to the group of Albino Rabbits applied with *Ehretia Laves Roxb*. There are not many difference noted for scar on the day 7 of biopsy.

The semi quantitative & quantitative histological parameters assessed are shown in Table-2

Table-2 Assessment of other semi quantitative & quantitative histological parameters

Semi-quantitative method	Date of burn injury 0 day	Biopsy done on 7th day		Biopsy done on 15th day	
Parameters	-	**Group A Ehretia Laevis Roxb.**	**Group B Phenytoin**	**Group A Ehretia Laevis Roxb.**	**Group B Phenytoin**
Wound reepithelialisation;	-	Maximum	Minimum	Maximum	Maximum
Migration of keratinocytes,	-	Present	Present	Absent	Absent
Bridging of cells, keratin	-	Present	Present but feeble	Present	Present
Inflammatory cells: absence/ Presence: (mild/ moderate/ marked)	-	Mild	Moderate	Absent	Absent
Fibroblasts: absence/ presence	-	Present	Present	Rare	Absent
New vessels; absence/ presence	-	Present	Present	Absent	Absent
(mild/ moderate/ marked)	-	Moderate	Mild		
Collagen: absence/ presence (mild/ moderate/ marked)	-	Present Marked	Present Moderate	Present Moderate	Present Mild
Quantitative method					
Polymorpho nuclear leucocytes/ tissue macrophages ratio	-	Maintained Normal	Normal	-	-
Percentage of reepithelialisation	-	85%	80%	98%	92%
Area of the granulation tissue	-	Present	Occasional y present	Absent	Absent

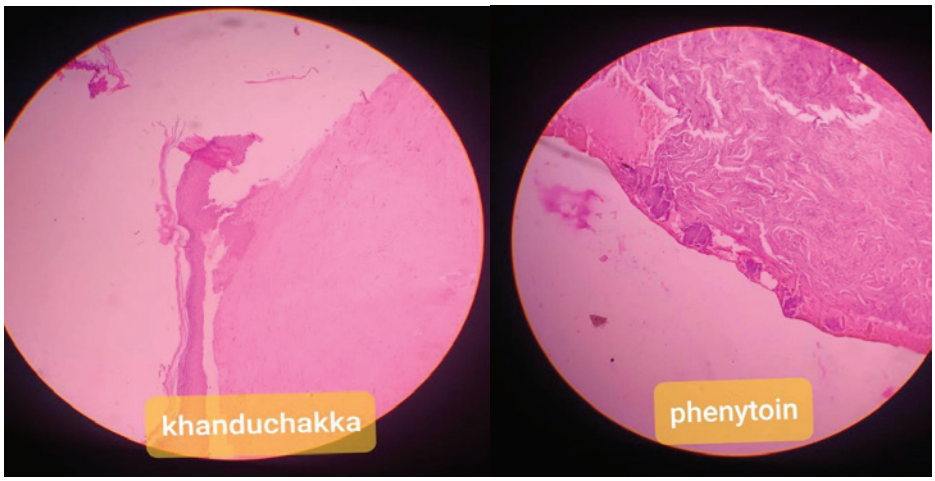
The overall epithelisation on the 15th day is much better in group of *Ehretia Laevis Roxb*.(98%) as compared to application of Phenytoin (92%). The presence of collagen was moderate on 15th day in the group of rabbits receiving *Ehretia Laevis Roxb*. as compared to group B receiving Phenytoin.

Histological changes for comparison between *Ehretia Laevis Roxb.*& Phenytoin on 7th day & 15th day have been shown in Figure- 3 A & B and Figure- 4 A & B. Figure- 4 A & B- Comparative Photographs of histology (15th day)

Figure 3:- Comparative photomicrographs of histology (7th day)

Group A:- Ehretia Laevis Roxb. Group B:- Phenytoin

Figure 3-A (Ehretia Laevis Roxb). Figure 3-B (Topical Phenytoin)



(H & E 10X)

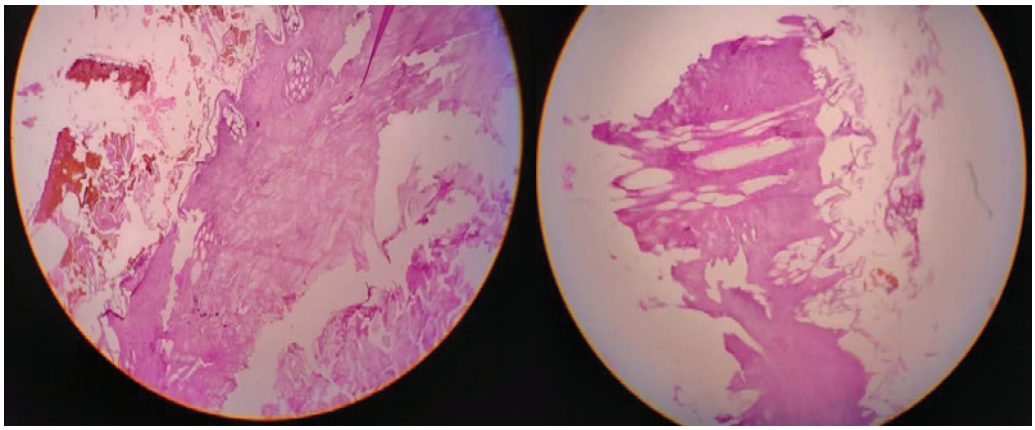
Figure 3-A Shows early epithelisation & appearance of sub epithelial fibroblast

Figure 3-B- Shows epithelisation & persistent granulation tissue.

Figure 4:- Comparative photomicrographs of histology (15th day)

Group A :- Ehretia Laevis Roxb. Group B:- Phenytoin

Figure 4-A(Ehretia Laevis Roxb) Figure 4-B(Topical Phenytoin)



(H & E 10X)

Figure 4-A- Complete epithelisation & proper organisation of mature collagen

Figure 4-B- Epithelisation marred incomplete dermal collagenosis.

Discussion

Part -I

The wound healing property has been documented. The similar observation were made in the studies but for the other drugs.

In another group Phenytoin ointment was taken, which promotes wound healing. Histological assessment was done on 7th and 15th day.

Granulation tissues were absent on date of injury and at fifteenth day from date of injury in both cases i.e. in group A and group B. Granulation tissues were

moderate in group A and scanty in group B on seventh day. Hence granulation enhances by *Ehretia Laevis Roxb.*plant compared to Phenytoin.

Inflammatory infiltrates were few at the date of injury and on fifteenth day from date of injury in both groups. Inflammatory infiltrate was few in group B and moderate in group A. Inflammatory infiltrate is more by *Ehretia Laevis Roxb.*plant as compared to Phenytoin.

Collagen fibre orientation was nil at date of injury in both groups, mixed on seventh day in both groups and on fifteenth day, horizontal in group A and mixed in group B.

Pattern of collagen was nil at the day of injury and mixed on the seventh and fifteenth day in both groups.

Quantity of early collagen was nil at the day of injury, profound in group A and moderate in group B on seventh day. Quantity of early collagen was profound in both groups on fifteenth day. Quantity of early collagen is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Quantity of mature collagen was nil at the day of injury, moderate in group A and minimum in group B on seventh day. Quantity of mature collagen is profound in group A and moderate in group B on fifteenth day. Quantity of mature collagen is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Part II: - As per semi-quantitative method and quantitative assessment:-

Wound reepithelialisation nil at the day of injury, maximum in group A and minimum in group B on seventh day and maximum in both groups on fifteenth day. Wound reepithelialisation is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Migration of keratinocytes nil at the day of injury, present in both groups on seventh day and absent in both groups on fifteenth day.

Bridging of cells keratin nil at the day of injury, present in group A and present but feeble in group B on seventh day and present in both groups on fifteenth day. Bridging of cells keratin is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Presence of inflammatory cells is nil at the day of injury, mild in group A and moderate in group B on seventh day and absent in both groups on fifteenth day. Presence of inflammatory cells is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Fibroblasts is nil at the day of injury, present in both groups on seventh day and rare in group A and absent in group B on fifteenth day.

New vessels is nil at the day of injury, moderate in group A and mild in group B on seventh day and absent in both groups on fifteenth day. Formation of new vessels is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Collagen is nil at the day of injury, present marked in group A and present moderate in group B and present moderate in group A and present mild in group B on fifteenth day. Formation of Collagen is significant by *Ehretia Laevis Roxb.*as compared to Phenytoin.

Part III- Quantitative assessment:-

Polymorpho nuclear leucocytes/ tissue macrophages ratio is nil at the day of injury, maintained normal in group A and normal in group B on seventh day and nil in both groups on fifteenth day.

Percentage of reepithelialisation is 85% in group A and 80% in group B on seventh day and is 98 % in group A and 92% in group B on fifteenth day. Percentage of reepithelialisation is significant by *Ehretia Laevis Roxb.* as compared to Phenytoin.

Area of the granulation tissue is present in group A and occasionally present in group B. And it is absent in both groups on fifteenth day. Area of the granulation is significant by *Ehretia Laevis Roxb.* as compared to Phenytoin.

Hence wound healed more significantly by *Ehretia Laevis Roxb.*Plant leaves paste as compared to application of Phenytoin ointment.

Anti microbial activity of plant useful in wound healing and promote healing. Anti oxidant activity plan promote the growth of tissue in wound healing. Hence both these activities help in wound healing.

The study of Sushma et al. has observed the role of natural medicine in wound healing on the basis of bio mechanical histological, biochemical, and molecular studies. However their studies included natural medicine of honey, ghee, Glycyrrhiza glabra, Nerium indicum. Histological analysis with all these application were found to have increase the rate of wound healing, rapid epithelisation, the normal collagenisation in dermis. The limited inflammatory cell response adjudged by ***Interleukin***-1β & enhances activity of myo-fibroblast & small blood vessels. The study has concluded that these natural traditional medicine brings enhances wound healing activity as observed in histological material. However the study of Sushma et al did not have group of sample, those were treated by *Ehretia Laevis Roxb.* [4]

Al-Henhena N et al evaluated topical application of Strobilanthes crispus ethanolic extract on the rat of wound closer & histology of healed wound. It was observed that group 3 & group 4 were. Strobilanthes crispus was used in 100 mg/ml & 200 mg/ml of ethanolic extract were found to have mean healing time 14.80 ± 0.37 days and 13.00 ± 0.37 days respectively. Histological evaluation when compared to control group had average healing time of 20.6 ± 0.37a days. The study concluded that Strobilanthes crispus leaf extract enhances wound healing as ascertained by histological study. [5]

The present study has found put that leaf extract paste of *Ehretia Laevis Roxb.* has enhanced the process of wound healing ascertained on histological parameters as compared to topical phenytoin application. There are not many studies that have experimental with *Ehretia Laevis Roxb.* leaves paste for the objective assessment for its property of enhance wound healing.

Conclusion

Ehretia Laevis Roxb. paste is found to be immensely useful at wound healing as compared to Phenytoin. *Ehretia Laevis Roxb.* is concluded to promote the healing significantly as observed by feature of granulation, collagenisation & re epithelisation of the wounds suggesting early healing by primary intension.

Ethical Clearance- Taken from institutional ethical committee of DMIMS(DU), Wardha

Source of Funding- Self

Conflict of Interest:- Nil

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A Study of Cheiloscopic Pattern in Relation to Gender and Blood Groups in a Tertiary Care Hospital at Chennai

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Abstract

Personal identification is of vital importance in forensic odontology for ethical, humanitarian and criminal enquiries. The applicability of Cheiloscopy (study of pattern of lip print), one of the easiest tools available in individual and medicolegal identification, is an area of extensive research in recent years. With the objective to investigate the uniqueness of lip patterns in relation to gender and blood groups, this study was conducted on 100 subjects, which included 50 males and 50 females. The lip prints obtained were studied by applying Suzuki and Tsuchihashi's classification.Type 2 (37%) was the most common lip print type in males and Type 3 (38.5%) was the most common lip print type in females. The Lower lip print can help in gender determination. No significant statistical correlation was found between the lip print patterns and blood group. Along with the traditional methods of identification like fingerprint analysis,Cheiloscopy can also be helpful in the identification of a person.However, the utility of the same in forensic medicine still remains largely untapped and unacknowledged. Similar studies with larger sample sizes are required to obtain statistically significant reports thereby allowing a more impactful emergence of lip print patterns usage in forensic analysis.

Keywords : *Cheiloscopy , Lip prints, Identification, Suzuki & Tsuchihashi classification, Gender, Blood groups.*

Introduction

Cheiloscopy,the study of lip prints analyses the furrows and grooves present on human lips for an affirmative identification. The lip prints comprising of the normal lines and fissures present in the zone of transition of human lip between the mucosa and skin are unchangeable, permanent even after death and unique to each person except in monozygotic twins. They are analogous to fingerprints. Lip grooves are formed in the 6th week of intrauterine life and remain unchanged throughout one's life.[1,2] The lip prints of parents and children and those of siblings have shown some similarities. It has the unique ability of reverting to its original pattern following minor trauma, inflammation and infections such as herpes.[3]

Suzuki and Tsuchihashi[12] in 1970 devised a classification of lip prints as :

Type I : Complete vertical pattern. (Clear cut grooves running vertically across the lip)

Type I' : Incomplete vertical pattern. (The grooves are straight but they disappear halfway)

Type II : Branching, Y- shaped pattern. (The grooves fork in their way)

Type III : Criss-cross pattern. (The grooves intersect)

Type IV : Reticular pattern.

Type V : Undetermined. (The grooves do not fall

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in any of the types from I to IV)

This is the most widely used classification used in literature. It was easy to interpret and was found to have a clear description of nearly all of the commonly encountered lip patterns.

Yet another biological record that remains unchanged throughout the life time of a person is the blood group. Determining the blood group of a person from the samples obtained at the site of crime, helps identify a person. Landsteiner classified blood groups under the ABO blood group system. [4]

The applicability of cheiloscopy in individual identification has been an area of extensive research in recent years. Lip prints can be a silent important evidence in the scene of crime to identify the culprit. [5]

The present study was conducted to determine if there is any correlation between lip print pattern, gender and blood group, which can be used in forensic identification of an individual.

Methodology

The study was conducted on 100 randomly selected individuals willing to participate in the study , belonging to age group between 18 to 24 years. The study population comprised of 50 females and 50 males. Any subject with malformation, deformity, inflammation, scarring of lips and known hypersensitivity to lipsticks was excluded. An informed consent for wilful participation in the study was taken from all the subjects.

For collecting Lip Prints: Bright red coloured non-glossy Lipstick, transparent cellophane tape of broad width with glue on one side, scissors, white paper, magnifying glass, cotton earbuds and swabs,soap,soft towels, cleansing milk were used.

The lips of the individuals were cleaned with soap &water and dried with a soft towel. The lipstick was applied uniformly using earbuds, starting at the middle of lips &moving laterally. The subject was asked to roll both lips inwards on each other to ensure an even spread of lipstick. The colour applied was allowed to dry for about 2 minutes. A strip of cellophane tape, 10cms long was cut with scissors. Keeping the oral fissure closed in normal resting position, the glued portion of cellophane tape was placed on the closed lips. It was held in place by dabbing in the center initially and later towards the corners of the lips. The tape was carefully lifted from the lips to avoid smudging. This strip was then stuck to a white paper. The lips were then cleaned with a cotton swab dabbed in cleansing milk.

Each lip print was divided into four quadrants and each quadrant was examined using a magnifying glass. To classify the lip prints , the classification proposed by Suzuki and Tsuchihashi was used.

The blood group of the individual was noted down by questioning and in case of people who were unaware, tests was done to determine their blood group.

For Recording of the Blood Groups of study population: Glass slides, anti–A serum& anti–B serum, Rh antigen serum, lancets, alcohol swabs.

The subject was asked to wash hands with soap and water & pat them dry. The pulp of middle finger was cleaned with a spirit swab and pricked with the help of a lancet. Two drops of blood was squeezed on to a glass slide. It was treated with anti A & anti B sera. A positive agglutination with anti A serum shows ‘A’ blood group; with anti B serum ‘B’ group; agglutination with both sera –‘AB’ blood group and no agglutination with any sera-‘O’ group. Agglutination with Rh antigen is ‘Rh positive’ and absence of agglutination is ‘Rh negative’

Statistical Analysis

The data obtained were entered in Microsoft Excel and analyzed using SPSS package version 24. The predominant lip patterns for the different groups were calculated using percentage method. Chi-square test was used to analyze and compare the lip print patterns with gender and blood groups. The level of significance was set at $P < 0.05$ and $P<0.001$.

Observation and Results

There were a total of 100 voluntary subjects in this study, comprising of 50 males and 50 females. Each lip print obtained was further divided into four quadrants, summing up to 400 quadrants for analysis.

In 50 males, out of 200 quadrant-wise patterns assessed, Type I, Type I’, Type II, Type III, Type IV,

Type V were seen in 53, 12, 74, 29, 28 and 4 quadrants, respectively. In 50 females, out of 200 quadrant-wise patterns assessed, Type I, Type I, Type II, Type III, Type IV, Type V were seen in 41,10,39,77,30 and 3 quadrants, respectively.

In males , the frequencies of lip print patterns : **Type 2>Type 1> Type 3 > Type 4 > Type 1' > Type 5.**

In females ,the frequencies of lip print patterns :**Type 3 > Type 1 > Type 2> Type 4 > Type 1' > Type 5.** [Table 1]

On application of Chi Square formulae, significant correlation between gender and lip print of the lower lip was found (P<0.001)

Among 100 individuals analysed, the percentage distribution of various blood groups is as follows:

O Positive blood group was seen in 36% individuals, B Positive in 25%, A Positive in 19 individuals, B Negative in 6% individuals, AB Positive in 5% individuals, A Negative in 4% individuals, AB Negative in 3% individuals, O Negative in 2% individuals.

On analysing the lip print patterns in relation to blood group the following observation was made:

· In individuals with **A Positive** blood group, **Type III** was more common (30.26%) followed by **Type II** (28.95%).

· In individuals with **A Negative** blood group: **Type I** was more common (37.25%) followed by **Type III** (31.25%).

· In individuals with **B Positive** and **B Negative** blood group: **Type II** was more common,32% and 37.5% respectively. It was closely followed by **Type III** (31%) in individuals with B positive blood group type and **Type IV** in individuals with B Negative blood group (25%).

· In individuals with **O Positive** blood group: **Type II** was more common (25.7%) followed by **Type I** and **Type III** (23.61% each).

· In individuals with **O Negative** blood group: **Type I, II and III** were most common accounting to 25% of quadrants each.

· In individuals with **AB Positive** blood group: **Type I, II and IV** were seen in25% of quadrants each.

· In individuals with **AB Negative** blood group: **Type II and III** was more common amounting to 33.33% of quadrants each. [Table 2]

On further analysis with Chi square, there was no significant correlation observed between the blood group and lip prints (P>0.05).

Table 1: Lip print patterns in relation to gender –

	Males		Females		Total	
	200 Quadrants	%	200 Quadrants	%	400 Quadrants	%
Type I	53	26.5	41	20.5	94	23.5
Type I'	12	6.0	10	5.0	22	5.5
Type II	74	37.0	39	19.5	113	28.25
Type III	29	14.5	77	38.5	106	26.4
Type IV	28	14.0	30	15.0	58	14.5
Type V	4	2.0	3	1.5	7	1.75

Table 2: Lip print patterns in relation to Blood group

Blood Group	Classification of lip prints						Total Quadrants
	Type I	Type I'	Type II	Type III	Type IV	Type V	
A Positive	17 (22.37%)	3 (3.95%)	22 (28.95%)	23 (30.26%)	11 (14.47%)	0	76
A Negative	6 (37.5%)	2 (12.5%)	2 (12.5%)	5 (31.25%)	1 (6.25%)	0	16
B Positive	22 (22%)	5 (5%)	32 (32%)	31 (31%)	7 (7%)	3 (3%)	100
B Negative	5 (20.83%)	1 (4.17%)	9 (37.5%)	3 (12.5%)	6 (25%)	0	24
O Positive	34 (23.61%)	9 (6.25%)	37 (25.7%)	34 (23.61%)	27 (18.75%)	3 (2.08%)	144
O Negative	2 (25%)	0	2 (25%)	2 (25%)	1 (12.5%)	1 (12.5%)	8
AB Positive	5 (25%)	1 (5%)	5 (25%)	4 (20%)	5 (25%)	0	20
AB Negative	3 (25%)	1 (8.33%)	4 (33.33%)	4 (33.33%)	0	0	12
Total	94	22	113	106	58	7	400

Discussion

The present study was conducted on 100 randomly selected volunteering subjects amongst which 50% were male and 50% were female.The classification proposed by Suzuki and Tsuchihashi of lip pattern was used as it is the most widely followed one globally and encompasses all types of lip patterns.The most predominant pattern observed was Type II (28.25%) when all the four quadrants in both the upper and lower lips of the entire study population were considered. This was followed by Type III (26.4%), Type I (23.5%), Type IV (14.5%), Type I' (5.5%), and Type V (1.75%). This was partially in accordance to a study conducted in North Kerala by Basheer et al [2] On the contrary, Verghese *et al*. in their study on the Kerala population found Type IV to be the most common pattern.[6]

Gender –In this study, it was found that no two individuals had similar type of lip prints, thus demonstrating the uniqueness of lip prints. The commonest lip print observed in both gender was Type II (n=113) followed by Type III (n=106). Type II lip print pattern was the most predominant pattern reported in studies conducted by by Verma P et al,Kataria et al. , Karki RK, et al. and Gondivkar et al. in their population from Rajasthan,Panchkula Nepal and Maharasthra respectively. [3,8,9,11] However,this is not in accordance with a study done by Gupta P et al., who in their study found that Type I was the most common lip print type in both males and females.[1]

On further analysis, the results showed that the most common lip print type found in males was Type II (37%)

followed by Type I (26.5%) whereas in females it was Type III (38.5%) followed by Type I (20.5%). Basheer S et al., in their study found that Type II was the most common lip print type in males and Type IV was the most common in females.[2]

The Lower lip print can help in gender determination (P<0.001) while the upper lip showed a more or less similar pattern distribution among males and females. This was similar to a study conducted by authors in a South Indian city.[10] .Another study by Gondivkar et al., predicted the accuracy of gender based on lip print patterns, which was stated to be 92% in males and 95% in females. It showed a statistically significant dimorphism in the lip print patterns among males and females. [11]

Blood groups –The most common blood groups found in the study population was O Positive followed by B Positive. Type II was the predominant lip print found in both the blood groups analysed. The present study showed no significant correlation between lip prints and blood groups on statistical analysis. This may be due to different areas or origins of lip prints and blood in intraembryonic life; however, further studies are needed to explicate the reason. The results of the present study are similar to those of studies by Karim B et al, which showed no correlation between lip prints and blood groups. .[7] Sandhu H et al., in their study found a correlation between lip prints and blood group except Type 1.[4].,

Conclusion

This study confirms the distinctiveness of Cheiloscopy and its potential usefulness in Forensic Medicine. To conclude , we observed a difference in lip print pattern types between males and females but it negates any statistical correlation of lip print pattern with ABO blood groups.The limitations of the study include small sample size, of a limited strata and age range of Kancheepuram district population. The lip prints and ABO blood group used in the study were obtained specially for the purpose and the overall quality would be far superior when compared to those obtained at the crime scene. Further studies with larger samples are required to obtain statistical significance of this correlation.

Conflict of Interest : Nil

Ethical Clearance: Obtained

Source of Funding- Self

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A Practical Perspective of Forensic Odontology in Age Estimation: 2 Medico Legal Case Studies

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Abstract

Forensic odontology is the branch of forensics that deals with human identification based on dental features. We discussed in this paper the medico-legal importance of different ages and the method of age estimation for legal obligations along with case studies. Dental structures along with the skeletal age and secondary sexual characteristics as a method of assessing age in different age groups is increasingly used in both criminal and civil proceedings, which falls within the purview of forensic odontology. Hence, a good knowledge for every dentist is necessary about various forensic methods like chronology of primary and permanent teeth, DNA studies, Cheiloscopy, Palatal rugae assessments etc for identification of an individual to assess factors like age, sex, and so can able to play vital role in practice of Forensic Odontology.

Keywords*: Forensic odontology; Human identification; Age Estimation; Forensic Medico-Legal cases*

Introduction

Assessment of age of an individual is very important in cases of civil and criminal matters.[1] A number of cases have been recorded for age assessment both in medico-legal and civil cases. The reason especially in developing and underdeveloped nations where there is no proper maintenance of an individual birth records, as many births takes place at home or in rural areas not in the hospitals. [1,2] In most of these individuals, for those who admit in the schools, the rough birth date was written by teachers in school records and for those who donot study will remain unaware of their birthdates.[1,2] This necessitates the age certificate by a doctor in living whenever legal issues arise. Certification from forensic physician, dentist, and radiologist from Government Medical institutions is necessary for issuing age opinion of an individual by assessing physically, dentally, skeletally, and secondary sexual characteristics.[2, 3, 4]

The following civil and criminal cases needs age verification usually [1, 4]

1. Criminal cases-Criminal responsibility, Rape section 375ipc, kidnapping section 361,366a, 366b, 369ipc, Infanticide etc

2. Civil cases-Employment, Marriage, Concession, Retirement benefits, older persons-question of fitness, Government pension and schemes, personnel Identification

CASE REPORTS: Herewith, we report 2 cases for age estimation of minors reported in RIMS Medical College, Kadapa, Andhra Pradesh, India.

MEDICO-LEGAL CASE REPORT I- AGE DETERMINATION OF A CHILD OF DISPUTED PATERNITY

A small boy was bought to the Forensic Department, RIMS Medical College, Kadapa for age estimation by the police. The history revealed that the boy was bought by a childless couple from a nurse when the boy was 3months old. They were taking care of the child since then. Now, a man came claiming that he is father of the child. In the police enquiry, the couple told the boy was of 5yrs age and the man told he was above 6yrs age.

1. Physical examination of the boy revealed normal general built & appearance, weighing around 14kgs.His height, chest and abdomen measurements were 107cm,50cm,49cm.

2. Intraoral examination revealed presence of all 20 temporary teeth .OPG revealed erupting permanent I mandible molar and resorbing root of mandibular right central incisor (picture 1)

3. Skeletal radiographs

a. Shoulder joint (picture 2) – Appearance of head of humerus, greater tubercle and lesser tubercle

b. Wrist joint (picture 3) - Appearance of lower end of radius & ulna with carpal bone

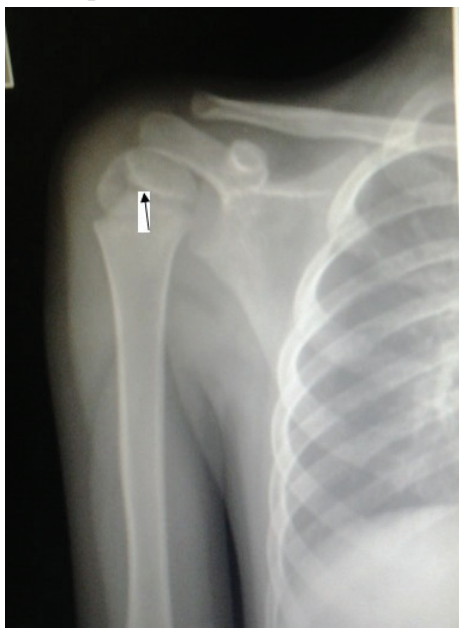
c. Ankle joint (picture3) - Appearance of lower end of fibula & tibia, naviculate and cuneiform bones

d. Pelvis &Hip joint - Appearance of head and greater trochanter of femur

We gave age opinion as 5-6yrs by correlating all the above features. We also witnessed the age of the boy in the court of law .Later, the DNA matching also came negative confirming the false claim of the man. The boy was handed over to the couple legally. Thus, our opinion was an important evidence to sort out the case.

Case Report-1

case report 1.Picture1-OPG



case report 1-Picture2.skeletal radiograph-shoulder joint

case report 1-Picture3skeletal radiograph-ankle joint and wrist joint

MEDICO-LEGAL CASE REPORT II-AGE DETERMINATION OF A BOY IN A SEXUAL ASSAULT.

Police came to the Forensic Department, RIMS Medical College, Kadapa for age estimation of a boy. The history revealed that the boy was involved in an unnatural sex practice with another boy in an orphanage. They want to confirm whether the boy is major or minor as the punishment for the offence in the court of law is less for minors. The boy was examined as follows

1. Physical examination of the boy revealed normal general built & appearance weighing around 40kgs.His height, chest and abdomen measurements were 163cm, 70cm, and 60cm.

2. Intraoral examination revealed presence of all 28 permanent teeth OPG revealed closure of root apex of second molars, missing maxillary 3rd molars buds and completed mandibular 3rd molars crown (picture4).

3. Skeletal Radiographs

1. Shoulder joint- Appearance of acaromion and coracoid, head of humerus under process of fusion. (picture 5)

2. Elbow joint- Medial epicondyles recently fused.

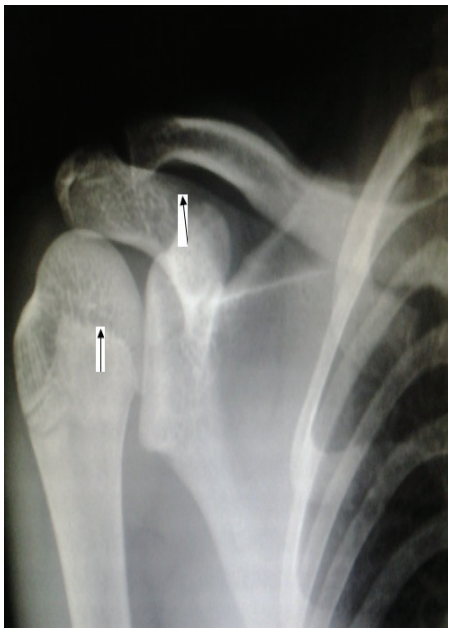
3. Wrist joint-Base of I metacarpal fused, lower end of radius &ulna under process of fusion (Picture6).

4. Pelvis-Triradiate cartilage fused, iliac cartilage crest & ischial tuberosity under process of fusion .

4 .Secondary sexual characteristics- Moustache, hoarseness of voice, pubic hair axillary hair were noticed and genitals appear normal.

We gave an age opinion of the boy as 15-16yrs by correlating above all features. We witnessed the age of the boy in the court of law. Later the boy was sent to juvenile home based on age certificate.

case report 2-Picture 4.-OPG



case report 2-Picture 5.skeletal radiograph-shoulder joint

case report 2-Picture 6.skeletal radiograph-wrist joint

Discussion

In the Government Medical and Dental colleges, AP, the age opinion of a living individual is given by a Forensic expert after consulting a Dentist and Radiologist. The dentist usually examine intraorally for number of teeth present and type of teeth. OPG will be of diagnostic to determine permanent teeth buds in case of primary dentition, for root apex closure of various teeth in case mixed dentition and for 3rd molars in case of permanent dentition (table 1).[5, 6, 7,8]. Dentists can give age opinion upto 25yrs by chronology of teeth of an individual approximately. But, if the age opinion has to be given beyond 25yrs of age, the individual is referred to the Radiologist as dentists were unable to assess depending on teeth. Radiologist give their age opinion by observing the appearance and completion of ossification of cartilage of different bones which takes place relatively fixed periods of life like shoulder joint, Elbow joint, Wrist joint, Pelvis with hip joint, and Ankle joint, Clavicles skull sutures (table2). [1, 4, 9] Thus, the medical opinions of age in above three cases are discussed below:

Medico-legal case report I

Physical characteristics showed age between 4-5yrs. Intra orally, 20 primary teeth were found. OPG revealed: I permanent Mandible molar was erupting which usually erupts by 6yrs of age Dental age opinion was around 5-6yrs.[5, 6] Shoulder joint reveals appearance of head of humerus , greater tubercle and lesser tubercle (by 5yrs). [1,4,9] The wrist joint showed appearance of 4 carpals and usually age is calculated as number of appeared carpals + 1, so, here 4+1=5. [1, 4, 9] Ankle joint showed an Appearance of lower end of fibula & tibia (by 1yr), naviculate and cuneiform bones (by 3yrs). [1,4,9] So, physical appearance, dental age and skeletal age were correlated and age opinion of 5-6yrs was given.

Medico-legal case report II

Physical examination and appearance of secondary sexual characteristics showed above 14yrs. Dental examination showed presence of 28 erupted permanent teeth in the intraoral cavity. The OPG revealed root apical closure of all erupted teeth (above 15yrs) and partial crown completion of unerupted 3rd molars . [5,6,7] Dental age opinion was between 15-16yrs. Shoulder

joint showed appearance of acromian and coracoid process(15-16yrs) [1,4,9] .Elbow joint revealed appearance of medial epicondyle but not fused (14-16yrs) [1,4,9] .Wrist joint revealed recent fusion of base of I metacarpal, Lower end of radius and ulna appeared and in the process of fusion (above 15yrs). [1,4,9] Pelvis showed fused tri radiate cartilage (>13yrs) [1,4,9], appearance of iliac crest but not fused (<16yrs) [1,4,9] and Ischial tuberosity not appeared (less than 16yrs) [1,4,9] .By correlating the above features, age opinion was given as between 15-16yrs.

Conclusion

These are a few examples that report in the Government Medical colleges, AP usually come for age certificates .The age of the individual can be assessed approximately up to the age of 25yrs by assessing and correlating development of physical growth, dental, skeletal and secondary sexual characteristics. But it is becoming difficult for us to assess the age above 25yrs in living as all above bodily characteristics would have fully developed by that age. So, we are looking forward for newer simpler, cost-effective and accurate techniques for age estimation in living for both below and above 25yrs of age.

Conflict of Interest – None

Source of Funding- Self

Ethical Clearance – Case Reports

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Sex Determination using the Ischiopubic Index in South Indian Population

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Abstract

The aim of this study was to use the Ischiopubic index to explore the sex differences in the pelvis between the male and female bones. The pelvis is characterized by sexually dimorphic features and thus it can be used for sex determination while establishing the biological profile in unknown skeletal remains. In this study we have assessed the sex prediction potential of the Ischiopubic index using 72 dry innominate hip bones (38 male and 34 female) belonging to South Indian population. The lengths of the pubis and ischium were measured in these bones and the Ischiopubic index was calculated as per Washburn's formula. The Ischiopubic index was calculated to be 85.46 ± 3.63 mm. in males (Range 74.15 - 95.28 mm.) and 97.75 ± 4.10 mm. in females (Range 89.56 - 116.37 mm.). Application of the Student's t test showed that there was a statistically significant difference in the Ischiopubic index between the male and female pelvic bones with a $p < 0.001$.

Keywords: *Pubic length, Ischial length, Ischiopubic index, Sex determination, South Indian population*

Introduction

The overarching goal of the forensic pathologist when confronted with undocumented skeletal remains is establishing the biological profile and thus confirming the identity of the decedent individual. Estimating the sex from skeletal remains is a key step in the identification process in this context and it is often the first component of the biological profile to be assessed owing to the fact that other aspects of identification such as age, stature, and ancestry are in turn dependent on the estimation of sex.[1] Furthermore, an incorrect estimation of sex precludes correct identification for the simple reason that if the estimation is erroneous, this will in turn mislead the investigators into looking into the wrong half of the population. Given the importance of sex in reconstructing the biological profile, forensic experts are engaged in research to improve the accuracy of sex estimation.[2,3]

There are several regions of the skeleton that can be utilized to estimate sex, including the cranium, however they pale in comparison to the pelvis.[4-6] The pelvis is generally accepted as the most sexually dimorphic region of the human skeleton thus making it the ideal bone for sex estimation.[7-10]The marked sexual dimorphism in the size and shape of the pelvis is a consequence of the adaptation of the pelvic bone in females to facilitate the biological function of parturition. A human maternal pelvis must be large enough to enable birth of a large-headed neonate under the constraints of maintaining optimal bipedal biomechanics. The very specific obstetric function of the pelvis is responsible for inhibiting drastic intrasexual variation in morphology.[11-14] These sexually diagnostic features begin to clearly exhibit themselves in the pelvis during adolescence and are most pronounced in late adolescents and adults.

The two main methodological approaches to sexing skeletal remains using skeletal traits are morphological analysis and metric analysis. More recently a more sophisticated approach using Geometric morphometric analysis has been used to do sexing of bones[15]. In this method, morphological features are converted into a quantifiable entity using three-dimensional scanning and coordinate mapping.

A study of the morphological traits focuses on the shape of the bone i.e. bony configurations that are macroscopically visible.[7,16-17] This approach is advantageous in that a particular form may be recognizable despite interpopulation variations and changes that occur over time. However, there is an element of subjectivity in this approach and the outcome cannot be ascertained with precision. Most of the older studies pertaining to sexual dimorphism employed this method. The most commonly used visual method of sex estimation from the pelvis is the one advocated by Phenice which involves examination of three traits of the pubic bone[7]. These three traits include the ventral arc, subpubic concavity, and medial aspect of the ischiopubic ramus on the pubic bone.

The second method is the metric method which is based on taking measurements of various dimensions of skeletal material which are prone to dimorphism.[12] Metric methods offer several advantages over the morphological methods. First, inter- and intra-observer error and subjectivity are typically lower in this type of analysis.[18] Second, they are more reproducible than morphological methods because they rely on standardized osteometric landmarks which are easy to locate and are more objective than non-metric methods because the landmarks are easier to locate and their assessment is not based on judgment. Examples of such metric methods applicable to the pelvis are the length of the pubis, length of the ischium, diameter of the acetabulum etc. These metric measurements can be transformed into indices such as the Ischiopubic index, the Sciatic notch index, Acetabulopubic index which can be subjected to statistical analysis. Metric analysis, however, despite its high level of objectivity and reproducibility, has its limitations.[17] Metric methods are population specific and the indices have to be established for each population. They have a tendency to yield error owing to the fact that they are dependent on absolute differences in measured dimensions of skeletons.

Washburn provided the first popularly utilized index, the Ischiopubic index.[12] He conducted pioneering studies on the differences between the sexes for the pubic and ischial bones using documented skeletal material of American White and Black adults from the Hamann-Todd collection held at the Natural History Museum in Cleveland. Washburn found both sex and populational differences, with the sex determination reliability rate over 90% when using the Ischiopubic index. In this study we have explored the utility of the Ischiopubic index in pelvic bones belonging to south Indian population.

Materials and Methods

The material for the study comprised of a total of 72 intact dry pelves of documented gender (38 male and 34 female bones) sourced from the skeletal collections in the Departments of Forensic Medicine and Anatomy at Chettinad Hospital and Research Institute (CHRI). The bones used for the measurements were intact, devoid of pathology, fracture or wear and tear. A digital sliding caliper was used for making measurements up to a least count of 0.01 mm.

This study entailed a correct identification of the acetabular landmark which serves as the base point for making the measurements. The acetabular landmark as described by Schulz is located in the anterior aspect of the bone and can be identified as an irregularity, a change in bone translucency or a notch.[19] This base point in the acetabulum was carefully located in each bone using the Schulz criteria before making the measurements.

The measurements made were the length of the pubis and the length of the ischium as described in standard protocols and defined as follows:[12,16]

Length of the Pubis: The greatest distance from the acetabular landmark to the symphysial surface of the pubis. While measuring the pubis, care was taken to hold the sliding calliper parallel to the long axis of the bone as detailed in the standard protocols.

Length of the Ischium: The greatest distance from the point in the acetabulum to the farthest point on the ischial tuberosity which is also called the Ischial point.[20]

The Ischiopubic index was calculated by using the equation of Washburn as follows:

$$\text{Ischiopubic Index} = \frac{\text{Length of the Pubis} \times 100}{\text{Length of the Ischium}}$$

Results

The lengths of the pubis and ischium were measured to the nearest 0.01 mm. with the help of a digital sliding

calliper and the Ischiopubic Index was calculated. A statistically significant difference was not observed in the values between the right and left side in both sexes and thus the data for the two sides was pooled. Computational and statistical analysis of the results was done using the SPSS software version 21 through which the mean and standard deviation (S.D.) were calculated for all three parameters. In addition, a calculated range was arrived at using mean ± 3S.D. and from this the demarcating point was established.

The range, mean, standard deviation, calculated range and the demarcating points for all three parameters are depicted in tables 1 and 2.

Length of Pubis:

The mean pubic length was found to be 74.26 ± 3.36 mm. in males (Range 62.14-85.23 mm.) and 80.28 ± 4.57 mm. in females (Range 65.76-91.36 mm.). The p value was found to be <0.001 thus implying that the difference in length between the male and female bones was statistically significant.

Length of Ischium:

The mean ischial length was found to be 80.55 ± 5.23 mm. in males (Range 70.16-93.23 mm.) and 77.49 ± 4.51 mm. in females (Range 63.35-87.63 mm.). The p value was found to be <0.001 thus implying that the difference in length between the male and female bones was statistically significant.

Ischiopubic Index:

The Ischiopubic index was calculated to be 85.46 ± 3.63 in males (Range 74.15 ± 95.28) and 97.75 ± 4.10 in females Range (89.56 - 116.37). The p value was found to be <0.001 thus implying that the difference in length between the male and female bones was statistically significant.

Table 1: Statistical analysis of Pubic Length & Ischial Length

Statistical Parameter	Pubic length (mm.)		Ischial length (mm.)	
	Male	Female	Male	Female
Range	62.14-85.23	65.76-91.36	70.16-93.23	66.35-87.63
Mean	74.26	80.28	80.55	77.49
Standard Deviation (S.D.)	3.36	4.57	5.32	4.51
3 S.D.	10.08	13.71	15.96	13.53
Calculated Range [Mean ± 3 S.D.]	64.18-84.34	66.57-93.99	64.59-96.51	63.96-91.02
Demarking Point	<66.57	>84.34	>91.02	<64.59

Table 2: Statistical analysis of Ischiopubic Index

Statistical Parameter	Ischiopubic Index	
	Male	**Female**
Range	74.15-95.28	89.56-116.37
Mean	85.46	97.75
Standard Deviation (S.D.)	3.63	4.10
3 S. D.	10.89	12.3
Calculated Range [Mean ± 3 S.D.]	74.57-96.35	85.45-110.05
Demarking Point (D.P.)	<85.45	>96.35
% of bones whose sex was identified using D.P.	35%	92%

Discussion

Analysis of the results obtained (refer tables 1 and 2) showed that the mean pubic length was greater in female pelvic bones when compared to males. The mean ischial length on the other hand was greater in males than in female pelvic bones. These findings are in agreement with that of researchers who have studied other ethnic populations. The results of our study, i.e. the pubic length, ischial length and Ischiopubic index have been tabulated for comparison with that of other researchers (Refer Tables 3 & 4).

Until puberty, male and female pelvis exhibit only moderate sexual dimorphism. The pubic region is most responsive to sex hormones and with the onset of puberty the sex-specific rise in oestradiol levels triggers a change in pelvic bone remodelling patterns.[21] Thus, there is a growth spurt in the feminine pubic length, resulting in rapid expansion of obstetrically relevant pelvic dimensions up to the age of 25–30 years. In the course of this growth spurt, the pubis grows faster than the ischium and this in turn increases the Ischiopubic index to a greater degree than in females than in males. The most significant sexual differences in the ischiopubic area are due to the feminine pubis length growth spurt.

The mean Ischiopubic index calculated in our study is higher in female pelvic bones and this confirms the findings reported by several other researchers. While there is a concordance in the pattern, the results show a significant difference in the actual mean values and demarking point between our study and that of other ethnic groups and this could be accounted for by various factors like heredity, race, climate, geographical variations etc. Thus, every population has its own metric standard values.

Table 3: Comparison of Ischiopubic Index in our study with that of other researchers

S. No.	Researcher	Ischiopubic Index			
		Range		Mean	
		Male	Female	Male	Female
1.	Present Study	74.15-95.28	89.56-116.37	85.46	97.75
2.	Adhvaryu A. V.22	86.84-116.92	101.2-136.07	100.52	115.52
3.	Kushal K. D.23	74.31-97.43	82.91-116.5	86.55	99.55
4.	Nirmala V. K.24	84.75-138.08	98.93-147.91	116.87	128.14
5.	Sandhya K.25	65.71-96.08	73.48-98.42	76.72	85.87
6.	Sachdeva K.26	70.11-122.71	92.13-139.18	98.27	117.97
7.	Jani C. B.27	77.01-97.53	88.39-108.96	86.72	96.87
8.	Sharma G. K.28	64.2-97.46	84.43-111.76	78.79	96.76
9.	Singh S.29	80.37-91.51	92.38-120.82	87.19	105.13

Table 4: Comparison of Average Pubic Length and Ischial Length in our study with that of other researchers

S. No.	**Researcher**	**Average Pubic Length (mm.)**		**Average Ischial Length (mm.)**	
		Male	**Female**	**Male**	**Female**
1.	Present Study	74.26	80.28	80.55	77.49
2.	Adhvaryu A. V.22	74.03	77.55	73.67	67.16
3.	Kushal K. D.23	75.32	78.89	81.36	78.44
5.	Sandhya K.25	66	66.8	86.6	78.2
6.	Sachdeva K.26	79.2	85.6	80.7	73.1

Conclusion

In conclusion, the present study has established the presence of statistically significant sexual dimorphism in the Ischiopubic index of South Indian population and comparison of the findings with that of other researchers also points to racial and regional variation. This underscores the need for each population group to have specific standards to optimize the accuracy of identification. The study attests to the utility of the Ischiopubic index in case of undocumented remains brought to the forensic expert for biological profiling given the need for more precise methods of identification from the skeleton because of the increasing number of decomposed remains that confront law enforcement agencies. The findings of this study are thus important in that they contribute to the database of anthropologically and obstetrically significant data for South Indian population and would also help in identifying unknown skeletal remains.

Conflict of Interest: None declared

Source of Funding: Nil

Informed Consent: Not applicable

Ethical Clearance: Not required as the study was done on bone specimens

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Estimation of Stature from Living Adult Females belonging to Tamil Population using Percutaneous Tibial Length

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Abstract

Establishment of the identity of an individual from dismembered remains or skeletal remains is a common problem encountered by the Forensic Pathologist and can be a challenging task. In addition to age and sex, stature is an anthropological parameter that is indispensable in establishing the identity of an individual. In the present study, we have analyzed the relationship between stature and percutaneous Tibial lengths (PCTL) and derived a regression equation for a specific population. The parameters namely, the stature and percutaneous Tibial lengths (PCTL) of both lower limbs were measured in 115 females belonging to Tamil population and recorded. The subjects were in the age group of 21-30 years. Analysis of the results involved the use of the Pearson's correlation to derive the relationship between the parameters and establish the dependence of the stature on the length of the left and right Tibia respectively in the study subjects. The results from this present study attest to the utility of the percutaneous Tibial lengths (PCTL) in determination of the stature in females belonging to the Tamil population.

Keywords: *Stature, percutaneous Tibial lengths (PCTL), Regression equation, Females, Tamil population.*

Introduction

The establishment of the identity of a decedent individual whose identity is unknown can be a challenging task to the Forensic Pathologist during post-mortem examination. The complete skeleton is not available at the scene of crime in many common forensic situations such as mass disasters, aircraft crashes and other transportation accidents and thus the problem can be more confounding when the dead body is mutilated, dismembered or skeletonized. In many scenarios, only the limbs or part of a limb is available.

Age, sex and stature are the three most important parameters that help in determining the biological profile and thus confirm the identity of the individual. The stature is an essential and useful anthropometric parameter that helps to determine the individual physical identity of the remains. Determination of stature involves two main methods, the first being the anatomical method when the skeleton is intact[1]. The second is the mathematical method, the application of which becomes incumbent on the forensic expert when the body is dismembered and the long bones are available. The mathematical method entails the use of regression formulae which have been hitherto established and are both race and sex specific[2].

A combination of factors such as genetic differences, isolation, climate and nutrition underlie these differences in regression formulae between various populations. This necessitates research to establish regression formulae that are population specific.

The greatest contributor to standing height in anatomical terms is the lower limb and most regression formulae use the long bones namely the Tibia or the Femur[3]. The lengths of the long bones of the lower limb are more reliable than bones of the upper limb for predicting stature[4]. The Femur length is the best predictor of an individual's stature but its measurement in living subjects is fraught with methodological constraints, in that the femur head is inaccessible and cannot be reached. It is more useful in studies using documented cadavers, i.e. that of known individuals whose height was recorded before death and at autopsy the Femur length can be measured and used for deriving regression equations. The Tibia on the other hand is a different

prospect. As the landmarks are subcutaneous and easily accessible, the percutaneous Tibial length (PCTL) can be measured in living subjects. Besides the fact that the Tibia is amenable to percutaneous measurement, it also resists degradation and is able to retain its anatomical form long after death and thus plays an important role in anthropological research[5-7].

Our study involved living female subjects belonging to the Tamil population to measure the percutaneous tibial length (PCTL) and assess its mathematical correlation with stature. In a situation where the body is dismembered, the same parameter i.e. PCTL can be measured and the height determined using regression equations. We have thus derived regression equations to determine the stature using the percutaneous Tibial length in female individuals of the Tamil population.

Materials and Methods

The study was conducted after obtaining approval from the institutional human ethics committee of Chettinad Hospital and Research Institute. A total of 115 females in the age group of 21-30 years were selected for this study. Research participants with congenital or acquired skeletal defects, previous history of fractures involving the lower limbs or the spine, malnourishment, endocrinopathies etc. that could introduce inaccuracies in the study were carefully excluded.

The objectives of the study and the procedure was clearly explained to the subjects and the measurements were made after obtaining a written informed consent from them.

The anthropometric measurements for this study comprising of the stature of the individual and the percutaneous Tibial lengths of both the left and right lower limb were made using standard protocols. The measurements were made by the same observer at fixed time in the day between 9 to 11 am to avoid diurnal stature variation and to rule out inter-observer errors[8-9].

The standing height was measured using a stadiometer with the barefooted participant standing upright on the baseboard of the instrument[10-11]. The subject's head was maintained in the Frankfort horizontal eye-ear plane by supporting the subject's chin and the height was measured as the distance between the utmost point on the vertex and the heel.

The percutaneous Tibial length (PCTL) is the measured distance between the Tibiale Mediale (the medial most superficial point on the upper border of the medial condyle) and Spherion Tibiale (tip of the medial malleolus). Standard protocols were employed to measure the PCTL on both the left and right side independently in cm using a pair of spreading calipers[11-12]. The subject was made to stand and keep one foot on a stool so as to create a 90^{o} angle between the flexor surface of the leg and the thigh and thus accentuate the bony landmarks on the medial aspect of the Tibia. The Spherion Tibiale was located at the lowest point on the medial malleolus. The specific location of the Tibiale Mediale was found by palpating the site bounded by the medial femoral condyle and the medial tibial condyle and the superior medial border of the Tibia located. Both landmarks were marked using a skin marking pencil and the distance between them measured using the spreading calipers.

Results

The observed values for the stature in female Tamil subjects with respect to the percutaneous Tibial length in both the right and left lower limb were recorded and statistical analysis was done using SPSS Version 21 software. The results of the analysis are tabulated in Tables 1 and 2. The estimated mean height of the female subjects was 156.7 cm with a standard deviation of 4.2 cm. The mean percutaneous lengths of the right and left Tibia were estimated to be 33.54 cm (with a standard deviation of 1.01 cm) and 33.56 cm (with a standard deviation of 1.02 cm) respectively.

The Pearson's correlation coefficient r for stature with respect to the length of the right Tibia was 0.816 with regression coefficient (b) of 3.36($p<0.01$). Similarly, the Pearson's correlation coefficient r for stature versus the length of the left Tibia was 0.807 with regression coefficient (b) of 3.34($p<0.001$).

The data was used to prepare scatter diagrams (Fig. 1 and 2) by plotting the stature versus the lengths of the right and left Tibia. From these plots, the Regression equations for stature were derived as 3.36 x [Right Tibial length] + 44.03 and 3.34 x [Left Tibial length] + 44.45 using the right and left Tibial lengths respectively.

Table 1: Significant statistical parameters for Right and Left Tibia in Females

Parameter	Dependent Variable	
Mean Stature (cm.)	156.7	
	Independent Variable	
	Right PCTL (cm.)	Left PCTL (cm.)
Mean Length (cm.)	33.54	33.56
Standard Deviation	1.01	1.02
Correlation Coefficient (r)	0.816 [p<0.001]	0.807 [p<0.001]
Coefficient of Determination (R2)	0.665	0.651
Regression Constant	44.03	44.45
Regression Coefficient (b)	3.36	3.34
Standard Error of Estimate (cm.)	2.45	2.50

Table 2: Regression equation for Stature estimation from PCTL in Females

PCTL	Regression equation for Stature
Right PCTL (cm.)	y1 = 3.36 x Right PCTL (cm.) + 44.03
Left PCTL (cm.)	y2 = 3.34 x Left PCTL (cm.) + 44.45

Fig. 1 and 2 are scatter diagrams prepared by means of plotting the values for stature against the lengths of the right and left Tibia from subjects and Table 2 shows the linear regression equations for determination of stature with respect to Tibia on each side.

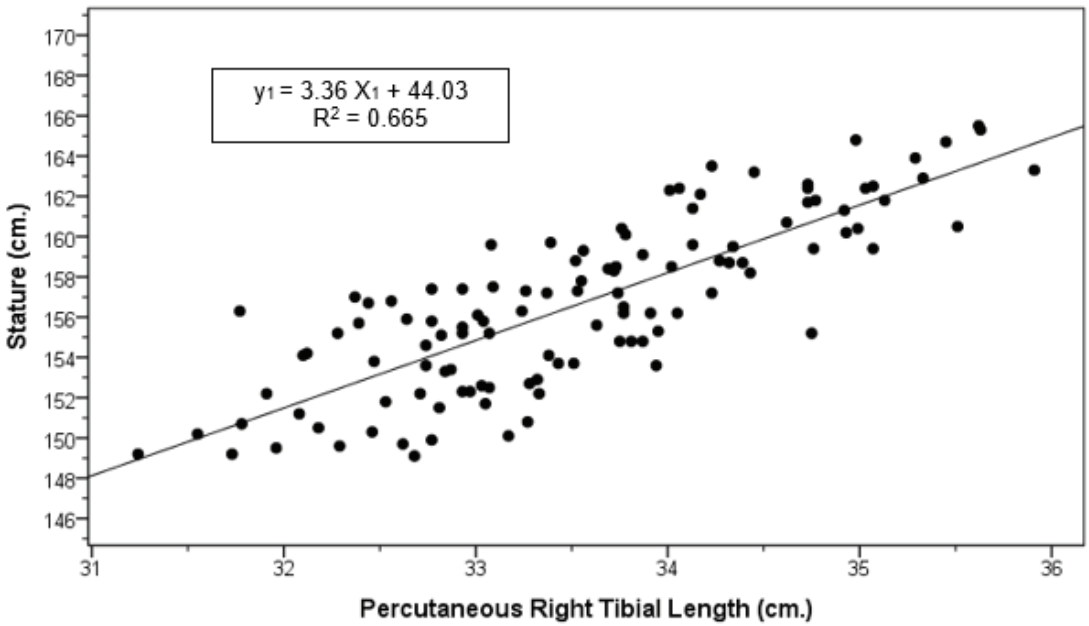

Fig. 1: Correlation between Right PCTL (X_1) and Stature (y_1) in Females

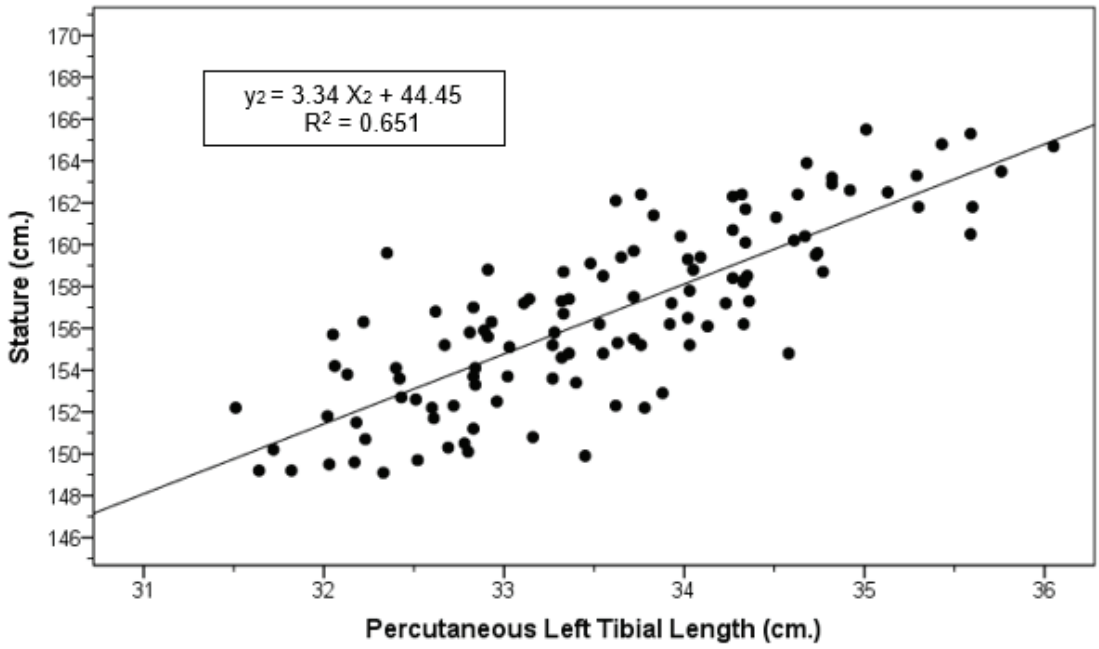

Fig. 2: Correlation between Left PCTL (X_2) and Stature (y_2) in Females

Discussion

The estimation of stature can be a challenging problem to the forensic expert when bodies are mutilated or fragmentary skeletal remains are available. In most medico-legal cases, the body is either dismembered or skeletonized and the anthropometric measurements are used to assess the stature of the decedent individual. Regression formulae for stature estimation are population specific and have been developed by several investigators from long bones using documented skeletal remains of decedent individuals whose stature was already recorded before death in populations of European White or African Black ancestry[13-15]. Such documented skeletal remains are not available in Indian populations and the alternative is to use percutaneous measurements of long bones using living individuals as the regression formulae developed for one population cannot be indiscriminately applied to another population.

This study was conducted on living adult female Tamilian subjects with the aim of deriving a linear regression equation that can be used for estimating the stature using the percutaneous Tibial length. We find a statistically significant positive correlation between Tibial length and stature and the proposed equations will predict stature from Tibial length, as attested by the lower p-values obtained while performing the regression analysis.

We compared our findings of stature, mean right and left percutaneous tibial length as well as the regression equations derived by us for Tamilian female population with the results of other investigators pertaining to different ethnic populations in the Indian subcontinent and the data has been tabulated for comparison (Tables 3 and 4). The equations derived by us for the Tamil population are different from that derived for other populations thus confirming the population specificity of the regression equations.

There are minor variations in the mean stature as well as the mean percutaneous Tibial lengths between the studied group and other ethnic populations in the Indian subcontinent. These differences could be attributed to a multitude of factors that have a bearing on the person's growth and body proportions such as nutrition and dietary habits, genetic factors, physical stress, lifestyle, environmental conditions etc. The distal segments of the limbs are more sensitive to environmental factors[16]. Research studies have suggested that the proportional change in the distal segments of the limbs is related to improvement of socioeconomic conditions, nutrition

and health status[17]. Given the fact that improvement in socioeconomic and health conditions will continue in the Indian subcontinent in the future, this will have an impact on bones of the lower extremities and specifically the Tibia in this context. Thus, the population will grow taller, the increase in length of the Tibia will be both absolute and proportional. Stature is a constantly changing target for forensic experts owing to secular trends and allometric changes in long bones. As per the Miller and Jantz study, the secular change is more marked in the lower limbs than the upper limbs, and the change in the distal bones is greater than that in the proximal bones. If the long bones change their proportion with time, then their relationship with stature will also change[18]. Thus, the older stature formulae that rely on a certain relationship of long bone length to stature may lose their efficacy over time. Linear regression analysis studies correlating stature to Tibial length will have to be done afresh every generation to ensure accuracy of stature estimation.

Table 3: Comparison of Mean Stature and Mean Tibial length in Females

Name of the Researcher	Year	Population for study	Mean Stature (cm.)	Mean Tibial Length (cm.)	
				Right	Left
Present Study	2020	Tamil Nadu	156.7	33.54	33.56
Kavyashree19	2018	Karnataka	158.87	34.96	33.03
Akilesh Trivedi20	2014	Madhya Pradesh	155.3	36.10	36.03
Ashita Kaore21	2012	Karnataka	156.187	32.19	32.14

Table 4: Comparison of Regression Formula for Stature (Y) in Females from length of Right Tibia (X_1) and Left Tibia (X_2)

Name of the Researcher	Year	Population for study	Regression Formula	
			Right Tibia	Left Tibia
Present Study	2020	Tamil Nadu	Y=3.36X1 + 44.03	Y=3.34X2+ 44.45
Kavyashree19	2018	Karnataka	Y= 1.069X1 + 122.54	Y=1.03X2 + 123.78
Akilesh Trivedi20	2014	Madhya Pradesh	Y=1.373X1 + 105.724	Y=1.35X2 + 106.64
Ashita Kaore21	2012	Karnataka	Y=1.678X1 + 102.16	Y=1.379X2 + 111.86

Table 4 shows a comparison of the regression formulae derived by us with that of other investigators for other ethnic populations in the country. All investigators were able to demonstrate a positive correlation between stature and Tibial length which thus affirms the relationship between the two parameters.

Conclusion

The conclusion from this study is that the regression equations that have been formulated by us can be utilized to estimate the stature with reasonable accuracy in adult female individuals belonging to the Tamil population in situations such as mass disasters where the body is dismembered and the lower limb bones are available. The formulae derived are both ethnic and sex specific and cannot be used for other ethnic groups.

We recommend further studies on a larger scale and involving stature groups to develop separate equations separately for short, medium and tall statured individuals. Such stature group specific regression equations will further reduce errors in stature estimation and provide more accurate height estimates than a single general formula[22].

Conflict of Interest: None declared

Source of Funding: Nil

Informed Consent: Obtained from all the participants

Ethical Clearance: Necessary ethical approval was obtained from the Institutional Ethics Committee, Chettinad Academy of Research and Education, Kelambakkam – 603103.

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Type of the Manuscript: Research article

Retrospective Study of Profile of Deaths Due to Fatal Electrocution Injuries in the Jammu Region of Union Territory of Jammu and Kashmir

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Abstract

Background: Electrocution injury is due to passage of a substantial electrical current through the tissue, which can cause skin lesions, organ damage, and death. Due to scarcity of typical autopsy findings in electrocution and unclear circumstances of death, many a times cause of death remains undiagnosed. Present study was retrospective, autopsy-oriented study, aimed to study profile of deaths due to fatal electrocution injuries in the Jammu region of union territory of Jammu and Kashmir. During study period 40 cases of electrocution were autopsied at our center. During study period, 1.23% incidence was noted for fatal electrocution deaths. In present study majority of the victims were men (78 %), with a male: female ratio of 3.5:1. Most common age group was 21–30 years (43 %)& upper limbs (58 %) were most common body parts involved. 53 % cases had both entry & exit marks while in 15 % cases no entry or exit marks were noted. Most of the electrocution deaths were accidental, common in males, in the rainy season & most of the deaths were either instantaneous or immediate. Most of the electrocution deaths are preventable in nature and they can be prevented by implementing proper education programs, widespread usage of safety appliances and insulators, keeping electric appliances in child proof cabinets.

Keywords: *Electrocution deaths, forensic Autopsy, Entry and Exit marks, accidental death*

Introduction

Electrocution injury is due to passage of a substantial electrical current through the tissue, which can cause skin lesions, organ damage, and death. Injuries due to electrocution depend on many factors such as voltage and frequency of current, duration of contact with body, atmospheric conditions, and route of current in the body.[1]

In India, electrocution caused 3.0% deaths during 2018.[2]In India though deaths due to electrocution are less, but the cases of injuries are quite common due to accidental touching of a current source.[3] The severity of electrical injuries is dependent upon several factors including voltage, type and amount of current flow, intensity of the electrical current, electrical source, and length of exposure to that source.

Fatal electrical injuries may occur in various ways: Direct contact with electricity, electrical arcs, and flash burns created by an electrical arc, or flame burns. They are mainly preventable with simple safety measures in most situations.[4]

Due to scarcity of typical autopsy findings in electrocution and unclear circumstances of death, many a times cause of death remains undiagnosed. Present study was aimed to study profile of deaths due to fatal electrocution injuries in the Jammu region of union territory of Jammu and Kashmir.

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Material and Methods

Present study was retrospective, autopsy-oriented study, conducted at the department of Forensic Medicine and Toxicology, Government Medical College Jammu , Union territory of Jammu and Kashmir. Study period was from July 2017 to June 2020 (3 years). Institutional ethical permission was obtained for present study.

All cases of electrocution deaths autopsied at our centre were considered for present study.

Detailed history of electrocution incident, demographic profile, referring hospital records, details from police inquest, autopsy reports and ancillary investigation (histopathological, chemical analysis) data was collected retrospectively from the records.

Gathered data was entered into Microsoft Excel sheet and analyzed for frequencies and percentages. Statistical analysis was done using descriptive statistics.

RESULTS

During study period 40 cases of electrocution were autopsied at our center. during study period, 1.23% incidence was noted for fatal electrocution deaths. In present study majority of the victims were men (78 %), with a male: female ratio of 3.5:1. Most common age group was 21–30 years (43 %), followed by age group 31–40 years (23 %) & age group 41–50 years (15%).

Table 1: Distribution according to age and gender

Age group (years)	Male	Female	Total
≤ 10	0	0	0
11–20	3 (8 %)	1 (3 %)	4 (10 %)
21–30	13 (33 %)	4 (10 %)	17 (43 %)
31–40	7 (18 %)	2 (5 %)	9 (23 %)
41–50	5 (13 %)	1 (3 %)	6 (15 %)
51–60	3 (8 %)	1 (3 %)	4 (10 %)
≥ 61	0	0	0
Total	31 (78 %)	9 (23 %)	40

With respect to the presentation of Fatal electrocution deaths were distributed according to the diurnal i.e day time (7 pm to 7 am) & night time (7 pm to 7 am) and seasonal variations. In present study 73 % cases were reported during the daytime. We noted higher deaths (50 %) during July–September period (rainy season), followed by 23 % cases in January–March period.

Table 2: Seasonal and diurnal variations

Month of incidence	Daytime	Night time	Total
January–March	7 (18 %)	2 (5 %)	9 (23 %)
April–June	3 (8 %)	3 (8 %)	6 (15 %)
July–September	15 (38 %)	5 (13 %)	20 (50 %)
October–December	4 (10 %)	1 (3 %)	5 (13 %)
Total	29 (73 %)	11 (28 %)	40

Upper limbs (58 %) followed by lower limbs (43 %) were most common body parts involved in fatal electrocution deaths.

Table 3: Distribution of cases according to body part involved*

Part of body	Number of cases	Percentage (%)
Head and Neck	5	13%
Upper limbs	23	58%
Lower limbs	17	43%
Trunk	11	28%
Whole body	9	23%

(* - one or more body parts can be involved simultaneously)

In present study 53 % cases had both entry & exit marks while in 15 % cases no entry or exit marks were noted. 10 % cases had burn injuries while 33% had other injuries.

Table 4: Type of electrical injuries noted during autopsy.

Type of electrical injuries	Number of cases	Percentage
Both entry & exit marks	21	53%
Only entry mark	7	18%
Only exit mark	6	15%
No entry or exit marks	6	15%
Burns	4	10%
Other injuries	13	33%

We noted that in electrocution deaths electrical wire (45%) was most common material followed by electrical cable (23%) & water heater in the bathroom (15%). Other causes were electrical stove (8%), electrical socket (5%) & generator (5%).

Table 5: Type of material causing electrocution.

Type of material	Number of cases	Percentage
Electrical wire	18	45%
Electrical cable	9	23%
Water heater in the bathroom	6	15%
Electrical stove	3	8%
Electrical socket	2	5%
Generator	2	5%

In present study, place of incidence was 60% in home, while 28 % occurred at working place & 13% at other places.

Table 6: Distribution of cases according to place of incidence

Place of electric injury	Number of cases	Percentage
Home	24	60%
Working place	11	28%
Other	5	13%

The manner of death was accidental in nature in all cases and no suicidal or homicidal death was reported.

Discussion

Electrocution injury causes passage of electric current through human body, it causes multiple effects, varying from a localized muscle spasm to sudden death of the person. Deaths due to electrical energy is a "functional" death -type in most cases, because fatalities may happen as a result of cardiac arrhythmia/ventricular fibrillation, asphyxia, or electrically induced respiratory muscle contractions.[5]

In present study with 40 cases of electrocution autopsies, we had 1.23% incidence of fatal electrocution deaths. Most common age group was 21–30 years (43 %) followed by age group 31–40 years (23 %). Electrocution was very rare in both extremes of ages. Similar findings were noted by other authors from different parts of the country.[4,6,7]

In present study majority of the victims were men (78 %), with a male: female ratio of 3.5:1. Death from electrocution is rare and generally an accidental occurrence. A majority of adult electrocution injuries occur to the male gender, are low voltage in nature, and happen in occupational settings.[8] Adult males are more often actively engaged in electricity dependent occupations, either at their workplace or home during their second to fourth decades, hence they are prone to electrocution hazards.

Regarding the seasonal and diurnal variations, higher incidence (50 %) was recorded in the rainy season and during the daytime (73 %). These findings are with the observations made by other indian studies by Gururaj B. et al.[9] and Kumar et al.[10] Human body is considered as a bad conductor of electric current but the wetness of ground or skin increases chances of electrocution injuries, which is commonly observed in rainy seasons. While Tirasci Y et al noted higher incidents of electrocution deaths during summer, which might be due to increased humidity and high usage of electric appliances.[11]

In present study 28 % cases had both entry & exit marks while in 15 % cases no entry or exit marks were noted. 10 % cases had burn injuries while 33% had other injuries. In study by Ragui S et al. they had 72% entry and corresponding exit wounds, while 20% cases had without any entry or exit wounds.[12] The distinct injury marks produced at the site of contact with electric wire (entry mark) and joule burns in electrocution cases can be considered as a classical external sign of electrocution.

The resistance offered by the tissue to the flow of electric current determines the amount and place of the heat formation. The skin is the human tissue that offers the maximum resistance to the electric current.[13] Skin is the most frequently involved tissue in electrocution injuries, but electrocution changes can also be seen in muscles, intra-abdominal organs, bone, neural and orbit.[14]

The hands and fingers of the upper extremities are the most frequent sites for electrical injuries (entry marks), while exit wounds were commonly located on the soles & toes of lower extremities, because extremities are the most common sites of contact with the source

of electric current. Similar findings were reported from other studies.[11,12]

All deaths were accidental in present study. The main reasons for accidental electrocution deaths are electrocution from tree trimming, contact with an electric switch board of the motor pump, overheating, aging of the material and use of sub-standard quality of electrical gadgets causing electrical short-circuit, etc.

Diagnosis of high voltage electrocution is sometimes difficult in the absence of history and circumstantial evidence, as the pathognomic features like electric marks and joule burns are often seen only with low or medium voltage current involvement and also both entry and exit marks are seen together only in 20% cases.[15] In such cases ancillary investigations such as histopathological, biochemical, chemical examination of skin & all relevant internal organs proves useful.

Even after a careful and painstaking autopsy, including investigations like the histopathological, biochemical, chemical examination of all relevant internal organs and the meticulous detailing of the circumstantial evidences, identifiable cause of death is not found. These autopsies are called negative autopsies. One of the important causes of the negative autopsy is death due to electrocution.[16]

Present study visit Limitations were small sample size, cause of death was declared based on the history and autopsy findings, crime scene was not visited in most cases & ancillary investigations were not performed in all cases. For detailed information large sample size with meticulous investigations will be helpful.

Conclusion

Most of the electrocution deaths were accidental, common in males, in the rainy season & most of the deaths were either instantaneous or immediate. Most of the electrocution deaths are preventable in nature and they can be prevented by implementing proper education programs, widespread usage of safety appliances and insulators, keeping electric appliances in child proof cabinets.

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Source of Funding: Nil

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A Protocol on Effect of Gaze Exercises and Balance Regimen on Quality of Life of Patients with Peripheral-Vertigo

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Abstract

Background: As vertigo is been one of the most common condition affecting the individual functionally, physically and emotionally and result in some form of disability. Most of physiotherapy intervention proves to be effective in vestibular rehabilitation of patients with vestibular disorders. Gaze stabilization exercises are effective and some suggest balance training is also effective in vestibular rehabilitation. But there is minimal literature on combination of both exercise protocol. **Aim:** To find out the effect of Gaze stabilization Exercises and balance training on quality of life of patients with Peripheral Vertigo. **Methods:** 30 participants with vertigo due to peripheral pathology will be selected on the basis of inclusion, exclusion and head impulse test. The patient will be evaluating using Visual analogue scale, Dizziness Handicap Inventory, Berg Balance Scale and Dynamic Gait Index Scale. The participants will be distributed into two categories. Group- A will receive gaze stabilization exercise and Group- B will receive balance training along with gaze stabilization exercise. Participants will be evaluating post-treatment. **Conclusion:** This study concluded that there may be significant reduction in vertigo symptoms and improved Quality of Life in peripheral vertigo by using combine effect of Gaze Exercise with Balance Training.

Keyword: *Peripheral-Vertigo, Physical therapy, Gaze Stabilization Exercises, Balance training.*

Introduction

Vertigo is the term used to refer to a self or environmental illusion, usually defined as spinning or whirling[1]. The prevalence of vertigo throughout the world is ~30%, with associated co-morbidity being 3.2%.[2]

A study in Germany found that vertigo prevalence was 22.9 percent and 3.1 percent[3]. According to an epidemiological study performed in France, the prevalence of vertigo was 48.3 per cent for one year[4]. The precise incidence and prevalence of the vertigo is still unknown in the general Indian population.

However, the overall prevalence of vertigo in India's adult population was stated to be 0.71%.[5] In the 5th decade following the 7th and 4th decades, incidence of vertigo found was more common in patient. Women (57.7%) were found to be more frequently affected than men (42.30%) in the 4:3 ratio[6].

Vertigo that is present as a symptom can have a central or peripheral cause[2]. The HEAD IMPULSE TEST is a bedside test for distinguishing between the central or peripheral causes of vertigo[7].Dizziness, nausea, and vomiting, sweating and spatial disorientation are the typical signs of vertigo[8]. Vertigo induces risk of falling, restricts mobility and daily life activities, and limits social involvement[9]. Vestibular rehabilitation is a clinical tool which is based on the core neuroplastic system[10].

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Gaze stabilization exercise is one of the steps that emerged in late 1980 for vestibular recovery. Gaze stabilization exercise is a key component of care for patients with the aim of enhancing the vestibular eye reflex, increasing visual acuity during head movement and reducing vertigo symptoms[11].

Balance exercise can have a positive effect on physical activity for elderly people with vertigo[12]. It is important to reduce prevalence and impact of vertigo as adult vestibular dysfunction can cause serious impairment with significant psychological morbidity[8].

Aim: To find out the effect of gaze exercises and balance regimen on quality of life of patients with peripheral-vertigo.

Method

The present experimental study will include the 30 adult participants which will be selected randomly from Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha (MS), India. on the basis of inclusion and exclusion criteria. The male and female participants between the ages of 20-60 years having Vertigo patient due to peripheral cause will be included. The participants below 20 years and above 60 years, having Vestibular schwannoma, Multiple sclerosis and Cervicogenic vertigo will be excluded. The informed consent need be obtained. They will be going through the following Test and measure prior to treatment and also post treatment. The participants will be divided into two groups. Group A will receive Gaze Stabilization Exercises alone and Group B will receive Gaze Stabilization Exercises with Balance Training. The study duration is of 6 month and intervention duration is 15 days. Assessment will be done on first day of visit and patient have to visit daily for the treatment, during the treatment period assessment will be done after every 2 days on Head Impulse Test, Visual Analogue Scale, Dizziness Handicap Inventory, Berg Balance Scale and Dynamic gait index.

Data Collection Tools

1. Visual Analogue Scale: It is an effective technique to obtain subjective intensity rating of vertigo, lightheadness, disequilibrium and Oscillopsia .The patients is asked to answer a question (e.g. How intense are your symptoms?)and mark on 10-cm line (on extreme of left indicate “non” to right “worst possible intensity”).The clinician then measure the line and obtain a quantified value.

2. Head impulse Test: This is a commonly recognized clinical method for analyzing function of semi-circular canal. cervical range of motion should be determined before performing the HIT and physical therapist should explain why the head must be move quickly. The HIT is conducted by first setting the patient to near-target (e.g., the nose of a clinician). When testing horizontal semi-circular canals, the head is flexed 30 degree. In the case of a low amplitude (5-15) of either a moderate amplitude (approximately 200/sec) and high amplitude with an angular momentum (3000-4000/sec), the eyes turn in a direction opposite to the head movement and the focus stays in a manual direction, while the Vestibular Reflex is working in a normal way. In a patient with a loss of vestibular function, the person will not move the eyes rapidly from the target as the head and eyes move away from target. The patient will then take a remedial saccade to reposition the eyes to the target. HIT is a test designed to provide a sensitive indication of vestibular hypofunction.

3. Dizziness Handicap Inventory (DHI) is a popular scale used in calculating the automatic impairment of a patient due to vestibular diseases. The DHI is highly accurate in tests and has good reliability internally. Patients respond to 25 question, sub-group into functional, emotional and physical component. The DHI quantifies the patient’s sense of disequilibrium and its effect on day-to-day activities. It provides Subjective improvements. Scoring are as: 16-34 -mild disable, 26-35-moderately disable and 54+indicate severely disable.

4. Berg Balance Scale: It is used to assess Balance ability of an individual (or incapacity) to manage them safely during a predetermined task. It is a set of 14 items each with an ordinal five-point scale of between 0 and 4. 0 is the lowest function level and 4 shows the highest function level. A. Functional equilibrium is indicated by 56 and score < 45 indicates that the person is more vulnerable to fall.

5. Dynamic Gait Index: This is important tool for persons of vestibular dysfunction. Because the test incorporates various head rotation action that challenge vestibular response to gait activities. The DGI is an

eight-item test with each item graded (0to30) as severely impaired, or normal, for a maximal score of 24. Then participant will be divided into two: Group-A: Gaze stability exercises and Group -B: Gaze stability exercises with Balance training under supervision. Both exercise protocol will be given for 15-40min for 15day depending upon treatment protocol. The exercise should also be progress in gradually so that it should start from simple gaze stabilization exercises (e.g.Patients advised to focus their eyes on close goal)and progressing gradually make it complex(e.g. Patients are advised to concentrate their attention on a near-target as the concentrate is held, the patient rotates the head and target horizontally in the opposite direction.The protocol for Balance should also progress in sequential manner from simple (e.g.Stand apart with feet about shoulder width, arm around the chest and move to near feet) and make it complex (e.g. standing on one leg).After 15days of treatment program again above test and measure will be performed to evaluate the treatment effects.

Statistical Analysis

The data will be collected and analyse utilizing inferential and descriptive statistics by using Chi-Square test and student's 't' test (unpaired and paired) & software for analysis will be SPSS 22.0 version and Graph pad prism 6.0 version & level of significance will be considered as $P<0.05$.

Discussion

Vertigo is the term used to refer to a sel for environmental illusion, usually defined as spinning or whirling[1]. Vertigo induces risk of falling, restricts mobility and daily life activities, and limits social involvement[9].Gaze stabilization exercise is a key component of care for patients with the aim of enhancing the vestibular eye reflex, increasing visual acuity during head movement and reducing vertigo symptoms[11].Balance exercise can have a positive effect on physical activity for elderly people with vertigo[12]. The study conducted on Effect of gaze stabilization exercise on vestibular function during postural control by Akiyoshi Matsugi, Yusuke Ueta et.al.(2017) on 18 healthy participant (13men and 5 women mean age) concluded that gaze stabilization exercise are helpful for improving balance and also suggest that the effect of gaze stabilization exercise can produced immediately after 5 min of training[15]. The study on Effectiveness of gaze stability exercise on balance in healthy elderly population by Vaishali Bhardwaj et.al.(2014) involving 30 participants without any definite balance disorder. The study suggests that the patient undergoing gaze stability exercise for 6- week shown to improve confidence for activities of daily living and balance than control group[16]. The study on Recovery of dynamic visual acuity in unilateral vestibular hypofunction by Susan J. Herdman et.al. (2003) on twenty-one patient with unilateral vestibular hypofunction, aged 20-86 years receiving gaze stabilization exercise four 4-week suggest that gaze stabilization exercise facilitate the recovery of gaze stability during head movement and also suggest that older patients and patient with chronic unilateral vestibular hypofunction may benefit from vestibular rehabilitation[14]. This study protocol detail two physiotherapy intervention in patient with peripheral-vertigo to improve quality of life and to reduce vertigo symptom: Exercises for Gaze stabilization and balance regimen. This study will focus on combine effect of both technique on quality of life and symptom of vertigo in peripheral-vertigo patient.

Conclusion

This study concluded that there may be significant reduction in vertigo symptoms and improved Quality of Life in peripheral vertigo by using combine effect of Gaze Exercise with Balance Training.

Ethical Approval and Dissemination

Ethical approval will be taken from institutional ethical committee. The DMIMS who will fund for research and the subjects who will be participating in the study can access the main findings of the research. Data held safely for the enrolled subjects a minimum of five years. After completion of data collection, statistical analysis a completion report will be formed and after review by institutional research cell will be send for publication.

Confidentiality

Participant and the relative will be informed about the study program and personal information will be taken by principal investigator only, in consent from the confidentiality statement will be mentioned. Signatures of both investigator and patient with two witness will

be taken and for the purpose of the study if needed to disclose some information complete assurance and consent will be taken from the patient.

Funding of Resources: No funding

Conflict of Interest: The author declares no conflict of interest.

Dissemination: After completion of data collection, statistical analysis will be done and completion report will be formed then after checking plagiarism, review by institutional research cell, will be send for publication.

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Mobile Phone Utility and Its Effects on Health among Medical Students in Rajahmundry, Andhra Pradesh

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Abstract

Background: Mobile phone has become important in everyday life and internet utility have increased especially among the student community. Overuse of mobile phone has been associated with many health issues. Objective was to know the mobile phone usage pattern and its health effects among medical students.

Methods: A cross sectional study was conducted among medical students of GSL Medical College, Rajahmundry including 287 students from first to third MBBS. Data was collected using a pre-tested questionnaire consisting of socio-demographic parameters, pattern of mobile phone usage like mode of use, time of maximum use etc. and the health effect variables like headache, fatigue, neck pain, painful fingers etc. Statistical analysis was done using MS-Excel 2007 and SPSS v 22, chi-square test was applied and $p<0.05$ was considered significant.

Results: 95% were using smart phone and 83% were having single phone. Maximum were using for 4-6 years (55%). Internet was used for academic purpose in 90%. Majority of them experienced eyes symptoms (55.23%) followed by sleep deprivation (46.21%) and headache (42.60%).

Conclusions: Mobile phone are having positive role in our daily lives but its overuse has bad impact on health of students.

Keywords: *Health Effects, Medical students, Mobile phone, Usage pattern.*

Introduction

Mobile phones are a modern-day communication invention, which has helped millions to reach in different parts of the world. Technological advancement in field of communication have made mobile phone even smarter to be able to surf the internet, make video calls, play games etc.[1]

The most important technological development in the last 30 years or so has been the arrival of the mobile phone also known as cell phone. In last 20 years, subscriptions of mobile phones all over the world have grown from 12.4 million to over 5.6 billion, penetrating about 70% of the global population.[2-5]**.** The usage of mobile internet has perceived more than three times increase since 2010, which has reached 6.319 billion in April 2019.[6] India holds second position in telecommunication, subscription, internet subscribers and application downloads globally.[7] Out of 830 million young people who are online, 320 million (39%) are in India and China**.**[8]

Textbooks have been the major source of information for medical students previously. However, with advances in information technology, the number of students using the electronic resources has increased evidently. Advances in technology have made it possible for people to easily get any information when needed [9-13]. Mobile phones took a revolutionary turn with the

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launching of the smartphone in 1994 [13]. Since that time mobiles have become an essential part in our day-to-day activities [14]. Mobile phones have great potential in the field of medical education if used rationally.[15] Smartphones are availed for various purposes by medical students viz., note taking, web browsing, cloud storage, imaging, clinical handbooks and text books, question banks, medical calculators, simulation apps etc.[16]

The contribution of mobiles in health care professions has become very strong and effective by providing access and provision of information to valuable advice in almost each specialty[17]. Over use of mobile phone has shown to be associated with health problems such as decreased concentration, headache, fatigue, heat sensations in and around ear, stress, sleep disturbances owing to night time use, and frustration.[18] Hence the study was conducted to evaluate the usage pattern of mobile phones and its health effects among medical students.

Objectives

- To find the pattern of mobile phone usage
- To find out the prevalence of health effects among the medical students.

Material and Methods

The present Cross-Sectional study was conducted among the medical students of GSL Medical College, Rajahmundry. It was two month study from 1st August 2019 to 30th September 2019. The study population consisted of 287 undergraduate medical students from first to third year MBBS selected randomly for the study as they have access to the Community Medicine department in view of the academic timetable. The students after the theory class were contacted & were oriented about the purpose & importance of the study & were instructed how to fill the predesigned proforma which was handed over to them. The questionnaires were distributed to the respondents and were asked to fill it up after giving the informed consent. It was a self-administered, pre-designed, pre-tested semi- structured questionnaire. The questionnaire comprised of socio-demographic characteristics, pattern of mobile phone usage (mode of use, time of maximum use, etc.), behavioral pattern (midnight checking of mobile, early morning checking, restroom usage), their opinion on mobile phones and the health effect variables experienced like headache, fatigue, neck pain, painful fingers etc.

Inclusion Criteria: All the medical students from 1st , 2nd & 3rd year MBBS were included to participate in the study.

Exclusion Criteria: Students who did not give informed consent.

Statistical Analysis: Data was analyzed using MS-Excel 2007 and SPSS v 22, proportion and Chi-square test was applied and p value <0.05 was considered significant. Results were presented in form of tables and figures.

Results

Out of a total of 287 student who took part in study, 62% were males and 38% were females. Most of them were in the age group of 21-23 years (57%) as shown in the (Table 1). 83% (237) possessed one mobile phone and 17% (15) were having two or more mobile phones and 95% were having smart phones whereas both keypad and smartphone was owned by 5% of them. 2% were using mobile phone for more than six years and 55% were using since 4 to 6 years. As far as the behavioral pattern is concerned a mere 10% of them switched off phone during sleep, 23% of them had habit of checking the mobile for missed calls or messages in midnight and 67% of them had habit of checking their phone as soon as they got up from sleep. The usage of mobile phone among students in the classroom during the class and break time was 56%, 2% used while driving whereas 42% used phone while using the restroom. (Table 2).

Table 1: Sociodemographic Profile Of Medical Students

PARAMETERS	NUMBER OFSTUDENTS (N%)
AGE (YEARS)	
18-20	118(41%)
21-23	165(57%)
>24	4(2%)
GENDER	
MALE	177(62%)
FEMALE	110(38%)
RELIGION	
HINDU	238(83%)
MUSLIM	28(10%)
CHRISTIAN	15(5%)
OTHERS	6(2%)

Students' view of mobile phone utilization was as follows- 47% had notion that it was academic hindrance and 63% as distractions but 94% of them had an opinion that mobile phones are beneficial for studies. 31% said that life without mobile phone is boring but normal. (Table 3).

Use of internet for purpose of academics was 90%, for purpose of entertainment was 93% and for social networking 88% participants used the internet (Table 4).

46% had sleep deprivation due to mobile use and 22% became late for classes and 16% had missed classes 47% of participants suffered decrease in concentration.

Sleep and academic disturbances were more common in participants who were using mobile phones more than five hours compared to participants using for less than five hours and their association was highly significant Table 5

Table 2: Pattern of mobile phone usage among medical students (n=287).

	NUMBER OF STUDENTS (N%)
NUMBER OF MOBLILE PHONES	
ONE	237(83%)
TWO OR MORE	50(17%)
DURATION OF MOBILE USAGE (YEARS)	
<2	6(2%)

Cont... **Table 2: Pattern of mobile phone usage among medical students (n=287).**

2-4	118(41%)
4-6	159(55%)
>6	4(2%)
TYPE OF PHONE	
SMART PHONE	272(95%)
KEYPAD	-
BOTH	15(5%)
MODE OF USAGE MOST OF THE TIME	
RINGING	104(36%)
VIBRATION	132(46%)
SILENT	51(18%)
USE AT PLACES	
CLASSROOM	161(56%)
RESTROOM	121(42%)
DRIVING	05(2%)
MONEY SPENT ON MOBILE PHONE (RS/MONTH)	
<300	106(37%)
300-1000	175(61%)
>1000	6(2%)
CHECKING PHONE AT MIDNIGHT	67 (23%)
SWITCH OF THE PHONE DURING SLEEP	30(10%)
CHECKING PHONE AS SOON AS GET UP FROM SLEEP	192 (67%)
EVER STAYED A DAY WITHOUT PHONE	114(40%)

Table 3: Opinion of Mobile Phone Use

	NUMBER OF STUDENTS (N%)
OPINION ON USAGE	
ACADEMIC HINDRANCE	134(47%)
DISTRACTION	180(63%)
BENEFICIAL FOR STUDIES	270(94%)
LIFE WITHOUT PHONE	
BORED AND NORMAL	88(31%)
BORED AND ALONE	37(13%)
NORMAL	62(22%)
BORED	38(13%)
ALONE	27(9%)
HAPPY	22(7%)
CLAM	33(11%)

Table 4: Other Than Communication Use of Mobile Phone By Medical Students

	NUMBER OF STUDENS (N%)
*OTHER PURPOSE	
HEARING SONG	267(93%)
GAMING	202(70%)
INTERNET	284(99%)
*USE OF INTERNET FOR	
ACADEMIC	257(90%)
ENTERTAIMENT	268(93%)
SOCIAL NETWORKING	253(88%)

Table 5: Association of sleep and academic disturbance with duration of mobile phone usage (n=287).

SLEEP AND ACADEMIC DISTURBANCES	DURATION OF MOBILE PHONE USAGE			
	<5 HOURS(n=119)	>5 HOURS(n=168)	CHI SQUARE	P value
	N%	N%		
Sleep deprivation (n=131)	38 (29%)	93 (71%)	15.40	0.000087
Waking time tiredness (n=112)	59 (53%)	53 (47%)	9.5188	0.002034
Difficulty in waking up (n=149)	79 (53%)	70 (47%)	17.0516	0.000036
Decline in study habits and grades (n=136)	42 (31%)	94 (69%)	11.9237	0.000554
Decrease in concentration (n=135)	38 (28%)	97 (72%)	18.62	0.000016
Increase in missed classes (n=46)	12 (26%)	34 (74%)	5.3363	0.020885
Being Late For Class (n=63)	13 (21%)	50 (79%)	14.4277	0.000146

Table 6: Association of health problem faced with duration of mobile phone usage (n=287).

HEALTH PROBLEM FACED	DURATION OF MOBILE USAGES			
	<5 HOURS(n=119)	>5 HOURS (n=168)	CHI SQUARE	P VALUE
	N%	N%		
EYE SYMPTOMS (n=156)	46 (29%)	110 (71%)	20.1968	0.00001
FATIGUE/TIREDNESS (n=153) 53	48 (31%)	105 (69%)	13.7478	0.000209
HEADACHE (n=130) 45	40 (31%)	90 (69%)	11.1977	0.000819
NECK PAIN (n=87) 30	15 (17%)	72 (83%)	30.1787	0.00001
PAINFULL FINGERS (n=65)	15 (23%)	50 (77%)	11.7044	0.000624
EARACHE (n=63)	11 (17%)	52 (83%)	19.1609	0.000012

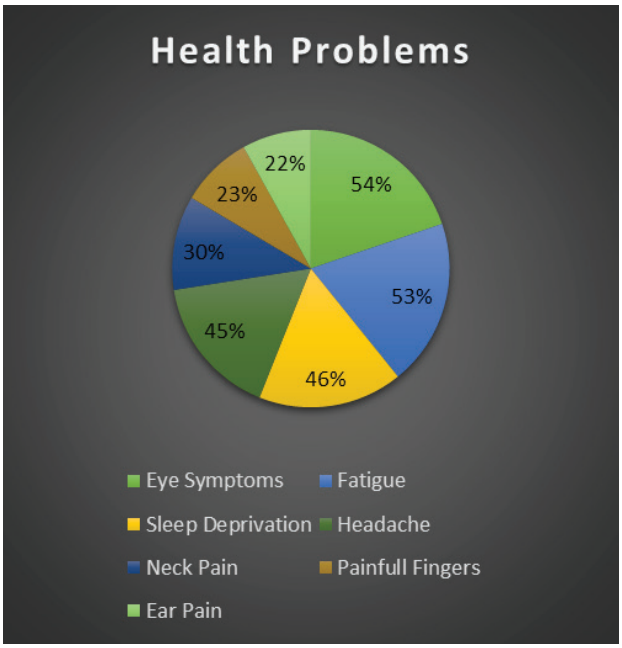

Figure 1: Health problems experienced due to mobile phone usage.

Discussion

In present study, everybody had mobile phones & nearly all of them were latest smart phones.. In our study 62% were male and 38% were female participants where as in a study conducted by Unnikrishnan et al in Coastal South India 56% were male and 44% were female students.[19] and in a study done by Sinha S[1] in Belgavi male constituted 62.09% of study participants whereas female made up 37.91% of the participants. In study done by Subha et al[20] and Sinha S et al[1] it was seen that 39.7%,37.55% students respectively were keeping their phone in ringing mode and 60.3% , 62.45% were keeping in vibrating or silent mode which was almost at par to our study which showed 36% kept phone in ringing mode while 64% of them kept in vibration or silent mode.

Result of our study showed that 59% were using their mobiles for more than five hours. A study conducted by Susila et al[18] among medicals students of Chennai saw that 29% spent more than three hours/day on mobile and 17.8% by Gupta et al in Punjab were using for more than three hours.[21]

Internet consumption for entertainment, academics and social networking was high in our study. Utilization of internet for academic purpose in present study was 90% whereas it was 11.11%, 19.4%, 76.9% in different studies done across India among the medical students by Damor et al, Ghosh et al, Aggarwal et al, respectively.[22,23,24] .The increased time duration on phone might be due to usage of mobile phone for academic purpose. Bagday et al study in Nagpur city showed 5.96% were using internet on mobile phones for chatting, 59.59% for shopping and 40.92% for games was less compared to our study.[25] Mobile phone was used by 14.4%, 5.8% for playing games and for watching movies and listening songs it was 21.81%, 8.3% according to Damor et al and Ghosh et al which was again less compared to our study.[22,23] A study conducted by Damor et al in Gujarat showed that 41.8% students said mobile phones hampered their academic performance which is almost similar to our study.[22]

According to George et al in Kerala, 34% were checking their phone at midnight which was more when compared to the our study.[27] In the present study 2% were using the mobile phone while driving while it was 17.4% in a study conducted in South India.[20]

Health problems faced in our study were eye symptoms (54%), followed by fatigue (53%), headache (45%) which was almost comparable to a study conducted by Arumugam et al where 64.3% had faced health issues like headache, sleep disturbance, ear pain, irritability.[27] Similar study conducted by Paul et al among the medical students found that 35.4% of the students complained of headache and spasms of arm or neck muscles (31.0%) or loss of attention (24.7%).[28] Among the medical students of Western Maharashtra, headache was 30.20% among girls, 15.60% in boys, eye symptoms 5.50%, sleep deprivation 21% by Yadav.[29]

The present study uncovered that maximum students used mobile phone for more than five hours , and everyone was using it for academic purpose, entertainment and social networking and they had experience health problems this same thing was reported by Sinha S et al[1] also. So, the students should be taught about the health issues due to the over usage of mobile phones, restriction rules of mobile phones use in the class and campus should be executed strictly by the administrative authority.

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Ethical approval: The study was approved by the Institutional Ethics Committee of GSL Medical College, Rajahmundry.

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Stability of Palatal Rugae after Rapid Maxillary Expansion

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Abstract

Introduction: Palatal rugae or plicae palatine are uneven ridges made of connective tissue situated beneath the incisive papilla. DNA analysis, finger prints analysis, are the most common techniques used for establishing person's identity but in situation where using these techniques are difficult palatal rugae can be used as a second source of identification. However, some habits like prolonged thumb sucking and orthodontic treatment for long duration could affect the rugae pattern. Hence, this study aims to evaluate the changes in transverse dimensions of palatal rugae in subjects treated with rapid maxillary expansion

Material and Methods: The palatal rugae patterns were measured and compared to evaluate the morphometric changes between pre-treatment cast (30 Pre-RME) and its post-treatment (30 Post-RME) counterpart. All pre-and post-expansion dental casts were traced and the intermedial and interlateral dimensions of first, second and third rugae were measured. The statistical analysis was done using paired t test.

Conclusion: The medial aspect of first primary rugae can be considered as a stable reference landmark while the lateral and medial aspects of the 2nd and 3rd rugae are liable to changes in transverse dimension. Hence, for forensic identification the stability of palatal rugae in patients who had undergone rapid maxillary expansion still remains questionable

Keywords: *Rapid Maxillary Expansion, Palatal rugae, Transverse dimension*

Introduction

Forensic dentistry or forensic odontology has large number of applications to forensic science. Even though DNA analysis, finger prints analysis, are the most common techniques used for establishing person's identity but in situation where using these techniques are difficult palatal rugae can be used as a second source of identification [1].

Palatal rugae or plicae palatine are uneven ridges made of connective tissue situated beneath the incisive papilla. Rugae starts from the anterior part of the hard palate to the mesial aspect of the first molars and never crosses the mid palatal raphae.[2.] They consist of keratinised stratified squamous epithelium with high density of merkael cells and develop by the third month of intrauterine life. Rugae aids in proper tongue placement during deglutition and mastication[3]. Once formed, their shape is maintained but due to palatal growth there can be changes in their size and acquires a unique pattern during puberty and stabilizes as the individual matures[4,5]. The rugae is protected by extraoral environmental factors, external temperature rise, trauma, palatal infections, chemical burns, and tooth exfoliation or eruption as these are anatomically located on the internal aspect of the oral cavity and hence it remains as a stable landmark.[6,7] However, some habits like prolonged thumb sucking and orthodontic treatment for long duration could alter the patterns of rugae, while in cleft patients surgical repair might lead to reduction in the number of rugae [8,9,10]

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People seek orthodontic treatment for improving facial aesthetics and function as the orofacial region is the main source of verbal communication and personal interaction, and is of significant concern. Transverse malocclusion can lead to facial asymmetry and subsequent aesthetic and functional problem. Irregularities in tooth position and jaws exerts significant impact on quality of life. Rapid maxillary expansion (RME) is a predictable evidence-based method for the correction of dentofacial abnormalities in transverse plane by expanding the maxillary arch [11,12,13]. RME leads to morphological recontouring of the maxilla and hard palate in the transverse dimension resulting in splitting of the mid palatal suture and separation of the two maxillary halves[11, 14]. However there is not much evidence with regards to other palatal changes, like structural alterations of the palatal rugae , changes in antero-posterior dimension, volumetric and surface palatal changes [15,16]. Many studies have focused on changes in sagittal or antero-posterior plane of palatal rugae related to non-extraction orthodontic treatment planning or maxillary first premolar extraction or even functional and orthopaedic appliances.[8,17,18,19] However, not many studies have compared the transverse dimension changes of the palatal rugae after rapid maxillary expansion which can have a significant effect on the palatal rugae

Hence, this study aims to evaluate the changes in transverse dimensions of palatal rugae in subjects treated with rapid maxillary expansion

Materials & Methods

· This retrospective study was conducted in Department of Orthodontics, Amrita School of Dentistry after the approval of the Institutional Research Ethics Committee Amrita Institute of Medical Science, Kochi.

· The study sample consist of records of adolescent subjects (10 ± 2.5years) with transverse maxillary constriction having posterior crossbite, without any habits like tongue trusting or thumb sucking and without any previous history of orthodontic treatment. Subjects with history of severe palatal trauma, syndromic patients, and patients with cleft palate or any surgical procedures performed on the hard palate were excluded from our study.

· Sample size was calculated based on results of a previous study[20]. With an effect size of 0.65 and 80% power, the minimum sample size was estimated to be 30 in each group.

· 30 Dental cast models of patients satisfying the inclusion criteria were selected from the Dept of Orthodontics. Bonded hyrax appliance was used for all 30 subjects. The appliance was activated 1 week after insertion with a protocol of 2 turns per day for 3-4 weeks activation was stopped once the maxillary palatal cusp of the upper first molars contacted the buccal cusp of the mandibular lower first molars allowing 2-3 mm of overexpansion to compensate for relapse. [figure 1]

· The palatal rugae patterns were measured and compared between pre-treatment cast (30 Pre RME) and its post-treatment (30 Post RME) counterpart for the evaluation of any morphometric changes. As this is a retrospective study there were no untreated control group.

Cast Analysis

· All pre-expansion and post-expansion dental casts were traced and analysed by two investigators independently to determine inter examiner reliability. Dental casts were drawn with a 0.3-mm graphite pencil (Faber Castell) according to Kapali et al classification of palatal rugae[21]

· The lateral and medial points of rugae were identified and marked on the lateral and medial ends of first, second, third rugae simultaneously on both pre and post cast and then traced (fig1&2).

· The line connecting the right and left medial rugae points is the intermedial dimension and the line connecting the right and left distal rugae points is considered as the interlateral dimension.

· The intermedial and interlateral dimensions of rugae were measured with the help of Vernier calipers with accuracy of 0.1mm and the readings were recorded.

Statistical Analysis

Statistical analysis comparing pre and post-expansion measurements of inter-medial and inter-lateral distances for first, second and third primary rugae was done using paired t test using SPSS software version 20. The inter

examiner reliability was assessed using Cronbach's alpha. First rugae showed Cronbach's alpha value of 1, second and third rugae 0.9 suggesting good agreement between the examiners.

Results

The study reveals an increase in inter medial and inter lateral values in all the post-expansion models. Minimum increase in values was recorded in the inter-medial distance of first primary rugae (0.29mm) and the maximum increase recorded in the inter-lateral distance of third primary rugae (2.58mm). A marked increase was also seen in the inter-medial distance of third primary rugae (1.27mm). Interlateral distance of second primary rugae was also increased(2.21mm). Statistical significance was calculated using paired t test. There was statistically significant($p<0.05$) increase in the intermedial and interlateral distance of second and third rugae whereas increase in the intermedial distance of first rugae was not statistically significant. Increase in the transverse changes was recorded maximum for third rugae and minimum for first rugae [table 1].

TABLE 1- Pre-expansion and post-expansion values of inter-medial and inter-lateral distances of first, second and third rugae

Rugae Number	Rugae Characteristic	Timepoint	N	Mean	Std. Deviation	P value
First rugae	Inter-medial	Pre-expansion	30	3.267	.5168	0.052
		Post-expansion	30	3.557	.6101	
	Inter-lateral	Pre-expansion	30	9.733	1.0273	0.008*
		Post-expansion	30	10.483	1.0716	
Second rugae	Inter-medial	Pre-expansion	30	5.247	.7736	0.000*
		Post-expansion	30	6.550	.9641	
	Inter-lateral	Pre-expansion	30	16.287	2.1770	0.000*
		Post-expansion	30	18.497	2.2986	
Third rugae	Inter-medial	Pre-expansion	30	9.080	1.2386	0.007*
		Post-expansion	30	10.357	2.1612	
	Inter-lateral	Pre-expansion	30	19.393	1.8857	0.000*
		Post-expansion	30	21.973	1.7352	

*Statistically significant

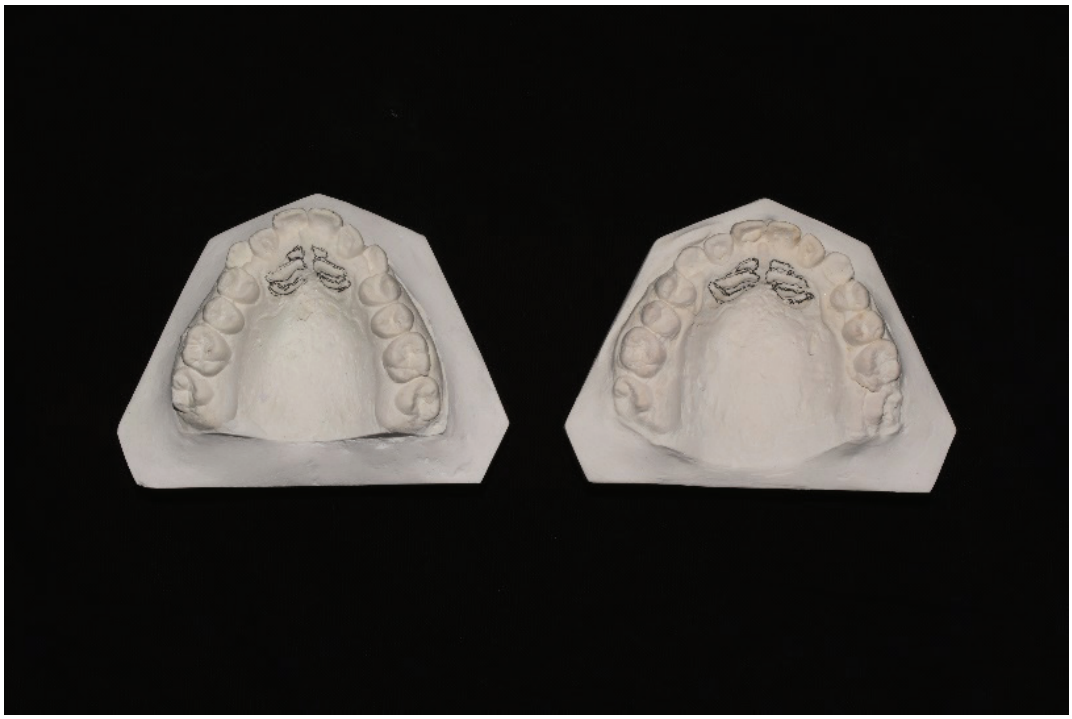

FIGURE 1 Pre and post expansion casts traced for first, second and third primary rugae with a 0.3 mm graphite pencil

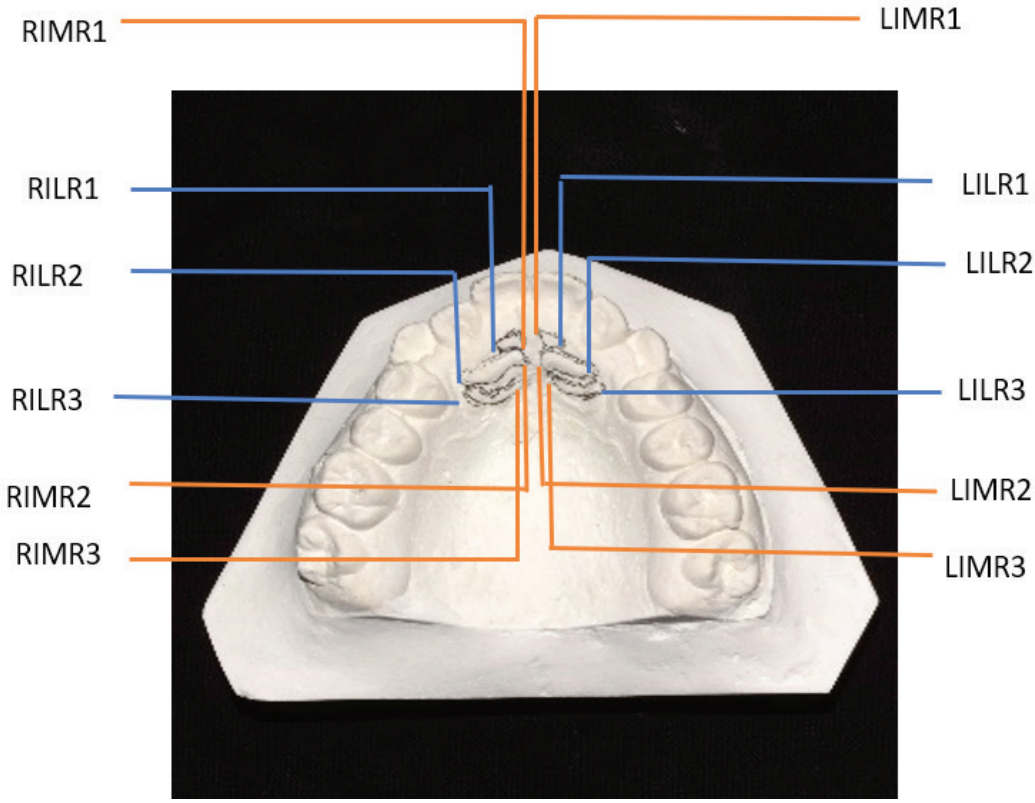

Figure 2 – Land marks for measuring of palatal rugae. RILR 1, RILR2, RILR 3- Right intermedial rugae points of first, second and third rugae. LILR1, LILR2, LILR3- Left interlateral rugae points of the first, second and third rugae. RIMR1, RIMR2, RIMR3- Right intermedial rugae points of the first, second and third rugae. LIMR1, LIMR2, LIMR3- Left intermedial rugae points of the first, second and third rugae

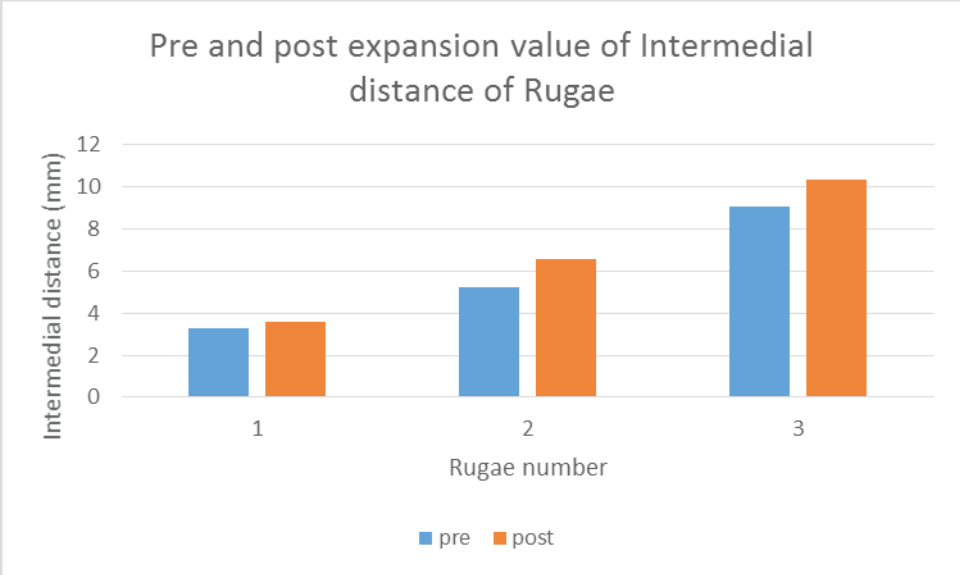

FIGURE 3- Pre and post expansion values of inter medial distance of Rugae

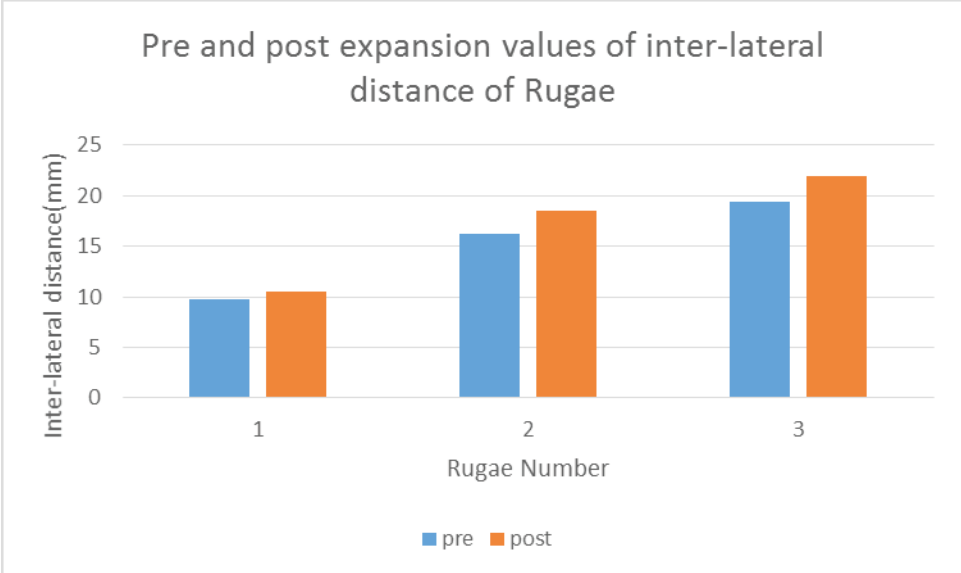

FIGURE 4-Pre and post expansion values of inter lateral distances of Rugae

Discussion

The palatal rugae are unique oral topographical structures of an individual, like his or her finger print and its characteristic pattern remains unchanged throughout life [22]. However, many studies have reported changes in the mean rugae count with age[23].The present study has been conducted to determine stability of palatal rugae in transverse dimension in adolescent patients subjected to rapid maxillary expansion and for the evaluation of stability of the palatal rugae for primary forensic identification criterion in these patients. Being a retrospective study there was no control group to monitor changes in palate due to growth in subjects who was not undertaking orthodontic treatment. Rapid maxillary palatal expansion can have a direct effect on palatal rugae as palatal expansion opens the mid palatal suture. This in turn will affect the connective tissue covering of palatal rugae and have an effect on the transverse dimension of palatal rugae.

Results of our study recorded an increase in transverse dimensions both in intermedial and interlateral distances of all first, second and third primary rugae, with least

changes shown by intermedial distance of first primary rugae (table 1).

Bailey et al[17] investigated on morphology of palatal rugae in non-extraction and extraction cases and concluded that the medial aspects of the 1st rugae remains stable in extraction and non-extraction group while the lateral and medial aspects of second rugae showed significant changes in extraction group .This is similar to the results of our study where the intermedial and interlateral distance of the first rugae did not show any significant changes while there were significant increase in the intermedial and interlateral dimension of the second and third rugae . This was also explained by Peavy et al [9] that "if the rugae are closer to the teeth, they are more prone to stretch in the direction related to the teeth movement".

Van der Linden [18] observed that medial point of the first rugae were not affected for the transverse values. However, in our study, intermedial distance of the first rugae

(0.29mm) were only minimally affected and it was statistically not significant.

Almeida et al [8] studied the stability of rugae during growth in class II patients and the effect of headgear or functional appliances treatment on position of rugae was studied and found that no significant change in transverse offsets and linear distances between medial points of the first rugae with respect to median palatal reference plane. Our study also supports this finding where the intermedial distance did not show any significant change in the context of first palatal rugae

Batool et al [19] investigated the stability of palatal rugae in expansion, extraction, non-extraction patients and found that the third rugae length was increased after RME which is similar to the results of our study where the intermedial and inter lateral distance of the third palatal rugae was increased and it was statistically significant($p<0.05$). Damstra et al[20] investigated the position of palatal rugae in antero-posterior and transverse dimension after rapid maxillary expansion and found significant change in transverse dimension of palatal rugae due to addition of RME to fixed appliance therapy .The third rugae showed significant increase in the transverse dimension, followed by the second rugae , and least for the first rugae which is identical to results of our study.

The RME could alter the position of palatal rugae because it increases the interpremolar and intermolar distance, and also affects the position of molar three dimensionally and can lead to elongation of palatal mucosa posteriorly. This was proved by Garrett et al[24] in their study, have shown total expansion at the level of 1st premolar was 55% (P1), at the level of 2nd premolar was 45% (P2), and at the level of 1st molar was 38% (M1). During expansion, Krebs et al[25]in his metallic implants study has proved the maxillary components rotates in the horizontal and frontal planes during expansion. The fulcrum of maxillary rotation during will be at the frontomaxillary as well as at the pterygopalatine suture during expansion. The increased intermedial and interlateral distance of palatal rugae proves that there is more of a skeletal expansion of the maxillary arch and at the mid palatal suture Moreover mid palatal expansion is associated with buccal tipping of posterior teeth which can stretch the lateral rugae points in outward direction, which in turn will increase the interlateral distance of the third rugae.

The mean transverse change between the third rugae (2.58) and second rugae (2.21) exceeds the 2.00 mm value considered to be significant statistically ($p<0.05$) and clinically. Therefore, the third and second rugae cannot be considered as stable anatomic marks for subjects undergone RME.

Conclusion

The quest for stability of medial and lateral rugae points following mid palatal expansion to be used as stable reference landmarks for forensic identification has led to the conclusion that only the medial aspect of first primary rugae can be considered as a stable reference landmark. While the medial and lateral aspects of the second and third rugae are liable to changes in transverse dimension. Hence, the ability of palatal rugae for forensic identification in patients who had undergone rapid maxillary expansion still remains questionable. However more studies with larger samples are required to confirm this finding.

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Effectiveness of Pictorial Health Warnings on Tobacco Products in Creating Awareness among 15-17 Years Old School Children in Lucknow

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Abstract

Aim: The present study was planned to assess the effectiveness of pictorial health warnings on tobacco products in creating awareness among 15-17 years old school children in Lucknow.

Materials and method: The present cross sectional study was conducted among 360 students studying in rural schools of Lucknow. Information about schools was obtained from DEO's. Sampling was done using multistage sampling technique. A specially designed questionnaire was administered to assess the effectiveness of pictorial health warnings on tobacco products in creating awareness given on the reverse panel of smoked and smokeless tobacco. Statistical analysis was done using chi square and test.

Results: The mean knowledge score among male and female was 6.37±1.56 and 6.50±1.73 respectively with statistically insignificant difference. Average and poor impact was reported among 21.11%, 1.94% and 14.17%, 0.28% of the boys and girls respectively. When different attitude grading was compared statistically among boys and girls, it was found to be statistically significant.

Conclusion: The present study threw light on some of the aspects of the young students towards the Pictorial Health Warnings on tobacco packs. The finding also highlights to improve the quality of the Pictorial Warnings and make them more robust and impactful

***Keywords**: Pictorial warning, Tobacco, Impact, Knowledge, Attitude, Practice*

Introduction

Tobacco smoking is the second leading cause of preventable deaths globally[1]. Even with such effects, it is surprising that tobacco is the only legal product that kills over a million people and impoverishes around 15 million people annually in India[2]. The total economic costs attributable to tobacco use from all diseases in India (2011) were estimated at US$22.4 billion[3]. Yet, India remains one of the largest consumers of tobacco products in the world, second only to China. It is also one of the major growers of tobacco in the world[4].

According to Global Adult Tobacco Survey India (GATS India), the mean age at initiation of daily tobacco use for tobacco users age 20–34 years is 17.8 years. The mean age at initiation of smoking as well as use of smokeless tobacco among users of respective products age 20-34 years is 17.9 years. Two in every five daily tobacco users age 20–34 had started using tobacco daily before attaining the age of 18[5].

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The cigarette package is a critical communication device for creating and reinforcing brand imagery, and is the link between other forms of tobacco advertising and the uptake of the addictive drug nicotine from a cigarette[6]. Using striking colors, distinctive fonts and carefully crafted materials, cigarette packaging is defined to be highly attractive, especially among young people[7]. On the other hand, cigarette pack warnings are able to disrupt brand imagery, an important factor for tobacco trial and use. This reality is acknowledged by the tobacco industry, which vigorously opposes such measures[8].

Package warnings are unique among tobacco control initiatives implemented to educate smokers and prevent smoking initiation as they cost little to produce and can be integrated with larger interventions such mass media campaigns[9,10]. Large comprehensive graphic warnings—that combine a picture embedded with a text message relevant to the depicted picture—are effective in increasing adult awareness of the dangers of smoking, aiding smoking cessation and are more likely to be noticed and rated as effective by adult smokers in comparison to plain text-only messages, while their size and emotional impact also play a vital role in their effectiveness[11-12].

On 1 April 2016, India implemented the much-awaited larger health warnings that cover 85% of the tobacco packs on both sides with 60% pictorial and 25% textual warning. The only other countries in the world with larger warnings are Nepal and Vanuatu at 90% and the other countries to equate such large graphic health warnings are Thailand (85%) followed by Australia at 82% and Uruguay as well as Sri Lanka at 80%. One of the most significant aspects of the new regulation is that it provides for a minimum size for the warnings, that is, not be <3.5 cm (width) and 4 cm (height) so as to ensure that the warnings are legible, prominent and conspicuous[13].

In Indian scenario, the Pictorial Warnings have changed periodically. Although stronger than the previous warnings, they are yet to prove their effectiveness. Since there is limited data regarding the impact of newer warning labels on smoking cessation in India. Hence, this study has been taken up as an opportunity to throw some light in this regard. As there is paucity of literature to assess the effect of newer pictorial warnings on quitting tobacco habits, the present study was planned to assess the effectiveness of pictorial health warnings on tobacco products in creating awareness among 15-17 years old school children in Lucknow.

Material and Method

The present cross sectional study was conducted at rural schools of Lucknow among 15-17 years old children. Information about schools was obtained from DEO's. Sampling was done using Multistage sampling technique. In stage two, four wards were selected randomly from each block. Then in stage three, from four wards, a simple random sampling method was used to select two private and two Government schools. In this way a total of sixteen schools (8 private and 8Government) were selected. The details of the schools were obtained from the Directorate of Elementary Education, Lucknow. 15-17 year old children were selected from each school to obtain the required sample size. Ethical clearance was sought from the Institution Ethics committee of "Career Post Graduate Institute of Dental Sciences & Hospital, Lucknow" explaining the study protocol along with the consent form.

Inclusion Criteria:

- Children in age group of 15-17 years.
- Children present on the day of examination.
- Children whose parents gave informed consent.
- Children who are permanent residents of that area.

Exclusion Criteria:

- Children of parents with poor compliance or who did not give consent.
- Children absent on the day of examination.
- Un-cooperative child.
- Child with congenital and developmental anomalies.

A Structured Questionnaire was developed by a committee in Hindi language, before being administered to the participant by principal investigator; it was then

reviewed by a committee comprising of experts, social and preventive medicine and public health dentist. If any differences of opinion, was discussed and sorted. Prior to being finalized, the questionnaire was pilot-tested on a group of 15 to 17 years old children to ensure clarity, feasibility, validity and to know baseline data for sample size calculation and necessary modifications were made.

A specially designed questionnaire was administered to assess the effectiveness of pictorial health warnings on tobacco products in creating awareness given on the reverse panel of smoked and smokeless tobacco. The questionnaire contained items about socio-demographic indicators like- name, age, gender, education and address of the school where the child studies. The questionnaire consisted of 10 knowledge questions dealing with the rules and regulations in relations to smoking and health warnings given on the smoking pack. The questionnaire assessed the salience (noticing, looking closely at warnings), depth of processing (thinking about warnings, discussing them with others), comprehension and credibility (warning comprehensibility, believability, and truthfulness), unaided recall, persuasiveness (warnings as a deterrent to smoking), avoidance techniques (e.g., hiding packs), and a behavioral indicator (forgoing cigarettes due to warnings) of the participants. A model containing the pictorial and textual warnings on panel of smoked and smokeless tobacco products were shown to the study subjects. This model was displayed in the rooms where the participants were supposed to see them and then complete the questionnaire. The response was recorded with the help of 10 point visual analog scale (VAS).

A pilot study was conducted among the students to evaluate the effects that statutory and pictorial health warnings had on individuals consuming these products. The questionnaire was reviewed by experts (who were active in the field of tobacco cessation) and content validity was checked. The test–retest reliability of the survey questions in the present scenario; 15 individuals who completed the survey their feedback was analysed. Cronbach's alpha co-efficient was found to be 0.84 and test-retest reliability was found to be 0.77.

Statistical Analysis

The collected data was entered into Microsoft Excel and analysed using SPSS 20 (Statistical Package for Social Sciences) package for relevant statistical comparison. Result will be presented in the form of tables and graphs. Descriptive statistics and inferential statistics was done. Statistical analysis was done using chi square and test. The level of significance was set at $p<0.05$.

Results

The present study comprised of 720 children, out of which 50% were males and 50% were females. The mean age of the boys and girls was 16.22±1.05 and 16.09±1.01 respectively. The overall mean age was 16.16±1.03. Table 2 shows the right knowledge score of each question. Excellent knowledge score was reported by 23.06% and 28.89% of boys and girls respectively whereas poor knowledge was found in 10.83% of the boys and 12.22% of the girls. When different grade of knowledge was compared statistically among boys and girls, it was found to be statistically significant (table 3).

Table 4 shows the distribution of attitude grading among boys and girls. High impact was more among girls (65%) as compared to boys (45%) while good impact was more in boys (31.94%) as compared to girls (20.56%). Average and poor impact was reported among 21.11%, 1.94% and 14.17%, 0.28% of the boys and girls respectively. When different attitude grading was compared statistically among boys and girls, it was found to be statistically significant ($p<0.01$).

Table 5 shows the question's response in terms of positive statement. When mean of different questions in relation to positive statement was compared among boys and girls, it was found to be statistically significant for two questions i.e. 'I am pretty sure that after seeing this picture I will never consume tobacco related products' and 'Is this picture able to impart information regarding cancer in relation to smoking'. Mean of negative statement was found to be 3.06 and 3.54 among boys and girls respectively with statistically significant difference ($p<0.01$).

Table 1: Demographic characteristics of the study population

Age	Boys		Girls		Total	
	N	%	N	%	N	%
15 years	102	28.3	123	34.2	225	31.25
16 years	125	34.7	122	33.9	247	34.31
17 years	133	37	115	31.9	248	34.44
Total	360	100	360	100	720	100
Mean±SD (years)	16.22±1.05		16.09±1.01		16.16±1.03	

Table 2: Right knowledge score of each question

Question	Boys		Girls		Total	
	N	%	N	%	N	%
Form of warning on cigarette packet?	304	84.44	287	79.72	591	82.08
How much the picture cover the percentage of cigarette pack?	73	20.28	64	17.78	137	19.03
Which picture is found on cigarette packet?	193	53.61	193	53.61	386	53.61
Tobacco products are allowed for sale in schools	25	6.94	14	3.89	39	5.42
Which legal implication in India has been proposed to stop tobacco habit?	210	58.33	222	61.67	432	60.00
Whether minor can sell the tobacco related products?	272	75.56	286	79.44	558	77.50
Consumption of tobacco products is allowed in schools?	314	87.22	314	87.22	628	87.22
Have you ever heard about message regarding quitting of tobacco?	322	89.44	335	93.06	657	91.25
Harmful impact of tobacco	287	79.72	295	81.94	582	80.83
Recognize this picture.	293	81.39	332	92.22	625	86.81

*: statistically significant

Table 3: Grading of knowledge scores

Grading	Boys		Girls		Total	
	N	%	N	%	N	%
Excellent (8-10)	83	23.06	104	28.89	187	25.97
Good (7-7.9)	98	27.22	117	32.50	215	29.86
Average (5-6.9)	140	38.89	95	26.39	235	32.64
Poor (<5)	39	10.83	44	12.22	83	11.53
Chi square	12.96					
p value	0.005*					

*: statistically significant

Table 4: Graduated scale of attitude among the study subjects

Grading	Boys		Girls		Chi square	p value
	N	%	N	%		
High Impact (8-10)	162	45	234	65	31.41	<0.01*
Good Impact (7-7.9)	115	31.94	74	20.56		
Average Impact (5-6.9)	76	21.11	51	14.17		
Poor Impact (<5)	7	1.94	1	0.28		

*: statistically significant

Table 5: Description of Positive and negative statement among the subjects

Positive Statement	Boys		Girls		t test	p value
	Mean	SD	Mean	SD		
I am pretty sure that after seeing this picture I will never consume tobacco related products	4.40	0.89	4.57	0.87	2.59	0.01*
Is it required to change the current picture with other picture so that it can have better impact	3.88	1.16	3.91	1.23	0.34	0.74
Is this picture able to impart information regarding cancer in relation to smoking	4.38	0.82	4.53	0.92	2.31	0.02*
Negative Statement						
It is not necessary to publish this picture on tobacco packings.	3.06	1.54	3.54	1.48	4.26	<0.01*

*: statistically significant

Discussion

The present study was undertaken to explore effectiveness of Pictorial Health Warnings on tobacco packs among the 15-17 years old school children. Previous studies have considered different study population; hence comparison is done wherever possible. No such study has been conducted in the state of Haryana.

The current research found that 82.08% of the students had seen the warning on the cigarette, bidi or gutka packet. Dimplejit Singh Mangat et al[14] stated that 81.6% of the participants had seen health warning labels on the cigarette packets. Similarly Shah VR et al[15] found that out of 776 tobacco users, 561 (72.3%) had observed warning over the tobacco products. However, study conducted by Talreja K et al[16] observed that 97.5% people had idea of the pictorial warnings on cigarette packs and 18.2% of them were not able to understand the clear picture of pictorial warning. These results were similar to the study conducted by Karibasappa GN et al[17], the reason behind not recognizing the warning label could be due to smaller size of packaging of the product.

Pictorial warning labels do have an impact on changing behavior pattern of the smokers participants and when they were asked about absolutely quitting this adverse habit, 84.45% gave a positive response. The mean score of the positive response was 4.49. These findings were in accordance with the study conducted by Karibasappa GN et al[17] in people consuming both smokeless and smoking form of tobacco. Similarly, Shah VR et al15 mentioned that 82.2% of the study participants had reduced the number of cigarettes after noticing the warning labels. Mangat DS et al[14] reported the same in 85% of the subjects. Parveen Dahiya et al[18] reported that 48.3% of tobacco users believe that presence of pictorial and text warnings had no impact on them. It showed that our responsibility regarding anti-tobacco programs should not be just limited to adding pictures and text warnings on tobacco products. Added to the reduced size, a large proportion of users are poorly educated, which would reduce the comprehension of the warnings. Additionally, there is wide variety and diversity of package design used by SLT manufacturers, which is a reflection of their use of packaging as a marketing venue to reinforce brand imagery, to minimize perceptions of risk, and to suggest incorrectly that some types of products are less harmful than others. SLT packaging designs can effectively counteract warning content through creative techniques that can undermine the salience and impact of the warnings.

The mean score regarding motivational impact of pictures on quitting tobacco and health related harmful impact of tobacco was 7.80 and 8.92 respectively among the study subjects in the present research. 64.47% of participants expressed that the current health warnings are inadequate to convey health impact of tobacco, and over half of participants in a study conducted by Monika Arora et al[19] felt that the pictorial warnings would neither motivate tobacco users to quit nor prevent nonusers from initiating. It has been experienced in other countries that strong pictorial health warnings on tobacco packs effectively inform people about adverse health effects of tobacco[20]. It is also known that lack of adequate knowledge about health consequences of tobacco use can lead to initiation and continuation of tobacco use[21].

Continued use of ineffective warnings represents a missed opportunity as the Government has failed to effectively utilize this evidence-based strategy to enhance knowledge about the effects of tobacco among the people, in addition to other educational interventions, e.g., anti-tobacco advertising employed by the Ministry of Health and Family Welfare in India.

Conclusion

The results of the present study shows that majority of the students were aware that the tobacco use is harmful to general health. Most were aware and noticed the presence of Pictorial warning labels on the Tobacco packets. The students also had a positive attitude regarding the presence of Pictorial Warning. Therefore the present study threw light on some of the aspects of the young students towards the Pictorial Health Warnings on tobacco packs. The finding also highlights to improve the quality of the Pictorial Warnings and make them more robust and impactful.

Conflict of Interest: Nil

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Effects of Body Position on the Outcome of Pulmonary Function Tests in Non-Mechanically Ventilated Adults: A Brief Review

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Abstract

Background: The most important focus of pulmonary physical therapy has been to improve pulmonary function. Studies have indicated that body position affects pulmonary function. Pulmonary Function Tests are a group of tests that are routinely performed to objectively assess lung volumes and capacities and are commonly performed in sitting position. However, positions other than sitting have to be considered in certain disease conditions when the patient may not be able to attain sitting posture. The result may provide objective data regarding positions suitable for pulmonary function measurements.

Methodology: A search to identify articles from the last five years from the period January 2015-January 2020 was conducted using free access search engines Medline, Pubmed and Google Scholar. Search was undertaken using both, key words and MeSH words for maximal retrieval of literature. Some older articles relevant to the topic were also included in this study.

Conclusion: The effect of sitting and lying positions on pulmonary function tests vary across study populations which makes it difficult to establish an optimal position for performing pulmonary function tests. However, supine lying position may be considered in those disease conditions where adopting a sitting position may not be possible for the subject.

Key words: *Body position, pulmonary function, pulmonary function tests, sitting position, supine position, lying position, ventilation, postural change.*

Introduction

A number of studies have indicated that body position affects pulmonary function by influencing ventilation perfusion matching and oxygen saturation. Body posture affects the length of the respiratory muscles.[1] There are many articles that have studied the respiratory function of normal individuals in sitting and standing position. Few studies have investigated the effects of different recumbent positions, viz. supine, right and left recumbent and prone position on pulmonary function. Pulmonary function tests are the most common investigation used by clinicians to assess the prognosis of a respiratory disease.[2] It assesses the effects of a respiratory drug and also the feasibility of a surgery. This is also the most common objective assessment tool to measure the effect of therapeutic intervention on pulmonary physiology and respiratory mechanics involving the lungs, respiratory muscles and the chest wall. Since body positions have an effect on lung volume and capacities, positions adopted during pulmonary function tests may also affect its results. The pulmonary function tests are usually done in sitting position due to factors like the comfort of the patient and ease of operating the device, lying position is also sometimes used in certain disease conditions as

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in neuromuscular conditions when the subject is unable to attain sitting position. There are many researches that show the effects of different body positions on pulmonary function in healthy adults. A change in the position of body affects the forced expiratory volume in one second and the percentage of expiratory volume in one second and also the forced vital capacity.[3] Few studies have investigated the effects of body position in a particular disease condition. However, there are not many systematic reviews that integrate the effects of body position on pulmonary function in healthy adults who are not mechanically ventilated as well as well as adults with certain disease conditions. This study is done with the aim to evaluate the effect of sitting, supine and left and right-side lying positions on pulmonary function and explore the possibility of the use of positions other than sitting in assessing pulmonary functions.

Aims of the Study

To find out the effects of body position on the outcome of pulmonary function test in non-mechanically ventilated adults.

Methodology

Articles from the last five years from the period January 2015-January 2020 and some older articles relevant to the topic were identified using free access search engines like Medline, Pubmed and Google Scholar using key words and MeSH words. key words that were used were body position, pulmonary function, pulmonary function tests, lung volume, lung capacities, ventilation perfusion match, change of body position, sitting position, supine position, standing position, postural change, position change. The MeSH words used were: adult, body position, posture, spirometry, pulmonary function, pulmonary diffusion capacity, respiration, FEV, PEFR, VC, lung volume measurements, heart failure. The search was undertaken by two researchers. 162 abstracts were identified that appeared to be relevant, 68 full texts were studied out of which 36 met the inclusion criteria and were included in the study. Studies not fulfilling the inclusion criteria were not included in the review. Inclusion criteria was: a. studies that included subjects more than 18 years of age b. studies on non-mechanically ventilated subjects c. studies that included at least sitting and supine lying positions d. outcome measures included Vital capacity(VC), Functional Residual Capacity(FRC), FEV1 or Forced Vital Capacity in one second , FVC or Forced Vital Capacity, Total lung capacity (TLC), Residual volume (RV), Peak expiratory Flow (PEF) and DLCO or the diffusing capacity of the lungs for carbon monoxide. Following were exclusion criteria: a. studies on mechanically ventilated subjects b. studies not including sitting and supine positions c. studies conducted on subjects less than 18 years of age d. studies including post-operative conditions. e. studies not including pulmonary function tests. Both the authors reviewed the articles included in this study and they were divided as: healthy adults, subjects with pulmonary disease, subjects with neuromuscular disease and subjects with cardiac disease.

Level of evidence was evaluated by using the Study Quality Assessment Tool by National Heart Lung and Blood Institute. A Quality assessment tool was used for before-after (pre-post) studies with no control group. Studies categorized as 'Good' and Fair' as per the Study Quality Assessment Tool only were included in the study. Risk of bias was assessed using the AHRQ checklist for Risk of Bias assessment. Studies categorized as 'Low Risk' were included in the review.

Results

Effect of body position on each of the outcome measures is as follows:

1. Vital capacity (VC): Most studies have not reported any difference between sitting and lying positions in healthy subjects. Most studies under the category of cardiac diseases reported higher VC in sitting as compared to supine and in the category of neuromuscular diseases, VC was higher in supine as compared to sitting.

2. Forced Vital Capacity (FVC): Most studies have found a higher FVC in erect position which included standing and erect sitting. In the category of subjects with pulmonary disease, significant increase in FVC was reported between supine and standing positions. The difference in value of FVC between sitting and supine was not significant. Significant difference was reported in one study in FVC between standing as compared to sitting and supine. In the category of subjects with cardiac diseases, most studies reported significant

differences in FVC between sitting and supine with FVC being higher in sitting. In subjects under the category of neuromuscular disease it was seen in most of the studies that there was no significant difference in FVC between supine position and the sitting position. However few studies done on spinal cord injury patients demonstrated that these patients had a higher FVC in supine lying as compared to sitting position.

3. FEV1: Most of the studies done on healthy adults have demonstrated a higher FEV1 in sitting as compared to supine. However, some researchers have also found that there was no significant difference in FEV1 between supine lying and sitting position. In patients with pulmonary disease, some studies reported a higher FEV1 in sitting as compared to lying whereas a few studies have not found significant difference in FEV1 between sitting and supine position. A few studies reported a higher FEV1 in standing as compared to sitting and supine positions. In patients with cardiac diseases, most studies reported a higher FEV1 in sitting as compared to supine position. One study has reported no difference FEV1 between these two positions. In patients with neuromuscular diseases, most studies have shown that FEV1 decreases from sitting to supine. However, in patients with spinal cord injury, the level of injury seems to affect the FEV1 values. Patients with tetraplegia showed a higher FEV1 in supine than in sitting position. Patients with injury at thoracic and lumbar regions showed higher FEV1 in sitting than in supine position.

4. FEV1/FVC: Most studies found that in healthy subjects, FEV1/FVC was higher in sitting than in supine. Few studies reported it to be higher in standing than in sitting. Few studies have also shown that there is no significant difference in FEV1/FVC between sitting position and supine lying. Studies on subjects with pulmonary, cardiac and neuromuscular diseases have demonstrated no significant difference in FEV1/FVC between sitting position and supine position.

5. FRC: in healthy subjects, FRC was found to be higher in standing as compared to sitting in most studies. FRC was found to be more in sitting than supine positions. Most studies did not find significant difference in DLCO values between sitting and supine positions in CHF patients. One study found the DLCO value to be more in supine than in sitting in CHF patients. No study included in the review evaluated FRC in subjects with pulmonary, cardiac and neuromuscular diseases.

6. Total Lung Capacity (TLC): No study reported significant difference in TLC between sitting and supine position in healthy subjects. No study included in the review evaluated TLC in different body positions in pulmonary, cardiac and neuromuscular diseases.

7. Residual Volume (RV): Studies have also found no significant difference in RV of healthy adults when comparing the sitting and supine positions. No study included in the review evaluated RV in different body positions in pulmonary, cardiac and neuromuscular diseases.

8. Peak expiratory flow (PEF): studies on healthy subjects and subjects with pulmonary disease compared PEF in standing and sitting positions with supine and found PEF to be higher in standing and sitting than in supine position. Patients with neuromuscular conditions and spinal cord injury patients have shown higher PEF values in supine lying as compared to the sitting position.

9. DLCO: the studies evaluating the diffusion capacity for carbon monoxide in healthy adults yielded variable results. Few studies found it to be higher in supine than in sitting. One study found DLCO to be reduced in both right and left side lying positions compared to sitting in healthy individuals. No significant difference was found in DLCO values between sitting and supine positions in patients with pulmonary diseases like COPD. Patients with cardiac diseases like CHF showed reduced DLCO value in sitting compared to supine.

Discussion

Sitting is considered to be the standard position for pulmonary function test measurements. The studies included in this review show that sitting position is associated with greater values of VC, FRC, FEV1, FVC, FEV1/FVC, TLC and PEF, indicating an improved pulmonary function in healthy adults and most diseased conditions. Studies have shown increase in FVC in sitting compared to supine position.[4] In patients having asthma the reason for an increase in FVC in sitting position when compared to supine lying might be the

increased diameter of the airways and reduced airway resistance in the sitting position.[5] In case of spinal cord injury patients, the level of injury influences the pulmonary function.[6] DLCO values were more in supine position than in sitting in healthy persons whereas it was more in sitting position than in supine lying in CHF patients.[4] In side lying position the heart compresses one lung and the airway causing a reduction in FVC and FEV1. In subjects with cervical spinal cord injury, there is an increase of FVC and FEV1 values in supine when compared to that of sitting as when lying in supine position the diaphragm is able to operate from a more mechanically advantageous position. FRC in healthy individuals was found to be more in sitting than in supine lying.[7]It may be due to the descent of diaphragm and reduced pulmonary blood volume. However, the change in FRC was not found to be significant between sitting and lying position in patients with CHF which shows that FRC is sustained possibly due to adjustments in respiratory muscles.

Upright posture is associated with higher lung volumes due to descent of diaphragm which causes greater elastic recoil of lung and chest wall.[8] Upright sitting elicits a stronger contraction of abdominal muscles which leads to an increase in intrathoracic pressure causing a forceful expiration. The excursions of the inspiratory muscles occur unhindered in sitting compared to lying position. FEV1 is higher in sitting position.

In supine position, the lung volume is reduced as the diaphragm ascends into the thorax. This reduces the VC, TLC and RV. Decreased lung volumes reduce the flow rates and increase airway resistance. The bases of both lungs are compressed due to the weight of the heart above and the thrust by the abdominal contents below. This compresses the alveoli and reduces lung compliance. This leads to reduced spirometric values in supine.[9]

In right and left side lying positions, the abdominal contents move forward, and may place the abdominal muscles at a more mechanically advantageous position than supine. However, thoracic expansion is hindered by the bed on one side which reduces lung volume and elastic recoil of the lung and chest wall compared to sitting and supine. This causes a decrease in VC, FVC and FEV1.

Studies have shown that PEF is higher in standing and sitting as compared to supine lying position.[10] More upright the posture, more is the PEF.[11] In patients with neuromuscular conditions, spinal cord injury patients have shown higher PEF values in supine than in sitting which may be due to a higher FVC and FEV1 in these positions.

DLCO values were more in supine position than sitting in healthy individuals. It was found to be higher in supine position than in sitting in patients with CHF.[12] DLCO is influenced by pressure of pulmonary circulation in CHF patients. In patients with CHF the increased size of heart and reduced elasticity of the lung reduces the diffusing capacity. In COPD, no change was found between both the positions due to reduced FVC.

Limitations of the study:

Most studies included in the review were experimental in nature and the sample size was small. Also, only those studies that were done on adult patients were included. Therefore, the results cannot be generalized.

Conclusion

Body position influences pulmonary function and these are manifested in the results of pulmonary function tests performed in different positions. In healthy adults and in many disease conditions, more upright was the posture, better was the pulmonary function. Sitting position was associated with improved pulmonary function as compared to supine and side lying positions. The side lying positions were shown to be least beneficial. Therefore, supine position may be the position of choice for pulmonary function tests. However, sitting and lying positions may also be considered in certain disease conditions.

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Heat treatment of Sodium Hypochlorite : A Comprehensive Review

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Abstract

Introduction - The primary objective of root canal therapy is reduction of the bacteria in the root canal. The effective debridement depends on thorough cleaning and shaping of the complex root canal system. Chemical débridements can be optimally achieved using various irrigants and intracanal medicaments. The primary irrigant of choice is Sodium Hypochlorite. It has excellent tissue dissolving ability along with antibacterial property. The purpose of this review is to discuss the need to heat Sodium Hypochlorite, techniques to heat Sodium Hypochlorite, effect of Sodium Hypochlorite on tissue dissolution, antibacterial efficacy, penetration into dentinal tubules, viscosity, and effect on Ni-Ti alloy and temperature rise on external surface of root.

Method- An electronic search was carried out using the certain keywords: "sodium hypochlorite", "heated sodium hypochlorite", "prewarmed sodium hypochlorite", "intracanal heating of sodium hypochlorite", to collect literature available on Heat treatment of Sodium hypochlorite.

Result- Fourty One articles published between 1936-2020 were selected. Four main categories of data were highlighted: Sodium Hypochlorite and Heat, the need to heat Sodium Hypochlorite, different techniques to Heat Sodium Hypochlorite, effect of Sodium Hypochlorite on various parameters.

Conclusion- The efficacy of lower concentration of sodium hypochlorite can be effectively enhanced by increasing its temperature. It enhance the pulp dissolution, antibacterial efficacy and flow without any adverse effect on periodontal ligament or outer surface of the root.

Keywords: *"heat treatment of sodium hypochlorite", "sodium hypochlorite", "prewarmed sodium hypochlorite", "intracanal heating of sodium hypochlorite", "heating sodium hypochlorite".*

Introduction

The primary goal of root canal therapy is the reduction/elimination of the bacteria and their by-products from the infected root canal space and to prevent the apical periodontitis.[1,2] This issue is addressed by disinfection of the root canal and subsequent sealing of the canal space. The canal is cleaned by the chemico-mechanical method. However, the root canal system posses the most complex anatomy in the human body, and thus, it is impossible to completely clean the canal with the conventional endodontic instrumentation.[3,4] The newer mechanical instruments and techniques though effective, cannot clean the root canal completely. Various studies dictate that large area of the canal is left untouched and unprepared, whatever might be the equipment used. This large area left untouched and

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unprepared harbors a large number of bacteria.[5,6,7] These shortcomings can be overcome by the use of irrigants. The irrigant can penetrate the uninstrumented area and effectively debride the root canal system. The irrigant can reach the delicate intricacies of canal space and clean the areas of webs, apical deltas, fins, isthmus, and anastomosis present in the root canal.[8] From past to present, variety of irrigants have been used from normal saline, hot water, anesthetic solutions, urea, urea peroxide, hydrogen peroxide to EDTA, Chlorhexidine, Hypochlorite, Qmix, and MTAD.[9]

Sodium Hypochlorite - Sodium hypochlorite is currently the most preferred irrigant for a maximum endodontist in the endodontic therapy. It would not be an overstatement to say that 'endodontic irrigation is incomplete without hypochlorite.' Sodium hypochlorite is a chlorine compound that was frequently used as a disinfectant or bleaching agent. Labarraque, in 1820, prepared sodium hypochlorite by chlorination of caustic soda. It was extensively used for irrigation of wounds by ' chemist Henry Drysdale Dakin and surgeon Alexis Carrel' at the time of First World War.They promoted the use of buffered '0.5 % sodium hypochlorite solution', later called as 'Dakin's Solution.' Hypochlorite showed necrotic tissue dissolving abilities, a wide spectrum of action, bactericidal and virucidal properties. These favorable properties enhanced the use of hypochlorite as the main endodontic irrigant in the 1920s.[10,11,12]The efficacy of sodium hypochlorite is not based on a single factor, but it is an interplay of multiple variable factors like - pH, temperature, contact time, concentration, and time. The currentlyavailable literature focuses majorly on the concentration, volume, pH, contact time, and ultrasonic activation method of the irrigant, but the review discussing the interaction of heat and hypochlorite is scarce. To our knowledge this is the first review to discuss heat treatment of Sodium Hypochlorite. Hence, this review aims to shed light on this seldom discussed facet of irrigation.

Material and Method - An electronic search was carried out using the following keywords: "sodium hypochlorite", "heated sodium hypochlorite", "prewarmed sodium hypochlorite", "intracanal heating of sodium hypochlorite", to collect literature available on till date. The abstracts and full text of relevant articles were obtained to collect comprehensive update on heating of sodium hypochlorite.

Discussion

This review is discussed under following headings -

Sodium Hypochlorite and Heat.

The need to heat Sodium Hypochlorite.

Different techniques to Heat Sodium Hypochlorite.

Effect of Sodium Hypochlorite on various parameters.

Sodium Hypochlorite and Heat -

Present data suggest numerous techniques to enhance/increase the efficacy of Sodium hypochlorite during in the root canal procedure.[13] The initial groundwork on heating of sodium hypochlorite can be traced back to 1980s. Cunningham and Balekjian (1980) stated that rise in temperature of hypochlorite from 22°C-37°C enhanced its tissue dissolution activity. Cunningham and Joseph (1980) stated that increasing the temperature also increases the antibacterial efficacy of hypochlorite. Elio Berutti and Marini (1996) mentioned about the action of hypochlorite to debride pulp tissue at various temperatures. Piskin and Turkun (1995) assessed the stability of hypochlorite at different temperatures and concluded that it was stable at higher temperatures.[14,15,16,17].

The need to heat Sodium Hypochlorite -

Chemical reaction rates are directly proportional to the temperature, pressure, and concentration of hypochlorite. Also, the intracanal pressure cannot be increased, and concentration beyond the certain limit of 6 percent is not advisable, and thus, it is thought to increase the temperature of the irrigating solution. The efficacy of hypochlorite is increased by heating the solution. The chemical reaction is accelerated and its effect is enhanced.[18] The heated hypochlorite solution can provide the same benefits as with the higher concentration, thus reducing the risk associated with using higher concentration hypochlorite.[19]

Different techniques to Heat Sodium Hypochlorite -

Sodium Hypochlorite can be heated either outside

the canal / prewarmed or inside the canal. It can be heated outside / prewarmed using a beaker on a hot plate, or kettle or with a baby bottle warmer.[20,21,22]and then introduced in the canal. The extraoral heating was ineffective as it could not maintain the rised temperature of the preheated hypochlorite inside the canal and attains the equilibrium within seconds.[21] The prewarmed irrigant inside the root canal will attain a neutral temperature between room and body temperature.[15] Ebtissam M. Al-Madi (2008) stated that heating can be effectively done with a fresh solution. He recommended preparing a stock solution and store them at low temperatures for chairside use.[23,24] The efficacy of a preheated hypochlorite decreases with few minutes and thus, if preheated hypochlorite is to be used, it should be replenished inside the canal continuously.[15] However, due to its short term efficacy or inadequacy, the new technique has evolved that heats the hypochlorite inside the canal. Dr. Woodmansey (2005) first advocated the technique for intracanal heating of sodium hypochlorite using Buchanan System B plugger. The plugger fit is verified by placing it within 3 mm of the apex. It should fit passively and not contact the canal wall. System B Heat Source is set to a power setting of 10 and a temperature of 200°C and placed in the canal for 3 to 5 seconds, with 5- second interval. This above technique was revised by Simeone M et al. They changed the temperature, plugger size, and the time. The temperature of the carrier was decreased from 200 to 150°C (boiling temperature of sodium hypochlorite is between 96 and 120 degree), plugger size 30/04, activation cycle of 10 sec (with an interval 10 sec,5 cycles) and the heat carrier makes a short up-down movement of 2 mm to shake the irrigant. [25] Recently, Prof. Angelo Putigano created a battery-based device called " Endowarmer ", having a thin 1 mm. probe with a temperature range of 45 to 60°C.[26]

Effect of sodium hypochlorite on various parameters -

1. Effect of Heat on the stability of sodium hypochlorite - Chemical stability of hypochlorite is generally measured by the amount of chlorine in the solution. Cunningham and Balekjian (1980) found that the available chlorine titer did not decrease at 37°C for up to 4 hours. However, he stated that deterioration of the warmed solutions does occur at 37°C if heated for an extended time.[14] Similar results were reported by Pişkin B and Türkün M (1995). Gambirini G et al (1998) showed that heating of NaOCl to 50°C did not affect its chemical stability.[27] Sirtes G et al. (2005) showed that the concentration of chlorine was not decreased for up to 60°C. Sodium hypochlorite retained 100 percent chlorine at the temperatures of 20°C, 45°C, and 60°C. [28] Trisha Dash et al. (2017) reported that both heated and nonheated solution showed similar chlorine concentration.[29]

2. Effect of Heat on tissue dissolution property of sodium hypochlorite -

It is a common consensus that heat increases the tissue dissolution efficacy of hypochlorite irrigant. Initially, this finding was confirmed by Cunningham and Balekjian (1980), and Abou-Rass & Oglesby (1981). Sirtes G (2005) reported that 1% hypochlorite at 45°C was equally effective as 5.25% at 20°C to dissolve the pulp.[28] Rossi-Fedele and De Figueiredo (2008) stated that the highest tissue dissolution occurs at 60 to 75 degrees. Sonja Stojicic (2010) showed that heated hypochlorite increased the organic matter dissolution by 30% to 300%. [31] Basaiwala AK (2018) reported a similar finding.[32] Furthermore, Kamburis JJ (2003) concluded that heated hypochlorite solutions are better in comparison to the unheated solution to remove organic debris from dentin shavings.[34] Amato M (2018) stated that pulp dissolution in lateral canals can only be achieved with intracanal heating followed by ultrasonic activation.[33]

3. Effect of Heat on antibacterial efficacy of sodium hypochlorite -

Costigam GM (1936) reported that hypochlorite heated to 60°C can kill M. Tuberculosis in a small time. [35] Cunningham and Joseph (1980) initially reported the increased bactericidal property of 2.6 % hypochlorite at 37°C against different planktonic bacteria.[15] Dychdala GR. (1991) reported that for every 5°C rise in temperature till 60 degrees, the antibacterial efficacy of hypochlorite is doubled.[36] Sirtes G et al. (2005) concluded that heating of hypochlorite by 25°C increased its efficacy by a factor 100.[28] Poggio C et al. (2010) compared the antibacterial property of different types of hypochlorite based irrigating solutions in unheated state versus solution preheated at 45 degrees. They concluded that a pre-warmed solution at 45°C was more effective than

the unheated solution.[37] Rakesh RR (2011) studied the antibacterial effect of different temperatures at of hypochlorite against E. faecalis and reported that 5% sodium hypochlorite at 45°C was more effective than 24 and 37°C.[24] Giardino L et al (2016) stated that hypochlorite at 45°C showed the lowest colony-forming unit with E. faecalis.[38] Yared G and Ramli GA (2020) was the first to study the ex vivo effect of hypochlorite heating on E.faecalis. They showed a significant reduction in the bacterial count when intracanal heating with System B at 150°C.[39]

4. Effect of Heat on viscosity, surface tension, and penetration of sodium hypochlorite -

Heat treatment of hypochlorite increases its temperature, which leads to the increase in kinetic energy of the molecules. This kinetic energy is greater than the intermolecular forces allowing the sodium hypochlorite to become more fluid (decrease in viscosity), less surface tension, more penetration into the dentinal tubules and thus greater reach to deeper situated microorganism. [53] Priyanka A et al. (2013) assessed the effect of heat on the viscosity of hypochlorite. The dynamic viscosity was evaluated at 25, 45, and 60°C. Irrigating solution molecules undergo thermal agitation on heating, which increases its flow properties. They said that heat application decreases the viscosity, thus increasing the flow characteristic which aids the penetration of irrigants into delicate spaces and canal complexity.[40] Kanfantari N (2019) compared the effect of heated hypochlorite at 20°C, 60°C, and 80°C. They found that the storage modulus was were reduced by 4.3 - 6.8 GPa and 5.1 - 9.7 GPa at 60°C and 80°C respectively.[22] Semra Sevimay et al. (2010) stated that the surface tension of hypochlorite decreases with an increase in temperature.[41] Ling Zou et al. (2010) measured the penetration of hypochlorite at 20°C, 37°C, and 45°C. They reported that the least penetration of 77 microns was seen with 1% for 2 minutes at room temperature and maximum penetration of 300 microns with 6% for 20 minutes at 45°C. Apart from temperature, time duration plays an important role concluding that longer exposure time was associated with deeper penetration with heated hypochlorite.[42] Yaghi A (2016) compared the penetration of heated and non-heated NaOCl by apical negative pressure irrigation into lateral canals of the root. They showed better penetration with heated irrigant and apical negative pressure and heated irrigant did not penetrate better in lateral canals.[43] Landolo A et al. (2019) in a study stated that heating of hypochlorite preceded by ultrasonic activation showed higher penetration of irrigant into dentinal tubules at both 3 mm levels and 5 mm levels. Intracanal heating of ultrasonically activated irrigation resulted in significantly cleaner canals than syringe-and-needle irrigation.[44] He conducted the similar study in 2018 that showed intracanal heating of NaOCl resulted in significantly less debris on the root canal walls, compared to irrigation with pre-heated NaOCl.[13] Similar results were reported by Semra Sevimay.[41]

5. Effect of heated sodium hypochlorite on the rotary instrument -

Endodontic files are used with the irrigant placed inside the canal. However, the fatigue life of NiTi instruments is also influenced by the irrigant used, its concentration, and temperature. The Ni-Ti rotary instruments at a lower temperature can be easily deformed which improves its fatigue crack growth resistance, flexibility and instrument have higher resistance to cyclic fatigue. However, the temperature inside the root canal is higher than room temperature (close to body temperature) and heating of irrigant might influence the properties of Ni-ti alloy. The heated hypochlorite when in contact with the Ni-Ti instrument causes microstructural defects.[45] Jamleh A (2015) stated that cyclic fatigue resistance decreases with an increase in temperature.[46] Demiral M et al (2017) reported similar results with the OneShape instrument. They stated that the number of rotations required to failure was less for NaOCl at 50°C compared to NaOCl at 37°C.[47] Huang X (2017) conducted a similar study with Distilled Water and hypochlorite at 22°C, 37°C, and 60°C. The results of their study were in accordance with others, but they found no significant difference between the two solutions.[48] Alfawaz et al. (2018) stated that at a higher temperature of NaOCl, the cyclic fatigue resistance of the Ni-Ti file decreased. This was more evident at higher concentration of 5.25% NaOCl, rather than 2.5%.[49] Keles A et al. (2019) studied the effect of preheated hypochlorite on thermal-treated Reciprocating files. They showed similar result as other studies. Preheated solution adversely affected the cyclic fatigue resistance.[50]

6. Effect of heated hypochlorite on the periodontal ligament and external surface of root surface -

The critical temperature on the external surface of root that affects the cells of the periodontal ligament was found to be 47°C-56°C because the alkaline phosphatase is denatured at this temperature. The cells of the periodontal ligament are adversely affected at this temperature. Temperature greater than 60°C may stop permanent blood flow and bone necrosis.[51] Macedo RG et al. (2014) stated that the highest temperature measure on the outer surface of the root was 39°C with heated hypochlorite.[52] Simeone M et al. showed that heating of hypochlorite inside the root canal till 150°C-200°C did not raise the temperature on the external root surface by more than 42.5°C.[25]

Conclusion

The efficacy of sodium hypochlorite can be increased by either using the pre-warmed irrigant or heating the hypochlorite inside the canal. The efficacy of lower concentration of sodium hypochlorite can be effectively enhanced by increasing its temperature. It enhance the pulp dissolution, antibacterial efficacy and flow without any adverse effect on periodontal ligament or outer surface of the root.

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Addressing Mental Health Issues During COVID -19 Pandemic

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Abstract

Objectives: analyzing psychological issues due to covid 19 and emphasizing psychological coping mechanisms.

Method: International journals found in two electronic databases were used to identify studies with large circulation: Scopus and Embase.

Findings: The present epidemic in Wuhan (China) triggered by the pandemic COVID-19, corona virus infection on human sites around the world, has a major impact on global health and mental health. In addition, given all instruments used to battle virus spread, additional strategies for addressing the mental health balance in India are needed. The general issues of mental health are growing, COVID-19 patients tainted, near contacts, older citizens, kids and health staff. Most health professionals who work in isolation units and hospitals are not trained on mental health. The consequence of mass quarantine seems more likely to be fear.

Key words: *Recognizing, COVID -19, Psychological problems, Coping*

Introduction

The latest pandemic of the corona virus (COVID-19) is unparalleled in the history of modern world health in 2019. In addition to the biological context and because of the enormous and permanent changes in everyday life, dealing with it is a challenge to psychological resilience. Earlier research demonstrated the dramatic individual and social psychosocial impacts of epidemics and contamination of diseases that gradually became more prevalent than [(1),(2)]. actually. the overall population has already been high anxiety and stress levels and depression as a result of this pandemic[(3),(4)]The rapid spread of the novel corona virus declared a pandemic by the World Health Organization has created a sense of fear around the globe.[(5)]

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During these changed times of COVID-19, several medical professionals, such as doctors, nurses and virologist, work in overtime or in altered routines, as with most medical workers, home IT professionals, regular work hours academics as well as work in special schedules and surroundings.[(6)]

Effect on general population due to COVID-19

During pandemics, when the world is facing a shutdown or a frequent slowdown, individuals are encouraged to create social separation, thereby reducing interactions among individuals. [(7)]

Evidence shows that sensitive groups confined to their homes during a pandemic can lead to adverse health outcomes. Children are less physically active and have much more observation time, uneven sleep cycles and poor diets, leading to increased weight and a cardio lack of respiratory fitness. [(8)]There are also other direct and indirect implications for school closures, such as unintentional childcare duties, which are particularly

significant in health care activities. [9] This can apply in most countries around the world not only to care for infants, but also to adults and geriatrics. [10]

Other facets of pandemic outbreak include stigma and terror. It may pose important barriers to healthcare seeking, social marginalization, lack of confidence in health authorities and the perception of public risks which lead to mass hysteria between citizens and to excessive health care allocations by politicians and healthcare professionals. [11]

Healthcare practitioners typically go the other way. As the require for healthcare is growing exponentially, they face long working shifts that can lead to physical pain and breathing difficulties, often with little resources and inadequate infrastructures8 and a need to wear Personal Protective Equipment (PPE). This can result in you isolating yourself from or expanding your family, changing your routine and limiting your social support network [12]

Over and above all this pandemic will impact the future policymaking at global , regional and national levels across the world starting from health, society , and economy. [13]

Any individual may experience symptoms of mental morbidity following a traumatic event and pandemics that cause much stress among large populations under appropriate stress..[14],[15]

Effect on COVID-19 Cases

Patients with confirmed or suspected 2019-nCoV can experience anxiety, isolation, and irritation from the effects, which may be life-threatening, of infection by a new virus. Symptoms like fever or hypoxia are also present cough and adverse drug effects such as corticosteroid insomnia may lead to a worse degree of anxiety and mental distress.[16]

Anxiety, anger, nervousness, emotionality, criticize and blame (everyone else), dissatisfaction, depression, mental addictions and failings to cater are echoing the particular problems during the COVID-19 pandemic. [17][18] Bio function, such as sleep impairment and intercourse. [19] (20)Cognitive disorders include impaired focus, poor memory, incapacity to make choices, lack of dignity, increased alertness, distortions of vision, distracting and inappropriate memories, decreased self-esteem, and denial. • Emotional outbursts, anger, arguments and the inability to settlement include the psychological and personality issues. Remission, failure to communicate with others, decreased and/or increased appetite (or coping) reduction, decreased or lost Libido, inability to control the use of drugs and increased risk-taking behaviour.[21]

Suicides in the COVID-19 pandemic were recorded from different parts of the world.[22]

Family Members and close connexions affect:

Families and closest contacts are faced with psychological problems, isolated or quarantine problems along with COVID-19, which cause people to feel insecure and guilty of the implications of infection, quarantine and stigma in their families or friends. [23] Families lose their loved ones as a result of the pandemic trigger resentment and rage. In addition, they also feel guilt, culpability or stigma for ill and/or quarantined members of the family and some studies have documented PTSD and family depression and close contact. [24] On the other hand, the incidence of an acute stress disorder, changes disorder, and grief in isolated or quarantined children is higher. 30 per cent of children registered PTSD and early loss or breakup of parents during childhood, including increased risks of developing mood disorder, psychosis and suicide, has long-term negative effects on mental illness.[25]

Mental Health Issues among Special Populations:

The mental health of the special populations is at very high risk in the case of COVID-19.

Elderly people: were particularly vulnerable to morbidity and death because of the COVID-19 pandemic; their problems are already at risk of autoimmune diseases and impaired immunity due to the lack of proper nutrition, are often affected by autoimmune disease and are more vulnerable to the development of dementia and depression.[26]

Young children : The social isolation of young children does not come into being; schools and play areas are closed and their classmates, friends, sleep-over and playing dates have ceased. Their children are the children who have no access. This can lead to

their tranquility and isolation.The protocols for social distancing and locks-downs may also increase, cause fear , anxiety, abnormalities in the behavior, irritability and agitation, and not just affect their perceptuals of the pandemic. Children can be watched and cried and teenagers can attempt to cope with the ingestion of drugs. These unhealthy management mechanisms can lead to short and long-term mental health problems.(27) (28)

Pregnant women: COVID-19 pregnant women are at severe risk because of sudden outbreak and an unexplored natural disease history. There was little evidence of how new coronaviruses can affect pregnant women worldwide. (29)

Even pregnant women's mental health is critical and needs to be taken seriously. Women are exposed to the virus during their initial stage of pregnancy which can cause unknown complications and pregnancy outcomes. In general women who are pregnant require emotional support, particularly during pregnancy, but protocols which distance them from social support usually received by them can prevent them. When a woman who is a health care worker is pregnant during these times, the issues are even greater. The already burdened system can be stressed unduely and women can be stressed.(30)

Marginalized groups: aboriginal and tribal people who have been marginalized from societies also are at high risk, typically have little access to healthcare standards and live where healthcare and facilities in normal times are difficult to meet. COVID-19 will provide such societies with a great challenge. Most of these people are employed in dangerous jobs and are unable to take advantage of paid leaves and face an eviction threat; they can lose employment and space, making them more prone to the illness and drive them to mental illness. You may encounter issues such as helplessness, desperation and perhaps strength to demand essential sustainability.(31)

The largest spreader of corona viruses is beggars living on the side of the lane. They keep their belongings on the brooks and sleep on the floor. Most do not wear masks and start at the nearest red light intersection. Stress and emotional disruptions also endured. Significant measures and methods for rehabilitating and supplying health services with mental health requirements for such citizens.(32)

People with comorbidities:

Because of their medical comorbidities, the risk to those already ill makes them very vulnerable to new coronaviral infections. This prevents them from being critically ill.

China and Italy studies have shown that COVID 19 has risen in severity and mortality and morbidity among the people who have medical comorbidity. This gives people with these conditions the sense of fear and anxiety, influencing their ability to deal with the disease. (33)(34).

Health care workers: The pandemic affects physicians in an emotional way and the vast majority says they experience one or more of the following consequences: fear of infection, anxiety, tiredness, sleeping trouble, loneliness, anguish, insecurity or irritability. The front-line people are particularly affected by fear of personal and family infection, workplace pressure and tiredness. (35)

Because of the exponential rise in demand for health care they have to cope with long shifts of work, often with little funding and fragile facilities 8 and with the need to wear Personal Protective Equipment (PPE), which may cause physical pain and breathing difficulty Moreover, a substantial number of clinicians may not be prepared to perform clinical intervention in new virus patients who are little known for, and for whom clinical guidelines or treatments are not well developed. (36)There is also fear of auto-inoculation and concern that the virus may spread to your family, friends or colleagues(37),(38) This can lead them to get alienated or extended, change their routine and reduce their social support network.

Taking care of confrontation with issues of mental health during COVID-19 pandemic

Tele psychiatry: Currently, tele psychiatric consultation has become increasingly popular with lock-downs as well as social distance, and fears of COVID-19. The advance of technology has made access to health care via networking possible with phones and internet. Different tele psychiatry models can be used in different national contexts. In lieu of COVID-19, India has already begun using psychiatric hotlines and

helplines. The most frequent services

Psychological can include the COVID-19 pandemic first aid, identification of mental health issues,

Checking and appropriate psychological symptoms referrals. This will lower the workload in

and a successful action to eliminate hospitals. Footfall in installations that lead to maintenance

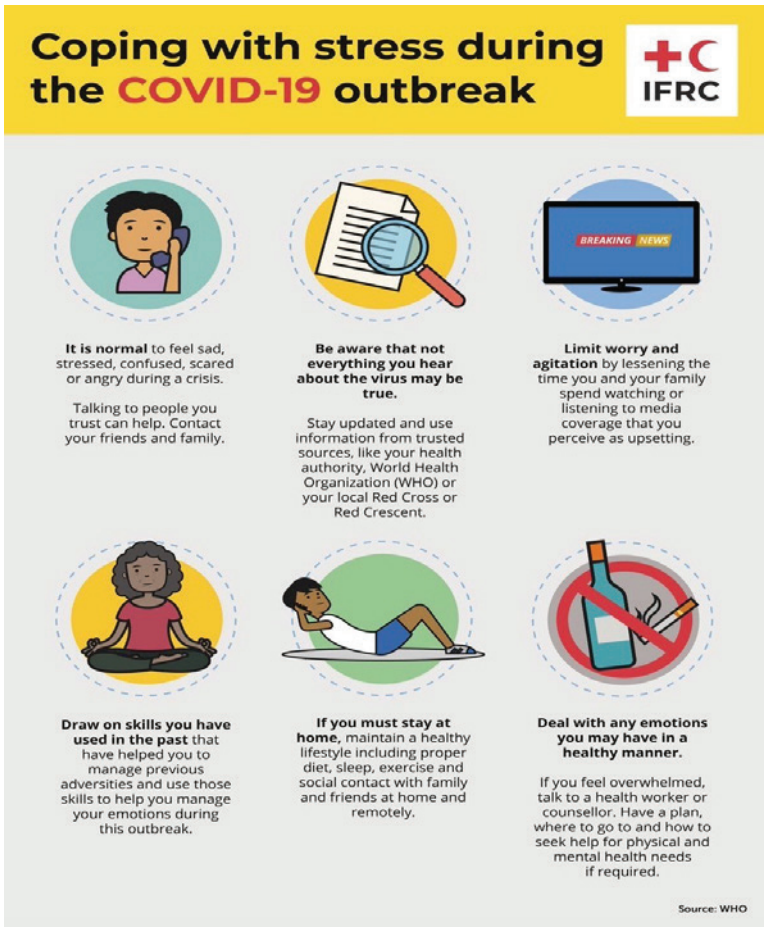
of the protocol of social distance. This will also help to make them think that somebody always listens,

Soon support is provided.[39]

E-teaching: E-teaching is one of the most common trends. The economic prices for the internet and better technology have created a great resource of knowledge about healthcare. This initiative in medical professionals can also be prepared to target the interventions of COVID-19 and can upgrade their knowledge[40]

Resilience: Although each person is affected and unnerved by the coronavirus pandemic and tries to adapt to it, not everyone can cope efficiently with stress and adapt quickly to new conditions. Resilience is measured by factors such as living conditions, poverty, lack of access to health care, potential future vulnerability (i.e. job risk), genetic make-up, previous experience, social interactions[41] and social help. Improved mental toughness helps to effectively fight the coronavirus pandemic[42].

Stress is a pandemic natural response. Possible stress-related responses may involve carelessness, irritability, anxiety, sleeplessness, reduced efficiency, and conflict between the members of society. This can be applicable to the larger population, but in particular to sectors directly involved (e.g. health workers). Depression and anxiety should be stressed as normal reactions to a dangerous situation [43]



Summary of coping with stress during COVID-19 (adapted from source international federation of Red Cross and Red Crescent societies)

Modalities to defeat mental health issues:

Maintaining relationships:

- Establish an online platform for mutual support
- Just sit down and enjoy the conversation
- Teams of staff have established online community gatherings, online book clubs and co-working areas in which others can (virtually) operate.

· **Your friend is Routine:**

· It helps to control fear and helps you respond more easily to this present reality.

· Setting coherent limits between working time and non-working time in your physical as in your head space.

· Identification of something that does not work and is not linked to viruses that bring happiness to you.

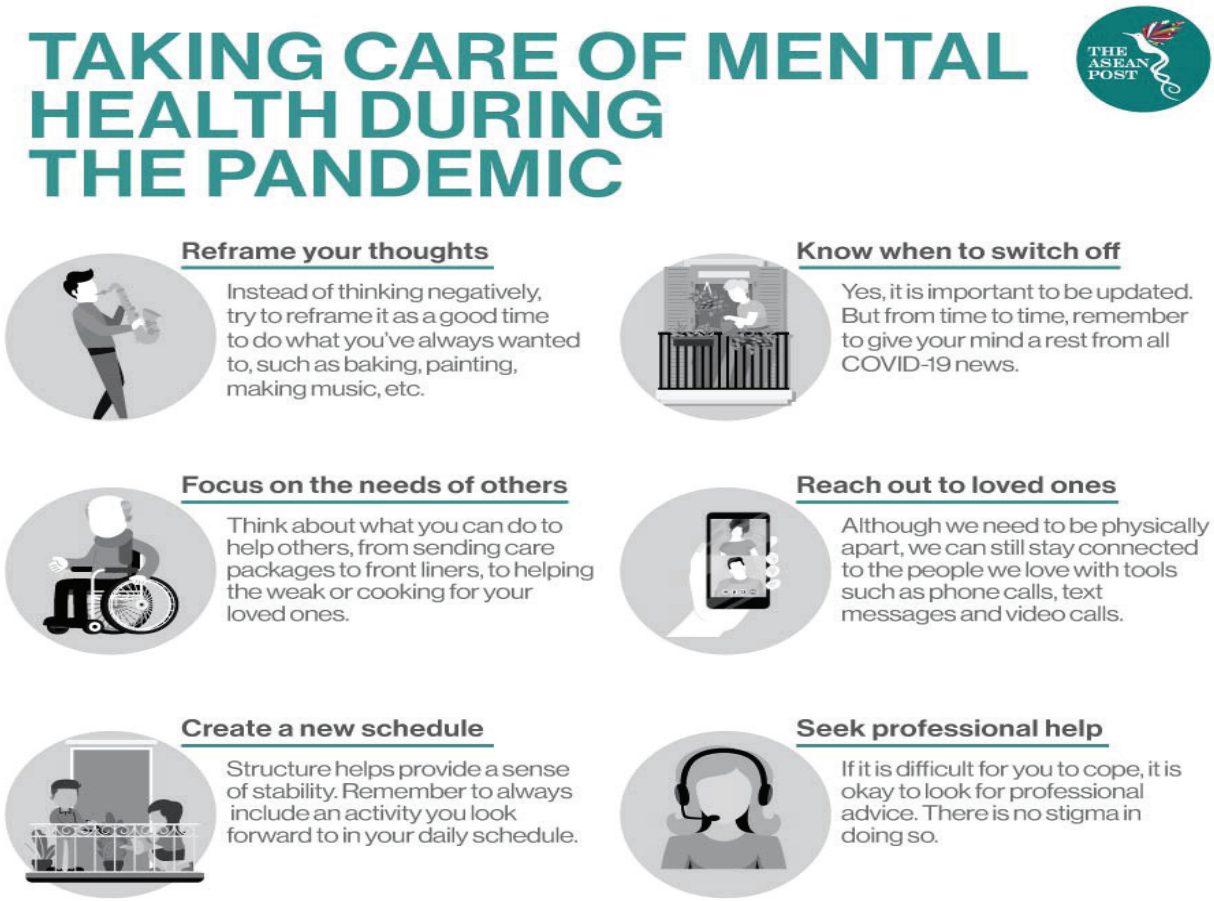
· Operating in brief blasts with consistent breaks can help to maintain continuity

· Restricting exposure to the pandemic can cause disturbances

· **Manage vulnerability by keeping in touch**

· • Take every day and focus on things you can deal with.

· Consciousness can be great tools and meditation



Taking care of mental health during COVID-19(adapted from resources Malaysian Health Coalition)

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To Assess the Effectiveness of Video-Assisted Teaching on Knowledge Regarding Lifestyle Modifications and Drug Regimen in Postoperative Coronary Artery Bypass Graft (CABG) Patients Attending OPD

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Abstract

Background: In patients suffering from coronary heart disease (CHD) after coronary artery bypass grafting (CABG), non-adherence to dietary guidelines, exercise, and prescription medication regimens is a major health care concern worldwide. **Aim of the Study:**To assess the effectiveness of video-assisted teaching on adherence to lifestyle modifications and drug regimen among postoperative coronary artery bypass graft (CABG) patients attending the outpatient department. **Objectives:**1. Assess the knowledge regarding lifestyle modifications and drugregimen among post-operative CABG patients attending OPD.2. Evaluate the effectiveness of video-assisted teaching on knowledge regarding lifestyle modifications and drugregimen among post-operative CABG patients attending cardiac and medicine OPD.3. Associate post-test knowledge score with selected demographic variables of CABG patients. **Material and Methods:** A hospital-based interventional study was used one group pre-test and post-test study design with a Quantitative research approach. This study was conducted among 100postoperative CABGattending outpatient departments of the age group from 55 years to 70 and above in Wardha city of Maharashtra state. The demographic data such asage, sex, educational status, monthly income, dietary pattern and duration after CABGalong with 30 knowledge questionnaire regarding lifestyle modifications and drugregimen in postoperativecoronary artery bypass graftwas obtained using a structured pre-tested questionnaire.Data collected were entered into the Microsoft Excel sheet. The statistical analysis was done using SPSS software. Frequencies and percentages were presented for categorical variables. **Result:** The pre-test findings show that 0-20%had a poor level of knowledge,21-40%had average knowledge,41-60% had good knowledge,61-80 % had very good knowledge. Aftervideo-assistedteaching the post-test scorewas26-50%had average knowledge,51-75% had good knowledgeand76-100 %had very good knowledge, thus it shows that after post-test the knowledge score was increased, applied to find the efficacy of video-assisted teaching't' test was applied and't' value was calculated, The post-test score was considerably higher at 0.05 level than that of pretest score. Hence it indicates that video-assistedefficient teaching.

Conclusion: In general, knowledge of lifestyle changes and drug regimen in post-operative patients (CABG) has been average in the pretest and after video-assisted knowledge of adherence to drug regimen and regulation of lifestyle factors like high fat, high cholesterol diet, obesity, smoking and lack of regular exercise has been increased and understanding of the disease dimension has therefore been generated.

Keywords:*Effectiveness, Video-assisted teaching, lifestyle modification, and post-operative CABG patients*

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Introduction

Coronary cardiac disease (CHD) is a major cause of global morbidity and mortality.[(1)(2)]In the year 2020, CHD caused 11.1 million deaths, according to the World

Health Organization (WHO) [3]The symptomatic relief and increases life expectancy of CHD patients since 1960 have included Coronary Arteries Bypass (CABG). [4]

CABG was allocated to the American and European Guidelines as a Class-1A indication for multi-ship revascularization.[5]An overall CABG death rate of 3.4 percent was estimated [6] Secondary prevention of CHD and cardiac rehabilitation Patients who have been revascularized are highly necessary to minimize their mortality, and maintain the best possible physical, mental and social well-being.Growing literature has shown that interventions to life style, beneficial modifications to the risk factor and selective use of prophylactic medication scans have decreased the risk of non-fatal and fatal in CHD patients.[7]The importance of dietary improvement, exercise and prophylactic drug therapies is clearly illustrated in existing guidelines.[8]

The individual's dedication remains, however the secret to achieving success in the general public with these guidelines. Compliance, as specified by WHO, is as follows from the accepted guidelines of a health care provider as a person's conduct (take medicine, retain a food and/or enforce change in lifestyles).[9]Compliance with lifestyle procedures and the prescribed drugs that enhance quality of life and happiness are crucial in preventing secondary heart injuries.[10][11]

Non-compliance following CABG is a complex issue and a significant public health problem. It can lead to poor health outcomes and imposes an increased health burden on national economies. It serves as an obstacle to achieving treatment goals and may exacerbate the illness. Despite several years of guidance, a variety of studies have shown that the guidelines and actual clinical practice vary considerably.[12]

Non-compliance has been motivated by a variety of factors: live alone, poor ties between patient and doctor, lack of understanding of the value of adherence, busy schedules, attitudes about practice and treatment, and psychological factors such as anxiety and depression[13] [14][15]

The most important factors contributing to drug non-compliance have been the reporting of several daily doses, over cost, forgetfulness and fear of side effects. [16]

Many studies have documented the prevalence of non-compliance after CABG among CHD patients, linked to lifestyle change and pharmacological care worldwide. Nevertheless, in our part of the world, there is a lack of data concerning non-adherence after coronary revascularization (post-CABG).Moreover, only a few studies have investigated possible food, exercise and substance non-consistency predictors. Given the absolute importance of adherence to CHD and mortality burden in Asia, information on lifestyle and drug regimes modifications in the after-operative bypassing artery graftCABG was the primary objective of this research)(17)

Methodology

Materials and methods: the present study was an interventional study conducted among 100 postoperative CABG patients attending OPD in AVBRHospitals at Sawangi Meghe, Maharashtrastate fromJanuary 2017 to March 2017.The research began after receiving authorization from the Committee of institutional ethical committee (IEC), Datta Meghe Institute of Medical Sciences (Deemed to be University) Sawangi (Meghe), Wardha. Purposive sampling technique was used to select the sample for this study. Based on the extensive review of various interventions, as well as keeping in mind, the cultural settings and resources available, the customized intervention was designed to impart knowledge regarding adherence to the drug regimen and lifestyle modification and this included the following components:

Pretested structured questionnaires were used for data collection. The questionnaire consisted of two parts. Part one consisted of demographic data of Post Operative CABG patients Such as age, gender, education& residential area, qualification, dietary pattern etc..

Part two consisted of 30 questions related to knowledge regarding lifestyle modifications and adherence to drug regimen after CABG surgery, for scoring the knowledge of each participant one score was allocated to each correct answer and zero to the incorrect answer. Furthermore, Patient who has already attended similar types of study and Patient those who are illiterate was excluded from the study.The subjects were

clarified about the study's existence and intent.Written informedconsent was taken from the participants before their recruitment. They had been assured that the data was confidential.

In the Microsoft Excel document, data collected was entered. SPSS software was used to carry out statistical analysis; Frequencies and percentages for categorical variables were provided.

Hypothesis:

H_0: There will be no significant difference between knowledge score regarding lifestyle modifications and drug regimen in postoperative (CABG) patients

H_1: There will be no significant difference between knowledge score regarding lifestyle modifications and drug regimen in postoperative (CABG) patients

Results

Demographic variables .shows that the Majority of 37 % of samples were from the age group of 51-55years,28% of samples were from the age group of 56-60 years. The majority of the 76% samples were males and the remaining24% samples were females. The majority of the31% belong to secondary education, 24% belong to higher secondary, and 18% graduate and above. The majority of subjects 68% of the urban area and the remaining 32% of samples were from the rural area. The majorityof the subjects 36% having monthly income 10001-15000,24% having 9000-10000 and 20% having monthly income 15000-20000 and above.53 % was mix vegetarian and 47 % was vegetarian.45% patients postoperative CABGduration was around 6months to 1 year and 55% patients postoperative period was above 1 year.

Table No. 1:Assessment of existing pretest knowledge regardinglifestyle modifications and drug regimen in postoperative coronary artery bypass graft (CABG)

n=100

Level of knowledge score	Score range	Percentage score	Pre Test	
			Frequency	Percentage
Poor	0-6	0-20%	1	1%
Average	7-12	21-40%	60	60 %
Good	13-18	41-60%	39	39 %
Very good	19-24	61-80 %	0	0 %
Minimum score	6			
Maximum score	18			
Mean score	12.20± 2.225			
Mean %	12.2 %			

Above table No.1 shows that the majority of 60% had average knowledge,39% had good knowledge the maximum score was 18, the mean score was 12.20± 2.22 with a mean percentage score of 12.2 %.

Table No. 2:Assessment of existing pretest knowledge regardinglifestyle modifications and drug regimen in postoperative coronary artery bypass graft (CABG)

n=100

Awareness score level	Range of score	Score of percentage	Post Test	
			Frequency	Percentage
Poor	0-6	0-25%	0	0 %
Average	7-12	26-50%	0	0 %
Good	13-18	51-75%	35	35 %
Very good	19-24	76-100 %	65	65%
Minimum score	15			
Maximum score	22			
Mean score	18.88 ± 1.499			
Mean %	18.88 %			

The above table no.2 shows that of the sample were had a poor level of knowledge score, none of them had a poor level of knowledge score, none of them had an average level of knowledge, 35(35 %) of them had a good level of knowledge score, 65(65%) have a very good level of knowledge. The minimum score was 15 and the maximum score was 22, the mean score was 18.88 ± 1.499 with a mean percentage score of 18.88 %.

Table No. 3: Effectiveness of video-assisted teaching on knowledge regarding lifestyle modifications and drug regimen in postoperative coronary artery bypass graft (CABG)

n = 100

Tests	Mean score	SD	't'-value	Degree of Freedom	p-value	Significant
Pre Test	12.20	±2.225	29.911	99	0.001	S, p<0.05
Post Test	18.88	±1.499				

The table No.3-Shows that there is a significant difference between pretest scores and posttest scores of knowledge interpreting the effectiveness of video-assisted teaching on knowledge regarding lifestyle modifications and drug regimen in postoperative coronary artery bypass graft (CABG)

The mean value of pretest is 12.20 and the posttest is 18.88 and a standard deviation value of pretest is 2.225 and the posttest is 1.499. The calculated t-value is 29.911 and the p-value is 0.001.

It is therefore interpreted statistically that the video-assisted teaching on knowledge regarding lifestyle modifications and drug regimen in postoperative coronary artery bypass graft (CABG) was effective. Thus the H_1 is accepted and H_0 is rejected in this study.

Table No. 4:Association of post-test knowledge score with selected demographic variables.

n=100

Demographic variables	Frequency	Percentage score%	Mean posttest knowledge score	F-value/ t-value	p-value
Age of parents in the year				F-value	
55-60	12	12	18.95± 1.618	1.957	0.126 NS, p>0.05
61-65	23	23	19.46±0.884		
66-70	37	37	18.61±1.576		
70 years and above	28	28	18.33±2.517		
Gender				t-value	
Male	76	76	18.85 ± 1.624	6.376	0.13 NS, p>0.05
Female	24	24	18.96 ± 1.138		
Residence				F-value	
Urban	68	68	18.77 ± 1.505	0.041	0.040 S, p<0.05
Rural	32	32	18.98 ± 1.502		
Educational status				F-value	
Primary education	27	27	19.06± 1.417	6.758	0.05 S, p<0.05
Secondary education	31	31	18.56±1.944		
Higher secondary	24	24	18.70±1.252		
Graduation and above	18	18	18.58±1.499		
Monthly income of the family				F-value	
9000-10000	24	24	18.70± 1.129	1.578	0.200 NS, p>0.05
10001-15000	36	36	18.74±1.643		
15001-20000	20	20	19.00±1.541		
20000 and above	20	20	18.88±1.499		
Dietary pattern				t-value	
Vegetarian	47	47	18.91 ± 1.502	0.035	0.851 NS, p>0.05
Mix	53	53	18.40 ± 1.517		
Duration after CABG					
6months -1 year	45	45	17.81 ± 1.502	t-value	
Above 1year	55	55	18.23 ± 1.517	0.335	0.743 NS, p>0.05

Table No. 4 shows how knowledge scores are associated with age, gender, monthly income and dietary pattern of samples was not statistically significant. The association of knowledge score was shown significantly to the demographic variable such as education and residence.It shows that integrating knowledge scores with the area of residence. The 'F' was calculated 0.041 at a 5% level of significance with df 98. Calculated value of 'p'=0.040 which was greater than the acceptable significance level, i.e.' p'=0.05.So it is perceived that the residential area of samples wasassociated with its scores of knowledge.

It demonstrates that the awareness scores are correlated with the education. The 'F' value was calculated 4.15 at a 5% level of significance with df 3 (97). Also the calculated 'p'

value is 6.758 that was more than the appropriate significance point i.e. 'p'=0.05.So it is perceived that education of parents associated with their knowledge scores.

Hence it is concluded that video-assisted teaching significantly brought improvement in the knowledge regarding lifestyle modifications and drug regimen in postoperative coronary artery bypass graft (CABG) patients.

Discussion

The research findings were addressed with regard to the study's objectives and the results of other studies in this section.This research was carried out to assess the efficacy of video assisted teaching on knowledge concerning the changes in lifestyles and the regimen of drugs for OPD patients after surgery coronary artery bypass graft (CABG)."The subjects in this study expressed an understanding of the seriousness of their medical condition— CAD. Among the reasons they consented to CABGS was a deterioration in the overall quality of their lives— expressed as a decrease in their functional ability; inability to perform job/family roles; angina symptoms/ feelings of illness; and a perceived option to the threat of a possible M.I., or imminent death. a crisis of an M.I or the urgent need for CABGS for unstable angina, or Left Main coronary disease, existed for most of the subjects in this study, even though subtle prodromal warning symptoms were present for many of them. To cope with the stressful situation of their anticipated CABGS and hospital recovery, the subjects expressed a belief in the hospital medical system, their expectations of a good outcome, and the support of their family and friends.

Identified "means of coping" of the present study groups included: family/friend support; religious beliefs; a positive attitude; pets; others with a heart condition/ or who had a CABGS; and work/hobbies/or sports. Similar "means of coping" were found in other studies of cardiac patient population groups (Kandheria U, Townsend K, Erickson SR, et al.)[18]Recommendations from the majority of these studies highlight the necessity to identify and provide counseling and guidance to those individuals whose "maladaptive" coping responses may lead to poor recoveries.

All of the subjects expressed an understanding of the limitations of their CABGS, i.e., it is not a curative treatment measure, and that lifestyle risk reduction behavioral changes were necessary to control their CAD and to maintain their overall QOL. Lifestyle behavioral changes (e.g., diet, exercise, stress reduction, etc.) often began within weeks of discharge from the hospital after CABGS, as evidenced by the Group

Most of the subjects reported that maintenance of a "cardiac healthy diet" was the most difficult part of their medication compliance.

The busy life of a patient contributes to the inability to perform physical exercise is a fascinating fact.Patients find it difficult to make time after an already hectic day because exercise takes a minimum of half an hour. However if time in certain situations isn't a limitation, exercise will make them exhausted because of which they are reluctant to start.CABG: CAB Furthermore it has also been documented that there are consistent "narrowness" and the reproaches of the partners in cases of exercise slacking or dietary discipline. In addition (Khanderia et al).In this study after providing video-assisted teaching the patients gain more knowledge about their disease aspects and the importance of adherence to the drug regimen and are to maximize coping with the "environment. Simplifying instructions or take down a patient's prescription doing the right dose on time would essentially make it easier for the patient [19]

Limitations:

The study findings must be interpreted with the knowledge that data were collected from a small purposive sample of CABGS patients.

Our analysis also has a downside because the study sample represented a small population selected by purposeful samples and the subjects were recruited from a single tertiary care hospital in Wardha City. More comprehensive research in a broader geographical region covering more tertiary hospitals to help draw conclusions

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Conclusion

It can be argued that failure to accept improvements in lifestyle and medicine is an emerging phenomenon worldwide.Despite the importance of secondary preventative measures, changes in patient behavior are still insufficient with heart disease due to sedentary behaviours and unhealthy diets in our days. Recognizing and addressing these predictors is important for the skilled health practitioners.

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Research Article

The Effectiveness of Breathing Exercises on the Physiological and Psychological Variables of Patients with Pulmonary Tuberculosis

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Abstract

A study to evaluate the effectiveness of breathing exercises on physiological and psychological variables of patients with pulmonary tuberculosis in selected hospital at Ambala, Haryana.

TB is a 7th killer disease in the world and one of the top 10 causes of death. Pulmonary tuberculosis (PTB) is an infection, which causes widespread pulmonary fibrosis and cavitation, and often leaves the patient with a chronic respiratory deficit as a result.

Aims and Objectives: The aim of the study was to evaluate the effectiveness of breathing exercises on physiological and psychological variables of patients with pulmonary tuberculosis.

Material and Methods: A quasi experimental design using non-equivalent control group pretest- posttest design was conducted on 110 pulmonary tuberculosis patients (55 in comparison group and 55 in experimental group) in DOTS center of UPHC RavidasMajriInderpuri colonyAmbala city, UPHC Baldev NagarAmbala city, UPHC Durga Nagar Ambala city and CHC Mullana. The dependent variables were physiological variables (dyspnea, spo_2, heart rate, respiratory rate) and psychological variables (anxiety, quality of life). Pretest was taken in UPHC Durga Nagar Ambala city and CHC Mullana on day 1 and posttest was taken inUPHC RavidasMajriInderpuri colonyAmbala city and UPHC Baldev NagarAmbala cityon day 30. In experimental group, breathing exercises were administered by the researcher two times a week. Each session was of 30 minutes including 4 breathing exercises (pursed lip breathing exercise, diaphragmatic breathing exercise, deep breathing exercise and segmental breathing exercise) and each exercise was performed for 6-8 times. Other days patients were estimated to perform breathing exercises themselves at home and on day 30, posttest was taken.

Results: Both the groups were homogenous with respect to physiological and psychological variables before the administration of breathing exercises. On day 30, the mean dyspnea score and anxiety score of experimental group was significantly lower than comparison group ($p<0.05$). The quality of life of patients in experimental group was significantly better than comparison group at day 30.

Conclusion: Based on the findings of the study, it can be concluded that breathing exercises are effective in reducing the dyspnea, anxiety, HR and RR. Breathing exercises are effective in improving theO_2saturation, blood pressure and quality of life among pulmonary tuberculosis patients.

Keywords: *Breathing exercises, physiological variables, psychological variables, pulmonary tuberculosis* patients**.**

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Introduction

TB is a 7th killer disease in the all over world. Tuberculosis (TB) is one of the top ten causes of mortality and the primary cause from a single communicable agent. Globally, ten million people developed tuberculosis disease in 2017; 5.8 million men, 3.2 million women and one million children. There were cases in all countries and age groups, but overall ninety percent were adults, ten percent were people living with HIV (72% in Africa) and two third were in eight countries: India (21%), China (14%), Indonesia (6%), the Philippines (3%), Pakistan (3%), Nigeria (5%), Ethiopia (3%), Bangladesh (4%) and (5%) in South Africa (figure 1.1).[1]

India is the 2nd most populous country in the world having more new TB cases annually than any other country and accounting for 1/5th of the global incidence. About 40% of Indian populations are suffered with tuberculosis bacillus. India has been ranked 17th among 22 high burden countries in terms of tuberculosis incidence rate.[2]

Pulmonary tuberculosis (PTB) remained a main health problem worldwide, most common in developing countries.In previous times tuberculosis was known as "consumption", "Phthisis pulmonary is" and "white plaque". In 19th century, it was also known as "the captain of all men of death".[3]

Pulmonary tuberculosis is an infection, which causes widespread pulmonary fibrosis and cavitation, and often leaves the patients with a chronic respiratory deficit, which often presents as an obstructive lung dysfunction. Pulmonary fibrosis and cavitation leads to thickening of the wall, and causes decrease oxygen supply in the blood and as a consequence, patients suffer from dyspnea.[4]

Dyspnea is a complex symptom that arises from physiological impairment and alerts one to the possibility of threatened homeostasis. Dyspnea not only manifests with respiratory difficulty that embarrasses and limits the patient's activity.[5]

Only a few studies were identified on the effect of breathing exercises on physiological and psychological variables among patients with respiratory illness. Majority of studies focused only on physiological parameters of patients. The researcher did not locate any study regarding the effect of breathing exercises on physiological and psychological parameters of tuberculosis patients. Most of the previous studies have assessed the effect of single breathing exercise on physiological parameters. Considering the effect of different breathing exercises on different lung function parameters and psychological parameters, a group of breathing exercises was clustered together and their cumulative impact was tested on selected physiological and psychological parameters of tuberculosis patients.

Materials and Methods

We enrolled inpatients of Pulmonary Tuberculosis (PTB) having dyspnea, willing to participate, able to understand Hindi or English and available during the period of data collection.

The present study was a quasi-experimental study using non-equivalent control group pretest-posttest design conducted at DOTS center of UPHC Ravidas Majri Inderpuri colony Ambala city, UPHC Baldev Nagar Ambala city, UPHC Durga Nagar Ambala city and CHC Mullana over the period of one monthJuly 2020

Total of 110 PTB patients were selected for the study and non-randomly divided into comparison group (55) and experimental (55) group.Convenience sampling technique was used to select PTB patients in the study who were come in DOTS center of UPHC Ravidas Majri Inderpuri colony Ambala city, UPHC Baldev Nagar Ambala city, UPHC Durga Nagar Ambala city and CHC Mullana. The dependent variables were physiological variables (dyspnea, spo_2, heart rate, respiratory rate) and psychological variables (anxiety, quality of life). Pretest was taken in UPHC Durga Nagar Ambala city and CHC Mullana on day 1 and posttest was taken in UPHC Ravidas Majri Inderpuri colony Ambala city and UPHC Baldev Nagar Ambala city on day 30. In experimental group, breathing exercises were administered by the researcher two times a week. Each session was of 30 minutes including 4 breathing exercises (pursed lip breathing exercise, diaphragmatic breathing exercise, deep breathing exercise and segmental breathing exercise) and each exercise was performed for 6-8 times. Other days patients were estimated to perform breathing exercises themselves at home and on day 30, posttest was taken.

Formal ethical permission was obtained from the Institutional Ethical Committee of Maharishi Markandeshwar (Deemed to be) University, Mullana, Ambala, Haryana**.** Informed consent was obtained from the pulmonary tuberculosis patients who werecome in DOTS center of UPHC Ravidas Majri Inderpuri colony Ambala city, UPHC Baldev Nagar Ambala city, UPHC Durga Nagar Ambala city and CHC Mullana.

Different tools like sample characteristics Performa, Modified Medical Research Council dyspnea scale (MMRC), physiological variables recording sheet, anxiety scale, respiratory disease quality of life (RD-QOL) and semantic differential scale were used to collect data.

Interview technique and bio-physiological measurement were used to collect data from pulmonary tuberculosis patients.

MMRC Scale [6] is a standardized tool developed for assessing the dyspnea level (0-4). Lower score 0 indicates mild dyspnea and higher score 4 indicates the severe level of dyspnea. Content validity of the MMRC dyspnea scale was 1 and test-retest reliability of the MMRC dyspnea scale was 1.Physiological variable recording sheet consisted of a recording of physiological variables like SPO_2, BP, heart rate and respiratory rate.

Anxiety scale is a ten items tool (I feel tense, I feel upset, I feel restless as I have to be on the move, Worrying thoughts go through my mind, I get sudden feelings of panic, I can sit at ease and feel relaxed, I feel confused, I feel fear of dying, I feel frightened, I get sudden feeling of heart pounding/ racing) developed by the researcher to determine the levels of anxiety among tuberculosis patients. It has been developed after reviewing hospital anxiety and depression scale (HAD) [7]. Only ten items pertaining to assess anxiety were included. The maximum score was 30 and the minimum score was zero. A higher score indicates an increased level of anxiety and a lower score indicates a lower level of anxiety. Content validity of the anxiety scale was 1 and the internal consistency of the anxiety scale was assessed using Cronbach's Alpha and found to be 0.81.

Respiratory Disease quality of life (RD-QOL) is 28 items developed by the researcher to assess QOL in PTB patients. Items of airway questionnaire 20 (AQ20) [8] were referred for developing the RD-QOL. The maximum score was 56 and the minimum score was zero. A higher score indicated the poor QOL and lower score indicated the good QOL. Content of the RD-QOL questionnaire was 0.9 and the Test-retest reliability RD-QOL questionnaire was assessed and found to be 0.8.The semantic differential scale was developed to assess the acceptability of breathing exercises among PTB patients. Content validity of the semantic differential scale was 0.9. All the tools translated into the Hindi language and then back-translated into English for semantic equivalence

The dependent variables were physiological variables (dyspnea, SpO_2, BP, heart rate, respiratory rate) and psychological variables (anxiety, quality of life).

Results

The results shows that both the groups were homogenous and comparable in terms of age in year, gender, marital status, type of family, previous history of pulmonary tuberculosis in family, socio-economic status, BMI, duration of TB in months, any comorbid disease, tubercular infection, treatment category of tuberculosis, performing any respiratory exercises, performing any physical exercises, smoking status, history of alcoholism, tobacco use and exposure to passive smoking before the administration of breathing exercises.

Therefore, it can be inferred that the comparison and experimental group were homogenous and comparable in terms of physiological and psychological before administration of breathing exercises**. [TABLE 1]**

Results of independent 't'-test revealed that breathing exercises are effective in reducing dyspnea, anxiety, HR and RR and improving SpO_2 , blood pressure and QOL. On day 30, the mean dyspnea score, anxiety score, heart rate and respiratoryrate of the experimental group were significantly lower than the comparison group ($p<0.05$). The quality of life of patients, blood pressure andSpO_2 in the experimental group was significantly better than the comparison group on day 30.**[TABLE 2]**

Majority of PTB patients in the experimental group were highly satisfied with the breathing exercises at day 30 (97.14%) respectively which shows acceptability of the intervention among patients.

Discussion

In the current study, the breathing exercises

administered for four weeks were effective in reducing dyspnea among PTB patients. A significant difference was found in dyspnea score of PTB patients in the comparison group and in the experimental group after administration of breathing exercises at day 30. These findings are in line with the study done by Brenda Morrow et.al (2016)[9]to determine the "effect of positioning and diaphragmatic breathing on respiratory muscle activity in people with a chronic obstructive pulmonary disease" which showed a significant decrease of dyspnea score in the experimental group than in comparison group respectively. The diaphragm helps to inflate the lungs, increase in vital capacity, functional residual capacity and oxygen consumption, which may result in a concomitant decrease in dyspnea.[10]

The breathing exercises were effective in improving oxygen saturation level score. The patients in the experimental group had higher oxygen SpO_2 level score than the comparison group on day 30. These findings are consistent with a study conducted by S.K. Sharma and GautamAhluwalia (2006) [11] who reported that mean oxygen saturation (SpO_2) improved after exercises in the experimental group. Purse lip breathing exercise improves the lungs' ability to get O_2 in and CO_2 out.

The breathing exercises were effective in reducing heart rate score. There was a significant difference found in heart rate of PTB patients in the comparison group and in the experimental group afterthe administration of breathing exercises at day 30. These findings are similar with the study done by Dr. Labiba Aba El-Kader Mohamed et.al (2013)[12] to examine the "effect of slow deep breathing exercise (DBE) on blood pressure and heart rate"on 120 newly diagnosed patients with hypertension in which heart rate score of patients before intervention was 97.06±10.65 and after intervention heart rate score of patients was 92.01±7.70. A deep breathing exercise improves vagal tone and decreases sympathetic discharge, by decreasing sympathetic discharge it helps to decrease heart rate.[13]

The breathing exercises were effective in reducing respiratory rate score. There was a significant difference found in respiratory rate score in the comparison group and in the experimental group after administration of breathing exercises at day 30. The patients in comparison and experimental group had a lower respiratory rate than the comparison group. These findings are similar with the study conducted by Ritu Adhana et.al. (2016) [14] to check the "effect of slow breathing training on spontaneous respiratory rate, heart rate and pattern of breathing" among 60 volunteers in which respiratory rate of patients before training was 20.0±2.0 and after training respiratory rate was 17.0±2.0. Diaphragmatic breathing exercise helps to inflate the lungs, increase in vital capacity and decrease in respiratory rate (RR).[15]

In the current study, the breathing exercises were effective in reducing anxiety. There was a significant difference found in anxiety score of PTB patients in the comparison group and in the experimental group after administration of breathing exercises at day 30. The patients in comparison and experimental group had lower anxiety score than the comparison group. These findings were similar with a study conducted by Li- Chi Chiang et.al (2009)[16] to evaluate the "effectiveness of relaxation breathing training on anxiety among moderate to severe asthmatic patients" in which after intervention anxiety score in the experimental group was 26.11±11.41 and the comparison group was 32.21±17.76.

Limitations

The limitations of the study is that the potential for comparison and experimental group patients to seek out DOTS or alternative treatment was not assessed which may be affecting physiological and psychological variables. The study did not use masking/blinding which might have increased risk of assessment biases.

Conclusion

Breathing exercises are effective in reducing dyspnea, heart rate, breathing rate, and anxiety and increasing the SpO_2 level and improving the QOL among PTB patients. These findings recommend that nurses should routinely provide breathing exercises to the pulmonary tuberculosis patients and must monitor the after the use of breathing exercises.Nurses should provide ongoing education to the tuberculosis patients regarding breathing exercises so that they are able to performand continue these exercises at home setting also.

Table 1: Mean, Mean Difference, Standard Deviation, Standard Error of Mean Difference and 't' value of Physiological and Psychological Variables in Comparison and Experimental group before administration of Breathing Exercises

N=110

Sr. No	Variables	Comparison Group (n=55) Mean ± SD	Experimental Group (n=55) Mean ± SD	M_D	SE_{MD}	t value	df	p Value
Physiological variables								
1.	Dyspnea	2.80 ± 0.80	1.02±0.30	1.78	0.05	0.15	108	0.87^{NS}
2.	SpO_2	94.18±2.10	94.02±3.97	0.16	-0.87	0.66	108	0.51^{NS}
3.	Systolic blood pressure	119.05 ±8.39	121.82±10.30	- 2.77	-1.91	0.96	108	0.34^{NS}
	Diastolic blood pressure	83.40±8.39	82.53±8.57	0.87	-0.18	0.33	108	0.74^{NS}
4.	Heart Rate	101.27±11.09	99.64±12.37	1.63	-1.28	0.31	108	0.75^{NS}
5.	Respiratory Rate	20.47±1.84	20.91±1.95	-0.44	-0.01	1.11	108	0.27^{NS}
Psychological variables								
6.	Anxiety	22.93±4.68	23.42±3.99	- 1.29	0.69	1.03	108	0.30^{NS}
7.	Quality of Life	48.44±3.63	47.55±2.69	0.89	0.94	1.64	108	0.10^{NS}

***-Significant (p ≤0.05)** **^{NS}- Not Significant (p >0.05)**

TABLE 2: Mean, Mean Difference, Standard Deviation, Standard error of Mean Difference and 't' value of Physiological and Psychological Variables among Pulmonary Tuberculosis patients in Comparison and Experimental group after administration of Breathing Exercises at day 6.

N= 110

Variables	Comparison Group (n=55) Mean ± SD	Experimental Group (n=55) Mean ± SD	MD	SEMD	t value	df	p Value
Physiological variables							
Dyspnea	1.51±0.54	1.02±0.30	0.49	0.24	2.8	108	0.01*
SpO2	95.37±1.89	96.60±1.24	-1.23	0.69	3.20	108	0.01*
S.B.P	118.47±7.30	116.24±8.50	2.23	1.20	1.56	108	0.03*
D.B.P	80.87±8.39	77.82±6.30	3.07	2.09	1.71	108	0.04*
Heart Rate	98.80±4.92	90.15±8.68	8.65	-3.76	2.16	108	0.01*
Respiratory Rate	20.20±1.78	19.13±1.62	1.07	0.16	2.80	108	0.01*
Psychological variables							
Anxiety	16.58±1.99	11.78±1.93	4.8	0.06	3.37	108	0.01*
Quality Of Life	42.91±1.78	38.82±2.43	4.09	-0.45	3.64	108	0.01*

***-Significant (p ≤0.05)** **^{NS}-Not Significant (p >0.05)**

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Original Article

Dermatoglyphic Patterns of the Medical Students and their Parents in Jammu Region of North India

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Abstract

Background and Aims: Fingerprints have a general flow to the ridges that translates into three major pattern types; a whorl, loop, or arch. Pattern types are considered to be genetically inherited, but the individual details that make fingerprint unique are not. This descriptive study was conducted to differentiate the fingerprint patterns of MBBS students to their parents and to look for the uniqueness of fingerprint patterns in these medical graduates.

Methods: To do this study, all ten fingerprints were examined from hundred first-year MBBS students of Acharya Shri Chander College Of Medical Sciences in the Jammu region of north India over 6 months from September 2019 to February 2020 along with their two biological parents. Rolled fingerprints were obtained by carbon ink in all 300 subjects divided into three groups(fathers, mothers, and students). Fingerprint patterns were examined by magnifying glass under the supervision of a forensic expert of our institute and classified as loops, whorls, or arches.

Results: The most frequent fingerprint pattern in all 3000 fingers examined is the loop pattern, which represents 64.8%, followed by whorl pattern 30.8% and the least frequent pattern is arch(4.3%).these results were almost similar in medical graduates fingerprints patterns(loops 63.7%,whorls32.5% and arches 3.8%)indicating no difference from other groups. Students show similar fingerprint patterns with those of their father's fingers in 659 fingers, 468 as matching loop patterns, 187 as a whorl, and 4 arch pattern types and the percent of similarity was 65.9% with higher similarity in the RL 76% and LL72% fingers. Students show similar fingerprint patterns as that of their mother in 685 fingers, 500 as matching loop patterns, 183 as a whorl, and 2 arch pattern types and the percent of similarity was 68.5% with higher similarity in the RT78% and RL finger 78%. Fathers and mothers groups unrelated genetically when observed among themselves for similarity in fingerprint pattern showed similarity in 584 fingers, 455 as matching loop patterns, 125 as a whorl, and 4 arch pattern types and the percent of similarity was 58.4% with higher similarity in the LL77% and RL73% fingers.

The statistical analysis for similarity correlation between students' and fathers' fingerprints shows a non-significant p-value found in the right index, left index, left middle, and left little fingers while other fingers show a significant p-value. Similarity correlation between students and mother shows a non-significant p-value found in the right middle, left thumb, left index, left middle, and left little fingers while other fingers show a significant p-value. Similarity correlation between father and mother showed no significant correlation in all fingers except in the LL finger, and negative correlation in RT, RM, and LM fingers.

Conclusions: We concluded that the most common fingerprint pattern is the loop followed by whorl then arch in MBBS graduates of our institute along with their parents. Moreover, the present study showed that fingerprint patterns do not 100% match one of the parents; nevertheless, there is a similarity between offspring and their parents.

Keywords*: Fingerprints, patterns, Medical, Students, Parents*

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Introduction

Fingerprint-based biometric systems are being increasingly used to determine the identity of the person that cannot be faked or stolen easily. Fingerprints have

been used for about 100 years as the oldest biometric signs of identity. The foundations of modern fingerprint identification were established by the studies of Sir F. Galton (Galton 1892)[1] and E. R. Henry (Henry 1905) [2]. Since then, fingerprints have been used for identification in many social conditions such as access control, crime investigation, personal trust, etc., since they will remain almost constant during people's lifetime [3].Fingerprints are very popular due to their lower changeability and easier accessibility than other methods such as signature and hand geometry [4]. The use of fingerprints for identification is based on the immutability and the individuality of fingerprints. Immutability refers to the permanent and unchanging character of the pattern on each finger, from the perinatal period until decomposition after death. Individuality refers to the uniqueness of ridge details across individuals. No two persons, even identical twins, have been found to have identical fingerprints, despite elements of similarity.[5] Fingerprints have two levels of structure: the Henry(1900) fingerprint pattern (with ridge count) and the Galton (1892) characteristics. The Henry Classification is the standard qualitative scheme for characterizing the global structure of ridge patterns and has traditionally been used to partition fingerprint databases.[5] At approximately ten weeks of embryonic development, the epidermal ridges on the palms and fingertips begin to develop precise, minute patterns, which are fully formed approximately at 25 weeks.[6] The study of such epidermal ridge pattern on fingers, palm, and soles is known as "Dermatoglyphics". Harold Cummins was the first to coin this word in 1926. [7] Development of these ridges were found to be affected by environmental and genetic factors. These patterns do not change throughout one's life once they are formed. [8] Although the worldwide average distribution of different fingerprint patterns is known, there is a paucity of published literature on the distribution of fingerprint patterns on individual digits, especially in this part of the world.

Material and Methods

This descriptive study was conducted at Acharya Shri Chander Medical College, Jammu (Jammu and Kashmir), India on the 100 first year MBBS students and their biological parents over six months.

Inclusion Criteria

Healthy first-year medical students of the 2019-20 batch of our medical college with no deformities or disfigurement in their fingers were included in the study.

Biological parents of first-year medical students were also included in the study

Exclusion Criteria

Medical students with deformity or disfigurement of fingers were excluded.

Medical students with single parents or no parents were also excluded.

However Medical students with parents having disfigured or lost fingers due to accidents or burns or trauma were not discouraged to participate and data of available digits included in our results.

Rolled fingerprints of 100 medical students are taken on plain paper after they consented for study .There were 72 males and 28 females. Hands were washed thoroughly with soap and water before taking the prints. Carbon ink was applied to all the ten fingertips, and prints are taken on plain papers (proforma) by simply rolling the fingers from one side to another. A pair of proforma along with instructions was given to each student to obtain fingerprints of their respective parents. Written consent from parents for the study was also taken on the consent form. Fortunately, there was no dropout, but unfortunately, the right thumb of one mother and Right index and Right middle fingers of one father was not available for fingerprint due to an accidental burn in former and accidental amputation in later. Fingerprints thus taken were studied with the help of a magnifying glass and categorized accordingly as loops, whorls, and arches under the supervision of an expert from the forensic department. Data obtained from the study were tabulated and analyzed for frequency and percentage of fingerprint patterns in students and their parents separately. Fingerprint patterns obtained were also analyzed in terms of their differentiation among different sexes and also on different digits. On the similarity of the fingerprint pattern between offspring and their parents, the percentage of matching fingers pattern type with father or mother were calculated and aggregated. Finally, the p-value and correlation between

offspring fingerprint pattern types and their parents were calculated using SPSS software version 22(Descriptive analysis, cross sheets, t-test, and chi-square test were used for validation and analysis of data collected as excel). A "p" value <0.05 is regarded as significant.

Ethical clearance has been obtained from the institutional ethical committee and informed written consent after the briefing objective of the study was taken from the subject.

Results

This study of 3000 fingers of 100 families is distributed into three groups, fathers group (100), mothers group (100), and one student group (100). The variation in the percentage of various patterns of fingerprint among subgroups is studied. The number/ frequency and percentage of various patterns of overall and different subgroups were calculated and compared in Table 1 to 6.

Legend:

RT= right thumb, RI= right index, RM= right middle, RR= right ring, RL= right little, LT= left thumb, LI= left index, LM= left middle, LR= left ring,

LL= left little finger

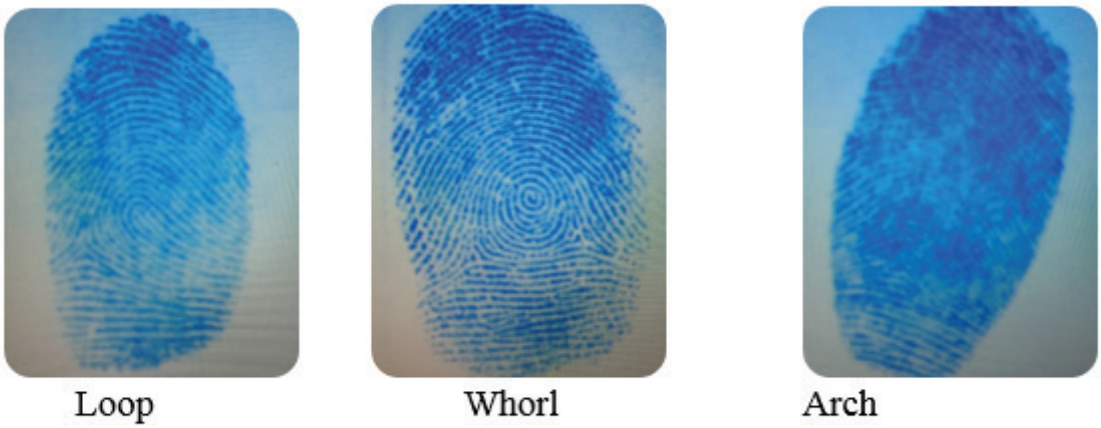

Figure 1: picture of various fingerprint patterns observed during the study

Table 1: Distribution of various fingerprint patterns in fingers of both hands in various subgroups .

GROUPS	LOOPS (%)	WHORLS (%)	ARCHES (%)	UNPRINTED	TOTAL
Father	626(62.6%)	325(32.5%)	47(4.7%)	2	1000
Mother	681(68.1%)	274(27.4%)	44(4.4%)	1	1000
Students	637(63.7%)	325(32.5%)	38(3.8%)	0	1000
Total (%)	1944 (64.8%)	924 (30.8%)	129 (4.3%)	3(0.1%)	3000

Out of total 2997 fingers studied , 1944 (64.8%) showed loops pattern,924 (30.8%) whorl pattern and129 (4.3%) as arch pattern. On comparing different groups we found that the father's group had 626 (62.6 %)fingerprints with a loop pattern whereas it was 681 (68%) in the mother's group and 637 (63.7%) in the student's group. Whorl pattern was observed in 325 (32.5 %) in the father's group, 274 (27.4%) in the mother's group, and 325 (32.5%) in the student's group. The arch pattern was seen in fewer numbers in all the three groups as 47 (4.7 %) in the father's group, 44 (4.4%) in the mother's group, and 38 (3.8%) in the student's group. It was also inferred from the data that medical students in our institute have the same fingerprint pattern as that of other groups with a predominance of loop pattern.

Table 2: Fingerprint patterns distribution among different sexes.

GROUPS	SUB GROUPS(NO.)	LOOPS (%)	WHORLS (%)	ARCHES (%)	TOTAL FPP
	Father (100)	626(62.6%)	325(32.5%)	47(4.7%)	1000
Male	Students (72)	451(63%)	236(33%)	33(4%)	720
Female	Mother (100)	681(68.1%)	274(27.4%)	44(4.4%)	1000
	Students (28)	186 (66%)	89(32%)	5 (2%)	280

On analyzing the distribution of fingerprint patterns in either sex, it was observed that there were 172 males and 128 females in the subgroups studied. The female group had a high percentage of loops pattern (mothers 681 (68%) and students186(66%) compared to the male group with 626 (62.6%) fathers and 451 (63%)students having loops.

Similarly, on analyzing whorls it was found that males(fathers 325 (32%) and male students236 (33%) showed a higher number of whorls compared to the female group(mothers with 274 (27.4%) and female medical students with 89 (32%)whorls.

Furthermore, the arch pattern was high in the male group (47fathers and 33 (4%)male) than the female group (44mothers and 5 (2%)females).

However contradictory findings concerning medical students of our institute were observed where the loop pattern was found to be slightly higher in males than females by 3%(66 versus 63), while the arch pattern was high in the female by 2%(4versus 2).

Table 3: Distribution of Fingerprint Patterns on Different Fingers.

FPP	RT	RI	RM	RR	RL	LT	LI	LM	LR	LL	TOTAL
LOOPS	181	176	217	168	233	184	176	203	166	240	1944
WHORLS	105	107	65	124	65	102	101	73	127	55	924
ARCHES	13	16	18	8	3	14	23	23	7	5	129

This study results showed a preponderance of loop pattern on little fingers(LL 240(80%) and RL 233 (77.66%), whorls on ring fingers (LR127 (42.3%) and RR 124(41.3%), and arches on LI and LM finger23 (7.6% each) in both hands and both sexes.

Table 4: Students' Fingerprint patterns similarity with father

FPP	RT	RI	RM	RR	RL	LT	LI	LM	LR	LL	TOTAL
LOOPS	44	37	58	34	64	43	39	49	36	64	468(46.8%)
WHORLS	21	26	12	25	12	20	21	17	26	7	187(18.7%)
ARCHES	0	0	1	0	0	1	1	0	0	1	4(0.4%)
TOTAL	65	63	71	59	76	64	61	66	62	72	659
%	65	63	71	59	76	64	61	66	62	72	65.9%

Students show similar fingerprint pattern with those of their father's fingers in 659 fingers, 468 as matching loop patterns, 187 as a whorl, and 4 arch pattern types and the percent of similarity was 65.9% with higher similarity in the RL 76% and LL72% fingers. . The non-significant p-value was found in the right index, left index, left middle and left little fingers while other fingers show significant p-value. All ten fingers had a positive correlation with the father's finger.

Table 5 : Students' fingerprint patterns similarity with mother

FPP	RT	RI	RM	RR	RL	LT	LI	LM	LR	LL	TOTAL
LOOPS	43	47	59	40	68	41	42	53	40	67	500(50%)
WHORLS	35	17	10	27	10	19	19	14	25	7	183(18.3%)
ARCHES	0	0	0	0	0	0	1	1	0	0	2(0.2%)
TOTAL	78	64	69	67	78	60	62	68	65	74	685
%	78	64	69	67	78	60	62	68	65	74	68.5%

Students show similar fingerprint patterns as that of their mother in 685 fingers, 500 as matching loop patterns, 183 as a whorl, and 2 arch pattern types and the percent of similarity was 68.5% with higher similarity in the RT78% and RL finger 78%. The non-significant p-value was found in the right middle, left thumb, left index, left middle, and left little fingers while other fingers show significant p-value. All the ten fingers had a positive correlation with the mother's fingers.

Table 6: Fathers and mothers fingerprint patterns similarity

FPP	RT	RI	RM	RR	RL	LT	LI	LM	LR	LL	TOTAL
LOOPS	34	36	49	36	64	46	38	47	35	70	455(45.5%)
WHORLS	11	14	7	18	8	15	14	9	24	5	125(12.5%)
ARCHES	0	0	1	0	1	0	0	0	0	2	2(0.2%)
TOTAL	45	50	57	54	73	61	52	56	59	77	584
%	45	50	57	54	73	61	52	56	59	77	58.4%

Fathers and mothers groups unrelated genetically when observed among themselves for similarity in fingerprint pattern showed similarity in 584 fingers, 455 as matching loop patterns, 125 as a whorl, and 4 arch pattern types and the percent of similarity was 58.4% with higher similarity in the LL77% and RL73% fingers. There was no significant correlation in all fingers except in the LL finger, and negative correlation in RT, RM, and LM fingers. Therefore, the present results showed that fingerprint patterns do not 100% match one of the parents in all populations; nevertheless, there is a similarity between offspring and their parents.

Discussion

Percentage of distribution of fingerprints

Keeping the aim of the study in mind it was found that the most commonly occurring fingerprint pattern type in overall fingers of all groups was the loop pattern in 64.8% of observations followed by whorl pattern in 30.8% and the arch pattern in 4.3%, which is quite similar to the prevalence reported by other authors as 60-70% loops,30-35%whorls and 5-15% arches.[9,10]

Percentage of distribution of fingerprints in medical students.

The overall preponderance of loops among medical students in our institute is (63.7%) followed by whorls (32.5%) and arches 38 (3.8%) which is as per that reported in other studies involving medical students. [11,12] Study conducted by Prateek R et al [13]shows (32.55%) whorls amongst medical students similar to our study. A study conducted by Desai B et al.[14], shows a similar distribution of loop pattern(64.6%) and whorl pattern (29.6%) just like worldwide distribution, whereas studies conducted by Sandeep V et al.[15]showed 24.34% of whorls and Sangita S Babu et al.[16] noticed 23.8% of whorls which is lesser number of whorls than worldwide distribution. Nagraj et al.[17], interestingly found equal numbers whorls and arches (both 14%) in their study, as compared to 64% of loops.

Sex distributions of various fingerprints

On analyzing the distribution of fingerprint patterns in either sex, the females were found to have a high percentage of loops (68%and 66%in mothers and female students respectively) in comparison to males (62.6% and 63%in fathers and male medical students respectively).

Contrary to the loops pattern whorls were found in high percentage in males (32% and 33% in fathers and male medical students respectively)than females (27.4%and 32%in mothers and female students respectively). Furthermore, the arch pattern was high in males (4.7% and 4%in fathers and male medical students respectively)compared to females (4.4%and 2%in mothers and female students respectively). This was found to be agreed with Bhavana, et al.[18]and also with Rastogi and Pillai et al[19] whose study of fingerprints about gender and blood group found that males have a higher incidence of whorls and females have a higher incidence of loops.

Therefore, loops were the predominant pattern in both genders, followed by whorls. The less common pattern in both genders was arch. This was found to be

agreed with Sam Rema et al.[20] who found the same result in the study of fingerprint patterns in the South Indian population.

Distributions of various fingerprints in different fingers

This study results matched the results of earlier studies conducted by Kanchan et al.,[11] and Mehta AA et al.[21] and demonstrated a preponderance of loop pattern on little fingers(80% in LL fingers and 77.66% in RL fingers)and whorl pattern on ring fingers (42.3% in LR and41.3% in RR fingers), However, arches pattern were observed with increased frequency in LI fingers (7.6%) and LM finger (7.6%) in both hands and both sexes.

The similarity in the distribution of fingerprints of students with their parents

On the similarity of fingerprint pattern between offspring and their parents, The percentage of matching fingers pattern type with father or mother were calculated and aggregated. Finally, the p-value and correlation between offspring fingerprint pattern types and their parents were calculated. We found that the similarity between offspring and their parents had a significant p-value in some fingers and non-significant in others:

Students show similar fingerprint patterns with fathers in 65.9% fingers and with mothers in 68.9% of fingers with matching loop pattern in maximum numbers(46.8%with father and 50% with mothers), indicating loop pattern in offspring fingers had the highest matching tendency to his/her parent. Furthermore, on analyzing the data it was found that whorl pattern was matching with fathers in 18.7% of fingers and with mothers in 18.3% of fingers which is consistent with the earlier study performed by Herman M. Slatis et al[22]. Arches were the least matching patterns with only 0.4% fingers matching with fathers and 0.2% with mothers indicating that it shows less similarity between siblings and parents, which affect the general p-value, we observed that when we exclude arch patterns the significance of the p-value increased. All ten fingers had a positive correlation with the fingers of both the parents.

When we compare the relation between fingers pattern type of genetically unrelated parents with each other it was found to be matching in only 58.4% of fingers with the maximum matching of loop pattern in 45.5% of fingers followed by whorls in 12.5% and least matching arches in only 0.4% of fingers. There was no significant correlation in all fingers except in the left little finger, and negative correlation in right thumb, right middle, and left middle fingers. Therefore, the present results showed that fingerprint patterns do not 100% match one of the parents in all populations; nevertheless, there is a similarity between offspring and their parents.

Fingerprint pattern types were similar to one of the parents more than non-related people according to this study and this agrees with Jacob B. Adler et al[23] who found that there is indeed a relationship between fingerprints of people in a family. All of the groups of related people had more features in common than non-related people.

The results of this study should be followed by other studies to find out more correlation within the family fingerprint-like inheritance of fingerprint pattern type, the relation between pattern types and other fingerprint details (delta, type line etc.), and twins fingerprint patterns similarity.

Conclusion

On the base of this study, we conclude that:

●The most common fingerprint pattern among MBBS graduates of our institute is the loop followed by whorl while arch was found to be the least fingerprint which was also the pattern observed in their parents as well.

●The loop pattern and the arch pattern were higher in female medical graduates, while the whorl Pattern was higher in male medical graduates just like in their mothers and fathers.

●Loops were found more in little fingers and whorls in ring fingers in medical graduates which is also a similar pattern in their parents.

● Loop patterns in medical graduates had the highest similarity percentage for their parents.

● The arch pattern of medical graduates does not match to their parents.

●Fingerprint patterns do not 100% match one of the

parents in all populations and MBBS graduates of our institute were no different justifying that fingerprints are unique to every individual.

●The similarity between fingerprints was more in genetically related groups (parents and students) than between father and mother group which were genetically unrelated.

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A Descriptive Study to Assess the Self-care Attitude and Practice among Nurses from Selected Hospitals of Pune City

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Abstract

Introduction: To learn Healthy coping strategy should be a part of self-care development for the nursing staff and the student nurses. It will encourage health and is also a fundamental value for the scope and standard of practice. The attitude of all the nursing different some are favorable and some are not favorable towards the positive attitude for the self-care. Some also denote that the attitude should be positive or negative. Attitude is one the essential part for nursing education[7,5,3]. **Objectives of the Study:** 1.To assess the self-care attitude among nurses from selected hospitals of Pune City.2. To assess the self-care practice among nurses from selected hospitals of Pune City. **Methodology:** Quantitative research approach was adopted for this study to accomplish the objectives of the study. **Result:** The analysis of the study suggests that nurses have positive attitude about their self-care in regards to spiritual wellness, Mental wellness, Emotional wellness, Nutritional wellness, Social wellness, Intellectual wellness,Physical wellness .But the practice regarding the self-care is very poor among nurses .which an important point to note and the researcher suggest that there should be proper awareness and practice regrading self-care among nurses.

Key Words: *Assess, Self-care, Attitude, Practice*

Introduction

Self-care practice is an professional anticipation which is to be underlying for the role of nurses in the clinical practice.

The importance of self-care for nursing staff should be included in the standards of practice of a clinical nurse. Various research studies shows the importance of self-care for all the nurses but the evidences shows that it is not been acknowledged[3].

Due to there is degrading in the implication for the development of standards of nursing practice across the globe and also creates a loophole in the existing standards.

To learn Healthy coping strategy should be a part of self-care development for the nursing staff and the student nurses. It will encourage health and is also a fundamental value for the scope and standard of practice[7].

The attitude of all the nursing different some are favorable and some are not favorable towards the positive attitude for the self-care. Some also denote that the attitude should be positive or negative[3]

.Attitude is one the essential part for nursing education.

It promote nursing strategies which may impact on their self-care .the self-care management is very rarely studied in nursing students. Because there clinical practice, professional identity if being focused more.

Statement of the Problem

A Descriptive study to assess the self-care attitude and practice among nurses from selected hospitals of Pune City

Objectives:

1.To assess the self-care attitude among nurses from selected hospitals of Pune City.

2. To assess the self-care practice among nurses from selected hospitals of Pune City.

Methodology

Research Approach

Quantitative research approach was adopted for this study to accomplish the objectives of the study.

Research Design

In this study, Non-experimental Descriptive survey design was used.

Variables.

Independent Variables:

The independent variable for the study was attitude and practice

Dependent Variables:

The dependent variables of the study was self-care.

SETTING OF THE STUDY

The investigator conducted the study in urban area of Pune City.

POPULATION OF THE STUDY

SAMPLE AND SAMPLE SIZE

In the present study, the nursing staff working in various hospitals of Pune City. Who fulfilled the inclusive criteria were the samples.

SAMPLE SIZE

The sample size was 194 .

SAMLING TECHNIQUES

Non probability convenience sampling technique was used to select the samples.

DEVELOPMENT AND DESCRIPTION OF THE TOOL

The instrument was developed and complied by the investigator with the guidance of experts and review of literature. The data collection questionnaire used in the present study had the following components:

Section A: Assessment of background variables

· Demographic variables of the nurse**:**

This included study participants age, religion, type of family, monthly income in rupees, educational status.

Section B: Structured Question to assess the self-care attitude among nurses .

Section C: Observational Checklist on self-care practice among nurses.

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CONTENT VALIDITY

Content validity of instrument was done by the panel of experts in the fields of Nursing Research and Statistics. The experts' suggestions were incorporated in designing the final tool for this study.

RELIABILITY

Reliability of the tool was measured using Karl's Pearson coefficient of correlation' test retest method for, **Structured Question to assess the** self-care attitude among nurses and **Observational Checklist on** self-care practice among nurses. And inter rater method for perceived practice.

The reliability r value were 0.78. These values were very high thus making it a reliable tool for assessing the self-care attitude and practice among nurses.

DATA ANALYSIS AND INTERPRETATION

Demographic Data

Table 1: Description of Demographic data in terms of Frequency and percentage distribution

N=194

SR	Demographic variable		Freq	%
1	Age	20 – 30 years	70	26.4
		31 – 40 years	85	32.1
		41 – 50 years	50	18.9
		51 and above	60	22.6
2	Gender	Male	112	42.3
		Female	153	57.7
3	Family	Nuclear family	160	60.4
		Joint family	96	36.2
4	Religion	Hindu	155	60.5
		Muslim	51	19.9
		Sikh	20	7.8
		Christian	10	3.9
		Other	20	7.8
5	Monthly family income in rupees	10-30 k/Month	60	23.4
		above 30 K/month	40	15.6
		Below 30 K/Month	155	60.5
7	Educational Status	GNM	50	19.5
		B.Sc Nursing	70	27.3
		P.B.BSc Nursing	80	31.3
		M.Sc Nursing	25	9.8

Table 2: Description of self-care attitude among nurses from selected hospitals of Pune City

N=194

Items		Mean	Std. Deviation	Scaled Response	Verbal Interpretation
Spiritual Assessment	Knows importance of life	3.81	0.495	Every Time	Positive
	Believes that some power is greater than myself	3.68	0.556	Every Time	Positive
	Believes on the perspective that life has value, meaning, and direction	3.49	0.655	Every Time	Positive
	Feel a part of life and living frequently	3.47	0.584	Every Time	Positive
	Knows that there is some level of connection with power	3.43	0.542	Every Time	Positive
	Recognize that there are different roles of my life are an expression of my true self	3.38	0.61	Every Time	Positive
	Know how to create a balance and feel a sense of connectedness	3.34	0.731	Every Time	Positive
Items		Mean	Std. Deviation	Scaled Response	Verbal Interpretation
Mental Assessment	Enjoy developing new skills and Talents	3.55	0.544	Every Time	Positive
	I am interested in and knowledgeable about many topics	3.4	0.577	Every Time	Positive
	I ask for suggestions and help when I need it	3.38	0.644	Every Time	Positive
	I am receptive to new ideas and life Patterns	3.38	0.573	Every Time	Positive
	Use my imaginations in considering new choices or possibilities	3.36	0.705	Every Time	Positive
	Prioritize my work and set realistic goals	3.23	0.598	Almost Every Time	Positive
	Read a broad range of subjects	3.02	0.707	Almost Every Time	Positive

Cont... **Table 2: Description of self-care attitude among nurses from selected hospitals of Pune City**

N=194

Items		Mean	Std. Deviation	Scaled Response	Verbal Interpretation
Emotional Assessment	Listen to and respect the feeling of others	3.62	0.61	Every Time	Positive
	Listen to inner self-talk	3.47	0.584	Every Time	Positive
	Assess and recognize my own feelings	3.4	0.825	Every Time	Positive
	Can remember and acknowledge most events of my childhood including painful as well as happy	3.38	0.573	Every Time	Positive
Nutritional Assessment	All meal should be taken on time	3.62	0.61	Every Time	Positive
	Balanced diet is important to maintain.	3.47	0.584	Every Time	Positive
	Maintains the regular water intake	3.4	0.825	Every Time	Positive
	Healthy and oil free snack is preferred in the mid-day snacks	3.38	0.573	Every Time	Positive
Social Wellness	Developing friendships	3.47	0.584	Every Time	Positive
	Healthy sexual behaviors	3.4	0.825	Every Time	Positive
	Ability to interact comfortably with others.	3.38	0.573	Every Time	Positive
Intellectual Wellness	Strong desire to learn from challenges and experiences.	3.62	0.61	Every Time	Positive
	uses the available resources, in and out of the classroom, to expand their knowledge and skills for the betterment of society.	3.47	0.584	Every Time	Positive
Physical Wellness	Involves regular physical activities	3.62	0.61	Every Time	Positive
	Proper nutrition and health care	3.47	0.584	Every Time	Positive
	Exercise or Sports	3.4	0.825	Every Time	Positive
	Personal hygiene.	3.38	0.573	Every Time	Positive

Table 2 : Describes the selfcare attitude among nurses from selected hospitals of Pune City. The first component related to the attitude of nurses is there attitude related to spiritual assessment, In regards to spiritual assessment, knows importance of life, Believes that some power is greater than myself, Believes on the perspective that life has value, meaning, and direction, Feel a part of life and living frequently, Knows that there is some level of connection with power,Recognize that there are different roles of my life are an expression of my true self, Know how to create a balance and feel a sense of connectedness and the result reflected that the nurses had positive attitude related to the spiritual assessment.

Analysis related to the mental assessment had covered the following parameters Enjoy developing new skills and Talents, I am interested in and knowledgeable about many topics, I ask for suggestions and help when I need it, I am receptive to new ideas and life Patterns, Use my imaginations in considering new choices or possibilities, Prioritize my work and set realistic goals, Read a broad range of subjects the result reflected that the nurses had positive attitude related to the mental assessment on selfcare.

Analysis related to the emotional assessment had covered the following parameters Listen to and respect the feeling of others, Listen to inner self-talk, Assess and recognize my own feelings, Can remember and acknowledge most events of my childhood including painful as well as happy result reflected that the nurses had positive attitude related to the emotional assessment on selfcare.

Analysis related to the nutritional assessment had covered the following parameters All meal should be taken on time, Balanced diet is important to maintain, Maintains the regular water intake, Healthy and oil free snack is preferred in the mid-day snacks, result reflected that the nurses had positive attitude related to the nutritional assessment on selfcare.

Analysis related to the social assessment had covered the following parameters Developing friendships, Healthy sexual behaviours, Ability to interact comfortably with others, result reflected that the nurses had positive attitude related to the social assessment on selfcare.

Analysis related to the Intellectual assessment had covered the following parameters Strong desire to learn from challenges and experiences, uses the available resources, in and out of the classroom, to expand their knowledge and skills for the betterment of society, result reflected that the nurses had positive attitude related to the intellectual assessment on selfcare.

Analysis related to the Physical assessment had covered the following parameters Involves regular physical activities, Proper nutrition and health care, Exercise or Sports, Personal hygiene, result reflected that the nurses had positive attitude related to the intellectual assessment on selfcare.

Table 3: Description of self-care practice among nurses from selected hospitals of Pune City"

N= 194

Self Care Practise		Very Good		Good		Poor	
		Freq	%	Freq	%	Freq	%
1	Spiritual Wellness	60	23.44	76	29.69	120	46.88
2	Mental/Psychology Wellness	19	7.42188	85	33.20313	150	58.5938
3	Emotional Wellness	56	21.875	70	27.3438	130	50.7813
4	Nutritional Wellness	26	10.1563	40	15.625	190	74.2188
5	Social wellness	75	29.2969	70	27.3438	111	43.3594
6	Intellectual Wellness	76	29.6875	100	39.0625	80	31.25
7	Physical Wellness	19	7.42188	85	33.20313	150	58.5938

Table 3 :Describes the self -care practise among nurses reflects that the spiritual wellness practise was performed in very good manner by 23.44 % nurses ,Mental wellness was practised by 7.4 % nurses ,Emotional wellness was performed by 21.8 % nurses, Nutritional wellness was 26 % in nurses ,Social wellness was 29 % among nurses ,Intellectual wellness was 29.6 % in nurses and Physical wellness was 7.4 % among nurses.

Conclusion

The above analysis of the study suggests that nurses have positive attitude about there self care in regrdas to spiritual wellness,Mental wellness,Emotional wellness,Nutritional wellness ,Social wellness,Intellectual wellness ,Physical wellness .But the practice regrading the self care is very poor among nurses .which an important point to note and the researcher suggest that there should be proper awareness and practice regrading self-care among nurses.

Recommendations

The study recommends the following

· A similar study may be conducted with large number of sample and population in various settings.

· A similar study may be conducted with large number of sample and population in various settings.

· A comparative study can be conducted between rural and urban population.

· A true experimental study with experimental and control group can be conducted.

Conflict of Interest – Nil

Source of Funding- Authors have no financial support to this project. The study was fully funded by authors.

Ethical Clearance – Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the professional included in the study.Informed the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential

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A Exploratory Study to Assess the Food Choices among College Students of Pune City

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Abstract

Introduction: Students studying in college make poor dietary choices which causes significant health problems to their health. Various students have their meals in the dining facilities of college were they have limited healthy food options.[1,2,3] If the students will not gain the adequate nutritional on daily basis this will be effect there academic and physical performance. The purpose of this study was to identify the food choices opted by the college students. **Objectives of the Study:** To assess the food choices among college students of Pune City.2. To associate the selected demographic variables with the food choices among college students of Pune City. **Methodology:** Quantitative research approach was adopted for this study to accomplish the objectives of the study. In this study, Non-experimental Descriptive survey design was used. The investigator conducted the study in urban area of Pune City. In the present study, the college students of Pune City. Who fulfilled the inclusive criteria were the samples. The sample size was 221. **Result:** The result of the above study reveals that the college students have and habit of consuming fast food during their lunch meal. During breakfast most of them take beverages and do not prefer fast food and majority of them almost take the full meal .It can be identified that they eat only one health meal during their dinner which is not enough for the students to fulfill their nutritional requirement of the day. Many students eat fast food and also there consumption of fast food was also very high and most of them eat this fast food during their lunch meal which is not good for their health. Future research and awareness should be created among college students to make them aware about the healthy eating habits and food which can be implemented in their diet in daily meals

Key Words: *Assess, food choices, College students.*

Introduction

Balanced diet is very vital component for marinating good health and wellbeing .Energy, protein, essential fats, vitamins and minerals are all essentials required for the growth and development of the health of students.

To provide right quantity of nutrients for a good health we require wide variety of foods .Students health as well academics sis effective if the nutrition is inadequate.

Students studying in college make poor dietary choices which causes significant health problems to their health. Various students have their meals in the dining facilities of college were they have limited healthy food options[1,2].

If the students will not gain the adequate nutritional on daily basis this will be effect there academic and physical performance.

The purpose of this study was to identify the food choices opted by the college students.

The behavior of eating is an important aspect of life because it will affect long term health outcomes,

Because if you have unhealthy habits like deficient nutrition food, meals skipping and lack of time for are main reasons which leads to unhealthy food choices ,health problem and nutritional deficiency

A well balanced diet and the also consumption of quality food can help to sustain the physical well-being and mental stability of students[3,4].

Likewise, It is understood that healthy diet play very significant role in the life of a college students which is one of the largest population were we can target to control health problems .The eating patterns and food choices are challenging for students residing away from home as they face various difficulties in new environment[1,2,3,4,].

STATEMENT OF THE PROBLEM

"A Exploratory study to assess the food choices among college students of Pune City"

Objectives:

1.To assess the food choices among college students of Pune City.

2. To associate the selected demographic variables with the food choices among college students of Pune City.

Methodology

RESEARCH APPROACH

Quantitative research approach was adopted for this study to accomplish the objectives of the study.

RESEARCH DESIGN

In this study, Non-experimental Descriptive survey design was used.

VARIABLES.

Independent Variables:

The independent variable for the study was college students

Dependent Variables:

The dependent variables of the study was food choices

SETTING OF THE STUDY

The investigator conducted the study in urban area of Pune City.

POPULATION OF THE STUDY

SAMPLE AND SAMPLE SIZE

In the present study, the college students of Pune City. Who fulfilled the inclusive criteria were the samples.

SAMPLE SIZE

The sample size was 221.

SAMLING TECHNIQUES

Non probability convenience sampling technique was used to select the samples.

DEVELOPMENT AND DESCRIPTION OF THE TOOL

The instrument was developed and complied by the investigator with the guidance of experts and review of literature. The data collection questionnaire used in the present study had the following components:

Section A: Assessment of background variables

· Demographic variables of the nurse**:**

This included study participants age, religion, type of family, monthly income in rupees, educational status, Stream of education

Section B: Structured Question to assess the food choices among college going students.

CONTENT VALIDITY

Content validity of instrument was done by the panel of experts in the fields of Nursing Research and Statistics. The experts' suggestions were incorporated in designing the final tool for this study.

RELIABILITY

Reliability of the tool was measured using Karl's Pearson coefficient of correlation' test retest method for, **Structured Question to assess the food** choices among college going students. Inter rater method for perceived practice.

The reliability r value were 0.78. These values were very high thus making it a reliable tool for assessing the food choices among college going students

DATA ANALYSIS AND INTERPRETATION

DEMOGRAPHIC DATA

Table 1: Description of Demographic data in terms of Frequency and percentage distribution

N=221

SR	Demographic variable		Freq	%
1	Age	20 – 30 years	70	26.4
		31 – 40 years	85	32.1
		41 – 50 years	50	18.9
		51 and above	60	22.6
2	Gender	Male	112	42.3
		Female	153	57.7
3	Family	Nuclear family	160	60.4
		Joint family	96	36.2
4	Religion	Hindu	155	60.5
		Muslim	51	19.9
		Sikh	20	7.8
		Christian	10	3.9
		Other	20	7.8
5	Monthly family income in rupees	10-30 k/Month	60	23.4
		above 30 K/month	40	15.6
		Below 30 K/Month	155	60.5
7	Educational Status			
		Graduation	70	27.3
		Post-Graduation	80	31.3

Table 2: Description of food choices of students in Breakfast among college students of Pune City.

N=221

Sr.No	Food items	Always		Sometimes		Never	
Break fast							
		Frequency	**%**	**Frequency**	**%**	**Frequency**	**%**
1	Poha	9	22	20	50	11	27.5
2	Allo paratha	11	27	25	62.5	4	10
3	Sandwich	14	35	17.5	22.5	9	22.5
4	Puff	13	32.5	20	50	7	17.5
5	Dosa	21	52.5	11	27.5	8	20
6	Chappati bhaji	9	22.5	16	40	15	37.5
7	Fruits	13	32.5	22	55	5	12.5
8	Rice	15	37.5	18	45	7	17.5
9	Idli samber	13	32.5	23	57.5	4	10
10	Upma	17	45.5	19	45.5	4	10
11	Thepla	15	37.5	17	42.5	8	20
12	Noodles	8	20	15	37.5	17	42.5
13	Samosa	10	25	23	57.5	7	17.5
14	Pulses	14	35	21	52.5	5	12.5
15	Vegetable	13	32.5	20	50	7	17.5
16	Milk	14	35	18	45	8	20
17	Tea	15	37.5	16	40	9	22.5
18	Coffee	14	35	20	50	6	15
19	Juice	14	35	18	45	8	20
Lunch							
1	Fruits	12	25	10	25	20	50
2	Manchurian rice	21	52.5	20	50	3	7.5

Cont... **Table 2: Description of food choices of students in Breakfast among college students of Pune City.**

N=221

3	Pizza	16	40	18	45	4	10
4	Sandwich	14	35	17	42.5	9	22.5
5	Pasta	14	35	18	45	8	20
6	Manchurian Noodles	16	40	20	50	0	0
7	Samosa	11	27.5	25	62.5	5	12.5
8	Dosa	18	45	19	47.5	3	7.5
9	Meat & Roti	3	7.5	5	12.5	32	80
10	Egg & Rice	4	10	8	20	28	70
11	Fish & Rice	1	2.5	5	12.5	34	85
12	Kachori	8	20	18	45	14	35
13	Frankie	15	25	15	37.5	10	25
14	Pastry	12	30	10	25	3	7.5
15	Pav	15	37.5	16	40	9	22.5
16	Samosa chat	18	45	15	37.5	7	17.5
17	Bhelpuri	10	25	20	50	8	20
18	Halva	18	45	20	50	2	5
19	Spring roll	18	45	17	42.5	5	12.5
20	French fries	15	37.5	17	42.5	8	20
21	Burger	20	50	10	25	10	25
22	Hotdog	18	45	18	45	4	10
23	Chips	14	35	17	42.5	9	22.5
24	Panipuri	13	32.5	17	42.5	10	25
25	Pakora	5	12.5	24	60	11	27.5
26	Cakes	12	30	20	50	8	20
27	Bread toast	15	37.5	18	45	7	17.5

28	Coffee	14	3	19	45.5	7	17.5
29	Cold drinks	7	17.5	25	62.5	8	20
30	Fruit juice	16	40	18	45	6	15
31	Full meal (dal, roti or puri, sabji, rice, curry, sweet, salad, buttermilk)	10	25	15	37.5	15	37.5
32	Half meal (roti or puri sabji or dal rice and buttermilk)	5	12.5	10	25	25	62.5
33	Biriyani and curd	10	25	18	45	12	30
34	Paratha and curd	10	25	15	37.5	15	37.5
Dinner							
1	Meat & roti	0	0	5	12.5	35	87.5
2	Fish & rice	0	0	3	7.5	37	92.5
3	Egg & rice	8	20	15	37.5	17	42.5
4	Friedrice	10	25	27	67.5	3	7.5
5	Noodles	15	37.5	23	57.5	2	5
6	Pizza	10	25	25	62.5	5	12.5
7	Burger	10	25	22	55	8	20
8	Sandwich	13	32.5	23	57.5	4	10
9	Pasta	12	30	22	55	6	15
10	Khakra	8	20	22	55	10	25
11	Frankie	8	20	25	62.5	7	17.5
12	Milk	13	32.5	12	30	5	12.5
13	Full meal (dal,roti,sabji,rice,curry, sweet,salad, buttermilk)	20	50	10	25	10	25
14	Half meal (roti sabji or dal rice)	5	12.5	15	37.5	20	50

Table 2 Describes about the breakfast intake choices 52.5% of the students always consume in their breakfast, 62.5% students consumes allo paratha ,45% of the students eat upma, 35% of them eat sandwiches, 37.5% of them preferred to have tea during breakfast and other 35% of them consumes coffee, juice and milk.

Lunch intake choices result says that 52.5% of the students always consumes Manchurian rice in their lunch and 60% of them consumes pakoda and 50% of them consumes burger and 45% of them consumes french-fries, Frankie, Kachori, spring roll, Manchurian noodles during their lunch time. Almost 62.5% and 25% of them were found that they consume cold drinks and 37.5 5 of students always take full meal. Also 45% of them sometimes consumes biriyani with curd and paratha with curd. Remaining 12.5% of them take half meal in their lunch.

Dinner intake choices result says 50% of the students always take full meal and 12.5% of them consumes half meal. Also 25% of the students always consumes noodles while 57% of them takes it sometimes, and 20% of them always consumes french-fries.

Table 3: Description of the association of Monthly family income in rupees with the food choices among college students. N=221

Food items		Monthly family income			p value
		10-30 k/Month	above 30 K/month	Below 30 K/Month	
Break fast	Poha	9	20	11	0.003
	Allo paratha	11	25	4	
	Sandwich	14	17.5	9	
	Puff	13	20	7	
	Dosa	21	11	8	
	Chappati bhaji	9	16	15	
	Fruits	13	22	5	
	Rice	15	18	7	
	Idli samber	13	23	4	
	Upma	17	19	4	
	Thepla	15	17	8	
	Noodles	8	15	17	
	Samosa	10	23	7	
	Pulses	14	21	5	
	Vegetable	13	20	7	
	Milk	14	18	8	
	Tea	15	16	9	
	Coffee	14	20	6	
	Juice	14	18	8	
Lunch	Fruits	12	10	20	0.001
	Manchurian rice	21	20	3	
	Pizza	16	18	4	
	Sandwich	14	17	9	
	Pasta	14	18	8	
	Manchurian Noodles	16	20	0	
	Samosa	11	25	5	
	Dosa	18	19	3	

Cont... Table 3: Description of the association of Monthly family income in rupees with the food choices among college students. N=221

	Meat & Roti	3	5	32	
	Egg & Rice	4	8	28	
	Fish & Rice	1	5	34	
	Kachori	8	18	14	
	Frankie	15	15	10	
	Pastry	12	10	3	
	Pav	15	16	9	
	Samosa chat	18	15	7	
	Bhelpuri	10	20	8	
	Halva	18	20	2	
	Spring roll	18	17	5	
	French fries	15	17	8	
	Burger	20	10	10	
	Hotdog	18	18	4	
	Chips	14	17	9	
	Panipuri	13	17	10	
	Pakora	5	24	11	
	Cakes	12	20	8	
	Bread toast	15	18	7	
	Coffee	14	19	7	
	Cold drinks	7	25	8	
	Fruit juice	16	18	6	
	Full meal (dal, roti or puri, sabji, rice, curry, sweet, salad, buttermilk)	10	15	15	
	Half meal (roti or puri sabji or dal rice and buttermilk)	5	10	25	
	Biriyani and curd	10	18	12	

Cont... **Table 3: Description of the association of Monthly family income in rupees with the food choices among college students. N=221**

	Paratha and curd	10	15	15	
Dinner	Meat & roti	0	5	35	0.02
	Fish & rice	0	3	37	
	Egg & rice	8	15	17	
	Friedrice	10	27	3	
	Noodles	15	23	2	
	Pizza	10	25	5	
	Burger	10	22	8	
	Sandwich	13	23	4	
	Pasta	12	22	6	
	Khakra	8	22	10	
	Frankie	8	25	7	
	Milk	13	12	5	
	Full meal (dal,roti,sabji,rice,curry,	20	10	10	
	sweet,salad, buttermilk)				
	Half meal (roti sabji	5	15	20	
	or dal rice)				

In proceeding to find out the association of demographic data income with the food choices among college students. The result says that there is significant association between the monthly family income and food choices made by students as the p values is less than 0.005

Conclusion

The result of the above study reveals that the college students have and habit of consuming fast food during their lunch meal. During breakfast most of them take beverages and do not prefer fast food and majority of them almost take the full meal .It can be identified that they eat only one health meal during their dinner which is not enough for the students to fulfill there nutritional requirement of the day. Many students eat fast food and also there consumption of fast food was also very high and most of them eat this fast food during their lunch meal which is not good for their health. Future research and awareness should be created among college students to make them aware about the healthy eating habits and food which can be implemented in their diet in daily meals

Recommendations

The study recommends the following

· A similar study may be conducted with large number of sample and population in various settings.

· A similar study may be conducted with large number of sample and population in various settings.

· A comparative study can be conducted between rural and urban population.

· A true experimental study with experimental and control group can be conducted.

Conflict of Interest – Nil

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Molecular Identification of epsA and ompA genes for *Acintobacter baumannii* Isolated from Burn Wounds

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Abstract

This study was designed to molecular detection of *epsA* and *ompA* genes for *Acinetobacter baumannii* Isolates that isolated from clinical. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from Ghazi Al Hariri Hospital and Baghdad hospital in Baghdad / Iraq. The results of the current study showed that seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %). From 70 samples inoculated on the Chromagar Acinetobacter, 10 isolate were grown on the medium, *A. baumannii* isolates on the Chromagar appeared as bright red colonies after 24h and incubation at 37 C . The results of *epsA* and *ompA* genes detection clarify that 8 isolate (80%) of *A. baumannii* isolates gave a positive result and carrying *epsA* and *ompA* genes while 2 (20%) of *Acintobacter baumannii* isolates were lack the gene and no cross reactivity could be observed with any of the AS-3 and AS-9 isolates. The identification of *A. baumannii* isolates were depended on culturing these isolates on CHROM agar Acinetobacter, MacConkey, blood, nutrient agar, biochemical tests and API NE 20 test VITEK 2 . Compact were carried out the confirms. Results showed the amplification of *epsA* and *ompA* genes for eight. *A. baumannii* isolates were AS-1, AS-2, AS-4, AS-5, AS-6, AS-7, AS-8 and AS-10. PCR product 531 bp of *ompA* gene and 451 bp of *epsA* gene.

Keywords: *Virulence genes, OmpA, EpsA, Acinetobacter baumannii*

Introduction

Acinetobacter baumannii is the most prevalent infection-causing organism in the hospital environment. *Acinetobacter baumannii* causes hospital acquired infections, such as ventilator-associated pneumonia, bacteremia, urinary tract infections, meningitis, and surgical wound infections [(1)] . *Acinetobacter baumannii* is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile bacteria; it has a high incidence among immuncommpromised *Acintobacter baumannii* is referred to as ‘’Iraqibacter’’ due to its seemingly sudden emergence in military treatment facilities during the Iraq War . *Acintobacter baumannii* produces high molecular weight capsular polysaccharide which surrounds the outer membrane [(2)]. Capsular polysaccharide forms a discrete layer on the bacterial surface providing protection from diverse environmental conditions, assisting in evasion of host immune defenses, and increasing resistance to a number of antimicrobial compounds [(3)]. *Acintobacter baumannii* is capable of entering and persisting inside host cells, it adheres to host cells, then translocate into nucleus. After killing host cells, it disseminates in bloodstream and tissues [(4)]. As a class of important virulence factors in bacteria, outer membrane proteins (OMPs) have attracted much more attention. Among those OMPs of *A. baumannii*, *OmpA* is the most deeply studied virulence factor which plays key roles in regulating the adhesion, aggressiveness, and biofilm formation of *A. baumannii* and immune response of host. Other virulence factors such as exopolysacharide also have an important role in the invasion of these bacteria . *Acinetobacter baumannii* produces a polysaccharide export outer membrane protein, called exopolysacharide or EPS, which is encoded by EpsA. EPS accumulates on the cell surface and provides protection to the cells against the harsh external environment [(5)] . The present work was aimed to molecular detection of some virulence genes (*OmpA* Gene and *EpsA* Gene) of *Acinetobacter* baumannii isolates

Materials and Methods

Sample collection

This study was conducted during the period from December 2019 to April 2020, During the study period, eighty clinical burn wounds swabs samples were taken. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from *Ghazi Al Hariri Hospital* (40 samples) and Baghdad hospital (40 samples) in Baghdad / Iraq.

Biochemical test

Biochemical test as Oxidase, Indole, Methyl red, Lactose fermentation, Citrate utilization, Catalase production and Urease test. were performed on the isolates to confirm their identification *Acintobacter baumannii*. All the tests were according to [6] .

API (Analytical Profile Index)

This test is used to identify *Acintobacter baumannii* bacterium, also used to determine and differentiate several types of Gram-negative bacteria .

Identification Using Vitek 2 System

The VITEK 2 is an automated microbiology system utilizing growth-based technology, according to the manufacturer's instructions (BioMerieux-France).

Molecular study

DNA Extraction

Genomic DNA was isolated from bacterial growth according to the protocol of ABIOpure . Primer sets used are shown in table (1) .

Table (1): Primer sets used in the present study

Primer name	Sequence	Annealing Temp. °C	Product Size (bp)	
epsA-F	5`-AGCAAGTGGTTATCCAATCG-3`	50	415	415
epsA-R	5`-ACCAGACTCACCCATTACAT-3`			
ompA-F	5`-CGCTTCTGCTGGTGCTGAAT-3`		531	531
ompA-R	5`-CGTGCAGTAGCGTTAGGGTA-3`			

Quantitation of DNA

Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5 min. incubation at room temperature, DNA concentration values were detected.

Polymerase chain reaction to check the quality of DNA

Polymerase chain reaction master mix was prepared (with final volume 20 µl per one reaction) containing 10µM forward and reverse primers, 2 X of master mix, and 5µl of nuclease-free water was added until the volume reached to 17 µl. Then, 3 µl of DNA was added to mixture .

Results and Discussion

According to table (2) , Seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %).

Table (2): Total number of burn wounds samples used for the isolation of bacteria

Clinical wounds samples	Positive (growth)	Negative (no growth)
80	70	10
Percentage	87.5%	12.5 %

The increasing body of literature on this subject suggested that *Acinetobacter* infections occur mainly in four patient populations and settings . At least 25% of healthy individuals may carry *Acinetobacters* as part of their normal skin flora, but carriage of *Acinetobacter* spp. By healthy subjects at other body site is normally low, in contrast, high colonization rate of the throat, skin, respiratory tract or digestive tract of hospitalized patients with clinically significant strains have been reported during outbreaks of infection [7] .

The majority of infections caused by *A. baumannii* are contracted in hospitals, most often in critically ill patients hospitalized in intensive care or surgery *Acintobacter baumannii* can resist dehydration, detergents, UV radiation and common chemical sanitizers, making it extremely difficult to eradicate [8] .

The emergence of multidrug resistance *A. baumannii* and carbapenam-resistant *A. baumannii* have cause hospital outbreaks and threat in treatment due to its wide variety of resistance mechanisms and high survival rate on a biotic surfaces [9] .

A. baumannii traumatic wound infections have become a topic of recent interest with reports of increasing incidences of outbreaks among victims of combat injuries and natural disasters . Recent data from Iraq and Afghanistan identify highly resistant strains of *A. baumannii* to be some of the most common organisms causing severe and often lethal wound infections [10] .

A. baumannii infection occurs when the immunological barriers of the host are breached (e.g. mechanical ventilation) and is hence considered an opportunistic pathogen . *A. baumannii* causes various types infections complicating burn wounds [11] .

From 70 samples inoculated on the Chromagar *Acinetobacter*, 10 isolate grew on the medium . CHROMagar Acinetobacter is a recently developed medium for selective and rapid identification of *Acinetobacter* spp. [12] .

Acinetobacter baumannii is rod-shaped which grows well on MacConkey agar (without salt). Although officially classified as not lactose-fermenting ,they are often partially lactose-fermenting when grown on MacConkey agar . Growth and purity of cultures of *Acinetobacter baumannii* were determined by culture on MacConkey agar and Blood agar. On MacConkey agar it's formed pale coloured, Non lactose fermenting colonies and on Blood Agar it's formed non-hemolytic colonies. There was only one type of colonies attesting to its purity [13] .

Ten isolates of *A. baumannii* grow at 44°C were positive on blood agar medium after 24 hrs of incubation. A appropriate temperature for the growth of most *Acinetobacter* spp were 37ºC whereas *A. baumannii* can grow well at high a temperature of 44 °C [14] .

The suspected all isolates of *A. baumannii* and were then subjected to the related biochemical tests. All isolates of *A. baumannii* were found to be catalase positive and oxidase / indole negative. Tests on Lactose fermentation and motility test gave negative results. The positive results for the test appeared in methyl red and Citrate utilization, finally we used the urease test which give negative results for *Acintobacter baumannii* . The isolates were identified as *A. baumannii* and have been confirmed by using Api 20 and VITEK 2 Compact, figure (1) .

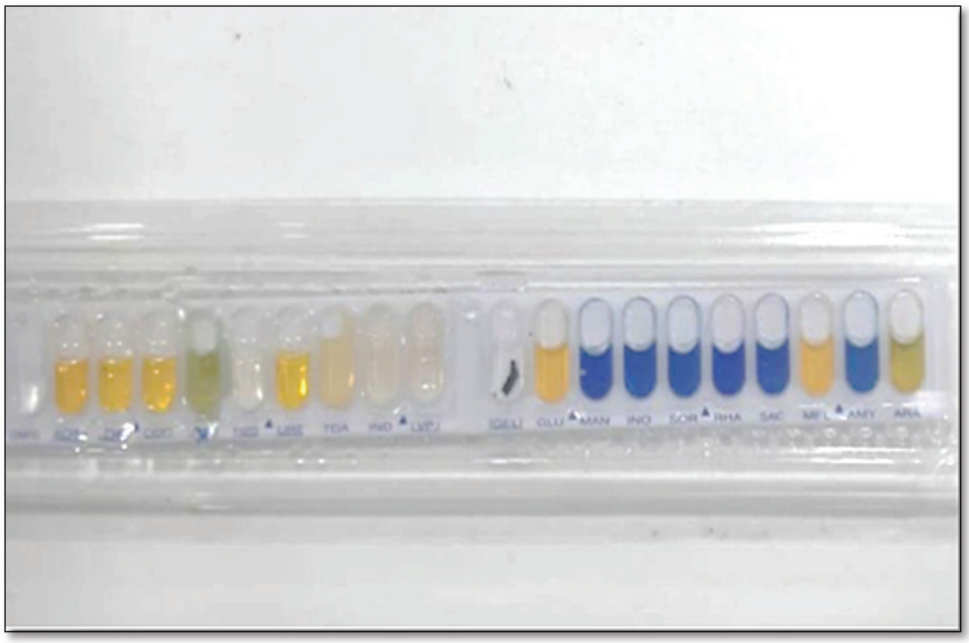

Figure. 1 : API test for identification of *Acintobacter bumannii*.

Acinetobacter was identified as non motile, oxidase negative and catalase positive. *Acinetobacter baumannii* is positive for citrate. Citrate in simmon citrate medium is important to detect weather the bacteria isolates able to grow on it as a unique carbon and energy source [15] .

Non-lactose fermenting and non-motile of *Acinetobacter baumannii* isolates [16] . Urease test was positive for *Acinitobacter baumannii* urease enzyme catalyzes the breakdown of urea, and the bacteria that can produce this enzyme is able to detoxify the waste products and to drive metabolic energy from its utilization which change the medium color from yellow to purple-pink, indicating urease positive test [6] .

Acinetobacter baumannii isolates were oxidase negative and catalase positive [17] . API NE 20 test VITEK 2 Compact were carried out confirms the biochemical tests regarding the species identification of the *A.baumannii* isolates. results showed that all 30 isolates were identified as *A.baumannii*, as shown in Figure (4). API 20 NE is a standardized system for the identification of non-fastidious, non-enteric Gramnegative rods, combining 8 conventional tests which include potassium nitrate (NO3), L-tryptophane (TRP), D-glucose (GLU), L-arginine (ADH), urea (URE), esculin ferric citrate (ESC), gelatin (GEL), and 4-nitrophenyl-β-D-galactopyranoside (PNPG), and also the 12 assimilation tests which include Dglucose (GLU), L-arabinose (ARA), D-mannose (MNE), D-mannitol (MAN), N-acetyl-glucosamine (NAG), D-maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malic acid (MLT), trisodium citrate (CIT), and the phenylacetic acid PAC [18] .

Genomic DNA was extracted from the most 10 isolates of *Acintobacter baumannii* using genomic DNA kit. Figure (2). The result shows that the recorded range of DNA concentration was 50 ng / µl and the DNA purity was 1.8-2.0.

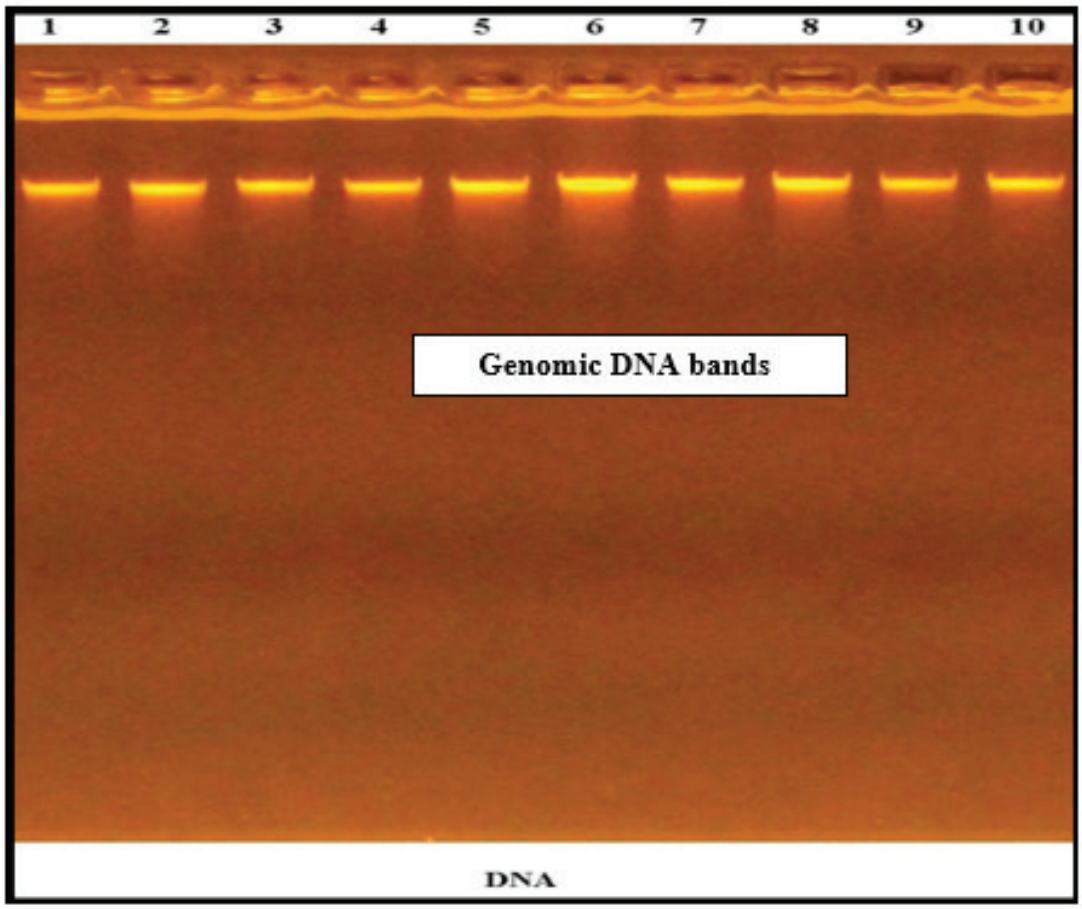

Figure. 2 : Genomic DNA bands extracted from *A. baumannii* isolates on (1%) agarose,75V, for 30 min stained with ethidium bromide. Lane 1-10: DNA of Lane.

The results of *epsA* and *ompA* genes detection clarify that 8 isolate (80%) of *A. baumannii* isolates gave a positive result and carrying *epsA* and *ompA* genes while 2 (20%) of *Acintobacter baumannii* isolates were lack the gene and no cross reactivity could be observed with any of the AS-3 and AS-9 isolates.

The results showed the amplification of *epsA* and *ompA* genes for eight *Acintobacter baumannii* isolates were AS-1, AS-2, AS-4, AS-5, AS-6, AS-7, AS-8 and AS-10. PCR product 531 bp of *ompA* gene figure (3) and 451 bp of *epsA* gene figure (4). The infection symptoms caused by *Acintobacter baumannii* are closely associated with its virulence factor. But the virulence genes distribution in clinical *A. baumannii* was rarely reported, except for *bap, omp* 33-36 and *traT* [19] .

This study examined the distribution of two virulence genes (*epsA* and *ompA* genes) in clinically collected *A. baumannii* isolates. The molecular investigation was performed to detect the amplification plots of the target genes and reference gene in order to find the threshold cycle value for each them as in figures (6) and (7).

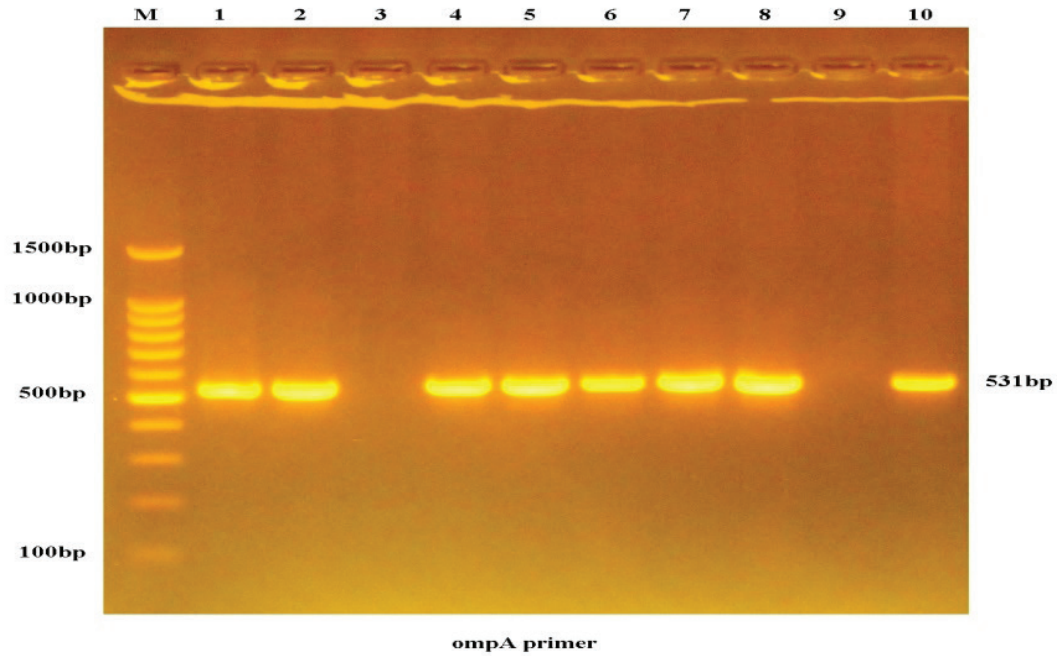

Figure. 3 : Results of the amplification of *ompA gene* of *Acinetobacter baumannii* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 531bp PCR products.

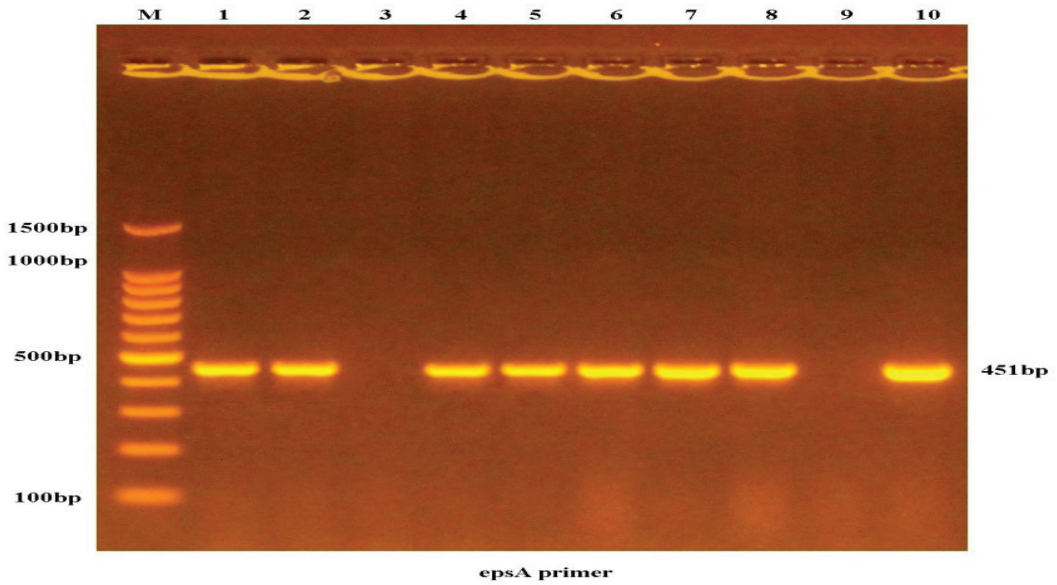

Figure. 4 : Results of the amplification of *epsA gene* of *Acinetobacter baumannii* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 451bp PCR products.

Several virulence factors involved in bioflm formation of *A. baumannii* such as the outer membrane protein A (*OmpA*), biofilm associated protein (*Bap*)chaperon-usher pilus (*Csu*), extracellular exopolysaccharide (*EPS*). The *OmpA* of *A. baumannii* is essential for the attachment to human epithelial cells, development of biofilms and antimicrobial resistance [20] .

Previous results showed that all *A. baumannii* isolates carried at least one biofilm related gene. The most prevalent gene was *csuE* (100%), followed by *pgaB* (98%), *epsA* and *ptk* (95%), *bfmS* (92%) and *ompA* (81%). 98% of isolates carried more than 4 biofilm related genes, simultaneously [21] .

Ethical Clearance: Taken from Al-Nahrain University ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Assess the Effectiveness of Self-Instruction Module on Knowledge Regarding Nipah Virus Infection and Its Prevention among General Population

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Abstract

Introduction: NipahVirus infection is a newly appear animal disease that develop a serious disease in both animal group and human being. NiV is a contagious agent which develops the serious illnesses by the Nipah (genus Henipavirus). NiV may be spread from animal (e.g. bats or pigs) or infected foodstuffs to man and may also be spread directly from mans to mans. Case mortality levels are reported at 40% to 75%, based on epidemiological monitoring and clinical intervention. This incidence can vary by epidemic. A self-instruction module on knowledge regarding Nipah virus infection and its prevention among general population helps to improve and update the knowledge regarding Nipah virus infection. Hence the investigator felt the need to develop a self instruction module for preventing Nipah virus infection.

Aim: To determine the effectiveness of self instruction module on knowledge regarding Nipah virus infection and its prevention among general population.

Materials and Methods: A quasi-experimental research design was used to conduct this study. Non probability convenient sampling technique used to collect data from general population on the basis of structured knowledge questioner. After collecting pretest data, self-instructional module was given for intervention on knowledge regarding Nipah virus infection and its prevention among general population. Seven days were given to the samples for utilizing self instructional module which was organized for 45-50 minutes. Post-test information was gathered after seven days from the day of intervention. The sample characteristics were described by frequency, percentage and t-test was used to describe the difference between pre-test and post-test knowledge score. Chi-square test was also used to find out the association between knowledge of general population regarding Nipah virus infection and its prevention with selected demographic variables.

Result: The findings showed that in pretest 112 (93.33 %) general population had average knowledge, 08(6.67%) general population had good knowledge, whereas post test 7(5.8 %) had good knowledge, 113 (94.17%) had excellent knowledge.

Conclusion: Nipah Virus is a newly emerging animal disease that leads to a serious disease in both animals and humans. Nipah virus may be transmitted to mans from animals (e.g. bats or pigs) or infected foods, and may also be transmitted directly from mans to mans so as this intervention will helpful to aware the general population to prevent Nipah virus infection and its complication.

Key word: *efficacy, general public, Nipah virus infection, self learning module, skill.*

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Introduction

Nipah Virus is a newly emerging animal disease that causes a serious disease both species as well as

in humans. Nipah Virus Infection (NiV), an infectious agent that has caused significant illnesses in humans and animals caused by Nipah (genus Henipavirus). [1]

Nipah virus may be communicated to mans from animals (e.g. bats or pigs) or infected foods, and may also be communicated directly from mans to mans. The mortality rate of cases is estimated at 40% to 75%. This rate may different by outbreak on the basis of epidemiological monitoring and clinical management. Infection with Nipah virus in humans causes a range of clinical occurrences; from asymptomatic (subclinical) infection to acute respiratory illness and fatal encephalitis. The Pteropodidae family of fruit bats are the natural hosts of the Nipah virus. Newly, no vaccine had been discovered for humans or animals. Strictly supportive care is the essential treatment for human cases .The 2018 annual review of the WHO R&D Blueprint list of priority diseases indicates that accelerated research and development on the Nipah virus is needed urgently. [2]

The earliest moment was found in fruit bats of the Pteropodidae family, Pteropus genus, i.e. besides natural hosts of the virus [3] and NiV was mainly discovered and acquired during a disease eruption in Kampung Sungai Nipah,Malaysia village in 1998 to 1999 Wherever the pig farmers get ill with encephalitis. In the case, the intermediate hosts were pigs. But, in later NiV outbreaks no intermediate hosts were present. In Bangladesh, the peoples got contaminated with NiV because of consuming date palm sap contaminated by infected fruit bats in 2004. Man-to man transmission had also been reported, including by the hospital situation in India. Out of a 582 human cases infected by Nipah virus, 54% were lethal.[4, 3]

Nipah virus infected humans has a variety of medical presentations, ranging from asymptomatic disease to the acute respiratory problems and fatal encephalitis. Pigs and other domestic animals also may infected by Nipah virus. Nipah virus is placed at "top of the list" and explores 10 priority diseases recognized by the World Health Organization as potentials for the next major outbreak.[5]

Nipah virus has caused only a few recorded outbreaks in Asian it infects a wide variety of species and causes serious disease and death in humans, thereby making it a public health concern. [1]

This virus was also identified and isolated during the outbreak of encephalitis among pig breeders and people with close contact with virus infected pigs in the following year. But shockingly this outbreak triggered a fairly mild disease in pigs, but almost 300 people were infected by NiV and more than 100 deaths were reported for the first time. No subsequent cases (in neither swine nor human) have been recorded in Malaysia or Singapore since this outbreak.[6] Study showed that the association of NiV to Hendra virus, bat species was quickly handpicked for investigation and flying foxes of the genus Pteropus were later identified as NiV reservoir.[7]

In India NiV outburst reported in 2001 and 2007 in West Bengal with symptoms of acute respiratory distress associated with febrile illness and/or neurological manifestations causing 50 deaths out of 71 cases (70% mortality rate). All fatal cases were identified to be NiV positive. In 2007 outbreak, a clump of bats were seen hanging from the trees around a patient's home.[8] The 2018 again there was an outbreak of the NiV in the Kerala pointed out its origin to fruit bats in that area specifically localized in the districts of Kozhikode and Malappuram districts which declared 17 lives.[9] This is the third outburst reported in India.[9] More than 2,000 people in these two districts were isolated and kept under observation during the period of the outburst.[10]

The epidemicity of NiV is distinct in different countries. In the 1998 outbreak in Malaysia, first the pigs were infected by the virus perhaps after consuming fruit contaminated with bat saliva.[11] After spreading widely on pig farms, the virus began advancing to humans who came in contact with the animals. As a result around 300 people fell ill but no person-to-person transmission seems to have occurred in Malaysia, unlike in Kerala.[12]

Human infections vary from asymptomatic infections to acute respiratory illness (mild, severe), and fatal encephalitis. Initially those people who having infection may develop symptoms like fever, headaches, muscle ache, nausea, vomiting and pharyngitis followed by unsteadiness, , somnolence, impaired consciousness, and symptoms of neurology suggesting acute encephalitis;. Some people may also develop atypical pneumonia and extreme respiratory illness, such as acute respiratory distress. In extreme cases, inflammation of brain and epilepsy occur, and progress to coma

within 24 to 48 hours. The incubation time is from 4 to 14 days. However incubation time of as long as 45 days is reported. The majority of people who survive acute inflammation of brain make a full recovery, but long term neurologic conditions have been reported in survivors. About 20 % of patients are left with residual neurological symptoms such as seizure disorder and changes in personality. A small number of people who recover subsequently relapse or develop delayed onset encephalitis.[2]

The mortality rate of cases is estimated at 40% to 75%.This rate may different by outbreak depending on epidemiological surveillance and clinical management. [1]

Newly, no vaccines discovered for Nipah virus. In 1999 outbreak of Nipah acquired involving pig farms as for preventing infection routinely thorough cleaning and disinfecting the pig farms with appropriate detergents can be effective.

If an outburst is suspected, the animal house should be immediately separated. Contaminated animals should be destroying – with close supervision of funeral or incineration of cadaver – may be important to reduce the risk of transmission to people. Strictly restricting the movement of animals from contaminated farms to other areas may reduce spread of infection.

In the absence of a vaccine, the only way to reduce or prevent infection in people is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the Nipah virus.

Newly, no vaccines discovered for Nipah virus so awareness program regarding risk factors and heath education regarding preventive measures can prevent the infection in people.

Before consumption fruits should be washed thourouly and fresh palm date juice should be boil for preventing bat to human transmission. Close unprotected physical contact should be avoided while giving care to the Nipah virus infected patients. Regularly hand washing is necessary after and before caring of sick people for preventing human to human transmission. While handling suspected or confirmed patients health team member should follow the standard infection control measures all time.[2]

While there is no vaccine that can avoid the spread of Nipah Virus infection, certain steps can also be taken to prevent it. Farmers with domesticated animals can avoid their animals from eating fruits that are vulnerable to bat infection, such as date palms. Individuals should also avoid eating date palms close to the areas affected by the bat. It is important to prevent close interaction with pigs and bats. In order to cope with NiV contaminated patients, hospitals should establish appropriate facilities so that it cannot be further spread to other individual. People in health care should wear gloves and high-quality masks. [13]

Research study showed that 17 cases were confirmed, the case fatality rate was 82%. 10 patients had a history of close contact with horses or of horse meat consumption. Deaths of 10 horses were reported in the same time period, of which nine showed neurological symptoms. However, samples from horses were not tested for NiV. Five patients, including two healthcare personnel, acquired the disease through person to person transmission. [14]

Research study concluded that the case-fatality varies from 40% to 70% depending on the severity of the patient's condition, such as encephalitis and availability of adequate healthcare facilities. Till now no antiviral drug available for Nipah virus disease and the treatment is just supportive. NiV infection can be considered an emerging disease and a public health problem as a consequence of the lack of effective vaccines and therapies and of the evidence that NiV can infect pigs.[15]

Aim of the study was to determine the effectiveness of self instruction module on knowledge regarding Nipah virus infection and its prevention among general population.

Assumption:-

General population may have some knowledge regarding Nipah virus infection and its prevention.

Hypothesis:-

H1: There will be significant difference between pre and post-test knowledge scores of general population regarding Nipah virus infection and its prevention.

H0: There will be no significant difference between pre and post-test knowledge scores of general population regarding Nipah virus infection and its prevention.

Materials and Methods: - A pre-experimental pre-test, post-test research design was used to conduct this study. The data gathering method began from July 2018 to December 2018 and the setting was selected in the Sawangi (M)Wardha (Maharashtra) after getting ethical permission.(Ref. no: DMIMS(DU)/IEC/2018-19/7306). By using a convenient sampling technique, 120 general populations were selected. General populations were informed and explained the objective of the study. The written informed consent dully signed individually by them was obtained. The inclusion criteria, (i)those who were willing to participate in the study.(ii)Those who were aged between 18-more than 58 years, (iii)those will be able to read and understand Marathi and (iv)included Male and female general population. (i)Those who had participated in similar type of research and (ii) those who were not available when the data was collected were excluded from the study. Demographic variables were collected in terms of age, gender, education, occupation and residence. A structured questionnaire, which is attached in Annexure 1, has 20 multiple choice questions was used. Each sample required mean time of 30 minutes to complete the pre test structured questionnaire. Then the self instructional module was intervened to the sample. The post test structured questionnaire was administered after 7 days. Based on the 20 questions each study participant was asked individually for his / her answers with the same questionnaire. As collected, the responses were arranged in tabular form to conduct statistical analyzes which are mentioned in the following sections.

Statistical Analysis

The collected data were coded, tabulated, and analyzed by using descriptive statistics (mean percentage, standard deviation) and inferential statistics. Significance difference between pre and posttest readings was tested by using a t-test; association of knowledge with demographic variables was done by one way ANOVA test and independent t-test. For statistical analysis SPSS version 16.0 was used.

Result

Table 1: Knowledge regarding nipah virus infection and its prevention. Pre test

n=120

Level of knowledge score	Score Range	Percentage score	Pre test	
			Frequency	Percentage %
Poor	1-5	1-25	00	00
Average	6-10	26-50	112	93.33
Good	11-15	51-75	08	6.67
Excellent	16-20	76-100	00	00
Minimum score			6	
Maximum score			13	
Mean satisfaction score			8.68 ±1.402	

The above table shows that 93.33% sample shows average score, and 6.67 % had good level of satisfaction score. Minimum satisfaction score was 6 and maximum satisfaction score was 13.Mean satisfaction score was 8.68 ±1.402.

Table 2: Knowledge regarding nipah virus infection and its prevention

Post test n=120

Level of knowledge score	Score Range	Percentage score	Post test	
			Frequency	Percentage %
Poor	1-5	1-25	00	00
Average	6-10	26-50	00	00
Good	11-15	51-75	07	5.8
Excellent	16-20	76-100	113	94.17
Minimum score			14	
Maximum score			19	
Mean satisfaction score			17.30±1.089	

The above table shows that 5.8% sample shows that good score, and 94.17 had excellant level of satisfaction score .Minimum satisfaction score in was 14 and maximum satisfaction score was 19.Mean satisfaction score was 17.30±1.089.

Table 3: Percentage wise distribution of effectiveness of self instructional module on knowledge regarding nipah virus infection and its prevention among general population.

Test	Mean	SD	t- Value	Degree of freedom	p- value	significan
Pre test	8.68	±1.402	67.84	119	0.000	S,P< 0.05
Post test	17.30	±1.089				

The table no.3 shows that there is a significant difference between pretest and post test knowledge score interpreting effective self instructional module on knowledge regarding nipah virus infection and its prevention among general population**.** Mean value of pretest is 8.68 and post test is 17.30 and standard deviation value of pretest ±1.402 and post test ±1.089. The calculated t value is 67.84 and p- value is 0.000. Hence it statistically interpreted that the self instruction module on knowledge regarding nipah virus infection and its prevention among general population.

Discussion

Researcher concluded that Nipah Virus outbreaks

are seen mainly in Asian countries. The effect has also been widely seen in India. Depending on the extent of the infection, the mortality rate varies from 35 to 65 %. The mortality rate attributed to the Nipah Virus 2018 The outburst crossed 17 in the Kerala district of India and separated persons associated with untreated patients, thus avoiding the dissemination of disease. So far there has been no vaccine or treatment created that can treat this lethal illness, but the study continues. Preventive steps should be taken in areas vulnerable to infection, and close contact with fruits that could be infected by fruit bats should be avoided for domesticated animals.[13]

In 2014, in two villages on Mindanao, an island in the Philippines, the Philippines National Epidemiology Center received a survey on human deaths. Additional human deaths and nonfatal illnesses with concurrent neurological illness, and unexplained deaths of some horses were reported through an epidemic investigation. 17 people fulfilled the meaning of the case by (11 with encephalitis, 5 with influenza-like illness, and 1 with meningitis. For all agents, except for henipaviruses, testing for a number of neurotropic pathogens was negative. Neutralizing antibodies were also found in 3 patients against NiV and IgM against NiV.[16]

Limitation:-

The study was limited to sample size i.e. 120, which might be inadequate to generalize the study findings. More time duration would give more relevant results with variations of any research, but the investigator planned to complete the research work within one week to get more feasibility of getting sample. Therefore, sufficient number of sample and time duration was required to establish the effect of structure instructional module, in general.

Conclusion

The study concluded that before intervention general population have some knowledge regarding nipah virus infection and its prevention but after intervention they improved their knowledge. So the self instructional module is proved to be improving their knowledge regarding nipah virus infection and its prevention.

Recommendations

- A research study may be conducted to assess knowledge and attitude regarding nipah virus infection among staff nurses.
- A research study may be compare in urban and rural area on knowledge regarding nipah virus infection and its prevention among general population.
- A research study may be conducted to assess knowledge regarding nipah virus infection and its prevention among staff nurses.
- A research study may be conducted to assess knowledge regarding nipah virus infection and its prevention among nursing students.

Ethical Clearance- Taken from ethical committee. Ref. no: DMIMS (DU)/IEC/2018-19/7306).

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Malpractice as a Leading Cause to Maternal Mortality in Egypt: A Postmortem Statistical Study

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Abstract

Background: Maternal deaths are quite common tragic event in developing countries where the main source of information on the causes of maternal death is clinical records and autopsies. Knowledge about the causes of these deaths are scarce in Egypt. Efforts to reduce maternal mortality in Egypt are mostly not driven by evidence. This autopsy study was performed to assess the cause of maternal mortality including malpractice, thus providing information which plays an important role in improving clinical practice in Egypt.

Methods: A retrospective, descriptive study was conducted at the Egyptian Forensic Authority (EFMA) in a 5-years perios between January 2008 and December 2012 to investigate maternal mortality cases alleged to be due to malpractice.

Results:. The total number of maternal deaths was 114 cases. The majority of cases (n=90, 79.0%) were negative concerning malpractice. Twenty-four deaths (21%) can be attributed to malpractice; 16 due to surgeon faults and 8 anesthesia-related deaths. The main cause of death was obstetric hemorrhage (n=46) followed by respiratory failure (n=30). Out of the 24 cases, 19 were proved to be due to negligence in the form of late intervention, improper preparation, unmanaged complication and unauthorized place of management.

Conclusion: Malpractice causes nearly one-fifth of alleged maternal deaths; the most frequenct type of which is negligence. Surgeon errors were double anesthesia related deaths. Hemorrhage was the most common cause of death in malpractice as well as incidental deaths. Quality of obstetric care in Egypt needs further improvement in the aspects of health facilities and care providers.

Key Words: *malpractice, maternal mortality, obstetric complications, cesarian section complications, anathesia complications, postpartum hemorrhage, MMR, MM.*

Backgrond

Globally, maternal deaths in 2015 were estimated to be 303 000, with a maternal death rate of 216 per 100 000 live births. In developing regions MMR was 239; about 20 times higher than that of developed regions. [1] According to the International Classification of Diseases (ICD-10), maternal death is defined as "death of a woman while pregnant or within 42 days of the end of pregnancy, irrespective of the duration and site of the pregnancy, from any cause related to or aggravated by the pregnancy or its management, but not from accidental or incidental causes".[2]

Despite long-lasting international commitments to reducing maternal mortality; as the Safe motherhood initiative, and the 5th Millennium Development Goal (MDG-5), the progress is unfortunately inadequate. Reducing maternal mortality (MM) is not just an issue of development, but also an issue of human rights.[3]

Obstetric complications are the leading causes of death among women of reproductive age, and for every woman who dies approximately 20 more suffer

injuries, infection and disabilities related to pregnancy or childbirth.[4] Most maternal deaths occur during the third trimester of pregnancy and up to the first week after the delivery.[5] Hemorrhage, hypertensive disorders, and sepsis are responsible for more than half of maternal deaths worldwide.[6]

Obstetrician-gynecologists (OB/GYNs) are frequently exposed to malpractice claims. During the past decade suggest, 74% of physicians practicing obstetrics and gynecology will face a malpractice claim.[7] It was found that litigation claims for obstetrics and gynecology procedures have higher average insurance payments than most medical specialties, and most frequently relate to gynecologic surgery.[8]

Almost all cases of maternal mortality are preventable. An estimated 74% of maternal deaths could be prevented if women had access to the interventions for prevention and treatment of pregnancy and delivery complications, especially emergency obstetric care.[9]

In developing countries the main source of information on the causes of maternal death is clinical records and autopsies.[10] Likewise, in Egypt, efforts to reduce maternal mortality are not driven by evidence.[11] According to available information, obstetric complications, including malpractice, are the most frequent cause of maternal deaths referred to The EFMA. Rupture uterus, puerperal sepsis, sepsis following caesarean sections, and postpartum hemorrhage; though preventable; are frequently encountered.[11]

This autopsy study was performed to assess maternal mortalities alleged to be due to malpractice to evaluate the possible causative complications of caesarean and of vaginal deliveries. The ultimate goal is refining the accuracy of cause-of-death reports and improving clinical practice in Egyptian health systems caring for females in the childbearing period.

Materials and Methods

Case Selection:

This retrospective study was conducted at the Egyptian Forensic Authority (EFMA). It included all forensic cases referred for autopsy to the EFMA in a five-years period between January 2008 and December 2012. It included all deaths fulfilling the standard definition of the World Health Organization (WHO) for maternal deaths.[2] Therefore, the study included women dying during pregnancy or within 42 days of completion of a pregnancy, irrespective of the cause of death.

Autopsy:

Maternal deaths underwent a complete dissection with macroscopic evaluation of each organ using a standardized macroscopic protocol. Special emphasis was taken to detect air embolism, acute fatty liver of pregnancy or evidence suggestive of pulmonary or amniotic fluid embolism. Samples from all grossly identified lesions and of viscera; especially from the female genital tract were collected from each woman for histopathlogical study. These included basically: heart, lungs, brain, female genital tract. Kidneys, liver and gastrointestinal organs, if relevant, are sent according to each case. Then, a dissection and preservation of the organs in 10% formalin was done. Blood culture and culture of other specimens was done as indicated.

Histopathological study:

Whole organ samples were sent for the pathology department for histopathologic evaluation. The final diagnosis was established by two pathologists after reviewing the histological slides and the macroscopic and clinical protocols.

Final autopsy report:

All cases were classified into direct or indirect causes which included subgroups based on the primary pathology evident on autopsy. The direct causes included hemorrhagic disorders of pregnancy, pre-eclampsia/eclampsia, pregnancy-related hepatic disorders, amniotic fluid embolism, pulmonary embolism, abortion-related causes, puerperal sepsis, and intra-uterine fetal death (IUFD)-induced maternal deaths. The indirect causes of death were hepatic, pulmonary (excluding tuberculosis), neurological, cardiovascular, renal, hematological, gastrointestinal, malignancy and infectious disease. The different diagnoses were classified as: (a) Malpractice causing the death; (b) Non preventable causes of death.

Statistical Analysis

Statistical analysis was done using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY,

USA). Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. A p-value < 0.05 was considered significant.

Results

Between January 2008 and December 2012, the total number of mortality cases alleged to be due to malpractice was 114 cases; 29 (25.4%) in 2008, 16 (14.0%) in year 2009, 22 (19.3 %) in 2010, 13 (11.4 %) in 2011 and 34 (29.8%) in 2012.

The majority of deaths were recorded in 3rd and 4th decades of life. Most of the patients were living in urban areas; only 10 patients (8.8%) were from rural areas. The main bulk of patients died during the third trimester of pregnancy (n=102). Among them, one women with eclampsia died undelivered due to respiratory failure. More deliveries of the remaining 101 patients were by cesarean section (n=66, 65.3%). Only 6/35 of the vaginal deliveries (17.1%) were attended by a midwife.

Twenty-four women delivered vaginally with no further intervention. The remaining 90 women had surgical procedures; 10 hysterectomies and one laparotomy followed vaginal deliveries due to intractable postpartum bleeding. Nine of the 12 first and second trimester pregnancies had surgical evacuation and 3 had laparotomy for complicated evacuation. Most of the deaths occur within 24 hours of the initiating incident.

Hemorrhage was the main cause of death in the studied group (40.3%) followed by respiratory failure . Postpartum hemorrhage was the main type of bleeding.

Death due to malpractice was diagnosed in 24 women (21%); in 8 of them anesthetic complications were considered the cause of death (Figure 1). All of the anesthesia-related deaths were caused by respiratory failure or asphyxia and occurred during cesarean deliveries. In the surgical malpractice cases (n=16), the cause of death was hemorrhagic shock (n=13, 81.3%) and septicemia (n=3, 18.7%) (Table 1).

Table 4 shows a significant difference in the cause of death between malpractice cases and incidental deaths (p < 0.001). In malpractice group, NVD was the least commonly done procedure (4.2%), while it was done in almost one quarter of incidental deaths group. Also, laparotomy following delivery or surgical evacuations was more common in malpractice group (p < 0.001) (table 2). Malpractice was more common in 1st and 2nd trimester deaths (p = 0.001).The type of malpractice is shown in (table 3); late intervention was the most frequent type (29.2%) followed by unmanaged complication (16.7%).

There was no significant difference between malpractice and incidental deaths in the distribution among urban and rural residences (p = 0.932). As shown in (table 4), more than one third of malpractice deaths occur within one hour, while the majority of incidental deaths occur between 1 and 12 of the triggering incident (p = 0.009).

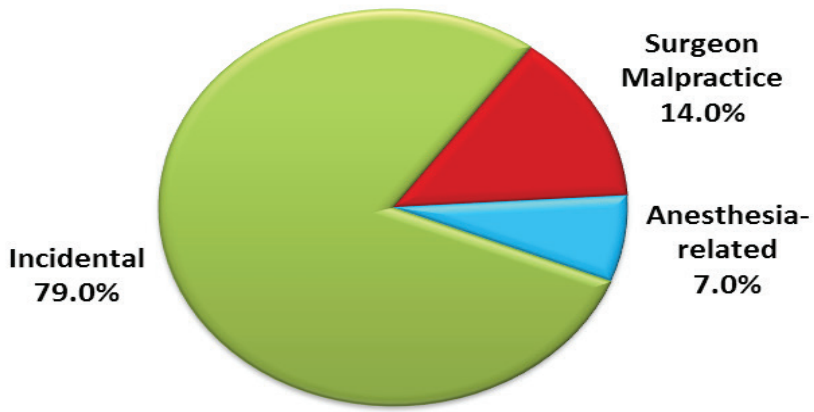

Figure 1: Classification of mortalities in the studied group

Table 1: Cause of death in relation to malpractice

Cause of death	Malpractice n=24	Incidental n=90
Hemorrhage	13 (54.1%)	33 (36.7%)
Respiratory failure	4 (16.7%)	26 (28.9%)
Septicemia	3 (12.5%)	9 (10.0%)
Asphyxia	4 (16.7%)	0 (0.0%)
Others	0 (0.0%)	22 (24.4%)

Table 2: Type of procedure done in in relation to malpractice

Procedure	Malpractice n=24	Incidental n=90
Surgical evacuation	6 (25.0%)	3 (3.3%)
Cesarean section only	10 (41.6%)	42 (46.6%)
Cesarean hysterectomy	3 (12.5%)	11 (12.2%)
Laparotomy after NVD/evacuation	4 (16.7%)	1 (1.1%)
NVD	1 (4.2%)	23 (25.6%)
Abdominal hysterectomy	0 (0.0%)	10 (11.2%)

Table 3: Type of malpractice diagnosed in the 24 diagnosed cases

	Frequency	Percentage
Late intervention	7	29.2%
Unmanaged complication	4	16.7%
Illegal abortion	3	12.5%
Improper preparation	2	8.3%
Anesthetic Overdose	2	8.3%
Unauthorized place of management	2	8.3%
Wrong procedure	2	8.3%
Combined Cause	2	8.3%

Table 4: The timing of death in relation to alleged malpractice

	Surgeon Malpractice n=16	**Anesthesia-related n=8**	**Incidental n=88**
Within 1 hour	3 (18.8%)	6 (75.0%)	16 (17.7%)
> 1 hour - 12 hours	4 (25.0%)	0 (0.0%)	35 (38.9%)
> 12 hours – 1 week	9 (25.0%)	2 (0.0%)	38 (26.7%)

The timing was not known in 2 women of the incidental death group

Discussion

Maternal mortality remains as a major Public Health challenge even with the various strategies developed by the international community to reduce it. For example, at the turn of this century, 189 countries signed up the Millennium Declaration to attain eight goals; one of which was to "*improve maternal health*". It aimed to reduce global maternal mortality by 75% in 2015.[12] Knowledge is a necessary step to solve any problem, therefore the analysis of maternal deaths is an essential instrument to improve obstetric care to avoid such a tragic event. It is essential to do periodical comparisons to detect changes and trends. This is the only way to improve and develop obstetrical protocols to save maternal lives.[13]

To save maternal lives is a fairly achievable goal as the majority of maternal mortality cases are preventable. In this context, the least responsibility of medical practitioners is averting malpractice which may represent a considerable part of mortality causes. This was the motive to perform the current study to assess the role of malpractice in maternal mortality in Egypt. The study encountered 114 deaths alleged to be caused by malpractice. It has been demonstrated that 24 deaths (21%) can be attributed to malpractice; 16 due to surgeon faults and 8 anesthesia-related deaths.

In a previous study, out of 108 pregnancy-related deaths; malpractice was implicated in 20.3% of these. However, they considered that these 22 cases were only among 41 preventable cases.[14] Similar report in Massachusetts reported that 41% cases of preventable maternal deaths were caused by care providers.[15]

The remaining 90 cases (79%) were due to medical mishaps; Intrapartum complications being responsible for near half of all maternal deaths. The leading cause of death was hemorrhage; it was the cause of 37% of incidental deaths and 54% of malpractice deaths.

Obstetric hemorrhage is one of the top three causes of maternal deaths worldwide.[16] It ranked 2 or 3 in USA, but in developing countries, hemorrhage usually comes on the top. The WHO statistics suggest that 60% of maternal deaths in developing countries are due to postpartum hemorrhage.[17] In Nigeria, severe hemorrhage (41.3%) was the cause of 41% of perinatal near misses.[18] Other studies confirmed that obstetric hemorrhage is the leading cause of maternal deaths.[19,20]

In a study in Singapore, Koh et al. stated that 75-90% of postpartum hemorrhage cases are caused by uterine atony.[21] Obstetric hemorrhage is considered a preventable cause of death if early recognition and treatment are started before signs of shock are more obvious.[22] Among common preventable errors are underestimation of blood loss, failure of timely blood volume restoration and decisive treatment of marked hypovolemia.[23]

The worst decision to manage postpartum bleeding was resorted to in about 12% of cases. It could not be determined if there was delay in deciding hysterectomy in the patients who died. It is impossible to know if the hemorrhage was in advanced stages when the decision

was made and if early invasive intervention could have protected the lives of patients.[24]

Medicolegal issues raised in the setting of obstetrical hemorrhage include failure to timely appreciate the amount of blood loss, failure to make a diagnosis, not recognizing delayed or concealed bleeding, delay in treating coagulopathy, and hysterectomy not considered or performed too late.[25]

In the current study, cesarean section was done in 64.7% of the 102 women who delivered in the third trimester. Fifteen of these 66 women (22.7%) proceeded to hysterectomy, with nearly equally proportion in incidental and malpractice cases. Cesarean delivery has been associated with maternal death in observational studies. Mechanisms of death include surgical hemorrhage, venous thromboembolism, perioperative infection, abnormal placentation in subsequent pregnancies, and anesthetic complications.[22] Nevertheless, a retrospective review of maternal deaths in the USA suggested that in 17% of cases, cesarean delivery may have potentially saved the mother's life.[26]

The decision to deliver by CS is sometimes stalled by fear of malpractice. In case of vaginal delivery, the doctor should prove that C.S would not have made any difference and should convince people that everything has been done.[27] Cyr noted that many OB/GYNs follow a "When in doubt, cut it out" philosophy. This encourages CS whenever the doctor has any concerns that a vaginal delivery may threaten the health of an infant or mother.[28]

Seven cases of hemorrhage in the current study complicated uterine tears. A ruptured uterus is a life-threatening obstetric complication of labor that contributes significantly to maternal and perinatal mortality. It is usually a result of poorly managed labor. It is associated with immediate complications, such as severe anemia, shock and a ruptured bladder. The prevalence of uterine rupture varies from country to country. While it is rare in high-income countries, it remains a common occurrence in low-income countries, particularly in Africa.[29]

The second most frequent cause of death was respiratory failure; affecting 30 cases. The majority of these cases had pulmonary embolism mainly thromboembolism. Only 3 cases showed trophoblastic emboli with signs of preeclampsia and one case had diffuse alveolar damage. Six cases died from amniotic fluid embolism.

The 2 types of embolism; thromboembolic disease and amniotic fluid embolism (AFE) are characterized by a sudden change in the anatomy and physiology of the patient. AFE usually presents as acute hypotension, hypoxia, cardiac arrest, and frequently with coagulopathy. Immediate therapy may prevent death, but not always. Malpractice issues that are raised include failure to recognize and diagnose in a timely manner and failure to promptly treat the embolism. However, in these cases, the risk of mortality is very high and even the best treatment may not be adequate.[25]

Twelve cases died from septicemia complicating therapeutic and illegal abortions, puerperal sepsis following vaginal delivery or cesarean sections in addition to one case following exploratory laparotomy. Four cases died from cardiac failure, two due to lymphocytic myocarditis and a case of chronic heart disease with tricuspid regurge. Cardiovascular disease complicates approximately 1-3% of pregnancies and is responsible for 10-15% of maternal mortality. Since more women with congenital and acquired heart disease reach fertile age due to improved medical care, the incidence of cardiovascular disease in pregnancy is increasing.[30]

In the current study, cases of asphyxia and neurogenic shock died less than an hour after the incident. On the other hand, the majority of cases of cardiac failure (66.67%), hemorrhage (52.78%) and respiratory failure (36.36%) died within 6 hours after the incident. The vast majority of anesthesia-related deaths occur within one hour, while surgeon malpractice cases occur mostly within 24 hours of the triggering incident.

In the current study, the majority of the cases (72.8%) died at or after the third trimester. Langlois et al. reported that maternal mortality mostly occurs within childbirth and the first week postpartum.[31] Ijadunola et al., stated that the postpartum period to account for the most maternal deaths.[32]

In the current study, 19 out of 24 cases proved to be considered due to negligence that was in the form of late intervention, Improper preparation, unmanaged complication and unauthorized place of management. Three cases were considered as malpractice due to the act of Illegal abortion. Three cases were attributed to wrong procedures; two wrong intubations and one wrong surgical procedure. Two cases died due to over dose of anesthetic medication in relation to mother's weight.

In fact, malpractice issues have been a major barrier to the provision of maternity care in the past decades. The problem has reached such crisis proportion that physicians are dropping obstetrics from their practices and hospitals are closing obstetric units, exacerbating the access problems for pregnant women.[33]

There is enough blame to go all around to everyone involved in medical malpractice. It is alleged that physicians are disregarding patient safety and have high rates of medical errors. Patients have become very litigious. However, we think that most filed malpractice suits represent medical mishaps with no accusation, as in most cases the patients were either late in seeking the medical care or there was no pregnancy follow up and the obstetrician was faced by a complicated labor without appropriate arrangements.

Conclusion

Malpractice causes nearly one-fifth of alleged maternal deaths; the most frequenct type of which is negligence. Surgeon errors were double anesthesia related deaths. Hemorrhage was the most common cause of death in malpractice as well as incidental deaths. Quality of obstetric care in Egypt needs further improvement in the aspects of health facilities and care providers.

Obstetrics is one of the highest litigated areas in medicine. It is also an area in which advances in technology have provided ways to reduce risk and improve outcomes while simultaneously giving rise to new legal and ethical concerns. It is vital to stay up-to-date of both the progressive science and the evolving case law associated with providing obstetrical care in Egypt.

Therefore, we recommend strengthening integrated health systems management and planning at district and provincial levels with improving district leadership, management, planning, and political engagement. Improving the functional capacity of health facilities for the delivery of routine maternity and emergency CS services are also crucially required.

Abbreviation:

ICD: international classification of disease

CS: cesserian section

MDG: Millennium Development Goalt

IUFD: intrauterine fetal death

WHO: world health organization

EFMA: Egyptian Forensic Authority

Ethical Clearance- Taken from Egyptian Forensic Authority committee

Source of Funding- Self

Conflict of Interest – no conflict of interest.

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Current Trends in Sudden Natural Deaths in South-Western Maharashtra: A Six Year Retrospective Study

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Abstract

Background: Sudden and unexpected nature of death is intriguing to the medical practitioner until a definite cause is found. Often a conclusive evidence of a natural cause is missing even after a thorough and elaborate autopsy. Material and methods: A six-year retrospective study of post mortem examinations conducted at a tertiary health care center to analyze the current trends in sudden deaths of natural cause. Results: Out of 1465 autopsies conducted ,40.60% cases were of sudden natural deaths with a male predominance (75.45%). Maximum number of natural deaths were found in the age group of 51 – 60 (19.28%) and least between 21 – 30 Years (1.98%). The most common system involved in these cases was Cardiovascular followed by Respiratory system. Conclusion: Analysis of causes of sudden natural deaths in various age groups garners epidemiological data and helps in prevention of hereditary, infective and metabolic disorders which form a majority of these deaths.

Key Words: *Sudden, Natural, Unexpected, Death, Forensic setting*

Introduction

Natural death means death occurring due to some natural disease or pathological condition, old age, debility or devitalization; here the death is not intended or attempted and also does not occur accidentally.[1] The World Health Organization (WHO) defines sudden death as death occurring within 24 hours of onset of symptoms. It also differentiates between instantaneous death, sudden death in infants, sudden cardiac death in the International Classification of Diseases and allotted codes accordingly.[2] Some authors however consider sudden death as an event occurring within one hour of onset of symptoms.

Whatever the time considered to define sudden death, the unexpected nature of death in an apparently healthy individual or the way the events have led to an abrupt end in a not so severe disease process intrigues the medical practitioner. And when these cases because of the sudden and unexpected nature of the event are brought to the medicolegal centers, it becomes the responsibility of the forensic pathologist to search for an answer. A thorough post mortem examination complete with investigations may lead to congenital, infectious, metabolic causes affecting various systems of the body. Often a negative autopsy might result which leaves no answer as to the cause of death. Deaths which may seem natural may turn out to be unnatural in origin after a thorough autopsy with a lurking evidence of injury or poison being discovered. The same was called 'Medicolegal Masquerades', the things are not always what they seem to be at first sight.[3]

The world's biggest killer remains ischemic heart disease accounting for 16% of deaths most of them sudden often followed by respiratory diseases.[4] Studies done by various authors have found sudden deaths in all age groups.Congenital causes, hereditary diseases form a majority of deaths in the children and young. Sudden infant death syndrome in less than a year-old child has been attributed to a variety of causes including infective,

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metabolic, genetic and accidental suffocation.[5,6] Infections especially with respiratory pathology are a major cause of sudden death in the young. [7] Sudden unexpected death in adolescents is often found to have a hereditary causation like in cardiomyopathies.[8] SUDEP refers to the sudden and unexpected death in a person with epilepsy who is otherwise well, whereby the death cannot be attributed to trauma, status epilepticus, toxins or drowning.[9] Very rarely environmental toxins lead to sudden deaths.

Evaluating a natural death in a forensic setting needs a systematic approach. A detailed medical history including family history, thorough postmortem examination with necessary ancillary studies would help in arriving at the cause. Some cases would need a scene investigation to differentiate from an unnatural death.[10] Lack of morphological evidence to the cause of death leading to a negative autopsy have prompted forensic pathologists to think about molecular analysis of a genetic cause of death .[11]

World Health Organization in its report on the top ten causes of death globally in 2019 says that it is important to know how people die to improve how people live.[4] Analyzing the current trends in the sudden natural deaths would help in the epidemiology, identifying the hereditary pattern of a disease thus alerting the family members. Knowing the severity of infectious and metabolic disorders which resulted in sudden unexpected death would help the medical practitioners to look into the preventable aspect of these diseases thus averting fatality in the future.

Material and Methods

This six-year autopsy based retrospective observational study was conducted in the Medicolegal center of a Tertiary Care hospital at South-Western Maharashtra. All cases of sudden natural deaths brought to mortuary and confirmed by autopsy between 01 January 2014 to 31 December 2019 were included in the study. Unnatural cases and those with pending reports were excluded.

Relevant data was obtained from history, inquest papers, postmortem reports, histopathological and toxicological analysis reports available at the center. The data obtained was studied and segregated using MS-Excel software and further analyzed using the same software.

Observation and Results

A total number of 1495 cases were received for autopsy at the center during the study period. Out of which 40.60 % (n=607) cases were of sudden natural deaths.

The most prone age group was found out to be 51 – 60 years with 117 cases (19.28%), followed by 61 – 70 years with 112 cases (18.45%). Least number of sudden natural deaths were between the age group of 11 – 20 years with only 12 cases (1.98%) compared to 35 cases (5.77%) in below 10 age group (Table 1).

Table 1. Distribution of Age

Age Group (in Yrs)	No. of cases	Percentage (%)
0 – 10	35	5.77
11 – 20	12	1.98
21 – 30	33	5.44
31 – 40	101	16.64
41 – 50	103	16.97
51 – 60	117	19.28
61 – 70	112	18.45
71 – 80	70	11.53
>80	24	3.95
TOTAL	607	100.00

Males constituted 75.45 % (n=458) while females were 24.55% (n=149), and the male to female ratio was 3.07:1 (Table 2).

Table 2. Distribution of Sex

Sex	No. of cases	Percentage (%)
Male	458	75.45
Female	149	24.55
Total	607	100.00
Ratio	Male: Female - 3.07: 1	

Out of 607 cases of natural deaths 42.01% (n=255) were already diagnosed with some premorbid condition related to death and data could not be retrieved in 2.47% (n=15) cases. (Table 3).

Table 3. Distribution of existence of Premorbid Conditions

Premorbid conditions	No. of cases	Percentage (%)
Yes	255	42.01
No	337	55.52
Not Known	15	2.47
TOTAL	607	100.00

Maximum number of deaths were documented on the morning hours between 0601 hours to 1200 hours (n=271, 44.65 %), followed by 1201 hours to 1800 hours (n=174, 28.67%) and least between 0001hours to 0600 hours (n=68, 11.20%) (Table 5).

Table 4. Distribution of Time of Declaration of Death

Time of Death	No. of cases	Percentage (%)
0001 - 0600 hrs	68	11.20
0601 - 1200 hrs	271	44.65
1201 - 1800 hrs	174	28.67
1801 - 0000 hrs	94	15.49
TOTAL	607	100.00

In most of the cases the death occurred at home (n=363, 59.8%) while 1.65% (n=10), cases died at workplaces (Table 5).

Table 5. Distribution of Place of Death

Place of Death	No. of Cases	Percentage (%)
Home / Residence	363	59.80
Work place	10	1.65
Enroute to hospital	76	12.52
Hospital	93	15.32
Outside residence	65	10.71
TOTAL	607	100.00

The Cardio-vascular (CVS) system was the leading system involved among the natural deaths under study with 72.98% (n=443), followed by Respiratory system (RS) with 22.90 % (n = 139), the Genitourinary system (GUS) was least diagnosed system in the causation of sudden natural deaths (n=04, 0.66%) (Table 6).

Table 6. Distribution of System involved

System	No. of cases	Percentage (%)
CVS	443	72.98
RS	139	22.90
CNS	09	1.48
GIT	12	1.98
GUS	04	0.66
TOTAL	607	100.00

Discussion

Our study found out that about 40% of deaths received in our postmortem centre had a natural cause with a male preponderence.Other studies done in Maharashtra [12,13] show a similar trend. About 35 % of deaths brought to the medicolegal centre in their study were of natural causation. Studies done in Kolkota,Imphal,South India and Maharashtra [15,17,19] and those done across the world showed male majority in sudden deaths.In all these studies males accounted for 75%-90% of deaths.Male to female ratio is 3:1 in our study whereas studies done across the world had a ratio of 4:1.

Chaudhari and his coworker [12] and Bhagora et al [14] in studies done in Maharashtra and Gujarat agree with our findings that the age group of 51-60 yrs has majority of sudden deaths while researchers from Nigeria,Malaysia,Kolkota and Southern part of India [17-21] found 41-50 yrs to be the most affected age group.A study from West Indies recorded most of the sudden deaths in the seventh decade of life.[16]

We tried to look into the existence of premorbid conditions which may or may not be severe enough to cause death and found that 42% had documented conditions.55 % of our cases did not have any preexisting

condition.Forensic pathologists from Imphal [19] documented that 25% of the deaths due to natural causes which they autopsied had premorbid conditions.Kruger et al in their study on sudden death commented that respiratory infections formed a majority of premorbid conditions .[7]

When time of death was profiled it was found that majority of deaths were in the first quarter of the day. We did not find any work which substantiated our correlation of death and the time of day .Most of these sudden unexpected deaths in our study occurred at home followed by hospital .Studies from Nigeria and Imphal [19,21] also followed a similar pattern.

Our study also ratified the most common system involved in the cause of sudden natural death which is the cardiovascular system followed by respiratory.We observed that the genito urinary causes were the least. Most of the work done in analysing sudden natural death across the globe also found that deaths due to cardiac causes formed a majority.Autopsy surgeons from WestIndies [16] differ in their opinion as they observed cerebrovascular accident was the most commom cause of death in their centre. Chaturvedi et al [22] evaluated young adult deaths and opined that infections resulted in a majority of deaths.

Conclusion

Analyzing the trends and causes of sudden unexpected natural death will aid in epidemiological profiling of diseases. Evaluating sudden unexplained deaths can help in timely intervention in familial diseases by instituting prevention strategies and treatment at the earliest. Infections and metabolic disorders also may be minimized with timely intervention and risk management techniques.

Limitations: This study took into account only those cases received at our center and may not represent the entire region.

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Correlation of BMI with Dynamic balance using Y-Balance Test in Young Adults with Flexible Flat Foot: A Pilot study

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Abstract

Introduction: - Increase in body mass index suggests an increase in adiposity, Excess body fat deposition leads to change in the Center of Pressure which leads to altered biomechanics of the body. Different systems work together to maintain balance. The present study finds a relationship between BMI and dynamic balance in young adults with flexible flat foot.

Method: - In this study, 30 participants were recruited based on inclusion and exclusion criteria**.** Of the 30 participants; 10 were of normal weight, 10 were overweight and 10 were obese. Y balance test was performed.

Discussion: - The study showed that dynamic balance significantly changes with an increase in weight in young adults with flat feet. It is more significant in overweight and obese subjects. Any changes in ankle and foot complex leads to changes in weight-bearing, muscle imbalance static as well as dynamic balance in ambulation.

Keywords: - *Body mass index, dynamic balance, Y balance test.*

Introduction

Body mass index is a way to quantify the tissue mass in the body of an individual. Individuals are classified as underweight, normal, overweight, and obese using this index. BMI identifies the adiposity of the body, excess fat deposition in a specific area may lead to altered biomechanics due to alteration in the center of mass. Adiposity has a significant effect on soft tissues like muscle, tendon, and cartilage. Evidence suggests that even modest increases in body mass index (BMI) are sufficient to induce musculoskeletal pain and difficulties in ambulation[(1)]. So, an increase in BMI will affect weight-bearing joints like the knee, ankle, and foot.

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The ankle and foot complex plays a critical role in maintaining erect posture, as also in adaptation to supporting surfaces, in correcting postural sway in single-limb stance, in shock absorption, and transition of ground reaction force (GRF) to aid the push-off during normal gait[(2)]. The foot is made up of two vertical and two horizontal arches when these arches collapse, individual develop a flat foot. Flexible flatfoot is the postural appearance of the foot, with depressed medial longitudinal arch, pronated subtalar joint, and the calcaneus assuming a valgus position under weight-bearing conditions[(3)]. Any disturbance in foot balance leads to disturbance in balance and gait pattern.

Balance is a constantly adjusting process of joints and muscles, involving integration between different systems such as detecting, transmitting, and processing sensorial and motor information, in the central nervous system and adapting motor responses to determine the body's posture in relation to the environment, thus

maintaining one's center of gravity within the support core [4]. Balance is of two types static and dynamic. . Static is the ability to maintain balance in a stationary position while the dynamic balance is the ability to maintain balance in a moving state. Dynamic balance can be assessed by the Y balance test.

The Lower Quarter Y-Balance Test (YBT-LQ) is a screening tool that measures single-leg balance and reach distances in 3 directions: anterior, posteromedial (PM), and poster lateral (PL). The YBT-LQ is modified version from the Star Excursion Balance Test (SEBT). Reliability of Y balance according to ICC is 0.85–0.93[5]

Material and Methodology

Methodology: - The study was carried out at Physiotherapy S&C, Government medical college and hospital, Nagpur. This is a correlational study and convenient sampling, snow ball method was used. Sample size was 30 of which 10 were of normal BMI, 10 were overweight and 10 were type1 obese.

Inclusion Criteria:

1**)** Females in the age group 20-30 years.

2) Those with flexible flat feet as per the navicular drop test.

3) Navicular Drop> 9mm.

4) Subject with BMI: Normal (BMI 25-16 kg/m2), Overweight (BMI, 25–30 kg/m2) and Obese (BMI>30 kg/m2)

Exclusion Criteria:

1) Subjects with an unstable ankle joint.

2) Congenital deformities of the foot and lower limb

3) Contracture

4) Sensory, vascular, and neurological problems.

5) Limb length discrepancy

6) Pain in the lower limb.

Outcome measure: Y balance test

Tools: -

1) Weighing machine (Max 150 Kilogram

2) Stadiometer

3) Marker pen

4) Measuring scale (15 cm scale)

5) Goniometer (0-180°)

6) Measuring tape (Upto150 centimeters)

7) Y Balance kit

8) Chair

9) Data collection sheet

10) Consent form

Procedure

The Subjects were assessed for the outcome measure, Weight and height was taken and BMI was calculated as per the formula body weight divided by the square of the height (kg/m2) for each individual. Classification of BMI will be Normal (18.5-24.5), Overweight (24.5-30), Obese (>30). Limb length was assessed. The navicular drop test was performed. The subject was in a sitting position with their feet flat on a firm surface with the knee flexed and ankle in neutral position. Navicular tubercle which is the most prominent surface of navicular bone was identified and marked with a pen w. The index card was placed on the floor vertically passing the navicular bone and the level of the most prominent point of the navicular tubercle was marked on the card. Without changing the position of the feet the individual was asked to stand and to distribute equal weight on both feet. In the standing position, the navicular tubercle was again been identified and marked on the card. The difference between the original height of the navicular tubercle in sitting position and the weight-bearing position was assessed with a tape measure rendering the ND amount in millimeter. 6-9 mm distance is considered as normal arched feet. More than 9mm is considered as flatfoot.

Y Balance Test: - Subjects were instructed. To keep their testing leg on the center of the grid. Trunk facing forward. Hands-on their hips throughout each trial. Must come back to the starting position. The patient was asked to reach in all three directions (anterior, posteromedial, and poster lateral by the great toe of testing leg as much

as she can. The distance covered by the subjects in all three directions were recorded. Then the same procedure was repeated for another leg. The score of the Y balance test was calculated by the following formula[6].

$$\text{Score of Y Balance Test} = \frac{\text{Anterior} + \text{Posteromedial} + \text{Posterolateral}}{3 \times \text{Limb Length}} \times 100$$

The scores were recorded and data collected and entered into a Microsoft Excel spreadsheet

Statistical Analysis

The data was entered in excel and was carry forwarded for statistical analysis. The summary of data was taken using descriptive statistics for mean and average. Pearson's method to calculate correlation coefficient was used

Ethical Clearance: The approval of the Committee on Institutional Ethics must be obtained prior to the start of the study. Patients must be treated with respect first. Upon meeting the requirements of

Inclusion and exclusion criteria, the patients are taken for review

Result

A total of 30 young adults took part in this study of which 10 had normal BMI, 10 were overweight and 10 were type1 obese.

There is statistically significant correlation between body mass index and dynamic balance for right limb (r= -0.7718, P value< 0.0001) for left limb (r= -0.7614, P value=0.0001). Positive correlation was seen between Navicular drop test and BMI for right limb (r=0.7089 P value<0.0001) for left limb (r=0.3967 P=0.0300).

Y Balance test was performed in three direction-anterior, posteromedial and posterolateral. Of which the result of anterior and posterolateral are statistically significant (Right r= -0.8375, P<0.0001, Left r= -0.8532, P<0.0001, Right r= -0.8023, P<0.0001, Left r= -0.8257, P<0.0001). For posteromedial direction the study was statistically non-significant (Right r= 0.0800, P=0.6753 Left r=0.0125, P=0.9475)

Discussion

The study showed that dynamic balance significantly changes with an increase in weight in young adults with flat feet. It is more significant in overweight and obese subjects. Structural deviations in the ankle and foot complex predispose the individual to changes in weight-bearing, muscle imbalance static as well as dynamic balance in ambulation. Previous studies have revealed obesity as being one moderating factor of foot shape. Some studies have found greater plantar pressures for the midfoot area in overweight children as well as a greater contact area derived from footprints and it has also been postulated that the flat feet of obese children may be caused by the existence of a plantar fat pad underneath the midfoot region which could imply a lowered medial longitudinal. In obese individual Flat Feet may be caused by a collapse of the medial longitudinal arch due to excessive loading of the feet as a result of continually bearing additional body mass, which affects the functional capacity of the longitudinal arch. Such a structural collapse can develop into a potentially disabling problem in later life, as proper mechanics of the longitudinal arch are critical to normal foot function[7]

Some authors observed a higher prevalence of obesity in individuals with flat feet[8] which may indicate that the increase in body mass associated with the weakness of the other structures responsible for the maintenance of the longitudinal arch collaborates with its drop. Obesity has a significant correlation with flat foot Increase in mass increased static and dynamic plantar pressure causing a significant change in the structure of the foot particularly in the forefoot and midfoot.

Overweight, central obesity and level of muscular fitness are strongly associated with balance. The static and dynamic posture requires the center of mass to be within the body's base of support (BOS), when the line of gravity falls outside of the BOS the body's balance is compromised. Although obesity and overweight are temporary, it can be the cause of flatfoot. Therefore subjects should pay attention to increasing weight that may cause flatfoot and also other problems in the future[9] .

Obesity increases the need for attentional resources to maintain postural stability and this may lead to compromised balance when the subject is required to maintain stability during daily activities. Maffiuletti NA, Agosti et al found that increased obesity has shown

to be positively correlated with the impaired postural balance in younger adults [10]. Yogi in their study stated that the dynamic body balance was highly affected in overweight and in obese subjects [11].

Conclusion

The study concludes that there is a negative correlation between obesity and dynamic balance in young adults with Flexible Flat Foot. So, this study concludes that any increase in BMI will lead to decreased in dynamic balance in the same way, any decrease in BMI will lead to increase in dynamic balance.

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Original Research

Correlation of body mass index with dynamic balance using Y-Balance Test in adults with the Flexible Flat Foot: An Analytical Correlation Research Protocol

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Abstract

Background: Balance is one of the primary requirements for both static and dynamic activities. Body Mass Index (BMI) is measurement for estimating excess body fat. The Lower Quarter Y-Balance Test (YBT-LQ) is a screening instrument that measures single-leg balance in different directions. Flexible flatfoot is the postural aspect with depressed medial longitudinal arch, pronated subtalar joint and valgus position of calcaneus, evident only in weight-bearing position.

Methods: An analytical correlation study with 255 participants (Purposive sampling) will be taken as per inclusion and exclusion criteria with study duration of 3 years. The BMI, sit to stand Navicular Drop Test (SSNDT), limb length and medial longitudinal arch angle (MLAA) will be measured and compared. The mean score of the Y Balance Test in different categories of BMI will be compared by performing one way ANOVA test. The mean score of the Y Balance Test between two groups in each category of BMI will be compared by Bonferroni t-test.

Conclusion: The study aims the correlation of BMI with the dynamic balance on Y-balanced test in adults with flexible flat foot. The expected outcome of study will concentrate on the significant correlation of Y Balance Test in different categories of BMI.

Keywords: *Balance, BMI, Flexible flat foot, YBT-LQ*

Introduction

Flatfoot is also called as pesplanus or fallen arches [(1)]. Acquired flatfoot develops from injury, illness, prolonged foot stress, obesity, and faulty biomechanics. The foot arch collapses in this postural deformity [(2)]. Flexible flatfoot is stated precisely as the postural aspect of the foot, with depressed medial longitudinal arch, pronated subtalar joint and valgus position of calcaneus under weight-bearing positions [(3)]. The expression "flexible" indicates that while the foot is flat when standing (weight-bearing) and the arch reappears in the non-weight-bearing state. One of the prevalent types of flatfoot is flexible flatfoot. Usually, it commences in childhood or puberty and persists on into adulthood. It ordinarily happens in both feet and advances in severity over the adult years. About 20-30% of the population

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frequently have flat feet reported a high prevalence of flatfoot in 18 to 25 years of young adults. Flatfeet is not dependent on gender [2,3].

The ankle and foot complex plays a significant purpose in keeping erect posture, as further in adjustment on supporting surfaces, in remedying postural sway in single-limb stance, in shock absorption and transition of ground reaction force (GRF) to assist the push-off throughout normal gait [1,4]. Minimal biomechanical alterations and functional variance in the ankle and foot complex, in turn, changes the peripheral sensory input in weight-bearing posture and contact with the surface area (5–7). The abnormalities occurring in the structure of the foot may influence the function in the static and dynamic state, and movement, thus altering the displacement of the body. They may intrude the motion-sensing receptors [8,9]. Since balance necessitates the processing of information from the motion-sensing receptors to estimate the body's position in space, it can affect posture in a complex interaction and affect the complete range of motion (ROM) of the joints required to obtain maximum balance [10,11]. Flat feet bring about variations in foot posture, foot mobility, and load distribution under the foot, which affects dynamic balance required for activities of daily living (ADLs) and optimal performance in the sports activity. Alterations in neuromuscular strategies change the ability to sustain an upright and stable posture, and anticipatory postural control is decreased, frequently making the person susceptible to falls and associated injuries[5,7]. Variations in the structures at the ankle and foot complex make a person susceptible to alterations in weight-bearing, and muscle imbalance in static and dynamic positions leading to compensatory strategies, which usually result in overuse injuries[2].

The World Health Organization, 1997 divided individuals into three categories depending upon body mass: normal (BMI<25 kg/m2), overweight (BMI, 25–30 kg/m2) and obese (BMI>30 kg/m2). BMI is a helpful measurement for estimating excess body fat. It is determined by dividing body-weight by the square of the height (kg/m2) per person[5,6] Balance is linked to the inertial forces acting on the body and the inertial components of body segments. It is stated as the process that keeps the centre of gravity within the body's support base and requires consistent changes with joint positioning and muscular activity[3]. Balance is one of the primary requirements for daily activities, both static and dynamic activities[11]. Balance is a physiological and mechanical state and the desire to move the body within the optimum level of support [11]. Factors that affect balance are sensory information received from the visual, somatosensory, and vestibular systems and motor responses that influence coordination, joint range of motion (ROM) and strength. Dynamic balance is the ability of an object to balance while in motion. Ability to sustain postural stability under dynamic conditions is an essential underlying element of physical activity execution.

The disadvantage presented by obese young adults occurred in dynamic activities, displaying less balance and an increment in the time to achieve these activities[12,13]. It has been recommended that fat mass may be the reason for the outward aspect of a "flat" foot in obese individuals. Bodyweight is considerably correlated to increase loading of the foot, especially the forefoot and midfoot, which infers that obesity enhances the stress applied to the foot directly via raised body weight and indirectly via changes to the foot structure, i.e., a comparatively pronated foot posture [12,14].

The navicular drop test (NDT) has been broadly used as a clinical method to estimate foot mobility. It has also been correlated to lower limb musculoskeletal injuries. Besides, it aided to estimate the amount of foot mobility, particularly pronation in athletes [15].

The Lower Quarter Y-Balance Test (YBT-LQ) is a screening instrument that measures single-leg balance and reaches distances in 3 directions: anterior, posteromedial (PM), and posterolateral(PL). The YBT-LQ was modified from the Star Excursion Balance Test (SEBT) [13,16].

Malik Manoj et al, 2018 studied navicular Drop Gender Differences among College Students in which the correlation of Right ND with left ND and age was significant, but no significant correlation was found between RND and height, weight, foot length and BMI [17]. Mohan Ganesan et al, 2018 found in their study that the individuals with high BMI have impaired dynamic balance control in AP direction reflected through higher COP amplitude and velocity. The COP changes during the double limb stance are unimpaired, both with eyes

opened and closed [18]. There are several studies which suggest that there is a correlation between increasing BMI and dynamic balance as it modifies body geometry and influences the biomechanics of weight-bearing areas of the foot [14,19].

Increase body mass may be a factor resulting in increased static and dynamic plantar pressures, which may cause significant change to the structures of the feet. Also, long-term body mass increase may appear to flatten the medial longitudinal arch of the subject which may change the line of gravity and action of forces on the foot in contact with the ground. Obesity thus may show influence over foot biomechanics and gait pattern [2,16]. However, there has been a significant lag in researches demonstrating a correlation of increased BMI on dynamic balance with individuals with flexible flat feet.

Parvez (2018) stated prevalence of flatfoot in normal working individuals in India according to the age. They stated the prevalence in subjects between 20 – 29 years to be 31.8%, 30 – 39 years to be 42%, 40 – 49 % to be 37.5% and above 50 years to be 37.5% flatfoot. According to gender, the flatfeet were found to have higher incidences in females[20]. Dare NW (2012) analysed prevalence of flatfoot in 510 adults (235 males and 275 females) aged 33±15 years of Bayelsia – Nigeria and they found it to be in 25.3% adults(20.4% in males and 29.5% in females) and incidence of flatfoot was found to be 1:4[21].

The study aims to study the correlation of BMI with the dynamic balance on Y-balanced test in adults with flexible flat foot.

The objectives include- Assessment of dynamic balance using Y-balanced test in normal, overweight and obese adults with flexible flat foot and correlation between the Y Balance score and the groups (Normal to Overweight, Normal to Obese, Overweight to Obese).

Methodology

Study design: An analytical correlation study

Study setting: The study will be carried out at Ravi Nair Physiotherapy College under the Datta Meghe Institute of Medical Sciences University. Permission and approval to carry out the research work shall be obtained from the Head of Institution and Institutional Ethical Committee.

Study duration: The duration of the research study will be three years.

Sampling method: Purposive sampling will be done for the study.

Sample size:

The sample size was estimated out as 255 using n Master version-2 Software based on the pilot study done on 50 subjects having 25 in each group with the following assumptions:

SD in Y Balance Test = 6.97

Mean score of Y Balance Test of flatfeet = 85.61

Relative precision (%) = 1

Desired confidence level (1- α) % = 95

Formula – $n = Z_{1-\alpha/2} \times \alpha^2 / \epsilon^2 \mu^2$

where,

α – Standard deviation

€ - Relative precision

μ - mean

1- α/2 - desired confidence level

Materials:

The study requires weighing machine, stadiometer, marker pen, measuring scale-10 cm scale, chalk, measuring tape and Y Balance kit. Half circle Goniometer, chair , plinth, white card

Eligibility criteria:

The inclusion criteria will comprise adults with age group 20-50 years[22], flexible flat feet according to navicular drop test (Navicular Drop> 10mm)[23], Medial longitudinal arch angle (MLAA) less than 130°, and BMI classification as normal, overweight and obese [5,6]. The exclusion criteria comprise the subjects with unstable/ postoperative ankle joint, Congenital deformities of the foot, Contracture (ankle joint), Pediatric and Geriatric

age group, and subjects with sensory, vascular and neurological problems. Selection of participants after diagnosing as per the BMI classification as normal weight, overweight and obese with Flexible flat foot diagnosed by the Navicular drop test and MLAA.

Recruitment and procedure:

The orthopaedics and health care practitioners working under DMIMSU are invited to refer the prospective patients to our out-patient department (OPD). The individuals fulfilling the inclusion criteria will be included in the study. Purposive sampling will be adapted for the selection of participants until the desired sample size is achieved. The similar criteria study on the effect of BMI (normal, overweight, obese) on Dynamic balance in individuals with flexible flat foot is referred. Written informed consent will be taken from the participants, explaining the study procedures, possible benefits of the study, the right to withdraw from the study and confidentiality in detail, in a language they understand. The pre-participation evaluation form will consist of a personal database, history, chief complaints and duration from the onset of symptoms.

Outcome measures:

1. Primary outcome measure: Y Balance Test is the primary outcome measure, in which the subjects will be asked to keep their non-testing leg in the centre of the grid, and reach in all three directions (anterior, posteromedial and posterolateral) by the great toe of the testing leg as much as he can. The primary investigators will record the distance covered by the subjects in all three directions. The subject will repeat the same procedure using the other leg.

2. Secondary outcome measures: The secondary outcome measures include body mass index (BMI), Sit to stand Navicular drop test (SSNDT), Lower limb length measurement, and Medial Longitudinal Arch Angle (MLAA). BMI is calculated as the bodyweight divided by the square of the height (kg/m2) for each individual. BMI will be classified as normal, overweight, and obese [24]. The SSNDT is useful to diagnose flatfeet. It will be calculated by the difference between the height of navicular from the floor when the subtalar joint is neutral in non-weight bearing (sitting position) and the height of navicular from the floor when in relaxed stance in a full weight-bearing position. The distance of 6-9 mm is considered normal, while less than 6 mm falls under flatfeet criteria [16,25]. Lower limb length measurement is performed in the supine position. Measuring tape will measure the distance between the anterior superior iliac spine and ipsilateral medial malleolus [25]. The MLAA is measured using a goniometer. The centre of the goniometer is placed at the navicular tuberosity, and the ends of the goniometer follow the landmarks on the centre of the medial malleolus and the head of the first metatarsal. The angle between the line from the medial malleolus to the navicular tuberosity and the line connecting the head of the first metatarsal bone and the navicular tuberosity will be measured in degrees. MLAA less than 131º is considered low arch foot.

Statistical Analysis and Data Collection And Management:

Statistical Analysis

The categorical variables will be expressed in frequency and percentages. Continuous variables will be presented in mean ± SD. The categorical variable will be compared by performing the chi-square test. The mean score of the Y Balance Test in different categories of BMI will be compared by performing one way ANOVA test. The mean score of the Y Balance Test between 2 groups in each category of BMI will be compared by Bonferroni t-test. Statistical significance will be set at $p< 0.05$. Data analysis will be done using statistical software STATA version 14.0.

Data Collection And Management

The principal investigators will collect and record Y Balance test score data, and enter in the pre-established spreadsheet for statistical analysis. The collected data will be entered into Microsoft Excel spreadsheet. Testing data will be put into a secure REDCap database. The non-electronic data, such as hard copies of assessment forms, signed consent forms, etc. will be stored securely in the study setting. Electronic data will be stored in DMIMSU's electronic repository as per participant ID. The administrator access rights will lie with the principal investigators (PI). PIs will supervise data collection and documentation. The documentation will be evaluated thoroughly for accuracy. The Excel spreadsheet will be released at the end of the study to an allocation blinded

statistician for conducting the necessary analysis. Checklists are used to prevent missing data due to the improper staff procedure.

Discussion

The study will benefit the subjects with increased BMI along with flexible flat feet to improve their dynamic balance and will prevent the risk of fall and injuries in future. J A do Nascimento et al, 2017 demonstrated that obese subjects exhibited postural alterations, such as head protrusion (47.6%), hyperkyphosis (46.7%) and hyperlordosis (26.7%) [11]. Besides, medial-lateral dynamic displacement, mean time to perform the limits of stability test and timed up and go test (TUGT), and the risk of falls were higher in obese subjects. In a study, Ashwini Chougala et al (2015) witnessed a significant relationship between obesity and flatfoot with a great predominance of flatfoot with high BMI [2]. Dong-Chul Moon et al 2014 demonstrated that short-foot exercise spontaneously raised the dynamic balance in individuals with remarkably pronated feet. The results of the study conducted by Karen P. Cote et al (2005) suggested that postural stability is affected by foot type under both static and dynamic conditions. Shyamala Shree et al(2018) conducted a study on health sciences and nursing students, which demonstrated an overall prevalence of flat foot among obese subjects as 44% [26]. Hannah C. Ded Porto et al, 2012 in their study observed significant biomechanical effects of obesity during conditions of static balance, perturbed balance and dynamic balance during gait [22]. Ajit Dabholkar et al 2012 in their Cross-sectional study compared Dynamic Balance Between Flat Feet and Normal Individuals using Star Excursion Balance Test to find extremely significant mean differences in sit to stand navicular drop test, calcaneum angle, width of the foot, great toe extension range of motion and SEBT was found in individuals with flat feet [27].

Sami S. Al Abdulwahab et al concluded from his study that an overweight BMI influences foot posture alignment and body stability and, BMI should be considered during rehabilitation management for lower extremity injuries and body balance [5]. If we found a positive correlation of dynamic balance with the flexible flat foot so the subjects will be advised to decrease the weight and improve the foot condition (flexible flat foot) by advising foot exercises and shoe modification.

Ethical Approval and Dissemination:

The protocol is designed as per the Declaration of Helsinki. The main findings of the study will be published in a peer-reviewed journal. The subjects participating in the study can access the main findings of the study. The data will be stored in the DMIMSU data repository after the completion of the study a minimum of 5 years, and the results are published.

Patient Consent: Principal Investigators will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality.

Author's Contribution: SMB and WMN led to the creation and design of the study. SMB wrote the manuscript of this article. SMB and WMN read and approved the final manuscript for publication.

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Estimation of Stature from Hand Dimensions

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Abstract

Background: The objective of this study was to estimate stature from hand dimensions in the South Indian population. It is a very useful tool in forensic anthropology where only incomplete skeletal remains or mutilated limbs are present.

Method: The study was done using standard measuring apparatus such as stadiometer to measure stature, and vernier callipers to measure hand length, palm length, hand breadth, and maximum hand breadth, and included both males and females. Regression formula and correlation was derived between hand measurements and stature.

Results: In this study, regression formula is derived with eight variables generalized for both males and females, S= 63.686+7.466RHL-2.298LHL-4.698RPL+3.697LPL+6.160RHB-9.622LHB+2.810RMHB+1.708LMHB ± 6.55977. Linear Regression equation for males and females have been derived separately with each of the hand parameters.

Conclusion: In conclusion, hand measurements can be an effective tool in estimating stature for individuals above 18 years of age.

Key words: *hand breadth, hand length, maximum hand breadth, palm length, stature estimation*

Introduction

In the field of forensic anthropology, stature estimation is an important parameter in establishing the identification of the individuals. It is an important element that provides useful data which narrows the pool of potentially matching identities.[1] Estimation of stature from the incomplete skeletal remains or from the mutilated or amputated limbs or parts of limbs or highly decomposed, fragmented human remains has obvious importance in personal identification in the events of murders, accidents or natural disasters considered as one of the biggest aspects of forensic science.[2] The hand dimensions, being genetically derived varies in different races and ethnic groups and is used to determine sex, age, stature and nutritional status of an individual.[3] Stature can be defined as the natural height of a person in erect position, and it is determined by many factors such as genetic and environmental factors.[4] The present study was undertaken to estimate any correlation between stature and hand dimensions by measuring stature as well as different lengths and breadths of hand in the South Indian population.[5]

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Materials and Methodology

It is a cross sectional study consisting of South Indian population. Subjects irrespective of sex, with age of 18 years, those who have given their consent were included in this study and those who have not given consent, those below 18 years of age were excluded from this study. Source of data is predominantly from the residents of Chennai in 2019-2020. The study was conducted in Saveetha Medical College Hospital; They

are measured for stature using stadiometer (Person should stand erect with head in the Frankfurt plane. Heels are together, with weight distributed equally on both feet; Shoulders and the upper extremities are kept relaxed), and for hand dimensions (using vernier calipers): Hand length (projected distance between the midpoint of a line joining the styloid process of radius and ulna bones of forearm and the tip of third finger), Palm length (maximum distance from the midpoint of the distal crease of the wrist and the palmar digital crease of the 3rd finger), Hand breadth (distance between the most remote points on the heads of the 2nd and 5th metacarpal bones), and Maximum hand breadth (distance between the most remote points on the heads of the 1st and 5th metacarpal bones). These measurements were taken for both hands. All the dimensions are recorded to the nearest centimeter using standardized measuring equipment and sliding calipers. Since stature is variable among population, age and sex, regression formula have been derived for the population. The data will be tabulated, analyzed and subjected to statistical calculation using SPSS software. It is then used to derive a relation between hand dimensions and height.

Implications:

The expected results from this study is knowledge of relation between stature and hand dimensions and its importance in medicolegal and forensic examinations.

Result

Table No: 1 Estimation of stature using hand measurements:

Parameter	Male	Female
Total Number	34	46
Mean of Stature	171.2294	155.0957
S.D of Stature	8.54207	5.01185
Mean of Right hand length	18.9735	17.2304
S D of Right hand length	1.34869	0.91381
Mean of Left hand length	19.0059	17.2826
S.D of Left hand length	1.38147	0.91124
Mean of Right palm length	9.9529	9.2891
S.D of Right palm length	.89856	1.008
Mean of Left palm length	9.9324	9.2565
S.D of Left palm length	.91840	0.98108
Mean of Right hand breadth	8.1029	7.5457
S.D of Right hand breadth	.81408	0.84871
Mean of Left hand breadth	8.0618	7.5413
S.D of Left hand breadth	.72531	0.83893
Mean of Right Maximum Hand breadth	9.7794	8.8783
S.D of Right Maximum Hand breadth	.62754	0.86997
Mean of Left Maximum Hand breadth	9.8382	8.9283
S.D of Left Maximum Hand breadth	0.73855	0.96001

Table No: 2 Correlation of stature with hand dimensions

	Correlation
Right hand length	0.761
Left hand length	0.755
Right palm length	0.370
Left palm length	0.375
Right hand breadth	0.278
Left hand breadth	0.262
Right maximum hand breadth	0.531
Left maximum hand breadth	0.494

	Male	**Female**
Right hand length	0.747	0.250
Left hand length	0.757	0.215
Right palm length	0.271	0.136
Left palm length	0.273	0.126
Right hand breadth	0.088	0.024
Left hand breadth	0.050	0.027
Right maximum hand breadth	0.232	0.337
Left maximum hand breadth	0.192	0.333

Correlations										
	Stature	**Right hand Length**	**Left hand length**	**Right palm length**	**Left palm length**	**Right hand breadth**	**Left hand breadth**	**Right maximum Hand breadth**	**Left Maximum Hand breadth**	
Stature	1	.761**	.755**	.370**	.375**	.278*	.262*	.531**	.494**	
Right hand Length		1	.997**	.572**	.573**	.404**	.397**	.551**	.519**	
Left hand length			1	.576**	.579**	.416**	.410**	.555**	.526**	
Right palm length				1	.989**	.832**	.827**	.682**	.719**	
Left palm length					1	.857**	.855**	.694**	.732**	
Right hand breadth						1	.994**	.776**	.816**	
Left hand breadth							1	.767**	.807**	
Right maximum hand breadth								1	.989**	
Left maximum hand breadth									1	
**. Correlation is significant at the 0.01 level (2-tailed).										
*. Correlation is significant at the 0.05 level (2-tailed).										

Table No: 3 Regression equation

Regression equation ± SEE

S= 63.686+7.466RHL-2.298LHL-4.698RPL+3.697LPL+6.160RHB-9.622LHB+2.810RMHB+1.708LMHB ± 6.55977

Linear Regression equation for Male

Regression equation ± SEE

S= 81.458+4.731RHL ± 5.7667

S=82.290+4.680LHL ± 5.6700

S=145.579+2.577RPL ± 8.3497

S= 145.998+2.540LPL ± 8.3447

S=163.761+0.922RHB ± 8.6410

S=166.507+0.586LHB ± 8.6638

S=140.300+3.163RMHB ± 8.4371

S=149.372+2.222LMHB ± 8.51297

Linear Regression equation for Female

Regression equation ± SEE

S= 131.465+1.371RHL ± 4.9075

S=134.672+1.182LHL ± 4.9501

S=148.796+0.678RPL ± 5.0211

S= 149.154+0.642LPL ± 5.0283

S=154.020+0.143RHB ± 5.0670

S=153.858+0.164LHB ± 5.0666

S=137.853+1.942RMHB ± 4.7718

S=139.571+1.739LMHB ± 4.7791

Discussion

Estimation of stature of an individual from extremities and their parts is useful in identifying the dead in forensic examinations. Estimation of stature from the incomplete skeletal remains or from the mutilated or amputated limbs or parts of limbs or highly decomposed, fragmented human remains has obvious importance in personal identification in the events of murders, accidents, or natural disasters considered as one of the biggest aspects of forensic science.

The hand dimensions, being genetically derived vary in different races and ethnic groups and are used to determine the sex, age, stature, and nutritional status of an individual. Stature can be defined as the natural height of a person in an erect position, and it is determined by many factors such as genetic and environmental factors. The present study was undertaken to estimate any correlation between stature and hand dimensions by measuring stature as well as different lengths and breadths of hand in the South Indian population.

Objective of the study is to measure hand dimensions in the population, calculate stature from the population and to examine the relationship between stature and hand dimensions.

The study design is cross sectional. It was conducted among the study population of the South Indian population, who gave their informed consent. The inclusion criteria comprised of adults aged above 18. They were involved in this study, irrespective of sex from the South Indian population. All those who did not give their consent or below 18 years of age were excluded from this study.

The material used for stature estimation is a stadiometer, and vernier callipers were used for measurement of hand dimensions. The various hand dimensions include- hand length, palm length, hand breadth, and maximum hand breadth. Hand length is the projected distance between the midpoint of a line joining the styloid process of radius and ulna bones of forearm and the tip of third finger. Palm length is the maximum distance from the midpoint of the distal crease of the wrist and the palmar digital crease of the 3rd finger. Hand breadth is the distance between the most remote points on the heads of the 2nd and 5th metacarpal bones. Maximum hand breadth is the distance between the most remote points on the heads of the 1st and 5th metacarpal bones. These measurements were taken for both hands.

The data was tabulated, analysed and subjected to statistical calculations using SPSS software. The study was undertaken with 80 adults of whom 34 were male (42.5%) and 46 were female (57.5%). The tabulated data include the mean and standard deviation values of stature, hand length, palm length, hand breadth, and maximum hand breadth derived from the entire sample space and based on sex distribution. Correlation was established and a linear regression formula was derived from the statistical analysis of the data.

Correlation of stature with respect to different parameters of hand dimensions is established. Males and females, have different values of correlation and that was separately derived. Aside from hand breadth, which has a significant correlation at the 0.05 level (2-tailed), the other parameters such as hand length, palm length, hand breadth, and maximum hand breadth have a significant correlation at the 0.01 level (2-tailed) with stature.

Stature estimation was undertaken by multiplication of the parameter with the multiplication factor or by using regression equation. The regression formula is derived with eight variables, generalised for both males and females,

S = 63.686 + 7.466RHL - 2.298LHL - 4.698RPL + 3.697LPL + 6.160RHB - 9.622LHB+2.810RMHB+1.708LMHB ± 6.55977.

Linear regression formula for males and females were derived separately with respect to each of the individual parameters. For males, the linear regression formula derived can be summarised as Right hand length (20.26%), Left hand length (20.30%), Right palm

length (10.63%), Left palm length (10.61%), Right hand breadth (8.65%), Left hand breadth (8.61%), Right maximum hand breadth (10.44%), Left maximum hand breadth (10.51%). For females, the linear regression formula derived can be summarised as Right hand length (20.05%), Left hand length (20.11%), Right palm length (10.81%), Left palm length (10.77%), Right hand breadth (8.78%), Left hand breadth (8.77%), Right maximum hand breadth (10.33%), Left maximum hand breadth (10.39%).

Conclusion

There is substantial evidence from the derived data that there is significant correlation between stature and hand dimensions. Hence, it can be used in forensic anthropology to estimate stature from hand remains. There may be discrepancies with respect to different demographical factors. In conclusion, the linear regression formula derived is a reliable indicator for the estimation of stature from hand dimensions in the South Indian population.

Ethical Clearance: Obtained from institutional ethical committee

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TNCS in the Context of Environment and Toxicology: A Critical Study

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Abstract

The research topic concerning environment and toxicology has its unique importance. Toxicology as a subject earlier seems confined to practice of diagnosing toxins and toxicants and treatment thereto. In its expanded horizon it started studying the industrial toxins and their emission into the environment. With the industrial growth and emergence of 'Transnational Corporations'(TNC's) countries across the globe are facing new challenges on environmental front. Accordingly, toxicology became interested in studying adverse effects of chemicals on living organisms with eco-system as carrier. The toxins released from industries and E-waste are cause of Toxic Jaundice, Toxic Cough, Toxic Paralysis, Toxic Shock Syndrome, besides Toxicosis and other diseases. International Conventions have been arranged to protect environment. Indian Constitution imposes duty on State towards improving public health, prevention of hazards, besides duty on citizens to improve environment. Responsibility on TNC's as visualised by leak of poisonous Methyl isocyanate from Union Carbide is a pointer towards this.The deliberations under the Research topic being relation between Industry, Environment, Health Hazard and Toxicology and areas of appropriate Management thereto.

Key Words: *Environment, Toxicology, TNC's, Health Hazard, Chemicals, Constitution, Industrial Growth*

Introduction

The topic has its importance in the context of expanded horizons of Toxicology by entering in Environment on one side and Industry on the other giving rise to Environmental Toxicology, besides Industrial Toxicology. The industry has shown its expansion with emergence of Transnational Corporations (TNCs)/ Multinational Corporations (MNCs). India is favouring globalisation by allowing the operation of TNCs/ MNCs. TNCs have to follow national laws and adhere to International laws including UN Code of Conduct for Transnational Corporations, 1983 especially its Article 41, 42 and 43 for safeguarding environment. In its grammatical context 'Toxicology' as noun means 'the scientific study of poisons'. As per medical science it is defined as 'the study of nature, effects and detection of poisons and treatment of poisoning'. It also connotes, 'the scientific dealing with the effects, antidotes and detection etc of poisons. In its present context many sub-divisions of Toxicology have emerged like a) Environmental Toxicology b) Food Toxicology and c) Medical/ Forensic Toxicology. In this way the Environmental & Industrial Pollution is cause of concern in spreading pollutants in atmosphere resulting toxins for humans and other living beings. The toxins so released are cause of Toxic Jaundice, Toxic Cough, Toxic Paralysis and Toxic Shock Syndrome, besides Toxicosis and other diseases. The Environment acts as medium to carry Industrial Toxins/Pollutants including the Industrial Waste from its source to recipients like animal and plant life especially Humans who inhale such polluted and

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toxic gases, chemicals etc. The topic covers interaction between the Industry especially the Industrial waste in its relation to atmosphere with ultimate consequence towards Biodegradation especially affecting Human and Animal life alike.

Material and Methods

The topic is dealt and deliberated on Doctrinal Methodology with the help of Primary and Secondary source of data whereby one source of data is corroborated with the other for arriving at conclusions.

Environment & Its Constituents:

The term Environment itself is derivative of a French word '*environer',* meaning *'to surround'*. Environment usually covers surroundings where the habitation exists. The surroundings maybe natural or artificial. The Natural surroundings may be living and/or non-living while the Artificial Environment comes into being through manmade developments by Industrialisations or otherwise. The National Industries as well as Transnational Industries are contributory to this. Industrial waste including E-Waste is contributory to the Environmental pollution by release of toxins from physical inorganic substances. Such release of toxins mixes with Lithosphere, Hydrosphere and Atmosphere paving the way to integrates with the biotic Environment affecting the life. In this way study of toxins in atmosphere and their impact on life comes within the study of Toxicology especially in its branch of Environmental and/or Industrial Toxicology having connectivity to human health. For health hazards Environment acts as conduit with Industry and its waste as source. The study of source, conduit and its impact assumes importance. The moment toxins enter human life, the study of toxicology stands ignited. In International arena there are number of cases[1] where on the strength of Environment pollution due to release of toxins, the Transnational Corporations were expropriated. As an instance, Metalclad v. United Mexican States reflects expropriation/non-grant of licence to TNC only on the ground of apprehended environmental pollution which might have resulted in health hazard due to release of toxins. On the other under Indian scenario, the TNC like Union Carbide has been cause of emission of toxic gases like Methyl Isocyanide causing death and destruction in and around the factory premises in Bhopal. People suffered lung damages and other pulmonary problems. Most of the affected people are still sufferers. The case has assumed international importance involving TNC's, Environment and Health Hazard issues. In Shriram industries case[2] where Oleum gas was released at industrial complex at Kirti Nagar, Delhi causing death and affected health problems for the many compelling the Supreme Court to pass number of guidelines to prevent health hazard and for protecting environment.

Toxins as Health Hazard Contributory:

Industrial waste among others is one of the contributory to health hazard by emissions of toxins in atmosphere. In addition to Industrial pollution and toxic emissions the scientific developments in technology opened new dimensions and challenges. **With** increase in electronic users and economic growth, India is projected to generate 5.2 million tonnes of E-waste by 2020 as observed by Associated Chambers of Commerce and Industry of India (ASSOCHAM). Leaving other pollutants and toxic emissions as to source aside, the E-waste generated from electrical equipment becomes un-useable leaving the metals, plastics, anode/cathode, circuit boards, cables and like for vagaries of nature. The discarded waste mainly belongs to Inorganic chemistry like copper, silver, gold and platinum. The presence of toxic substances like lithium, mercury, nickel, polychlorinated biphenyls(PCBs), selenium, arsenic, barium, brominated flame retardants, cadmium, chrome, cobalt, copper, and lead are cause of concern for Environmental pollution and as health hazard forming subject of Toxicology at Macro that is at Environmental level as well as at Micro individual human level.

Constitutional Concerns for Health & Environment:

Under the Indian Constitution Article 21 says "Nobody shall be deprived of his life or personal liberty except according to procedure established by law". This Article has been subject to wide judicial interpretation. It was observed by the Supreme Court that the conservation and protection of environment is an inseparable part of right to life as transpired from number of judgements including Centre for Environmental Law[3]. Reference may also be made to obligations on state for clean environment including pollution free water and air as held by the court[4]. The concern for public health is provided in Article 47 of directive principles forming part

IV of the Constitution of India. The State is also obliged under Article 39(f) of the constitution of India that the children are given opportunities and facilities to develop in a healthy manner. By Amendment of 1977 provision was added to Article 39 mentioning that the children are given opportunities and facilities to develop in a healthy manner. The Court has shown its concern for health and deliberated upon hazardous industries which now even include within the subject of Industrial Toxicology. In Vellore Citizens Welfare Forum[5], the issue was whether the tanneries should be allowed to continue to operate at the cost of lives of lakhs of people whereby the Supreme Court observed that these tanneries though are foreign exchange earners and employment providers but at the same destroy environment and poses health hazard to everyone.

The Constitutional (42nd Amendment) Amendment Act, 1976 makes it obligatory on citizens to protect and improve natural environment and in Article 51-A (g) says that "*It shall be the duty of every citizen of India to protect and improve the natural environment including forests, lakes, rivers and wildlife, and to have compassion for living creatures;"*

When taken with the Constitutional Provisions in totality and the 'Environmental Protection Act, 1986 it points towards the legislative efforts for clean Environment and prevention of Toxins in environment as health hazard.

Toxicology in Relation to Environment/Industry:

Since earlier times there have been cases confirming death or decease by poisons. The earlier physicians especially the Greeks were under control of symptoms in relation to poisons of different categories. Later in its systematic study the subject evolved under the title 'Toxicology' and formed a part of Medical Jurisprudence. In this sense for a medico-legal case Toxicology assumed importance for ascertaining the cause of death or disease which paved the way for investigator for identifying the criminal. The science of Toxicology as such evolved has been defined differently by different authorities and simplest way to define it is:

"A Study of Adverse effects of Chemicals, Physical, or Biological agents on living organisms and Eco-system, including prevention and amelioration of such adverse effecs"[6]

The evidence of medical expert before court of law is taken as Expert Evidence under Section 45 of the Indian Evidence Act, 1872 and as such reliable in absence of direct evidence. From mere use of Toxicology in crime detection, it is considered as subject for understanding the Industrial and/or Environmental pollution. The field is expanding for Toxicology. As such "Toxicology has been defined on the Corpus of Science devoted to the study of the harmful effects produced by chemical compounds on living Organisms[7]". C.P. Gebrs[8] has much of work on the subject including the review of Environmental Contamination and Toxicology. Loomis[9] classifies Toxicology into Environmental, Clinical & Forensic. There being other branches like Analytical Toxicology, Behavioural Toxicology, Aquatic Toxicology, Industrial Toxicology, Corporative Toxicology, Speculative Toxicology, Ethnic Toxicology, Immuno Toxicology, Wild Life Toxicology and Genetic Toxicology. However, modern Toxicology is referred as Multi-Disciplinary divided into four disciplines namely –i) Environmental ii) Economic iii) Clinical & iv) Forensic. It may be noted that during the later quarter of 20th century Curtis Klaassen[10] added three more disciplines to Toxicology like Descriptive, Mechanise and Regulatory. Accordingly, it can be assumed that the subject of Toxicology and its study is expanding but yet is not given its due which it attained in its close relation with human health and crime detection.

Conclusion & Suggestions

The research topic leads to deductions that Industry, Environment and toxic emissions have a definite relation. The constitution makers seem to have been aware and government efforts are visualised in legislations. But serious concern remains in non-involvement of Toxicology for the purpose. There ought to be at least two or more Toxicologist in all such Environmental and Industrial bodies/boards to suggest preventive and curative measures including as an expert for grant or denial of Industrial Licence. The hazardous emissions from Industries including those from Transnational Corporations with Environment as carrier enters the human body causing deceases like Toxic Jaundice and like other diseases which in some cases become fatal causing death. In addition, the Transnational

Corporation like Union Carbide alone was cause of death of thousands of humans and lakhs became deformed and/or lost their normal immune system. Followed by other toxic emissions from like industries including oleum gas leak has become subject of environmental moments in India, besides for judicial adjudications. The laws to protect the environment emerged but not with sufficient sanction and/or Regulatory mechanism. The law stood easy to breach whosoever desired to do so which warrants effective measures for implementation. The Industrial activity is need for development but not at the cost of disease by poisonous emissions. The Medical Jurisprudence though entered in Toxicology as early as 19th Century but by now it is forced to expand its horizons to cover Industrial and Environmental Toxicology and its affect on Human body for causation of disease or death. Need for integration of Environment, Industry and Toxicology and a corresponding Expert Board thereto seems one among other answers. Now the time has approached to address the issues, lest it may not be late.

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Source of Funding: From own resources.

Ethical Clearance: The approval of Institutional Ethics Committee is not required as the research does not involve any in-vivo study.

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A Review of India's Preparedness to Tackle the Covid19 Crises?

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Abstract

The novel Coronavirus, first appeared in Wuhan province of China in late December 2019, and gripped the whole world in 2020, growing to be a full-blown pandemic and changed every individual's life completely. India recorded its first Novel Coronavirus case on 30th January 2020 in Kerala, a student from Wuhan, China. In this article, the question about whether India is ready to tackle the crisis, i.e., Covid-19, was answered. Three mathematical models were reviewed and then compared to the number of beds available in the country. The third mathematical model seemed to model the country's curve much closer than the second one. The first one's prediction actually came out to be true. Furthermore, the paper discusses the health infrastructure in the country and the strategies adopted by states to prevent the spread. It talks about two specific states Kerala and Maharashtra and how the country needs to deal with the crisis. It is important to note that according to the models and numbers currently, India is geared, However, if precautions are not taken the situation can get much worse. This paper also discusses the fatality rates currently in India and where we stand for treatment. Currently, the treatment protocols keep changing from time to time.

Keywords: *Covid-19, Mathematical Modelling, Health Infrastructure, Future of India*

Introduction

The novel Coronavirus, first appearing in Wuhan province of China in late December 2019, gripped the whole world in 2020, growing to be a full-blown pandemic. As of 5th July, 11 million cases and about 5 lakh deaths were recorded worldwide[1]. The virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes or contact with contaminated surfaces, so it is important to maintain proper hygiene and respiratory etiquette. However, A disease causing pathogen is transmitted based on its infectibility, which depends on the R-naught value i.e., the number of persons who can possibly contract the virus from an infected person on an average. And, in case of COVID 19, it is found to be between 1.4 and 2.8 [2]. Community transmission being the worst-case scenario, where the source of the infection cannot be traced, and if unchecked, can easily turn to an endemic in the region. While studies have been going on about effective treatment and vaccines, behavioral intervention is the preventive measure that can be taken by everyone, and screening and testing by the government. India recorded its first Novel Coronavirus case on 30th January 2020 in Kerala, a student from Wuhan, China. A national lockdown was imposed on March 25th to curb the spread. However, starting from the first case, the country has recorded more than 6 lakh cases, placing itself in the top ten worst affected countries.

The corona virus has affected everyone's lives in a massive manner. With the case count growing everyday, it is important to answer questions like; Will we have enough beds and ventilators for critical cases in the future? Will some states be worse than the others? What precautions should each state take? When will all this be over? This paper sheds some light into these questions.

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It also looks at predictions of various mathematical models, extrapolates those peak numbers and compares them with the number of beds available in the country. Furthermore, it discusses some of the strategies taken by various states.

Materials and Method

In this review article, a lot of mathematical modelling papers from various online publishing sources were reviewed where the Covid-19 peak case count in India was predicted. It is important to note that all of these papers used different types of mathematical models and also had different data available to them at the time of publishing. Different conditions and assumptions were taken into account for each model. Specifically, three papers and the mathematical models were chosen to be reviewed. After extrapolating data from these papers and manipulating them, they were compared to the number of beds available in India and the main question whether India is prepared to tackle the crises was answered.

Study Design

I. Stochastic Mathematical model - April 2nd, 2020 [3]

This model uses a classic SEIR type model which divides the population into four compartments: Susceptible, Exposed, Infectious, and Recovered. Furthermore, the model then uses simple mathematical differential functions on Matlab to predict the covid curve for India. This paper really focuses on the exponential nature of the virus. It also compares the difference of the impact of lockdown on the case count of covid 19. It gives the case counts based on the effectiveness of India's lockdown.

II. Gaussian Mixture model - June 16th, 2020 [4]

In this paper, the author uses two mathematical methods to predict the curve for India. Data from the second Fourier based model was focused on. This model takes previous data for 14 days into account and predicts data for the future based on the previous data. Certain parameters were used for different countries in this model.

III. Regression Analysis - June 22nd, 2020 [5]

Finally, this paper also was based of covid 19's exponential behaviour. For this study the R-naught(reproduction) value was around 1.4 - 3.9. This model was applied to two countries, China and Korea, who are much further ahead as the coronavirus hit them earlier. The model seemed to fit both the countries and was adapted with specific constants for India. It is important to note that this model was also a sort of compartmental model as paper I. However, it was written a lot after with a lot more data.

Results

I. Stochastic Mathematical model - April 2nd, 2020

Even though this model was written a while ago i.e., there was not enough data at the time. It predicted that by 25th May, 2020 if 50% of the people quarantined, there would be around 241,000 cumulative cases in India. That date has already passed and on 25th May, India actually had a total of 144,000 cases[6] which means that more than 50% of the people quarantined and helped us flatten the curve.

II. Gaussian Mixture model - June 16th, 2020

The fourier model in this predicts that, there will be around 1.1 million cases at the peak

of the curve. It seems like this model does not accurately represent the current situation in India as we are nearing 1 million cases and the case count is still increasing at a staggering rate everyday[6].

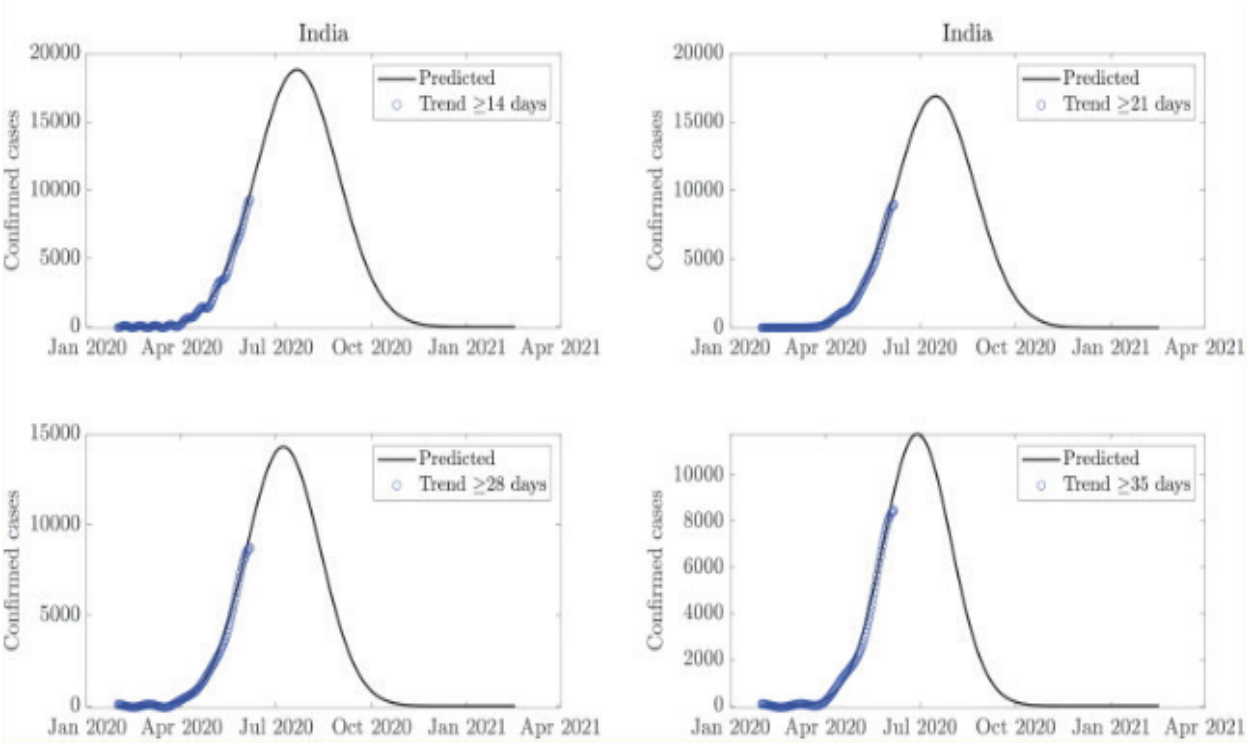

Figure 1 : Trend estimation using FDM[4]

III. Regression Analysis - June 22nd, 2020

Finally, the model that was derived from other countries and seemed to fit their data

estimated that the peak cases in India would be around 2.4 million cases. This makes a lot more sense mathematically.

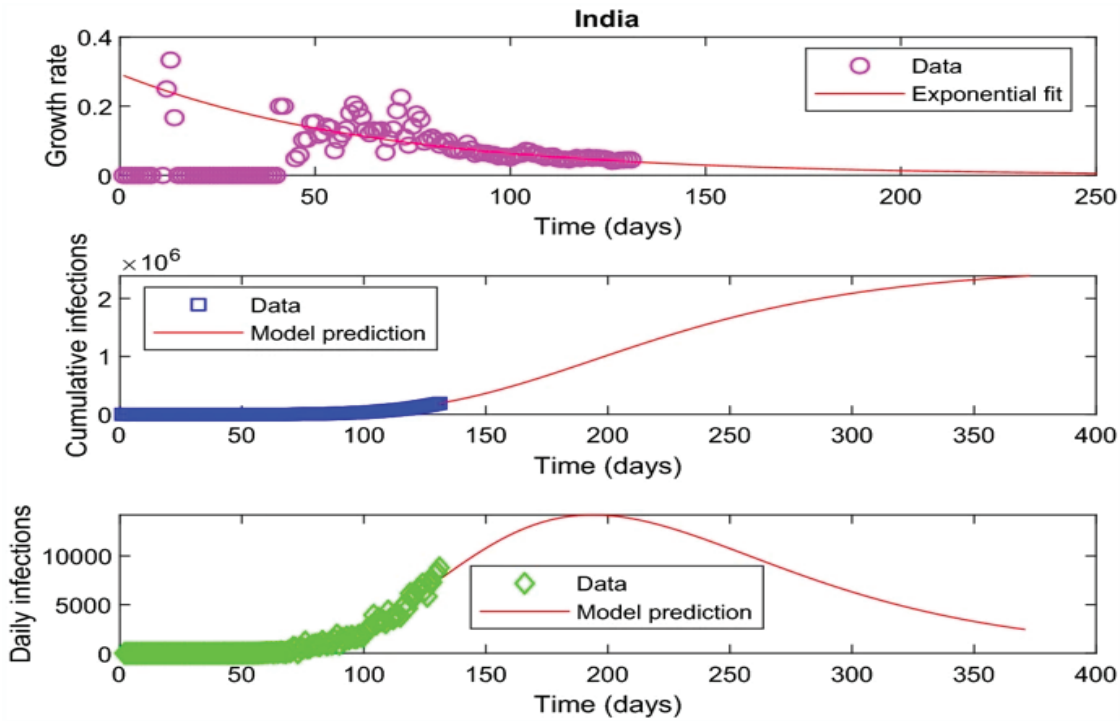

Figure 2: Forecast for India based on the third model[5]

Discussion

The predictions from the first test were pretty close to the numbers in the sense that we know that a lot more people quarantined at the start due to the restrictions set by the Government of India. The case count was a lot lower than what was predicted. The second study predicts about 1.1 million cases which seems a bit off now that the cases are going up whereas the model predicts that they should have already reached their peak and must be going down by mid-July. Lastly, the third study is the value that needs to be compared to the number of beds available as it seems to make a lot more sense compared to the current trend.

Table 1: Summarizing all the results

Study	Peak Case Count	Date written
Stochastic Mathematical model	241,000 - by 25th May if 50% people quarantined	April 2nd, 2020
Gaussian Mixture model	1.1 million	June 16th, 2020
Regression Analysis	2.4 million	June 22nd, 2020

According to recent Indian Government based covid-19 data, only 6-7% of the cases need hospitalization out of all the cases[7]. This implies that at the peak of 2.4 million we would need about 2,00,000 beds rounded off. To get an actual comparison of how well prepared the country is we need to understand India's health infrastructure first.

Health Infrastructure

For many poor households in India, public healthcare is the only available option since private healthcare is too expensive. And, isolation wards are required for the confirmed positive patients and intensive care for the critical patients. So, it is important to assess the medical capacity to formulate how to go about handling the situation.

The public health facilities used for COVID management were classified into 3 categories:

1. Dedicated COVID Hospital (DCH) - for the clinically assessed severe cases

2. Dedicated COVID Health Centre - for the clinically assessed moderate cases

3. Dedicated COVID Care Centre - for the mild or suspect cases. These are mainly make-shift facilities set up in hotels, hostels, stadiums etc.

According to the National Health Profile 2019, there are a total of 7,13,986 government beds available in India, i.e., 0.55 per 1000 population. As for the elderly population, the availability of beds is 5.18 per 1000 population [8].

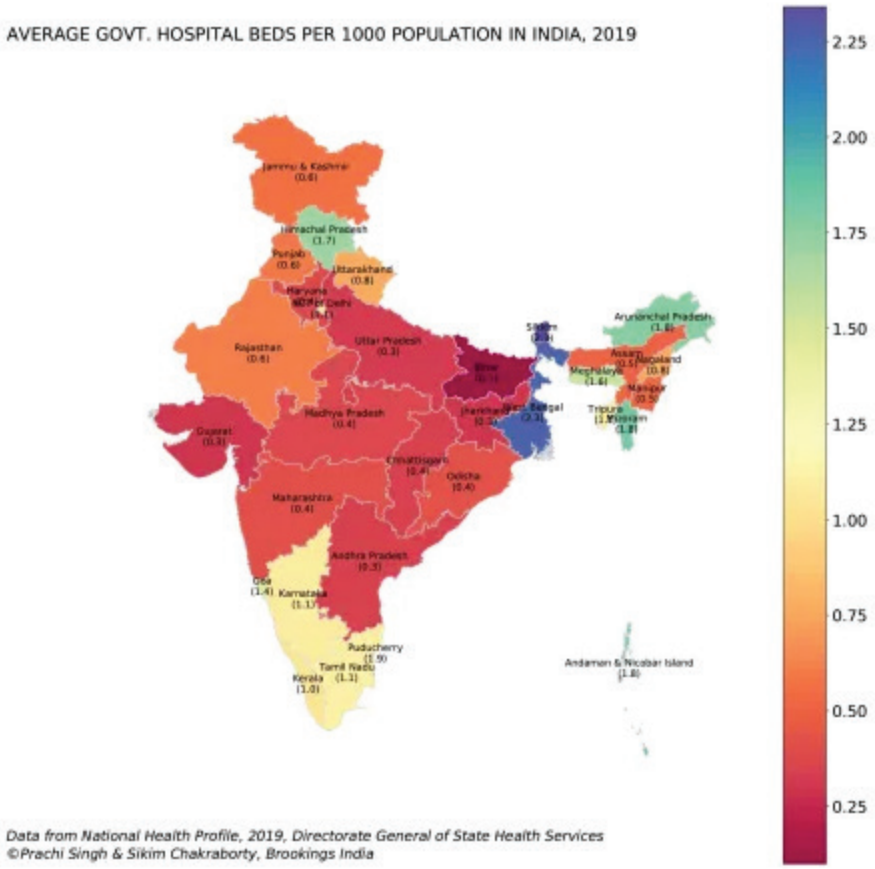

Figure 3: Heat map of government beds in India [8]

From the maps, many states lie below the national average of 0.55 per 1000 population. Bihar has a shortage with just 0.11, whereas Sikkim, West Bengal and Delhi seem to be better off with 2.34, 2.25 and 1.05 per 1000 population, respectively.

The availability of government beds is extremely low and an epidemic can make the situation much worse. Though measures are being taken to contain the spread, and early detection and treatment is supposed to prevent the cases from getting critical, the supply of beds and intensive care equipment like ventilators, ICU and oxygen support need to be amped up. Also, the government can coordinate with the private healthcare to meet the needs.

Adding to the stress on health infrastructure is the shortage of PPE kits for the healthcare workers. The lockdown imposed by many countries interfered with the imports and so, the government decided to stop exports to make sure there was adequate supply for the country, however, the demand far exceeds the supply. Shortage of PPE kits and other protective equipment is a matter of growing concern as it puts all the healthcare workers at great risk.

Finally, we extrapolated based on the third model that we need around 2,00,000 beds. It is

important to note that this is based on the current statistic, that only 6-7% of the people get hospitalised. Once the cases rise, the demand will become much higher which puts our healthcare system under a lot of pressure. We need to ensure that this does not occur and keep, "flattening the curve," so as to not put any pressure on the Indian healthcare system.

Discussion II - Where is India at right now?

Population, poverty, illiteracy and an overwhelmed

health care system are the main worries that prove to be deciding factors in this battle against COVID 19. The nationwide lockdown, a historic decision put into action on March 24th 2020, was the first step taken by the country to fight this, and thereby moving forward with a 'cluster containment strategy' to contain the disease within a defined geographic area by early detection of cases, breaking the chain of transmission and thus preventing its spread. The 21 day lockdown was first extended to May 3rd and then to May 18th. While there was still a steady rise in the number of cases and fatalities, the growth rate was lower than the other affected countries. Studies also show that the results of the lockdown can easily be negated if people go back to normal activities post lockdown, showing the need for measures of suppression post lockdown.[9] On June 8th, the country took a tentative step out of a 75 day lockdown, opening up malls, restaurants, religious places and offices with strict measures limiting the number of people, use of masks, social distancing and mandating the sanitization of premises.

In a country like India with a population of 1.2 billion, with a major sector being migrant workers and daily wage earners, the lockdown resulted in millions of workers returning home by foot or by train. This showed a steady rise of cases in countries like Assam, Chhattisgarh, Uttarakhand, Himachal Pradesh, with most of the new cases to be people who were travelling back from work places around the country. One important factor in deciding what will help our country is how rapidly we test people around the nation. It will help isolate breakouts and warn people that are affected by the virus to not spread it more.

Testing

India's testing strategy changed multiple times throughout the past 6 months. And in an attempt to ramp up the testing, ICMR approved a total of 1049 laboratories, both public and private. However, the access to testing still remains a huge challenge in many parts of the country. Approval of the use of point of care rapid antigen test, in containment zones as well as hospitals is also aimed at increasing the outreach of testing, thereby reducing the spread since 'test-track-treat' is the key strategy to contain the pandemic.[10] The average testing rate as of June 15th was 4972 per million, Ladakh having the highest number of testing, followed by Goa, Jammu and Kashmir and Delhi.

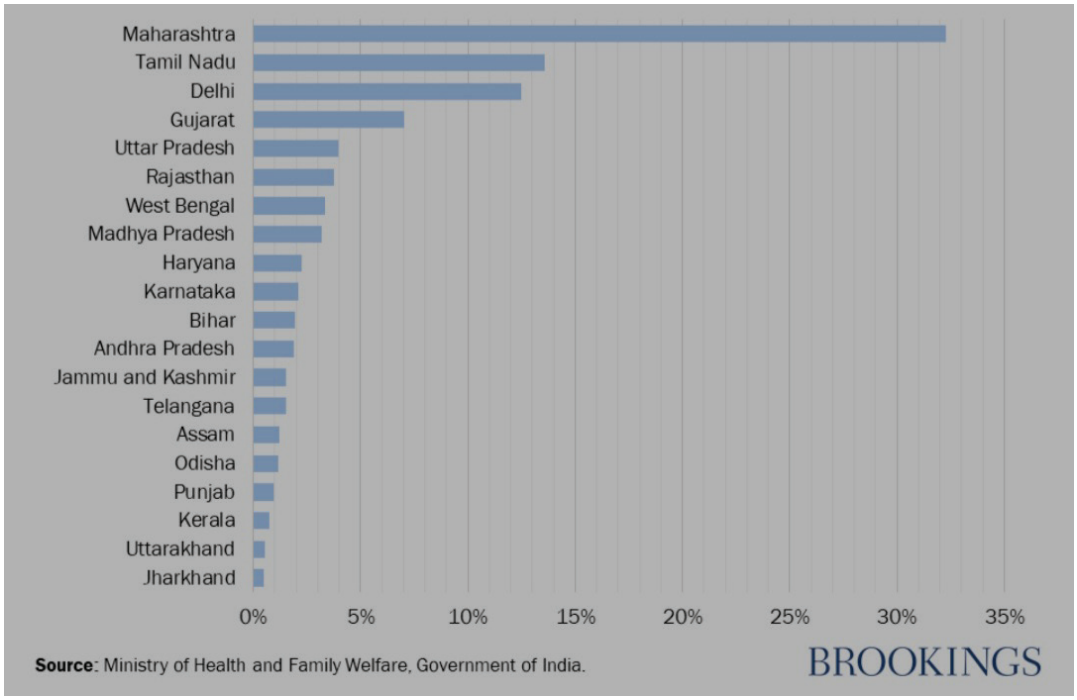

Figure 4: States with the greatest share of total COVID-19 cases in India as of June 15, 2020

Though Maharashtra seems to have the highest number of cases, it's testing rate stands at just 5445 per million, indicating the need to test more and control the spread.[11] The state recorded 6330 new cases as of July 2nd , and also recorded its highest number of recovered and discharged at a count of 8018 on the same day.[12]

There is also the problem of cases not being added in the state database by considering they are not native or residents of the area.[13] This can grow to be an even bigger problem and the authenticity of the numbers published can be raised. These stray cases need to be identified, recorded and treated appropriately.

At the same time when compared to the testing rates of 20 other countries most affected by the virus, India ranks in the bottom quartile with 5.63 tests per 1000 people till June 26th.[14] However, the testing rate has increased during the course of the pandemic, and if the trend keeps up, India could move up on the list, but the testing needs to be ramped up extensively to keep up with the surge in cases per day to contain the disease.

Strategies Adopted by Different States Kerala

The state which first detected the virus in the country in late January, declared a health emergency after 2 more cases were recorded on February 2nd and 3rd. Surveillance and screening of all incoming passengers from China and people who were in close contact with the travelers was the first response. With the increase in cases, the state ramped up the testing, contact tracing and strengthened the surveillance and control measures against the disease. The state also built thousands of shelters for the migrant workers stranded due to the nationwide shutdown, and established COVID care centers in all districts to accommodate the non-residents. Some of the key strategic interventions that helped keep the disease in control are the strong community engagement and addressing the psychosocial needs of the vulnerable population. As of June 30th, the state had sent 171,846 samples for testing, and in addition samples were collected from health care workers as a part of sentinel surveillance, to assess community transmission.[15] The experience and learnings of the state from the past Kerala floods of 2018 and the NIPAH outbreak in 2019 proved to be useful to prepare in the fight against COVID. Furthermore, the state also extended the COVID regulations – to wear masks, maintain social distancing and avoid public gatherings till July 2021, to cut the chain of transmission and to reduce the spread.

Maharashtra

Home to India's largest city and financial capital, Mumbai, the state recorded its first case on March 9th, and is now a red zone accounting for 32% of India's cases and 41% deaths.[16] The state slashed the COVID testing rates by private labs providing relief to people. The government also implemented an order in April, making it compulsory to wear a mask outside, and anyone violating the order would be fined or arrested.

However, the battle seemed to be not just against the virus but also other deep seated issues which were blunting the government's efforts. The health care system was found to be overwhelmed and burdened with having to choose between patients to save due to the shortage of beds and ventilators, and also there seemed to be piling up of cases that died due to COVID, with families not willing to take them being afraid of the infection.

The state also houses one of the largest slums of Asia, Dharavi, where 80% of the population depends on community toilets and 8-10 people live in a house all in narrow lanes with 2-3 storied houses, social distancing can only be considered a luxury with no possibility of effective home quarantine. The first case was confirmed in the beginning of April and by the end of the month, there were about 491 cases with a doubling period of 18 days. The BMC was quick into action to contain the spread, and the main components were effective containment strategy, conducting comprehensive testing and ensuring uninterrupted supply of essentials to the community. Also, quarantine facilities were set in colleges and nature parks and 90% of the patients were treated in Dharavi and only the critically ill were moved out. The proactive measures taken successfully reduced the growth rate to 4.3% in May and 1.02% June.[17]

Case Fatality Rates

Case fatality rate, ratio of confirmed deaths to confirmed cases. It is usually used as a measure of the severity of a disease, and also to predict its prognosis, where high rates indicate that a high fraction of COVID affected patients are succumbing to the disease. CFR has

seen to be increasing in most of the affected countries throughout the course of the pandemic. The rates in India went up from 1.9 in February, to 3.6 in March and then declined to 3.2% in April.[18] However, when compared to other South Asian countries, India has a significantly high CFR, which highlights the limitations of the healthcare strategies like care of critical patients, early detection of cases, proper triage etc.

Another concept, Lag case fatality rate which takes into consideration a 14 day delay in reporting COVID 19 deaths, is being looked into since CFR data only includes deaths till a particular date and there is usually some delay in reporting.

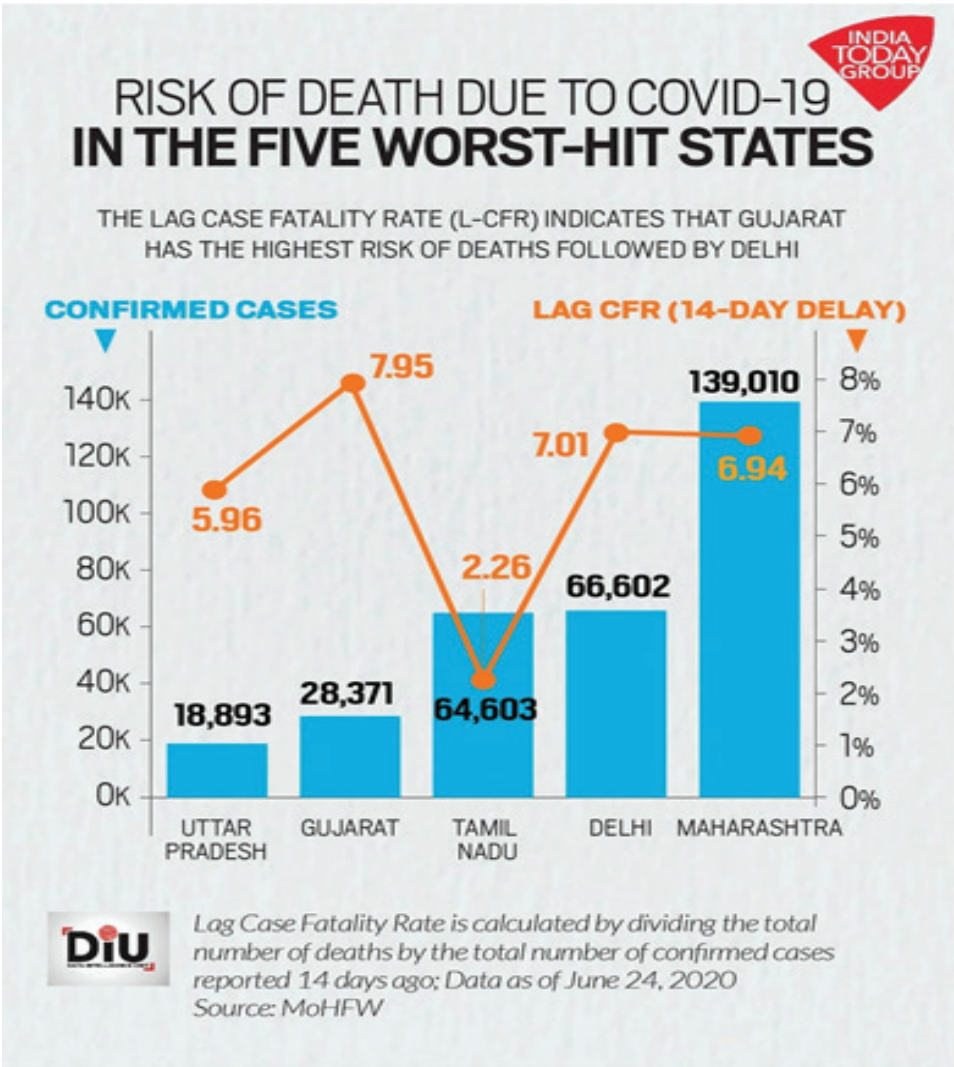

Figure 5: Risk of death due to COVID-19 in the five worst hit states

The disease wise fatality rate of COVID 19 calculated based on any comorbid disorders present, indicated that diabetes, cardiovascular ailments, hypertension, respiratory ailments and kidney diseases were the highest cause of COVID related death, in the order mentioned. And around 31.8% deaths without any underlying health condition, indicating how everyone needs to be vigilant and not just the elderly or the ones with underlying health conditions.[19]

Treatment[20]

The treatment protocol keeps changing from time to time based on new evidence. The various drugs Indian Council of Medical Research (ICMR) approved are:

1. The use of Hydroxychloroquine for chemoprophylaxis in asymptomatic health care workers treating confirmed cases of COVID 19 and also asymptomatic household contacts of confirmed cases.

2. Remdesivir – for patients in a moderate stage (on oxygen)

3. Tocilizumab – for moderate cases with increasing oxygen demands, and also for mechanically ventilated patients showing no improvement with steroids.

4. Dexamethasone – as an alternative to methylprednisolone in moderate to severe cases. The drug was shown to have benefits in critically ill patients and also reduced mortality by one third for patients on ventilators and one fifth for patients maintained on oxygen therapy.

5. Convalescent Plasma Therapy – is used as a post exposure prophylaxis where plasma containing neutralizing antibodies collected from a previously infected and cured patient is transfused into infected patients. It is considered in moderate cases not improving despite the use of steroids. To ease access to plasma, India got its first plasma bank set up in Delhi on July 4th.

Ayurveda is a medicine system originating from India. This type of system believes that prevention is better than cure. It includes eating a healthy diet with various herbs and doing specific exercises related to our respiratory system to build immunity against COVID-19.[21] Prevention is definitely better than the cure and one of the only ways to fight against covid until the vaccine is made.[22] Additionally, the role media plays is very important in keeping us connected in times like this. However, there is a lot of misinformation going around on social media that hinders people from preventing covid correctly. It is important to ensure the information we get about preventive measures from trustworthy sources with a proper scientific background.[23] Furthermore, we need to ensure no other diseases originate like obesity during quarantine due to lack of exercise. So it is important to maintain a healthy diet with a specific caloric intake. The diet will also help in building immunity against Covid 19. [24]

Conclusion

In conclusion, the first study showed that more than 50% of the people quarantined and India managed to flatten the curve. The second study seemed to miss the mark however showed good early data. The third was used to compare the number of beds and serious cases predicted and see how geared India is. Furthermore the strategies from a couple different states were analyzed to discuss what measures we are taking currently to flatten the curve. This review article shows that India's healthcare is prepared but that is just based on theoretical numbers. People need to be cautious and take preventive measures otherwise, the situation could get out of hand.

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Sociodemographic Profile and Pattern of Injuries Due to Fall From Height in a Tertiary Hospital of South India- A 2 Year Retrospective Study

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Abstract

Globally, falls from height (FFH) are a substantial public health peril and are among the foremost causes of serious and fatal injuries sustained to a victim. Falls are the second leading cause of accidental or unintentional injury deaths worldwide. This retrospective study consisting of 182 cases was undertaken to study the sociodemographic profile and injury pattern in victims of fall from height for a period of 2 years . Males outnumbered the females (3.04:1). Most of the victims were construction workers (43.40%) by occupation . Most of incidents 117 (64.28%) took place in semi urban area and 45 (24.72%) victims occurred in the Urban locality. 49.45% of the cases (90) occurred in the summer season.37.91% of the events were seen between 12 Noon to 6 PM. In majority of cases, side of the body was the primary site of impact (25.27%) followed by head injury (21.43). 12.08% of the cases turned fatal. Implementation of adequate safety measures would effectively reduce the incidence of mortality and morbidity associated with fall from height .

Keywords: *Fall , height, accident, construction, head injury, sociodemographic profile*

Introduction

A fall is defined as an event which results in a person coming to rest inadvertently on the ground or floor or other lower level. An estimated 6,46,000 fatal falls occur each year, making it the second leading cause of unintentional injury death, after road traffic injuries. Over 80% of fall-related fatalities occur in low- and middle-income countries, with regions of the Western Pacific and South East Asia accounting for 60% of these deaths. In all regions of the world, death rates are highest among adults over the age of 60 years. About 37.3 million falls occur each year that are severe enough to require medical attention. Such falls are responsible for over 17 million DALYs (disability-adjusted life years) lost. The largest morbidity occurs in people aged 65 years or older, young adults aged 15–29 years and children aged 15 years or younger.[1]

According to the Accident and suicide statistics of National Crime Records Bureau (NCRB) for the year 2019, a total of 12048 cases of fall from height were registered in India. Out of this, 11997 cases were fatal with male : female ratio being 5.4:1.The burden was seen more in victims belonging to age groups between 30-45 years of age. [2]

Many factors affect the mortality and morbidity of falls, such as age of the victim, height of fall, cause of fall, type of ground on which the victim fell, and body parts injured. This study was aimed to analyze the demographic characteristics of victims of falls from height , the injuries sustained and their effects on morbidity and mortality.

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Methodology

A retrospective study of 182 cases due to fall from height for a period of 2 years, from January 2018 to December 2019 was studied in the Department of Forensic Medicine and Toxicology, Saveetha Medical College and Hospital, Chennai. Data pertaining to sociodemographic profile like age, sex, occupation, location of accidents, season , socio-economic status and injury patterns was collected from the records from the emergency medicine and medical records departments related to victims of fall from height. The obtained data was entered in excel sheet and analyzed using appropriate statistical tests using SPSS Software Version 24.

Observations and Results

Out of 182 victims studied, Males (137) outnumbered the females (45) in the present study, with the ratio being 3.04:1. The mean age in the present study was 30.11 years. Majority of cases were in 21-30 years of life (47.25%), followed by cases between 31-40 years (17.04%) and 11 -20 years (12.09%) of life respectively. Least number of cases was reported in the geriatric age group (more than 70 years of age) followed by 1st decade of life. The only age group where females outnumbered the males was in the age group between 41-50 years. However, the maximum incidence of female patients was in 21-30 years of life. The youngest was a baby of 1 year and the oldest being 71 years. [Table 1]

Majority of victims in the present study were construction workers (43.40%) followed by students (26.38%) and housewives (8.24%). Among the study population, retired workers (3.29%) were the least in number. [Fig 1]

Maximum cases were noted between mid noon to 6 pm (69, 37.91%) followed by that between 6 PM to midnight (40, 21.98%). 39 (21.43%) cases were noted between 6 am to 12 noon and from midnight to 6 am, 34(18.68%) of them sustained injuries. The maximum cases presented themselves from semi urban locality, 117 (64.28 %) followed by those which occurred in Urban area, 45 (24.72%) and a very minimal case belonged to the village accounting to 11 % (20) cases. In this retrospective study patients sustained injuries most commonly in summer season 90 (49.45%) followed by winter season 63 (34.62%) and rainy season 29 (15.93%). According to Modified Kuppuswamy scale 2020, majority of the cases belonged to upper lower socioeconomic class 85 (46.70%), followed closely by lower middle socioeconomic class 40 (21.97%). The least numbers belonged to Upper socioeconomic status 9 (4.95%). [Table 2] Injury to the side of the body was seen in 46 victims (25.27%) followed by head region in 39 cases (21.43%). [Fig 2]

Among the patients who sustained injuries to thorax, soft tissue injuries (19.78%) was the maximum followed by fracture of ribs (17.58%) and fracture of sternum (7.69%). Soft tissue injuries to abdomen (24.18%) were seen in majority of the cases followed by splenic rupture (14.29%) and injury to liver (12.64%). Fracture of pelvis had the least incidence (2.20%). Among the patients who sustained injuries to head and neck, soft tissue injuries (26.37%) was the highest followed by intracranial hemorrhages (19.78%) and skull fracture (17.03%). [Table 3,4,5] In this study, mortality was seen in 22 cases.

TABLE 1: AGE AND GENDER WISE DISTRIBUTION

AGE IN YEARS	MALE	FEMALE	TOTAL	RATIO
0-10	1	1	2	1:1
11-20	17	5	22	3.4:1
21-30	69	17	86	4.06:1
31-40	25	6	31	4.16:1
41-50	4	11	15	0.36:1
51-60	13	3	16	4.33:1
61-70	7	2	9	3.5:1
71-80	1	0	1	
TOTAL	137	45	182	3.04:1

TABLE 2: PROFILE OF FALL FROM HEIGHT INJURY CASES (N=182)

CHARACTERISTICS		NO. OF CASES	PERCENTAGE (%)
TIME OF ACCIDENT	12:01AM-06:00AM	34	18.68%
	06:01AM-12:00PM	39	21.43%
	12:01PM-06:00PM	69	37.91%
	06:01PM-12:00AM	40	21.98%
SEASON	SUMMER	90	49.45%
	MONSOON	29	15.93%
	WINTER	63	34.62%
PLACE OF ACCIDENT	URBAN	45	24.72%
	SEMIURBAN	117	64.28%
	RURAL	20	11%
SOCIO ECONOMIC STATUS (MODIFIED KUPPUSWAMY SCALE 2020)	UPPER CLASS	9	4.5%
	UPPER MIDDLE CLASS	32	17.58%
	LOWER MIDDLE CLASS	40	21.97%
	UPPER LOWER CLASS	85	46.7%
	LOWER CLASS	16	9.25%
PARTS OF THE BODY INJURED ON PRIMARY IMPACT	UPPER LIMB	14	7.69%
	LOWER LIMB	29	15.94%
	HEAD AND NECK	39	21.43%
	FRONT OF THE BODY	36	19.78%
	BACK OF THE BODY	18	9.89%
	SIDE OF THE BODY	46	25.27

TABLE 3 : DISTRIBUTION OF STUDY POPULATION ACCORDING TO INJURIES TO THORAX

SL. NO.	PRIMARY IMPACT	NUMBER OF CASES	PERCENTAGE
01	Soft tissue injuries	36	19.78
02	Fracture of ribs	32	17.58
03	Fracture of sternum	14	07.69
04	Lungs	10	05.49
05	Heart	08	4.40
06	Diaphragm	07	3.85

TABLE 4 : DISTRIBUTION OF STUDY POPULATION ACCORDING TO INJURIES TO ABDOMEN

SL. NO.	PRIMARY IMPACT	NUMBER OF CASES	PERCENTAGE
01	Soft tissue injuries	44	24.18
02	Fracture of Pelvis	04	2.20
03	Spleen	26	14.29
04	Liver	23	12.64
05	Kidney	16	8.79
06	Intestines and mesentry	08	4.40
07	Fracture of lumbar vertebrae	07	3.85

TABLE 5 : DISTRIBUTION OF STUDY POPULATION ACCORDING TO INJURIES TO HEAD AND NECK

SL. NO.	PRIMARY IMPACT	NUMBER OF CASES	PERCENTAGE
01	Soft tissue injuries	48	26.37
02	Fracture of Skull	31	17.03
03	Intracranial haemorrhages	36	19.78
04	Fracture of facial bones	05	2.75
05	Fracture of cervical spine	09	4.95

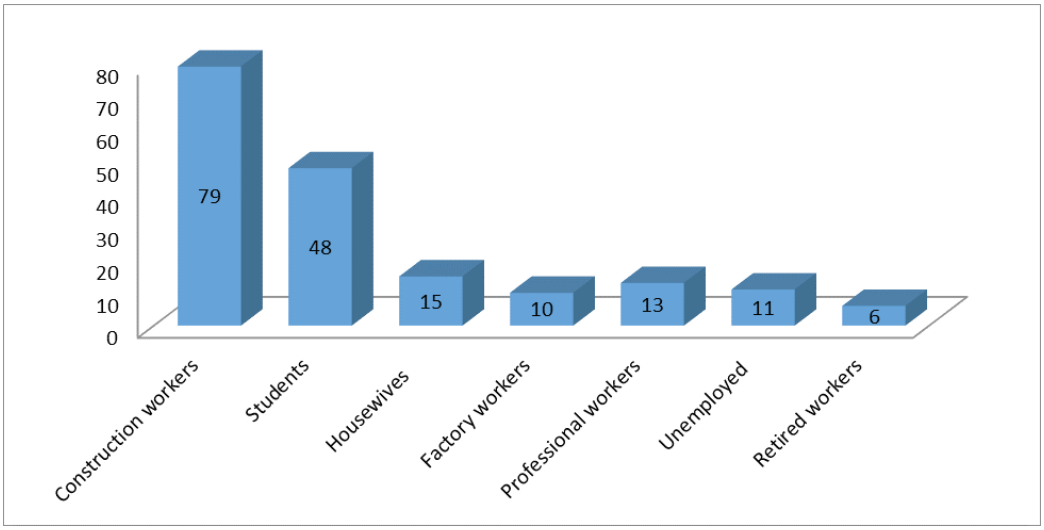

FIG 1: DISTRIBUTION OF STUDY POPULATION BASED ON OCCUPATION

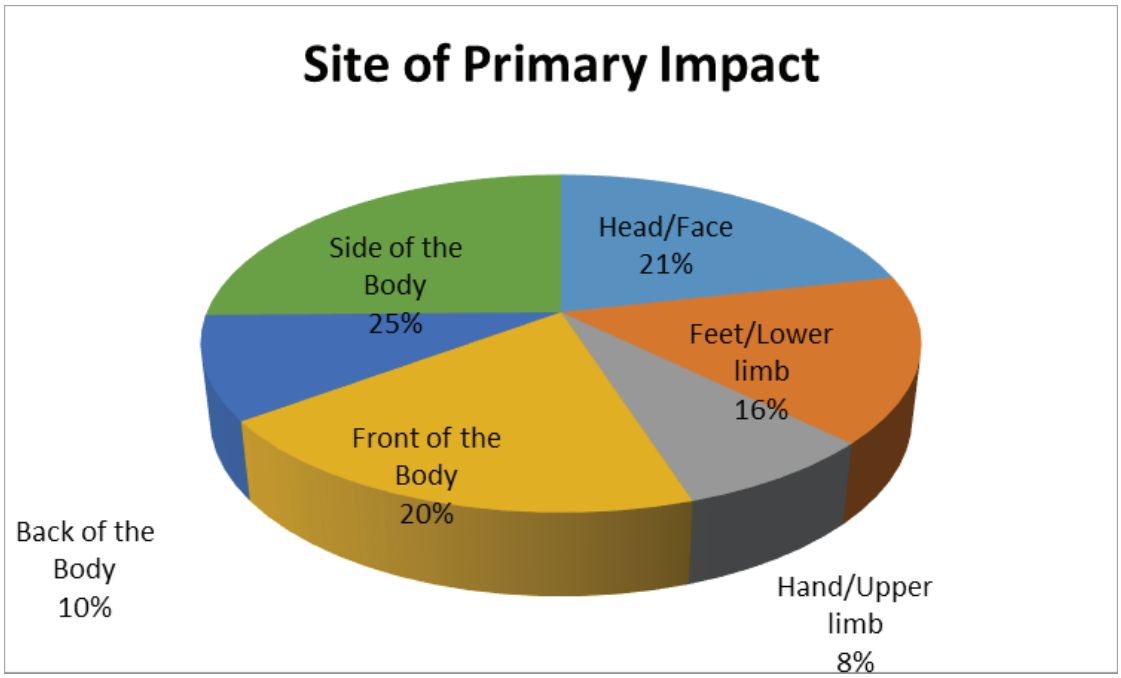

Fig 2: DISTRIBUTION OF INJURIES BASED ON SITE OF PRIMARY IMPACT

Discussion

This study was undertaken to assess the injury patterns of fall from height cases at Saveetha Medical College and Hospital, Chennai. 182 cases who presented over a period of 2 years were retrospectively analyzed.

The victims of fall from height in the present study ranged from 1 to 71 years of age. Majority of victims belonged to 21-30 years of age which was similar to that of the study by Jagannatha SR et al.[3] This may be probably due to reduced work efficiency and expertise in younger age group. Also, younger people with their stressful and ambiguous lifestyles are more vulnerable to falls. However, this finding was dissimilar from the studies done by of authors who reported the highest incidence in 4th and 5th decades of life.[6, 7]

The results of the present study with male: female ratio of 3.04:1 is lesser than that of few authors [3,8]. This variation can be attributed to the regional variations of female and male population of a specific region. The high incidence in males could be due to them being the major breadwinner of the family thereby being exposed to more stress, strain and occupational hazards and also in comparison to females, they have greater amount of eagerness in handling the work at heights.

On looking at the occupation aspect, most of the victims were construction workers (43.40%) which are similar to findings of many authors. [3,4,5,9,10]. Chennai being a developing city, there are lots of emerging buildings employing workers and this could be the main cause behind the accidental falls involving construction workers. Non adoption of basic safety measures, poor working skills, careless attitude, and overconfidence in doing the unusual work could be the reasons causing fatal injuries. Poor sleep hygiene, depression due to excessive workloads, exertion due to rigorous physical activities and working at heights for long intervals are few other predisposing factors for fatigue causing injuries especially in the summer season during the mid noon time period.

Side of the body was the site of primary impact in most cases (25.27%) followed by head (21.43%), and front of the body (19.78%). This result is different from other studies where impact to head had the highest incidence. The present study showed intracranial bleeding in 19.78% cases. Subdural bleeding was the commonest amongst the cases with intracranial hemorrhages (63%). It was often associated with fracture

of skull .Fracture of spine was seen in 16 cases of the present study. Maximum number of cervical spine was noted in primary head impacts (4.95%). Fracture of the vertebrae was usually due to the jackknifing of the torso resulting in flexion/extension of the spine at more than one site. The present study revealed injuries to various parts of skeleton. Fracture of ribs was noted in primary thoracic impacts (17.58%).Regardless of the nature of primary impact, various injuries to internal organs like lungs, heart, liver, spleen and intestines are sustained. However maximum number of lung injuries was in the group of trunk impacts. Maximum incidences of liver injuries were seen in primary trunk and buttock impacts. Spleen was found ruptured in 14.29% cases. [3,8,10]

Conclusion

In conclusion, it was observed that accidental fatalities due to fall from height are mainly preventable. The severity of injuries largely depends on the height from which the person falls, the surface and area of impact, presence or absence of safety gears in case of workplace injuries and age. Head injury is the primary cause of mortality. Most of the victims were construction workers. Pre employment risk assessment should be made mandatory and safety gears like Nets, airbags, harnesses, helmets should be provided for the workers. Limitation of the study is that a small population was studied in a semiurban locality and extrapolation of the data to larger community may not bring out exact burden of the issue. In addition, assessment of substance abuse pattern was not determined due to lack of adequate data.

Conflict of Interest : Nil

Ethical Clearance: Obtained

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Original Research Article

A Cross Sectional Study to Assess the Knowledge, Attitude and Practice towards Voluntary Blood Donation amongst Undergraduate Students of a Medical College in North India

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Abstract

Background: The youth of the country especially college students can serve as a readily available pool of voluntary blood donors and help tide away some of the scarcity of blood and blood products. This study was conducted to determine the Knowledge, attitude and practice (KAP) regarding Voluntary Blood Donation among medical undergraduate studentsand to assess the factors which can lead to a subsequent increase in the same.

Method: A cross-sectional descriptive study was conducted amongst 500 undergraduate medical students studying at Government Medical College, Patiala, Punjab using a structured, self-administered questionnaire regarding KAP.

Results: The mean age of the 500 participating students was 20.46 years with a standard deviation of 1.5 of which 55.2% were females.71.2% and 61.4% students had correct knowledge regarding interval between blood donation for males and females respectively but the knowledge regarding common causes of deferral was less. Posters and pamphlets were the most common sources of information regarding blood donation.92.6% students had a good attitude towards blood donation. 127 (25.4%) students had donated blood previously and 17 (3.4%) of them had donated blood more than once in a year.

Conclusion: The students had a fair knowledge about VBD and the majority had a favourable attitude towards it. Steps should be taken to increase the awareness regarding VBD since early sensitisation towards its importance can motivate students to become voluntary blood donors, making them major contributors to the blood donor pool throughout their adulthood and help in overcoming blood shortage in the country.

Keywords: *Knowledge, Attitude, Practice (KAP), Voluntary Blood Donation (VBD), medical students*

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Introduction

Blood transfusion is an essential component of healthcareservices since blood and blood products are essential to care for people with traumatic injuries in emergencies, disasters and accidents; patients undergoing advanced medical and surgical procedures;

women during pregnancy and childbirth; children with severe anaemia due to malaria and malnutrition as well as patients with blood and bone marrow disorders or inherited disorders of haemoglobin and immune deficiency conditions.[1]Hence, the importance of blood can't be undermined in this modern era. Despite this fact, we see a dearth in the amount of the voluntary blood donors. WHO estimates that blood donation by 1% of a nation's population is generally the minimum needed to meet the nation's most basic requirements for blood.[2]As per the above norm, India's minimum demand for blood is around 13.1 million blood units (1% of 1.3 billion population). But as per a report by the National AIDS Control Organisation (NACO) India, in 2017, the annual collection of blood in the country was 11.1 million units.[3] This unmet need for blood not only affects the patients in critical need of blood and its components, butalso leads to a trend of paid and professional donors, who carry along with them a high risk of transmitting several blood borne infections called transfusion transmitted diseases (TTIs).

In such a grim scenario where there is a disparity between the amount of blood required and the amount of blood readily available for transfusion, adequate and reliable supply of safe blood can only be assured through a stable base of regular, voluntary and unpaid donors who are the safest group of donors because the prevalence of blood borne infections is lowest amongst them.[4]

In total, 79 countries collect over 90% of their blood supply; however 56 countries collect more than 50% if their blood supply from family/replacement or paid donors.[5] To overcome this situation and to progress towards WHO's goal of 100% voluntary blood donation, the youth of the country, especially the college students can play a major role in providing a large pool of potentially safe blood donors since they are usually healthy and more likely to be disease free.

Several studies have been undertaken in the past to assess the knowledge, attitude and practice of blood donation among college students.Such studies help in projecting the perceptions regarding blood donation among the youth of the country and help the policy and decision makers to take the correct measures to increase awareness about blood donation and promote voluntary blood donation in the country. This can help in overcoming the shortage of blood and its components and ensure that timely and safe blood transfusions can be provided to all the patients in need.

This study was conducted with the objective of determining the knowledge, attitude and practice (KAP) regarding voluntary blood donation (VBD) among medical undergraduate college students in Government Medical College, Patiala, Punjab, India and to assess the factors which can bring about a subsequent increase in the same.

Methods

Study design and Population**:** A descriptive cross sectional study was conducted among the undergraduate MBBS students of Government Medical College and Rajindra Hospital Patiala, Punjab, India, in the month of March 2019. The study population consisted of 500 MBBS students in the age group of 17-25 years from 1st, 2nd, 3rd and 4th professional years.

Pretesting of Questionnaire: A self-administered, structured questionnaire was developed by 5 Academicians. The investigator conducted a pilot study on 20 MBBS students to gain feedback on the overall acceptability of the questionnaire in terms of length and language clarity. Based on their feedback, the questionnaire did not require any corrections. These students were not considered as part of the data collection for the study.

Questionnaire: The questionnaire, designed to obtain the students' knowledge, attitude and practice towards voluntary blood donation consisted of four sections. It was based on blood donor selection and referral guidelines-2017 by National AIDS Control Organization (NACO), New Delhi; India and Transfusion Medicine Technical Manual by Directorate General Of Health Services (DGHS), Ministry of Health and Family Welfare, India.[6,7] Section I solicited general demographic details of the students. Sections II to IV consisted of multiple choice questions from which the students selected their responses. Section II consisted of 22 questions which assessed knowledge of the students regarding blood donation. Section III comprised of 4 questions, aimed to assess the attitude of the students towards blood donation. Section IV consisted of 6

questions regarding the practice of blood donation among the students.

Methodology: The questionnaires were distributed amongst the students who were requested to fill in a written consent form and explained briefly regarding the objectives of the study and asked to choose the single best answer for the questions from the multiple choices given. Confidentiality of personal data was assured to the participants.

Statistical Analysis: 500 completed questionnaires were then coded and spreadsheets were created for data entry using Microsoft Excel. The data was analysed using SPSS 20 (SPSS Inc., Chicago, IL, USA) Windows software program.

Results

A total of 500 students with the mean age of 20.46 ± 1.5 years participated in the study. Demographic data showed that 276 (55.2%) of the participants were females and 224 (44.8%) were males.

71.2% students had correct knowledge regarding interval between blood donation for males and 61.4% knew about the same for females.55.2% were aware about the donor weight eligibility criteria, whereas 89.8% had the knowledge about the age eligibility for blood donation. Majority of the students were not aware of the deferral period for common causes of deferral for blood donation like tattooing and ear piecing, antibiotic intake and dental manipulation with only12.6%, 12.2% and 6.4% of the students respectively being aware of the same. Only 18% of the students were aware of the average time taken for blood donation. (Table 1)

Table 1: Knowledge assessment regarding blood donation practice and donor eligibility criteria.

S.no.	Question	Correct Responses	Percentage
1.	Minimum time interval between two blood donations in males	356	71.2%
2.	Minimum time interval between two blood donation in females	307	61.4%
3.	Average time duration during a blood donation process	90	18%
4.	Age eligibility criteria for blood donation	449	89.8%
5.	Weight eligibility criteria for donation	276	55.2%
6.	Minimum quantity of blood donated at one time	223	44.6%
7.	From where is blood usually collected?	389	77.8%
8.	How long after alcohol consumption can a person donate blood	297	59.4%
9.	Number of components produced from a unit of blood	195	39%
10.	Duration for deferral after ear piercing or tattooing	63	12.6%
11.	Duration for deferral after Immunization with Hepatitis B immunoglobulin or rabies	115	23%
12.	Duration of deferral after dental manipulation	32	6.4%
13.	Component transfused in a person suffering from anaemia	298	59.6%
14.	Component transfused in a person with INR>1.5	231	46.2%
15.	Component transfused in a person with platelets<10000/µl	308	61.6%
16.	After how long can a person on antibiotics donate blood?	61	12.2%
17.	Knowledge about health conditions leading to permanent deferral	175	35%
18.	Knowledge about when a person can donate blood at Rajindra Hospital	392	78.4%

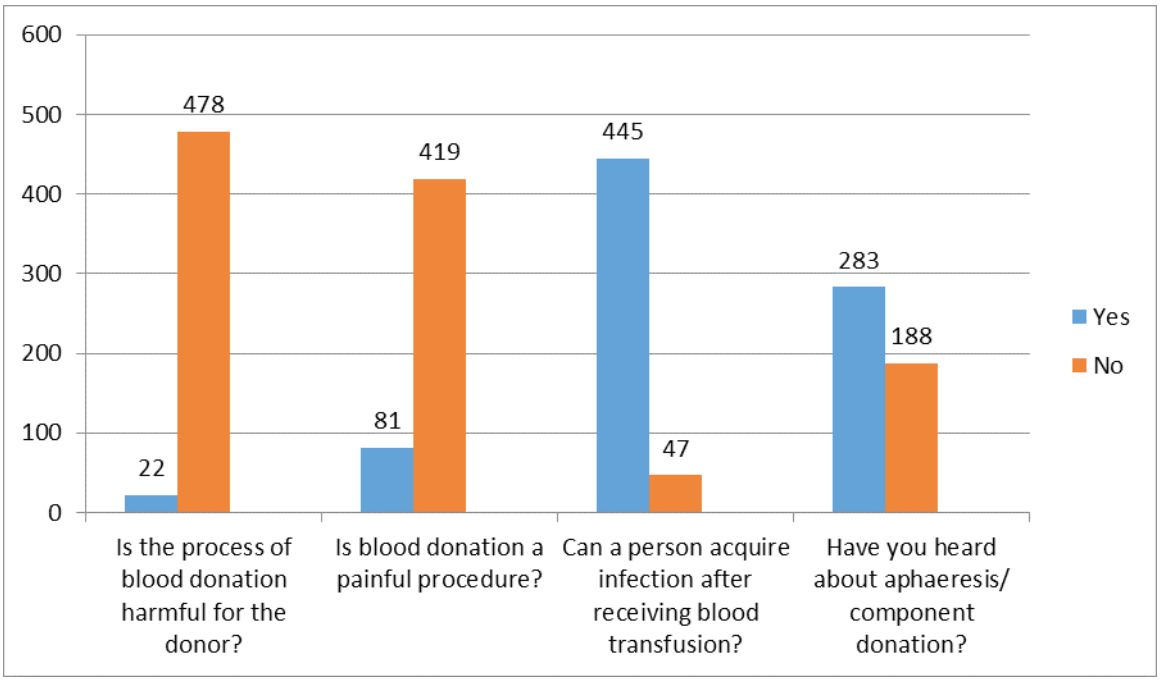

Figure 1: Knowledge regarding blood donation

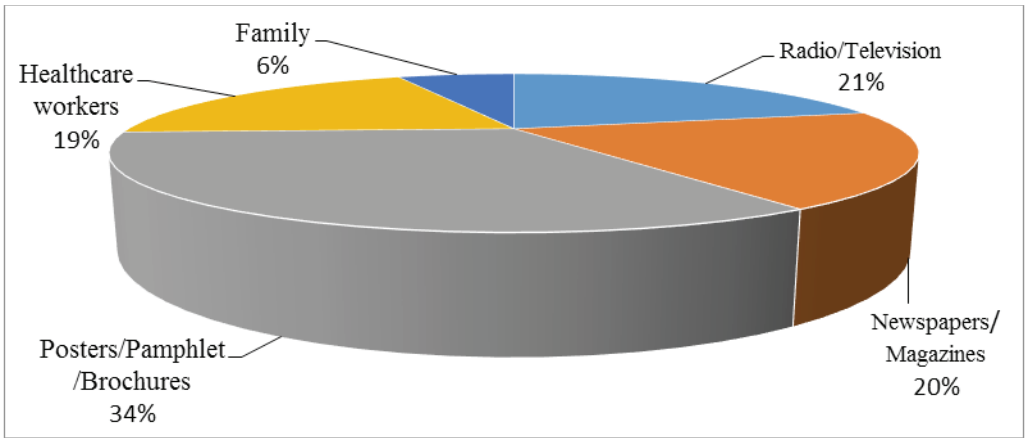

Figure 2: Sources of information about voluntary blood donation

On assessing the source of information regarding voluntary blood donation, it was found that most of the students (34%) knew about voluntary blood donation from posters and pamphlets followed by radio or television (21%), newspapers and magazines (20%), healthcare workers (19%) and only 6% had known about the same from a family member as depicted in Figure 2.

Table 2: Attitude of students regarding blood donation

S.no.	Attitude Based Questions	Response	Percentage
1.	What do you think about blood donation?		
a	Good	463	92.6%
b	Bad	27	1.4%
c	Neutral	7	5.4%
2.	What do you think is the best source of donor blood?		
a	Voluntary donors	444	88.8%
b	Replacement donors	24	4.8%
c	Paid/ Professional donors	14	2.8%
d	Don't know	14	2.8%
3.	What would you do if you were asked for blood donation to strangers/family members in dire need for blood?		
a	Would readily donate	321	64.2%
b	Would hesitate	28	5.6%
c	Depends on the situation	140	28%
4.	What was your reason for not donating blood?		
a	I didn't think of it	98	26.2%
b	Lack of opportunity	90	24.1%
c	Fear of needle/ fear of sight of blood	52	13.9%
d	Blood donation can lead to anaemia	28	7.5%
e	Lack of time	47	12.6%
f	No one has ever asked me to donate blood	38	10.1%
g	Objection from family members	20	5.3%

Attitude regarding blood donation

92.6% students had a positive attitude towards blood donation. Majority of the students (64.2%) were willing to donate blood to family members or strangers if needed. In our study, 88.8% thought that the best source of donor blood is a voluntary donor. The data indicates that the most common reason (19.6%) for not donating blood was that they had never really thought about it and 18.8% students had not donated blood due to the lack of opportunity. (Table 2)

Table 3: Practice of blood donation

S.no.	Practice Based Question	Yes	No
1.	Have you donated blood earlier?	127	373
2.	Do you want to be a regular voluntary donor?	301	199
3.	Have you previously been deferred for blood donation?	70	430
4.	Did you face any problem after blood donation?	28	99
5.	How often do you donate blood?		
a	One time in a year	40 (8%)	
b	More than one time in a year	17 (3.4%)	
c	Rarely	70 (14%)	
d	Never	373 (74.6%)	
6.	What was your reason for blood donation?		
a	Regular donor	17 (13.3%)	
b	Voluntary occasional donor	90 (70.8%)	
c	Donor to help friends/family	20 (15.7%)	

Out of all the students who had participated in this study, 127 (25.4%) had donated blood in the past and of these, 17 (13.3%) students had donated blood more than one time in a year in the past. 90 (70.8%) students had donated blood on a voluntary basis. 70 (55.1%) students had been previously deferred from blood donation.Out of those who had donated blood, 28 students i.e. 22% of the students had experienced some type ofan adverse donor reaction following blood donation. The findings are depicted in Table 3.

Discussion

Provision of safe blood and its components to all the patients in need of transfusion is a global concern. However, many people are ignorant of the blood donation process and fears and misconceptions regarding blood donation are major deterrents against voluntary blood donation. This can only be overcome by encouraging the

young and relatively healthy population of the country like the college students to become voluntary blood donors. Thus, if sensitized early in the course of their education regarding the significance of blood donation, they can set a good example for the society and by being blood donors themselves,can help motivate and encourage the masses towards this noble act and allay any fears or doubts that have taken home in the minds of the people. This can help in creating a crucial reservoir of safe blood for transfusion in the country. Thus, knowing the level of their knowledge, attitude, beliefs and practices regarding blood donation can help us in taking the correct measures for formulating better blood donation promotional activities, to encourage more and more people to come forward and donate blood.

In our study, majority of the students had good knowledge regarding the eligibility criteria for blood donation although knowledge regarding deferral criteria was less. Regarding the interval between two blood donations, 71.2% and 61.4% students respectively were aware of interval between donations in males and females. In an Ethiopian study by Melku et al it was found that 67.8% students knew about the interval between blood donations while Chopra et al found 48.9% students were aware of the same.[8,9] Desai et al reported only 30.2% and 15% medical students to be familiar with interval between blood donation for males and females respectively.[10]Majority of the respondents in our study i.e. 89.8% knew about the suitable age group of blood donation.Similar findings were observed in the studies by Chopra et al (90%), Kowsalya et al (87.5%) and Aslami et al (85%) whereas Syiemlieh et al reported that only 50% students were aware of the same.[9,11,12,13]However, in our study, the knowledge regarding weight eligibility was less and only 55.2% students knew about it, which was similar to the findings by Meinia et al (51.3%).[14] On the other hand, Desai et al and Chauhan et al found that most of the students, 80% and 82% respectively were aware of the weight eligibility for blood donation.[10,15]Mishra et al conducted a KAP study on college students and found that students who were blood donors had more knowledge and positive attitude regarding blood donation than those who were non donors and the differences were statistically significant.[16]

Our study showed 89% students believed that there were chances of acquiring an infection while receiving a blood transfusion similar to the reporting by Malako et al where 87.2% people believed in this possibility.[17] To counter this risk of TTIs, it is necessary to provide specific training on communication and counselling skills for all staff involved in any stage of blood donor counselling so that deferral can be done properly increasing the chances for safe blood collection. Majority of the students (95.6%) in our study believed that blood donation is not a harmful process which was similar to the findings by Chopra et al (83.8).[9] The college curriculum should consist of teaching the basics of voluntary blood donation (VBD) to the students so that with increased knowledge and awareness, they become regular voluntary blood donors and also motivate their peers and general population to come forward and donate, by serving as role models for them.

In our study, the majority of the students (92.6%) had a good attitude towards blood donation and 64.2% of the students were willing to readily donate blood to patients in need. Malako et al in their study on healthcare workers in Ethiopia found that 58.5% of the respondents had a good attitude towards blood donation with 99.1% feeling that blood donation is good and 56.4% of the healthcare workers were willing to donate blood.[17] Chauhan et al reported 91% students having a positive attitude towards blood donation with 84% willing to donate to strangers if required.[15]Melku et alin their study on health sciences students in Ethiopia found that 79.2% students had a positive attitude towards blood donation with 85.5% students expressing intent to donate blood in future.[8]

Even though most of the studies reported favourable attitude towards blood donation, the actual practice of the same was drastically lower. We found in our study that only 127 (25.4%) students had donated blood earlier. Similar results were seen in the studies conducted in different parts of Indialike Chopra et al, Syiemlieh et al, and Chauhan et al where 23%, 27.3% and 22.9% of the students had ever donated blood respectively.[9,13,15]Even lower blood donation practice was seen in studies by Kowsalya et al and Verma et al where only 13.2% and 16.5% students respectively had donated blood earlier.[11,18]However, relatively higher donation practice was reported byMeinia et al,Sahoo et al and Sabu et

al who reported that 43.4%,47.7%, and 38% students respectively had donated blood earlier.[14,19.20]Thus, differences exist between the levels of donation in different parts of the country and low levels of blood donation were also reported in other parts of the world as byMajdabadi et al in Iran where just 9.74% students had donated blood.[21]In a study by Siddiqui et al in Pakistan, 15.2% medical students and 20% non-medical students were found to be blood donors.[22] Malako et al reported blood donation in students in Ethiopia to be 21.6% , whereas studiesby Elias et al in Tanzania showed 30% and Ugwu et al in Nigeria reported 35.4%students had donated blood earlier.[17,23,24] Thus even though the levels of blood donation stay in different parts of the world, a large disparity can be also be seen in the equitable distribution and availability of blood even though WHO strives towards the universal access to safe blood and blood products for the universal health coverage and advocates for the rational use of blood.[25] We should also work on providing timely access to those who need blood via effective organization and integrated blood supply networks.

Most of these studies were conducted on medical or health sciences students. But a few comparative studies between medical and non-medical students or only on non-health sciences students have also been conducted. Gebresilase et al conducted a study on undergraduate Health Science and Non-Health Science University students in Ethiopia and reported a significant good level of knowledge difference between health science students (79.4%) and non-health science students (13.9%). In addition, significant favourable attitude differences were observed between health science students (46.7%) and non-health science students (35.6%). But increased knowledge in the Health Science students was not seen to have any positive correlation with practice with similar blood donation levels seen in both groups.[26]Raghuvanshi et al conducted a study on medical and non-medical students and found that as expected, knowledge was better among students in medical field as compared to non-medical field but blood donation was more common among non-medical students as compared to medical students. The medical students' primary reason for non-donation was that nobody had asked them to donate and lack of opportunity whereas for the non-medical students it was fear of donation and the procedure.[27]Kittu et al conducted a study among students in a polytechnic college and found that the majority had inadequate knowledge regarding blood donation and 58.8% students had never donated blood and only 30.7% donors were willing to become regular donors. Majority (33.1%) reported lack of opportunity followed by fear of getting infected (22.1%) as reasons for not donating.[28] This shows that although the medical or health sciences students have better knowledge regarding blood donation as compared to non-health sciences students, it does not necessarily translate into better practice.

In our study, the most common reason for not donating blood was that the donors had never thought about it and due to a lack of opportunity. Similarly, Chopra et al and Chauhan et al also reported that 56.5% and 53% students respectively quoted lack of opportunity and not being called upon for blood donation to be the reasons behind not donating.[9.15]According to Verma et al, the most common reason (50.5%) among students for not donating blood was the lack of awareness regarding when and where to donate.[18] Mishra et al reported that the most common reason (27.4%) for not donating blood was fear of needles.[16] Thus there is a need to create more opportunities for blood donation by conducting more blood donation awareness camps and by encouraging the students to actively participate in these activities. Steps should be taken to ensure easy accessibility of blood donation centres and convenient timing for donation so as to accommodate all types of donors especially the students and the working class.At the same time efforts should be made by blood bank staff to allay the donors' fears and apprehensions and ensure that blood donation is a comfortable and fulfilling experience for them and for this, specific training on communication and counselling skills should be provided to all the blood bank staff involved in any stage of blood donation process.

Conclusion

Majority of the students in our study were cognizant of the basic eligibility criteria for blood donation along with a positive attitude towards blood donation. 60.2% students were willing to readily donate blood in the future although only 25.4% had donated blood in the past. The most common reason for not donating blood was that the students had never really thought about it. Broadcast and print media were the most common sources of VBD

information. Keeping in mind the large population of healthy students in colleges and universities across the countrythat are eligible to donate blood, blood donation campaigns targeted at them would help in motivating increasing numbers to donate blood.

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Declarations

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Conflict of Interests: Nil

Ethical approval: The study protocol was reviewed by the Ethical Committee of Government Medical College and Rajindra Hospital and was granted ethical clearance.

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Evaluation of Stress Levels in Patients Suffering from COVID 19 Infection Admitted in Isolation Ward of a Tertiary Hospital at Hapur as Assessed by Perceived Stress Scale

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Abstract

The present study aimed to evaluate the stress levels in patients suffering from COVID 19. 100 COVID 19 patients with age >15 years of both genders were included in the study after taking written informed consent. All the subjects were administered Perceived Stress Scale questionnaire to fill and scores were calculated. 82% of the subjects were suffering from high stress while 16% were in moderate category and only 2% reported low perceived stress. As far as age wise distribution of subjects was considered, 36% were < 40 years of age, 32% were between 40 – 60 years and 32% were more 60 years of age. Out of the females 43% had high stress, 3% had moderate and none were in low stress category. While in males 39% had high stress, 13% had moderate and 2% reported low stress. There was a significant increase in the perceived stress score in females as compared to males (33.08 ± 4.29 vs 30.57 ± 5.27; $p < 0.01$). So, there is a need to assess the stress in all COVID 19 patients and regular counselling and psychotherapy should be done specially in female patients.

Keywords: *COVID 19, stress, Perceived stress scale*

Background

Coronavirus disease 2019 (COVID-19) was first identified in December 2019 in Wuhan City in central China. From then, it has spread to all parts of the world and is still spreading in many places. [1] In India, the first case of COVID 19 was reported in late January 2020, ever since it is spreading at a high speed and India has the second highest number of World cases and highest in Asia.[2] Though recovery rate of COVID in India is quite high but the fear of death, mental stress, anxiety and panic associated with being COVID positive and living in isolation ward away from family is also very high, quarantined family.[3] Despite the acquisition of knowledge about coronavirus, its clinical manifestation and diagnostic criteria, its confirmed treatment is still awaited and availability of vaccine in India is still a few months away. Although world has already confronted with extensive epidemics of acute respiratory illness like SARS in 2003, but this outbreak was aptly managed by quarantine measures. Apart from ensuing social instability, COVID-19 is persistently influencing all aspects of human lives. The spreading of the Severe COVID-19 pandemic could be associated with development of stress in patients.[3,4,5]

The Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress. There are 10 questions which are general in nature, easy to understand, and the response alternatives are simple to grasp. In each case,

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respondents are asked how often they felt a certain way.[6]

So, this study is designed to evaluate the stress levels in COVID 19 patients.

Objectives:

1. To evaluate the stress levels in COVID 19 patients using Perceived Stress scale.

2. To determine stress levels in different age groups.

3. To compare stress levels between males and females

Methods

Study sample:

The present study was conducted in the Saraswathi Institute of Medical Sciences, Hapur. The study included 100 patients with age >15 years of both genders admitted in isolation ward of the Hospital after being diagnosed positive for COVID 19 by RT PCR test. Ethical clearance was obtained from Institutional Ethical Committee and informed consent was taken from all subjects. Subjects with terminal illness, SPO_2 < 90 %, known cases of cardiovascular diseases or hypertension were excluded from the study.

All the subjects were divided into 3 groups according to age viz < 40 years, 40 – 60 years and > 60 years. They were administered Perceived Stress Scale (English/ Hindi Version based on their preference) at the time of admission stress score was calculated. Scores ranging from 0-13 were considered low stress. Scores ranging from 14-26 were considered moderate stress. Scores ranging from 27-40 were considered high perceived stress.[3]

Analysis

Data was analyzed using descriptive statistics and stress levels between different groups were analyzed using independent t test and one way ANOVA

Result

The mean age of the subjects was 49.02 $\pm$ 18.5 years. 54 % of the subjects were males and rest were females. 82% of the subjects were suffering from high stress with stress scores $\geq$ 27 while 16% were in moderate category with stress score $\geq$ 14 and only 2% reported low perceived stress with score $\leq$ 13. As far as age wise distribution of subjects was considered, 36% were < 40 years of age, 32% were between 40 – 60 years and 32% were more 60 years of age. Age wise distribution of stress levels is given in table 1. The mean stress scores in different age is given in table 2. Out of the females 43% had high stress, 3% had moderate and none were in low stress category. While in males 39% had high stress, 13% had moderate and 2% reported low stress. There was a significant increase in the perceived stress score in females as compared to males (33.08 $\pm$ 4.29 vs 30.57 $\pm$ 5.27; p <0.01).

Table 1: Age wise distribution of stress levels

	Low	Moderate	High
< 40 years	1%	7%	28%
40 – 60 years	1%	7%	24%
>60 years	0	2%	30%

Table 2: Age wise comparison of stress scores.

	< 40 years	40 – 60 years	>60 years	P value
Stress scores (mean + SD)	32.08 + 5.15	30.9 + 5.6	32.15 + 4.13	Not significant

Table 3: Gender wise distribution stress levels

	Low	Moderate	High
Males	2%	13%	39%
40 – 60 years	0	3%	43%

Table 4: Gender wise comparison of stress scores:

	Males	Females	P value
Stress scores (mean + SD)	33.08 + 4.29	30.57 + 5.27	< 0.01

Discussion

Due to COVID-19 pandemic and essentiality of isolation and quarantine, the stress levels in the COVID patients is drastically increasing. Though the recovery rate is very high in India, but the associated anxiety and stress is still high.

Our study included 100 patients admitted in isolation ward with mild symptoms of COVID 19. According to our study high perceived stress was prevalent in most of the subjects irrespective of age group. Statistically there was no significant difference in stress scores in different age groups. As far as gender wise distribution of stress is concerned in both males and females most of subjects were under high stress. But the mean stress scores were significantly higher in females than in males.

Our study results were consistent with the results of other studies. A study done by Raza et al, also showed females had higher stress scores than males. While contradictory to our study, Raza et al found normal anxiety and stress scores in COVID patients. [3]A study done by Parker et al also showed 25 % of patients with acute stress disorder. [7] An Italian study of 402 patients with COVID-19 evaluated in the emergency department and then screened for psychopathology approximately showed 28% of patients screened for stress disorder.[8] Al-Rabiaah et al explored the impacts of the Middle East respiratory syndrome coronavirus (MERS-CoV) epidemic by examining medical students and found that all of these students experienced stress; however, female students were found with higher levels of stress. [9]

Though researchers across the world are rigorously searching for the cure or developing vaccine but the impact of COVID-19 pandemic on the psychology of people is a neglected facet and should be brought to the attention of stakeholders for timely intervention. Our results suggest that screening of all COVID 19 patients for stress and other psychiatric disorders should done on regular basis. All the patients should be counselled and psychotherapy should be done for them. Stress relieving practices like yoga and meditation sessions and music therapy are recommended for all patients to prevent stress related complications. Special attention should be paid in female patients as they are more prone to stress.

Conflict of Interest: None

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Efficacy of Static Stabilization Exercises along with Progressive Resistive Exercises on Balance and Quality of Life in Elderly Population: A Study Protocol

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Abstract

Background: Balance impairment is one of the most prevalent concern with increasing age. Globally, One in five older adult experiences balance problem. Prevalence of fall has been estimated at 14-53 %in India. Various intervention has already been use to prevent fall and improve quality of life. Static Stabilization Exercises and Progressive Strength training have proven to be effective as a single intervention however minimal literature is available on combined effect of both. The main objective of study is to find out efficacy of combined Static Stabilization Exercises and Progressive Resistive Exercises on balance and quality of life of elderly population and also establishment of dose response relationship of balance training.

Methods/Design: The study will be designed as experimental study. 100 participants will be selected as per inclusion and exclusion criteria and will be allocated in two groups, Combined Intervention Group (A) and Traditional Balance Intervention group (B). Both intervention include 30-45 min session which will be carried out 3-5 times in week for duration of 8 weeks structured into different components. Outcome will be Balance and Quality of Life and outcome measures will be evaluated at baseline, at midway (4th week) and at the end (8th week) of intervention period. Statistical Analysis will be done using SPSS24. Oversion and $p<0.005$ will be considered as level of significance.

Discussion: The study protocol details two Physiotherapy Interventions to improve balance and quality of life in elderly population. In current literatures there are minimal evidence on combined effect of Static Stabilization Exercises and Progressive Resistive Exercises on balance and quality of life. Successful Completion of trial will provide evidence for best strategy targeting balance and quality of life and relationship of dose response and balance training.

Key words: *Static Stabilization Exercises, Progressive Resistive Exercises, Balance, Quality of life, Elderly, Older adults*

Introduction

Postural control (balance control) is the ability of body to control it's position for stability and orientation in space. It plays an important role in activities of daily living [(1)] Static postural control (static balance control, stability) is the ability to maintain stability and orientation with the Centre Of Mass (COM) over the Base Of Support (BOS) with the body at rest. Dynamic postural control (dynamic balance control, controlled mobility) is the ability to maintain stability and orientation with the COM over the BOS while parts of the body are in motion [(2)].

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Balance control requires interaction of nervous, sensory and musculoskeletal system and is affected by impairment in any of them. Aging and changes in vestibular, visual and somatosensory systems coincides which leads to reduced, improper or irrelevant feedback to higher centres. Similarly, muscle effectors also lack the ability to react adequately in response to postural instability as advancing age results in decline of cross-sectional area and muscle strength. Hence strength of muscle and postural control has direct relationship (

In basic terms, balance loss results when the COM falls out of alignment with the BOS. Age related alteration in postural is well established, significant studies demonstrated increased in postural sway with advancing age [7]. Globally, one in five elderly individuals experience balance impairment problem.[8] Prevalence of fall has been estimated at 14-53 % in India [10].

Different types of Traditional Balance Exercises (TBE) have been utilized for balance improvement in older adults. One of Cochrane Systematic Review summarizes these exercises as effective, in enhancing balance in elderly population which includes Co-ordination exercises, exercises involving functional tasks, Tai Chi, Yoga therapy, General Physical activities etc. Combination of any above exercises as multiple exercise intervention has also been used widely [11].

Studies have shown relationship between strength training and functional abilities in older adults. In Past, various studies and meta-analysis have demonstrated effectiveness of Progressive Resistive Exercises (PRE) as sole intervention on balance and functional abilities in elderly (Gillespie et al., 2012; Liu and Latham, 2009; Westhoff et al., 2000)2016; Liu and Latham, 2009; Westhoff et al., 2000 . However, recent studies suggests strength training when combined with different interventions improves balance and as an isolated intervention it hasn't been shown effective in balance improvement [13,16–18]mean age 80 (SD±3.9

Various components of Static Stabilization Exercises (SSE) had been utilized in different studies as part of intervention and has positive role in improvement of balance in elderly population [19–22]. Different types of exteroceptives and proprioceptive stimulation techniques (Sensory Stimulation Techniques) such as vibration,joint approximation etc. have been found effective in improving static balance [23–25].

In residential care, different approaches aren't found consistent which have been proven effective in reducing falls or enhancing balance in community dwelling older adults. Example, exercise as single intervention prevent falls [17]. For older adults, statistics have been reported of various effective exercise programs for balance enhancement and fall prevention[26] however preliminary dose response relationship of balance training hasn't been specified yet [27].

Need For Study

Balance impairment has been identified worldwide as one of the leading causes of morbidity and mortality amongst the elderly. Various interventions have proven to be effective in balance improvement. Several literatures have identified that progressive resistive exercises and static stabilization exercises are effective in improving balance but there are minimal studies on combination of both. Purpose of this study is to incorporate PRE and static stabilization exercises together to determine its effect on balance in elderly population.

Aim & Objective

Study aims to find out the efficacy of combined Static Stabilization Exercises and Progressive Resistive Exercises on balance in elderly population and to establish dose-response relationship of balance training

Methodology

Study setting:-

The trial will be carried out in the two Geriatric Homes of Wardha city (Matoshree and Mahila Ashram) and if participants lacks community dwelling older adults will be recruited from Sawangi M., Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University.

Study design and Sample **size**:-

The design of study is experimental, which will include two different intervention groups (SSE-PRE and TBE) of elderly individuals. The participants number, enrolled in the experimental study will be 100 (n = 100).

Randomization of participants will be done in a 1:1 ratio, Group A (Static Stabilization Exercises & Progressive Resistive Exercises) and Group B (Traditional Balance Exercises) each for the duration of 8 weeks. The informed consent will be obtained, subjective information will be gathered and initial assessment will be done and after that eligible individuals as per inclusion and exclusion criteria will be recruited. Recruited participants will be explained about the type of study in their own language and written patient consent form will be taken. The study procedure is shown in Figure 1 and the schedule of enrolment, screening, assessment, allocation, intervention, post intervention assessment and follow-up of the study (as recommended by Standard protocol items: recommendation for intervention trials) [28] reporting, and external review of clinical trials. However, the completeness of trial protocols is often inadequate. To help improve the content and quality of protocols, an international group of stakeholders developed the SPIRIT 2013 Statement (Standard Protocol Items: Recommendations for Interventional Trials is illustrated in Figure 2.

Participants

Inclusion criteria of participants

Age above 60

Both genders

Individuals willing to participate

MMSE score 24-30

Bergs Balance Score < 41/56

Functional Reach Test score < 11 inches

Dynamic Gait Index score < 22/24

Time UP and GO score > 14 second

Exclusion criteria of participants

Individuals with Cognitive impairments

Individuals with Neurological issues

Individuals with Musculoskeletal issues

Individuals with visual deficit

Individuals with hearing deficit

PARTICIPANT TIMELINE:

Study duration is of 6 months and intervention duration is 8 weeks so participant will be enrolled during first 3 months of study so 8 week intervention will be completed successfully. Assessment will be done on 1st day of visit then in midway (4th week) and last (8th week) of intervention.

Implementation

Research coordinator and principal investigator will supervise randomization. Participants will be asked to manually select from the envelope, sealed group allocation for the recruitment into either group.

Blinding

Tester(s) will be blinded to assign the subjects to the group. To ensure binding, subjects will be mandated not to reveal any details of their treatment to the tester.

STUDY PROCEDURE:-

Participants will be allocated in two groups

Intervention of Group A (SSE & PRE):

Participants of this group will be going to perform Static Stabilization Exercises (SSE) starting from day one of 1st week and Progressive Resistive Exercises (PRE) starting from day one of 2nd week of intervention in graded progressive manner for 30-45 mins each day, 3-5 days a week, continue for duration of 8 weeks. SSE will include selection of different postures (functional, antigravity), utilization of different tools to facilitate static stabilization (quick stretch, joint approximation, resistance etc) and sequential transition of movements. Complexity will be increase progressively. Progressive Resistive Exercise program will be designed on basis of FITT principle which will include warm-up, conditioning and cool down period. Exercises will mainly focus on targeting key muscles of lower limb (Hip flexors, knee extensors, ankle dorsiflexors etc.). Intensity will be based on REPITATION MAXIMUM (RM) and progression will be according to DeLorme and Watkins protocol.

Intervention of Group B (TBE):

Traditional Balance Exercises OR Conventional Balance Exercise group will be performing TBA, 45

minutes a day, 3-5 sessions per week for the duration of 8 weeks. TBA program comprises include 8 components (sitting balance exercises, standing balance, weight shifts, tandem standing, one leg standing, figure of eight walking, crossover walking, backward and sideways walking). Based on ability of participants complexity will be increased.

PROGRAM VARIABLE	DOSAGE
Sets	3 sets (per exercise, per muscle group)
Repetition	10 repetition in a set
Intensity	REPETITION MAXIMUM
Frequency	3-4 times in weekly
Modality	Free weights

Outcome measures:

Balance: -

Outcome measures will be evaluated by Bergs Balance Scale (BBS), Functional Reach Test (FRT), Dynamic Gait Index (DGI), Time Up and Go Test (TUG)

BBS is a clinical tool to access balance (static and dynamic). The scale contains fourteen everday functional tasks. Scoring is based on a 5-point ordinal scale. A maximum score of 56 points is possible. Inter-rater reliability (ICC = 0.98) and intra-rater relative reliability (ICC = 0.97) of BBS have proven to be excellent.

FRT is clinical screening tool of balance problems in elderly population. Normative value with reference to age has been given. It has shown good validity and correlates with speed of walking, walking with one foot in front of other, and one leg standing in community dwelling elderly. Reliability: Inter-rater and test-retest- has shown to be excellent for 20 – 87 years of age group.

DGI examines a ability of an individual to perform different alterations in walking with respect to command. The scale consists of 8 tasks with maximum possible score of 24. The DGI appears to bensensitive in predicting likelihood for falls with older adults .

TUG is quick and easy test used to assess mobility of a person. It has been proven to have excellent interrater (intraclass correlation coefficient [ICC] = .99) and intrarater reliability (ICC = .99).

For Quality Of Life: - SF 36 (36 Item Short Form Survey)

The SF-36 contains 36 items based on questions used in the RAND Health Insurance Study. SF-36 is a set of comprehensive, rational, simple and easy to administer measures. The SF-36 demonstrated high reliability and validity (correlation coefficients ranging from 0.81 to 0.88).

Data Collection And Management

Data collection

Information about study given at time of recruitment (elaborating the purpose, nature, procedure, benefits and after effects of the intervention) with all baseline tests and assessment will be repeated on 2 more occasions.

2^{nd} – midway of intervention (4^{th} week), 3^{rd}- end of intervention (8^{th} week)

Data will be collected in designed database and Primary Investigator will supervised study and make sure t accuracy, precision, authenticity of data and safety of participants. All the data (hard copies of assessment forms, signed consent forms, etc.) will be stored securely in the study setting. The administrator access rights will lie with the principal investigators (PI).

Data management

Principal investigators will supervise documentation & data collection. For precision and accuracy brief evaluation of the study documentation will be done. After completion of study, Excel spreadsheet will be released to an allocation blinded statistician for conducting the analysis, following which unblinding of the groups will be done.

Statistical Analysis Plan

Effect of training interventions on outcome measures will be determined via linear mixed affect model across 'time' (pre-intervention vs post-intervention) and 'group'' (Experimental vs Control). To assess which intervention will result in effective improvement. Data

analysis will be done by using descriptive and inference statistics using Chi-square test and student's unpaired t test.

Discussion

Balance impairment and fall has been estimated as one of key factor deteriorating Quality Of life and cause of mortality amongst elderly. Our Study aims to estimate the efficacy of combined static stabilization exercises and progressive resistive exercises on balance in elderly population and dose-response relationship of balance training. According to study by Horlings et al., loss of balance in elderly population involves multiple risk factors where muscle weakness tends to be a significant risk factor for falling and strength training can improve weakness, particularly when given as a component of a multifactorial intervention [(29)]. Multiple studies found that a program consisting multiple intervention (mobility, strength and balance training) found to be the prime approach for enhancing functional independence (gait, balance and strength) and reducing fall rates in older adults [(16,26)].

Despite abundance of research in domain of falls and balance impairment, there are few which concluded effective ways and dose response. As per study of Lesinski et al., even though numerous studies have been conducted in this area very few provides detailed information hence, preliminary dose response relationship of balance training hasn't been proven and specified yet [(27)]evidence-based dose–response relationships in BT modalities (i.e., training period, training frequency, training volume. Again, Literatures suggests Static Stabilization Exercises as an effective intervention for balance [(30)] but very few studies has utilized it as sole intervention or as part of multiple intervention. This study is incorporating PRE and static stabilization exercises together for determining its effect on static as well as dynamic balance in elderly population. Outcome measures will access efficacy of intervention and successful completion of trial will provide evidence for best strategy (detailed and effective) targeting balance and quality of life and establishing relationship of dose response of balance training in elderly.

Ethical Approval and Dissemination: The participant individuals of the study and DMIMSU who will fund it will be able to retrieve findings of study. Enrolled subject's data will be stored securely for atleast 5 years. After completion of study and publication of results data will be stored in the DMIMSU data repository

Patient Consent

Principal Investigators will obtain the written informed consent from the participant on a printed form (local language) with signatures and give the proof of confidentiality.

Confidentiality

The study program will be explained to the participant, the principal investigator will take subjective information. The consent form will include the confidentiality statement and signatures of the principal investigator, patient and a witnesses. If required to disclose some information for the study, consent will be taken from the patient with complete assurance of his confidentiality.

Author's contribution

MIQ suggested the design of the study. SM and MIQ led to the creation and design of the study. SM wrote the manuscript of this article.NC gave final touches to the manuscript. MIQ read and approved the final manuscript for publication

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Comparative Study on Effectiveness of Peppermint Oil and Hot Fomentation For Joint Pain among Sickle Cell Anemia Patients

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Abstract

Introduction: Sickle cell anemia may cause pain such as stiffness of the joint, bone pain, muscle weakness, chest or abdominal pain. It is often not recommended to use over - the-counter painkillers (such as ibuprofen, mortin, advil, or aleve) because they can adversely affect the function of the kidney and/or liver. Try to use hot compresses for about 15 minutes up to 2-3 times per day to treat pain spontaneously. Beyond healing irritated skin, increasing endurance and encouraging relaxing, herbs Are always an effective way to manage pain. It is possible to apply peppermint essential oil on the skin to decrease muscles sore or joint pain[1].

Objectives:-

1. To assess the level of joint pain among sickle cell anemia patients.

2. To assess the effectiveness of peppermint oil for joint pain among sickle cell anemia patients.

3. To assess the effectiveness of hot fomentation for joint pain among sickle cell anemia patients.

4. To compare the effectiveness of peppermint oil and hot fomentation for joint pain among sickle cell anemia patients.

5. To associate the effectiveness of peppermint oil for joint pain among sickle cell anemia patients with their selected demographic variables.

6. To associate the effectiveness of hot fomentation for joint pain among sickle cell anemia patients with their selected demographic variables.

Material and Methods: For this research, true experimental pre-test post-test research design has been adopted. The study was conducted in selected area of Wardha district. Simple random sampling technique was used to select 60 participants and 30 were recruited for each group. To evaluate joint pain, the tool used for data collection was a scale of (0-10 scale of pain severity) pain scale. Both each groups were given peppermint oil and hot fomentation for seven days for three times a day.

Result:- The after intervention mean score of hot fomentation 2.90±0.71was higher than that of peppermint oil 1.53 ±0.68 and the calculated ' t' value 7.59 was significant at $p<0.0001$.

Conclusion:- The study proved that peppermint oil was effective than the hot fomentation for reduction of joint pain among sickle cell anemia patients.

Keywords :- *Sickle cell anemia; Assess ; Effectiveness*

Introduction

Sickle cell anemia is linked with a series of inherited blood disorders triggered by a type of hemoglobin named HbS. People afflicted by sickle cell anemia has dual forms of this type of beta globin and HbS are the only hemoglobin present in its blood cells. Sickle cell anemia is especially common in people who paternal ancestry from Sub-Saharan Africa, the peak areas of Spain (Cuba, South America, Central America), India, Morocco, Pakistan, Saudi Arabia and European countries like Greece, Italy, and Turkey[1] .

Sickle cell anemia may cause pain such as stiffness of the joint, bone pain, muscle weakness, chest or abdominal pain. It is often not recommended to use over – the counter painkillers (such as ibuprofen, mortin, advil, or aleve) because they can adversely affect the function of the kidney and/or liver. Try to use hot compresses for about 15 minutes up to 2-3 times per day to treat pain spontaneously. Take warm baths or sitting in a hot tub / jacuzzi, massage therapy, exercising or seeing a physical therapist also is a safe solution to prevent deterioration of debilitating symptoms. Beyond healing irritated skin, increasing endurance and encouraging Relaxing, herbs are always an effective way to manage pain. It is possible to apply peppermint essential oil on the skin to decrease muscles sore or joint pain. Some oils that assist relieve problem such as occurrence to decrease; lavender oil to treat with stress management; as well as citrus oils such as grapefruit or orange to help reduce tiredness[2] .

The use of fomentation is on the rise throught the world. Fomentation is used in clinics, spas and medical studies. Fostering is more popular than cold fostering. Pain is the most major problem to be handled by hot fomentation. Hot fomentation can be used in a variety of ways , including electric sheets, hot water bottle, hot gel packets or hot baths. Heat vasodilate increases blood flow, supplying cells with oxygen and nutrients, helping to reduce joint pain .

Sickle cell anemia patients who are selected in selected area of Wardha district. The multidisciplinary approach to the management of sickle cell anemia pain includes routine pain assessments, which have been incorporated into the inpatient protocols and allow for application of peppermint oil or hot fomentation. Pain status includes assessments of pain intensity and pain relief. The present study is based on compare effectiveness of peppermint oil and hot fomentation for joint pain among sickle cell anemia patients in the selected area of Wardha district over a 3 week period[3].

Meterial and Methods

Interventional research approach with True experimental pre and post -test design (two group before intervention, after intervention)was used for the study. Research study was conducted in 12th August to 31th September 2019. At selected area of Wardha district. A sample of 60 sickle cell anemia patients was selected using simple random sampling technique and then assigned the samples in experimental group I(peppermint oil) and experimental group II (hot fomentation). 60 samples were collected. Before intervention pain assessment was done. After that 30 sample were selected for peppermint oil and 30 samples for hot fomentation. Before intervention sensitivity test was done of both group experimental group I (peppermint oil) and experimental group II (hot fomentation). If sensitive to peppermint oil or hot fomentation then they were exclude from the study and informed to physician for further management. If not sensitive to peppermint oil then apply peppermint oil to the experimental group I for 30 sickle cell anemia patients thrice a day for 7 days. Same as if sickle cell anemia patients was not sensitive to the hot fomentation then apply hot fomentation to the experimental group II for 30 sickle cell anemia patients for thrice a day for 7 days.

On the end of the 7th day after intervention assessment of joint pain related symptoms in both groups were done by using pain scale and then compare the effectiveness of peppermint oil and hot fomentation for joint pain.

The study was carried after obtaining permission from the Institutional Ethics Committee (IEC), Datta Meghe Institute of Medical Science (Deemed University) Sawangi (Meghe), Wardha.

The tools used for data collection was pain scale (0-10 scale of pain severity)this was developed based on the objetives of the study and through review of literature. Instrument consists of two sections

Section - A :- Demographic variables.

Interview guide which consists of questions to collect the demographic data like age, gender, type of sickle cell anemia, occupation, major health problem, on medication of sickle cell disease, attended any such type of activity in the past.

Section –B :- Pain scale (0-10 scale of pain severity)

Pain scale was used to assess the level of joint pain. It consists of 0-10 score scale of pain severity with each statements. Categories to respond are mild, moderate, severe, and extreme in each statement. Total score is 10.

Table no.-1 (0-10 scale of pain severity) pain score

Level of joint pain	Score
Mild	0-2
Moderate	3-4
Severe	5-9
Extreme	10

Results

Distribution of sickle cell anemia patients with regards to demographic variables.

In experimental group I (peppermint oil) with regard to age Majority of sickle cell anemia patients 30.00 % were from the age group of 18-22 year, 53.33% were from the age group of 23-27 year and 10.00% of then belonging to the age group of 28-32 and remaining 6.67% were more than 32 years age.with regard to gender 33.33% were males and remaining 66.67 % were females. With regard to type of sickle cell anemia 56.67% of them had AS patterns and 43.33% had SS patterns. With regard to occupation 10.00% were farmers, 23.33% were labourer, 10.00% were doing private job, 6.67% were doing government job,16.67% were homemaker and remaining 33.33% were students. With regard to any major health problems 23.33% had major health problems and remaining 76.67% not have any major health problems. With regard to on medication of sickle cell anemia 23.33% had on medications of sickle cell disease and remaining 76.67% had not on medications of sickle cell disease.

In experimental group II (hot fomentation) with regard to age Majority of sickle cell anemia patients 23.33% were from the age group of 18-22 years, 26.67% in the age group of 23-27 years and 20.00% of then belonging to the age group of 28-32 and remaining 30.00% were more than 32 years age. With regard to gender 53.33% were males and remaining 46.67 % were females. With regard to type of sickle cell anemia 50.00% had AS pattern and 50.00% had SS patterns. With regard to occupation 23.33% were farmers, 20.00% were labourer, 6.67% were doing private job, 20.00% were doing government job, 26.67% were homemaker and remaining 33.3% were students. With regard to major health problems 10.00% had major health problems and remaining 90% not have any major health problems. With regard to on medication of sickle cell anemia 46.67% had on medications of sickle cell disease and remaining 53.33% had not on medications of sickle cell disease.

Comparison of effectiveness of peppermint oil and hot fomentation for joint pain among sickle cell anemia patients.

Table No.2 : Significance of difference between pain score of hot fomentation and peppermint oil among sickle cell anemia patients.

Overall	Mean	SD	Mean Difference	t-value	p-value
Hot fomentation	2.90	0.71	1.36±0.17	7.59	0.0001 S,p<0.05
Peppermint oil	1.53	0.68			

The above table no.2 shows the comparison of difference in before intervention and after intervention pain scores of sickle cell anemia patients in hot fomentation and peppermint oil. Mean, standard deviation and mean difference values are compared and student's unpaired 't' test is applied at 5% level of significance. The tabulated value for n=30-2 i.e. 28 degrees of freedom was 2.05. The calculated 't' value i.e. 7.59 are much higher than the tabulated value at 5% level of significance for overall pain score of sickle cell anemia patients which is statistically acceptable level of significance. Hence it is statistically interpreted that peppermint oil is more effective than hot fomentation among sickle cell anemia patients.

Association of level of after intervention joint pain score of peppermint oil and hot fomentation among sickle cell anemia patients in relation to demographic variables

In experimental group I (peppermint oil) finding shows that demographic variable type of sickle cell anemia, major health problems, and on medication of sickle cell disease had shown significant association with pain score and the other demographic variables are age, gender and occupation had not shown statistically significant association with after intervention pain score.

In experimental group II (hot fomentation) finding shows that demographic variable only age had shown significant association with pain score, and the other demographic variables are gender, type of sickle cell anemia, occupation, major health problems, on medication of sickle cell disease had not shown statistically significant association with after intervention pain score .

Discussion

In present study table no.4 shows comparison of effectiveness of peppermint oil and hot fomentation for joint pain in sickle cell anemia patients, in experimental group I (peppermint oil) mean score 1.53 with standard deviation 0.68 and in experimental group II (hot fomentation) mean score 2.90 with standard deviation 0.71. Hence it is statistically interpreted that peppermint oil is more effective than hot fomentation among sickle cell anemia patients.

Study conducted by Nikita H Chauhan (2017) effectiveness of the hot application and castor oil application in clients with joint pain, among 50 old osteoarthritis patients, result revealed that in experimental group I (hot application) mean score 2.60 with standard deviation 1.155 and in experimental group II (castor oil application) mean score 2.40 with standard deviation 1.041. Hence it is statistically interpreted that castor oil is more effective than hot application among clients with joint pain [4].

In present study in experimental group I (peppermint oil) finding shows that demographic variable type of sickle cell anemia, major health problems, and on medication of sickle cell disease had shown significant association with pain score and the other demographic variables are age, gender and occupation had not shown statistically significant association with after intervention pain score.

In experimental group II (hot fomentation) finding shows that demographic variable only age had shown significant association with pain score, and the other demographic variables are gender, type of sickle cell anemia, occupation, major health problems, on medication of sickle cell disease had not shown statistically significant association with after intervention pain score .

Study conducted by Nikita H Chauhan (2017) in a study to evaluate the impact of hot application and castor oil application on joint pain among 50 old osteoarthritis patients in old age homes. In this research, it was observed that in both experimental group demographic variables are age, gender weight, occupation, diet duration of joint pain, daily living activity and habits in patients with joint pain had not shown significant association with after intervention pain score [4].

Limitation

The study was limited to sample size i.e 60 (30= experimental group I (peppermint oil), 3= experimental group II (hot fomentation). This might be inadequate to generalize the study findings. More time duration would give more relevant results with variations of any research, but the investigator planned to complete the research work within one month to get more feasibility of getting sample. Therefore, sufficient number of sample

and time duration was required to establish the effects of peppermint oil and hot fomentation for manage pain in sickle cell anemia, in general.

Conclusion

The study compared the effectiveness of peppermint oil and hot fomentation for joint pain among sickle cell anemia patients. From the above findings, it was evidenced that peppermint oil was effective than hot fomentation on relieving joint pain among sickle cell anemia patients.

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Effects of Ballistic Six Exercises and Theraband Exercises on Physical Performance in Badminton Players: A Randomized Controlled Trial

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Abstract

Background – Badminton is one of the most active recreational sport played in India. Different training programs have become common to boost the athletic performance and advantages. Some of them are plyometric workouts, gyms, free body weight exercises and resistance exercises. Ballistic Six training is given to strengthen the muscles of the rotator cuff with overhead throwing activity to prevent injury to the shoulder whereas therabands with a combination of physical strength and warm-up movements are done for medical issues

Objective – To compare the effects of Ballistic Six exercises and thera-band exercises on strength, agility, speed and function in badminton players.

Method - 40 participants aged between 18 – 25 years, playing on regular basis will be targeted in this study. Players will be split randomly into groups A (plyometric group) and B (thera-band group). All the training sessions will be initiated after a 10 minutes of warm up session. Protocol given will be twice a week for 8 weeks. Main outcomes will be Seated Medicine Ball Throw for shoulder strength, Closed Kinetic Chain Upper Extremity Stability Test for agility, Plate Tapping Test for speed and Kerlan – Jobe Orthopaedic Clinic Shoulder & Elbow Score questionnaire will be used to assess the shoulder function. All assessments will be done at baseline and at the end of 8 - weeks training session. Follow – up will be taken at end of 6 – month.

Results – This study aims to compare effectiveness of two strengthening protocols, that is, Ballistic Six exercise and Thera-band exercises, in young badminton players. Results of this study will depend on outcome measures that are shoulder strength, agility, speed and function.

Conclusion – To conclude, this research may help badminton players to enhance their performance through the better exercise protocol.

Keywords *– Badminton Players, Plyometric exercise, Thera-band exercise, Physical performance*

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Introduction

Badminton is one of the most frequent racquet sports of the world and the second greatest performed sport after Cricket in India. Badminton in India is governed by India's Badminton Association. Saina Nehwal, Srikanth Kidambi, Parupalai Kashyap, P.V. Sindhu and Jwala Gutta are currently successful Indians players [1].Daily badminton workouts improve physical health, specifically

speed, strength and aerobic fitness. Badminton often needs a continuous study of the changing position on the court, concentrating the player clearly and rapidly on racketing, refining his estimation and planning the next pass [2]. The kinetics chain mechanism helps badminton gamers to pace up the racquet as well as shuttlecock to the determined speed. The mechanism of the Kinetic chain is based on neuromuscular synchronization (that is, parts of a body move in a specific sequence) to shift energy from the ground to the thighs, knee, lower back, upper back, neck, forearm, hand and eventually to racquet[1]. Badminton players need to be fast and agile around the court. Muscle power, strength, coordination, agility, flexibility and balance are important components for a player[3].

There are six basic moves, in badminton. The most every and efficient offensive badminton technique for the player to defeat the opponent is smashing among these moves. Smash is known for being the most common killing shot. Smash is played around 53.9 percent of all offending shots that are normally played in badminton, so badminton smash in tests is decisive. To smash, the participant hit the shuttle clearly hard and slams the shuttle down as steeply as possible to make sure it overflows the net [1]. According to Omosegaard et al 1996, on a badminton court, an agile competitor usually needs to be able to leap long, turn directions easily and typically look too fast and versatile. Shoulder strength is a muscle's ability to bury the resistant to execute a shoot. The higher the shoulder's strength, the greater the smash's speed, the stroke's strength also influence its course.[1].

Tiwari et al. (2011) noted that physical agility is output at badminton match (r=0.83) whereas R. Jeyaraman et al. (2012) concluded that they were agile, explosive legs power, pace and leg strength where there is a significant positive connection with the playability. Sakurai and Ohtsuki (2000) found that smacking accuracy was higher and more stable temporal regulation of upper limb muscles in skilled athletes and non-skilled individuals was also determined. Good temporal control by the muscles was an important quality for great smashing performance

Strength training programs designed to maximize athletic success and benefits have gained popularity. In badminton a player's success is determined by strength and not restricted by strength [2]. Individuals tend to engage in conventional resistance training programs, to increase their muscle strength. Those workouts are accomplished by athletic instruments from dumbbells or devices, elastic bands or by its own weight of body [4]. Kim Yew Tiong and Chin Ngien Siong (2019) carried a study on 12 year old badminton players to examine the effectiveness of Plyometric and Resistance Band Training on Badminton Overhead Clear through 6- week training and concluded that that 6 weeks of plyometric training was more effective in enhancing badminton overhead simple stroke efficiency compared to resistance band training[5].

Ballistic Six training is given to enhance the efficacy of the throwing exercise and to strengthen the muscles of the rotator cuff with overhead throwing activity to prevent injury to the shoulder. This training protocol involves six upper extremity plyometric exercises developed with quick, powerful movements needed to enable the shortening process of the stretches. This consists of three stages of eccentric phase, amortization and concentrated phase[6] The nervous system therefore trained to react faster. Thus, plyometric training seems to be an appropriate intervention to improve the ability of the neuromuscular to increase power, stamina and reaction time [7].

Exercise resistance bands with a combination of physical strength and warm-up movements are done for medical issues. Some of the finest examples of such a method is the thera-band (resistance rubber) [8]. These resistance bands are low cost, safe, portable and easily available elastic band with different grades or thickness. These bands never operate against gravity and can be used at the same time to train one or more joints. Therefore, they could also theoretically be used as a realistic substitute for resistance training. Elastic resistance bands have been reported as a useful tool for improving muscle strength in young and elderly populations, both genders individuals with and without musculoskeletal pain.[4].

Strength, agility, speed and function are the main components for the enhancement of physical performance and for preventing injury in badminton players. To date, there is no study that examines the

effect of Ballistic Six exercises and thera-band exercises on shoulder performance in young badminton player (18 – 25 age), although there are studies suggesting that there is improvement in agility and strength of lower extremity in players who underwent plyometric programme[3]. In this context, the aim of this study is to determine the effects of an 8-week (2 d.wk-1) Ballistic Six exercises and thera-band training protocols aimed at developing strength, agility, speed and upper limb coordination on young badminton players.

Methods/Design

Aim

To compare the effects of Ballistic Six exercises and thera-band exercises on strength, agility, speed and function in badminton players.

Study design

This study will be carried out in the Sports Clubs in Wardha that will selected according to feasibility, after clearance by the Datta Meghe Institute of Medical Sciences Institutional Ethics committee, Deemed to be a University. Before inclusion, all participants will be informed regarding the aim and procedure of research. Those participants who will meet the inclusion criteria must give the written informed consent. In this comparative study, participants (N= 40) will be enrolled for 8 weeks.

Trial Design:

In this randomized controlled parallel group single-blind trial the subjects will be randomized into two group independent design (Group A and Group B) through envelope method of randomization. Group A will be Ballistic Six Exercise group while Group B will be Thera-band group.

Participants:

40 participants meeting the inclusion criteria will be involved in this study.

Inclusion [1,3]:

Subjects of both genders between the age of 18 – 25, who are active recreational players and have been playing badminton for over a year will be included, also participant's BMI will be about 18.5 kg / m2-24.9 kg / m2 of standard range.

Exclusion:

Subjects of both genders between the age of 18 – 25, who are active recreational players and have been playing badminton for over a year will be included, also participant's BMI will be about 18.5 kg / m2-24.9 kg / m2 of standard range.

Recruitment Procedure:

Participants who regularly play badminton at sports clubs in Wardha and meet the eligibility requirements will be eligible to engage in the RCT analysis. The training coach of the clubs will be given detailed information of the study and benefits so as to increase the recruitment rate.

Sample Size Consideration:

Sample size calculation was done from a study which compared the effects of plyometric exercises and thera-band exercises in 12 year old badminton players [5]. In power calculation, we calculated the sample size required per group to be 20 players with 95% confidence interval, power of 0.84 and standard deviation (SD) of group 1 and group 2 is 0.50 and 0.36 respectively. Therefore, 20 subjects are selected in each group.

Randomization:

Following the baseline evaluation, participants who fullfill the requirement of inclusion will then be assigned randomly to either group (GROUP A or GROUP B). A computer-generated randomisation schedule in randomized permuted blocks will be prepared by an independent statistician, to ensure the number of players undergoing the two treatments within each group are closely balanced and the allocation numbers will be placed in invisible sealed envelopes. These envelopes will be made available to the player after signing the consent form.

Intervention Design:

Participants will receive the protocol twice a week for 8 weeks. Data collection will continue after 10 minutes of warm-up session with includes low intensity exercises such as calf stretch, toe touch, reach ups,

jogging, shoulder circles, neck clock, arm hug, windmill (1). All the athletes will be given the appropriate trials of the tests for strength, agility and speed to familiarize them with the test and most importantly reduce the probability of error.

GROUP A (BALLISTIC SIX PROTOCOL) [9]

To reduce the deceleration phase and maximize the stretch-shortening cycle (SSC) training results, subjects are advised to ballistically use maximum effort and exercise. Exercises of 3 sets of 10–20 repetitions should be completed, with each series offering 30 seconds of rest. The Ballistic Six exercise uses thera-band latex tube (red) and fitness balls (2-lb for one extremity training / 6-lb for dual extremity training).

Exercises:

1) External rotation with latex tube.

2) 90/90 external rotation with latex tube.

3) Overhead soccer throws using a 6-lb (2.7kg) fitness ball.

4) 90/90 external rotation side-throw using a 2-lb (0.9kg) fitness ball.

5) Deceleration baseball throws using a 2–lb (0.9kg) fitness ball.

6) Baseball throws using a 2-lb (0.9kg) medicine ball.

GROUP B (THERABAND EXERCISE) [4]:

Participants will learn the techniques for the exercises during the first familiarisation session. They will be taught to use a thera-band, and will be given correct guidance. Thera-band colour is the resistance indicator for the thera-band. Player's level of strength will decide the form (colour) of the thera-band to be used. For training exercises, they'll use six feet of thera-bands. The training strength can be changed by adjusting the thera-band colour, which means increasing the band's resistance. The strength of the exercises will be determined every two to three weeks by adjusting thera-band colours. All repetitions must be done in the appropriate way by the participants. The duration of training sessions shall be 3 sets of 10–20 repetitions. Week wise training protocol:

a) 1-3 weeks: Standing Shoulder Press, Chest Fly, Triceps French Press, Biceps Curl, Rhomboid Squeeze

b) 4-6 weeks: Trunk Twist, Reverse Flies, Elbow Kick Back, Concentration Curl Scapular, Retraction

c) 7-8 weeks: Straight Arm Pulldown, Chest Press, Triceps Kick Back, Rear Deltoid, Dead Lift.

The players participating will be motivated to maintain the workout schedule by adequate physical instruction up to 8 weeks, but there might also be reasons for some to leave. Participants that experience discomfort due to wrong workout procedure will not be allowed to engage any more. Participants who plan to quit will also be contacted with respect to the reason for discontinuation. Furthermore, if they are discontinuation, replacements will be selected.

Data Collection Procedure:

Data will be collected at baseline and at the end of 8 – week training. Follow – up will be taken at 6 month. Demographic data will be collected and all the assessments will be done by physical performance tests and a questionnaire to assess function.

Outcome Measures:

1) Sitting medicine ball throw (SMBT)

Sitting medicine ball throw test is reliable with ICC value 0.98 [10]. The participants must sit up erect against the wall on the ground. Lower extremities will be stretched and fitness ball weighing 2 kg will be grasped at 90 degrees of shoulder abduction with both limbs and the elbow flexed. The medicine ball will be over coated with gymnastic chalk after each throw to leave a clear mark on the floor. A measuring tape is mounted on the surface, reaching more than 10 metres. By throwing the exercise ball in a straight line participants will ensure maximum elbow wall contact, feet to back as long as possible. The medicine ball is thrown with arms spread out from the body to fit the varying arm length. The angle connecting wall and closest point of chalk mark will be abstracted from overall angle of throw. 3 tests shall be performed with a rest of 1 min between tests.

2)
Closed Kinetic Chain Upper Extremity Stability Test (CKCUEST)

This test is reliable with an ICC value of 0.85 to 0.96 [10]. Subjects must push up 91.4 cm apart with their hands (marked with two strips of tape on the floor) with both shoulders perpendicular to their knees. The back and the bottom are stabilized with the feet isolated from the shoulder width. From this position the dominant hand extends over the body to contact the non-dominant hand and return to its original position. The non-dominant hand would later perform the same procedure. Participants will be told to do as many alternating touches as possible in 15 seconds, while keeping the correct push up posture.

3) Plate tapping test (PTT) [4]

Plate taping test is both an efficient and even a precise tool for measuring the upper limb movement coordination and speed for the dominant arm movement rate. This test is reasonably accurate and has an ICC value of 0.57 [11]. Two yellow (20 cm) disks are placed on smooth floor, 60 cm apart in the middle plate tapping test. White rectangle measuring 30/20 cm will be put in between the two disks. Participants are told to put non-dominant palm on rectangle and push back and forth the dominant hand between the two yellow disks. 2 touches are recorded as 1 loop while subjects are asked to perform 25 cycles as fast as possible. Time needed to complete assessment shall be calculated using a stopwatch. The subject must do the test twice in a standing posture, with a 5-minute rest time, and show the better result.

4) Kerlan-Jobe Orthopaedic Clinic Shoulder & Elbow Score

The KJOC is a reliable questionnaire with an interclass coefficient of 0.93 [12] developed to assess the upper extremity's functional status, especially in athletes engaged in overhead sport. Researchers have confirmed that the KJOC score can differentiate between professional baseball athletes who have no pain, play with pain or don't play because of pain. The questionnaire contains 10 questions on health and work within the EU. Participants will be instructed to place a 'x' on a line of 10 cm linked to each of the 10 questions. The x location will indicate their current output or role level, or both. An x placed nearer to the right shows a higher rating for performance, function or both [13,14].

Data Management:

Data of this study will be stored in a safe, secured store room with limited access by a biostatistician for later examination

Statistical Analysis

Data collected will be noted down and then will be placed in a tabular format. It will be analyzed with the help of SPSS latest version. Both statistical analyzes should be conducted with a 95% confidence interval (p-value < 0.05) to assess effect of two measures. Homogeneity of the two study classes will be tested for individual studies using the Student's t test. Mann-Whitney U will be used for comparing Groups at baseline.

Results

This study aims to compare effectiveness of two strengthening protocols, that is, Ballistic Six exercise and Thera-band exercises, in young badminton players. Results of this study will depend on outcome measures that are shoulder strength, agility, speed and function.

Discussion

The protocol is going to be conducted for a single-blinded randomized clinical trial to test incorporation of which procedure during training session would increase the performance of badminton players on the shoulder. Resistance training is a type of strength training where any action is carried out against a particular resistance-generated external force (i.e. resistance to being pulled, squeezed, stretched or bent)[5]

Plyometric practice provides promising outcomes in most sports like football, karate, handball, badminton, and tennis [15,16]mass: 81.5 kg. This can also improve as well as impact the players' vertical power on badminton technique such as overhead simple stroke smashes [17,18]. Plyometric training is a process that teaches strength and velocity to produce additional muscular power [5]. Samuel et al. addressed that plyometric training and the use of the stretch shortening cycle improve the capacity of the neural and musculotendinous system to generate maximum force in the shortest period of time, thereby merging a difference between speed and strength [19].

Ballistic Six plyometric training procedure at the upper extremity includes a series of high volume functional exercises to mimic the overhead-throwing motions, locations and forces [9]. When comparison was made between the two independent groups, i.e. ballistic six exercise and free weight exercise, in cricket players, there was a substantial difference between the two groups with a p value < 0,05 which showed that ballistic six exercise improved more than free weight exercise [6].

The elastic band was used by Ellenbecker and Roetert to reinforce muscles of the shoulder in tennis [20]. According to Zhang et al. effective throwing emerges from all body segments which give dominant hand maximum absolute velocity and pass the energy to your racket in single smooth motion [21]. Escamilla et al. recorded that baseball players increased their 2.2-mph throwing pace during 4-week (3 d.wk-1, 75 min. d-1) elastic tubing, throwing and stretching strength training [22, 23, 24]. Transferability of strength gains from general resistance training approaches to sport-specific results remains a critical problem [4]. Moreover, Carter et al. observed that both resistance training and upper extremity plyometric training succeeded in strength improvements during 8 weeks, just the plyometric exercise group increased the throwing speed by 2.0 mph in college baseball players [9].

In addition; the effects of therapeutic measures in cases of shoulder strengthening suggest significance of using these techniques, at court as well as at one's own pace. Procedures used in the study have less negative consequences and are less cost-effective. The hypothesis may or may not reflect the results of this study. In addition, this longitudinal study will allow the badminton players to examine the long-term impact of strength training.

Research Ethics Approval:

Ethical approval received from institutional ethical committee. The trial will be performed in accordance with the Declaration of Helsinki.

Dissemenation and Protocol Amendments:

The primary RCT findings will be sent to a foreign peer-reviewed journal for publication, regardless of whether the findings are positive, negative or inconclusive about the research hypothesis.

Confientiality:

Specific player information will be kept separate from the central dataset, and will not be exchanged. All personal data will be stored securely before, during and after the court to preserve the confidentiality.

Funding:

There will be no direct support for this research from public and private organizations. The Department of Physiotherapy, at Datta Meghe Institute Of Medical Sciences, Deemed to be University will provide material needed for research.

Perspectives of the Study:

The results of this RCT study will provide quality evidence supporting a practice of one of the training protocol to increase strength and will provide a scientific support to trainers to incorporate these protocols in training circuits.

Conflict of Interest: Nil

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Epidemiological Study Evaluating Type of Labial Frenum, Attachment Level and Gingival Biotypes in Chennai Population

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Abstract

Background: Periodontal diseases are multifactorial and while formulating a diagnosis multiple factors must be taken into account and considered which may cause the disease or alter the treatment plan and prognosis. Some of the factors affecting surgical prognosis include the gingival biotype and labial frenal level, type, morphology and attachment. The frenal attachment and level may causes additional pull and force on gingiva affecting healing, gingival biotype affects the kind of flap to be raised or flapless technique, time taken for healing and also the amount of tissue required while procuring connective tissue and free gingival grafts. The present study was undertaken to get a better understanding of the relation between Gingival Biotype and Labial Frenal attachment by conducting an Epidemiological survey in a Chennai population. **Methodology:** A total of one hundred and fifty systemically healthy patients were screened at random from the outpatients of a dental college and hospital between age groups 22 to 60 years. Each patient was screened by a single clinician, where the patients demographic data, type of frenal attachment, the medial maxillary labial frenal classification based on morphology, morphology of labial frenum, level of frenal attachment, Miller`s grade of gingival recession, gingival biotypes, visibility of the periodontal probe through the gingival sulcus and gingival thickness measured using an endodontic reamer. **Conclusion:** Among all the patients who had Simple single frenum with no nodule and elongated parallel margins with gingival recession, two thirds of them had a Thin gingival biotype.

Keywords- *labial frenum morphology, attachment, gingival biotype, gingival recession, periodontal, survey, transgingival probing.*

Introduction

Periodontal diseases are multifactorial but at the same time there are a wide range factors affecting the ultimate outcome of a disease or altering the prognosis. One of the factors that affects the surgical outcome of a disease included the gingival thickness and thereby the biotype.[1] The term gingival biotype is defined as the thickness of gingiva in the facio-palatal and facio-lingual dimension, which is broadly classified as thick flat and thin scalloped. Thick gingival biotype refers to a resilient type of tissue, supported extensively by underlying bone architecture, making it a lot more favourable for surgical interventions. Thin architecture similarly refers to minimal or thin underlying support from bone architecture with poor resistance to occlusal force and inflammation.[2–4] It has thus been suggested that inflammation in thick gingival biotype results in increased periodontal pocket formation while a thin biotype results more in gingival recession and anaesthetics zone of poor papilla coverage[4] A gingival thickness of greater than or equal to 2 mm was referred to flat thick gingival biotype while less than 1.5 mm was referred to as thin scalloped biotype. These assumptions

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also suggested the underlying bone depicts the contour of the gingiva above.[5]

Modern technologies which aid in the measurement of hard and soft tissue is the magnetic resonance imaging, but due to the expensive nature of such a scan it cannot be routinely used to make such measurement without any underlying pathology.[6,7] In case of epidemiological studies with a large population simpler methods such as a the transparency of periodontal probe through the gingival sulcus along with measurement of the gingival thickness by transgingival probing using endodontic files have been utilised.[7–9] The aim of this study was to assess the relation between labial frenal type, levels of attachment of frenum and gingival biotypes in Chennai population.

Methodology

This study design was initiated after obtaining approval from the scientific and ethical review board (SRB/SDMDS11/18/PERIO/26) of the Saveetha University, Chennai. A total of one hundred and fifty patients were screened at random from the outpatients of a dental college and hospital in Chennai. Patients included in the study were between age groups 22 to 60 with no underlying systemic diseases such as diabetes mellitus, asthma, hypertension, desquamative gingivitis, connective tissue disorders, respiratory and Cardiac problems. Patients excluded from this study were completely edentulous patients, patients with partially edentulous space in relation to the upper and lower front tooth region, pregnant women, lactating mothers, smokers.

All patients were explained the nature of the study in detail and their informed consent was taken to utilise their pictures for the purpose of scientific research only. Each patient was screened by a single clinician, where the patients demographic data , chief complaint, past medical and dental history were also collected. The following clinical parameters were assessed for every patient including type of frenal attachment by Swerin et al, the medial maxillary labial frenal classification based on morphology by Mohan R. et al, morphology of labial frenum by Monti et al, level of frenal attachment by Placek et al, Miller`s grade of gingival recession, gingival biotypes based on Siebert and Lindhe`s classification. .[1–3,10,11]The other clinical parameters recorded include visibility of the periodontal Probe through the gingival sulcus, which has previously been used as a means to clinically assess gingival biotype and the thickness of gingiva measured by an endodontic file. The site recorded was initially anesthetised using 2% Lignocaine with 1 in 80,000 units of Adrenaline administered as local anaesthetic infiltrate.[12,13] A No.25 size endodontic reamer along with its stopper were used such that the stopper covered the tip of the file, this file was then placed along the mid labial aspect of the attached gingiva roughly 3 mm from the base of the gingival sulcus of required teeth namely 11,12,21,22,31,32,41,42.[14,15] Mild amount of preasure was applied ensuring that the file did not bend but instead penetrated the anesthetised gingiva to record the thickness of gingiva using the depth of penetration of the rubber stopper. All obtained values were recorded using a 150mm/6inch stainless steel digital Vernier calliper with an LCD display. A single file was used or one patient only after which it was discarded.

Results

Data was collected as per the above-mentioned study protocol and study design which was then analysed to reveal the following results illustrated in table 1. It was observed that among a Chennai population of one hundred and fifty patients who were examined 78.4% had a mucosal labial frenum attachment, 7.8% had gingival labial attachment and 1.90% had papillary penetrating labial frenum attachment. It was also found that 80% of the population had healthy gingiva while 18.6% had gingival recession and the remaining 1.4% had gingival enlargement. Based on morphology of labial frenum given by Monti et al revealed 72% of the study population had an elongated parallel labial frenal margin while 22% of the population had a triangular labial frenum with an apical base. (table-1)

While comparing Labial frenum morphology with gingival status it was found that 60.5% of the population had a simple single labial frenum without Nodule with healthy gingiva while 23.6% of the population had gingival recession with simple single frenum without nodule whose morphology was elongated parallel margins. (Figure 1)

Gingival biotype is one of the important factors that determines the ultimate fate of surgical treatment plans

and prognosis of periodontal disease as well as any root coverage or restorative procedure.[16–18] There are several advantages and disadvantages affecting both thin and thick gingival biotype. This study combines information gathered from various aspects of clinical examination including level of labial frenum, morphology of labial frenum, type of labial frenum, gingival recession, enlargement, health along with gingival biotype, clinical thickness and visibility of periodontal instrument through the sulcus making it very comprehensive and resolute.

Parts of the recorded data coincide with previous studies in regards to the commonly seen level and type of labial frenal attachment population in a Chennai population.[19,20] Other studies findings which suggested majority of the population had a thick gingival biotype 85% while thin biotype only accounted for 15% of the population[21] did not seem to coincide with the findings observed nor was there any relation with age of the patient.[22] Based on all the findings observed the following assumptions can be made, epidemiologically speaking the most common type of labial frenum is Simple single frenum with a nodule present on the middle 3rd accounting for about 44% of the examined population followed by simple single frenum without nodule. The most commonly seen morphology of labial frenum amongst the examined population was elongated frenum with parallel margins which was seen in 72% of the population. Seventy eight percentage of the examined population appears to have a healthy gingiva with no signs of recession or enlargement while 18% had gingival enlargement.

Table-1 depicts the data gathered and tabulated for analysis from the above-mentioned epidemiological survey

Modified Swerins Classification and Medial Maxillary Labial Frenum Classification on Morphology	
Feature	**Number Of Patients**
Simple Single frenum with no nodule	57
Simple Single frenum with nodule at middle 3rd	67
Simple Single frenum with nodule at alveolar 3rd	18
Tectolabial frenum	8
MORPHOLOGY OF FRENUM (Monti et al)	
Elongated with Parallel margins	108
Triangular with apical base	33
Triangular with alveolar base	9
ATTACHMENT LEVEL (Placek et al)	
Mucosal	120
Gingival	12
Papillary	6
Papillary penetrating	2
Tectolabial	10
ATTACHMENT LEVEL	
Enlargement (Attachment coronal to CEJ)	2
Recession (Attachment apical to CEJ)	28
Attachment At CEJ	120
GINGIVAL BIOTYPE (Siebert and Lindhe)	
Thick Flat	87
Thin Scalloped	63

Image-1(a) Image-1(b) Image-1 (c)

Image-1 depicts Simple single papillary penetrating labial frenum with a nodule along with triangular morphology and coronal base.

Image-1 (b) depicts Tactolabial frenum

Image-1(c) depicts transgingival probing using No. 25 endodontic reamer with stopper

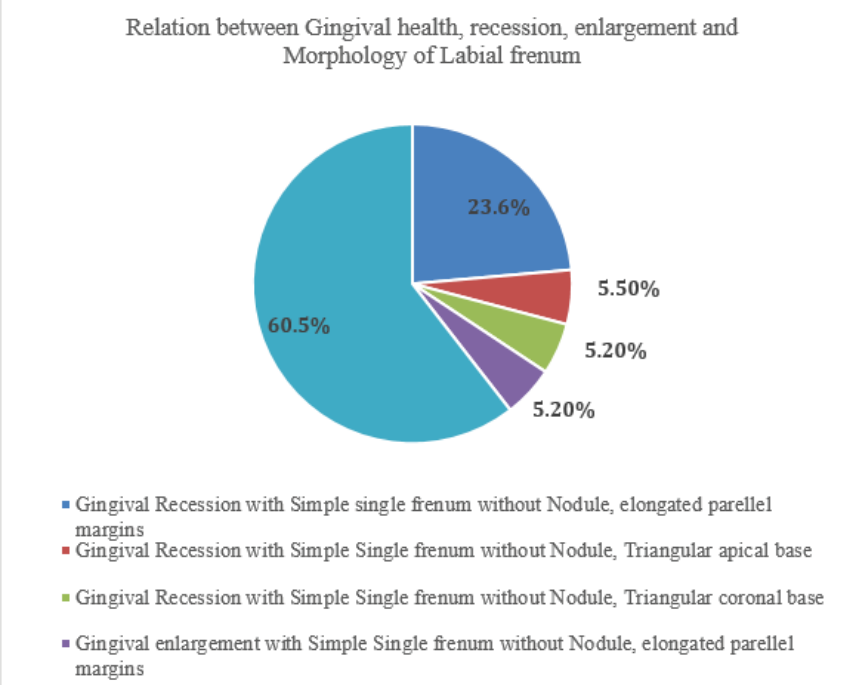

Figure-1 depicts the relation between Gingival health, recession and enlargement with morphology of labial frenum in a Chennai Population.

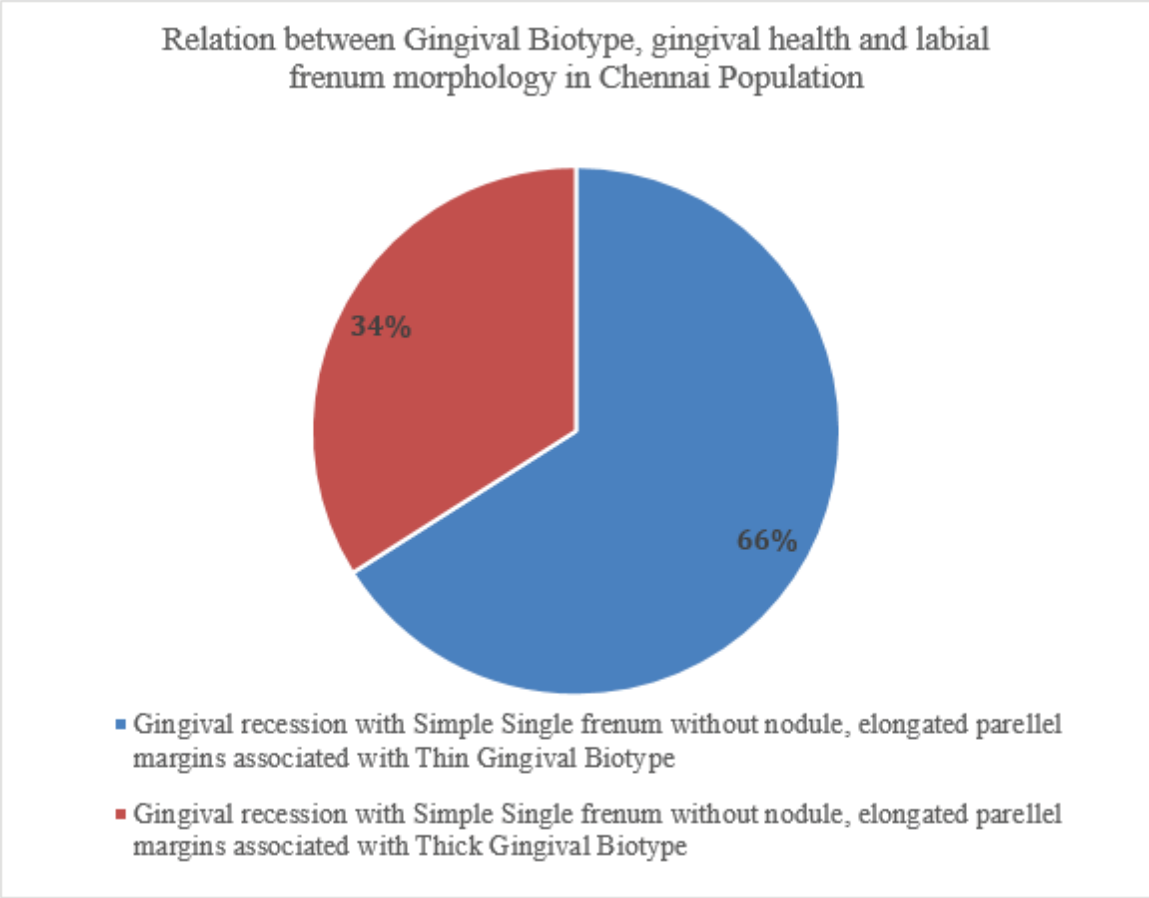

Figure 2-depicts the relation between gingival biotype, gingival health and labial frenum morphology in Chennai population.

While comparing and analysing all the above mentioned Data, one of the interesting features noted was among the population which had simple single labial frenum without nodule (38%), about (34.3%) of the population had recession and of those 23.6% had Elongated labial frenum with parallel margins and 66% of those patients had an underlying Thin gingival biotype. Despite the following findings one still cannot predict the level and type of labial frenum, gingival health or recession and the gingival biotype seen. However, based on the above-mentioned observation one can safely assume that individuals with recession in the upper and lower anterior teeth could have a thick or thin gingival biotype aggravated by the type and level of labial frenal attachment which affect the treatment plan, and overall periodontal prognosis. It is of benefit of the operator that all clinical parameters must be recorded and considered while forming an ideal patient specific comprehensive treatment plan.

Conclusion

In conclusion, among all the patients who had Simple single frenum with no nodule and elongated parallel margins with gingival recession, two thirds of them are likely to have a Thin gingival biotype instead of thick biotype commonly associated with thicker and flatter underlying bone.

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Conflict of Interest: All authors involved in the present study declared No conflict of Interest.

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Ethical Clearance: Approval was taken from the Saveetha University Scientific Review and Ethical Committee (Ethical Approval Number- (SRB/SDMDS11/18/PERIO/26).

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A clinical Study of Obstructive Jaundice Due to Biliary Calculi And Its Management

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Abstract

Introduction: Jaundice can be classified into prehepatic, hepatic or post hepatic. Out of these, the post hepatic jaundice caused by obstruction to the outflow of bile, also known as obstructive or surgical jaundice is the most relevant to surgeons. Materials and methods: it is a prospective study done over 100 cases of obstructive jaundice. **Results**: Highest frequencies (33%) were observed in the age group of 31-40 years with male and female ratio 1: 3.7. Serum bilirubin level in calculous obstructive jaundice cases < 4(mg%) was observed in 60% of the cases. USG detected stone in 90 patients out of a total of 100(90%). Cholecystectomy with Choledocholithotomy and T-tube Drainage was done in 47% cases. Conclusion: For proper management clinical evaluation of obstructive jaundice due to biliary calculi in adults is essential.

Keywords*: Pain abdomen; Cholecystectomy; Ultrasonography; Choledocholithotomy.*

Introduction

The word jaundice or icterus is the yellowish staining of the skin, sclera and mucous membrane by deposition of a bile pigment named bilirubin in these tissues.[1]

Jaundice, caused by elevation of the serum bilirubin level, can be demonstrated in the sclera, the frenulum of the tongue, or the skin. Serum bilirubin levels above 2.5 mg/dl are necessary to detect scleral icterus routinely, and levels above 5 mg/dl will be manifested as cutaneous jaundice.[2]

Jaundice or cholestasis can be classified into prehepatic, hepatic or post hepatic. Out of these, the post hepatic jaundice caused by obstruction to the outflow of bile, also known as obstructive or surgical jaundice is the most relevant to surgeons. Obstructive jaundice is not a definitive diagnosis and early evaluation to establish the aetiology of the cholestasis is crucial to avoid secondary pathological changes (e.g. Secondary biliary cirrhosis) if the obstruction is not relieved.[3]

Obstructive jaundice occurs due to intra or extrahepatic obstruction to biliary outflow. It should be differentiated from intrahepatic cholestasis in hepatocytes or biliary canaliculi (non-obstructive) and the bile conduits within the liver (obstructive form). Both these forms of cholestasis have a similar pathophysiological basis and biochemical changes and it is crucial to differentiate these two, as the extrahepatic cholestasis is potentially amenable to surgical correction.

The present work is an attempt to study and evaluate the clinical aspect of obstructive jaundice due to biliary calculi in adults with special reference to its management in the light of the review of pieces of literature, as obstructive jaundice due to CBD stone is quite common in our region.

Materials and Methods

This prospective study was carried out in the department of general surgery, Guwahati Medical College and Hospital, Guwahati from 1st July 2016 to

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30th June 2017. Out of all surgical admissions, 100 cases of obstructive jaundice with USG proved calculi in CBD or suspected of calculi of different age group were selected randomly.

A detailed history and clinical examination were done and appropriate investigations recorded. Patients were assessed pre-operatively and later subjected to surgery or endoscopic procedure depending on the need. Post-operatively patient condition was assessed and complications were documented. Patients were followed up for a mean period of three months where patients underwent surgical or endoscopic intervention.

Results

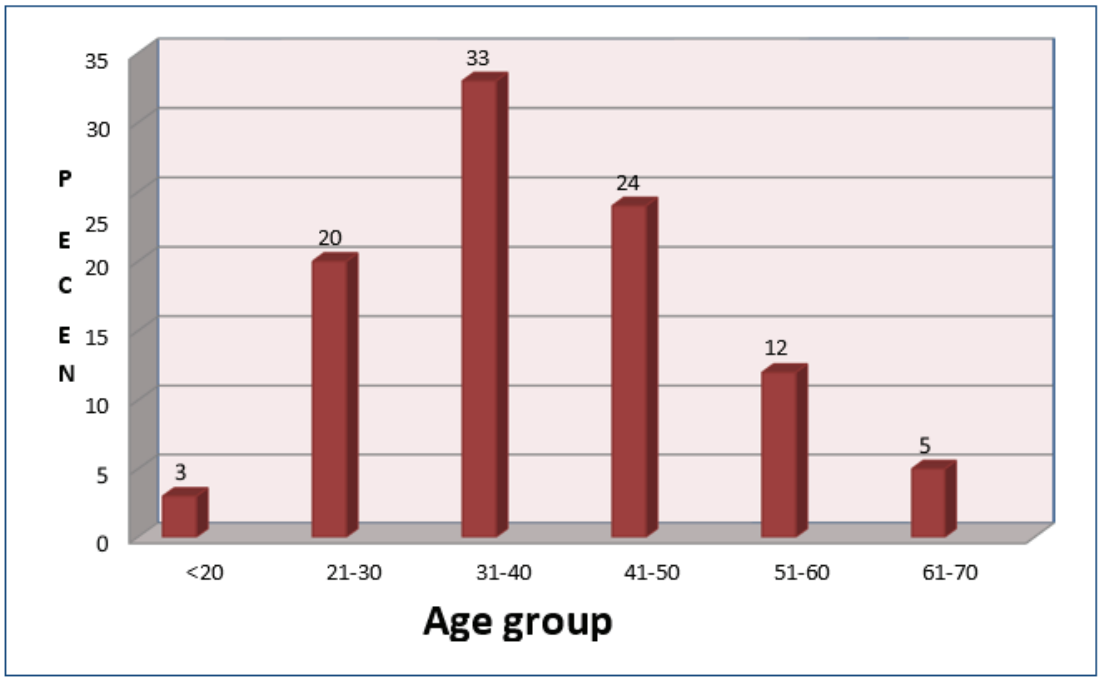

Fig. 1 Age incidence of obstructive jaundice due to choledocholithiasis

Table 1 reflects the sex-wise distribution of obstructive jaundice cases due to choledocholithiasis. The male and female ratio was 1:3.7.

Table 1 Sex incidence of obstructive jaundice due to choledocholithiasis

Sex	Number of cases	Percentage (%)	Ratio (male: female)
Male	24	24.00	1: 3.7
Female	76	76.00	
Total	100	100.00	

The presence of biliary colic associated with intermittent abdominal pain, fever with chills and rigour and intermittent jaundice (**Charcot's Triad**), was found in 22 cases out of 100 patients (22%) of calculous obstructive jaundice in the present study.

The clinical features of obstructive jaundice due to choledocholithiasis were narrated in **Fog. 2**.

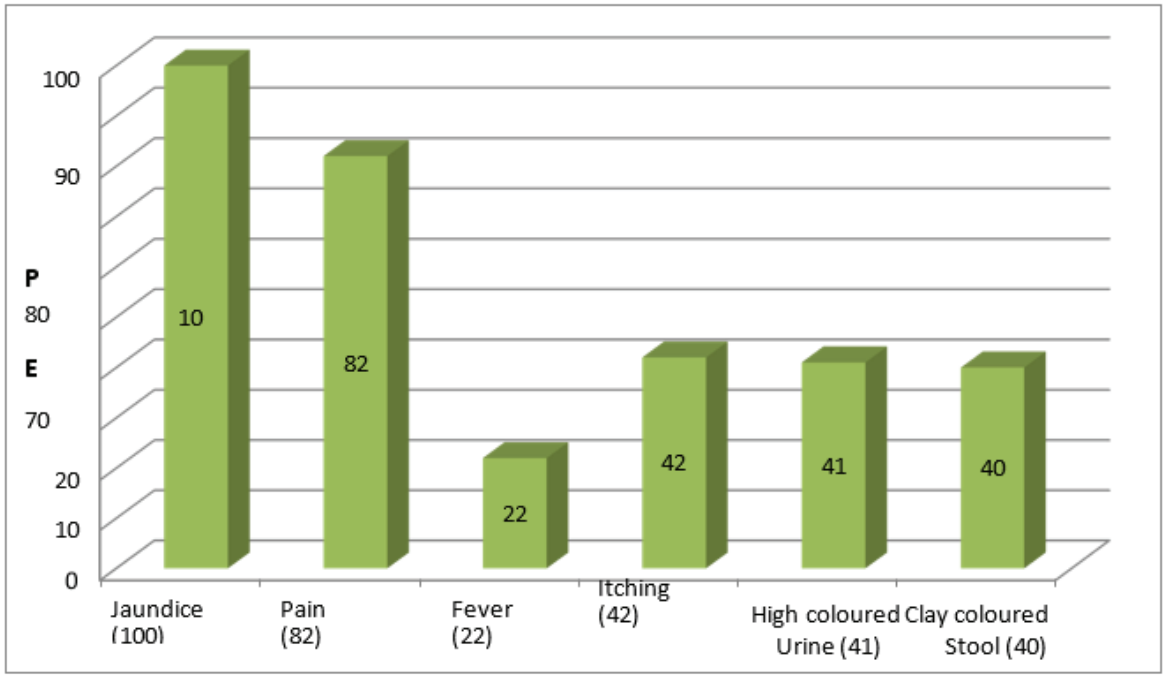

Fig. 2 Clinical features of obstructive jaundice due to choledocholithiasis

Table 2 Shows serum bilirubin level in calculous obstructive jaundice cases. Its level < 4(mg%) was observed in 60% of the cases.

Table 2 Serum bilirubin level in calculous obstructive jaundice cases

Serum bilirubin level	No of cases	Percentage (%)
< 4(mg%)	60	60
4 -10(mg%)	29	29
>10(mg%)	11	11

In the present study 63 out of 100 patients (63%) showed a high value of serum alkaline phosphatase. Serum gamma-glutamyltransferase (GGT) level was higher in 96% of cases. A total of 22 patients showed raised total leukocyte count (TLC) along with other features of cholangitis. However, the serum albumin level was within the normal range in 94 patients with a mean value of 3.94 gm%.

USG detected stone in 90 patients out of a total of 100(90%). In 4% of patients, USG showed GB stone with CBD normal in diameter without any stone and rest 6% patients showed GB stone with dilatation of CBD without visible calculi in CBD as shown in **Table 3**.

Table 3 Findings of Ultrasonography of the cases

USG finding	No of Patients	Percentage (%)
GB stone or sludge +CBD stone	40	40
Only CBD stone	40	40
GB stone or sludge+Normal CBD	4	4
GB stone or sludge + Dilated CBD	6	6
Postcholecystectomy status, Residual Stone in CBD	10	10
Total	100	100

The different procedures adopted in the present study for calculous obstructive jaundice is shown in **Table 4**.

Table 4 Procedures employed in this present study of calculous obstructive jaundice

Diagnosis	Procedure undertook	No of cases	Percentage (%)
Cholelithiasis with Choledocholithiasis or only choledocholithiasis/Retained stone	ERCP followed by Lap cholecystectomy/ERCP	34	34
Cholelithiasis with Choledocholithiasis / choledoch olithiasis	Cholecystectomy with Choledocholithotomy and T-tube Drainage	47	47
Cholelithiasis with choledocholithiasis (CBD >1.5 cm)	Cholecystectomy with choledocholithotomy & Choledochoduodenostomy	12	12
Cholelithiasis with choledocholithiasis (CBD > 1.5 cm)	Cholecystectomy with choledocholithotomy & Chole dochojejunostomy	2	2
Cholelithiasis with Impacted stone in CHD, LHD	Cholecystectomy with Hepaticojejunostomy	1	1
Choledocholithiasis (single calculus, normal CBD diameter)	Cholecystectomy, CBD Exploration and Primary Closure	4	4
Total	**-**	**100**	**100**

Discussion

In the present study, the youngest patient was 18 years of age and the eldest was 72 years, with the mean age being 38.78 years which is contrary to Way et al.,[4] who in their study observed that the incidence of common bile duct stones causing obstructive jaundice was highest amongst the age of 61-70 years of age. Baker et al.,[5] in their study found the age for calculus obstruction to be in the range of 51-60 years of age. In the same study, the male to female ratio of 1:3.8 was revealed supporting the present results. Women are three times more likely to develop gallstones than men as reported by Nakeeb et al.[6]

Rubin et al.,[7] in their series of 60 patients of choledocholithiasis found this triad in 26.6% of cases and Leung JW et al.,[8] in 21% of patients are in support with the current study.

Various authors reported incidence of different clinical features of obstructive jaundice due to biliary calculi consistent with the current study are given in **Table 5**.

Table 5 Clinical features of obstructive jaundice as reported by different authors

Clinical features	Prabhakar A et al.9	Agarwal et al.10	Anand et al.11	Phillipo Chalya et al.12	Nageeruddin Shaik et at.13	Present study
Jaundice	60	100	53.6%	58.6	100	100
Pain abdomen	100	79.1	71.4	17.2	95	85
Itching	50	50	39.28	43.1	35	42
Fever	14	12.5	10.71	-	25	22
Nausea/Vomiting/ dyspepsia	70	70.9	35.71	-	50	37
Loss of appetite/ weight	50	-	32.14	56.9	30	50
High coloured urine	60	-	39.28	-	-	
Clay-coloured stools	40	41	32.14	-	10	42

Total serum bilirubin was higher to 5.68+0.473 mg/dl (Mean+SD) in the current study is in support of Elzeiny Mmet et al.,[14] and Kumar PN et al.[15] The mean conjugated serum bilirubin level in the present study was 4.16+0.471 mg/dl agree Prabhakar A et al.,[9] and Elzeiny Mmet et al.[14]

Western authors Mullen et al., and Lygidakis[16] found high values of serum alkaline phosphatase (ALKP) in 53% and 36% of patients with cholelithiasis which is almost similar to our study. The study of Prabhakar S et al.,[17] and Elzeiny Mmet et al.,[14] support the current findings of ALKP level, i.e., 292.86+12.358 IU/L.

The high level of (mean) Gamma Glutamyl Transferase (GGT), i.e., 233 of the present study agree Peng WK et al.,[18] and Anciaux ML et al.[19] Also, the mean serum albumin level of 3.94 gm% of the present study is in support of Anand S et al.,[11] and Kumar PN et al.[15]

In our study, USG of abdomen detected biliary calculi in 90 out of 100 patients screened and thus showed a sensitivity of 90%. Stones were also found during interventions in these 90 patients. Thus showing the specificity of 100%, which is similar to the studies of Caddy GR et al.,[20] M Sugiyama et al.,[21] and Blumgart.[22]

Since the introduction of laparoscopic cholecystectomy and endoscopy, preoperative ERCP has become the standard approach for patients with suspected CBD stones.[23] In all patients who were considered fit for ERCP were counselled for ERCP first. But in 40 patients only, ERCP could be done due to lack of patient's compliances.

In 47 cases where moderately dilation of common bile duct with multiple stone or sludge or fragmented stone, choledocholithotomy along with T-tube drainage was done. The rubber T-tube was secured in place by 3-0 chromic catgut and kept in place for 8-12 days. In one study by Anand S et al.,[11] 41% of patients were treated with T-tube while this procedure was performed in 33% patients in a study by Kumar PN et al.[15] In the present series of study, this procedure was performed in 47% of patients, showing the value is close to the other studies.

In the remaining 12 cases where multiple stones or biliary sludge was found, along with hugely dilated (>1.5 cm) common bile duct, Choledochoduodenostomy after removal of the stone was done. A wide enough (at least 2.5cm) anastomosis between the common bile duct and duodenum was done, so that free entrance and progress of bile were possible and any stasis or stone formation was avoided. Maingots found that the size of stoma of more than 2 cm is essential for the success of the operation.

In two patients Choledochojejunostomy was done as it was difficult to mobilize the duodenum. In one patient with impacted stone in CHD and Left Hepatic duct Hepaticojejunostomy done.

Drainage of the sub-hepatic area was done in all the cases with Abdominal safe tube drain brought out through a stab wound on the right flank. The drain was removed after 2-3 days after ensuring that the drain output was almost negligible.

All the patients were followed up for the first time after one week, thereafter two weekly for three months. No complication developed during the follow-up period. In four patients post ERCP complications developed, which were treated conservatively and discharged and lap cholecystectomy was done after six weeks. In those patients ERCP stents were placed, the stents were removed along with one removal after 6 weeks by the endoscopic method. Those patients came during the last quarter of the study period they could not be followed up completely.

The percentage of surgical site infection reported by Lygidakis[16] was 8.9%. In the present series, seven patients out of 66 cases operated developed surgical site infection postoperatively (10.6%). One (1.51%) patient developed bile leakage after removal of T-tube which is comparable with other studies. Bile leak was found in 1.51% of cases in the present study which was similar to the study of Gharaibeh KI et al.,[24] Wills VL et al.,[25] and Maghsoudi H et al.[26]

One study by TCK Tham et al.,[27] found post ERCP complications as pancreatitis occurred in nine of 208 patients(4.5%), haemorrhage in two(1%), and fever in one (0.5%).[27] According to Blumgart, acute pancreatitis, 1.5% to 5.4%; cholangitis, 1% to 2.7%.[28] In the present study, acute pancreatitis was in 5% and cholangitis was in 5% of patients, which is very close to the above studies.

The study shows that statistically there is no significant difference between endoscopic treatment and open surgical treatment of obstructive jaundice due to choledocholithiasis (p-value 0.8328).

Conclusion

The common presentation of surgical jaundice is jaundice and the common cause of surgical jaundice is CBD calculi. USG remains the cheapest, safest and most reliable diagnostic tool in the management of surgical jaundice. Endoscopic treatment and open surgical

treatment both are equally good treatment modality in the management of obstructive jaundice.

Ethical Clearance: Taken.

Conflict of Interest: None.

Source of Funding: None.

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Original Research Paper

Evaluation of Sociodemographic Characteristics of Pregnant Women without Any Medico-Legal Issues

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Abstract

Background and Objectives: In light of the pregnancy, identifying sociodemographic issues is crucial for optimising antenatal care. The period of pregnancy is very critical, as each of the women expects a usual outcome. Any inconvenience caused to both mother and unborn fetus arising out of the mismanagement may invite litigation as prescribed by the land rule. Therefore, we aimed to analyse different sociodemographic factors associated with pregnancy without medico-legal issues (MLI).

Materials and Methods: It is a prospective, cross-sectional study carried out on 325 participants of pregnancy without any MLI. All the participant were observed and interviewed to know the inconvenience faced during their antenatal period if any. The medical record was physically verified, and sociodemographic data were recorded for analysis. Descriptive statistical methods were computed, and the statistical significance was tested by chi-square test and student's t-test. eth

Results:Majority of patients, i.e. 48% were within the age group of 21 to 25 years. The number of nulliparous women was the highest being 143 (44%). Majority of the cases belonged to the middle socioeconomic class. 189 (58%) cases came from a rural background while 136 patients (42%) came from urban areas, and 65.5% of cases were the booked cases. Majority of 197 (60.6%) cases comprises Hindu.

Conclusion: The pregnancy cases without any medico-legal complication show average sociodemographic findings. Such findings are useful for targeted health policies aimed at attaining healthy pre-pregnancy care.

Keywords*: Social inequalities; adverse pregnancy outcomes; pregnant women; medico-legal issues (MLI).*

Introduction

The relation between social factors and the populations' health conditions has been investigated in the literature for some years.[1-5] Acknowledging socioeconomic inequalities as determinants of increasing health inequities and identifying the magnitude of these inequalities is essential for promoting public policies that can reduce these differences.[3]

Individuals with more significant social deprivation have fatalistic beliefs about their health and less awareness of the need for care. In this context, health and wellbeing are concepts that express social and population thoughts, which, in turn, are influenced by cultural and demographic values resulting from how they relate to a territory and its characteristics.[6]

Social demographic factors significantly influence individuals' habits and behaviours and their knowledge, perception, and ability to self-manage health.[7] Economically disadvantaged people are disproportionately affected by the due care. Concerning family income, scientific evidence shows that low family

income is linked to the more flawed perception of one's pregnant condition. The lower the income, the lower the proportion of people who have access to ante-natal services,[1,8] affecting the pregnancy.

Thus, social and demographic factors can influence health conditions and even give rise to subgroups of greater vulnerability, highlighting prenatal care. The literature considers pregnant women a strategic population group for applying for educational programs, recognising pregnancy as a favourable phase for establishing healthier habits. Pregnant women are psychologically receptive to new knowledge, which makes them prone to adopting new and better health practices, the benefits of which extend to the rest of the family.[6,9-12]

The incorporation of healthier behaviours by pregnant women depends on socioeconomic factors, such as the number of children, and age.[13-15]

For all parents and grandparents, birth is a joy, a wonder and a renewal of hope. One of the most devastating, life-changing events for parents is finding out their child suffered from damages or maternal loss.[16] Any embarrassments caused to both mother and fetal loss following treatment may create medical litigation against the service providers.

Therefore, this paper analyses the social-demographic factors of pregnant women without having any MLI.

Materials and Methods

It is a perspective and cross-sectional study in a tertiary care centre on 325 pregnant women cases without MLI. The study population was drawn from different parity between rural and urban areas and various socioeconomic classes and other ethnic groups.

Thus, samples were physically examined, and data were collected for statistical analysis, which was performed using a Statistical Package for the Social Sciences (SPSS) software version 20.0. Descriptive statistical methods were computed, and the statistical significance was tested by chi-square test and student's t-test. A p-value of less than 0.05 was considered statistically significant. Ethical clearance was taken from ethics committee (Human).

Results

Age group

The ages of the patients studied were in the range of 18 to 35 years. Majority of patients, i.e. 48% were within the age group of 21 to 25 years, followed by 39% in the age group of 26 to 30 years (**Table 1**).

Table 1 Showing the age distribution

Age in years	No. of cases	Percentage (%)
18 – 20	36	11%
21 - 25	156	48%
26 - 30	127	39%
31 - 35	6	2%

Parity

The number of nulliparous women was the highest being 143 (44%). The number of women with parity one, parity two, parity three was 128 (39.38%), 41 (12.62%), and 10 (3%) respectively. The parity four groups had only three cases (**Fig. 1**).

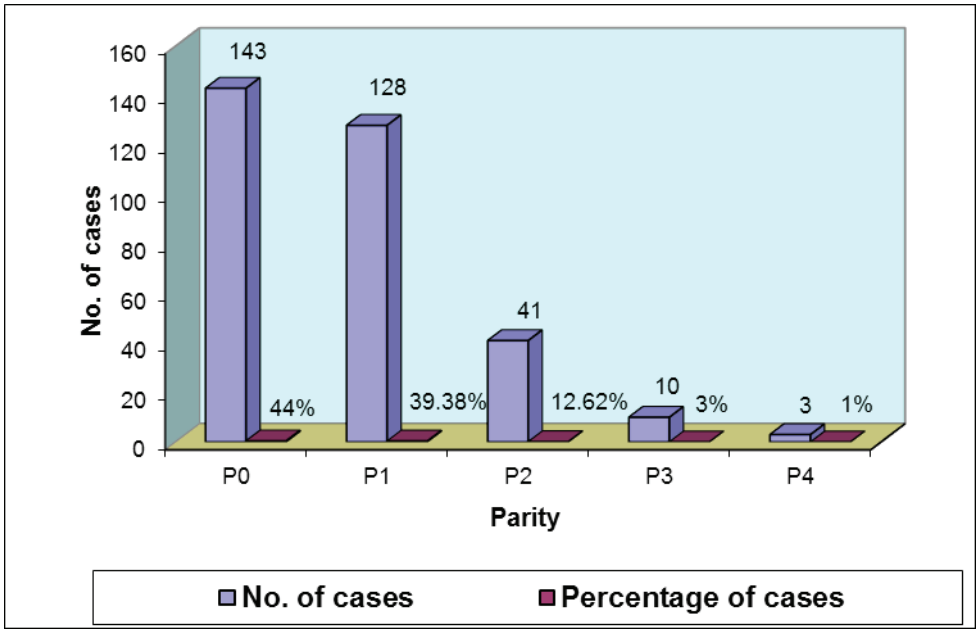

Fig. 1 Showing the parity of the patients

Socio-economic status

The socioeconomic status is determined by the annual income of the patient and her husband combined. Low socio-economic group is income below Rs/- 40,000.00 per annum, Middle is between Rs/- 40,000.00 to Rs/- 1,60,000.00 per annum and High is above Rs/- 1,60,000.00. Majority of the cases belonged to the middle socioeconomic class. There were 153 cases (47%) from a middle class, 136 cases (42%) from the lower class and 36 cases (11%) form the upper class. (**Fig. 2**)

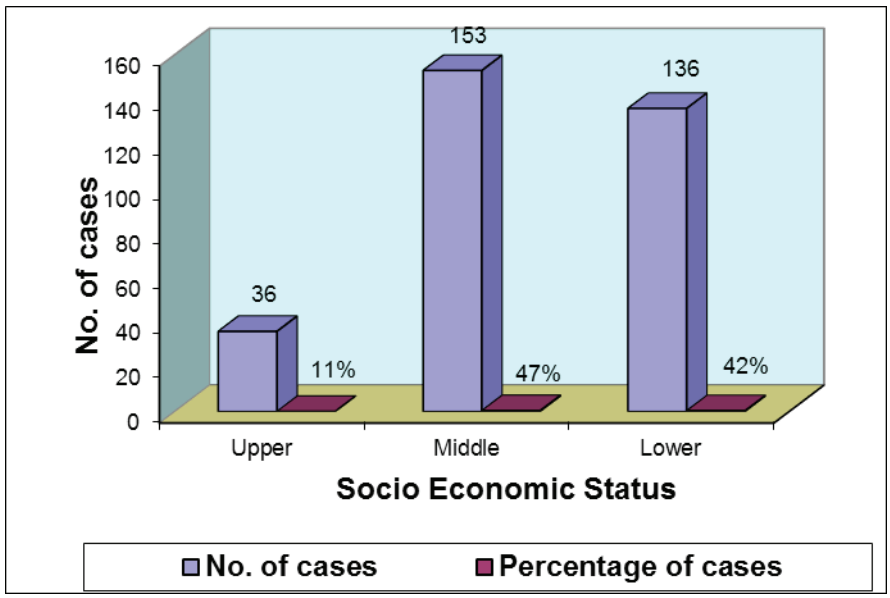

Figure 2 Diagram showing the socioeconomic status of the patients

Locality

One hundred eighty-nine cases (58%) came from a rural background, while 136 patients (42%) came from urban areas (**Fig**. **3**).

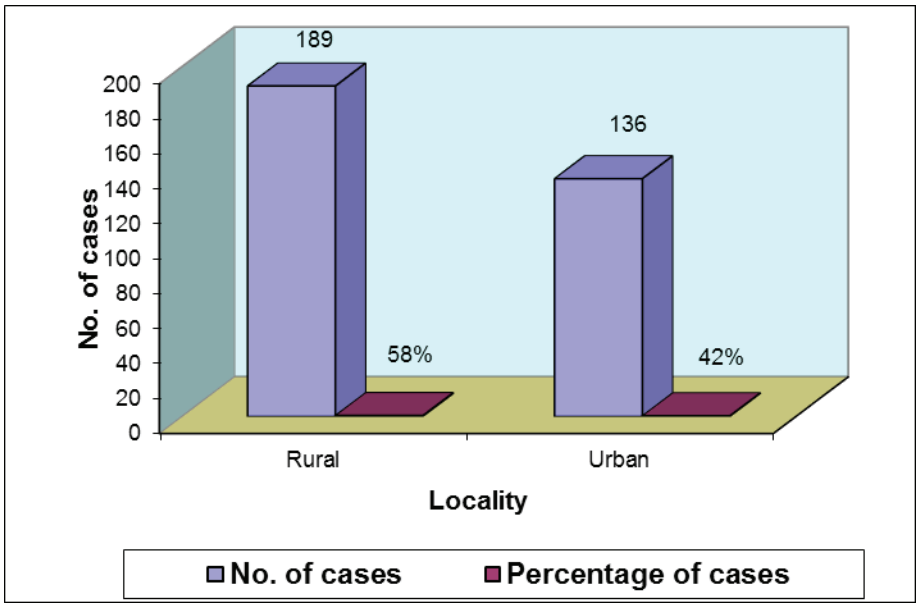

Figure 3 Diagram showing the locality of the patients

Booked or un-booked cases

There were booked and un-booked cases. The un-booked patients had no or less than three antenatal check-ups. The booked patients had regular antenatal check-up (at least a minimum of 3 antenatal check-ups) as shown in **Fig. 4**).

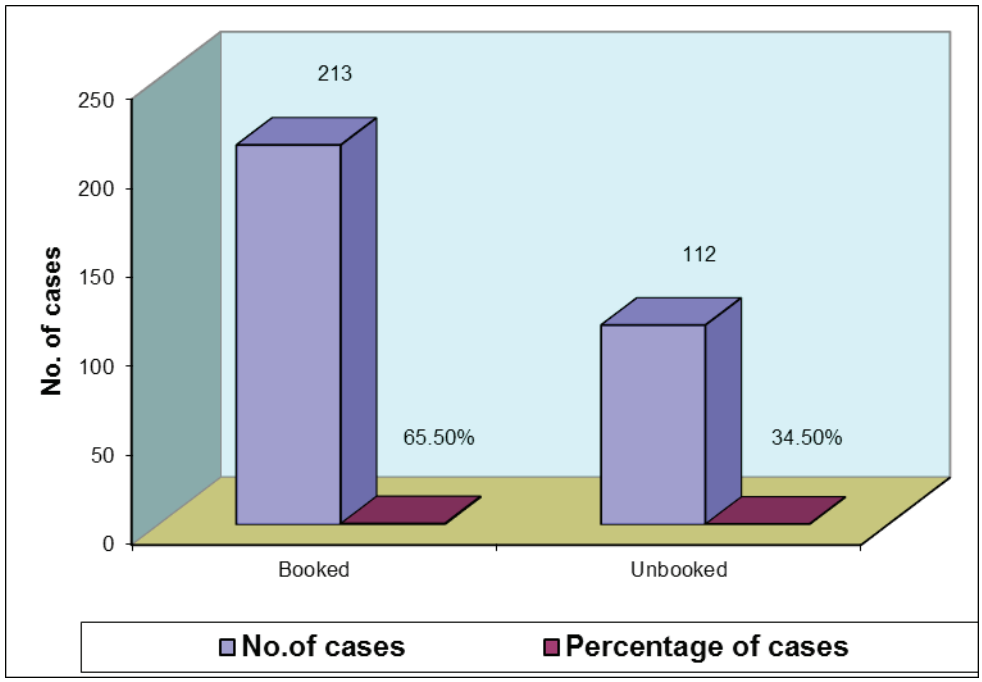

Figure 4 Showing the Booked or Un-booked status of the patient

Religion

Majority of the patients were Hindu, followed by Muslims. One hundred ninety-seven cases (60.6%) were Hindu, 115 cases (35.4%) were Muslim, 8 cases (2.5%) were Christian, and five patients (1.5%) were Sikh as shown in **Table 2**.

Table 2 showing the different religion of patients

Religion	No. of cases	Percentage of cases
Hindu	197	60.6%
Muslim	115	35.4%
Christian	8	2.55%
Sikh	5	1.5%

Discussion

In the current study, most pregnant women, i.e. 48%, were within 21 to 25 years that has given birth of a normal baby. In contrast, inconsistent results have been found in maternal obesity with age at giving birth, as revealed by some recent study.[13-18]

The number of nulliparous women having normal pregnancy was the highest being 143 (44%). Some studies[13-15] have revealed that the incorporation of healthier behaviours by pregnant women depends on socioeconomic factors, such as the number of children, and age is in the current study's agreement.

Majority of the standard pregnancy cases belonged to the middle socioeconomic classes agrees the fact revealed in some review.[1,7,8]

One hundred eighty-nine cases (58%) of typical pregnancy cases came from a rural background while 136 patients (42%) came from urban areas though the findings are not significant. However, this fact can be explained that rural women engage themselves in physical activities to withstand the stressful pregnancy period, which is essential. Also, supplementation of less adulterated food and hygienic environment can be a factor for good health.

The current study revealed 65.5% booked cases to have normal pregnancy implying the importance of antenatal check-up. Though the majority 197 (60.6%) of the patients were Hindu, suitable support was not found to compare with the present results.

Conclusion

This survey recommends that the women's usual sociodemographic factors positively influence their perception about ante-natal care. Thus, the identified clinical predictors serve as the standard criterion beyond which women need specific antenatal attention to avoid any medico-legal inconveniences to the service providers.

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Contribution of authors: (1) The article is original with the author(s) and does not infringe any copyright or violate any other right of any third party. (2) The article has not been published (whole or in part) elsewhere and is not being considered for publication elsewhere in any form, except as provided herein. (3) All author(s) have contributed sufficiently in the article to take public responsibility for it and (4) All author(s) have reviewed the final version of the above manuscript and approved it for publication.

Ethical Permissions: Taken from the institutional ethics committee (Human).

Informed consent: Before the collection of the data, informed consent was also taken from the participants.

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Oral Health Awareness Among Parents of Differently Abled Children : A study in North Indian Population

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Abstract

Introduction Good oral health is of paramount importance not only for chewing but also plays a major role in overall healthy development of a child. Differently abled children may not be able to look after their oral health and hygiene themselves. Every child has the equal right for healthy development and opportunities in various fields of life. As most of the differently-abled children perform their regular activities with the help of or under the supervision of a family member or caregiver, it is important for the parents or caregivers to have sufficient knowledge about oral hygiene measures in order to guide the child efficiently.

Materials and Method: A structured modified oral health awareness questionnaire having two components- sociodemographic data and questionnaire- related to oral hygiene habits, sugar exposure, and oral health seeking behaviour was constructed. The parents and guardians of 200 institutionalized physically/mentally challenged students were asked to fill up the questionnaires and were then educated regarding proper oral hygiene measures with the help of posters and audio-visual aids.

Conclusion: Differently children, due to limited ability and inadequate guidance on oral hygiene maintenance, are prone to poor oral health. There is lack of knowledge of importance of oral health in parents of differently abled children. It is the duty of oral health care workers to educate the parents of differently abled children about significance of oral health and how they can train their child for maintenance of proper oral health and hygiene for their overall healthy development.

Key words: *Oral heath, awareness, differently abled children*

Introduction

It is a human right of children to become empowered to their full potential and attain sustainable growth. In view of this, the dental health care like many other fields has an essential role and responsibility to enable proper care for their healthy development. Although every child has the right to get equal chances and opportunities, the differently abled children do not get the proper care required from the society and sometimes even by their parents.

Mental and physical disability has a rising trend with the population rise and aging process. More than 80% of the mentally or physically challenged persons come from the developing countries. As per the National Sample Survey Organization, in 2002 about 1.8% of the National Population in India was affected by disability. It is true that disability has seldom been related to physiological deficit. The American Health Association defines a child with disability as "a child who for various reasons cannot fully make use of all his or her physical, mental and social abilities".

In 1995, the Government of India under the "Persons with Disabilities Act" described "handicapped" as a person with one or more of the following disabilities: impaired vision, leprosy-cured, hearing impaired, orthopaedic disability, mental retardation and mental illness. Unlike Disability Act, 1995, which defines disability in relation to medical conditions and degrees of impairment, the UN Convention, sees disability as an interaction of impairments and barriers that hinder effective participation in a society. United Nation Convention on Rights of Persons with Disabilities

(UNCRPD, 2008) was brought into force in India in May 2008. With growing understanding, the term disability has undergone a paradigm shift from merely a medical one to a meaning that accounts for a person's physical, as well as social aspects. It includes the person's physical health or impairment along with a variety of environmental factors.

Oral health is important for maintaining good overall health and well-being throughout life. Proper oral hygiene is important not only for masticatory and nutritional functions but it also plays a major role in communication and social well-being of a person. The mouth is known to be a looking mirror into the overall health of the body, giving important indicators for a multitude of disorders. In drafting a healthcare programme or strategy, it is important to take into account the oral health of a person for the successful implementation of the programme or policy. Oral disease also affects the quality of life for children with disabilities, who have a higher prevalence and severity of oral diseases. It also has a negative social impact due to the aesthetic aspects of dental health.

As most of the differently abled children perform their regular activities with the help of or under the supervision of a family member or caregiver, it is important for the parents or caregivers to have sufficient knowledge about oral hygiene measures in order to guide the child efficiently. In light of these facts, the present study was conducted with the aim to assess the oral health awareness and knowledge among parents of differently abled children in the North Indian population.

Materials and Method

A descriptive cross-sectional study was carried out with the aim to assess the knowledge of oral hygiene measures among the parents or caregivers of differently abled children attending special schools of North India. The study was carried out over a period of 1 year from June 2019 to May 2020 among differently abled children in Delhi NCR. Ethical approval was obtained from the Institutional Ethics Committee (SU/SMS&R/76-A/2019/62). The sample size for the present study was calculated based on the data obtained from census, 2011 for disability in Delhi. According to the census, the total number of physically challenged children with visual impairment was 4170, hearing and speech impaired children was 8684 and orthopedically challenged was 7084. The sample size for this study was determined to be at a 95% confidence interval. Sample size was calculated to be 200. The addresses of the institutionalized physically challenged students were obtained from the Department of Social Welfare, Delhi. Parents/guardians of institutionalized physically/mentally challenged students were included in the study. Illiterate or uneducated parents/guardians and parents/guardians of children staying at full-time boarding institutes for differently abled students were excluded from the study. A self structured modified oral health awareness questionnaire consisting of 16 questions based on the WHO Basic Oral Health Survey 2013 and 1997 was used to collect the data. The questions were written in both English and Hindi languages. The proforma had two components: sociodemographic data such as gender, type of handicap, duration of disability etc and questionnaire related to oral hygiene habits, sugar exposure, and oral health seeking behaviour. The parents/guardians were asked to fill up the questionnaires and were then educated regarding proper oral hygiene measures with the help of posters and audio-visual aids.

Results

A total of 200 parents/care givers of differently abled children participated in the survey.There was found to be a higher percentage of male students enrolled in the special schools. Seventy percent of the children whose parents/caregivers undertook the study were physically disabled, while 30% were suffering from mental disability. The educational qualifications of the parents/care givers were found to be average with 37% graduates and 34.5% having intermediate level qualifications. Most (51%) of the children carried out tooth cleaning by themselves followed by 35.5% doing it under supervision and 13.5% doing with the help of other person (fig. 1).

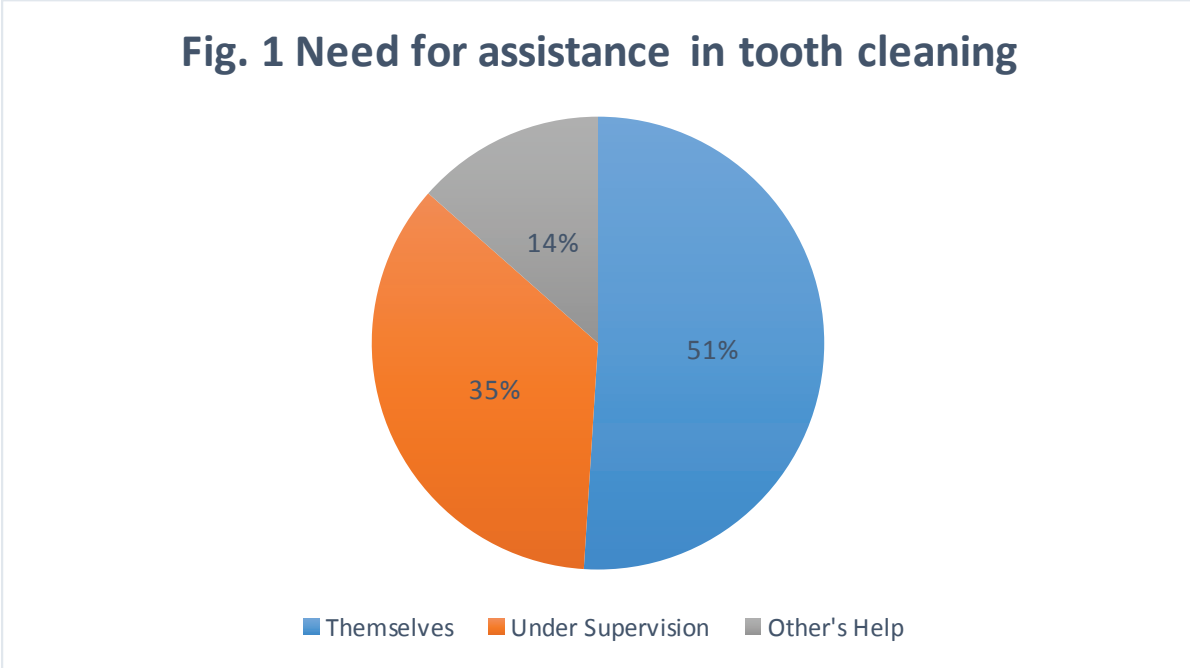


Fig. 1 Need for assistance in tooth cleaning
14%
51%
35%
Themselves
Under Supervision
Other's Help

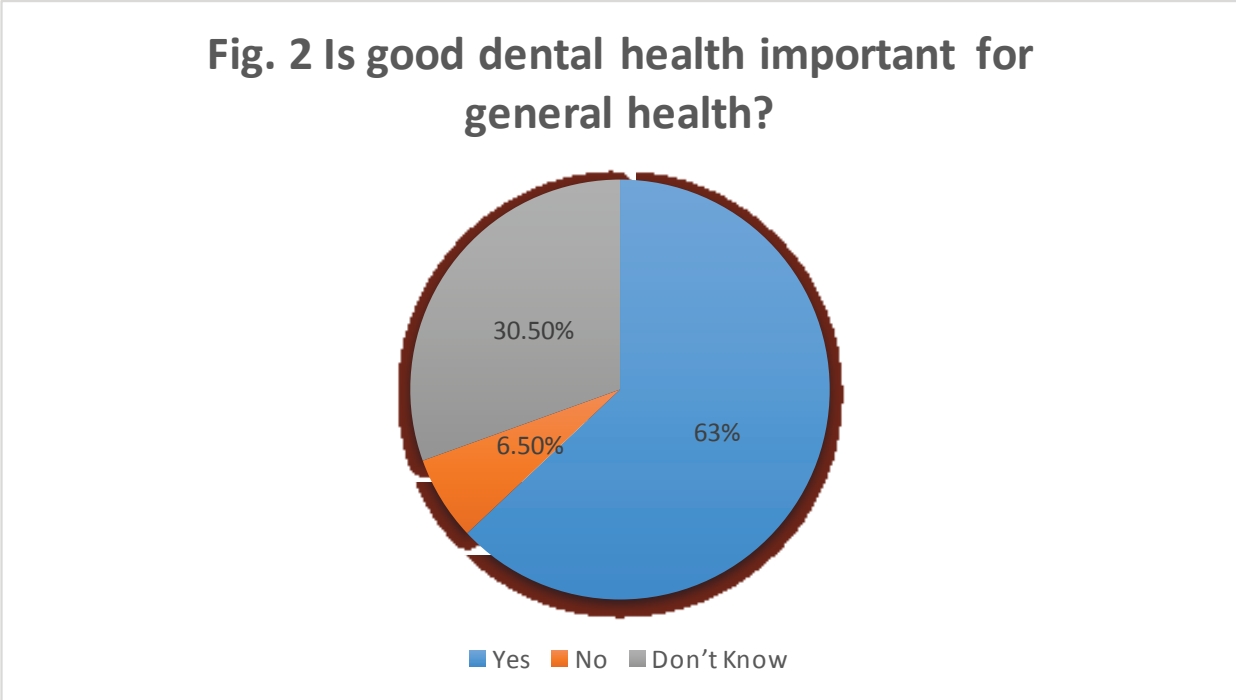
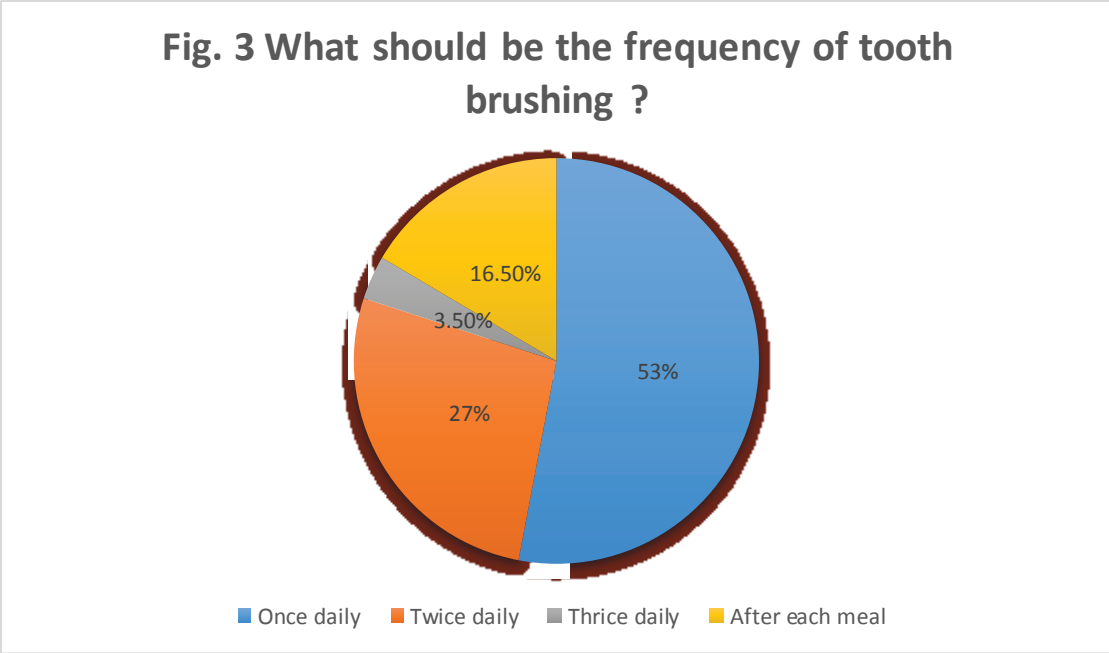
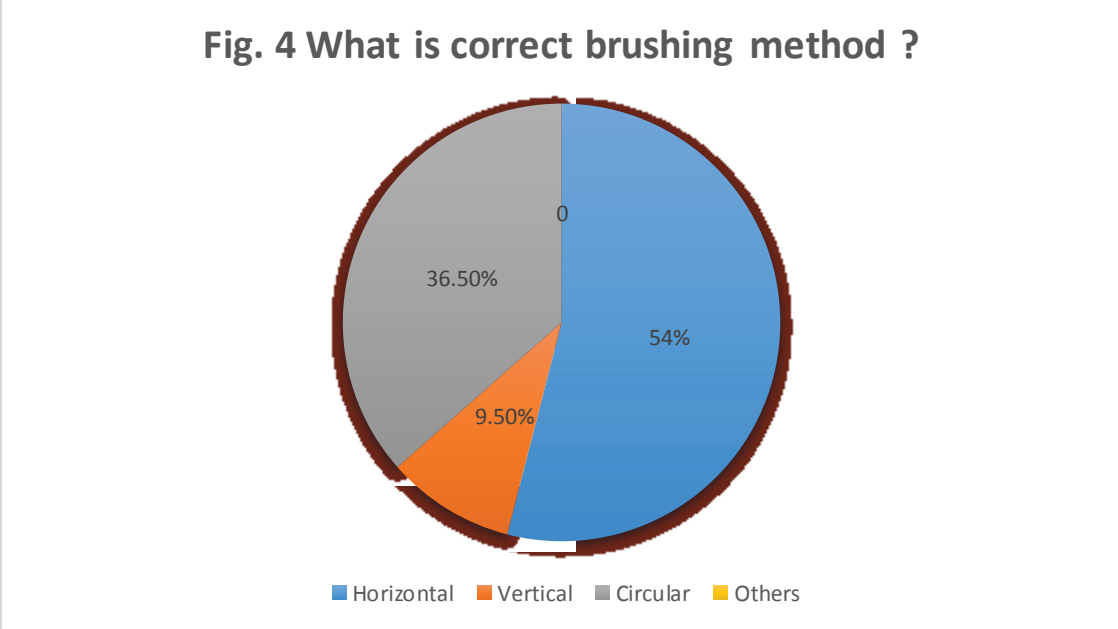


Fig. 2 Is good dental health important for general health?
30.50%
6.50%
63%
Yes
No
Don't Know





All of the study participants agreed that good dental health is an essential prerequisite for chewing, however, only 63% of them were aware of the link between oral and general health (fig. 2). Forty three percent of the respondents were of the view that dental visits should be made twice per year. A majority (53%) of them also believed that teeth should be brushed only once daily (fig. 3). Fifty four percent of the participants stated that their child brushes with a horizontal motion while 36.5% chose circular motion (fig. 4). Sixty one percent of the respondents were found to be aware that sugary food causes dental decay as well as 74.5% agreeing that soft drinks are detrimental to tooth integrity. While 51.5% of the participants had heard of fluoride, 43.5% accepted that

they do not know the function of fluoride in maintaining oral health. On discovering a pit or discoloration in a tooth, 50.50% study participants mentioned that they would go to their dentist immediately. Forty percent of the respondents were aware that bleeding gums is the reason behind blood on toothbrush while brushing.

Discussion

As the disabled child belongs to a vulnerable section of the community, it is essential to set a comprehensive policy, addressing not only the requirements of the disabled child, but also of the relations to enable them to steer a traditional life. Oral hygiene awareness in India is subject to social attitude, beliefs and traditions. Therefore a scientific analysis of their awareness is the need of the hour for charting out any healthcare policy. Majority of the parents could identify the importance of good oral health for both function and aesthetics as well as systemic health.

A considerable number of parents were of the opinion that, it is necessary to take the child for regular dental visits which shows a positive attitude. It has been seen that dental check-ups from an early age significantly reduce the risk of dental caries and other oral health problems.[1,2] This was in line with other studies by Nagarajappa et al, Sakai et al and Talekar et al.[3,4,5] However 36% the participants believed that there is no need to get a dental check-up done unless there is pain or discomfort. Reasons for lack of visit to a dentist maybe fear of pain, expenses and poor knowledge of the importance of dental health. This delay in proper care results in added morbidity to the patient which in turn leads to increased costs, thus continuing the vicious cycle of the perception that dental treatment is expensive. These hindrances need to be evaluated and eliminated by oral health education.

Respondents had partial knowledge on the frequency of brushing the teeth. Most of them were of the opinion that brushing once daily in the morning is sufficient to take care of their child's oral health. There was also low awareness about the proper brushing technique as horizontal brushing habit was found to be most prevalent among children. According to the American Dental Association, proper brushing technique requires the placement of toothbrush at a 45-degree angle to the gums followed by gentle back and forth movement in short strokes. All the surfaces of the teeth must be properly cleaned. Two minutes of brushing two times a day has been recommended.

Majority of the parents were aware that sugary items and sweetened beverages can lead to dental caries. Similar results were seen in studies conducted by Moulana et al.,[6] Wyne et al.,[7] Kamolmatyakul and Saiong[8] and Chan et al.[1] However, the participants showed poor knowledge on the different forms of sugary items, which are harmful to the teeth. This exposes inadequacy of oral health awareness regarding the various sources of food that contain sugar and pose a risk of dental caries. In this study even though majority of mothers had heard about fluoride, they had negligible knowledge about its role. This was similar to the study done by *Moulana et al.*,[6] *Suresh et al.*[9] whereas studies done by *Gussy et al.*,[10] *Franzman et al.*,[11] Kamolmatyakul and Saiong[8] reported good knowledge about fluoride. About half of the parents stated that they would visit their dentist immediately on discovering a pit or discolouration in a tooth. The parents were aware that the blood on toothbrush while brushing was due to bleeding gums indicating poor gingival health.

Oral health of children is associated with knowledge of their parents/guardians as oral health-related habits are established during infancy and maintained throughout early childhood.[8] *Lamba, et al.* in a study showed that children who were dependent on parents or care givers for oral hygiene maintenance had poorer oral health status.[12] It has also been previously shown that children requiring tooth brushing assistance have poorer oral hygiene than those able to brush their teeth. This displays the inadequacy or discrepancy in the efficiency with which oral care may be provided by care providers.

Parents with higher education have a better knowledge regarding the oral hygiene practice and importance of deciduous teeth. *Szatko et al.* in a study reported that mothers having low level of education also showed low levels of oral health related knowledge.[13] It has been suggested that the parents with a general, improved level of education may be able to assess appropriate source of information and understand that information more completely.[14,15]

Overall parents and caregivers showed insufficient oral health knowledge and awareness. All these findings

indicate the need for an effective oral health education program. Keeping in mind the changing attitude in society, it is important to plan appropriate oral health programs targeting different groups through the strategies designed for specific requirements. More emphasis should be placed on improving the level of knowledge of parents, which would be reflected in their child oral health behaviour.

Conclusion

It is unquestionable that only with the support of their parents can children truly understand their self-worth. This is even truer in case of differently abled children who need special care and support for their comprehensive development and success. These children are prone to dental problems due to limited dexterity or inadequate guidance on oral hygiene maintenance. Poor dental health may have a significant impact on an individual's overall well-being. In spite of the media, most of the people trust their dentists for gaining accurate knowledge regarding oral health. Therefore, it is the duty of the dentists to educate parents or care givers about the proper oral hygiene measures and clear their misconceptions regarding dental care in order to have a positive impact on their children's health.

Ethical Clearance: Taken from Instituitional Ethical Committee

Conflict of Interest: None

Source of Funding: Seed fund, Sharda University, Greater Noida

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COVID…19 is it an agent for Bioterrorism?

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Abstract

Biological weapons are referred to as a weapon of mass destruction including chemical, nuclear and radiological Weapons as well.COVID-19 are an infectious disease caused by corona virus, which has recently been discovered. Before the epidemic started in Wuhan, China, in December2019, this sort of new virus and disease was unknown. Chemical and radiation terrorist attacks are early signs of a bioterrorism threat. They have lodged a petition against China before the United Nations Human Rights Council (UNHRC) for suspected crimes against humanity by unleashing COVID-19 on the world, the London International Council of Jurists and all the Indian Bar Association. The complaint lodged against the People's Republic of China, the people's Liberation Army and the Virology Institute in Wuhan charged the novel COVID-19. The health crisis in Covid-19 reminds us that, unlike nuclear or chemical weapons

Keyword: *COVID-19, Bioterrorism, United Nations Human Rights Council, Ministry of Health and Family Welfare.*

Introduction

Biological weapons are a branch of a broader class of weapons called mass destruction weapons that also include chemical, nuclear, and radiological gun. The use of biological agents is a serious issue, and there is an growing risk of using these agents in a bioterrorist attack.[1]

Corona viruses are broad family viruses that derive from animal or human diseases.COVID-19 is the infectious disease caused by corona virus that was most recently discovered. This newest virus and disease was unknown in December 2019, prior to the outburst in Wuhan, China. [2]

Francis Boyle is a professor of international law at the College of Law at the University Of Illinois. He introduced the U.S. domestic Biological Weapons Convention implementing law, known as the 1989 Biological Weapons Anti-Terrorism Act.

The causes of agricultural bioterrorism are that they can theoretically be used to manipulate the food supply. That includes toxin Clostridium botulinum, toxin Clostridium perfringens, the organisms Salmonella, and Escherichia coli O157:H7, Shigella, and B enterotoxin Staphylococcal. Clostridium botulinum toxin is the one that is most harmful and will most likely be used in bioterrorism.Bioterrorism warning signs [3]

Bioterrorism Agent Categories are as follows

Class A: The high-priority agents which include organisms or toxins which pose the highest risk to the public and national security because: they easily spread high death rates, trigger public panic and social disruption. Category B: The second highest priority is these agents, because: moderately spread disease rates and low death rates. Class C: These third priority agents involve the advent of pat. [4]

According to an ET Prime story, a group of Chinese scientists in Canada is suspected of spying and deprived of access to the National Microbiology Laboratory of Canada (NML), which is considered to work on some of the most deadly pathogens. [5]

According Economic times of India Covid -19 is an experimental germ that mistakenly escaped a Chinese laboratory. In any case, the Chinese owe the world a huge apology for having so long kept a dark secret like Covid-19, making it too late for other nations to batten their hatches down. Covid-19 is the canary in the coal

mine that warns people against trying to exploit the destructive power of pathogens whose deadly presence [6]

For conspiracy theorists the Wuhan Institute was already the suspect. Novel coronavirus was already in discussion as a bio-weapon that escaped or permitted to escape from the lab. This gained more currency from reports of being expelled from a virology center in Canada by a Chinese scientist couple, which denied the connection between their disciplinary action and Covid-19 spread. [7]

The lack of preparedness for the ongoing COVID-19 pandemic showed how non-state actors could access deadly stresses to perpetrate a bioterrorist assault, Secretary-General of the United Nations, Antonio Guterres, notify the UN Security Council. "The vulnerabilities and lack of preparedness exposed by this pandemic provide a window on how a bioterrorist assault might unfold – and could increase its risk.

This involves researching the normal benefit of function that occurs in nature when viruses evolve or recombine and their potential for use in the delivery and production of drugs and vaccines. On the other hand, a bioweapon used by a non-state actor (i.e., a bioterrorist) may be a fully natural virus, although a malicious state would be likely to try a more efficient weapon via bioengineering. [8]

India is at risk from bioterrorism

Bioterrorism: Alertness in the health field. This analysis article provides us with an overview of bioterrorism, the agents used, and counteracting steps, with a related note on India's current monitoring system scenario, laboratory response network, and preparedness need. [9]

India began educating its medical staff to deal with the eventualities of bioterrorism attacks as far back as in December 1998. Since ratifying the Biological and Toxin Weapons Convention of the United Nations in 1972, India has not initiated a Bioweapon Program. Defense Research and Development Establishment (DRDE) located at Gwalior in Madhya Pradesh. It is India's go-to studies institution. The Hyderabad Institute of Preventive Medicine's Biosafety Level 2 laboratory offers guidance in preparing the government for a biological attack.

Two non-conventional, lethal threats to war threaten global stability.The chemical agents that terrorists produce. Toxic weapons used for killing dissidents at home and abroad by authoritarian governments; India said the effects of the COVID-19 pandemic underlined the need for international cooperation, including improving the World Health Organization's institutional structure. India has been collaborating with other Convention countries to create a database that can play a significant role in resolving bio-threats and bio-emergencies. India also cited the rapidly spreading corona virus. [10]

The fight against bioterrorism

MoHFW and WHO deliberated on further strengthening and additional steps that can be taken to respond to COVID-19 in the country in a video-conference held on 15 April 2020.Dr Harsh Vardhan, Minister of Health and Family Welfare of the State, said in his address that the WHO Country Office for India worked closely with MoHFW on COVID-19 preparedness and response measures, including surveillance [11]

In the United Nations Human Rights Council (UNHRC), the London-based International Council of Jurists and All India Bar Association have lodged a complaint against China for committing alleged crimes against humanity by unleashing COVID-19 on the world. The petition lodged against the People's Republic of China, the People's Liberation Army and the Virology Institute in Wuhan alleged the novel corono. [12]

At the U.S. President's regular White House briefing on Wednesday, Fox News reporter John Roberts, asking Trump about the allegations, said, "Multiple sources inform Fox News today that the U.S. now has high confidence that while the corona virus is a naturally occurring virus, it emanated from a virology laboratory in Wuhan that an intern was infected because of poor safety procedures [13]

Conclusion: The health crisis in Covid-19 reminds us that, unlike nuclear or chemical weapons, biological weapons are exceedingly difficult to locate and identify in adequate time to react to.Covid-19 is the biological agent, or has inadvertently jumped the virus from the

Wuhan province bio research facility.

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Effect of Non-Computerized Cognitive Remediation and Risperidone to Improve Disability Function in Schizophrenia

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Abstract

The aim of the study was to find out the effect of non-computerized cognitive remediation and risperidone therapy to improve cognitive function and disability condition in schizophrenia patients. This study was an experimental research design, with fourteen participants divided into two group as the experimental group and the control group. The experimental and control groups both received Risperidone. In addition, the experimental group was given the non-computerized cognitive remediation (CR). The non-computerized cognitive remediation will be carried out in 12 sessions (3 sessions per week) using mainly paper and pencil tasks and is based on cognitive strategy instruction. Moreover, all samples will be measured for PANSS (Positive and Negative Symptom Scale), WHODAS 2.0 (World Health Organization Disability Assessment Schedule), and SCoRS Vi (Schizophrenia Cognition Rating Scales Indonesian Version) scores. As the results, both experimental and control group showed a significant decrease in the SCoRS Vi and WHODAS 2.0 score (p=0.001). However, the experimental group showed greater improvement compared to the control group. Synergistic effect of Non-computerized Cognitive Remediation and Risperidone can improve disability condition in schizophrenia patients.

Keywords: *Schizophrenia, Non-computerized Cognitive Remediation, Risperidone, Disability*

Introduction

Schizophrenia is a chronic mental illness characterized by hallucination, delusion and thought disorder as well as cognitive and psychosocial impairments.[1] Cognitive deficits are core features of schizophrenia, contribute substantially to poor functional outcome of the patients and are likely as important as positive and negative symptoms for clinical treatment of the illness.[2] Schizophrenia is a psychotic disorder that affects about 26 million people around the world. It is typically diagnosed in early childhood and mostly persist through-out people's live. Several long-term follow up studies have challenged the view about schizophrenia poor outcome and proved that varying degrees of improvement is still one of the main causes of disability worldwide.[3] Patients with schizophrenia have difficulty succeeding at school, obtaining or maintaining a job, having social relationship, living independently and even for some taking care of their basic daily needs.[4]

The decline occurring in schizophrenia had been broadly classified as something related to neurocognition and social cognition.[5] While all domains of cognition are affected in schizophrenia, there are selective areas of increased impairment – particularly verbal and visuo-spatial memory, attention, executive function and speed of processing.[6] Antipsychotic drugs can relieve the main symptoms of schizophrenia but have no therapeutic effect on this cognitive impairment and the overall outcome is generally unsatisfying which explains the increasingly widespread use of cognitive remediation for schizophrenia.[7,8] Alternate theories of schizophrenia point to neurodevelopmental abnormalities as a potential cause. Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor essential for development of the

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central nervous system and modulation of neuronal connections that may be involved in the pathophysiology of schizophrenia and its associated cognitive impairment, especially immediate memory.[9,10]

In 20 clinical trials for cognitive enhancement, atypical antipsychotic medication moderately improved some aspects of cognitive functioning in patients with schizophrenia. Next, the influence of neurocognitive rehabilitation on neurocognitive functioning come to attract attention. Cognitive remediation was associated with a medium effect size for cognitive performance.[11] Then, are there any synergistic effect of cognitive remediation and antipsychotic that can improve disability in schizophrenia patients? It was generally thought that atypical antipsychotic would improve neurocognitive functioning but some published reports suggest that the magnitude of neurocognitive functioning for antipsychotic is lower than previously expected.[11] Thus, developing medications and cognitive therapies to treat the cognitive deficits associated with schizophrenia is a high priority.[12,13]

According to the WHO-ICF, disability encompasses impairments of body functioning, activity limitations, or participation restriction, arising as interconection result between health condition and contextual factors. A person with schizophrenia, might experience disability due to impairment of thought or perceptual functions.[3] In previous research, showed the significant improvement (p=0,002) in cognitive function after given 12 session of non-computerize cognitive remediation in schizophrenia treated with atypical antipsychotic.[14] However, the study did not link the benefits or impact of cognitive function improvement with disability condition. Therefore, we were interested to conduct further research to determine the effect of non-computerized cognitive remediation and risperidone on disability improvement in schizophrenia patients.

Materials and Methods

Location and Time: The study was conducted at the Rumah Sakit Khusus Daerah (RSKD) Hospital in Makassar, South Sulawesi, Indonesia, from April to July 2018.

Ethical Clearance: Ethical approval for the study was given by the Ethics Commission on Biomedical Research in Humans, Medical Faculty of Hasanuddin University, Makassar, Indonesia.

Participants

The participants were diagnosed schizophrenia by psychiatric, in a stable state (PANSS score 50-70), irrespective gender or race, age 18 to 45 years, minimal level of education is elementary school, no evidence of head injury or organic disorder, who were hospitalized in Rumah Sakit Khusus Daerah (RSKD) Hospital. The participants were taken from April 2018 until July 2018 by Consecutive sampling method.

Procedure

All participants gave informed written consent (N=14). All assessments were carried out by medical graduation. Sociodemographic data were collected from case note, nurses and participants. All participants were assessed on the measures of PANSS, WHO DAS 2.0 and SCoRS Vi before and 4 weeks postbaseline. Every participant received (TAU) treatment as usual (Risperidone) and randomly assigned to receive non-computerized cognitive remediation. The participants were divided into 2 groups, experimental group (TAU plus CR, n=7) and control group (TAU, n=7).

Interventions

Participants in experimental group have received CR for 12 sessions (3 sessions per week). The intervention should have been performed in a clinical setting by a medical graduation with qualification in CR. The CR was either a paper and pencil format and the length of therapy should be from 45 minutes to 1 hour.

Data Analysis

All data were presented in mean value and statistically analyzed with SPSS computer software. The data were analyzed using Mann-Whitney test and Wilcoxon test, with the significant value was determined if the $p< 0.05$.

Results

The study was conducted at the Rumah Sakit Khusus Daerah (RSKD) Hospital in Makassar, Indonesia, from April to July 2018. In Table 1, most subjects in experimental group were male (57,1%), in contrast with

mostly female in control group (57,1%). Most subjects were between 30 – 39 years old (57,1%) in both groups. In the experimental group, the education level was equally for Elementary School and Senior High School (42,9%) and in control group was mostly Elementary School (57,1%). For occupation, unemployed subjects were 57,1 % in the experimental group and 85,7% in the control group. For marital status, unmarried were 42,9% in the experimental group and 85,7% in the control group. Moreover, for the diagnosis, schizophrenia unspecified (F20.9) was dominated (57,1%) in both groups.

Table 1. Subjects Profile

Variable	Experimental Group		Control Group	
	N	(%)	n	(%)
Gender				
Male	4	(57,1)	3	(42,9)
Female	3	(42,9)	4	(57,1)
Age				
≤ 20	0	(0)	0	(0)
21-29	2	(28,6)	1	(14,3)
30-39	3	(42,9)	4	(57,1)
≥ 40	2	(28,6)	2	(28,6)
Education				
Elementary School	3	(42,9)	4	(57,1)
Junior High School	1	(14,3)	1	(14,3)
Senior High School	3	(42,9)	2	(28,6)
Occupation				
Employee	3	(42,9)	1	(14,3)
Unemployment	4	(57,1)	6	(85,7)
Marital Status				
Married	4	(57,1)	1	(14,3)
Unmarried	3	(42,9)	6	(85,7)
Schizophrenia Subtype				
Paranoid Schizophrenia	3	(42,9)	3	(42,9)
Schizophrenia Unspecified	4	(57,1)	4	(57,1)

In Table 2, we conclude the comparison of SCoRS Vi and WHODAS 2.0 scores in the experimental group and control group. In the experimental group, the baseline SCoRS Vi scores was higher than the scores on the fourth week. Meanwhile, WHODAS 2.0 scores decreased on the fourth week of treatment and this was also the same in the control group. Using the Independent sample t-test analysis, the baseline SCoRS Vi scores in both groups, did not show any significant difference (p=0,056), as well as the result on the fourth week of treatment (p=0,831). There was also no significant difference in the baseline of the WHODAS 2.0 scores between the two groups (p=0,094), but there was a significant difference in WHODAS 2.0 scores on the fourth week in both groups (p=0,000).

Table 2. The Comparison of SCoRS Vi and WHODAS 2.0 Scores in Experimental And Control Group

Scale	N	Treatment Group		Control Group		P
		Median (Minimum-Maksimum)	Mean ± SD	Median (Minimum-Maksimum)	Mean ± SD	
SCoRS Vi Baseline	7	72 (68-76)	71.7143 2.49762	65 (60-75)	66.1429 6.51738	0.056
SCoRS Vi On the 4th wk	7	63 (55-70)	61.8571 5.36745	62 (55-70)	62.5714 6.77882	0.831
WHODAS 2.0 Baseline	7	37 (30-40)	36.571 3.4572	39 (36-45)	39.714 2.9841	0.094
WHODAS 2.0 On the 4th wk	7	28 (20-35)	27.8571 4.52506	37 (34-40)	37.1429 2.34013	0.000

In Table 3, in the experimental group, there was a significant difference in SCoRS Vi and WHODAS 2.0 scores at the baseline and after fourth week of treatment (p=0,001).

Table 3. The Differences of SCoRS Vi and WHODAS 2.0 Scores in Experimental Group

Scale	n	Experimental Group		P
		Median (Minimum-Maksimum)	Mean±SD	
SCoRS Vi Baseline	7	72 (68-76)	71.71±2.49	0.001
SCoRS Vi On the 4th wk	7	63 (55-70)	61.85±5.36	
WHODAS 2.0 Baseline	7	37 (30-40)	36.57±3.45	0.001
WHODAS 2.0 On the 4th wk	7	28 (20-35)	27.85±4.52	

In Table 4, in the control group, there was also a significant difference in SCoRS Vi and WHODAS 2.0 scores at the baseline and on the fourth week of treatment (p=0.001).

Table 4. The Differences of SCoRS Vi and WHODAS 2.0 Scores in Control Group

Scale	n	Control Group		P
		Median (Min-Max)	Mean±SD	
SCoRS Vi Baseline	7	65 (60-75)	66.14±6.5	0.001
SCoRS Vi On the 4th wk	7	62 (55-70)	62.57±6.77	
WHODAS 2.0Baseline	7	39 (36-45)	39.71±2.98	0.001
WHODAS 2.0 On the 4th wk	7	37 (34-40)	37.14±2.34	

In Table 5, there were an improvement of SCoRS Vi and WHODAS 2.0 scores and were equally significant (p=0,003) in both groups. However, the improvement of SCoRS Vi and WHODAS 2.0 scores in the experimental group was higher than in the control group. It means that the administration of Risperidone in combination with non-computerized cognitive remediation showed a better outcome compared to Risperidone only.

Table 5. The Deviation of SCoRS Vi and WHODAS 2.O Scores in Experimental and Control Group

Scale	n	Control Group		Experimental Group		P
		Median (Min-Max)	Mean ± SD	Median (Min-Max)	Mean ± SD	
Deviation SCoRS Vi	14	3 (2-5)	3,5 (1,39)	8 (5-16)	9,8 (4,37)	0.003
Deviation WHODAS 2.0	14	2 (2-5)	2,5 1,13	9 (3-16)	8,7 (4.11)	0.003

Discussion

Cognitive impairment has emerged as an important new target in schizophrenia therapeutics in light of evidence that cognitive deficits are critically related to the functional of disability that is characteristic of the illness.[15] Schizophrenia has a range of cognitive deficits that may evolve from decreased BDNF.[10] This study showed that there was a significant decrease in the SCoRS Vi score, both in the treatment and control groups, as well the WHODAS 2.0 score. However, the improvement in the experimental group was greater than in the control group.

One of the targets, the treatment of schizophrenia is improvement of cognitive function. Cognitive remediation is a therapeutic process to increase or improve the capacity of individuals to process and use information, and enable improvement of daily life functioning.[16,17] Cognitive remediation is a training activity on the cognitive function of schizophrenia patients. By giving cognitive remediation, it will indirectly improve the cognitive function of schizophrenia patients. Thus, it will improve the disability of the schizophrenia patient, especially in the daily life functioning (such as bathing, dressing and participating in other group activities). Cognitive remediation is the gold-standard practice to address cognitive deficits in schizophrenia.[18]

In this study, it appeared that both the experimental and control group had the improvement of the SCoRS Vi and WHODAS 2.0 scores and were equally significant (p=0.005). However, the degree of improvement in the experimental group was greater than in the control group. It meant that the improvement of SCoRS Vi and WHODAS 2.0 scores was better with the addition of remediation compared to risperidone treatment only. The scores of SCoRS Vi and WHODAS 2.0 were improved after 12 sessions of non-computerized cognitive remediation which was given 3 times per week for 4 consecutive weeks. Previous study showed that addition of non-computerized cognitive remediation could influence neurobiological changes.[19] It was reported that patients receiving the non-computerized cognitive remediation showed an increase in BDNF serum levels and activation of the inferior frontal gyrus which was assessed through fMRI examination compared to the control group.[17,18] This result was consistent with the results of other studies, showed that BDNF levels began to increase in the second week,[20] and reached high levels at the end of the third week.[21] Brain derived neurotropic factor (BDNF) is considered to a putative biomarker for cognitive recovery in schizophrenia.[22]

Conclusions

This study concluded that the addition of risperidone, in combination with 12 sessions of non-computerized cognitive remediation was better to improve the disability condition in schizophrenia patient. We suggested that the non-computerized cognitive remediation therapy to be given in addition with risperidone treatment. Limitations in this study are that it is only examined the diagnosed type of schizophrenia which did not represent to all types of schizophrenia and that some participants were not interested in remediation process.

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Ethical Clearance: Ethical approval for the study was given by the Ethics Commission on Biomedical Research in Humans, Medical Faculty of Hasanuddin University, Makassar, Indonesia.

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Role of TE in MR Spectroscopy for the Evaluation of Brain Tumour with Reference to Choline and Creatinine

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Abstract

Background: The brain tumour is the most typical neurological disease in the human. The brain tumour causes partial paraplegia, memory loss and various kinds of neurological disorder which causes changes of metabolic process in the brain, integrity of neurons, cells proliferation and detoriation. For accuracy in detection, patient will undergo MR Spectroscopy (MRS) based on MR imaging will enhance more widely used clinical applications and is capable to gives details information about metabolite properties in the brain of normal and abnormal tissue morphology. MRS is the non-invasive procedure may provide details and accuracy about the metabolites in the brain tumour. This study aims to detect efficacy of short and intermediate TE in evaluation of brain tumour with reference to choline and creatinine using multivoxel MRS technique.

Method: The total participants (n = 75) with the brain tumour will be included randomized scan under the MRI with a customised protocol. We will be applying two various types of multivoxel MR sequence with a short TE and intermediate TE. Both sequence applying on each participant of brain tumour with cover proper saturation band and after the post processing we will evaluate choline and creatinine metabolite and measured the exact variation between given values.

Expected Results: MR Spectroscopy may allow the noninvasive separation of high-grade tumour from the low-grade tumour. Choline and Creatinine ratios may show the steady results in concluding the grade of tumour. The Intermediate TE may show more accuracy, sensitivity and specificity as compared with short TE.

Keywords: *Brain Tumour, Spectroscopy, Short TE, Intermediate TE, Choline, Creatinine, Metabolites, Multivoxel.*

Background/ Rationale

Brain tumours are significant health issues that rapidly expansion yearly. all the initial condition of brain tumours, such as gliomas, astrocytoma and meningioma etc. Gliomas are the most typical brain tumour. The tumour grading is important and it helps to determine appropriate treatment strategies [1].

There are various types of tumours occurring inside the brain parenchyma those includes tuberculoma, neurocysticercosis, lymphoma, neoplasms, demyelinating lesions, primary tumours, secondary metastasis etc. Most of these lesions are found at the grey-white matter interface, come across the subcortical area in the brain parenchyma. Among various infective causes, tuberculosis is the most common in India followed by neurocysticercosis (NCC) [2].

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The advanced modalities used under the radiology like computed tomography and magnetic resonance imaging with more and more recent advances are taking their place in today's world when it comes to detection of early changes of the brain tumours. Considering a specific class of these, from the extra-axial compartment, generally encountered tumours are meningiomas and schwannomas [3]. Whenever it helps to diagnose the rarest tumour as Pleomorphic xanthoastrocytoma which is the rare benign tumour of CNS, this is the type of tumour considering for lesser than 1% of all astrocytoma's [4]. They possess characteristic properties and attributes on imaging modalities which helps us in conclude respective diagnosis [3].

Magnetic resonance spectroscopy (MRS) has a huge ability for the various and corresponding quantity of different chemical metabolites present in the brain due to this, it is a useful method to determine different intracranial disorders, it specifically determines the major range of disorder which is revealed by the lesion under observation, for example, neoplastic, ischemic inflammatory [5].

MRI (Magnetic Resonance Imaging) spectroscopy provides greater clinical diagnostics. MR spectroscopy (MRS) is a non-invasive method that provides complete knowledge regarding metabolic processes like energy metabolism, the integrity of neurons, proliferation of cells, and deterioration, it also provides information about changes occurring in necrotic tissue. In MR spectroscopy the chemical structures and metabolites in the tissue, measured and analyzed without contrast agents [6]. which allows the estimation of selective biological compounds in vivo condition, MR spectroscopy (MRS) provides details and accuracy about the possibilities of the extent and significant changes on the standard MRI scan by processing the present rate of tissue metabolites like NAA, creatine, and choline, etc. Worldwide usage of advanced MRS applications with the strongest SNR ratio and high spatial resolution, to permit us to identify changes of functional metabolites, which gives more information to study the detailed nature of the various tumours. It also gives data to understand exact morphological as well as physiological variations which occurs surrounding the brain parenchyma [7]. The Proton MRS technique that is obtaining an important role play in the detection of brain tumours before the surgery. The MRS technique gives knowledge of metabolic process about the tissue being studied that gives more complements from the anatomic knowledge obtained with MR imaging [8].

The echo time (TE) is an important parameter that can influence on a large scale and it is responsible for the quality of MRS. Use of exact TE is very important to obtain accurate MR spectra of brain tumours. The main aim of studying different TE is to learn its impact on brain tumours, its characterization done by correlating the performance of MRS retrieve at two different TE (In between 35 ms to 144 ms) [8].

The short echo time (TE) MRS it has the ability to detect more metabolites but there are few drawback water contaminations and the distortion of the spectrum below the effect of eddy current and superimposed of lactate along with lipid peaks resulting it has high shimming demands [1]. The range for short TE between 18-45ms and long TE between 120 and 288 ms [9]. Short and long echo time (TE) spectra in terms of accuracy in the clinical characterization of brain tumours [8].

Intermediate TE has advantages over short TE, it is capable to detect metabolites like (Cho)/N-acetyl aspartate (NAA), Cho + Cr/NAA, Cho/Creatine (Cr). Intermediate TE can be performed without baseline noise also it didn't require high shimming inherent. Short TE has chances of background noise in MRS due to contamination of lipids and water [8]. The proportion of metabolites like Cho + Cr/NAA, Cho/Creatine (Cr), Choline (Cho)/N-acetyl aspartate (NAA) furthermore lactate and lipid are calculating at short and intermediate echo times (TEs) [1].

In the MRS used wide advanced application of the chemical shift Imagine using Multivoxel sequences according to the brain tumour. also gives high spatial resolutions with a large coverage over the tumour and surrounding area. Multivoxel MRS may be more effective than single-voxel MRS technique for acquiring a map of metabolites of a wide area of tumours [1].

Aim

To study the efficacy of short and intermediate TE in evaluation of brain tumours with reference to choline and creatinine using multivoxel MRS technique.

Objectives:

· To study the efficacy of short TE in evaluation of brain tumours with reference to choline using multivoxel MRS technique.

· To study the efficacy of intermediate TE in evaluation of brain tumour with reference to choline using multivoxel MRS technique.

· To study the efficacy of short TE in evaluation of brain tumour with reference to creatinine using multivoxel MRS technique.

· To study the efficacy of intermediate TE in evaluation of brain tumours with reference to creatinine using multivoxel MRS technique.

· To compare the concentration of choline – short TE vs intermediate TE

· To compare the concentration of creatinine – short TE vs intermediate TE

Hypothesis:

We hypothesized that as echo time of intermediate TE is more that short TE, we may get better knowledge about grade of tumour with intermediate.

Methodology

The MRI Spectroscopy scan will be carried out in the MRI unit under the department of radio-diagnosis of Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe) Wardha. Relevant data on the demographics and treatment history as well as the indications will be recorded. Counselling of all participants for research work will be done. The routine protocol in our set up is as follows: History of the patient will be taken. after approval from the Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences, (Deemed to be University).

Study design and sample size:

The design of the study is an MR Spectroscopy for the brain tumour will be included randomized scan under the MRI with a customized protocol. The number of participants registered in this research study is 75 (n = 75). The participant will be scan individually. Participant entered the strong magnetic field will use advanced MRI dashboard and then we will be applying further customized scanning protocol. For tumour, scanning will be implanted three plane T1 weighted fat suppressed sequence after given gadolinium-based contrast media. After acquiring contrast images, it will be applied to two different multivoxel MR sequence with a short TE beside the intermediate TE. Both sequence applying on each participant of brain tumour with cover proper saturation band and after the post-processing, we will evaluate choline and creatinine metabolite and measured the exact variation given values. Before scanning, the participant will be counselling about the research study and the objectives and approaches of the study, and written patient consent forms will be signed by them. History and indication will be recorded for further assessment.

SR NO	STUDY	SHORT TE (TR-1500 / TE-35 ms)		INTERNIDIATE TE (TR-1500 / TE-144 ms)	
		Choline	Creatinine	Choline	Creatinine

Fig: Proforma for the enrolment and assessment

Setting:

Department of Radio-diagnosis Acharya Vinobha Bhave Rural Hospital, Datta Meghe institute of medical sciences, Sawangi (Meghe), Wardha Relevant dates, including periods of recruitment: August 2019 – August 2021

Statistical Analysis Plan:

For the study of MR spectroscopy to assess the statistical analysis will be done by using descriptive and inferential statistic using **Z-test**. To find the difference between two means **Chi-square** and **Wilcoxon Signed Rank test** for paired test before and after test. and for this study software used in this analysis will be.

SPSS 24.0 version and **GRAPH PAD 7.0** version and P<0.05 is considered as the level of significance. The comparison will be done between the two different groups using two-way **ANOVA** for the demographic measures and initial scores on outcome measures.

Sample size:

This study total number of 75 (n = 75) participant will be enrolled for in this research study of MRS with short TE and intermediate TE (Echo Time) referred to Acharya Vinobha Bhave Rural Hospital, Sawangi (Meghe).

Sample will be calculated by the following formula:

$$N = \frac{\mathbf{Z\alpha/2}^{\mathbf{2}}\mathbf{.P(1-P)}}{d^2}$$

Expected Outcomes:

MR Spectroscopy may allow the noninvasive separation of high-grade tumour from the low-grade tumour. Choline and Creatinine ratios may show the steady results in concluding the grade of tumour. The Intermediate TE may show more accuracy, sensitivity and specificity as compared with short TE.

While using intermediate TE, for Cho/Cr Ratio it may be possible that choline metabolite peak is greater than creatine, although, at short TE, the choline peak may be lesser than creatine because it has a short T2 and decays faster than choline metabolite. The cause of these differences in the appearance of Cho peak at short and intermediate TE may result in variation in Choline and creatinine ratio-dependent of TE. Choline and Creatinine ratio is high in brain tumours and is best correlated at higher TE.

Participants:

The inclusion criteria for the participants are as under:

· Patients referred to Radio-Diagnosis department for MRI spectroscopy of brain for the evaluation of the brain tumour

· Patients with history of brain tumour

· Patients of all age groups irrespective of sex

The exclusion criteria for the participants are as under:

· Non consentine patient.

· Uncooperative patient in MR Spectroscopy cannot be performed.

· Claustrophobic patients

· Small sized lesions where voxel placement is not possible for application of MRS technique.

Discussion

Differentiation of high-grade tumour from low-grade tumours is very essential to establish an effective treatment against it. Traditional Magnetic Resonance imaging technique can be hard & sometimes it can't be trustworthy in getting complete knowledge of tumour grade [11,12]. Use of MR Spectroscopy increasing day by day for non-invasive method, Identification of tumour grade can help to improve the diagnostic accuracy during tumour grading [13,14]. In this study, we will try to evaluate the functionality of combined short and intermediate TE for grading of brain tumour. There is a study performed in 2008, they evaluated the value of MR Spectroscopy in glioma tumour grading, the researcher stated that Choline & some other measures associated with it (Cho / Cr and Cho / NAA) are major indices for grading glioma tumour when correlated with the ratio of some additional metabolites [15]. In this study, the ratio of Choline + Creatinine ratio will be calculated for to differentiate between Choline and Creatinine peaks in heterogeneous tissues. There are very few studies who did research about the role of Choline and Creatinine ratio in this aspect [16,17]. Correlating intermediate TE and short TE, our study may reveal insights about diagnostic efficiency of metabolite ratio better for intermediate TE to those at short TE [1]. In regard to our study, they stated that Choline and Creatinine ratio could differentiate between the low and high grade of brain tumours.

Conclusion

Magnetic resonance spectroscopy may allow the noninvasive differentiation between high grade and low-grade tumours. Choline and Creatinine ratio may play the most reliable in determining the tumour grade. This study may give better knowledge about the use of short TE and intermediate TE for specificity, sensitivity, and accuracy of diagnosis. at the end of this study, we may use a better combination of short and intermediate TE for proper grading and diagnosis of brain tumours.

Patient Consent:

Every before MRS study will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality as well as along with consent will be take proper clinical history about brain tumor and to explained about the research study and the objectives with approaches of the study.

Ethical Committee:

The study approval was obtained from Institutional Ethics Committee at DMIMS (DU), Nagpur. all patients were asked to go through informed consent. Total 75 patients performed under magnetic resonance imaging.

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Xenografts in Periodontal Regeneration: A Viable Alternative

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Abstract

Periodontal disease is categorized by the destruction of periodontal tissues. Over the years, there have been several clinical techniques and material options that have been investigated for periodontal defect repair or regeneration. The development of improved biomaterials for periodontal tissue engineering has significantly upgraded the available treatment options and their clinical results. Bone replacement graft materials, barrier membranes, various growth factors and combination of these have been used. Autografts have been used as the bone graft material of choice since many years. But this type of grafts has many limitations to its use. In order to overcome its drawbacks, xenografts may be used as a viable alternative. Xenografts also known as heterologous graft tissues, is obtained from different species, and has been used in various different periodontal procedures. The characteristic properties of xenografts and the benefits of using it has made this type of graft to emerge as an appropriate choice for periodontal regeneration. The objective of this article is to a review xenograft as a bone replacement graft material to be used in periodontal therapy. The source, mode of bone regeneration and the respective advantages and disadvantages of xenogenic materials have been discussed in detail.

Key words: *Bovine derived grafts; Coralline derived grafts; Equine derived grafts; Periodontal Regeneration; Porcine derived grafts; Xenografts*

Introduction

Bone grafts have been used extensively in various periodontal applications. It may be used either along with barrier membranes or without, for the regeneration of periodontal tissues or reconstruction of alveolar ridge before the insertion of implants. In the early 1990s, autogenous bone or fresh frozen allografts were used for bone grafting procedures, but the emergence of efficient processing and sterilization techniques, resulted in the increased use of bone graft substitutes - Xenografts for various different periodontal procedures. The most important benefits of using bone graft substitutes is the excess availability and reduced morbidity, taking into consideration that procurement of autogenous bone leads to a second intraoral or extraoral surgical site.(1)

There are four main hard tissue replacement graft materials that are used for periodontal regenerative applications. These include autografts, allografts, xenografts and alloplasts. Autografts refer to the graft material which are obtained from the same individual. This type of graft material is considered as the "gold standard". Nevertheless, there are a few limitations related to autografts. These include, morbidity of the donor site, limited volume of bone available, and the unpredictable replacement rate(2). Xenografts on the other hand are obtained from different species. In recent times this type of graft material is being widely used in clinical periodontal regenerative applications. These grafts have the advantage of being relatively inexpensive as well as readily available.(3)

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Xenograft

Xenografts are also known as heterologous graft tissues; this is because they are obtained from different species. This type of graft material is usually osteoconductive in nature and has limited resorptive potential. The origin of the xenogeneic scaffolds which are available for periodontal regeneration, may be bovine, equine, or porcine. These materials undergo thorough processing techniques, resulting in biocompatible products. These are similar to human bone based on their structure. (4)

Xenografts have various advantages. These are readily available as they can be manufactured in excess amounts at comparatively lower processing expenses, and free of disease transmission. The overall performance for bone regeneration is related to the physicochemical characteristics of the graft. Using Xenografts eliminates the requirement for a second surgical site, as well as takes lesser time to heal, hence preferred by several patients.(5)

The comparison of the different properties of other types of grafts has been shown in Table 1.

[Table 1] Comparing properties with other types of grafts:

GRAFT MATERIAL	ORIGIN	BONE REGENERATION POTENTIAL	RESORPTION TIME
AUTOGRAFT	Patients own tissue	Osteogenic, Osteoinductive, Osteoconductive	Weeks to months
ALLOGRAFT	Tissue from individuals of the same species	Osteoconductive, Osteoinductive (DFDB)	Months (4-12 months)
XENOGRAFT	Tissue from other species	Osteoconductive	Months to a year
ALLOPLAST	Synthetically produced material	Osteoconductive	Wide range (rapidly resorbable to non resorbable)

Processing And Purification

Xenogenic bone grafts constitute of de-proteinized cancellous skeletal bone tissue. This is procured from different species, and is then placed in the recipient site. In case of unavailability of human source grafts, the preferred type of graft is considered to be the xenografts. This is used after thorough sterilization and processing techniques in order to cancel out the inadequacies of the graft.(6)

The organic contents present in the xenogenic grafts are eliminated in order to prevent any kind of immunological reaction or transmission of pathogens. The inorganic component that remains after the processing and purification techniques, serves as a structural matrix for the formation of new bone. In addition, it acts as a good source of calcium, which is crucial for the formation of bone.

At present there are two kinds of methods used for the processing of bovine bone. The first method includes a chemical extraction process which is a low temperature method. The second method uses extreme high temperatures for the removal of residual organic material. The latter method results in longer hydroxyl-apatite crystal with higher crystalline structure. (6)

These biomaterials undergo a process consisting of deproteinization and demineralization using sodium hydroxide. These processes are not completely effectively in elimination of prions.(7) In order to eliminate the probability of transmission of disease, the manufacturers utilize animals from those countries in which these kinds of diseases have not appeared.

Prions are misfolds of protein which play the role of a carrier of various disease are mainly found in the medullary tissues. Hence, tissues located distant to the medullary tissues are generally chosen to be processed. After the deproteinization process, the residual mineral component consists of calcium phosphate along with calcium carbonate. This is present in a reticular format which is made up of apatite crystals. Such architecture helps in clot stabilization as well as bone apposition. (1)

Bovine Derived:

Deproteinized bovine bone mineral is the most frequently used xenograft in periodontal applications. Bovine bones was the first bone derived from different species to be used for xenograft production. Currently there are several bovine bone xenograft products being used. Bio-Oss® (Geistlich Biomaterials, Switzerland) is a well-established xenograft. It consists of a mineral matrix of osseous origin which is acquired from bone of the spine [5]. Cerabone® (Botiss Biomaterials, Germany) is another bone grafting material which is acquired from the condyles of cattle femur, which is cancellous in nature. [8]

Previously bovine xenografts have cause graft rejection problems. This may be attributed to the fact that earlier materials would undergo chemical detergent extraction, which caused residual proteins, thus causing adverse reactions and clinically unacceptable results. Presently available bovine-derived material is thoroughly deproteinated, leaving its natural microporous structure. This residual structure supports cell mediated resorption. This is necessary for the replacement of new bone. [9]

Bovine-derived hydroxyapatite grafts act as an osteoconductive scaffold. There is expanded available surface area owing to their porosity. The mineral content of the graft is comparable to human bone, thus causing proper integration with the host bone. These have been used for the treatment of intrabony defects as well as in ridge augmentation.[10]

A study was conducted by ***Baldini et al (2011)*** in order to analyse the histological and clinical outcomes of deproteinized bovine bone in various periodontal procedures. This includes procedures such as periodontal regeneration, socket preservation, peri-implant reconstruction and alveolar bone augmentation. It was noticed that deproteinized bovine bone was effective in case of periodontal regeneration with as well as without barrier membranes. This was observed in case of favourable containing defects.[11]

A study conducted by ***Cosyn (2012)*** evaluated the clinical as well as aesthetic outcome of regenerative periodontal therapy (RPT) using minimally invasive surgery and a collagen-enriched bovine-derived xenograft. The study demonstrated that even though preservation of soft tissue aesthetics was not possible, the results revealed favourable clinical outcome.[12]

Bruyckere et al (2018) conducted a study to assess the clinical outcome of regenerative periodontal therapy in 5 years, using minimally invasive surgery along with a collagen enriched bovine-derived xenograft. This study also identified the predictors for clinical attachment level gain as well as vertical radiographic bone gain. This study concluded that patients with perfect oral hygiene along with good compliance showed significant Clinical attachment level gain when treated with xenograft.[13]

Kollati et al (2019) conducted a study to analyse the efficiency of naturally derived bovine hydroxyapatite (Cerabone) combined with platelet-rich fibrin matrix in the preservation of extraction socket. This study indicated that PRF (platelet rich fibrin) in combination with xenograft and collagen plug, reduced the resorption activity at the test site. [14]

Matteo et al (2020) conducted a retrospective 10-year follow- up study of implants placed in ridges grafted using autogenous mandibular blocks covered with bovine bone mineral and collagen membrane. It was concluded that implants placed in areas that had been reconstructed using bovine bone graft showed comparable survival rates with those placed in native bone.[15]

Porcine Derived

Porcine bone graft tissue is a porous bone graft material which is devoid of organic component. Calcium phosphate is a major part of its composition. Porcine bone is similar to human bone with regard to structure and formulation. [2] The category and structure of lipid content is similar in both types of bones. [5] These come in granular formulations, where in the size of each particle is approximately 0.25–1 mm and 1–2 mm (Gen-Os®). This bone graft material is produced by eliminating all the organic substances. [2]

This anorganic bone mineral matrix is biocompatible. It contains interconnecting macroscopic and microscopic porous architecture which enhances new bone formation at the implantation site. [2]

A study conducted by ***Giovanna Orsini et al (2006)*** related to the "histologic and ultrastructural evaluation of

porcine bone derived biomaterial in the form of granules in maxillary sinus augmentation". The bone-biomaterial interface demonstrated a close contact between the biomaterial derived from porcine and the adjacent surrounding bone. There were characteristic features of mature bone with elevated numbers of osteocytes observed. It was concluded that the biomaterial was biocompatible, and could be used in periodontal treatments such as maxillary sinus augmentation procedures.(16)

Antonio Scarano et al (2010) conducted a retrospective clinical study to evaluate the usage of porcine bone in maxillary sinus augmentation. The study consisted of one hundred twenty-one healthy patients There were no major complications reported during the healing phase of all the grafted sinuses. The study concluded that porcine bone could be in sinus augmentation procedures. It also demonstrated that rougher-surfaced implants are preferred to be used.(17)

Jung-Im Park et al (2014) conducted a study of which the aim was "to determine the efficacy of crosslinked collagenated porcine bone (CPB) for the repair of surgically prepared one-wall intrabony defects". The study concluded that by a process of crosslinking, the collagenated porcine bone could be confined in the defect during the period of wound healing. The collagenated porcine bone along with a barrier membrane combination use resulted in more effective periodontal regeneration than when applied without a barrier membrane.(18)

A clinical study conducted by ***Renzo Guarnieri (2017)*** in humans in which implant site development using porcine-derived graft was investigated, showed reduction in vertical as well as horizontal bone resorption after tooth extraction. (19)

CORAL DERIVED

The coral bone graft substitutes are derived from the exoskeleton of corals, for example Biocoral® (Inoteb, Saint Gonnery, France). The evaluation of the potential of corals being used as bone grafts was initiated in the early 1970s. Commonly used coral- Porites resembles cancellous bone in terms of structure as well as mechanical properties. (20)

Porous coralline hydroxyapatite materials have been fabricated using the microstructure of corals of the marine. (21). These grafts are manufactured by subjecting the corals to extreme temperatures in an aqueous phosphate solution kept under pressure, thus resulting in formation of calcium hydroxyapatite. This process conserves the organized, penetrable and interconnecting pore structure. The average pore diameter is approximately 200 μm, consisting of roughly 50 - 60% porosity.(2)

Coralline calcium carbonate grafts have characteristic properties of high potential of osteoconductive characteristics, thus causing rapid new bone formation in the implantation sites.(22) These grafts show refined defect filling in cases of periodontal regeneration. These grafts do not undergo fibrous encapsulation.(2)

For initial bone formation, unlike synthetic coral material, natural coralline graft material does not need surface transformation into a carbonate phase. Thus, these grafts initiates bone formation more rapidly. They have a high osteoconductive potential. The disadvantages of this graft include its brittle texture and the fact that it is difficult to handle this material. Coralline calcium carbonate provides significant clinical attachment gain, reduction of probing depth and defect fill when compared to other bone replacement grafts.(9)

Change- Sung Kim et al (2005) conducted a study to assess the healing of periodontal tissues in one-wall intra-bony defects post implantation of autogenous bone or a biomaterial derived from coral. It was observed in this study that placement of coral-derived biomaterial cause proper periodontal healing. The healing process did not involve aberrations such as root resorption and ankylosis. (23)

Puvaneswary et al (2013) conducted a study to assess the morphological as well as chemical composition of coral graft (CG) and also their osteogenic differentiation potential, by using rabbit mesenchymal stem cells (rMSCs) *in vitro.* Coral graft proved to have better surface roughness. There were higher levels of osteogenic differentiation markers in case of the coral grafts this included alkaline phosphatase, Osteocalcin and Osteonectin levels. It was concluded that coral grafts showed superior osteogenic differentiation when compared to other bone graft culture system. (24)

A study conducted by ***Yoshifum Matuda et al (2019)*** aimed to evaluate the Periodontal Regeneration Using Cultured Coral Scaffolds in Class II Furcation Defects. Coral scaffolds were found to have the potential to regenerate periodontal tissue. There was evidence of new bone as well as cementum formation in the furcation spaces. (25)

Erlina Mahanani et al (2020) conducted a study to investigate the porosity of a coral scaffold and its biocompatibility while it is attached to human gingival cells. There were various compositions of synthetic coral scaffold prepared. Measurements related to the porosity percentage as well as gingival cell attachment were carried out. It was observed that having the highest porosity percentage could provide life time favourable microenvironment to the cells.(26)

Equine Derived Bone Graft

Equine bone grafts, which are enzyme-deantigenic in nature have been used frequently for the reconstruction of bone in various fields. (27) It has been used successfully in the correction of periodontal defect, for the reconstruction of horizontal as well as vertical atrophic ridge, and sinus augmentation procedures. (28)

Equine bone mineral (EBM) is a sterile, natural bone graft substitute. It is nonantigenic in nature. It constitutes of a porous structured bone mineral matrix. The equine bone graft is manufactured by entirely getting rid of the organic components including the proteins present. It is similar to human bone in both physical as well as chemical aspects. The mineral matrix consists of a macroporous and microporous structure with a trabecular architecture. This kind of texture supports the osteoconductive formation of new bone. (27)

Type I collagen is preserved during the enzymatic process for production of equine bone graft. Type 1 collagen is proved to be the most abundant protein of its extracellular matrix. This is what differentiates it from other types of xenografts, where in, in case of the latter all forms of organic components including all proteins are removed during the processing. (29) This property could lead to faster new bone formation. (30)

Myron Nevins et al (2012) conducted a study to test the effectiveness of hydroxyapatite and collagen bone blocks of equine origin (eHAC), infused with recombinant human platelet-derived growth factor-BB (rhPDGF-BB), to augment localized posterior mandibular defects in non-human primates (*Papio hamadrya)*. There was increased regeneration in case of the test sites, but these findings were not statistically significant. This study concluded that the combination including equine bone blocks and growth factors could be used for the vertical augmentation of severely resorbed ridges.(31)

Nevins et al (2013) conducted a study "to examine the bone regenerative potential of a newly introduced equine-derived bone mineral matrix (Equimatrix) to provide human sinus augmentation for the purpose of implant placement in the posterior maxilla". The qualitative as well as the quantitative results suggests better bone regenerative results at 6 months when compared to other types of xenografts. (27)

Di Stefano et al (2016) conducted a study to assess the formation of bone over time post maxillary sinus augmentation with an enzyme-deantigenic, bone collagen-preserving equine bone graft by evaluating the histomorphometric data. This was a retrospective human study including 77 patients with bone loss of 4 to 7 mm. Formation of new bone took place earlier, when equine bone was used for sinus augmentation. Some sites showed new bone formation within 3-5 months after surgery.(32)

Ji-Young Lee et al (2017) conducted a study "to evaluate the efficacy and safety of equine-derived bone matrix as a carrier for recombinant human platelet-derived growth factor BB (rhPDGF-BB) versus beta-tricalcium phosphate (β-TCP) for the treatment of intraosseous periodontal defects in adult patients". Thirty-two adults suffering from severe periodontal disease were included in this study. Significant gain in the clinical attachment level was observed in the test group. It was confirmed in this study that equine derived bone matrix was effective as well as safe, and could be used to treat periodontal defects. (33)

COMBINATIONS WITH XENOGRAFTS

Xenografts can be used alone or in combination to enhance the osteoinductive property. They tend to integrate well in the site of placement. It is histologically confirmed that xenografts have a direct contact with the

parent bone and it tends to resorb slowly.

Nevins et al (2012) conducted a study "to evaluate the potential for periodontal regeneration of a critical-sized defect with the application of recombinant human platelet-derived growth factor (rhPDGF-BB) combined with either a particulate equine or a β-tricalcium phosphate (β-TCP) matrix". The study concluded that the combination showed potential for the regeneration of periodontal attachment apparatus.(34)

Yasuko ***Nemoto et al (2018)*** conducted a study to assess the use of enamel matrix derivative along with deproteinized bovine bone mineral in periodontal regeneration therapies. There was superior influence in the healing process of soft tissue when the combination with collagen membrane was used. This also proved to be effective in decreasing pocket depth.(35)

A randomized, controlled, split-mouth study conducted by ***Andy Temmerman et al (2019)*** compared the clinical outcome between "Bovine-derived xenograft in combination with autogenous bone chips and xenograft alone for the augmentation of bony dehiscence around oral implants". It was concluded in this study that both these treatment options were equally effective. (36)

Silvio et al (2019) conducted a prospective study aimed at investigating clinically and histologically the effectiveness of a biomimetic magnesium-enriched-hydroxyapatite (MgHA) compared to a collagen-based deproteinized bovine bone matrix for alveolar socket preservation. Both the materials proved to show similar effectiveness. It was concluded in this study that either of the two materials could be used as a viable option for bone substitute. (37)

Kollati et al (2019) conducted a study aimed to compare the efficacy of natural bovine hydroxyapatite and platelet-rich fibrin (PRF) matrix along with collagen plug to unassisted natural healing in extraction sites. The study concluded that addition of PRF along with bone graft for socket preservation provided additional growth factors which enhanced the wound healing process and maintained the dimensions. (14)

Limitations/ Disadvantages

Xenografts tend to have high osteoconductive potential, but on the other hand are brittle in texture. These grafts lack toughness. Thus making them more susceptible to failure during surgeries, such as screw fixation procedures and/or after implantation. (2) Xenografts are frequently unable to gain adequate height and width in case of large bone defects. It is not always available in formulations that allow easy adaption or modelling, hence may be difficult to use in comparison to autogenous grafts / allografts. (38)

Xenograft is bone tissue which is harvested from one species and placed into another species. Hence a vigorous immune response is the main cause of concern when using it. Deproteinated and defatted xenograft bone have shown to decrease immune response. However, these processing techniques tend to destroy valuable osteoinductive matrix proteins. The main drawback with these materials is the risk of transmission of infective agents. It can cause disease transmission, which was evident in the case of bovine spongy form encephalopathy reported in Great Britain

Conclusion

Bone grafting is the most commonly used treatment modality used in periodontal regenerative therapy. It has been established that bone replacement grafts can be utilized for adequate amount of periodontal regeneration. Autografts have the disadvantage of causing morbidity at the site of harvestation. It also has limited availability. Xenografts on the other hand has the advantage of being manufactured in excess amounts at comparatively lower processing expenses. There is no need for a second surgical site when xenografts are used. It has also been observed that the healing process takes lesser time in case of xenografts.(5) It is structurally similar to human bone and has relatively superior osteoconductive capability. These grafts have excess available surface area due to their porosity. Xenografts have proved to be effective in the treatment of intrabony defects and ridge augmentation. Xenografts has been found to be clinically effective and is being brought up as a new avenue to treat advanced periodontal defects. (9)

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A Comparative Study of Corneal Complications in Small Incision Cataract Surgery and Phacoemulsification

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Abstract

Methods: The present study was done among 80 patients from them 40 patients underwent SICS (group-1) and 40 underwent phacoemulsification(group-2) ,both with posterior chamber intraocular lens implantation. Post-operative corneal complication were reviewed with slit lamp examination on the 1st, 7thand 30th post-operative day.

Results: In our study 80 eyes from 80 patient included from which 40 eyes in group-1 and 40 eyes for group-2. Mean age of patients was 58 years. Among 80 eyes 34 eyes developed corneal complication after cataract surgery. Total corneal complication rate after cataract surgery (SICS+ Phacoemulsification) was 42.5%.out of them 17.5% and 25% corneal complication occur in SICS and phacoemulsification respectively. 22% ,7%,3%,3% rate of mild striate keratopathy, moderate striate keratopathy, edema, descemet's membrane detachment respectively in group-1(SICS). 20%,15%,10%,5% rate of mild striate keratopathy, moderate striate keratopathy, micro cystic edema, descemet's membrane detachment respectively in group-2(phacoemulsification).

There was statistically significant difference of corneal complication between manual small incision cataract surgery and phacoemulsification surgery at 1st postoperative day. After 1month follow-up there was no statistically difference of corneal complication in both groups.

Conclusion: Corneal complication on follow-up were almost similar in both phacoemulsification and small incision cataract surgery.

Keyword*: SICS, Phacoemulsification, Striate keratopathy, Microcystic edema, Descemet's membrane detachment.*

Introduction

Cataract is the most common cause of reversible blindness in India[1]. Manual SICS and Phacoemulsification with posterior chamber intraocular lens implantation are preferred method to treat cataract[2]. In cataract surgery our main concern towards cornea are to maintain anterior corneal surface and corneal endothelium. Risk factors of corneal complications include preoperatively (hyper mature and mature cataract ,brown cataract, poorly dilating small pupil) , intra-operatively (premature entry in anterior chamber, descemet membrane detachment, posterior capsular rent ,marked manipulation in anterior chamber during surgery, prolonged duration of surgery), postoperative (retained viscoelastic material inanterior chamber) [3][4][5]. Corneal epithelial surface and endothelium are responsible for corneal transparency. Endothelial

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damage occur secondary to surgical trauma that may be due to mechanical cause include (phacoemulsification probe tip heating, high ultrasound energy, high flow rates etc.) or chemical cause include (irrigating fluid, viscoelastic substance) and during nucleus delivery (in SICS)[6][7].

Endothelial cell loss is more after cataract surgery than in un operated eyes.[8] Most common corneal complications after phacoemulsification is transient corneal edema that lead to decrease visual acuity in the post-operative periods.[9]

The advances in recent cataract surgery have reduced these complications. This study wasdone to compare corneal complications between MSICS and phacoemulsification and assess the final visual outcome in thepatients.

Material and Methods

This prospective hospital-based follow up study was done in 80 eyes of 80 patients, among them 40 patients undergone MSICS and 40 patients undergone for phacoemulsification with posterior chamber intraocular lens implantation (PCIOL) at Dhiraj hospital, Vadodara during 5 months period from October 2019 and February 2020. All the patients with clinically significant cataract were enrolled in this study conducted in Ophthalmology Department of Dhiraj hospital, Vadodara. Exclusion criteria were taken – pre-existing corneal decompensation, corneal scar, any history of ocular trauma, previous intraocular surgery, patients unwilling to participate in the study, traumatic cataract, uveitis, and a known case of glaucoma.

Detailed history and detailed ocular examination was done on the day of admission.

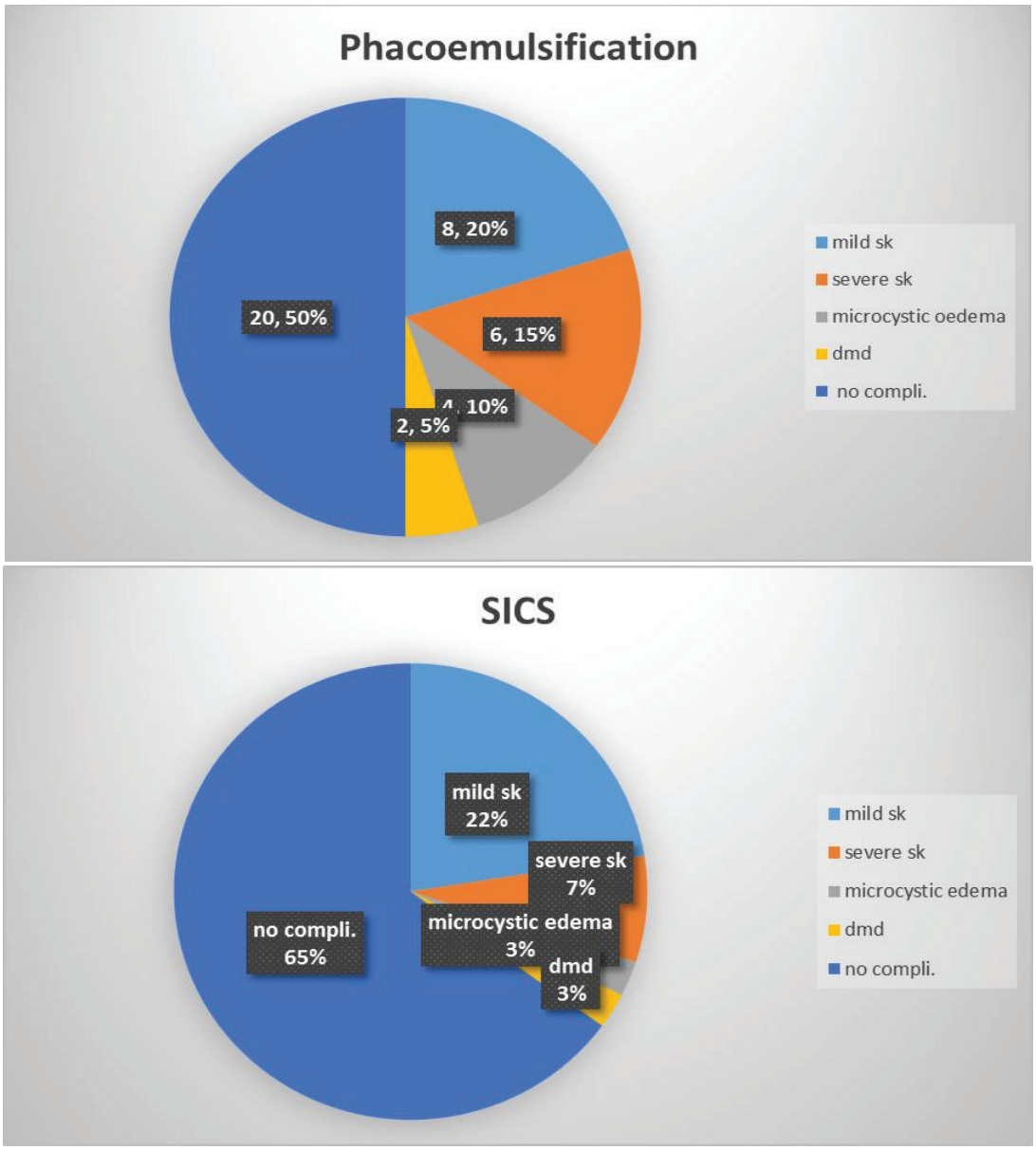
All 80 patients were given antibiotic prophylaxis from a day prior to surgery. Standard steps of MSICS and phacoemulsification were being followed and standard postoperative treatment was started.Post-operat ivecomplicationsandBCVAandslitlampexaminationwer ereviewedonday 1st, 7thand 30thpost-operative day.

Findings

This study enrolls 80 eyes of 80 patients with various stages of cataract who had been admitted for surgery at Dhiraj hospital. Patients were divided into two groups Group-1 included 40 patients who underwent for SICS and group-2 included 40 patients who underwent for phacoemulsification. This study includes 48 males and 32 female patients. Mean age of patients was 58 years. Out of 80 patients 34 patients developed corneal complication where 14 from SICS and 20 from phacoemulsification.

Table1. Age and Sex distribution of patients enrolled in this study.

Age group (year)	Males	Females
45-55	6	5
56-65	18	8
>65	24	19
Total	48	32



Graph 1: Corneal complications in phacoemulsification vs in small incision cataract surgery.

Table 2: Visual Outcomes for SICS and Phacoemulsification on followups.

Visual Acuity (logMar equivalent)	Phaco			SICS			
	1stpostop day	7th day	30th day	1st postop day		7th day	30th day
>20/200 or 1.1	4	2	0	6	3		0
20/200 -20/63 or 1.0- 0.5	22	16	5	10		5	4
20/63 – 20/20 or 0.5-0.0	14	22	35	24		32	36
Total	40	40	40	40		40	40

Table 3: Corneal complications in SICS (group1) and Phacoemulsification (group2).

	CORNEA COMPLICATIONS			
POST OP DAY	STRATE KERATOPATHY		MICROCYSTIC EDEMA	DESCEMET MEMBRANE DETACHMENT
GROUP-1 SICS	MILD	SEVERE		
DAY-1	9	3	1	1
1WEEK	0	1	0	0
1MONTH	0	0	0	0
GROUP-2 PHACO				
DAY-1	8	6	4	2
1 WEEK	0	3	2	0
1 MONTH	0	0	0	0

In group 1(SICS) 09 Patients developed mild(<10 Descemets' membrane Folds) and 03 patients developed severe striate keratopathy.(>10 Descemets' membrane)01 patients had microcysticodema and 01 patients had descemet membrane detachment.

In group 2(phacoemulsification) -08 patients developed mild and 06 patients developed severe striate keratopathy.04 patients had microcystic edema and 02 patients had descemet's membrane detachment.

The most common corneal complication in our study was mild striate keratopathy which resolved with treatment by 7th postoperative day in both groups.

And severe striate keratopathy which resolved by the first post-operative week in 03 patients in group-1 and 03 patients in group-2.

01 patients from group-1 and 04 patients group-2 developed microcystic corneal edema which resolved after anti-glaucoma medication by one week in group-1 and by more than one week in group-2.

01 patients from group-1 and 02 patients from group-2 developed descemet membrane detachment which was resolved by first week post-operatively.

Total corneal complication rate after cataract surgery(SICS + Phacoemulsification) was 42.5%.out of them 17.5% and 25% corneal complication occur in SICS and phacoemulsification respectively out of which 22% ,7%,3%,3% rate of mild striate keratopathy , moderate striate keratopathy,microcystic edema, descemet's membrane detachment respectively in group-1.

20%,15%,10%,5% rate of mild striate keratopathy , moderate striate keratopathy, microcystic edema, descemet's membrane detachment respectively in group-2.

On the 1st post-operative day, most patients had BCVA (logMar equivalent) of 1-0.5. On 30th post-operative day, most patients had attained a best corrected visual acuity of 0.5 -0.0 So good post-operative visual outcomes depend on timely management of corneal complications.

Discussion

Manual SICS is a popular technique in the developing countries due to its low cost, machine independent and short learning curve. Phacoemulsification remains the preferred method in developed countries. Corneal complication in the immediate postoperative period after phacoemulsification and manual SICS leads to patient's dissatisfaction and worsening of visual outcome. Corneal complication particularly corneal edema after cataract surgery is a common and avoidable complication in most of the cases and usually are transient and resolve by the end of 1 week.

In our study the incidence of Microcystic edema, striate keratopathy and descemet membrane detachment is seen more with phacoemulsification compared to manual small incision cataract surgery in this study. By the 30th post-operative day, most of the patients obtained best corrected visual acuity of logMar 0.5 to 0.0.

Similar results to this study have been obtained by the studies done by Sudhakar et al and Venkatesh R et al, their study results showed MSICS is with lesser corneal complications and more economical than phacoemulsification [9][10].MSICS also has an advantage of shorter learning curve. Study by **Venkatesh et al** which included 100 patients who undergone small incision cataract surgery at Aravind eye hospital,

Pondicherry had the following complications - 6 eyes (6%) developed corneal edema with<10 Descemets folds and 7 eyes (7%) had corneal edema with >10 Descemets folds on the 1st postoperative day which resolved with medical therapy by day 3. **Sudhakar et al**,evaluated the complications in 1000 cases of small incision cataract surgery with PCIOL implantation and reported that the most common post- operative complication on day1 post surgery was striate keratopathy (7.3%) and corneal edema (7%)[9]. **Gogate P.M., Kulkarni, S.R.et al** study showed that postoperative corneal edema was present in 4.5% of cases on 1st day in phacoemulsification surgery and 2% in small incision cataract surgery patients [11]. Thus MSICS is ideal for developing countries.

In this study total corneal complication rate after cataract surgery(SICS+ Phacoemulsification) was 42.5%.out of them 17.5% and 25% corneal complication occur in SICS and phacoemulsification respectively, from which the rate of mild striate keratopathy , moderate striate keratopathy, microcystic edema, descemet's membrane detachment were 22%,7%,3%,3% respectively in group-1(SICS) and 20%,15%,10%,5% in group-2 (phacoemulsification).

Conclusion

Both phacoemulsification and MSICS achieved comparable and excellent visual outcomes for treatment of cataract, with lower complications rates and earlier postoperative visual rehabilitation in small incision cataract surgery. To reduce the rate of corneal complications a proper preoperative workup, careful surgery and timely management of these complications with regular follow-up of patients to be done. In this study immediate post- operative corneal complication in phacoemulsification were more than SICS. So good post- operative visual outcomes depend on timely management of corneal complications.

Conflicts of Interest: There is no conflict of interest in this study.

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Effectiveness of Kaltenborn Posterior Glide and Coracohumeral Ligament Positional Stretching on External Rotation Range of Motion in Patients with Adhesive Capsulitis

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Abstract

Background: Adhesive capsulitis is a common and debilitating medical condition which is treated by various surgical and non-surgical treatments but there is no significant method universally. Physical therapists use various shoulder mobilization techniques to improve the ROM. Radiographic and surgical evidence presents tightened CHL to be a prime contributor to inadequate shoulder external rotation in individuals diagnosed with adhesive capsulitis.

Objective: To determine the combined effectiveness of Kaltenborn posterior glide and coracohumeral ligament (CHL) positional stretching on external rotation in patients with adhesive capsulitis.

Methods and Measures: A number of 30 subjects, both Male and Female with a primary diagnosis of shoulder adhesive capsulitis by the physician were recruited. 15 subjects each were randomly assigned to Group-A(Experimental) and Group-B(control). All subjects received 6 therapy sessions consisting of moist heat, shoulder pendular and ROM exercises, Kaltenborn posterior glide and Coracohumeral ligament positional stretching in Group-A. Moist heat, shoulder pendular and ROM exercises in Group-B. Pre-test and post-test were carried out for both the groups and analysed using paired and independent t tests in an SPSS software.

Results: The experimental group(Group-A) mean VAS score had decreased from 6.27 to 3.73, SPADI mean score decreased from 0.534 to 0.380 and also Goniometer (Shoulder external ROM) showed an improvement from 29.2 to 35.33 but when intergroup analysis was done, it resulted in significance only for SPADI where $p < 0.05$. Through this measure, we can state that the treatment protocol for group A had a significant improvement in disability but not much on pain and ROM in patients with adhesive capsulitis.

Conclusion: All the interventions has brought about some improvement in each group post-treatment based on the mean score but its significance vary. So, this study can be concluded that, the treatment protocol, Kaltenborn posterior glide and CHL positional stretching could bring a significant difference with regard to disabilty but not much of difference in pain and ROM in patients with adhesive capsulitis.

Key words: *Coracohumeral Ligament, Adhesive capsulitis, Kaltenborn, Positional stretching, CHL.*

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Introduction

Adhesive capsulitis, is a disease form, of unspecified origin of cause[1] portrayed with a loss of glenohumeral joint motion in all directions, both active and passive movements in an agonizing and steady-rate manner,

affecting the external rotation predominantly as a result of progressive fibrosis and contracture of the glenohumeral joint capsule.[2] Various ailments, trauma, muscular disproportion, degenerative changes of the shoulder complex may also lead to adhesive capsulitis but is usually considered as acquired or secondary.[3] AC occurs in 2% to 5% of the population which is more frequent in women aged between 40 and 60 years and in about 20-30% of cases this condition is bilateral. Many pathological disorders can be associated with AC as diabetes mellitus, thyroid dysfunctions, dupuytren contracture, cardio respiratory and autoimmune diseases.[2,4]It was in the year 1945 in a histological paper published, Neviaser[5] was the first to attempt to convey the adhesive nature of the shoulder capsule as the attribution of the cause in frozen shoulder. And labelled this condition as 'Adhesive capsulitis' subsequently.[5]

Neviaser et al.[6] and Hannafin et al.[7] identified this condition to possess 4 stages, which have been stated after a thorough study on the corresponding features between clinical examination and histological findings. The first one is the 'Painful stage', characterised by gradual onset of symptoms that continues for less than 3 months. Second stage is the 'Freezing stage', where symptoms are present from 3-9 months and are marked with nocturnal pain intensified when patient seem to have laid on the affected side, further portrayed with loss of both active and passive ROM. Symptoms persists from 9-14 months with shoulder stiffness primarily and painful end range movement in the 'Frozen stage'. And the last stage is the 'thawing stage', that presents with a minimal pain and a gradual improvement of ROM occuring between 15-24 months.[6,7]The pathophysiology remains unspecified till date. But the most appealing findings considered often is the inflammation that occurs within the joint capsule and synovial fluid leading to pain at the initial stage which is then, followed by reactive fibrosis and adhesions of the synovial lining of the joint resulting in decreased ROM.[8] Fibrosis and hyalinization of connective tissue at the rotator interval including CHL were shown to be one of the pathogenesis of adhesive capsulitis.[9]

Bernard et al. found a thickened CHL of 4mm or more which had a specificity of 95% and a sensitivity of 59% for diagnosis of frozen shoulder along with a thickened capsule in the rotator cuff interval of 7mm or more had a specificity of 86% and sensitivity of 64%.[10] In a study done by Wen Zhao et al., it was concluded that MRI can be used to show characteristic findings in diagnosing AC that includes thickening of the CHL and the capsule at the rotator cuff interval and complete obliteration of the fat triangle under the coracoid process have been the most prominent characteristic in MRI findings seen with AC.[11]

The thickening, contractures and fibroplasia of CHL has been noted where the CHL remain taut in shoulder external rotation. However, quite a number of favourable outcomes have been reported in studies targeting the CHL through surgical release, microadhesiolysis and stretching.[12] According to the anatomical fibre orientation of CHL, stretching can be applied to improve the extensibility of the tissue.[13] CHL stretching had a positive impact on calcification, which further prevented fibrotic changes and facilitated clinical improvement and also found that including positional stretching in a rehabilitation protocol improved disability outcomes and results in better ROM.[14] Feland et al. classified a stretch known as 'Long-duration' stretch greater than 30 seconds for one repetition and a 'lowintensity' as well according to the patient's perception and found that long-duration and low-intensity stretching increased ROM especially in the elder population with the concept of more the ageing process, more the tautness of the tissues leading to loss of elasticity and poor state of connective tissues thereafter.[15]

The use of ice while stretching or cryostretching provided enhancement in flexibility of the hamstring than with heat or stretching alone.[16] It was stated that the rationale for using cryotherapy along with stretching is based on the concept of cold possessing the capability to contract the tissues in the new lengthened position.[17] A study stating the effects of superficial thermal agents and shoulder stretching exercises in normal subjects concluded that the use of superficial heat in conjunction with low-load prolonged stretching produced more long-lasting changes in the extensibility of soft tissue than did stretching alone.[18]

Grade 3 posterior translation has a great impact in improving range of motion of external rotation of shoulder in patients with adhesive capsulitis by the distraction force on posterior capsule that eases

both the pain and increases ROM of external rotation of shoulder. Kaltenborn mobilization has found to have greater evidence in increasing range of motion. Stretching of fibrous tissue allows the tissue to undergo creep which relates to the ability of tissue to change shape over time as a constant load is applied.[19] And joint mobilization along with active exercises were found to be more helpful in hypomobility of shoulder adhesive capsulitis.[20] Andrea J. Johnson et al. portrayed that a posteriorly directed Kaltenborn mobilization was more effective in increasing external ROM in subjects with shoulder adhesive capsulitis.[21] Also, Jose Orlando[22] practiced a positional stretching of CHL and have found to improve the DASH and SPADI scores significantly with improved external rotation and overall mobility and function.[22] But to our knowledge, there are no studies stating the combined effectiveness of Kaltenborn posterior glide and CHL positional stretching. Therefore, this study has been carried out with an aim to find the combined effectiveness of these two manoeuvres in patients with adhesive capsulitis.

Methodology

An experimental design conducted for a duration of 12 months and a number of 30 subjects, both genders with a primary diagnosis of shoulder adhesive capsulitis by the physician were randomly assigned to Group-A(Experimental) and Group-B(control) after meeting the inclusion criteria. Both males and females of age between 45-65 years withIdiopathic or Primary adhesive capsulitis, painful stiffshoulder for at least 4 months, restricted glenohumeral external rotation when measured at 90° of shoulder abduction without history of shoulder surgeries to the affected shoulder and manipulation under anaesthesia of the affected shoulder, willingness to perform and complete the treatment regimen as explainedand patients who have provided written and verbal informed consent were included. Patients with secondary adhesive capsulitis, history of fracture and dislocation in and around the shoulder joint, inflammatory diseases such as rheumatoid arthritis, sensory impairment, subjects with any cognitive and perceptual disorders, concurrent severe cervical signs and symptoms, any musculoskeletal pathology in the upper limb other than AC, malignancy, subjects under any oral steroidal medication or intra-articular steroid injections and subjects not willing to participate in the study were excluded from the study.The study proposal has been accepted by the ethical committee and the samples were collected from Assam Down Town University OPD and Physiotherapy Department, Down Town Hospital.

Outcome Measures

VAS, SPADI and Goniometry were used for assessing pain, functional ability and ROM for the shoulder joint.

Procedures

The samples were randomly divided into, Group-A(Experimental) and Group-B(control), each group containing 15 subjects. A pre-test and post-test were conducted by assessing range of motion. The interventions in Group-A included: moist heat, pendular and shoulder ROM exercises, Kaltenborn posterior glide and CHL positional stretching with ice pack. And Group-B received moist heat, pendular and shoulder ROM exercises. Each group received 6 therapy sessions every alternate days.

The tissue is preheated with moist heat for 5mins prior to any intervention as heat is very commonly used before exercise with its advantage of being able to increase tissue metabolism the muscle which is prepared for the metabolic challenge of exercise.[23] This intervention is received by both the groups.

Kaltenborn posterior glide

Initial position for the posterior mobilization. At the end range of abduction and external rotation a lateral humeral distraction in its midrange position is maintained, while the posterior stretch mobilization is performed to its end range. **(Figure 1)**

Progression of the posterior mobilization. At the end range of flexion and external rotation, a lateral distraction in its midrange is maintained, while the posterior stretch mobilization is performed to the end range.**(Figure 2)**

The end range position is held for at least 1 minute. Each stretch repeated so that a total of 15 minutes of sustained stretch is performed. And no oscillatory motions were performed.[21]

Figure 1: Position 1 of Kaltenborn Posterior glide.

Figure 2: Position 2 of Kaltenborn Posterior glide

Coracohumeral Positional stretching

Patient is asked to lie on the unaffected side. 20-22 inches of dowel is made to hold with the affected arm. Forearm supination to encourage external rotation. Extension to 10 degrees and adduct close to the body. Positional CHL stretching is repeated along with a cold pack, building up from 5 minutes at the initial visit and expected to increase upto 15minutes by the third treatment session. **(Figure 3)**

Figure 3:Coracohumeral ligament positional stretching on patient.

Pendular exercises:The patient used a chair or a table to perform this exercise. Patient leaned forward and firmly grip the chair or table with the non affected hand and slowly bring the affected arm down so it is hanging freely. Once in this position, the patient will slowly swing his affected arm forward, backward, circles and from side to side. These exercises should be done in repetition of 15 times in each direction by the affected hand.

Shoulder ROM exercises: Forward shoulder flexion and extension, where Subject stands with trunk keeping upright, with clasped hands lift both the arms straight up as much as possible in front, letting the unaffected arm assist the affected arm. Followed by Subject standing with trunk keeping upright, push steadily both the arms behind as much as possible and bring back gently. In standing position, lift both the arms at the side of the body and return gently to start intending to perform an abduction and adduction.

Subject in sitting with arms relaxed, gently lift(shrug) both the shoulders up and gently relax. Also, set shoulder blades in neutral position. Keep arm at the side with elbow at 90 degrees, forearm at mid-prone position and wrist in neutral. Rotate the upper arm towards the body and away from the body simultaneously without moving the elbow forward or backwards. Three sets of 15 repetitions of all exercises were performed daily.

Data Analysis

A paired t test and independent t test were used to analyse the variables pre-intervention and post-intervention in an SPSS software. Level of significance with p value was set at 0.05 less than this is considered as statistically significant difference.

Results

Table 1: Intra – group analysis of Group A and Group B of VAS.

Group	VAS	Mean ± SD	N	t	df	p	Remarks
Group A	Before Treatment (Pre-Test)	6.27 ± .799	15	13.20	14	0.000	S
	After Treatment (Post-Test)	3.73 ± 1.223	15				
Group B	Before Treatment (Pre-Test)	5.47 ± 1.302	15	5.26	14	0.00	S
	After Treatment (Post-Test)	4.33 ± 1.676	15				

*S =Significant

Table 2 : Intra-Group analysis of Group-A and Group-B of SPADI

Group	SPADI	Mean ± SD	N	t	df	p	Remarks
Group A	Before Treatment (Pre-Test)	0.534± 0.17	15	6.83	14	0.000	S
	After Treatment (Post-Test)	0.380 ± 0.167	15				
Group B	Before Treatment (Pre-Test)	0.579± 0.148	15	4.37	14	0.00063	S
	After Treatment (Post-Test)	0.523 ± 0.188	15				

*S = Significant

Table 3 : Intra-Group analysis of Group A and Group B of Goniometer (Shoulder External ROM)

Group	Goniometer (Shoulder External ROM)	Mean ± SD	N	t	df	p	Remarks
Group A	Before Treatment (Pre-Test)	29.2 ± 18.42	15	-4.97	14	0.0002	S
	After Treatment (Post-Test)	35.33 ± 21.41	15				
Group B	Before Teatment (Pre-Test)	35.33 ± 25.42	15	-4.18	14	0.0009	S
	After Treatment (Post-Test)	36.67 ± 25.65	15				

*S = Significant

Table 4: Inter-group analysis between Group A and Group B to compare and find the effectiveness of interventions on external rotation ROM in patients with adhesive capsulitis

SCALES	GROUP	Mean ± SD	t	df	p	Remarks
VAS	Group A	3.73 ± 1.22	-1.120	28	0.272	NS
	Group B	4.33 ± 1.670				
SPADI	Group A	38.074 ± 16.75	-2.178	28	0.038	S
	Group B	52.25 ± 18.84				
Goniometer- Shoulder External ROM	Group A	40.00 ± 21.414	0.386	28	0.702	NS
	Group B	36.67 ± 25.656				

*S = Significant, *NS = Non Significant

This was a 12 months structured study performed to determine the effects of Kaltenborn posterior glide, CHL positional stretching, moist heat , shoulder ROM and pendular exercises and was received by Group A(Experimental group). Also, a control group, Group B was set at a treatment protocol of moist heat fomentation, shoulder ROM and pendular exercises. Subjects of both the groups were assessed to find the VAS core for pain, SPADI for disability and goniometer for shoulder external ROM specifically. A pre-test and a post-test score of each outcome measures were recorded.

It is seen that in group A there is a significant difference between pre-test and post-test since $p < 0.05$ and the protocol (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises) is effective since mean VAS score decreases from 6.27 to 3.73.In group B, there is a significant difference between pre-test and post-test since $p < 0.05$ and the protocol (Moist heat fomentation, shoulder ROM and pendular exercises) is effective since mean VAS score decreases from 5.47 to 4.33**(Table 1).** For SPADI, it is seen that in group A there is a significant

difference between pre-test and post-test since $p < 0.05$ and the protocol (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises) is effective since mean SPADI score decreases from 0.534 to 0.380.In group B, there is a significant difference between pre-test and post-test since $p < 0.05$ and the protocol (Moist heat fomentation, shoulder ROM and pendular exercises) is effective since mean SPADI score decreases from 0.579 to 0.523**(Table 2).**

In group A, a significant difference has been found between pre-test and post-test since $p < 0.05$ and the protocol (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises) is effective since mean Goniometer (Shoulder external ROM) score increases from 29.2 to 35.33.In group B, there is a significant difference between pre-test and post-test since $p < 0.05$ and the protocol (Moist heat fomentation, shoulder ROM and pendular exercises) is effective since mean Goniometer (Shoulder external ROM) score increases from 35.33 to 36.67**(Table 3).**

It is found that the VAS mean score of group A, post treatment (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises) is 3.73 and the mean VAS score of group B post treatment (Moist heat fomentation, Shoulder ROM and pendular exercises) is 4.33. Therefore, group A treatment protocol results better in alleviating the pain according to the overall VAS mean score. But, since $p > 0.05$ it is found to be non significant.**(Table 4)**

The mean SPADI score of group A, post treatment (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises) is 38.074 and the mean SPADI score of group B post treatment (Moist heat fomentation, Shoulder ROM ad pendular exercises) is 52.25. Therefore, based on the overall mean SPADI score, the group A treatment protocol found effective in improving the disability and also significant since $p< 0.05$.**(Table 4)**

It is found that the mean Goniometer (Shoulder external ROM) score of group A, post treatment (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises) is 40.00 and the mean Goniometer (Shoulder external ROM) score of group B post treatment (Moist heat fomentation, Shoulder ROM ad pendular exercises) is 36.67. Therefore, according to the overall Goniometer (Shoulder External ROM) mean score, the group A treatment protocol responded better in increasing the shoulder external ROM. But, since $p > 0.05$ it is found to be non significant.**(Table 4)**

Therefore, we can conclude that based on the inter-group analysis, only the interventions for SPADI is found to be significant. And out of which, the mean SPADI score decreased in group A post treatment (Kaltenborn Posterior glide, CHL positional stretching, Moist heat fomentation, shoulder ROM and pendular exercises). Hence, we can state that the disability is decreased in this protocol but not much of difference in pain and ROM in patients with adhesive capsulitis.

Discussion

Adhesive capsulitis, being known as a debilitating ailment of the shoulder joint commonly, has an unknown origin of cause but there are quite a good number of literature that portrays a good response post physiotherapy treatments. Simultaneously, the difficulty to completely combat the pain and disability through a specific treatment approach in a timeframe is observed even today. And this difficulty was once acknowledged by Codman in 1934: "This is a class of cases which I find it difficult to define, difficult to treat and difficult to explain..."[24(p216)]which exists even today.

This study aimed at determining the combined effectiveness of Kaltenborn posterior glide and CHL positional stretching,moist heat shoulder ROM and pendular exercises(Group A) on external rotation in patients with adhesive capsulitis by measuring VAS for shoulder pain, GONIOMETER for external rotation range of motion and SPADI for shoulder disability. The study also had a control group(Group B) who received a treatment protocol of moist heat fomentation, shoulder ROM and pendular exercises. Each group consisted of 15 subjects which were randomly assigned and every single subject successfully completed their therapy session. Therefore no drop-outs were recorded. Luciano Andrés Rossi et al.,[25] has also discussed a few current concepts in the treatment of adhesive capsulitis of the shoulder wherein, the importance and positive result of physical therapy combined with home based exercises

were found to be the mainstay. And other approaches like intra-articular steroid injections provided a significant result in alleviating the pain but only for a short-term.[25]

So, in this study, the experimental group(Group A) mean VAS score had decreased from 6.27 to 3.73, SPADI mean score decreased from 0.534 to 0.380 and also Goniometer (Shoulder external ROM) showed an improvement from 29.2 to 35.33 but when inter-group analysis was done, it resulted in significance only for SPADI where $p < 0.05$. Through this measure, we can state that the treatment protocol for group A had a significant improvement in disability but not much on pain and ROM in patients with adhesive capsulitis.

Conclusion

All the interventions has brought about some improvement in each group post-treatment based on the mean score but its significance vary. So, this study can be concluded that, the treatment protocol, Kaltenborn posterior glide and CHL positional stretching could bring a significant difference with regard to disabilty but not much of difference in pain and ROM in patients with adhesive capsulitis.

Limitation

This study consisted of a short course of treatment of 6 therapy sessions, result only showed about short-term effects of the interventions, there was no follow up for the interventions, the sample size was small (Group A, n = 15; Group B, n = 15) and the strength of the shoulder complex musculature were not taken into account.

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Analysis of Defence Injuries in Homicide Cases Categorised in Accordance to Homicide Injury Scale

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Abstract

Introduction: The instinct of self-preservation drives a person to defend themselves from any violent attack which in itself may produce injuries on the body. These are termed as defence injuries. This study aims to analyse defence injuries in homicide cases which are categorised based on its severity as determined by Homicide Injury Scale (HIS).

Methods: This is a retrospective study which included all homicide cases which were autopsied during a twelve year period in a tertiary care hospital. Each of these cases were categorised according to the HIS. The presence, type and distribution of defence injuries were then tabulated and analysed.

Results: A total of 85 homicide cases were autopsied during the specified study period. Out of these 85 cases, 53 cases showed defence injuries (62%) and majority of these cases fell under higher categories of HIS. Cases categorised under HIS 2,3 and 4 had defence wounds which were more blunt force in nature while cases under HIS 5 and 6 had more sharp force defence injuries. Passive defence injuries were found uniformly across all categories of HIS, however active defence injuries were found more in higher categories of HIS than in lower ones.

Conclusion: This is rare study where the pattern and distribution of defence injuries was analysed based on a standardised scale. Active defence injuries were more commonly seen in homicide cases placed on higher scales of HIS.

***Key words**: Defence injuries; Homicide; Homicide Injury Scale*

Introduction

As unsavoury as it may seem, homicide rates have seen a constant uptick in the modern era. There may be many reasons for a human to violently end the life of another human being including rage, jealousy or greed. Every living being has a natural instinct for survival and it is this instinct that makes a person defend himself/herself from violent assault. The very act of defending oneself may cause numerous injuries to the body. These injuries, which may be incurred either by blunt or sharp force trauma, on any part of the body are termed as defence injuries. These defence wounds can be classified as active wounds or passive wounds. [1,2] Active defence wounds are the injuries produced when a person tries to stop the attack by catching hold of the offending weapon and therefore are seen in the palms of the hands. Passive defence wounds are acquired while shielding vital parts of body by using limbs and so are commonly seen in the back aspect of forearm or outer aspect of the arm. [1,2] Passive defence wounds can also be present on other parts of body like legs, feet or back. Presence of defence wounds indicates that the person was conscious and could comprehend the attack. The

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pattern and distribution of defence wound depend upon numerous factors such as speed of the attack, position of the assailant in relation to the victim, the weapon used and the level of intoxication of the victim. [2]

Homicide Injury Scale (HIS) was formulated by Safarik and Jarvis in 2005 using injury data from medical examiner's report. [3] The main aim of this scale was to add more specificity and accuracy in the analysis of victims' injuries in homicide cases. It was found to be particularly useful while comparing injuries in a large sample of cases where there are different and multiple causes of death with or without related injuries. HIS quantifies only those injuries which are directly related to the cause of death. It has a scoring range of 1 to 6 where score 1 represents a single cause of death with no external injuries and score 6 was given when there were multiple causes of death with multiple external injuries amounting to overkill [Table 1.]

Safarik and Jarvis defined overkill as given in the Crime Classification Manual. According to 2006 edition of this manual, overkill is defined as excessive trauma beyond that which is necessary to cause death. [4]

This study aims to categorise homicide cases according to Homicide Injury Scale and analyse the characteristics of defence injuries found in these cases.

Objectives

1. To classify homicide cases according to its severity based on Homicide Injury Scale.

2. To analyse the presence, type and distribution of defence injuries in each severity group

Method

This is a retrospective study which includes all homicide cases which were autopsied at a private medical college in Pondicherry from 2004 to 2016. Ethical clearance for the study was obtained from Institutional Ethical Committee. The data for this study was obtained from post-mortem report and police inquest report of the cases. In each of these cases, the fatal injuries were studied and categorised according to the Homicide Injury Scale. The presence, type and distribution of defence injuries in all cases were noted for each group. The results were analysed using IBM SPSS Statistics software version 22.0.

Inclusion criteria:

1. All homicide cases with defence injuries which were autopsied in the hospital from 2004-2016

Exclusion criteria:

1. Homicide cases which showed advanced decomposition changes obliterating the injuries

2. Homicide cases where the cause of death could not be ascertained

Results

A total of 85 homicide cases were autopsied during the specified study period. These cases were categorised into 6 groups according to homicide injury scale based on the injuries mentioned in the autopsy reports. Of the 85 cases, none of them fell under the category of HIS 1. For each of the other categories, the total number of cases with defence injuries, types and regional distribution of defence injuries were tabulated.

Out of 85 cases, 53 cases showed defence injuries (62%) and majority of these cases involved male victims within the age group of 20-40 years. Cases which were categorised under HIS 5 and 6 had more defence injuries than the lower categories. (Table. 2)

Nature of Defence Injuries

Both blunt force and sharp force injuries were noted as defence wounds. Blunt force injuries include abrasions, contusions and lacerations while sharp force injuries are incised wounds, chop wounds and stab wounds. 60% of the cases had blunt force defence injuries while 53% had sharp force defence injuries. Cases categorised under HIS 2,3 and 4 had defence wounds which were more blunt force in nature while cases under HIS 5 and 6 had more sharp force defence injuries. (Table.3)

Type Of Defence Injuries

Active defence injuries are seen when victims catch hold of the offending weapon to stop the attack and are seen in the palms while passive defence wounds are seen when the victims shield their vital parts such as head or neck with other parts of the body. In our study passive defence injuries were found in 89% of the cases while

49% of cases had active defence injuries and 38% had both. Passive defence injuries were found uniformly across all categories of HIS, however active defence injuries were found more in higher categories of HIS than in lower ones. (Table.4)

Table 1: THE HOMICIDE INJURY SCALE (HIS)

HIS score	Definition
HIS 1	Single cause of death only: internal injuries only with no visible related external injuries (e.g., smothering, strangulation, ruptured organs resulting from blunt force trauma)
HIS 2	Single cause of death only: internal injuries only with minor related external injuries (e.g., smothered with related abrasions and/or contusions of mouth and face, strangled with related abrasions or ligature marks)
HIS 3	Single cause of death only: related external moderate to serious injuries not identified as either excessive or overkill
HIS 4	Two or more causes of death: related internal and/or external injuries not identified as either excessive or overkill
HIS 5	Single cause of death only: related external injuries identified as either excessive or overkill
HIS 6	Two or more causes of death: related internal and/or external injuries in at least one of the causes of death identified as either excessive or overkill

Table 2: GENDER AND DEFENCE INJURIES

HIS category (n)	MALE [n (%)]	FEMALE [n (%)]
6 (22)	21 (95%)	1 (5%)
5 (5)	4 (80%)	1 (20%)
4 (12)	6 (50%)	6(50%)
3 (13)	11 (85%)	2 (15%)
2 (1)	0	1 (100%)
TOTAL (53)	42 (79%)	11 (21%)

n- number of cases %- percentage of cases

Table 3: NATURE OF DEFENCE INJURIES

HIS Category (n)	BLUNT FORCE INJURIES [n (%)]	SHARP FORCE INJURIES [n (%)]
6 (22)	6 (27%)	21 (95%)
5 (5)	3 (60%)	3 (60%)
4 (12)	11 (92%)	2 (17%)
3 (13)	13 (100%)	2 (15%)
2 (1)	1 (100%)	0 (0%)
Total (53)	32 (60%)	28 (53%)

n- number of cases %- percentage of cases

Table 4: TYPE OF DEFENCE INJURIES

HIS category (n)	ACTIVE DEFENCE INJURIES [n (%)]	PASSIVE DEFENCE INJURIES [n (%)]	BOTH [n (%)]
6 (22)	16 (73%)	21 (95%)	15 (68%)
5 (5)	2 (40%)	5 (100%)	2 (40%)
4 (12)	5 (42%)	10 (83%)	3 (25 %)
3 (13)	3 (23%)	10 (77%)	0
2 (1)	0	1 (100%)	0
TOTAL (53)	26 (49%)	47 (89%)	20 (38%)

n- number of cases %- percentage of cases

Discussion

Apart from being a violent crime, homicide also has a considerable impact on the psychosocial, political and socio-economic parameters of a country. [5] The United Nations Office on Drugs and Crime (UNODC) considers homicide is an indicator for determining a country's level of violence and safety. As per the global study on homicide published in 2017 by UNODC, more people were killed by homicide rather than armed conflict. [6]

Several scoring systems have been developed to categorise injuries based on their severity. Fredik et al has published a paper in which 7 injury scoring systems were compared and had concluded that Sum of Abbreviated Injury Scale (SAIS) score is the closest to a gold standard for injury severity scoring in homicide victims. The authors also concluded that if their proposed

definition of overkill is used, then Homicide Injury Scale (HIS) can be used as a valid alternative method for SAIS while quantifying injuries in a homicide victim. [7] In a study which was published earlier, various attributes of homicide cases were correlated with its severity based on Homicide Injury Scale. [8]

While many researches on pattern and distribution of defence injuries in homicide victims has been published, we could not find any article which compared or analysed defence injuries when the severity of homicide cases are categorised based on any standardised scale. In our study we found that majority of victims with defence injuries were males in the age group of 20-40 years. These findings were consistent with the studies by various other authors, where young adult males were more often victims of violent homicides. [9-20] Our study also found that these victims fell into homicide cases of higher severity. This may be because young adult males, in their prime are able to fight back and resist a violent assault resulting in multiple injuries.

Majority of cases in our study had blunt force type of defence injuries. This can be attributed to the fact that majority of homicide cases included in the study involved some form of blunt weapon rather than sharp. This corresponds with the findings of other studies conducted by Rančić N, Djurović G, Pilipović F and Savić S,[9] Vinoth K V and Sangeetha R,[14] Lakmali MGN et al. [15] and Mohite PM and Mohite DP. [19] But contradictory findings were found in other studies. [11,12,13,16,17,18] Our study also found that homicide cases of lower HIS category had more blunt force defence injuries while higher categories like HIS 5 and 6 had more sharp force defence injuries. This may be because most of the cases with overkill involved either a light or heavy sharp cutting weapon as the offending weapon.

Another finding in our study was that 89% of cases had passive defence injuries while active defence injuries were found in 49% of cases and 38% had both active and passive defence injuries. This is consistent with the findings of other studies where passive defence wounds were found to be more predominant than active defence wounds. [12-15] Our study also found that passive defence injuries were seen uniformly, across all categories of HIS irrespective of its severity whereas active defence injuries were found more in the higher severity categories of HIS. This may be because as the violence of assault increased, the victim became desperate enough to stop the assault by catching hold of the offending weapon.

Conclusion

Defence injuries found on the bodies of victims tells the story of the fight they fought to defend themselves from the violent onslaught. It depends upon a number of factors like the age, physical attributes, level of consciousness and sobriety of the victims. This study concludes that the nature of defence injuries shift from blunt force to sharp force as the severity of the homicide increases on the scale. Though passive defence injuries were found uniformly across all categories of HIS, active defence injuries were predominant in cases categorised under higher scales of HIS.

Though the sample size used in this study was small, this is rare study where pattern and distribution of defence injuries was analysed based of a standardised scale. This study will be a stepping stone towards a wider research in various other attributes of homicide cases in future.

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Haplotype Profiling of Y-STRs among Northern Population in Thailand

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Abstract

Background: The investigation of Y-short tandem repeats (Y-STRs) has been widely performed in forensic caseworks. Due to variation between populations, understanding of genetic information within individual a population could provide the database and scientifically reliable results. This study was to investigate allele and haplotype frequencies of Y-STRs among localized people who lived in northern Thailand.

Methods: A retrospective descriptive study was conducted by gathering medical reports of Y-STRs typing. The allele frequency and gene diversity as well as haplotype frequency and diversity were calculated. Pairwise genetic distance (R_{st}) was also calculated based on haplotype pattern by using AMOVA and Multidimensional Scaling (MDS) tools.

Results: The result showed that DYS389II, DYS439, and DYS392 represented the highest number of allele patterns. The highest and lowest allele frequency was found to be allele 14 of DYS437 and allele 13 of DYS437, respectively. The highest and lowest gene diversity was observed in DYS389II and DYS437, respectively. One-hundred and sixty different haplotypes were defined where 144 carried a unique haplotype and 16 carried a replicate haplotype. A significant R_{st} value was obtained between the studied population and central Thai population.

Conclusion: This study provided genetic database of Y-STRs among localized population in northern region of Thailand. The genetic structure of our subjects also represented the significant close relationship with some other populations in Southeast Asia.

Key words: *Allele frequency, Forensic genetic, Haplotype frequency, Y-STRs*

Introduction

DNA fingerprinting performed on the specific region on the DNA strand known as short tandem repeats (STRs) has been widely used for human identification in forensic genetic casework[1]. Because of variation in STRs lenght and high meutation rate, it promoted the genetic polymorphism of STRs markers and therefore useful in human identity testing. The investigation of Y-STRs is a useful reliable marker for personal identity in forensic genetic cases including paternity testing in paternal lineage, historical cases, special cases of the missing person or disaster victim identification involving men, ancestor study, as well as sexual assault with mixed DNA profile between male and female DNA[2, 3]. Moreover, Y-STRs analysis can be crucial for sex confirmation in the case of amelogenin dropout[4].

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According to the Scientific Working Group on DNA Analysis Methods (SWGDAM), the interpretation of Y-STRs is based on the comparison of haplotype between question and known samples. The SWGDAM further recommended to calculate Y haplotype frequency based on the ratio of number of observed haplotypes and the total number of haplotypes in a specific population database. The additional statistical parameter might be performed by calculation of the upper confidence limit for the probability of the Y haplotype within a population and match probability[5, 6]. Therefore, the localized population allele and haplotype frequencies are essential for more accurate calcualtion of Y-STRs result.

Previous studies demonstrated the diversity of Y-STRs on different populations between Africa, Asia, Europe, Latin America, and North America including the different ethnic groups[7, 8]. Since Thailand is located on Mainland Southeast Asia which was a route for people migration from southern China and other nationalities during historical migration and settlement, it promoted ethnic diversity. As expected, it was confirmed by the finding of 70 different recognized languages in people who lived in Thailand[9]. Therefore, it was possible that factors for example different ethnic groups and geographic regions could affect a diversity in population probably influenced genetic variation in Y-STRs. Likewise, there is insufficient information of Y-STRs data on certain specific northern Thai populations. This study was to evaluate the genetic profile of Y-STRs in the northern Thai population in order to discover allele and haplotype frequency database for use in related populations. The understanding of genetic profiles within individual a population could provide the precise database and scientifically reliable results.

Materials and Methods

Study design

A retrospective descriptive study was conducted by gathering information from of Y-STRs typing report from Faculty of Medicine, Chiang Mai University, Thailand. This study was performed in accordance with the Code of Ethichs of the World Medical Association (Declaration of Helsinki). Ethical approval for this study was obtained from the Research Ethics Committee of Faculty of Medicine, Chiang Mai University, Thailand (Number 6109/2019).

Data collection

Y-STRs loci containing DYS19, DYS385a/b, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, and DYS439 were recorded. The criteria for data enrollment consisted of 1) male living in 17 provinces of northern Thailand according to Department of Provincial Administration who were declared ethnicity as northern region of Thai 2) each subject must not be blood-related.

Allele frequency and gene diversity

Y-STRs data was transferred to calculate the allele frequency and gene diversity (GD) using the counting method according to the formula supplied by Nei, 1987[10] as follow equations:

$$\text{Allele frequency} = \frac{\text{Number of observed allele}}{\text{Number of total allele}}$$

$$\text{Gene diversity (GD)} = \frac{n(1 - \Sigma Pi^2)}{(n-1)}$$

where n: number of populations, Pi: allele frequency in tested population

Haplotype frequency and diversity

The number of haplotypes was estimated by counting method. Haplotype frequency and diversity were calculated as follow equations[11]:

$$\text{Haplotype frequency} = \frac{\text{Number of observed haplotype}}{\text{Number of total haplotype}}$$

$$\text{Haplotype diversity} = \frac{n(1 - \Sigma Pi^2)}{(n-1)}$$

where n: number of populations, Pi: haplotype frequency in tested population

Genetic Distance

Pairwise genetic distance (Rst) was also calculated based on haplotype pattern by using AMOVA and Multidimensional Scaling (MDS) tools of YHRD (www.yhrd.org).

Results

Totally 53 alleles were detected at the all tested Y-STRs loci in 180 samples. The most recorded allele was found to be DYS389II, DYS439, and DYS392 loci with 7 patterns. The most allele frequency was observed at allele 14 of DYS437, whereas the least allele frequency was observed at allele 15 of DYS389I, allele 21 of DYS390, allele 11 of DYS393, allele 12 of DYS391, allele 15 of DYS439, allele 16 of DYS392, and allele 13 of DYS437. The greatest polymorphic marker was DYS389II with a genetic diversity (GD) value of 0.74057 and the least polymorphic was DYS437 with a GD value of 0.41239. Moreover, nine loci including DYS389I, DYS390, DYS389II, DYS19, DYS393, DYS391, DYS439, DYS392. and DYS438 were classified to high polymorphism (gene diversity > 0.5) as shown in Table 1.

Regarding DYS385a/b loci, the dominant allele combination at DYS385 locus (13/19) had a frequency of 0.0778. Gene diversity for the entire sample was 0.97058. We found the tri-allelic pattern of DYS385a/b locus with 16/17/20 as shown in Table 2. This tri-alleic pattern was no matching with reported information form two databases (YHRD database (www.yhrd.org) and NIST: National Institute of Standards and Technology).

A 160 distinct haplotypes were observed in the total data set, 144 (90%) of which were unique haplotypes and 16 (10%) of which were replicated haplotypes. The replicated haplotypes can be classified into 13 haplotypes (8.125%) of them were shared by two individuals, 2 haplotypes (1.25%) of them were shared by three individuals, and 1 haplotype (0.625%) was shared by four individuals. Haplotype diversity for the entire sample was 0.9984 (99.84%) meaning that the probability of finding an individual sharing this haplotype is 1 in 625 (0.16%). The common haplotype observed in this study on DYS389I, DYS390, DYS389II, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS392, DYS437, and DYS438 loci was 14, 23, 31, 14, 11/12, 13, 11, 12, 14, 14, 11. This haplotype pattern was matched 52 times in total 266,542 haplotypes in global populations, and 3 times in total 1,170 haplotypes in Thai populations according to haplotype pattern in YHRD database.

To determine the genetic flow among populations, pairwise genetic distance (R_{st}) for each locus were estimated between previously reported populations using YHRD calculation online. The observed haplotype frequency in this study was compared with databases in YHRD consisting of population from central Thailand[12], Hongkong[13], Japan[14-16], Laos and Nepal[17, 18], South Korea[19-21], Taiwan[22], and Vietnam[7, 23]. Haplotypes of the northern Thai population were significantly different from those of the other 8 populations ($P < 0.05$). The R_{st} values for genetic distance was ranging from 0.0118 – 0.5092. It indicated that the genetic differentiation of each populations was classified as low to very high differentiation according to the previous report[24]. The lowest R_{st} value indicating the smallest genetic distances was observed between central Thai population and Vietnam population; whereas the highest R_{st} value indicating the largest genetic distances was found between population from Laos and Taiwan. It is clear that the our studied population was found to cluster more closely with populations from the central Thai population ($R_{st} = 0.0154$) as shown in Table 3. As demonstrated in the MDS plot, it showed the close relationship between populations from northern Thailand, central Thailand, Hongkong, Vietnam, and Laos. These clusters form a conspicuous cluster standing far apart from populations from South Korea, Japan, Nepal, and Taiwan as shown in Figure 1.

Table 1 Allele frequency and gene diversity of Y-STRs loci

Alleles	DYS389I	DYS390	DYS389II	DYS19	DYS393	DYS391	DYS439	DYS392	DYS437	DYS438
9						0.0500	0.0278			0.0111
10						0.6167	0.0444	0.0167		0.5944
11					0.0056	0.3278	0.3167	0.1500		0.3278
12	0.3500				0.2611	0.0056	0.3444	0.0222		0.0667
13	0.3722			0.0611	0.3167		0.1944	0.4444	0.0056	
14	0.2722			0.3167	0.3389		0.0667	0.3500	0.7222	
15	0.0056			0.4222	0.0778		0.0056	0.0111	0.2611	
16				0.1722				0.0056	0.0111	
17				0.0278						
21		0.0056								
22		0.0167								
23		0.2444								
24		0.3889								
25		0.3333								
26		0.0111								
27			0.0278							
28			0.2611							
29			0.3167							
30			0.3000							
31			0.0611							
32			0.0167							
33			0.0167							
Gene diversity	0.66855	0.68128	0.74057	0.69112	0.71456	0.51254	0.74018	0.66025	0.41239	0.53765

Table 2 Haplotype pattern and frequency of DYS385a/b locus

Alleles	Frequency	Alleles	Frequency	Alleles	Frequency	Alleles	Frequency	Alleles	Frequency
7/16	0.0056	12/13	0.0056	13/19	0.0778	14/22	0.0056	16/18	0.0167
11/11	0.0056	12/16	0.0111	13/20	0.0222	14/23	0.0056	16/19	0.0444
11/12	0.0500	12/17	0.0167	13/22	0.0056	15/15	0.0222	16/20	0.0056
11/13	0.0278	12/18	0.0111	14/14	0.0111	15/16	0.0333	16/21	0.0056
11/14	0.0222	12/19	0.0333	14/15	0.0056	15/17	0.0111	17/17	0.0056
11/16	0.0056	12/20	0.0167	14/16	0.0056	15/18	0.0278	17/18	0.0056
11/17	0.0056	12/22	0.0056	14/17	0.0278	15/19	0.0500	17/19	0.0056
11/18	0.0056	13/13	0.0111	14/18	0.0333	15/20	0.0222	17/21	0.0056
11/19	0.0167	13/14	0.0111	14/19	0.0222	15/21	0.0333	18/18	0.0111
11/20	0.0056	13/17	0.0722	14/20	0.0056	16/17/20	0.0056	18/19	0.0056
12/12	0.0111	13/18	0.0611	14/21	0.0056	16/17	0.0278	21/21	0.0056

Table 3 The genetic comparison by pairwise R_{st} value between different populations

Populations	Northern Thai	Central Thai	Hongkong	Japan	Laos	Nepal	South Korea	Taiwan	Vietnam
Northern Thai	-								
Central Thai	0.0154	-							
Hongkong	0.0512	0.0198	-						
Japan	0.1120	0.0899	0.0977	-					
Laos	0.0244	0.0492	0.1044	0.1437	-				
Nepal	0.0931	0.0800	0.0661	0.0809	0.1689	-			
South Korea	0.1395	0.1027	0.0970	0.0252	0.1681	0.1035	-		
Taiwan	0.3792	0.3092	0.2218	0.3545	0.5092	0.3345	0.3337	-	
Vietnam	0.0239	0.0118	0.0565	0.0882	0.0278	0.1180	0.1076	0.3905	-

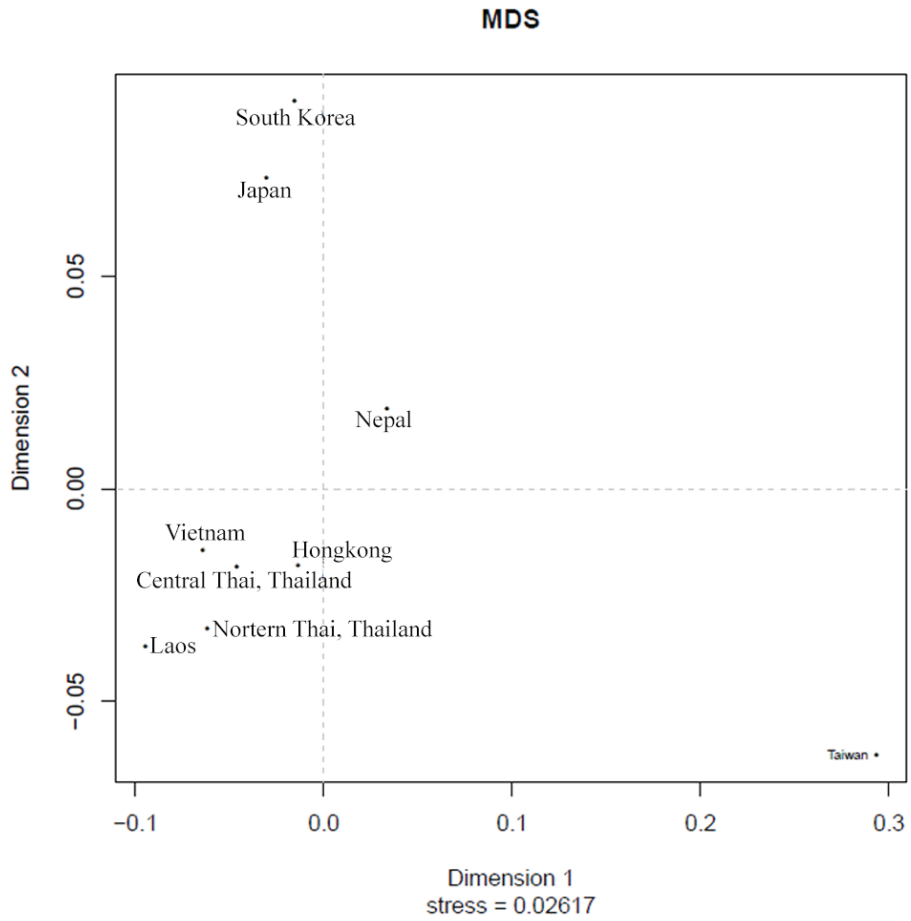

Figure 1 A MDS plot based on R_{st} between northern Thai population and eight reference populations.

Discussion

Our study discovered the genetic structure of localized population in northern region of Thailand in terms of Y-STRs. Such information is of essential importance for forensci casworks. We found that allele 14 at DYS437 locus had the highest frequency which was in concordance with previously report observation in the population from lower northern and central Thailand as well as China[12, 25]. We observed the highest allele frequency on DYS391, followed with DYS549 and DYS437 loci that was concordant with the population from central, west, and southern Thai populations[26]. The value and highest locus of gene diversity among studied Y-STRs loci were similar to the gene diversity of central Thai populations which was previously described[27]. The gene diversity of Y-STRs among different continents and geographic regions was caused from mutation, including random genetic drift. It affected allele frequency in small populations without natural selection resulting in some allele being unable to transmit into the next generation. Additionally, the factors related with social and cultural activity were reported to affect the allele frequency of Y-STRs[28]. It is interesting that one sample of DYS385a/b allele was found to be a tri-allelic pattern (16/17/20). The tri-allelic patterns of DYS385a/b were sometimes observed into 9/13/14, 11/12/13, 11/12/14, 11/14/15, 12/16/17, 13/14/15, 13/14/18, 13/18/19, 13/19/20, 14/16/17, and 15/16/17[29]. Howoever, the allelic pattern of DYS385a/b could be found to have a five-allelic pattern and a six-allelic pattern in the population from China[30]. In the present study, we found that studied population had a close genetic relationship with the population from central Thai, Laos, and Vietname; whereas the difference between the northern Thai population and Taiwanese population was large. It is probable that the patrilineal genetic structure could be from the same ancestral population. Thai population

was reported to have a close genetic relationship with East Asian populations who are native Austroasiatic and Sino-Tibetan speakers[31]. The native Austroasiatic speakers have covered and spread over Asia, India, and Southeast Asia[32]. According to the report of Kutanan *et al.*, 2017, the ancient origin of the Thai and Laotian populations was derived from Austroasiatic groups and some sub-population from Taiwan was separated from the Thai population[33]. The native Sino-Tibetan speakers originated among people in northern China and spread over on the east, west and south of China[34]. The migration of people from Southern China into the northern region of Thailand influenced the relationship between the Thai population and other Southeast Asian populations[35, 36].

Conclusion

We provided the precise database of genetic information of Y-STRs loci in specific northern Thai population to be applied for forensic genetic casework. The limitation of this study is that it does not represent the whole genetic structure of all populations in Thailand. To clear the understanding about the different genetic structure between other populations, the information of population among other regions of Thailand should be collected to compare the genetic polymorphism between individual people.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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Effectiveness of *Pippalyadi* Ointment in Management of *Arsha* (Haemorrhoids)

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Abstract

Arsha is a common anorectal disease so associated with bleeding per anum . It can be compared to Haemorrhoids accoding to modern science. Haemorrhoids are dilated veins in the anal canal. Through all the modern modalities of treatment in haemorrhoids are universally accepted but all have some or other limitations. In *Ayurveda* various measures have been mentioned for the management of Abhyantar *Arsha* , among which *lepa karma* is one of them . Various study have been done on the application of various *lepa* so prepared have been conducted depending upon the sign and symptoms. **Objective-** This study was carried out to find the effectiveness of *Pippalyadi* ointment in Ptients of *Arsha* .**Materials and Methods-** Here the patient of *Arsha* were selected as per criteria and *Pipplyadi* ointment was advised for 15 days twice a day as local application.

Keywords*: Arsha ,Haemorrhoids, Pippalyadi ointment, Lepa.*

Introduction

Ano-rectal disorders are progressively increasing in the society. Out of many of the causes, some important are sedentary lifestyle, irregular and inappropriate diet, prolonged sitting or standing and certain psychological disturbances too. *Arsha* is one among the ano rectal diseases which occurs in *Gudapradesha*, which is a *sadhyopranahara Marma*[1]. In *Arsha* bleeding per anum is the principal symptoms . It is manifested due to multifold factors viz. disturbed lifestyle or daily routines, improper or irregular diet intake, prolonged standing or sitting, faulty habits of defecation etc. which results in derangement of Jatharagni leading to vitiation of Tridosha, mainly Vata Dosha.[2]

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Charaka has enumerated a detail list of dietic ingredients, therapeutic abuses, habits and mechanical factors in two group comprising mainly dietic ingredients leads to accumulation of Mala and second group leads to vitiation of *Dosha* specially *Apana vayu*, which is responsible for physiological function of *Guda*, which plays major role in development of *Arsha. Sushruta* has enumerated the causes leading vitiation of *Dosha* including excessive and improper dietic indulgence, posture which create pressure on *Guda* region e.g. sitting on hard objects, riding on vehicle for long duration, suppression of natural urge of micturition and defecation, which suppress the normal digestive power i.e. *Mandagni*, leads to development of *Arshas*[3].

In modern medical science *Arsha* can be compared with haemorrhoids. Hemorrhoid often described as "varicose veins of the anus and rectum". Hemorrhoid are dilated, tortuous or varicose veins occurring in relation to the anus and originating in the epithelial plexus formed by radicals of the superior, middle and inferior rectal veins. Some of the causes includes straining, diarrhea , constipation, overpurgation, carcinoma rectum,

pregnancy, portal hypertension (rare cause).[4]

Haemorrhoids are divided into two categories- internal and external haemorrhoids. Internal haemorrhoids means it is within the anal canal and internal to the anal orifice and the external haemorrhoid is situated outside the anal orifice and is covered by skin. The two varieties may coexist and the condition is called intero-external haemorrhoids[5].

In this present research work efforts were made to provide the treatment which is non-invasive, easy to implement, effective and were not need any hospitalization. To fulfil the above criteria the present research study were conducted to know effects of *Pipplyadi lepai* in the management of *abhyantar Arsha*. *Acharya Sushruta* has mentioned four treatment modalities to combat this condition i.e *Bhesaja, Kshara, Agni* and *Shastra*. *Bhaishaja chikitsa* is mentioned mainly for *abhyantar Arsha* in their earlier stages when involved *doshas*, symptoms and complications are not severe[6] .

Case Study

Present complaint

A male patient of 30 years came to our OPD with complaints of bleeding per rectum after defecation and generalized weakness since 7 days.

History of present illness

The patient was apparently well before 7 days and suddenly he started suffering from complains of bleeding per rectum after defecation and generalized weakness . Hence he vistited MGACH and RC for his treatment.

Past history

v No H/O - HTN, DM

v H/o Trauma

Personal History

v Appetite - moderate

v Sleep – Reduced sleep

v Bowel - unsatisfactory stools

v Micturition – Normal, 4-5 times a day

Habits

v Non alcoholic

v Regular smoker

Asthavidha Pariksha

v Nadi – 80/min, regular

v Mala – saam, irregular

v Mutra- 4-5 times a day

v Drik – spastha

v Jiwha - Niraam

v Shabda- spastha

v Sparsha – Anushnasheeta

v Akrithi - Madhyam

Examinations

v BP - 130/80 mm of Hg on supine position

v Pulse - 80/min regular

v Temp. – Afebrile

v Respiration rate – 20/min

Systemic Examination

v CNS - conscious oriented

v RS – air entry both side unilateral

v CVS - S1, S2 normal, No added sounds

v P/A - soft & normal

v P/R- 2nd Degree Haemorrhoidal mass was found at 11 O Clock position after proctoscopic examination

Local examination

v Inspection – No such findingds

v Palpation – no anal spasm , no tenderness, no active bleeding

Blood Investigations

v Hb – 11.1gm/dl

v Total WBC Count - 6200/cumm

v RBC count – 3.10 millions/cmm

v PCV/Hematocrit – 30 %

v MCV - 72fL

v Platelet Count – 3.77 Lakh/cmm

v Bleeding Time - 02 min 02 sec

v Clotting Time - 05 min 11sec

v Blood Sugar Random – 104 mg/dl

v HbsAg - Nonreactive

v HIV 1 (Antibodies) - Non Reactive

v HIV 2 (Antibodies) - Non Reactive

Methodology

v The patient was diagnosed with the help of criteria of diagnosis.

v The treatment protocol included application of Pippalyadi ointment twice a day with applicator from per rectum route.

v The total duration of the treatment was 4 weeks with regular follow up at 7th day,15th day and 30th day.

v The patients was advised to take light and easily digestible diet and avoid incompatible foods.

Observation

The observation was done on the basis of subjective as well as objective criteria

Subjective Criteria-

Presence of clinical signs and symptoms of *abhyantar Arsha.*

1.Constipation

1) Subjective Criteri

Sr.no	Grade	Explanation
1	0	Absent
2	1	Present

2. Bleeding per rectum

Sr.no	Grade	Explanation
1	0	Absent
2	1	Present

Objective criteria

Grade 1	No prolapsed. Just prominent blood vessels.
Grade 2	Prolapsed upon bearing down but spontaneously reduce.
Grade 3	Prolapsed upon bearing down and require manual reduction.
Grade 4	Prolapsed and cannot be manually reduced.

Result

On the basis of above said criteria and the symptoms so mentioned the patient have shown better improvement.

Discussion

v *Pipplyadi Ointment* contains *Pippali* having *Laghu, Snigdha, Tikshna guna* and *madhura, katu rasa* which is acts as *Vatakaphashamaka*.

v It is also having properties like Antibacterial, Antifungal, Anthelmatic.

v *Saindhav lavan, Kushta* is having properties like *Kaphavatajita, Raktashodhaka.*

v *Shirish* acts as *Shothahara, Tridoshahara*.

v Shirish and *Snuhi* are having properties like *kashay rasa* and astringent by nature immediately helps in stopping bleeding due to its *Rakta Stambhana Karma.*

v So due to combine effects of all drug bleeding, inflammation , pain is stopped due to this Ointment which was act locally to relive the pain and bleeding in *arsha*.

Conclusion

The above study concluded in showing effective results in management of Arsha with no adverse effect.

I.E.C Permission taken from committee - DMIMS(DU)/IEC/2017-18/7252

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Ayurvedic Protocol on Effectiveness of *Pippalyadi* and *Suranadi* Ointment in Management of *Arsha* (Haemorrhoids)

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Abstract

Background- Ano-rectal disorders are progressively increasing in the society. Out of many of the causes, some important are sedentary lifestyle, irregular and inappropriate diet, prolonged sitting or standing and certain psychological disturbances too. *Arsha* is one among the ano rectal diseases which occurs in *Gudapradesha*, which is a *sadhyopranaharaMarma*[1]. In *Arsha* bleeding per anum is the principal symptoms .Arsha is a gift of modern diets and busy lifestyles and many people are suffering from some sort of Anorectal disorder, it may be simple constipation to complex carcinoma, in which prominent disorder is Arsha. It is manifested due to multifold factors viz. disturbed lifestyle or daily routines, improper or irregular diet intake, prolonged standing or sitting, faulty habits of defecation etc. which results in derangement of Jatharagni leading to vitiation of Tridosha, mainly VataDosha.

Keywords- *Arsha, Gudapradesha, sadhyopranaharaMarma.*

Introduction

Ano-rectal disorders are progressively increasing in the society. Out of many of the causes, some important are sedentary lifestyle, irregular and inappropriate diet, prolonged sitting or standing and certain psychological disturbances too. *Arsha* is one among the ano rectal diseases which occurs in *Gudapradesha*, which is a *sadhyopranaharaMarma*[1]. In *Arsha* bleeding per anum is the principal symptoms .Arsha is a gift of modern diets and busy lifestyles and many people are suffering from some sort of Anorectal disorder, it may be simple constipation to complex carcinoma, in which prominent disorder is Arsha. It is manifested due to multifold factors viz. disturbed lifestyle or daily routines, improper or irregular diet intake, prolonged standing or sitting, faulty habits of defecation etc. which results in derangement of Jatharagni leading to vitiation of Tridosha, mainly VataDosha. These vitiated Doshas get localized in GudaVali and PradhanaDhamani which further vitiates Twak, Mansa, and MedaDhatus due to Annavahashrotodushti leads to development of Arsha.

In modern medical science *Arsha* can be compared with haemorrhoids. Hemorrhoid often described as "varicose veins of the anus and rectum". Hemorrhoid are dilated, tortuous or varicose veins occurring in relation to the anus and originating in the epithelial plexus formed by radicals of the superior, middle and inferior rectal veins. While other scientist considered it as displacement of anal cushions. Haemorrhoids are divided into two categories-internal and external haemorrhoids. Internal haemorrhoids means it is within the anal canal and internal to the anal orifice and the external haemorrhoid is situated outside the anal orifice and is covered by skin. The two varieties may coexist and the condition is called intero-external haemorrhoids[3] .

In the management of Haemorrhoids, the procedures, which are in practice at present in modern surgery, are laser surgery, rubber band ligation, sclerotherapy, bipolar diathermy, infrared photocoagulation, cryosurgery, infra-red coagulation and hemorrhoidectomy, but all procedures have their limitations.[4]Though all the

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modern modalities of treatment in haemorrhoids are universally acceptable but all have some limitations. In ancient *Ayurvedic* compendia various palliative measures have been mentioned in the management of *AbhyantarArsha ,lepa karma is* one among them.

Need of Study-

Though all the modern modalities of treatment in hemorrhoids are universally acceptable but all have some limitations To find out a suitable solution there is need to finf noninvasive treatment for management. In this present research work efforts were made to provide the treatment which is non- invasive, easy to implement, effective and were not need any hospitalization. To fulfil the above criteria the present research study was conducted to compare effects of *Pipplyadilepa*and *Suranadilepa*mentioned in *BhaishajyaRatnavali*in the management of *abhyantarArsha.*

Aim-

Evaluate the efficacy of *Pipplayadi Ointment* over *Suranadi Ointment* in management of

Arsha.

Objective

· To evaluate the efficacy of *Pipplyadi*Ointment in management of *Arsha*.

· To evaluate the efficacy of *Suranadi*Ointment in management of *Arsha*.

· To compare the effects of *Pipplyadi*Ointment and *Suranadi*Ointment in *management* of*Arsha*

Material-

The raw materials will be collected from reliable source and will be authenticated from Department of *Dravayguna* and analysed in Pharmaceutical Laboratory, SawangiWardha.

Method-

*Pippaly*adi ointment was prepared with collection of drugs including *Pippali, Saindhav, lavan,Kushta, Shirish*and*Snuhi* are were made into a fine powder. *Til tail* is taken into clean stainless vessel and placed over mild heat until it starts foam appearing. Then beewax is added to 1/5th of til tail, when all the wax is completely melting in oil, it is filtered and kept in another vessel. It was used as base for preparation of ointment called as *siktha tail*. In this *tail* fine powder of all above drugs were added and stirred. This mixture then attains thicker consistency as wax cool down and made into a soft paste.

Suranadi ointment was prepared with collection of drugs including. *Suran, Haridra, Chitrakmool, Sudhha Tankan* and *Guda*are made into a fine powder. *Til tail* is taken into clean stainless vessel and placed over mild heat until it starts foam appearing. Then beewax is added to 1/5th of *til tail*, when all the wax is completely melting in oil, it is filtered and kept in another vessel. It was used as base for preparation of ointment called as *siktha tail*. In this*Siktha tail* fine powder of all above drugs along with *Guda* were added and stirred. This mixture then attains thicker consistency as wax cool down and made into a soft paste.

Both the ointments will be prepared in *Rasa Shala*of MGACHRC under supervision.

PLACE- OPD and IPD of *ShalyaTantra*M.G.A.C.H and R.C

SAMPLE SIZE- 30, 15 for *pippalyadi ointment* and 15 *for**suranadi ointment*

SAMPLE SELECTION TECHNIQUES-

GROUPS – 2 groups with 15 patient each

Group A- *Pippalyadi*ointment

Group B- *Suranadi*ointment

STUDY DESIGN- Double arm

STUDY TYPE- Interventional study

Inclusion Criteria-

§ Diagnosed without any major systemic disorder's cases of first and second degree internal haemorrhoids.

§ Age group of 20 to 50 years, irrespective of their sex, occupation, & economic

Exclusion Criteria-

§ Haemorrhoids that are thrombosed , third

degree and fourth degree.

§ Haemorrhoids associated with fissure in ano, fistula in ano, perianal abscess, rectal polyps, rectal prolapse and rectal CA.

§ Patients having tuberculosis, AIDS & Hepatitis, Ulcerative colitis, Crohn's disease & Pregnant women

INVESTIGATIONS

Routine hemogram

Blood sugar

Routine and microscopic examination of urine and stools

Dosage-

Sufficient quantity of ointment will be taken for local application twice a day during the treatment period.

Any other departments expertise required- No

7. Observation-

A]Subjective criteria-

Constipation

Bleeding per Rectum

B]Objective criteria- Healing of *Arsha*

8. Assessment Criteria

A] Subjective Criteria-

1.Constipation

1) Subjective Criteria

1Constipation

1) Subjective Criteria

Sr.no	Grade	Explanation
1	0	Absent
2	1	Present

2. Bleeding per rectum

Sr.no	Grade	Explanation
1	0	Absent
2	1	Present

B] Objective Criteia

Grade 1	No prolapsed. Just prominent blood vessels.
Grade 2	Prolapsed upon bearing down but spontaneously reduce.
Grade 3	Prolapsed upon bearing down and require manual reduction.
Grade 4	Prolapsed and cannot be manually reduced.

Data management: Principal investigator will do coding of data.

Ethics and dissemination: Permission for research has been taken from Institutional Ethical Committee .

Consent or assent: Written informed consent will be obtained from the patient.

Dissemination policy: For future research results will be disseminated and research will be published in reputed journal

Informed consent materials: All the research related document and consent form will be given to the patients.

Discussion: In *Ayurveda, pippali* is used for the treatment of *vrana, gulma, bhagnasandhana, grahani, arsha, gudaroga*[7]. *Rasapanchak* of *pippali* is *Rasa- Katu, tikta, kashaya, Virya-Ushan, Vipaka-Katu, Karma -Vata-Kaphahara*. *pippali* has anti-inflammatory activity, analgesic, antifungal activity and anti-ulcer activity It is known for *Vranaropak* properties.In modern medical sciences Haemorriodectomy is ideal procedure but after procedure pain bleeding are common complication To sort out these problem we need treatment which is easily applicable and non surgical.

Conclusion-After the study is completed suitable conclusion will be withdrawn on the basis of results.

Ethical Clearance- Taken from Institutional Ethical committee

Conflict of Interest: NIL

Source of Funding- Self

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Risk Factors of Suicides: A Retrospective Study

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Abstract

Introduction: "Suicide" is a fatal act that represents the person's wish to die. The "psychological autopsy" is a "procedure for investigating a person's death by reconstructing what the person thought, felt and did preceding his or her death. The present study aimed to find out the profile of those completing suicide with regard to various factors to know the circumstances leading to suicide. **Materials and method:** Retrospective diagnoses of psychiatric disorders were made by questions on 120 deceased cases of suicides, over the ages of 15 years, both urban and rural, which were sent for medical autopsy. All these data was collected by interviewing the close relatives and friends of the patient. **Results:** Most of people involved in suicide were males belonging to age group 16-30 years while very few of them were above 60 years. Most of them were illiterates, working as daily labourers or self employed. Among women most of them were housewives. They were from low economic background, from the rural areas and married. The most common cause of suicides was financial loss, stress and illness. **Conclusion:** Proper counselling and monitoring the intended victims may help to reduce the number of deaths. Also the families need to be counselled regarding the importance of family values and integrity among the family members.

Keywords: *Suicides, risk factors , burns, psychological autopsy, hanging*

Introduction

An act which result in a person taking his own life is called suicide. However, thinking about suicide and acting on it are not the same. Some of the people may thing of committing suicide, but may never act on it , while some may plan about it for days and weeks and sometimes even for years, before actually committing the act. This is called premeditation. Some may take their lives on impulse without a previous plan[1]. It is estimated that every year, over 1 lakh people commit suicide in India[2]. A population rise of 15% was seen between 2003 and 2013 in our country and a rise of 5.7% was seen among suicides[2].

There may be various reason for suicides, such as psychological, sociological biological, genetic and twin factors. Sociological factors are further classified into altruistic, egoistic, and [5]anomic[3]. Psychological factors among the suicides may be due to aggression which is turned inwards against introjection, ambivalently cathected love object. Diminished serotonin levels, may play a role in the suicidal tendencies and constitute the biological factor[1-4].

After the death of the person, the "procedure for investigating a person's death by reconstructing what the person thought, felt and did preceding his or her death", is called "psychological autopsy"[5]. This is one of the major tool for investigating the cause of a suicidal death.

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In the strategy of reconstruction of a suicidal death, there are four major constructs in the psychological autopsy. They are precipitants or stressors, lethality, motivation and intention. It has been established in various studies that psychological autopsy in suicidal deaths is a very useful tool to confirm the cause of death thereby being an instrument in various clinical assessment and research[5].

The present study was therefor aimed to assess the profile of those completing suicide with regard to various circumstances leading to suicide.

Materials and Methods

This descriptive cross sectional study was conducted at OSMANIA GENERAL HOSPITAL MORTUARY over a period of 1year.

120 deceased cases of suicides, over the ages of 15 years, both urban and rural, which were sent for medical autopsy were included into the study. Retrospective diagnoses of psychiatric disorders were made by questions, which presumably fit into the diagnostic criteria for the particular disorders. The families, relatives and friends of the deceased were assessed at the hospital and also at their homes.

Demographic details of the deceased such as age, sex, marital status, education, income, family history were taken. Various psycho-social variables associated with suicide time of death, substance abuse, psychiatric illness, chronic illness, change of behavior before suicide, suicide note, method used, and previous attempts, treatment details if any were also noted. All these data was collected by interviewing the close relatives and friends of the patient.

Results

Of the 120 cases, 78 cases were males (65%) and 42 were females (35%) (Fig: 1).

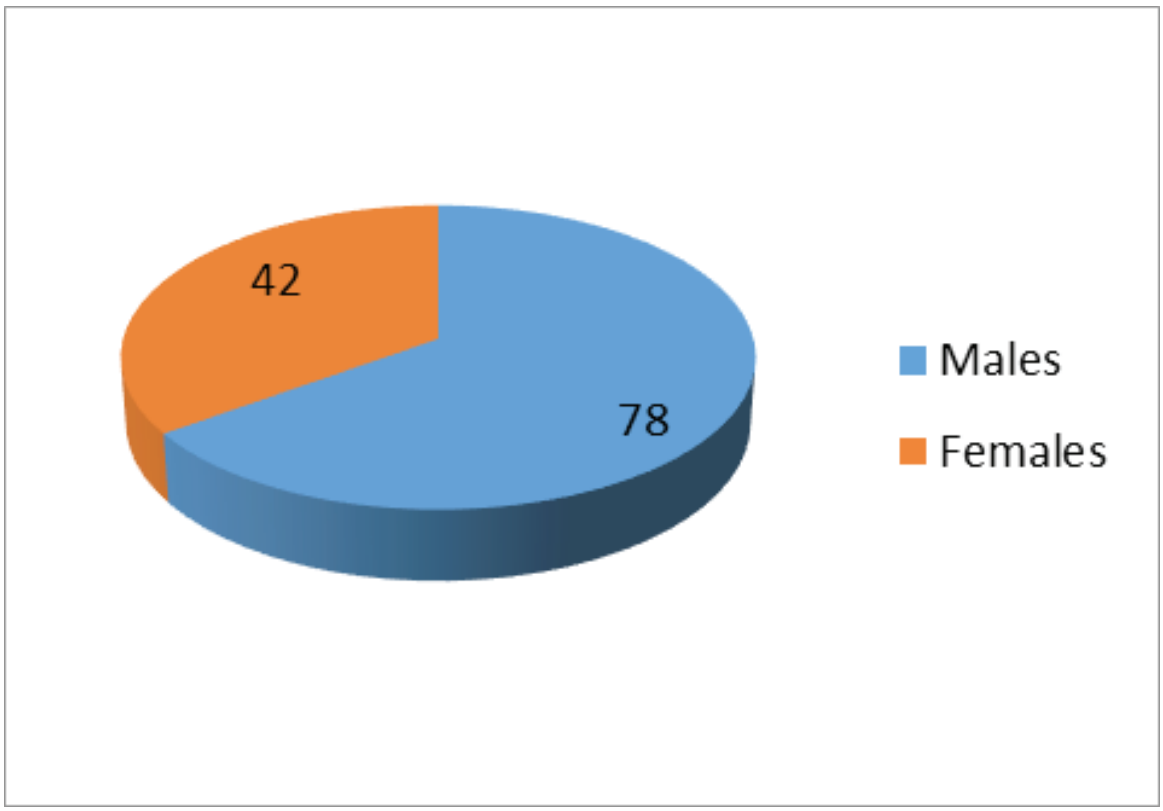
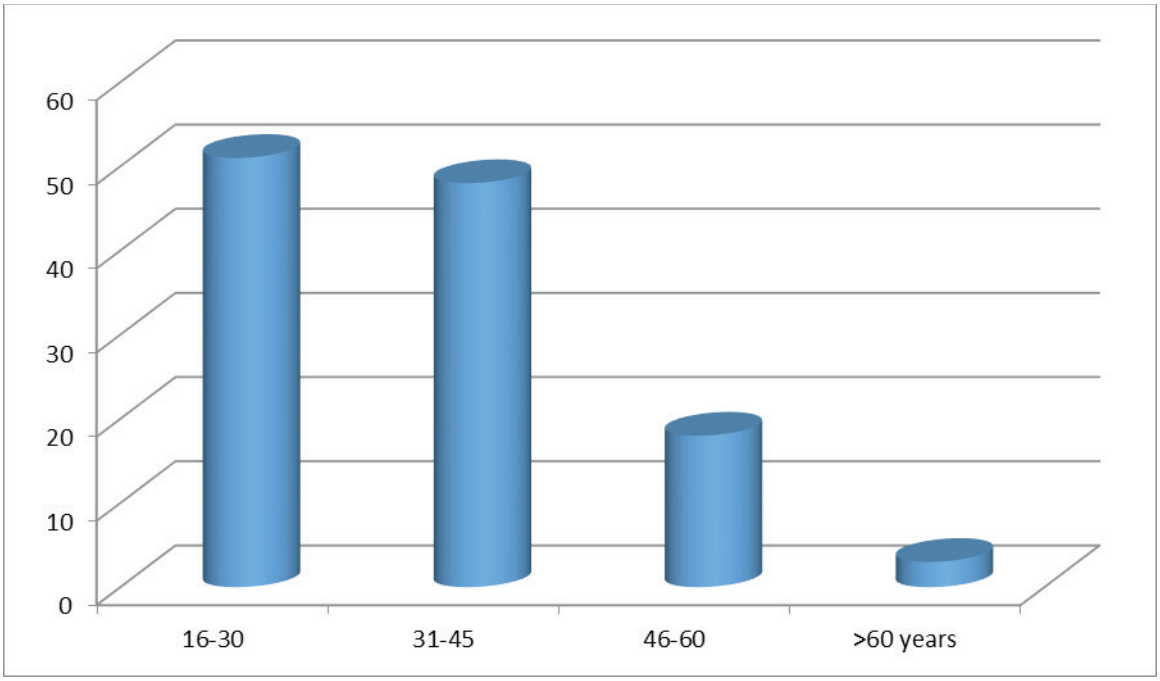


Fig: 1 : Sexwise distribution of suicide victims

Most people who committed suicide belonged to age group 16-30 years with 51 of them (42.5%), 48 (40%) were in the 31-45yrs age group, 18 (15%) were between 46 and 60 years and 3 (2.5%) belonged to above 60 years of age (Fig:2).

Fig: 2 : Agewise distribution of the suicide victims



In most of the cases with completed suicides, the patient was illiterate (36in number, 30%), 30 of them had primary education (25%) while 29 had secondary education (24.2%). 24 (20%) had done their graduation and 1 person (0.8%) had completed post graduation. Most of the patients especially the illiterate ones (30%) were daily labourers and 30 (25%) were self employed. 19 (15.8%) were government employees and 11 (9.2%) were private employees. 24 920%) of them were housewives. 66 cases (55%) were from the lower economic status, while only 2 (1.7%) were flom the upper class. 52 (43.3%) were from the middle class. 69 (57.5%) were married and 30 (25%) were unmarried. 21 (17.5%) were single either because they were separated or were divorced (Table: 1).

Table: 1 : Demographic detail of the patients

Parameter	Number	Percentage
Education		
Illiterate	36	30
Primary education	30	25
Secondary education	29	24.2
Graduation	24	20
Post Graduation	1	0.8
Work		
Daily labourers	36	30
Self Employed	30	25
Govt Employee	19	15.8
Pvt Employee	11	9.2
House wives	24	20
Socioeconomic status		
Lower	66	55
Lower middle	42	35
Upper middle	10	8.3
Upper	2	1.7

Cont... **Table: 1 : Demographic detail of the patients**

Marital Status		
Married	69	57.5
Unmarried	30	25
Single/Divorced	21	17.5
Background		
Urban	72	60
Rural	48	40
Type of family		
Nuclear	102	85
Joint	18	15
Family History		
Yes	12	10
No	108	90

Majority of the suicides (52.5%) occurred in the night, when it is quite around. Most of them i.e 93 (77.5%) of them, did not leave a suicide note. The most common method for suicides was hanging, followed by poisoning, with poisoning by drinking pesticides being most common (Table: 2).

Table: 2 : Details of Suicides

Detail	Number	Percentage
Time of suicide		
Night	63	52.5
Daytime	57	47.5
Suicide note		
Yes	27	22.5
No	93	77.5
Method of suicide		
Hanging	48	40
Poisoning	36	30
Self immolation	18	15
Drowning	10	8.3
Falling from height	8	6.7
Psychiatric illness		
History	15	12.5
No History	105	87.5

Discussion

It was observed in the present study that most of the people who committed suicides were in the 15-29 year age group, while the least number were above 60 years of age. A similar result was seen in a study by Gururaj et al in Gujarat, who also found 115-29 years to be the most predominant age group for suicides[6]. Patton et al, reported that over 2.6 million deaths occurred among the 10-24 year age group[7], which was similar to reports by NCRB[2]. The most probable cause for the lower rates among the elderly was the fact that they were well respected persons who were well integrated into the society.

The most common factors which lead to suicides at a very young age is emotional instability, financial troubles, not performing well in education and career. In women the most common causes were sex abuse, child birth and post partum depression, early marriage, dowry etc.

In the present study, majority of the persons committing suicides were males, and the male to female ratio was 2:1. This was similar to results by Patel et al, wherein, 15-29 years age group was the most predominant age and the male to female ratio was 3:1, slightly higher than our study[8]. A study y Gururaj et al showed similar results[6]. In contrast, in a study by Bannerjee et al showed suicides among women were more common than in the men and most of the victims were below 25 years of age[9]. In case of suicides by men more lethal modes was common and the plan was acted upon more meticulously in order to avoid detection. In contrast, women commonly use less lethal modes, and are more impulsive, less well planned, and more likely to be found and rescued.

In the present study, very few people studied up to graduation and above. Most of the people had no education or had primary educational qualification and comprised of 55%. Less than 1% of the people had an educational qualification up to the post graduate level. Similar results were reported in the NCRB data with 25.3% of the victims educated upto the primary level[2].

Most of the persons who committed suicides in our study were labourers or self employed with financial instability. House wives accounted for around 20% of the cases. The next largest group, comprising of 11.9% of the cases were farmers and people involved in agriculture. Most of the victims, around 90%, belonged to the lower and lower middle socioeconomic class. Our study was corroborated by other studies by Banerjee et al, Patel et al and Vijaykumar et al, where the lower middle class and middle class were more prone to suicides[7-9].86.8% and 79.72% of the victims who committed suicides were from the lower class in studies by Nilamadhab Kar and Mohanty et al[10,11].

Most of the victims in our study were married (57.5%). Similar was the case in a study by Mohanty et al who reported a huge number of 76.04 % of the victims to be married. So were 56.2% of the victims in a study by Srivatsava et al[12] Sharma et al[13] and Shukla et al[14].

Very few people were from joint family who were victims of suicide and most of victims are from nuclear family (85%). This was the case in most of the studies from India where the majority of suicide victims were from nuclear families[12,15]. Social integration and family support seems to play a major role in preventing suicides[15-18].

The most common cause of suicides was financial loss followed by 25% having unknown reasons. An NRDB report in 2013 also reported similar findings[2]. Unemployment was another common cause according to a study by Srivatsava et al[12]. Past stressful events also was a cause according to other studies[13-18].

Most of the suicides in our study occurred during the night which was in accordance to studies by Gunnell et al and Chavan et al. This could be as the person may feel more alone at night, when other a re asleep. Moreover, there may not be anyone around to stop him from committing the act[19,20].

Conclusion

More number of men compared to women, in the younger age group tend to commit suicides. The reason most often is marital discord, less interpersonal relationships, financial loss or illness. Most of the men are from rural background, less education and from nuclear families. Family history of suicides had no significant role in either committing or attempting suicides. Proper counselling and monitoring the intended victims may

help to reduce the number of deaths. Also the families need to be counselled regarding the importance of family values and integrity among the family members.

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Source of Funding- self

Conflict of Interest – Nil

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Awareness about Acts and Amendments in Bills Related to Sexual Offences amongst Medical Students and Interns In A Private Medical College and Hospital at Chennai

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Abstract

Women and Children constitute one of the vulnerable sections of our society. Proactive scheduling and stipulation of services to them is an asset for the future of our country. Their care and protection has emerged as a precedence for the Government .The objective of the study was to assess and analyze the awareness about the amendments in acts and introduction of new bills related to sexual offences amongst Medical students and Interns in teaching medical colleges,hospitals at Chennai. 300 participants took part in this cross sectional study which revealed that almost one fifth of them (20%) felt that only children below 16 years came under the POCSO Act 2012. Nearly 55% of the participants were unaware of the exact features of the POCSO Act 2012. Only one fourth were aware that as per the new amendment in the proposed MTP bill regarding requirement of only a single RMP's opinion for termination of pregnancy up to 20 weeks, while 56% of the participants were unaware of the minimum punishment for the rape of a girl under 16 years.

The study result implies the need to have more educational sessions for the students regarding the amendments in the acts.

Keywords: *Awareness, Amendment,Sexual offence, POCSO Act, MTP bill, Rape.*

Introduction

Child Sexual Abuse is a heinous crime which is being increasingly reported in India as well as globally. Indian law makers took a step ahead and espoused the Protection of Children from Sexual Offences Act (POCSO) in 2012. It is a comprehensive law on sexual abuse, which expands the scope and range of forms of sexual offences and makes reporting of abuse mandatory. It defines guidelines for the investigation in a friendly manner and examination of victims .[1] The protection of children from sexual offences act (POCSO ACT 2012) is an act to protect children (below 18 years, Gender neutral) from offences of sexual assault, sexual harassment, pornography and recommends establishment for special courts for speedy trial of such offences. The Act provides for rigorous punishment to the offenders. [2] A study conducted by World Vision India shows that in Tamil Nadu ,only 13.7% of the children were aware of POCSO Act(2012).

The medical termination of pregnancy (MTP) act was founded in 1972 .Medical Termination of Pregnancy (MTP) represents an important aspect of women's reproductive health and right. In Women's access to safe abortion services is essential to safeguard their health and is one of the important components of Reproductive and Child Health Programme.[3] In India, an ICMR study documented that the rates of safe (legal) and unsafe (illegal) abortion were 6.1 and 13.5

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per 1000 pregnancies, respectively.[4]Rape, however it is defined falls under the wider umbrella term of 'sexual violence', which also includes acts such as flashing, sexual harassment, forcing someone to watch pornography, sexual bullying and other forms of non-consensual sexual contact.[5] On an average, 88 rapes take place every day in India, according to the National Crime Records Bureau (NCRB) data for 2019.

However, the conviction rate is as low as 27.8%. [6] These statistics show that despite amendments being made in the various acts related to sexual offences, a vast majority of the population was still unaware of the provisions of the laws. Keeping that in mind, this study was conducted to ensure that the medical students and interns of private medical college in Chennai were aware of the various laws , acts and proposed amendments in bills related to sexual offences.

Methodology

This is a questionnaire-based study conducted amongst the students and Interns of private medical colleges in Chennai. A pretested semi structured questionnaire was distributed amongst the 300 randomly chosen participants after explaining about the study and obtaining consent to be a part of this study. Confidentiality of the volunteers was ensured. The study was conducted between April 2020 to September 2020. The data obtained was statistically analyzed with appropriate statistical tests using SPSS software version 24.

Observation and Results

A total of 300 students in medical colleges in Chennai were included in the study .The participants from 1st year to internship were 66, 112, 55, 49 and 18 respectively. [Fig 1]

This study had a female preponderance with 55% participation (165 females) with F: M ratio being 1.22:1.

It was found that 78% of the study population was aware that statutory rape refers to the rape of a person below the age of consent. 39.9% of them neither agree nor disagree that a husband who has sexual intercourse with his own wife, who is living separately while divorce proceedings are pending in the court, can be punished. The statement that as per section 375 IPC, rape refers to only vaginal penetration by penis was considered to be false by 66.5% of respondents. 96.5% felt it is true that free treatment must be provided to all the survivors of sexual abuse. 57% of the people believed that anyone below the age of 18 should be considered a child, as per the POCSO act 2012. 44.5% of the people were aware that special courts for a speedy trial was a feature of the POCSO act 2012. 69% of the participants felt that transsexualism doesn't come under Child Sexual Abuse. 82% of the respondents knew that the age of consent for sexual intercourse for females has increased to 18 years. 44% of the participants knew that the minimum punishment for the rape of a girl under 16 years is 20 years. 32.7% of the people thought that the minimum punishment, if found guilty of using children for pornographic purposes is 10 years while 24.1% of them felt that it was life time imprisonment. 86.4% felt that it was true that storage of child pornographic material for commercial purposes can be penalised with a minimum imprisonment of 3 years.80% of the people know that the MTP act came into effect in 1971. 37.5% of them strongly agreed that a woman should always have the right to abortion in case of an unwanted pregnancy. 24% of them believed that a woman needs to have her spouse's approval to have an abortion. 27% of them disagree that the consent of the father is a must in all cases of abortion. 76% of them were aware that for termination of pregnancy up to 20 weeks of gestation, the opinion of 2 RMP's was required. 43% of the people stated that the MTP (amendment) bill 2020 sought to increase the upper limit for termination of a pregnancy from 20 weeks to 24 weeks.66.5% of the people felt that the statement was false regarding MTP in case of contraceptive failure being allowed only for married couples.

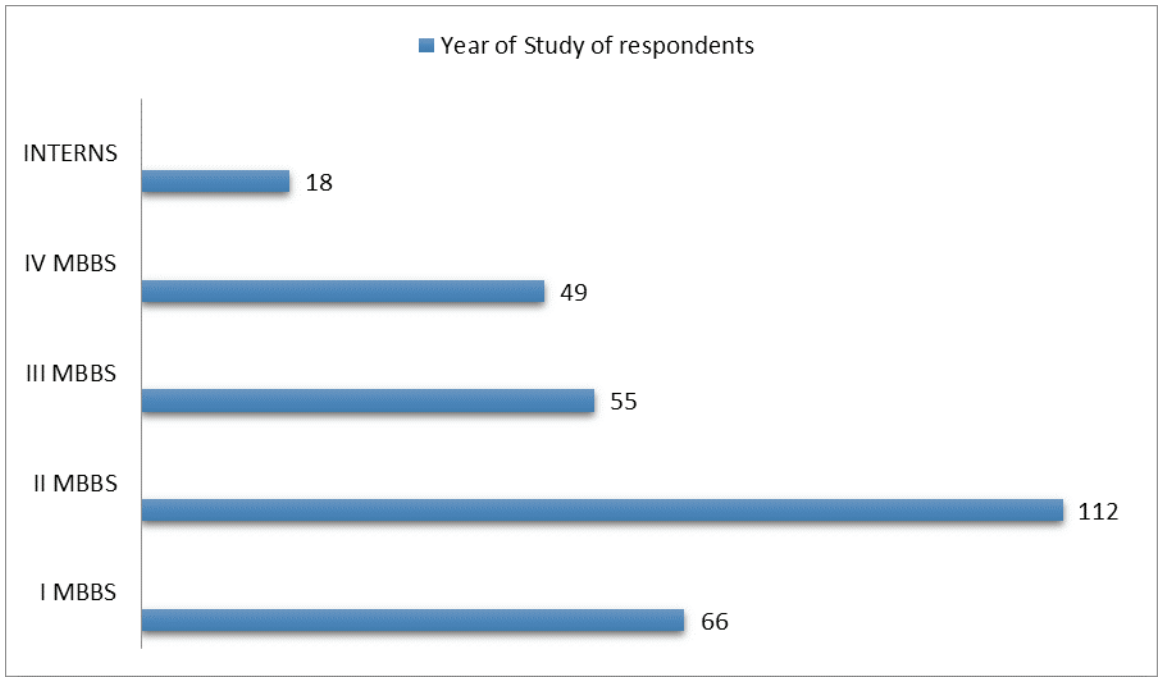

Fig 1: Year of Study of respondents

Discussion

Almost four fifth of the respondents in this study were aware about the MTP act 1971 and over one third of them strongly agreed that a woman should always have the right to abortion in case of an unwanted pregnancy and not require spousal agreement in it. A little over three fourth of the respondents were aware that for termination of pregnancy up to 20 weeks of gestation, the opinion of 2 RMP's was required. Over half of the study population was not aware that the MTP (amendment) bill 2020 sought to increase the upper limit for termination of a pregnancy from 20 weeks to 24 weeks. The Abortion Assessment Project, a multicentre survey, estimates that of the 6.4 million abortions performed annually, 3.6 million or 56% are unsafe. Deaths from unsafe abortion are estimated to constitute 10%–13% of the total maternal deaths in India[7]. These deaths can be partly attributed to lack of awareness of the legality of abortion. These statistics show that awareness about the legalities of medical termination wasn't sufficient enough even though there were several amendments made to the MTP act. [8] over half of the respondents (55%) were unaware of the exact features of the POCSO act 2012. This finding is almost similar to a study conducted in Chennai[2] but lower in comparison with a study conducted by a group of medical students in medical colleges in Maharashtra. Their study showed that 89% of medicos did not know the prevalence of CSA in India and only 20.4% were aware about the special act to deal with children suffering from CSA.[9] In India nearly 1 in every 3 rape victims are below 18 years of age. As per a study conducted by Maplecroft, India ranks as the 7th worst in terms of sexual crimes against minors[10]. These statistics indicate the need for thorough education of the people regarding the laws pertaining to sexual offences and the amendments proposed to be made to it as well as the punishments in case any of these laws are violated. The spread of awareness has to start at the grass root level, where the people are most vulnerable. Although the results of the study were above average in comparison to other studies conducted elsewhere, there is always scope for improvement.

Conclusion

The study showed that the awareness about amendments proposed in acts related to sexual offences amongst medical students and Interns were not satisfactory and can be further improved. Hence more sensitization programs are the need of the hour.

Limitation :

The limitations of the study comprise only a limited

population was studied and the results may not be representative of all the medical students across the city. Further studies with more volunteers are required to attain statistical significance.

Conflict of Interest : Nil

Ethical Clearance: Obtained (SMC/IEC/2020/03/288)

Source of Funding: Self

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A Study on the Histopathological Changes in Cases of Pesticide Poisoning and Proposed Diagnostic Algorithm

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Abstract

Pesticide poisoning in developing world is a global public health problem. Incidences of poisoning are increasing with socio-economic hurdles. Often reporting and chemical examination of samples collected from viscera are difficult to precisely diagnose cases and therefore extensive studies on histopathological corroborations of vital organs such as stomach, liver and kidney is necessary along with socio-demographic data. The common autopsy findings were cyanosis in the lips, in the fingertips and nose. Histological findings in stomach revealed congestion, erosion of muscularis mucosa, submucosal petechial haemorrhages and necrosis. Liver shows congestion, sinusoidal dilution, degeneration of hepatocytes and haemorrhages. Kidney shows tubular necrosis and glomerular congestion. However, history of poisoning along with autopsy and histological findings are considered as strong evidence for acute organophosphorous poisoning.

Keywords: *autopsy, histopathology, pesticide poisoning*

Introduction

Deaths due to pesticide poisoning are an important public health issue. It is a concern to the forensic pathologist as consumption of pesticides and poisoning is a common issue in emergency admission at hospitals with a high prevalence for mortality. This is global problem with maximum cases reported from developing countries like India. Agricultural pesticide poisoning is a global problem with at least 250,000- 370,000 people getting affected each year [1].

In India, due to its easy availability, pesticide poisoning is the most common. Poisoning can occur due to suicidal or accidental ingestion. Suicidal pesticide poisoning is the most common method of self destruction. Accidental as well as homicidal poisoning by pesticide is also reported.

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According to the Food and Agriculture Organization of the United Nations (FAO), pesticides are defined as any substance or mixture of substances intended in preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs or which may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant-growth regulator, defoliant, desiccant or fruit- thinning agent or agent for preventing the premature fall of fruit and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Pesticides are considered as either biodegradable which may be broken down by microbes or persistent pesticides which usually take years to be broken down and therefore accumulate in the food chain viz. DDT (dichloro-diphenyl-trichloroethane).

Organophosphates affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter. Most organophosphates are insecticides. They were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Some are very poisonous. However, they usually are not persistent in the environment.

There is a pressing need for meticulous study on autopsy findings in pesticide poisoning cases as common changes are often non-specific and depends on chemical examination of viscera for further diagnosis. In relation to the factors like decomposition, transport, non-availability of tests for all pesticides, postmortem alteration in chemical nature of poison etc., it is necessary to study the impact of poisoning on vital organs both for morphological and histopathological changes in order to establish diagnosis.

The aim of the present study is:

· To investigate the socio-demographic profile of the cases of death due to pesticide poisoning examined at autopsy in a tertiary care hospital morgue.

· To analyze the pattern of various poisoning deaths

· To study the morphological and histopathological changes in stomach ,liver and kidney in death due pesticide poisoning

· To study the risk factors with special reference to socio-demographic determinants of suicide by pesticide poisoning

Materials and Method

In the present study all fatal cases of pesticide poisoning either admitted to the Emergency department of Burdwan Medical College & Hospital, or brought dead in the Police Morgue, **in** Department of Forensic & State Medicine Burdwan Medical College & Hospital. Burdwan, West Bengal, during February 2015 to January 2016 were evaluated. This cross sectional study is done which included total 268 cases of fatal pesticide poisoning.

Some pesticides with organophosphorous compounds have characteristics petroleum have a characteristic petroleum or garlic like odour, which is helpful in establishing diagnosis. Further toxicological evaluation report of gastric content helped in confirming the type of pesticides. The age, sex, cause of ingestion, compound involved, time elapsed between ingestion and admission to the hospital, duration of hospital stay are recorded. A descriptive study was designed to explore the socio-economic profile of the fatal cases of poisoning.

Poisoning cases with decomposed bodies were excluded from the study. History of poisoning, manner of death, type of exposure was recorded from autopsy findings in standard formats (PM report vide West Bengal form number 5372). Meticulous autopsy findings of all the cases were noted and analyzed along with histopathology of the vital organs namely stomach, liver and kidney.

Specimens collected for the study were stored in buckets with 10% buffered neutral formalin for the process of fixation. Histological slides were prepared according to standard laboratory protocols for Hematoxylin and eosin counter staining protocols. DPX mounted permanent slides were observed under compound digital microscope. Data was analyzed using 'SPSS software for WINDOWS' Descriptive statistics was applied wherein the mean, mode median and standard deviation and range of the values were noted. For inferential statistics hypothesis was tested using students-t test. Association was tested using non parametric tests like Chi square test. P value of less than .05 was considered statically significant.

The study was conducted after receiving approval from the clinical ethical committee, Burdwan Medical College & Hospital.

Results

In the present investigation of total 2423 unnatural deaths autopsied during the study period 351 cases ware due to poisoning (14.38%). Out of those poisoning cases 268 were due to pesticide amounting to 76% of poisoning deaths. It was found in the present study that pesticide poisoning constituted 9.04% of all unnatural deaths (268 out of 2423).

a) **Socio-demographic profile of the study**:

As per distribution for age and occupation; it was found that out of 268 poisoning cases (n=268) 67.2% were male and among them 95.9% were in the age group of 20-29 years. Amongst the occupational distribution, agriculture (96.3%) is more susceptible.

As per distribution for sex and locality (n=268), 69.8% were from rural area. As per the distribution of sex and marital status of the studied population, 174 (96.7%) were married male.

As per the distribution of sex and educational status, out of 268; 153 (85%) were male and have completed secondary school education with females 76 (86.4%) as shown in table1. On contrast educational status below secondary education and for graduates the incidences are less.

As per distribution of study subjects according to frequency of admission in hospital, 81% of study population admitted in hospital and rests (19%) were not as shown in table2. According to the time of incidence most of the incidents (68.6%) occurred between 12 PM to 6 AM.

As per distribution of study subjects for the type of poison (n=268), it has been found that most of the cases (86.2%) were of organo phosphorous poisoning.

b) **Post-mortem histological findings**:

i) **Stomach**

The post-mortem histological findings as shown in figure 1 and 2 of the study subjects, the stomach haemorrhage were present among 93.65 % and among them 86.5% were from the cases of organophosphorus poisoning. Stomach congestion with mononuclear infiltration was present among 96.26% of cases and among them 86% was shown in organophosphorus poisoning. Stomach necrosis was found among 95.55 % study population and among them 86.3% was present among organophosphorous poisoning. Erosion of lamina propria were present in 97.01 % cases and among them 86.2% were present in organophosphorous poisoning .4.6 % were present among aluminium phosphide poisoning cases.

ii) **Liver**

The post-mortem histological findings showed in figure 3 and 4 that 97.76 % have liver haemorrhages and among them 84.32% had been associated with with organophsphorus poisoning. Out of 268 poisoning cases, liver congestions were associated with 98.50% and among them 84.70% had poisoned with organophsphorus compounds. Bile pigments in the hepatocytes were presents among 96.3 % population and among them 83.58% have organophosphorus poisoning cases. Degeneration of hepatocytes were present in 97.76% of cases and among them 84.7 % were organophosphorus poisoning cases. Liver sinusoidal dilatations were present among 98.13 % of cases with associated liver steatosis among 97.10 % of cases.

iii) **Kidney**

The post-mortem histological findings as showed in figure 5 that kidney haemorrhages were associated with 98.5 % cases and 85.07% were presents among organophosphorus poisoning cases. The glomerural congestions were found among 98.13% cases. Tubular necrosis findings were presents among 97.76 %.

As per distribution of study subjects according to frequency of post-mortem histological findings among total poisoning cases (n=268) are summarized in table 3.

Table1: Distribution of study subjects according to their sex and educational status (n=268)

SEX	EDUCATIONAL STATUS			TOTAL
	< SECONDARY SCHOOL EDUCATION	SECONADARY SCHOOL EDUCATION	GRADUATE	
MALE	7(3.9%)	153(85%)	20(11.11%)	180
FEMALE	3(3.4%)	76(86.4%)	9(10.22%)	88
TOTAL	10(3.7%)	229(85.44%)	29(10.82)	268

Table2: Distribution of study subjects according to frequency of admission in hospital (n=268)

FREQYENCY OF ADMISSION IN HOSPITAL	NUMBER	PERCENTAGE
ADMITTED	217	81.0%
NOT ADMITTED	51	19.0%
TOTAL	268	100%

Table 3: **Distribution of study subjects according to frequency of post-mortem histological findings (n=268)**

Frequency of post-mortem histological findings	**Present**	**Absent**
Stomach haemorrhage	251(93.7%)	17(6.3%)
Stomach congestion with mono nuclear infiltration	258(96.3%)	10(3.7%)
Stomach necrosis	256(95.5%)	12(4.5%)
Erosion of lamina propria	260(97%)	8(3%)
Liver haemorrhage	262(97.8%)	6(2.2%)
Liver congestion	264(98.5%)	4(1.5%)
Bile pigment in the hepatocytes	258(96.3%)	10(3.7%)
Degeneration of hepatocytes	262(97.8%)	6(2.2%)
Liver sinusoidal dilation	263(98.1%)	5(1.9%)
Liver steatosis	260(97%)	8(3%)
Kidney haemorrhage	264(98.5%)	4(1.5%)
Glomerurlar congestion Tubular necrosis	265(98.9%) 262(97.8%)	3(1.1%) 6(2.2%)

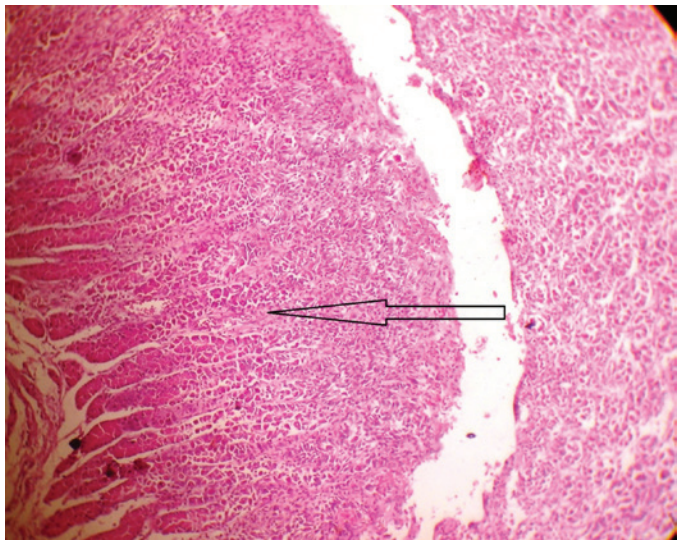


Figure 1-Intestine-40x: Congested intestinal mucosa with chronic inflammatory cell infiltration.

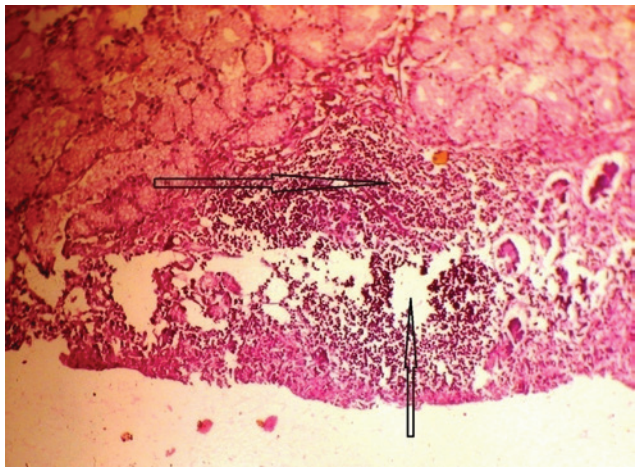


Figure 2-Intestine-40x: Ulcerated denuded mucosa with areas of necrosis & chronic inflammatory cell infiltration.

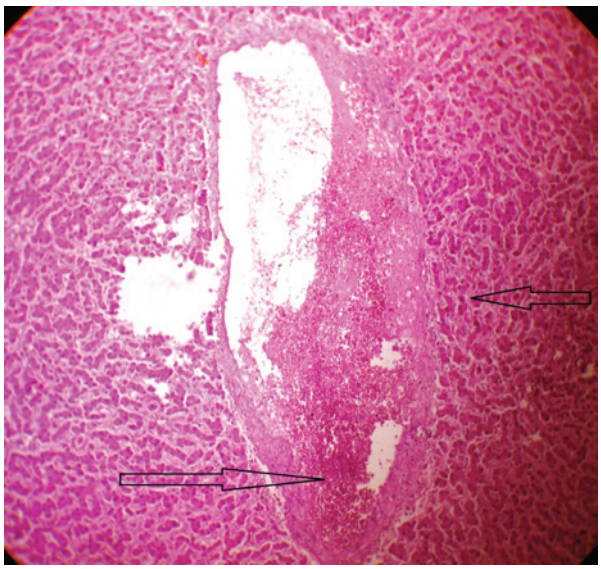


Figure 3-Liver-40x: Dilated congested central vein with areas of hemorrhage and chronic inflammatory cell infiltration in sinusoids

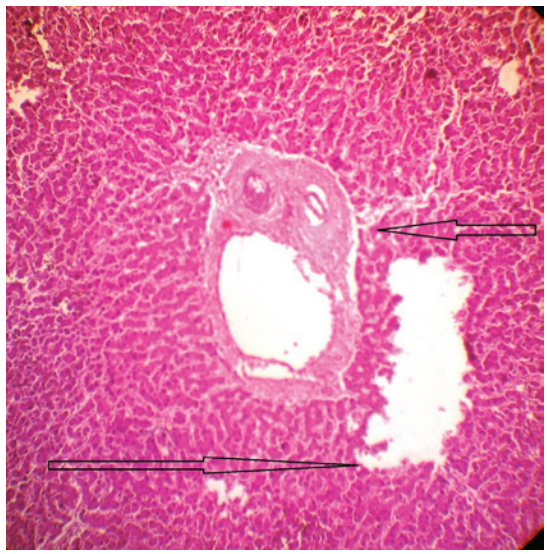


Figure 4-Liver-40 x: Dilated portal vein with periportal inflammation and areas of edema

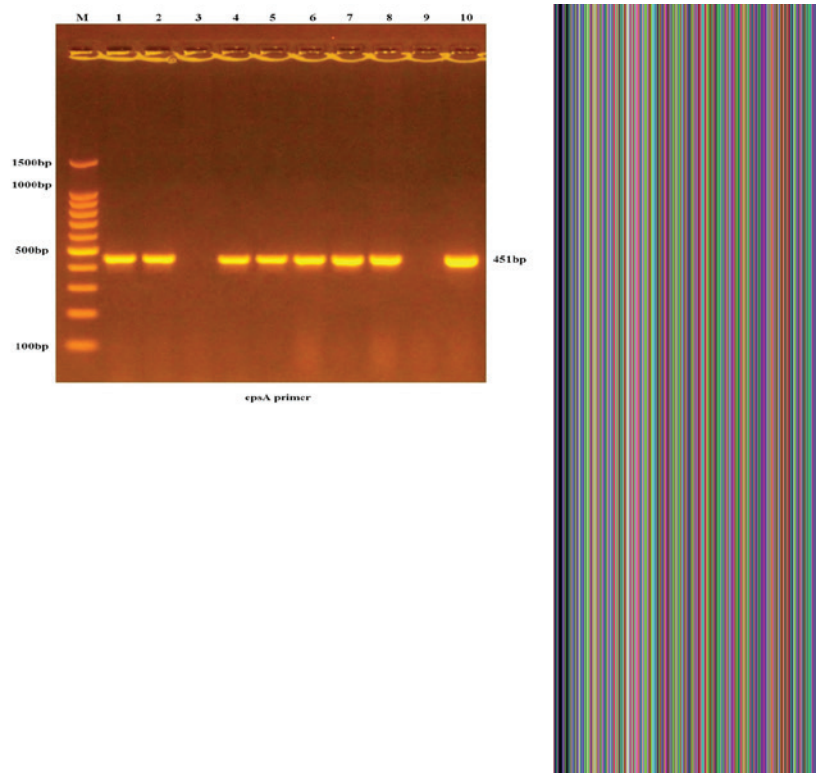


Figure 5-Kidney-40 x: Dilated and congested blood vessels with areas of hemorrhage and chronic inflammatory cell infiltration

Discussions

In the present investigation of total 2423 unnatural deaths autopsied during the study period 351 cases ware due to poisoning (14.38%). Out of those poisoning cases 268 were due to pesticide amounting to 76% of poisoning deaths. It was found in the present study that pesticide poisoning constituted 9.04% of all unnatural deaths (268 out of 2423) which was consistent with earlier studies [2].

In the present study poisoning deaths were higher as 67.2 % in males than female deaths 32.8 %. Males are exposed to stress, strain and occupational hazards to a greater extent when compared to females because they are bread earners.

In the present study higher incidence of death due to poisoning was found in young age group (20 to 29 years) followed by 30 to 39 years of age which was also observed in previous studies[3]. Stress of the modern lifestyle, family problems, financial problems and failure in the exams could be explained as major causes of suicidal poisonings [3, 4].

Increased farming activity like spraying of pesticides contributed for maximum number of poisoning cases at rural areas (69.8%). Illiteracy or less education, less availability of immediate treatment may be causes of such poisoning. The most commonly affected age group was 20 to 29 years. Poverty, unemployment, early marriage are some of the major stress factors responsible for higher incidence of deaths in these rural areas.

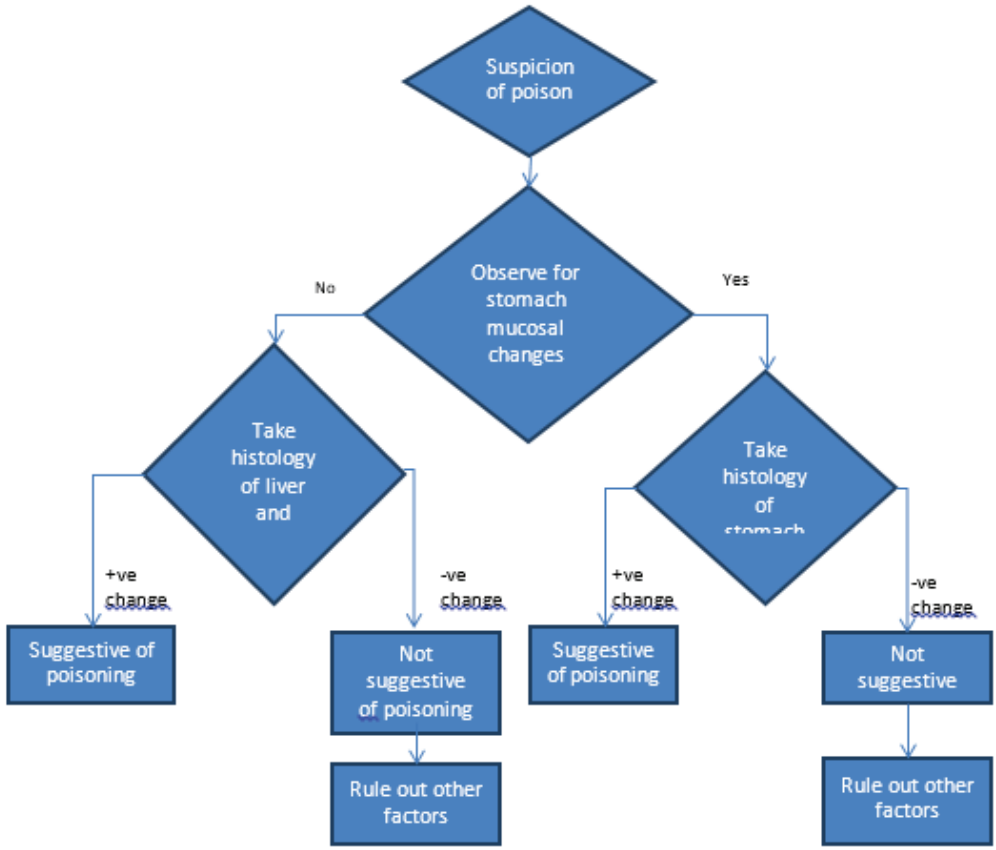
Organophosphorus compounds were the most commonly used poisons, because of their wide used in agriculture Thus results of the present study were consistent with the previous studies, except for some differences in the kinds of poison preferred [5, 6 and 7]. Trends of the poisons seem to be a function of need and availability of specific substances.

The common autopsy findings were cyanosis in the lips, in the fingertips and nose. All the internal organs were congested in 100% of cases. Dissection revealed congestion of the mucous membrane of stomach with scattered submucosal petechial haemorrhages (98. 3% cases).

Histological corroborations in stomach revealed congestion, erosion of muscularis mucosa, submucosal petechial haemorrhages and necrosis. Liver shows congestion, sinusoidal dilution, degeneration of hepatocytes and haemorrhages. Kidney shows haemorrhages, tubular necrosis and glomerular congestion.

Conclusion

It was found in the present study that pesticide poisoning fatal cases constituted 9.04% of all unnatural deaths (268 out of 2423). Meticulous autopsy findings including histopathological corroborations of stomach, liver and kidney is the central consideration of the present study to establish acute poisoning fatal cases. This is necessary because often reporting and chemical examination of viscera is difficult due to several technical problems, whereas histology is readily available in all hospitals because they have pathology centre even in the subdivision levels. The findings of the present investigation will supplement the methods commonly available to establish the cause of death in poisoning specially pesticide poisoning in a setup where resources of analytical work are not easily available. The methodical findings in order to diagnose acute poisoning cases may be represented through the following flowchart.



Therefore, thumb rule for diagnosing acute poisoning cases from histopathological considerations may be stated as follows:

History of poisoning + autopsy including triad of positive finding of histology = strong evidence of acute pesticide poisoning.

Therefore, thumb rule for diagnosing acute poisoning cases from histopathological considerations may be stated as follows:

$$\text{Triad of positive findings of histology} = \begin{array}{l}\text{+ve finding of stomach histology} \\ + \\ \text{+ve finding of liver histology} \\ + \\ \text{+ve finding of kidney histology}\end{array}$$

History of poisoning + autopsy including triad of positive finding of histology = strong evidence of acute pesticide poisoning.

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The Effectiveness of Computational Thinking Based Learning (CTBL) and Problem Based Learning (PBL) Learning Models on Learning Achievements Reviewed From Brain Dominance

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Abstract

This study aimed to determine: (1) which one is more effective between Computational Thinking Based Learning (CTBL) and Problem Based Learning (PBL) on learning achievement, (2) which one is better, students with left brain dominance or students with right brain dominance, (3) for each brain dominance, which one is better between CTBL and PBL. This study used a quasi-experimental research method with a 2x2 factorial design. The population in this study were all students of class VIII SMPN 1 Bendo in the Academic Year 2020/2021, totaling 9 classes. The research sample included 45 students (two classes) who were taken using cluster random sampling. The instrument used was a questionnaire on the students' brain dominance and a mathematics learning outcome test. From the results of this analysis, it can be concluded that CTBL was more effective to implement compared to PBL in odd semester class eight students at SMP 1 Bendo Magetan in the Academic Year 2020/2021. There were differences in the results of mathematics learning achievement in students who were given CTBL and PBL in terms of the brain dominance of odd semester class eight students at SMPN 1 Bendo Magetan in the Academic Year 2020/2021.

Keywords : *computational thinking; computational thinking based learning (CTBL); problem based learning (PBL); brain dominance*

Introduction

Lately, the world of education in various countries around the world is incessantly incorporating or carrying Computational Thinking (CT) into the lesson curriculum in schools. Including in Indonesia, the Ministry of Education and Culture (Kemendikbud) launched two new competencies in the Indonesian children's learning system. The two additional competencies are Computational Thinking and Compassion. CT has begun to be applied by several educational institutions. Even the government itself has included it in the national curriculum known in the subject of Informatics, although actually CT can be applied in almost all subjects depending on the extent of the creativity of the teacher in making or creating questions/cases that lead to CT.

CT is one of the things proposed to complement the 4 C's (Critical thinking & problem solving, Creativity, Communication & Collaboration) which has been issued by UNESCO as a skill needed by future generations or more commonly referred to as the digital generation [1–4]. CT is a thought process that is needed in formulating problems and solutions, and therefore these solutions can become effective information processing agents in solving problems. Computational thinking as a basic skill for all people's analytical abilities as well as those for reading, writing, and arithmetic[5–9]. CT helps someone solve a problem by breaking the problem into smaller parts so that it can be easier to find solutions to solve the problem [10]. CT will train students in solving problems/ cases that exist and they will definitely need in their lives because every human being will never be separated from a problem [11–14]. Students who have mastered or are

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accustomed to CT will be tougher in facing challenges in the future and are not easy to give up [15,16]. The way to implement Computational Thinking is to understand the problem, collect all the data, and then start looking for solutions according to the problem. In Computational Thinking, there is what is called decomposition, where we break down a complex problem into small problems to be solved. In this study, learning which implements CT is called Computational Thinking Based Learning (CTBL).

Previously, learning that was being developed especially in Indonesia was problem-based learning (PBL). Problem-based learning will certainly strengthen computational thinking in getting solutions and learning that is designed to form a team to make students more confident and able to share all the advantages of each individual [17–23]. A research on the PBL model that has been carried out by Ratnaningsih (2016) was learning mathematics with PBL to improve students' mathematical problem solving abilities. The results of the research concluded that the increase in mathematics problem solving abilities of students who received mathematics learning with the PBL model was better than direct learning. Furthermore, the result of research who conducted by Mashuri, Djidu, & Ningrum (2019) show that PBL be able to increase the interest to learn of students, because students be active in learning.

In learning, students use their cognition to determine the answers to the questions given by the teacher. This, of course, is inseparable from which brain dominance exist in each student. A research on brain dominance showed that different types of information are processed in the left and right cerebral hemispheres and the functions of these two cerebral hemispheres are different [26]. According to a research, the left cerebral hemisphere showed a more analytic approach, while the right cerebral hemisphere showed a holistic and spatial approach. Each cerebral hemisphere has special mental abilities. The left cerebral hemisphere is analytical, abstract, verbal, digital, logical, sequential, and rational, while the right cerebral hemisphere is holistic, concrete, non-verbal, visual spatial, intuitive, simultaneous and analogical [26–28]. The two cerebral hemispheres interact with each other under normal circumstances. However, each of them has a different basic function. The roles of the left brain and right brain are related to one another, so it is very important to have a balance between the two brains.

So far, learning has not been found in Indonesian schools that pay attention to the dominance of the brain as the basis of learning. In addition, it is necessary to see whether learning with CTBL can be applied in Indonesia and there needs to be research on how the application of CTBL is compared to other lessons. Therefore, this study aimed to see the extent to which CTBL can be applied compared to PBL learning in terms of students' brain dominance. The dominance of the students' brain which is meant here is the dominance of the right brain and left brain.

Methods

This research was conducted at SMP Negeri 1 Bendo in the odd semester of the Academic Year 2020/2021. This type of research is quasi experimental research with a 2x2 design. The population in this study were all students of class VIII SMP Negeri 1 Bendo which consisted of 9 classes. The sampling technique was carried out using cluster random sampling, because the sampling of members of the population was carried out randomly without considering the strata in the population. The research sample included 45 students consisting of 22 students (class VIII A) as the experimental group 1 which applied CTBL and 23 students (class VIII D) as the experimental group 2 which applied PBL.

The variables in this study were divided into two, namely the dependent variable and the independent variable. The dependent variable in this study was the achievement of learning mathematics in the sequence and series material, while the independent variables in this study were learning (CTBL and PBL) and brain dominance (right brain dominance and left brain dominance). Data collection techniques used questionnaires and tests. The research instrument consisted of a brain trend questionnaire called the Open Hemispheric Brain Dominance Scale (OHBDS) and a mathematics learning achievement test on sequence and series material.

The trial of the mathematics learning achievement test instrument refers to the criteria, namely validity, level of difficulty, differentiation and reliability. The number of items on the OHBDS was 20, while the number of

items in the mathematics learning achievement test was 20. The data on the students' initial ability in mathematics learning achievement were obtained from the midtest scores for the even semester of the experimental class 1 and the experimental class 2. Before conducting the experiment, the normality test, homogeneity test and balance test were carried out on the mathematics initial ability data. Mathematics learning achievement data were analyzed using two-way analysis of variance with different cells after the normality and homogeneity tests were carried out. The normality test for initial ability data and mathematics learning achievement data was carried out using the Lilliefors method and the homogeneity test of population variance using the Bartlett method. Furthermore, if the hypothesis test used two-way analysis of variance with different cells resulting in H_0 rejected, a double comparison test was carried out using the Scheffe' method.

Results and Discussion

1. Prerequisite Analysis Test

a. Normality test

The normality test was used to determine the random data obtained by the researcher came from a normally distributed population. In this study, the researcher chose the normality test using the Lilliefors method with a significant level of $\alpha = 0,05$. The summary of the results of the normality test that has been carried out is as follows:

Table 1. Summary of Normality Test

Group	L_{obs}	DK	Decision	Conclusion
CTBL	0.0701	0.1437	H_0 Accepted	Normally distributed
PBL	0.1222	0.1477	H_0 Accepted	Normally distributed
Right brain dominance	0.1035	0.1419	H_0 Accepted	Normally distributed
Left brain dominance	0.0960	0.1498	H_0 Accepted	Normally distributed

From the summary of the analysis, the results of the normality test showed that the samples from the CTBL class and the PBL class came from a normally distributed population. Likewise, the sample of students who belonged to the right and left brain dominance came from population that were normally distributed.

b. Homogeneity Test

The homogeneity test was used to determine whether the variances of the population under study are the same or not. The homogeneity test used was the Bartlett test with a significant level of $\alpha = 0,05$. The summary of the results obtained from the homogeneity test is as follows:

Table 2. Homogenity Test Summary

Group	χ^2_{obs}	DK	Decision	Conclusion
CTBL and PBL	1.2205	3.841	H_0 Accepted	Both classes were homogeneous
Right and left brain dominance	1.3095	3.841	H_0 Acecpted	Both categories were homogeneous

Based on the summary results of the homogeneity test, it showed that the data obtained in the study on the CTBL and PBL classes had homogeneous variances. Likewise, the two categories of students who have right and left brain dominance have homogeneous variances.

2. Hypothesis Test Analysis

Based on the prerequisite test analysis that has been carried out, it showed that the observed data in the sample taken came from a population that was normally distributed and had homogeneous variance. This means that the prerequisites for conducting hypothesis testing with the ANOVA test technique were met. The summary of the results of the hypothesis testing on ANOVA 2 × 2 with a significant level of $\alpha = 0.05$ is as follows:

Table 3. Summary of Variance Analysis

Source	JK	dk	RK	F_{obs}	F_{α}	Conclusion
Brain Dominance (A)	4712.77	1	4712.77	41.5296	3.987	H_0 Accepted
Learning (B)	1963.04	1	1963.04	17.2986	3.987	H_0 Accepted
Interaction (A*B)	308.188	1	308.188	2.7158	3.987	H_0 Accepted
Galat	7943.59	70	113.48	-	-	-
Total	14927.588	73	-	-	-	-

Based on the summary of the analysis of variance, it showed that:

a. There were differences in the effect between rows on the dependent variable.

b. There were differences in the effect between columns on the dependent variable.

c. There were no row and column interaction with the dependent variable.

3. Post Anava Follow-Up Test

Based on the results of the hypothesis test, it showed that H_{0A} was rejected, H_{0B} was rejected and H_{0AB} was accepted. This means for H_{0A} and H_{0B}, it is necessary to do a post-anava follow-up test to see the differences that occur for each category. The follow-up test used was the Shceffe test with a significant level of $\alpha = 0.05$. The summary of further tests obtained is as follows:

Table 4. Shceffe Test of H_{0A} and H_{0B} Summary

H_0	F_{obs}	$F_{0,05:1,70}$	p	Conclusion
$\mu_1 = \mu_2$	41.57	3.987	< 0.05	H_{012} Rejected
$\mu_1 = \mu_2$	17.35	3.987	< 0.05	H_{012} Rejected

The summary of the Shceffe test showed that the factor a_1 had a significant difference in the mean with the factor a_2. Meanwhile, factor b_1 also had a significant difference in mean with factor b_2.

B. Interpretation of Data

1. The Differences in Students' Mathematics Learning Achievement with CTBL and PBL.

From the main research data, it was obtained that the average mathematics learning achievement of students with CTBL in the sequence and series material was 68.5526. Meanwhile, for students with PBL the average achievement test was 58.4722. Analysis of the existing data obtained value of t_{hitung} = 3.2425 and the value of t_{tabel} at a significant level of 0.05 for $n_1 = 8$ and $n_2 = 36$ is 1.960. Because $t_{hitung} > t_{tabel}$, and therefore H_o was rejected, so it can be concluded that CTBL was more effective than PBL.

Based on this description, it showed that students achievement in the CTBL class was better than that of the PBL class. Then seen from the average score obtained in each class or the marginal average of each class, it can be seen that the mean score of students with CTBL was better than the average score of students with PBL. Thus, it can be concluded that the mathematics learning achievement of students who were given CTBL learning was significantly different from students who were given learning with PBL.

2. Differences in Students' Mathematics Learning Achievement Based on Students Brain Tendencies.

Based on the main research data, it showed that the average learning achievement of students who have a right brain dominance was 71.1538 or 71.15%, while the average learning achievement students who have a left brain dominance was 55.2857 or 55.29%. This means that students who have a right brain dominance were able to solve the questions correctly 71.15% and students who have a left brain dominance were able to solve the questions correctly 55.29% of the 20 test questions given.Based on this description, it showed that the chances of students who have a right brain dominance to solve questions correctly were better than students who have a left brain dominance.

The result of hypothesis testing showed = 41.5296 DK so H_{0A} was rejected. This means that there were differences in mathematics learning achievement obtained between students who have right and left brain dominance. Based on the results of the post-Anava follow-up test, it showed that the average difference in mathematics learning achievement in the hypothesis for students who have a right-brain dominance was significantly different from students with left-brain dominance.

3. The Differences in Students' Mathematics Learning Achievement based on CTBL and PBL

The results of the hypothesis analysis test showed F_{obs} that = 17.2989 $\in$ DK so H_{0B} was rejected. This means that there were differences in mathematics learning achievement obtained between students taught using CTBL and students taught using PBL. Based on the results of the post-Anava follow-up test, it showed that the difference in the average mathematics learning achievement in the hypothesis for students taught using CTBL was significantly different from students taught using PBL.

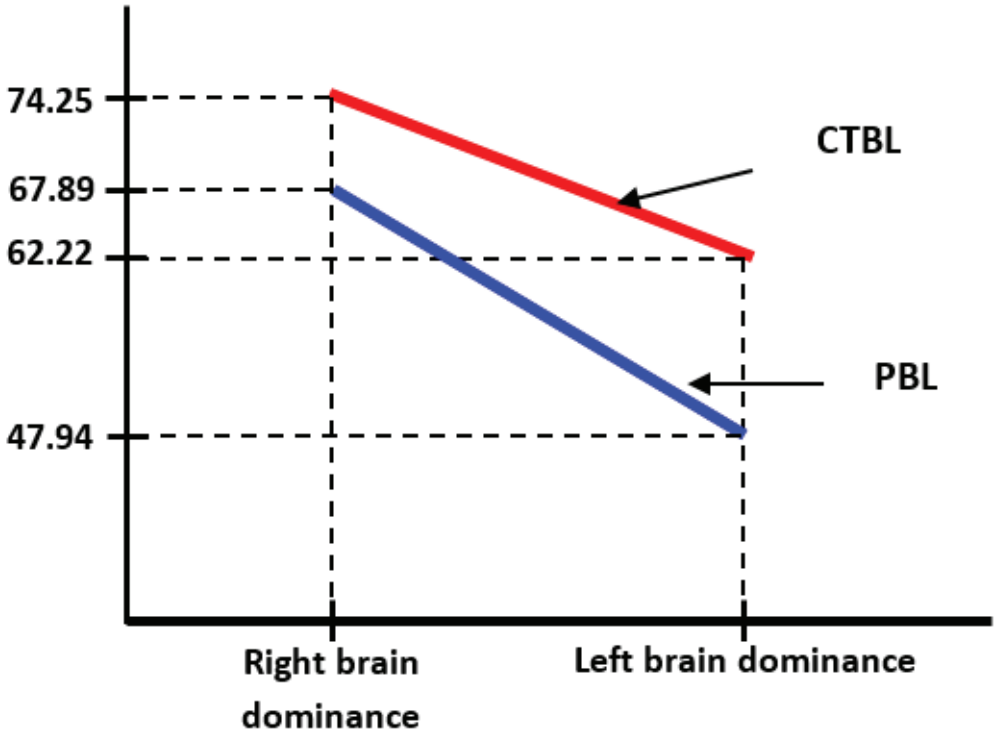

Figure 1. Learning Variables Effect Profil

E. Hypothesis Testing Conclusion

Based on the data analysis, data description and interpretation above, it can be concluded that:

1. The mathematics learning achievement of students who were given learning with CTBL was more effective than students who were given learning with PBL.

2. Mathematics learning achievement among students who were given learning with CTBL was better than students who were given learning with PBL both for students who have a right or left brain dominance.

Conclusion

Based on the analysis and hypothesis testing that has been carried out, it can be concluded in this study that CTBL was more effective to apply compared to PBL in class VIII students of odd semester at SMPN 1 Bendo Magetan in the Academic Year 2020/2021 year. There were differences in the results of mathematics learning achievement in students who were given CTBL and PBL in terms of the brain dominance of odd semester class eight students at SMPN 1 Bendo Magetan in the Academic Year 2020/2021.

Ethical Clearence: Yes.

Conflict of Interest: No

Source of Funding : Kementerian Riset Dan Teknologi / Badan Riset dan Inovasi Nasional (RISTEK-BRIN)

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Investigating the Causes of Maternal Mortality in Abadan School of Medical Sciences During 2012 - 2017

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Abstract

Introduction and Background: : Maternal mortality is one of endangering hazards of human life and is the index of women health in society. The purpose of this study was investigating causes of maternal mortality in Abadan School of Medical Sciences during 2012 - 2017.

Material and Methods: : This is a cross sectional study that all maternal death during 2012 – 2017 were assessed by a questionnaire and information obtained from investigation files and autopsy report. data were analyzed through SPSS software and descriptive statistics reports. Chi-Square and logistic regression were employed.

Finding: the maternal mortality ratio was 27 per 100000 live births in 2012 to 2017. The most common cause of maternal mortality was bleeding. In 70% of deaths, the way of childbirth was cesarean. Fifty percent of dead mothers were illiterate or had primary study degrees and 58% were urbane residents. Seventy-two percent of this mothers lacked cares before pregnancy and in 71% of deaths, prenatal care was low.

Conclusion: Increasing support and quality of cares before pregnancy, during pregnancy and after pregnancy and optimizing the skills and knowledge of medical and midwifery staff in caring and optimal treatment of mothers will be of the most important effective actions in reducing maternal mortality.

Keywords*: Health index, maternal mortality, Pregnancy period, Postpartum*

Introduction and Background

Maternal mortality during pregnancy or during childbirth and 42 days after childbirth because of any cause except for events is assumed as maternal death caused by pregnancy and childbirth[1]. MMR[1] associated with complications of pregnancy and childbirth is one of the most important development indexes that show the developing status of each country through quantifying the number of pregnant mothers' death per 100000 live births[2-4] . In Iran, in 1975, this index has been 274 and in the latest report in 2014, it has reached to 25 per 100000 alive births[3-5]. The difference of number of maternal mortality between under-developed and developed countries is higher than the difference of any other health problem[6]. This issue testifies this message that maternal death is a fundamental problem of public health in these countries[7]. The maternal mortality ratio in Mexico has been reported 72 per 100000 live births and the most common causes of maternal death have been preeclampsia, eclampsia and then obstetric hemorrhage respectively[8]. Another study in Sudan showed that the most common causes of maternal mortality were blood disseminated infection, bleeding, embolism and malaria, respectively[9]. A great number of maternal mortalities

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happen immediately after childbirth or at the beginning of puerperium that mostly the cause is bleeding[10]. in Iran, the four main reason of maternal mortality have been reported, respectively, bleeding, hypertension, infection[11]. In Mohammadinia et al. study, the causes of maternal mortality were reported, respectively, bleeding, preeclampsia and underlying diseases[12]. Different studies in the world and Iran reported that mother's literacy level, pregnancy more than four times, living in rural areas; lack of receiving intensive cares during pregnancy and lack of accessibility to emergency obstetric services are the most important effective factors on maternal mortality[13-16]. Other studies showed that unwanted pregnancy had the main role in death of pregnant mothers and the most common causes of death were bleeding 25% and amniotic fluid embolism 17.5%[17-21]. Therefore, with regard to the importance of maternal mortality in the country's health care system and insufficient classified information in line with preventing avoidable maternal deaths, necessity of determining the spread and exact causes of maternal mortality and effective factors on it with the aim of running an intervention program is felt. So, this study is done with the purpose of determining the causes of pregnant mothers' death associated with pregnancy complications and childbirth during years 2012-2017 at the level of Abadan School of Medical Sciences.

Material and Methods

In a cross sectional study, all files related to the death of all mothers during pregnancy and 42 days after childbirth at Abadan School of Medical Sciences through census sampling were investigated. In a way that the list of all dead patients from the April 2012 till March 2017 were taken from deputy treatment and the file of each patients was received. Each file was scrutinized from the reception of patient by the first center until his or her death in that hospital or another center. In investigating each file, all points such as demographic characteristics, biography, autopsy report and proceedings of maternal mortality were collected to determine the real cause of death clearly. Collecting data done by using maternal mortality table that has been extracted from 2001 maternal mortality surveillance system has been implemented in Iran[22]. Also, by investigating autopsy report a harmony between the clinical causes of death and autopsy was determined. It should be mentioned that in this study, related policies to saving information has been fulfilled. To determine the relationship between demographic factors and maternal death χ^2 test and logistic regression were used. This article obtained from project with NO IR.IGUMS.REC.1394.96.

Finding

According to the findings of the study, the most amount of maternal mortality in Abadan School of Medical Science was occurred in 2015 (8 deaths) (Table 1). The maternal mortality ratio in this study was 27 per 100000 live births in a 5-year period between 2012 to 2017. Other finding of the study was reported in Table 2 And Figure 1 and 2. In this study, the chi-square test demonstrated significant relationship between cause of maternal mortality and underlying diseases, high risk pregnancy and having optimal pregnancy and postpartum care (P>0.001).

Table1- The Maternal Mortality rate in Abadan School of Medical Sciences in 2012-2017

Number of deaths	Number of Births	Year
3	15137	2012
3	14513	2013
5	14969	2014
8	15189	2015
4	15038	2016
23	74846	2012-2017

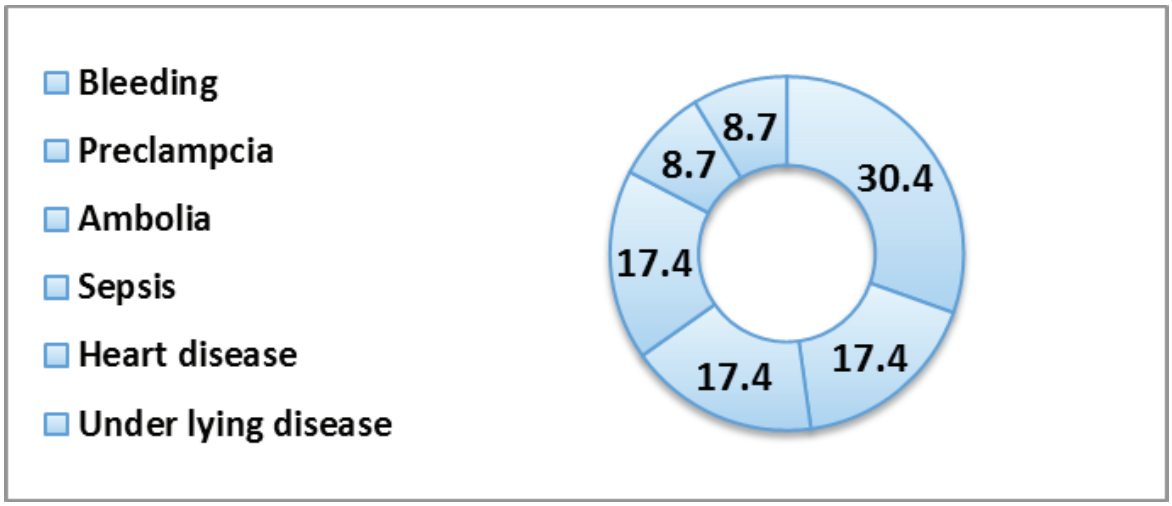

Figure1- The relative frequency of different causes of death of pregnant women in Abadan School of Medical Sciences in 2012-2017.

MMR

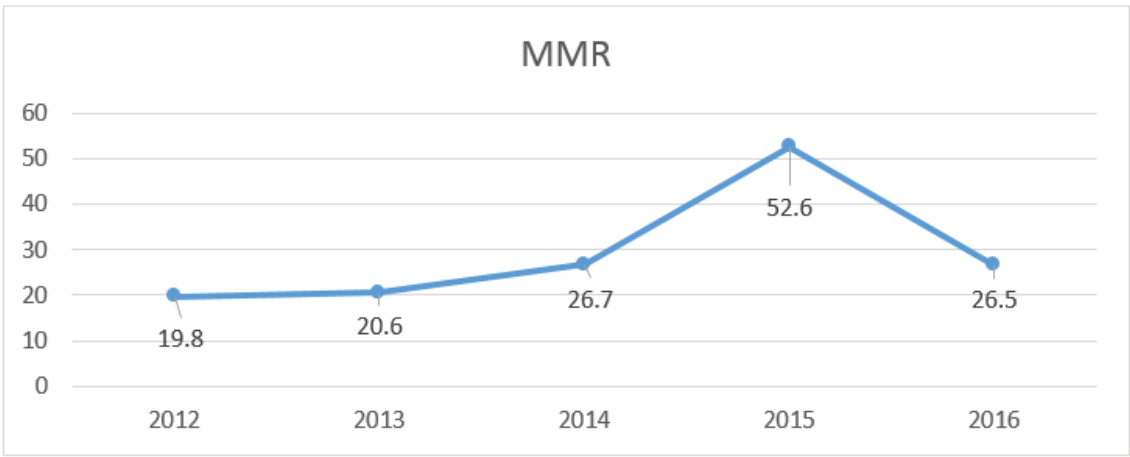

Figure2- The Maternal Mortality rate in Abadan School of Medical Sciences in 2012-2017.

Table2- The relationship between some demographic factors and maternal death

Variable		Number	Present	p- Value	EXP(B)	CI 95%
Age Of Maternal Death	>18	0	0	0. 3		
	18-35	20	86%			
	<35	3	13%			
Education	No Formal or Primary Education	11	48%	0.5		
	Secondary	12	52%			
Living Area	Rural	35	38%	.159		
	Urban	15	65%			

Cont... **Table2- The relationship between some demographic factors and maternal death**

parity	0	6	26%	0.03	2.3	(1.2,4.6)
	>4	5	22%			
Wanted Pregnancy	Yes	18	78%	0.2		
	No	5	22%			
Preconception Care	Yes	9	39%	0.2		
	No	14	60%			
Adequate Number of Pregnancy Care	Yes	15	65%	0.119		
	No	8	34%			
Having optimal pregnancy care	Yes	7	29%	0.005	0.93	(0.03,0.3)
	No	16	71%			
Having optimal Delivery care	Yes	11	48%	0.15		
	No	12	52%			
Having optimal postpartum care	Yes	11	78%	0.012	2.0	(1.0,4.0)
	No	12	52%			
Delivery mode	Vaginal	6	20%	0.148		
	Caesarean section	13	56%			
Death location	Hospital	20	90%	0. 2		
	Home/other	3	13%			
Period of Death	pregnancy	4	17%	0.08		
	<24hr of Delivery	6	26%			
	>24 hr. of Delivery	13	56%			
Having Underlying Disease	Yes	13	34%	<0.001	20.803	(6.6,65.8)
	No	10	67%			
Drug Addiction	Yes	3	13%	0.372		
	No	20	90%			
Having Risk Factors	Yes	20	87%	<0.001	4.8	(1.3,18.1)
	No	3	13%			

Generalized linear models were used to evaluate the relationship of potential factors and maternal mortality and to develop point and interval estimates of relative risk associated with these factors.

Discussion

Based on the findings of the present study, maternal mortality ratio at the level Sciences Abadan School of Medical Science in a four-year period was reported 27 per 100000 live births. The mentioned index has reported the following ratio in different provinces

of Iran, 17/2 per 100000 in ChaharMahal Bakhtiari province in a 10-yeaar period (2002-2011) (23), 53.3 in Semnan city in a 10-year period (2000-2009)[24], 57.7 in kohkiluye and Boiyer Ahmad in a 10-year period (1997-2007)[15], 82.6 in Sistan and Baloochestatn in an eight-year period[12], 39.8 in Hormozgan in a five-year period (2007-2011)[25]. However, with regard to the date of doing studies, health conditions and quality promotion of cares in recent decades has led to reduction in death all around the country. in other countries, different ratios have been reported, such as, a study was done in Jordan this ratio was 19.1[26]. 20 in South Korea in 2009 and has been reported 10 in France and 9 in Japan. According to the latest repot of the world Health Organization in 2014, in the world in general, this index was less than 16 in developed the countries and was 400 in the under-developed countries[2].

It can be mentioned that complications of pregnancy and childbirth are observable in the developed countries. But, because of more facilities and immediate treatment its ratio in these countries is lower[11].

Unfortunately, trend of death in medical Sciences University of Abadan has had an ascending trend and it has got to 8 deaths in 2016 while it was 3 in 2012 that according to the analyses, the 37.5 percent deaths are because of N1H1 flu virus. Other causes of death were similar to the past ones. This issue shows the need for vital intervention for reduction of the related index.

Therefore, bleeding and hemorrhagic shock with DIC were recognized as the most common causes of death. The most used way of childbirth was emergency cesarean (61%) and of predisposing causes of death associated with bleeding, the most common was the age between 18-35 years. Doctor and midwife's lack of knowledge and information, shortage and inappropriate use of blood and blood products, delay in recognizing bleeding by midwife, volume replacement, realization and DIC treatment, and bleeding control by operation were of the main predisposing causes of maternal mortality because of midwifery bleeding.

In studies in other provinces, the similar results have been attained. In a study by Gholami (2008) in kohkiluye and Boiyer Ahmad[20], the most common cause of maternal mortality was bleeding. Sedighani et al. (2002) in Azerbaijan Gharbi, bleeeding was 54%, hypertension was 21% and infection was 6.6%. In Mansouri (2005) in Mashhad, bleeding, Eclampsia and embolism, respectively, were mentioned[27]. With regard to the report of the office of Maternal Health Ministry in 2015, bleeding was the first cause of death and preeclampsia and eclampsia were in the next ranks.

The results of the present studies were a manifestation that a high percentage of deaths (83%) have happened Postpartum. In Mohammadi's study, most of cases of death were reported after childbirth which is in line with the result of the present study[12].

As it was mentioned, in the most cases, childbirth had been done through cesarean that in 34 percent cases, maternal underlying diseases and in 12.5 percent mothers' previous cesarean were the reason of cesarean. It should be mentioned that childbirths associated with cesarean are mostly those kind of childbirths that health of mother and embryo are at risk which are called high risk childbirth. Moreover, childbirth through cesarean surely will have repeated cesarean in follow and these repeated cesareans increase some complications such as adhesions pair, bleeding and death in the end.

According to the results of this study, 71 percent of dead mothers lacked pre-pregnancy cares. 34 percent of them had underlying diseases and a significant relationship among underlying diseases; high risk pregnancy and cause of death were observed. This issue shows the importance of improving the qualitative and quantitative cares and consulting before the pregnancy since with appropriate consulting and realizing the mother with underlying disease, the pregnancy is prevented until disappearing the disease[28].

According to the findings of this study, 50 percent of dead mothers were illiterate or with primary degrees. These findings are in line with Emami Afshar et al. work. Therefore, high rate of illiterate dead mothers in country is a manifestation of importance of the role of literacy in promotion the maternal health level[17].

The current research was contained all numbers of maternal death, which is a strentgh of this study. However, if the duration of data collection was longer, this study could be strengther, which highlights the importance of performing further studies. Finally, another limitation was lack of control over the mothers' condition because

it was a cross sectional study that limits researchers to monitor mothers condition.

Conclusion

With regarding high number of death in postpartum and bleeding period, to optimize present services including increasing services' quality related to postpartum, accessibility to blood products, accessibility to expert staff, optimized caring, balance between the clients and personnel, improving hospital instruments, being careful in recording patients biography and treating the patients, careful and complete recording of information, and necessary consulting in appropriate time can be useful in reducing maternal mortality.

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Ethical Clearance: This study was extracted from a research project, approved on 29/10/2012 by Abadan University of Medical Sciences (code: 910732), the authors appreciate the cooperation and assistance of the University Vice Chancellor for Research.

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Hypolipidemic Effects of *Rosa Damascena* Mill. Extract in Streptozotocin-induced Diabetic Rats

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Abstract

Background: Diabetes mellitus is a chronic hyperglycemic condition with a lot of complication which can lead to death. Vascular complication in diabetes commonly caused by dyslipidemia which characterized by decreased HDL, elevated LDL, Cholesterol and Triglyceride. *Rosa damascena*- an ornamental plant-thoughts to have anti-hyperglycemia and antioxidant effects because of its large amount of polyphenolic components . This study analyzed hypolipidemic activity properties of an ethanol extract of *Rosa damascena* by measuring the lipid profile using various doses. **Methods**: This research is experimental study with randomized post-test only control group design. Twenty male Wistar rats divided into 5 groups; The groups were Healthy Control Group (HCG), Diabetes Group (DG), and Extract Group: P1 (250 mg/kgBW), P2 (500 mg/kgBW), P3 (1000 mg/kgBW). All treatment groups were injected by single-dose streptozotocin 50 mg/kgBW to induce diabetes, and given the *Rosa damascena* ethanolic extract oral treatment for 2 weeks. Statistical results showed that *Rosa damascena* significantly decrease cholesterol (58.2±15.19), LDL (11±2.44) optimally in dose 250 mg/KgBW and triglyceride (96.7±44.2) optimally in dose 500 mg/kgBW after 14 days. **Conclusion**: *Rosa damascena* extract seems to be great candidate for anti-hyperlipidemic drugs.

Keywords: *Lipid profile, Rosa damascena, Streptozotocin*

Introduction

According to WHO database in 2016, 8.5% diabetes mellitus patients are adults within range 18 years to 65 years old. Diabetes mellitus is a chronic hyperglycemic condition that caused by disruption of insulin secretion, insulin works or even both[1,2].

This condition escalate morbidity and mortality of diabetes patients which caused by its vascular complication itself [3]. Vascular complication in diabetes divided into microangiopathy and macroangiopathy. These angiopathy could get worse due to atherosclerosis lesion which formed faster than non diabetes patients [2]. Microvascular complications in diabetes are neuropathy, nephropathy and retinopathy; while macrovascular complications are coronary artery disease, peripheral artery disease, artery renalis sclerosis and even stroke [4].

Dyslipidemia is one of the main factor that contribute forming atherosclerosis lesion marked by poor high density lipoprotein (HDL), elevating low density lipoprotein (LDL) and rising Triglyceride (TG) [2,5]

One of the plant that claimed has potency to decrease blood glucose and control lipid profile was red rose (*Rosa damascena* Mill.) [6]. Red rose contain a large amount of polyphenolic components; some of them are kaemfrol, *cyanidine 3,5* , *D-glucoside*, *quarcetin*, and gallat acid [7]. The highest antioxidant activity of anthocyanin in *Rosa damascena* was *cyanidine 3,5, D-glycoseides* or known as *cyanidine* [8]. The *cyanidin* found to have anti-hyperglicemia and antioxidant effects [7,9].

Roses are easy to grow in tropical country like Indonesia, they sprout under upland and lowland between 1500 height above mean sea level [10]. Indonesian Central

Bureau of Statistic mentioned that roses is one of the ornamental plants which has the second largest harvest area after *Chrysanthemum* [11].

The present study aimed at investigating lipid lowering agents of ethanol extract of *Rosa damascena* with various doses in diabetic rats.

Material and Methods

Experimental criteria and animals care

The animals which used in this research were 20 male white rats Wistar Strain, age ± 12 weeks with body weight between 150-200 grams, all animals were healthy with no handicap which characterized by shining and clean fur, pink mucous membrane around the eyes and agile. The criteria for the exclusion were sick and disabled. If the experimental animals were dead along experiment they went to dropout criteria.

All animal samples were obtained from Department Pharmacology, Faculty of Medicine, Universitas Airlangga. They were kept under laboratory standard condition with temperature 12 hours light/dark cycle. There were 5 cages with size 500 x 300 x 150 mm (length x width x height). Each cage consists of 4 rats. They were fed on standard pellets with water *ad libitum*.

Animal labs were injected with low dose streptozotocin 50 mg/KgBW protocol. Fasting blood glucose levels were measured 5 days after the injection, if their glucose level are above 200 mg/dl then it will be considered diabetic. Easy touch glucometers and glucostrip were used to measure fasting glucose levels [12].

Animals were divided into 5 groups with random sampling. The groups were Healthy Control Group (HCG), Diabetes Group (DG), *Rosa damascena* Group: P1 (250 mg/KgBW), P2 (750 mg/KgBW), P3 (1000 mg/KgBW). All experiment procedures in this study were approved by the Ethics Committee Faculty of Dental Medicine Universitas Airlangga number 456/HRECC.FODM/X/2020, Surabaya, Indonesia, and were performed in an ethical manner with strict adherence to the animal research guide and purpose.

Rosa damascena Extract

100 grams of dried *Rosa Damascena* was soaked in 96% ethanol solution for 3 x 24 hours before it was evaporated at temperature of 50˚C to get viscous extract. This extract was added with CMC-Na 0,1% to increase viscosity and prevent particle from clotting [13].

The extract will be given in three different doses which are P1 as much as 250 mg/kgBW, P2 as much as 500 mg/kgBW, and P3 as much as 1000 mg/kgBW. Ethanol extract dissolved with destilled water before giving to rats orally.

Biochemical Analysis

After 14 days treatment, blood plasma was examined to measure the lipid profile each unit sample. Procedure was required anesthesia and termination since it was using cardiac puncture technique. Blood was collected into vacutainer plastic serum tube using 5 ml syringe with 23G needle. All blood samples were sent to Surabaya Health Center Laboratory to be analyzed using automatic analyzer.

Statistical Analysis

Lipid profile from each group were expressed as mean±SD. The data were statically analyzed using *Anova* (normal distribution) and *Kruskall Wallis* (abnormal distribution) continue with post hoc test with multiple comparison every each group. Values of $p<0.05$ were considered significant[14].

Results

a. Cholesterol

Table 1. Effect of ethanol extract of *Rosa damascena on* cholesterol (mg/dl) in streptozotocin-induced diabetic rats.

Group	N	X ± SD	Median (Min-Max)
HCG (Health Control Group)	4	64.5±3.10a	65.5 (60-67)
DG (Diabetes Group)	4	138.5±38.72b	147 (87-173)
P1 (Extract RD 250 mg/kg BW)	4	58.2±15.69a	59.5 (41-73)
P2 (Extract RD 500 mg/kg BW)	4	65±7.74a	67 (54-72)
P3 (Extract RD 1000 mg/kg BW)	4	61.7±13.2a	57.5 (51-81)

Noted: superscript showed significance difference between groups with α=0.05

The lowest cholesterol level was found in group P1 (60.4±14.5) followed with group P3 (62±11.4) and P2 (65.8±6.9). All groups cholesterol mean was analyzed with *Anova* followed with *Brown-Forsythe* and post hoc *Games-Howell*. Since *Games-Howell* test showed there was no differences, *independent sample t* test was carried out. The result was there are significant differences between diabetes group, health control group and treatment group.

b. High Density Lipoprotein (HDL)

Table 2. Effect of ethanol extract of *Rosa damascena* on HDL (mg/dl) in streptozotocin-induced diabetic rats

Group	N	X ± SD	Median (Min-Maks)
HCG (Health Control Group)	4	30.8±0.81	30 (29-31)
DG (Diabetes Group)	4	31.2±3.40	30.5 (28-36)
P1 (Extract RD 250 mg/kg BW)	4	28.2±5.12	28 (23-34)
P2 (Extract RD 500 mg/kg BW)	4	31.5±5.97	32 (24-38)
P3 (Extract RD 1000 mg/kg BW)	4	29.5±3.87	29.5 (25-34)

HDL level shows the data was normally distributed so it was analyzed with *Anova*. It was found that there was no significant differences between groups, the highest HDL level was in P2 group (31.5±5.97) while the lowest was in P1 group with (28.2±5.12) as shown as in table 2.

c. Low density Lipoprotein (LDL)

Table 3. Effect of ethanol extract of *Rosa damascena on* LDL (mg/dl) in streptozotocin-induced diabetic rats.

Group	N	X ± SD	Median (Min-Max)	P
HCG (Health Control Group)	4	10.7±1.25a	11 (9-12)	0,031*
DG (Diabetes Group)	4	29.2±11.44b	32 (14-39)	
P1 (Extract RD 250 mg/kg BW)	4	11±2.44ac	10.5 (9-14)	
P2 (Extract RD 500 mg/kg BW)	4	13.2±1.25cd	13 (12-15)	
P3 (Extract RD 1000 mg/kg BW)	4	11±2.70ad	10 (9-15)	

Noted: superscript showed significance difference between groups with α=0.05

Low Density Lipoprotein data was abnormally distributed, therefore *Kruskall- Wallis* test was carried out and followed with *Mann whitney U* test to find differences between groups. Table 3 showed that there was significant differences between LDL levels in all groups.

d. Triglyceride

Table 4. Effect of ethanol extract of *Rosa damascena* on Triglyceride (mg/dl) in streptozotocin-induced diabetic rats

Group	N	X ± SD	Median (Min-Maks)
HCG (Health Control Group)	4	122.2±13.74a	120 (108-141)
DG (Diabetes Group)	4	1179.2±362.77b	1145 (772-1654)
P1 (Extract RD 250 mg/kg BW)	4	175±114.4a	183.5 (49-284)
P2 (Extract RD 500 mg/kg BW)	4	96.7±44.2a	100 (43-144)
P3 (Extract RD 1000 mg/kg BW)	4	207.2±81.9a	177 (149-326)

Noted: superscript showed significance difference between groups with α=0.05

The lowest triglyceride level was found in group P2 (96.7±44.2) followed with group P1 (175±114.4) and P3 (207.2±81.9). All groups triglyceride value was analyzed with *Anova* followed with post hoc *Tukey* HSD. From this test, it was found that there were significant differences between HCG, diabetes group and extract group.

Discussion

Streptozotocin is a chemical compound which often used to induce diabetes in animal labs. It enters beta pancreas cells through Glucose Transporter 2 (GLUT2) and cause DNA alkylation. This lead to poly ADP-rybosilation and make DNA damage which set off beta

cell necrotizing [15]. Furthermore, mononuclear cells that infiltrate Langerhans islets make the beta cell damage much worse and conduct hyperglycemia [15,16] .

While T cells and macrophages are infiltrating, adipose cells activating pro inflammation cytokine which makes insulin resistance in adipose tissue. Hormon sensitive lipase are activated and cause triglyceride breakdowns into free fatty acids and glycerol. Free fatty acids went to bloodstream to get to the liver and synthesized into triglyceride again [17].

In this study we found that *Rosa damascena* could lowering cholesterol, LDL and triglyceride significantly but not increasing HDL. 250 mg/KgBW of *Rosa damascena* extract have better effect in lowering cholesterol (58.2±15.19) and LDL (11±2.44) than other doses. On the other hand 500 mg/KgBW of *Rosa damascena* extract optimally decrease triglyceride (96.7±44.2) much better than other doses.

Several in vitro studies on cell culture have shown pancreatic *α-glucosidase* and *α amylase* inhibition activates by anthocyanins [6,18]. Antocyanin were mentioned could increase insulin sensitivity and glucose uptake in vital organs such as muscle and adipose tissue and suppress lipogenic factors [19]. Other study revealed that administration of *cyanidine-3-O-glucosidase* able to reduce glycerol and free fatty acids released by mouse embryonic cells during hyperglycemia phase [20]. Anthocyanin can also reduce body weight by decreasing levels of SREBP-1 mRNA (Sterol Regulatory Element-Binding Protein) and inhibiting enzyme that synthesize fatty acids and triglycerol [21].

There are several possibly mechanism of action of *Rosa damascena* extract in reducing triglyceride and cholesterol in this study. Gholamhusein in 2010 found that *Rosa damascena* as pancreatic *lipase* inhibitor. *Rosa damascena* has been shown to have strong anti porcine pancreatic *lipase* activity, measured by turbidimetric assay[22]. Another mechanism was *Rosa damascena* induce the activation of *Peroxisome Proliferator Receptor γ (PPAR γ)* in adipose tissue. This condition will deactivate hormone sensitive lipase, breakdown triglyceride and reduce free fatty acids [23]. *Rosa damascena* also has *3-hydroxy-r-methyl-glutaryl-CoA reductase* activity. Another study showed that 0.15mg mL^{-1} of rose extract has 60% inhibitory effect on activity of *HMG-CoA reductase* [24].

Although the real mechanism of *Rosa damascena* as pancreatic *lipase* inhibitor and *HMG-CoA reductase* inhibitor are not known yet, *Rosa damascena* extract is able to control dyslipidemia. All three doses of *Rosa Damascena* extract showed better effect in lowering cholesterol, LDL and triglyceride.

Conclusion

The result of this study showed that *Rosa damascena* ethanol extract can decrease cholesterol, LDL and Triglyceride but not rising HDL in diabetic rats. This anti-hyperlipidemic effect was probably exerted at least by three mechanism including inhibits pancreatic *lipase*, increase *PPAR γ* and inhibits *HMG-CoA* reductase. Therefore, *Rosa damascena* extract seems to be great candidate for anti-hyperlipidemic drugs.

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Awareness and Attitude Towards Organ Donation Under Transplantation of Human Organ Act 1994 amongst Medical Students in Teaching Hospital at Chennai

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Abstract

Organ transplantation, a treatment procedure to save the lives of patients with end stage organ failure is a grey zone about which the multitude is still unaware. The attitude of the Indian population towards organ donation is very bleak accounting to a rate of 0.26 per ten lakh people. A cross-sectional study was conducted among 300 medical students with a pre-tested, semi structured questionnaire to assess their knowledge and attitude regarding organ donation. Data collected was statistically analysed using SPSS software. 96% of the study participants were aware about organ donation and 98.5% believed that organ donation should be promoted. 4.8% participants of this study had already signed up the organ donation form .Willingness for organ donation was significantly associated with knowledge about organ donation. When compared to boys, girls reported higher mean scores in knowledge (8.48±1.11) and attitude (7.38±0.85). Though most of the students had adequate knowledge, gaps existed in their practices. By providing adequate knowledge and orientation about the topic, all the Medical graduates can become the potential organ donors in the future and also abide by the law of land by motivating the patients to donate their organs.

Keywords : *Organ donation, THOA, Knowledge, Attitude, Practice, medical students.*

Introduction

Chronic diseases are replacing the infectious diseases as the major cause of morbidity and mortality worldwide. The prevalence of chronic kidney disease in Indian population varies from 0.78% to 1.39%, heart failure ranges from 1.3 to 4.6 million and chronic viral hepatitis is 400- 500 million. [1] Organ transplantation is the most chosen treatment of many of the end-stage organ diseases as it offers a better quality of life and has a better long-term survival benefit. However, organ donor shortages are a global problem, but Asia lags behind much of the rest of the world. There is a poor organ donation rate of 0.26 per million in India, compared to some of the better performing countries such as America's 26, Spain's 35.3, and Croatia's 36.5 per million respectively. With a 1 per million-donation rate, India would have 1,100 organ donors or 2,200 kidneys, 1,000 hearts, 1,100 Livers, 1,100 Pancreas and 2,200 Eyes. However, there is a need of roughly 200,000 kidneys, 50,000 hearts and 50,000 livers for transplantation each year.[2] Majority of the patients die while on the waiting list as they do not get an organ on time. According to a survey in India every year, about 5 lakh people die because of non-availability of organs and 1.5 lakh people await a kidney transplant but only 5,000 get among them. [3] Lack of awareness along with myths, misconceptions and certain religious beliefs add to the low percentage of organ donation. In India, due to the imbalance between availability and demand, kidneys were harvested from the poor by exploiting their poverty. To ban this illegal sale of organs, in the year 1994, the Government of

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India passed the Transplantation of Human Organs Act, which aimed at regulation of removal, storage, and transplantation of human organs for therapeutic purposes and for prevention of commercial dealings in human organs. Many states in India adopted this act, particularly Tamil Nadu and Andhra Pradesh (undivided) lead the list. As future doctors, medical students, interns as well as residents can play a major role in promoting organ donation by educating and motivating the public to pledge their organs for donation. Therefore, this study was undertaken to assess the awareness and attitude of medical students and residents regarding organ donation which is the need of the hour.

Methodology

A cross sectional study was undertaken after obtaining Institutional Ethical Clearance at Saveetha Medical College and Hospitals,Chennai. Sample of 300 volunteers (consent obtained) inclusive of medical students were chosen randomly. Anonymity and confidentiality of respondents were maintained and participation was voluntary. Confidentiality regarding the participant response for the questions was ensured. A pretested, semi-structured questionnaire was administered. The questionnaire had details about demographic data like age, gender and religion, followed by few questions to assess knowledge. Score was allotted to each question based on the response. Subjects who secured 50% or more were considered as having adequate knowledge, and those with less than 50% were considered as having inadequate knowledge towards organ donation. Other segment of the questionnaire was used to assess attitude, practice,barriers to practice with regards to organ donation. This study was carried out for 6 months from April 2020 to September 2020. Statistical analysis was done using Microsoft excel and SPSS statistical package version 24.

Observation and Results

The total of 300 study population constituted in this study with female and male subjects 574% and 43% respectively .[Fig 1] The girls reported higher mean scores in knowledge (8.48±1.11) and attitude (7.38±0.85). The study consisted of 85, 112, 56 and 47 students belonging to 1st, 2nd, 3rd and Final year MBBS. [Fig 2] 96% of the study participants were aware about organ donation and 98.5% believed that organ donation should be promoted. It was observed that 91% of the participants strongly approve organ donation as being ethical. Participants of our study were aware about eye (97.6%), kidney (99.2%),liver (91.3%) and heart (96.9%) donation. Only 29% of the study participants had identified all the organs that can be donated.73.4% of the participants were aware of kidney being the first organ to be transplanted. It was observed that 90.4% of participants of our study felt that organs could be donated both during and after life. Another 9.6% believe that organ can only be donated after death. However, 52.4% of the participants believed that live donors had an advantage over cadaveric donors.4.8% participants of this study have already signed up the organ donation form pledging to donate an organ/body donation. More than half participants of our study (65%) are interested in becoming a potential donor. Also, 40.5% knew someone who had donated their organs. 4.7% of participants religion did not allow organ donation and 43% were doubtful about it. That being said, 96% of the participants were willing to donate to anyone in need irrespective of their religion.57.9% of the study participants believed that the organs donated meet the requirements of the organs needed. Another observation noted that 94.6% believed in the possibility of the organs donated being misused, abused or misappropriated.82% of the participants said that their donated organs could go to anyone in need without any preference to family or friends and 15.8% preferred to donate their organs to family members. More than 80% preferred that they donated their organs to a non-smoker or/ and a non-drinker. 76.2% preferred to donate to a young person. 87.5% preferred to donate to a mentally sound person and 39.5% preferred to donate to a physically disabled person.

For a living donation, it was observed that 79.1% of the population believed that the consent of the donor alone was sufficient and for donation after death, 58.5% believed that the consent should be taken from their family. In the event of the family member resisting donation of a dead person with an organ donation card, 78.5% of the participants have said that the best way to proceed is to convince the family to accept. In cases of unclaimed dead bodies, 44.1% believed that medical colleges / doctors should make decisions about organ donation.

Few other values recorded state that 80.9% believed that there is a need for having effective laws to govern the process of organ donation. Even though laws have been enforced to make sure organ donation is done with the right measures, 62.6% are neutral about the current measures taken by the government to promote organ donation are effective. Moreover, 64.9% of the participants felt that giving a monetary benefit to the donor family, giving awards to the donors or/ and giving free health treatment to the donor family will be more effective in promoting organ donation.

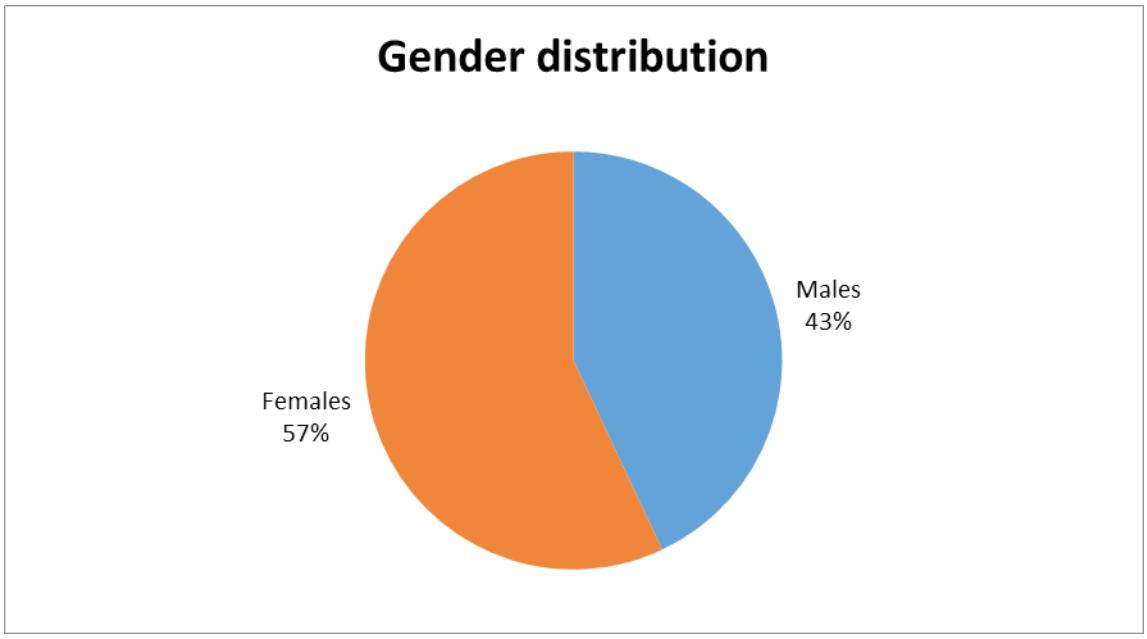

Fig 1: Gender distribution of study population

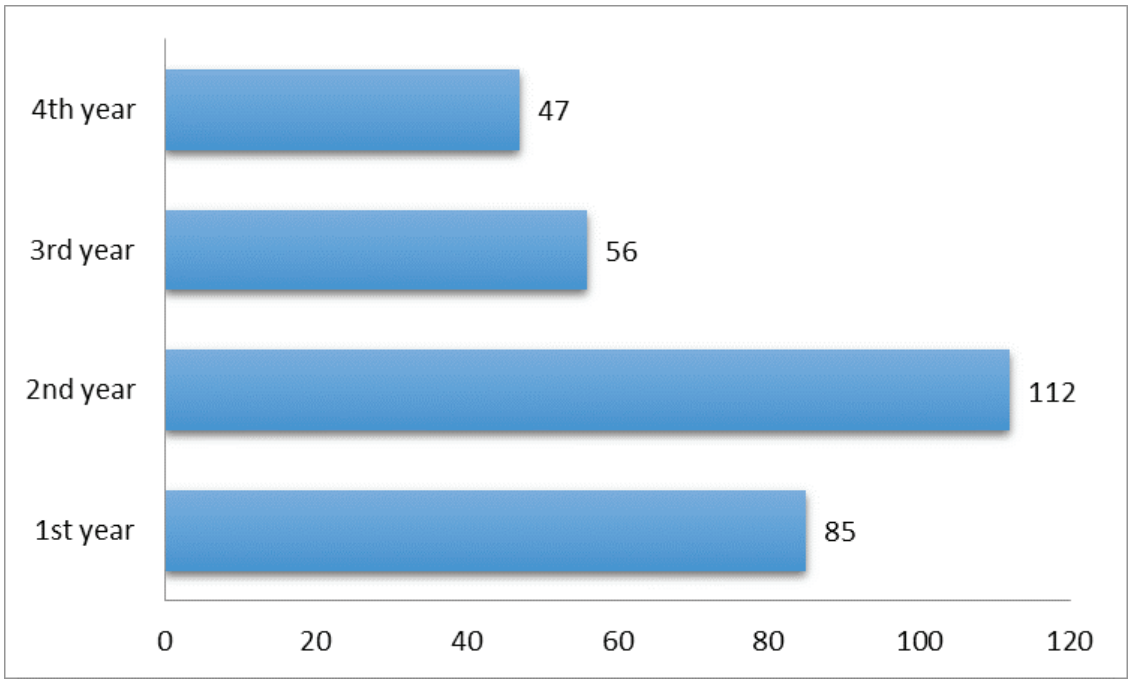

Fig 2: Year of Study of MBBS Students

Superior Thyroideal Vasa Ligation as Distally as Possible Method to Avoid External `Branch of

Discussion

Despite all advances in organ and tissue transplantation over the past decades, there are many patients waiting for an organ to become available for transplantation and the gap between available organs and patients awaiting transplantation is widening.[4] A study by Bapat et al on organ donation, awareness, attitudes and beliefs showed that majority (97%) of the medical students were aware of organ donation and transplantation.[5] This was in accordance with our study where we found awareness amongst 96% of the study participants and belief in 98.5% of them towards promotion of organ donation. The present study found that there is a significant association between knowledge acquired regarding organ donation and willingness to donate organs.Our study showed that females significantly had more knowledge than males which is in accordance with study done by Alex et al and Adithyan et al. [6,7] Only 29% of the study participants had identified all the organs that can be donated, whereas it was only 26% according to Karini et al[8]

More than three fourth (89.9%) of the students knew that there is shortage of organs in India for transplantation which is similar to the findings of Payghan et al (75.29%).[9]

4.7% of participants religion did not allow organ donation and 43% were doubtful about it. Even though all the religions are not against organ donation there seems to be presence of myths and misconceptions even among medical students as is revealed.79.1% of the participants believed that the donor consent was sufficient for donation after death, 58.5% believed that the consent should be taken from their family. In the event of the family member resisting donation of a dead person with an organ donation card, 78.5% of the participants have said that the best way to proceed is to convince the family to accept. Regarding the consent, 58.5% were aware that family's consent is required for donating organs even if the deceased has signed for organ donation. Our results also go along with the study done by Karini et al(53.7%). [8]

65% participants of our study were interested in becoming a potential donor which is similar to an observation made by Karini et al and Bathija et al. [8,10] These findings reflect the importance of bringing awareness about organ donation among medical fraternity and general public as well.

Our study found that the most common reason for not being an organ donor was due to the fear of possibility of the organs donated being misused, abused or misappropriated as stated by 94.6% of the study population. Similar results were observed by Bathija et al. [10]

Few other values recorded state that 80.9% believed that there is a need for having effective laws to govern the process of organ donation. Even though laws have been enforced to make sure organ donation is done with the right measures, 62.6% are neutral about the current measures taken by the government to promote organ donation are effective. This result was dissimilar to the findings of studies by Jothula et al (22.5%) and Ali et al (15.2%) done in Telengana and Karachi respectively. [1,11]

With respect to practice, 4.8% participants of this study have already signed up the organ donation form pledging to donate an organ/body donation. Similar results were found in studies done by Jothula et al (5.6%), Adithyan et al (7.2%), and Chung et al (27%) which specifies a need for constant motivation to turn knowledge and attitude into practice.[1,7,12]

Conclusion

In conclusion, it can be said that the knowledge of this study group is found to be satisfactory and their attitude towards organ donation is appropriate. This study shows that there is willingness of participants for organ donation; however, percentage of the participants who had registered for donation so far was meagre. The limitations of the study are that the population represents only a small part of the medical community. So, the results available from this cannot be extrapolated for the whole community. As there is a huge gap between requirement and availability of organs for transplantation in India, it becomes necessary to actively educate people about organ donation, its procedure and legislature. This would require active involvement of healthcare, media,

religious and political persons, and such an informed awareness would go a long way in improving the expectancy of patients in need.

Conflict of Interest : Nil

Ethical Clearance: Obtained

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Superior Laryngeal Nerve Injury in Thyroidectomy Surgery

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Abstract

Introduction: The thyroid gland is one of the endocrine glands with the most frequent disorders or diseases compared to other endocrine glands. Treatment of these disorders is generally performed by surgery or called a thyroidectomy. Thyroidectomy surgery can pose a risk of complications, both early complications, and late complications. Common complications are hemorrhage, hypoparathyroidism, recurrent laryngeal nerve injury, and external branch of the superior laryngeal nerve.

Purpose: To analyze the effect of the surgical method of superior thyroideal vasa ligation as distally as possible on the prevention of injury to the external branch of the superior laryngeal nerve (EBSLN) in thyroidectomy.

Methods: Clinical trial study with pre and post-test design in patients undergoing thyroidectomy surgery. Patients were examined using a questionnaire of the injury-related voice of EBSLN (SKC-EBSLN), which tested the validity and reliability of the previous test and measured 1-octave sound ability before and after thyroidectomy surgery using the superior thyroidectomy vasa ligation method as distally as possible to see its effect on EBLSN function and tone ability function.

Results: There were differences in the scores of the SKC-EBSLN questionnaire before and after thyroidectomy surgery using the method of superior thyroideal vasa ligation as distally as possible ($p < 0.05$). Further analysis showed that clinical diagnosis, surgery, tumor size, and AP outcome did not have a statistically significant effect on the postoperative SKC-EBSLN score ($p > 0.05$). In this study, the SKC-EBSLN score had a significant effect on the 1-octave tone ability ($p < 0.05$).

Conclusion: As many as 51.9% of patients had a normal voice condition with few complaints and were able to sing 1-octave tone after surgery using the method of superior thyroidal vasa ligation as distally as possible. The SKC-EBSLN questionnaire is a valid and reliable questionnaire to evaluate the results of thyroidectomy surgery.

Keywords: *external branch of the superior laryngeal nerve, superior thyroideal vasa, thyroidectomy, SKC-EBSLN*

Background

The thyroid gland is one of the endocrine glands with the most frequent disorders or diseases compared to other endocrine glands. Abnormalities in the thyroid gland include birth defects, inflammation, malfunctioning, goiter, and benign or malignant tumors. Treatment of these disorders is generally performed by surgery or what is called a thyroidectomy.

Types of thyroidectomy surgery may include lobectomy, isthmolobectomy, subtotal thyroidectomy, near-total thyroidectomy, total thyroidectomy, and Dunhill Procedure. Any thyroidectomy surgery will pose a risk of complications, both early complications, and late complications. Common complications are hemorrhage, hypoparathyroidism, recurrent laryngeal nerve injury, and external branch of the superior laryngeal nerve. Nerve injury can be in the form of

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temporary neuropraxia and permanent paralysis as a result of transection, traction, entrapment, thermal damage, or vascular injury during the release of thyroid tissue.

The recurrent laryngeal nerve (RLN) measures 3.2-4.0 mm, while the external branch of the superior laryngeal nerve (EBSLN) is 0.82 + 0.18 mm in diameter, which is 5-6 times smaller (Veraldo et al. 2014; Gavid et al. 2017). RLN, one of which invades the vocal cords, which functions motorically to move the vocal cords so that they function to produce sound. If this nerve is injured, it will result in hoarseness symptoms. During thyroidectomy, identification and preservation of RLN are relatively easy because of its visible dimensions and anatomical location that is in the tracheoesophageal groove, medial to the inferior thyroid artery, and on the inside of the Berri ligament. The frequency of occurrence of RLN injuries during thyroidectomy surgery ranges from 2% - 13%. In stark contrast to the EBSLN, which invades the cricothyroid muscle and has motor functions to lengthen and shorten the vocal cords to produce high-pitched sounds. If this nerve is injured, it will cause paralysis and/or weakness of the cricothyroid muscles, resulting in changes in sound quality, sound projection, and production of high-pitched sounds.

The surgical implications of this classification system, namely type 2a and 2b, are considered to be the high risk of EBSLN injury during thyroidectomy. During thyroidectomy surgery and when superior thyroideal vasa ligation action, identification of this nerve is a little difficult because of its very fine dimensions (0.3 - 0.8 mm), and its anatomical location that is medial to the superior thyroid vasa, and may be above or behind the upper polar cross-section of the thyroid. The frequency of occurrence of this nerve injury during thyroidectomy surgery ranges from 0-58%.

Efforts to avoid EBSLN injury during the arctic release of the thyroid are by ligating the superior thyroid vasa by identifying the nerve by cutting the sternothyroideus muscle at its base and by slightly pressing the upper pole of the thyroid, but this action can be done if the thyroid mass is not too large and is rarely done. Another action to identify this nerve is using intraoperative nerve monitoring (IONM) device. This monitoring action is rarely performed because not all hospitals have these facilities. Moreover, from some literature studies, monitoring the nerve will not guarantee that the nerve is free from injury.

Based on the efforts to identify EBSLN and the high risk of nerve injury despite using nerve monitoring device, it is necessary to find a way or method of how to ligate the superior thyroid vasa when releasing the pole over the thyroid without identifying the nerves and using a nerve monitoring tool safely so that no injury to the nerve occurs. So the act of ligation of the superior thyroid vasa as distally as possible (close to the thyroid capsule) at the upper pole of the thyroid to avoid EBSLN injury during thyroidectomy surgery, is a method that is expected to be able to secure the nerve from injury without identification and use of nerve monitoring (IONM) devices.

To prove that this procedure is safe to perform in thyroidectomy surgery, patients who will undergo thyroidectomy surgery using this method in the polar liberation of the thyroid (as research subjects) can undergo a clinical examination both pre-operatively and post-operatively using a questionnaire of the injury-related voice of EBSLN (SKC-EBSLN) as a means of screening (self-assessment) whether there is a sound disturbance. Other tests that can be used to objectively detect EBSLN disorders are acoustic analysis, video laryngostroboscopy, and laryngeal electromyography (L-EMG). The technical questionnaire examination can be done after thyroidectomy surgery in a certain period of time (2 weeks, 1 month, 3 months, and 6 months) for a period of 6 months, which is the time the wound healing process takes place in the tissue.

Methods

The method used was a clinical trial study with pre and post-test design in patients undergoing thyroidectomy surgery. Patients who have undergone thyroidectomy surgery in several hospitals that have been determined by several operators from the Head and Neck Surgery Division staff with the surgery method according to the research material and meet the inclusion and exclusion criteria were carried out their data collection including identity, preoperative diagnosis, date of operation, type of surgery, results of anatomical pathology examination, and contactable telephone/cellphone numbers.

After the patient's data was complete, then the researcher contacted the patient directly via telephone for an interview to fill out a modified TVQ questionnaire and test the vocal ability of 1-octave power from low to high notes by saying: do-re-mi-pa-sol-la-si-do. After all the required data were complete, including the results of testing the vocal skills of the voice, the data was then recapitulated. Furthermore, the data was processed and analyzed in accordance with the statistical program to obtain the conclusions of the study.

Results

In the last 6 months of 2020, the researchers found 27 patients who had undergone thyroidectomy surgery in several hospitals in the city of Surabaya with the doctor in charge of the patient (DPJP) who were several doctors from the staff of Head Neck Surgery Division of Faculty of Medicine of Unair / RSUD Dr. Soetomo Surabaya with the surgery method according to the research, which is the standard thyroidectomy operation procedure in the Head and Neck Surgery division. Researchers collected demographic data of patients who became the subjects of the study including gender, age, occupation, date of operation, date of the interview, and home address.

From gender data, the number of female patients was more dominant than males, specifically 22 female patients (81.5%) and 5 male patients (18.5%). According to the age group with a range between 15-81 years, most data appeared in the age group >50 years for 15 patients (55.6%), followed by the age group between 31-50 years for 8 patients (29.6%), and the age group <30 years for 4 patients (14.8%).

Other demographic data were patient occupation. Patients working in the private sector/entrepreneurship was the largest group for 10 patients (37.0%), followed by the unemployed group for 7 patients (25.9%), students for 4 patients (14.8%), civil servants for 4 patients (14.8%), and the teacher as a professional group for 2 patients (7.5%).

In this study, the basic clinical characteristics included clinical diagnosis, type of surgery, tumor size, and results of anatomical pathology (AP). In clinical diagnosis before surgery, the most were bilateral non-toxic multinodosa struma for 16 patients (59.3%), followed by thyroid carcinoma and non-toxic uninodosa struma for 4 patients (14.8%) of each, and diffuse toxic struma for 3 patients (11.1%).

The majority of actions performed in this study were total thyroidectomy in 19 cases (70.4%), hemithyroidectomy and completion of total thyroidectomy in 3 cases (11.1%) of each, and subtotal thyroidectomy was the least surgical operation performed in this study, which was 2 cases (7.4%).

After measuring the mass of the removed thyroid tissue, the largest number was found in 20 patients (74.1%) with a size between 5-10 cm, then 5 patients (18.5%) with a size >10 cm, and 2 patients (7.4%) with a size <5 cm. In this study, the results of most anatomical pathology (AP) examinations were benign in 18 patients (66.7%) and malignant in only 9 patients (33.3%).

This study used the SKC-EBSLN questionnaire as a research parameter. The results of the SKC-EBSLN questionnaire score are normal if the score is <5 and abnormal if the score is >5. From the pre-operation data, it was found that the normal value was 26 patients (96.3%) and only 1 patient (3.7%) with abnormal value. After thyroidectomy surgery, there was a decrease in the number of patients with normal values by 13 patients (48.1%) and abnormal values by 14 patients (51.9%).

The results of the SKC-EBSLN questionnaire score could also be divided into 3 groups based on the severity of voice disorders due to thyroidectomy surgery, namely:

a. Normal/Mild Group (0 -8) = 15 patients (55,6%)

b. Moderate Group (9-23) = 9 patients (33,3%)

c. Severe Group (24-32) = 3 patients (11,1%)

This vocal ability test was performed by the patient after filling in all the SKC-EBSLN questionnaire data by following the direction of the researcher over the telephone. The test was carried out by asking the patients to sing a tone voice: Do-Re-Mi-Pa-Sol-La-Si-Do, without stopping and with starting a low note to a high note with a 1-octave scale. There were 3 categories of ability assessment, namely: 1) capable, 2) less capable and 3) incapable. For the preoperative assessment of the ability of this vocal test, the assessment is only based on the subjective recognition of the patient with a fixed value of 3 categories, namely: 1) capable, 2) less capable

and 3) incapable.

In this study, preoperative data showed that 24 patients (88.9%) admitted to having the ability to produce high-pitched sounds, 3 patients (11.1%) said they were less capable to produce high notes, and not a single patient claimed to be incapable to produce high notes. Moreover, from the postoperative data, there were only 14 patients (51.9%) who were capable to produce a 1-octave tone sound, then 10 patients (37.0%) were considered to be less capable, and 3 patients (11.1%) felt incapable to produce a 1-octave tone sound.

In this parameter, further analysis was carried out with a comparative test using the Mc Nemar Test. The results indicated that there was a significant difference between the pre-operative and post-operative sound quality ($p = 0.003$; $p < 0.05$).

Table 1 - The results of the comparative test analysis of patients who performed the superior thyroid vasa ligation surgery method against the pre and post-operative SKC-EBSLN scoring

		Post Operative		Total	Nilai P
		Normal	Abnormal		
Pre Operative	Normal Abnormal	13 0	13 1	26 1	0,003*
Total		13	14	27	

Postoperative SKC-EBSLN scoring was also analyzed for comparison of basic clinical characteristics such as clinical diagnosis, surgery, tumor size, and AP results in this study. The comparative test used in this study was the independent t-test, chi-square, and Fisher's exact test. The results of the analysis showed that clinical diagnosis, surgery, tumor size, and AP results did not have a statistically significant effect on the postoperative SKC-EBSLN scoring ($p > 0.05$).

Table 2 - Results of comparative test analysis on basic clinical characteristics and postoperative SKC-EBSLN

		Post		Operative		Total		Nilai P
		Normal		Abnormal				
Clinical Diagnosis	Non Toxic Uni Nodular Struma Non Toxic Multi Nodular Struma Struma Diffusa Toxica Carcinoma Thyroid	2 7 1 2	(75,0%) (43,8%) (33,3%) (50.0%)	1 9 2 2	(25,0%) (56,3%) (66,7%) (50,0%)	4 16 3 4	(100%) (100%) (100%) (100%)	0.671
Operation	Hemithyroidectomy Subtotal Thyroidectomy Completion Total Thyroidectomy	3 1 1	(66,7%) (50,0%) (33,3%)	0 1 (50,0%) 2 (66,7%)	(0,0%)	3 (100,0%) 2 (100,0%) 3 (100,0%)		0,287
	Total Thyroidectomy	8	(42,1%)	11 (57,9%)		19(100,0%)		
Mass	< 5 Cm	2	(100,0%)	0	(0,0%)	2 (100,0%)		
Size	5– 10 Cm >10 Cm	7 2	(40,0%) (40,0%)	11 (60,0%) 3 (60,0%)		20(100,0%) 5 (100,0%)		0,306
Anatomical Pathology Result	Benign Malignant	9 4	(50,0%) (44,4%)	9 5	(50,0%) (55,6%)	18(100,0%) 9 (100,0%)		1.000

In this study, the correlation between SKC-EBSLN scoring and postoperative test of 1-octave tone ability was also sought. After the statistical analysis was carried out through the independent t-test and chi-square test, the results of the analysis showed that there was a significant difference in results ($p = 0.002$; $p < 0.05$) between the SKC-EBSLN score and the results of postoperative 1-octave tone ability.

Table 3 - Correlation between SKC-ESBLN and Postoperative 1-Octave Tone Ability Test

			1-OCTAVE TONE ABILITY			TOTAL
			NOT CAPABLE	LESS CAPABLE	CAPABLE	
MTVQ	NORMAL	count	0	0	9	9
EBSLN PASCA-OPERATION		% within MTVQ EBSLN Postoperative	0,0%	0,0%	100,0%	100,0%
	ABNORMAL	Count	3	10	5	18
		% within MTVQ	16,7%	55,5%	27,8%	100,0%
		EBSLN Postoperative				
TOTAL		count % within MTVQ Postoperative	3 16,7%	10 55,5%	14 51,9%	27 100,0%

Discussion

Thyroidectomy surgery has been performed since 956 AD by Albucasis. Thyroidectomy surgery is performed to remove the tumor mass or abnormalities in the thyroid gland. Types of thyroidectomy surgery include lobectomy, isthmolobectomy, subtotal thyroidectomy, near-total thyroidectomy, total thyroidectomy, and Dunhill Procedure. Any thyroidectomy surgery carries a risk of complications. Common complications include hemorrhage, hypothyroidism, hypoparathyroidism, recurrent laryngeal nerve injury, and external branch of the superior laryngeal nerve (EBSLN). Nerve injury can be neuropraxia which is reversible and paralysis which is non-reversible resulting from transection, traction, entrapment, thermal damage, or disrupted blood supply during the release of thyroid tissue [(1)].

The external branch of the superior laryngeal nerve (EBSLN) has the function of invading the cricothyroid muscle which functions as a motor to lengthen and shorten the vocal cords to produce high-pitched sounds. If this nerve is injured, it will cause paralysis and/or weakness of the cricothyroid muscles, resulting in changes in sound quality, sound projection, and the inability to produce high-pitched sounds.

The anatomical location of the EBSLN lies at the junction between the superior thyroid vasa and the upper pole of the thyroid [(2)]. The surgical implications of this classification system, namely type 2a and 2b, are considered to be the high risk of EBSLN injury during thyroidectomy. During thyroidectomy surgery and the superior thyroid vasa ligation action, identification of this nerve is a little difficult because of its very fine

dimensions (0.3–0.8 mm), and its anatomical location that is medial to the superior thyroid vasa, and may be above or behind the upper polar cross-section of the thyroid. The frequency of occurrence of this nerve injury during thyroidectomy surgery ranges from 0-58% [3].

Efforts to avoid EBSLN injury during the arctic release of the thyroid are by ligating the superior thyroid vasa by identifying the nerve by cutting the sternothyroideus muscle at its base and by slightly pressing the upper pole of the thyroid, but this action can be done if the thyroid mass is not too large and is rarely done [4]. Another action to identify this nerve is by using intraoperative nerve monitoring (IONM) device, this monitoring action is rarely performed because not all hospitals have these facilities. Moreover, several literature studies stated that monitoring this nerve will not guarantee that the nerve is free from injury [5].

Based on the efforts to identify EBSLN and the high risk of nerve injury despite using nerve monitoring device, it is necessary to find a way or method of how to ligate the superior thyroid vasa when releasing the thyroid pole without causing injury to the nerve. Furthermore, the superior thyroid vasa ligation as distally as possible (close to the thyroid capsule) at the upper pole of the thyroid to avoid EBSLN injury during thyroidectomy surgery, is a method that is thought to be able to secure the nerve from injury without identification and use of nerve monitoring (IONM) devices.

Recurrent laryngeal nerve (RLN) injury is the main cause of voice changes after thyroidectomy, while EBSLN injury is another cause of changes in both sound quality and high-pitched sound production. However, the effects that occur on sound and swallowing as common complaints often occur after thyroidectomy despite the absence of decreased function of the RLN and EBSLN.

The aim of this study was to assess the success rate of the surgical method of superior thyroid vasa ligation as distally as possible to avoid EBSLN injury without the identification of these nerves using a thyroidectomy-related voice questionnaire (TVQ). The modified score became the injury-related voice of EBSLN (SKC-EBSLN) score. The SKC-EBSLN questionnaire scoring was used as a research parameter by comparing the scores before and after thyroidectomy surgery. The results of this questionnaire can predict the occurrence of EBSLN injuries and have been studied to have sensitivity and specificity values of 86% and 88%. [6]. Researchers have also tested the validity and reliability of the modified SKC-EBSLN questionnaire through the statistical test method, the value of Corrected Item-Total Correlation was >0.3 for validity, and the values of Cronbach's Alpha was >0.6 for reliability. The result was that the pre-operative SKC-EBSLN questionnaire scoring was invalid but reliable while the post-operative SKC-EBSLN questionnaire was valid and reliable. This means that the postoperative SKC-EBSLN scoring can be used as a research parameter.

In this study, each thyroidectomy surgery performed by surgeons from the Head and Neck Surgery staff of Faculty of Medicine of Unair / RSUD Dr. Soetomo Surabaya is considered to use the surgical method of superior thyroid vasa ligation as distally as possible when releasing the upper pole of the thyroid to avoid EBSLN injury. In accordance with the inclusion and exclusion criteria, during the last 6 months of 2020 carried out at Dr. Soetomo and several hospitals in the city of Surabaya and its surroundings, it is found that 27 patients as research subjects with a minimum sample size of n = 22.

From the data on patient demographic characteristics, female patients were more dominant than males, which was 22 patients (81.5%): 5 patients (18.5%). The age range of 27 patients was 15-81 years, mostly in the >50 years age group for 15 patients (55.6%), followed by the 31-50 years age group for 8 patients (29.6%), and the <30 years age group for 4 patients (14.8%). In accordance with the research of Kwon et al. 2017, which reported that 180 female patients as the study sample and their age range were between 18-82 years, the mean age of 51 + 12.6. The impression is that thyroid gland disease is more dominant in women than men with the largest age range between 50-60 years.

From 27 patients, the basic clinical characteristics of the patients were examined in the form of clinical diagnosis, type of surgery, size of tumor mass, and results of AP. The most clinical diagnoses in this study were bilateral non-toxic multinodosa struma followed by non-toxic uninodosa struma, thyroid carcinoma, and toxic diffuse struma. Previous studies have stated that non-toxic multinodosa struma was one of the most

common thyroid gland disorders worldwide. A study conducted in Shandong province of China stated that the prevalence of non-toxic multinodosa struma in the area was 40.1% [7].

The majority of types of surgery performed in this study were total thyroidectomy, while subtotal thyroidectomy was the least type of surgery performed. A meta-analysis study states that total thyroidectomy has a lower tumor recurrence rate than subtotal thyroidectomy, but with more side effects [8]. However, this is not in line with the research conducted by Giles et al. which stated that the tendency for transient recurrent laryngeal nerve pareses in total thyroidectomy did not differ significantly from subtotal thyroidectomy, even in the study by Pappalardo et al. stated that the incidence of permanent recurrent laryngeal nerve paresis in patients undergoing total thyroidectomy was significantly lower than that of subtotal thyroidectomy [7,8].

Tumor sizes in this study were classified in the <5 cm group, between 5-10 cm and >10 cm. Tumors with a size of 5-10 cm were the largest group in this study. As for the results of AP, this study stated the most results were benign tumors.

The difference in the basic clinical characteristics of patients in this study was thought to provide variability in the analysis results. However, from the results of the analysis of each patient's basic clinical characteristics such as clinical diagnosis, type of surgery, tumor size, and the results of the AP examination on the results of the postoperative SKC-EBSLN score, the result indicated that there was no significant difference in any variability of patient characteristics on the results of the postoperative SKC-EBSLN scoring. This means that the clinical diagnostic factors, the type of surgery, the size of the tumor mass, and the AP results did not determine the post-operative SKC-EBSLN scoring results, therefore, the surgical method of superior thyroid vasa ligation for the release of the upper thyroid pole in thyroidectomy for 27 patients was not affected by the factors mentioned above.

The TVQ questionnaire score was developed by Chun et al. in 2012, which was used to measure the sound ability related to thyroidectomy surgery. Ideally, the measurement of the TVQ questionnaire score is carried out before and after surgery, to screen for voice abnormalities before thyroidectomy surgery. This questionnaire has been extensively researched, has been validated, and has a specificity of 86% and a fairly high sensitivity of 88% [9,10].

In this study, EBSLN injury was detected or not by using the SKC-EBSLN questionnaire score performed before and after thyroidectomy surgery. There was 1 patient (3.7%) who had an abnormal SKC-EBSLN score before surgery. After surgery, the abnormal SKC-EBSLN score increased to 14 patients (51.9%). There was a significant difference between the data that was assessed from the SKC-EBSLN questionnaire scores before and after surgery.

The post-operative SKC-EBSLN questionnaire was valid and reliable, has 8 questions with the lowest score was 0 (without complaints and voice symptoms) and the highest was 32 (mood disorders with severe symptoms). Based on the index score of complaints and symptoms that arise after surgery, the normal or mild scores were 0-8, moderate scores were 9-23, and severe scores were 24-32. From the 3 scoring groups, it was found that 15 patients (55.6%) had normal or mild symptoms, 9 patients (33.3%) had moderate symptoms, and 3 patients (11.1%) had severe symptoms.

At the time of this study, the method of superior thyroid vasa ligation as distally as possible may not have been assessed as a safe surgical method to avoid trauma to the EBSLN. There is still a need for follow-up time to assess the success of the method of surgery in 3 abnormal patients with symptoms of severe voice disorders. So it is recommended to do a video diagnostic examination of laryngostroboscopy and EMG of the larynx, in order to prove the presence or absence of abnormalities in the vocal cord and EBSLN paralysis. This may imply that post-operative thyroidectomy sound quality disturbances often occur even though EBSLN does not necessarily occur.

Finally, in this study, the correlation between the SKC-EBSLN questionnaire score and the postoperative 1-octave tone ability test was also sought, namely a significant difference in the scores between the post-operative SKC-EBSLN questionnaire scores and the postoperative 1-octave tone ability test results. This ability test is more objective in assessing EBSLN function than filling out the SKC-EBSLN questionnaire

because the researcher directly listens to the patient's voice and can even be recorded for acoustic analysis. However, in this study, acoustic analysis was not included in the dependent variable. The results of the correlation were as follows:

a). There were 9 patients with normal and capable conditions, meaning that these patients had no problems with high-pitched sounds.

b). There were 5 patients whose condition was abnormal and capable, meaning that these patients were able to sing the high notes but there were still other complaints.

c) There were 10 patients whose conditions were abnormal and were still incapable to sing the high notes, therefore, these 10 patients still needed a follow-up to assess the ability to sing high-pitched sounds.

d) There were 3 patients whose conditions were abnormal and incapable, thus, these patients took time for having complete healing.

It is necessary to do video laryngostroboscopy and EMG of the larynx after 6 months postoperatively to diagnose the presence of vocal cord and EBSLN paralyze as prognostic factors and seek appropriate treatment.

There was a significant difference between the SKC-EBSLN questionnaire score and the postoperative 1-octave tone ability test. This means that; a) these test tools are very useful for assessing the current condition of the postoperative EBSLN, and b) the two tools can work together when used as a test tool to assess the condition of the postoperative EBSLN.

Voice changes after thyroidectomy surgery in the absence of injury to RLN or EBSLN often go unnoticed. Possible causes could be a laryngotracheal fixation with decreased vertical motion, cricothyroid muscle dysfunction, surgical injury, or temporary dysfunction of the extralaryngeal strap muscles, trauma to orotracheal intubation and laryngeal edema, severe retractile cervical scar, local neck pain, and psychological factors [11].

Researchers have used the SKC-EBSLN questionnaire to 27 patients as research subjects at the same time after surgery including data collection before surgery with the results described above.

In a previous study, this TVQ score has been used to evaluate the sound condition before and after thyroidectomy surgery for 4 periods of time, namely, before surgery, and after surgery (2 weeks, 2 months, and 8 months) with the result of a change in value [8]. Abnormal values were higher at 2 weeks to 2 months after surgery and there was a significant decrease in abnormal values at 8 months after surgery. So that there was a significant difference between normal values before surgery and abnormal values 8 months after surgery.

Although this questionnaire score has been shown to have high sensitivity and specificity, it is necessary to carry out a similar study using more standard parameters in assessing the presence of EBSLN injuries using video laryngostroboscopy and laryngeal electromyography (LEMG). The samples used in this study were few and no controlled randomization was carried out, for this reason, the sampling time span also needs to be extended so that a larger number of samples will be obtained.

In this study, 3 patients (11.1%) were suspected of having EBSLN injury after thyroidectomy surgery, compared to previous studies, the results varied depending on the method of surgery performed, those were:

a. Thyroidectomy without knowing the course and identification of EBSLN was 9-28% [2,8]

b. Thyroidectomy with EBSLN identification was 8-14% [2,8]

c. Thyroidectomy with EBSLN identification and using intraoperative nerve monitoring (IONM) was 0-1% [2,8].

As a comparison, the results of the study by Hurtado-Lopez et al. 2017, from 100 patients undergoing thyroidectomy without EBSLN identification and with nerve identification using IONM who experienced EBSLN injuries was 28% with details of 20% EBSLN injuries that were transient/temporary and 8% permanent EBSLN injuries.

In this study, although the ligation method has been carried out well, if there are still EBSLN lesions, it may be due to excessive use of monopolar electrocautery for homeostatic in the upper arctic area of the thyroid, nerve

ischemic due to the disrupted blood supply, possibly the anatomical location of the EBSLN according to Cernea 2a and 2b criteria (37, 5%) which often led to the entanglement of EBSLN when performing superior thyroideal vasa ligation, especially during the large goiter mass for >25 cm in diameter. Therefore, to prevent the occurrence of EBSLN lesions, the researchers suggested that before performing superior thyroideal vasa ligation, do EBSLN tracking in the "Jolles area" or use a neurostimulator and IONM.

Conclusion

As many as 51.9% of patients had normal voice conditions with few complaints and were capable to sing 1-octave tone after surgery using the superior thyroidal vasa ligation method as distally as possible. The SKC-EBSLN questionnaire is a valid and reliable questionnaire to evaluate the results of thyroidectomy surgery.

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Effect of Wobble Board Training with Mirror Feedback and Computer based Balance Board Training on Static and Dynamic Balance and Quality of Life in Elderly Population

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Abstract

Background: The extent of the risk of falling rises with ageing, and the number of falls and accidents that occur strongly contributes to admission to a nursing home. Fall is mostly caused due to lack of balance and also affect the quality of life, so the various Physiotherapy approaches are available for prevention and treatment of fall but very few studies utilized computerized balance board training program to improve the balance, hence need for the study is to find out the efficacy of balance board training & to enhancing balance and gait as well as the significance of these training on static and dynamic stability and quality of life in the geriatric population.

Method: Total n=96 elderly people will be targeted who are about 60 years of age or older. Participants will be randomly separated into two groups (control or experimental group).The training session design for a 6 weeks and each session for a 20-30 minute. Outcome measures will be used to assess the static and dynamic stability and quality of life in the elder population group.

Discussion: This clinical work seeks to equate the impact of traditional wobble board balance instruction in the elderly community and computer-based balance board preparation. To conclude, this work will help elderly people strengthen their static and dynamic equilibrium, and also increase their quality of life.

Keywords *– Wobble board, Computer-based balance board, Static and Dynamic balance.*

Introduction

India is the second-most populous country in the world for the geriatric population. More than one third of the group-dwelling people aged 60 years and older in the geriatric community are at risk of declining once a year on average. Upon aging, the level of anxiety of dropping grows, and the frequency of slips and injuries that arise also lead to entry to a nursing home.(1)

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Aging is defined as a process that is genetically determined and environmentally modulated or it is simply getting old.(2)

The number of cells that comprise the body decreases and the physical adaptability is slowly lost, resulting in death. It is related to a detriment of muscle strength and peripheral motor and sensory nerves, impairment of bilateral vision, head movement coordination through the sensory and vestibular cortex. These disabilities may lead to a loss in balance and poor gait in the older population, Secondary to the ordinary ageing process.(3)

There are various strategies for enhancing balance and gait within the geriatric people. Conventional wobble-board stability techniques are used to improve older people's equilibrium.(4) Conventional wobble board balance training used to effect on physical

functional ability by controlling their centre of gravity and maintains a static and dynamic stability postures in unstable surface condition, also it helps to enhance the strength and endurance in elder population during training programme. Wobble board balance activity incorporates an understanding the movement techniques (i.e. hip, knee, ankle and walking techniques) and muscle function trends arising from an unpredictable individual standing on a wobble board surface.[5]

Falls and fall-related injuries can cause limited mobility and functional decline leading to disability and may have a negative effect on the socioeconomic status and quality of life (QOL) in elderly individuals. Daily living functions are often affected due to weakness in core muscles such as rectal abdominals, abdominal transversals, and oblique inner and outer muscles. This will result in a lack of ability to expand the objects away from the body.[6]

Physical factors affecting falls include weakening of lower extremity muscle strength, decreasing gait capacity, decreasing balance capability, and declining sensory-motor control. The training exercise is an effective activity to improve bone health, muscle strength, and balance, thereby reducing the risk of falling.[7] Static and dynamic balance requires the integration of various sections including the sensory systems, the central nervous system, and the motor system. The integrity of joints, ligaments, tendons, and muscles plays a major character in maintaining a proprioceptive response to maintain the balance.[8]

Balance impairment was associated with a greater incidence of falls. For the patient, the cost of falling causes both in terms of physical and psychological damage, losing confidence, as well as the financial burden on the health care system, is increased. Of these reasons, it is important to look at approaches that helps for improving the individual balance and can avoid such fall risks.[9]

The benefits of conventional wobble board training in young players have been described in numerous reports; however, the effectiveness of balancing board instruction for the elderly has remained uncertain. Hence the essentiality for the training is most in the elder population.

Aim

To find out the Effect of wobble board training with mirror feedback and computer based balance board training on static and dynamic balance and quality of life in elderly population.

Methodology

Study setting:

This study will be conducted with a sample size of 96 participants, selected according to feasibility in the Department of Community Health Physiotherapy, RNPC, Sawangi (Meghe), Wardha, on approval by the Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University.

Study design and sample size:

The design of the study is a single-blinded randomized controlled trial of a computerized balance board training and conventional balance board training for elder individuals with fall risk. In this trial total 96 participants (n=96) will be enrolled in this experimental study, the subjects will be randomized into two group independent design (Group A and Group B) through cheat method of randomization. Group A will be receive wobble board training with mirror feedback while Group B will be receive computer based balance board training. Participants will receive the training protocol for alternate days in a week continue for a 6 weeks. Before inclusion, the participants will be explained about the objectives and approaches of the study, and written patient consent forms will be signed by them. The study procedure is shown in Figure 1 and the schedule of enrolment, interventions, and assessments of the study (as recommended by Standard protocol items: recommendation for intervention trials (spirit), (2013) is illustrated in fig 2.

Procedure

Fig. 1 Illustrating the study procedure.

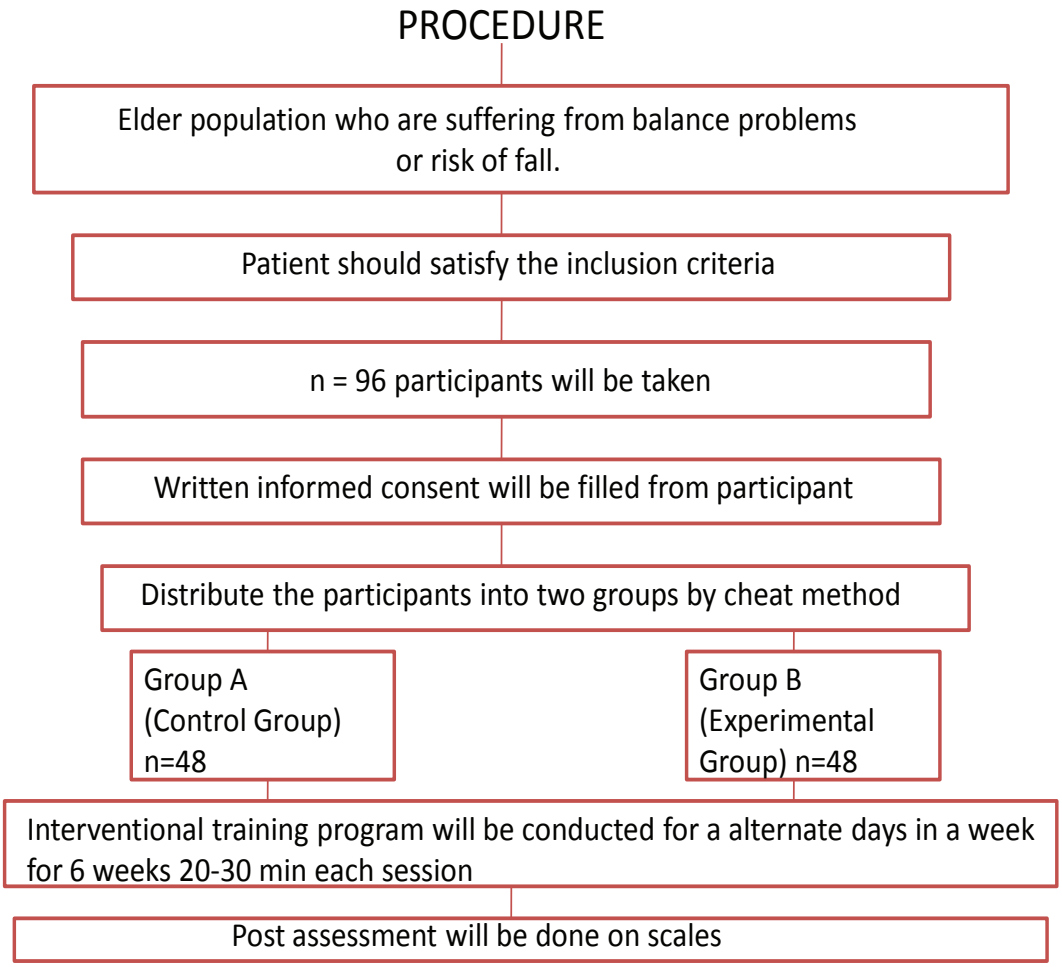

Fig. 2 Schedule of enrolment, interventions and assessments.

	STUDY PERIOD				
	Enrolment	**Allocation**	**Post- Allocation**		**Follow up test**
TIME POINT	**-t1**	**0**	**Intervention (t1-10)**	**Post-test (t11)**	**Tx**
ENROLLMENT:					
Eligibility screen	X				
Informed consent	X				
Allocation		X			
INTERVENTIONS:					

Cont... **Fig. 2 Schedule of enrolment, interventions and assessments.**

[Conventional]			X		
[Computerized Balance board]			X		
Baseline Variables: Tinetti Scale, QOL	X				
Outcome Variables: Tinetti Balance scale, Quality of life	X			X	X

PARTICIPANTS

This analysis would include 96 participants who meet the inclusion requirements.

Inclusion criteria:

Age group: above 60 years healthy individual able to understand and follow the simple information; male and female; [4] Can mobile and execute their functions of daily livings minimum; [4] male and female.[8]

Exclusion criteria:

A significant cognitive disability that impeded their comprehension of instructions, Musculoskeletal condition (fracture of lower limb, amputation), neurological (Parkinson disease, severe stroke, multiple sclerosis, vertigo) or visual, auditory impairments and severe conditions that might affect the measurements, Head injuries with or without loss of consciousness, Use of foot orthotics or walking aids.

Recruitment Procedure:

Participants who are 60 years of age and older and who have a balance issue that affects their quality of life and suits eligibility criteria will be enrolled in the RCT analysis. Sample size calculation was done from a study that compared the effects in geriatric population of the conventional balance board training and the electronic balance board training. In power calculation, we calculated the sample size per group required being 48 elderly people with 95 percent confidence interval, 0.14 is prevalence and 0.50 and 0.36 standard deviation (SD) of group A and group B respectively. Hence, in each group 48 subjects are selected.

Once the eligibility criteria for their enrolment in the sample have been achieved, the subjects must experience the examination of performing difficult tasks under safety measures. To measure their chance of dropping and being acclimatized to the equipment. And certain people with a greater probability would be exposed to randomisation. Informed patient consent will be cumulated before the allocation and after elaborating the purpose, nature, procedure, prospective benefits and after effects of the intervention.

Randomization:

Following the baseline evaluation, participants who fulfil the requirement of inclusion they will be assigned randomly to either group GROUP A or GROUP B. An independent statistician will prepare a computer-generated randomization schedule in randomized permuted blocks to ensure that the number of participants undergoing the two types of training is closely balanced within each group and that the allocation number is placed in invisible sealed cheats. After signing the consent form, certain cheats will be made available to the participants.

Blinding:

Tester(s) will be blinded to assign the subjects to the group. To ensure binding, subjects will be mandated not to reveal any details of their treatment to the tester.

STUDY PROCEDURE:

The participants will be categorised into 2 groups:

GROUP A: (WOBBLE BOARD TRAINING WITH MIRROR FEEDBACK)

To improve the static and dynamic balance, wobble board training will support, by which they will observe and monitor the balance frequently to maintain an unstable surface position. Conventional wobble board training with mirror feedback will give the external feedback to control upright standing posture and accommodates to provide visual feedback which increases the effectiveness in the upright position. And balance training protocol consists of different exercises with repetitions, if the patient get tired, offering 1-2 min rest. Complete training protocol will do under the supervision and under adequate safety measures for alternate days in a week (3-4 days) for 6 weeks;

Training Program:

The participant will initially be given a small warm-up period to reduce the chances of any incident.

1) Balancing on balance board with open eyes and closed eyes.

2) Lateral tilt exercises

3) Anterior – Posterior tilt exercises

4) Functional reach-outs

5) Forward and backward bending or sway

6) Forward and backward step balancing

7) Single leg stance

8) Sitting to standing

9) Stepping

GROUP B: (COMPUTER BASED BALANCE BOARD TRAINING)

Participants will learn the techniques for the training protocol during the first familiarisation session. They will be taught for the handling of how to operate a computer-based balance board and correct guidelines. First, the participants will be explained and understand the procedure. A computer-based balance board visualizes a screen that gives feedback during training about the balance board and positioning. It helps to provide electronic feedback which increases the effectiveness in the posture and participants can adjust them according to the feedback. Then balance training protocol will include different exercises with repetitions. Complete training protocol will be done under supervision and under adequate safety measures for a minimum of alternate days (3-4 days) in a week continue for 6 weeks.

Training Program:

Initially, a small warm-up period will be given to the participant to reduce the chances of any incident.

1) Balancing on balance board with open eyes and closed eyes

2) Lateral tilt exercises

3) Anterior – Posterior tilt exercises

4) Functional Reach outs

5) Forward and backward bending or sway

6) Forward and backward step balancing

7) Single leg stance

8) Sitting to standing

9) Stepping

Elder people will be motivated to maintain the training schedule by adequate balance training instructions for up to 6 weeks, but there might be some reasons for leaving. Participants that experience discomfort due to physical inability or pain, the procedure will not be allowed to engage anymore. Participants who plan to quit will also be contacted with respect to the reason for discontinuation. Furthermore, if they are discontinued early, replacements will be selected.

Outcome Measures:

Tinetti balance assessment scale (performance-oriented mobility assessment)

The Tinetti balance assessment scale is used to assess balance and gate in older adults to assess static and dynamic stability during activities of daily life and

fear of falling. The Tinetti test uses a 3- point ordinal scale of 0,1and 2. Gait is scored over 12 and balance is scored over 16 total is 28. The lower the score on the Tinetti test, the higher the risk of falling. The score is ≤ 18 has a high risk of fall, 19-23 has moderate risk fall, and ≥24 has a low risk of fall. The pre-assessment and post-assessment will be done on the Tinetti balance assessment scale.

Quality of life Scale (QOL)

Quality of life assessment scale is a test that measures health status and commonly used in health economics as a variable. This scale includes physical functioning, physical health problems, energy/fatigue, bodily problems, role limitation due to personal or emotional problems and well being, social functioning, and general health perceptions. Here 36 items are distributed by the International Resource Centre for a health care assessment. Scoring is done in 0 to 100 ranges so that the lowest and highest possible scores are 0 and 100 respectively. Maximum the score better the quality of life and vice versa. Hence the quality of life will assess before and after the training period.

Data Collection And Management

Data Collection

At baseline and at the conclusion of a training cycle of 6 – weeks, data should be compiled. Demographic details will be gathered and all evaluations will be performed by physical skill testing and an appraisal system of functions. In the setting of the study, non-electronic data such as hard copies of evaluation forms, signed consent forms, etc. will be stored securely. Financial incentives for attending test sessions will be given to enhance participant retention. In addition, regular feedback on adherence and call-back (for in-person therapy attendance) will be used. Access rights of the contractor must rest with the principal investigators (PI).

Data Management

Data collection and documentation will be done under the guidance of the principal investigators. The study documentation will be evaluated thoroughly for accuracy. The Excel spreadsheet will be released at the end of the study to an allocation blinded statistician for conducting the necessary analysis, following which unbinding of the groups will be done. Checklists are used to prevent missing data due to the improper staff procedure.

Statistical Analysis

Data obtained will be written down and then will be put in a tabular format. It will be analyzed using the new edition of SPSS. All statistical analyzes will be carried out with a trust interval of 95 per cent (p-value < 0.05) to determine the influence of two tests. The homogeneity of the two sample groups would be evaluated using the Student's t check for the individual studies.

Bias

The data obtained is written down and then inserted into tabular format. Using the latest SPSS version it can be analysed. To assess the effect of two variables, all statistical analyzes will be performed with a confidence interval of 95 per cent (p-value < 0.05). The homogeneity of the two study classes will be measured using the person studies t test of the Student's.

Discussion

The protocol is going to be conducted for a single-blinded randomized clinical trial` to test the effect of wobble board training with mirror feedback and computer-based balance board training, incorporation of which procedure during training session would improve the static and dynamic balance stability, strength, endurance in elder peoples to reduce the fall injury and improve the quality of life. Conventional wobble board balance training used to effect on physical functional ability by controlling their centre of gravity and maintains a static and dynamic stability postures in unstable surface condition. When it is associate with mirror feedback it helps to give a visual feedback and maintain upright posture, and it also helps to build up participant confidence.[10], [11]

Computer based balance board training is a modified electronic balance board which have sensors with visualising screen. It is new technology based device which used for balance training in elderly people.[12] It also helps to improve static and dynamic posture which is used to enhance compatibility in day to day life.[13] Which will ultimately helps in increasing

functional ability and quality of life by visual feedback, balance board sensors which gives accuracy during training, and helps the participant to make confident as well as motivated during the training.[14] As per the study, Mhaske GC, et.al. Discussed in there about the conventional balance training and physical exercises to increase flexor and extensor muscle strength in the geriatric population. Although the impact on the general for decrease the pain and incapacity improving joint function, the integrity of muscle, and ligaments which proportionally affect balance.[1],[15] Aditi Khot, et.al. Involved in their computer-based balance board training study which is a modified electronic balance board with visualization screen sensors. This is a modern technology-based system being used to educate elderly people in equilibrium.[2], [4] It also helps improve static and dynamic posture that is used in day-to-day life to improve compatibility. Ultimately, this will help improve functional ability and quality of life through visual feedback, balancing board sensors that provide accuracy during training, and help the participant to become confident and motivated during training.[16]

Procedures used in the study have fewer negative consequences and are more productive. The hypothesis may or may not reflect the results of this study. In addition, this longitudinal study will help elder people and make an impact on their quality of life.

Ethical Approval and Dissemination:

The Procedure is planned according to the Helsinki Declaration. The key results about the efficacy of wobble board training with mirror input and computer-based balancing board testing on static and fluid equilibrium and quality of life in the elderly population will be reported in a peer-reviewed journal and discussed at an international conference. The test participant participants and the DMIMSU which will finance this analysis will access the study's main findings. The details of the subjects enrolled stored securely for at least 5 years. After the analysis is complete, the data will be deposited in the DMIMSU data repository and the findings will be released.

Patient Consent

Principal Investigators will obtain the informed consent from the patient and one of the relatives on a printed form with signatures and give the proof of confidentiality.

Confidentiality

The study program is explained to the participant and one of his / her relative, and personal information is taken from the principal investigator. Information of specific participants will be kept separate from the central dataset, and will not be exchanged. To preserve confidentiality, all personal data will be stored securely before, during and after the tribunal.

Author's Contribution: All authors made the best contribution for the concept, assessment and evaluation, data acquisition and analysis and interpretation of the data.

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Stevens Johnson Syndrome in Babylon

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Abstract

SJS and TEN are emergency medical conditions, rare but with high mortality rate. They start as flu like illness with high fever. They are mucocutaneous disorders Affect mainly the skin, eye, nose, mouth and genitalia, with characteristics rash, blistering, peeling of the skin. Two accused as a cause: certain drugs, and infections like Mycoplasma pneumonia. In order to overcome the lack of informative studies about these syndromes, and to lower the high mortality rate associated, we followed the occurrence, diagnosis, management and prognosis of these syndromes in one year field study in the province of Babylon, Iraq from the 1st of March 2018 to the 1st of March 2019. The data were taken from the medical records of 4 major hospital in the center of AL Hilla city, Iraq (Al Hilla teaching hospital , Merjan teaching hospital Babil teaching hospital, and Al Noor pediatric hospital). Five cases of this syndrome where reported, 3 males and 2 females with age range 23-30 years. One female survived. Four patients died. Full typical autopsy with necessary laboratory tests where achieved in the department of Forensic Medicine in Babylon. The occurrence of this syndrome was 5 /2 million /year, the diagnosis was delayed and accordingly the management and lines of treatment were delayed too, leading to bad prognosis (four deaths, one survival). The mortality rate was 80%. The direct causes of death were pneumonia and encephalitis associated with Stevens Johnson Syndrome. This syndrome should be kept in mind when prescribing a medication. Early management is leading to good prognosis.

Key words: *Mortality; Occurrence; Steroids; TEN (toxic epidermal necrolysis);*

Introduction

Steven Johnson syndrome Is a mucocutaneous necrosis with at least two mucosal sites are involved Skin involvement may be limited or extensive. Drugs or combination of infections and drugs are the main associations. Epithelial necrosis with inflammatory cells seen on histopathology. Steven Johnson syndrome may have features overlapping with toxic epidermal necrosis including a prodromal illness.[1]

On the other hand, Toxic epidermal necrolysis is a medical emergency because the extensive mucocutaneous blistering is associated with a high mortality rate. It is usually induced by drug, 1 to 4 weeks after intake, the patient becomes unwell and often feverish and the blistering develop on the trunk but rapidly involving all skin, an early warning sign is cutaneous pain. By pressing gently on the lateral side of the affected skin, this can result in epidermal detachment(Nikolsky sign), demonstrating the severity of skin fragility. Blistering of the skin and mucosae maybe hemorrhagic.[2]

Several drug may induce TEN or SJS including: Allopurinol, Trimethoprim-sulfamethoxazole, and other sulfonamide antibiotics, aminopenicillins, cephalosporin, quinines, carbamazepine, phenytoin, phenobarbital, and NSAIDS of the Oxicam type [2,3,4,5,6,7,8,9,10,11,12], cases were encountered also with paracetamol and Metronidazole [13,14].

Toxic epidermal necrolysis has a skin reaction with a large degree of separation more than 30%of the skin area while Steven Johnson syndrome has a skin reaction with a degree of skin separation less than 10% of the skin area. There is an intermediate degree which is an overlap between Steven Johnson syndrome and the toxic epidermal necrosis sizes With a degree of cutaneous separation between 10 to 30% [2,15].

Mycoplasma pneumonia and Herpes simplex virus, Herpes zoster, HIV, and Hepatitis A infections are documented as clear causes. There are rare cases of unknown etiology. Genetic susceptibility to SJS and TEN is likely. There is a strong association observed in Han Chinese, Southeast Asian or Indian between a genetic marker, the human leukocyte antigen HLA-B*1502, and SJS induced by carbamazepine. In children, unlike adults, infections may be the only cause for SJS [16,17,18,19,20].

The risk factors in SJS: AIDS infection, people infected with HIV infection have incidence of Steven Johnson syndrome about 100 times greater than among the general population, weakened immune system may have an increased risk of SJS. The immune system can be altered by increased age, malignancies, an organ transplant or HIV infection, prolonged intake of steroids and auto immune disease, history of a Steven Johnson syndrome (if you have SJS and related form of such condition you are at risk of a recurrence). A family history of SJS in a relative had Steven Johnson syndrome or TEN you may be at a high risk to develop Steven Johnson syndrome. Genetic factor: by having HLA-B 1502 gene, there is an increased risk of SJS particularly with drugs for seizures, gout or mental illness [20,21].

Diagnosis of SJS: physical examination; diagnosis relies mainly on the clinical signs So the doctors can identify SJS based on medical history and physical examination. Skin biopsy, histological analysis of a skin biopsy showing typical full thickness epidermal necrolysis due to extensive keratinocytes apoptosis. Lesion biopsy to rule out other possible causes. Skin Culture, oral culture, to detect infections. X ray Imaging: may be neede in suspected infection like pneumonia. Blood culture: to isolate suspected infection. Differential diagnosis: linear IgA dermatosis, Para neoplastic pemphigus, pemphigus vulgaris, bullous pemphigoid, acute generalized exanthematous pustulosis (AGEP), disseminated fixed bullous drug eruption, and staphylococcal scalded skin syndrome (SSSS), erythema multiform associated with herpes simplex [20,22].

The signs and symptoms in SJS are: fever, unexplained widespread skin pain, red or purple spots has dark center like arrow throwing discs, blisters on the skin and the mucous membranes of mouth, nose eyes and genitalia, shedding of skin within days after a blisters form, Sore mouth and throat and Fatigue, cough, and Burning eyes [20].

The management of TNE and SJS should started in burn unit or an ICU [21,23,24,25,26]. General measures: immediate withdrawal of all suspected drugs(drugs with long half-lives have a high mortality rate. Supportive measures by maintaining the body temperature, proper fluid and electrolyte balance within aseptic conditions. Medical measures: systemic and oral steroids should be used 24-48 hours [27]. Cyclosporine is the 2nd common immunomodulator used in treatment of TEN and SJS[28]. Immunoglobulin was used in the treatment and had higher mortality rate than Cyclosporine [29]. While it is still the commonest immunomodulator used around the world for treatment of TEN and SJ-S [30]. Pain killer should be restricted to certain cases. Antibiotic should be used when there is a need. Medication for treatment of eye and the mucous membrane maybe used [20]. The occurrence of SJS and TEN is 1 or 2 in every million/ year [3,31]. To predict the prognosis of the disease, a severity score is used:

Factor

- Age more than 40 years
- Pulse above 120 beats/min
- The presence of malignancy
- body surface area involved more than 10%
- urea in blood above 10 mmol/L (28 mg/dL)
- bicarbonate less than 20 mmol/L
- Blood sugar above 14 mmol/L (252 mg/dL)

Factor	**Mortality rates**
0-1	**3%**
2	**12%**
3	**35%**
4	**58%**

Factor 5 and above, mortality rate is 90%[2].

Mortality rate in SJS is 5-12%, while in TEN is more than 30%. The direct causes of death are sepsis, multi-system organs failure and electrolytes imbalance [1].

Measles is a highly contagious disease caused by a virus. Before the introduction of measles vaccine in 1963 and widespread vaccination, major epidemics occurred approximately every 2 to 3 years, and measles caused an estimated 2.6million death each year. High fever is the most prominent sign at first, which begins 10-12 days after the viral invasion, and continues to about 7 days. Running nose, inflamed eye, and tinny white color spots inside cheeks, after few days the characteristic rash appears on the face, neck and spreads to extremities. Most deaths occurred in measles are caused by complications associated with the disease. Serious complications are more common in children under the age of 5 or adults over the age of 30. The most serious include blindness and encephalitis severe diarrhea and dehydration, ear infections or severe respiratory infection such as pneumonia [32].

Material and Methods

One year field study was conducted in the province of Babylon from the 1st of March 2018 to the 1st of March 2019. The data were collected from the medical records of 4 major hospitals in the center of Al Hilla city (Al Hilla teaching hospital, Merjan teaching hospital, AL Noor pediatric hospital and Babil teaching hospital). The cases were collected, sex, age, type of specialist who treated them, their signs and symptoms, the received drugs and prognosis. The dissection and accompanied necessary tests were achieved in the Forensic Medicine department of Babylon. The diagnosis of SJS and TEN was done according to the medical records of the hospitals, signs and symptoms (by history from relatives) and typical autopsy dissections with direct examination of the skin lesions and biopsy with histopathological tests, lungs lesions with histopathological examination, blood culture to isolate Mycoplasma pneumonia. To exclude other causes of death, general histopathological examination and toxicological tests were employed.

Results

Five cases of severe skin reactions were recorded (Stevens Johnson Syndrome overlapped with toxic epidermal necrolysis). 3 males, 2 females. Age range 23 - 30 years. All were treated by physicians. The days of admission to the hospitals were in range of 5-10 days in the (internal diseases lounge) . The last level of blood urea range mentioned in the medical records 50-65 mg/dL, and heart rate 100-110 beat/min. The patients at the onset had a complain of flue like illness with high fever and a prodromal period. Received antibiotics, mainly penicillin derivatives, ciprofloxacin, antipyrexial (NSAIDS, paracetamol). 1 - 2 days later, the rash appeared all over the body and started to blister and peel. By a dermatologist consultation, they were diagnosed as SJS and TEN. They received supportive treatments mainly, antibiotics, steroids and eye preparations beside stopping the suspected drugs. Four patients died within 4-7 days after the appearance of the rash. One female patient aged 30 years survived. On autopsy: all four diseased patients appeared as 100% burn, at least one lung was affected by pneumonia (Mycoplasma pneumonia) in each cadaver, two with encephalitis. Mycoplasma pneumoniae was isolated by Blood cultures in all cases. The direct causes of death were pneumonia associated with Stevens Johnson Syndrome with or without encephalitis. SJS occurred in a rate of 5 in every 2millions/year (the population of Babylon is 2millions [33] . The mortality rate was 80%.

Discussion

Simple cases of skin reactions (eruptions) not to be hospitalized usually, so there may be bias in our study.

All cases were of that type where there is an overlap between SJS and TEN, diagnosed by dermatologists when consulted few hours before the death of the four patients, and at the appearance of rash coincidently in the case of the survived one, all in the (internal diseases lounge), while it is mandatory to be admitted into the ICU or burn units [21]. At the onset of the disease, they were diagnosed as common cold with high fever. This is accepted and concurs with literatures[1,2]. The medications they received (penicillin derivatives, ciprofloxacin, NSAIDS, paracetamol), as many literatures mentioned [2,5,6,7,13], may be the trigger to SJS and TEN. After the appearance of the rash, the cases were diagnosed as measles, while the rash in SJS and TEN is characteristic in shape and distribution[20], and additional unnecessary antibiotics continued. The SJS

and TEN are too rare diseases to put in mind while examining rash [4], and there are atypical cases of SJS associated with Mycoplasma pneumoniae without typical rash [34]. The rash appearance may necessitate a dermatologist consultation as early as possible instead of wasting critical hours. When blistering started, the physicians consulted dermatologists to put the final diagnoses SJS and TEN, and treatment started but it is too late. In order to lower the morbidity and mortality, the treatment should be started early [35].

In the case of the survived one, early diagnosis by dermatologist, early cessation of the culprit drugs, early treatment, good prognosis, and this is compatible with literatures [1,3,36]. All cases were young adults, males affected more than females, and this coincide the study of Theresa N. [37]. Medications used in treatment of the survived case seem to be effective in spite of the opinion of considering steroids a controversy [16,20]. Mycoplasma pneumoniae was isolated in all cases with clear lung involvement both grossly and histopathologically. These findings enforce the studies that pointed to the strong association between SJS and Mycoplasma pneumoniae infection [17,18,19], and be at odds with others [37]. The mortality rate was 80% which is high and goes with elevated blood urea, increased heart rate, high percentage of body surface area involved [2]. The occurrence of SJS in Babylon is approximate to that mentioned in similar studies [3,31].

Conclusion

- Stevens Johnson Syndrome is not too rare to think about when prescribing a medication.
- Stevens Johnson Syndrome and toxic epidermal necrolysis are associated with high mortality rate 80%, so they are an emergency condition in dermatology and should be treated in ICU or burn unit under the supervision of a dermatologist
- SJS usually affect young adults and males more than females.
- Rash is a dermatological problem and a dermatologist should be consulted.
- There is approved strong association between Steven Johnson syndrome and Mycoplasma pneumoniae infection, and this infection is treatable.
- Early diagnosis, cessation of unnecessary drugs With supportive and symptomatic treatment with steroids all may lead to good prognosis.
- The direct causes of death were pneumonia and encephalitis associated with Stevens Johnson Syndrome.
- SJS and TEN occurrence in Babylon is approximately the same in other places.

Conflict of Interest: Nil

Ethical Clearance: Human Rights Committee in the Babil health Directorate .

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Impact of a Mental Health Gap (mhGAP) Module on Behavioral and Psychological Symptoms of Dementia (BPSD) and Caregiver Burden in a South Indian Community

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Abstract

Background: Non pharmacological interventions are the first line treatment recommended for behavioral and psychological symptoms of dementia and mental health gap module is a non-pharmacological interventions based program developed by Word Health Organization for dementia patients especially in low and middle income countries. This study was conducted to evaluate the impact of mental health gap module on Behavioral and psychological symptoms of dementia and caregiver burden in a south Indian rural community.

Methods: A quasi experimental one group pretest posttest time series design study was conducted among 61 elderly with dementia in a south Indian rural community who were selected using purposive sampling. Pre interventional assessment was done with Neuropsychiatric Inventory Questionnaire and Zarit Burden Interview. A multifaceted mental health gap intervention was delivered to the patients through monthly home visit by community health workers for one year and impact on behavioral and psychological symptoms and caregiver burden was tested at six weeks, three months and 12 months.

Conclusion : There was a significant improvement in behavioral and psychological symptoms of dementia and caregiver burden during study period. This study proved that behavioral and psychological symptoms of dementia and caregiver burden can be managed though home based non pharmacological interventions using community health workers which is a beneficial and cost effective model for low and middle income countries.

Key words: *Behavioral psychological symptoms of dementia (BPSD), caregiver burden, mhGAP module, South India, Rural community*

Introduction

Dementia is a disease in old age marked by progressive cognitive impairment and by 2050 about 132 million people in the world will have dementia[1]. There were 3.7 million elderly in India with dementia in 2010 and will rise to 7.6 million by 2030[2]. South Indian states have the highest proportion of elderly with Kerala at the peak of 12.6%[3]. These states will be going to face a significant rise in dementia cases in the near future.

Behavioral and psychological symptoms of dementia (BPSD) are a group of non-cognitive, distressing symptoms found in 90% of patients with dementia and associated with a poorer prognosis, rapid cognitive decline, increased institutionalization, increased health care costs and loss of quality of life[4,5]. Caregiver burden

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is another phenomenon which threatens the physical, psychological, emotional and functional health and increases morbidity and mortality of the caregiver[6]. Majority of dementia caregivers reports high levels of stress, depression and anxiety[7].

Antipsychotics prescribed for BPSD has serious adverse effects, [8] thus non pharmacological methods (NPIs) are advised by various clinical guidelines[9]. World Health Organization (WHO) developed Mental Health Gap (mhGAP) intervention guide[10] to deal with the mental health gap occurred due to the unavailability of services in rural areas in low and middle income countries. In India, mhGAP is more than 90%[11], so community health workers (CHWs) can be utilized to manage community dwelling patients. The mhGAP guide has to be adapted to local situations depending on the contextual differences in prevalence and availability of resources and would be helpful for developing countries where the scarcity of the specialists is high in rural areas. Though some studies were conducted in many middle or low income countries[12], not many Indian studies were found regarding the effectiveness of mhGAP module on community dwelling dementia patients.

Materials and Methods

This study was conducted to evaluate the impact of modified mhGAP module on BPSD and caregiver burden. A quasi experimental one group pretest posttest time series study was conducted among elderly above 60 years of age who were living in Thalikkulam Panchayath at Thrissur district of middle Kerala. Seventy one patients living with a primary caregiver for a minimum period of one year were selected using purposive sampling after screening with Brief Community Screening Instrument for Dementia (CSID-BRIEF). Patients with vascular dementia and with severe medical or psychiatric illness were excluded. The study was conducted in three phases.

First phase (pre intervention):

Tool 1: A validated and pre-tested socio personal variables questionnaire with 16 items for patients and caregivers.

Tool 2: Neuropsychiatric Inventory Questionnaire (NPI-Q) is a standardized informant-based instrument measuring both the severity and distress of 12 BPSDs. The severity scale scores from 1 to 3 (mild, moderate and severe) and distress scale scores from 0 to 5 (no distress; minimal distress; mild distress; moderate distress; severe distress and extreme distress). Tool was validated by subject experts and reliability was established by inter rater method.

Tool 3: Zarit Burden Interview (ZBI) is a 22 item standardized tool to measure subjective burden of caregivers with item score ranges from 0 to 4 (never, rarely, sometimes, quite frequently and nearly always). Total score ranges from 0 to 88 and are categorized to no burden (0-20), mild to moderate burden (21-40), moderate to severe burden (41-60) and severe burden (61-88). Tool was validated by subject experts and reliability was established by test retest method.

Second phase (intervention)

The original mhGAP module 2.0 with seven sections was translated to Malayalam and modified according to cultural affiliations, from additional literature review from dementia care guides and palliative care modules and contributions from three focus group discussions with five CHWs. Evaluation and validity was done by independent experts from geriatric psychiatry, geriatric psychiatric nursing, community medicine, and community health nursing. Conceptual adequacy, appropriateness and presentation style was revised before finalizing. The flow and ease of understanding and simplicity of the language was validated by language experts. Pilot study was done with ten patients from the target population, following which vocabulary indicating the difficult terms and appropriateness of illustrations was reviewed.

We have selected seven CHWs and a multifaceted intervention was conducted as shown in table 1. We divided the 71 patients among CHWs and the interventions were delivered through monthly home visits. Each CHW kept a field diary and they recorded the interventions given and improvements noticed on each visit. Follow up meetings were conducted by researcher with CHWs every month and necessary guidance were given. Reinstitution of intervention was given after six months.

Third phase: (post intervention)

Evaluation of the effect of mhGAP module was carried out through home visits by researcher after six weeks, three months and 12 months. The impact was assessed by change in NPI-Q and ZBI scores. Ten patients dropped out during the study period and final sample size was 61.

Data Analysis

Data were tabulated and analyzed using EpiInfo 7.0 software. The test of normality (Kolmogorov–Smirnov test) was applied and data was found to be non-normally distributed (P <0.05), hence median and interquartile range were used to describe the data. Friedman's test was used to find the association between pre and post-test scores and Wilcoxon signed rank test was used to ascertain the differences between pre and post-test changes.

Results

Sociopersonal variables: Most (75%) of the patients were females with a mean age of 80.03+7.67 years and 64.8% were educated up to primary school. Majority (70.4%) were widows and 69% were living in a three generation family and 77.5% were living in their own house. Mean duration of dementia was 38.04 months.

The mean age of the caregivers was 50.38+13.21 years and most were females (98.6%), married (84.6%) and educated up to primary school (36.6%). Most were daughter- in- laws (66.2%) and 39.4% of patients were partially dependent for day to day functioning. Mean duration of caregiving was 38 months and 70.4% of the caregivers did not have any assistance in care giving.

BPSD and caregiver burden: When analyzed the pretest data, delusion (77%), apathy (75.4%) and agitation (72.1%) were the most common and hallucination (32.8%) and euphoria (26.2%) were the least common BPSD seen. When assessed for BPSD distress, apathy (74.6%), delusion (70.4%) irritability (69%) and agitation (69%) were the most common and hallucination (31%) and euphoria (25.4%) were the least common distress producing symptoms found. Regarding burden, 40.8% of the caregivers experienced mild to moderate burden and 38% of them were having moderate to severe burden (Fig 1).

Effect of mhGAP on BPSD and caregiver burden: After the mhGAP intervention, there was a significant difference in all BPSD severities (Fig 2) except for motor disturbances, all BPSD distress (Fig 3) and caregiver burden after 12 months (p<0.05). (Table 2,3). Post hoc analysis revealed that most of the significant changes in BPSD and burden happened during longer periods pretest to 12 months and 6 weeks to 12 months.

Table 1: Interactive session on mhGAP module interventions

Section	Content	Teaching learning techniques	Duration
Introduction	Dementia, Definition of dementia, Types of dementia, Stages of dementia	PowerPoint Presentation	5 mins
Creating awareness	Introduce the concept to family Revealing diagnosis of dementia Explain the treatment plan of dementia	PowerPoint Presentation	2 mins
Managing behavioral and psychological problems	Types of BPSD, Causes of BPSD Strategies to manage BPSD	Think-pair-share (scenically represented question was asked, participant was allowed to think alone and then discuss with neighbour before settling on a final answer)	5 mins

Improving social life and daily routine	How to schedule daily routine, Promoting independence, Exercises , Falls prevention, structural modifications at home	Vignette (a patient scenario was given) and discussing possible interventions	3 mins
Improving intellectual abilities	Reorientation techniques, Communication techniques	Group discussion	3 mins
Physical and mental abuse	Identifying signs of abuse, Reporting abuse	Lecturing with PowerPoint Presentation	1 min
Managing caregiver burden	Identifying signs of burden, Structuring caregiving schedule, Training caregivers, Counselling caregivers, Social support and self-help groups	Role playing using participants on specific situations.	5 mins
Instructions to be given directly to caregivers	Safety at home, Wandering, Incontinence, Diet modifications, Personal hygiene, dressing, Insomnia, Infections, Constipation, Visual impairments, Palliative care	Brain storming	6 mins
Summary/Conclusion	Summarizing, clarifying doubts, follow up instructions	Distribution of leaflets/pamphlets	

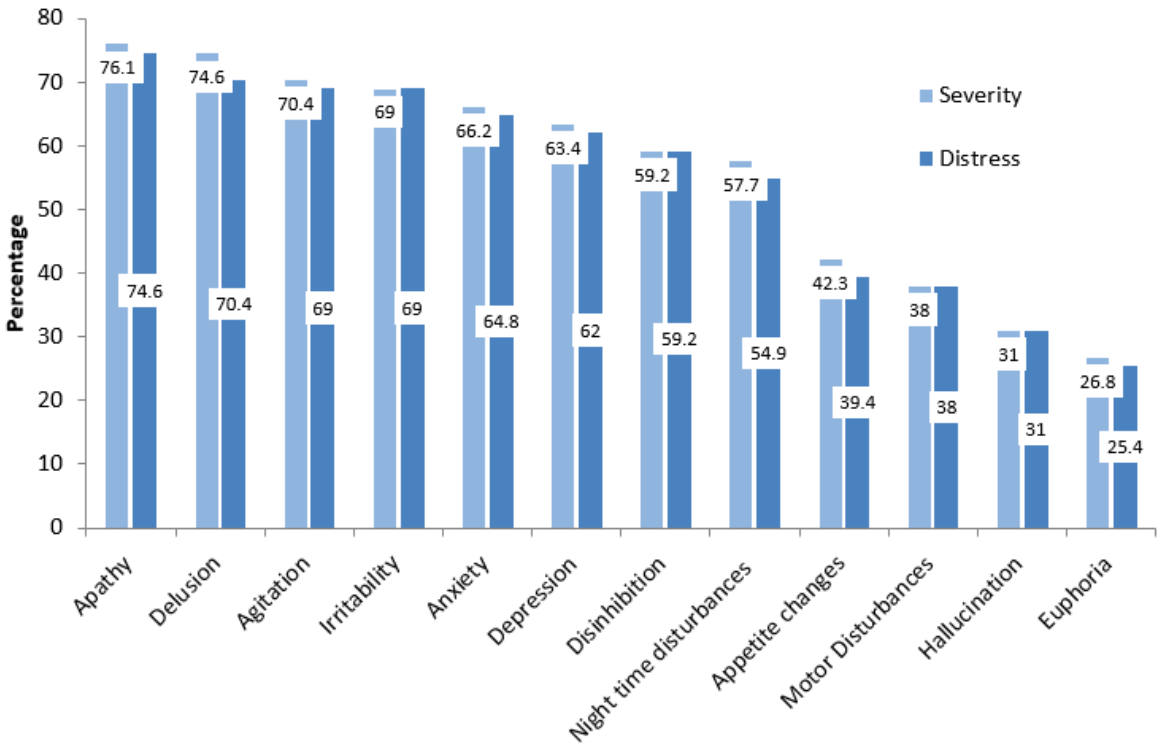

Fig 1: BPSD severity and distress among patients with dementia

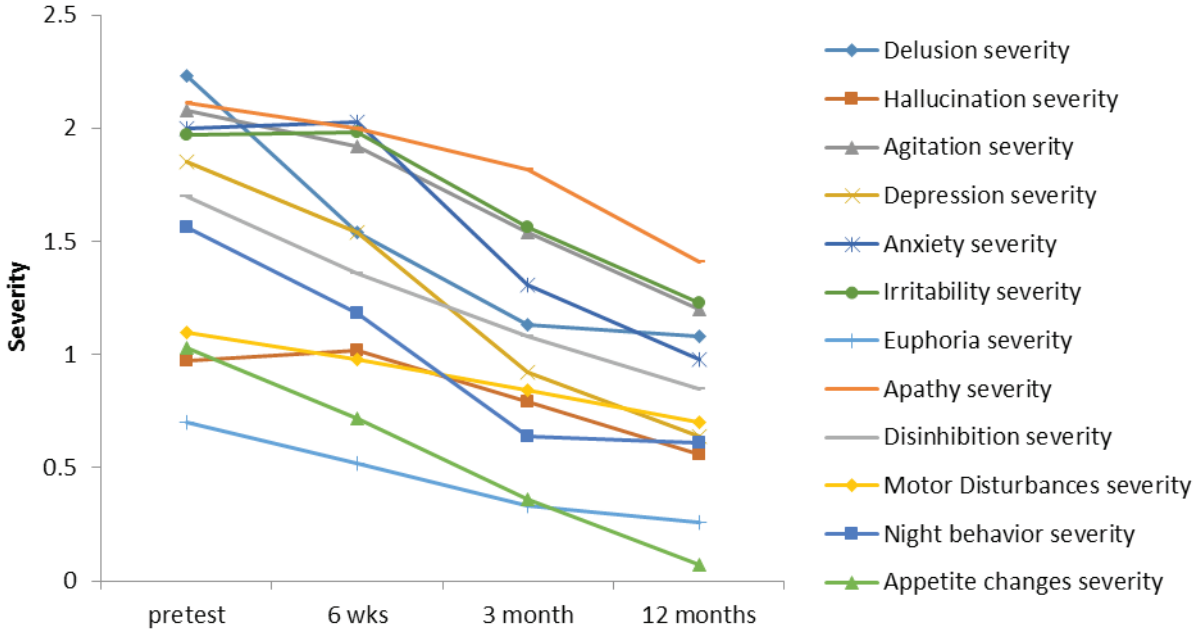

Fig 2: Change in BPSD severity over the study period n=61

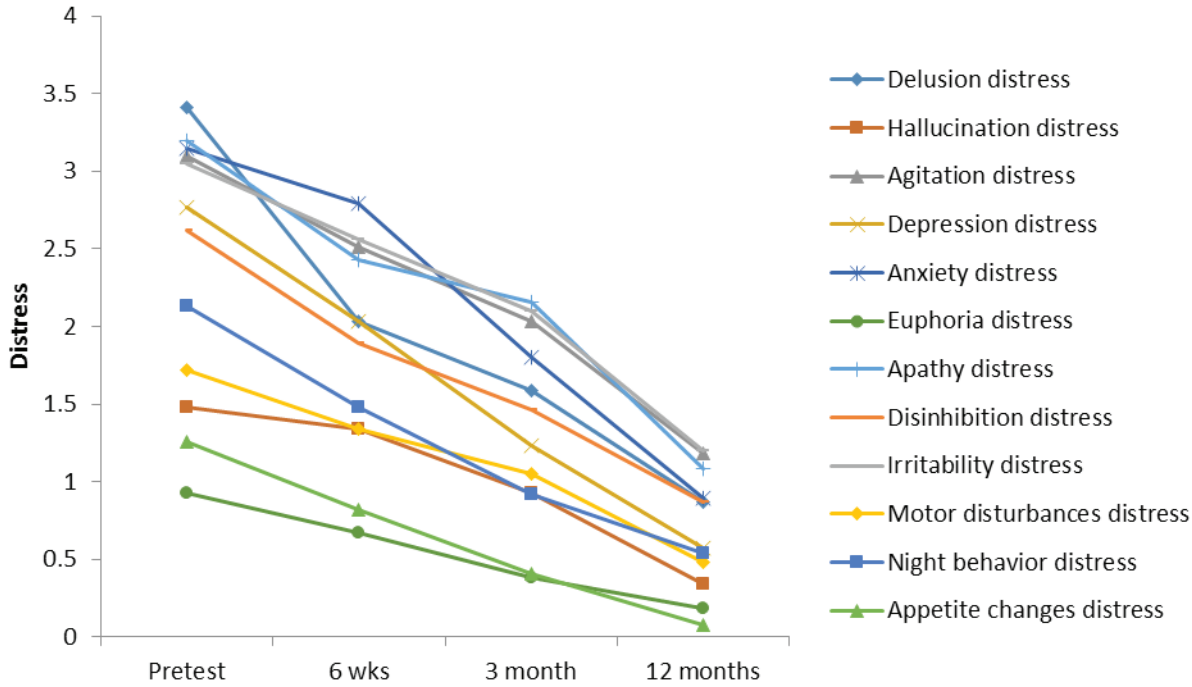

Fig 3: Change in BPSD distress over the study period n=61

Table 2: Comparison of BPSD severity scores before and after the implementation of mhGAP module

Variables	Pre test Median (IQR)	6 weeks Median (IQR)	3 months Median (IQR)	12 months Median (IQR)	χ2#	p value
BPSD total	19 (14.5 to 24)	16 (10.5 to 22)	10 (6 to 17)	8 (5 to 15)	75.18*	<0.0001
Delusion severity	3 (2 to 3)	2 (0 to 3)	1 (0 to 2)	1 (0 to 2)	42.84*	< 0.0001
Hallucination severity	0 (0 to 3)	0 (0 to 3)	0 (0 to 2)	0 (0 to 1)	9.06*	0.03
Agitation severity	3 (0 to 3)	3 (0 to 3)	2 (0 to 2.5)	1 (0 to 2)	29.48*	< 0.0001
Depression severity	3 (0 to 3)	2 (0 to 3)	0 (0 to 2)	0 (0 to 1)	39.08*	< 0.0001
Anxiety severity	3 (0 to 3)	3 (0 to 3)	2 (0 to 2)	1 (0 to 2)	46.18*	< 0.0001
Euphoria severity	0 (0 to 1.5)	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	9.91*	0.019
Apathy severity	3 (0.5 to 3)	3 (0 to 3)	2 (1 to 3)	1 (1 to 2)	19.17*	< 0.0001
Disinhibition severity	3 (0 to 3)	1 (0 to 3)	0 (0 to 2.5)	0 (0 to 2)	23.85*	< 0.0001
Irritability severity	3 (0 to 3)	3 (0.5 to 3)	2 (1 to 2)	1 (1 to 2)	30.28*	< 0.0001
Motor Disturbances severity	0 (0 to 3)	0 (0 to 3)	0 (0 to 2)	0 (0 to 2)	7.31ns	0.063
Night time behavior severity	2 (0 to 3)	0 (0 to 3)	0 (0 to 1)	0 (0 to 1)	30.91*	< 0.0001
Appetite changes severity	0 (0 to 2)	0 (0 to 2)	0 (0 to 0)	0 (0 to 0)	34.82*	< 0.0001

N=61

Friedman's test *p<0.05

Table 3: Comparison of BPSD distress scores before and after the implementation of mhGAP module

Variables	Pre test Median (IQR)	6 weeks Median (IQR)	3 months Median (IQR)	12 months Median (IQR)	χ2#	p value
Delusion distress	5 (0 to 5)	3 (0 to 4)	2 (0 to 3)	0 (0 to 1.5)	56.10*	< 0.001
Hallucination distress	0 (0 to 5)	0 (0 to 3)	0 (0 to 2)	0 (0 to 0)	21.00*	< 0.0001
Agitation distress	5 (0 to 5)	3 (0 to 4)	2 (0 to 3)	1 (0 to 2)	49.02*	< 0.0001
Depression distress	3 (0 to 5)	2 (0 to 4)	0 (0 to 3)	0 (0 to 1)	50.99*	< 0.0001
Anxiety distress	5 (0 to 5)	3 (0 to 4)	2 (0 to 3)	0 (0 to 2)	61.8*	< 0.0001

Cont... **Table 3: Comparison of BPSD distress scores before and after the implementation of mhGAP module**

Euphoria distress	0 (0 to 0.5)	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	11.44*	0.010
Apathy distress	5 (0 to 5)	3 (0 to 4)	3 (1 to 3)	1 (0 to 2)	40.11*	< 0.0001
Disinhibition distress	3 (0 to 5)	1 (0 to 4)	0 (0 to 3)	0 (0 to 1.5)	33.06*	< 0.0001
Irritability distress	5 (0 to 5)	3 (0.5 to 4)	2 (1 to 3)	1 (0 to 2)	41.06*	< 0.0001
Motor disturbances distress	0 (0 to 5)	0 (0 to 3.5)	0 (0 to 2)	0 (0 to 0)	21.78*	< 0.0001
Night time behavior distress	1 (0 to 5)	0 (0 to 3)	0 (0 to 1)	0 (0 to 0.5)	29.74*	< 0.0001
Appetite changes distress	0 (0 to 2.5)	0 (0 to 2)	0 (0 to 0)	0 (0 to 0)	32.07*	< 0.0001
Caregiver burden	41 (25.5 to 54.5)	42 (33.5 to 53.5)	36 (27 to 46)	15 (6 to 28.5)	81.07*	< 0.0001

Friedman's test *p<0.05

Discussion

The findings of this study reveals an extensive yet cost effective impact of mhGAP module on managing BPSD and caregiver burden among community dwelling patients with dementia. Systematic reviews and clinical practice guidelines recommended that NPIs are to be preferred in managing BPSD[13,14].In current study, we tested the effectiveness of an NPI based modified mhGAP module on BPSD and burden among patients and caregivers living in a rural community. In comparison, this finding is significant with a number of studies from several countries like Ethiopia, Nigeria, Pakistan and Sri Lanka[12] where mhGAP was found effective. So, this study is adding to the existing evidence that mhGAP module is suitable for low and middle income countries in training health workers and managing patients at community itself.

Current study reported delusion, apathy and agitation as most common, which is coherent with previous study findings[15, 16, 17].There are findings that motor disturbances[18] were also common but our findings did not support this. Regarding caregiver burden, we got a mean ZBI score of 42.25 which was found as high compared to the previous studies[15,19,20.] This could be because of social factors operating in India, where caregivers are predominantly females who are also responsible for household works and rearing children.

Current study demonstrated significant improvement in all domains of BPSD except motor disturbances among patients who received mhGAP interventions. Since this method is cost effective in terms of money and manpower and has a larger impact on community with minimal training of nonprofessionals, policy makers can focus on this area to widen the reach of dementia care. Evidences also support community interventions which resulted in less burden and institutionalization[21]. The common trend of decreasing BPSD over 12 months was observed and it also could be because the caregivers might have learned to handle BPSD over time. Another reason could be that when patient gets weak or bed ridden, BPSD may be less expressed and they will not be able to force caregivers to act on BPSD. In spite of all these factors considered, there was a significant reduction in BPSD after implementing mhGAP module and the researcher recommends that all caregivers must be trained in managing BPSD.

The caregiver burden scores showed a slight increasing trend initially in six weeks and later considerably reduced from six months to one year as a result of mhGAP interventions. The initial increasing

trend could be attributed to the fact that when the caregivers recognize BPSD for the first time, they feel more burdened. Subsequent decreasing trend in burden is observed as the caregiver learned to manage BPSD as the result of mhGAP interventions. It is also postulated that when the patients become physically weak and bed ridden over months, they mostly need palliative care and caregivers are less engaged, and they can divert to other activities which patient could not interfere which might have resulted in less burden.

From the post hoc analysis, it was observed that most of the significant changes in BPSD and burden happened during pretest to 12 months and 6 weeks to 12 months. Hence it can be interpreted that long term interventions are more effective than short term interventions to manage BPSD and burden. This study suggests training of all CHWs in long term dementia management for patients' living in community. Thus the study proves the effectiveness of a community based model for management of dementia patients in low and middle income countries.

The present study has the limitation of quasi experimental design as there was no control group. But quasi-experimental study strategies are commonly utilized in the studies testing effectiveness of intervention programs when random assignment is not feasible. Another limitation was purposive sampling which might have introduced a selection bias which limits the generalizability of findings.

Conclusion

The findings of this study highlighted the need of community based strategies to improve dementia care in rural communities. Low and middle income countries need to focus on cost effective NPI based interventions and training of non specialists to manage dementia patients in the community.

Conflict of Interest: NIL

Source of Funding: Self

Ethical Clearance : The research proposal was submitted to the institutional ethics committee and obtained permission. (IEC NO.B1/312/2015/ CONTSR(2) Dated 15.07.2015). Written informed consent from patients and caregivers were obtained.

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The Role of Doctors in Drug Administration and the Execution of Penal Death

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Abstract

Although until now Indonesia still applies capital punishment, efforts to limit its application are still being made, including improving the procedures for its implementation. One way to ensure that the execution of the death penalty is still carried out with respect for life is to involve doctors in treatment and the execution of the death penalty. In addition, in the reform of Indonesian criminal law (Draft KUHP), a policy for postponing death penalty has been formulated. Those sentenced to death are given the opportunity to improve themselves for a certain time (10 years). If within the grace period the convict can show improvement in himself, then the death penalty need not be carried out and replaced with deprivation of liberty.

Keywords: *The Role Of Doctors, Drug, Execution, Penal Death*

Introduction

Execution of the death penalty can be carried out in several ways, either by shooting or injecting with medicinal drugs, including using sodium thiopental or Pentothal, Pancuronium bromide, Potassium chloride, etc. with high doses of doctors and medical teams. The responsible for the execution to determine whether the convict is really dead.

Not many people understand that the role of doctors in providing treatment and execution of penal death in Indonesia is very important. His presence is very much needed, because doctors have a role to ensure the death of the convict. In the provisions of Article 4 letter c juncto Article 15 letter m Regulation Of The Head Of The State Police Of The Republic Of Indonesia Number 12 Year 2010 Concerning Procedures For Execution Of Penal Death, it is determined that the doctor puts a black mark on the shirt of the convict at the position of the heart as the target of shooting, then the Doctor and Team 2 keep away from the convict. Even in the event that after the execution the convict still shows signs of life, the doctor has the authority to order an end shot to ensure that the convict is really dead.

Embryonally, the debate onpenal deathin Indonesia has lasted for a long time. The debate has placed at least two opposing perspectives, the perspective that rejects penal deathand either by shooting or administering drugs that are injected into inmates the perspective that supports capital punishment.(1) The perspective that rejects the penal deathis based on several rationalities as follows:

a. Treatment of soul consciousness through penal death is not a punishment because it does not meet all the criterias required for a punishment. According to Modderman, the penal deathis not equal with the criminal's wrongdoing, with its imposition the possibility to improve oneself for the perpetrators is completely closed. The certainty of the judge's verdict that is right, correct and fair is difficult to guarantee because after all the judge is still a human.The penal deathcloses altogether the possibility of reviewing the verdicts that may be wrong, and the sentence as well as

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the executionof penal deathhave a bad effect on society.

b. A human's life, even a criminal, should not casually be taken away by the execution of capital punishment or attempts to provide a treatment that can kill.

c. Had treatment the penal death been considered as a tool to scare the prospective criminals, it would be difficult to accept the idea of sentence commutation.

Meanwhile the perspective that supports the penal death is based on several rationalities as follows:

a. Penal death guarantees that the criminal will not commit crime anymore, hence the people will no longer be disturbed by the criminal.

b. Penal death is a powerful repression tool for the government, hence the people's interest is guaranteed and the peace and the order of the community are protected.

c. Penal deathis also functioned as a means of general prevention, hence the prospective criminals will abort their intention to commit crime.

The Role of Doctors in the Execution of Penal Death

Even though Indonesia is still apply death penalty, its implementation still respects the values of life by including doctors in executions. Doctors who play an important role in treatment and the execution of the death penalty in Indonesia are explicitly stated in Article 4 in conjunction with Article 15 which states that is determined that the doctor puts a black mark on the shirt of the convict at the position of the heart as the target of shooting, then the Doctor and Team 2 keep away from the convict. Meanwhile, in the provisions of Article 15 letter w it is also stated that the Executive Commander, the Executing Attorney, and the Doctor examined the convict's condition and according to the Doctor that the convict was still showing signs of life, the Executing Prosecutor ordered the Executive Commander to carry out the final shooting. Apart from gunfire, there are countries that are still using drugs capable of transmitting, the convicted person will die within one or two minutes after the drug is injected.

Doctors have a very important role in treatment and the execution of capital punishment because doctors play a role in determining the death of the convict after being executed. The involvement of doctors in the execution of capital punishment can also be interpreted as an effort to ensure that the execution of the crime does not conflict with human values. The role of doctors can then be seen in the provisions of Article 15 letter y Regulation Of The Head Of The State Police Of The Republic Of Indonesia Number 12 Year 2010 Concerning Procedures For Execution Of Penal Death which states, this final shooting can be repeated, if according to the doctor's statement there is still signs of life. Based on the provisions of Article 15 letter y, it can be seen that the position of doctors in the execution of capital punishment plays a key role in ensuring the death of the convicted person. Such an important role for doctors in the execution of capital punishment is also evident from the provision of Article 15 letter z which states that the execution of capital punishment is declared complete, if the doctor has stated that there are no more signs of life in the convict.

Penal Death in the Perspective of Islamic Law

Talking about penal death in an Islamic perspective is seen as urgent in its relation to thepenal deathissue in Indonesia. Its urgency is at least due to several reasons. ***First***, although it is not mentioned in the constitution as an Islamic state[2]—hence it cannot be formally referred to as an Islamic state[3]—Indonesia is known as the largest Muslim nation in the world. Apart from that, most people are lacking the intellectual sophistication in understanding Islamic teachings, both due to historical and cultural factors.[4] ***Second***, even though Indonesia does not describe itself as a religious state, constitutionally, Indonesia has established itself as a state based on the principle of Belief in One Supreme God.[5] Therefore, according to Hazairin, as quoted by Jimly Asshiddiqie, he stated that in Indonesia something that contradicts the religious rules must not occur or apply.[6] All legitimate religions in Indonesia have the same opportunity to fill the formation of legal system—including criminal law—in Indonesia based on *Pancasila*.[7] In its relation to Islamic law, Islamic values—whether taken from the textual sources of Al-Qur'an and al-Hadith, from the sources inspired from socio-historical or empirical experiences regarding the

application of *fiqh* law throughout the Islamic history, or the local sources of Indonesian people—are the sources of law that can color the reformation of national law.[7] ***Third***, the law essentially is not an institute that falls from the sky, but it is rooted in a certain socio-cultural community.[8] The law is a reflection of the values that live in its society.[9] The law is essentially an extension of the will and the desire of the people. It is essentially more a reflection of certain social ideal.[10] The law that isout of its social context will be a burden on its society.

Based on the consideration of religious demography on one hand, and the mandate of the constitution on the other hand as explained above, the penal deathdiscussion in the Islamic perspective is seen as urgent in its relation with the penal deathdiscussion in Indonesia. Given that there are so many Islamic legal sources—including those relating to capital punishment—on this occasion, it is sufficient to refer to the main and first source of law in Islam, namely the Al-Qur'an. Some verses of the Al-Qur'an which explicitly provide the basis for the penal deathapplicationincludingAl-Qur'an Surah Al-Ma'idah verse 45 and Al-Qur'an Surah Al-Baqarahverse 178.

Based on the two verses of the Qur'an mentioned above, it is concluded that Islam provides legitimacy for penal deathin the form of *qishaash*. *Qishaash*means taking the same retaliation. However, it should be noted[11], that the concept of *qishaash* in the Islamic law contains a certain "uniqueness". Although its type and method have been explicitly determined in the Qur'anic text—thus qualifying as a hudud crime—but in *qishaash*, law enforcement is highly dependent on the final attitude shown by the victims of *qishaash* crime (in the case of*qishaash* crime of persecution) or by the victim's family (in the case of*qishaash* crime of murder).[12]

Conceptually, the punishment commensurate with the action (*qishaash*) also developed in the western world. This concept in the western world is known as retributive theory,[12] which starts from the idea that the punishment must be based on a crime (let the punishment fit the crime).[13] According to this theory, the punishment becomes a fair retribution for the perpetrator's action. The only justification for a crime according to this theory is because the perpetrator has committed a crime. It is often said that this theory has a backward-lookingorientation.[14] Hence, the orientation is towards the criminal act—which has been—committed by the perpetrator. The retribution concept in punishment is more aimed at providing a general prevention effect, which is to prevent other people from doing the same thing as the perpetrator.

Penal Death in the Perspective of Indonesia's Penalreform

Until this study written, the applicable criminal law in Indonesia is essentially a Dutch inheritance which was enforced on January 1, 1918. It is a criminal law that is much influenced by classical criminal law. At this point, it is understandable why the principles of criminal law enforced in Indonesia feel so stiff and rigid. Therefore, since the independence—precisely starting in 1964—Indonesia has prepared a reformation of national criminal law in the form of Criminal Code Draft.There are several ideas behind the reformationof criminal law in Indonesia. They are: *First*, political reason, sociological reason, and practical reason.[15]

Based on the background of the aforementioned idea, then in the concept of reforming the Indonesian criminal law, the penal deathis positioned as a specialpunishment, not as a mainpunishment as in the current Criminal Code. With its status as a special punishment, the penal deathin the reformation of Indonesian criminal law is also not formulated rigidly. It is formulated as a punishment who'sthe treatment and execution can be postponed (suspended penal death) with special conditions. To provide an overview of penal deathin the concept of reformation criminal law in Indonesia, the following are its formulation in the Indonesian Criminal Code Draft (2015), namely:

Article 89

Penal deathis alternatively sentenced as the last resort to protect the community.

Article 91

(1) The execution of penal deathcan be postponed with a probation period of 10 (ten) years, if:

a. the people's reaction to the condemned inmate is not great;

b. the condemned inmate shows regret and there is hope for improvement;

c. the condemned inmate position in the criminal offense is not very important; and

d. there are mitigating reasons.

Conclusion

Based on the brief study above, it can be concluded that:

Execution of prisoners can be done by using lethal drugs and gunfire. Although until now Indonesia still applies penal death, the execution of penal death still respects the values of life. One of the methods taken is by calling for doctors to giving drugs on and carries off penal death. The tendency to keep the penal death in Indonesia is based on the values of Islamic law as a religion that is believed by the majority of Indonesian people.

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Conflict of Interest: No

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Identification of *Giardia lamblia* Genotypes among Children in Tikrit City by Using Nested PCR

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Abstract

The parasite *Giardia lamblia* is one of the most widespread intestinal protozoa that infects humans all over the world, causing giardiasis. It was first diagnosed in the duodenum and ileum in human digestive tract, where its effect is primarily on children. Giardia disease affects more than 280 million people every year worldwide. It is also one of the most important parasitic infections in many countries in terms of incidence and morbidity. 615 stool samples were collected from children attending Tikrit Teaching Hospital and private laboratories during the study pehriod to investigate the spread of *G. lamblia* among children, and the total infection rate was 14.30%. Where the rate of infecting children in the city was 11.54% of the total of 50 samples. Through the molecular examination in this study, the parasite *G. lamblia*, the target gene TPI triose phosphate isomerase was used to identify clusters of G. lamblia. All positive samples were positive with group B and no infection was registered with group A.

Keywords: *parasites, Giardia lamblia, Giardiasis, Nested PCR.*

Introduction

The parasite *Giardia lamblia* is one of the most common intestinal protozoa that infects humans all over the world, causing Giardiasis [(1)], it was first diagnosed in the duodenum and ileum in humans digestive tract, where its effect is primarily on children[(2)]. Giardiasis affects more than 280 million people every year [(2)] and is also one of the most important parasitic infections in many countries in terms of incidence and morbidity[(3)]. Clinical signs of the infection with the parasite *G. lamblia* are variable. They symptoms could be absent until their appearance, which include fatty diarrhea, abdominal cramping, malnutrition, weight loss, vomiting and nausea, among the most obvious symptoms when infected with this parasite [(3)].

G. lamblia competes with its host for its food, including basic nutrients such as proteins, fats, vitamins and mineral elements, which causes malnutrition and disease among the population of developing countries, especially children [(4,5)]. *Giardia Lamblia* is one of the most common causes of diarrhea in the world [(6)]. Diarrhea is one of the most serious pathological symptoms facing public health, and the state of fluid loss is associated with this disease, and returning lost fluids is an effective treatment to reduce dehydration. However, the rate of spread of giardia infections in the world rises in societies with poor economic and environmental conditions and areas with crowded population [(7)] .

Several methods are used for diagnosing intestinal parasites, including direct smear microscopy, which diagnoses primary movement without changing its shape, as well as the use of lugol's iodine solution, which works at stabilizing the parasites, as well as the solution used to dye the polycystic stages of primary and other parasitic eggs [(8)]. The microscopic examination has some difficulties in diagnosing *G. lamblia* due to the difficulty in diagnosing the accuracy of the cysts being intermittently, or to the difficulty in distinguishing between the cysts of this parasite with other parasite cysts that are not pathogenic [(9)], and also as a result of an overlap between the parasite shape and other excretions of the host such as mucus or vegetable fibers or fats, and all of these hinder the examination and diagnosis,

as a result, it is preferable to use focus methods as an integral part of the examination over the direct smear method for diagnosing parasites in clinical laboratories and scientific research that depends on the difference in specific gravity.

Modern diagnostic methods based on molecular techniques have been used. The method of polymerase chain reactions using nested PCR assay. Nested PCR is a modification of conventional PCR reactions, which is used to amplify and measure the target DNA molecule at the same time. The parasite *Giardia lamblia* has a high degree of genetic diversity that has been assembled. In genotypes from A to H, as well as the presence of other secondary genotypes within these main groups [10-12]. The aim of this study is to detect *Giardia lamblia* in feces. Using Nested PCR technique by using the target gene (bg) beta -giardin. Genotyping of the *Giardia lamblia* parasite in infected subjects using the triosephosphate-isomeras target gene (TPI).

Methodology

This study was conducted during the period from December first 2018 to December first 2019. 615 stools were collected from patients attending Tikrit Teaching Hospital Laboratory, and from private laboratories, from both sexes, with ages between 1-16 One year to investigate the spread of the *Giardia lamblia* parasite in Tikrit, and all the fecal samples that were collected were kept in sterile, dry plastic bottles with a sealed seal and prepared for this purpose.

The optical microscope examination of the samples was conducted within half an hour from the time of their arrival to the laboratory by the direct wet mount method to detect the feeding and cystic phases of the parasite using the small magnification force 10x and then the largest 40X, with two scans per sample. Part of the samples was preserved after extraction for molecular assays.

After performing the microscopic examination of the samples, the blood samples were examined by molecular methods by extracting the DNA from the stool samples using the QIAamp® Fast DNA Stool Mini Extraction Ki extraction kit. The feedstocks were supplied by Macrogen in a lyophilized form. The primers were dissolved in nuclease-free water to give a final concentration of 100 µL as the effective starting solution of 10 Pmol / µL.

After PCR amplification, agarose gel electrophoresis was relied on to confirm the presence of the amplification (32).

Statistical Analysis

The results were analyzed statistically according to two tests, the chi-square test (X2) and the (T-test), with a significant level of 0.05 and 0.01 (Narrator, 2000) using the statistical program Minitab.

Results and Discussion

The results of the current study showed that there are differences between the diagnostic methods of flattening using $ZnSo_4$ sulfate with a percentage of 8.94%, and the diagnosis by the direct method using lugal-iodine and the physiological solution, where the percentage was 5.36% of the total samples. The results of the statistical analysis using X^2 showed a significant difference between the methods of diagnosing the parasite at a probability level ($P < 0.01$) as in Table (1).

Table (1): Efficiency of different diagnostic methods to detect *G.lamblia* parasite.

Type of test	Total	Positive	%
Direct smear method (wet smear)	615	33	5.36
Floatation method by ZnSo4	615	55	8.94
Total	615	88	14.30

The results of this study were in agreement with the results of a number of studies, including Zangana [13], where the highest percentage was recorded by the method of flattening using zinc sulfate, where the percentage reached 7.74%, then the direct method followed by 5.99%, they agreed with the results of the Qaryagdi study (14) regarding its use. The method of flattening and wet smear in the diagnosis of infection with the *G. lamblia*, where the highest rate of infection was recorded by the method of flattening using zinc sulfate, reaching 13.5%, followed by the wet smear method by 1.8% in Kirkuk.

The reason is that the detection using the flattening method depends on the principle of the specific density difference between the solutions the primary cysts and the worm eggs, in addition to that it depends on examining a large amount of faeces compared to the direct wet swab that is done by taking a small amount during the biometric examination [15].

Therefore, more sensitive diagnostic methods were required, using the β-gardian gene and by the technique of nested PCR. A piece of the β-gardian gene for a portion (511 bp) was amplified by the β-gardian technique to detect *Giardia lamblia* from a total of 17 samples. The results of the amplification showed that 6 samples were positive from a total of 17 samples as shown in Figure (1).

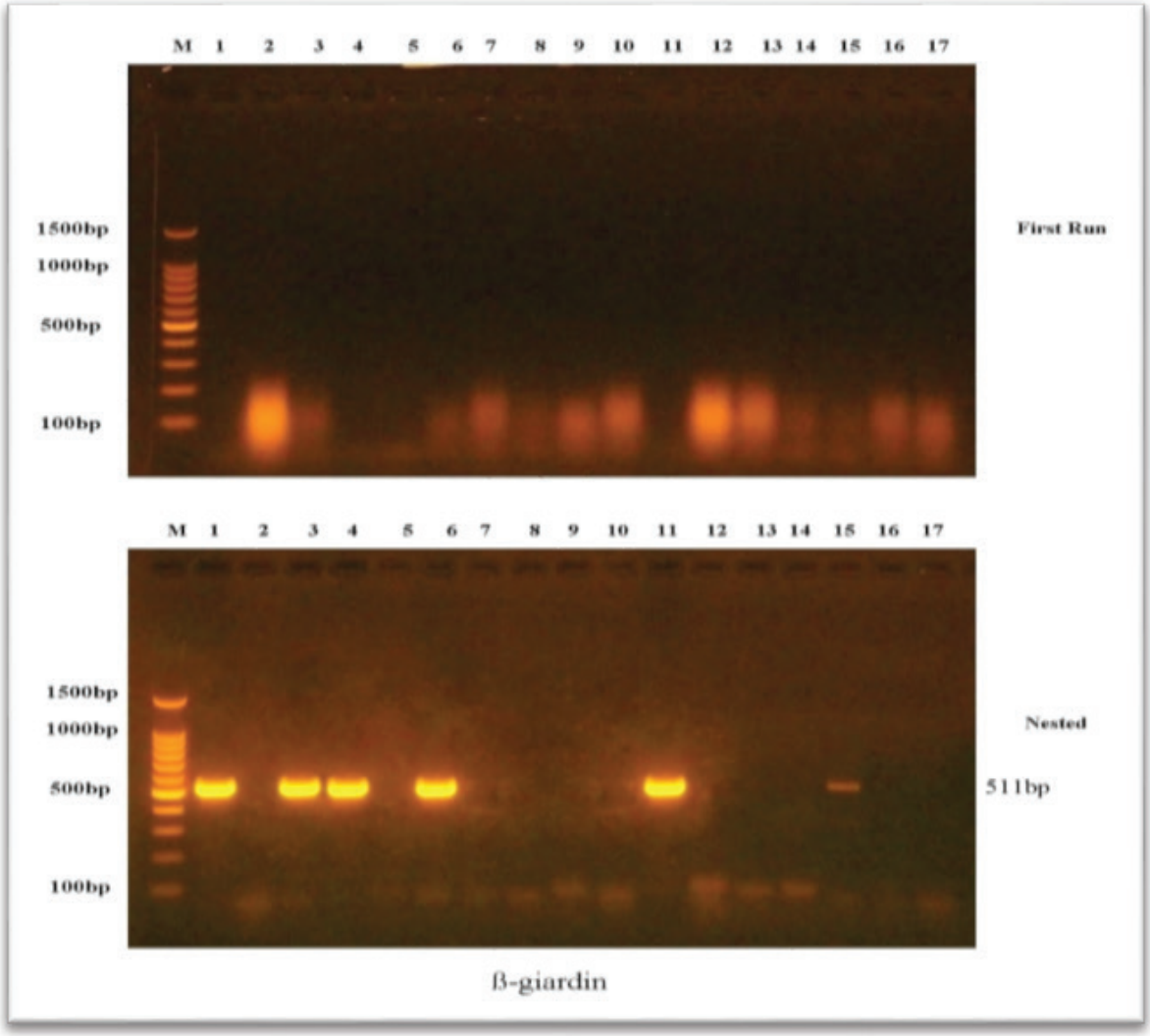


Figure (1): Detection of *G. lamblia* mediated by the β-gardian gene using Nested PCR technique.

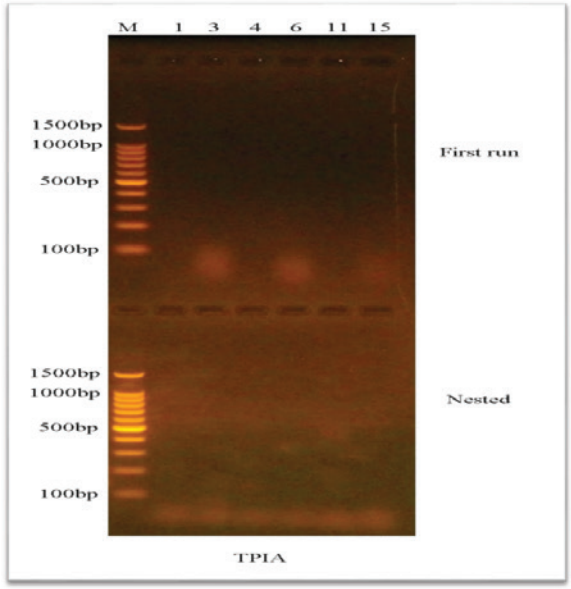
Despite the fact that all of the 17 samples were positive under microscopy, it did not give a result when tested with Nested PCR technique, although the polymerase chain reaction has great benefits in increasing the speed, sensitivity and specificity more than microscopy and immunological detection in the detection of microorganisms in a significant difference [16], the failure to amplify a specific gene in samples has been confirmed by several previous studies [17].

There are a number of factors that affect the efficiency of PCR-based techniques, which leads to non-amplification of gene segments and these inhibitory substances (polysaccharides, hemoglobin degradation products, bilirubins, and bile salts), cyst quantity and quality, type of target gene, prefixes selection and spin conditions. Thermography and the low concentration of DNA in the sample from which the gene is to be amplified are among the main reasons that lead to negative states [18].

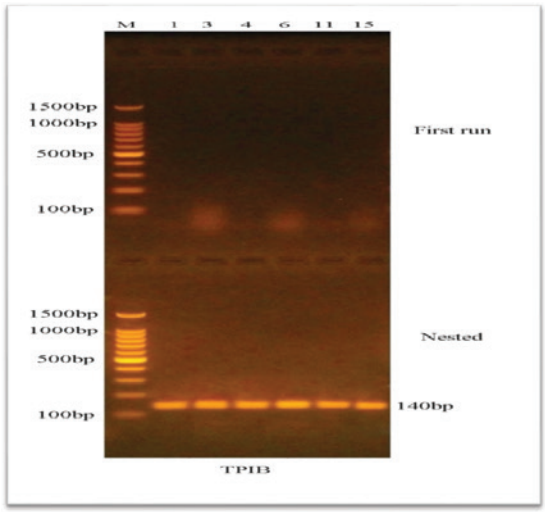
To reduce the effect of DNA inhibitors, alternatives were used in the process of extracting DNA from them, commercial kits were used. These kits could reduce the amount of inhibitors, as well as among other reasons for the small number of the parasite [18], and it may be due to the confusion that occurs in distinguishing other species of parasites upon microscopic examination, or sometimes the time between sampling and analysis, all these factors lead to negative results.

Lalle [19] stated that despite the fact that gene primers are designed to bind to specific regions conserved in the gene, mismatches in the initiator sequence, such as being too long are among the reasons that lead to the failure of gene amplification in many stool samples. The β-gardian gene is characterized by high specificity in detecting giardia and determining genotypes. Among these studies is the study that was conducted by [20], and in another study, the β-gardian gene was used to reveal the molecular and epidemiological characteristics of Giardia populations. .

To determine the genotypes of *G. lamblia* according to TPI gene amplification using the Nested PCR technique. A piece of the TPIA gene was amplified for 6 samples from a total of 17 samples that were analyzed from examined human isolates using Nested PCR, the current study showed that the samples do not contain genotype A. As the initiator pair showed the required package with a size of (476 bp) as it shown in figure (2).



While a piece of the TPIB gene was amplified, where the initiator pair showed the required package with a size of (140 bp) for 6 samples, and the amplification of the TPIB gene showed the presence of genotype B in all samples, as shown in figure (3).



Studies conducted on DNA extracted directly from stool samples from different countries confirmed that only the A and B genotypes are associated with human infection [21]. It is known that genotype A is most often responsible for zoonotic infections as it is transmitted by a wide range of animals that act as reservoir hosts. The genotype B is most likely transmitted from human to human and may represent a zoonotic pattern. Determining the different genotypes that contribute to disease events can determine the specific hosts,

transmission routes and sources. Infection, and thus it may lead to controlling parasitic infections [22]. Also, failure to detect common infections of both genotypes together in some studies does not mean the absence of mixed infection, but because one of the two genotypes was preferred to the other in replicating in one place [23]. The predominance of the B group in this study is compatible with the study of [24] as well as [25]. Minetti *et al*. [26] indicated that patients with group B showed a higher rate of elimination of cysts and this may lead to a higher transmission rate, and consequently a higher rate of infection where cluster B was prevalent. These results are also consistent with its record AL-Obaidi [27], which recorded the superiority of genotype B over genotype A, and social and environmental conditions may have contributed to the differences in the distribution of clusters. Therefore, the detection of these factors leads to the control of parasitic infection.

There are also many studies that show the predominance of type B over A, meaning that B is more prevalent than A, and among these studies also is a study of Dixon *et al.* [28], and a study by Traub, *et al*. [29] and Kohli *et al*. [30,31] Thy confirmed that children with group B get rid of cysts more than children with group A.

Conclusion

G. lamblia parasite was detected using β-gardian and using Nested PCR technique, 6 samples were found from a total of 17 positive samples. Genotypes were detected using the target gene Triose phosphate isomeras (TPI) and all samples positive for Giardia were genotype (B).

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

Funding: The work was supported by the researchers themselves.

Conflict of Interest: The author declare no conflict of interest regarding this paper.

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Stature Determination from 3D Foot Impression among Telugus in Malaysia

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Abstract

Personal identification is the primary aim of anyforensic investigations. Foot impressions, either 2D or 3D are used for person identification. Foot impression found on soft surfaces is three dimension in nature (3D), also namedfoot outline which shows the boundaries of the foot left by the offenders on soft surfaces. It is a fact that 3D impressions can also be used to determine stature, body weight and gender. In the developing countries, people are still walking barefooted. Earlier foot impression studies were conducted on mixed population and currently researchers are insisting the importance of ethnicity consideration.Malaysian Indians are an important ethnic group living in Malaysia, with minor sub-ethnicgroups such as Malayali, Telugu, Sikh, Tamil, and others. Hence this study intends to examine the relationship between stature and 3D foot lengths among Malaysian Telugus, mostly living in peninsular Malaysia. The stature and foot outlines (3D) were collected from consented Telugu 140 subjects, comprising 73 males and 67 females, age ranging from 18 to 60 years old. The data obtained were analysed statistically using SPSS software version 21.0. There exists a strong positive correlation between stature and 3D foot impression lengths and the correlation coefficient (R) values are found to be higher in unknown gender/pooled samplefollowed by males and females. This study developed regression formulae to determine stature from foot outline (3D) lengths among Malaysian Telugus for crime scene investigation. The equations are population specific and should not be used for any other populations.

Keywords: *Forensic science, Stature, Foot outline, Telugus, Malaysia*

Introduction

Foot impressions are used to develop individual's profile such as stature, body weight and gender[1-3]. Generally, examination of barefoot impression is very important and in Asian countries like Malaysia, India people used to walk barefooted due to socioeconomic and climatic condition[4].Also during the indoor stay like home, temples, people walk without foot wears. Foot impressions can be recovered from crime scenes in the form of two dimensional(2D) and three dimensional 3D (also foot outline). Footprintis created when the plantar surface of the foot touches any hard surfaces like mosaic floor, dust or blood termed as 2D footprint whereas 3D (foot outline) shows the line tracing or size parameter of the foot which formed when the foot touches any soft surfaces like soil, snow, and mud [5-6].

Stature determination is an important key componentfor personal identification which help to solve a crime and 2D or 3D foot impressions are used to estimate stature[1,4], also footprints show individual characteristics[7-8].These characteristic features can be used as inclusion or exclusion criteria whenever dealing

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with suspects during the process of identification. Researchers have shown that foot outline/ 3D foot impression found on soft surfaces in the crime scenes can also be used to determine stature [1,4,9]. Anthropological researchers have reminded that ethnicity is an important key factor to be considered before started anthropological research relatingfoot impression and stature, since the morphology of human feet varies in terms of their lifestyle, food habits, climate, and ancestry [10].

Researchers have confirmed that stature determination formulae developed for a particular population is unsuitable to apply on any other population in the country or world. Malaysia shows lack of study in stature determination from 3D foot impression (foot outline).Hence this research was aimed to conduct foot outline study to estimate stature among Telugu population in Malaysia for forensic application.

Methodology

The study subjects consist of 140 adult consented Malaysian Telugus (73 male and 67 female) representingthe population in Malaysia.To start with, the subject's name, age and sex are obtained. The height of the subjects was recorded using a portable stadiometer. Then the feet of the subjects were cleaned with wet tissues and dried with clean white towel. Then foot outline was obtained by tracing method by placing a foot on A4 size white paper and drawn outline using a sharp pencil [1,4,9].

Foot outline samples were analysed following Robbin's Diagonal method[9]. The designated longitudinal axis (DLA) and base line (BL) were drawn, perpendicular to each other. Then, five diagonal lines frompternion L for left and R for right to most anterior tip of all toes were drawn. The left foot outline length measurements were labelled as LOT1, LOT2, LOT3, LOT4, and LOT5, the length from pternion Lto anterior tip of toes from OT1 to OT5, while for the right foot outline lengths were labelled as ROT1, ROT2, ROT3, ROT4, and ROT5. Figure 1 shows the foot outline lengths and the land marks.

The data obtained was computed and analysed using SPSS (Statistical Package for Social Sciences) version 21.0 computer software. The linear andmultiple regression analysis method were employed to derive regression equations for stature determination from various foot outline lengths.

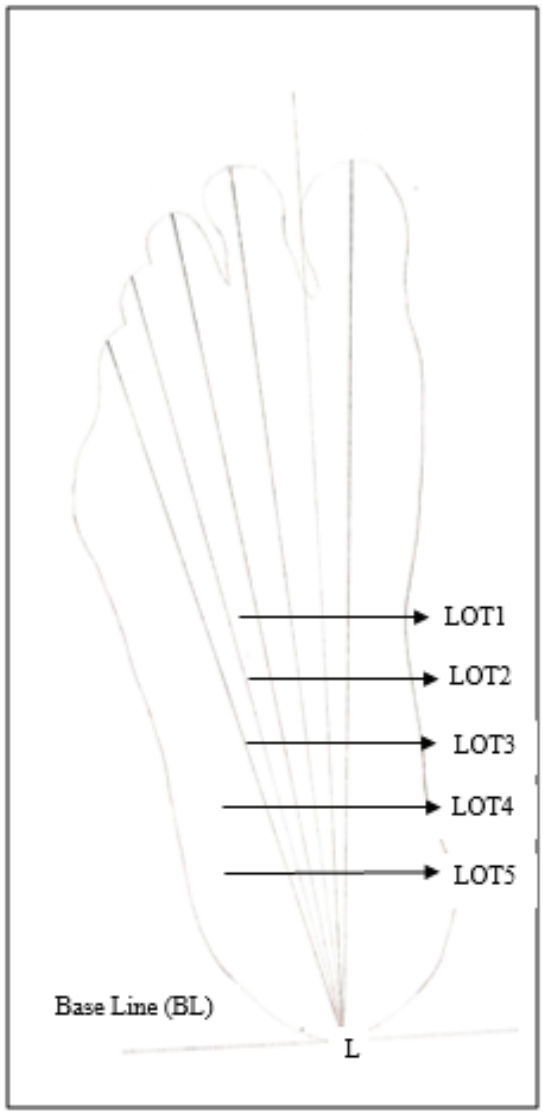

Figure 1: Landmarks and diagonal length measurements on left foot outline, from posterior point of the heel L,pternion to the anterior tip of all toes.

Results

Table 1 shows the descriptive statistics of stature in males, females and unknown gender/ pooled samplewhich represent the combination of male and female samples and considered unknown gender. The table shows that the mean stature of males found to be comparatively higher (168.9 cm) than females (156.9 cm). The stature ranges from 143.6 cm to 186.0 cm in males and 142.7 cm to 170.7 cm in females.

Table 1: Stature (cm) in males, females and unknown gender in adult Malaysian Telugus

Variables	N	Mean	Std. Deviation	Minimum	Maximum
Male	73	168.863	8.1031	143.6	186.0
Female	67	156.934	5.5427	142.7	170.7
Unknown gender	140	163.154	9.1849	142.7	186.0

Table 2 presents the foot outline lengths among males, females, and unknown gender respectively. In Table 2, the male right foot outline lengths are found to be higher (ROT1 – ROT5) than left foot outline (LOT1 – LOT5) except for first left toe (LOT1), showing the bilateral asymmetry. Table 3 shows the foot outline lengths of females shows that left foot outline lengths (LOT1 – ROT5) are found to be higher (LOT1 – LOT5) than right foot outline lengths (ROT1 – ROT5), existence of bilateral asymmetry.

Table 3 shows the linear regression formulae for stature determination from various foot outline lengths for both left and right sides in males, females, and unknown gender. The correlation coefficient (r) values of foot outline lengths are higher in unknown gender (0.704-0.785) followed by males (0.584-0.680) and females (0.381-0.622).Also that, the SEE values found in males (5.9821-6.4614) are comparatively higher, followed by females (4.3750-5.1689) and unknown gender of foot outline (5.7095-6.2382). All the foot outline length measurements among males, females and unknown gender found to be statistically significant as p=0.000when analysed through linear regression method.

Table 4 shows the multiple regression equations for stature determination from foot outline length in males, females and unknown gender. The correlation coefficient (r) values of foot outline lengths found to be higher in unknown gender (0.803-0.807) followed by males (0.672-0.702) and females (0.635-0.675).For the SEE values are lower in females (4.4547-4.2512), than unknown gender (5.5200-5.5802) and males (5.9796-6.2195). All the foot outline length measurements are statistically significant and correlation coefficient (R) values are significant with p=0.000 in multiple regression analysis method. The p values are found lesser than 0.002 in linear regression analysis methods.

Figure 2presents scatter graphs showing the relationship between left and rightfoot outline lengths and stature of male, female and pooled sample (unknown gender). The analysis of scatter diagrams exhibiting a strong positive correlation between foot outline length and stature as the stature (dependent variable, Y) increased when foot outline length (independent variable, X) increased.

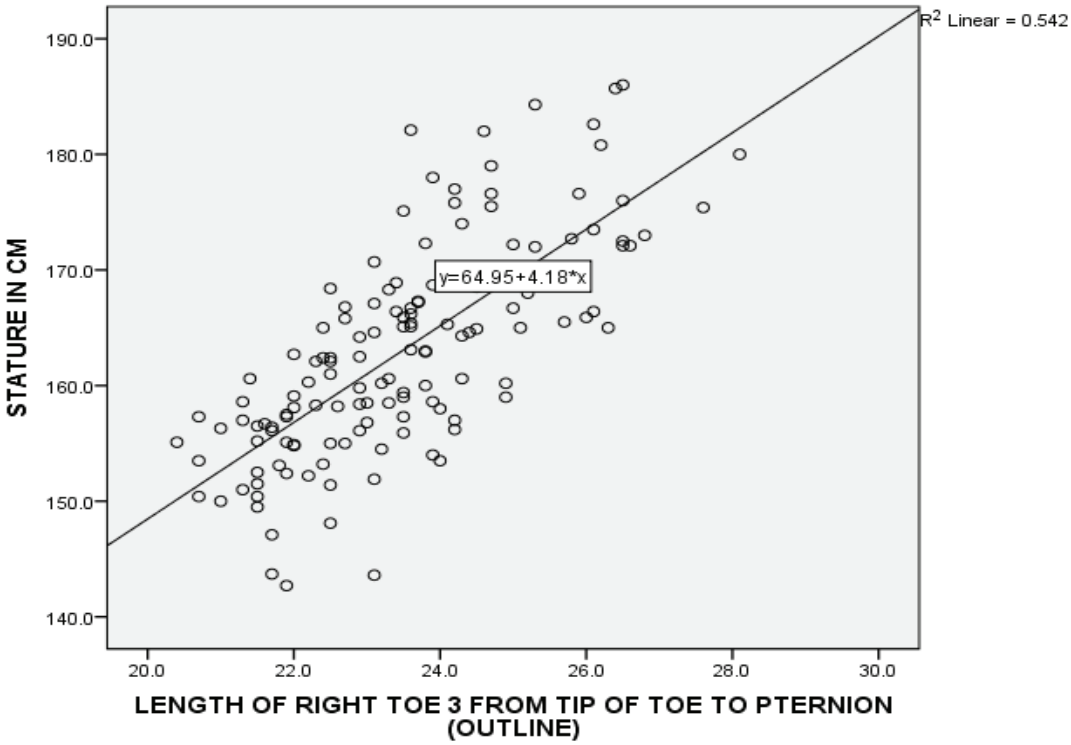

Figure 2: Example of scatter graph showing the relationship between right foot outline length with toe 3 and stature in Telugus in unknown gender/pooled sample

Table 2: Descriptive statistics of foot outline length measurements (in cm) in adult Malaysian Telugus

Variables	Male (N=73)			Female (N=67)			Unknown gender (N=140)		
	Min-Max	Mean	Std. Deviation	Min-Max	Mean	Std. Deviation	Min-Max	Mean	Std. Deviation
LOT1	22.8-28.6	25.801	1.4881	20.0-26.6	23.604	1.1215	20.0-28.6	24.750	1.7196
LOT2	21.8-28.9	25.332	1.4941	20.0-26.2	23.112	1.1042	20.0-28.9	24.269	1.7243
LOT3	21.8-27.9	24.529	1.4405	20.0-25.0	22.337	1.0407	20.0-27.9	23.480	1.6722
LOT4	20.3-26.7	23.240	1.3694	19.0-25.2	21.237	1.1485	19.0-26.6	22.281	1.6141
LOT5	18.9-26.2	21.505	1.3596	17.7-25.3	19.761	1.3808	17.7-26.2	20.671	1.6210
ROT1	22.6-28.7	25.611	1.4955	22.0-26.4	23.594	1.0225	21.2-29.2	24.646	1.6363
ROT2	22.5-29.2	25.345	1.4779	21.2-26.0	23.210	1.0814	22.0-28.7	24.324	1.6829
ROT3	21.7-28.1	24.511	1.4080	20.4-25.0	22.434	1.0377	20.4-28.1	23.517	1.6193
ROT4	20.1-27.0	23.358	1.3585	19.5-23.3	21.279	0.9056	19.5-27.0	22.363	1.5592
ROT5	19.0-27.1	21.711	1.3836	18.1-22.1	19.824	0.9077	18.1-27.1	20.808	1.5093

Table 3: Linear regression formulae for stature determination from various foot outline lengths (in cm) in adult Malaysian Telugus

Variables	Male (N=73)			Female (N=67)			Unknown gender (N=140)		
	Linear regression equations	R	SEE	Linear regression equations	R	SEE	Linear regression equations	R	SEE
LOT1	S= 75.446 + 3.621(LOT1)	0.665	6.0950	S= 103.582 + 2.260(LOT1)	0.457	4.9669	S= 61.976 + 4.088(LOT1)	0.765	5.9325
LOT2	S= 78.684+ 3.560(LOT2)	0.656	6.1561	S= 103.696 + 2.303(LOT2)	0.459	4.9624	S= 64.357 + 4.071(LOT2)	0.764	5.9449
LOT3	S= 79.892 + 3.627(LOT3)	0.645	6.2369	S= 111.641 + 2.028(LOT3)	0.381	5.1646	S= 66.487 + 4.117(LOT3)	0.750	6.1021
LOT4	S= 78.321 + 3.896(LOT4)	0.658	6.1417	S= 117.341 + 1.864(LOT4)	0.386	5.1516	S= 69.464 + 4.205(LOT4)	0.739	6.2107
LOT5	S= 81.693 + 4.053(LOT5)	0.680	5.9821	S= 126.883 + 1.521(LOT5)	0.379	5.1689	S= 80.650 + 3.991(LOT5)	0.704	6.5429
ROT1	S= 78.682 + 3.521(ROT1)	0.650	6.2019	S= 77.429 + 3.370(ROT1)	0.622	4.3750	S= 54.541 + 4.407(ROT1)	0.785	5.7095
ROT2	S= 83.789 + 3.357(ROT2)	0.612	6.4521	S= 89.168 + 2.920(ROT2)	0.570	4.5905	S= 61.644 + 4.173(ROT2)	0.765	5.9406
ROT3	S= 86.466 + 3.362(ROT3)	0.584	6.6232	S= 101.132 + 2.487(ROT3)	0.466	4.9426	S= 64.945 + 4.176(ROT3)	0.736	6.2382
ROT4	S= 83.777 + 3.643(ROT4)	0.611	6.4614	S= 91.581 + 3.071(ROT4)	0.502	4.8311	S= 62.801 + 4.488(ROT4)	0.762	5.9717
ROT5	S= 91.148 + 3.580(ROT5)	0.611	6.4614	S= 90.857 + 3.333(ROT5)	0.546	4.6796	S= 67.136 + 4.615(ROT5)	0.758	6.0098

p =0.000

Table 4: Multiple regression equations for stature estimation from various foot outline length measurements in adult Malaysian Telugus

Gender	Side	Multiple regression equations	R	SEE
Male (n=73)	Left	S = 73.243+ 2.445(LPT1)- 1.078(LPT2) + 2.230(LPT3)- 1.065(LPT4) + 1.694(LPT5)	0.692	5.9989
	Right	S = 80.228 + 1.065(RPT1) + 1.256(RPT2) - 2.247(RPT3) + 2.067(RPT4) + 2.001(RPT5)	0.629	6.5839
Female (n=67)	Left	S = 89.006+ 2.209(LPT1)+ 2.736(LPT2) - 2.907(LPT3)+ 1.323(LPT4) - 0.302(LPT5)	0.609	4.5436
	Right	S = 78.661 + 4.671(RPT1) + 0.149(RPT2) - 1.661(RPT3) - 1.262(RPT4) + 1.811(RPT5)	0.680	4.1131
Unknown Gender/ Pooled Sample (n=140)	Left	S = 57.520+ 2.269(LPT1)+ 1.362(LPT2) - 0.348(LPT3)+ 0.444(LPT4) + 1.059(LPT5)	0.790	5.6074
	Right	S = 56.168 + 1.605(RPT1) + 1.201(RPT2) - 2.376(RPT3) + 1.862(RPT4) + 2.929(RPT5)	0.776	5.9560

p=0.000

Discussion

Malaysian Telugus are sub ethnic group of Malaysian Indianmostly living in Peninsular Malaysia[11]. According to population and census of Malaysia 2020, the population of Telugus in Malaysia is 1, 19,000 with 0.36%. During British regiment, Telugu people were brought to Malaysia as labourers, professionals and traders, from what is now Andhra Pradesh and Telangana states in India. Malaysian Telugus have "Telugu Association of Malaysia (TAM)" to safeguard the cultural identity and heritage.

The age range of the subject in this research chosen as 18-60 years, since the adult's foot attained by the age of 16 years in male and 14 years in Space between[12] and whereas height shrinkage occurs after 60 years old[13]. Therefore, the minimum age of the subjects was set at 18 years for this research. The foot outline lengthsare well correlated with the stature. Stature found to be higher in males (143.6cm – 186.0cm) than females (142.7cm – 170.0cm) showing the general sex difference in Malaysian Telugus and the finding is accordance with Chinese, Indonesia Minangs, Malays and Indian populations [1,4,14-15]. The mean stature of Telugu male and female are 168.9 cm and 156.9 cm respectively, whereas the stature of Philippine Kagay-Anon (male: 161.4 cm, female: 151.3 cm) and Indonesian Minang (male: 163.9 cm, female: 152.4 cm) are shorter than Malaysian Telugu population. On the contrary, the stature of Malaysian Chinese is taller (male: 171.5 cm, female: 158. 2 cm) than the study population. Thus ethnicity consideration is an important factor to be considered whenever initiated anthropological study of stature, gender and body weight[6,17]relating foot[9] and hand anthropometry [4,16].Linear and multiple regression analysis are the common statistical tool used to study the relationship between stature and foot length measurements[1].In this study three types of regression equations were derived to determine stature from foot outline lengths exclusively for males, females and unknown gender. In real crime scenes, sometimes, the gender of foot impression remain unknown, and hence the formulae developed for unknown gender may be used to determine the stature and to proceed the investigation.

The result of this study reveals that the correlation coefficient (R) between stature and foot outline length measurements is higher in unknown gender compared to male and female individually. But, when compare both regression models, the derived multiple regression equations to estimate stature from foot outline length measurements in both male and female are reliable and accurate since the correlation coefficient (r) and prediction accuracy (R^2) value for the multiple regression equations were higher while the standard error of estimate (SEE) values are found to be low[18-19].

Conclusion

In is concluded that the present study developed regression equations to determine stature from 3D foot impression (foot outlines) among Malaysian Telugus for crime scene application. The equations are population specific and should not be used for any other populations for stature determination.

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Conflict of Interest: NIL

Funding for this Research: Self

Ethical Clearance: Obtained from MSU research committee

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Gender Determination from Toe Prints Among Kagay-Anons of Philippines for Forensic Application

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Abstract

Footprint is a physical evidence, often found at the crime scenes. Due to its ability to provide more information than fingerprint, it has been considered as a valuable clue. Sometimes, partial footprints or toe prints are found in crime scenes. Hence the present study used toe prints among Kagay-Anons of Philippines population for gender determination. A total of 4000 toe prints were collected and analyzed by "Acree method", using 5mm x 5mm square area transparent film. The number of ridges within the square was calculated as ridge density. The finding has shown that females havecomparatively higher ridge density than malesin all five toes. The present study shows that partial foot print found at the crime scene can be still collected and analyzed for identification.

Key words: *Forensic science, toe print, ridge density, gender, Kagay-anon, Philippines*

Introduction

Footprint has uniqueidentifying characteristiclike fingerprint that can be used for identification[1] regardless of complete or partial footprint[2]. But unlike the criminals hide the fingerprints by wearing gloves, it is cumbersome to avoid thefootprint at the crime scenes, while standing, walking or running[3-4]. During crime operations, some criminals tend to avoid wearing footwear to preventnoise that produces while walking and for better grip while climbing walls[5]. These crime scene footprints aid in the investigation for determination of stature[6-8], and body weight[9-10]. Unlike fingerprint, footprint provides more information such as gender [4,7], age[4], number of individuals[3,4,7], walking speed[1,4], carrying load[1] and direction of movement[1]. Dermatoglyphics is the study of impression of hands and feet that have been utilized by the anthropologists and forensic scientists[11]. The friction ridge pattern presents on finger, palm, sole, and toe that remain unchanged intheir lifetime[12]. Friction ridge density refers to the number of ridges in a defined area on a print which is influenced by the ridge width and the distances between ridges[13]. There were incidences wherein only toe impressions (partial footprint) found in the crime scenes, rather than full footprint and the crime scene investigator have neglected the evidence, considering unimportant. Hence the present study was aimed to determine gender from toe print ridge density among Kagay-anon population, an indigenous ethnic group in Philippines.

Methodology

The present study was conducted on the consented 400 (201 males, and 199 females) Kagay-anon peopleresiding at Cagayan de Oro City in Philippines. Toe prints were collected by using "inking technique"adopted in earlier research[1,6,8,14-15].

The study area on the toe prints was then analyzed by "Acree method", in which 5mm x 5mm area transparent film was placed on the designated area (Figure 1)[16]. Linen tester, a strong magnifier was used to count the number of ridges present in the designated space. The value obtained represents the number of ridges in 25mm² area and reflects the ridge density value. The data were statistically analyzed using SPSS version 26.

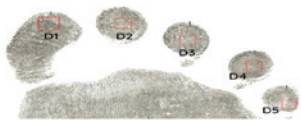

Figure 1: Designated study area on toe prints

D1: Great toe, D2: Second toe, D3: Third toe, D4: Fourth toe, D5: Little toe

Results

Table 1 shows the descriptive statistics of toe print ridge density in males and females in all five toes on both left and right sides viz. great toe (D1), second toe (D2), third toe (D3), fourth toe (D4), and little toe (D5). The result shows that the mean ridge density in females was comparatively higher than males in all areas of toeprints.

Table 1: Descriptive statistics for toe print ridge density for both gender and side

	Great toe (D1)		Second toe (D2)		Third toe (D3)		Fourth toe (D4)		Little toe (D5)	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Right toe prints										
Mean	9.98	10.82	13.25	14.15	14.33	16.16	13.87	15.79	15.01	16.37
S.D.	1.845	1.884	2.611	2.572	2.743	2.630	2.734	2.673	2.179	2.338
Range	6-16	7-17	7-22	8-22	7-23	9-23	6-21	10-24	6-23	11-27
Left toe prints										
Mean	10.20	10.49	13.01	13.52	14.35	15.16	13.97	14.88	14.44	14.76
S.D.	1.848	1.819	2.627	2.628	2.616	2.579	2.540	2.481	2.322	2.235
Range	7-17	6-16	8-22	6-20	9-22	9-21	6-21	9-22	6-19	8-21

Table 2 presents the result of independent T-test used to determine the sex differences in toe print ridge density. The result indicatesthat statistically significant sex differences were observed in all five toeson right footprint, and only third toe (D3) and fourth toe (D4) for the left toeprints. The results also shows that the maximum gender variation appeared on right fourth toe.

Table 2: Sex difference in toe print ridge density

Toes	Right toe prints		Left toe prints	
	t-value	**p-value**	**t-value**	**p-value**
D1	4.532	0.000	1.592	0.112
D2	3.460	0.001	1.907	0.057
D3	6.795	0.000	3.086	0.002
D4	7.051	0.000	3.603	0.000
D5	6.014	0.000	1.435	0.152

Table 3 shows the correlation analysis of the ridge density among five toes. The p values are 0 in all toe prints andthus statistically significant. Table 4 shows that statistically significant differences were evident for toe print ridge density for all five toes. The frequency distribution of ridge density in great toe, third toe, and little toe for the left footprint is shown in figure 2.

Table 3: Pearson Correlation (r) of toe print ridge density between toe regions

Inter Toes	Right toe print		Left toe print	
	r	p-value	r	p-value
D1 – D2	0.229	0.000	0.222	0.000
D1 – D3	0.272	0.000	0.224	0.000
D1 – D4	0.334	0.000	0.347	0.000
D1 – D5	0.326	0.000	0.225	0.000
D2 – D3	0.500	0.000	0.478	0.000
D2 – D4	0.411	0.000	0.376	0.000
D2 – D5	0.285	0.000	0.227	0.000
D3 – D4	0.532	0.000	0.514	0.000
D3 – D5	0.333	0.000	0.279	0.000
D4 – D5	0.368	0.000	0.306	0.000

Table 4: Differences between toe regions

	Right toe print	Left toe print
Chi-square	735.048	662.333
df	4	4
p-value	0.000	0.000

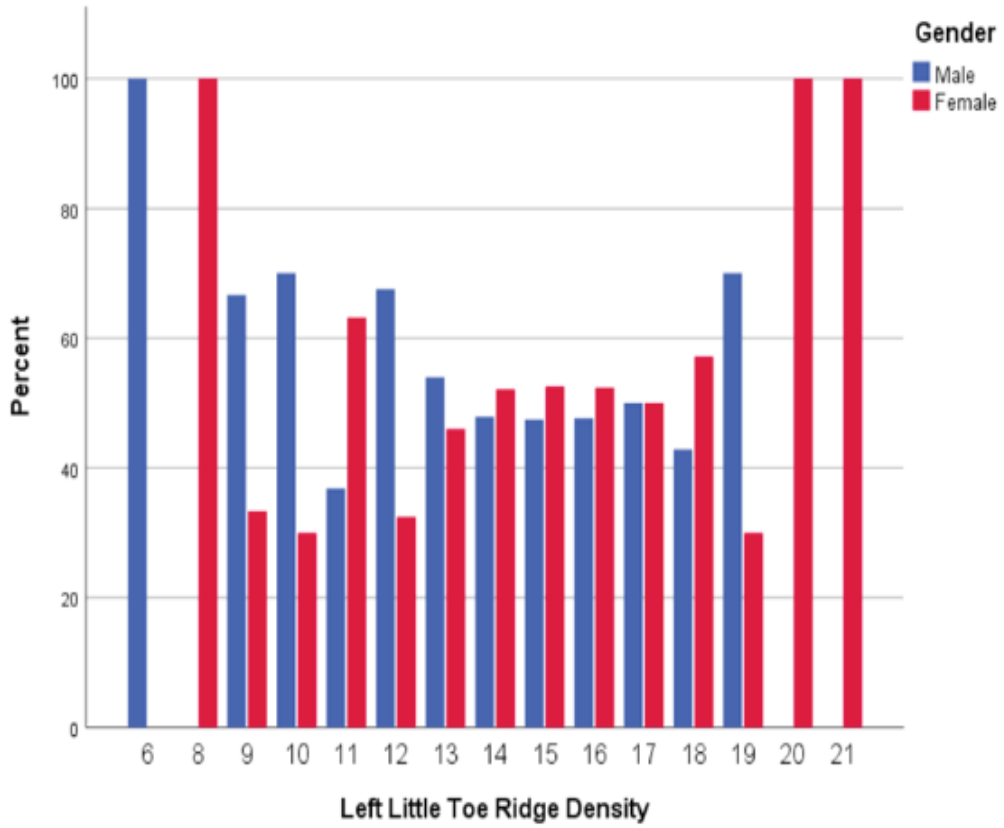

Figure 2: Example of frequency distribution of ridge densityin left little toe print

The ROC curve analysis shows that right toe printsreflect higher sexing potential for dimorphism. The AUC in ROC curve for the right toe prints show the maximal sexing potential on the third toe which is 68.8%, while the maximal sexing potential for the left footprint is on the fourth toe which is 59.3%.

Discussion

Literature review shows that many studies have been conducted on fingerprint[17-18] and palm print [19-20] ridge density for gender determination, but there are limited study conducted on the footprint [21-23] and toe printridge density[4, 24]. In most of the crime scenes, partial footprints are commonly found rather than complete footprint. However, due to lack of footprint database, investigators tend to neglect the partial footprint, i.e., presence of toe prints, present at the crime scene consideringunfit for comparison[24]. Considering this point,the present study has been conducted for investigating partial footprint found at the crime scenes.

Just like fingerprint, toe prints show ridge density and ridge pattern in every toe. Thus, present study had been conducted on all 5 toes and hence the presence of a single toe in a crime scene may help in narrowing down the suspects of the crime. The present study shows the mean ridge density of great toe for female (R: 10.20, L:10.49) is comparatively higher than male (R: 9.98, L: 10.82). While study on great toe ridge density among North Indian population from Himanchal Pradesh State showed the mean ridge density in female (R: 8.1, L: 7.7) to be higher than male (R:6.4, L:7.0)[21]. Another toe print study was conducted onIban population in Malaysia Borneo showed that the mean ridge density of great toe for males (R: 12.6, L:12.9) are comparatively lower than females (R:13.5, L:13.4) [24]. Similarly a footprint ridge density was studied among South African population using two different ethnic groups, cape colored (R: 10.06, L:10.56) and white Afrikaans (R:10.82, L:10.4) [22] and the ridge density is different from other populations. Hence, the ridge density of Kagay-anon population is comparatively higher than North Indian population, butlower than Iban population.

Conclusion

The present study provided useful information in determining gender using toe print ridge density among Kagay-anon population in Philippines. Hence, the findings can be applied in analyzing the toe prints found at the crime scenes.

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Funding for this Research: Self

Ethical Clearance: Obtained from MSU research committee

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Sexual Dimorphism from Fingerprint Ridge Density among KagayAnons of Philippines for Forensic Application

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Abstract

Hand morphology and fingerprint patterns form a valuable tool for person identification. Sex identification is a vital role in forensic and medico legal investigations. Determination of sex or gender of an individual is important because it can help in the forensic investigators to identify the suspect if male or female based on the ridge density of handprint or palm print. The present study used fingerprints collected fromKagay-anon population for determination of gender through ridge density. A total of 4000fingerprints (200 males and 200 females) were collected and analyzed using the "Acree" method of ridge density counting within 5mm x 5mm square area calculated the ridge density. The mean ridge density iscomparatively higher in females (12.39-14.14) than males (11.06-11.87) in the fingerprints of the study population. The results are presented in the form of tables and graphs.

Key words: *Forensic science, fingerprint, ridge density, sex, Kagay-anon, Philippines*

Introduction

In our day today life, so many crimes are reported that involved murder, terrorism, burglary, and sometimes natural disasters such as earthquake, floods causing mass fatalities. During the investigation at the crime scene, the suspect or perpetrators tend to leave their identity in the form of physical evidence, that can be used to link the crime and suspects or criminals. Physical evidence such as fingerprint, footprint, bloodstains, paint, and seminal stains have been used for person identification[1-2]. Hand morphology and fingerprint patterns form a valuable tool for human identification. Fingerprint is unique where it has their own pattern that are constituted by the ridges on the surface of fingers. The friction ridge pattern presents on fingers, palms,soles, andtoes remain unchanged throughout their life[3]. In the crime scene, determination of gender is the initial step in the investigation process that ease the investigators and impression evidence such as fingerprint, handprint and footprint are found useful in gender determination[4]. Some researchers have developed formulae to estimate the stature from fingerprint, handprint, and hand anthropometry during the process of crime investigation [5-7].Recently researchers have been conducting study on sexual dimorphism from footprint, toe print, fingerprint, and palm print ridge density [8-11]and the researchers aware the fact that ethnicity should be considered whenever conductinganthropological based research. Therefore, present study was aimed to study on sexual dimorphism from fingerprint ridge density among Kagay-anon population, an indigenous ethnic group in Philippines.

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Methodology

The study sample consists of 400 Kagay-anon

people living in Philippine. Out of 400 study subjects, 200 are males and 200 are females with age ranged from 18 – 65 years. Subjects with hand disorder or disease were excluded from this study. Kagay-anon people is an indigenous ethnic group that reside at Cagayan de Oro City in Philippines. An informed consent from the participants and ethical approval from MSU obtained. Before sample collection, subjects were advised to wash their hands and dry. Using a plain clean glass plate, fingerprint roller, fingerprint ink, the fingerprints were collected in A4 size white papers by inking technique as done in earlier research [10-11].

Five designated areas were taken from the tip of the fingerprints for this study. The radial area in the fingerprint was used to study ridge density. The five areas in right sidesare thumb (R1), index finger (R2), middle finger (R3), ring finger (R4) and little finger (R5). Similarly, the left-hand fingerprints are designated as L1, L2, L3, L4, and L5. Ridge density count was conducted and analyzed using "Acree" method in which a square box of 5mm x 5mm was drawn on transparent film and placed on designated areas for counting[12].A linentester, strongmagnifier (Figure 2), was used to count theridges. The values that obtained represents the ridge density in 25mm² area and the data were statistically analyzed using SPSS software, version 25. Student's t test was used to study the gender variation in the designated areas.

Figure 1: Five designated areas on right fingerprintsR1: thumb, R2: index finger, R3: middle finger, R4: ring finger, R5: little finger

Figure 2: Linen tester used

Results

Table 1 shows the meanfingerprint ridge density and standard deviationsamong male and female fingerprints of the participants. The study examined 1000 right and 1000 left fingerprints. The result indicated that the mean ridge density of male ranged from 11.06 to 11.87 while female from 12.39 to 14.16. The female ridge density is comparatively higher than males. The ridge density of the left hand and right hand is not same in both gender and showing bilateral asymmetry. The ring finger reflected higher ridge density than other fingers in both genders.

Table 1: Descriptive statistics for fingerprint ridge density among Kagay-anon population(Males= 200, Females = 200)

Right fingers	Mean		SD		Left fingers	Mean		SD	
	Right side					Left side			
	M	F	M	F		M	F	M	F
Thumb (R1)	11.48	13.28	1.64	1.89	Thumb(L1)	11.57	13.38	1.77	2.18
Index finger (R2)	11.34	12.64	1.68	1.90	Index finger (L2)	11.17	12.49	1.52	1.75
Middle finger (R3)	11.40	13.03	1.99	1.93	Middle finger (L3)	11.41	12.99	1.53	2.17
Ring finger (R4)	11.87	14.16	1.61	2.03	Ring finger (L4)	11.61	13.91	1.77	2.19
Little finger (R5)	11.21	12.87	1.57	2.07	Little finger (L5)	11.06	12.39	1.78	1.89

M: Male, F: Female, SD: Standard deviation, R1-R5: Right fingerprints, L1-L5: Left fingerprints

Figure 1 and 2 shows the mean ridge densities of the designated area in both sides among male and females. It showed that the mean finger ridge density is higher in female than male.

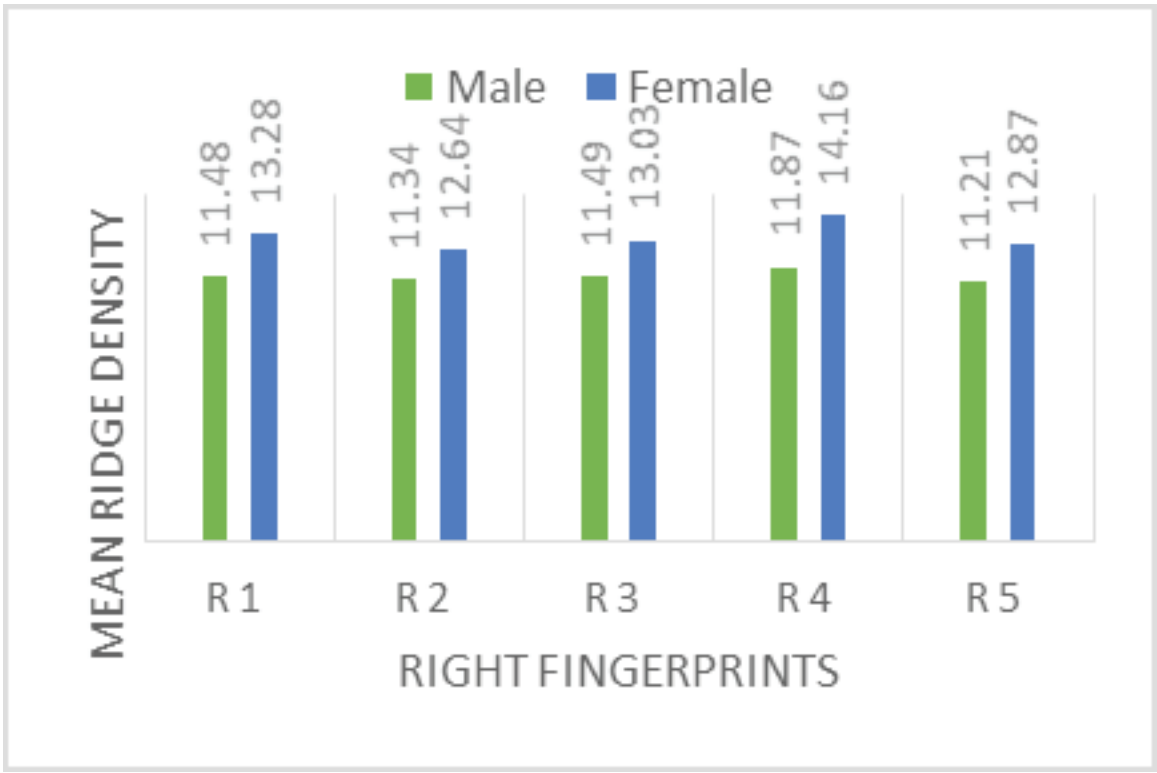

Figure 3: Mean ridge density in rightfingerprints

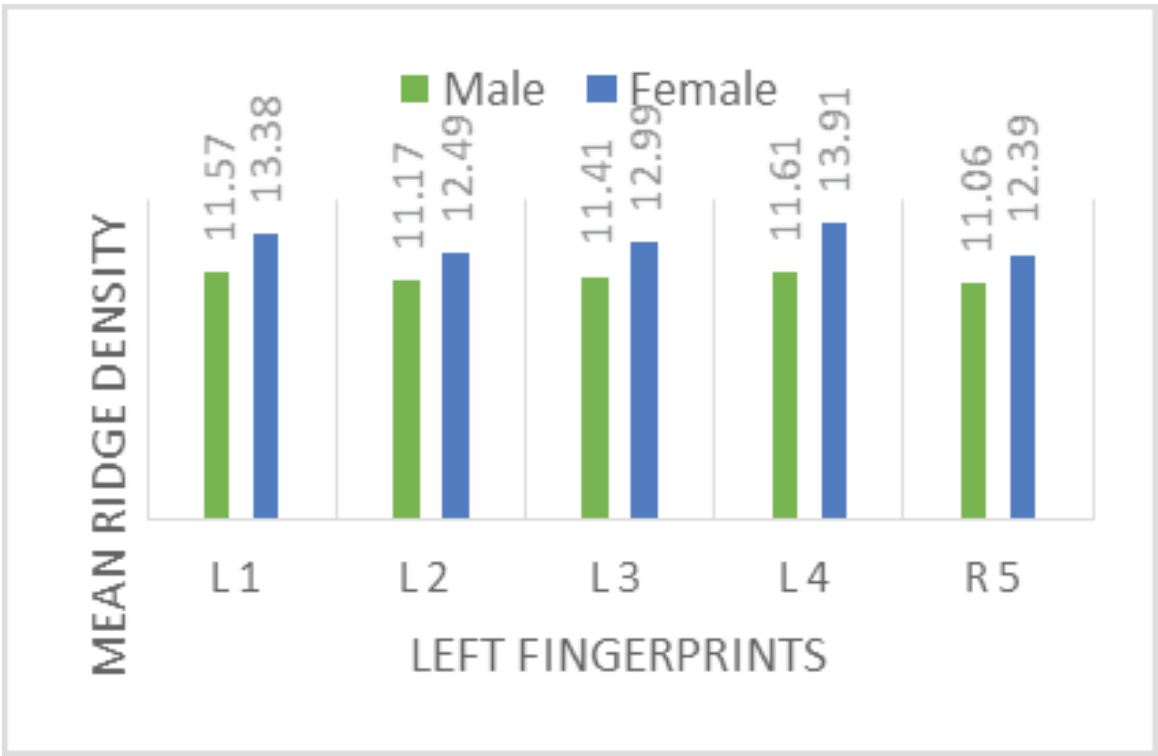

Figure 4: Mean ridge density in left fingerprints

Table 2: Sex differences in ridge density

Fingers	Right Finger		Left Finger	
	t value	*p* value	*t* value	*p* value
1	10.14	0.000*	9.10	0.000*
2	7.26	0.000*	8.03	0.000*
3	8.27	0.000*	8.39	0.000*
4	12.33	0.000*	11.57	0.000*
5	9.06	0.000*	6.76	0.000*

**p* is significant at the 0.01 level (2-tailed)

Table 2 showed that the *p*-values were found to be lesser than 0.01 and statistically significant. Sex differences found to be maximal in L4,R4and minimal in R2, L5.

Table 3 shows the frequency distribution of mean fingerprint ridge density in Kagay-anon population. 64% of males have a ridge density of 11 or lesser than 11, while 80% of females have 12 and above. From the frequency distribution mean of fingerprint ridge density, probability densities and likelihood ratio were calculated using Bayes' theorem. Assuming the probabilities of 50%, the odd was calculated.

The probability density and likelihood ratio based on Kagay-anon population was shown in Table 4. A ridge count of 10 ridges/25mm ² is more likely to be male (p=0.79) whereas ridge density of 13 ridges/25mm ² is more likely to be female (p=0.77). There is a high probability that fingerprint ridge density with 9 or lesser ridges to be male (p=1.00) and high probability of handprint ridge density with 14 or more ridges to be female (p=1.00).

Table 3: Frequency distribution of mean handprint ridge density of Kagay-anon population

Mean ridge density range	Male	Female
≤ 8	2	0
8 – 8.99	6	0
9 - 9.99	19	0
10 – 10.99	38	10
11 – 11.99	63	30
12 – 12.99	56	51
13 – 13.99	16	56
14 – 14.99	0	37
15 – 15.99	0	13
16 – 16.99	0	2
17 – 17.99	0	1
Total	200	200

Table 4: Probability densities and likelihood ratio in the handprint of Kagay-anon population

Ridge count	Probability density		Likelihood ratio		Odds
	Male (C)	Female (C')	(C)/ (C')	(C')/(C)	Male = 0.5, Female = 0.5
≤ 8	0.010	-	-	-	1.00 ≥ 0.00
8	0.030	-	-	-	1.00 ≥ 0.00
9	0.095	-	-	-	1.00 ≥ 0.00
10	0.190	0.050	3.800	0.263	0.79 ≥ 0.21
11	0.315	0.150	2.100	0.476	0.68 ≥ 0.32
12	0.280	0.255	1.100	0.912	0.52 ≥ 0.48
13	0.080	0.280	0.286	3.500	0.22 ≤ 0.77
14	-	0.185	-	-	0.00 ≤ 1.00
15	-	0.065	-	-	0.00 ≤ 1.00
≥16	-	0.015	-	-	0.00 ≤ 1.00

Discussion

Individualidentification isa mainstay in forensic investigation and impression evidence like fingerprint, footprint, palmprintor toe prints are playing a vital role for gender determination. Dermal friction ridges that present on finger or palmer appears during our intrauterine life and remains unaltered until death[13]. KagayAnons are people living mostly in Cagayan De Oro, Philippines. Cagayan De Oro region is noted for the tourists[14]. As a well-known biometric, fingerprint is globally used for identification of live and dead.Fingerprint patterns were studied to understand the inheritance factor that may find use in crime scene application [15-17].

A friction ridge is a raised portion of the epidermis on fingers, toes and palm or sole consisting of one or more connected ridge units of friction ridge skin. Ridge density study is the latest tool used for gender determination for various populations and hence researchers are showing interest in gender determination from footprint, fingerprint, toe print and palm print ridge density.Earlier period, the chance fingerprints were lifted by the fingerprint experts from the crime scenes like house breakings, homicides, sexual assaults and then compared crime scene prints with the fingerprints in the records by using hand magnifier. In the advance of science, currently automated fingerprint identification system (AFIS) is used. It is a process of using a computer match the crime scene fingerprints against the database. In the data base, the fingerprints of ex-convicts and suspects were recorded for criminal identification purposes.

But there are instances, wherein the involvement of first offender in crime involvements pose challenges in the AFIS, since the first offender fingerprints are not found in the system and the crime scene fingerprint is

questionable. In such complicated scenarios, the gender determination from fingerprint ridge density paves a way to investigation. Initially, the gender can be determined by using ridge density followed by stature determination and finally individual identification. The total ridge count could be influenced by the genetic components of individual such as sex chromosome, particularly in Y-chromosome[18]. In this study, radial area was chosen forridge density calculation.

The present study shows the mean ridge density for female is 13.11 while male is 10.27. Sudanese population study showed the mean ridge density for female has 14.50 while male 12.80[19].Another ridge density study on Filipino population showed that the ridge density for female and male were 15.89 and 14.57, respectively. Again, it is scientifically proved that ethnicity is a key component to be considered in the ridge density study on gender determination.

Some of the crime scene investigators have been underestimating the value of footprint, handprint and fingerprint evidence and neglected it from the initial stage of investigation[20]. This is because of the lack of knowledge on investigatorsabout the value of physical evidence found in crime scenes.

Conclusion

It is concluded that the present study done on Kagay-anon population revealed that there is existence of sexual dimorphism from the fingerprint ridge density. The result from fingerprint ridge density may be used as acorroborative evidence in the initial stage of investigation for person identification.

The present fingerprint ridge density study conducted on Philippine Kagay-Anon population, form a data base for gender determination. The ridge density results are different from other population hence can be used in real crime scenes for identification.

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Suicide Bombing in a Money Lending Shop – A Rare Non-Terrorist Explosion Scene Report

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Abstract

Suicide is an intentional actof a person causing one's own death. Suicide bombing is the choice of an individual or organization member to embrace death for achievement. Suicide bombers are mostly from terrorist organization. It is a crime scene report wherein a normal citizen, a lorry driver in India triggered suicidal bombing in a money lending shop. Suicidal death with explosive by normal citizens is rare. The author, then Government forensic crime scene investigator had investigated the suicidal bombing scene wherein a lorry driver aged about 42 years committed suicide bombing in a money lending shop. It was not a terrorist attack,from any terrorist organization. The author examined the blasting site and identified the type of explosive used in this act. After the bombing, the bomber survived for a while and subsequently collapsed within a shop, a rental house.The dead body found on the floor, within the shop with injuries on abdomen with exposed viscera. The body was intact, nor blown to pieces as appeared in terrorist suicide attack. An improvised devise found attached in abdomen region and the chemical substance used was low power explosives, never used by the terrorists for the suicide attack.The author concluded with a finding that the local lorry driver had committed suicide bombing under stress, because of his inability to pay the lorry loan instalments for many months. It was an act of psychological stress rather than terrorist attack since the moneylender was safe, even in his close range of the bomber.

Keywords: *Crime scene, suicide bomber, non-terrorist, low power explosive*

Introduction

Suicide is an intentional act of a person causing one's own death which is a serious problem to public health and society[1].The choice of committing suicide is based on access or available source to end their life. Most common method of suicide is the deliberate consumption of insecticides and pesticides that easily available in the market used for agricultural usage. Hence in many countries, hazardous pesticides and insecticide have been banned to reduce the number of suicides[2]. Suicideswhile in traffic are also reported, while a person jumps under a moving train or heavy vehicles[3]. The other means of committing suicide is jumping from heights such as multi-storey buildings and towers, mostly by young people suffering from schizophrenia[4]. Researchers have indicated that suicide by jumping from a bridge is the choice of some people, mostly males [5-6]. Suicide committed by firearms to the heads are also occurred in developed countries because of their usage and easy accessibility[7-8]. Death due to explosions are mostly accidentaland sometimes they are by homicidal act. But suicide by explosives isexceedingly rare and literature review showed the incidences as non-terrorist suicides[9-11]. Suicide operations by terrorist organization are used by fanatic religious and nationalistic groups

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and regard them as a kind of holy war by divine command[12-13]. There are about ten terrorist groups that are using suicide terrorism as a tactic against their own or foreign governments and VIPs to achieve their target by using improvised high power explosive devices[14-15]. Explosives involved suicidal deaths are rare and they used crackers, contain low power explosives like black powder or gun powder. The use of low power explosive can be ascertained with the analytical report obtained from forensic science laboratory. The present crime scene report is a non-terrorist suicidal death involving low power explosive, the chemical substance used in crackers.

Leaping from heights in general is a method with high lethality (Rock et al., 2005). It is the fourth most frequent suicide method in Switzerland, represent-ing 10.7% of all suicidesAlso jumping from high buildings, also into well and rivers are other ways of suicides. In the developed countries, firearms like revolver and pistol are the easy available source for suicidal deaths. The most common risk factors for suicide is depression and mood disorders

Suicide by jumping in general appears to be more often chosen by subjects suffering from schizophre-nia. Among those who jump, young subjects, in particular, choose bridges to end their lives. suicide by jumping from a bridge may be the choice of a population which differs from people jumping from other sites in certain demographic characteristics

Method and Observation

The author, then Government Forensic Crime Scene Investigator in India, investigated this suicide bombing, wherein a lorry driver initiated the improvised explosive device (IED) in a money lending shop, that occurred in Tamilnadu state, South India.

Figure 1: Suicide bomber was alive for a while after the blast and subsequently collapsed

Figure 1 shows the presence of dead body of the suicide bomber lying on the floor after the blast and alive for a while.Even the bicycle of the owner, parked near the deceased within the shop did not show any mark of damage or dent in any place of the bicycle. As the police station was extremely near to the money lending shop, the police arrivedimmediately whilethe lorry driver was still alivefor a while with sitting posture. Under low voice he informed the police that he was unable to pay the instalments for many months for the loan obtained and bought a lorry. He planned to get the money from a money lender under threat using bomb but in vain.

Observation and Reconstruction

As requested by the Deputy Superintendent of Police, the forensic crime scene investigator (the author) rushed to the money lending shop, the blasting scene. The money lender hired a house as money lending shop. He used to come to this shop from his residence through bicycle and park the cycle within the hall as seen in the figure. The suicide bomber was found dead near the bicycle. The body was intact, unlike body pieces found in terrorist suicide attack, wherein high-power explosives like RDX, PETN with missiles are tied in the body under dressingand after the blast the body of the terrorist maybe thrown away into pieces with damages in the surrounding area. But on examination of the body in the money lender shop, the hands, legs, and chest areas have no noticeable injuries. The shirt and the dhoti have shown characteristic damagesand the inner blue trouser went to pieces and two pieces found on the body. I noticed burned match sticks on the floor and charred cloth pieces. In a terrorist suicide attack, the bomber used missiles like nails, glass pieces, iron balls and other metal fragments within the high-power explosive to cause more casualties to the people who are far away from the blasting site. But the author did not observe any such missiles near the dead body, or any missile strike marks on the walls nearby. The owner who was sitting on a chair, away from the bomber did not sustain any injuriesor damages in the furniture he used. The lower abdomen region of the body showed the protruding of intestine with excess bleeding that caused the death.

Based on the crime scene observation, the bomber might have collected black powder from the crackers and made an improvised bomb with a wick and tied around

the abdomen region. He entered into the money lending shop and demanded money but the money lender scolded him to get away from the shop. Then the lorry driver, suicide bomber might have triggered the improvised country made bomb with fire. The improvised explosive device exploded in the abdomen region that caused injury with a hole through which a part of intestine projected out. The dress in the abdomen region burned, as found in the crime scene. The charred pieces and bloodstain evidence were collected for the onward transmission to the Forensic Sciences Laboratory, Chennai, India for analysis. The body was sent to Department of Forensic Medicine for autopsy examination. The forensic laboratory identified and reportedthe presence of black powder used in firecrackers, a pyrotechnic chemical substance. The autopsy report confirmed the explosive injuries caused by the explosion of firecracker chemical substance that classified as low power explosive. The author reconstructed the blasting scene based on physical evidence in the crime scene.

Discussion

Suicide bombing forms a tool for many terrorist organizations and using as a tactic against their own government or other governments [16]. Terrorist organization preferred young women to participate in the suicide bombing operations, considering the gender advantage[17].Social networks play a role of predicting the terrorist organization, who triggered the suicide bombing.Explosives associated deathsoccur at work places or homicidal discharge during terrorist activity[18]. Non-suicidal deaths are reported infrequently but require keen crime scene observation and investigation. Literature review shows only countable number of non-suicidal death cases reported[19-20].

Collection of physical evidence in the death scenesand crime reconstruction is an important key to solve the mystery andto arrive a conclusion[21]. Examination of injuries, distribution, and pattern of the victim in the crime scene provides valuable information for the manner of death[22]. In the developing countries, it is generally considered that depressions, mental illness, economic burden, and unemployment are the causes for committing suicide[23].

Conclusion

In the present case report a lorry driver turned to be a suicide bomber due to stress andfinancial crisis and ended his life. Based on the keen crime scene examination and autopsy examination, this fact was identified and solved the mystery by eliminated the possibility of terrorist attack.

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Ethical Clearance: Not required since it is my crime scene report

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Hanging Deaths at Tertiary Care Teaching Hospital

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Abstract

Background: Hanging signifies an asphyxiation form which, by a noose or another constricting band tightened by body weight, is secondary to compression or restriction of the neck structures. All hangings are suicidal. Accidental and homicidal hangings are rare and uncommon.

Objective: This research was performed on deaths attributed to neck strain related to hanging and strangulation carried out for post-mortem analysis in the mortuary of Osmania General Hospital

Methods: Detailed information regarding the deceased and the circumstances of the death were collected from the police and relatives by a questionnaire. For certain situations, the detail was matched by an on-site observation or by photos of the incident location.

Results: Knot was present in the right side of the neck in 97(48.5%) cases, over the left side of the neck in 62(31%) cases, and over the center of the occipital region in 41(20.5%) cases. In the present study, it is observed that in 167(83.5%) cases, the level of ligature mark was above the thyroid cartilage, below the thyroid cartilage in 11(5.5%) cases, and overriding the thyroid cartilage in 22(11%) cases. parchmentization was present in 135(67.5%) cases and absent 65(32.5%) cases. The causes for these observations are the ligature material type and the length of suspension which in the majority of cases are parchmentized. 169 cases (84.5%) did not show any changes around the ligature mark, but in 31 cases (15.5%). Fracture of hyoid bone was seen in 3.5% of the cases and fracture of thyroid cartilage was seen in 4.5% of the cases

Conclusion: Our society has a great socio-economic burden due to the high incidence of suicidal hanging among young adults particularly women. The private essence of hanging and convenient access to ligature points and ligature supplies renders suicide avoidance challenging. Marital disappointment, organic disease issues, and dowry abuse are the primary reasons behind suicide hanging.

Keywords: *Hanging, Ligature strangulation, Hyoid Bone, Thyroid Cartilage*

Introduction

For all living things, death is inevitable, but only people prematurely end their lives by suicide.. One of the most widely used techniques, since materials are readily accessible and have a good chance of success, by hanging.

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Hanging is the asphyxia that induces a ligature around the necks due to the suspension of the body, the force that is restrictive is the body's weight (complete hanging), or part the body's weight (partial hanging).[1]

In action, hanging has been since ancient times and prior to the coming of civilization. Death was found especially disgraceful in ancient Rome, and the citizens who died by this means were denied a funeral. As a result of this activity, Greeks assumed that many women were dead. In Roman and Greek cultures, 1.5-10% and 30% of suicides are by hanging. Different causes such as marital disharmony, financial difficulties, mental conditions,

persistent physical pain due to malignancy, and improper evaluation, are the liability of an individual.[2]

Although the majority of the cases are suicide, Hanging can be homicidal or accidental which can often lead to suspicions among family, police and even the surgeon. In such situations when the noose is too tight, there are too many twists, and when the perpetrator is a kid in an odd spot such as sitting or kneeling when it is a complete ligature, there is a controversy.[4,5]

This study is carried out using systematic analyses of the hanging and contrasts the outcomes with other studies and to assess the relevance of post-mortem observations of hanging.

Materials and Methods

All 200 cases of hanging brought to Osmania Medical College and General hospital mortuary for post-mortem examination between the period November 2017 to October 2019 are included in the study.

Detailed information regarding the deceased and the circumstances of the death was collected from the police and relatives by a questionnaire. For certain situations, the detail was matched by an on-site observation or by photos of the incident location. Permission of the ethical committee on the use of human material for research purposes was obtained.

A meticulous autopsy was conducted with special reference to neck structures, the bloodless field of dissection was carried out in all the cases. All the important findings were noted down in the master chart.

Inclusion criteria:

All 200 cases of hanging brought to Osmania Medical College and General Hospital mortuary for post-mortem examination between the period November 2017 to October 2019 are included in the study.

Exclusion criteria:

Cases other than hanging as cause of death.

Statistical Analysis: SPSS software version 22 has been used for statistical analysis. Data were presented as statistical tables and charts.

Results

Table No 1: Distribution of the study population according to age, sex, martial status, locality and fracture of thyroid cartilage.

Age in years	No. of cases	Percent
<10	1	0.5%
11-20	30	15%
21-30	94	47%
31-40	41	20.5%
41-50	20	10%
51-60	10	5%
61-70	3	1.5%
>70	1	0.5%
Sex		
Male	135	67.5%
Female	65	32.5%

Cont... **Table No 1: Distribution of the study population according to age, sex, martial status, locality and fracture of thyroid cartilage.**

Marital status		
Married	117	58.5%
Single	83	41.5%
Locality		
Urban	175	87.5%
Rural	25	12.5%
Treatment		
Treated	24	12%
Not treated	176	88%
History of previous suicidal attempt		
Yes	31	15.5%
No	169	84.5%

The most vulnerable age for hanging is found to be between 21-30 years, wherein 47% deaths occurred. The next vulnerable age group is 31-40 years in which 20.5% deaths occurred. In 11-20 years age group, 30 deaths (15%) have occurred. In 41-60 years age group, 30 deaths occurred. In old age, i.e. after 61 years, incidence came down to1.5%.

Table 2: Distribution of the study population according to the Type of Hanging (typical/atypical)

Marital status	**Type of hanging**		**Total**
	Typical	**Atypical**	
Married	28	89	117
Unmarried	7	76	83
Total	35	165	200

Table 3; Distribution of the study population according to the Type of Hanging (complete/partial)

Gender	**Type of hanging**	**No. of cases**	**Percent**
Male	Complete	41	20.5%
Female	Complete	17	8.5%
Male	Partial	96	48%
Female	Partial	46	23%

Table 4: Distribution of the study population according to the Postmortem Staining

Post Mortem staining	No. of cases	Percent
Post Mortem staining in lower limbs	51	25.5%
a)With Petechiae	14	27.45%
b)Without Petechiae	37	72.54%
Post Mortem staining at the back	149	74.5%

Table 5: Distribution of the study population according to the Position of Knot, Level of ligature mark, Peri ligature injuries, Effusion of blood

Position of knot	No.of cases	Percent
Right	97	48.5%
Left	62	31%
Centre of Occipital region	41	20.5%
Total	200	100%
Level of ligature mark		
Above the thyroid cartilage	167	83.5%
Overriding the thyroid cartilage	22	11%
Below the thyroid cartilage	11	5.5%
Parchmentisation of the ligature mark		
Present	135	67.5%
Absent	65	32.5%
Periligature injuries		
Present	31	15.5%
Absent	169	84.5%
Effusion		
Present	3	1.5%
Absent	197	98.5%

Knot was present in right side of neck in 97(48.5%) cases, over left side of neck in 62(31%) cases and over centre of occipital region in 41(20.5%) cases. In the present study, it is observed that in 167(83.5%) cases, the level of ligature mark was above the thyroid cartilage, below the thyroid cartilage in 11(5.5%) cases and overriding the thyroid cartilage in 22(11%) cases. parchmentization was present in 135(67.5%) cases and absent 65(32.5%) cases. The reasons for the above observations being the form of ligature material and the duration of suspension leading to parchmentization in majority of the cases. 169 cases (84.5%) did not show any changes around the ligature mark, but in 31 cases (15.5%)

Table 6: Distribution of the study population according to the fracture of hyoid bone, thyroid cartilage

Fracture of hyoid bone	No. of cases	Percent
Present	7	3.5%
Absent	193	96.5%
Fracture of thyroid cartilage		
Present	9	4.5%
Absent	191	95.5%

Fracture of hyoid bone was seen in 3.5% of the cases and fracture of thyroid cartilage was seen in 4.5% of the cases

Discussion

Each suicide is a horrific tragedy that has the premature end of the life of a person and an unabated effect that has a profound effect on the lives of family and community.[6] The study indicates that males are more frequent victims relative to females. The findings have demonstrated that men are the most frequent victims compared to females. One of the common strategies for committing suicide is to remain hanging. It is notably a lethal suicide procedure with an expected fatality rate of more than 70%.[7] There is no chance of changing mind since death usually comes quickly after the hanging in contrast to overdose.[8] During the last 30 years, hanging suicides have risen, particularly among young men throughout the world.[9,10] Males record the maximum number of hanging. The age ranges aged 21-30 years had the largest number of hanging deaths. In the urban areas and in the low socioeconomic community, the majority of hanging deaths occurred. Married men sometimes take their lives by hanging. Studying stress for excellence and success is one aspect that cannot be overlooked in causing hanging deaths. People prefer to hang up at night or choose midday time. Closed inside room has been turning into a safe hanging area. Ceiling rod and fan are also often used as suspension points. Typical ligature marks with hanging partly overnumbered typical ligature marks and hanging complete. Foot hitting the ground is often seen during partial hanging. The ligature marks were disrupted and dominant in majority. In maximum numbers of cases a single ligature above the thyroid cartilage stage with a 1-2cm diameter is found.

Coarse parchment ligature patterns with colour stains varying from red to yellowish brown to dark brown are most often found in hanging deaths. In a few cases, peripheral ligature damage involving abrasions, bruising and cord burns have been observed. Peri-ligature wounds, contusions and abrasions have been seen. Peri-ligature injuries are caused by nodal contusions, by the projection of fibres from the ligature material and by nail claw marks inflicted by the victim fighting in order to free itself at death. They also often used soft ligature products, such as saree, dupatta, lungi and bed sheets. The ligation mark was dark brown and parchmentization with hard and soft ligament materials with an improvement in suspension length.

In situations in which a narrow, strong or hard ligature material is added, a distinct ligature mark groove of width and pattern is found.

In cases of complete hanging, a conspicuous ligature mark is found. When use of softer and more wide ligature fabrics, a less distinctive mark is noticed on the neck. The research revealed characteristics such as dribbles of saliva, transverse tears of carotid affection, abrasions, bruising and rope burns around the ligature mark as well. The ligature mark was usually above the thyroid cartilage, made upward and backward, depending on the location of the knot, to the right or left of the occipital zone. It has been found that the tissues below the ligature mark were pale and gleaming in 197 cases, with blood effusion seen in 3 cases. After the ligature around the neck has been bonded the blood effusion experienced following a lengthy drop. Post-mortem staining happened in 74.5% of the posterior and in 25.5% of the lower limbs based on the duration of the hanging. 10,77 percent of cases have been reported to have accidental discharge from anal and external urethral meat. The most frequent cause for hanging was marital disharmony. The rate of hyoid bone fracture was 3.5%. Thyroid cartilage fracture was 4.5%. The Delayed deaths were minimal.

Since the study was limited to a specific region, the areas confined to Osmania Medical College and hospital jurisdiction were covered. Deceased information is focused primarily on the records of the police, family, inquiries and photographs from the incident scene.

Conclusion

The high suicide rate among young adults, particularly women, is a major socio-economic burden for our society. Avoidance of suicide is challenging because of its private existence and easy access to ligature points and supplies. The main cause behind suicide by hanging is marital discontent, organic diseases and dowry abuse.

Ethical Clearance: Ethical clearance was obtained from College institutional ethics committee of Osmania Medical College and General Hospital prior to the commencement of the study.

Source of Funding: Self

Conflict of Interest: Nil

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Toxicological Assessment of Ethanolic Leaves Extract of Kalanchoe pinnata in Rats

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Abstract

Kalanchoe pinnata is a perennial plant that is widely used in the folklore treatment of kidney and gall stones and urinary insufficiency. The present study aims to evaluate the potential toxicity of the leaf extract of this plant upon acute and sub-acute (28 day) exposure in Wistar rats when administered orally. In Acute oral toxicity(n=3) Female Wistar rats were treated with a single dose of 2000mg/kg b.wt and observed for 14 days and Sub-acute toxicity(n=6) Male and Female Wistar rats were treated with the dose of 500 and 1000mg/kg b.wt of leaf extract for 28 days consecutively. No mortality, morbidity or adverse clinical signs of toxicity were observed during the experimental period. No significant changes in the body weight was observed. Gross necropsy did not indicate any treatment-related pathological changes in any of the animals in the acute and sub-acute toxicity studies. Histopathology of the Liver, Heart and Kidney did not show any remarkable lesions that could be related to the administration of the leaf extract. Ethanolic leaf extract of *Kalanchoe pinnata* is relatively safe with an LD_{50} value greater than 2000 mg/kg. From the sub-acute study, the no-observed adverse effects level (NOAEL) of the extract can be derived as 1000 mg/kg b.wt in male and female Wistar rats.

Keywords: *Kalanchoe pinnata, traditional medicine, safety, acute toxicity, sub-acute toxicity.*

Introduction

Kalanchoe pinnata (*K. pinnata*) is a medicinal plant of ethnomedical importance and used by the Asian folklore for various ailments.[1] The leaf extracts of this plant have been routinely used for ailments like microbial infections, kidney stones and wound healing. A number of active compounds, including flavonoids, glycosides, triterpenoids, steroids, bufadienolides and organic acids have been identified in *K. pinnata* that have been shown individually to possess variety of biological activities.[2-6]

Rajsekhar et al.[7] reviewed the pharmacological potentials of this plant which included wound-healing, antioxidant, anti-cancerous, anti-proliferative, antimicrobial, antiviral, anti-protozoal, anti-leishmanial, anthelmentic, insecticidal, anti-allergic, analgesic, antinociceptive, anti-edematogenic, anti-inflammatory, muscle-relaxant, antipyretic, anticonvulsant, antidepressant, sedative, antilithiatic, hepatoprotective, gastroprotective, antidiabetic, nephroprotective, haemoprotective, antihistamine, anti-hypertensive and immunosuppressant activities. Fernandes et al.[8] illustrated the presence of novel bioactive molecules in *K. pinnata* against bothropic venom. Zakharchenko et al.[9] explored the fungicidal potential of transgenic *K. pinnata* extract containing CecP1 and recommended their use as a candidate drug for treatment of wounds infected with *Candida albicans*.

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Despite the fact that medicinal plants such as *K. pinnata* have immense therapeutic value, the safety profiling needs to be performed using validated protocols so that it can be continuously utilized in folklore treatment.[10] To address this, a safety evaluation study of the ethanolic leaf extract of *K. pinnata* was conducted in Sprague-Dawley rats following the methodology described in the OECD guidelines for testing for acute (OECD 423) and repeated dose toxicity (OECD 407) as an initial screening for safety.

Materials and Methods

Plant material

Fresh leaves of *Kalanchoe pinnata* were collected from ITWWS (Irula Tribe Women's Welfare Society) Thandarai village, Chengalpattu, Tamil Nadu, India. The plant material was authenticated by Dr.P. Jayaraman, Director, Plant Anatomy Research Center, West Tambaram, Chennai. [PARC/3213]. The voucher specimen was deposited in the herbarium of PG and Research, Department of Plant biology and plant Biotechnology, Presidency collage, Chennai.

Extraction

The extraction was performed according to the method adopted by Azwanida.[11] Fresh leaves of *K. pinnata* were washed to remove any impurities present and shade-dried. The dried leaves were pulverized and the preparation was taken for extraction. The coarse particles were cold macerated with ethanol and kept in a shaker for seven days at 37 ºC and later filtered (using Whatman filter paper No.1 (11 µm pore size)) and dried on the hot plate at 40ºC. The crude extract was stored in refrigerator (2 -8ºC) until use.

Animals

Healthy young adult animals (8 to 12 weeks old) of Wistar strain were obtained from Sainath agencies, Hyderabad, India. The females were certified to be nulliparous and non-pregnant. The health status of the animals was provided by the vendor. All the animals were found to be health and active. The animals were acclimatized for a period of six days and housed individually in polypropylene cages with stainless steel top grill. Sterilized paddy husk bedding was provided to the animals. Standard laboratory diets (Rodent pellet) and purified drinking water was provided *ad libitum* throughout the experiment except when fasting was necessary. The animals were maintained under controlled environmental conditions at a temperature of 22°C (± 3°C) and relative humidity of 36-64% with 12 h light and 12 h dark cycle. Individual animals were identified by tail marking with unique numbers.

Acute oral toxicity

The evaluation of acute toxicity in a limit dose test was performed using the procedures described by the Organization for Economic Cooperation and Development (OECD 423). A limit test at one dose level (2000mg/kg body weight) was carried out with 6 female animals (3 animals per step). The ethanolic leaf extract of *K. pinnata* was administered as a single dose orally to overnight fasted rats. Food was withheld for a further 3 hours after dosing. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. Individual weights of animals were determined shortly before the test substance is administered and weekly thereafter. All test animals were subjected to gross necropsy. Gross pathological changes (if any) were recorded for each animal.[12]

Repeated dose toxicity

Following the acute toxicity study, the effect of 28-day repeated oral dosing of the extract in Wistar rats was evaluated following the OECD 407 Test Guideline. The method used provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on the nervous, immune and endocrine systems.

In the repeated dose oral toxicity study, 18 animals were randomly allocated to the control and treatment groups with 3 males and 3 females in each group. The animals in the treatment group were dosed with the ethanolic extract of *K.pinnata* at 500 and 1000mg/kg body weight daily 7 days each week for a period of 28 days. Animals in the vehicle control group received purified drinking water at 10ml/kg body weight. The extract/vehicle was administered in a single dose to the animals using a suitable intubation cannula.[13]

General clinical observations were made at least once daily. Mortality and morbidity observations were made twice daily. Body weight and feed measurements were made weekly. At the end of the 28-day treatment period, animals were euthanised. At gross necropsy, blood samples were collected from the retro-orbital sinus for haematology and biochemistry analyses.

Hematology and Biochemistry

Blood was collected from the retro-orbital sinus under mild anaesthesia using diethyl ether, into heparinized and nonheparinized tubes for hematology and biochemistry analyses respectively. Hematological parameters analyzed include Hematocrit, Clotting time, Hemoglobin concentration(Hb), Red blood cell count(RBC), White blood cell count(WBC), Differential count(DC) and Platelet count. Serum samples were analysed for the following biochemical parameters: Sodium, Potassium, Glucose, Total cholesterol, Urea nitrogen, Creatinine, Total protein, Albumin, Total bilirubin, Alanineaminotransferase (ALT) and Aspartate aminotransferase (AST).

Histopathology

The animals were later euthanised by CO_2 asphyxiation. Gross necropsy (External and internal) was performed; Major organs (Liver, Heart and Kidney) were collected for histopathological evaluations.

Statistical Analysis

Data obtained were subjected to paired Student's *t*-test to evaluate the difference between the treated and control groups. P value was set at 0.05. Values were represented as Mean ± SD.

Results

Acute oral toxicity

No mortality/morbidity was observed any of the animals treated with the ethanolic extract of *K. pinnata* at 2000mg/kg body weight during the 14-day observation period. All animals showed an increase in body weight relative to that recorded prior to treatment. Based on the observations, the median lethal dose (LD_{50}) of *K. pinnata* extract in female rats was estimated to be greater than 2000 mg/kg body weight.

Repeated dose toxicity

Repeated oral administration of the extract did not induce any adverse clinical signs of toxicity in any of the treated animals in both the sexes. No mortality or morbidity was observed during the course of the treatment period until termination. No significant changes in body weight or feed consumption were observed. Gross necropsy did not reveal any treatment-related pathological lesions. All treated animals gained appreciable body weight relative to that recorded prior to dosing (Table 1).

Table 1. Repeated dose toxicity study – Mean Weekly Body weight (in grams)

Treatment	Days					BWC
	0	7	14	21	28	
Control 10mL/kg b.wt	128.25 ± 2.12	134.3 ± 1.71	141.3 ± 2.08	146.65 ± 3.06	152.75 ± 3.70	24.5 ± 2.78
KPEE 500mg/kg b.wt	127.27 ± 3.40	133.73 ± 2.53	139.95 ± 2.52	145.62 ± 2.31	152.43 ± 2.91	25.1 ± 1.49
KPEE 1000mg/kg b.wt	125.73 ± 2.43	132.25 ± 2.27	138.87 ± 2.59	144.53 ± 2.12	152.75 ± 3.70	24.5 ± 2.78

Values are expressed as Mean ± S.D, N=6(both 3male&3female), KPEE – *Kalanchoe pinnata* Ethanolic Extract, BWC - Body Weight Change

Hematology and Biochemistry

The effects of sub acute oral administration of *K.Pinnata* extract on hematological and biochemical parameters are presented in Tables 2 and 3. No significant changes were observed in the levels of Hematocrit, Clotting time, Hemoglobin concentration(Hb), Red blood cell count(RBC), White blood cell count(WBC), Differential count(DC), Platelet count were observed with respect to the untreated control. No statistically significant changes were observed in the biochemical parameters assessed.

Table 2. Effect of ethanolic extract from *K. pinnata* leaves on Hematological parameters

Hematological Indices		Control 10mL/kg b.wt	KPEE Treated 500 mg/ kg b.wt	KPEE Treated 1000 mg/ kg b.wt
RBC(10^6/µL)		8.22 ± 0.24	8.21 ± 0.48	7.88 ± 0.59
Hb(g/dL)		13.73 ± 0.38	13.82 ± 0.35	13.33 ± 0.29
HCT(%)		44.37 ± 0.86	43.92 ± 2.21	43.36 ± 1.37
WBC(10^3/µL)		11.3 ± 0.34	11.02 ± 0.35	10.68 ± 0.65
PLT(10^3/µL)		949.94 ± 21.91	944.7 ± 31.94	947.33 ± 16.14
CT(s)		133.01 ± 6.7	132.97 ± 8.64	136.92 ± 3.01
Differential Count	L (%)	74.35 ± 2.37	72.17 ± 2.21	73.18 ± 0.83
	M (%)	5.48 ± 0.32	5.46 ± 0.43	5.36 ± 0.28
	G (%)	22.54 ± 1.37	21.62 ± 2.27	20.33 ± 0.71
MCH(pg)		16.03 ± 0.84	15.95 ± 0.45	16.55 ± 0.59
MCHC(g/dL)		29.55 ± 1.48	30.19 ± 1.47	30.61 ± 0.69
MCV(fL)		54.31 ± 1.35	55.01 ± 2.19	55.71 ± 1.93

N=6(both 3male&3female), KPEE – *Kalanchoe pinnata* Ethanolic Extract Values are expressed as Mean ± S.D; Hb, hemoglobin; RBC, red blood cell count; HCT, hematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; WBC, white blood cell count; L, lymphocyte percentage; M, monocyte percentage; G,granulocyte percentage; PLT, platelet count; CT, clotting time.

Table3. Effect of ethanolic extract from *K. pinnata* leaves on Biochemical parameters

Biochemical Indices	Control 10mL/kg b.wt	KPEE Treated 500mg/kg b.wt.	KPEE Treated 1000mg/kg b.wt.
Sodium(mEq/L)	145.41 ± 2.05	144.95 ± 2.35	146.84 ± 1.34
Potassium(mEq/L)	4.89 ± 0.29	5.17 ± 0.31	5.32 ± 0.44
Glucose(mg/dL)	106.32 ± 2.86	105.11 ± 1.82	105.01 ± 2.29
Cholesterol(mg/dL)	99.35 ± 2.19	97.54 ± 3.76	98.05 ± 3.88
Total Bilirubin(mg/dL)	0.37 ± 0.07	0.42 ± 0.04	0.34 ± 0.05
Total Protein(g/dL)	6.89 ± 0.18	6.89 ± 0.37	6.44 ± 0.47
Albumin(g/dL)	3.73 ± 0.32	3.97 ± 0.28	3.84 ± 0.36
Urea(mg/dL)	16.6 ± 0.53	16.39 ± 0.75	16.79 ± 0.29
Creatinine(mg/dL)	0.56 ± 0.05	0.63 ± 0.11	0.58 ± 0.09
AST(IU/L)	75.53 ± 2.52	75.41 ± 2.35	76.91 ± 1.09
ALT(IU/L)	19.2 ± 1.11	19.34 ± 1.13	20.5 ± 0.89

N=6(both 3male&3female), KPEE – *Kalanchoe pinnata* Ethanolic Extract Values are expressed as Mean ± S.D; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

Euthanasia and Gross necropsy:

The animals were euthanized by carbon dioxide asphyxiation and gross necropsy of the external and internal organs did not reveal any treatment-specific changes.

Histopathological Examination

Figures 1 represent the microscopic images of the stained tissue sections of liver, kidney and heart in the control group (A) and treatment groups (B 500mg/kg b.wt and C, D 1000mg/kg b.wt.). No treatment-specific lesions, inflammation or pathological changes related to were observed in the organs of the animals from the treatment group compared to the untreated group. In general, microanatomy of liver, kidneys and heart did not present any treatment-related adverse toxicological effects in the animals that received 500mg/kg b.wt. and 1000mg/kg b.wt.

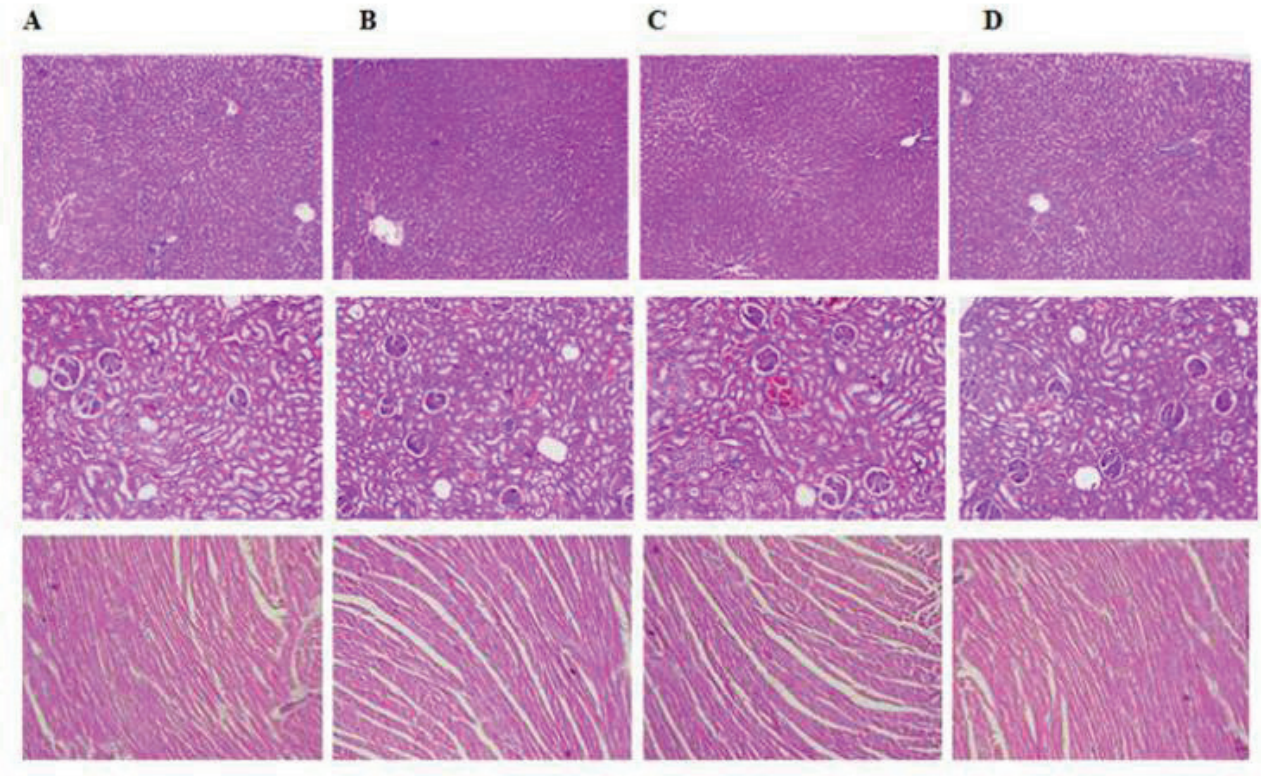

Figure 1: Hematoxylin and eosin stained cross sectional liver, kindney and heart views of control(A) and .KPEE extract treated 500 mg/kg b.wt(B) and 1000 mg/kg b.wt (C and D) in sub-acute toxicity study in male and female rats (magnification, x40)

Discussion

Medicinal plants are an important source of substances which are claimed to induce anti-inflammatory and antioxidant effects. *K. pinnata* found in the temperate and tropical regions and largely used in Indian system of medicine(Folk, Siddha and Ayurvedic) for the treatment of kidney stones, gastric ulcers, pulmonary infections, rheumatoid arthritis.

Ozolua et al.[14] studied acute and sub-acute oral toxicity of the aqueous extract of *K. pinnata* in Sprague Dawley rats. No mortality was observed at the acute oral dose of 5 g (5000 mg) per kg body weight by the oral route. The medial lethal dose following intraperitoneal administration was 1.8 g (1800mg) per kg body weight. Sub acute treatment did not significantly alter animal weights, organ-to-body weight ratios, fluid intake, hematological indices and the levels of AST, ALP and albumin. ALT level was significantly reduced in the treated group. Total bilirubin and conjugated bilirubin levels remained unaffected.

Zakharchenko et al.[9] indicate that ethanol extracts have become increasingly popular form of *Kalanchoe* application in therapy. The therapeutic efficiency is attributed to the presence of lipophilic constituents such as the bufadionolides, polyphenols and flavonoids.

Nayak et al.[15] studied the wound healing potential of the ethanolic extract of *K. pinnata* leaf in rats and concluded that there was increased wound retraction and hydroxy proline content in the extract treated groups indicating a significant wound healing potential consistent with the traditional healing practice. Mathew et al.[6] reported the analgesic and anti-inflammatory potential of the ethanolic extract of the stem of *K. pinnata* in rat models, anti-depressant potential in mice, and anti-diabetic potential in alloxan-induced diabetic rats. In all these studies, the extract as such which served as the control did not exhibit any adverse effects in the treated animals, indicating its safety.

Nevertheless, an independent safety evaluation of the ethanolic extract has not been reports so far. The

present study attempted to evaluate the safety of the ethanolic extract of *K. pinnata* leaves during acute and sub-acute treatments in Wistar rats. The results obtained are similar to those obtained for the aqueous extracts performed by Ozolua et al.[15] in terms of the absence of mortality and the haematological and biochemical indices studied. In addition, histopathological evaluation of the major tissues (Liver, Heart and Kidney) did not indicate any remarkable lesions that could be correlated to the repeated dosing of the plant extract. The results therefore indicate the safety of the ethanolic extract of *K. pinnata* leaves complementing its use in traditional medicine.

Conclusion

Toxicological evaluation of the ethanolic extract obtained from leaves of *K. pinnata s*howed no signs or symptoms of acute toxicity. The median lethal dose (LD_{50}) value of the extract was derived to be higher than 2000 mg/kg b.wt indicating that the extract is non-toxic. Repeated dose toxicity study with the extract did not induce any adverse clinical signs of toxicity in the treated animals; in addition, hematology and biochemistry parameters remained unaffected in the treated animals. No histopathological changes (in the organs studied) related to treatment with the plant extract were recorded. The extract is therefore considered safe for repeated administration up to 1000mg/kg/day. In conclusion, the ethanolic extract of *K. pinnata* does not contain any toxic phytochemical constituents as evidenced by the lack of adverse clinical effects in acute and repeated dose toxicity studies in rats and can therefore be concluded as safe for oral consumption.

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The Dessert Dimension: Genes related to Sweet Taste Receptors, Sweet Taste Preferences, its Association with Alcohol Consumption and dental Caries Incidence – A Literature Synthesis and scoping Review

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Abstract

Sweet taste is considered to be the most desirable taste category. With recent advances and gene mapping techniques, the genes associated with sweet taste were identified, namely, TAS1R family and the Sac locus. In this review, we have tried to summarise the association of sweet taste and genetics, involving the studies related to sweet taste receptors, sweet taste preferences and how sweet taste preference can become a potential phenotype that influences alcohol intake and dental caries. The PubMed and Google Scholar databases were searched for articles using keywords 'sweet taste', 'genes', 'sweet taste preferences', 'sweet taste and alcohol', 'dental caries', etc. The process also involved hand searching using references.

Keywords*: sweet preference, genetics, dental caries, alcohol.*

Introduction

Sweet, sour, bitter, salty and umami are the universally accepted 5 well-characterised tastes that humans experience. This sense of taste has allowed us to differentiate and consume "good" and fresh food, pinning itself on the massive pin board of evolution. Taste of this landscape of nutrients is also the reason why people love to eat. Anatomically, the taste receptors are located in the beginning of the alimentary canal. Hence become the determining gateway to homeostatic systems dealing with choice of nutrient and fluid intake.[1] As our perception and preference of food gets wired over time, this food compass points to the direction of genome and genetics.

Sweet food substances being calorically rich are consumed as sources of energy. Although sweet food substances are avidly consumed, there is considerable variation in sweet taste perception and preferences within and among species.[2] Multiple variables affect sweet preferences for example age, race, culture, genetics, etc.[3] Genetic studies in humans and experimental animals strongly suggest that the liking of sugar is influenced by genotype.[4] With recent advances and strong armamentarium, genetic mapping studies were able to identify the locus of sweet receptor.[5] Which are the located sweet taste receptors? Do genes determine sweet perception and preferences? What are the animal model studies pointing towards? As this area of study

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is seeing increased attention, it is worthwhile to pursue these questions. Understanding the interplay of genetics and sweet taste will also yield further information on how sweet taste preferences are associated with predisposition to dental caries or alcoholism. Thus, helping healthcare professionals tackle these concerns and tailor diets.

Sweet Taste

Apart from being a natural taste category, "sweet" taste is also reckoned as something desirable or pleasurable. In many languages, the word for "sweet" translates to pleasant experience.[6] In addition to evoking behavioural responses, sweet taste stimuli can elicit pre-absorptive cephalic phase responses, such as insulin release, activate endogenous opioidergic, dopaminergic and serotonergic systems and produce analgesic effects in children and young animals.[7,8] The structures of compounds that are perceived as sweet include a wide group of polyalcohols such as sugars and glycerol, some amino acids, peptides, proteins, and many artificial and organic sweeteners.[9]

The Sweet Taste Receptors: The Sac Locus and Tas1r Gene.

Taste papillae on which most sweet receptors are present can be seen on the tip of the tongue. Taste receptors that interact with food and drinks are located majorly on the tongue and soft palate. 50 to 150 cells form a taste bud that reside in projections called papillae. Information from the interaction is carried to the brain by Cranial nerves VII, IX, and X.[10]

The quest to identify the sweet taste receptor and understand sweet taste perception and preferences was possible because of extensive studies and discoveries, as shown in Table 1. [9, 11-23] The identification of Sac locus and the Tas1R gene has majorly increased our understanding of the sweet receptor's gene constellation. The mammalian T1R gene family consists of three genes named 'taste receptor, type 1, member 1, 2 or 3'. TAS1R1, TAS1R2 or TAS1R3 are used as gene symbols in humans and Tas1r1, Tas1r2 or Tas1r3 are used for non-human animals.

Table 1: Animal Model Studies

Author(S)	Study Description And Findings
Fuller	By studying two inbred strains of mice, the results of his experiments about inheritance pattern of sweet intake suggested a dominant mode of inheritance for the Sacb allele.
Stockton and Whitney	Investigated sucrose and glucose preferences of several inbred strains of mice and their F1 generations. Inbred strains of mice showed differences in their preference and intake of both sucrose and glucose solutions.
Lush	Confirmed the existence of sac locus. His studies on mice model suggested that a single locus was responsible for much of the strain difference.
Bachmanov et al	Using the F2 generation from a high-saccharin preferring strain (C57BL/6ByJ) and a low-saccharin preferring strain (129/J), mapped the Sac locus to a small region of distal chromosome 4. Conducted a high-resolution linkage analysis of the chromosomal locations of Taslrl and Sac. Concluded that Taslrl and the saccharin preference locus, Sac, are coded by linked but different genes. Positionally cloned the Sac locus. Concluded that Taslr3 was very likely a sweet taste receptor. Later, also concluded that the dpa locus and the Sac locus are separate based on their chromosomal positions.
Blizard et al	Also suggested that the Sac locus maps to distal chromosome 4 subsequently.
Ninomiya, Y et al	suggested that a single genetic locus largely determined the phenotype (D-phenylalanine) with breeding experiments using tasters and non-tasters of Dphe and their F, and F2 hybrid generations.

Cont... **Table 1: Animal Model Studies**

Capeless and Whitney	Resolved the issue of whether sac locus and dpa locus are alleles of the same genes by testing five inbred strains of mice for their preferences for D-phe and saccharin.
Hoon et al	Identified a putative sweet taste receptor called TR1- G protein coupled receptor (Taste Receptor 1 now renamed T1R1).
Max M et al	Suggested that Taslr3 may dimerize with the other two members of this receptor family, Taslrl and Taslr2 and that these dimers may be the actual sweet receptor.
Nelson et al	Reported that when Taslr3 and Taslr2 are simultaneously expressed in a non-taste cell system, certain sweeteners activate the system.
Kitagawa et al., 2001; Li et al., 2002; Kim et al., 2006; Wendell et al., 2010	Determined that sweet perception is determined via a G-protein-linked heterodimer which is encoded by TAS1R2 and TAS1R3 gene receptor proteins.
Kim et al., 2006	Concluded that TAS1R and TAS2R genes function in taste perception mechanism via coding taste receptor proteins. TAS1R gene subunits are located on chromosome 1, which is TAS1R1, TAS1R2 and TAS1R3.

Sweet Taste Preference

Sweet taste happens to be the most appealing choice of taste category for most people and makes them call out their "cravings" or "sweet tooth". However, considering this preference to be a uniform pattern would be inaccurate. Ponderous variation is reported within families and even individuals themselves over time; age acting as a variable. For instance, children consume sweets more often. Sweet preference is affected by multiple variables like genetics, age, sex, culture, etc.[3] Measuring taste behaviour takes into account aspects such as intensity, quality and hedonic value of the taste sensation. In humans, verbal information is assessed.[1] Multiple studies have been performed to understand the relation of sweet perception and genetics better, as summarised in Table 2. [24-29] These studies involve family and twin model (monozygotic and dizygotic) subjects. Digging in deeper on how gene structure and variation affects sweet perception helps define better models that will in turn help prevent people from getting driven towards caries-inducing sweet diet or predisposed alcoholism.

Table 2: Studies Related to Sweet Taste Preferences

Author(S)	Study Description And Findings
Thompson et al	Divided subjects into one of two types: Type I and Type II, on the basis of their reaction to a series of sucrose concentrations.
Meiselman	Noted the extreme degree of individual variation in sweet preferences.
Witherly and Pangborn	Observed that large within-group variation overshadowed between-group differences when obese and lean adults gave hedonic ratings for sweet stimuli.
Faurion et al	Concluded that variation within individuals is small compared with variation between individuals.
Ritchey & Olson	Concluded that pre-school children's degree of liking for sweet foods was not co-related to the degree of parental liking for sweet foods.
Cavalli-Sforza	Concluded, food preferences are largely determined by cultural transmission and individual experience by reviewing family and twin studies.
Pérusse & Bouchard	Concluded that 20% of the variance associated with fat and carbohydrate preference is genetic. And, "the literature indicates a rather moderate role of heredity in energy intake and food preferences."

Sweet Taste Preferences and Alcohol Consumption

Due to its pharmacological effects, alcohol (ethanol) is avidly consumed and just over 50% of adults living in the United States are regular drinkers.[4] Humans perceive certain concentrations of alcohol (ethanol) as sweet.[30] Rewinding time, there is evidence to suggest that the liking for sweetness co-evolved with that for ethanol, because fermented fruit contains both.[31] Electrophysiological recordings indicate that lingual application of ethanol activates sweetener□responsive neural fibres in the gustatory nerves .[32,33] Although genes responsible for the association of sweet taste and alcohol are unknown, several studies have shown that in humans sweet liking is associated with proclivity to drink more alcohol. [31,34]Data suggest that Tas1r3 alleles influence perception of the sweet taste component of ethanol flavour.[2] Tas1r3 gene is identical to the Ap3q locus (alcohol preference 3 QTL) and that its pleiotropic effect on ethanol consumption is mediated by genetic differences in perception of the sweet taste component of ethanol flavour; more the hedonic attractiveness of ethanol sweetness, more the ethanol intake by B6 mice. Considering recent animal model studies, the role of the T1R3 receptor in alcohol consumption was exhibiting that mutant mice lacking the Tas1r3 gene have diminished ethanol intakes and preferences. In studies accordant with mice, a more sensitive Tas1r3 allele makes the pleasant sweet taste component of ethanol stronger and facilitates consumption of ethanol by them. Consistent with this, alcohol consumption is associated with allelic variants of the Tas1r3 sweet taste receptor gene in mice and TAS2R bitter taste receptor genes in humans.[35] Therefore, a fuller understanding of the phenotype of sweet taste has a potential to be used as biological marker for diagnosing predisposition to alcoholism.[31,34]

Sweet Taste Preference and Dental Caries

Dental caries is a multifactorial and complex disease. It is also the most prevalent diseases in industrialized and developing countries. Heredity has been linked with dental caries incidence in scientific literature for many years.[36] Although dental caries is a multifactorial disease, a model looking into genetic basis becomes a requisite to cognise the disease with a better approach. Genetic sensitivity to taste is an inherited trait in children, Kulkarni et al. demonstrated that TAS1R2 was associated with dental caries in Canadian adults and polymorphisms in the sweet taste receptor (TAS1R2) and glucose transporter (GLUT2) genes individually and in combination are associated with caries risk. [37] Sweet taste preferences have been associated with genetic polymorphisms in sweet taste receptors in humans.[38, 39] Studies centring on sweet taste preferences and not on taste thresholds have shown a positive correlation between sweet taste and dental caries in both urban and rural populations, but this was found to be stronger in the rural groups. Pidmale et al. observed that tasters (sweet dislikers) had lower dmfs values compared to non-tasters (sweet likers) which was statistically significant in 119 children aged 36–71 months.[40] It is also noteworthy that previous studies have shown that genetic polymorphisms in taste receptors such as rs35874116, rs3935570, rs9701796, and rs307355 were associated with dental caries.[41, 42] Additional studies may be able to define the pattern of sweet taste preferences and genetics among respective populations. When this phenotype will be considered in identifying and screen patients who are at risk of caries due to genetic factors, a detailed etiological model can made. Therefore, host genetics projecting the phenotype of sweet taste preferences has the potential to be conceived as a part of rapidly changing disease model of caries.

Conclusion

The interplay of sweet taste and genes is complex. Yet, our understanding of many aspects of sweet taste preferences and its association with alcohol intake and dental caries has increased over time. The gene mapping techniques have set the stage to demystify better models and implement them for diet-related diseases. The future of the taste perception and genetics arena holds promising research and discoveries.

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Assess the Effects of Back Massage to Improve Sleeping Pattern of the Postoperative Cardiothoracic Patients among the Selected Hospitals

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Abstract

Background : During the recovery time after cardiac surgery, some patients find that they have disturbed sleep. This is due to a combination of the effects of anesthesia, pain or discomfort in the area of the incisions, back, shoulder, and neck pain from manipulation of body during surgical procedure and the post-operative changes to daily routine and stress. Lack of sleep may cause fatigue; delay in recovery process and lead to prolonged hospitalization Therapeutic back massage has gained support as an intervention that improves the overall relaxation of the body. Patients experience back massage as a calming, stimulating and effective measure of sleep-inducing influence. **Objectives:** To assess the sleeping pattern of post operative cardiothoracic patients before giving back massage, To assess the sleeping pattern of post operative cardiothoracic patients after giving back massage, To compare the difference between before and after massage effect on sleeping pattern of post operative cardiothoracic patients, To correlate the effects of back massage with selected demographic variables and to find out association between before and after back massage effects on sleeping pattern of post operative cardiothoracic patients. **Material and Methods:** An experimental method research with a configuration which is quasi experimental (non-randomized control group). This research was carried out in selected hospitals of the Vidharbha Region on postoperative cardiothoracic patient. **Result:** Statistically significant difference was found in sleeping pattern score of postoperative cardiothoracic patients, before and after massage effect in experimental group. **Conclusion:** Massage evokes an aura of acceptance, body-respect and treatment. The intimate personal contact suggested in massage improves the bond between the nurse and the patient and this in effect provides an environment for wellbeing and healing. The present study concludes that back massage has an effect on improving the sleep pattern of postoperative cardiothoracic patients in order to cope with the problem of postoperative sleep disruption that helps to recover early after surgery.

Key Words: *Assess, Back Massage, Effects Postoperative cardiothoracic patient, sleeping pattern.*

Introduction

During the recovery time after cardiac surgery, some patients find that they have disturbed sleep. This is due to a combination of the effects of anaesthesia, pain or discomfort in the area of the incisions, back, shoulder, and neck pain from manipulation of body during surgical procedure and the post-operative changes to daily routine and stress. Lack of sleep may cause fatigue; delay in recovery process and lead to prolonged hospitalization.[1] Sleep and rest are fundamental human needs which are essential for the physical and psychological well-being of every individual. Approximately one third of our lives spent asleep. The purpose of sleep is a mystery, but health and a sense of well-being are necessary.[2]

The commonly performed cardiac surgeries are categorized into three types. They are reparative, reconstructive and substitution procedures. Cure and prolonged improvement are the goals of reparative procedures like closure of patent ductus arterious, atrial septal defect, repair of mitral stenosis and tetrology of

fallot. Coronary artery bypass grafts, reconstruction of incompetent mitral, tricuspid and aortic valves are belonging to more complex reconstructive procedures, whereas, valve replacements and cardiac transplantations are known as substitution procedures. Among these coronary artery bypass grafting is the widely performed one.[3]

Sleep can be described as a normal state of altered consciousness during which the body rests; it is characterized by reduced responsiveness to the environment, and external stimuli can excite a person from it.[4]

A person's lifelong need for rest and sleep shifts. A young adult typically requires less sleep than an older or middle-aged adult. A chronically ill patient needs more rest than a healthy person of the same age.[4]

The average daily sleep rate varies greatly between adults. Most adults in the 20 to 50 age group sleep an average of 6 to 8 1⁄2 hours. However 5 % to 10% of this age group sleeps more than 9 hours and 2% to 5% sleep less than 6 hours without difficulty.[4]

The manipulation of the soft tissues of the body to stimulate the nervous, muscular, respiratory and muscular tissue is known as therapeutic back massage. It relives the mental stress and anxiety by the therapeutic value of touch.

It reduces the pain by stimulating the release of endorphins. Back massage stimulate the level of neurotransmitters like serotonin and dopamine and hormone like oxytocin. Adequate level of serotonin produce a general sense of wellbeing.

Dopamine helps the human body sleep, concentration and mood. Oxytocin, also known as "hugging hormone," induces a sense of peace and happiness.[5]

In post-operative heart surgery patients, nursing professionals can no longer disregard the sleep disturbance. Appropriate therapeutic regimens and experiments need to be investigated. Studies have shown that therapeutic back massage is one of the most important means of mental recovery, pain relief and is very useful in sleep promotion.[6]

The most important effects of therapeutic massage are relieving the muscle tension, increased circulation of the blood and relaxation response initiation. The release of muscle tension will enhance balance and coordination, leading to more restful sleep and less pain medication need. The improved circulation increases the nutrition of the tissues and eliminates waste products from the tissues, decreases inflammation, increases skin tone, relieves dryness and itching, and facilitates rapid healing etc.[6]

The research was performed with 30 hospice clients to explore a non-pharmacological means of relief. The aim was to investigate the effects on systolic and diastolic blood pressure, sleep, heart rate, and skin temperature of slow stroke back massage (SSBM). Slow Stroke Back Massage was associated with decreased blood pressure, diastolic blood pressure and heart rate, increased skin temperature, enhanced sleep and relaxation. It has been shown that Slow Stroke Back Massage produces modest clinical, but statistically significant changes in vital signs which indicate relaxation. This is a cost effective service that adds to the comfort of hospice customers.[7]

Some patients find they have interrupted sleep during the recovery period following cardiac surgery. This is due to a combination of the effects of anaesthesia, pain or irritation in the area of incisions, back, shoulder and neck pain arising from body pressure during surgery and post-operative changes in daily life and stress. Sleep deficiency can lead to fatigue; delay in recovery phase and prolonged hospitalization.[1] Massage is one of the most common methods of healing. The tissues of the body are engineered to reduce muscle spasm, promote relaxation, enhance blood flow and increase venous drainage. It is the systematic manipulation of body tissue[9], back massage is an alternative or complement to pharmacological care, it is a clinically successful nursing technique for sleep promotion.[10]

The investigator herself during her working period came across with post cardiac surgery patients; who were experienced pain, anxiety and sleep disturbance. The personal experience of the student researcher and the above mentioned factors regarding the importance of sleep in post-operative cardiac patients and the significant effect of massage therapy on sleep inspired the student investigator to help the postoperative cardiothoracic

patients with sleep disturbance to improve sleeping pattern through the application of back massage for early postoperative recovery and leading a healthy life

Aims and Objectives

1) To assess the sleeping pattern of post-operative cardiothoracic patients before giving back massage.

2) To assess the sleeping pattern of post-operative cardiothoracic patients after giving back massage.

3) To compare the difference between before and after massage effect on sleeping pattern of post-operative cardiothoracic patients.

Material and Method

Research Approach-Experimental research with quasi experimental (**nonrandomized control group**) design.

Population – postoperative cardiothoracic patients admitted in selected hospitals of Vidharbha region.

The inclusion criteria- Postoperative cardiothoracic patients, who have undergone cardiothoracic surgery via sternotomy, who gave consent and medically fit to participate in back massage, patients from the postoperative day 2-10.

Exclusion criteria - Patient with chronic pain syndrome. Patient with significant history of psychiatric disorder, patient with postoperative complications.

The content validity of the tool was done by 11 various experts from different fields. Suggestions proposed were incorporated in tool and appropriate changes were made. **Reliability** analysis was done by Intra class Correlation.

The **pilot study** was conducted at Awanti Heart Institute Nagpur and shrikrishana hridayalaya and critical care , Nagpur, from 12th August to 17th August 2013 as per the criteria laid down and

Sample was selected by non-probability convenience sampling technique.

The process of data gathering was from 2ndSept. to 22nd Sept. 2013. Prior to initiation of the research, ethical approval was obtained from the Datta Meghe Institute of Medical Sciences Institutional Ethics Committee, considered to be university, Sawangi (Meghe) Wardha. The details of the procedure were explained to the participants in their vernacular language and an informed written consent was obtained from them. Data on Name, age, sex, occupation, and personal habits of the subjects was recorded using questionnaires. The researcher visited the selected hospitals in the Vidharbha region prior to data collection and obtained the necessary permission from the concerned authorities. The data were analyzed using different statistical tests based on the objectives and hypothesis.

Statistical Analysis

Collected data were organized in tabular form for analysis. The collected data was coded, tabulated and analyzed by using descriptive statistics (mean, percentage and standard deviation) and inferential statistics. The analyzed data was presented in graphs and tables. Significance difference between the experimental and control group were tested using students paired and unpaired "t" test, association was made with the help of paired and unpaired "t''test. The data was presented in the form of tables and graphs.

Table No I-: Plan for data analysis

S. No	Data Analysis	Method	Remark
1.	Descriptive statistics	Frequency, Percentage	Assess the sleeping pattern of postoperative cardiothoracic patients.
2.	Inferential statistics	Mean, standard deviation, Students paired 't' test, Students unpaired 't' test.	Compare the effects of back massage on sleeping pattern of postoperative cardiothoracic patients

Table No. II Scoring Of Sleeping Pattern

Level of sleeping pattern disturbance score	Percentage on Groningen sleep pattern assessment scale
Mild	1-25%
Moderate	26-50%
Severe	51-75%
Very severe	76-100%

Results

Table III: Assessment with sleeping pattern disturbance score before giving back massage

Level of sleeping pattern disturbance score	Percentage score	Sleeping Pattern Score	
		Control Group	Experimental Group
Mild	1-25%	2(6.67%)	0(0%)
Moderate	26-50%	1(3.33%)	1(3.33%)
Severe	51-75%	18(60%)	27(90%)
Very Severe	76-100%	9(30%)	2(6.67%)
Minimum score		3	6
Maximum score		13	12
Mean score		10.06 ± 2.58	10.06±1.22
Mean Percentage		67.11±17.23	67.11±8.19

The table No III shows that 6.67% of the patients of control group had mild level of sleeping pattern disturbance score, 3.33% in each control and experimental group had moderate level of sleeping pattern disturbance score, 60% in control and 90% in experimental group had severe level of sleeping pattern score and 30% in control and 6.67% in experimental group had very severe level of sleeping pattern disturbance score. Minimum sleeping pattern score of the patients of control was 3 and maximum sleeping pattern was 13, mean score was 10.06 ± 2.58

Whereas in experimental group minimum sleeping score was 6 and maximum score was 12 with mean percentage score of 10.06±1.22

Table IV: Assessment with sleeping pattern score

Level of sleeping pattern disturbance score	Percentage score	Sleeping Pattern Score	
		Control Group	Experimental Group
Mild	1-25%	5(16.67%)	14(46.67%)
Moderate	26-50%	6(20%)	12(40%)
Severe	51-75%	14(46.67%)	3(10%)
Very Severe	76-100%	5(16.67%)	1(3.33%)
Minimum score		3	0
Maximum score		12	12
Mean score		8.10±2.86	4.30±3.13
Mean Percentage		54±19.12	28.66±20.87

The above table No.IV shows that 16.67% of the patients of control group and 46.67% in experimental group had mild level of sleeping pattern disturbance score, 20% in control and 40% in experimental group had moderate level of sleeping pattern disturbance score, 46.67% in control and 10% in experimental group had severe level of sleeping pattern disturbance score and 16.67% in control group and 3.33% in experimental group had very severe level of sleeping pattern disturbance score. Minimum sleeping pattern score of the patients of control was 3 and maximum sleeping pattern was 12, mean score was 8.10±2.86 whereas in experimental group minimum sleeping pattern score was 0 and maximum score was 12 with mean percentage score of 4.30±3.13

Table V: Significant difference between before and after massage effect on sleeping pattern of post-operative cardiothoracic patients.

		Mean	N	Std. Deviation	Std. Error Mean	t-value	p-value
Control Group	Before t/t	10.06	30	2.58	0.47	3.30	0.003 S,p<0.05
	After t/t	8.10	30	2.86	0.52		
Experimental Group	Before t/t	10.06	30	1.22	0.22	8.71	0.000 S,p<0.05
	After t/t	4.30	30	3.13	0.57		

Table No. V the mean sleeping pattern disturbance score in control group before massage effect was 10.06±2.58 and at after t/t it was 8.10±2.86. Statistically significant difference was found in sleeping pattern disturbance score (t=3.30, p-value = 0.003) before and after massage effect whereas in experimental group in before massage effect it was 10.06±1.22 whereas in experimental group after t/t it was 4.30±3.13. Which shows that sleeping pattern is improved by decreasing the sleeping pattern disturbance score. Statistically significant difference was found in sleeping pattern

score (t=8.71,p-value=0.000)

Discussion

The assessment of sleeping pattern of post-operative cardiothoracic patients before giving back massage was assessed by the level of sleeping pattern disturbance divided under following heading mild, moderate, severe, and very severe , 60% in control and 90% in experimental group had severe level of sleeping pattern disturbance score. Statistically significant difference between the pretest and posttest sleeping pattern scores of experimental group found in the pretest of experimental group postoperative cardiothoracic,90% had severe, 6.67% had very severe level of sleeping pattern disturbance score, with mean percentage score of 10.06and standard deviation 1.22.whereas the post test score after the back massage in the experimental group shows that only, 10% postoperative cardiothoracic patients having severe, 3.33% very severe level of sleeping pattern disturbance score, with mean percentage score of 4.30 and standard deviation 3.13,Shows the level of sleep disturbance is decreased. Statistically there is a significant difference between the pretest and posttest sleeping pattern scores of experimental group. Hence research hypothesis $H_1$is accepted.

The comparative difference between before and after massage effect on sleeping pattern of post operative cardiothoracic patients was found Mean sleeping pattern score in control group before massage effect was 10.06±2.58 and at after t/t it was 8.10±2.86. Statistically significant difference was found in sleeping pattern score(t=3.30, p-value=0.003) before and after massage effect whereas in experimental group in before massage effect it was 10.06±1.22 whereas at after t/t it was 4.30±3.13, which shows that, the sleeping pattern is improved by decreasing the level of sleep disturbance. whereas the in the post test assessment the level of sleeping pattern disturbance of the control group was 8.10 and standard deviation 2.86comparing with the pretest value 10.06 and standard deviation 2.58. Whereas the post test score of the experimental group shows that the level of sleep disturbance was 4.30and standard deviation 3.13 comparing with pretest score 10.6 and standard deviation 1.22.Statistically significant difference was found in sleeping pattern scores(t=8.71,p-value=0.000) of experimental and control group, Hence the research hypothesis $\mathbf{H_2}$ was accepted.

The following literature supports the above findings of the research.

An experimental study was done in Arizona to assess the effects of the use of back massage therapy on inpatient pain levels in a Medical Center among 53 hospital inpatients. The samples are cardiac surgery patients selected by convenient sampling. The pain levels before and after massage therapy were recorded using a 0-10 visual analogue scale. In this study the patients received one or more massage therapy sessions averaging 30 minutes each.

Results of this study showed that the mean pain level prior to treatment was 5.18 (S.D:2.01). The mean amount of pain following relaxation was 2.33 (S.D:2.10). The pain relief observed has been statistically significant: paired samples t 52 = 12.43. R=.67, d=1.38, and p<.001. They concluded that massage therapy has major effects on the overall level of discomfort, mental well-being, relaxation and sleeping capacity. The study showed not only a substantial reduction in pain rates, but also the interrelationship between pain, relaxation, sleep, emotions, rehabilitation and, ultimately, the hospital stay healing process.[14]

A randomized study was conducted to test the effect of massage therapy for the alleviation of pain, tension and anxiety in the post-operative cardiovascular patients.

The sample size was 113 and divided into massage group (n=62) and control group (n=51). Tools used were visual analogue scale and Beckon anxiety scale. Research results indicated that patients receiving massage therapy had substantially decreased discomfort, anxiety and stress compared with control group. Patients were extremely pleased with the care and no significant obstacles were found for implementing massage therapy. This research concluded that after cardio-vascular surgery massage therapy can be incorporated as an essential component of the recovery process for patients.[6]

In 2010 Brazil-based research focused on the effectiveness of massage to improve sleep in patients following cardiopulmonary bypass artery graft surgery.13 Poor sleep and exhaustion are normal after thoracic surgery and may hinder treatment and recovery.

Poor post-operative sleep quality may be attributed to many factors including discomfort from surgical incision, presence of thoracic drain, discomfort from extended bedtime, and high rates of anxiety. However, muscle pain, particularly in the neck, shoulders and back, can make breathing, cough, movement and sleep difficult for patients.[7]

A research was undertaken to assess the impact of relaxation and visualization on adults suffering from serious illness. The research was a randomized clinical trial with two groups being selected at random. Research made use of repeated ANOVA steps. Thirty-six adults (17 males and 19 females) in three critical care units in two large metropolitan hospitals with a variety of physical diagnoses were studied. The outcome tests were scores calculated on three mornings on a visual analog sleep scale. The therapy was a blend of relaxation and creativity that was given on two days. Over time the sleep of all the subjects changed. There were major interaction effects between intervention, gender and time, with the scores of males rapidly improving and the scores of females falling first, and then rapidly improving. A combination of relaxation and visualization is successful in improving the sleep of critically ill people, with men instantly responding to relaxation and imagery with better sleep, and women taking more time to respond to the intervention.[3]

Pinto (2001) at KEM Hospital, Mumbai, examined the effect of selected nursing strategies in sleep promotion of patients with congestive heart failure. The data was collected using a semi-structured schedule of interviews and an analytical checklist of 40 subjects who met the necessary criteria. The research employed a concise assessment approach. The intervention group provided 10-minute back massage and 6-minute deep breathing exercise, and the test results revealed that 80 percent of the sample in the research group slept well and that their standard of sleep in the control group only slept well at 50 percent. In the control group, sleep quality was 45 percent lower than in the study group.[4]

Conclusion

So the present study concludes that back massage has effects on improving the sleeping pattern of the postoperative cardiothoracic patients to cope up with the problem of postoperative sleep disturbance.

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Synopsis

A Study Protocol on Effect of Postural Deviation on Functional Capacity in Patients with Chronic Respiratory Disease

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Abstract

Background: According to WHO Chronic respiratory diseases are diseases of the airways and lung. Chronic obstructive pulmonary disease , asthma, occupational lung diseases and pulmonary hypertension are among the most severe. Chronic respiratory disease is generally divided among adults into obstructive and restrictive conditions. Restrictive lung disorders are characterized by decreased volumes of the lung, either due to a lung parenchyma change or due to pleura, chest wall or neuromuscular apparatus disease. Unlike obstructive lung diseases such as asthma and chronic obstructive pulmonary disease, which exhibit normal or elevated total lung capacity (TLC), restrictive diseases are correlated with a reduced (TLC). These presenting symptoms of chronic respiratory disease results in the postural deviations. It mainly affect the cervical and lumbar region of the spine which include forward head posture, upper thoracic kyphosis which occurs due to continues use of accessory muscles of breathing.The functional capacity of the patients are assessed by performing the 6-minute walk test (6MWT) it is fairly easy for evaluating the status of cardiac and pulmonary diseases.

Methoud/Design: The study is designed as observational and correlation study. The participants will be selected as per inclusion and exclusion criteria from AVBRH , sawangi Meghe for the study. The duration of the study will be 6 months. Study include evaluation of the posture with the help of kinect azure and evaluation of functional capacity using 6 MWT. Correlation of 6MWT,Vo2 peak with severity of the postural deviation.

Discussion: This study details about effect of postural deviation on functional capacity in patients with chronic respiratory disease. Many studies have been done on assessment of the postural deviation in COPD patients but there is scarcity of literature which focus on the other respiratory conditions also. Kinect azure is the objective measure for assessment and gives the detail evaluation of posture. Since deviated posture puts extra load on musculoskeletal system which indirectly affects the functional capacity. So the this study aims to observe degree of postural deviation and relate it to the functional capacity. The present study can provide us the baseline idea data about the importance of maintaining good posture in chronic respiratory diseases patients for improving functional capacity and also importance of good postural habit in exercise program.

Keywords- *chronic respiratory disease, 6 minute walk test, postural deviation*

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Introduction

According to WHO Chronic respiratory diseases are diseases of the airways and lung. Chronic obstructive pulmonary disease , asthma, occupational lung diseases and pulmonary hypertension are among the most severe.

Many risk factors include air pollution, industrial contaminants and recurrent lower respiratory infections during childhood. (1) Chronic respiratory disease is generally divided among adults into obstructive and restrictive conditions. Obstructive diseases are further classified into reversible and irreversible. In individuals diagnosed with chronic lung disease such as COPD, cystic fibrosis, asthma, bronchiectasis, dyspnea, fatigue and reduced quality of life are typical symptoms. (2) An significant feature of obstructive respiratory disease is increased workload of the respiratory muscles. It also includes muscle hyper tonicity, shortened chest wall and upper extremity. This can result in decreased strength, soft-tissue contracture formation and muscle imbalance[3]

Restrictive lung disorders are characterized by decreased volumes of the lung, either due to a lung parenchyma change or due to pleura, chest wall or neuromuscular apparatus disease. Unlike obstructive lung diseases such as asthma and chronic obstructive pulmonary disease, which exhibit normal or elevated total lung capacity (TLC), restrictive diseases are correlated with a reduced TLC . Expiratory airflow controls are maintained and airway resistance is normal and the ratio of forced expiratory volume (FEV1)/forced vital capacity (FVC) is increased (4) The prevalence of these diseases is increasing everywhere particularly among children and adults.(5)

The posture is a controlled structural configuration of the body. It is a measure of the body segment's location with the joints and muscles in the balance state, which requires minimal musculoskeletal system effort and load, and maximum performance.(6)

These presenting symptoms of chronic respiratory disease results in the postural deviations. It mainly affect the cervical and lumbar region of the spine which include forward head posture, upper thoracic kyphosis which occurs due to continues use of accessory muscles of breathing.(7) Such postural changes can impose excessive pressure on the musculoskeletal system that may manifest as cervical pain or clinically diminished physical function. Pain can also be associated with concomitant musculoskeletal conditions such as osteoarthritis and osteoporosis that have been related to COPD, Cystic Fibrosis and asthma (8)

Respiratory and peripheral muscle dysfunctions found in chronic respiratory disease result in airflow limitation, dyspnea, and inactivity, resulting in decreased functional capacity.(9) The functional capacity of the patients are assessed by performing some tests which give us a measure of functional status of the patient. The 6-minute walk test (6MWT) is a fairly easy for evaluating the status of cardiac and pulmonary diseases compared to other exercise tests like shuttle walk test, has been commonly used in COPD patient management. In a number of studies, distance of the 6-minute walk test has been taken as an important methoud of exercise tolerance in patients with COPD. (10) The 6MWT is a self paced walking test. Patients are asked to walk along a flat path as far as possible in 6 min. Walking distance is the primary outcome and is measured in feet or metres. Standardized guidance and encouragement are usually given during the test.(11)

Objective:

- To evaluate the postural deviation using Kinect azure
- To evaluate functional capacity using 6MWT
- To correlate 6MWT Vo2 peak with the severity of the postural deviation.

Methouds, Participants, Interventions and Outcomes

Study Setting: Ravi Nair Physiotherapy College, DMIMS, Sawangi (Meghe), Wardha, Maharashtra, India.

Eligibility:

Inclusion Criteria:

- patient diagnosed with complaint of chronic respiratory disease by pulmonologist

Exclusion Criteria:

- patients with complaint of cardiovascular disorders
- Patients with complaint of major neurological dysfunction
- Patients with complaint of severe

musculoskeletal dysfunction

- Patients with complaint of organ failure
- Patients who are haemodynamically unstable

Procedure:

Patients suffering from Chronic respiratory diseases diagnosed by pulmonologist coming in physiotherapy OPD will be screened according to the inclusion and exclusion criteria and will be selected for the study. The total no. of subjects filling the criteria will be taken. The participants of the study will be explained the study procedure and written consent will be obtained from each participant. The demographic data including name, age, address, occupationwill be collected and will be entered in case record forms. Then posture will be evaluated with Kinect azure which will provide the detailed evaluation of posture. Then with the help of 6 minute walk test functional capacity will be evaluated

Outcome measures:

- The 6-minute walk test has been taken as an important methoud of exercise tolerance in patients with COPD. (10)
- The 6MWT is a self paced walking test. Walking distance is the primary outcome and is measured in feet or metres. Standardized guidance and encouragement are usually given during the test.(11)

Participant Timeline:

As the study duration is of 6 months observation of posture will be done the first day and 6 min walk test will be done the next day.

Sample Size: Convenient Sampling

Sampling Method is Convenient sampling method as the duration of the study is of 6 month.

Recruitment:

Regular visit to Cardiorespiratory OPD, Cardiorespiratory Wards will be done, record of maintaining office of cases will check out that, contact will be maintain with doctors to reach target sample size.

Discussion

This study details about effect of postural deviation on functional capacity in patients with chronic respiratory disease. Many studies have been done on assessment of the postural deviation in COPD patients but there is scarcity of literature which focus on the other respiratory conditions also. Kinect azure is the objective measure for assessment and gives the detail evaluation of posture. Since deviated posture puts extra load on musculoskeletal system which indirectly affects the functional capacity. So the this study aims to observe degree of postural deviation and relate it to the functional capacity. The present study can provide us the baseline idea data about the importance of maintaining good posture in chronic respiratory diseases patients for improving functional capacity and also importance of good postural habit in exercise program.

Result

We Hypothesize that there can be association between the degree of postural deviation and level of functional capacity. With severe postural deviation the level of functional capacity would be reduced . Or there will be no relationship between both.

Ethics and Dissemination: Institutional ethics committee (IEC) of Datta meghe institute of medical sciences Deemed to be University, Sawangi (Meghe)

Conent:

Consent form will be obtain from the patient and one of the relative on a printed form with signatures by principal investigator and giving proof of confidentiality.

Confidentiality:

Participants and the relative will be informed about the study program and personal information will be taken by principal investigator only, in consent form the confidentiality statement will be mentioned. Signatures of both investigator and patient with two witness will be taken and for the purpose of the study if needed to disclose some information complete assurance and consent will be taken from the patient.

Declaration of Interests: None

Dissemination:

After completion of data collection, statistical analysis will be done and completion report will be formed then after checking plagiarism, review by Institutional research cell, will be send for publication.

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Original Research Article.

Assessment of Awareness and outlook of a Pathological Autopsy among Medical Students

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Abstract

Aim: This study assesses the awareness, outlook & knowledge toward Pathological autopsy amongst medical students at tertiary care teaching center.

Objectives: To assess medical student's opinions, feelings and attitude towards autopsy and its importance in medical education.

Material and Method: We conducted a study of 300 students studying 3rd and 5th semesters where they taught about the subject. We used questionnaire containing questions relating to autopsy to assess the awareness, outlook & knowledge towardPathological Autopsy.

Results: Amongst the class of 300 students no statistically difference is observed in the views of male and female students on any item in the present study.

Conclusion: The Forensic Medicine is important subject for medical profession. Teaching how to perform an autopsy is relevant and plays a positive role in medical education, and autopsy training from both theoretical and practical perspectives must be included in the forensic medicine curriculum. Medical institutes must take responsibility to increase awareness about the effect of media on medical education.

Key Words: *Medical Students,Pathological Autopsy, Forensic Medicine, awareness and outlook.*

Introduction

The educative value of autopsy is so clearly appreciated by the students and most students agreed that autopsy is an educationally useful and necessary procedure.Over a centuries in a medical education postmortem examination in one of the important concept.In nineteenth century Virchow and Osler used to understand the pathology of fatal disease like Endocarditis and pulmonary embolism by coordinating the value of pre and post mortem findings.[1]

The autopsy is beneficial in determining, the cause of death, the time of death, various medico legal issues surrounding death, and providing data on disease and injury.[2]

However, the non-forensic or academic autopsy has been broadly considered a "dying art"[3] or a "vanishing" medical procedure[4] mostly because of the

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worldwide dramatic fall in autopsy rates during the last decades.[5]Autopsy as a teaching medium has additional advantage that it provides an opportunity for medical students to come to term with death.

Consented autopsies are conducted at the request of either the familymembers of the deceased or a clinician in cases where the cause of death is known and, although there no additional examination and or investigation are required by law.Consent of the family of the deceased is always required in case of consented autopsies.

Medico-legal autopsies require no consent from the family of the deceased, and there is no mechanism by which families can prevent a coroner's autopsy taking place.

However, in any aspect of medico-legal autopsy that concern the cause of death of the deceased, such as removal of tissue for testing purposes, can take place only with additional consent given by the relatives. In undergraduate medical training there is no mention of their use.

The use of the autopsy as an educational tool is in diminished and there is evidence suggest that many recent undergraduates students and even junior doctors have never attended an autopsy.[6]

Aim and objectives

1) This study assesses the awareness, outlook & knowledge toward autopsy among medical students at tertiary care teaching center.

2) To determine the thoughts, feelings & attitude of medical students towards autopsy & its value in medical education

Material and Method

This study approved from institutional Ethical Committee.

This cross-sectional is proposed to be conducted at the tertiary care teaching hospital and research center. The sample size is 300undergraduate medical students of second MBBS of 3rd& 5th semester for one year duration. All these students will be asked to fill questionnaire and this was voluntary for them. The survey consisted in several questions relating to the autopsy practice, the knowledge of the procedure attitude, various important aspect of the postmortem examination. The responses were made anonymously. Remainders were subsequently given to present this proforma. A valid consent of each student regarding filling all questionnaires was taken. Critical evaluation of the results will be analyzed using SPSS software.

Results

Amongst the class of 300 students there were 168 males and 132 females. The age of students range from 21-25 years with mean age of 22 years.

Statistically no difference is observed in the views of male and female students on any item in the present study.All of the medical students attained these questionnaires, their valuable feedback explained in table 1

Table 1: Questionnaires and feedback

Pathological autopsy is mandatory in all unnatural and sudden unexpected and suspicious death cases:	Yes=290(96%)
	No. n=7(2.33%)
	Ignorant .n=3(1%)
Taking out of viscera for histopathological examination and toxicological analysis for pathological as well as medico legal autopsy:	Yes. n=295 (98%)
	No. n=3(1%)
	Ignorant=2(0.6%)

Cont... **Table 1: Questionnaires and feedback**

Pathological autopsy examination is:	Done to rule out the cause of death. n=296(98.66%)
	It is a legal formality. n=90(30%)
	Mental harassment of the relatives of the deceased n=20(6.66%)
	Helpful in reaching the culprit of crime. n= 275(91.66%)
The reaction of the students on the first exposure to pathological autopsy procedure n=280(93.33%)	Comfortable. n=80(28.56%)
	Slightly uncomfortable. n=77(27.5%)
	Moderately uncomfortable. n=33(11.78%)
	Very uncomfortable. n=70(25%)
	Indifferent. n=20(7.14%)
Students should watch more such type of autopsies	Yes. n=277(92.33%)
Wished to have pathological autopsy examination on self/relative when required	Yes .n=260(86.66%)
	No. n=40(13.33%)
Given a chance would you choose not to watch more such autopsies	No. n=215(71.66%)
	Yes=85(28.33%)
The utility of such autopsy examination in medical education.	Yes. n=265(88.33%)
	No. n=5(1.66%),
	Should be scrapped from medical education. n=30(10%)
Defacement by pathological autopsy examination. n=248(82.66%)	Yes. n=80(32.25%)
	No. n=168(67.74%)
Pathological autopsy is disrespect to human body	Yes. n=35(11.66%)
	No. n=265(88.33%)
Source of information and knowledge about pathological autopsy	Medical curriculum. n= 290 (96.66%)
	Magazine and newspaper. n=70(23.33%)
	T.V. n=90(30%)
	Internet. n=140(46.66%),
	Friend and relative. n= 25(8.33%).

Discussion

In a long history of medical education the word autopsy is obtained from theancient Greek word "autos" (oneself) and "opsis" (sight); literally meaning "to see for oneself".[7]

The utility of the autopsy as a tool in both medical practice and medical education has remained well-recognized in more recent times.[8,9,10]

In view of this usefulness, throughout the early and middle part of the last century, the autopsy was widely and regularly used as a teaching tool, and regular attendance by medical students was expected.

Despite the high importance put on the autopsy as a teaching tool in the past, its use in this context has decreased significantly, leading to a situation where many students during their under graduation period will no longer see any autopsies at all.[11,12]

The observation of autopsies by students is elective in some medical schools and college in the world because of religious and cultural ground. Based on this, some students may complete their training in these centers without observing single autopsy.

The degree to which learners were actively promoted seemed to be differ. Particularly where attendance was encouraged, postmortem examination opportunities tended to be based on particular conditions like being on placement and engaging with a patient on whom a postmortem was being conducted and employers attitudes and opportunities.

In view of medico-legal awareness, it was understood that all doctors at some stage in their medical carrier would come into contact with dead patients and that autopsy attendance was given a chance for them to understand the legal system that applies to the dead patient. Particular importance was given to the creation of knowledge of consent issues and the role of legal authority among medical students.

It was seen that attending postmortem exams had beneficial effects on diagnostic and communication skills, potentially improving future patient care.

Some have seen postmortem observation as likely to promote wider differential diagnosis and the related investigative methodologies for problem solving.

The usefulness of autopsy in the provision of the hidden curriculum in medical education has been highlighted in several studies. Certain aspects of medical practise that cannot be taught by formal teaching sessions, but are subconsciously learned by observing teachers and peers attitudes and activities. [12,13]

Even if other methods have failed to demonstrate a definite cause of death, because their own expertise and experience about the subject is lacking and those students who passed their graduation without autopsy experience would refuse to request an autopsy. [14]

A subset of medico-legal autopsies take place where there is the suspicion of criminal act forensic or special autopsies, and such cases involve the police service and may utilize other professionals such as forensic scientists. Like all medico-legal autopsies, forensic cases require no consent from the family of the deceased, and there is no mechanism by which families can prevent such an examination taking place.

There is no statistical difference in the views of male and female students on any item in the present study possibly because they all had similar educational background and socioeconomic status. Since, this study is based on an academic task this could be why the students responded in almost the same way; efforts of the accompanying faculty to make this meaningful could be another.

In the present study 86.6% of the student answered positive on wished to have postmortem examination on self and or relative when required is favored by Study of Sanner et al[15] in Sweden where 90% of the students would not mind autopsy to be performed on them.

JoonJoon et al[16] indicated 77.5% of the students recommended making autopsy session compulsory during undergraduate medical training.

In our study 92.33% of the students recommended that students should watch more postmortem examination favored by different studies.[17,18]

96% of the students recommended making autopsy session compulsory during undergraduate Medical training in all unnatural and sudden unexpected and

suspicious death cases.

Taking out of viscera for histopathological examination and toxicological analysis for pathological as well as medico legal autopsy reported in 98% in our study.

Only 25% of the students were very uncomfortable for when they were first time exposed to pathological autopsy procedure.

In present study majority of the students acquire knowledge and information about postmortem examination from their medical curricula in spite of development of print, electronic mass media and internet accessibility.

Very few students (11.66%) were of opinion that postmortem examination is disrespect to human body, which is favored by various studies.[17,16]

Just 2.5% of the students felt that postmortem examination was harassment to the relatives of the deceased.

This study showed that the medico-legal autopsy demonstration is still valued by medical students as a learning opportunity. Most students in the current study were aware of the circumstances where medico-legal autopsy and preservation of viscera is mandatory under Indian law for chemical analysis and histopathological examination.

It was also found that attending postmortem examination strengthened the perception of the pathologists function and that of other practitioner in health care.

It helped students to understand the impact of life style, social deprivation, and other socio-economic variables on health, well-being, and death.

A literature review shows that the potential importance of the autopsy in undergraduate teaching is very high, but its significance has declined in recent decades, and medical students can qualify in some schools without even entering a mortuary.[19]

The reasons for this decrease in autopsy based teaching include; insufficient curriculum time, inadequate training of doctors on values of autopsy, conflicting departmental requirements and insufficient hospital autopsies, an improvement in the medical diagnostic technology available; and difficulties in obtaining consent from relatives.[20, 21]

Conclusion

The Forensic Medicine is important subject for medical profession. Teaching how to perform an autopsy is relevant and plays a positive role in medical education and autopsy training from both theoretical and practical perspectives must be included in the forensic medicine curriculum. It must be duty of Medical institution to raise awareness about the effect of media on medical education.

Ethical Clearance: Taken from institutional ethics committee.

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Child Abuse and Neglect: Role and Obligation of Pediatric Dentist in India

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Abstract

Background: Child abuse is a form of behavior that involves treating someone (child, under the age of 18years) with cruelty or violence resulting in actual or potential harm to the child. It has no boundaries and may occur in any geography, culture, or in any socioeconomic strata. Many studies reveal that there is inadequate knowledge and awareness regarding child abuse recognition and reporting among health workers.

Method: Most of the studies reported that trauma to head and facial region occurs in approximately 50% or more of physically abused children. Therefore Pediatric Dentist has major chance of witnessing the physical abuse in clinical practice. On the same time they are not the authority to make diagnosis of child abuse & neglect, rather identify and report the suspected cases, so that a process for effective protection of the victimized children can be initiated. It is the responsibility of the child protection agency/commission to confirm the crime and do the needful. Hence, the role of pediatric dentist in the identification and reporting of suspected child abuse is crucial. He is expected to practice 4R's: Recognize, Refer (if any), Record and Report

Conclusion: Understanding the peculiar role of Pediatric Dentist in recognizing and reporting child abuse and neglect which ultimately contributes towards making better world for children.

Key Words: *Child abuse, India, Neglect, Obligation, Pediatric Dentist, Role.*

Introduction

Child abuse is a form of behavior that involves treating someone (child, under the age of 18years) [1] with cruelty or violence resulting in actual or potential harm to the child. It is a complex phenomenon and may be exercised by parents, caregivers, relatives or persons responsible for the child and can occur at home or in the organizations, schools or communities, the child interacts with. It has no boundaries and may occur in any geography, culture, or in any socioeconomic strata. ***Journal of Child Abuse and Neglect,*** describes Child Abuse as "any recent act or failure to act on the part of a parent or caretaker which results in death, serious physical or emotional harm, sexual abuse or exploitation, an act or failure to act which presents an imminent risk of serious harm" [2]. The terms child abuse and child maltreatment are often used interchangeably, although some researchers make a distinction between them, separating child maltreatment as neglect, exploitation and trafficking [3]. WHO distinguishes child abuse/maltreatment into five types: physical, sexual, emotional (psychological) abuse; exploitation and neglect & negligent treatment [4] (Table I). While physical and sexual abuse leave some signs, which can be differentiated from an accidental injury, emotional abuse can severely damage a child's mental health and

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social development. Child neglect is a separate entity, where the parent/guardian fails to provide the basic need of a child, although he/she is in a position to do so. Regardless of the types of abuse, the result is serious emotional harm, which can leave deep long lasting scars in children.

Despite long past record of history of child abuse, actual professional investigation on this issue considered to have begun in 1960s, following publication of an article "The Battered Child" by C Henry Kempe and his co-workers [5] . Over time there has been an exponential increase in consciousness among the public for the same, yet studies reveal that there is inadequate knowledge and awareness regarding child abuse recognition and reporting among health workers, specifically dental professionals [6].

India is home to the largest child population in the world [7] and child abuse here is widespread. However, response towards reporting the incidence is inadequate. Fear of social stigma, unfriendly behaviour of police and long judicial process are the deterrents to seek justice [8] . There is neither any provision for training the health workers on child abuse examination, interviewing and care taking, nor any protocol has been developed with regard to documentation and reporting [8]. Therefore the objective of this article is to review the role and obligation of pediatric dentists in identifying the suspected case of child abuse and neglect in India.

Table I: Recognizing child abuse and neglect [10] (Reproduced with permission)

Category	Definition9	Examples of signs the dental team may observe
Physical Abuse	Hitting, shaking, throwing, poisoning,burning or scalding, drowning,suffocating or otherwise causing physical harm to a child;fabricating or inducing illness(formerly known as Munchausen by proxy syndrome).	Bruises;abrasions and lacerations;burns;bite marks;bone fractures;intra-oral injuries.SeeAlso Figures 1 A and 1 B
Emotional Abuse	Persistent emotional maltreatment such as to cause severe and persistent adverse effects on the child's emotional development;conveying to children that they are worthless or unloved ;imposing inappropriate expectations;preventing normal social interaction;seeing or hearing the ill-treatment of another (eg domestic violence).	Interaction with parents,eg parent using threatening or abusive language;poor growth;developmental delay;social immaturity;aggression;indiscri minate friendliness;challenging behaviour.
Sexual Abuse	Forcing or enticing a child to take part in sexual activities, whether or not the child is aware of what is happening; either involving physical contact or non – contact activities (eg involving children in looking at or producing pornographic material	Direct allegation(disclosure);sexually transmitted oral infections;pregnancy;trauma including oral trauma; emotional and behavioural changes.
Neglect	Persistent failure to meet a child's basic physical and/or psychological needs, likely to result in the serious impairment of the child's health or development;failing to provide adequate food, clothing,shelter,failing to protect from danger;failure to ensure access to appropriate medical care.	Failure to thrive;frequent injuries;inappropiate clothing;ingrained dirt;developmnetal delay;behavioural changes, eg withdrawn or attention-seeking; untreated dental disease with repeated episodes of dental pain.

Prevalence

According to World Health Organization(WHO), prevalence of child abuse/maltreatment vary widely by country, depending on country's definition of child abuse, the type of study, the scope & quality of data gathered and the scope & quality of surveys [4]. Despite these limitations, WHO report on international studies reveal that, one fourth of all adults having been physically abused as children; 1 in 5 females and 1 in 13 males were also sexually abused as children [4]. Emotional abuse and neglect were also frequently experienced during childhood. Naidoo.S (2000), accounted that boys had higher prevalence (72%) of child abuse than girls (65%), relations perpetrated approximately 89% of the crimes and majority of the incidents (70%) remain unshared and unreported [11]. Therefore, it is assumed that the actual prevalence of child abuse and neglect would be much higher than the reported prevalence. With regards to site and type of injury observed in child abuse, most of the studies reported that trauma to head and facial region occurs in approximately 50% or more of physically abused children[12] - [17] and most common soft tissue injury in these areas is bruise [18].

In India, Ministry of Women & Child Development, in conjunction with UNICEF conducted a study over 12000 children, and reported the prevalence of child abuse of concern as; physical(66%), sexual(50%) and emotional(50%) [19]. A recent study by the National Commission for Protection of Child Rights (NCPCR), conducted in 7 states of India among 6,632 child respondents, showed that 99% children face corporal punishment in schools [20].

RISK FACTORS

Table II: Risk Factors

Parental Characteristics	Child Characteristics	Environment Characteristics
§ Violence § Poverty § Parental history of abuse § Socially isolated § Low self-esteem § Less adequate maternal functioning	§ Unwanted or unplanned child § More number of children in the family § Child's nature § Child's position in the family § Physically challenged or disabled § Activity level or degree of sensitivity to parental needs.	§ Chronic stress § Problem of divorce § Poverty § Unemployment § Poor housing § Frequent relocation, § Alcoholism, § Drug addiction.

In an attempt to understand the causes of child abuse and neglect, the following risk factors have been identified, which provide an overview on the social and cultural contexts of child abuse and neglect [2] (Table II).

CHILD ABUSE AND PEDIADRIC DENTIST: IS THERE AN OBLIGATION ?

Children of all ages are susceptible to physical abuse, although majority of incidents occur under the age of two years [21]. This may be partly because they cannot comply with the instructions of the abuser, owing to their intellectual development and partly because they are more vulnerable. In contrast, observations affirm that non-physical abuse (sexual, emotional &exploitation) is more common in elderly children and mostly remain unshared and unreported [19] [22].

Physical abuse, as reported mostly involves the head and face region, thus any signs of inflicted injury to these areas can be readily identifiable by the dentists. Additionally, diagnosis of non-physical type of child abuse warrants knowledge on child's cognitive, psychological, social development and behavior. Pediatric Dentists, having adequate understanding on these aspects are in a superior position than general dentists in analyzing the behavioural change of the child

victim as well. Moreover, pediatric dentists have the convenience of spending at least 30-45 minutes with the child, conversing and providing dental treatment, thereby maintaining a cordial relationship with the child and his parents [22]. This makes their role even more critical in the process of diagnosis, both physical and non-physical type of maltreatment so that the general well-being of the child can be facilitated. Therefore, pediatric dentists have a moral and legal obligation to recognize and report the suspected case of child abuse.

Child Abuse and Pediatric Dentist In India: The Role Defined

Pediatric dentist is not the authority to make diagnosis of child abuse & neglect, rather identify and report the suspected cases, so that a process for effective protection of the victimized children can be initiated. It is the responsibility of the child protection agency/ commission to confirm the crime and do the needful. In India, the data on offences or crimes against children as reported by MWCD (Minister of Women and Child Development) is lacking [19]. Notably the only source of data available in India is with the National Crime Records Bureau. Although this data is useful, it constituted a miniscule of the total crime/violence against children. It may be due to the fact that, crimes against children in India are either not recorded, or cognizance is not taken. Moreover, the absence of a comprehensive and common definition of the term 'child' creates confusion and a dilemma. Children have limited protection from physical violence and abuse under the Indian Penal Code 1860 (IPC), and the Juvenile Justice (Care and Protection of Children) Act, 2000 [24]. Hence, the role of pediatric dentist in the identification and reporting of suspected child abuse is crucial. He is expected to practice **4R's** [10].

Recognize

Refer (if required)

Record

Report

RECOGNIZE:

Recognition of child abuse and neglect should be initiated at the reception area while the child is being carried by the parents or family members. Each member of the dental team, irrespective of its position, plays an important role in the identification of abuse. Valuable information provided by the observant receptionist will certainly facilitate the Pediatric dentist to diagnose both the physical and non-physical type of abuse. On the dental chair, child should be assessed for his general & oral health and hygiene, signs of proper nourishment and clothing. Following initial assessment, the indicators of child abuse should be looked for. First one is physical, which includes trauma to head, face, neck and hands [25]. Second indicator is behavioral, which is difficult to judge at first instance, unless not accompanied with physical abuser. Two types of extreme behaviors are observed. Either, child avoids/wary of adult contact, displays passive behavior to avoid conflicts, frightened, afraid to go home, loyal to parent, fear of being punished; or he/she seems overly aggressive, violent, demanding, displaying abusive behavior, exhibit dramatic mood changes and uncooperative [23][26].

Refer For Assessment

This step is optional and is applicable, if pediatric dentist is not in a state to identify a suspected form of abuse, but has an apprehension, specifically for sexual (oro-genital injury) or emotional abuses. She/he is required to refer the case to a Pediatrician/Gynecologist/ Psychologist for a better assessment and further investigations. Physical, radiological and laboratory tests, if required would be carried out by them to confirm the initial impression [29]. If a decision is made to refer the child, it is necessary to discuss his/her concern with the parents and seek consent. A detailed documentation should be made in child's record [10].

Record & Documentation

The next step following recognition is proper documentation of evidences and maintaining a record for future reference. The process begins with an overall assessment of the child in a sequence of History, Physical Examination, Radiological and Laboratory investigation and if required appropriate photographs should also be taken [23].

2a) *HISTORY: (Must be witnessed)*

v Interview the parent/custodian and child

separately, record and document their explanation in their own words, dully signed by interviewer and witness on completion of the documentation.

v Ask; open ended question, non-threatening type and which require descriptive answers.

v Note for any risk factors, difference in history and lack of consistency between severity of the trauma and the story told by parents/custodian. These may point towards abuse.

v Record, when where and how it happened and if any witness is there

v Who lives with the child/ takes care of the child?

v Note history of past injuries, hospitalizations and medical conditions, which might mimic abuse pattern.

v Do not interrogate or attempt to prove an abuse or neglect.

To record the history, five questions need to be asked and five observations to be made.

Table III: Check list for child abuse [21]

Five Questions:	
1)	Could the injury have been caused accidently and if so how?
2)	Does the explanation for the injury fit the age and the clinical finding?
3)	If the explanation of cause is consistent with the injury is this itself within normally acceptable limits of behaviour.
4)	If there has been any delay in seeking doctor's advice, then reasons for this?
5)	Does the story of the accident vary?
Five Observations:	
1)	The general demeanour of the child
2)	The relationship between parent and child.
3)	The child's reaction to other people
4)	The child's reaction to any medical/dental examinations
5)	Any comments made by child and/or parent that give concern about the child's upbringing or lifestyle.

2b*) PHYSICAL EXAMINATION:*

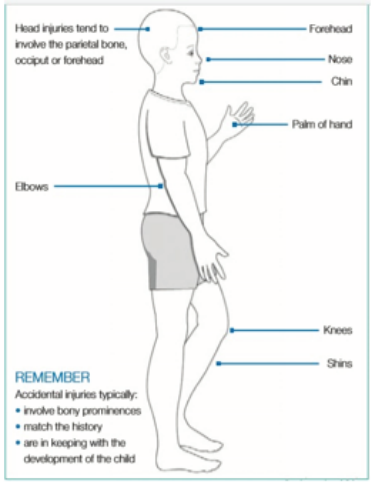
In case of any suspicion, proceed for physical examination in a systemic order starting from extra oral (hand to toe) onto intra oral as,

1) Extra oral: Location and type of injury differentiating the accidental and non-accidental injuries [27] (Figure 1 A and B).

v Triangle of safety

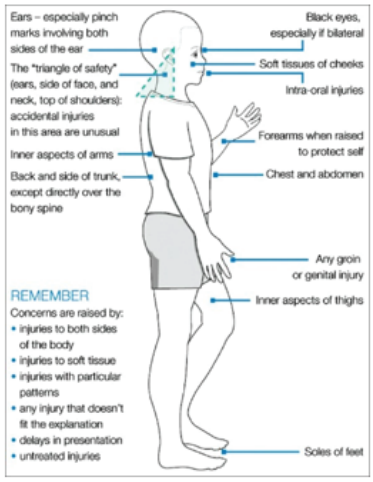
v Trauma on both sides

v Check for ***6 B's – Bruises, Breaks, Bonks, Burns, Bites, Baby blues (Non –accidental)*** (Figure 2)



A

Figure 1A: Typical sites of accidental injury (Reproduced with permission) [27]



B

Figure 1B: Typical sites of non-accidental injury (injuries that should raise concerns) (Reproduced with permission) [27]

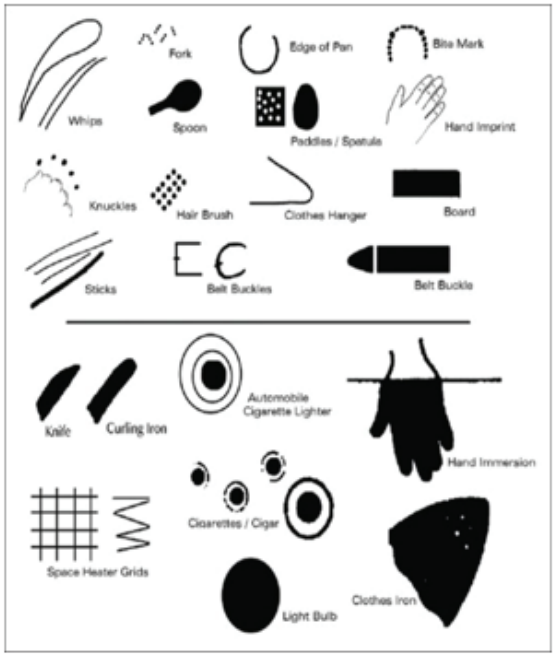


Figure 2: Common patterns of bruise marks (Reproduced with permission) [29]

2) Intra Oral: Soft and hard tissues [2]

v Lacerations or scar marks may present on lip, on labial Frenum from forced feeding, on tongue from forcibly biting down, and sometimes a severe form may present on edentulous ridges.

v Burns marks from heated instruments, or rope marks on the corners of the mouth from a gag being placed over the mouth may present.

v Bruises may present on Palate from forced oral sex called as fellatio (unexplained petechiea or bruises particularly at the junction of the hard and soft palate) or

on edentulous ridge.

v Contusions may present on floor of the mouth.

v Non accidental trauma induced fractured or nonvital teeth, missing or displaced teeth for which there is no obvious explanation.

v Appearance of white slough due to necrotic epithelium caused from caustic substances or scalding liquids.

v Additionally, the child may report with excessive salivation, even drooling, and may have difficulty in swallowing.

v Venereal warts, HIV-associated lesions, or any STDs.

IF DENTAL NEGLECT:

v Untreated, rampant caries.

v Untreated pain, infection, bleeding or trauma in orofacial region

v Delayed seeking of dental help after clear diagnosis.

2c) PHOTOGRAPHS:

American Board of Forensic Odontologists (ABFO) made a standard reference scale ABFO Number 2 used by forensic odontologists for bite mark measurement and photography [30]. Millimeter reference scale may be placed if required close to the area being photographed (Figure 3A and 3B). Images should be captured at a distance of 3-5 feet from the subject [31].

A tag with date and reference number can be added on each photograph.

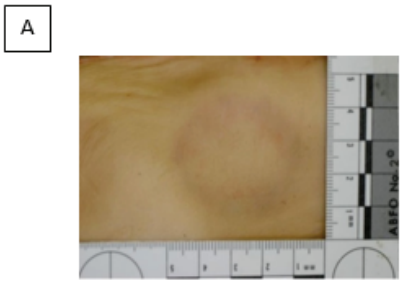
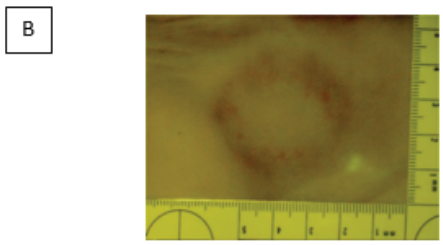


Fig. 3A Visible light image of two-week old bite on arm



Alternate light image of same bite as seen in Fig. 3B

Report

Mandatory reporting mandates certain professionals to report suspected cases of child physical and sexual abuse to appropriate authorities. Designated professionals (including pediatric dentist) are required by law to report all suspected cases of child abuse & neglect and under Juvenile Justice Act (Care and Protection) Act, 2000 (amended 2006) [32], proof is not required. Rather, they are protected by law in case of an erroneous reporting as long as it was in good faith. In contrast, they can be legally penalized in case they fail to report. In India, such provisions have not yet been introduced. But Government of India has launched a Childline (dialing number-1098) which is a 24 hours free phone service and can be accessed by child himself/herself in any distress situation or emergency assistance whenever required. It can also be used by adult on child's behalf. This service responds to call for medical assistance, child protection from abuse, emotional support or any referral services [33]. However, for proper reporting three "Ws" are important. They are: WHY ? WHOM ? and WHAT ? [33][34]

A. WHY to report? (Table IV)

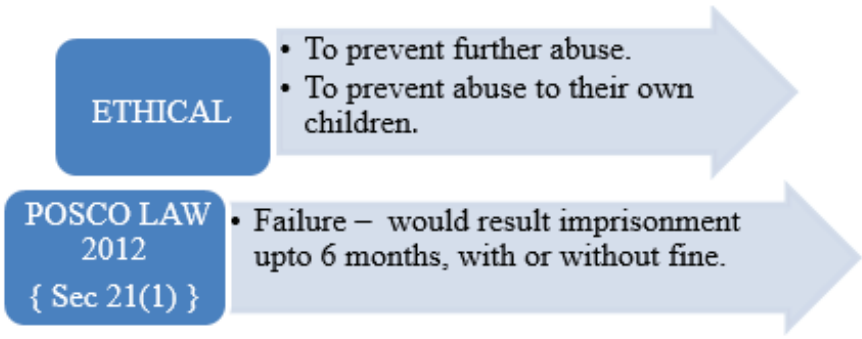


Table IV: Why to Report? [33]

B. WHOM to report? In the absence of 'mandatory reporting', child protection services in India constitute an important decision. Usually the reporting can be done to the police, the local Child Welfare Committee and even to the Child line. Regular follow up to monitor and assess child abuse and development [33].

C. WHAT to report?

1) Name, age, home address of the affected child

2) Observations as documented with signature and date. Also, obtain the signature of a witness to injuries and interview.

3) The name, age and condition of the other children in the same household, if available.

4) Identify the person or persons responsible for abuse or neglect, if known

5) Any other information that you believe may be helpful in establishing the cause of the abuse to the child.

6) Record verbatim the comments made by the child and parent explaining the injury.

Conclusion

Child abuse and neglect is observed in every social, cultural and economic background, both in developed and developing countries. It depends on the person's mental state and attitude, who indulges in this type of abusive behaviour. Although literature reveals that, dental professionals do not have the adequate training and knowledge on recognition and reporting of suspected case of child abuse and neglect, pediatric dentists have the potential to recognize it in their clinical practice, owing to their comprehension on child's cognitive, psychological and social development. However, most of them do not report such cases, probably because they are unaware of their legal responsibility or existence of social barriers which complicate the task, thereby putting the child at risk of further harm. Nevertheless, the country like India, with the existing legal system and the perceptions in the society, the moral of pediatric dentists on this issue is certainly not high. Hence, a change in our attitude and sense of responsibility is required to safeguard children against child abuse and neglect.

Conflicts of Interest: Nil

Financial Support And Sponsorship: Self

Ethical Clearance: This is a review article therefore Institutional Ethics Committee clearance is not required

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Type of manuscript: *Case report*

Fracture of Implant Prosthesis at Abutment Neck- A Rare Phenomenon

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Abstract

Implant dentistry has become common treatment modality for total and partial edentulous arches. Complications arising after placement and eventual functioning of implant prosthesis are also increasingly encountered in routine clinical practice. This case report is about implant prosthesis where fracture of neck of abutment occurred at internal hex level of implant after 5 years of optimal functioning in oral cavity.

Keywords: *Implant, Prosthesis,Abutment, Fracture, Stress*

Introduction

Titanium dental implants are widely used in the restoration of partially edentulous and fully edentulous patients thus offering the functional and esthetic rehabilitation. Complications arising due to implant loss, abutment loss are also encountered by clinicians in some cases after few years of optimal functioning of the prosthesis. These complications may compromise the outcome of treatment to various degrees. This case report discusses an extremely rare phenomenon where fracture of abutment neck occurred at internal hex level of implant with abutment mobility. In the past only one clinical study has reported fracture of abutment at neck level without screw fracture and consequent successful new abutment placement.

Case Report

A 72-year-old reported to Dept of Prosthodontics complaining of loose prosthesis in lower right back teeth region. On intraoral examination it was found that the implant prosthesis in the region of 45, and 46 were found loose. Implants (AdinTouareg -S 3.5x10mm. 5x10mm respectively) were placed 5 years back. They had been restored with cement retained splinted full metal crowns. Radiograph revealed osseo-integrated implants with apparently no fracture of screw at implant level. There was a saucer shaped bone loss around implant in 45 regions. (Figure 1)

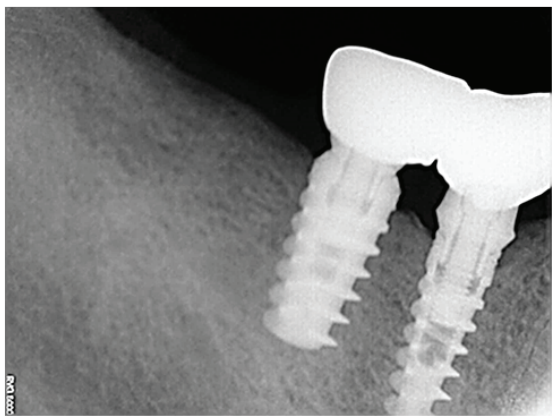

Fig.1 Bone loss around anterior implant

Remaining natural teeth had moderate to severe occlusal wear. There is no self-reported history of bruxism. A hole was made through the center of crowns of 45 and 46 to gain abutment screw access. Once the access was gained, an attempt was made to unthread the screws in both abutments. The screws were removed without any fracture from 45 and 46 implant regions. But close examination revealed that the abutment at 46 implant positions had fractured at neck level with the fractured neck remaining on internal hex of implant. The fractured part of abutment neck at internal hex of

implant at 46 implant position was removed using an endodontic probe (DG16, Dentsply).This fortunately prevented an inadvertent damage to the internal threads of implant which could have otherwise happened if rotary instrumentation was used. (Figure 2 and Figure 3)

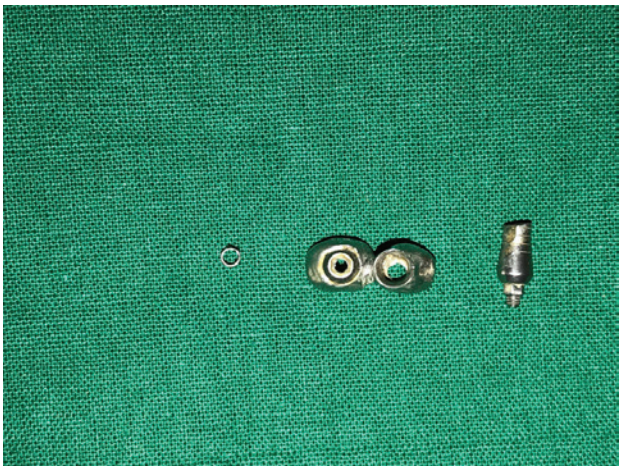

Fig.2 Fractured neck of molar abutment and prosthesis with abutment of premolar separated from super structure

Fig.3 Fractured neck assembled on molar abutment for illustrative purpose

Patient was offered the suggestion of an extra implant for molar to distribute the occlusal load which he refused. Implant level impressions were made, and implants were restored with new prosthesis with light contacts in centric occlusion and no contacts during any excursive movements. Patient was instructed to wear soft splints at night since he was not compliant with hard splints.

Discussion

According to Mariana et al the lack of passive fit and generation of shear forces are the most frequently reported causes of prosthetic failures of implants.[1]Ramtin et al have listed the following six categories of technical or mechanical complications that are associated with single implant restorations and partial fixed implant-supported prostheses: (1) loosening of screws, (2) fracture of screw,(3) fracture of framework, (4) fracture of abutment, (5)chipping or fracture of veneering material, and(6) decementation.[2]Katona recommended that in posterior regions implants should be centered mesiodistally and as perpendicular as possible to the occlusal surface so that leverage can be minimized during function.[3] Several studies have addressed the effect of occlusal overload on implants .Guinchet in a comparative study between screw and cement retained prosthesis found that, after function, the marginal gap of screwed dentures was significantly smaller than that of cemented prostheses. The transmission of load in screwed prostheses was more yet transmitted to the apical portion of the implant, while in cemented prostheses it was less yet concentrated at the coronal portion of the implant. [4] The saucer shaped one loss around implant in 45 is typical of the loss which happens after few years of functioning. This is evident in the present case report wherein increased stress at the crestal level of implant has led to fracture of abutment neck in cement retained prosthesis.The smaller marginal gap of screwed prostheses is related to a higher load transmission, and the greater marginal gap of cemented prostheses is related to a lower load transmission. Finite element analysis of implant-supported restorations has shown that the largest amount of stress is concentrated at the coronal part of the alveolar bone at the implant-bone interface. [3] This can also be appreciated in the present case report where there is fracture of abutment at neck level with surprisingly no fracture of screw. The abutment could not be separated from the splinted crowns of 46 possibly due to intensification of cold work over years of occlusal load. The abutment in premolar was easily separated from the crown.

Anders nd others in their critical review mentioned that when prosthetic intervention is indicated for a patient with heavy masticatory load, it is mandatory to optimize occlusion in such a way that structural integrity of prosthesis is not compromised. [5]

Bragger remarked that bruxism and heavy masticatory load were associated with more technical failures. [6] Metal fatigues can be influenced by various factors, such as the implant diameter, implant-prosthesis

structural design, occlusal force magnitude, and marginal bone loss. Manda et al suggested that extensive non-destructive testing of metallic dental prosthesis before application may induce cracks. Such pre-existing cracks could intensify due to the chemical action of hydrogen cations in the oral cavity or they may be overloading during screw fixation process. They suggest that a single factor cannot be pointed out in failure of dental prosthesis.[7] According to a prospective study of mandibular implant-supported fixed prostheses with 15 years follow up, Lindquist and Carrlson observed that factors such as smoking and poor oral hygiene had a significant influence on bone loss, while occlusal loading factors such as bruxism, maximal bite force and length of cantilevers were of minor importance.[8] Multiunit prosthesis supported by one or two implants and replacing missing posterior teeth are at a greater increased risk of bending overload according to Rangert et al .[9]Hence abutment/screw fracture may always have a latent risk of implant failure because of the inability to retrieve fractured parts within the implant body according to Freitas-Júnior et al.[10]The placement of implants on posterior mandibular regions, close to the temporo-mandibular joint, yields an adverse condition due to the magnitude of load transmission in this area. Likewise, the wide occlusal platform avoids load transmission following the implant long axis. [1].As a general rule, a calibrated torquing device should be used for adequate preload. Inadequate preload is also cited as a reason for prosthesis screw loosening.Nithyapriya et al suggested that repeating the torquing of abutment screws after 10 min of the initial torque must be performed during implant abutment connection..[11]

Naveau,Renault Pierrisnard.in their finite element analysis of implant-supported restorations have shown that the largest amount of stress is concentrated at the coronal part of the alveolar bone at the implant-bone interface. [12] This can also be appreciated in the present case report where there is fracture of abutment at neck level with surprisingly no fracture of screw. Shim and Yang reported that middle-aged patients, the molar position, and a large implant diameter were associated with a high incidence of abutment fracture based on their study oven long term survival of single tooth implants. [13]. A conservative mode of managing abutment screw loosening without sacrificing the crown with review of literature on the various reasons for abutment screw loosening has been described. In a case report by Vinod Krishnan et al the crown and the abutment with the connecting screw were kept in the ceramic furnace at 250° C for 15 min which disintegrated the luting agent. [14].According to Bolshi and Wolfinger, use of two implants simulating the two roots of a missing molar can minimise prevent implant overload.[15]

Conclusion

Heavy masticatory load plays significant role in the longevity of implant prosthesis. Proper diagnosis and treatment planning and placement of two implants in case of missing molar may help in stress distribution and prevent such uncommon complication to a large extent.

Ethical Clearance- Not required.

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Pharmacology and Toxicology Teaching Patterns for Undergraduate Students in Pharmacy and Medical College at Umm Al-Qura University, Makkah, Saudi Arabi: A Survey

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Abstract

Background: Pharmacology and toxicology is the field of biomedical sciences that cares with the study of medication and the drug action, where a drug can be defined as any chemical substance, that, when taken by a living organism, produces a therapeutic effect and promote well-being.

Objective : The Aim of this study was to assess the students' attitudes toward, perceptions of and feedback surrounding pharmacology and toxicology teaching methods.

Method : This was a cross-sectional study involved a survey given to 3rd years and 4th years pharmacy (Pharm D + B Pharm) students and medical students with a total number of (108) participants. The study was administered through a pre-validated questionnaire containing questions based on the internationally accepted "Likert Scale".

Results : The study showed that students favored many changes in pharmacology and toxicology, including the trend of teaching them to third year and fourth year students (48%), instruction on how to choose drugs rationally in future practice (48%), distribution of handouts before lectures (43%), clinical pharmacology (49%), teaching certain topics during pre-final or final year (35%), and a combination of multiple choice questions (MCQs) and written and oral rating evaluation methods for examination (40%). Students were in favour of that pharmacology should be integrated horizontally with other paraclinical subjects and emphasis on problem solving exercises rather than on didactic (teaching) lectures.

Conclusion : It is evident that there is an imperative need to implement radical changes in the teaching of pharmacology and toxicology, changes that would allow the pharmacist and/or doctor of tomorrow to render better health services**.**

Keywords: *Pharmacology; toxicology; Likert scale; questionnaire; teaching; undergraduate students.*

Background

Pharmacology and toxicology is a field of biomedical sciences that cares with the study of medication and the drug action,[1] where a drug can be defined as any chemical substance, that, when taken by a living organism, produces a therapeutic effect and promote well-being. More specific definition, it is the study of the mechanism of interactions between a human body and chemical substance which affect the biochemical function. Moreover, it describes two mean subjects: pharmacodynamic which discuss the mechanism of action of drugs and pharmacokinetic that is related to areas of absorption, distribution, metabolism and excretion within the body.

The pharmacology discipline can be classified into different sub- branches each with a certain interest, such

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as

a- According to Systems of the body [2]

• Neuropharmacology: the study of neurons of the central and peripheral nervous systems.

• Immunopharmacology: the study of the immune system.

• Cardiovascular: thee study of the heart and blood vessels.

• Renal pharmacology: deal with kidney function.

• Endocrine pharmacology: the study of the endocrine glands.

• Psychopharmacology: is the study of the effects of drugs on the psyche and behavior. [3]

b- According to clinical practice and drug discovery [4]

· Clinical pharmacology: the branch of pharmacology that focus on the application of principles of pharmacology in caring of patient.

For example, posology that is concerned with how medicines are dosed.

c- Experimentation and analysis

• Theoretical pharmacology is a branch of research uses computational techniques. It aims to interpret the association between the experiential efficacy of a certain drug to its structural composition. which results to know the structure activity relationship. [5, 14]

d- Wider contexts

• Pharmacology branches which deal with wider contexts than the body physiology. For example pharmacoepidemiology that deal with the efficacy of drugs in large population and associated with the broader fields of epidemiology and human health.[6, 7]

The drugs studying requires high knowledge of the nature of biological system. The field of pharmacology has also changed substantially upon the knowledge of cell structure and cell biology. It has become easier due to the molecular analysis of biological receptors that is considered an important step for drug design as understanding the structure of receptors help to determine the cellular signaling. [7, 10, 11]

The story of synthetic organic chemistry started in 1828, when Friedrich Wohler synthesized urea from inorganic substances and therefore demolished the vital force theory. The birth date of pharmacology is not as clear-cut. In the early 19th century, physiologists performed many pharmacologic studies. Oswald Schmiedeberg (1838–1921) is generally recognized as the founder of modern pharmacology. In 1878, he published a classic text, Outline of Pharmacology, and in 1885, he introduced urethane as a hypnotic. [8, 12, 13]

University of Umm Al-Qura is teaching students pharmacology that includes the main classes of drugs in universal clinical department such as drugs effecting the autonomic nervous system, drugs effecting the central nervous system, drugs effecting the cardiovascular system, drugs effecting the endocrine system, drugs for respiratory disorder, drugs for anemia, drugs for gastrointestinal disorder, analgesic, and antibiotics. Therefore, these are the basic important classes of drugs, in which can have beneficial and harmful.

Pharmacology and toxicology is taught as a basic science to students to know how a drug works and what is the desirable effect we want to treat a patient, for this reason we discuss pharmacology and start this research to ensure that students favourable of the patterns technique teaching of pharmacology in Umm Al-Qura University.

Needless, to mention that undergraduate students should have an excellent foundation of pharmacology to using their knowledge of drug class, mechanism of action, pharmacokinetic, adverse effect, route of administration, dose, frequency, and the duration of the treatment to make the right decisions and choosing the desirable and effective drugs for each specific condition for each patient in clinical practice.The Aim of this study was to assess the students' attitudes toward, perceptions of and feedback surrounding pharmacology and toxicology teaching methods.

Methods

This was a cross-sectional study involved a survey given to 3rd year and 4th years pharmacy (Pharm D + B Pharm) students and medical students with a total

number of (108) participants.

The study was administered through a pre-validated questionnaire containing questions based on the internationally accepted "Likert Scale". the trend of teaching pharmacology and toxicology to 3rd and 4th years pharmacy and medical students, instructions on how to choose drugs rationally in future practice, distribution of handouts before lectures, clinical pharmacology, and a combination of multiple-choice questions (MCQs) and written and oral rating evaluation methods for examination.

Results

From the survey the total number of participants were 108 students, in which 81% were females and 19% were males . Table 1 shows the median scores and percentage distribution of individual statements (Questions 1-8). Participants strongly agreed (28%) with the statement that pharmacology and toxicology is one of the favorite subjects during their studying years. Moreover, participants agreed (48%) that studying pharmacology and toxicology in the third and fourth years would help in rationally choosing the right drugs in future of their practice. Our results demonstrated a preference for horizontal integration of pharmacology with other paraclinical subjects (49%). However, participants agreed that there should be more emphasis on problem-solving exercises rather than on lectures (41%). Participants (43%) agreed on the importance of prior distribution of handouts due to its crucial benefit in providing road maps for upcoming lectures. Students agreed (43%) that problems based on learning and prescriptions would be extremely useful in clinics or hospital settings. The response was very good (46%) with regards to correlating drugs with a specific disease unless pharmacology and toxicology is simultaneously learnt along with clinical conditions.

Table 1. Percentage wise distribution and median score of various parameters

N= 108 Item no	Percentage wise distribution	Median score
1	SDA - 6.0 (5.00 %); DA – 17.0 (14.0 %); NS – 36.0 (31.0 %);	3
	A – 26.0 (22.4 %); SA – 33.0 (27.8 %)	
2	SDA – 12.0 (15.8 %); DA - 1.0 (2.00 %); NS – 2.0 (3.6 %);	4
	A – 22.0 (48.0 %); SA – 9.0 (19.8 %)	
3	SDA – 8.0 (7.1 %); DA – 11.0 (10.1 %); NS – 21.0 (20.3 %);	4
	A – 53.0 (49.0 %); SA – 15.0 (14.3 %)	
4	SDA – 18.0 (16.0 %); DA – 11.0 (10.7 %); NS – 18.0 (17.3 %);	4
	A – 44.0 (41.3 %); SA – 17.0 (16.3 %)	
5	SDA – 11.0 (20.1 %); DA – 11.0 (7.3 %); NS – 16.0 (17.1 %);	4
	A – 26.0 (34.2 %); SA – 6.0 (13.3 %)	
6	SDA – 19.0 (17.1%); DA – 3.0 (3.3 %); NS – 22.0 (21.4 %);	4
	A – 46.0 (42.9 %); SA – 17.0 (16.3 %)	
7	SDA – 7.0 (7.2 %); DA – 4.0 (4.0 %); NS – 17.0 (16.5 %);	4
	A – 48.0 (46.0 %); SA – 18.0 (17.4 %)	
8	SDA – 8.0 (8.0 %); DA – 11.0 (10.0 %); NS – 43.0 (40.0 %);	3
	A – 37.0 (35.0 %); SA – 8.0 (8.0 %);	

Note: SDA Strongly Disagree; DA Disagree; NS notsure; A Agree; and SA Strongly Agree

Table 2 shows the response rate and percentage distribution of statements (9 to 12). Around half the participants (50%) preferred using multiple choice questions [MCQs] only, written questions (3%) and oral questions (7%) as evaluation techniques for examination purposes. When students were asked to state the best tool through which to study pharmacology and toxicology, their responses varied between preferring to use a combination of studymethods (35%) and preferring lecturing as their primary study tool (37%), 17% of the participants preferred textbooks. Around (44%) of the students in this survey study preferred the understanding method when learning pharmacology. When asked whether they wished to become pharmacologists, the answers were varied: 11% said they did not know, 39% selected maybe, 31% said no and 19% answered yes.

Table 2. Percentage wise distribution and median score of different parameters

Item in questionnaire	Responses %			
	MCQs only	Written only	Oral only	Combination
Q09	57.0 (50.00 %)	8.0 (7.00 %)	3.0 (3.00 %)	49.0 (40.00 %)
	Text Book Only	Teaching Class	Self-Prepared	Combination
Q10	24.0 (17.00 %)	35.0 (37.00 %)	16.0 (11.00 %)	51.0 (35.00 %)
	Cramming	Understanding	Grasping	Combination
Q11	11.0 (7.00 %)	46.0 (44.00 %)	19.0 (13.00 %)	52.0 (37.00 %)
	Don't know	May be	No	Yes
Q12	14.0 (11.00 %)	47.0 (39.00 %)	37.0 (31.00 %)	23.0 (19.00 %)

9 = Rating evaluation methods for examinations: Choose only one option using tick (√).

10 = Study material to learn pharmacology: Can choose more than one option using tick (√)

11 = Pharmacology learning methods: Can choose more than one option using tick (√).

12 = Wish to be a pharmacologist: Choose more than one option using tick (√)

Discussion

This survey was set out to investigate the impact of pharmacology and toxicology teaching patterns and gather students' recommendations. An "Likert Scale" based questions was used to collect data for the study, as mentioned in the methods. From the results, it is clear that first question showed that although 31 percent of students were not sure that pharmacology and toxicology is their favorite subject among the basic sciences and 19 percent did not support that, 50% on the other hand prefered it, this result appears in some way to explain why only 19% of students wished to become pharmacologists in the future and around 39 percent of them placed the profession of pharmacologist among the options that are not excluded.

Another promising finding that only 28 percent of students did not find studying pharmacology and toxicology in third and fourth year of medical faculty helpful in choosing drugs rationally in their future practice.

About two-thirds of respondents stated that pharmacology should be horizontal integrated with other paraclinical subjects this is vital as it lead to understand the reason to see more than half that said it would be difficult to correlate the drugs with the disease unless pharmacology and toxicology is simultaneously learnt along with clinical conditions that covered in other approaches, such as Therapeutics. A survey of Italian physicians by Furlanut determined the pharmacology and toxicology teaching they had received was mainly theoretical and suggested that more time and attention

should be devoted to issues more closely related to clinical practice [16, 17,18]

Our results also was in agreements with other similar surveys that concluded that more than 50% of medical students want more clinically-oriented lectures. [15, 16, 17]

Those who preferred to receive lectures in a didactic (teaching) method were below average compared to the 57% who nominated focusing on problem-solving exercises, this yields a negligible impact increase in results on the answer of question six, which appeared as a 59 percent of reply-ers went to noted that problems based on learning and prescriptions are extremely useful in clinics.

Our research explored that 56 percent of pharmacy students see that lecturers should distribute handouts giving the outline of the topic before the lecture classes, perhaps the reason for this is that it will increase their awareness of the general framework of the subject.

While 42% students agreed with teaching some topics during the pre-final or final year, forty percent aren't sure about that. Late results casts a new light on rating evaluation for examinations in which half of the percent favor multiple choice question methods and 40% in side of combination, but only 7% for short essay questions.

The (teaching class notes) were the most common study material to learn pharmacology with 37 percent , combination study material come next with 35% , however 17% used textbook only for memorizing their lessons and 11% depended on self-prepared notes. Overall, these results are in line with the oenions on the methods used for learning pharmacology which were divided into the followings: 44% for understanding, 36% for combinations, 13% for grasp thinking and 7% of cramming/mugging.

Although our study survey was widely accepted, our implementation suffers from some limitations due to obstacles to accessing pharmacology and toxicology marks for students.

It would be good if future researchers consider investigating more deeply of positive outcomes of distribute handouts giving the outline of the topic before the lecture classes.

Conclusion

The results obtained from the present study revealed that pharmacology and toxicolgy is a corner stone of the medical sciences subjects. More specifically, this exploration of the students' preferences regarding pharmacology and toxicology teaching and its outcomes could potentially modify undergraduate teaching patterns in them. It is accepted that reviewing the teaching and evaluation methods through feedback from students, then modifying methodologies accordingly, is vital to cooperate with the undergraduate teaching adapt to changes in the profession . It is evident that there is an imperative need to implement radical changes in the teaching of pharmacology, changes that would allow the pharmacist and/or doctor of tomorrow to render better health services.

Although our study survey was widely accepted, our implementation suffers from some limitations due to obstacles to accessing pharmacology and toxicology marks for students. It would be good if future researchers consider investigating more deeply of positive outcomes of distribute handouts giving the outline of the topic before the lecture classes.

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Sexual Dimorphism from Foot Morphometry : A Study among Two Endogamous Groups of North India

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Abstract

Background: The present study aims to exhibit sexual dimorphism from foot morphometry and to find if it can be used as a tool for sex determination in forensic sciences among two endogamous groups (baniya, jats) of North Indian population. It is also aimed at providing anthropometric values for foot length and foot breadth and comparing them to existing data needed for the establishment of reference standard for the study groups on the basis of which various endogamous groups can be compared with one another and sexual differences established.

Material and Methods: The present study was carried out at Department of Anatomy , MMIMSR Mullana –Ambala, on 400 subjects(100 males,100 females of each group) belonging to baniya and jats community of North India between 21-35 years of age. Foot morphometry was done with the help of osteometric board and results were statistically analysed.

Results: The mean Foot length in males was 3 cm more than females with highly significant p value. The mean foot breadth of males was 1 cm higher than females with statistically significant p value. The mean foot length and foot breadth of jat group was more as compared to Baniya group with highly significant p value.The Mean Foot index ranges from 37(jats) – 36.9 (Baniya) males. In females,the range of mean foot index is between 37.73 (jats) -38.25 (baniya) .

Conclusion: Males belonging to jat and baniya community had higher mean foot length,foot breadth than females. Among the study groups,jats males and females have longer and broader feet than baniya males and females.Foot index <37 is suggestive of male foot and >37 female foot and can be used as a reference point for sex determination among study groups.

***Key words**: Foot length, Foot breadth, baniya, jat*

Introduction

Quantitative analysis of foot anthropometry is important in the study of forensic sciences,ergonomics and anthropology .[1] Anthropometry is the typical and the traditional tool of physical anthropology which provides the scientific methods and techniques for estimating the various measurements and the observations on the living as well as skeleton of man.[2]The word anthropometry is of greek origin. It is derived from “anthoros” meaning “ human” and “metron” meaning “measure”(National Health and Nutrition Examination Survey ,2007)[3] Foot anthropometry is the measurement of size and proportion of foot and the parameters often include foot length and foot breadth.

Human foot, the foundation of bipedal locomotion, is a highly complex multibone structure with 26 major

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bones and more than 30 synovial articulations.[4] Foot gives support to the body in standing,resilient for walking and accommodation to variations of surfaces on which it is placed.The normal foot shows great individual variation in length,and breadth in males and females.[4,5] Morphology of human feet is greatly influenced by the combined effects of hereditary and living style of man that determines the size and shape of feet or foot prints and thereby makes them unique data to establish human identity.[6,7] Besides age,race and stature sex is considered as one of the big four parameters in forensic identification. Sexual dimorphism refers to sex related variations that exist between males and females .These variations not only include external genitalia but also body size, appendages and specific cellular components as well. Sex determination can be done from foot index and foot shape. Although bilateral symmetry is a feature seen in human beings yet there is asymmetry seen in foot length of an individual.[8] The foot shapes are said to be corresponding to different weight bearing conditions. Both the foot length and foot width are usually found increased during weight bearing. The shapes of human feet change due to their habit,climatic conditions and the presence of any disease.

Studies on the anthropometry of foot shape used different protocols and measurement devices. Most approaches directly measure the foot length,breadth,height and girth dimensions using vernier caliper,cloth tape flat ruler[4,9,10] and foot prints Various Anthropometric studies on the foot to evaluate gender differences from foot morphometry were reported among various populations. [4,5,13] Body dimensions vary from person to person,male to female and in various racial groups. The present study mainly aims to exhibit sexual dimorphism from foot morphometry and to find if it can be used as a tool for sex determination in forensic sciences among two endogamous groups(baniya,jats) of North indian population.It is also aimed at providing anthropometric values for foot length and foot breadth and comparing them to existing data needed for the establishment of reference standard for the study groups on the basis of which various endogamous groups can be compared with one another ,and sexual differences established.Further,in this study an effort is made to provide baseline data for design and manufacture of shoes for correct shoe fit among males and females belonging to baniya and jat community.

Material & Methods

The present study was carried out at Department of Anatomy,MMIMSR Mullana –Ambala, on 400 subjects(100 males,100 females) of each group belonging to known endogamous group (baniya and jats) of North india age group ranged between 21-35 years. Prior written consent for the study was taken from all the subjects both in English and in vernacular. Cases with foot anomalies, trauma , inflammations and surgery was excluded for the investigation.

Equipments used : Osteometric board

Foot measurements were taken with the help of osteometric board_

§ Osteometric board :-Osteometric board is an anthropometric instrument that consists of a flat board with two ends, one of which is movable and travels along a routed track. This is a preferred instrument for measuring long bones. The object to be measured is placed between the two end pieces and the movable end brought up to the object, where the measurement scale can be read.

§ Foot length - Foot length was taken as a straight distance between the most posteriorly projecting points of heel (pternion) to the most anterior projecting point (Acropodion) of the first or the second toe whichever will be bigger when the foot is fully stretched.

§ Foot breadth – Foot breadth was measured as a straight distance from metatarsaletibiale (the most medially placed point on the head of first metatarsal) and metatarsalefibulare (the most laterally placed point on the head of the fifth metatarsal) when the foot will be fully stretched.

§ Foot index = foot breadth / foot length x 100

Statistical Analysis :Data was analyzed using Statistical Package for Social Sciences (SPSS, version 20). Descriptive statistics including mean, standard deviation, minimum and maximum value for foot morphometry of both sides were calculated separately for both sexes. Bilateral asymmetry between various foot measurements was calculated separately for both sexes and paired t test was used to test it .

Results

The mean Foot length in males was 3 cm more than females with highly significant p value. The mean foot breadth of males was 1 cm higher than females with statistically significant p value. (Table -1) The mean foot length and foot breadth of jats was more as compared to Baniya males and females and with highly significant p value.(Table -2)The Mean Foot index ranges from 37(jats) – 36.9 (Baniya) males. In females,the range of mean foot index is between 37.73 (jats) -38.25 (baniya) .(Table 3) Foot index <37 is suggestive of male foot and >37 female foot and can be used as a reference point for sex determination.

Table 1. Descriptive statistics of foot anthropometric measurements among the study population

Parameters	Side	Male (Mean ± SD) N=200	Female (Mean ± SD) N=200	p value
Foot Breadth	Right	9.59 ± 1.136	8.60 ± 0.913	<0.001
	Left	9.96 ± 1.251	8.86 ± 0.986	<0.001
Foot length	Right	26.26± 1.600	23.48 ± 1.846	<0.001
	Left	26.60 ± 1.609	23.79 ± 1.868	<0.001
Foot Index	Right	36.54 ± 4.125	36.73 ± 4.067	0.6455
	Left	37.48 ± 4.483	38.235 ± 3.539	0.7497

Table 2 . Descriptive statistics of foot morphometry among endogamous groups

Parameters	Side	Jat			Baniya		
		Male	Female	p value	Male	Female	p value
Age		24.4 ± 3.31	24.78 ± 3.02	0.423	25.18 ± 4.140	25.04± 3.576	0.798
Foot Breadth	Right	10.05 ± 1.100	9.22 ± 0.803	0.000HS	9.12 ± 0.970	8.51 ± 0.888	0.000HS
	Left	10.46 ± 1.267	9.49 ± 0.838	0.000HS	9.46 ± 1.019	8.70 ± 1.023	0.000HS
Foot length	Right	27.55 ± 0.949	24.64 ± 1.685	0.000HS	24.97 ± 0.941	22.44± 1.207	0.000HS
	Left	27.89 ± 0.916	24.97 ± 1.691	0.000HS	25.30 ± 0.986	22.65± 1.164	0.000

p value $\leq$ 0.001 Highly Significant

Table 3. Descriptive statistics of foot index of males and females among endogamous groups

Parameters	Side	Jat		Baniya	
		Male	Female	Male	Female
Foot index	Right	36.57 ± 4.551	37.45 ± 2.159	36.51 ± 3.673	38.05 ± 4.462
	Left	37.57 ± 5.104	38.01 ± 2.26	37.39 ± 3.786	38.46 ± 4.813

Discussion

In the present study,the mean Foot length in males was more than females with highly significant p value. The mean foot breadth of males was higher than females with statistically significant p value. (Table -1) This suggests that males have longer and broader feet than females for a given age.Various previous studies have been conducted on gender differences supports our present study.Obikilli and Didia[14] found that mean values of foot length and foot breadth are significantly higher than females.Wunderlich and Cavanagh[4] analysed gender differences in foot shape found that men have longer and broader feet than women .Another study done by Singla et al,[15] on gender differences of foot dimensions among rajasthani jats concluded males of this group have higher foot length (2cm) and foot breadth(1cm) as compared to females and p value was highly significant.

On comparison among study groups,it was seen that the mean foot breadth and mean foot length of the males and females of the Jat community is higher than the baniya community.This may be due to the hereditary factors, racial and dietary factors as Jat community are taller and well built than baniya, belongs to dominant land owners being physically active, and known for their strenuous work under extreme weather conditions. Jat community have healthy dietary habits in the form of high intake of milk products and non- vegetarian food. On the contrary, Baniya group belongs to high socio-economic status,lead a sedentary lifestyle, their dietary habits are in the form of high fat intake, and they indulge in less physical activities. On comparison with the previous studies our findings for Jat population correlates with the study done on Nigerian Population. In Baniya community the measurements of foot length and foot breadth are correlating with the study done by Singla et al[16] , Rai R[17] (Table – 4,5)

Table 4. Comparison of foot morphometry in males of the study population with the previous studies

Study	Population	No. Of Subjects	Foot Breadth		Foot length	
			Right side	Left side	Right side	Left side
Present study	Jat	100	10.05±1.100*	10.46 ± 1.267	27.55 ± 0.949*	27.89±0.916**
	Baniya	100	9.12 ± 0.970*	9.46 ± 1.019*	24.97±0.941**	25.30±0.986**

Table 4. Comparison of foot morphometry in males of the study population with the previous studies

Manuel I et al18 (2008)	Nigerian	249	9.87±0.53***	9.73±0.55**	26.92±1.02***	26.92±0.13**
Rani et al19(2011)	Delhi	150	8.524±0.67ns	8.666±0.68**	23.34±1.58**	23.46±1.59**
Singla et al16(2012)	Haryanvi Jats	150	9.51±0.55***	9.48±0.611***	25.79±1.27**	25.76±1.22**
	Rajasthani Jats	150	9.53±0.56***	9.60±0.57***	25.77±1.19**	25.80±1.16***
	Northen India Mixed Population	150	8.98±0.71**	9.60±0.57***	26.04±1.26**	25.98±1.15***
Rai R and Shreshtha S17 (2014)	Uttar Pradesh	33	9.94±1.00***	9.89±0.94***	25.44±1.68ns	25.46±1.75ns

Table 5 . Comparison of foot morphometry in females of the study population with the previous studies

Study	**Population**	**No. Of Subjects**	**Foot Breadth**		**Foot length**	
			Right side	**Left side**	**Right side**	**Left side**
Present study	Jat	100	9.22 ± 0.803**	9.49 ± 0.838**	24.64 ± 1.685**	24.97 ± 1.691**
	Baniya	100	8.51±0.888**	8.70±1.023**	22.44±1.207**	22.65±1.164*
Manuel I et al18(2008)	Nigerian	228	9.14±0.58***	8.52±0.59***	25.00±1.33***	24.75±0.17***
Rani et al19(2011)	Delhi	150	7.816±0.776*	7.962±0.79ns	20.59±1.25***	20.69±1.44***
Singla et al16 (2012)	Haryanvi Jats	150	8.68±0.58***	8.72±0.55***	23.46±1.15*	23.5±1.1***
	Rajasthani Jats	150	8.8±0.543***	8.81±0.48***	23.68±1.0***	23.67±1.0***
	Northen India Mixed Population	150	8.4±0.48***	8.81±0.48***	23.14±1.1ns	23.19±1.8ns
Rai R and Shreshtha S17 (2014)	Uttar Pradesh	17	8.63±0.70***	8.73±0.71***	22.47±0.81ns	22.4±0.70ns

On comparison of foot index among study groups it was seen that the Mean Foot index ranges from 37(jats) – 36.9 (Baniya) males. In females,the range of mean foot index is between 37.73 (jats) -38.25 (baniya) .(Table 3) Foot index <37 is suggestive of male foot and >37 female foot and can be used as a reference point for sex determination.The present study is supported by a study done by Agnihotri et al[20] on Mauritius population, stated that foot index more than 37 is suggestive of females and less than 37 of males. Another study done by Singla et al[16] on Haryanvi,Rajasthani and North indian mixed population concluded that in Haryanvi and North indian mixed population,Foot index more than 36 is suggestive of females and less than 36 is that of males.

Conclusion

This study is able to establish that males belonging to jat and baniya community had higher mean foot length,foot breadth than females. Among the study groups,jats males and females have longer and broader feet than baniya males and females. Foot index <37 is suggestive of male foot and >37 female foot and can be used as a reference point for sex determination in study groups. Further in this study, an effort is made to provide baseline data for design and manufacture of shoes for correct shoe fit among study groups.

Conflict of Interest – Nil

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Ethical Clearance - Ethical clearance and approval was obtained from the institutional Ethics Committee

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Environmental Maintenance through the Application of Adat (Criminal) Sanctions on Baduy Communities

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Abstract

This article aims to describe the implementation of customary criminal sanctions used by the Baduy community in maintaining and preserving their nature and the effectiveness of the use of customary sanctions in preventing and overcoming actions that damage the environment of the Baduy community. It is a qualitative research to produce descriptive data, where the approach used is socio-legal research using secondary data as a normative or doctrinal approach, and uses a social approach to explore primary data in the form of values and interpret the behavior of local communities in solving customary cases. The results of this study indicate that the life of the Baduy community which is supported by agriculture and cultivation is very dependent on natural preservation. The maintenance and preservation of the environment in the Baduy community is highly respected. Various ways to preserve nature through avoidance of goods and modern lifestyle are carried out from generation to generation so as not to change the order of nature and the environment. In preserving nature, the Baduy community provides guidelines on certain actions that their indigenous people should not take, such as burning forests, cutting trees in certain areas.

Keywords: *Environmental Maintenance, Baduy Communities, Customary law, criminal sanctions*

Introduction

Indonesia as a country consisting of several ethnic groups with various customs is full of values of peace and harmony/balance in life, namely the balance between human relations with God, with the universe and the environment, with fellow humans as individuals and with fellow humans in his society. In the beliefs of indigenous peoples, spiritual life is very dominant and is placed in the most sacred place. The belief in the existence of the universe is controlled by spirituality which manifests as a macrocosm very much characterizes the lives of indigenous peoples, where every human behavior and behavior in a customary environment is always monitored by something magical. With this belief, humans in their lives who are in the microcosm circle must maintain harmony and balance between macrocosm and microcosm.

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The form of consistency in maintaining the harmony and balance of the relationship between macrocosm and microcosm in indigenous peoples is how indigenous peoples take care of the natural environment so that it is both spiritually and factually preserved in their lives, so that nature is fully preserved, as practiced by the Baduy community The principle instilled by the Baduy community in preserving nature as a gift from God to them is. “mountains may not be destroyed, and valleys may not be destroyed,” and “short should not be joined, length may not be cut.” This principle underlies people’s understanding and awareness that forests, mountains, valleys, and their contents are the lungs of nature. The awareness of the Baduy community that life must be one with nature, implies that using nature as a source of life must also be done very wisely, including by dividing the natural types of forest, namely cultivated forest (which is taken for life), Panisan forest (forest that becomes water sources), and prohibited forest (forest that should not be touched) (Rifa’i, 2017).

In preserving nature, the Baduy community rejects modernization and the use of products and objects that come from outside, which are believed to destroy real

life, such as the prohibition of using bath soap and toothpaste and replacing them with *kecombrang* or *honje* to replace bath soap and coir coconut or betel to replace toothpaste. This is based on the assumption that soap and toothpaste contain chemicals that can destroy nature. There is a prohibition on eating goat meat because it is considered bad for health, while chicken and beef are only eaten in religious ceremonies. Another uniqueness is in treating the dead, namely being buried in the family land and after 7 (seven) days the graves are used again as cultivated gardening land, with the principle that the dead will return to land (Rahma, 2017). In using the land, not using cows or buffalo for plowing, the prohibition of four-legged animals other than dogs from entering the Baduy area is an effort to preserve nature. The nature conservation process is used strictly when building traditional houses made of wood and bamboo, using river stones as the foundation for the foundation, it can be seen from the land contour that is still sloping and not excavated to protect nature (Somantri, 2012).

In preserving nature, the Baduy community has a mechanism for customary sanctions imposed on violators. It is interesting to study related to how the customary sanctions system is applied and its effectiveness in preserving the environment in the Outer Baduy community.

This article is the result of research, which aims to describe the implementation of customary criminal sanctions used by the Baduy community in maintaining and preserving their nature and the effectiveness of the use of customary sanctions in preventing and overcoming actions that damage the environment of the Baduy community.

Research Methods

This research is a qualitative research in which this research uses qualitative methods which produce descriptive data in the form of words, notes related to the meaning and value, and understanding (Kaelan, 2012). The approach used is socio legal research by using secondary data as a normative or doctrinal approach as well as using a social approach to explore primary data in the form of values and interpret the behavior of local communities in solving customary cases, which resulted from interviews with traditional leaders.

In exploring primary data, the research was carried out in the Banten area in the interior of the Outer Baduy tribe community. The informants consisted of the Outer Baduy tribe community leaders, who were determined purposively, based on the ability to know and understand the social, cultural and religious situation in the research object under study. Secondary data were analyzed deductively, namely the process of searching for general truth by using the theories used in this study, while primary data were analyzed qualitatively through data reduction, data presentation, and conclusion drawing. Triangulation techniques in the form of checking the validity of the data for checking data or comparing data are used to assess the validity of the data in this study.

Results and Discussion

Moeljatno (1984) said that certain acts which were strictly prohibited in small communities throughout Indonesia, because they brought disaster not only to those who violated them, but also to society in general, which were known as acts of abstinence, taboo, punishment etc. The concept of this action is based on spiritual beliefs that bind and control people's behavior in indigenous communities (Moeljatno, 1984; Sudarto, 1990).

Unwritten legal systems, also called customary, folk, or indigenous law, do not merely belong to the past. In many societies they are part of a complex legal constellation. They govern such diverse areas as rights to land, water and forests, kinship and inheritance, but also political offices. These systems have undergone, and still are subject to, much change, under the influence of state legal systems, and due to economic and political change. They have been subject of both heated political and scientific controversy. For indigenous peoples, customary law is an important source of identity (Benda-Beckmann, 2001).

The importance of the Customary Law System for indigenous peoples is as an important source of identity, not only in the past but also in the present through various adjustments to the legal and political system merging into the country's legal system.

The importance of customary values in filling national legal policies as a state legal system can also be studied with the mirror theory that law is a mirror

of society, which functions to maintain the social order (Tamanaha, 2001). Law must be a reflection/mirror reflection of society, as a mirror of society, the law must reflect the values that live, grow and develop in the society.

Karl Von Savigny in Chambliss & Seidman (1971) argues that:

"*Law is a function of the volksgeist, custom evolved out of people, the judges merely enforce the custom. The guiding force is the common consciousness of the people, an unitary, mystical force that, like Parson's common set of value orientation is supposed to tie together the entire community."*

The view that law is a function of the spirit of the nation (volkgeist), so that local values which are the realization of the soul of the nation in custom must fill the national legal policy as a reflection of the spirit of the nation that must be upheld.

The Baduy community is a local community with an original culture that has not been mixed with modern culture from outside. Local values that are very positive and full of policies in managing society and the environment are based on living principles. The principles of life above which are believed from generation to generation by the Baduy community have positive implications in maintaining the harmony and balance of life between humans and nature as a manifestation of spirituality that must be kept sacred. The fear of natural anger which is considered to bring disaster or disease or death for actions violating customs is deeply embedded in the minds of the Baduy community. This creates awareness in the Baduy community to use nature as a source of life very wisely. The awareness that forests, mountains, valleys and their contents are the lungs of nature causes the Baduy people to be very careful in treating the lungs of life so that they are always able to pump the breath of life. The principles of preserving nature are closely held with the awareness that nature and its environment as gifts from God which have social, economic and spiritual meanings, must be preserved and must not be changed.

The simple life of the Baduy community is supported by the awareness of managing nature independently for farming (Ngahuma) based on the concept and practice of the original natural conservation system from their ancestors, such as managing various kinds of biological wealth based on a spatial system colored with regional sacredness. The Baduy community practices an environmentally friendly farming system based on organic farming with a native agroforestry system that is reliable in dealing with pests, weather and climate anomalies, and the penetration of the global economy that enters the Baduy rural area (Iskandar, 2012).

This principle of life is very relevant in preserving nature, where the management of agricultural land and plantations (agriculture) is the mainstay of the life of the Baduy community as a livelihood, the results of which are used collectively in the Baduy community. Management of agricultural land and plantations is carried out in a sustainable manner. As stated by Sutisna (2019) that:

"The Baduy depends entirely on the agricultural sector, where the availability of agricultural land becomes very important. Baduy people have their customs related to the existence of agricultural land and have customary rights to the area and agricultural land. Baduy people have their customs related to the existence of agricultural land and have customary rights to the area and agricultural land. To ensure sustainable agriculture, the regulation of the local agricultural system, especially land use and management of the agricultural system is essential".

In preserving nature, the Baduy community realizes this by dividing forest types, namely cultivated forest (which is used for life), Panisan forest (forest which is a source of water), and prohibited forest (forest that cannot be touched) (Rifa'i, 2017). The wise use of the forest environment will result in a natural balance that provides benefits, peace, prosperity and serenity for its inhabitants. On the other hand, natural disasters will arise if nature is used carelessly (Senoaji, 2004).

The attachment of the Baduy community to the environment in which they live and the relationship between community members makes each individual feel the same awareness to maintain the environment in which they live. This is in line with the research results of Hay (1998); Uzzell et al., (2002); Devine-Wright (2007); Low & Altman (1992); Giuliani, (2003); Hidalgo & Hernández (2001); Hernández et al., (2010) that:

"Place identity and place attachment are bonds that people establish with the surroundings in which they carry out their daily activities and go about their personal lives. Place identity, on the other hand, refers to a conception of the self that has been constructed on the basis of the place to which individuals belong and incorporates elements related to the public image of that place."

"*Place attachment implies affective bonds between people and their surroundings and the desire to maintain the relationship with the place over time and at different stages in their lives."*

The awareness of living with the Baduy community to preserve the natural environment and natural purity is agreed upon by the following customary prohibitions; a). It is forbidden to meet strangers; b). Use of transportation and vehicles is prohibited; c). Footwear is prohibited; d). Using electronic devices is prohibited; e). It is forbidden to nail the building; f). Machine sewing is prohibited; g). It is forbidden to build a bathroom at home; h). Building a school building is prohibited, and i). It is forbidden to build mosques (Interview with Ayah Murshid, 2017).

Baduy customary prohibitions in maintaining the purity of nature are related to preventing environmental pollution and the risk of violating the principles of life that are believed by the community. This is in line with the fact that (Ashford & Caldart, 2015):

"The manufacturing, processing, and use of chemicals and materials in industrial workplaces are often accompanied by environmental, health, and safety hazards and risks. Occupational and environmental factors cause or exacerbate major diseases of the respiratory, cardiovascular, reproductive, and nervous systems and cause systemic poisoning and some cancers and birth defects."

In protecting and preserving the environment against environmental pollution in the Baduy community, prohibitions on the use of chemical charcoal are stipulated and the construction of buildings or roads that are permanent in nature using building materials processed by factories. All actions that change the integrity of the natural construction are considered crimes against nature in the Baduy community, which includes (Interview with Sarpin, 2020):

1. Burning or utilizing mountains and protected forests for life;

2. To make modern buildings from permanent building materials, such as: walls with cement and sand. Buildings must use environmentally friendly materials such as wood for walls and coconut fibers as roofs;

3. Road construction with asphalt is prohibited because it changes nature;

4. Use of modern household appliances, such as gas stoves, use of soap and toothpaste, plastic or ceramic or glass cups.

5. Prohibition of clearing fields by hoeing, because it has the potential to cause landslides.

6. The use of footwear for indigenous people.

7. The burial of the body may not be made permanent, after 7 (seven) days the burial place can be opened for plantation or agricultural land;

8. It is prohibited to raise four-legged animals, such as buffalo, goats, cows and others, which are considered to have the potential to make the forest deforested to feed the livestock.

All of the aforementioned customary prohibitions must be obeyed, if they are not obeyed or violated, the customary law enforcement officers of *Baduy dalam* (inner Baduy) and *Baduy luar* (outer Baduy) will take persuasive action in the form of a warning. However, if the warning is not heeded, a trial or deliberation of the customary court will be held to impose a sentence in the form of expelling the perpetrator and his family from the Inner Baduy Traditional Village to move to the Outer Baduy Traditional Village (Interview with Ayah Murshid, 2017).

Punishment is not just to trap perpetrators who violate the principles of environmental preservation, but more than that punishment is needed to prevent violations of environmental offenses by other community members. This is as stated by Bentham (1962) who takes a crime approach with an economic approach, where by Ross Harrison (1989) explains in his brief outline of Bentham's thought:

"[...] punishment is not retribution for past action, but prevention of future harms; obedienceto the state is not because of some past promise, but to prevent future harms."

K. Von Benda-Beckmann (2001) said that:

Unwritten legal systems, also called customary, folk, or indigenous law, do not merely belong to the past. In many societies they are part of a complex legal constellation. They govern such diverse areas as rights to land, water and forests, kinship and inheritance, but also political offices.

The customary sanctions imposed on violators of environmental conservation are in the form of:

1. Birth Sanctions

This birth sanction consists of sanctions according to custom in the form of being excommunicated for 40 days at the Customary Head's House. Violators here are required to stay at the Customary Head's House to help with the work of the Customary Head. In addition, criminal sanctions are also imposed as threatened in the Law on Environmental Management as state law.

2. Inner Sanctions

This mental sanction is in the form of a Customary Ritual Ceremony called *Ngabokoran* in front of the customary institution. This Ngabokoran is followed by a statement that he will not repeat his actions which is called *Sumpah Batin*. With this inner vow it is believed that it will result in death, life without blessings, and sickness to the offspring. This sanction is believed to be more effective in protecting the environment because it is related to the mystical. It is the Ngabokoran sanction that makes environmental violations in Baduy very rare. If the perpetrator of the Baduy environmental customary violation dies before carrying out the Ngabokoran, then the *Serah Pati* sanction is carried out by the offspring of the perpetrator. Serah Pati sanctions are also imposed on perpetrators who repeat violations against the environment.

In the Baduy Customary Law, violations against the environment are categorized as serious offenses, because they are related to disturbances to the natural environment. So the settlement is also through customary courts for serious cases. The belief in karma or a reward in kind for the perpetrator and his descendants if they commit a violation of nature preservation underlies a serious offense and the imposition of severe punishment for the perpetrators.

The resolution of these serious offenses must be carried out by customary courts, and it is not allowed for the settlement of deliberations between families. This is because a serious offense is considered an offense that is not only related to the individual perpetrator and victim, but this offense disturbs society and shakes or destroys the cosmos or spirituality and invites the anger of nature or ancestral spirits.

The resolution of this serious offense was carried out by the customary court which involved all customary court officials, the families of the perpetrators and the families of the victims, community leaders, and all structures of the indigenous peoples. Where the perpetrator must repent asking forgiveness from God and Nature which he destroyed through a Ngabokoran Traditional Ceremony.

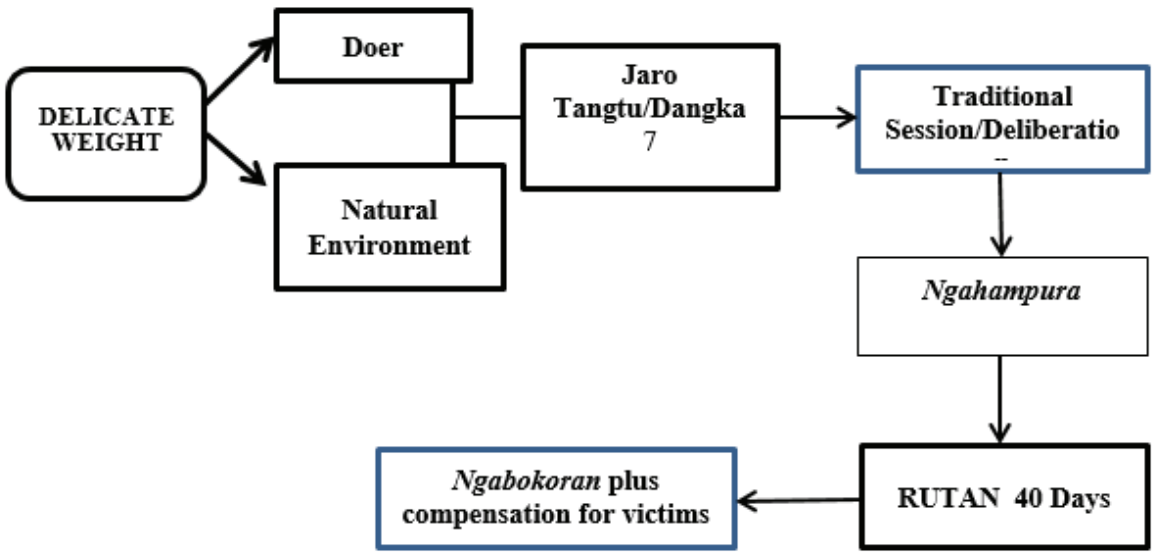
Customary Sanctions held by the Baduy Customary Court, accompanied by criminal sanctions as stipulated in the Law on Protection and Management of the Environment Number 32 of 2009, which aims at keeping the environment in the Baduy community, namely protecting the territory of the Republic of Indonesia from pollution and/or environmental damage. In the context of realizing a preserved living environment, environmental protection and management shall be carried out based on the principles of state responsibility, the principles of sustainability and the principles of justice. In the context of economic, social and cultural utilization of the environment and natural resources, it must be carried out with the principles of prudence, environmental democracy, decentralization, recognition and respect for local and environmental wisdom.

The process of imposing penalties for perpetrators of violations of nature conservation is carried out by customary officials. David Garland defines crime as a legal process in which offenders of a criminal law are reprimanded and subject to sanctions according to certain categories and legal procedures. This process is very complex, differentiated and composed of a chain of processes from the making of laws, the criminal process,

the imposition of crimes and the execution of crimes. Furthermore, it is said that:

"*it involves discursive frameworks of authority and condemnation, ritual procedures of imposing punishment, a repertoire of penal sanctions, institutions and agencies for the enforcement of sanctions and a rhetoric of symbols, figures, and images by means of which the penal process is represented to its variation audiences.*

The settlement scheme for Customary Environmental Violations in the Inner and Outer Baduy Communities



The serious offense settlement scheme through the Baduy Dalam and Outer Baduy customary courts shows that there is a settlement of offenses based on customary conferences for serious offenses that occur in the Outer Baduy and Inner Baduy areas which involve both the outer Baduy and Inner Baduy customary law enforcement officers. Even though they are in a different customary area, in resolving the serious offense, the customary law enforcers in the Outer Baduy and Inner Baduy areas are jointly administering the customary court/customary deliberation in resolving the offense.

In the event of serious offenses such as violations of the preservation of the natural environment involving spiritual interests, the roles of PUUN Cibeo, PUUN Cikertawana and PUUN Cikeusik as judicial leaders for cases brought by Jaro Tangtu Cibeo, Jaro Tangtu Cikertawana and Jaro Tangtu Cikeusik as public prosecutors. PUUN in the village where the case where the case took place will lead the judiciary in assembly with PUUN from other villages, in deliberations to take decisions, PUUN is assisted by Baresan, namely representatives from each village consisting of 9 (nine) people (each Cibeo village, Cikertawana, and Cikeusik 3 (three) people). Baresan is the party that gives consideration to PUUN on the charges and demands of Jaro Tangtu, in making decisions on the results of customary deliberations. In the implementation of customary justice, it must be attended by all structures of indigenous peoples and community leaders of Outer Baduy and Inner Baduy, as well as victims and perpetrators as well as victims 'families and perpetrators' families are required to attend.

Conclusions

The life of the Baduy community which is supported by agriculture and cultivation is very dependent on natural preservation. The maintenance and preservation of the environment in the Baduy community is very unique, where the principle of life from generation to

generation is upheld. Various ways to preserve nature through avoiding goods and modern life styles are carried out from generation to generation so as not to change the natural structure of nature and its environment. In preserving nature, the Baduy community provides guidelines for certain actions that their indigenous people should not do, such as burning forests, cutting trees in certain areas. The offenses were made into serious acts of which the punishment was in the form of *birth punishment* related to society and *inner punishment* which was spiritual in nature.

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Concealment of Crime Revealing by Detailed Autopsy: A Study

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Abstract

Ancient literature suggests a link in between modus operandi of different crimes in different parts of universe, but there is a wide variation in motive and execution of crime. However assailants are very keen to conceal crime by different methods. Defacement of body, electrocution , throwing into river, burial , burning the body , dismembering the body parts and throwing body on railway track are more common methods of concealment of crime. Among various method of concealment of crime some of are discussing in this article with other related factors.

Keywords: *Modus Operandi, Concealment, Crime, Assailants.*

Introduction

Concealment is a common attribute of criminal acts for example many robberies take place at night because there will be lesser witnesses, thus lowering the probability of apprehension.[1] homicidal means cause death of a person by another person. This homicide may be lawful or unlawful. Lawful homicide includes justifiable and excusable homicides. Unlawful homicides include section 299 IPC, culpable homicide and section 300 IPC, amounting to murder [2, 3& 4]. There may be several reasons for committing a murder it is jealously, rivalry, mind war etc. There are various methods used for homicide like stabbing, cut throat, burning, uses of dangerous weapon, ligature strangulation and smothering. As per survey by Canadian Centre for Justice Statistics, stabbing, shooting and beating were the common methods of homicide during 2008-2012.[5]. With ever increasing knowledge, availability to people fear of conviction and new technologies, assailants used to try to showcase the crime in other ways to mislead the investigation and escape conviction. Many times accused try to hide the identity of body in that ways which leads improper tracing of crime. There are many methods used to hide the crime include Defacement of body, electrocution , throwing into river or remote place like jungle, burial , burning the body , dismembering the body parts and throwing body on railway track. As Kota city situated on the bank of river, long railway track of connecting Delhi to Mumbai and there is dense jungle of Chambal also present, due to that it is very easy to hide the crime by concealment. These factors are favorable to dispose the dead body of offence, so this study is conducted in Kota. In such instances thorough details postmortem, proper history, collection of corroborative evidences and crime scene visit are necessary. A careful postmortem examination helped the investigating authority to relate the segregated parts of the crime, generated by the accused to conceal his act.[6] The autopsy provided valuable key points to conclude the case.[7] Here are the some important cases which was brought to mortuary of Government Medical College, Kota from the period of 1st January 2019 to 28th February 2020. In these all cases concealment of crime was conducted by the accused but meticulous autopsy, evidence collections, details history and visit of crime scène changes the whole investigation.

Case no: - 1

An unknown decomposed female body was taken by the police in which was packed in polythene bag. Mouth of polythene bag was tied by aluminum wire. Body was kept in deep freeze for identification. Two families came for identification as body was decomposed one of the family was confused, at last denied that same body related to them as no clothes over body and wound over abdomen which was stitched by aluminum wire confused them because they assume that deceased was pregnant. As it was month of June the changes over body was very fast, After 4 days hours police came with requisition

of medical board to conduct postmortem. Rigor mortis was passed off, postmortem lividity was unable to elicit and skin is peeled off at places. Nailed loosen with dark blue nail pant, teeth intact and hairs easily pluckable. It was very shocking that distension of abdomen and bluish discoloration was not present. Aluminum wire was around the neck and it was tightly encircled, tongue present inside oral cavity. Stitched wound over abdomen was opened it was vertically placed; postmortem was wound also seen at chest. No injury seen around peri-vaginal region. During internal examination of vagina parts clothe observed. So we prefer to examine while internal examination. When we opened the abdomen it was seen that in abdominal cavity ghaghra, kurti and bra were present. Most large intestine, uterus, spleen, right kidney and most of liver part were missing. It means concealment of identity was done by concealing the clothes by putting them inside the abdominal cavity. Neck examination reveals that there was fracture of hyoid bone with hematoma in muscles. Other organs like lung and brain shows decomposition changes. Stomach contains pinkish color fluid with disagreeable smell, mucosa shows decomposition changes. Sample for poison, sexual offence, DNA and other corroborative evidences collected. It was shocking case in which accused try to conceal the identification by putting her clothes inside abdominal cavity and try to hide the body at lonely place. This case was dealt by author.

Case no 2:-

A body 35 years old male was found by the side of railway track. Both upper and lower limbs were tied. Mark of strangulation was present around the neck. Facial skin was missing with avulsion of skin at places, muscle also missing, facial bone easily appreciable. It seems to be that some material was used to conceal the identity of the deceased. But body was identified by the clothes and tattoo make on the body. Cause of death was Asphyxia as a result of strangulation. Remaining skin and muscle was sent to FSL for presence of material which was used to ruin the face. Author did the postmortem of deceased. Identification of person was achieved by tattoo mark over right forearm. Concealment of crime was done by using hiding the identity of person. This case was also dealt by author.

Case no 3:-

A young male body was found by the side of road, which was semi-packed in poly bag. It is in decomposition state no clothes over body. Body was stained with blood and dust places. Semisolid powder also present over clothes, especially over face of deceased. Body was unidentified since two days as most of the facial muscles and skin peeled off. During postmortem it is found that decomposition sign present but they are different on particular body parts as face shows removal of skin but skin over lower limbs healthy. Distension of abdomen is not present, blue discoloration of right side flank is present. Semi- solid material which was present over body seems to be salt which was collected for FSL. Reports of FSL suggested the material was salt. There was cut throat injury over neck and open head injury, which was filled with semisolid powder and dust. Cause of death was absolutely clear cut hemorrhagic shock as a result of ante mortem cut throat injury with ante mortem head injury. Time since death was little bit confusing as face showed decomposition signs but other body parts decomposition sign was not resemble. As forensic pathologist I suggest that body was kept in salt for fast decomposition and it is also suggested that crime scene was not the same as no specific powder was found at the crime scene where body was found. It was case of try to concealment of crime by putting body in salt for fast decomposition. This case was also dealt by author.

Case no 4:

A 50 years male brought to the emergency department with history of chest pain on early morning of the same day. He declared dead after providing the emergency treatment by cardiac experts. ECG shows some cardiac changes. Emergency doctor denied to give cause of death as ECG changes was not confirmed by the cardiac experts. Emergency doctor handed over the body to police. Post mortem of deceased conducted after some hours. Same history was given by the relatives and punchnama suggest the same that cause of death was sudden cardiac arrest. During postmortem a trame line bruise was present over the right scapular region which was fresh. Swelling was also seen over occipital region. During skull cavity examination, sub-scalp hematoma was seen at occipital region. There was subdural, subarachnoid hemorrhage present. No fracture of skull

vault present. Brain shows intraventicular hemorrhage. Organs for histopathology forwarded. No specific findings were suggested by the histopathology. Police investigate the case strictly, as the Postmortem reveals the head injury. Relatives accept history of assault and complain of head injury. It was a case of concealment of crime by hiding the history. This case was also dealt by author.

Case no 5:-

A young female was taken to private hospital with history of fall from height. She declared dead on arrival at private hospital. Doctors of private hospital called to police handed over the body to relatives as she was brought dead. Body was taken to mortuary where police send the requisition for postmortem. External examination revealed that body was wrapped in hospital bed sheet, and she wear only blouse. Lower part was naked. When I asked for same relatives said that she was missing since morning and body was found at around 2 pm today itself. When they took the body she wear ghaghra, but it was stained with mud so they removed and taken to private hospital. During postmortem it was found that body was clean not wet oozing of blood from both ears, deceased had bruise at inner thigh and at peri-vaginal region and graze abrasions at both forearms. She had lacerated wound at mid parietal region. Tear of spleen present, fracture of pelvis bone with fracture of right femur bone and about 800 ml blood and blood clots present in lower abdominal cavity. There was subdural and subarachnoid hemorrhage. Vaginal examination revealed some smell with oozing of sticky fluid. Perivaginal, intravaginal swab, slides and nails, nail scraping samples collected. Investigating officer is advised to collect the ghaghra from first treating doctor or from crime scene for FSL examination. Cause of death was hemorrhagic shock as result of polytrauma. It was shocking that how an injury over mid parietal region occurred due to self fall or fall from height. Same was revealed to police but police ignored any foul play. After two months police came with FSL reports. It was shocking that FSL reports mentioned the presence of semen in the vagina. Now police investigate the case very seriously which revealed that she was forced for sex, afterwards she was hit by blunt weapon on head. When she tried to run, she slipped down from the construction site. It was a case of concealment of crime by hiding the evidence of sexual assault by giving the suggestion of removing wet clothes from the deceased body. This case was dealt by author.

Table no : 1 (cause of death and motive)

Case no	Cause of death	Motive
1	Asphyxia as result of strangulation	Hid identification by hiding clothes
2	Asphyxia as result of strangulation	Hid identification by putting material on the face
3	Hemorrhagic shock as a result of antemortem cut throat injury	concealment of crime by putting body in salt for fast decomposition
4	Shock as result of antemortem head injury	concealment of crime by hiding the history
5	Shock as result of polytrauma	concealment of crime by hiding the evidence of sexual assault by giving the suggestion of removing wet clothes from the deceased body

Table no: 2 (case no 1 sample collected and their results)

Sr no	Sample collected	Results
1	Vaginal slide, swab	Inconclusive
2	Clothes and poly bag	Presence of semen
3	Aluminum wire	Cross matched with crime scene
4	Nails and foreign body	Inconclusive
5	Bone for DNA	Matched with son
6	Viscera for poisoning	No poison
7	Uterus for histopathology	Part of uterus

Table no :3 (Case no 2 sample collected and their results)

Sr no	Sample collected	Results
1	Remaining skin and muscles of face	Presence of hydrochloric like material
2	Viscera	No poison
3	Rope use for tying upper and lower limb	Inconclusive

Table no 4 (Case no 3 sample collected and their results)

Sr no	Sample collected	Results
1	Clothes	Presence of foreign material which is salt
2	Semisolid powder present over body	Salt
3	Foreign body from back of body	Not matched with first crime scene
4	Polybag	Presence of blood matched with victims
5	Blood	For cross match

Table no 5 (Case no 4 sample collected and their results)

Sr no	Sample collected	Results
1	Viscera	Negative for presence any common poison
2	Sample for histopathology (heart, piece of liver , spleen and kidney)	Normal histopathology

Table no 6 (Case no 5 sample collected and their results)

Sr no	Sample collected	Results
1	Clothes which was above	Nil
2	Vaginal slide and swab	Presence of semen which is inconclusive
3	Blood	For cross match with crime scene
4	Nail with foreign bodies	Nil
5	Ghaghra collected from relatives	Presence of semen but inconclusive

Discussion

There is important correlation between concealment of crime and meticulous autopsy. Detailed autopsy with corroborative evidences changes the line of investigation. Many times evidences collected from the dead body helps the investigation authority to reveal the crime and its causes. Homicidal incidences take places due to several reasons and by several methods. Concealment of crime is situation which used to arise in front of accused to hide his deed. The above discussed case focuses on the method of murder but also focuses on the manner of concealment of crime. In above mentioned all cases accused tried to hide the crime, but as meticulous autopsy, crime scene visit, corroborative evidences change the whole scenario. As it is now clear in all cases motive was predominantly escape from conviction. Things are always not same as they seen in first sight. Many cases of homicide go undetermined because of lack of proper investigation and incompatible autopsy surgeon. Real cause of death established after proper history, crime scene visit and detailed autopsy. A complete autopsy is necessary to substantiate the truth of the evidence of eye witness. A poor autopsy is leads miscarriage of justice. We expose concealment of homicide by proper history, meticulous autopsy and trace evidences.

In case no 1 female was taken by accused for aim of sexual gratification but accused was serial killer. Accused murdered the victim and he tried to hid the crime by conceal her identity by putting her clothes inside her abdominal cavity. It was rare case in which aim was common but hiding of identification was uncommon even if till date such type of crime not registered or discussed in any article, book of police and forensic. Meticulous autopsy specifically neck dissection was done step by step. It was very important to differentiate out the antemortem and postmortem injuries. As a result of perfect postmortem and collected corroborative evidences case was solved, psychopath killer was caught and satisfaction achieved by us that accused got death penalty by district as well as from state high court.

All cases of death should be recorded an unnatural until proved otherwise. In case no 2 deceased was young male but concealment as done by hiding the identification of victim. Facial skin was avulsed out due to use of hydrochloric acid and postmortem artifacts occur due to creature ate the remaining skin. Detailed autopsy and evidences reveal the case; we are waiting for the judgment.

In case no 3 male body was tried to conceal by using salt, accused follow some serial on television and they tried their best, but autopsy with details collection of evidences removed the chances of escape of accused. It was rare case in which salt was used. Even if crime scene was also different as which was confused, investigation completed till time we are waiting for the final outcome.

All death should be considered as unnatural if a case is brought dead to emergency and the same body should not be handed over to relatives, In case no 4 male brought to the emergency department with history of chest pain on early morning of the same day. In this case deceased was taken by relatives with history of chest pain and due to some query postmortem was conducted. Injury over back and internal injury to head changed the whole investigation of police. Even if relatives changed the history afterwards, defined autopsy and detailed

examination of head with histopathological reports cause of death results final outcome.

In short this study give the conclusion that meticulous autopsy by competent autopsy surgeon may explode concealment of homicide as it changes the pathway of investigations. The criminal are adopting various scientific means to conceal their crimes and destroying evidences to escape from conviction. Sometimes it becomes a challenge for the autopsy surgeon to distinguish both suicide and homicide because in many situations, there is specific finding supporting the manner of death, which mostly relies on circumstantial evidences

Conclusion

Life is never ever static it changes day by day, after every dark night we found the new sun with new brightness. Every day we used to observe newspaper fulfill with murder cases and murder mysteries. The above discussed cases were being the part of local newspaper, some cases made top lines of newspaper. Verdicts also sends message amongst society that crime never pays. Moreover by considering the televisions, shows criminal tried to hide their deed. Cinema television shows provide the false mind a new thought how crime can conceal. As a forensic expert it is the duty that he has to do detailed postmortem and provide the corroborative evidences to investigating authority. They can also give suggestion which may help to open out the crime. As a team of forensic expert and investigation authority may open the path of stopping the crime. It is the duties of government to trained and update the doctors and investigating agencies regarding new developments.

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A Survey to Assess Oral Hygiene Awareness, Difficulties Faced and Satisfaction Level During and after Orthodontic Treatment

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Abstract

Introduction: The foremost goal of orthodontic treatment is to improve dental occlusion and alignment of teeth, which eventually results in a healthy functioning of dentition and facial aesthetics. The aim of the present study was to assess the oral hygiene awareness, difficulties faced and satisfaction level among a group of students undergoing orthodontic therapy during and after treatment.

Materials and Methods: A sample of 200 students from Melaka Manipal Medical College, who received orthodontic treatment were included in the study. A cross - sectional study in the form of self-administered questionnaire was carried out. The questions were based mainly to assess the difficulties faced, awareness with regard to oral hygiene and satisfaction level of the participants during and after orthodontic treatment.

Results: The difficulties faced by orthodontics patients were in the range of 65 to 85%, which were trouble sleeping, food lodgement and difficulty in maintaining oral hygiene. Study participants were not aware of various cleaning modes though more than 50% used floss. In the post treatment phase, aesthetic appearance was found to be better.

Conclusion: The present study showed that the participants who were undergoing orthodontic treatment did not have sufficient knowledge about oral hygiene practices. The participants were satisfied with the aesthetic appearance post treatment.

Key words- *Aesthetics, Interdental brush, Oral Hygiene awareness, Orthodontic treatment, Satisfaction levels.*

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Introduction

Orthodontics is a well-proclaimed branch of dentistry. It is majorly related to diagnosis, prevention and correction of malpositioned teeth and the dentofacial complex .[1] An individuals' requirement for a healthy and functional bite with pleasing esthetics necessitates an orthodontic treatment. Although, recent era has seen

an upsurge in use of clear aligners but its superiority over conventional methods is still controversial.[2]

In addition to being grueling for the orthodontist, the treatment is equally challenging for the patient as well, especially in terms of maintenance of oral hygiene. The conventional wires and brackets make even the simpler everyday practices of maintaining oral hygiene difficult. Although, special interdental and proximal brushes are available for cleaning food entrapped between the brackets and the wires, the awareness to use the same is not universally present. Thus, leading to enormous amount of food lodgment in these patients undergoing orthodontic treatment. Further, this becomes the root cause for accumulation of plaque, calculus, resulting in gingival and periodontal problems. The long duration of an orthodontic treatment also can cause demineralization of the teeth eventually making it prone to decay. The other problems associated are the malodor and essentially an unsightly appearance.[3] Malocclusion is not only harmful for the oral cavity but also affects the psychological well-being of the patient related to social acceptance. An orthodontic treatment is usually a poignant part of adolescence and because of all the above stated issues; it becomes a scarring part of the growing up stage.

According to few studies, it is seen that majority of the population needs orthodontic treatment but only a few research projects have looked at patient satisfaction with treatment outcome and factors contributing to satisfaction.[3,4] Since, the patient satisfaction after orthodontic treatment is influenced by a number of factors, such as sex, age, duration of treatment, compliance, and dentofacial improvement, so it is imperative that a certain correlation must be worked upon in between treatment expectation, motivation, and satisfaction with treatment outcome.[4]

The main goal of orthodontic treatment is to improve dental occlusion and alignment of teeth, which ultimately results in a good functioning of dentition. Orthodontic treatment helps the patients to improve dental and facial aesthetics; above all, it also builds up self-esteem. An oral hygiene awareness program must, therefore, be a part of the orthodontic treatment in order to counteract adverse factors. This study was carried out to assess oral hygiene awareness among patients wearing fixed orthodontic appliances. The aim of the present study was to identify and assess the difficulties faced, Oral Hygiene awareness and satisfaction level during or after orthodontics treatment.

Materials and Method

A cross-sectional study was conducted after obtaining ethical approval from the Institutional Ethical Committee, Kasturba Medical College, Manipal. The participants were explained in detail regarding the importance of the study and written informed consent was obtained from all the students who were willing to participate in the study. A total of 203 students from Melaka Manipal Medical College, Manipal, participated in this study.

A self - administered multiple-choice questionnaire was used, the first part of questionnaire enquired about the participants social demographic background and the rest was regarding the issues elicited. The questions given were based on difficulties faced, Oral Hygiene Awareness and Satisfaction during or after orthodontic treatment. In self-assessment of oral hygiene, question regarding cleaning modes i.e use of interdental brushes, floss, and frequency of brushing were included. In assessing, the difficulties faced during the treatment such as pain, ulcers, restrictions in eating certain food and debonding of bracket were assessed. In assessing the level of satisfaction questions regarding esthetic and functional outcomes were asked.

Each option was given score from 1 to 4, with 4 being strongly agree and 1 being strongly disagree. Certain close-ended questions with Yes / No option were included. The scores were summed and analyzed to assess the knowledge of the students regarding oral hygiene and the difficulties faced as well as the level of satisfaction achieved during and after orthodontic treatment. The social demographic data obtained was also analyzed to determine its relation with the level of knowledge of oral hygiene and difficulties faced, satisfaction during and after orthodontic treatment. The data obtained was tabulated and were statistically analyzed using SPSS-15.0 with descriptive statistics and the Chi-square test.

Results

A total of 203 students participated in the study, with 69.46% of female subjects and 30.54% of male subjects (Table1). On analyzing the self-reported knowledge on oral hygiene during orthodontic treatment, 81% of the participants were aware and 18.23% were not aware (Table 2). Table 3 and table 4 shows the mean and standard deviation derived from the questionnaire concerning the outcome of the Orthodontic treatment in terms of Aesthetics and functionality respectively. Table 5 shows the correlation between the treatment outcome (Aesthetics) and the Problems faced before and during orthodontic treatment. On assessing the correlation between the treatment outcome (Functionality) and the problems faced before and during orthodontic treatment, it was seen that there were few problems that showed significant positive correlation with the outcome, however few problems showed significant negative correlation. (Table 6)

Table 1: Frequency distribution of participants

Subjects	N (%)
Male	62 (30.54)
Female	141 (69.46)

Table 2: Frequency distribution of participants with knowledge on Oral Hygiene during orthodontic treatment

Oral Hygiene during orthodontic treatment	N (%)
Yes	37 (18.23)
No	166 (81.77)

Table 3: Questionnaire concerning the outcome of the Orthodontic treatment in terms of Aesthetics

Aesthetics (Outcome)							
1. Does your teeth look more aligned?		2. Does it improve the appearance of your facial features?		3. Do you feel more confident than before?		4. Are you satisfied with the orthodontic treatment?	
Mean	SD	Mean	SD	Mean	SD	Mean	SD
3.36	0.77	3.39	0.75	2.64	0.96	3.17	0.79

Table 4: Questionnaire concerning the outcome of the Orthodontic treatment in terms of Functionality

Functionality (Outcome)													
1. Was it easier to chew food after undergoing orthodontic treatment?		2. Did it help to improve your occlusion (closing of mouth)?		3. Has it helped you speak clearer?		4. Did your breathing improve after undergoing orthodontic treatment?		5. Are you satisfied with your treatment or were you expecting something more than what you have received?		6. Do you think the money you have spent on orthodontic treatment was worth it?		7. Do you have any regrets after undergoing orthodontic treatment?	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2.90	0.83	3.01	0.81	2.82	0.90	2.57	0.92	2.81	0.87	3.02	0.80	2.06	0.95

Table 5: Correlation between the treatment outcome (Aesthetics) and the Problems faced before and during orthodontic treatment

	Aesthetics (Outcome)							
	1. Does your teeth look more aligned?		**2. Does it improve the appearance of your facial features?**		**3. Do you feel more confident than before?**		**4. Are you satisfied with the orthodontic treatment?**	
Difficulties during orthodontic treatment (Experience)	r	P-value	r	P-value	r	p- value	r	p-value
1. Do you have trouble falling asleep or experience pain in the middle of the night caused by the treatment?	0.106	0.133	0.032	0.649	0.106	0.134	-0.002	0.971
2. Do you often find food particles lodged on your teeth & was it difficult to remove it?	-0.0814	0.248	0.185	0.008*	-0.043	0.543	0.142	0.044*
3. Did you develop ulcers during the orthodontic treatment?	0.0265	0.708	0.155	0.027*	0.089	0.205	0.069	0.325
4. Were you able to manage the restrictions on eating certain foods? (nuts/popcorn/chewing gum)	0.0101	0.886	0.151	0.031	0.054	0.443	-0.035	0.619
5. Did you experience any malocclusions (cannot clench your teeth properly) after each appointment?	-0.046	0.508	-0.008	0.9	-0.035	0.618	0.091	0.194
6. Did any of your brackets come out during your orthodontic treatment?	0.102	0.146	0.095	0.174	-0.015	0.831	0.027	0.703
7. Did you find it difficult to maintain oral hygiene throughout?	0.030	0.665	0.023	0.74	-0.051	0.47	-0.065	0.353
8. Do you have trouble falling asleep or experience pain in the middle of the night caused by the treatment?	-0.011	0.871	0.034	0.629	0.067	0.336	-0.013	0.85

Table 6: Frequency distribution of participants with knowledge on Oral Hygiene during orthodontic treatment

	Functionality (Outcome)													
	Was it easier to chew food after undergoing orthodontic treatment?		2. Did it help to improve your occlusion (closing of mouth)?		Has it helped you speak clearer?		Did your breathing improve after undergoing orthodontic treatment?		Are you satisfied with your treatment or were you expecting something more than what you have received?		Do you think the money you have spent on orthodontic treatment was worth it?		Do you have any regrets after undergoing orthodontic treatment?	
Problems before and during orthodontic treatment (Experience)	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
1. Do you have trouble falling asleep or experience pain in the middle of the night caused by the treatment?	0.147	0.036*	0.056	0.422	0.167	0.017*	0.078	0.267	0.063	0.367	-0.090	0.198	0.015	0.832
Do you often find food particles lodged on your teeth & was it difficult to remove it?	0.005	0.933	0.17	0.015*	-0.031	0.659	-0.009	0.898	-0.0009	0.99	0.066	0.344	-0.189	0.006*
Did you develop ulcers during the orthodontic treatment?	0.083	0.238	0.12	0.087	0.090	0.199	0.061	0.387	0.165	0.018*	0.074	0.292	0.004	0.944
Were you able to manage the restrictions on eating certain foods? (nuts/ popcorn/chewing gum)	0.082	0.24	0.065	0.356	0.164	0.019*	-0.026	0.711	0.141	0.044*	0.008	0.907	0.032	0.649
Did you experience any malocclusions (cannot clench your teeth properly) after each appointment?	-0.072	0.302	0.025	0.723	-0.099	0.158	0.041	0.558	-0.067	0.338	-0.064	0.36	-0.137	0.051
Did any of your brackets come out during your orthodontic treatment?	0.157	0.025*	0.119	0.091	0.175	0.012*	0.090	0.198	0.002	0.971	-0.003	0.963	0.099	0.158
Did you find it difficult to maintain oral hygiene throughout?	0.068	0.333	-0.016	0.82	0.022	0.748	0.018	0.793	0.109	0.123	-0.114	0.104	0.144	0.039*
Do you have trouble falling asleep or experience pain in the middle of the night caused by the treatment?	0.085	0.224	0.002	0.972	0.081	0.248	0.035	0.611	0.090	0.197	-0.050	0.474	0.104	0.141

Discussion

Patients undergoing orthodontic treatment usually face difficulty in maintaining oral hygiene. Previous studies have shown a rapid fall in oral hygiene maintenance after the initial bonding as the appliance favours plaque build-up and also hinders the oral hygiene practices like tooth brushing and flossing.[5,6] The satisfaction level after treatment is mainly related to patient expectations prior treatment. This study assessed the difficulties faced, Oral Hygiene awareness and satisfaction level during or after orthodontic treatment.

Maintenance of oral hygiene is important during orthodontic treatment. This study assessed the oral hygiene awareness among participants during and after orthodontic treatment. Only 18.23% of the students had sufficient awareness in maintaining oral hygiene during treatment. 81 % of the students had no sufficient awareness about oral hygiene maintenance during treatment. Question regarding different cleaning modes and frequency of brushing after every meal and use of dental floss and interdental toothbrushes were asked to check the knowledge of the participants regarding oral hygiene. The results of our study are similar to study done by Shah et al, where 62% of patients did not follow the oral hygiene instructions, and despite being given instructions; most of them hesitated in practicing them consistently.[3]

Previous literature states that patient satisfaction with orthodontic treatment had no outcome. This can be explained by the difficulties faced during treatment and expectations of patients receiving orthodontic treatment.[4] However, no major correlations were found between treatment-outcome satisfaction and patient expectations of orthodontic treatment in some studies. The outcome of the treatment in terms of aesthetics and functionality were compared with the satisfaction levels in the present study. On comparison of the difficulties faced during the orthodontic treatment and aesthetic outcome, many problems showed a positive correlation with the treatment outcome. Many of the patients were satisfied with the aesthetic outcome of the treatment and with the facial appearance post treatment. On comparison between the treatment outcome in terms of functionality and the problems faced before and during orthodontic treatment, it was seen that there were few problems that showed significant positive correlation with the outcome, however few problems showed significant negative correlation. However, it is interesting that the participants reported higher correlations with functional aspects of their treatment outcomes compared with aesthetic aspects. The results of other studies that showed that aesthetic considerations were the primary reason for orthodontic treatment.[7,8] It can be considered that functional and aesthetic factors influence the participants to undergo orthodontic treatment. Previous research showed that the facial aesthetics is crucial in the development of a person's self-esteem and self-image.[9,10]

These findings offer an important strategy to improve patients' orthodontic treatment satisfaction level. Given that possible pre-treatment self-considerations correlate with post treatment satisfaction, a first step would be to assess patients' expectations during the screening appointment. The limitations of the study should be noted. The findings reflect only the satisfaction levels of students during and after orthodontic treatment in Melaka Manipal Medical College. Therefore, the results cannot be generalized to orthodontic patients in general. The questionnaires had only a few questions to assess the satisfaction level in term on aesthetics and function, which means that issues were dealt with only superficially.

Conclusion

The participants did not have sufficient knowledge about various cleaning modes, but they showed a positive attitude to learn about the same. The participants were satisfied with the aesthetic appearance post treatment.

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Conflict of Interest - Nil

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Telemedicine : Guidelines in India and its Importance in COVID Pandemic

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Abstract

Social Distancing became the new norm in the COVID 19 pandemic. While at one side the world-wide medical fraternity is finding ways to combat the viral infection, there were patients with existing illness who needed follow-up care. The role of Telemedicine became important under such circumstances.

A study conducted by Earnst & Young (EY) in association with Indian Pharmaceutical Alliance (IPA) [Mar'20], estimated the Telemedicine market in India to reach $5.5 billion By 2025[3]. These telemedicine companies offer services like teleconsultation, e-prescription, e-pharmacy and online ordering & scheduling of laboratory investigations.

Just after the start of statutory lockdown in India in view of COVID pandemic, on 25 March'2020, the BOARD OF GOVERNORS in supersession of the Medical Council of India notified the "Telemedicine Practice Guidelines" to enable R.M.P.s to provide healthcare using Telemedicine [7]. [This constitutes Appendix 5 of the Indian Medical Council (Professional Conduct, Etiquette and Ethics Regulation, 2002]

Although the practice of Telemedicine was going on in some form or the other in the last two decades, but there was a question with respect to its legitimacy and acceptance by the medical fraternity at large. The notification of these guidelines has cleared the picture to a large extent and lays the foundation for this new tool of medicine practice.

This article is a peak into the current guidelines of Telemedicine in India and its usefulness in the COVID pandemic.

Keywords *: telemedicine, telemedicine overview, telemedicine india, telemedicine guidelines, telemedicine covid, telehealth*

Introduction

Social Distancing became the new norm in the COVID 19 pandemic. While at one side the world-wide medical fraternity is finding ways to combat the viral infection, there were patients with existing illness who needed follow-up care. It is a challenging task to screen patients before attending them and for the patients, it is a difficult decision whether to visit a health facility or not for their ailment. The role of Telemedicine became important under such circumstances.

Telemedicine in India was formally launched on March 30th, 2000, when Bill Clinton— the then President of the United states— commissioned the first telemedicine unit in the village of Aragonda in Southern India, about 200 km from the tertiary care centre in Chennai while he was witnessing a live cardiac teleconsultation [1].

Professor K. Ganapathy, an eminent neurosurgeon, is a member of the Indian National Task Force on Telemedicine and a founding member of the

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Telemedicine Society of India [2].

A study conducted by Earnst & Young (EY) in association with Indian Pharmaceutical Alliance (IPA) [Mar'20], estimated the Telemedicine market in India to reach $5.5 billion By 2025[3].

The following is a list of top telemedicine companies in India based on total number of visitors: - [4]

Company Name	Total Visitors (in millions)
Practo	47.48
1 mg	26.18
Lybrate	25.18
Portea Medica	7.3
Medlife	2.54

These telemedicine companies offer services like teleconsultation, e-prescription, e-pharmacy and online ordering & scheduling of laboratory investigations.

In the months from April to June'2020, around 5 crore Indians accessed healthcare online; 80% of the users accessed it for the first time; 44% consultations were from non-metro cities and in-person doctor visits decreased by 67% [5].

Telemedicine Guidelines in India refer to the case of Deepa Sanjeev Pawaskar vs. The State of Maharashtra Criminal Anticipatory Bail Application No. 513 of 2018 dated July 25, 2018, in which the judgment passed by the Bombay High Court created uncertainty about the place and legitimacy of telemedicine [6]. In this case, two doctors, a husband and wife ("Applicants") were held liable for criminal negligence resulting in the death of a woman, one Dnyanada.

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Although the practice of Telemedicine was going on in some form or the other in the last two decades, but there was a question with respect to its legitimacy and acceptance by the medical fraternity at large. The notification of these guidelines has cleared the picture to a large extent and lays the foundation for this new tool of medicine practice.

As per the guidelines Telemedicine is defined as, *"The delivery of health care services, where distance is a critical factor, by all health care professionals using information and communication technologies for the exchange of valid information for diagnosis, treatment and prevention of disease and injuries, research and evaluation, and for the continuing education of health care providers, all in the interests of advancing the health of individuals and their communities."*

The various advantages of Telemedicine are[7]:-

1. Fast and timely access

2. Access to service which may not be otherwise available

3. Saving of cost and effort for rural patients as they need not travel long distances

4. Useful when there is no need of physical check-up. Eg. Continuous monitoring, regular & routine follow-up.

5. Higher likelihood of maintenance of records and documentation à Increased legal protection for both parties

6. Patient and healthcare worker safety à no spread of contagious infections

Purpose of the Guidelines [7]

The guidelines will assist the medical practitioner in pursuing a sound course of action to provide effective and safe medical care founded on current information, available resources, and patient needs to ensure patient and provider safety

They will help to realize the full potential of these advancements in technology for healthcare delivery

It provides norms and protocols relating to :

i. Physician-patient relationship

ii. Issues of liability and negligence

iii. Evaluation, management and treatment

iv. Informed consent

v. Continuity of care

vi. Referrals for emergency services

vii. Medical records à Privacy and security of patient records and exchange of information

viii. Prescribing

ix. Reimbursement

x. Health education and counselling

The guidelines specifically explicitly exclude the following [7]: -

1. Specifications for hardware or software, infrastructure building and maintenance

2. Data management systems involved; standards and interoperability

3. User of digital technology to conduct surgical or invasive procedures remotely

4. Other aspects of telehealth such as research and evaluation and continuing education of healthcare workers

5. Does not provide for consultation outside jurisdiction of India

The Telemedicine Applications can be divided into four basic types [7]: -

1. Mode of Communication – Audio, Video, Text

2. Timing of Information transmitted

a. Real-time audio/video/text

b. Asynchronous exchange of relevant information

3. Purpose of Consultation

a. First consult

b. Follow-up consult

4. Interaction between the individuals involved

a. Patient to RMP

b. Caregiver to RMP

c. RMP to RMP

d. Health-worker to RMP

e. Emergency Situations

The guidelines accept all forms of digital communication – audio, video and text and various online platforms like whatsapp, email, skype, sms etc.

The following seven elements have to be considered in every telemedicine consultation [7]: -

1. Context

2. Identification of the R.M.P. and Patient

3. Mode of Communication

4. Consent

5. Types of Consultation – First/ Follow-up

6. Patient Evaluation – technology-based tools, reports, video examination

7. Patient Management – Health education, Counselling and Prescribing Medicines

Various Drug Lists and Prohibited List as per the Guidelines [7]

List O: It will comprise those medicines which are safe to be prescribed through any mode of tele-consultation. This list included commonly used 'over-the-counter' medications such as Paracetamol, Oral Rehydration Solution (ORS) packets, Antacids etc. This list also includes medicines that may be deemed necessary during emergencies and would be notified from time to time.

List A: These medications are those which can be prescribed during the first consult which is a video consultation and are being re-prescribed for re-fill, in case of follow-up. This list includes usually prescribed medications for which diagnosis is possible only by video consultation such as antifungal medications for Tinea Cruris, Ciprofloxacin eye drops for Conjunctivitis

etc. and Re-fill medications for chronic diseases such as Diabetes, Hypertension, Asthma etc.

List B: Is a list of medication which RMP can prescribe in a patient who is undergoing follow-up consultation in addition to those which have been prescribed during in-person consult for the same medical condition. This list includes 'add-on' medications which are used to optimize an existing condition. For instance, if the patient is already on Atenolol for hypertension and the blood pressure is not controlled, an ACE inhibitor such as Enalapril

Prohibited List: An RMP providing consultation via telemedicine cannot prescribe medicines in this list. These medicines have a high potential of abuse and could harm the patient or the society at large if used improperly. E.g. Drugs listed in Schedule X of Drug and Cosmetic Act and Rules or any Narcotic and Psychotropic substance listed in the NDPS Act, 1985. Anti-cancer drugs are also prohibited.

A Standard format for prescription is also suggested in the guidelines which is quite in line with the format which is used in offline practice.

Medical Ethics, Data Privacy and Confidentiality [7]

The R.M.P should maintain the Digital Trail/ Documentation of Consultation.

R.M.P.s may charge an appropriate fee for the Telemedicine consultation and should also provide a receipt/ invoice for the fee charged.

Examples of Professional Misconduct [7]

1. R.M.P.s insisting a telemedicine consultation when the patient is willing to travel to a facility and/or requests an in-person consultation

2. R.M.P.s misusing patient images and data, especially private and sensitive in nature (e.g. RMP uploads an explicit picture of patient on social media)

3. Prescribing medicines from specific restricted list

4. R.M.P.s are not permitted to solicit patients for telemedicine through any advertisements or inducements

Penalties: As per I.M.C. Act, Ethics and Other Prevailing Laws

Guidelines for Technology Platforms enabling Telemedicine [7]

1. Technology platforms (mobile apps, websites etc.) providing telemedicine services to consumers are required to ensure that the consumers are consulting with qualified RMPs. Technology Platforms are required to conduct their due diligence before listing any RMP on its online portal.

2. Technology platforms based on artificial intelligence or machine learning are not allowed to counsel patients or prescribe any medicines to a patient. Only an RMP is entitled to counsel a patient or prescribe medicines to a patient.

3. Technology Platforms must ensure that there is a proper mechanism in place to address any queries or grievances that the end-customer may have.

4. In case any specific technology platform is found to be in violation of the T- Guidelines, the Board of Governors of the MCI may blacklist such technology platform, and no RMP may then use such platform to practice telemedicine.

Conclusion

How Telemedicine is adapted in day-to-day practice, only time will tell. But it is showing promising signs for now. Like any other technology, the technology used for telemedicine services can be abused. It has some risks, drawbacks and limitations, which can be mitigated through appropriate training, enforcement of standards, protocols and guidelines. These guidelines should be used in conjunction with other national clinical standards, protocols, policies and procedures.

Conflict of Interest : None

We certify that the manuscript has been read and approved by both of us and we agree with the ranking of the authorship.

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Ethical Clearance : The content of this article are personal views and comments of the authors. There are

no ethical issues involved.

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Impact of Covid 19 Pandemic on Dentists: Psychological Evaluation using DASS 21

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Abstract

Background: The outbreak of coronavirus disease 2019 (COVID-19) in the area of Wuhan, China, has evolved rapidly into a public health crisis. Despite having high standards of knowledge and practices, dental practitioners around the globe are in a state of anxiety or fear. Being on the list of high-risk professions, dentists are very much expected to develop stress due to the current pandemic situation indicating a need to evaluate the effect of COVID 19 on the psychological status of Indian dentists.

Methodology: The data was collected through well-structured Google form from 500 dentists working in Delhi NCR. The entire form had two components. The first part of the form collected the information regarding the demographic profile of the dentists whereas the second part of questionnaire consisted of psychological evaluation using well established psychological scale DASS 21.

Result: The results showed that 8% of Indian dentist showed signs of severe depression and 17% had moderate signs. 9% showed moderate and 13% showed mild anxiety. 8% showed severe stress and 24% had moderate signs of stress. Significant correlation was found between depression and anxiety with stress.

Conclusion: Dentists are undergoing stress which in turn can have adverse effects on their physical and mental well-being in a long run. There is a need to evolve with the current COVID 19 pandemic and brainstorming the ways to encourage the safer practice and decrease the levels of depression, anxiety and stress among dentist.

***Keywords**: COVID19, Stress, Indian dentists, DASS 21.*

Introduction

The coronavirus disease 2019 (COVID-19) whose onset is reported from Wuhan province of China, has spread rampantly to almost every part of the world and has evolved very swiftly into a public health catastrophe.[1] The genome sequence for this virus has a close similitude with other Beta-coronaviruses such as SARS-CoV and MERS-CoV. The Coronavirus Study Group of the International Committee on Taxonomy of Viruses has scientifically named it SARS-CoV-2, though commonly it is referred to as the COVID-19 virus.[2]

This virus belongs to a clan of single-stranded RNA viruses group called Coronaviridae , known to be transferred from animals to humans beings and include Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), in 2002, and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), reported in 2012[3,4] There is strong affirmation about the zoonotic nature of this novel coronavirus being similar to coronavirus species seen in bats and also pangolins. On January 30, 2020, the World Health Organization

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(WHO) declared the widespread spread of COVID-19 a global pandemic.[5] According to the WHO situation report (June 5, 2020) update on COVID-19, there have been more than 6,700,000 reported cases and 387000 deaths worldwide and 225,000 cases with 6800 deaths in India and this number continues to increase at a rapid rate.

Till date there is no specific antiviral drug against this virus and though large scale efforts are on to develop a vaccine against it, still that seems to be at least eight to ten months away. Currently the approach against this rapidly spreading virus is to minimize cross infection and prevent community spread. The approach is to wear masks, maintain social distancing, and practice hand hygiene. It is equally important to do rapid testing to diagnose those infected and isolate them to minimize spread and flatten the epidemic curve. The treatment has mainly been supportive though use of hydrochloroquinone and remdesivir drugs has also been suggested but with limited success.

Dental clinic due to their work environment are particularly at a higher risk of infection as they involve face to face communication and direct contact to oral mucosa.[6] Also the procedures involve the use of three way syringes, ultrasonic scalers and air motor all well capable of generating aerosols, a spray of water and air particles that contains saliva, blood and microorganisms. This spatter can easily transit on to the patient , dentist , assistant and also the operatory surface and the floor causing spread of infection.[7] This is particularly more dangerous in patients that are either asymptomatic or in incubation period, as they can inadvertently be potential COVID 19 spreaders.

Considering the rapid spread of pandemic and it closeness to the oral route of transmission the dental clinics were mostly closed during the national lockdown. This long duration of not working and staying at home had an overall effect on the daily routine of every individual and dentists also were no different. The working was required only for emergency procedures and with extensive guidelines issued by the World Health Organization, Dental Council of India and the Indian Dental Association. These guidelines also suggested the need of procuring few new equipment and certain pre and post procedures for treating any patient.

Dentists being amongst the professions with higher risks of getting infected are obviously under tremendous stress due to this current pandemic.[8] Through dental clinics globally maintain a strict infection control and sterilization protocols still there is bound to be fear and anxiety amongst them while working on a patient during this COVID-19 pandemic.[9] There is a tremendous pressure from the patient's perspectives and dentist stands in an indecisive state of mind regarding their current means of practice.[10] This makes them prone to stress which in turn is known to be linked to the development of other disorders and can potentially affect a person's ability at work and even in life.

COVID-19 (Corona Virus Disease 2019) has resulted in a significant number of psychological effects.[11] In a study done in China during the initial phase of the epidemic more than fifty percent of the subjects reported a moderate-to-severe psychological impact and one-third had moderate-to-severe anxiety.[12] Hence there is a current need to evaluate the effect of COVID 19 on the psychological status of Indian dentists. The aim of this study is to identify components associated with a low level psychological impact of COVID-19 on their mental health and to assist policy makers in formulating specific policies.

Methodology

The data has been collected through well-structured Google form and was sent to 500 dentists working in Delhi NCR. The form was mailed to them as it was the safest method during this pandemic. The consent was obtained from the recipients of the form for conducting this survey. The entire form has two components. The first part of the form collected the information regarding the demographic profile of the dentists like age, gender and place where they practiced. The type of practice and number of emergency procedures handled by them were also noted. A view was also taken on their awareness of WHO guidelines and the newer equipment which they seem necessary for any form of dental practice in near future. Their concern with COVID 19, government support and expectation to return to a regular practice were also noted.

The second part of questionnaire consisted of psychological evaluation using DASS 21 scale. DASS (Depression, Anxiety, and Stress Scale) is a survey

which include the 21 question adaptation of the DASS (Lovibond and Lovibond, 2004). The original 42-item DASS of Lovibond was reduced into a shorter variant having 21-items. Several studies have been done to check how reliable and valid it is; all have shown that the DASS-21 is a very reliable instrument to measure manifestation of depression, anxiety and stress in a group of adults both clinically and non-clinically. Each question measured the prevalence of indication of depression, anxiety, or stress over the prior week. Answers are registered on a well-established Likert scale. The relevant questions scores are summed up to calculate the prevalence of depression, anxiety and stress. DASS-21 being a shorter version of the DASS (42 items), each sub-scale is multiplied by a factor of two to reach to a final score according to the given severity rating index.

Results

The Google form was sent to around 500 dentists out of whom a response was received from 480 dentists. Out of them 280 were males and 180 were females. 20 of the respondents did not want to disclose their gender. The average age of the responding dentist was 41 years. A majority of the dentists, around 66.7% were in nuclear family while rest had a joint family. The majority of the dentist contacted (62.5%) were having a general practice, performing relatively all dental procedures and 37.5% were doing only a specialized practice. A majority of the dentist i.e. 440 of them did believe that the COVID 19 has affected their daily routine.

Regarding the number of emergency calls from patient per day 75% received less than 5 calls per day. The total number of emergency patients handled by them during the lockdown period, 79.2% had attended less than 10 patients. Among the type of cases reported for emergency treatment the highest percentage was for endodontic problems (83.3%) followed by swelling or trauma at 50 %. (Fig.1)

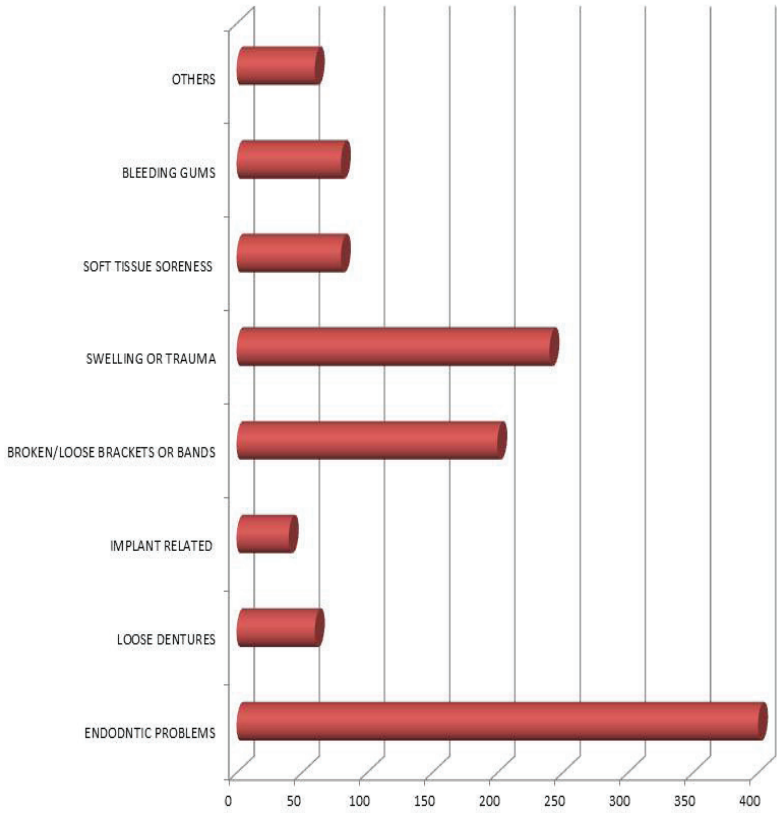

Figure 1: Dental problems leading to emergency calls to the dentist

Majority of the dentist around 87.5% was aware of the WHO guidelines for safety of healthcare workers and 58.3% have even attended an online course or a workshop during the pandemic period. The respondents did feel the need of PPE for themselves (91.7%) as well as for their assistant (87.5%). Regarding modification in the overall practice the respondents felt highest need for face shield and disinfectant machine, both at 75% (Fig. 2).

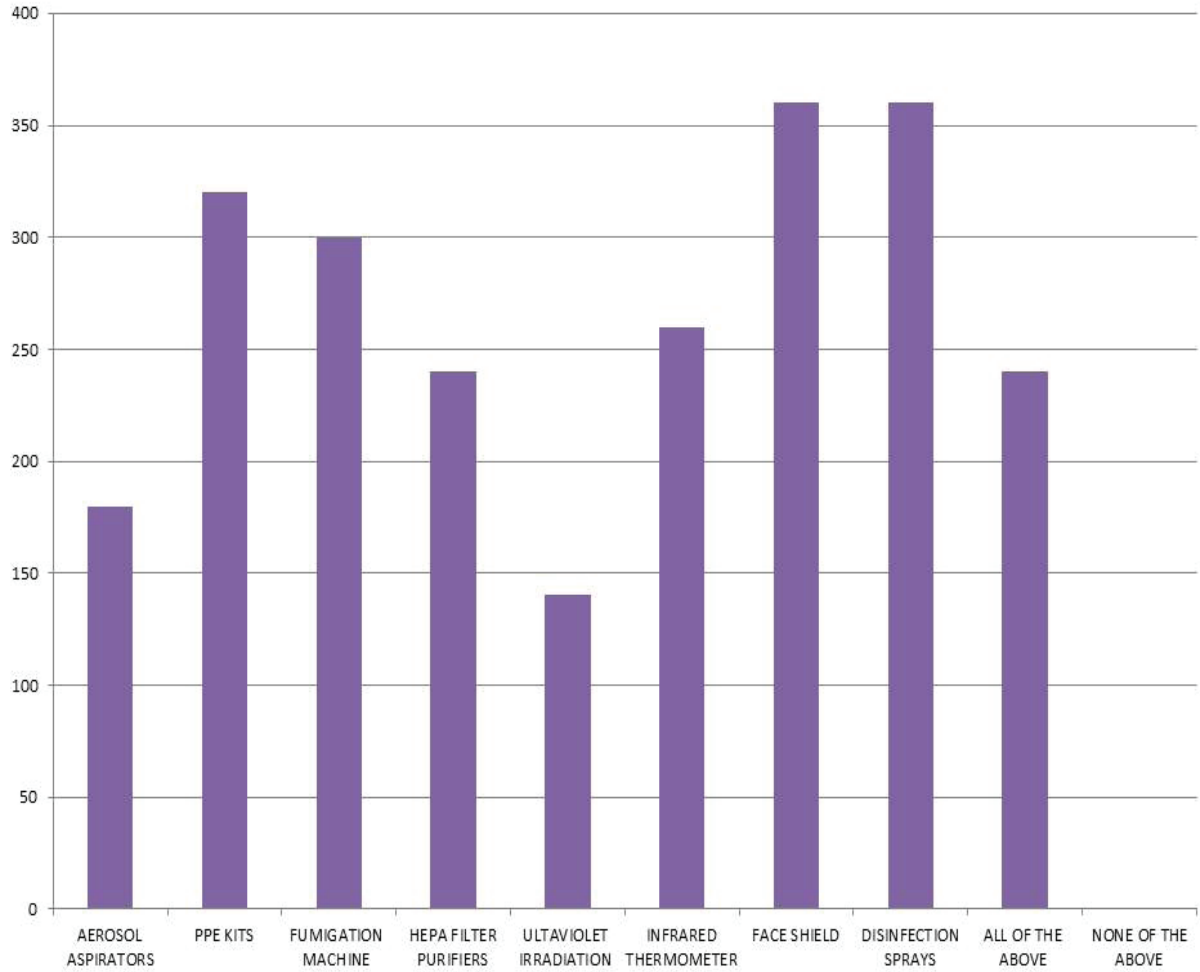

Figure 2: Equipment deemed necessary for dental practice with Covid 19

Only 54.2% of the respondents believed that they will be able to transfer the extra equipment's cost to the patient. Most dentists (87.5%) believed that with the decline in income of the people and fall of Global economy there is bound to see an overall fall in the generation of dental revenue.The DASS 21 scale ranks each participant on the basis of his or her scores of depression, anxiety, and stress levels, as either "normal," "mild," "moderate," "severe," or "extremely severe." Of those surveyed, 8% reported symptoms of severe levels of depression, 9% indicated moderate level of anxiety, and 8% had severe stress (Fig. 3).

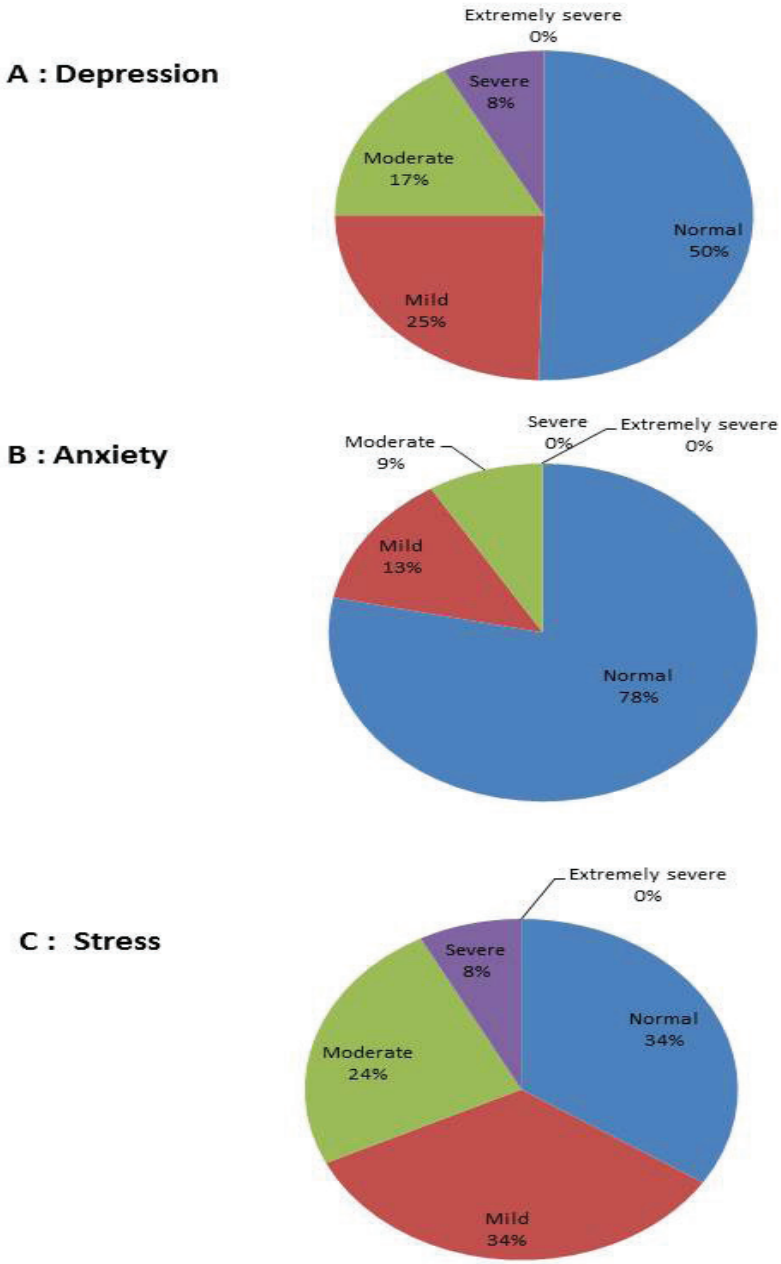

Figure 3 : The proportion of participants whose answers on the DASS 21 indicated a normal, mild, moderate, severe or extremely severe amount of Depression (A), Anxiety (B), and Stress (C)

On statistical analysis significant correlation was found between stress and depression (Table 1) as well as stress and anxiety (Table 2) in the dentist during the COVID 19 .

Table 1 : Correlation between Stress and Depression in Dentists during Covid 19

			Stress	Depression
Spearman's rho	Stress	Correlation Coefficient	1.000	.883**
		Sig. (2-tailed)	.	.000
		N	480	480
	Depression	Correlation Coefficient	.883**	1.000
		Sig. (2-tailed)	.000	.
		N	480	480
**. Correlation is significant at the 0.01 level (2-tailed).				

Table 2: Correlation between Stress and Anxiety in Dentists during Covid 19

			Stress	Anxiety
Spearman's rho	Stress	Correlation Coefficient	1.000	.883**
		Sig. (2-tailed)	.	.000
		N	480	480
	Anxiety	Correlation Coefficient	.883**	1.000
		Sig. (2-tailed)	.000	.
		N	480	480
**. Correlation is significant at the 0.01 level (2-tailed).				

Discussion

The dentist is subjected to a numerous type of physical and mental ailments that are either induced or increased by their work environment. Dentists always knew the dentistry is not an easy job. There is a tremendous physical and mental stress amongst them. When talking about physical disorders we have to take into account musculoskeletal problems, backache and neck and shoulder stiffness all very commonly seen in dentists. The prevalence of musculoskeletal complaints among dentists is high and well documented.[13, 14] 83 percent of dentist's perceived dentistry as being "very stressful", nearly 60 percent perceived dentistry as a profession which is comparatively more stressful than others.[15]

The present survey reported depression, anxiety and stress among dental professionals while working during this global pandemic. To ascertain this, a questionnaire of closed-ended questions was used to evaluate dentists for any changes in their practice or any fear amongst them due to COVID-19 outbreak. Psychological ramification such as fear and anxiety are quiet common in pandemics, especially when both the number of infected persons and overall mortality rates is so high. Researches on previous epidemics such as severe acute respiratory syndrome (SARS) demonstrated psychological agony in healthcare workers including the worry of either getting themselves infected or infecting their families.[16, 17]

The virus has an incubation period extending up to 14 days making it extremely difficult to locate the causative infection source and also due to absence of a vaccine or any confirmed treatment; there is every reason to be anxious from even the thought of getting infected. The droplets and aerosols are established leading routes of spread of this virus which by nature increases the chances of oral healthcare workers of getting infected and spread it to other persons they may come in contact.[18] The current guidelines on the COVID-19 outbreak recommends delaying all non-emergency dental treatment, and only patients with acute pain, swelling or trauma are advised to visit a dentist.[19] In the present study also there was clear evidence that most people understood the delay in their routine treatment, with more than 300 dentists observing that over 80 % of their patient were cooperative for delay in treatment. Around 75% of the dentists received less than 5 emergency calls per day while 20.8% received 5 to 10 calls per day suggesting that overall the volume of emergency dental need was less. In the current study the maximum emergency calls were related to endodontic problems 83.3%, followed by swelling or trauma at 50 % and loose band and brackets at 41.7%. Bleeding gums, swelling and issues related to implants were also reported for emergency but not in high numbers. This finding can help the governing body to concentrate on guidelines regarding these procedures only, making it more specific for the dentists.

Most of the dentists in the present study were aware of the mode of transmission of the COVID-19 which is very crucial for controlling spread of infection from the dental practice. It is essential during this pandemic phase to follow the guidelines and measures to reduce effectively the volume of aerosol generated from the clinics. Similarly, it was quite promising to see that 87.5% of dentists were aware of the WHO guidelines for cross-infection control like recording patients' travel history and body temperature. If dental healthcare workers comprehensively follow the recommendations suggested by these regulatory authorities then there is a minimum chance of them getting infected. These include the universal cross-infection control protocols along with some additional measures like face shield and disinfectant spray getting the highest number of response, followed by PPE kits, fumigation machines, infrared thermometer, UV irradiation etc. Over 240 respondents suggested the need for all of the above equipment for a safer dental practice.

This research provided an assessment of the level of psychological affliction experienced by Indian dentists during the COVID-19 pandemic outbreak. The findings validated our hypothesis implying that dental professionals would show an increased chance of developing psychological distress. Similar observation was also recorded among professional involved with healthcare in the 2003 outbreak of SARS in Canada[20]and current studies on Covid 19 pandemic in Israel[21] and China.[22] In the present study on Indian dentist 8% of them showed signs of severe depression, 17% moderate and 25% had mild signs. Though extremely severe and severe level of anxiety was not seen in dentists but 9% showed moderate and 13% showed mild anxiety. Stress level was expectedly high 8% showing severe stress, 24% moderate and 34% having mild signs of stress. Significant correlation was found between depression and anxiety with stress , p value greater than .001 for both suggesting that psychological effects of COVID 19 lead to depression or anxiety among the dentist eventually contributing to stress among them.

Conclusion

The current pandemic has resulted in a state of uncertainty and fear among all and the dentist also have been influenced by the situation. It has not only effected the growth and demand of the profession but has also arisen various issues related to the practice and safety of the dentist at work. Dentists are undergoing stress which in turn can be having adverse effects on their physical and psychological well-being in a long run. There is a need to evolve with the current COVID 19 pandemic and brainstorming the ways to encourage the safe modes of practice and eventually finding ways to decrease the levels of depression, anxiety and stress among dentist.

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A Study to Analyze the willingness of Smokers to Quit Smoking Following Dentist's Advice

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Abstract

Aim: To analyze the patient's perceptions about the role of dentists in smoking prevention, counseling and cessation and to analyze the willingness of smokers to quit smoking following dentist's advice.

Materials and Methods: A descriptive study was conducted among dental patients visiting Dental College and Hospital in the month of November 2019 using a self administered questionnaire. Convenient sampling was done. Among five hundred patients who participated in the study, 486 completed it with a response rate of 97.2%. The descriptive statistics and chi square analysis was performed to analyze data.

Results: The patients had good knowledge about the effects of smoking on general and oral health. However its effect on hair loss, ageing and wound healing can be reinforced. Non smokers had significantly higher ($p<0.05$) knowledge about the effects of smoking on ageing, bad breath, gum diseases and oral cancer compared to smokers. Patients had positive perception about the role of dentist in smoking cessation activities. Smokers exhibited willingness to quit if suggested by the dentist.

Conclusion: Patients perceive that dentists can play an important part in smoking cessation activities. Hence dentists should not hesitate to give smoking advice to their patients and grasp this opportunity to improve the general and oral health of the community.

Key words*: smoking, oral health, patient knowledge, patient perception.*

Introduction

Smoking is responsible for 100 million deaths worldwide.[1] It has been established as a risk factor for death from several systemic diseases including lung cancer, respiratory diseases and cardiovascular diseases.[2] About 30% of all cancer diseases and deaths, 80% lung cancers, 30% ischemic heart diseases and strokes, 80% of myocardial infarctions before the age of 50 years and 70% of chronic lung diseases are caused by smoking.[3,4] It causes large number of oral diseases and conditions such as staining of teeth & restorations, halitosis, impaired wound healing, periodontal diseases, failure of implants and surgical treatments, ANUG and life threatening precancerous and cancerous lesions.[5,6]

According to WHO the prevalence of tobacco habits in India is high with 34% using bidis, 31% cigarettes, 19% chewing tobacco, 9% hookah & 7% other forms.[7] The cancer patients' aid association of India revealed prevalence of cigarette use as 20%, bidis 40% and chewable tobacco 40%.[8] It has huge physical, mental, social and economic implications for our country. Preventive strategies are need of the hour to prevent and reduce the menace of smoking and tobacco.

The scope of preventive dentistry is constantly expanding and can be as far reaching as a professional's imagination, sense of responsibility and efforts. Dentists

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have been recognised as "ideally positioned to counsel against the use of cigarettes and smokeless tobacco products".[9] Most of the studies in the past have focused on dentists' or dental student's knowledge, attitude and practices regarding the various aspects of the smoking and smoking cessation activities.[10,11] These studies have completely sidelined how the recipient of such interventions feel and what are their views. Very few studies have focused on patient's knowledge, awareness and perceptions regarding smoking and smoking cessation interventions.[12,13] Those available stress more on oral cancer rather than comprehensively covering all the aspects related to oral health and general health. There is paucity of patient centred studies in the Indian scenario on such a sensitive issue. Hence the present the present study was conducted to assess dental patients' knowledge about effects of smoking and their perceptions regarding role of dentists in smoking cessation interventions.

Material and Method

A descriptive study was conducted in the year 2019 in the month of November to assess dental patient's perceptions about smoking cessation as a part of oral health care delivery. Ethical clearance was obtained from Institution Review Board of Career Post Graduate Institute of Dental Sciences & Hospital, Lucknow to conduct the study. Informed consent was obtained from participating patients. Patients attending the department of oral medicine and radiology in Career Post Graduate Institute of Dental Sciences & Hospital constituted study population. A total of 500 patients participated in the study using convenient sampling technique. Among five hundred patients who participated in the study, 486 completed it. The response rate was 97.2%.

Inclusion criteria

Ø Subjects 18 years and above.

Ø Subjects giving consent for participation.

Exclusion criteria

Ø Subjects unable to read the questionnaire.

A self administered questionnaire given by M Terrades et al[14] was used in the present study. The face validity was checked by ten individuals who were not the part of study. The content validity obtained for the present study was 0.88 which was above the minimum value of 0.85 for a panel of eight experts. The final questionnaire consisted for four sections namely sociodemographic, knowledge about effects of smoking on general and oral health (eleven questions), perception of patients towards role of dentist in smoking cessation and attitude towards smoking cessation counseling (eight questions). The fourth section consisted of three questions, meant only for smokers and assessed their willingness to quit and consulting a specialist on dentists' advice.

Statistical Analysis

Descriptive statistics was used to analyze the data. Chi square analysis was done to find association between knowledge, attitude of study subjects and response to the questions. The value of $p< 0.05$ was accepted as statistically significant. The analysis was performed using SPSS (23.0) software.

Results

Out of 486 patients 311 (64%) were males and 175 (36%) were females. Non smokers constituted 74% (N=360) of the patients, while 20% (N= 99) of the subjects were smokers and 6% (N= 27) were ex- smokers. Regarding effects of smoking on oral health, 407 (84%) & 457 (94%) patients agreed it causes discoloration of teeth and bad smell from the mouth respectively. 274 (56%) patients believed smoking can cause tooth decay, while 154 (32%) subjects didn't know its effect on tooth decay. 318 (65%) patients agreed that smoking can cause gum diseases, while 132 (18%) didn't know about it. 388 (80%) patients agreed that smoking can cause oral cancer. Statistically significant difference ($p<0.05$) was observed in the knowledge of smokers and non- smokers about the facts that smoking causes wrinkled skin, bad smell from mouth, gum diseases and oral cancers. Non-smokers were more aware of the above facts (Table 1).

411 (85%) believed that dentists can help patients stop smoking and 408 (84%) agreed that dentist should advice regarding how to stop smoking. 411 (85%) patients will appreciate a dentist who provides smoking cessation advice to smokers. 409 (84%) patients will not get offended and shift to the other if asked about their smoking habits. 369 (76%) patients think that a dentist should ask about the smoking status of their

patient's every time they visit. There was no statistically significant difference ($p<0.05$) in the perception of smokers, ex- smokers and non smokers about the role of dentist in smoking cessation activities (table 2).

Smokers showed positive attitude towards smoking cessation counseling given by the dentist. 88(89%) smokers agreed to try to stop smoking if suggested by the dentist, while 5 (5%) said no to dentist's suggestion. 91 (93%) smokers agreed to put more efforts to quit smoking if the dentist showed its effect on the oral cavity. Only 3 (3%) were not willing to put no efforts and another 4 (4%) didn't know if they will put more efforts or not (table 3).

Table 1: Knowledge about effects of smoking on general & oral health depending upon smoking status

Question Smoking Causes	Response	Non smokers N (%)	Ex- smokers N (%)	Smokers N (%)	Total N
Lung cancer	Yes	331(91.4)	26 (96.29)	82(82.8)	439
	No	8 (2.2)	1 (3.7)	6 (6.06)	15
	Don't know	21 (5.8)	0	11 (11.11)	32
Lines on the skin*	Yes	185 (51.3)*	12 (44.44)	35 (35.35)*	232
	No	27 (7.5)	2 (7.4)	18 (18.18)	47
	Don't know	148 (41.11)	13(48.14)	46 (46.46)	207
Stained teeth	Yes	303 (84.16)	24 (88.88)	80 (80.8)	407
	No	21 (5.8)	0	12 (12.12)	33
	Don't know	36 (10)	3 (11.11)	7 (7.07)	46
Bad smell from the mouth*	Yes	342 (95)*	26 (96.29)	89 (89.89)*	439
	No	2 (0.5)	0	6 (6.06)	8
	Don't know	16 (4.4)	1(3.7)	4 (4.04)	21
Tooth decay	Yes	202 (56.11)	15 (55.55)	57 (57.57)	274
	No	37 (10.2)	5 (18.5)	16 (16.16)	58
	Don't know	121 (33.61)	7 (25.29)	26 (26.26)	154
Affect healing of wound	Yes	95 (26.38)	5 (18.55)	21 (21.21)	121
	No	63 (17.5)	5 (18.55)	20 (20.20)	88
	Don't know	202(56.11)	17 (62.96)	58 (58.58)	277
Gum disease*	Yes	244(67.77)*	16 (59.25)	58 (58.58)*	318
	No	21 (5.8)	2 (7.4)	13 (13.13)	36
	Don't know	95 (26.38)	19 (70.37)	28 (28.28)	142
Cancer of mouth*	Yes	297 (82.5)*	24 (88.88)	67(67.67)*	388
	No	9 (2.5)	0	11(11.11)	20
	Don't know	54 (15)	3 (11.11)	21(21.21)	78

***Statistically significant at p<0.05**

Table 2: Perceptions about role of dentist in smoking cessation according to smoking status

Question	Response	Non smokers N (%)	Ex- smokers N (%)	Smokers N (%)	Total N
I would expect my dentist to provide treatment & tell about smoking	Yes	306 (85)	22 (81.48)	77 (77.77)	408
	No	22 (6.1)	1 (3.7)	9 (9.09)	32
	Don't know	32 (8.88)	4 (14.8)	13 (13.13)	49
I'd expect my dentist to be interested in smoking status of their patients	Yes	281 (78)	20 (74)	80 (80.80)	381
	No	61(16.94)	6 (22.22)	15 (15.15)	82
	Don't know	18(5)	1 (3.7)	4 (4.04)	23
I think dentists should explain effects of smoking on oral health	Yes	353 (98)	25 (92.59)	94 (94.94)	472
	No	2 (0.5)	2 (7.4)	2 (2.02)	6
	Don't know	5 (1.5)	0	3 (3.03)	8
Dentists can help patients stop smoking	Yes	301(83.6)	24 (88.88)	24 (24.24)	349
	No	25 (6.9)	2 (7.4)	33 (33.33)	60
	Don't know	34 (9.4)	1 (3.7)	42 (42.42)	77
Dentists should give advice regarding how to stop smoking	Yes	295(81.9)	26 (96.29)	87 (87.87)	408
	No	13 (3.6)	0	2 (2.02)	15
	Don't know	52 (14.44)	1 (3.7)	10 (10.10)	63
Do you like dentist who provides smoking cessation advice to smokers	Yes	304 (84.44)	22 (81.48)	85 (85.85)	411
	No	19 (5.2)	1 (3.7)	5 (5.05)	25
	Don't know	37 (10.2)	4 (14.8)	9 (9.09)	50
Would you change to the other dentist if one dentist asks you about your smoking status	Yes	30 (8.3)	2 (7.4)	4 (4.04)	36
	No	297 (82.5)	23 (85.18)	89(89.89)	409
	Don't know	33 (9.1)	2 (7.4)	6 (6.06)	41
Dentist should ask about patient's smoking status on each & every visit	Yes	268 (74.4)	20 (74.07)	81 (81.81)	369
	No	66 (18.33)	5 (18.5)	12 (12.12)	83
	Don't know	26 (7.2)	2 (7.4)	6 (6.06)	34

Table 3: Response of smokers towards smoking cessation counseling

Question	Yes N (%)	No N (%)	Don't know N (%)
I would try to stop smoking if a dentist suggested so.	88 (89)	5 (5)	5 (5)
I would put more efforts to quit smoking if a dentist showed me its effects on the mouth	91 (92)	4 (4)	4(4)
I would go to a specialist for consultation if a dentist suggested so	84 (85)	10(10)	5 (5)

Discussion

The present descriptive study assessed patients' knowledge about the effects of smoking, perceptions about the role of dentist and willingness of smokers to quit following dentist advice.

90% of the patients in the present study agreed that smoking causes lung cancer. It is comparable to the studies done by Richard et al[15] and Terrades et al[14] where 91% and 98% patients respectively reported that smoking causes lung cancer. The high knowledge may be because many campaigns and advertisements have focused on the smoking as a causative factor for lung cancer.

Patients in the present study demonstrated fairly good knowledge (76%) about the oral effects of smoking. It was comparable to that reported by Lung et al[12] (78%), however less as compared to 95% reported by Shammari et al[13] and Terrades et al[14] 84% patients believed that it causes discoloration or staining of teeth.

59% believed that it causes bad taste from the mouth. 31% did not know if smoking causes bad taste or not. For a non smoker it may not be easy to appreciate this fact and smokers may become used to change in the taste and hence unable to appreciate it. This may be responsible for the above finding in the present study. However Terrades et al[14] reported a relatively high percentage (92%) of bad breath.

65% of the patients in this study believed that smoking can cause gum diseases while 27% were not aware of this. Shammari et al[13] and Terrades et al[14] reported relatively higher percentage (76% & 80%respectively). There was significant difference ($p<0.05$) in the knowledge of smokers and non- smokers regarding effect of smoking on gum diseases which was similar to that reported by Terrades et al[14].

Both smokers and non smokers had a positive perception about the role of dentist in smoking cessation activities. 83% of the patients believed that dentist should do health education apart from routine dental treatment. It was comparatively higher than 28% reported by Terrades et al[14].

In the present study 78% and 97% patients believed that dentist should be interested in the smoking status of their patients and should explain about the effects of smoking on oral health respectively. This finding was comparable to 77% & 95% reported by Terrades et al[14] and higher than 73% reported by Richard et al[15].

85% of the patients in the present study believed that dentists can help patients stop smoking and should advice how to do it compared to 60% reported by Terrades et al[14].

84% of the patients would not shift to the other dentist if asked about their smoking status, whereas 79% reported this in Terrades et al[14] and 60% in Richard et al[15] study. Though many patients feel it is embarrassing to tell about their smoking status, however it is not the

reason for the change of dentist.

In the present study 89% of the smokers agreed that they will try to quit smoking if suggested by a dentist. It was comparatively higher than 30% reported by Richard et al[15] & 60% reported by Terrades et al[14] 92% of the smokers showed willingness to put more efforts to quit if oral effects of smoking are explained compared to 69% reported by Terrades et al[14]. 95% smokers agreed to go to a specialist for consultation if suggested by a dentist compared to 81% reported by Terrades et al[14].

The present study reflects the perceptions of patients attending a dental hospital, so the results should be interpreted carefully as it may not reflect the perceptions of general population. The individuals reporting to hospitals usually have more knowledge of various conditions and diseases and hence may have good knowledge about effects of smoking than general population.

Conclusion

It can be concluded from this study that dental patients had a good knowledge about the general and oral effects of smoking. However they had poor knowledge about the effects of smoking on hair loss, ageing and wound healing. Smokers exhibited willingness to follow the dentists' advice about quitting smoking. It is recommended to conduct such surveys among general population covering different age groups. Dentists should inform their patients about the effects of smoking and strongly advice and help them in quitting.

Conflict of Interest: Nil

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The Clinical Effect of Super Oxidized Water Mouthwash on The Periodontal Parameters (Plaque Index, Gingival Index And Bleeding on Probing) for Patients with Dental Biofilm Induced Gingivitis

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Abstract

Background: One of the most predominant periodontal diseases is the dental biofilm induced gingivitis. : For the past 20 years, super-oxidized solutions have been shown to be powerful antimicrobials and disinfectants via oxidative damage. The taste is better than chlorhexidine and doesn't stain the teeth. Microsafe®, a super-oxidized solution for gingival care, offers a completely improved approach to treatment of gingivitis.

Aim: To evaluate the efficacy of super oxidized water mouthwash by improving gingival health in dental biofilm induced gingivitis. .

Materials and Methods: 45 adult male patients with generalized dental biofilm induced gingivitis participated in the double blinded randomized controlled parallel study divided into three groups, two mouthwashes and distilled water(negative control) used during seven days periods as adjunctive to regular mechanical oral hygiene(brushing and interdental aids cleaning), one group received super oxidized water mouthwash (Microsafe®) three times daily and the second group received 0.12% alcohol-free chlorhexidine mouthwash (Kin gingival®) twice daily and the third group received distilled water twice daily (negative control).

Results: According to the plaque index, highly significant improvement of all treatment groups was observed in the second visit one-week post treatment. Microsafe® (SOW) showed to have clinical outcomes closed to the effect of CHX (the positive control) than the negative control group (D.W group). According to the GI, there was a significant difference between the study groups in the2nd visit after treatment and CHX was appeared to be most effective when compared to Microsafe®.

According to BOP , all the treatment groups showed reduced BOP one-week between the 1st visit and the 2nd visit ; although, CHX showed better outcomes (the mean difference was 14.258) when compared to D.W and Microsafe® groups(the mean differences were 10.838, 9.9 respectively).

Conclusion: There was a positive effect of topical application of Microsafe® mouthwash as adjunctive to regular mechanical oral hygiene on gingival inflammation three times daily after one week. This was improved by clinical periodontal parameters (PlI , GI and BOP).

Keywords: *super oxidized water, chlorhexidine, distilled water, plaque index, gingival index, bleeding on probing, Microsafe®, Kin gingival®.*

Introduction

Irrespective to the gender, race or age, about more than 90% of the people have dental biofilm induced gingivitis which is the most common periodontal diseases. Gingivitis is originated by the noxious materials result from the accumulation of microbial

plaque at or near the gingival sulcus[1]. The important thing of gingivitis is that it can lead to periodontitis, though not all gingivitis will lead to the progress of periodontitis [2], so the importance of avoiding gingivitis and periodontitis is further highlighted. The maintenance of good oral hygiene is then of dominant importance, in spite of epidemiological studies representing that most part of the people does not reach adequate mechanical hygiene control[3].

This fact has led to the increase in attention in the expansion of oral hygiene products based on chemical plaque control, mainly those effective antimicrobial mouthwashes that may prevent the development of gingivitis by having a major effect on the supragingival and subgingival establishing of teeth by oral bacteria,[3]. Microsafe®, a super-oxidized solution for gingival care, offers a completely improved approach to treatment of gingivitis. The neutral pH, super oxidized water was certified as an antiseptic in México in 2004[4].The active components of this solution include 99.97% super oxidized water and < 0.03% of numerous reactive species of chlorine and oxygen including sodium chloride 0.023% , hypochlorous acid 0.003% and sodium hypochlorite 0.004%,. However, the total content of free available chlorine is low and its ranges between 50 and 80 ppm. This SOW has bactericidal, fungicidal virucidal, and sporicidal actions by oxidative damage and it is ready to use with no additional mixing or dilution [5] .

It does not need special disposal or handling. Super-oxidized solutions have been shown to be powerful antimicrobial agents and disinfectants through oxidative damage[5] Chlorhexidine is the typical mouthwash used for chemical plaque control [3]. Inappropriately, CHX has some unwanted effects such as extrinsic stain on the teeth and dental restorations, interfering with function of the taste, it has a bitter taste, increasing calculus formation[6]. CHX mouth rinses holding anti discoloration agents were reported to have no reliable useful effects on plaque control and gingivitis[7] . This inspires many researchers to find replacements for CHX. This study was conducted to record the effect of Microsafe® (super oxidized water) on dental biofilm induced gingivitis by monitor its effect on the PLI, GI and BOP.

Materials and Methods

Study sample

A double blinded randomized controlled parallel study was conducted from November 2019 to March 2020 at Karbala dental specializing center. This study approved by ethical committee/ college of Dentistry/ University of Baghdad, followed the guidelines of Helsinki and Tokyo for humans (the reference no. 136619 in 2\12\2019). It was registered at (http:// clinicaltrials.gov. (the reference no. NCT04328753), and followed the consolidation standards of reporting trials (CONSORT 2010).A total of 45 Iraqi male patients aged 15_55 years, who met the eligibility criteria were participated in this study for the non-surgical treatment of generalized dental biofilm induced gingivitis. Inclusion criteria included patients with generalized gingivitis, no antibiotic treatment during the three months period prior to the start of the trial, no regular medication with anti-inflammatory compounds, no history of allergy to oral care products and no regular use of oral antiseptics. Exclusion criteria included patients who refuse to write an informed consent form, smokers, and patients with systemic diseases that may have effect on periodontal tissue. In the first visit, PlI,GI and BOP were measured , scaling and polishing were done then inform the patient to use the coded bottle which gave to the participant by the assistant not involve in the study so the researcher did not know the type of mouthwash that was given to the participant(CHX 15 ml for 1 minute twice daily while SOW 15 ml for 1 minute three times daily and distilled water 15 ml for 1 minute twice daily) [8][9] .The mouthwashes was given for one week with routine mechanical dental home care(brushing and interdental aids cleaning).In the second visit the PlI ,GI and BOP was measured again.

Results

In the first visit

The comparison among the study groups by clinical periodontal parameters (PlI,GI &BOP) before treatment is shown in table [1]. In this study, there were no statistical significant differences ($P \geq 0.05$) among study groups regarding PlI and GI clinical parameters before treatment and highly significant difference regarding BOP $p= 0.004$.

Table (1) statistical analysis of the clinical periodontal parameters (PLI,GI and BOP) at the first visit.

		N	Mean	SD	Minimum	Maximum	F	P value
PlI	DW	15	1.212	±0.119	1.050	1.460	.485	.619 NS
	CHX	15	1.194	±0.193	1.000	1.710		
	Super oxidized water	15	1.159	±0.125	1.030	1.510		
	Total	45	1.188	±0.148	1.000	1.710		
GI	DW	15	1.234	±0.143	1.040	1.480	.727	.489 NS
	CHX	15	1.197	±0.143	1.060	1.530		
	Super oxidized water	15	1.172	±0.138	1.050	1.500		
	Total	45	1.201	±0.140	1.040	1.530		
BOP	DW	15	37.245	±11.246	15.170	60.710	6.326	**.004 HS**
	CHX	15	27.739	±13.707	10.700	59.800		
	Super oxidized water	15	21.535	±11.447	10.700	43.750		
	Total	45	28.840	±13.579	10.700	60.710		

In the 2nd visit after treatment

Plaque index (PLI)

Table (2) showed the comparison of mean values of PLI among groups between first and second visits. Means of plaque index were highly significantly decreased in the 2nd visit compared to 1st visit in the D.W, CHX and SOW groups ($p<0.01$). The mean differences were also taken between the 1st visit and the 2nd visit regarding D.W, CHX and SOW (0.196, 0.502 and 0.471 respectively).Highly significant difference at $p<0.01$among the study groups in PlI in the2nd visit compared to 1st visit.

Table (2) Statistical analysis of PlI at the 1st and 2nd visits in each study group.

Groups	**1st visit PLI**			**2nd visit PLI**			**Mean differences**	**Paired t-test**	**P value**
	Mean ±SD	**F**	**P value**	**Mean ±SD**	**F**	**P value**			
DW	1.212 ±0.119	0.485	0.619 NS	1.016 ±0.074	22.040	**0.000 HS**	0.196	6.714	**.000**
CHX	1.194 ±0.193			0.692 ±0.225			0.502	8.066	**.000**
SOW	1.159 ±0.125			0.688 ±0.214			0.471	11.492	**.000**

Gingival index (GI)

Table (3) shows the mean values of GI among groups between 1st and 2nd visits. Means of gingival index were significantly decreased in the 2nd visit compared to 1st visit in the D.W , CHX and in the SOW groups (p<0.01). The mean difference were also taken between the 1st visit and the 2nd visit in D.W ,CHX and SOW(0.169 , 0.179 and 0.152 respectively).

Significant difference among the study groups in GI in the2nd visit (P= 0.029) compared to 1st visit

Table (3) Statistical analysis of mean values of GI at the 1st and 2nd visits in the study groups.

Groups	1st visit GI			2nd visit GI			Mean differences	Paired t-test	P value
	Mean ±SD	F	P value	Mean ±SD	F	P value			
DW	1.234 ±.0143	0.727	0.489 NS	1.065 ±0.038	3.856	0.029 Sig.	0.169	5.187	.000 HS
CHX	1.197 ±0.143			1.018 ±0.025			0.179	5.004	.000 HS
SOW	1.172 ±0.138			1.020 ±0.024			0.152	4.576	.000 HS

Bleeding on probing

Table (4) shows the comparison of the mean values of BOP among groups in 1st and the 2nd visit. Means of BOP were significantly decreased (p<0.01) in the 2nd visit compared to 1st visit in all study groups. The mean difference were also taken between the 1st visit and the 2nd visit in D.W ,CHX and SOW (10.838 , 14.258 and 9.9 respectively). Highly significant difference at p<0.01 among the study groups in BOP in the 2nd visit compared to 1st visit

Table (4) Statistical analysis of BOP at the 1st and 2nd visits in the study groups.

Groups	1st visit BOP			2nd visits BOP			Mean differences	Paired t- test	P value
	Mean ±SD	F	p	Mean ±SD	F	p			
DW	37.245 ±11.246	6.326	**0.004 HS**	26.407 ±6.911	21.278	**0.000 HS**	10.838	6.980	**.000**
CHX	27.739 ±13.707			13.481 ±7.181			14.258	7.168	**.000**
SOW	21.535 ±11.447			11.635 ±6.139			9.9	6.872	**.000**

Discussion

Scaling and polishing can reduce the pathogenicity of the plaque significantly by disruption of biofilm, and it should be maintained by personal consistent oral hygiene commitment [10]; thus, plaque accumulation which can be expressed by plaque index is related to patient's ability to control individual oral hygiene post professional mechanical intervention. This study evaluated the clinical parameters and the analysis of the results showed that Microsafe® as adjunctive treatment can be a potent additive to improve and facilitate the clinical outcomes. Microsafe® reduced gingival inflammation, it was achieved statistical significance. Significant improvement of all treatment groups was observed in the second visit one-week post treatment.

Microsafe® (SOW) showed to have clinical outcomes closed to the effect of CHX (the positive control) than the negative control group(D.W group). The mean difference in plaque accumulation taken between the 1st visit and the 2nd visit in Microsafe® was 0.471; whereas, the group treated by CHX was 0.502 and the D.W group was 0.196 . All the treated groups had a significant reduction compared to the baseline scoring. The result of CHX was similar to the result of the research of Grossman and others that the CHX is the gold standard chemical agent appears to be the most effective antimicrobial agent for reduction of both plaque and gingivitis [11].

The valuation utilized the gingival index as the parameter to estimate the extent of inflammation, which was reduced with adjunctive use of Microsafe®. This research demonstrated that according to the mean difference in the gingival index, Microsafe® reduced gingival inflammation by 0.152 after one week between the 1st visit and the 2nd visit which was less than the mean differences of CHX and D.W groups (0.179 , 0.169 respectively).

According to the GI, there was a significant decrease between the study groups p= 0.029 and CHX was appeared to be most effective when compared to Microsafe® ,so we need an extended period of follow up to evaluate the influence of Microsafe® on gingivitis by the gingival index.

The results in plaque index and gingival index between D.W group and SOW group were coincide with other study done by SUN Peng and ZHANG [9], who studied the clinical therapeutic efficacy for gingivitis using Super-Oxidized Solution(SOS) by taking fifty cases with gingivitis were divided into two groups randomly.

According to BOP, all the treatment groups showed reduced BOP one-week between the 1st visit and the 2nd visit ; although, CHX showed better outcomes (the mean difference was 14.258) when compared to D.W and Microsafe® groups(the mean differences were 10.838, 9.9 respectively).also so we need an extended period of follow up to study the influence of Microsafe® on gingivitis by the bleeding on probing .

Conclusion

According to the results of this study, there was a positive effect of topical application of Microsafe® mouthwash as adjunctive to regular mechanical oral hygiene on gingival inflammation three times daily after one week. This was improved by clinical periodontal parameters (PLI, GI and BOP)

This study demonstrated that Microsafe® can attain an influential effect on improving the outcomes of periodontal diseases; Although that CHX showed more potent impact on clinical outcomes, Microsafe® proved to enhance the results of the treatment as well.

Conflict of Interest: No

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Synthesis and Identification and Biological Studies of New Azo Dyes Derived from Imidazole and their Chelate Complexes

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Abstract

Anew azo ligand (E)-2-((4- fluoro phenyl)diazenyl)-4,5-bis(4-methoxy phenyl)-1H-imidazolehas been synthesized from coupling 4,5-bis(4-methoxyphenyl)imidazole with para-chloro-anline. The structures of the new ligand and their transition metal complexes ware investigated and characterized using different physicochemical studies as elemental analysis, IR spectra, ^{1}HNMR,, UV-Vis spectra, mass spectra, Molar conductance ,and magnetic susceptibility .The complexes were found to have the general formula $[ML_2Cl_2].H_2O$ where M = Co(II), Ni(II), Cu(II) ,Mn(II),and Fe(II), $[MLCl_2].H_2O$where M=Zn(II),Cd(II),Hg(II), and Pd(II). The IR results demonstrate that the co-ordination sites are the azomethine nitrogen of the imidazole ring and azo nitrogen atom of the azo ligand. The electronic spectral and magnetic measurement data indicate that the complexes exhibit octahedral geometry,except the Zn(II),Cd(II),andHg(II) complexes suggest tetrahedral and Pd(II) a square planar geometry.The cytotoxicity of Pd(II) complex on human Lung Cancer (SCLC) and normal cells.

Key word: *Synthesis, Biological activity, Lung Cancer.*

Introduction

Azo dyes are among the compounds with continually increasing application in analytical chemistry, because they show specific color changes in reactions with metal ions. Azo-dyes [1]. In addition, the presence of nitrogen along with reduce the solubility ofthe complex) es and making the isolation of these complexes easier Interest in complexes ofthese ligand systems now covers veral areas, ranging from general considerations of theeffect and electron delocalization in transition metal complexes to potential biologicalactivity and practical application[2]. Azo dyes compounds which are having the hetero atoms in the ring system such as sulfur and nitrogen exhibit a more shift in the absorption maxima λmax indifferent solvents compared to the plan ring system , especially azo dyes derivedfromheterocyclic compounds such as imidazol andBenzimidazole[3]. Many azocompound complexes were reported and the results indicated that their biological activities are more active than free azodyes. [4]. In Recent decades, literature survey showed that many researchers in worldwide focused on oxazolones and imidazolones for drug discovery[5].The imidazole unit is a key building block that has been widely used in medicine, ionicliquids, anion sensors, as well as electronic and optical materials ,Hence, it convincesus to incorporate an imidazole unit into an azo dye, it is anticipated that the azo-imidazoleswith excellent photochromic properties will be obtained.Because of the advantages of short reaction time, high efficiency, improving reaction yields,and reducing thermal degradation byproducts, microwave-assisted synthesis has beensuccessfully and routinely applied in organic and medicinal chemistry[6].it is ossible to synthesise compounds with ligands bearing functional groups such as –NH2, –COOH, i.e. groups open for further modification or cleaving to biological molecules .Moreover, the study of octahedral complexes with simple heterocycles that are related to biological molecules such as nucleotides and amino-acids may uncover the potential of these compound as, for example, anticancer agents[7-8]. Imidazole can be tailored at the 2, 4, 5 positions thereby giving tri-substituted imidazole derivatives. In the literature various tri-aryl imidazole have been reported. However, the compounds with aryl substitution at the 4, 5 positions of imidazole, as

the biological behavior of cancer cell is concerned, it is nothing but an increasing of abnormal in human body. Moreover different types of tests [9].

Materials and Method

All chemicals and solvents used in this work were high purity supplied from different companies such as Fluka, BDH and Sigma Aldrich and used without further purification, chemical products were purchased from commercial sources : .$6H_2O$(99.99%, BDH),$ZnCl_2.2H_2O$ (99.99%, BDH),$CdCl_2.1/2H_2O$ (≥99%,BDH), $CuCl_2.6H_2O$ (99%, BDH), ethanol (≥99.5%).

Preparation of 4,5-bis(4-methoxyphenyl) imidazole

The imidazole derivative obtained from the reaction of benzyl derivative and hexamethylenetetramine in the presence of glacial acetic acid .In a round flask (250 mL) was added 50 mL of glacial acetic acid to a mixture of benzyl derivative (2.90 gm, 0.01 mol), hexamethylenetetramine (0.256 gm, 0.005 mol) and ammonium acetate (6.0 gm, 0.23 mol). The solution reflux was heated for 90 min, by using reflected condenser. The solution was diluted after cooling by adding 400 mL of distal water. The imidazole derivative was precipitated by adding the solution of ammonium hydroxide. The white precipitate was collected by filtration, washed several times with distal water**.**

Preparation of ligand: [(E)-2-((chlorophenyl) diazenyl)-4,5-bis(4- methoxyphenyl)-1H-imidazole

A(1.27gm,0.01mol) frompara-chloro-anlinewas dissolved in 3ml of con.HCl and 50ml of water, it is then cooled in ice bath. then add slowly with continuous stirring solution of sodium nitrite prepared by dissolving (0.75g , 0.010 mol) in 10 mL of distilled water. Then add the diazonium salt solution, which is gradually formed with continuous stirring to a solution (2.80g, 0.01 mol) from the base of the 4-5 bis (4-methoxy phenyl) imidazole dissolved in a mixture of(150ml) of ethyl alcohol and 5 ml (1) sodium hydroxide solution(10%)

Preparation of the complexes

The chelate complexes were prepared at optimal pH by dissolved (0.4185gm, 0.001 mole)from ligand in ethanol (15ml) and then (0.001mol) metal chloride with 1:2 (metal:ligand) Mn(II),Fe(II) Co(II), Ni(II),Cu(II), and 1:1 with Zn(II), Cd(II),Hg(II) and Pd(II) chlorides salts .The product was isolated after reduction of volume by evaporation . It ware filtered off , washed with ethanol and dried under vacuum and purified by recrystallization from hot ethanol and left to dried at room temperature . The som physical properties of ligand and its metal complexes are collected in Table (1).

Table (1):- Analytical and som Physical properties for ligand (L) and its metal complexes

Compound	Color	m.p C°	Yield %	(M.wt)	Found (Calc.)%			
					C	H	N	M
(C23N4O2H19Cl)	Red	106-108	94	418.5	(65.94) 65.63	(4.54) 4.33	(13.38) 13.63	—
[Mn(C23N4O2H19Cl)2Cl2]. H2O	Deep-red	180-182	72	980.948	56.272)) 56.269	(4.077) 4.046	11.420)) 11.444	(5.600) 5.623
[Fe(C23N4O2H19Cl)2Cl2]. H2O	yello	170-172	73	981.84	(56.220) 56.263	(4.073) 4.083	(11.407) 11.471	(5.687) 5.757
[Co(C23N4O2H19Cl)2Cl2]. H2O	brouwn	189-190	88	984.933	(56.044) 56.153	(4.061) 4.057	(11.371) 11.331	5). (983 5.993

Cont... **Table (1):- Analytical and som Physical properties for ligand (L) and its metal complexes**

[Ni(C23N4O2H19Cl)2Cl2]. H2O	red	207-208	89	984.69	(56.058) 56.165	(4.062) 4.063	(11.374) 11.463	5).960) 5.965
[Cu (C23N4O2H19Cl)2Cl2]. H2O	violet	162-163	94	989.546	(55.783) 55.722	(4.042) 4.056	(11.318) 11.322	(6.42) 6.44
[Zn(C23N4O2H19Cl) Cl2].H2O	red	280-281	79	572.909	48.175)) 48.173	(4.665) 4.571	(9.774) 9.815	(11.416) 11.323
[Cd(C23N4O2H19Cl) Cl2]. H2O	yello	217-219	83	619.9	(44.523) 44.343	(3.387) 3.396	(9.033) 9.212	(18.131) 18.111
[Hg(C23N4O2H19Cl) Cl2].H2O	violet	131-132	84	708.09	(38.97) 38.69	(2.965) 2.996	(7.908) 7.668	28).32) _____
[Pd(C23N4O2H19Cl)Cl2].H2O	Violet	------	73	613.92	44.956)) 44.835	(3.420) 3.4 91	9.121)) 9.133	(17.339) 17.345

Combination Cytotoxicity Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 × 10^4cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compound. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of(3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide**)** reagentMTT (and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a micro plate reader at 492 nm (test wavelength); the assay was performed in triplicate.

Result and Discussion

All the complexes are stable in air . They are Freely soluble in DMSO and DMF and sparingly soluble in methanol and ethanol . The metal complexes were characterized by elemental analysis , molar conductivities , IR, UV-Vis and ^{1}HMNR spectra . The analytical data of the complexes are in agreement with the experimental data . the value reveal that the metal to ligand ratio is (1:2)of Mn (II), Fe (II), CO (II), Ni (II), Cu (II) and(1:1) ofZn (II), Cd (II), Hg (II) and Pd (II). Are presented in table 1 . The magnetic susceptibilities of the complexes at room temperature were consistent with octahedral geometry for Mn (II), Fe (II), CO (II), Ni (II), Cu (II)and tetrahedral geometry forZn (II), Cd (II), Hg (II) expected the Pd(II) complex is square planer.

IR spectroscopy

In order to clarify the mode of bonding and the effect of the metal ions on the ligand, the IR spectra of the free ligand and the metal complexesware studied and assigned based on carful comparison of their spectra with that of the free ligand. Relevant IR bands for the ligand and metal complexesware presented in Table 2. The spectrum of free ligand show absorption band at 1639 cm^{-1}due to ν(C=N)of theN_3 imidazole nitrogen.In

the complexes, this band are shifted to lower and hight frequency [10] .These differences suggest the linkage of metal ions with nitrogen imidazole ring. The spectra of the metal complexes showed the a location, shape and intensity different from the free ligand spectrum, The sharp band in the range (788 cm^{-1}) are due to ν (C–Cl) [11]. In the metal complexes, the aromatic C-H stretching bands were observed between 3066 and 3005 cm^{-1}[12]. The infrared spectra of the complexes showed a weak band at frequencies (3414-3332 cm^{-1}), which are due to the vibrations of the O-H that indicate the presence of water molecules[13].The bands in the region 3416 cm^{-1} due to stretching mode of NH in the spectra of the ligand undergo some charge in the spectra of the complexes. The spectrum of ligand show band 1463cm-1 due to (N=N) group.This band appearing at 1463-1469cm-1withdifferentin shape and reduced in intensity of the spectra of complexes. Band shifted and reduced intensity due to complexes formation[14]. Also bands in the regions (466 – 416)cm^{-1} which to υ(M-N) bands [15].

Table(2): IR frequencies (in cm^{-1}) of the azo- ligand and their complexes

Compounds	ν(N-H) imid +υ(O–H(H2O	ν(C-H)or.	ν(C-H)al.	ν(C=N) Im	ν(N=N)	ν (C-Cl)	ν (M-N)
(C23N4O2H19Cl)	3414b.	3066w.	2951w.	1639s.	1463w.	788s	----
[Mn(C23N4O2H19Cl)2Cl2]. H2O	3446b.	3010w.	2953w.	1610s.	1436w.	786s.	466w.
[Fe(C23N4O2H19Cl)2Cl2]. H2O	3313b.	3086w.	2962w.	1622s.	1442w.	788s	447w.
[Co(C23N4O2H19Cl)2 Cl2]. H2O	3404b.	3088w.	2943w.	1608m.	1435w.	790s	455w.
[Ni(C23N4O2H19Cl)2 Cl2]. H2O	3525b.	3383w.	2933w.	1635m.	1440w.	788s.	455w.
[Cu (C23N4O2H19Cl)2 Cl2]. H2O	3419 b.	3001w.	2960w.	1610m.	1436w.	792s	459w.
[Zn(C23N4O2H19Cl) Cl2]. H2O	3334b.	3008w.	2953w.	1610w.	1438w.	788s	460w.
[Cd(C23N4O2H19Cl) Cl2]. H2O	3419b.	3066w.	2949w.	1610m.	1436w.	790s.	450w.
[Hg(C23N4O2H19Cl) Cl2]. H2O	3332b.	3005w.	2935.w,	1608s.	1438w.	788s	468w.
[Pd(C23N4O2H19Cl)Cl2].H2O	3442b.	3066w	2922w	1606s	1438w	792s	464w

Electronic Spectra

UV-ViS spectra data for the ligand (L) and its metal complexes withMn(II),Fe(II) Co(III) , Ni(II) , Cu(II) , Zn(II) , Cd(II) and Hg(II) were recorded at 300 – 1100 nm using absolute ethanol as a solvent. .The UV-ViS. spectra of the ligand (L) and its metal complexes are shown in figure (2) and the absorption regions, bands assignment and the proposed geometries are given in Table (3).

The electronic spectrum of the ligand is characterized by three absorption bands. These bands are show at the position 230nm, 281 nm and 484nm. The first band can be attributed to a n→π* transition of the azo group(-N=N-) while the second band assigned to n →π* of the π-electrons of the phenyl system[16]. The third band may be attributed to the π →π* transition resulted from the hetro imidazole ring through the azo group(-N=N-) [17]. This band was shown at a red shift on coordination [18].

Table (3):- Electronic spectra (nm and cm^{-1}),electronic transition, magnetic moments, proposed geometry and hybridization of the prepared ligand and its metal Complexes

Compounds	λ max (nm)	Transitions	μ eff (B.M)	Geometry	Hybridization
Ligand =L	309	n →π*	---------	_____	_____
	461	π →π*			
[Mn(L)2Cl2].H2O	462	M→L,CT	5.61	Ohedral	Sp3d2
[Fe (L)2Cl2].H2O	464	M→L,CT	4.90	Octahedral	Sp3d2
[CO(L)2Cl2].H2O	472	M→L,CT	4.19	Octahedral	Sp3d2
[Cu (L)2Cl2].H2O	508	M→L,CT	1.73	Octahedral	Sp3d2
[Cd(L) Cl2].H2O	464	M→L,CT	Dia.	Tetr hedral	Sp3
[Hg (L)Cl2].H2O	467	M→L,CT	Dia.	Tetr hedral	Sp3
[Pd (L)Cl2].H2O	594	1A1g→1B1g	Dia.	Square planer	dsp2

Mass spectrum of ligand

Characterize the mass spectrum of the azo compound (L) Figure (3) has a range of different peaks in their relative abundance. The top peak at (m/z+ =419.5) was attributed to molecule $[C_{23}H_{20}N_4O_2Cl]^+$, which is equivalent to the molecular weight of the azo dye after the acquisition of protonin. The process of breaking the organic molecule continued to show another part at (m / z + =366) and low abundance and perhaps returned to the molecule $[C_{22}H_{17}N_2O]^{+\cdot}$The peak at ((m / z + =280) is due to the derivative of imidazole $[C_{17}H_{16}N_2O_2]^+$, which is the composition of pairs It also illustrates many of the mass fragmentation peaks of other parts [19;20].

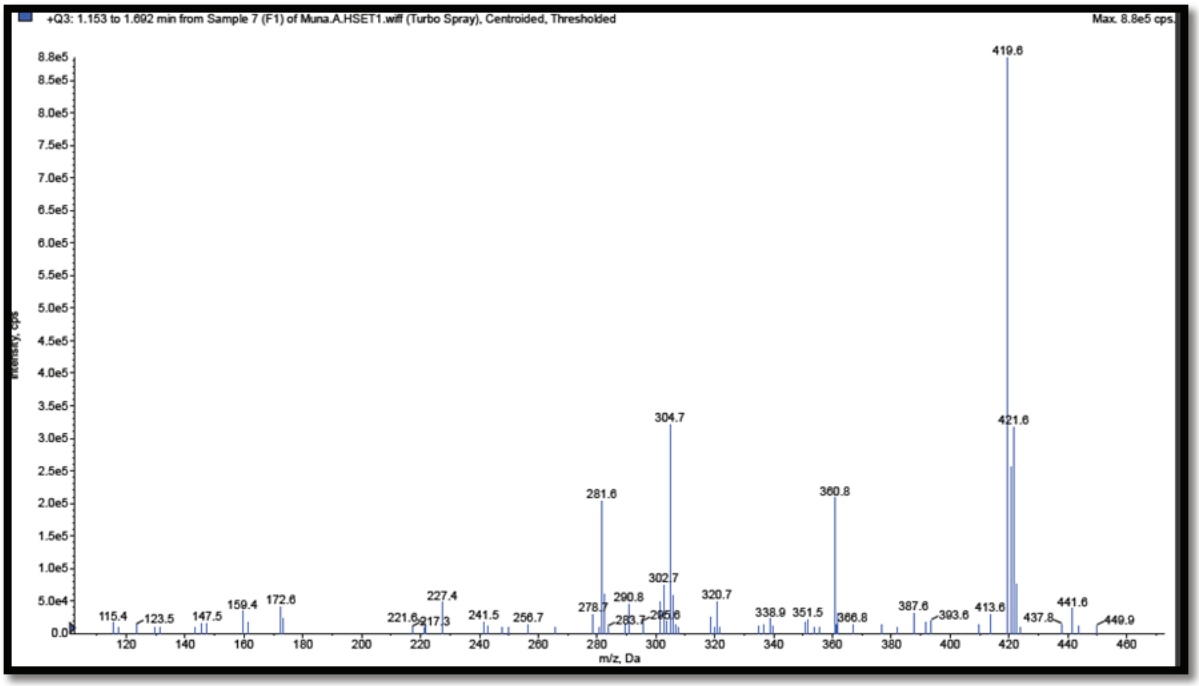

Figure (1):- Mass spectrum of imidazolylazo dye ligand (L)

^{1}H-NMR Spectrum of ligand

The newly synthesized ligand gave a satisfactory spectral data and the molecular structure was assigned on the basic of ^{1}H NMR chemical shift. ^{1}H NMR spectra were determined in solution of DMSO with tetra methyl saline as an internal reference. The ^{1}H NMR spectrum of the ligand showed clear signals involved singlet at δ (2.5) ppm belong to the proton of solvent (DMSO) and multiples signals at δ (7.3-7.9) ppm which were assigned to aromatic protons of phenyl rings.The proton of amine group (N-H) for the imidazole ring appeared in the chemical displacement(13.3 ppm)[21;22]. while the protons of the two methoxy group appeared at the chemical displacement(3.75 ppm)[23] as shown in Figure.(2).

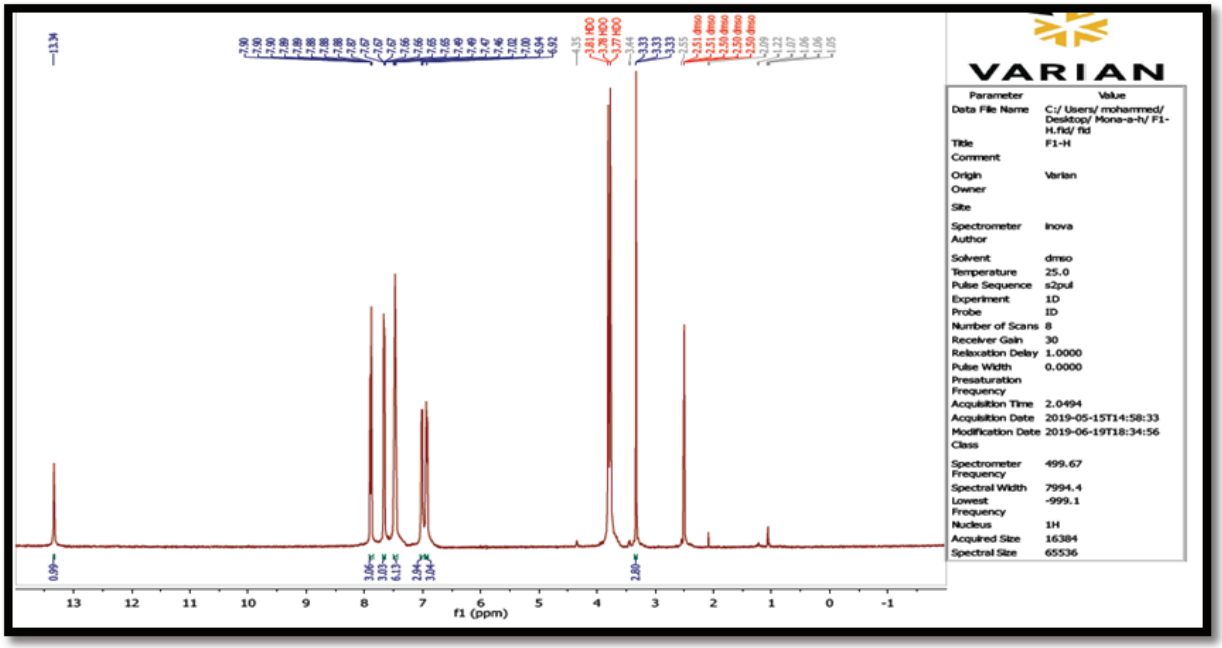

Figure (2):- ^{1}H-NMR spectrum of imidazolazo ligand

Cell viability and cytotoxicity assay

Chemotherapy is the use of anticancer drugs designed to slow or stop the growth of rapidly dividing cancer cells in the body. Table (3) shows the effect of Pd(II)Complexon cells growth of Lung Cancer SCLC as well as healthy cells (WRL). The concentrations used to inhibit cell growth ranged from (6.25- 400 µg / ml) to Lung Cancer SCLC and thus to healthy cells (WRL).The best rate of inhibition of cell growth of the cancer lines of the remaining living cells and healthy cells after Pd(II) Complexreactivation was at 400 µg / ml concentration. The results showed that the concentration of the compound used was an important factor in determining the rate of inhibition of cells, as detailedin [24].

Conductivity measurement

Molar conductance (Λm) measurements of the metal complexes carried out using DMSO as solvent at the concentration of 10^{-3} M in room temperature. All chelate complexes prepared in this work showed conductivity values ranged between (5.43-12.48) $s.mol^{-1}.cm^{2}$ that non-electrolyte and no conductive species [25]. we can deduce the probable structures of thecomplexes.

Conclusion

In this paper, new azo ligand and its metal complexes were prepared and characterized by spectral and analytical data. Depending upon all results was suggested an octahedral geometry for complexes Co(II), Ni(II),Cu(II),Mn(II),and Fe(II) ions, and tetrhedral geometry with each of Zn(II), Cd(II), and Hg(II) ions except for Pd(II) ion which has square planer geometry. In the biological activity studies. The cytotoxicity of Pd(II) complex on human Lung Cancer (SCLC) and normal cells were studied using MTT assay. Pd(II) complex appeared higher selective cytotoxicity versus cancer cell line , whereas it was very secure on normal cells line . The results signalize undoubtedly the possibility of utilize them as Lung Cancer drugs in the domain of medicine .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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The Effects of Mouth Bacterial Infection on Saliva Flow

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Abstract

Background: Mouth bacterial infections most probably due to dental cares are a prevalent disease. Salivary flow rate is affected by many diseases but there is not enough published data about the effect of mouth bacterial infection on the salivary flow rate. **Objective:** this study examines the relationship between salivary flow rate and mouth bacterial infection in adult population. **Methodology:** cross sectional study. **Result:** salivary flow rate is affected by mouth bacterial infection as it is founded to be very low flow rate in 17% and low flow rate in 40% of the sample population. **Conclusion** Salivary flow rate is affected by oral bacterial infections that need urgent medical intervention to improve the outcome of those patients.

Keywords: *Mouth; bacterial infection; health care; toxicity*

Introduction

Oral infections are defined as a problem for patients suffering from chronic conditions as cancer and infection with human immunodeficiency virus and patients with ventilator associated pneumonia. There are intrensic bacteria in the mouth that are associated with two major oral diseases, caries and periodontal diseases **(10).**

In a survey done in the United States from 2011 to 2014, there is about one in each four adults aged from 20 to 64 years and one in each six adults aged 65 years or older had untreated mouth cares. the prevalence of periodontitis in the United States which is reported in the National Health and Nutrition Examination Survey from 2009 to 2014, was 42% among adults 30 years or older **(5).**

The oral cavity containing most types of vast flora in the all human body and is the main entrance port for two major systems which considered vital to human function and physiology, the gastrointestinal system and respiratory system. Also a specific pathologic condition in the oral cavity such as periodontitis which is an inflammation of the periodontal attachment of the teeth and the alveolar bone can be present in the oral cavity. Infection organisms which arise from peridonditis can affect many body systems and salivary gland secretion also **(21).**

Oral bacterial infection can also be associated with many effects in body systems. It also can affect salivary flow by many mechanisms. Salivary flow reduction is a condition that affects oral health. Its prevalence is not published in adults and there is no known treatment for increasing the salivary flow rate. Reduced salivary flow is related to dental caries and mouth bacterial infections which are considered the most common oral disease. Salivary flow rate is reduced in people with inadequate food intake and subsequent insufficient nutrition carried to the salivary glands **(6).**

The prevalence of low salivary flow rate was ranging from 10.9 to 17.8% and 17.3 to 22.7%, respectively and the reduced salivary flow rate in adults was related to caries and oral infections **(6).**

There is lack in the published data about effect of mouth bacterial infection on saliva flow. So this study tries to address this issue.

Aim of the Work

To improve the medical care service provided to patients suffering from oral bacterial infections.

Objectives of the Study

This study will examine the relationship between salivary flow rate and oral bacterial infection in adult

population.

Research Question

Does mouth bacterial infection can affect the stimulated salivary flow rate?

HYPOTHESIS

Null hypothesis

Bacterial mouth infections has no effect on salivary flow rate

Alternative hypothesis

Bacterial mouth infections associated with reduced salivary flow rate

Research Design and Methods

ý Study Design:

Cross sectional study will be conducted from November 2019 to January 2020

ý Study Setting:

The study will be conducted at the outpatient of private clinics at Al-Hilla City.

ü Target population:

The target population will be adult patients who suffering from bacterial mouth infections at Al-Hilla City.

Inclusion Criteria:

Adults aged from 18 to 65

Adults Suffering from bacterial mouth infections

Exclusion Criteria:

Patients who refuse to participate in the study.

Patients suffered from debilitating disease which can affect saliva as diabetes mellitus.

Sampling

Frame: patients with bacterial mouth infections who fulfilled the inclusion criteria will be enrolled in this study.

Method: systematic random sampling

Sample Size: The sample size was calculated and about 100 patients.

Data Collection Tools

Data about socioeconomic characteristics and medical history will be fulfilled. Oral examinations will be done.

Saliva samples un-stimulated will be collected at 7 AM and 12 PM and salivary flow rate, pH, and viscosity will be assessed.

Salivary flow will be categorized to:

Very low salivary flow rate was below 0.1 mL/min

Low 0.1-0.2 mL/min

Normal > 0.2 mL/min

Data Management:

The data was collected, coded and entered into the computer via Excel 2018 program. SPSS program version (21) will used for data analysis. Data will analyze and presented as numbers and percentages using tables and graphs with the CI at 95%. P value <0.05 were used as the limit of statistical significance.

Ethical Considerations:

· Approval of the responsible authorities was obtained from Al-Mustaqbal University College.

· Participant was informed with the aim of the study and its benefit to them as it could help in improving the medical care provided to patients with oral bacterial infections.

· Written informed consent was obtained from the participant.

· Ensure the confidentiality of data collected, and that no data were going to be used outside this study without personal approval.

· The researcher's phone number was provided to the participant for any enquiries.

· The participant had the right to withdraw from the research at any time or even refuse to participate

from the beginning with no effect on the decisions taken for the plan of the management.

Budget

Table 1: The budget of the study was offered by the researcher.

Item	Estimated Costs
Data Collection	2000 D.
Data Management	500 D.
Preparation of final book and presentation	500 D.
Others (Transportation, Printing, Internet access … etc.)	200 D.
Total	3200 D.

Time Table

Table 2: Time needed for preparation of the thesis:

Duration/weeks	1-3	4-6	7-8	9-10	11	12
Protocol Preparation						
Literature review						
Data collection						
Data analysis						
Editing						

Statistical Analyses

Statistical analysis was done by using SPSS version 20 in which, mean and standard deviation were used as descriptive statistics and analysis of variance with LSD for comparison between groups. P value ≤ 0.05 regarded significant.

Results

According to socio-demographic characteristics, it is founded that the number of males were 30 and the females were 70 with the median age of 45 years, the age ranged from 18 to 65 years. 60% of the sample population was from urban areas and 40% were from rural areas. From the studied 100 patients with mouth bacterial infections, about 17% of them had very low salivary flow rate and 40 % had low salivary flow rate and the remaining 43% had normal salivary flow rate. Multiple logistic regression analysis revealed that very low salivary flow was common in females, obese, above 45 ages and with low socioeconomic status.

Table.3. basic characteristics of all patients

Baseline characteristics	N/value	N/ Value
Female	30	
Male	70	
Urban	60	
Rural	40	
Age (years, median)	45	

Table.4.Classification of salivary flow rate

Salivary flow rate	**N**
Very low salivary flow rate	17
Low salivary flow rate	40
Normal salivary flow rate	43

Discussion

This study revealed that about 17% of patients with mouth bacterial infections had very low salivary flow rate and 40 % had low salivary flow rate and the remaining 43% had normal salivary flow rate which is inconsistent with [6] .who founded that the prevalence of salivary flow rate in dental cares patients was very low and low in 17.8% and 17.3 respectively. This may be due to low prevalence of mouth bacterial infection in Flink sample population [14].

This study is inconsistent with [19] .who mentioned that there were no significant differences in saliva flow rate between dental caries and infection calculus and non-calculus groups but it is consistent with [22] who found low saliva in cares.

This study revealed that salivary flow rate is more in females who is obese which is consistent with the study of [16] who found that The obese patients exhibited higher number lower salivary flow rate of the stimulated whole saliva ($P < 0.001$) which may prove the effect of obesity on oral health [13].

It is founded that patients with poor oral hygiene were about more than eight times more likely to suffer from obesity than patients with good oral hygiene which may be related to bacterial inflammatory cytokines in the infected saliva which may be related to disturbances in the metabolism as discussed by [15,18].

This study revealed that low salivary flow rate is mostly in low socioeconomic females with malnutrion which is inconsistent with [21] and [20] who found that there is no correlation between salivary flow rate and nutritional status [2].founded that there is a relation between salivary flow and socioeconomic status.

Conclusion

Mouth bacterial infection is a prevalent disease and has a negative effect on bacterial flow rate as it decreases the flow rate and this flow rate reduction is associated with low socioeconomic status and obesity

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Histogenesis of Skin in Fetal Period of Rabbits

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Abstract

This study was conducted on skin samples from New Zealand white rabbits during the period E15 days prenatal to E25. to investigate the histomorphological development of the skin, hair follicle, Sebaceous glands.

Early in the fetal life (E15) the epidermis is in the form of simple squamous epithelium or stratified Non-keratinized squamous epithelium of two or more layers, depending on the region studied, while the dermis is in the form of loose connective tissue composed of mesenchymal cells in a loose and irregular shape. (E20) prenatal, the epidermis is stratified squamous epithelium non-keratinized composed of more than three layers including the periderm, the intermediate layer and the basal layer in all regions, except Fingered hand and foot that appearance keratinized squamous. the cells of the mesoderm begin to condense under the basal layer, indicating the beginning of the formation of hairs placodes. Stratification became evident in(E25) of prenatal with a marked keratinized and extension of hair follicles in the dermis, accompanied by the beginning of the development of sebaceous glands in the form of a group of cells. During our study of the development of the skin of the New Zealand rabbit before birth, we could not notice any sweat glands in the examined skin samples.

***Key words**: epidermis, dermis, hair follicle, sebaceous gland, sweat gland.*

Introduction

Rabbits are laboratory animals that are used as an experimental sample for research [(1)]. The skin is the largest member in the body, which acts as a permeable barrier between the external environment and the body allows the exchange of air, heat and liquids containing a substance of very little molecular weights [(2)]. The skin consists of two complex layers, the epidermis and the dermis. The epidermis has cells that are in various stages of differentiation, as cells emigrate from the basal layer to the superficial where they become cornify. The dermis is made up of connective tissue that contains blood vessels, lymph, nerves, sebaceous glands, hair follicles, and sweat glands [(3)]. indicated that the development of skin layers and appendages mainly arises in the prenatal stage with few growth events occurring in the postnatal period[)4)]. Epidermis development begins from a single layer of the ectoderm to a multilayered keratinized [(5)]. The dermis produces Skin annexes such, sweat glands, sebaceous glands, and hair follicles. Dermis have multiple embryonic origins [(6)] [(7)] [(8)]. Regardless of the origin of the dermis, the development of the skin and appendages requires interaction between mesenchymal cells and epithelial cells [(9)] [(10)]. In mammals, the development of hair follicles is divided into three phases: a- hair placode genesis b-hair follicles organogenesis c-cytodifferentiation in the future [(11)12)).]

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Material and Method

The study was conducted on twenty-eight embryos of New Zealand white rabbits obtained from the College of Biotechnology / Al-Nahrain University, divided into four groups (E15, E20, E25). The age of the embryos was estimated according to the first day of Intercourse, which is considered the first day of fetal life. The crown-rump length(CRL) of the fetus was measured prenatal only [(13)]. Female rabbits at different stages of pregnancy

E15, E20 and E25 were anesthetized, dissected, and embryos extracted. Embryos were washed with 0.9% saline (Nacl) and fixed in 10% with formalin solution for 2-5 days. Skin samples taken from different region and embryos at E15 are dehydrated by graduated chain from ethanol After that, the samples (skin and fetus at E15) are clearing by transferring them to xylene for 5-15 minutes, depending on the thickness of the skin. Finally, the specimens are infiltered in melted wax at a degree of 58-60 ° the molten wax is poured into special molds and the samples are placed vertically and horizontally to prepare paraffin blocks, the blocks are left to harden and ready for cutting by microtome device with a thickness of) 5µm (. It should be noted that the embryos at the age of E15 were completely sliced. Finally stained by hematoxylin eosin [14(, mason trichrome [15] and Verhoeff stain [(16. And studied under light microscopic.

Result

Embryonic Age E15: early fetal life, the epidermis consists of a single layer of simple squamous cells called periderm in all regions of the body, (fig1a, b) except for the neck and upper lip, which appears to be made of stratified squamous epithelium (two layer or more) (fig1c, d), The cells of the basal layer are cubic while the surface layer is squamous cells. The dermis is a loose connective tissue made of mesoderm cells loosely without any special pattern of arranging.

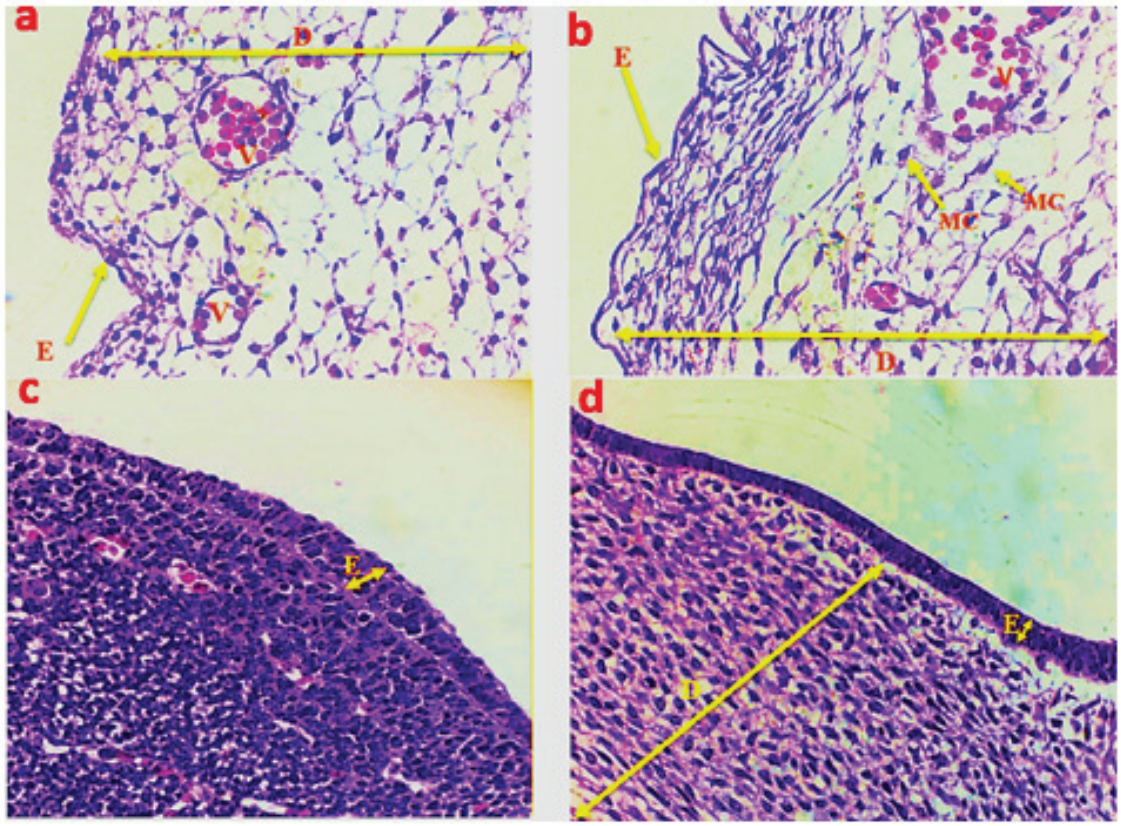

Fig 1: panel a, micrograph of skin of a rabbit fetus from the abdominal region at E15 shows the developing epidermis (E), the dermis (D), the blood vessels (V). panel b, micrograph of Skin of a rabbit fetus from the dorsal region at E15. It showed the epidermis as simple squamous epithelium (E), mesoderm cell (MC), and dermis (D). panel c, micrograph of Skin of a rabbit fetus from the neck region at E15. The epidermis is shown as a two-layer squamous stratum epithelium (E), panel d, micrograph of Skin of a rabbit fetus from the upper lip region atE15. The epidermis(E) shows up as a squamous stratified epithelium consisting of more than two layers. (H&E.X400).

Embryonic Age E20: Most regions the stratification of the epidermis is more clearing where the epidermis is stratified squamous non-keratinized epithelium composed of more than 3 layers, (fig 2 a) except Fingered hand and foot that appearance keratinized squamous epithelium(fig2b). the upper layer is called the periderm its cells are flat, the intermediate layer is oval-shaped cells with a round nucleus while the basal layer is cuboid to columnar cells. The columnar cells of the epidermis cluster to form hair follicle buds that down growth toward the dermis in all areas(fig2c). Mesenchymal cells are condensed under the epidermis with many blood vessels in the dermis. In addition, the mesodermal cells have begun to differentiate into muscle cells(fig2d).

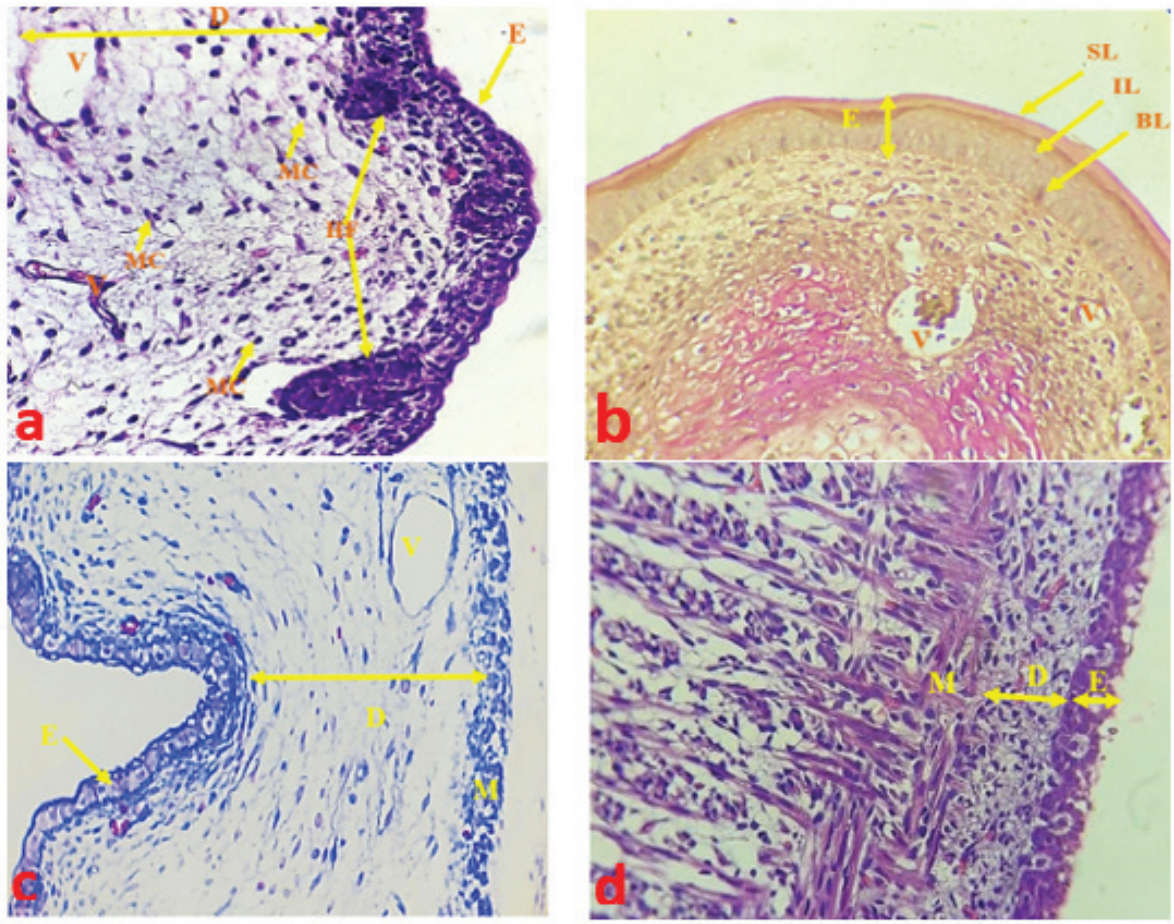

Fig2: panel a, micrograph of Skin of rabbit fetus from the abdominal region at of E20. It shows hair follicles (HF), epidermis (non -keratinized) (E), dermis (D), blood vessels (V), and mesenchymal cells (MC). (H& E, 400X). panel b, micrograph of Skin of a rabbit fetus from of the finger of the hand at E20. The surface layer(keratinized) (SL), the middle layer (IL), the basal layer (BL), epidermis (E). (Verhoff's stain, 400X). panel c micrograph of Skin of rabbit fetus from the dorsal region at E20. It illustrates the epidermis (E), muscle (M), dermis (D). (Masson Trichrome stain, 400X). panel d, micrograph of Skin of a rabbit fetus from the head region at E20. shows stratification of skin, epidermis (E), dermis (D), and muscle (M). H&E stain, X400).

Embryonic Age E25: The epidermis is a keratinized stratified squamous epithelium in all regions, at this stage it is distinguishing irregular basement membrane and extension of the hair follicles inside the dermis without the protrusion of the hair shaft on the surface of the epidermis (fig3 a, b). The main feature at this stage is the differentiation and development of the sebaceous glands as a group of cells from the upper third of the hair follicle (fig3c, d).

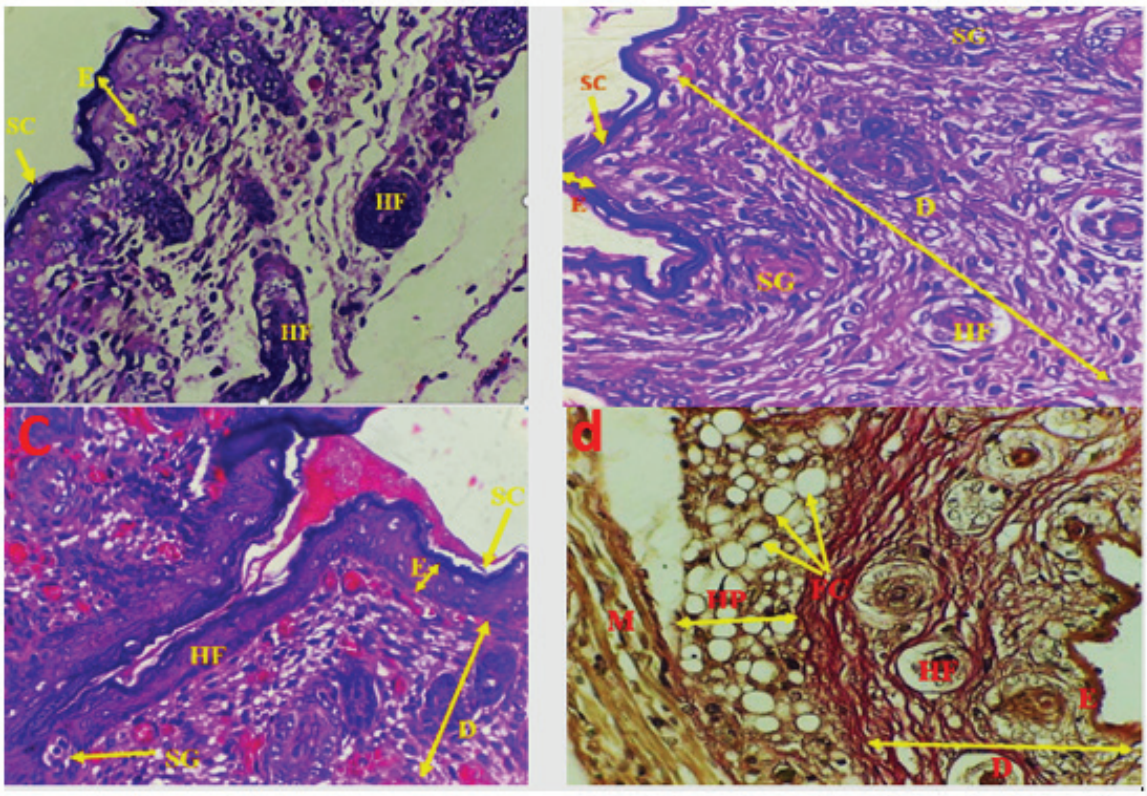

Fig3: panel a, micrograph of Skin of rabbit fetus from the thorax region at E25. Explains development stratum corneum (SC), hair follicles (HF), dermis (D). (H&E stain, 400X). panel b, micrograph of Skin of a rabbit fetus from the head region at E25.showed extension of Hair follicles (HF) in the dermis (D), stratum corneum (SC), epidermis (E) the sebaceous glands (SG). (H&E stain, 400X). panel c, micrograph of Skin of the rabbit fetus from the dorsal surface region of the foot at age E25. Demonstrates the development of the sebaceous gland adjacent to the hair follicle (HF) with an increase in the thickness of the stratum corneum (SC) of the epidermis (E). (H&M, 400X). panel d, micrograph of Skin of a rabbit fetus from the abdominal region at of E25. shows Skin stratification, epidermis (E), dermis(D),hair follicles (HF) cells (Verhoeff stain, X400).

Discussion

Day E15 The epidermis consists mainly of a single layer of simple squamous cells) periderm) in most areas of the body except for the neck and upper lip, in which the epidermis is composed of stratified squamous epithelium which is basal layer and periderm. These results were confirmation with [17] in rabbits, reported that epidermis at E15 consisted of a single layer in most regions of the body, the epidermis in a few areas consisted of a double layer that could be distinguished into a basal layer and periderm. While in goat embryos, the surface epithelium on day E23 is mainly consist of only one layer flat cells [18(. Also in rats, the epidermis was a double layer of cells at day E12[(19.

During the results of present study, mesenchymal cells appear loosely arranged at E15 without any distinction between the reticular layer and the papillary layer with the presence of many blood vessels. Similar results were reported by [20(Mesenchymal cells are found in few number in the dermis between E10-E15. Note

also that the increase in the number of mesenchymal cells in the dermis is proportional to the development of neovascularization. accordingly, explained [21] in human fetus, dermis was differentiated from non-specific mesenchymal cells, and the distinction between the layers of the compact dermis became gradually recognizable.

At embryonic age E20 the stratification of the epidermis becomes more evident in some areas of the body where epidermis has consisted of 2-3 layers. These results were acceptable with observation of [17[in rabbit, they mentioned that stratification of the epidermis has become a common feature of E21, it consists of superficial, intermediate and basal layers everywhere. Previous results are in agreement with [22] in guinea pig fetus, indicated that the epidermis in the dorsal region consisted of three layers including the periderm, intermediate layer and basal layer at day E40 before birth. While the results were contrasted toward [[23in the rat embryos, the epidermis was composed of three layers on the day at E17 of pregnancy including the basal layer, spinal layer and granular layer respectively, epidermis appearance a lot similar mature skin at E19.

In addition, at E20, the mesoderm cells collect below the epidermis and this event is followed of the initiation of formation of the hair follicle buds by clustering of the columnar cells of basal layer of epidermis and down growth in the form of invaginations into dermis. This was supported by [20[those who reported that the stimulation of hair primordia to initiate by the aggregation of mesenchymal cells with the capillaries under the epidermis and the rapid differentiation of the epidermis, including the down growths into dermal, led to the development of hair primordia in rabbit. It is widely agreeable that a coordinated chain of interactions occurs between mesenchymal- epithelial interactions necessary for hair follicle formation. [24[. While in mice, the morphogenesis of primary hair follicles begins at about E14.5 days [25[.in rats, begin formation hair follicle buds at E16[19[.in Dog, it is possible to observe the initial formation of a hair bud in the embryo at E44 before birth. These buds are formed as a result of the proliferation and division of the basal cells of the epidermis towards the mesenchymal cells [[26. In humans, hair follicle development is observed early at 14 weeks old fetal [[27. This variance in the development of hair follicles is due to the difference in the periods of pregnancy between species.

in present study, at E25 epidermis was 4 layered in most areas of the body. superficial layer was keratinized. These findings were concurrence with of [20[indicated exfoliate and degradation of the periderm at age E25-26 and this related with the differentiation of the stratum corneum of the epidermis in rabbits. Also, in the guinea pig explained [22] the epidermis at E60 consist by three layer, in which the corneum layer is describe by flatty dead cells without nucleus, filled with keratin, composing thin keratin surface stratum. While in rat fetus, early epidermis maturation was evident by the appearance of keratinized cells at E20[[19.

At E25, Hair follicles begin to growth and elongated inside into dermis, with the development of the sebaceous gland observed for the first time at this stage. These results were similar to a study conducted by [17] in rabbit, that observed the development of the sebaceous glands as growth from the hair follicles beginning at E25 before birth. While the previous results contradicted [28], who indicated that the sebaceous glands did not develop at E25, but the sebaceous glands were seen at E0 day after birth as a group of poorly developed cells in the papillary region of the upper lip connected to hair follicles in all areas of the body in rabbit. In dog's fetuses, also formation of sebaceous glands associated with hair follicles was not observed at day E44 of gestation[26)]. Accordingly, in rats, the sebaceous gland develops as a growth from the outer root sheath of the follicle at the during postnatal stage [19)] Moreover, the present results were consistent with the study conducted by [29] in pig, where they reported that the appearance of sebaceous glands was closely related to the development of hair follicles.

Finally, it can be **conclusion** that the development of the rabbit's skin layers and its appendices occurs rapidly and mainly during the prenatal stage. The interaction between the dermis and the epidermis cells might directly have related to the development of hair follicles.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER

in Iraq

Conflict of Interest: None

Funding: Self-funding

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Molecular detection of *Escherichia coli* Cause Urinary Tract Infections among Pregnant Women at Thi-Qar province, Iraq

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Abstract

The prevalence of antibiotic resistance in Enterobacteriaceae has increased sharply in recent years. Extended-spectrum ß-lactamase-producing Enterobacteriaceae include *Escherichia coli* has become especially common. Although traditionally linked to risk factors such as prior hospitalization and antibiotic use, these bacteria have become increasingly recognized in the community, especially as pathogens in urinary tract infections (UTIs). In this study urine samples from 150pregnant women clinically diagnosed with UTIs were used for Gram staining, culture, API 20 E and singleplex PCR. Singleplex PCR was performed with primers targeted to chuA and yjaA genes and anonymous DNA fragment TspE4C2 of *E. coli*. The positive singleplex PCR products were identified by presence of 279 bp, 211 bp and 152 bp amplicons of chuA and yjaA genes and anonymous DNA fragment TspE4C2 for of *E.coli*. Conventional methods of Gram staining, culture and API 20E test showed positive result for *E. coli* in 35 (40%) out of 150 pregnant clinically diagnosed with urinary tract infection. PCR detected 24 (27.5%) out of the 35 (40%) samples that were positive for *E. coli*. The majority of UTIs caused by spectrum ß-lactamase-producing Enterobacteriaceae include *E. coli* was acquired in the community, so rapid, specific and sensitive molecular are urgently needed to better prevalence, prevent and treat these infections in Iraq.

Keywords: *UTIs, E. coli, pregnant women, ChuA gene, yjaA gene, TspE4C2 fragment*

Introduction

Urinary tract infections are either acquired from the acquired infections community, or Nosocomial Infections which is the second largest cause of Bacteraemia in hospitalized patients(1). Urinary tract infection (UTIs) is one of the most common diseases caused by different types of pathogens. Women are often more likely to be infected than men for physiological and anatomical reasons of the urinary system(2). Urinary Tract Infections (UTIs) are important and common health problems in developing and developed countries and the major proportion of them are bacterial pathogens. UTIs are an infection caused by the presence and growth of microorganisms anyplace in the urinary tract. It is usually causes by bacteria from the digestive tract, which the reach the opening of the urethra and begin replication to cause infection(3) Pregnancy is one of the factors that increase the risk of urinary tract infection, which is one of the most common complications that women suffer during pregnancy, especially when the injury develops to kidney infections, which leads to complications and risks to the mother and fetus, especially when not treated(4). Urinary tract infection occurs in all ages in pregnant women and in various stages, and the third stage of pregnancy is the most common stage of pregnancy with urinary tract infections(5). Bacteria are the most frequent cause of UTIs and aerobic Gram negative bacilli predominant(6) Urinary tract infections occur when bacteria invade the tissues of the urinary system, which extends from the opening of the urethra to the renal cortex, and that clinical symptoms, diagnosis, treatment, complications and their long-term significance vary depending on the site of the injury and the presence or absence of birth defects in the urinary system(7).

Identification of bacterial isolates is an essential task of clinical microbiology laboratories, and its`

relies on phenotypic tests. Traditional phenotypic identification is difficult and time consuming, while genotypic identification is emerging as an alternative or complement to established phenotypic methods. Typically, genotypic identification of bacteria involves the use of conserved sequences within phyelogenetically informative targets[8]. Phylogeny is the study of the evolutionary history and relationships among individuals or groups of organisms. Molecular phylogeny has also revealed that horizontal transfer plays an important and unexpected role in evolution[9]. The aim of present study is to review the impact of *E. coli* in causes of urinary tract infections in pregnant women, and to study the role of chuA and yjaA genes and anonymous DNA fragment TspE4C2 in the molecular detection of *E. coli* diagnosed with urinary tract infections.

Materials and Method

Mid-stream urine samples were obtained from pregnant women clinically diagnosed with urinary tract infection in Al-Hussain Teaching Hospital, Thi-Qar, south of Iraq for a period from March to July 2020. The samples (150) of urinary tract infections were investigated. Patients' ages ranged from 18 to 42 years.

Identification of the bacteria

Urine samples were obtained from150 pregnant women clinically diagnosed with urinary tract infection and directly processed for bacterial isolation and identification using standard methods according to[10]. The urine samples were cultured on blood and MacConkey agars as an enrichment and deferential media for *E. coli* from other Enterobacteriaceae members and incubated aerobically at 37°C for 24 hrs. After that the bacterial isolates from positive samples were activated using brain heart infusion broth and incubated at 37°C for 18 hrs. Gram stains were performed. API 20 E was used as a further conventional diagnosis for the *E. coli* isolates from other Enterobacteriaceae isolates.

DNA extraction.

DNA was extracted from the isolates using Quantiphore® Bacterial DNA Extraction spin kit according to manufacturer's instructions (Anatollia, Turky)

The primers for ChuA and YJa genes and anonymous DNA fragment TspE4C2 of E. coli as the target genes for this study were selected according to [11].

Table 1: Primer name, sequence and expected product size of ChuA and YJa genes and anonymous DNA fragment TspE4C2 of *E. coli*

Primer name	sequence (5'→3')	Size product
chuAF	GACGAACCAACGGTCAGGAT	279bp
chuA R	TGCCGCCAGTACCAAAGACA	
yjaA F	TGAAGTGTCAGGAGACGCTG	211bp
yjaA R	ATGGAGAATGCGTTCCTCAA	
TspE4C2 F	GAGTAATGTCGGGGCATTCA	152bp
TspE4C2 R	CGCGCCAACAAAGTATTACG	

Table (2):- PCR conditions of chuA, yjaA and TspE4C2.

PCR step	Temp. °C	Time	Repeat
Initial Denaturation	94	3mim	1
Denaturation	95	30sec	
Annealing	4	30sec	35cycle
Extension	72	5min	
Final extension	72	5min	1

Results

The result of conventional methods showed positive result for *E. coli* in 35 (40%) out of 150 pregnant women clinically diagnosed with urinary tract infection. PCR detected 24 (27.2%) out of the 35 (40%) samples that were positive for *E. coli* samples that were positive using Gram staining, culturing on MacConky and blood agar, The result of ChuA and YJa genes and anonymous DNA fragment TspE4C2 amplification by singleplex PCR using ChuAF, ChuAR, YJaF, YJaR, TspE4C2F and TspE4C2R primers, respectively. Pregnant women (150) clinically diagnosed with urinary tract infection, a positive result for E. coli was detected in 24 (27.5%) out of the 35 (40%) samples who were gave positive result by the conventional methods. The singleplex PCR products and 50-1500 bp DNA ladder were resolved by electrophoresis, signleplex PCR product were loaded on 1.5% agarose gel and run at 70 volt/ cm for one hour. The gel was stained with ethidium bromide solution (0.5 µg/ ml) for 15-30 minutes.

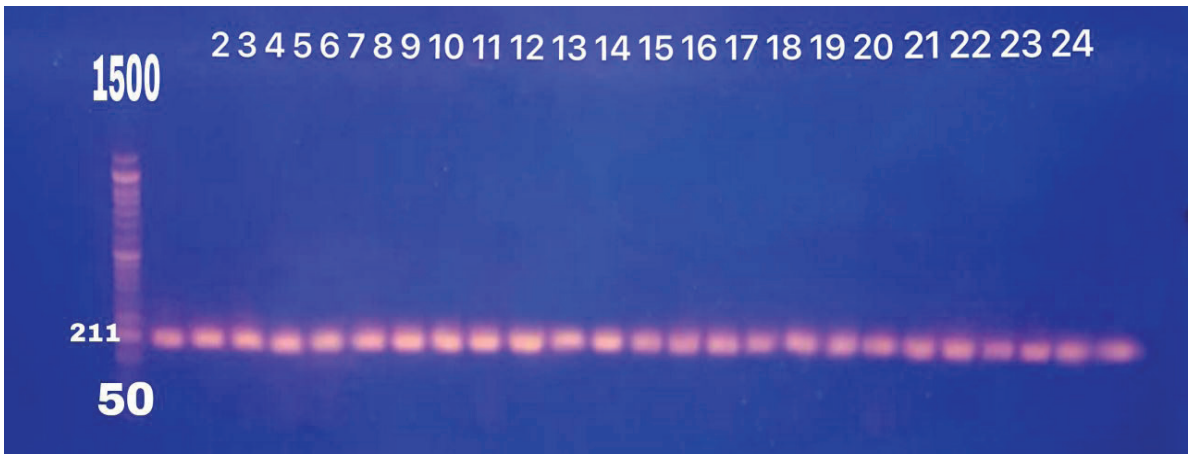

Figure 1: Gel electrophoresis of singleplex PCR products 211 bp of YJa gene for *E. coli* isolates using 1.5% agarose gel at 7 volt/ cm for 1hour. Lane 1: 50-1500bp DNA ladder, lane 2-24: PCR products of YJa gene.

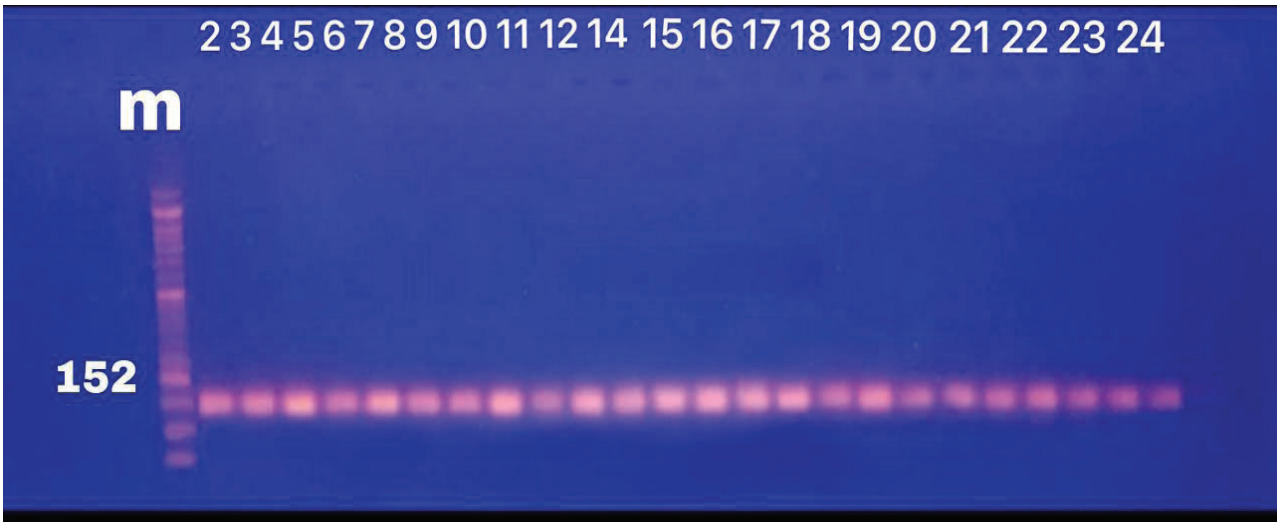

Figure 2: Gel electrophoresis of singleplex PCR products (152 bp) of TspE4C2F DNA fragment for E. coli isolates using 1.5% agarose gel at 7 volt/ cm for 1 hour. Lane 1: 50-1500 bp DNA ladder, lane 2-24: PCR products of TspE4C2F DNA fragment

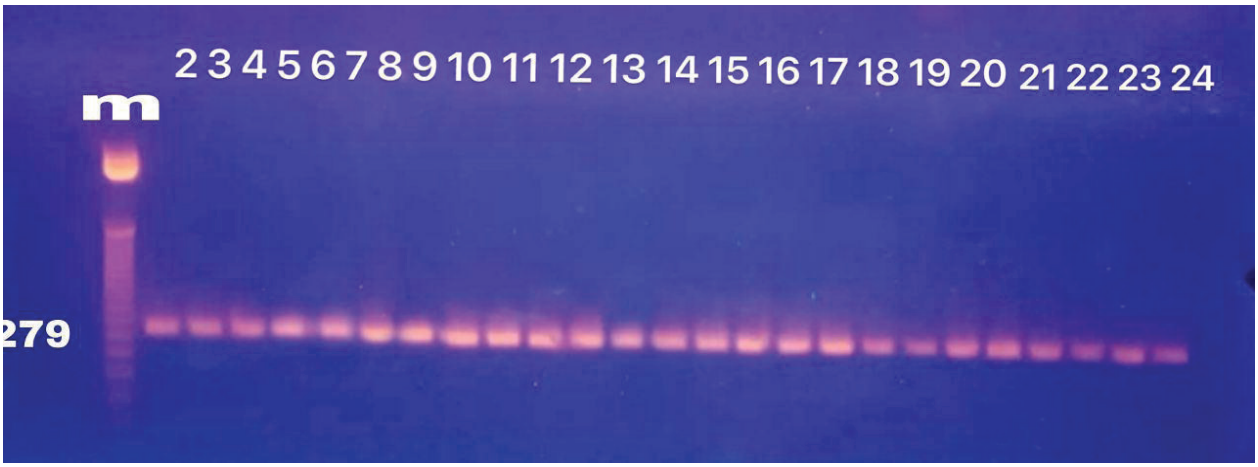

Figure 3: Gel electrophoresis of singleplex PCR products of ChuA gene (279bp) for *E.coli* isolates using 1.5% agarose gel at 7 volt/ cm for 1 hour. Lane 1: 50-1500 bp DNA ladder, lane 2-24: PCR products of ChuA gene.

Discussion

The results showed that *E. coli* was the most isolated bacteria that causes UTIs in pregnant women. these results came close to the results obtained by the researcher[11] showed that E. coli was the most common causative agent as a cause of UTI in pregnancy (46%), also came close to the result recorded by[11] study on adult patients, the largest isolation rate of E. coli was 31.5%. and in the study of the researcher [12] that have conducted on pregnant women with diabetes, the incidence of E. coli was(37.7%.). Also these results agree with data from various medical records showed that the epidemiology of the pathogens of UTI is represented by E. coli the most common causative agent which was 51.70%, In Iraq[13], have found that the most prevalent bacteria in the urinary tract infections were E. coli. Another study by[14] on UTIs, revealed that the E. coli was (52.2%) which represent the most common bacterial isolates .The benefits of molecular methods are more sensitive, more qualitative for results, materials available, but the drawback of molecular methods is costly. These explanations made molecular methods relatively more accurate than conventional methods [15]. The data of present study agrees with the study conducted by [16] that confirms the efficacy of the PCR

assay compared to conventional methods of diagnosis in the clinical setting [17].

In conclusion, this study has demonstrated the efficacy of the ChuA and YJaF genes and TspE4C2F DNA fragment as molecular markers to detect of the *E. coli* in pregnant women with urinary tract infection offers the possibility of reducing the risk to the mother and fetus, also the present study referred to possibility of using the singleplex PCR-based test as a rapid, specific and sensitive method to prevalence the E. coli

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Molecular Identification of *T. mentagrophytes* and *M. canis* Isolated from Children Infected with Tinea Capitis in Basra Governorate by Using AP-PCR Technique

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Abstract

The current study included molecular identification of dermatophytes causing Tinea capitis in children in Basrah governorate, including T. Mentagrophytes&M. canis, using AP-PCR or RAPD –PCR polymerase chain reaction technique. By using EZ-10 Spin Column Fungal Genomic DNA Mini –Preps Kit used to extract DNA from dermatophytes fungi, where DNA was obtained from these two dermatophytes fungi. The DNA bands appeared clearly after the electrophoresis of the DNA extract showed the results of DNA amplification .

The efficiency of the primeres used in the diagnosis of these two types of dermatophytes, called: random primeres OPAA11, OPAA17, OPU15, OPD18, was obtained by the positive results obtained when amplified using these primeres.Used for both dermatophytes The results obtained by using OPAA17 showed success in DNA amplification - obtained from M.canis and T. Mentagrophytes, with six bands for M.canis. As for T. Mentagrophytes, the number of bands obtained was 13, and the amplification results obtained using OPAA11 showed the efficiency of this primer.

Five bands were obtained when used to amplify the DNA of the dermatophytes. While seven bands belonged to T. Mentagrophytes, The amplification results of OPU15 indicated that there were six bands for M.canis, while only one bande for T. Mentagrophytes showed the results of amplification using OPD18 used in diagnosis of dermatophytes to four bands for M.canis while the number of bands for T. Mentagrophytes has ten bands. This method for identifecation is fast and has a high diagnostic efficiency of dermatophytes, as the diagnosis based on genetic characteristics is more accurate than resulting on phenotypic characteristics .

Keywords *: tinea capitis , dermatophytes , molecular identification*

Introduction

The infection of dermatophytic fungi in the head is known as tinea capitis.This dermatophytes affects the scalp and hair[(1)], also known as scalp ring worm. Although it is observed at all stages of life, the incidence of males is higher than females [(2)], it is one of the most prevalent disease in the world. Which is the higher percentage in the chilidren in the Poor Communities [(3)].

There are a number of laboratory methods that can diagnose dermatophytic fungi leading to skin diseases, including tinea capitis. The methods currently used in the diagnosis of dermatophytic fungi are based on phenotypic charachteres [(4)]. The culture is then carried out on the media [(5)].

It is clear from the above that for the diagnosis of dermatophytic fungi that causing to tinea capities caused by dermatophytic fungi requires a microscopic examination method was found that this method is inaccurate as it may give false results false(False negative results)by more than 15% of cases [(6)]. Also the culturing of some type of dermatophytes that slow-growing species such as ***T.verriucosum***, may take several weeks after which several diagnostic tests are required, and usually require different culture media [(7)].

Therefore, the development of techniques used in the diagnosis of this type of dermatophytes, such as molecular techniques based on the diagnosis of genetic differences in pathogens and not on phenotypic Morphological characteristics, as these methods are more accurate and faster than traditional methods based on phenotypic that require long periods of time[8]. Methods based on geneticMorphological characteristicsare less affected by external conditions affecting phenotypic Morphological characteristics, and these methods are done using DNA amplification techniques. These techniques are PCR, including AP - PCR (Arbitrarily primed PCR) or Random ampli. fied poly morphic DNA or RAPD [9, 10]. This technique accelerated the diagnosis process and the ability to diagnose skin fungi increased significantly [11]

PCR Techniquehas helped in the diagnosis of dermatophytic fungi in a very fast and accurate manner. Random amplification (RAPD) has added a great benefit through which it can be diagnosed without having to know the exact sequence of the area occupied by the gene. DNA has a special sequence, the random amplification method (RAPD) in which different types of microorganisms can be diagnosed accurately and quickly [12,9].

This method of amplification is carried out using a short random primer, usually 10 nucleotides. However, the exact technique in which this process is performed is not fully understood (11), and this method of diagnosis has been used in a number of studies, including in the diagnosis of dermatophytic fungi [13, 14].

Several studies have shown that the use of a type of random primers in the AP-PCR technique can be diagnosed by several types of dermatophytic fungi [15], where one of the types of random primers, the primer((- GAGCCCGACT -3)) Arbitrary primed polymerase chain reaction (AP-PCR) in diagnosis of ***T. mentagrophsytes var. interdigitale*** and ***T. mentagrophsytes var. mentagrophytes*** very quickly by obtaining different DNA bandes for each, and the ease and speed of using AP-PCR technique made it a common method in laboratories for diagnosis of human dermatophytic fungi [7].

In another study, the primer was used to diagnose several types of dermatophytes, including ***T.mentagrophytes***, ***T.rubrum***, and ***T.tonsurans*** [12]. Another study used four primer types (OPAA11). OPD18, OPAA17, OPU15 (AP-PCR) technique. One of these primers can be used to diagnose approximately 25-20 dermatophytes, either at the Species or sub-species levels, by obtaining DNA-specific diagnostic bandes. It was found that when migrating using agarose gel, AP-PCR was found to be a quick and easy technique and a practical method that could be used in the rapid diagnosis of dermatophytic fungi [11].

Materials and Methods

The materials in the diagnostic kit were used to extract DNA from fungi processed by BIO BASIC INC. Canadian known as EZ-10 Spin Column Fungal Genomic DNA Mini – Preps Kit (Ft 82012)Version4.0 ISO9001 Certified.

Isolated fungal colonies were renewed during the study at the age of 10-7 days on the culture of saproid dextrose agar(SDA) with chloromphenicol and cyclohexamide. Then, 12 ml of isopropanol was added to Universal PW Solution and 22.5 ml of ethanol (100-96%) was added to Universal Wash Solution before use as per the manufacturer's instructions. The electrophoreses conducted according to the method of [16].

Table (1) Sequences of nitrogen bases in primers used in the amplification process

Primers	Primers Sequences	Length
OPAA11	ACC CGA CCT G-3 - ′5	10 Base
OPAA17	5-GAG CCC GAC T -3′	10 Base
OPD18	5- GAG AGC CAA C -3 ′	10 Base
OPU15	′5- ACG GGC CAGG T -3 ′	10 Base

The amplification process took place in two cycles, the first cycle lasted 15 minutes while the second cycle lasted two and a half hours as shown below:

Table (2) amplification process for the PCR program used

Stage	Temperature(c)	Time (sec.)	No. of cycles
Denaturation Annealing Elongation	94	60	3
	36	45	
	72	90	
Denaturation	94	30	32
Annealing	36	45	
Elongation	72	90	

Finding

In this study, DNA extraction of dermatophytic fungi was performed for molecular diagnosis to Molecular identification using APR PCR (PCR) or RAPD –PCR. Which are *M. canis* and *T. Mentagrophytes*. The results of this study showed the efficiency of the kit supplied by BIO BASIC INC. Canadian known as EZ-10 Spin Column Fungal Genomic DNA Mini –Preps Kit used to extract DNA from dermatophytic fungi where DNA was obtained from these two dermatophytic fungi. Figure 1 shows the DNA bands obtained.

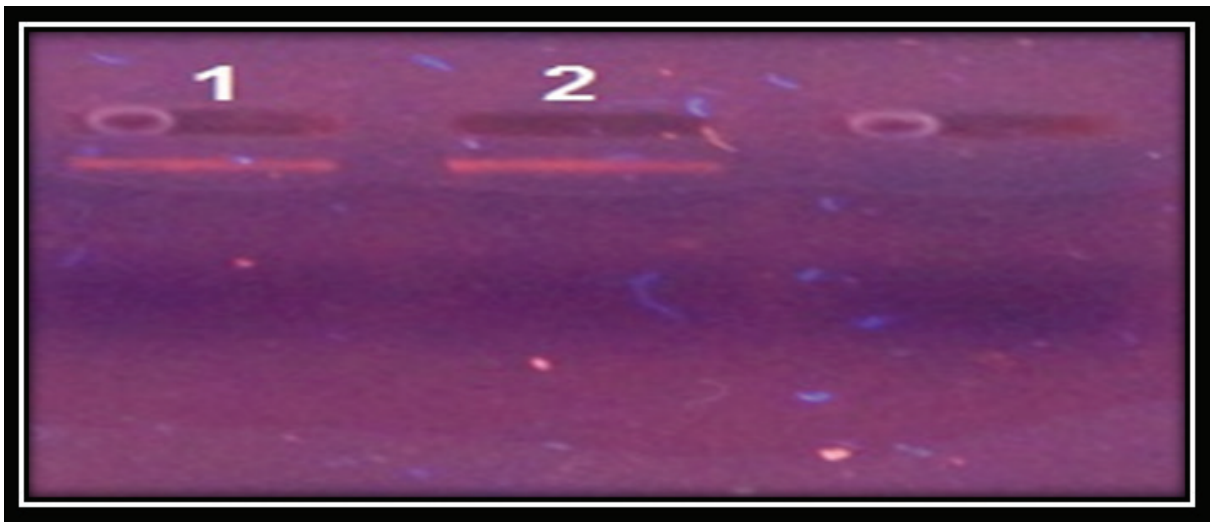
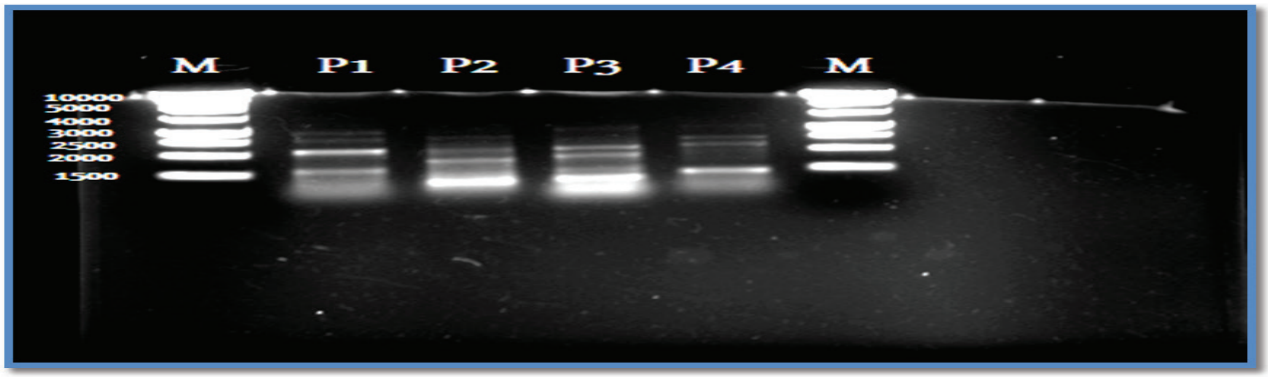


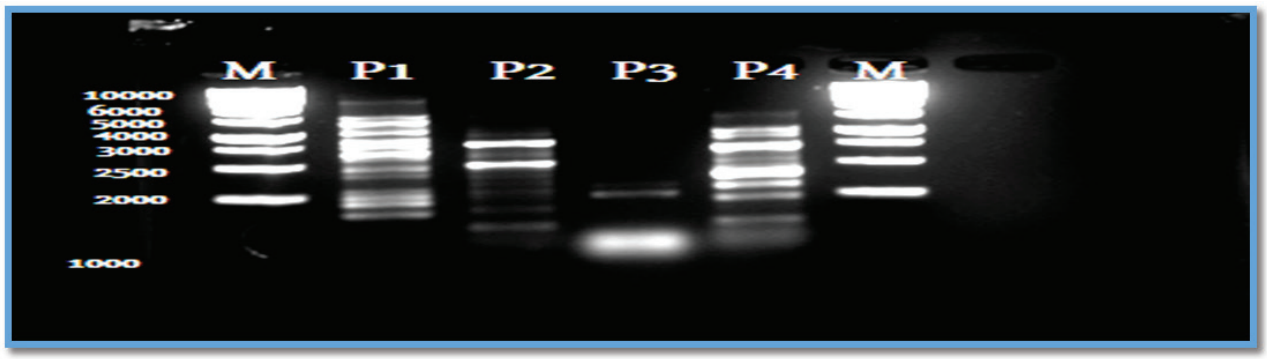
Fig. (1) 0.8 agarose gel electrophoresis of DNA extracted from dermatophytes

1- ***M. canis***, 2 - ***T. Mentagrophytes***

The results of DNA amplification obtained showed the efficiency of the primers used in the diagnosis of these two types of dermatophytes, namely, the primers OPAA11, OPAA17, OPU15, OPD18, through the positive results obtained when amplified using these primers were obtained Different bandes differ depending on the prefixes used for both dermatophytes to be diagnosed and shown clearly in Fig. 2



M. canis



T. mentagrophytes

Fig. (3) 3% agarose electrophoresis results for PCR using four primers: M Ladder (10 000 bp): P1 OPAA17, P2: OPAA11, P3: OPU15, P4: OPD18

The results obtained by using OPAA17 showed success in DNA amplification process obtained from ***M. canis***and ***T. Mentagrophytes***. As for ***T. Mentagrophytes***, the number of bands obtained was 13, as shown in the picture P1 (3) and tables 3 and 4. The amplification results obtained by using OPAA11 showed the efficiency of this primer, where five bands were obtained when used to amplify the DNA of ***M.canis*** while seven bands belonged to ***T. Mentagrophytes*** as shown in Fig. 3 (P2). Tables 3 and 4

The amplification results when using OPU15 indicated six bands for ***M.canis*** and only one for ***T. Mentagrophytes***, as shown in Fig. 2 (P3) and Tables 3 and 4.

The results of the amplification using OPD18 used in the diagnosis of dermatophytic fungi showed that there were four bands of ***M.canis*** while the number of bundles for ***T. Mentagrophytes*** was ten, as shown in Fig. 3 (P4) and tables 3 and 4.

Table (3) Total number of bands and bands size of RAPD technique for *M. canis*

Dermatophyte M.canis												
Bands size bp										Number of bands	Primers	Sequence
				0.7	1.2	1.6	2.1	2.5	3.0	6	OPAA17	1
					1.2	1.5	2.0	2.5	2.6	5	OPAA11	2
				1.0	1.5	1.6	2.0	2.2	3.0	6	OPU15	3
						1.2	1.5	2.5	3.0	4	OPD18	4

Table (4) Total number of bands and the size of the bands technique RAPD primers *T. mentagrophytes*

T. Mentagrophytes															
Bands size bp													Number of bands	Primers	Sequence
0.8	0.9	2.0	2.1	2.8	2.5	3.0	3.2	3.5	4.0	4.5	5.0	8.0	13	OPAA17	1
						0.9	1.7	1.9	2.3	2.0	2.5	3.0	7	OPAA11	2
												1.4	1	OPU15	3
			0.8	0.9	1.0	1.2	1.5	1.7	2.0	2.5	2.7	3.2	10	OPD18	4

Discussion

The results obtained in this study show the efficiency of the kit used in the extraction of genetic material of dermatophytic fungi where the results showed when examined using ultraviolet radiation to the presence of DNA bundles clearly for both dermatophyte used in the study, ***T. mentagrophytes*** and ***M. canis***.

The primer used OPAA11, OPAA17, OPU15, OPD18 have shown high efficacy in the diagnosis of these dermatophytic fungi by obtaining DNA bands that appeared visible under ultraviolet light. This finding is consistent with the findings of [11] which diagnoses 25-20 species of dermatophytes using one of these four primers using AP-PCR technique based on the diagnostic bands that appeared with these types of primers when electrophoresis in agaros gel, this method is a fast and highly diagnostic method for dermatophytes. Depending on the characteristics A number of researchers have used this technique to diagnose pathogenic fungi, including dermatophytes, in their studies [14, 13].

When the first primer OPAA17 was used to diagnose ***M.canis***, the results indicated that there were six bands, three of which were identical to those of [11]. The size of these three bands is(3.0 ,1.2, 0.7) bp, in addition to three new bundles. This isolation obtained in this study is (2.5, 2.1 and 1.6) bp, and this may be due to genetic variation between the two isolates, as well as when using

this primer for the skin dermatophyte ***T. mentagrophytes*** has shown the results of these In the study of [11], the number of the resulting bands was two (3.5 and 2.8) bp. The two results in the previous study are identical to those reached in addition to new bundles that may be due to genetic mutations and new traits possessed by dermatophyte depending on the time period.

Also, for the second primer OPAA11 for ***M.canis***, the number of bands produced by using this primer was five, two of which are identical to the findings of[11] (2.6 and 1.2) bp. While the use of this primer for ***T. mentagrophytes*** results showed the presence of seven bands while the results of [11] showed the presence of three bands (2.5, 1.9 and 0.9) bp and were identical with the bands obtained in these studying .

The results indicated that when using OPU15 for ***M.canis***, there were six bands, two of which were identical with [11], which obtained three bands using this primer, 2.2 and 1.0 bp. ***T.mentagrophytes*** The results of this study showed that there is one band which is identical with one of the bands obtained in the study of [11] which is (1.4) bp.

When using the fourth primer OPD18 with ***M. canis***, four bands were found, one of which was identical with [11]. Their study showed that only 1.2 bp was present. As for T.mentagrophytes, the results showed that there were ten bundles when [11] showed that there were three bundles which were all identical to the results of the present study (3.2, 2.0 and 0.9) bp. Variations in the number of bands obtained may be due to differences in the time periods in which these results were obtained, which could result in a genetic variation between the isolates obtained.

while the matching in some of the resulting bands can indicate the genetic similarity between these isolates, indicating the possibility of genetic diagnosis based on the identical bands obtained in the two studies and this confirms the high diagnostic efficiency of AP- PCR and within a short period of time where the diagnostic result can be obtained within 24 hours of the amplification and relaying of the results obtained.

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Isolation of Bacteria and Detection of Cephalosporin Antibiotics Effects among Patients with UTI in al-hilla City

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Abstract

90 urine samples were collected randomly from patients whose visit the Urology department in Murjan Medical City in Al-Hilla in period between April to July 2020 , the patients included 35 males and 55 females and in age between 17-77 years old. The urine samples divided into two parts , the first part using for general urine examination and the second part using for culture and sensitivity . The results of current study appeared to 35 (39%) males among total patients infected with UTI while 55(61%) females among total patients infected with UTI . In field of effects of age on infection the current results appeared in females the infection of urinary tract increase in young age group (17-27 years) in percentage (33%) in comparison with other age groups (20 %) in (27-37 years) , (16%) in (37-47 years) , (14%) in (47-57 years) , (10%) in (57-67 years) and (7%) in (67-77 years) and in males the results appeared increase the UTI with age , (67-77 years) in percentage (32%) in comparison with other age groups, (3%) in (17-27 years) , (9%) in (27-37 years) , (11%) in (37-47 years) , (20%) in (47-57 years) , (25%) in (57-67 years) . the results of API20 test that using for identification of types of bacteria which cause UTI appeared to 34 isolates of E.coli , 15 isolates of Enterobacter spp. , 18 isolates of Klebsiella spp. , 11 isolates of Pseudomonas spp. , 13 isolates of S.aureus and 4 isolates of Acinetobacter spp. bacteria .In field of antibiotic sensitivity the results appeared the effects of third and fourth generations of cephalosporin more than other generations .

Key Words: *UTI , Cephalosporin antibiotics ' toxicity; healty; urine samples*

Introduction

A urinary tract infection (UTI) is an infection in any part of your urinary system — your kidneys, ureters, bladder and urethra. Most infections involve the lower urinary tract — the bladder and the urethra [(18)] . Women are at greater risk of developing a UTI than are men. Infection limited to your bladder can be painful and annoying. However, serious consequences can occur if a UTI spreads to your kidneys [(8)] .Doctors typically treat urinary tract infections with antibiotics. But you can take steps to reduce your chances of getting a UTI in the first place. Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. Although the urinary system is designed to keep out such microscopic invaders, these defenses sometimes fail [(2)]. When that happens, bacteria may take hold and grow into a full-blown infection in the urinary tract. The most common UTIs occur mainly in women and affect the bladder and urethra. Infection of the bladder (cystitis). This type of UTI is usually caused by Escherichia coli (E. coli), a type of bacteria commonly found in the gastrointestinal (GI) tract [(7)]. However, sometimes other bacteria are responsible. Sexual intercourse may lead to cystitis, but you don't have to be sexually active to develop it. All women are at risk of cystitis because of their anatomy — specifically, the short distance from the urethra to the anus and the urethral opening to the bladder. Infection of the urethra (urethritis) [(17)]. This type of UTI can occur when GI bacteria spread from the anus to the urethra. Also, because the female urethra is close to the vagina, sexually transmitted infections, such as herpes, gonorrhea, chlamydia and mycoplasma, can cause urethritis [(4).]

Patients

90 urine samples were collected randomly from patients whose visit the Urology department in Murjan Medical City in Al-Hilla in period between April to July 2020 , the patients included 35 males and 55 females and in age between 17-77 years old .

Methodology

The Urine samples in sterile containers transported to the laboratory directly after the collection and each sample which divided into two parts , the first parts to making general Urine examination to indicate the UTI and the second one to making urine culture , the first part of urine which put in centrifuge in 3000 cycle/ 1 Min for 15 Minutes ,after that the precipitate examined under microscope in 40 power to detection pus and bacteria [20] . While the other part of urine sample cultures directly on nutrient agar and MaCconky agar and incubated for 24 hours ,after incubation period the bacterial colonies identified by Api20 test to detect the species of bacteria in urine sample , the antibiotic sensitivity test done [6].

Statistical Analyses

Statistical analysis was done by using SPSS version 20 in which, mean and standard deviation were used as descriptive statistics and analysis of variance with LSD for comparison between groups. P value ≤ 0.05 regarded significant.

Results and Discussion

1-Patients Sex

In current results showed to 35 (39%) males among total patients infected with UTI while 55(61%) females among total patients infected with UTI , and the results agreed with [10]. and the cause belong to the long of urethra in male longer than urethra in female and the bacteria difficultly reaching to the urinary tract and to other part of urinary system while in female there are easy in reaching and spread to all parts of urinary system [5,6].

2-Age Groups

Table (1): Age group among patients

Age groups	Female No.	percentage	Male No.	Percentage
17-27 years	18	33%	1	3%
27-37 years	11	20%	3	9%
37-47 years	8	16%	4	11%
47-57 years	7	14%	7	20%
57-67 years	5	10%	9	25%
67-77 years	4	7%	11	32%
Total	55	100%	35	100%

In current study table (1) referred to in females the infection of urinary tract increase in young age group (17-27 years) in percentage (33%) in comparison with other age groups (20 %) in (27-37 years) , (16%) in (37-47 years) , (14%) in (47-57 years) , (10%) in (57-67 years) and (7%) in (67-77 years) , and this results showed to decrease of infection with increasing in age , this results agreed with (10). The cause belong to hormonal changes and sexually activation in young in comparison with old female [20]. While the results opposite in males

where the table (2) showed to increase the UTI with age , (67-77 years) in percentage (32%) in comparison with other age groups, (3%) in (17-27 years) , (9%) in (27-37 years) , (11%) in (37-47 years) , (20%) in (47-57 years) , (25%) in (57-67 years) , and the current study agreed with Abraham Soman N. and Miao (2015) . the cause belong to the decrease of immunity and hygiene with age in additional to the chronic disease and some problems in involuntary urination in some old males and prostate infection, each of this reasons help the infection with UTI [10].

3-Bacterial isolates

The current study results referred to differences in genus and species of bacteria which cause UTI, *E.coli* bacteria take the first place in 35% percentage while (15.5%) in *Enterobacter spp.*, (18.5%) in *Klebsiella spp*. , (11.5%) in *Pseudomonas spp.,* (13.5%) in *S.aureus* , (5%) in *Acinetobacter spp.* This current study agreed with [3] .

3-Antibiotic sensitivity

Table (2): Antibiotic sensitivity for First generation

Bacteria	Cefazolin	Cefalothin	Cefaloridine	Cefradine	Cefadroxil
E.coli	-	-	+/-	+/-	-
Enterbacter spp.	+	+/-	-	-	-
Klebsiella spp.	-	-	+	-	-
Pseudomonas spp.	+	-	-	-	-
S.aureus	-	-	-	-	-
Acinetobacter spp.	-	-	-	-	-
Proteus spp.	-	-	-	-	-

In field of antibiotic sensitivity and resistance table (2) referred to using of first generation of cephalosporin antibiotics among all bacterial isolates and the results showed in *E.coli* appear the completely resistance to Cefazolin ,Cefalothin and Cefadroxil antibiotics while moderately sensitivity of Cefaloridine and Cefradine antibiotics .In *Enterbacter spp*.bacteria appeared sensitivity to Cefazolin , moderately sensitivity to Cefalothin and completely resistance to Cefaloridine , Cefradine and Cefadroxil. In *Klebsiella spp*. appeared sensitivity to Cefaloridine while completlt resistance to Cefazolin, Cefalothin, Cefradine and Cefadroxil .

In *Pseudomonas spp*. appeared sensitivity to Cefazolin while appeared completely resistance to Cefalothin, Cefaloridine, Cefradine and Cefadroxil. In *S.aureus* , *Acinetobacter spp.* and Proteus spp. appeared completely resistance to all antibiotics in first generation of cephalosporin . current study agreed with [11,14] .

Table (3): Antibiotic sensitivity for Second generation

Bacteria	Cefaclor	Cefuroxime	Cefotetan	Cefmetazole	Cefprozil
E.coli	+	+	-	-	+/-
Enterobacter spp.	-	-	+	+	+
Klebsiella spp.	-	+/-	-	+	-
Pseudomonas spp.	-	-	-	-	-
S.aureus	-	-	-	-	-
Acinetobacter spp.	+	-	-	-	-
Proteus spp.	-	+	-	+	-

Table (3) referred to using of second generation of cephalosporin antibiotics among all bacterial isolates and the results showed in *E.coli* appear the completely resistance to Cefotetan and Cefmetazole antibiotics while moderately sensitivity of Cefprozil and sensitivity to Cefaclor and Cefuroxime antibiotics .

In *Enterbacter spp.*bacteria appeared sensitivity to Cefotetan , Cefmetazole and Cefprozil , while appeared completely resistance to Cefaclor and Cefuroxime antibiotics . In *Klebsiella spp*. appeared sensitivity to Cefmetazole while completely resistance to Cefaclor, Cefotetan and Cefprozil , and moderately sensitivity to Cefuroxime antibiotic.

In *Pseudomonas spp*. and *S.aureus* bacteria appeared completely resistance to all antibiotics in second generation of cephalosporin. In *Acinetobacter spp.* appeared completely resistance to Cefuroxime Cefotetan, Cefmetazole and Cefprozil , so appeared sensitivity to Cefaclor antibiotic.In Proteus spp. bacteria appeared sensitivity action to Cefuroxime and Cefmetazole antibiotics and completely resistance to other antibiotics in this generation of cephalosporin. current study agreed with [13,14].

Table (4): Antibiotic sensitivity for Third generation

Bacteria	Cefixime	Cefdiner	Cefotaxim	Ceftriaxone	Cefodizine	Ceftazidim
E.coli	++	+	+++	+++	+	+
Enterbacter spp.	+++	+	+	+++	++	++
Klebsiella spp.	+++	++	+	+++	+/-	+
Pseudomonas spp.	++	+	++	+++	++	+
S.aureus	+	-	+/-	+	+	-
Acinetobacter spp.	-	-	+/-	+/-	+/-	-
Proteus spp.	+/-	+/-	+/-	+	+/-	+/-

In current study table (4) referred to using of third generation of cephalosporin antibiotics among all bacterial isolates and the results showed in *E.coli* , *Enterbacter spp*. and Pseudomonas spp. appeared sensitivity action for all antibiotics of third generation of cephalosporin . In *Klebsiella spp*. bacteria appeared sensitivity action for Cefixime, Cefdiner, Cefotaxim, Ceftriaxone and Ceftazidim , while moderately sensitivity to Cefodizine. In *S.aureus* bacteria appeared sensitivity for Cefixime ,Ceftriaxone and Cefodizine antibiotics , and completely resistance for Cefdiner and Ceftazidim , while appeared moderately sensitivity for Cefotaxim . In *Acinetobacter spp.* bacteria appeared completely resistance for Cefixime, Cefdiner and Ceftazidim , while appeared moderately sensitivity for Cefotaxim Ceftriaxone Cefodizine antibiotics . In *Proteus spp.* bacteria appeared moderately sensitivity for Cefixime ,Cefdiner, Cefotaxim, Cefodizine and Ceftazidim while appeared sensitivity for Ceftriaxone antibiotics in third generation of cephalosporin. Current results agreed with [9 ; 14] .

Table (5): Antibiotic sensitivity for fourth generation

Bacteria	Cefepime	Cefquinome
E.coli	++	+
Enterbacter spp.	+	+
Klebsiella spp.	+	+/-
Pseudomonas spp.	+	+/-
S.aureus	+/-	-
Acinetobacter spp.	+/-	-
Proteus spp.	+/-	-

Table (5) referred to using of fourth generation of cephalosporin antibiotics among all bacterial isolates and the results showed in *E.coli* and *Enterbacter spp.* bacteria appeared sensitivity action for Cefepime and Cefquinome antibiotics. While *S.aureus ,Acinetobacter spp. and Proteus spp*. bacteria appeared moderately sensitivity for Cefepime and completely resistance for Cefquinome . in *Klebsiella spp.* and Pseudomonas spp. bacteria appeared sensitivity for Cefepime and moderately sensitivity for Cefquinome antibiotics , current results agreed with [9,12].

Table (6): Antibiotic sensitivity for fifth generation

Bacteria	Ceftobioprole	Ceftaroline	Ceftolozane
E.coli	+	+	+
Enterbacter spp.	+	+	+
Klebsiella spp.	++	+	+
Pseudomonas spp.	+	+	+
S.aureus	+/-	+	-
Acinetobacter spp.	+/-	-	-
Proteus spp.	+/-	-	-

In current study table (6) referred to using of fifth generation of cephalosporin antibiotics among all bacterial isolates and the results showed in *E.coli , Enterbacter spp. , Klebsiella spp. and Pseudomonas spp.* bacteria appeared sensitivity action against Ceftobioprole, Ceftaroline and Ceftolozane antibiotics . While *Acinetobacter spp*. and Proteus spp. bacteria appeared moderately sensitivity against Ceftobioprole and completely resistance against Ceftaroline and Ceftolozane antibiotics among fifth generation of cephalosporin. Current study agreed with [18].

Conclusion

The conclusion in current study summarized in following points

Females infected with UTI more than males .

In Females the UTI decrease with age while in males the UTI increase with age.

E.coli bacteria considered the first causative agent of UTI than other bacteria isolated .

Third generation more effect to treat UTI than other generations among cephalosporin antibiotics.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Iraqi Dentist Response to the Covid-19 Spreading

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Abstract

Introduction: These new generations of virus "COVID -19" now are not the chines nightmare only however soon as speedy as feasible spread all over the world and grew to be the most existence threating health disorder in the world.

Aim: This descriptive research aim to measure the commitment of Iraqi dentists to the Iraqi dental association recommendations at the time of lockdowns during the corona virus infection.

Result: In this study the Iraqi dentists who had participated in it were 169 from all over the country, this descriptive research shows great response of the Iraqi dentists to control infection and reduce the spread of "COVID-19".

Discussion: "COVID -19" is a pandemic disease lead to large changes all over the world, actually dentistry were one of the careers affected by this disease, the dentist during the daily work with dental slow speed handpiece or high speed turbine produce droplets and aerosols which probably are contaminated with the virus "COVID -19".

Conclusion: This study had been showed that the Iraqi dentists have a great commitment toward the society during the period of curfew they had been attend to close their privets clinic in order to reduce the viral spread and control the infection.

Keyword: *COVID-19, infection control, Iraqi dentist, toxicity; patients*

Introduction

In the late December 2019, a number of signs and symptoms of pneumonia infection have been noticed in Wuhan City which is one of a vital chine's metropolis [(1)], The medical sings have been stated fever, cough, and myalgia or fatigue with bizarre chest CT, and different minor symptoms have been sputum production, headache, hemoptysis, and diarrhea[(2,3,)]. Some of the clinical symptoms had been distinctive from the severe acute respiratory syndrome (SARS) triggered with the aid of SARS coronavirus (SARS-CoV) that befell in 2002–2003 [(4,5,6)]. On January 8, 2020, formally announced that a novel coronavirus is the causative pathogen of "COVID-19" by way of the Chinese Center for Disease Control and Prevention [(7)].

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These new generations of virus now are not the chines nightmare only however soon as speedy as feasible spread all over the world and grew to be the most existence threating health disorder in the world [(8, 9)]. The novel coronavirus used to be initially named 2019-nCoV and formally as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

According to previous researches, this virus recorded as zoonotic in the mode of transmission which is comparable to 'SARS-CoV and MERS-CoV" and as a consequence suggested the virus have the potential to transmitted from animals to human, then the virus had been shown the capacity to transmit from human-to-human, It is now believed that its interpersonal transmission takes place in most cases through respiratory droplets and contact transmission [(10, 11).] Moreover fecal-oral transmission may be reflecting in consideration as a hazard in the spreding of the infection, as researchers have recognized SARS-CoV-2 in the stool of patients

from China and the United States [12,13,14].

The virus indicated exceptional rote of transmission with a high survival potential in a variety conditions in assessment with 'SARS-CoV and MERS-CoV" moreover the length of incubation for the contaminated affected persons have been reported to be (1–14 days), however it had been verified that the human without sign and symptoms can lead to spread the infection [4,5,15], so in according to all of these previous presentation the most individual who exposed to the infection with the virus have been the medical group of workers and the human whose been in contact with the affected persons carrying the virus and the most vital job in the medical worker force who face the hazard of contamination with the corona virus is the dentist and his assistant [16], these worker forces exposed to pathogenic microorganisms, such as viruses and micro-organism that infect the oral cavity and respiratory tract. Infection can take place due to face-to-face conversation with patients, and widespread exposure to saliva, blood, and variety of body fluids, also the dealing with sharp instruments. The pathogenic microorganisms can be transmitted in dental settings by inhalation of airborne microorganisms that can stay suspended in the air for lengthy interval [17,18]. To minimize the contamination of this viral infection the dentist has to guard himself by way of numerous procedures as wearing mask, gloves, face shield, use appropriate ventilation in the private clinic and sterilize the instrument with autoclave in the health center daily [19].

Aim

This descriptive research aim to measure the commitment of Iraqi dentists to the Iraqi dental association recommendations at the time of lockdowns during the corona virus infection.

Material and Methods

The study had been performed as a descriptive survey of private dental practices in Iraq, several questions had been designed to gain statistic about whether the Iraqi dentist have been committed to the recommendation of their association during the period of viral unfold and what is the most appropriate ways that the dentist follow in their private clinic in Iraqi country to minimize or prevent cross-infection of corona virus. These questionnaires had been pre-tested, revised before used. The study population included several dentists in all over the country in April 2020 (n=169) to whom the questionnaires was submitted. Questionnaires data collected via a special program have been designed for this research in which the questioners send to the dentists through a web site for collecting the data on it, because the research had been done in the time of viral spread and to reduce face-to-face interviews. The questionnaires involve several items as if the Iraqi dentist have been committed to the curfew and completely close their private clinic or partially open the clinic to receive the emergency cases only or whether they receive all the cases, additionally which protocol of cross infection control they have been depend on it in their private clinic as wearing gloves, mask, use of rubber dam, the use of high suctioning, the method of storing instruments and disposal, the methods of contaminated material etc. and method of sterilization utilized in the clinic.

Result

This chart (figure1) had been shows the response of the Iraqi dentist to the Iraqi dental association during the period of crew and the spred of virus in percentage

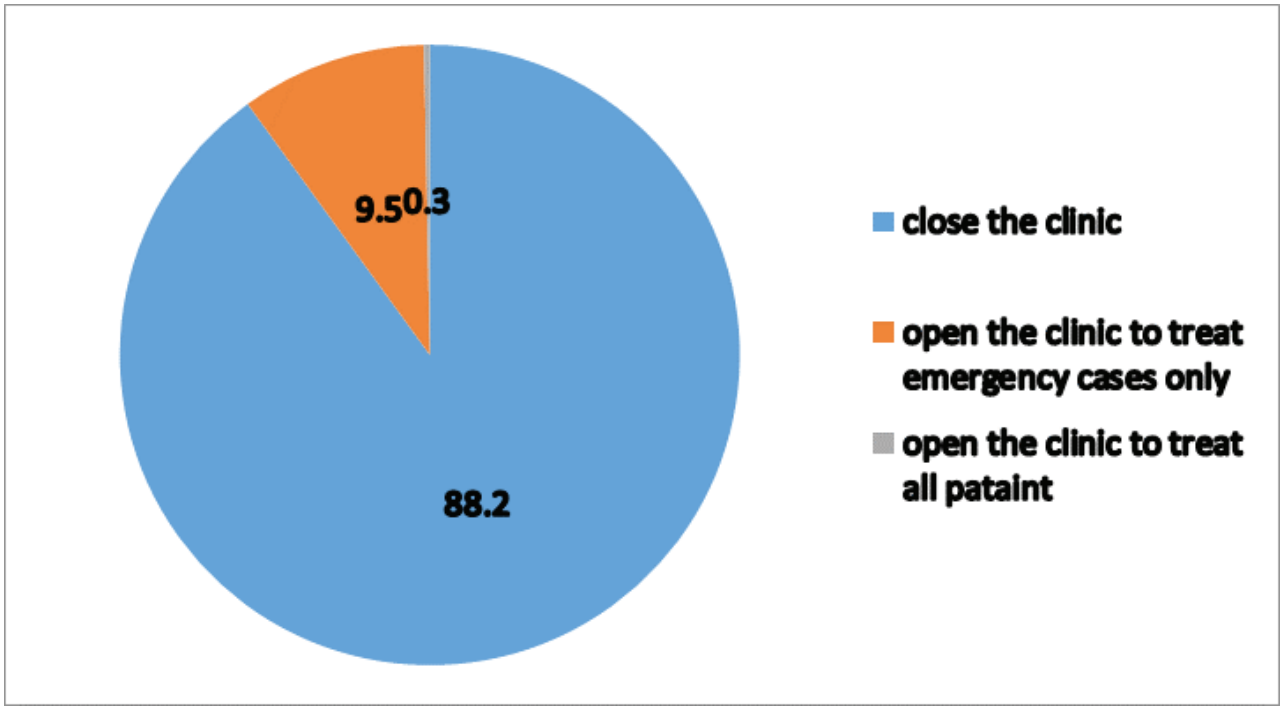

Fig:1 opening of the private clinic during the curfew in Iraq

This chart explains the rote of sterilization and the substance which the Iraqi dentists and their team workers utilized in the private clinic (figure2).

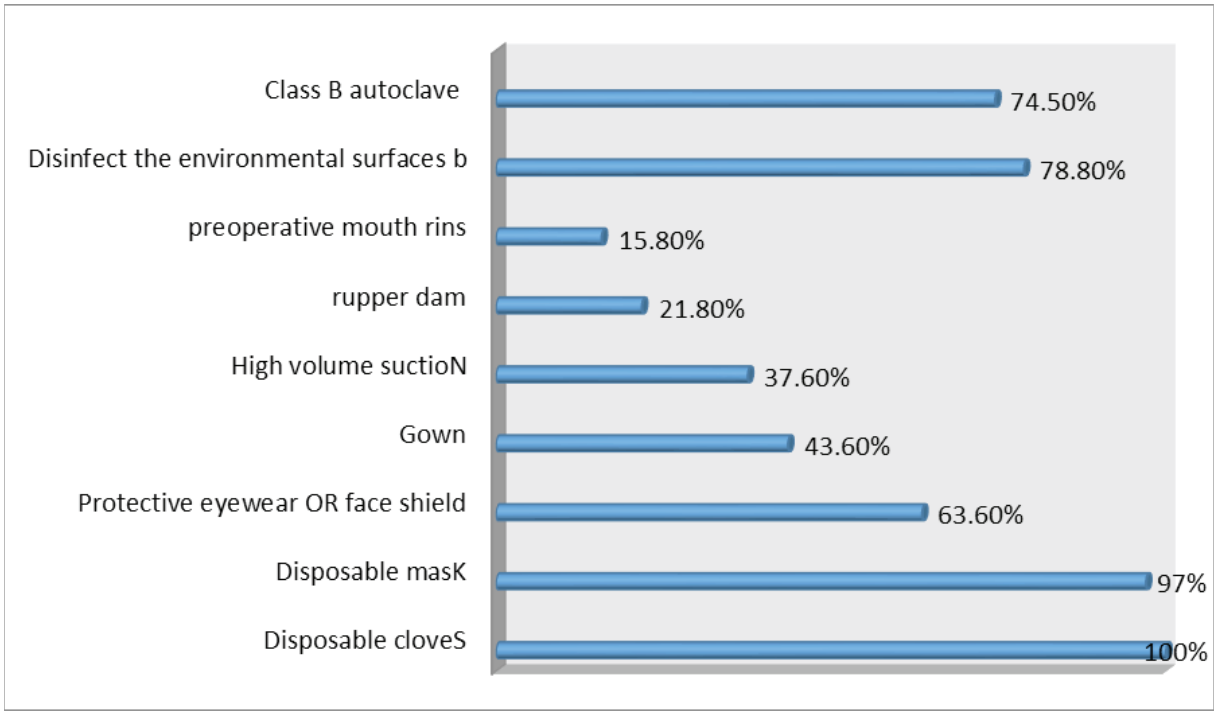

Fig: 2 Protective measures used by the dentists on their daily work

Discussion

"COVID – 19" is a pandemic disease lead to large changes all over the world, actually dentistry were one of the careers affected by this disease the dentist and their team works during the daily work with dental slow speed handpice or high speed turbine produce droplets and aerosols which properly are contaminated with the virus "COVID – 19" .[21] Avery small Particles of droplets and aerosols enough to stay airborne for an extended period before they reach any surfaces in the clinic or dental units or enter the respiratory tract of the dentist and the workers team.[20]

Control strategies have been needed to prevent the spreading of "COVID – 19" especially for dental clinic because it is the most suitable area for viral transmission and infection. [21]

Several recommendations have been introduced from WHO, ADA, CDA and the Iraqi dental association promoted the dentist to close the private clinic and to restricted the work in the private clinic with treating only highly emergency cases if there is no national hospital receive the patient and these rules have been placed to control the viral infection and minimize the rout of transmission. [22]

Some of dental clinics established measuring the temperature of every patient and consider it as routine procedure before the diagnosis for any patient in the same time take an illness history from the patient and if the patient had been travel or in contact to a person who were travel in the last few days [23]

Emergency treatments should be done in a short period and immediately. Dentist should apply a rubber dams and saliva ejectors highly suction with running water to minimize aerosol in dental treatment. Furthermore, face shields and goggles are essential with use of high- or low-speed drilling with water spray [24]

In this descriptive statistic, the study showed a highly response from the Iraqi dentist (88.2%) to close their private clinic during the period of curfew in order to reduce the viral spread and control the infection thus refer to the highly attitude and knowledge of those population and their aim to control the infection and return to the normal life as fast as possible, in the same time the study shows in about (9.5%) of the dentist who participate in the research receive only emergency cases who difficult to reach the national hospital because of lockdown while very few percentage (0.3%) open the private clinic and not response to the instruction of the dental association at the time of infection spreading which could be due to financials issues. Moreover the result of infection control procedure whose the dentists and their workers follow in their daily work were promising with positive result, all of the dentists who take part in this study have been wearing to minimize transmission of infection from the hands of operator to the patients, also wearing gloves prevent contact of blood and saliva with the hands of operator [25] and (97%) of the dentist participate in the study wearing face mask, while unfortunately only (63.6%) of the dentist use face shield or eye goggle, the use of face shields or eyewear as protection against splatter during dental treatment also the study explained that most of the dentist wearing mask, face shield or eye goggle and follow the CDC and ADA recommendation in cross infection control [26,27,28] several studies have been found that most affected area in the face involve the nose and inner corner of the eyes are at high risk of contamination so wearing face shields or eye goggle very important to reduce the cross infection [29] This study shows (15.8%) of dentist motivate the patient to use mouth wash before any dental treatment, this can reduce the number of oral microbes [30] also only (37.6 %) of the dentist participate in the research have been wearing gown which consider low percentage in compromise to its important role which have been utilized to reduce the transmission of the viral infection through the cloth according to the guideline for isolation precautions of the CDC and Prevention the health care, workers should be instructed to wear isolation gowns during patient-care activities [31], while using a high volume suctioning approximately (37.6%). Research shows that aerosols and splatter have been produced via ultrasonic and polishing treatments mostly contain saliva, blood and bacteria. Once these particles can remained in the operation field for an hour or more till finally lands on the surfaces in the clinic. This poses a risk for the spread of the common cold and influenza viruses, herpes viruses, pathogenic streptococci or staphylococci, severe acute respiratory syndrome (SARS), and tuberculosis (TB) [32,33], additionally the number of clinician who`s used rubber dam and have been participated in this research in about (21.8%), the rubber dam improved better cross-

infection control for the clinicians, team workers and the patients, especially in cases of communicable diseases such as HIV (AIDS) and hepatitis [34], while (78.8%) represent the clinician who's disinfect the surface and dental unit after each patient according to CDC infection control guidelines[35], otherwise the research shows only (74.5%) used the class B autoclave to sterilize the instrument. Several studies found that utilize of autoclave in the sterilization process relatively the best method to sterilize of the instrument Proper cleaning and sterilization prevent cross-contamination in dental instrument.[36,37]

Conclusion

This study had been showed that the Iraqi dentists have a great commitment toward the society during the period of curfew they had been attend to close their privats clinic in order to reduce the viral spread and control the infection.

Recommendations

A better the acknowledgement in control infection process have been essential for the safety of clinician, teamwork and the patient. Also, the acknowledgment will certainly ensure the provision the improvement and development of dental health-care services for the people.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Bioremediation of Chlorpyrifos Insecticide by using *Aeromonas Hydrophila* Bacteria

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Abstract

Several bacteria species may have the ability to degrade and digest soundly the residuals of certain insecticide with causing any deteriorated consequences. This work was designed to examine the ability of *Aeromonas hydrophila* bacteria for biodegrading of chlorpyrifos insecticide from contaminated soil. However, soil sample was collected from home garden at depth of 10 cm while the chlorpyrifos insecticide was obtained from the local market. Meanwhile *Aeromonas hydrophila* bacteria were isolated from the garden soil. The bacterial cultural media were prepared to consist of $(NH_4)_2SO_4$ (1g), NaCl (6g), K_2HPO_4 (0.5g), yeast extract (1g), $MgSO_4$ (0.1g) and CMC (5g). This work has examined the efficiency of *A. hydrophila* bacteria to remove insecticide from the soil under various growing conditions such as five different pH values, five termperature degrees and four insecticide concentrations. The obtained results showed that the highest bacterial efficiency (98.0 ± 7.8%) was recorded as pH value of 6 and it was 91.1 ± 6.0% at temperature of 40 ° C while it was 99.1 ± 6.8 % at insecticide concentration of 25 ppm.

Key words: *chlorpyrifos, Aeromonas hydrophila, pH, temperature, insecticide concentrations.*

Introduction

Significant quantity of works hase examined thoroughly the various chemical synthetic insecticides in terms of agricultural. (1) Health and environment Chlorpyrifos insecticide belongs to organophosphate class and its' chemical formula is and also known as lorsban with an average molar mass of 350.59 g/mol and a density of 1.4 g/cm3. (2) However, it is intensively used worldwide on various plant crops such as citrus, corn, soybean, cotton, nuts, almonds and other crops. (3) However, various works have examined the possible toxic effects of Chlorpyrifos insecticide and reported elevated contents of the insecticide residues in plants .(4) On the other hand, various techniques were used for the removal of the pesticide residues such as conventional method (4) Phytoremedation is a unique technique for the removal of various environmental pollutants including the pesticide residues being environmentally sound method, almost costless and very accissable and it was intensively and widely used in the treatment of various environmental contaminants. However, this technique consists of using certain plant species (5) Several bacterial species are well known being capable of controlling soil contamination with hydrocarbon and heavy metal (6) However, these bacterial species are used successfully to face the different environmental challinges. So, the current work was designed to use *A. hydrophilla* bacteria to clean up the soils from the residues of applied chlorpyrifos insecticide.(7)

Materials and Methods

Soil samples were collected from home garden at depth of 10. The collected soil sample was air dried under room temperature and sieved via 0.8 mm stainless steel sieve.(8) A 100 ml of chlorpyrifos insecticide of 50 ppm was obtained from the local market. *Aeromonas hydrophila* bacteria was isolated from the garden soil by using bacterial cultural media consisting of 1 g ammonium sulfate [$(NH_4)_2SO_4$], 6g of sodium chloride [NaCl], 0.5 g dipotassium phosphate [K_2HPO_4], 1 g yeast extract, 0.1 g magnesium sulfate [$MgSO_4$] and 5 g CMC. (9) This synthetic media was placed in

volumetric flask containing 1 liter distilled water. A 100 growing mineral salt media with toleum of 125 g /l were placed in 250 ml sterilized volumetric flask. Each of these flasks has received 1 g sieved soil and placed in shaker incubator for one week under pH of 7 and temperature of 28 °C at RPM of 120. [10] The bacteria was isolated in nutrient agar petri dishes and after the purification, each isolated bacteria was planted in small glass vials by slant method and kept for subsequent tests. However, three sets of experiments were carried out during the current study where all of them were related to the factors affecting bacteria effiency for the removal of insecticide from the soil. [11] The first factor was growing pH with five values (4,6,7,8 and 10) were examined with fixing insecticide concentration at 50 ppm and temperature of 28 °C. [12] Saline culture media was placed in volumetric flask with adjusted five pH values where each volumetric flask received 50 ppm and the isolated *A. hydrophila* bacteria was planted on each of these petri dishes. The experiment was replicated 3 times giving a total of 15 volumetric flask and incubated for two weeks and the bacterial effiency for the removal the insecticide was calculated. [13] The second factor was growing temperature which were 5, 10, 20, 30, and 40 °C. The test was done with fixing growing pH at 7 and insecticide concentration of 50 ppm. Again similar saline culture media explained above were used and the same testing procedure was followed and the experimental unites were incubated for one week where the bacterial effiency for the removal the insecticide was again calculated. Finally, the third testing variable was the insecticide concentration and four levels (10, 25, 50 and 100 ppm) were examined using similar growing media described above and following the same method in case of testing both growing pH and temperature. But, these two variables were fixed at 7 for pH and 28 °C for temperature. [14] The experiment was incubated for one week and the bacterial effiency for the removal the insecticide was recorded.[15]

Results and Discussion

Table 1 displays the mean value ± standard deviation of bacterial effiency for the removal of chlorpyrifos insecticide from the contaminated soil under various values of pH, temperature and insecticide concentration. It seems clearly that insecticide removal effiency of *A. hydrophila* bacteria from the contaminated soil is affected significantly ($P \leq 0.05$) by the examined variables. In general, highest value of the insecticide removal effiency was recorded in bacteria under different growing conditions such as soil pH, incubation temperature and insecticide concentration.

Table 1 The mean value ± sd of bacterial effiency for the removal of chlorpyrifos insecticide from the contaminated soil under various values of pH, temperature and insecticide concentration

Examined Variable	Values	Mean Effiency ± SD
pH	4	0.9 ±0.05
	6	98.0 ± 7.8
	7	90.9 5.5
	8	90.0 ± 4.8
	10	0.6 ± 0.03

Cont... **Table 1 The mean value ± sd of bacterial effiency for the removal of chlorpyrifos insecticide from the contaminated soil under various values of pH, temperature and insecticide concentration**

Temperature	5	25.3 ± 2.6
	10	36.3 ± 4.2
	20	86.0 ± 5.2
	30	90.0 ± 5.9
	40	91.1 ± 6.0
Insecticide concentration	10	90.3 ± 7.2
	25	99.1 ± 6.8
	50	50.8 ± 4.5
	100	9.0 ± 1.7

The effects of various pH values (4,6,7,8,10) on the bacterial insecticide removal efficiency were found being the best removal efficiency was 98.0 ± 7.8 % recorded at pH of 6 followed by 90.9 ± 5.5 % at pH 7 and 90.0 ± 4.8 at pH 8 while the lowest efficiency percentage of 0.6 ± 0.3 % was recorded at pH of 10 followed by 0.9 ± 0.05 % detected at pH 4 (Table 1; Figure 1).

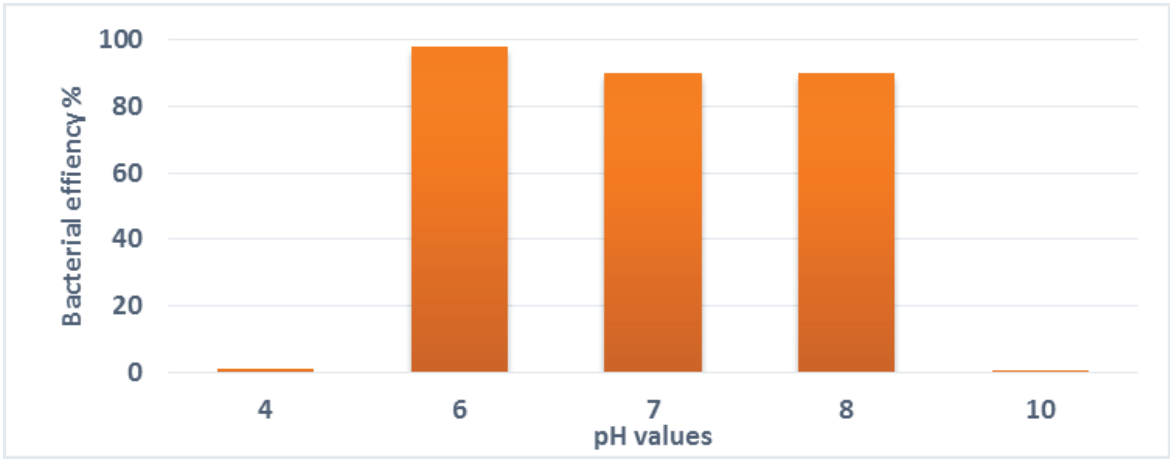

Figure 1: The effect of differenr pH values on the bacterial efficiency forf the removal of insecticide from the soil.

In case of the temperature, the obtained results showed that the highest mean value (91.1 ± 6.0 %) of insecticide removal effiency by examined bacteria was recorded at temperature of 40 °C followed by that detected at 30°C which was 90.0 ± 5.9 while the lowest mean value (25.3 ± 2.6 %) was found at ° C followed by those recorded at 10 °C which was 36.3 ± 4.2 % and at 20 ° C, it was 86.0 ± 5.2 % (Table 1; Figure 2)

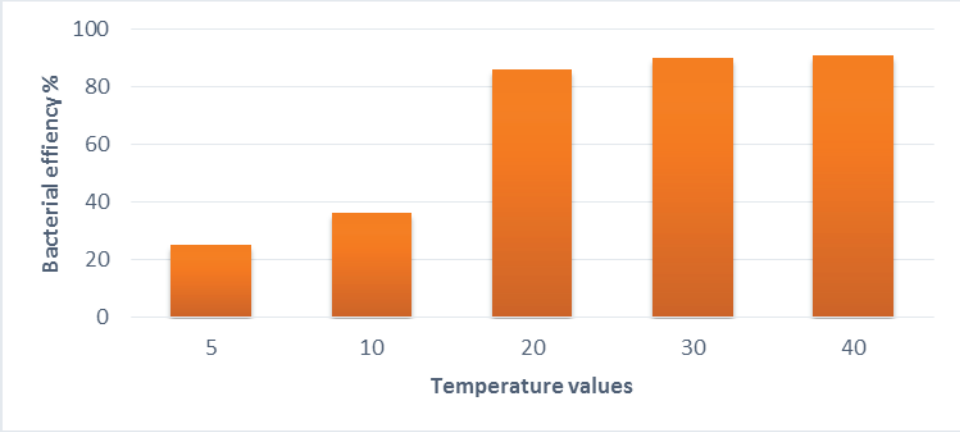

Figure 2: The effect of different temperature values on the bacterial efficiency forf the removal of insecticide from the soil.

Regarding the insecticide concentration, this work has found that the highest mean value of bacterial effiency for the removal of insecticide residues from the soil was 99.1 ± 6.8 % which recorded at 25 ppm followed by that detected at 10 ppm which was 90.3 ± 7.2 % while the lowest mean value (9.0 ± 1.7 %) was found at 100 ppm and at 50 ppm, the mean value was 50.8 ± 4.5 % (Table 1; Figure 3).

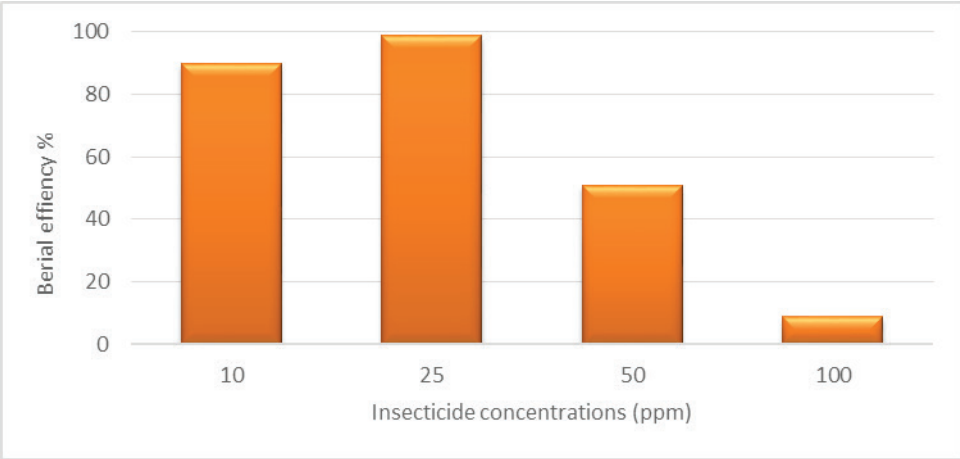

Figure 3: The effect of different insecticide concentrations on the bacterial efficiency forf the removal of insecticide from the soil.

Previous study has examined the effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium and reported the bacteria at pH greater than 6.7 was able to maintain its biodegradation of chlorpyrifos insecticide. Such pH effects was also reported by other works [1] However, it seems that the soil pH affects sorptive behavior of pesticide molecules as reported by previous study [3] Regarding the effect of temperature on the biodegradation of insecticide in general, it was reported that the biodegradation is increased with increasing the temperature up to 40 °C .[5]) For insecticide concentration, it was found that the insecticide concentration had the greatest impact on the degradation of chlorpyrifos inseciticide as reported by previous study [7] but at very higher insecticide concentration (1000µg/ g) , the biodegradation of of chlorpyrifos is inhibited as reported by an early work . [9] On the other hands, other study has reported that that the pH variable needs to be associated with temperature for better biodegradation of chlorpyrifos insecticide [3]. However, *A. hydrophila* bacteria has wide thermal range (5 – 50 °C) and also its ability in secretion of various enzymes that assiss to get environmental adaption . [6] Also, this bacteria has the ability to degrade chlorpyrifos insecticide via excreting excellular hydrolysis phosphotriesterase enzyme [9].

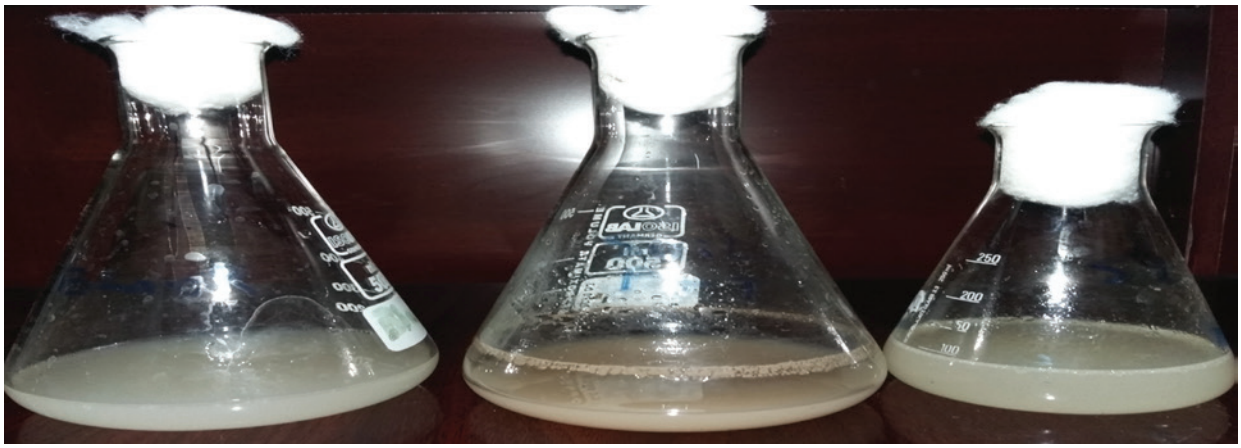

Figure 4 A picture showing the transformation of the pesticide into an emulsified solution two weeks after the experiment

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Molecular Co-Localization of Human Epstein Barr Virus Infection Associated with the Expressed Protein of Cyclin D1 \Cyclin Dependent Kinase 4 in Hodgkin's Lymphoma Tissues

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Abstract

This retrospective case-control study to assess the impact of Cyclin D1 and CDK4 expression along with EBV infection with Hodgkin's lymphoma (HL).80 lymph node included in this study; (40) formalin-fixed, paraffin- embedded biopsies from Hodgkin's lymphoma (HL), and (40) apparently healthy lymph nodes as control group. EBV detection was done by *CISH* whereas IHC detection system was used to evaluate both Cyclin D1 & CDK4 gene expression.

The positive –*EBERs* CISH reactions detected in 16 / 40 (40 %) of Hodgkin lymphoma tissues while positive - *EBERs* reactions detected in 3 out of 40 tissues (7.5%) in control lymph nodes tissues. Statistically was found highly significant between study groups (P value = < 0.05). Cyclin D1 & CDK4 -IHC were detected in 40% and 52.5% of HL, respectively. Statistically, significant differences noticed among negative, low and moderate scoring of tissues (P<0.05).

Significant EBV gene expressions along with higher percentage of tissues in which Cyclin D1 & CDK4 genes being expressed may be indicator their possible role in the pathogenesis and / or the carcinogenesis of a subset of our Hodgkin's lymphoma cases.

Key word: *EBV; Hodgkin's lymphoma (HL), Cyclin D1;CDK4, CISH, IHC.*

Introduction

As an unusual malignancy, HL is characterized of Hodgkin/ Reed Sternberg cells by the inflamation infiltrates. Two types of HL are recognized on morphologic, immunophenotypic, and clinical bases; classical (cHL) as well as and nodular lymphocyte-predominant Hodgkin lymphomas [(1).]

As a ubiquitous human herpetic virus, EBV has a global seroprevalence of 95%. Usually, EBV infection is asymptomatic in childhood, while in adolescents, infection frequently results in infectious mononucleosis[(2)].

The three forms of cHL, pediatric as well as older adults HL are EBV-positive types, while HL of young adults is EBV-negative type. Moreover, EBV is also associated with the development of other lymphomas, including natural killer / T-cell lymphoma and diffuse large B-cell lymphoma [(3).]

Reed-Sternberg cells are EBV positive in 40% of the times and demonstrate latency II pattern.It was showed that consistent presence of 1 of the 3 chromosomal translocations in BL deregulate the c-myc expression [(4).] Although still controversial, it has revealed a presence of EBV in small proportion of nodular lymphocyte-predominant HL cases · However, the link of classical Hodgkin lymphoma with EBV is most clearly

established, and was first implicated in the pathogenesis of such lymphomas in patients who had raised antibody titers to EBV preceded development of these lymphomas by several years [5].

EBV-positive BL cells have gene expression profile similar to the latency form resembling memory B cells[6] with switching from one to other EBV – latency forms in B-cells might regulate the viral life cycle and redirecting the EBV-positive B cells towards long-term persistency in their memory pools. LMP1 replaces the survival as well as differentiation signals provided by an activated CD40 receptor [7].

The CCND1 gene in hematological malignancy , due to either translocation, amplification, deletions, point mutations of 3′-UTR of t(11;14)(q13;q32) gene, or genetic alteration. Cyclin D1, with cyclin-dependent kinases (CDKs), play a key role in regulation of cell cycle during the G1/S transition [8].

The cyclin D1 gene expression is associated classically with lymphoma while abnormalities of cyclin D1 & CCND1 gene expression were not well studied in nodular Hodgkin lymphomas [9] .

The oncogenic potential of *CDK4* gene was experimentally shown necessary for the transforming and immortalizing effect of *cyclin D1* [10].

Here, we aimed in the present research work was explore both the possible impact of EBERs-EBV as well as to assess the effects of expression of cyclin D1 and CDK4 proteins on the cellular proliferation of lymph node tissues in order to gain further insight into the proliferation profile of a group Iraqi patients with Hodgkin's lymphomas.

Materials and Methods

Few other 4µm - tissue sections were mounted on charged slides for application of CISH & IHC techniques.

For the detection of EBERs by CISH kit (purchased from Zyto Vision GmbH. Fischkai, Bremerhaven. Germany), the Chromogenic In Situ Hybridization procedures were performed according to the manufacturing company instructions.

Immunohistochemistry / Detection system (purchased from Abcam. England) was used to estimation the protein genes products of Cyclin D1 & CDK4 by using specific monoclonal antibody for that antigen determination on the targeted Cyclin D1 & CDK4 protein.

In the current study, and for statistical analysis, Chi –square test was used via Version– 21 SPSS program and P value was significant when p <0.05.

Results

I-Results of EBERs-EBV in Hodgkin Lymphomatous tissues:

Sixteen out of 40 tissues with Hodgkin lymphomas showed positive chromogenic in situ hybridization reactions (constituting 40% of the total Hodgkin lymphoma tissues) (Table 1). The healthy lymph nodes tissues revealed seven and half percent (3 / 40). The differences between the percent of study groups showed statistically highly significant difference (P value = < 0.05).

Table (1): Results of EBERs - Associated HL Using CISH Technique

EBERS-EBV-	HL (no.=40)	%	A.H Control (no.=40)	%	P-value
Negative	24	60%	37	92.5	Z test P=0.003 sign. (P<0.05)
Positive	16	40%	3	7.5	

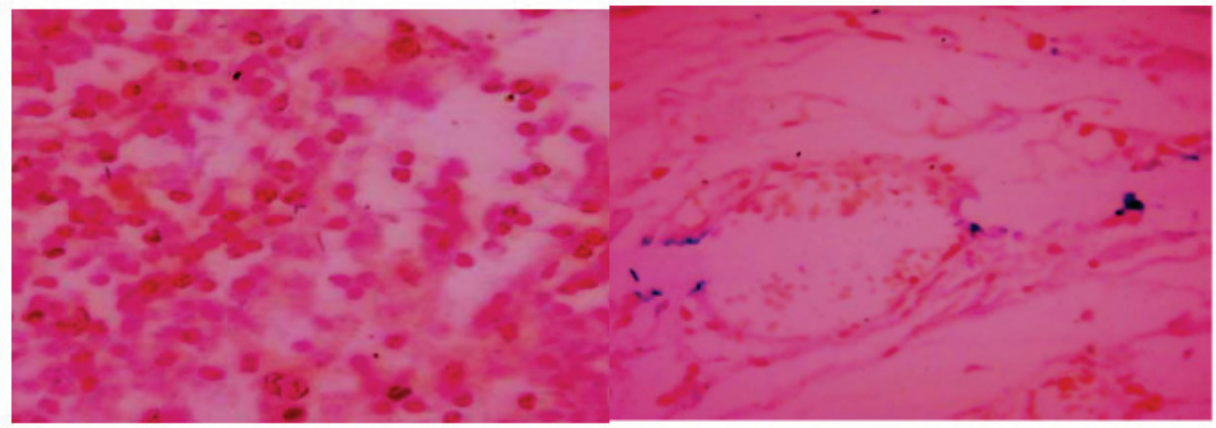

Figure 1: CISH reaction for EBERs- EBV detection in lymph node tissue sections with Hodgkin Lymphoma: (40X). (A) Negative reaction; (B) positive reaction.

II. The Results of Cyclin D1 -IHC Signal Score in tissues with Hodgkin Lymphoma.

Table (2) shows the positive result of Cyclin D1 IHC detection where 52.5 % from HL showed positive signals including 22.5 % in the low score (I) followed by 17.5 % and 12.5% in the moderate score (II) and high score (III), respectively, while the percentage of positive signals in AHC was (7.5%), including 5 % score (I) followed by 2.5 % in score (II) (Fig. 2 A& B). Statistically, significant differences were found among scorings of IHC signals (P<0.05).

Table (2): Cyclin D1 -IHC Score Signaling in Hodgkin Lymphoma

Cyclin D1 -IHC signal scoring		Hodgkin lymphoma(n=40)		A.H. Control (n=40)		P-value
		No.	%	No.	%	
Negative		19	47.5	37	92.5	
Positive		21	52.5	3	7.5	
Scoring	I	9	22.5	2	5	χ2test P=0.03 sign. (P<0.05)
	II	7	17.5	1	2.5	
	III	5	12.5	0	0	

III.CDK4 -IHC expression in patients with Hodgkin lymphoma:

Among the 40 Hodgkin lymphomatous tissues, 45% exhibited positive CDK4-IHC reaction, while in control group 12.5% (Table 3) .

The signal scores were as follows: 22.5% (9/40) low score (I) followed by 17.5% and 5% in the moderate score (II) and high score (III), respectively, The intensity signal was shown as intensity I in 25%, and 12.5% as intensity II, and lastly 7.5% as intensity III while in AHC 7.5% as intensity II, and 5% as intensity I (Table 3 & Fig. 2 C& D). Statistically significant difference (P<0.05) found between various intensities (Table 3).

Table 3: The grading of CDK4-IHC score and intensity signals in Hodgkin lymphoma

CDK4-IHC		A.H control		Hodgkin lymphoma (n=40)			P-value (χ2test)
		No.	% out of 40	No.	% out of 40	% out of 17	
Negative		35	87.5	22	55		
Positive		5	12.5	18	45		
Signal Score	I	1	2.5	9	22.5	47.1	0.02
	II	3	7.5	7	17.5	35.3	
	III	1	2.5	2	5	17.6	
Signal Intensity	Weak	2	5	10	25	52.9	0.04
	Moderate	3	7.5	5	12.5	35.3	
	Strong	0	0.00	3	7.5	11.8	

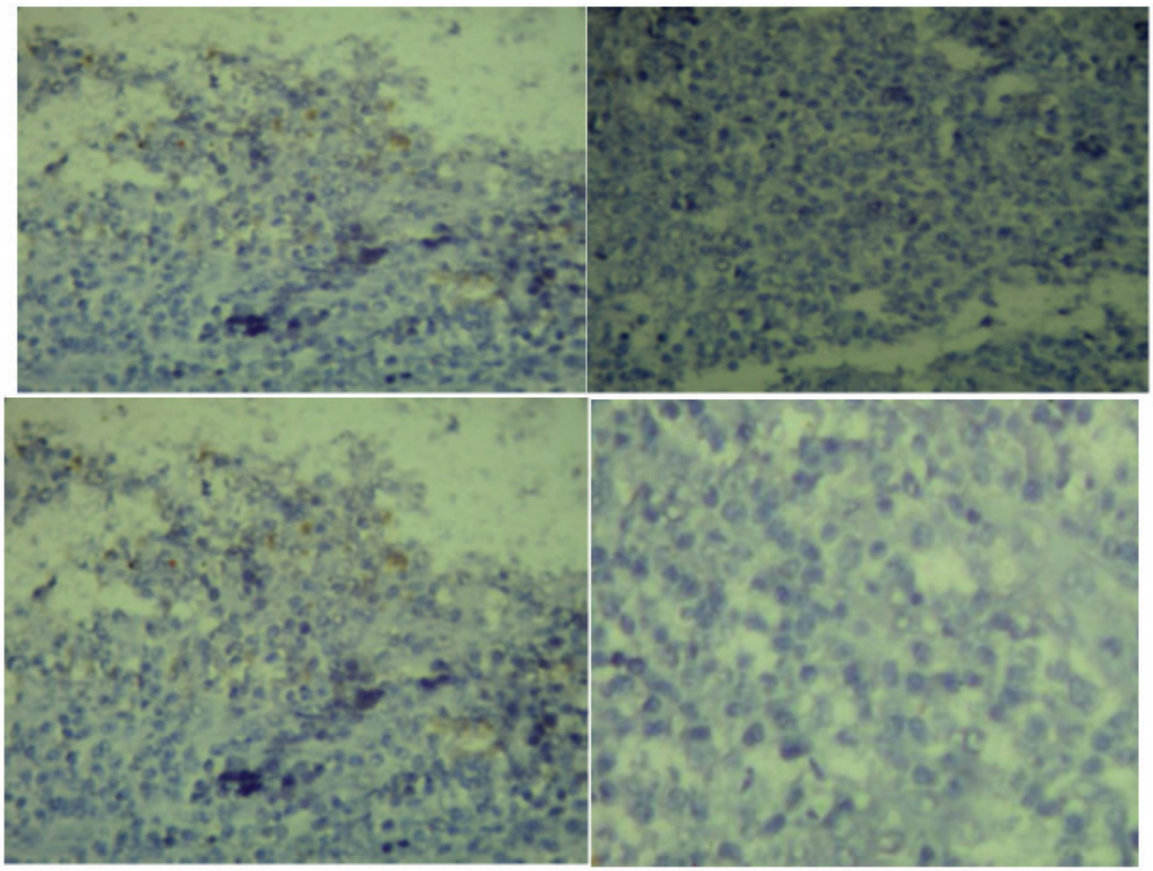

Figure 2: IHC staining of Cyclin D1 and CDK4 protein in lymph node sections with Hodgkin Lymphoma (×40): (A) Negative reaction Cyclin D1; (B) positive reaction Cyclin D1. (C) Negative reaction CDK4; (D) positive reaction CDK4.

IV. Correlations among the studied markers (EBERs-EBV; Cyclin D1 and, CDK4) in lymph node tissues from patients with Hodgkin lymphoma:

A significant was highly between EBERs / EBV and Cyclin D1 in Hodgkin lymphoma (r = 0.443, P = 0.003). Similarly, between CDK4 & grade of Hodgkin lymphoma (r = 0. 398; p= 0.044) was noticed. No significant correlations among EBERs / EBV and other parameters (Table 4).

Table 4 Spearman's rho of the studied parameters in Hodgkin lymphoma.

Spearman's rho		Age groups (years)	Grade	EBERs-EBV	Cyclin D1	CDK4
Grade	r	.165				.398*
	P	.104				.044
EBERs-EBV	r	0.040	0.133			
	P	0.765	0.412			
Cyclin D1	r			.443**		
	P			.003		
CDK4	r	0.158	0.432	0.483		
	P	0.347	0.006*	0.003*		

* highly significant

Discussion

The present results revealed that grade I - malignant lymphomas have affected our Iraqi studied patients at an earlier age than that those expected globally, a finding that could be attributed either low mean age & inadequate screening of these patients or to the small enrolled samples in the current study, when compared to those abroad studies. More researches are to be done to elucidate the causes for such frequency of these grade I - lymphomas, as fronted by the majority (54.5%) of low-grade lymphomas results in western countries [11].

In Iraq, Epstein-Barr virus and hCMV were studied in tissues from Non-Hodgkin lymphomas, [12,13] while in Hodgkin's Lymphoma tissues and up to our best knowledge only one study on Human T Cell Lymphtropic Virus Type-1- genes was studied [14] but no research study on EBV was found.

A pathogenic role for EBV in HL has been suggested by the finding of monoclonal EBV genomes in HRS cells indicating role for EBV as an early event, prior to clonal expansion, expressing type II latency genes (i.e. EBNA-1, LMP-1, LMP-2 , EBERs and BARTs RNAs) [15].

The loss of a functional BCR and its signaling components are combined to prevent virus replication during tumor development and maintenance of EBV-associated HL [16].

The epidemiologic studies demonstrated an increased risk to HL in those patients with infectious mononucleosis and further showed that they had an elevated antibody titers against EBV antigens preceding their disease ,and EBV -positive tumor cells in a subset of their tissues [17].

In the current study, we found 16 \ 40 tissues with HL showed EBV positive CISH (40%) .Flavell and Murray,[18] , who found in the HL triggering mechanisms of pathogenic, or as reflection to the presence of depression in the immune regulation, rather than immunosuppression.

Another form of latency of EBV infections that are lacking EBER expression might present and as several previous studies by Bonnet *et al*.,[19] & Sugawara *et al*.,[20] have suggested. Another possibility is that this virus transformed the progenitor cells of Hodgkin's lymphomas by a "hit and run" mechanism .The EBV relation to this disease depends on many factors, including gender, age , ethnicity, resident country, and histological subtype.

Cyclin D1 partners are also present in breast tumors and colorectal cancers [21]. In Cho *et al*.,[22] study of NLPHL cases in correlated with CCND1 gene abnormalities, "20%" of LP cells were positive for "CD20"/"cyclin D1". The cdk4 hyperactivity has been well reported in many types of cancers, particularly melanoma, lung cancer and lymphoma [23].

The current result of cdk4-IHC detection showed that 45% (18/40) exhibited positive CDK4-IHC reaction in Hodgkin lymphoma group, while in control group 12.5% (5\40). Hernandez *et al*., [24] found overexpressed cdk4 in 21% of MCL cases. The results are consistent with Bockstaele *et al*. [25], was studied over two decades ago the biochemical and genetic characterization of D-type cyclins, their cyclin D-dependent kinases (cdk4 and cdk6), and the polypeptide CDK4/6 inhibitor $p16^{INK4}$ and revealed role of mammalian cells in regulation of the entry into regulation of pRb dependent manner in cell division cycle.

EBV along with *Cyclin D1* and *cdk4* over- expression in Hodgkin lymphoma patients of the current study are supporting an etiologic roles for that virus along with these genes in Hodgkin lymphoma development.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Histological Features of the Vomeronasal Organ in New Born Lambs (*Ovis aries*)

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Abstract

Background: vomernasal organ is an auxiliary olfactory organ which located at the base of nasal septum composed of olfactory epithelium and being enclosed by cartilaginous capsule**.** The current study was aimed to investigate the histological structure of vomernasal organ in new born lamb.

Methods: six heads of new born lamb were used for this study. The nasal cavity including the hard palate was sward up into four transvers sections. The sections were processed with paraffin embedding technique and tissue sections were stained with H&E stain.

Conclusions: The rostral and middle portions of vomeronasals duct had medial and lateral walls with crescent shape lumen and enclosed completely by hyaline cartilage. The medial wall was lined with still diffentiating neuro-epithelium, possessed many of intra epithelial capillaries and composed of supporting, bipolar neuron & basal cells. The lateral wall was lined with differentiating respiratory epithelium. The lamina propria-submucosa beneath both neuro and respiratory epithelia composed of ruminant of embryonic mesenchymal tissue contained newly formed glands, blood vessels and nerves. A significant ($p \leq 0.05$) difference was recoded between the diameters of the rostral, middle portions and caudal portion of both gender and there were no differences between genders. The epithelial height of the neuro epithelia of the middle portion in both gender had significant value ($p \leq 0.05$) in compare with epithelia of other portions.

***Key Word**: Vomernasal organ, lamb, Jacobson gland, neuro-epithelium, respiratory epithelium.*

Introduction

Vomernasal organ is an auxiliary olfactory organ in most mammals and has an important function in sexual pheromones sensation [(1)], [(2)], [(3)], [(4)]. The vomernasal organ is narrow bilateral tubular structures and located at the base of nasal septum of nasal cavity composed of olfactory epithelium being enclosed by cartilaginous capsule [(5)], [(6)]. In adult mammals the functional vomernasal organ is bilateral tubal epithelial organ lined by two types of epithelia (neuro epithelium & respiratory epithelium) and protected by extendable hyaline cartilage, the neuro-epithelium is composed supporting cells, olfactory bipolar cells and basal cells, and the respiratory epithelium has composed ciliated pseudo stratified columnar epithelium heavily with goblet cells. The sub epithelial tissue is well organized glandular and vascular loose connective tissue [(7)], [(8)], [(9)], [(10)]. The vomer nasal organ of different adult mammals were described by several authors like [(9)], [(6)], [(10), (11)], [(12)],[(13)]. This study was aimed to investigate the histological structure of vomernasal organ in new born lamb.

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Materials and Methods

A six heads of healthy newly born lambs (3male & 3 females) up to five days old were used for this study. After slaughtering, the heads sample were removed immediately and infused with 10% formalin throughout the orifices of incisive papilla. Then the samples were immersed in 10% formalin for 72 hr. The hard palate including the nasal cavity was sawed up into four transverse sections. The serial sectioning was made

up through the level of the incisive papilla into the 7th transverse palatine ridge that included the vomernasal organ as the following: The 1st section has made through the incisive papilla. The 2nd is section represented the incisive duct (At the levels of 2nd and 3rd palatine ridges). The 3rd section was represented the vomernasal duct (At the levels of 4th and 6th palatine ridges). The 4th showed the end of the vomernasal duct (At the level of the 7th palatine ridge) [13], [14]. The sections were processed with up grading concentrations of ethanol alcohol in routine paraffin technique and clearing with xylen. The paraffin blocks were sectioned serially at (5-6) µm by oratory microtome. The prepared tissue sections were stained with the Hematoxylin and Eosin stain [15]. The Histometrical measurements included the diameters of vomernasal duct and the height of the epithelial lining in all portions of vomernasal organ. The tissue sections were examined by light microscopy and microphotography has been done by using Future Win Joe microscopic camera, the images have analyzed and scored by using Fiji image analyzer system [15]. The statistical analysis was done by using SPSS statist version (24). One way analysis of Variance (ANOVA) was used to detect significant difference at level ($P<0.05$).

Results and Discussion

In new born lamb the vomernasal organ has composed of still-differentiating olfactory part (Vomeronasal duct). The rostral and middle portions of the vomeronasal duct showed crescent shape lumen (fig.1). The diameters of the duct at rostral portion were 514.93±9.27µm in male, and 512.93±8.33µm in female, meanwhile those of middle portion were 574.54±6.76µm in male and 571.13 ±7.88µm in female. The vomernasal duct has composed of medial and lateral walls (fig.2). The ciliated pseudo stratified columnar epithelium was lined the lateral wall and part of medial wall, meanwhile the medial wall was lined with pseudo stratified columnar epithelium (Neuro-epithelium) (fig.3). The neuro-epithelium was still diffentiating pseudo stratified columnar epithelium devoid of goblet cells, possessed many of intra epithelial capillaries and composed of three layers cells (Supporting, olfactory bipolar neurons & basal cells) (fig.3). The epithelia were similar those in adult mammals which mentioned by all authors with exception in newly born lamb the epithelia were revealing still differentiation (Late morphogenesis, transformation and growth). This result has recorded by [13] in Gazelle, [6] in Ox, [17] in buffalo, [18], [19] in camel, [5], [9] in sheep, [20], [21] in canine, [12] in equine, [22] in male red fox, [23] in Adult Guinea pig, [24] in giraffe, [25] in bear. On other hand this result disagrees with [26] in goat, [12] in horse who mentioned that the respiratory epithelium is only type in rostral portion of organ. This result suggests that, the neuro-epithelium which lining medial wall of vomernasal duct (rostral and middle portions) has been given these portions specify with olfaction, subsequently called "olfactory part".

The hyaline cartilage was enclosing the vomernasal duct completely with except small silt like opening which seen at the dorsal aspect of the cartilage (fig.1), similarly to all mammals, the vomeronasal organ of newly born lamb is enclose and supported by cartilage which at the pre rostral portion, meanwhile at vomernasal duct the cartilage has completely enclosed the vomernasal duct, this result paralleled with results of [5] in sheep, [26] in goat, [20], [21] in doges, [25] in the brown bear, [27] in monkey, and in male red fox [22]. This result suggest that the incomplete condition of cartilage serve to gives the vomernasal duct a required distention during pumping mechanism [6], [28], so is give an idea that the vomernasal organ had functional statues also the variable shape of the lumen of vomernasal duct, on the other hand the incomplete cartilage lead to allow the blood supply into the soft tissue of the vomernasal organ from nasal mucosa, this agrees with results [5], [8]and [6]

The lateral walls respiratory epithelium was pseudo stratified columnar epithelium showed few of goblet cells and composed of (Ciliated columnar cells, non-ciliated goblet cells & basal cells) and revealed poor ciliogenesis (fig.4). The heights of the respiratory epithelia were 31.15±0.71 µm in male and 29.75±0.79 µm in female at the rostral portion, meanwhile those in middle portion were 31.09±0.86 µm in male and 31.09±0.80 µm in female. This result dissimilar to results of adult mammals recorded by [5], [6], [17], [20], [21] , [22] [23], [25], [29] and [30]. The current results suggested that the respiratory epithelium has displayed few population of goblet cells, this feature revealed that the function of new born lamb vomernasal organ could be limited because that the mucous secretion of the goblet cells is so important as a part of mechanical apparatus of pumping system, this observation is supported [10] and [26].

The lamina propria-submucosa beneath both neuro and respiratory epithelia has composed of remnant of still differentiating embryonic mesenchymal tissue which revealed newly differentiating fibroblasts, newly produced of collagen fibers, angiogenesis with newly formed blood vessels and nerve bundles. The lamina propria submucosa showed few scattered clusters of newly differentiating compound tubular acinar Jacobson glands (fig. 3, 4 & 5). The current results are disagree with the results of all authors whom refereed that the lamina propria submucosa of the adult vomernasal organ is composed of mature glandular and vascular loose connective tissue that make the newly born vomernasal organ with a limited function. On the other hand there were some specialization of epithelial apical surface were never seen in new born lamb vomernasal organ as those seen in adult by [6],[31] whom mentioned that, the neuroepithelium epithelium has abundant with cilia and or microvilli. The caudal potion showed narrower lumen of vomeronasal duct and their lateral and medial walls showed still differentiating epithelium (fig.6).

The data analysis revealed non-significant differences ($p \leq 0.05$) between both sexes in diameters of the rostral and middle portions of vomernasal duct. The epithelial height of the neuroepithelia of the middle portion of the both male and female were revealed significant differences ($p<0.05$) in compare with other rostral and caudal portion.

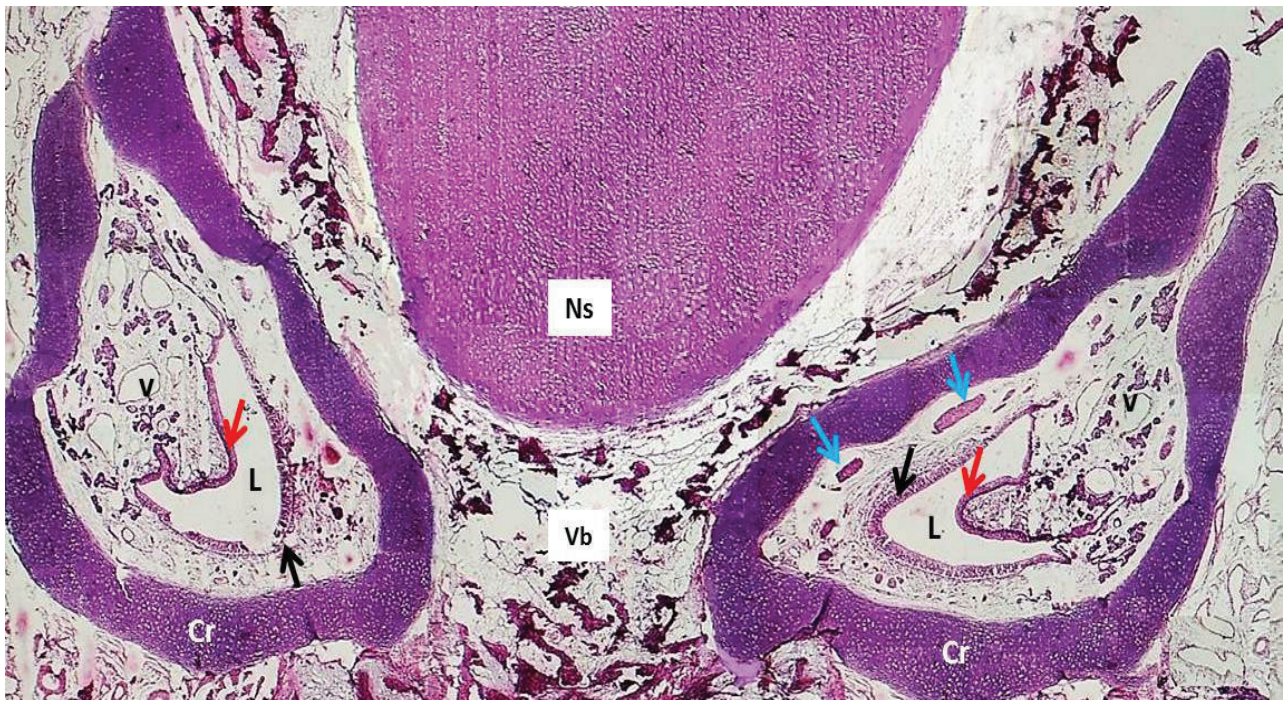

Figure 1: Transvers section of vomernasal duct (Rostral portion) shows: nasal septum (Ns), vomer bone (Vb), convex lateral wall (Red arrows), concave medial wall (Black arrows) , hyaline cartilage (Cr), wide vein (v), axons of the accessary olfactory nerve (Blue arrows) H&E stain. 40x

Figure 2: The vomernasal duct (Rostral portion) shows: medial wall which lines with neuro-epithelium (Black arrows) & lateral wall which lined with respiratory epithelium (Red arrow). H&E stain.100x

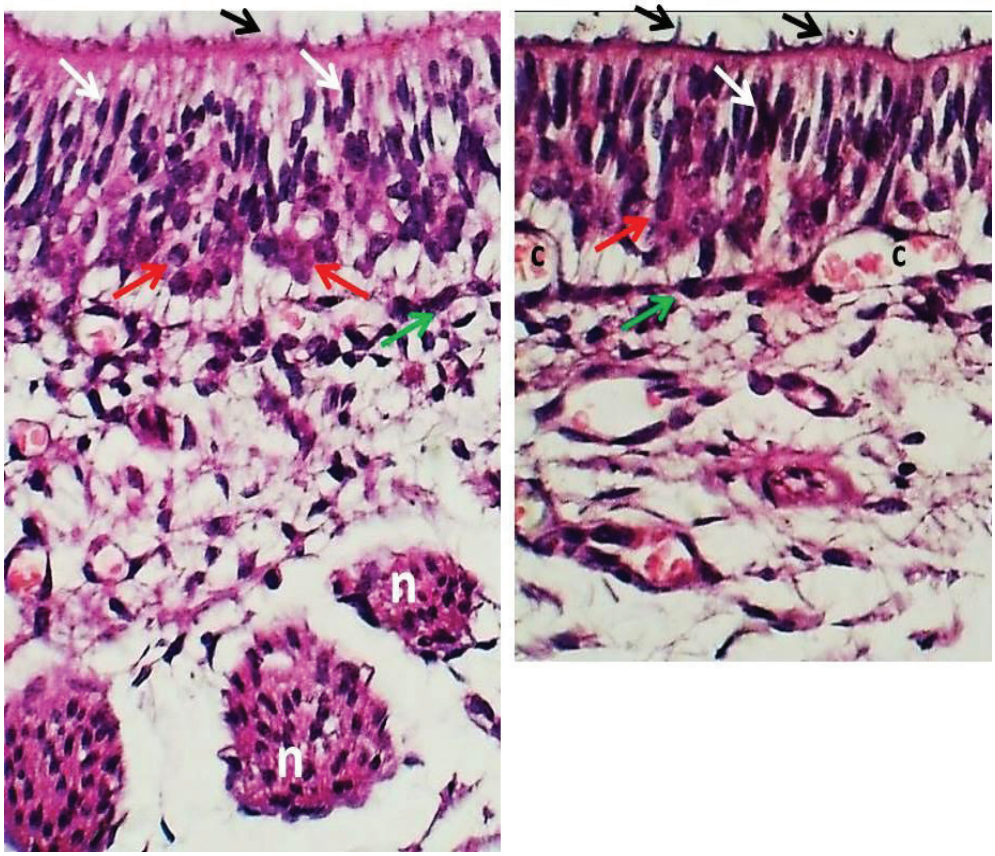

Figure 3: The neuroepithelium of the rostral portion) shows: sustentacular cells (white arrows), bipolar neurons (Red arrows), basal cells (Green arrows) & nerves bundles of accessary olfactory nerve (n). H&E stain. 400x.

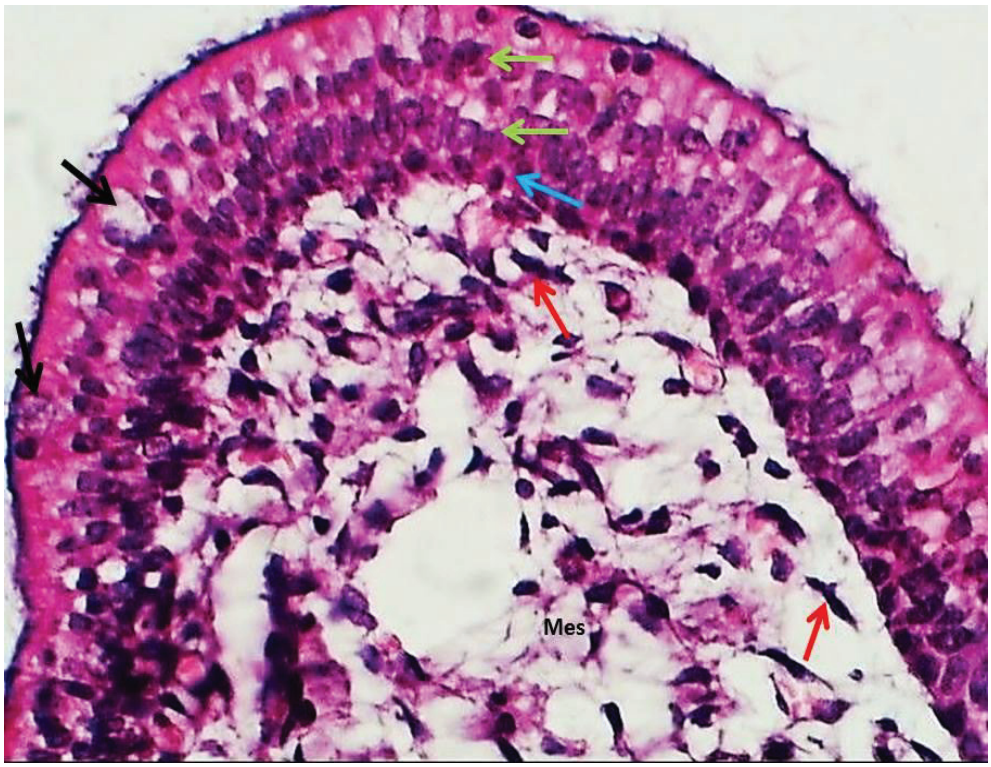

Figure 4: Mucosa of lateral wall (Rostral portion) shows: layer of columnar cells (Green arrows), nuclei of basal cells (blue arrow), goblet cells (Black arrows), and fibroblast (red arrows) within mesenchymal tissue (Mes). H&E stain.400x.

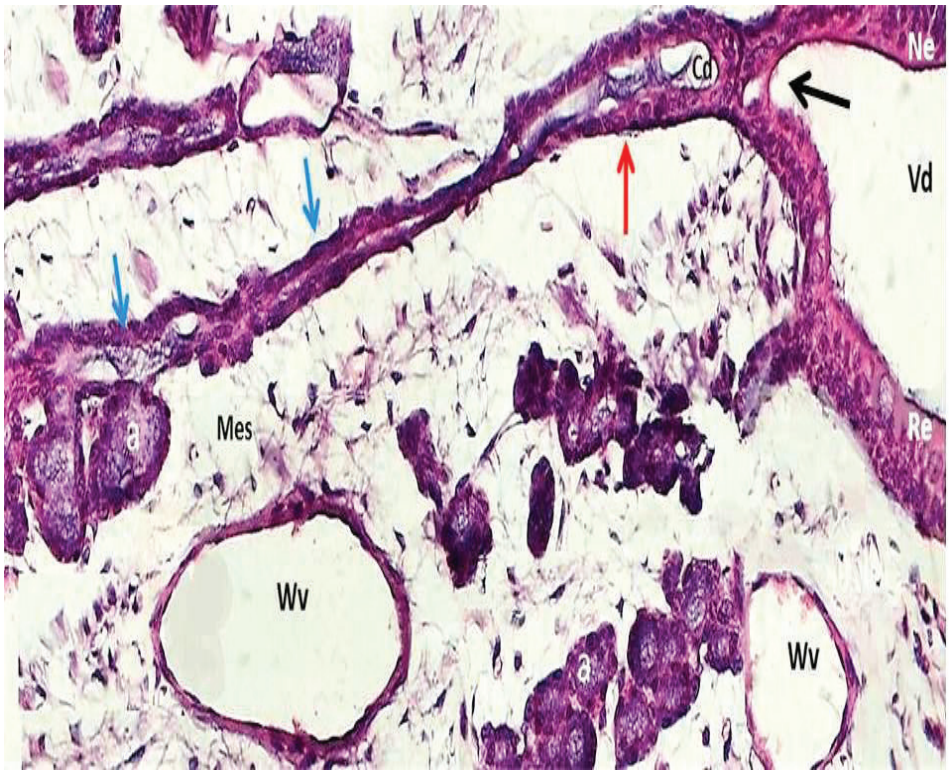

Figure 5 : Jacobson glands (Rostral portion) shows: neuro-epithelium (Ne), respiratory epithelium (Re), lumen of vomernasal duct (Vd), common duct (Cd), dorsal commissure (Black arrow), acini of Jacobson's gland (a), striated duct (Red arrow), intercalated duct (Blue arrows), wide veins (Wv)& mesenchymal tissue (Mes) . H&E stain. 400x.

Figure 6: Transvers section of vomernasal duct (Caudal portion) shows: vomernasal lumen (Vd), convex lateral wall (Red arrows), concave medial wall (Black arrows) , oval shape hyaline cartilage (Cr), wide vein (Wv), Jacobson's glands (Blue arrows) H&E stain.40x.

Conclusion

The results concluded that the vomeronasal organ of new born males and female lambs (up to five days) was still differentiation despite it had some features of that in present in adult small and large ruminants.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Association between Genetic Polymorphism of *ADH3* exon 8 and *ADH2* exon 9 Genes and Specific Enzymes with Alcoholism in Iraq

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Abstract

A retrospective cohort study of the relationship between *ADH2*- exon9 and *ADH3* exon 8 genes polymorphisms with specific enzymes SGOT, SGPT, and catalase among Iraqi individuals identified with alcohol abuse was conducted during period from December 2018 to June 2019. Blood samples of the research subjects have extracted DNA. The genetic polymorphisms analyses were conducted by Restriction Fragment Length Polymorphism (RFLP-PCR) for *ADH2*- exon9 and *ADH3* exon 8 genes. The current study shows that the presence of a catalase was significantly associated with genotype of *ADH2*- exon9 (AA, AC, CC) among cases at ($p < 0.05$). Whereas the presence of an SGOT, SGPT and catalase were non significantly associated with genotype of *ADH3* exon 8 (AA, AG, GG) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$).

Key Words: *Alcohol, ADH2- exon9, ADH3 exon 8, SGOT, SGPT and catalase.*

Introduction

Excessive consumption of alcohol is an growing public health issue in relation to psychoactive legal use and is a global problem , affecting both an individual development, family life and social life.

Alcohol is typically absorbed by mouth, and because it is such a small water soluble substance, it can move through the walls of the stomach and intestines and surface in the bloodstream within minutes. Alcohol requires no absorption and by quick diffusion moves through the body's different membranes. Consumption of alcohol induces various medical problems including hepatic disease. Alcoholism is an important cause of terminal hepatic diseases which is world-wide in distribution [(1)].

Enzymes such as ADH and LDH play a significant role in the metabolism of alcohol in the liver. Drinking of alcohol is followed by processing with ADH1B which turned it into the highly reactive substance acetaldehyde. This toxic substance is oxidized by ALDH2 to acetic acid which through the Krebs cycle is converted into CO_2 and H_2O products which leave the body [(2)]. The three main pathways of alcohol metabolism show in Figure (1). After consumption, alcohol from the stomach and intestines is absorbed to the blood.

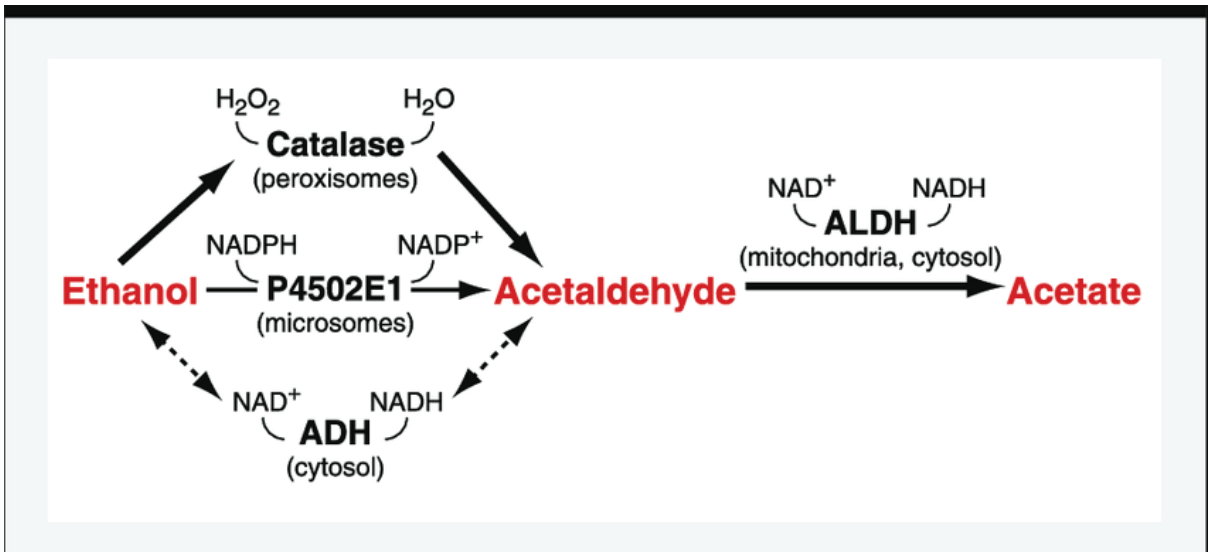

FIG. 1 Alcohol Metabolism [3].

Catalase, ADH, and ALDH may be affected through genetic polymorphisms that affect their activities in converting ethanol to acetaldehyde and then to acetate. ALDH2 is the most important alcohol- metabolizing enzyme that predisposes Asian populations to alcoholism. The prevalence of the ALDH2 * 2 allele, which codes for the physiologically inactive mitochondrial ALDH form, is lower among the alcoholic than for non-alcoholic individuals [4,5,6]. ADH enzyme in humans is a dimeric molecule, resulting from the various subunits of the seven genes. Therefore, over 20 ADH isozymes variants involved in metabolizing different types of alcohols [5].

Material and Methods

Design of the Study

A retrospective cohort study was conducted at the Institute of forensic medicine, and different hospitals in Baghdad, Iraq, during the period from December 2018 to June 2019.

Study population

A total of 50 alcoholic male subjects and 50 nonalcoholic male as a control.

Blood Sampling

Sera were collected from five milliliters of venous blood samples from each study subject. Following conventional methods

Enzymatic assay

The liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic and non-alcoholic males were measured by Reflontron/ Germany as previously described [7].

Genotyping of *ADH2*- exon9 and *ADH3* exon 8 Polymorphisms

Preparation of Genomic DNA

The DNA from genome was prepared from blood samples (gSYNC™ DNA Extraction Kit) following the manufacturer's instructions. Purity and concentration of the DNA was assessed using Nano drop spectrophotometer (Apel/Germany).

Polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP)

The primers were selected, table (1) to amplify fragments of (202 and 130) bp for the detection of alleles for each of *ADH2*- exon9 and *ADH3* exon 8 respectively. PCR mixtures was in 20 µl including 10 p mole/µl of each primer, 1x Master mix (PCR premix / Bioneer/ Korea), and 0.15µg/µl genomic DNA. The mixture was processed in thermal cycler (Cleaver Scientific, UK).

Table (1): List of primers sets

Gene	Prime sequence (5′-3′)	product Size / bp	Reference
ADH2- exon 9	F- TGGACTCTCACAACAAGCATGT R- TTGATAACATCTCTGAAGAGCTGA	202	(8)
ADH3- exon 8	F- CTTTAAGAGTAAAGAATCTGTCC R- ACCTCTTTCCAGAGCGAAGCAG	130	(8)

To detect *ADH2*- exon9 and *ADH3* exon 8 variants the PCR programs were mentioned in table (2).

Table (2): Thermal cycling conditions

Temperature/ Time	ADH2- exon 9/ No of Cycles		ADH3- exon 8/ No of Cycles	
First denaturation(ºC)	94/ 2 min	1	95/2 min	1
Second denaturation (ºC)	94/30 sec	30	95/ 1 min	35
Annealing(ºC)	54/ 30sec		53/45 sec	
Extension (ºC)	72/30sec		72/45sec	
Final extension(ºC)	72/5 min	1	72/5 min	1

The PCR product for each *ADH2- exon9* and *ADH3* exon 8 were digested with restriction endonucleases (*ALwN1* and *SspI*) respectively in a volume of 20µl including10 units of enzyme with buffers supplied by the manufacturer's instructions. The resulted products were analyzed for their molecular size on 2% agarose gel. The bands were stained with ethidium bromide and visualized under ultraviolet light. DNA molecular weight marker of 100bp (BioNeer/Korea) was included to reveal the sizes of the amplicon fragments [9].

Biostatistical consideration

The [10] program was used to analyze the differences in variables among cases and controls. Means, standard Error, One – sample T test was used to compare differences among percentages at a P-value of < 0.05 probability.

Results and Discussion

The present study included blood samples collected from fifty alcoholic Iraqi males registered with the Institute of forensic medicine, and several hospitals / Baghdad, Iraq. The study subjects were men of age mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic individuals, as control group, with mean age of 34.30 years ± 10.86. All the identified frequencies of allele and genotype were in accordance with the Hardy–Weinberg equilibrium ($P<0.01$) as below.

Genotyping of ADH2

The results of present study of *ADH2* genotype were organized into three groups based on polymorphism presence or absence: AA wild homozygous (*ADH2*.1 allele) have one band expected to be 202 bp, the second genotype was CC mutant homozygous (*ADH2*.3 allele) which has both 132 bp and 70 bp bands. While the third

group AC heterozygous exhibited three bands 202 bp, 132bp, and 70bp. Differences in the distributions of the variant ADH2*1(AA) and ADH2*3(CC) alleles among case and controls were significant. Whereas the differences in the frequency distributions of the heterozygous (AC) allele were not significant. The result revealed that the risk of alcohol consumption associated with ADH2*3(CC) alleles (36% among cases vs 16% among control) was greater than the risk associated with ADH2*1(AA) (54% among cases vs 70% among control). ADH2 belongs to the family of alcohol dehydrogenase which converts alcohol into acetaldehyde. ADH2 gene changes were hypothesized to affect genetic susceptibility to *Alcoholic* liver disease (ALD)[11].

Association between genotype of *ADH2*- exon9 and parameters

The association between *ADH2*- exon9 and SGOT, SGPT and catalase were investigated. The current study shows that the presence of a catalase was significantly associated with genotype of *ADH2*- exon9 (AA, AC, CC) among cases at ($p < 0.05$), while SGPT and SGOT were non significantly among control and cases at ($p < 0.05$). Regarding the association between genotype of *ADH2*- exon9 and catalase, there were no differences among control groups, table (3).

Table (3): Relationship between genotype of *ADH2*- exon9 and parameters in cases and control

Group	Genotype of ADH2- exon9	Mean ± SD		
		SGOT	SGPT	Catalase
Case	AA	38.49 ± 24.15	50.51 ± 24.02	242.61 ± 163.84 b
	AC	33.86 ± 11.84	46.08 ± 9.78	446.80 ± 215.84 a
	CC	50.79 ± 23.06	46.70 ± 19.79	521.45 ± 324.16 a
	LSD value	18.93 NS	19.79 NS	202.79 *
Control	AA	19.34 ± 11.39	15.52 ± 10.15	97.69 ± 51.71
	AC	23.86 ± 13.94	14.10 ± 3.30	121.81 ± 58.63
	CC	18.87 ± 8.95	19.35 ± 8.61	100.45 ± 47.44
	LSD value	10.48 NS	7.57 NS	48.87 N.S
* (P<0.05), N.S: Non-Significant. Means that varied significantly with different letters in the same column				

CAT catalyzes hydrogen peroxide to water and oxygen, which are commonly present in livestock, plants and micro-organisms. CAT, which is an endogenous antioxidant enzyme, protects cell injury from ROS and plays a significant role in protection against oxidative stress [12]. Oxidant-antioxidant system may be affected by Alcohol [13].

While Catalase may scavenge hydrogen peroxide resulting from processing alcohol to water, it may decomposes alcohol which harmful that to liver, [14] which attenuates alcohol□induced acute liver injury may inhibit catalase.

The increased activity of catalases following ingestion of ethanol and its impact in the CNS is correlated with low activity of ADH. This increase in CNS catalase activity may be due to adaptive processes induced by hydrogen peroxide increase by animals consumption of high concentrations of ethanol in the CNS [15].

Genotyping of ADH3

ADH3 genotypes were distributed in three classes

based on the existence or absence of the polymorphism: AA wild homozygous (*ADH3*.1 allele) contain restriction site for *SspI* have both 67 bp and 63 bp bands; the second genotype was GG mutant homozygous (*ADH3*.2 allele) with have one band expected 130 bp. While the third group AG heterozygous exhibited three bands 130 bp, 67bp, and 63bp.

The ADH3 genotype for the homozygous AA was not present in samples from cases and control. While the heterozygous AG has genotype frequency as (40%) for case and (36%) for control, while the GG genotype frequency was (60%) and (64%) for case and control respectively, as shown in table (4.7). The current results showed that the ADH3 frequency distributions have not varied significantly. Although there was no significant variations between case and control on ADH3 polymorphism, the result show protective role for ADH3 among individuals with alcoholism, (O.R: 0.084; C.I. 95%:0.77-1.48) for both AG and GG genotypes. Several studies have shown that ADH3 enzyme genetic polymorphism reduces the alcohol risk, and ADH3 * 1/*1 enzyme polymorphism raises the risk of colorectal adenoma [16, 17]. In addition, further studies have reported by [16] and [17] who indicated that genetic polymorphism of ADH2 * 2 and ADH3 * 1 enzymes reduces the risk of alcoholism, and that ADH3 * 1/*1 enzyme polymorphism raises the risk of colorectal adenoma.

Association between genotype of *ADH3* exon 8, and parameters

The association between *ADH3* exon 8 and SGOT, SGPT and catalase were investigated. The current study shows that the presence of an SGOT, SGPT and catalase were non significantly associated with genotype of *ADH3* exon 8 (AA, AG, GG) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$), table (4).

Table (4): Relationship in case and control between the ADH3 exon genotype 8 and the enzymes identified.

Group	Genotype of ADH3 exon 8	Mean ± SD		
		SGOT	SGPT	Catalase
Case	AG	47.82 ± 23.10	52.46 ± 22.46	391.40 ± 279.22
	GG	38.88 ± 23.28	46.18 ± 20.49	344.76 ± 241.97
	LSD value	12.37 NS	12.94 NS	130.59 NS
Control	AG	21.63 ± 11.93	20.37 ± 9.87 a	94.21 ± 31.90
	GG	18.92 ± 11.02	13.43 ± 8.03 b	105.62 ± 60.08
	LSD value	6.94 NS	5.02 *	32.39 N.S
* (P<0.05), N.S: Non-Significant. Means that varied significantly with different letters in the same column				

Serum enzymes are the most widely used and reliable biochemical indicators for liver disease evaluation. [18], who has reported that alcohol is a liver-destroying toxin, one of the leading causes of death due to alcohol disease. While [19], found consumption of alcohol causes several pathological changes in the liver. A percentage distribution of abnormal (GPT, GOT, ALP) in alcohol users was reported to be greater than that of non-alcohol users; (GPT, GOT, ALP) normal levels in non-alcoholic users were lower than those in alcohol users [18].

Conclusion

Excess alcohol intake is a significant concern of public health, especially given the severe damage done by chronic or prenatal alcohol exposure affecting various physiological processes. The current study suggests that genotypes of both ADH2 and ADH3 influence the metabolic rate of alcohol, and are susceptible to alcohol development. The current study shows that the presence of a catalase was significantly associated with genotype of *ADH2*- exon9 (AA, AC, CC) among cases at ($p < 0.05$). The present research indicates the presence of an SGOT, SGPT and catalase were non significantly associated with genotype of *ADH3* exon 8 (AA, AG, GG) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Pharmacological Effects of Topical Dapsone Gel in Experimentally-Induced Thermal Injury in Rabbits

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Abstract

Burn injuries have been a global health issue due to their multiple widely present causes and the burden of their consequences. There have been many chemical agents used topically to accelerate the burn wound healing process and avoid developing infections but the desirable satisfactory level has not been reached. Dapsone, a century-old antibiotic with anti-inflammatory effects has been investigated as a potential topical treatment for burn wounds compared with standard silver sulfadiazine.

Forty-two apparently male rabbits were divided into four groups each of twelve animals except for the negative control group which was 6 animals: intact healthy animals (HA), burn without treatment as a positive control (BWT), dapsone 5% topical and silver sulfadiazine cream 1%, the latter three group' animals were treated twice daily for 24 days.

Burn wound contraction, the levels of tumor necrosis factor-alpha TNF-a in skin tissue homogenates and histopathological changes were assessed at days 8,16, and 24 in addition to in-vitro antimicrobial sensitivity test.

Dapsone has shown a comparable antibacterial activity against gram-positive bacteria to SSD while showing poorer activity against gram negative bacteria.

Dapsone has also shown an earlier and more significant burn size contraction compared to SSD since day8 and until the end of the study and significantly lower levels of TNF-a compared to SSD while showing lower inflammatory and higher granulation, re-epithelization and fibrosis compared to SSD in histopathological examination.

Our study demonstrates that dapsone is a promising candidate as a topical burn treatment due to its antibacterial, anti-inflammatory, and wound healing properties.

Key words: *Skin; Burns; Dapsone ; toxicity; anti-inflammatory; histopathological examination*

Introduction

Burn injuries are one of the most dangerous and potentially lethal skin wounds, they are coupled with several serious local and systemic complications including infections, end-organ damage, disabilities, and psychological problems, and are considered a major cause of death [(1).]

Huge progress was made in the last century in burn wound treatments that reduced much of their burdens and led to a notable drop in morbidity and mortality. One of the major and most effective and widely used interventions is the topical formulating of anti-infective and healing promoting agents [(2)] Major criteria for choosing a topical application include: anti-infective, anti-inflammatory, healing enhancement, and scarring prevention properties as those effects will reduce burdens and long-lasting consequences [(3)].

Despite the availability of a big variety of interventions, more researches are still required to obtain more efficacious treatments to obtain better results for such a stubborn widely prevalent issue [1].

Dapsone is an important anti-infective approved as a first-line treatment for leprosy in combination with rifampicin and clofazimine and is a second-line medication for pneumocystis pneumonia and toxoplasmosis prevention in immune-compromised individuals [4], while in topical form; dapsone is FDA approved for acne and dermatitis herpetiformis [5] and found to have several pleiotropic effects like anti-inflammatory and wound healing actions [6],as this combination of effects is essential in burn wound healing; this study has been done to investigate the topical formulation of dapsone as a 5% gel in burn wound healing and revealed promising positive results.

Materials and Methods

Dapsone 5% gel preparation:

Dapsone 5% gel was prepared following the composition in the brand "Aczone®" gel, according to Gorle & Pawara as follows:

Carbapol 980 gelling agent powder was accurately weighed using a sensitive balance to obtain 3.5 g and gradually dissolved in 170 ml of deionized water under continuous stirring by a magnetic stirrer at room temperature for 30 minutes, until a uniform solution was formed.

Separately, an accurately weighed 10 g of dapsone powder was dissolved in 20 ml of methanol and then added to the above solution under continuous stirring, finally; a small amount of methylparaben preservative and a few drops of triethanolamine buffer were added to the mixture under stirring that was later left to settle as a 200 mg viscous gel of dapsone 5% [7].

Evaluation of the physical properties like color, appearance, and aggregates was done visually and has shown clear white appearance while pH was measured three times(from different preparations) using a digital pH meter, pH records of the formulation were 6.8, 7.1, and 7.3, which are compatible with human skin [8].

Antimicrobial Susceptibility Test (AST) (*in vitro*):

The experimental chemotherapeutic agents were investigated for their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the well diffusion method in Muller-Hinton agar.

The plates were gently and evenly swabbed across their walls with standard normal saline dilutions of mentioned bacteria and inoculated for 6 hours at 37 °C to solidify, and after that; four holes (wells) of 5 mm diameter were made with sterilized biceps and 2 wells in each plate were filled with dapsone 5% gel while the other two were filled with an equal volume of Silver sulfadiazine 1% cream (9), and the plates were re-incubated for 24 hours at 37 °C.

Animal model (In-Vivo):

Forty-two apparently healthy male rabbits weighing (1-1.5) kg and of 8-14 months of age were bought from a local farm and housed in a calm room with standard conditions and were given free access to food and water.

Animal grouping:

The animals mentioned above were divided into 4 groups with 12 rabbits in each except for the negative control which had six rabbits, as below:

- **Group1:** Apparently healthy animals (HA) as a negative control (no burn, no treatment).
- **Group 2:** Induced burn animal without treatment as a positive control, only base gel was applied for lubrication.
- **Group 3:** Induced burn animals treated with dapsone 5% topical gel.
- **Group 4:** Induced burn animals treated with silver sulfadiazine cream 1%.

Chemotherapeutic agents and base gel were applied topically on the burned area twice daily for 24 days.

Burn wound contraction size (Burning zone diameter), inflammatory marker tumor necrosis factor-alpha (TNF-α) level in skin tissue homogenate were studied in addition to histopathological examination.

Experimental procedures:

The animals' backs were shaved 2 days before the procedure and anesthetized by intramuscular injection of Ketamine 50 mg/kg plus Xylazine 5 mg/kg [10]. After that, A specially designed bar with a brass circular end of 3cm diameter was immersed for 15 minutes in a 100°C boiling water and applied to the animals' shaved backs for 45 seconds with no pressure but the bar's weight to produce a full-thickness (Third-degree) burn injury [11], after that, each animal received a single SC dose of meloxicam 1 mg/kg for analgesia [10].

The samples were taken on days 8, 16, and 24 from the burned area for examination after euthanizing the animals by locking them in a closed container with ether-soaked gauze

Burn size contraction (Burn wound diameter contraction):

The diameter of the induced burn was measured 4 times using a ruler, and then the average of these measurements was taken from all burned animals at days 8, 16, and 24 and photographed using smartphone camera. [12] as shown in **Figure 1**.

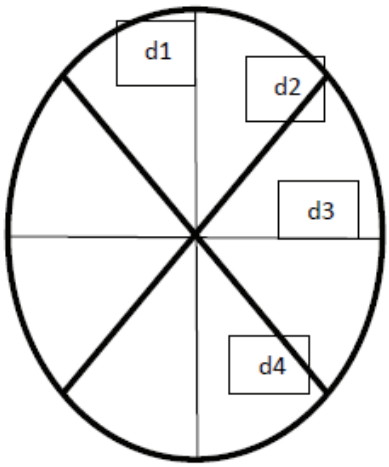

Figure 1 Burn wound diameter measurement method [13]

After the euthanasia of animals, five cm diameter full-thickness skin samples were cut using a sterile sharp blade and washed with distilled water then cut to two equal pieces, the first one is immediately preserved in a cooling box to prevent autolysis of proteic biomarkers and transported to the medical research unit at Al-Nahrain University, College of Medicine for the preparation of skin tissue homogenate while the second piece at day 24 was immersed in the bottom of a 10 sterile ml test tube filled with neutral buffered formalin for histopathological study.

Skin tissue homogenate

A full-thickness piece of burn wound skin tissue was rinsed with PBS and weighed; a 100 mg piece was homogenized with 1 ml of PBS using an electrical homogenizer and (TNF- α) levels were assessed using ELISA.

Histopathological investigation

Skin samples were stained with hematoxylin and eosin and inspected for histopathological changes under the supervision of a skilled independent histopathologist.

Statistical Analysis

Experiment data were analyzed as the mean of a group ± standard deviation Differences between groups' data were performed using a one-way analysis of variance (ANOVA), $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Antimicrobial Susceptibility Test AST (In-vitro)

Dapsone showed significant inhibition in the growth zone of gram-positive Staphylococcus aureus that was at least non-inferior to that of SSD while showing poor activity against gram-negative *Pseudomonas aeruginosa* see **Figure 2**.

These results match with the findings of Zhanel & Rosso who reported that dapsone demonstrated poor activity against Gram-negative bacilli while showing significant inhibition against Gram-positive cocci including resistant strains [14].

This weak activity against Gram-negative bacteria may represent a limitation to using dapsone in burn wounds, however, the effect of dapsone metabolites, including hepatic metabolites like monoacteyldapsone (MADDS) and dapsone hydroxylamine (DDS-NOH) which were found of pharmacological value in both anti-infective and anti-inflammatory effects [15] must be considered, In addition to the hepatic pathway, Drayer

et al. and Uetrecht et al. both reported polymorphonuclear leukocytes (PMN) metabolism of dapsone producing (–NO_2) and chlorodapsone (–Cl) from the non-enzymatic oxidation of (DDS-NOH) [16] [17] while Khan et al. found that dapsone is metabolized in human keratinocytes under the influence of several cytokines to yield DDS-NOH [18]. The pharmacological activities of those metabolites were not assessed yet.

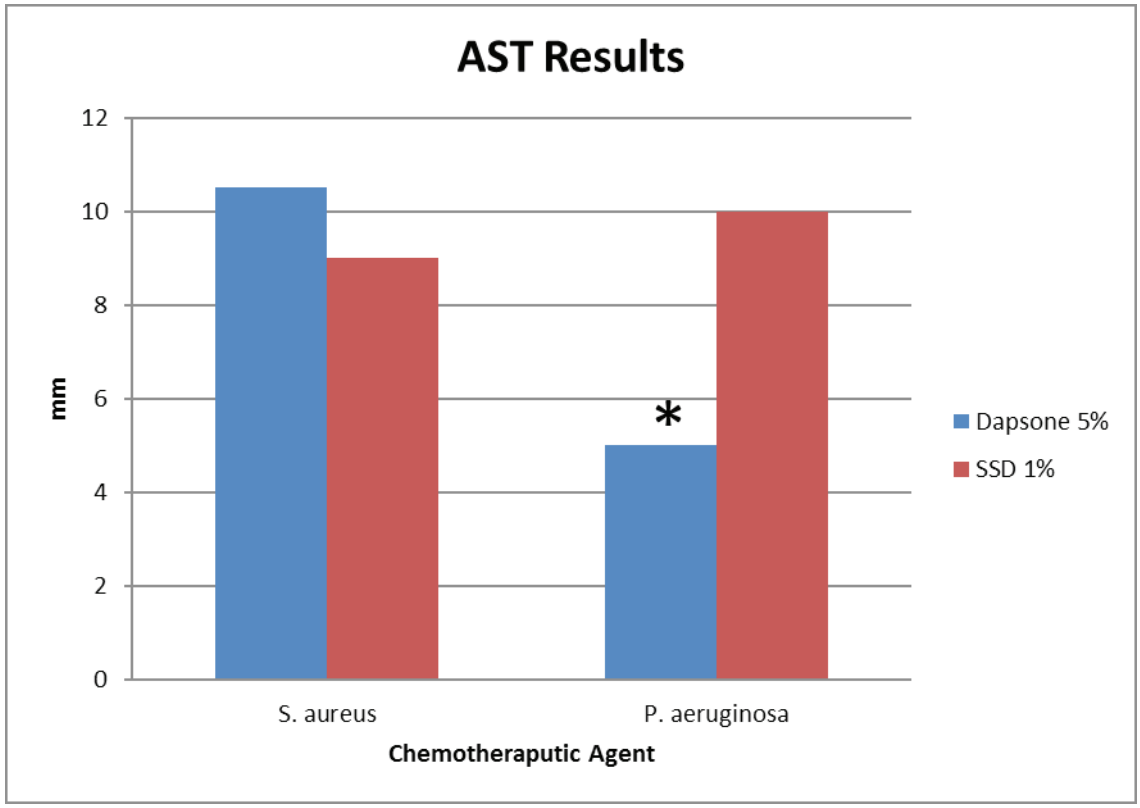

Figure 2 Inhibition zone in mm/24 hours for study agents BWT: Burn without treatment, SSD: Silver Sulfadiazine*: Statistical significannce Burn Wound Contraction:

On day one, there was no observable reduction of burn wound size in any of the experiment's groups, in fact; the wound had expanded for several millimeters around the area of injury.

On day 8, all groups showed a significant reduction compared to day 1 while dapsone and BWT groups have shown significantly smaller wounds sizes compared to SSD with no significant difference among them.

On day 16, there were no significant differences between the groups.

On day 24, the SSD group witnessed a significantly smaller burn size compared to BWT, while Dapsone has shown a significantly smaller burn size compared to both SSD and BWT.

Dapsone demonstrated superior healing effects compared to SSD which can be attributed to its multiple mechanisms of actions, these findings go in line with Chandrashekar et al. experiment that showed superior wound healing effects for dapsone compared to rifampicin and clofazimine [19], and Martins et al. study that showed a better wound contraction demonstrated with dapsone in comparison with mesenchymal stem cells [20].

See (**Figures 3 and 4**).

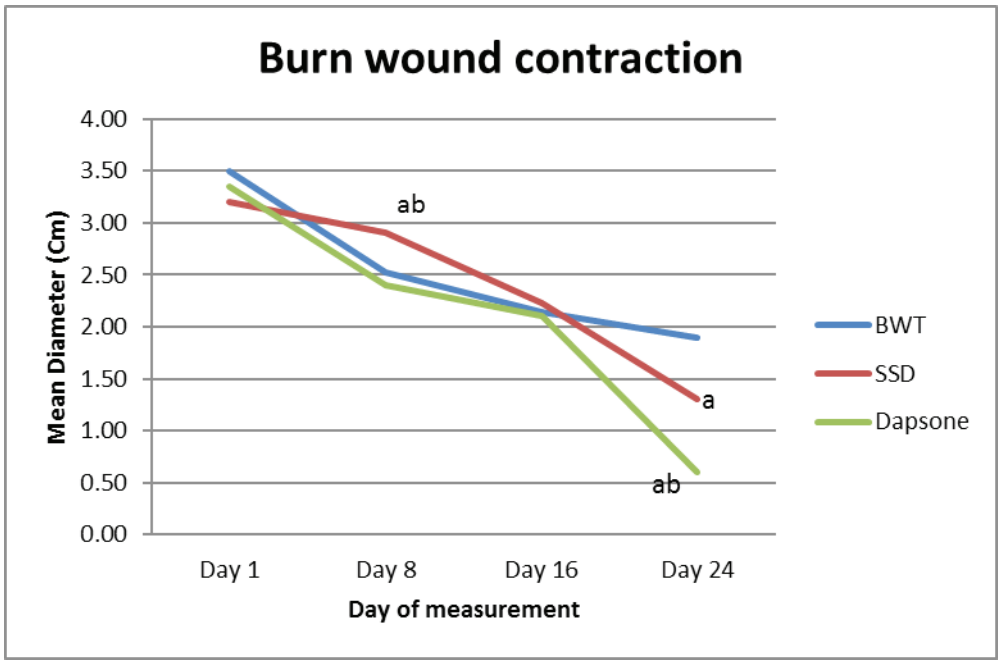

Figure 3 Burn Area Diameter Contraction among the three groups BWT: Burn without treatment, SSD: Silver Sulfadiazine *a*: Significant to BWT *b*: Significance between DDS and SSD

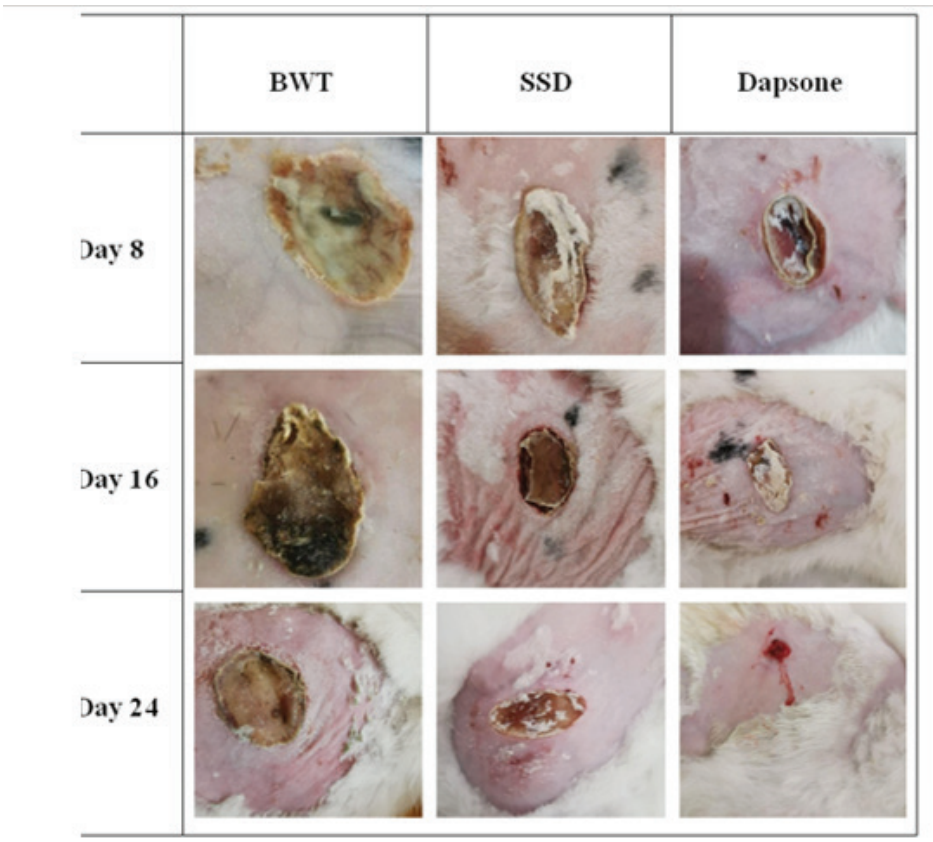

Figure 4 Burn wound contraction among experiment groups BWT: Burn without treatment, SSD: Silver Sulfadiazine

Tumor Necrosis Factor-alpha (TNF-α):

On day 8, all groups have shown a significant spike in TNF-α level compared to AH.

Dapsone group has shown significantly lower levels when compared to SSD with no significant difference among other groups.

On day16, Dapsone and SSD group has shown significantly lower levels than BWT and no significant difference was found between them.

On day 24, SSD group levels were significantly lower than BWT's, and dapsone group levels were significantly lower than SSD.

Dapsone group TNF-α levels at days 16 and 24 were comparable to the AH group levels with no significant difference between them, see (**Figure 5**). It is well verified that major burn injuries stimulate an acute systemic and localized inflammatory response that might be partially attributed to the release of various cytokines [21] and inflammatory markers like TNF-α, C-Reactive Protein (CRP), and several interleukins and factors [22].

The spike of TNF- α is notable in body fluids within the first twelve hours post-injury as an essential component of inflammatory response.

The dapsone effects demonstrated in this study are in line with its various and well-known anti-inflammatory properties and it's mentioned superiority in burn wound contraction and it fits with the findings of Abe et al. , Kwon and Geyfman et al. which have shown significant TNF- α levels' reduction associated with dapsone [23] [24] [25].

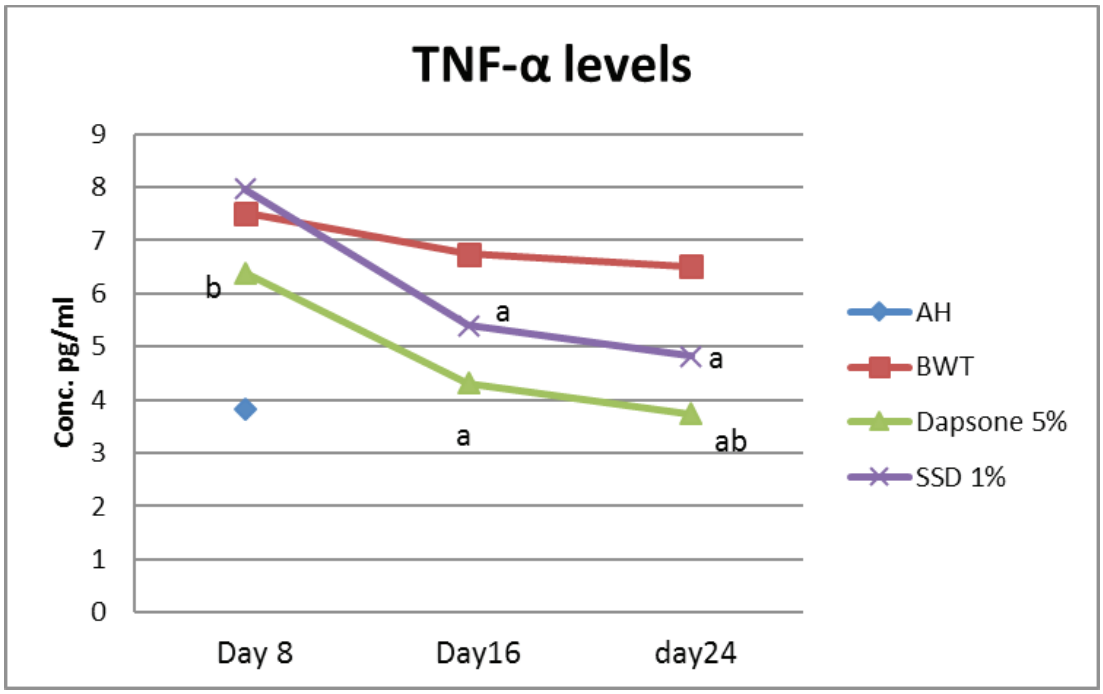

Figure 5 TNF-αlevels in experimental groups throughout the study
AH: Apparently healthy animals, BWT: Burn without treatment, SSD: Silver Sulfadiazine
***a*: Significant to BWT *b*: Significance between DDS and SSD**

Histopathological Examination:

Dapsone has shown significantly lower inflammatory presence (Polymorphonuclear cells) and higher granulation, re-epithelization, and fibrosis on day 24 as shown in **Figure 6.**

Those findings agree with Chandrashekar et al. who showed a significant reduction in inflammatory cells, marked rise in granulation tissue, and earlier fibrosis compared to the control group in skin wounds ([19]).

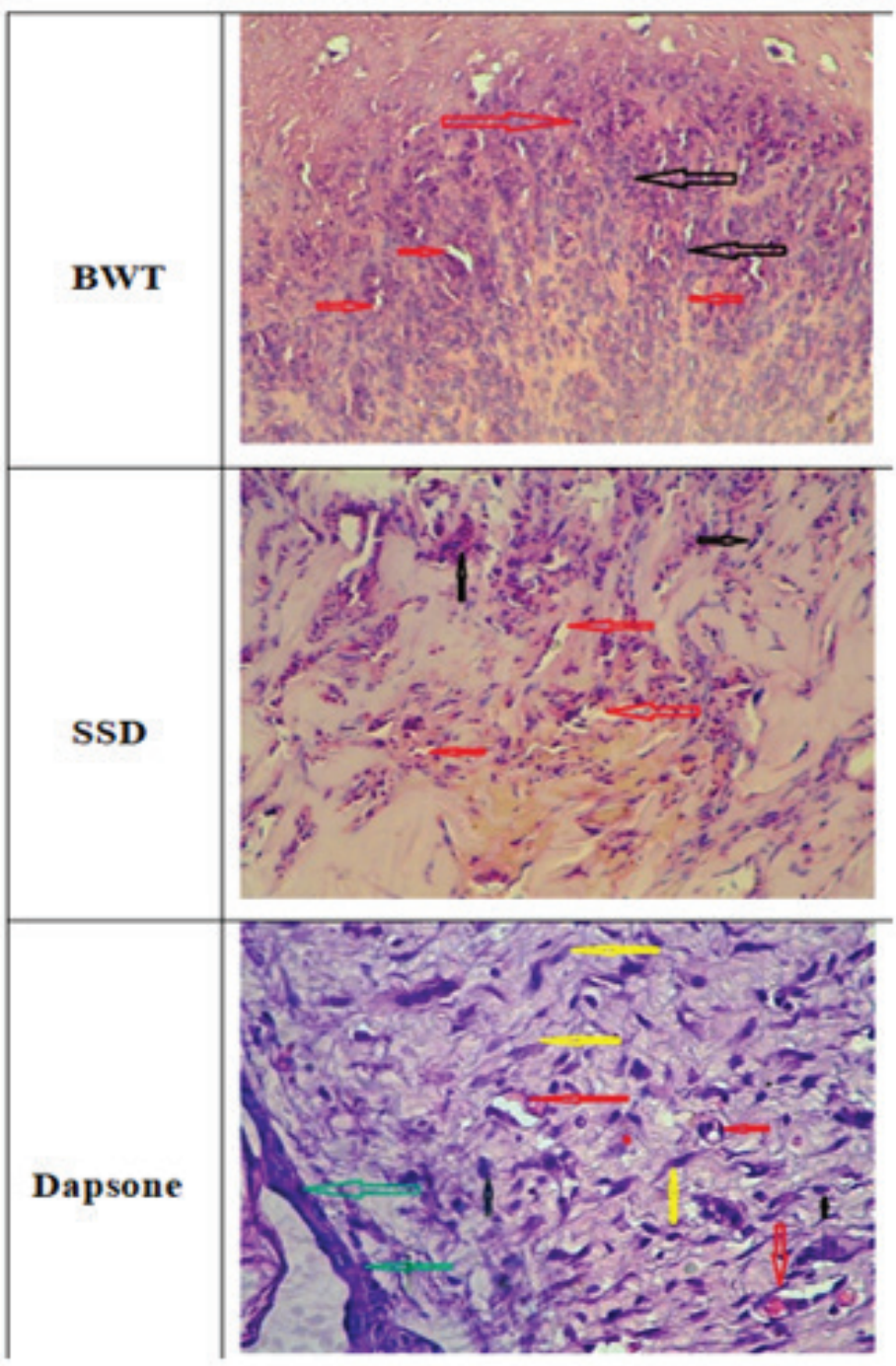

Figure 6 Histological changes at day 24: Black arrows: inflammatory cells, Red: granulation, Yellow: Fibrosis and Green: Re-EpithelizationBWT: Burn without treatment, SSD: Silver Sulfadiazine

Conclusion

Dapsone is a promising candidate as a topical burn treatment due to its antibacterial,anti-inflammatory, and wound healing properties.

Further studies are required to assess its effects on scar tissue formation, the effects of higher doses, different formulations, and the possible pharmacological effects of its metabolites.

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The impact of Specific Exercises by Means of Assisting in Developing some Bio-Movement Abilities and Accuracy of Some Offensive Skills for Young Basketball Players

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Abstract

The current research aims to identify the following: Preparing specific exercises using assistant means . Knowing the effect of specific exercises by using assistant means in developing some bio-movement abilities and accuracy of some offensive skills in basketball for youth. The experimental design is used, also the search community is identified by the young basketball players in the Mahawil Sports Club, which numbered (24) players. Then, the offensive skills test was applied to them, as well as the appropriate statistical treatments were used to reach the results, then the results were presented, analyzed and discussed, as it is reached the following:

1. By using of the specific exercises has a positive effect in developing some bio-movement abilities and offensive skills of young basketball players.

2. Using of the teaching means is better than the used method, which increased the effectiveness of learning some offensive skills in the basketball game.

3. The qualitative exercises by using the educational methods had an effect in controlling the accuracy of performance of skills with the correct way.

Based on the results of the research, the researchers have reached the most important recommendations:

1. Adopting a qualitative exercises approach by using educational methods in the process of learning youth the basketball skills as well as the possibility of using them for students.

2. Relying on educational means as an essential part of the content of the educational curriculum for skills in youth basketball.

3. Emphasizing on generalizing the contents of the qualitative exercises in learning basketball and benefiting from the experiences it provides in the educational process.

Keywords: *specific exercises, assisting, bio-movement abilities, offensive skills*

Introduction

Learning considers one of the important aspects that play an important role in the people progress because it affects both positively and comprehensively in the formation [1] of a new generation, especially if it is based on advanced and modern scientific foundations, that the learner is the centre of the educational process, that the development of his capabilities and ability is the primary goal, as it requires comprehensive and careful attention in providing the requirements which serve the learning process and provide the opportunity to achieve the optimum performance of the various mathematical skills that reflect the learner's ability to understand the

parts and path of movement or skill, that many of the exercises [2] that are used to learn movement skill that which are still using success rates in skill and physical performance, so experts and researchers have sought to find appropriate educational methods that use games and various sporting events to suit the capabilities of the individual. Basketball won wide popular attention and popularity due to the speed of the game and the strength it contains in defense and skill in defense and because it contains many basic and motor skills as it is necessary to reveal [3] it to achieve the required level, proficiency and excel in it, as the educational curriculum that includes various exercises which works to develop them well, that we take into consideration the educational means that serve the goal of the curriculum that we aim to reach through the use of movement exercises by using teaching means to help developing some bio-movement capabilities and learn some offensive skill for basketball players [4].

The Problem of the Research:

Through the researchers' following-up to most of the educational units of the clubs and see the items of the curriculum and exercises which ae used in the learning process, They noticed that most of the units do not contain innovative or modern methods that help and increase the speed and accuracy of learning, as well as reduce the effort in the learning process, also the lack of giving specific exercises that are similar to the playing conditions of the learners, in other words, a combination of modern or innovative exercises, devices and means to reduce the effort and the length of time devoted to learning, as well as the accuracy in learning, from the foregoing, the researchers decided to prepare exercises by using means to develop some bio-movement ability and learn some offensive skill for young basketball players.

Research goals:

1-Preparing exercises with assistant means.

2- Knowing the effect of the specific exercises by using assistant means in developing some biotic capabilities and the accuracy of some offensive skill.

The Research hypothesis:

-There is an effect to the specific exercises by using assistant means to develop some biotic capabilities and learn some skills.

The Research Domains:

- The human Domain: Players defined at Al-Mahawil Sports Club for the sports season 2018-2019.

-**Temporal Domain**: The Duration from 1/9/2018 to 28/7/2019

-**Spatial Domain**: closed hall in Mahawil - Babil Governorate.

3- The Research methodology and field procedures.

3-1 Research Methodology: It was used the experimental approach with the experimental design of the two equivalent groups (experimental and control).

3-2 The Research community and its sample.

The research community included Al-Mahawil Sports Club players for the sports season 2018-2019, as (5) players were selected to conduct the Exploratory experiment on them.

Collecting Information:

1- The questionnaire.

2- Scientific sources and references.

3- The tests.

3-3-2 The Devices and tools which were used:

1. (1)Legal stadium number.

2. (15)Balls.

3.Computer (1) type Dell (5040)

4. Signs.

The Field procedures

1- Determining some offensive skills for basketball players and bio-movement and its tests:

Some skills were selected (high Dribbling, chest handling, aiming from stability), as well as bio-movement which were identified by the researchers after consulting with basketball experts and approved by

agreement (100%).

2- The Exploratory experience:

The special experiment (explosive capacity of the arms , the strength of the arms and legs, high dribbling, handling and aiming from stability) was performed on (5) players, that is at four o'clock in the afternoon on 12/30/2018 and the performance was evaluated by the arbitrators, after (5) days passed, the skills were evaluated again and from the experts themselves as the results were fixed in the evaluation form.

3- The Scientific basis:

First: The Validity: it was used (validity of content) in the research after conducting an interview with experts and specialists, as it was presenting tests for young basketball players (see Appendix 1) as "the validity of the content is one of the most usable types of validity." [(1)]

Second: Stability: It is that the test gives the same results if it is reapplied to the individuals themselves under the same conditions. Accordingly, the test stability coefficient was found by testing and re-testing the exploratory sample, as it was performed on 12/30/2019 and repeated on 3/1/2019 on the same sample in similar conditions as the stability coefficient of the tests was high, that the test stability coefficient, the explosive test for the two arms (0.88), the exploded strength test for the two arms (0.84) and the strength of the two legs (0.90), the handling test (0.93), the dribbling test (0.91), and the steps test, it reached (0.93), which are high coefficients.

Third: The Objectivity: The objectivity of the test "is the test in which there is no discrepancy between the opinions of the arbitrators if the arbitration of the individual tested more than one judgement"[2(2)] as it was used the correlation between the results of the arbitrators * that it was found from the results that the test is highly objective.

The Describing of the selected tests:

First: the test: throwing a ball of 2 kg from sitting farther away[3(1)].

The Goal of the Test: Measuring the exploded strength for the two arms

The Used Tools: flat space, small rope, ball weighted (3 kg), chair.

The Specifications of the performance :

The recipient sits on the chair and the back is straight, the medical ball is held by hands in front of the chest and below the chin, then the recipient is tied with a rope around its chest and held from behind the chair in order to prevent the movement of the body forward with the ball, then the player throws the ball forward with two hands.

Conditions: Each recipient has two attempts that count toward the best.

Registration: Registered the distance of the moving ball in the direction in front of the chair for the best of two attempts and registration the distance to the nearest centimeter.

Second - the test: bending and stretching the arms (the maximum number in 10 seconds). [(1)]

The purpose of the test: to measure the distinct force at the speed of the arms

Tools: handball field, stopwatch, whistle to give the start and end signals.

The Performance descriptions: from the position of bending, stretching the arms, the maximum number possible (10 seconds).

The Test Evaluators:

1- **Registered**: One who calls on names and records the number of times performed.

2- **Timer**: The timing of the test performance.

Registration: The recipient is registered the number of the times in the performing of bending and stretching arms within 10 seconds.

Third: The test: The jumping forward test with the two legs through (10 seconds). [4( )]

The purpose of the test: to measure the strength

1

2

3

4

which is marked by the speed of the two legs.

Tools: flat space ground, measurement tape, stopwatch, whistle, chalk

The way of performance: The recipient stands on the starting line as when hearing the starting whistle and the starting of the stopwatch is began, the recipient starts with the maximum speed bouncing forward with the two legs from the moment of the first whistle until the arrival of the timing as the distance traveled by the player is calculated during time.

The Registration: the covered distance is measured in meters and its parts during time

Fourth- The Test name: The accuracy and speed of passing. [5( )] ()

The purpose of the test: to measure the accuracy and speed of passing for basketball players.

The Tools: Basketball balls

The Procedures:

- Drawing (6) squares on the wall, the length of its sides (60 x 60) cm, three upper squares in one horizontal level and the distance between each square to the other (1.80) m, then three squares below the previous squares, also in one horizontal level.

The Specifications of the Performance:

- The recipient stands behind the starting line, the player is receiving the ball in front of square (1) passes on square (1) and receives it and then passes it on square (2) and receives it, continuously, passes are made by moving to the right side, when it has finished passing the ball over square (6) who passes it again and then receives the ball, and passes it on square (5) and so on the rest of the boxes, until it reaches square (1).

- Continuing to pass the squares as many times as possible during (30) seconds, as he must perform the passes during the movement with noticing that the ball is not passed twice on any of the squares, except for the last square, where it is passed toward it twice.

The Registration:

- The timer starts operating when the ball touches the square (1) and stops the clock at the end of the time of (30) seconds.

- A score is calculated for each ball touching the square and its limits.

Fifth: The Test: High Dribbling. [( )]

The Purpose of the test: Measuring the speed of the dribbling

Tools: stadium, 6 funnels.

The specifications of Performance: the player takes the high start, as upon hearing the starting signal, the recipient runs with the dribbling by changing the direction between the characters(signs), then he revolves around the last person and continues to run with the dribbling between the characters until it crosses the finish line.

The Recording: the time is calculated which the recipient took from the moment the signal was given until he crossed the finish line.

Sixth: The test: The free throw test.

The purpose of the test: to measure aiming or scoring. [(6)]

Tools: stadium, basketball balls.

- Each player is given (10) attempts.

The registration (Scoring): points are calculated, for each player scores a point (for each ball entering the basket) as the player is not counted for any ball that does not enter the basket.

5- Pre-tests:

The sample was organized and distributed on the unit timings and their names were registered according to the totals of the research sample on Tuesday 8/1/2019, in cooperation with the team coach to explain the test procedures and the players 'performance to them, as the special pre tests for both groups were conducted.

6- The Parity:

The Parity of the variables was verified, see Table (1).

Table (1) shows the two means of the experimental and control group, their standard deviation and the (t) value in the pre-test in the research variables.

No	Measurement / Tests	Experimental		Control				Significance
		-S	P	-S	P	t	Sig	
1	The explosive strength of the arms	4.87	0.26	4.65	0.88	1.24	0.06	Not Significant
2	Strength of the arms	6	0.46	6.15	0.77	1.13	0.543	Not Significant
3	Strength of the legs	7.26	0.46	6.79	0.83	0.78	0.082	Not Significant
4	The Dribbling	16.76	0.89	17.57	0.62	0.92	0.123	Not Significant
5	Scoring	3.5	1.01	3.87	0.79	1.05	0.07	Not Significant
6	Passing accuracy	13.75	1.12	12.93	0.45	1.18	0.063	Not Significant

7- Implementing the educational curriculum:

After conducting the pre-tests of the research groups, the researchers did the following:

-The total units are (10) educational units.

-The number of units per week (one educational unit).

-The time of the educational unit is (90) minutes.

-The main section time (60 minutes) (see Appendix 2).

-The time of the practical section (45) minutes.

- The exercises were applied to the experimental group.

8- The Post-tests: The tests were implemented on Tuesday 19/3/2019 for both groups.

9- The Statistical Analysis: The Statistical Bag for Social Sciences is used, Seventeenth Edition:

-Arithmetic mean.

-The Standard deviation.

- The (t.test) for correlated samples.

- The (t.test) for independent samples.

-The Correlation coefficient.

The Presentation and the analysis of results: -

1- Showing the results of the experimental group: -

Table (2) shows the results of the experimental group

No.	The Tests	The Pre		The Post				Statistical Significance
		-S	P	-S	P	t	Sig	
1	The explosive strength of the arms	3.87	0.26	4.67	0.82	4.5	0.001	significant
2	Strength of the arms	4	0.46	6.01	0.32	7.64	0.000	significant
3	Strength of the legs	7.26	0.46	9.01	0.32	9.64	0.000	significant
4	The Dribbling	16.76	0.89	13.54	0.48	3.98	0.004	significant
5	Scoring	3.5	1.01	6.37	1.4	3.05	0.001	significant
6	Passing	13.75	1.12	18.37	1.06	7.88	0.000	significant

By presenting the results in Table (2), it was found that there are differences in the arithmetic mean and standard deviations of the results which are concerned with the tests before and after the experiment, as for the researcher to get to know the truth of these differences and the variables that occur between doing the tests before and after the experiment, The T-test was used, as the results indicated that all results were significant, indicating that there is a difference.

2- The Presenting the results of the control group: -

Table (3) shows the results of the control group

No.	The Tests	The Pre		The Post				Statistical Significance
		-S	P	-S	P	t	Sig	
1	The explosive strength of the arms	3.65	0.88	3.88	0.66	1.5	0.081	Not Significant
2	Strength of the arms	3.98	0.77	4.56	0.59	1.64	0.0689	Not Significant
3	Strength of the legs	6.79	0.83	7.98	0.68	2.08	0.349	Not Significant
4	The Dribbling	17.57	0.62	15.64	0.53	1.98	0.504	Not Significant
5	Scoring	3.87	0.79	4.74	0.67	1.05	0.086	Not Significant
6	Passing	12.93	0.45	13.87	0.78	2.01	0.079	Not Significant

Table (3) Shows the statistical analysis of the control group in the tests of biotic-movement abilities and the skill for the pre and post tests for the basketball players by presenting the results, it was showed the absence of differences in the arithmetic means and the standard deviations of the results of the tests.

3- The Discussing of the results:

There are significant differences in the experimental group in all tests and which tend to the post tests, as the researchers attribute the emergence of such differences to the use and follow of the group to the specific exercises by means of assisting that affect effectively, as the "qualitative exercises are very important in various of sporting activities, as they must be practiced without exception, especially qualitative exercises by means of assistant in order to overcome the effort and reduce it, as well as the presence of the elements of suspense and excitement"[7]

The researchers believe that the use of exercises contributes greatly to the development of capabilities, because the ability to make decisions and proper performance is based on a good vision, so when the player practices specific exercises with assistant means and for various skills, as a result of the repetition of these exercises, they contribute to the acquisition of these capabilities[8].

As for the control, noticing Table (3) shows that there are differences which are not significant, as this is what the researchers attribute to not subjecting members of this group to the exercises as they were only on the trainer method.

The researchers can attribute the reason for the significant differences due to the exercises, which facilitated and increased the clarification of the required skills, which was reflected positively by the results of an understanding and a good awareness of the features, characteristics and parts of the skill, as this was not available for the control group.

The modern learning creates a clear visualization of the movement because "the assistant means of learning has been able to clarify difficult skills, giving them clear explanations and perceptions, enriching them with illustrations, alert the learner to the errors that may occur in performance." [1]

Conclusions

Through the research results, the researchers were able to reach to:

1. The use of specific exercises had a positive effect in developing some bio-movement capabilities with the accuracy of the offensive skills for young basketball players.

2. The use of teaching assistants educationally the method used, which increased the effectiveness of learning.

3. The Specific exercises by using the educational methods have had an effect in controlling the accuracy of the performance of the researched skills and in the correct manner.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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The Impact of the Applied Demonstration Strategies on Learning Referee Signals for Female Basketball

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Abstract

Our research aims to: Preparing exercises for the applied demonstration strategies in learning referee signs in female basketball. Knowing the effect of the applied demonstration strategies on learning referee signals in female basketball. The researchers have used the experimental design with equal groups, as the research community included the second stage students who are (24) female students for the academic year 2018-2019, that they were randomly divided (lots) into two groups by (12) female students, after that, educational units were prepared, using the application strategies as exercises were applied for 8 educational units. The results were extracted and the appropriate statistical treatments were used to reach the results. In light of the results, the researchers reached the following:

1- The applied demonstration strategies had an effective role in learning the referee signals basketball for female students.

2- Using the strategies, save the effort and time for the teacher and the student in learning the referee signals in the basketball for the students well, which it is suitable for teaching this category.

3- The use of the applied demonstration strategies has a positive impact on the interaction of female students during the exercise and their attendance.

Keywords: *applied demonstration strategies, learning, referee, female basketball*

Introduction

The Faculties of Physical Education are interested in using applied teaching methods because they have a positive impact on the speed of learning and in the optimal investment of the effort and time, adopting these strategies and methods as a primary axis in the lesson that qualifies the students to learn and practice the arbitration mechanism. Through this development which is taking place in sport in our time, teaching [1] methods and strategies have emerged, among these strategies is the applied demonstrations strategy, which is one of the methods that helps in the learning process which is required to be learned, as the idea and the image approximate to learn. As basketball is one of the high speed games which requires a high effort by the referees and excellent skill in decision-making with the accuracy in the referee mechanism, that what concerns our study is the referee signals because they are the cornerstones of the game because of its influence and control of the course of play inside the field. Therefore, the researchers wanted to know the importance of applied demonstration strategies and their effect on learning referee signals for female students[2]. The problem: By noticing the researchers that they are specialists and teaching in the College of Physical Education and Sports Science, they noticed a problem for female students in learning signs, which is one of the basic axis in referee signs, as this imbalance has limited the performance of this mechanical that led to the emergence of difficulties for students in their learning because of the difficulty of these signals and signs, the fact that most of the female students are not playing the game [3], thus lead to the emergence of difficulties they have, so the researchers

decided to prepare educational exercises by using the applied strategies in learning the signals for students.

The two Goals of the research:

-Preparing exercises for the applied demonstration strategies in learning the referee signs.

-Knowing the effect of applied demonstration strategies on learning the referee signs.

The Research hypothesis:

There is a positive impact of the applied demonstration strategies in learning the referee signs.

The Research Domains:

The Human Domain: Students of the College of Physical Education and Sports Science - for the second stage of the academic year 2018-2019

The temporal domain: from 10/2/2018 to 9/11/2019.

The Spatial domain: The closed hall in the College of Physical Education and Sports Science.

The Research methodology and the field procedures.

1- The Research Methodology: The experimental design of the two equivalent groups (experimental and control) was used to suit the research problem.

2- The research community and its sample: The research community included students of the second stage, who numbered (24) for the academic year 2018-2019, (5)students were chosen for the survey, as the sample was divided into two groups.

3- Data collection methods, devices and tools which are used.

Data collection methods: (questionnaire - scientific sources - test and measurement).

The Devices and tools which are used: (Legal stadium (1) - legal balls (4) - computer type (Dell) 5040 (1) - Japanese-made digital camera (Nikon) - holder camera (1) - electronic projector (1) - CDs.

Methodology

A- Evaluating the referee signs: A special form was prepared for the evaluation of referee signs, after which a number of experts * were consulted to ensure the clarity of the form, after the consultation it was approved.

B - The exploratory experiment for the referee mechanics form: The exploratory experiment for the refereeing mechanism was conducted on (5) female students, at exactly ten o'clock in the morning on Tuesday 2/10/2018 at the basketball hall in the College of Physical Education and Sports Science / University of Kufa, as the performance was evaluated by the arbitrators, after (5) days, the mechanism of the refereeing signals was evaluated again and from the experts themselves. The results were fixed in the evaluation form and the experiment was useful because it revealed the ability of the evaluator to understand the items of the prepared form.

3- The scientific basis of the evaluation form:

A: The Validity: It was verified in the research by interviewing experts and specialists, viewing the form as "the validity of the content is one of the most usable types of truthfulness."

B: The Stability of the test: A stable test is "that the test gives the same results if it is reapplied to the individuals themselves under the same conditions", accordingly, the test stability coefficient was found by testing and re-testing on, as it was applied on 2/10/2018 and it was repeated 7/10/2018 on the same sample with the same conditions as the stability of the form was (0.98), which indicates a high correlation.

C: The Objectivity: the objectivity of the test "is the test in which there is no discrepancy between the opinions of the arbitrators if the arbitration of the individual tested more than one referee" . As the researchers have used a "Pearson correlation coefficient" between the results of the two arbitrators' evaluation * that the correlation coefficient was (0.99), which is a high correlation.

4-Strategies for the applied demonstration which are used in the research: The strategies of the applied demonstration were used through the application of the following means:

The minimizing stable pictures:They were prepared in two ways:

-Using the appropriate graphics programs that the designers use to prepare various drawings that match with their needs.

- Pictures that are added from external sources by using some cameras, then the pictures have been processed. As it was used by displaying pictures of refereeing signs, that they show the most important details of the signal, its sequence and what its purpose was, as it was put in the form of a booklet, so that each student plays her role in the reference.

* The Computer: The distinctive features of our time and what reveals the amazing developments in the computer world. also its entry into all the joints of life gave the interested people the possibility to take advantage of the many facilities which were offered by this device, the computer-assisted learning system embodies this important role by providing data that contribute in a way to achieve learning as experiments have proven its effectiveness for applications by presenting concepts, presentations and analyzing different systems

Here, the researcher has benefited from this device, which has had a positive impact on his experimental sample, as he has watched the games that include this skill on them through the computer, as he noticed that the sample was affected by the behavior of the students inside the stadium, that this increased the students ’learning of the referee signs.

* The alive model: The researchers have performed the mechanical refereeing signals, where he performed the required signals, as the researchers explained them in detail and explained when to use them and what are the common mistakes when performing these signals, after that, the study sample was given enough time to implement these signals, with the increasing the enthusiasm of the students when they watched the alive model.

* The CD: The researchers designed an educational video that included a refereeing signals, as well as how to give them, the correct sequence of the signal, converting it on a disk, giving it to students of the experimental group.

5- The Pre-tests: The sample was organized and distributed to the unit timings, as their names were recorded according to groups. The pre-tests for the research sample were conducted on Tuesday 9/10/2018, in cooperation with the teachers of the material to explain the test procedures and the performance of female students, that the special pre-tests were conducted for both groups.

The Parity in the research variables: To verify the parity of the two groups (experimental and control) in the mechanism of refereeing signals in basketball for students, see Table (1).

Table (1) shows the two means of the degrees of the experimental and control group, their standard deviation and the (t) value in the pre-test in the search variables.

	Group	S^-	±P	Value of (t)	Value of sig	Significance
The Mechanism of Refereeing signs	Experimental	33.77	2.55	1.78	0.457	Not significant
	Control	33.66	2.84			
* Freedom value (22) and significance level (0.05).						

6- Implementing the educational exercises by using the applied demonstration strategies:

After the implementation of the pre-tests of the research groups, the researchers did the following:

- (8) units of the mechanical teaching for refereeing signals were implemented in basketball for the female students by using the applied demonstration strategies.

-The number of units per week (1).

-Its time is (75) minutes.

-The main section time (60 minutes).

-The time of the practical section (45) minutes.

- The educational exercises were used by using the applied demonstration strategies and applying them on the experimental group, as it included exercises for the mechanics of refereeing signals in the basketball for female students.

- The exercises were done.

7- The Post-tests: The tests were conducted in 11/12/2018 for both groups.

8- The Statistical means: The researchers have used the Statistical Package for Social Sciences SPSS the 17th Edition to extract the following appropriate means:

-The Arithmetic mean.

-The standard deviation.

- (t.test) for correlated samples.

- (t.test) for independent samples.

- Pearson simple correlation.

The Presentation, analysis and discussion of results.

Table (2) shows the statistical means of the experimental and control group in the research variables.

The Group	Variables	Pre Measurement		The Post measurement		(t)	sig	Significant
		S	P	S	P			
The Experimental	The Refereeing Mechanism of signs	33.62	2.55	86.53	1.49	15.51	0.000	Significance
The Control	The Refereeing Mechanism of signs	33.20	2.84	70	1.64	12.86	0.000	Significance
The Degree of freedom (11) and significance (0.05).								

From the Table (2), the experimental group for refereeing signaling mechanics achieved an arithmetic mean (33.62) with a standard deviation (2.55) in the pre-test, while the arithmetic mean (86.53) with a standard deviation (1.49) in the post test. To find the differences between the arithmetic means between the pre and post tests of this group, the researchers adopted a test (t) to verify the significance of the differences. As the calculated value (t) reached to (15.51), which is the significance because the value of (sig) is smaller than the level of significance, which indicates the presence of a significant difference.

The control group achieved the mechanics of refereeing signals between the arithmetic mean (33.20) with a standard deviation (2.84) in the pre-test, while the arithmetic mean (70) with a standard deviation (1.64) in the post test, to know the differences between the arithmetic means between the pre and post tests of this

group, the researchers have adopted a test (t) to verify the significance of the differences. As the calculated (t) value reached to (12.86), which is significant since the value of (sig) is smaller than the significance level.

Since the researchers have noted that there was a significant difference in the research variables, even in the control group, so it went toward finding out if the exercises had an effect that differs from the control group, so the results were as in Table (3).

Table (3) shows the statistical means of the experimental and control group degrees in the research variable

The Group	S	P	(t)	sig	Significance
Experimental	86.53	1.49	9.13	0.000	Significant
Control	70	1.64			

From the Table (3) the experimental group for the mechanics of refereeing signals between the arithmetic mean (86.53) with a standard deviation (1.49) the post-test achieved, while the control group achieved an arithmetic mean (70) with a standard deviation (1.64), while the (t) value is (12.86) which is significant as what we observe from the results of the arithmetic mean and standard deviation is that there is a significant difference.

Discussion

It appears in Tables (3 and 3) that the amount of the difference was clear and good for the experimental group and the control in the mechanism of refereeing signals in the post test compared to the pre-test, the achieving an amount in the development of good performance, as the researchers attribute the difference in the experimental group to the use of (educational exercises by using the applied demonstration strategies) as it had an effect on learning, that the use of new methods and keeping pace with the scientific progress which it is used by researchers, that led to the improvement in the performance of female students by increasing the motivation in learning and increasing desire, suspense and excitement that were among the items of the educational exercises. This was confirmed by the study of Hossam Ghaleb Abdel Hussein , where the results of the two experimental groups were developed.

Conclusions

In the light of the results that have reached by the researchers through the field experiment, the following conclusions were reached:

1- The applied demonstration strategies had an effective role in learning the refereeing signs.

2- Using the applied demonstration strategies saved the effort and time for the teacher and student in learning the refereeing signals very well which it is suitable for the teaching this class.

3-The use of the applied demonstration strategies has a positive effect on the interaction and attendance of learners during exercise.

4- For the educational exercises by using the applied demonstration strategies have a great impact on learning.

Recommendations:

1- The necessity to use the applied demonstration strategies for learning refereeing signs.

2- The need to use the applied demonstration strategies during educational and training applied to ensure the diversity of experiences for the learner.

3- The necessity of making optimal use of the devices and tools to ensure that they benefit from them in the learning process.

4- Learning methods and methods must be in line with the learner's needs and capabilities.

5- Conducting other studies and researches on different age groups and different skills and for both sexes in basketball and other games.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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The Effect of the Kinetic Collections According to the Outcome of Movement Performance in Learning Some Skills in Basketball for Young Players

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Abstract

The Study Aimed to: The numbers of movement collection according to information about the result. Knowing the effect of movement collection on the basis of information about the result in learning some skills for basketball for young players. The design of the two equivalents groups was used, as the research community is determined the players of Al Mahaweel Club in basketball for the youth category for the sports season (2018-2019) by (20) players, that the research community is divided into two equal groups (control, experimental) then items foe collection of movement is designed, so the total educational units reached (8) units, in light of the results that the current research reached:

1. The trainer's approach has a positive effect in learning some basketball skills.

2. The group which used the kinetic collections was better than the other group in learning some skills in basketball.

In light of the results of the research and its conclusions, the current research has reached:

1. Adopting the kinetic collections to learn the basic skills of basketball as being successful and effective.

2. Work to take advantage of the results of the study to improve the level of skill performance in basketball for other groups.

Keywords: *kinetic collections, outcome, movement, performance, learning, skills*

Introduction

The information about the result consider one of the basic and necessary axes for the success of any learning as information is presented, or the skill [1] to the learner who is given during the educational curriculum, as information about the result is presented after each exercise, or given after each educational unit , its meaning is the number of information which received feedback, as the learner can know his mistakes in detail which is very important in modifying the movement path of the player or the learner [2]. Information about the result is important in the learning process and correcting mistakes.

Basketball is difficult to perform as it contains many skills, including defensive and offensive, because the defensive skills need speed and interdependence of many skills, which constitute the kinetic collections that require high performance and high speed, that this requires disciplined and coherent performance to form kinetic collections that [3] give the learner the freedom to use different types of skills together. kinetic collections should be used according to the information about the result in learning some basketball skills for young players. The Problem of the Study : Basketball game considered one of the fast and accurate games, as it is characterized by the difficulty of being fast and requires dynamic performance at a high level of speed, especially in the performance of defensive skills because it is one of

the basics of the game, which contains a great importance in forming the attack, that it needs to be important in his learning, so the researchers noted that as [3] they are specialized in basketball, the inability of the educated players to organize and link defensive skills with each other firstly, then linking it with offensive skills, especially one of the characteristics of the basketball game that more than one dynamic duty is learned at the same time, as this is in line with the requirements of play for the multiplicity of situations that occur in the playing environment [4], which it is open, therefore it is imperative that more than one skill be linked, So the researchers decided to study and find the necessary solutions by placing kinetic collections which based on information about the result. The Two Goals of the research

1-The numbers of the kinetic collections according to information about the result.

2- Knowing the effect of the kinetic collections on the basis of information about the result in learning some skills about basketball for young players.

Methodology

- Exercises with kinetic collections, according to information about the result, as a positive effect on learning some skills in basketball.

The Research Domains:

The Human Domain: Al Mahweel Basketball Club Young Players for the Sports Season (2018-2019)

-Temporal Domain: From 1/15/2019 to 5/11/2019.

- The Spatial Domain: The hall in Al-Mahaweel Club. Babil Governorate.

The Field procedures: -

The Research Methodology: - The experimental approach was used by designing the two groups (control and experimental).

The research community and its sample: - The research community was determined by the players of Al-Mahaweel Club in the young basketball team, which numbered (20) players, that are divided into two groups.

Devices and tools: -

The Research means: -

• Sources.

•Interviews.

• The questionnaire.

• The Tests.

Tools and devices: -

• Basketball court.

• Basket Balls (15).

• Sticky tape.

• Plastic cones, number (3) and length (30) cm.

Determining the Researched Skills:

The researchers identified the skills which are (handling, scoring, defensive moves, pulling balls).

Determining the basic skills tests in basketball: - The researchers determined the tests, which are:

First: The test: the accuracy of passing

Test purpose of the test: To measure the accuracy and the passing speed in basketball.

Tools: basket balls - Sticky tape.

The Procedures:

- Drawing (6) squares on the wall, the length of its sides (60 x 60) cm, three upper squares in one horizontal plane and the distance between each square and another (1.80) m, also three squares below the previous squares in one horizontal plane and the distance between each square and another is (1.80) M.

The Characteristics of the Performance:

- The player stands behind the starting line and receives the ball in front of square (1) and passes on square (1) and receives it, then he passes it on square (2) and receives it, then moves it to the right side, when he finishes passing the ball on square (6), he passes it again and then receives the ball, passes it on square (5) and so on the rest of the boxes, until he reaches square (1), this

is done by moving to the left side.

- He continues to pass the squares within (30) seconds, as he must pass the passes during the movement, noting that the ball is not passed twice on any of the squares, except for the last square, where it is passed twice when the player changes direction.

Registering:

- The timer starts operating on the hour when the ball touches the square (1) and stops the clock at the end of the time of (30) seconds.

Second: The name of the test: **The Scoring**

Tools: basketball court, basket balls.

Registration: The player is awarded two scores for each ball that enters the goal and one score for each ball that touches the ring and does not enter the goal. As the player's score is the sum of scores which obtained in the (15) attempts that the the maximum score for the test is (30).

Third: The Name of the test: Measuring the defensive steps:

The Purpose of the test: to measure the movement of steps of a basketball defender

Tools: legal basketball court, (5) characters, timing clocks (2) tape of measure.

The Procedures: The first sign is placed (on the edge of the free-throw circle), the second and third signs are placed outside the free zone, as the fourth and fifth signs are placed in the specified location on both sides of the stadium near the corner of the stadium and each sign places 7 meters from the starting point.

The Description: The player moves with defensive steps from the middle point of the distance between the fourth and fifth lower sign, the target to the forward until he touches the first sign and then returns to the back with the same steps until the starting point, then he moves with defensive steps to the forward until he touches the second sign and returns with defensive steps until the starting point, then to the third sign, returning to the starting point, then take side steps to the fourth sign, going back to the side steps and touch the fifth sign.

The total time is calculated for the five signs. The recipient performs with two attempts and computes the best attempt.

Fourth: The name of the test: Pulling the ball from under the goal during (10) Sec.

Fifth: The purpose of the test: to measure the speed of pulling the ball from under the goal during (10) sec.

Tools: Basketball Court, 10 Basket Balls, Whistle, Registration Form.

The Performance description: Upon instructing the player to pull a ball from the basket which is thrown on the board by the researcher and then jump to pull another ball and so on.

Registration: counting the number of times the pulling ball during the set time.

The Exploration Experience

It was conducted on a sample consisting of (8) from the research community at exactly (4) pm on (2/22/2019) in the gymnasium hall and after (5 days) the experiment was conducted on the members of the same sample, through which the tests were re-applied.

The Main procedures:

The Pre-tests were conducted with the assistance of the assistant team at 4:00 pm in the closed hall at Al-Mahaweel Sports Club on 5/3/2019.

- The principle of parity between the two groups was achieved as shown in Table (1).

Table (1) shows the parity of the research sample

No.	The Variable	Control		Experimental		t	sig	Type of significance
		S	P	S	P			
1	Passing	1.64	0.84	1.71	0.72	0.20	0.543	Not significant
2	Scoring	19.50	1.65	19.64	1.73	0.28	0.143	Not significant
3	Moves	23.57	2.82	23.71	2.81	0.21	0.284	Not significant
4	Defensive follow-up	3.64	0.74	3.85	0.86	0.61	0.193	Not significant

Table (1) shows that all tests are not significant, which indicates the achievement of the principle of parity in the tests under research discussion.

-The Implementation of the items of the curriculum:

Through the personal interviews, the output of the skill performance was presented according to the relative time of the learners and presenting it, as 25% of the skill performance of (60) minutes was determined, which is the applied part of the educational unit as the time of the educational unit reached to (90) minutes.

- The Post-tests: - After applying the educational units, the Post-tests were conducted, as they were done for defensive and offensive skills in basketball for young players.

-The Statistical medium: The statistical program was used to process data.

- Showing the results of the control group:

Table (2): shows the Statistical analysis of the control group

No.	Variables	Pre-test		Post-Test		(t)	sig	Type of Significance
		S	P	S	P			
1	Passing	1.64	0.84	2.85	0.66	6.49	0.000	Significant
2	Scoring	19.50	1.65	21.64	1.44	12.09	0.000	Significant
3	Various defensive moves	23.57	2.82	27.28	1.93	4.75	0.000	Significant
4	Defensive follow-up	3.64	0.74	4.71	0.72	6.51	0.000	Significant

Table (2) showed, all tests were significant.

-Presentation of the experimental group:

Table (3) shows the statistical analysis of the experimental group

No.	Variables	Pre-test		Post-test		(t)	sig	Type of Significance
		S	P	S	P			
1	Passing	1.71	0.72	4.90	0.49	7.87	0.000	Significant
2	Scoring	19.64	1.73	23.50	0.28	9.88	0.000	Significant
3	Various defensive moves	23.71	2.81	31.26	1.25	8.44	0.000	Significant
4	Defensive follow-up	3.85	0.86	7.28	0.61	9.55	0.000	Significant

Table (3) showed, all tests were significant.

-Presenting the post results of the control and experimental groups:

Table (4) shows the statistical analysis of the control and experimental research groups

No.	Variables	Control Group		Expremental group		(t)	sig	Type of Significance
		S	P	S	P			
1	Passing	2.85	0.66	4.90	0.49	7.06	0.000	Significant
2	Scoring	21.64	1.44	23.50	0.28	5.16	0.000	Significant
3	Various defensive moves	27.28	1.93	31.26	1.25	8.12	0.000	Significant
4	Defensive follow-up	4.71	0.72	7.28	0.61	8.29	0.000	Significant

Table (4) shows the results of the experimental and control groups in the post-tests, as all of them showed significant tending to the experimental group through the above of the presentation and analysis of the tables, which shows that there is significant indication in the tests, that the reason for these variables is the impact of the curriculum that which is used, as well as the continued and regularity of the players in the learning process, which had a clear role in the development of the skills, likewise, the effectiveness of kinetic collections with the experimental group as it is tried with them as much as possible to be similar to the cases of playing for the sake of practice and motivation which generated by the players, as well as gives the spirit of actual participation and speed in action and reaction ”In other words, introducing elements similar to the conditions of playing, because this type of exercise helps a lot in the cohesion and integration of game skills, so that they will ultimately be in the service of the learning process” also it was emphasized by (Hanafi Mahmoud) ” playing is the continuation of learning

As for the presentation of Table (4), it was appeared that there are significant differences due to the effectiveness of kinetic collections which are prepared by the researchers, that led to the remarkable progress, the exercises which were used in the service of the

kinetic performance itself for the skills "Choosing the approprate exercises enable the development of physical charecteristics and at the same time improves the player's skills"

Likewise, the repetition contributes to developing performance, reaching the right thing, also leads to relative change. This means giving opportunity to weak emerging players, repetition and stability in performance, what completes these is that the kinetic collections that were given to the young players are appropriate for the flow of movement as "The player must perform the kinetic collections that are very similar to the basic requirements in the game, whether they are functional, kinetic, physical, tactical or technical requirements"

Moreover, the exercises have led to the interaction of the players, so that the kinetic collections motivated their performance "in the kinetic performance through the kinetic collections by linking the two movements to each other, or moving within the same movement, between the parts of the movement when it contains more than a kinematic part, as one of its most important features is kinetic compatibility and flow.

That the kinetic collections consider one of the best ways to develop and raise the level of achievement in defensive and offensive skill methods, which is reflected in the level of skill performance during the competition.

As Muhammad Abdul-Ghani, 1987 indicates that the return of information is a basic condition of the educational process, it has been proven in the experiment that the educated individual is in urgent and continuous need of trial as the error is necessary in acquiring skillful learning

Likewise, the researchers attribute the reason for this to the effectiveness of the exercises that the researchers had been prepared had a prominent impact on the players, also that these exercises add excitement during the lesson, as well as the tools that were used.

Conclusions

1. The kinetic collections which were prepared by the researchers have a positive effect on learning some basic basketball skills for young players.

2. The use of the kinetic performance result before the new educational unit is helped to inform the educated player about the result performance with for the form of skills which he performed and contributed to learn the offensive and defensive skills under discussion.

3. The use of the relative time to provide information about the performance result for learners about the result of their performance during the curriculum, which represents 25% of the total repetitions of the educational unit, which represents 15 minutes, was sufficient time to provide information about the performance result for learners.

Recommendations:

1-Adopting the kinetic collections to learn the basic skills of basketball because it is successful and effective.

2- Working to benefit from the results of the current study to improve the level of skill performance in basketball.

3-Inserting the kinetic collections in the teaching process.

4-It is possible to use the relative or absolute time in information about the performance of learners, as this is very important, to inform the learner about the result of his performance and identifying his mistakes.

5-Various other proportions of the relative time can be used to provide information about the result, or they can be used to provide feedback on the performance of each player.

6. Conducting similar studies to provide information about the result of the kinetic performance in other skills according to the kinetic collections in other sports and a different sample.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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Building the Causal Attribution Scale of Winning and Losing for Basketball Coaches

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Abstract

Sports psychology is concerned with studying human behavior as a living being that seeks to adapt to the stimulation and the sports coach is one of the samples to which this applies, as he has to follow a certain behavior to deal with his players, which requires him to form his own philosophy and this philosophy appears clearly through his training profession As the work in training aims to gain more respect and self-esteem and good social status.

All of this appears through the sporting competition that ends either with winning or losing, and here comes to the coach's mind the evaluation of many justifications and the reasons behind the gain or loss, including personal and external or administrative reasons, and here the issue of causal attribution emerges, and there is no study that has identified this topic and for this This study aimed to build a measure of the causal attribution of victory and loss for basketball coaches.

Keywords: *Scale, Causal Attribution, Winning, Basketball, Losing*

Introduction

The recent years are witnessing a growing interest in the scientific study of human behavior in the athlete, with the aim of achieving maximum performance and personal development for exercise, which is known as the field of sports psychology [1]. And since psychology deals with studying human beings as a living being that seeks to adapt to the environment, it is well established that the apparent behavior reflects the personal characteristics that represent human behavior, so psychologists and education have studied scientific behavior in order to understand the best characteristics of this behavior and guide it to the good of man and the well-being of society [2]. When we deal with others, we are merely curious, and at other times to define how to deal with them in the context of life, as this knowledge often depends on observing subjectivity and personal opinions, making it characterized by inconsistency, objectivity and possibly bias. Here Ratib confirms that "every person in life has goals that he seeks to achieve or a philosophy that directs his behavior, and this is the case for the coach. He must have a philosophy that directs his behavior and is interested in defining it and developing it, and it is then more than just knowing the technical aspects of sport [3]. It helps in testing The appropriate method for dealing with players in different situations The coach's philosophy is general principles and beliefs that guide his behavior in sports-related situations and they represent levels that are (life philosophy, training philosophy, and specialized sports philosophy in which he undertakes training) [4] This philosophy emerges into existence through the profession of sports training adopted by the sports coach, as the work in training aims to gain more respect and self-esteem and a good social situation, all of which appears through sports competition that is characterized by winning or losing, so winning is usually a share A little while the loss is a lot [5]. Sports competition is seen not as a test position to show the best players, but a test position to show the best coaches, but when players succeed or fail, this is a kind of success or failure for him [6]. Because of the increased interest in winning and achieving, the athlete-trainer proposes goals that increase his capabilities and therefore difficult to achieve despite the use of different training methods and by that effort, which makes the athlete-coach more vulnerable to the phenomenon of

excess training. And when he fails, he becomes more vulnerable to negative psychological stress, and here (Allawi 1998) confirms. "The sports training profession is one of the most different professions related to stress, as the coach's work is related to many factors that are characterized by emotions, such as anxiety, tension, physiological arousal, and psychological pressure.

Here, comes to the mind of the athlete-coach and offers many justifications and reasons for gain or loss, including personal and external or administrative reasons related to the nature of his work in order for players, administrators, the public and the media to understand the methods (causal attribution) of winning and losing.

1-2 Problem of the study :

Sports coaches with different specializations of their sports activities during sports competition are exposed to the principle of winning or losing, which leads to suspension of this for various reasons including negative and positive ones specific to the coach or player.

Through the researcher's experience and currency in the field of basketball, he noticed that there are reasons that lead to winning or losing behind the coach. In view of the lack of psychological field studies that dealt with this topic and to supplement the mathematical scientific library in general and basketball in particular, the researcher resorted to - building the causal attribution measure of winning and losing for basketball coaches.

1-3 Aims of the study

1) Building a causal attribution measure of victory and loss for a basketball coach.

2) Knowing the level of the causes of winning and losing from the point of view of basketball coaches.

1-4 Limits of the study:

1-4-1 Human Limits : 1st division basketball clubs managers

1-4-2 place limits: sports clubs – sports halls / In the country's provinces

Section Two

3- Procedures of the study

3-1 The methodology:

The descriptive method was used in the survey method to suit the nature of the research.

3-2 Research community and sample:

3-2-1 The sample of building the scale:

The sample attribution of the basketball attribution scale for basketball coaches has been tested in the intentional method, represented by the coaches of the country basketball clubs, as they reached (30) coaches.

The causal attribution scale has been applied to basketball coaches after completing the steps of building it according to building psychological standards on the research community represented by the coaches of the Baghdad Governorate basketball clubs, which are (4) clubs, to become the research by (11) coaches and the machine table shows that and some variables about the research community.

The table represents statistical parameters and variables of the research community

Training Certificate	Academic Achievement	Average age Training ةنسلاب	average age Chronological year	Statistical processors
International first	Intermediate	9	41	-M
	Bachelor of Physical Education	4.53	5.61	± s.d

-3 3 Research tool:

The causal attribution measure for basketball coaches "building and applying»

This part includes the scientific procedures that the researcher undertook during the construction of the causal attribution scale for the handball coach, which included:

3-3-1 Numbers and phrasing of phrases:

3-3-2 Truthfulness and validity of the statement

3-4 Statistical Analysis of Phrases (Distinguishing Power of Phrases):

3-4-1 Statistical analysis by way of cracking groups

The table represents calculated statement numbers and calculated T values

calculated T value	no. of statement	calculated T value	no. of statement	calculated T value	no. of statement
4.08	25	7.81	13	2.86	1
8	26	17.6	14	11.75	2
6.08	27	2.82	15	7.35	3
4.59	28	12.4	16	2*	4
2.73	29	1*	17	17.6	5
5.89	30	9.69	18	5.86	6
1.43*	31	5.87	19	3.59	7
2.41	32.	11.73	20	1*	8
2.41	33	4.82	21	0.89*	9
5.89	34	12.25	22	3.79	10
		12.28	23	4.18	11
		4.15	24	3.35	12

* Not significant at 0.05 error rate

In front of the degree of freedom (22) Tabular value (T) = 2.04

* Unmarked phrases carry numbers (31.17.9.8.4)

3-4-2 Statistical analysis using the factor analysis method:

The global honesty of (the causal attribution measure of a basketball coach)

The researcher used global honesty, which is an advanced statistical method that relies on global analysis to prove the sincerity of the tests to obtain the global honesty of the sample for excellence which is (30) trainer forms. Conducting the distinction process in two ways (extremist groups and internal consistency) of (28) words. The following is the sequence of the statistical procedures that used the global analysis:

1. Correlation matrix calculated

2. After analyzing the correlation matrix, the in-kind values (latent root) and the ratio of variance and variance combined to the correlation matrix variables were found, as these labels are important statistical indicators that confirm the accuracy of the tool and the legitimacy of its use, as the number of extracted factors is based on the number of latent roots equal or greater than the correct one for each Factor

The following table Shows the actual values, the percentage of variance, and the variance combined for causal attribution scale factors

The combined variance	the percentage of variance	The actual value	no. of factor
38.169	38.169	10.687	A1
61.988	23.818	6.669	A2
84.488	22.5	6.300	A3

5-3 The stability of the scale

3-6 Description of the scale and its correction: (The final image of the scale)

3-7 The final application of the scale:

3-8 Statistical means:

- Arithmetic mean
- standard deviation
- Simple correlation coefficient
- T-test

Results view and Discussion

After collecting the data obtained by the researcher through his application of the scale and in order to ascertain the extent to which the research hypothesis was achieved, the results were presented according to a mechanical image:

The following table represents the mean, the standard deviations and the calculated test (T) value or the lifetime variable

Result	calculated (T) value	+-s.d	M-	amount	Age Chronological year
* moral	2.94	6.32	47	4	30-39
		5.27	36	7	40-above

*** Moral at the error rate of 0.05 and in front of the degree of freedom (9) value (T) tabular = 2.26**

From the table it is clear that:

· The highest mean arithmetic mean for search personnel at ages (30-39) years is (47), with a standard deviation of (6.32). Whereas, the arithmetic mean for ages (40 years and over) was (36), with a standard deviation of (5.27).

· There were significant differences in the degree of causal attribution among basketball coaches according to the age variable in favor of minimum ages (30-39) years.

The researcher attributes the appearance of the results to the experience factor of the sports coach regardless of the gain or loss, as experienced coaches are described as more balanced and calm and less concerned with their peers, the coaches whose experience is limited or less than it, stressing that "the coach can face many problems related to the qualification process and provide capabilities The players and the wrong perceptions that are considered causes of psychological stress and differ according to the training age "This is if we know that most of the coaches of the research sample are those who practiced the game as a player and for many years formed him as coach currently previous experiences to his current experiences. This result is consistent with what he mentioned (Ratib, 1997) that "more experienced trainers have better skill to adapt to the pressures of training and competition than coaches with less age or experience" as well as what he indicated to him that "The experience of the athlete - the coach - the important and effective role in controlling unwanted emotional states before, during and after the competition

Conclusions and Recommendations

Conclusions

1. The causal attribution measure for basketball coaches - prepared by the researcher as a scientific indicator and as a tool to measure the degrees of causal reasoning of victory and loss for handball coaches exclusively during sports competition

2. The basketball coaches who were included in the research agreed their interest in the factors (self, external and administrative) as basic factors for the reasons for the causal attribution (the causal explanation) for winning and losing for the sport competition.

3. After applying the scale, the researcher concluded:

4. Chronological age (training experience) and educational attainment have a positive impact on explaining the degree of causal attribution of victory and loss. (More experienced coaches have better skill in attributing accuracy (causal reasoning) to winning and losing.

5-2 Recommendations

According on what the research results showed, the researcher recommends the following:

1. Adopting the research tool - the causal attribution of winning and losing for basketball coaches - as a research tool and used exclusively for basketball coaches.

2. Since the behavior was that in an acquired condition acquired by the individual - the trainer - through its preparation and refinement, it must be entered into continuous training courses commensurate with its training and scientific level in order to inform them of the latest methods and strategies in team management and leadership, whether in training or competition.

3. Get coaches into local and international courses and tournaments to coexist with the different sporting competition conditions.

4. Create requirements for the success of the training process and sports competition and give the coach the freedom to make the appropriate decision for all cases.

5. The distribution of tasks and duties between the players, coaches and administrators during both training and competition according to its competence and responsibilities, non-duplication and authoritarianism in it.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under The General Directorate of Dhi Qar Education and all experiments were carried out in accordance with approved guidelines.

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An analytical Study of the Reality of Legal Knowledge of Some Group Games among Physical Education Teachers in Babylon

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Abstract

The research objectives to identify the level of legal knowledge of physical education teachers in Babylon governorate, as for the research methodology, the researcher used the descriptive approach in the style of survey studies because it is the best and easiest curriculum to reach the achievement of the objectives to be researched. The research community of physical education teachers in the Babylon education directorate for the academic year 2020-2021 has been identified, their number (699) distributed into four departments each: From (Al-Hilla Al-Markazi, Al-Mahawil District, Al-Hashemiya District, Al-Musayyib District), as for the research sample, it was determined randomly by (223) teachers and schools, and it formed a percentage of (31,90) of the research community. As for the most important conclusions, they were that the current scale is able to reveal the level of legal knowledge of physical education teachers, as the scale shows that there is a clear weakness in the legal knowledge of physical education teachers in some group games resulting from not following up on the amendments to the law of these games. As for the most important recommendations, they were as follows: Paying attention to the law for all games in a more serious manner by physical education teachers, it is also necessary to give part of the lesson time in which the legal aspect is addressed and to hold courses or training sessions to explain and interpret the articles of law for some group games, and also directing teachers by their supervisors upon their visits to a simplified explanation of the law during the implementation of the lesson plan, with the follow-up of sports activity through field visits to find out the legal level of physical education teachers, especially (female teachers).

Keywords: *analytical study, legal knowledge, physical education, Babylon*

Introduction

Physical education, with its various activities, is one of the important and vital aspects of the educational process in the school, as it is concerned with the teacher and student acquiring values by virtue of its nature and objectives because it participates in its various aspects of activities that deal with social life in addition to its concern for health, theoretical skills and physical abilities. Knowledge of sports, especially group games, is one of the important matters that physical education teachers, a practitioner or a spectator, should understand and absorb an appropriate and sufficient amount of them, an efficient physical education teacher is the one who combines information and practical skills, including legal knowledge, without the knowledge associated with it, it is a deficient process, the profession of a teacher is great because he is the person who carries out the systematic teaching process, and which most groups of society go through, as every individual receives some kind of education, the major message of the teacher requires a great effort to develop and develop their information and acquire various skills and knowledge so that they can influence those who teach them and create positive interaction between them and the students. The importance of school sport, one can deny its importance as the basis for the development and success of any sports game, and over the course of the sporting process the school had an indispensable role in the development of Iraqi sports, and therefore we see that the physical education teacher is the cornerstone of all this development, get to school sports, on the other hand, we see today the physical education lesson as a neglected lesson in most schools and not given its importance, so there has become an urgent necessity for a teacher that

is constantly evolving in line with the spirit of the age to keep pace with development and meet the needs of the lesson. Here lies the importance of research by preparing several paragraphs that include legal knowledge of some of the groups games most practiced by the student and loved by students and teachers through which we can identify the information level, in terms of understanding and interpreting legal cases and the extent to which teachers enjoy them to put the results in the hands of officials to develop and evaluate the specialist .

Research problem:

The preparation of a physical education teacher project from the researchers' point of view starts from the school, so if there is a good teacher who has the ability to properly communicate the information to the student through the interpretation of cases in a good and correct manner based on scientific foundations free of errors, which leads to our obtaining a teacher project a successful person is aware of the important role he will play and demands to fulfill it, We know that the teacher is a role model for his students, and if the theoretical, cognitive and scientific level of this example is weak, it will definitely be a bad example that will reflect negatively on the student's desire to practice sports in the future, and vice versa, Researchers noticed during their participation, judging, and their attendance in school races held annually by the sports activity for schools and being federated for some team games, they found a clear weakness in the legal knowledge of most teachers of sports education, especially in groups games.

Research objective :

Identifying the level of legal knowledge of physical education teachers in Babylon governorate

Search fields :

- **The human field**: Physical education teachers in Babylon governorate, which constitute the governorate center of Al-Hilla, Al-Mahawil, Al-Hashemiya and Al-Museeb.

- **Time field**: From 1/11/2020 until 10/1/2021.

- **Spatial field**: The schools of the Babylon education directorate.

Terms used in the search:

Legal knowledge: The researchers defined it procedurally, which is the teacher's familiarity with the articles and vocabulary of law for all games, especially the group games most practiced and traded among students, and the interpretation of errors that occur in races and matches, and following up on all new amendments to the laws of games.

Research methodology and field procedures:

Research Methodology:

The nature of the research problem is aware of the great interest in legal knowledge of physical education teachers, so the researcher used the descriptive approach in the style of survey studies because it is the best and easiest method to reach the achievement of the objectives to be researched.

The survey "provides the researcher with information that enables analysis, interpretation and decision-making, and reveals for him the relationships between the studied variables" [(1)].

Community and sample research:

Determining the research community is one of the important steps and stages in the process of conducting the research, as the researcher can deal with the whole community with research if this community falls within the limits of its capabilities and capabilities, and the research community knows "all the individuals, events, or things who represent the problem of research" [(2)], the research community included physical education teachers in the Babylon education directorate for the 2020-2021 academic year, their number (699) distributed into four departments (Hilla Al-Markaz, Al-Mahawil District, Al-Hashemiya District and Al-Musayyib District).

The research sample: The research sample was determined randomly by (223) teachers and schools, and it formed a percentage of (31,90) of the research community, as follows: Hilla (center), where the total number of the community was (304). As for the survey sample, it was (5) and the main sample was (97), with a percentage of (43.49), as for the district of Mahawil, the total number was (106), while the survey sample (5) and the main sample (39), with a percentage (17.48) as for

the Hashemite district, the total number was (172). As for the survey sample (5) and the main sample (47), with a percentage of (21.07), and the Al Musayyib district, the total number was (117). As for the survey sample (5) and the main sample (40), with a percentage of (17,93).

Devices, tools and methods used in the research:

Any research from the research is not without means to collect data that the researcher can use to solve a problem and achieve his goals, and based on the researcher's use of the following research methods:

Observation:

Through the researchers attending the training lessons, their participation in the school races held annually by the sports and scouting activity in the governorate, and because they are certified referees in the Iraqi central federation for team games (football, basketball and volleyball), they noticed a clear weakness in the legal knowledge of most teachers and physical education teachers, especially in differential games, and through it the problem of the present study was identified.

Interview:

The interview is defined as "a friendly dialogue between the researcher and one or more specialists to consult his specialist to provide information that the researcher can use in the diagnosis to determine the methods of guidance and means of treatment" [(1)], The researcher conducted a dialogue with a professor at the College of physical education* to collect information on how to reach appropriate solutions to the research problem and what are the important procedures on how to place questions related to legal knowledge in its own form.

Scientific sources and references:

Researchers reviewed many scientific sources and references that dealt with legal knowledge of researched games, general psychology, sport psychology, and scientific research to support the research steps.

Tools and devices used:

- Calculator
- Stationery (papers - pens)
- DELL laptop.

Field research procedures:

Specify Team games to represent legal knowledge:

The researchers distributed questionnaires and questionnaires (9) from sports activity directors and supervisors of a sport education specialty, to show which of the differential games most participated by physical education teachers in the annual races held by the directorate of sports activity and Scouting in each of the sports activity departments affiliated with to the Babylon education directorate, as follows: Basketball, the number of agree were (9), the disagree was (0) and kai square was (9), and the level of significance was (0.001), and the significance of the differences was (sig), and football, the number of agree were (8), while the disagree (1) and kai square was (5,44), the level of significance was (0,000) and the significance of the differences was (sig), Track and field were the number of agree (3), the disagree were (6) and kai square was (1) and the level of significance was (0,411) and the significance of the differences was (non sig), and Futsal, the number of agree were (8). As for the disagree was (1), and kai square was (5,44), the level of significance was (0,000), and the significance of the differences was (sig), and handball, the number of agree were (4), the disagree were (5), and kai square was (0.05), the level of significance was (0.283) and the significance of the differences was (non sig).

Numbers of legal knowledge paragraphs:

After the group games that participated in the sports races held by the sports and scout activity for the academic year (2020-2021) were identified (basketball, volleyball and futsal), accordingly, it became necessary to define the legal paragraphs of those games, as researchers worked to prepare a questionnaire for each of the aforementioned games, as each questionnaire contains (15) questions from the law of the game to be measured and mentioned above, and the researcher put the answers in the form of choices and answered them by placing any a sign or indication of the correct choice after reading the question.

Find the validity of the paragraphs of legal knowledge:

In order to find the validity of the paragraphs, the researchers worked to present these paragraphs to the experts from the directors of the sports activities departments in Babil Governorate and the specialized supervisors for sports education, as follows:

Paragraphs (1,3,5,8,10,11,16,13,17,19,20,21,38, 78) were agree by (9) as for those who disagree (0) and the chi-square (9) and the indication significance of the differences (sig), as for the paragraphs (2,26,29,6,7,31, 32,35,39,42,43,41,36,24), those who agreed upon them (7) and disagree (2) and the Chi-square (4,5). And the significance of differences (sig), about the paragraphs (6,9,12,14, 15,18,25,34,35,45,44,2,23) were agree by (8) as for those who disagree (1) and the chi-square (5,44) and the significance of differences (sig).

Exploratory experience:

The researchers worked on conducting an exploratory experiment on Sunday 11/11/2020, on a group of (20) teachers and schools research sample for the purpose of knowing the time it takes to answer the scale and what are the difficulties and obstacles that may appear and work on how to overcome them. The results of these teachers.

The main experience:

The researchers conducted the main experiment on Sunday, 18/11/2020, on the research sample, whereby all schools in the governorate covered by the research were visited by the researcher personally according to the book on facilitating the task for the researcher to enter middle and middle schools issued by the directorate of preparation and training in Babylon, on Sunday 22/12/2020.

Correct legal knowledge test:

After completing the collection of the forms for the members of the research sample, their total score was extracted, each game separately using the correction key, prepared for this purpose "because it is the tool with which the researcher reveals the answers that indicate the existence of the result that is being measured" [3] .

The testers were given one score on the correct answer and zero for the wrong answer, the process of correcting one questionnaire took (3 - 5) minutes, and the testers 'scores ranged from (10-25) degrees, with a mean of (19,311), and a standard deviation (4,379) the iterations distributions of the building sample's knowledge scores were as follows:

1- Category (15) had a iterations of (85) and its percentage (38.11%).

2- Category (10) had a iterations (5) and percentage (2,24%).

3- Category (20) had iterations (113) and percentage (50.67%).

4- Category (30-25) had a iterations (20) and percentage (8.96%).

Where the total number of iterations was (223).

Ease and difficulty extracting paragraphs:

The difficulty factor for the paragraphs is of special importance, which is the use of the degree of difficulty to find the test vocabulary. The easy questions most of the sample members can answer them and the difficult questions the majority of the sample members do not agree to answer, and to extract the difficulty factor, the difficulty factor index [4] was used, the designers of tests and metrics recommend excluding items with difficulty coefficients less than (10%) or more than (90%) [5] and did not exclude any paragraph of legal knowledge because the transactions are naturally difficult, as shown in the following table:

Table (1) shows the difficulty coefficients of the legal knowledge items for physical education teachers

N	Basketball		N	Volleyball		N	Futsal	
	Ease coefficients	Difficulty coefficients		Ease coefficients	Difficulty coefficients		Ease coefficients	Difficulty coefficients
1	0,46	0,54	1	0,41	0,59	1	0,41	0,59
2	0,49	0,51	2	0,33	0,67	2	0,29	0,71
3	0,43	0,57	3	0,32	0,68	3	0,43	0,57
4	0,51	0,49	4	0,51	0,59	4	0,51	0,49
5	0,56	0,44	5	0,45	0,55	5	0,55	0,45
6	0,32	0,68	6	0,53	0,57	6	0,46	0,54
7	0,35	0,65	7	0,39	0,61	7	0,61	0,39
8	0,39	0,61	8	0,32	0,68	8	0,51	0,49
9	0,43	0,57	9	0,33	0,67	9	0,34	0,66
10	0,41	0,59	10	0,41	0,59	10	0,36	0,64
11	0,31	0,69	11	0,52	0,48	11	0,43	0,57
12	0,47	0,53	12	0,43	0,57	12	0,45	0,55
13	0,48	0,52	13	0,45	0,55	13	0,31	0,69
14	0,35	0,65	14	0,43	0,57	14	0,35	0,65
15	0,41	0,59	15	0,32	0,68	15	0,34	0,66

Extract the coefficient of distinguish :

The discrimination coefficient means "the ability of the paragraph to distinguish between individuals with higher relationships and individuals with lower relationships" [6] , and to find the coefficient of discrimination [7] , the following was done:

1- The overall correction of the scale paragraphs was made to obtain the total score obtained by each individual on the test.

2- The overall scores are ranked from highest to lowest for PE teachers.

3- The total grades were divided into two parts by 27% of the research sample of 223, and by 60 teachers for the total of the higher and 60 teachers from the lower group.

4- Extracting the number of male and female teachers who answered correct answers to the paragraph from the two groups, as for the (Abel)criteria that were used to distinguish.

The Abel criteria for comparing the discriminatory power of paragraphs were as follows:

1- 40% and above were very good paragraphs.

2- 0.30 - 0.39 paragraphs were reasonably good but could be subject to improvement.

3- 0,20 - 0.29 were marginal paragraphs that needed improvement.

4- 0.19 and fewer were weak paragraphs deleted or improved.

The researchers adopted the paragraphs whose indication of distinction is from (0.30) and above, so the higher the value of the discrimination factor, the better

in terms of quality, and no paragraph was excluded and the above, so the scale paragraphs made up of (45 paragraph) for physical education teachers, and as shown in the table below.

Table (2) shows the parameters of distinguishing the articles of legal knowledge of physical education teachers.

N	Basketball		N	Volleyball		N	Futsal	
	Discrimination coefficient	Evaluation Paragraph		Discrimination coefficient	Evaluation Paragraph		Discrimination coefficient	Evaluation Paragraph
1	0,40	Characteristic	1	0,43	Characteristic	1	0,63	Characteristic
2	0,40	Characteristic	2	0,66	Characteristic	2	0,36	Characteristic
3	0,43	Characteristic	3	0,36	Characteristic	3	0,42	Characteristic
4	0,50	Characteristic	4	0,34	Characteristic	4	0,33	Characteristic
5	0,50	Characteristic	5	0,37	Characteristic	5	0,38	Characteristic
6	0,50	Characteristic	6	0,36	Characteristic	6	0,52	Characteristic
7	0,46	Characteristic	7	0,46	Characteristic	7	0,43	Characteristic
8	0,36	Characteristic	8	0,43	Characteristic	8	0,46	Characteristic
9	0,34	Characteristic	9	0,45	Characteristic	9	0,50	Characteristic
10	0,30	Characteristic	10	0,60	Characteristic	10	0,39	Characteristic
11	0,35	Characteristic	11	0,41	Characteristic	11	0,45	Characteristic
12	0,39	Characteristic	12	0,36	Characteristic	12	0,33	Characteristic
13	0,32	Characteristic	13	0,50	Characteristic	13	0,36	Characteristic
14	0,44	Characteristic	14	0,30	Characteristic	14	0,53	Characteristic
15	0,50	Characteristic	15	0,47	Characteristic	15	0,40	Characteristic

Internal consistency:

Internal consistency is the most common type in the field of physical education, as it is achieved when the ability or the characteristic to be measured includes multiple tests and the sum of the scores of these sub-tests gives a picture of the test score as a whole, availability of internal consistency of the test, the sincerity of internal consistency may be achieved through the following indicators:

The correlation coefficient between the paragraph score and the sum total of the measure:

To find the validity of the internal consistency, the formula for the Point-Piseral correlation coefficient was extracted between the paragraph score and the total score of the scale, and for the members of the legalization community, who numbered (223) teachers and schools. The calculated value was greater than its tabular value of (2,312) at the degree of freedom (221) and the level of

significance (0.05), and the following table shows that.

Table (3) shows the correlation coefficient between the paragraph score and the overall score for the legal knowledge scale.

Paragraph number	Correlation coefficient	T test	Significance type	Paragraph number	Correlation coefficient	T test	Significance type
1	0,41	4,99	Sig	24	0,48	6,07	Sig
2	0,22	2,50	Sig	25	0,49	6,23	Sig
3	0,42	5,13	Sig	26	0,29	3,36	Sig
4	0,50	6,40	Sig	27	0,30	3,49	Sig
5	0,34	4,01	Sig	28	0,23	2,62	Sig
6	0,49	6,23	Sig	29	0,34	5,28	Sig
7	0,60	8,32	Sig	30	0,27	3,11	Sig
8	0,37	4,42	Sig	31	0,40	4,84	Sig
9	0,27	3,11	Sig	32	0,47	5,91	Sig
10	0,32	3,75	Sig	33	0,46	5,75	Sig
11	0,23	2,62	Sig	34	0,34	4,01	Sig
12	0,25	2,86	Sig	35	0,39	4,70	Sig
13	0,36	4,28	Sig	36	0,28	3,23	Sig
14	0,30	3,49	Sig	37	0,37	2,42	Sig
15	0,22	2,50	Sig	38	0,23	4,99	Sig
16	0,34	4,01	Sig	39	0,41	5,28	Sig
17	0,26	2,99	Sig	40	0,34	6,23	Sig
18	0,49	6,23	Sig	41	0,49	7,69	Sig
19	0,21	2,38	Sig	42	0,57	2,86	Sig
20	0,49	6,23	Sig	43	0,25	3,88	Sig
21	0,28	3,23	Sig	44	0,33	4,28	Sig
22	0,46	5,75	Sig	45	0,42	0,13	Sig
23	0,39	4,70	Sig				

Correlation coefficient between paragraph score and total sum of the field:

To find the validity of the internal consistency, the formula of point payseral correlation coefficient was used between the paragraph score and the total sum of the field (where each game was considered a field in the scale), as shown in the table below:

Table (4) shows the correlation coefficient between the paragraph score and the overall score for the legal knowledge scale.

Paragraph number	Basketball field			Paragraph number	Volleyball field			Paragraph number	Futsal field		
	Correlation coefficient	T calculated	Significance		Correlation coefficient	T calculated	significance		Correlation coefficient	T calculated	significance
1	0,49	6,23	Sig	1	0,42	5,13	Sig	1	0,51	6,58	Sig
2	0,25	2,86	Sig	2	0,35	4,14	Sig	2	0,41	4,99	Sig
3	0,41	4,99	Sig	3	0,40	4,84	Sig	3	0,48	6,07	Sig
4	0,40	4,84	Sig	4	0,52	6,75	Sig	4	0,43	5,28	Sig
5	0,44	5,43	Sig	5	0,58	7,90	Sig	5	0,29	3,36	Sig
6	0,59	8,10	Sig	6	0,33	3,88	Sig	6	049	6,23	Sig
7	0,38	4,56	Sig	7	0,37	4,42	Sig	7	0,32	3,75	Sig
8	0,32	3,75	Sig	8	0,43	5,28	Sig	8	0,47	5,91	Sig
9	0,41	4,99	Sig	9	0,45	5,59	Sig	9	0,51	6,58	Sig
10	0,51	6,58	Sig	10	0,41	4,99	Sig	10	0,44	5,43	Sig
11	0,45	5,59	Sig	11	0,33	3,88	Sig	11	0,44	5,43	Sig
12	0,34	4,00	Sig	12	0,35	4,14	Sig	12	0,36	4,28	Sig
13	0,34	4,00	Sig	13	0,38	4,56	Sig	13	0,33	3,88	Sig
14	0,35	4,14	Sig	14	0,44	5,43	Sig	14	0,42	5,13	Sig
15	0,44	5,43	Sig	15	0,29	3,36	Sig	15	0,28	3,23	Sig

Correlation coefficient between the field score and the total sum of the scale:

To find the validity of the internal consistency, the formula for the Point-Piseral correlation coefficient between the degree of the field and the subject of the scale was used for the scale and for the members of the legalization community, which numbered (223). Greater than its tabular value of (2,312) at the degree of freedom (221) and the level of significance (0.05). As shown in the table below:

Table (5) shows the relationship of the field to the total score of the scale.

N	Field name	Correlation coefficient	T calculated	Significance type
1	Basketball field	0,731	4,531	Sig
2	Volleyball field	0,881	4,331	Sig
3	Futsal field	0,767	4.321	Sig

The scientific foundations of the scale of legal knowledge:

The conditions for building the scale are of great importance in confirming the safety and process of this construction, and the general conditions for measuring tools are of great importance for the success of the measurement process, and the most important of these conditions are the purpose, stability and objectivity of the measurement tool [8] , in what follows, explain to that:

Validate the scale:

The degree of validity is the most important factor for the tests' quality and scale tests. Validity is defined as "a test that measures with sufficient accuracy and the phenomenon that it is designed to measure and does not measure anything in place of it or in addition to it" [9], The more evidence the researcher provides of the validity of his scale, the greater the confidence used in his being really measuring what he intended to measure.

As for the validity of the content, it means (the degree to which the test measures and what it is designed to measure in society), and it is considered one of the most important types of validity in achievement tests and is related to the answer to the question.

And for the validity of the hypothetical formation, it is also called the validity of the construct or the validity of the concept, because it depends on investigation and experimentation of the extent to which the degrees of the scale correspond to the concepts that the researcher relied on in constructing it, and it is one of the most important types of honesty in the stages of building the scale, because it constitutes the theoretical framework of the scale and its calculation is considered More complex than other types of honesty because it depends on theoretical assumptions that are verified experimentally [10].

Stability of scale:

"It is the test that gives results of an approach or the same if applied more than once in similar circumstances." [11], it is distinguished by a high degree of accuracy, proficiency, consistency and objectivity, and the concept of consistency indicates that the grades are not affected by the irregular errors that have multiple sources, some of which relate to the measuring tool or procedures for applying and correcting the test, and others relate to the tested individuals[12].

Halftone segmentation method:

It is one of the most used methods of stability, due to its economy in effort and time (1), and the paragraphs were divided into even (22) and individual items (23), and to confirm the homogeneity of the two halves of the physical education teachers scale, the (F) test was used and its calculated value was (1,371), with a degree of freedom (221) and a level of significance (0,05), which is less than the tabular amount of (2,223).

The simple correlation coefficient between these two halves was used and its amount was (0.791), and this value indicates the stability of the half of the test, and the (Spearman - Brown) equation was used [13], to find the reliability coefficient, the test as a whole, and the reliability coefficient as a whole was (0.883), which is a good indicator of the reliability of the scale.

Statistical means: The researcher used the SPSS statistical bag to access the research results.

- Chi-squar.
- Percentage .
- Correlation coefficient.
- T calculated.

Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached:

1- The current scale is able to reveal the level of legal knowledge of physical education teachers.

2- The scale shows that there is a clear weakness in the legal knowledge of physical education teachers in some group games.

3- Failure to follow up on amendments to the law of these games.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

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Study the Relation of Copeptin and Some Biochemical Parameters in with Obesity in Kirkuk City

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Abstract

In this Case-Control Study, One hundred thirty six adult subjects with obesity were separated to take part in the current research. At the Kirkuk General Hospital in Kirkuk City- Iraq, Applying this study had done for the duration of the 16th of February to the end of June 2020 Adult subjects with obesity outpatient attended to Kirkuk General Hospital were enrolled in this research. Ages of adult persons were amid 18 to 62 years and they were from the center and periphery of Kirkuk City. Thirty one adult persons with obesity were considered as a study group, thirty adult persons were measured as control, sixty-three adult persons didn't assembly the inclusion criteria and 20 adult persons refuse to participate in this study because of the in agreement of their people. Blood sample were collected from each adult person for determination of copepetin, lipid profile and blood sugar determination**.** The study revealed that the mean of copeptin was higher in obese group as compared with healthy control group (166.4±81.8 *vs.* 35.01±12.3 pg/ml). The study showed a significant difference between obese and the control group regarding fasting blood sugar (FBS) (119.3±12.5 and 98.2±11.6 mg/dl) respectively. The study showed that, the mean serum triglyceride was recorded to be higher in obese group (110.4±45.3 mg/dl) as compared with healthy control group (90.9 ±29.1 mg/dl). The study showed that, the mean of serum cholesterol was higher in obese group (170.7±36.3 mg/dl) as compared with healthy control group (151.7 ±29.6 mg/dl). The study showed that, the lowest mean of serum HDL was recorded in obese group (41.6±14.5 mg/dl) as compared with healthy control group (61.44 ±7.63 mg/dl). The study showed that, the highest mean of serum LDL and VLDL was recorded in obese group as compared with healthy control group. The study showed negative correlation between serum copeptin and FBS in obese individuals. It was concluded that, Serum copeptin was significantly higher in obese group as compared with healthy control group and there was a negative correlation between copeptin and blood sugar levels

Keywords: *copeptin; BMI; Obesity; Lipid profile; Kirkuk*

Introduction

People are commonly look at obese once their (BMI) body mass index, a value calculated via splitting weight of persons via the square of the height of persons, is more than 30.0 $kg.m^{-2}$; the variety 25.0 to 30.0 $kg.m^{-2}$ is considered as overweight.(1). The sickness associated to loudly body fat is type 2 diabetes, obesity, depression, osteoporosis, colon, and cancer of the breast (2). Obesity is a worldwide health problem majority due to lifestyle alterations such as diet, insufficient physical activity or socioeconomics factors. However, several clues invariably showed that genetics assists markedly to the weight-acquisition susceptibility (3). A few cases are due to several factors firstly endocrine disorders, genes, mental disorder, or drugs (4). The opinion that overweight individual eat slight likewise acquire weightiness because of a metabolism isn't clinically enhanced (5). Obese persons have bigger energy disbursement matched with their natural corresponding persons because of the energy demands to preserve an elevated body mass (6). Succeeded weight loss attempt involve a multifactorial program that includes a healthful low calorie diet and regular physical activity (7). In the present situation, the specialized of dietary variety and the scanty data to tell guidelines on particular sides of dietary variety that might be helpful or hurtful to the right weight, it

is suitable to encourage the manner of healthy eating that confirms sufficient capacity of resources of protein, plant foods, dairy products which has a few-fat, nuts, and vegetable oils ,in the same time reduces counsumption of drinks which are sweetened by sugar- , red meats and candies [8]. Weight loss outcomes in amelioration of much of the obesity□related morbidity. The compounds of neurophysin II, copeptin, and he C-terminus of pre-pro-hormone of arginine vasopressin can be provide a 39-amino acid-long peptide named Copeptin or CT-proAVP. The hormone of antidiuretic which is called (Arginine vasopressin (AVP)), is included in different pathways of renal and cardiovascular and abnormally AVP concentrations are concerned with different maladies. Therefore, the AVP determination will be helpful, nonetheless isn't usually executed in medical application due to half-life is very small cause hard to measure its concentration. Whereas, copeptin can be immunologically determined with facilely and so can be applied as a vasopressin alternate indicator [11]. So, the aim of the study was to evaluate the role of copepetin in occurrence and development of obesity in relation with other biochemical factors.

Materials and Methods

In this Case-Control Study, One hundred thirty six adult subjects with obesity were separated to take part in the current research. At the Kirkuk General Hospital in Kirkuk City- Iraq, Applying this study had done for the duration of the 16th of February to the end of June 2020 Adult subjects with obesity outpatient attended to Kirkuk General Hospital were enrolled in this research. The diagnosis of obesity was prepared via the BMI determining from every woman and men included in this study, that is mean that the diagnosis of obesity was according to measuring weight and height. Ages of adult persons were amid eighteen to sixty two years and the center and districts of Kirkuk is their habitation. Thirty one adult subjects with obesity were considered as a study group, thirty adult subjects were considered as control, sixty-three adult subjects did not meeting the inclusion criteria and 20 adult subjects refuse to participate in this study because of the in agreement of their families. Thirty apparently healthy adult persons devoid of disease of hyperglycemia, obesity, and thyroid gland were in view of as a group of control. These ages were from 18 to 43 year.

Inclusion Criteria

The cases include any adult subject who was proved to be suffering from obesity who agrees to participate in this study.

Exclusion Criteria

Each adult subject with any of the next diseases was left out from the survey.

- Thyroid gland disease.
- Ischemic heart diseases.
- Any endocrine disease that affect the weight of person.
- Diabetes mellitus.

Five ml of sample of venous blood was pulled via utilizing a sterile one-use syringe from the antecubital vein of every adult person at morning, and was preserved in normal tube, then permitted to coagulate at temperature of room. Every sample was filtered by centrifugal at roughly 3800 rpm for duration of ten minutes to attain serum. Finally, the serum was divided in plastic tubes and kept at – 20 ºC till the assessment time. The Serum of the obese and control persons has been tested via: ELISA for Copeptin, Spectrophotometer for Fasting blood glucose, and lipid profile

Physical Test

The study included the measurement of the height and weight of each individual included in this study and BMI was calculated by using the following formula: weight divided by square of height, where weight in kilograms and height in meter. Quantifying obesity via the categorization of Body Mass Index of World Health Organization, and international obesity task force. [60]

Results

The study showed non-significant difference between obese individuals and healthy control regarding age with mean age (35.24±9.17 vs. 33.85±4.36 year), Table 1.

Table 1: Distribution of the Studied Groups According to Their Age

Age Groups (years)	Obese Group		Control Group		P. value
	No.	%	No.	%	
18-27	3	9.68	6	20	0.81* NS
28-37	12	38.71	11	36.67	
38-47	11	35.48	10	33.33	
48-62	5	16.13	3	10	
Total	31	100	30	100	
Mean	35.24		33.85		0.09 NS
SD	9.17		4.36		
Minimum	18		15		
Maximum	62		38		

* P value >0.05: non-significant (NS)

The study showed no significant difference between obese males and females regarding the mean of BMI (P= 0.63), Table 2.

Table 2: Relation of Obesity with Gender

BMI	Female	Males	T. Test	P Value
No.	12	19	0.47	0.63
Mean	39.52	38.77		
SD	4.2	4.1		

The study revealed that the mean of copeptin was higher in obese group as compared with healthy control group (166.4±81.8 *vs.* 35.01±12.3 pg/ml). The difference was highly significant (P= 0.01), Table 3.

Table 3: Levels of Copeptin in Obese and Control Groups

Copeptin (pg/ml)	Obese Group	Control Group	T. test	P. value
No.	31	30	9.24	0.01
Mean	166.4	35.01		
SD	81.8	12.3		
Minimum	21.19	14.25		
Maximum	503.02	69.34		

The study showed a significant difference between obese and the control group regarding fasting blood sugar (FBS) (119.3±12.5 and 98.2±11.6 mg/dl) respectively (P= 0.047), Figure 1.

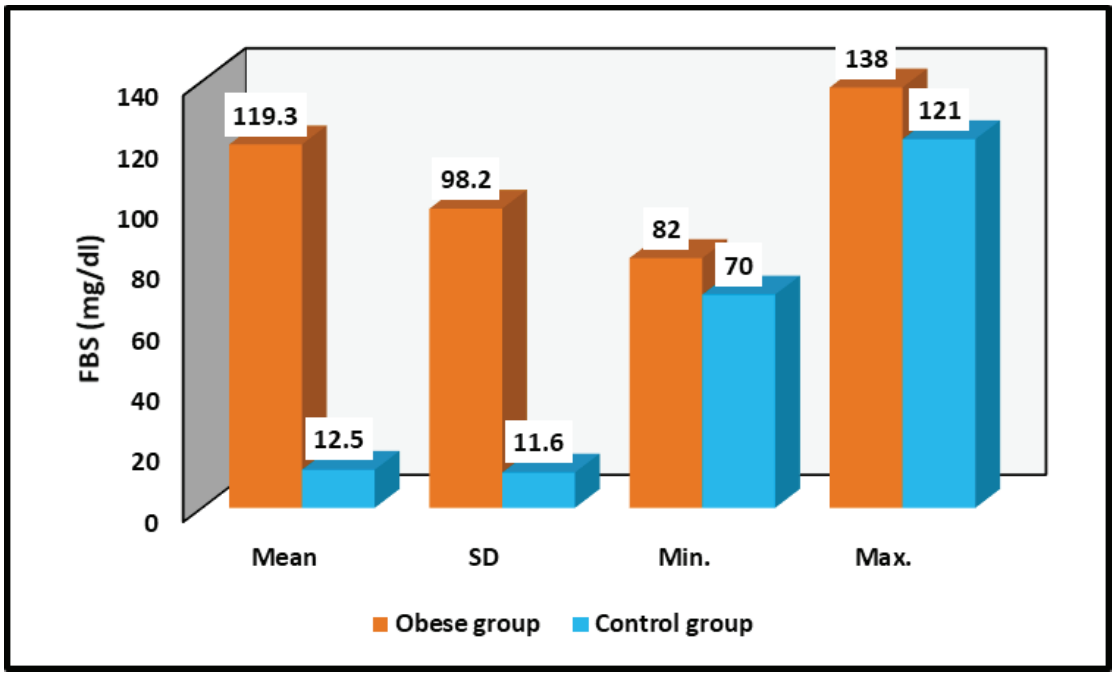

Figure 1: Levels of Blood Sugar in the Studied Groups.

The study showed that, the mean serum triglyceride was recorded to be higher in obese group (110.4±45.3 mg/dl) as compared with healthy control group (90.9 ±29.1 mg/dl). The result was significant (P:0.046), Figure 2.

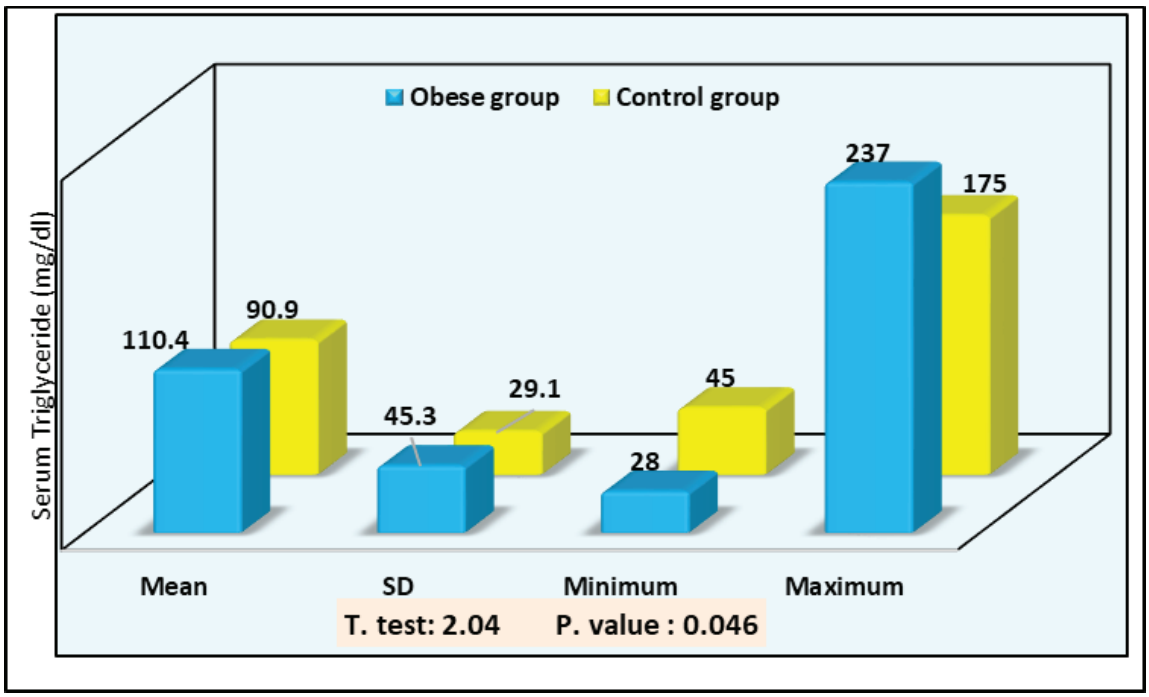

Figure 2: Levels of Serum Triglyceride in the Studied Groups

The study showed that, the mean of serum cholesterol was higher in obese group (170.7±36.3 mg/dl) as compared with healthy control group (151.7 ±29.6 mg/dl). The result was significant (P= 0.022), Table 4.

Table 4: Levels of Serum Triglyceride in the Studied Groups

Serum Cholesterol (mg/dl)	Obese Group	Control Group
Mean	170.7	151.7
SD	36.3	26.9
Minimum	139	115
Maximum	266	234

t. test: 2.35 P. value 0.022

The study showed that, the lowest mean of serum HDL was recorded in obese group (41.6±14.5 mg/dl) as compared with healthy control group (61.44 ±7.63 mg/dl). The result was highly significant (P= 0.01), The study showed that, the highest mean of serum LDL was recorded in obese group (105.4±40.7 mg/dl) as compared with healthy control group (72.3 ±28.1 mg/dl). The result was highly significant (P= 0.01) The study showed that, the highest mean of serum VLDL was recorded in obese group (22.93±8.32 mg/dl) as compared with healthy control group (17.89 ±5.92 mg/dl). The result was significant (P= 0.027) The study showed negative correlation between serum copeptin and FBS in obese individuals (r= -0.21), Figure 4.

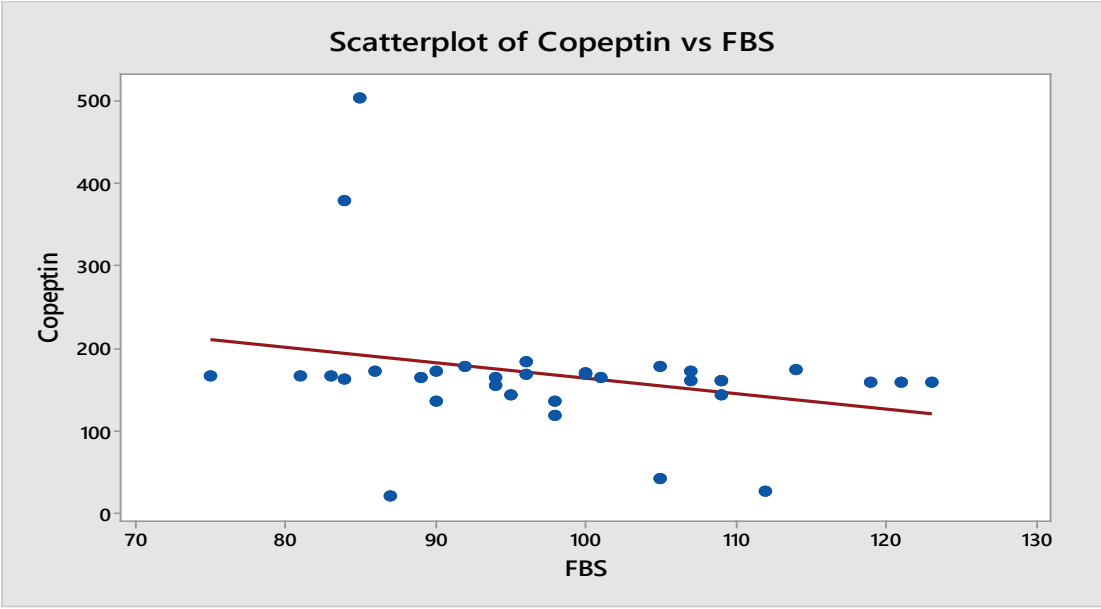

Figure 3: Correlation between Serum Copeptin and FBS of Obese Group.

Discussion

The study revealed that the mean of copeptin was higher in obese group as compared with healthy control group (166.4±81.8 *vs.* 35.01±12.3 pg/ml). The difference was highly significant (P= 0.01). These finding was in agreement with Aly *et al* [12] indicated that, serum copeptin levels were significantly higher in the obese group than they were in the control groups ($p < 0.01$). Vintilă *et al* [13] have shown a cross-sectional association between plasma copeptin and obesity. Other studies that associate diabetes mellitus, cardiovascular risk, central obesity, and copeptin levels [14]. Frøssing *et al* [15] have recently reported that copeptin is associated

with cardiovascular risk in in obese women. Rich *et al* [16] have reported that copeptin independently predicts abdominal obesity; it also shows increased risk of micro- albuminuria. Tenderenda *et al* [17] reported that higher serum copeptin levels are associated with systolic and diastolic blood pressure and several components of metabolic syndrome including obesity, elevated triglycerides, albuminuria, and serum uric acid.

Obese men had higher concentrations of copeptin and glucagon than men of normal weight. Copeptin was positively associated with glucagon. Our data suggest that increased arginine vasopressin-stimulated glucagon secretion might contribute to higher glucagon concentrations; therefore, increased arginine vasopressin secretion, in addition to other factors, could further aggravate the hyperglucagonaemic state found in obese individuals [9,10].

With regard to the differences in copeptin levels in men and women, the higher plasma copeptin level in men was consistently observed in our study and in previous studies [15]. Sex is one of the major determinants of plasma levels of copeptin[16].

Recent studies suggest that inflammation of visceral adipose tissue, ectopic fat deposition and adipose tissue dysfunction mediate insulin resistance in human with obesity [18]. A significant relation between BMI and blood sugar level was report also BMI was an influential factor for FBS [19]. One mechanism to explain the relationship to an increase in blood glucose levels in obese people which resulted in HbA1c levels also increased is through an increase in body weight supporting the results of this study [20].

In agreement with thesis findings, Kuwabara *et al* [21] showed that, obese subjects had higher levels of serum total cholesterol, triglycerides, LDL-C, compared with lean control subjects. Yan *et al* [22] also showed that, triglyceride, cholesterol and LDL were elevated significantly in obese group than in normal persons. In humans, one of the characteristics of obesity is dyslipidemia which includes high levels of triglycerides (TG) in very-low-density lipoproteins (VLDL) and low levels of high-density lipoprotein cholesterol (HDL-C) [13,15]. In agreement with the current study, Feingold *et al* [23] found a statistically significant increase in serum triglyceride in obese subjects . Lubis *et al* [24] also revealed that there was increase in triglyceride with increasing BMI, the relationship of triglyceride to BMI was (P= 0.001). Significant relation of serum concentrations of triglycerides, cholesterol, and LDL-cholesterol with BMI were recorded also by several studies done earlier [25].

Conclusions

It was concluded that, Serum copeptin was significantly higher in obese group as compared with healthy control group and there was a positive correlation between copeptin and blood sugar levels

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Medical Biochemistry and all experiments were carried out in accordance with approved guidelines.

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Assessment of Type-2-Diabetics' Patient Knowledge toward Diabetics' Control Methods at Baghdad Teaching Hospital/ Medical City

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Abstract

The study aims to assess the level of type (2)diabetic patients' knowledge toward diabetes control methods and to find out the relationship between diabetic patients' knowledge toward diabetes control methods and their demographic characteristics that include (age, gender, and level of education). A quantitative descriptive study design was carried out at medical wards in Baghdad teaching hospital, for the period of January, 20th 2019 to April 20th 2019, data was collected7th-30th March2019. The study sample is composed of (30) patients who were treated in medical wards at Baghdad Teaching Hospital. The questionnaire consists of two parts; first is concerned with the demographic and personal data for patients; and the second part is concerned with patient knowledge toward diabetes control methods, that include diet, exercise and weight loss. The data have been analyzed through the application of: descriptive analysis that include frequency, percentages; mean of scores, and the inferential analysis that include: Analysis of variance and the researcher used the SPSS version 20 to analysis of data. The study identified that more than half of the study sample (53.3%) were male at age(45-64), in relation to level of education, most of the patients were illiterate. most patients have regular follow up, always use self- blood glucose monitoring practice, (73.3%) were receiving information about diabetes mellitus (DM). (83.3%) have information about diet. Polyuria and Hunger is most specific signs and symptoms of hyperglycemia and hypoglycemia determined by patients, (66.7%) don't have physical exercise and walking is chosen exercise of patient for 30< min in day. Most patient use has diet adjustment especially in dinner meal. There is no significant difference between patient knowledge and demographic characteristics.

Keywords: *Type 2 Diabetes mellitus, Physical exercise, Weight loss, Diet*

Introduction

Diabetes mellitus (DM) defines a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. This chronic hyperglycemia is associated with long term damage, dysfunction and failure of various organs [1]. Diabetes self-management education can target medication education and compliance, goal setting, foot care and interpretation of laboratory results [2]. Lifestyle modification programs should be developed using a patient centered approach. Each plan should focus on physical activity [3].

Methodology

A quantitative descriptive study design was carried out at medical wards in Baghdad Teaching Hospital/ Medical City. The study sample is composed of (30) patients who were treated in medical wards at Baghdad Teaching Hospital. The questionnaire consists of two parts; first is concerned with the demographic and personal data for patients; and the second part is concerned with patient knowledge toward diabetes control methods, that include diet, exercise and weight loss. Data was collected from 7th- 30th March 2019. The content validity of the study instruments are established through a panel of (15) experts, they were (10) from medical ward in Baghdad city, and (5) from college of

nursing/university of Al-bayan. Test-retest has been obtained through evaluating 10 patients selected from Baghdad Teaching Hospital (medical wards). According to the knowledge test questionnaire, and Pearson correlation coefficients is used which = (0.84). Data have been analyzed through the use of simple statistical analysis and the inferential analysis that include: Analysis of variance and the researcher used the SPSS version 20 to analysis of data.

Results

Table (1): Socio-Demographic Characteristics for Patients with Diabetes Mellitus No=30

Variables	Classification (N)	Percentage (%)
Sex		
Male	16	53.3
Female	14	46.7
Total	30	100
Age in years		
12-24	1	3.3
25-44	5	16.5
45-64	22	73.6
64>	2	6.6
Total	30	100
Education Level		
Illiterate	9	30
Read and write only	7	23.3
Primary	8	26.7
High school	1	3.3
Graduate	3	10
Post graduate school	2	6.7
Total	30	100

F=Frequency; % = Percentage

Table (1) presents the demographic characteristics of the study sample were (16 (53.3%) of patients was male, (22(73.6%) of them at age (45-64) years old, (9(30%) was illiterate.

Table-2- Clinical Characteristics for Patients with Diabetes Mellitus

Variables		
	F	**%**
Duration of DM (years)		
1-2Yr	0	0
3-4Yr	0	0
Y r5≤	30	100
Total	30	100
Follow up of DM		
regular follow up	19	63.3
Sometimes	10	33.3
No follow up	1	3.3
Total	30	100
Patients self- blood glucose monitoring practice		
Always	16	53.3
Sometimes	9	30
Don't know	5	16.7
Total	30	100

F=Frequency; %= Percentage

Table -2-shows that the highest percentage of the patients (30(100%) have 5≤years' experience with diabetes mellitus (DM). According to follow up of DM highest percentage of the patients (19 (63.3%) have regular follow up, concerning the Patients self- blood glucose monitoring practice highest percentage of the patients(16 (53.3%) always use self- blood glucose monitoring practice.

Table-3- Distribution of Patients According to Received Health Information about Diabetes Mellitus

Variables	F	%
Do you receive information about DM		
Yes	22	73.3
No	4	13.3
Not sure	4	13.3
Total	30	100

Cont... **Table-3- Distribution of Patients According to Received Health Information about Diabetes Mellitus**

If yes what is the Topic		
About foot Care	10	33.3
About diet	25	83.3
About physical exercise	12	40
Total	50*	100
Reason for not received information about DM		
No book about DM	15	50
No subjects in TV	9	30
Un readiness of health care provider to provide information	6	20
Total	**30**	**100**
Which Topic is more clear for you		
Foot care	31	43.3
Diet	20	66.7
Exercise	14	46.7
Drug intake	14	46.7
Total	**61***	**100**

F=Frequency; %= Percentage; DM= diabetes mellitus; *= sample have chance to answer more than one choice

Table -3-shows that the highest percentage of the patients (22 (73.3%) have receive information about DM. According to topic of information highest percentage of the patients (25(83.3%) have information about diet, concerning the reason for not received information about DM highest percentage of the patients (15 (50%) don't find book about DM, related to topic that consider more clear for patients highest percentage of the patients (20(66.7%) was about diet.

Table-4-Distribution of Patients According to Clinical Information about Diabetes Mellitus

Variables	F	%
Do you know signs and symptoms of hyperglycemia and hypoglycemia		
Yes	27	90
No	3	10
Total	30	100
If you know what is signs and symptoms of hyperglycemia		
Polyuria	27	90
Polydipsia	13	43.3
Polyphagia	9	30
Blurred vision	11	36.7
Headache	18	60
Lethargy	8	26.7
Coma	6	20
Total	92*	100*
If you know what is signs and symptoms of hypoglycemia		
Sweating	15	50
Tremor	12	40
Hunger	21	70
Blurred vision	10	33
Confusion	7	23.3
Seizures	10	33.3
Coma	5	16.7
Total	80*	100*
Do you know normal fasting test		
Yes	18	60
No	12	40
Total	30	100
Do you know normal random test		
Yes	14	46.7
No	16	53.3
Total	30	100
Do you know normal glycosylated hemoglobin (Hb A1C) test		
Yes	13	43.3
No	17	56.7
Total	30	100

F=Frequency; %= Percentage; *= sample have chance to answer more than one choice

Table -4-shows that the highest percentage of the patients (27(90%) know signs and symptoms of hyperglycemia and hypoglycemia. According to signs and symptoms of hyperglycemia highest percentage of the patients (27(90%) know polyuria is signs and symptoms of hyperglycemia , concerning the signs and symptoms of hypoglycemia the highest percentage of the patients(21(70%) know hunger is signs and symptoms of hypoglycemia, related to normal fasting test highest percentage of the patients(18(60%) know normal fasting test, related to normal random test the highest percentage of the patients(16 (53.3%) don't know normal random test; according to normal glycosylated hemoglobin (Hb A1C) value test the highest percentage of the patients(17(56.7 %) don't know normal glycosylated hemoglobin (Hb A1C) value test.

Table-5- Distribution of Patients According to Physical Exercise to Control Diabetes Mellitus

Variable	F	%
Having physical exercise		
Yes	10	33.3
No	20	66.7
Total	30	100
Type of physical exercise		
Walking	14	46.7
Running	3	10
Devices in home or special	4	13.3
Other	9	30
Total	30	100
How long do you exercise(day)		
30< min	23	76.7
30-60 min	4	13.3
60> min	3	10
Total	30	100

F=Frequency; %= Percentage

Table -5-shows that the highest percentage of the patients (20(66.7%) don't have physical exercise. According to type of physical exercise the highest percentage of the patients (14(46.7%) work walking as exercise, concerning the how long do you exercise in day the highest percentage of the patients (23 (76.7%) use 30< min in day as exercise.

Table-6- Distribution of Patients According to Dietary Adjustment for Recommended Foods for Control Diabetes Mellitus

Variables	F	%
Do you have diet adjustment		
Yes	21	70
No	9	30
Total	30	100
Which meals		
Breakfast	17	56.7
Lunch	18	60
Snack	3	10
Dinner	19	63.3
Total	57*	100*
Special diet when blood glucose level is high		
Yes	21	70
No	9	30
Total	30	100
Which type of diet		
CHO	17	43.3
Protein	19	63.7
Fat	12	60
Cellulose rich food	10	33.3
Total	58*	100*

F=Frequency; %= Percentage; *= sample have chance to answer more than one choice

Table -6-shows that the highest percentage of the patients (21(70%) have diet adjustment. According to type of meals the highest percentage of the patients (19(63.3%) adjustment the dinner to control DM, concerning the special diet highest percentage of the patients (21(70%) use special diet when blood glucose level is high; related to type of diet highest percentage of the patients (19 (63.7%) use Protein as special diet when blood glucose level increase.

Discussion

Our study revealed that the study sample (16 (53.3%) of them was males, (22 (73.6%) of them was at age (45-64) years old, regarding their level of educational, most of the patients (9 (30%) were illiterate (Table-1). Aldossari and others, (2015) revealed that(163) patients ,slightly more than half of them (51.9%) were males whereas (41.5%) were in the age group 40-59 years whereas seventy five patients (19.1%) were over 60 years. and (28.8%) were house wives. Moreover the highest percentage of the patients (30 (100%) have 5≤ experience with DM. According to follow up of diabetes mellitus (DM) highest percentage of the patients (19(63.3%) have regular follow up; concerning the Patients self- blood glucose monitoring practice highest percentage of the patients (16(53.3%) always use self-blood glucose monitoring practice(Table-2-).Krishnan and Thirunavukkarasu,(2016) revealed that out of

153 patients surveyed, only (37(24.1%) (20 males, 17 females) patients were aware and have been following self-blood glucose monitoring appropriately. Also the study revealed that the highest percentage of the patients (22 (73.3%) have receive information about DM. According to topic of information highest percentage of the patients (25 (83.3%) have information about diet, concerning the reason for not received information on DM highest percentage of the patients (15(50%) don't find book about DM, related to topic that consider more clear for patients highest percentage of the patients (20 (66.7%) was about diet(Table-3-). Xu and others (2016) revealed that Newspapers and books (61.4%), television and the internet (46.3%) were the major sources of knowledge about type 2 diabetes, and more than half of the participants (55.9%) considered that medical staff was the most reliable source.(Table-4-) revealed that the highest percentage of the patients (27(90%) know signs and symptoms of hyperglycemia and hypoglycemia. According to signs and symptoms of hyperglycemia highest percentage of the patients (27(90%) know polyuria is signs and symptoms of hyperglycemia, concerning the signs and symptoms of hypoglycemia the highest percentage of the patients(21(70%) know Hunger is signs and symptoms of hypoglycemia, related to normal fasting test highest percentage of the patients(18 (60%) know normal fasting test, related to normal random test the highest percentage of the patients(16 (53.3%) don't know normal random test; according to normal Hb A1C value test the highest percentage of the patients(17 (56.7%) don't know normal Hb A1C value test. Konduru and others ,(2017) revealed that the only 1% diabetic were aware on diabetic symptoms. Majority of responders (71%) diabetic were aware on Polyuria as a diabetic symptom for where (37%) diabetic were aware on polydypsia as a diabetic symptom, numbness of hands as 38% diabetic, (6%) diabetic were aware on abnormal sensation of feet.(Table-5-) revealed that the highest percentage of the patients (20(66.7%) don't have physical exercise. According to type of physical exercise the highest percentage of the patients (14(46.7%) work walking as exercise, concerning the how long do you exercise in day the highest percentage of the patients (23 (76.7%) use 30< min in day as exercise. Palermo and Sandoval (2016) revealed that the majority of the patients in UP-PGH Diabetes Clinic had moderate to high physical activity (68.9%). A higher percentage of subjects aged 60 years and above (68.1%; p=0.022) and with poor glycemic control (89.4%; p=<0.001) had low physical activity level as compared to subjects aged 30 to 59 years (31.9%) and with good glycemic control (10.6%) respectively. Moreover the highest percentage of the patients in our study (21 (70%) have diet adjustment. According to type of meals the highest percentage of the patients (19 (63.3%) adjustment the dinner to control DM, concerning the special diet highest percentage of the patients (21(70%) use special diet when blood glucose level is high, related to type of diet highest percentage of the patients (19 (63.7%) use protein as special diet when blood glucose level increase (Table-6-). Laissaoui and Allem(2016) revealed that their food was mainly characterized by high amounts of fats, the green salads and the desserts (fruits) represent only a secondary amount. Statistically, Overweight + obese patients with diabetes had significantly higher level of consumption of the bread. As well the highest percentage of the patients (16 (53.3%) don't know what mean CHO. Bano and others findings that (41.5%) had positive attitude with diabetic diet while the remaining (58.5%) were having negative attitude. (Table-8-) revealed that the highest percentage of the patients (24 (80%) know fat in obese person increase resistance cell to insulin. According to which body weight the patient have the highest percentage of the patients (17 (56.7%) believe that the weight no appropriate to criteria; concerning to score of body weight highest percentage of the patients (24 (80%) don't know what score of body weight. Kutbi and others (2018) revealed that the compared to normal-weight participants, participants who were underweight, overweight, or obese, did not differ with regards to knowledge about diabetes symptoms. Adjusted models showed that overweight and obese participants had lower odds of being in the lowest tertile of knowledge about diabetes complications compared to normal-weight participants**.** Likewise the study finally revealed that there is no significant difference between patient knowledge toward control on diabetes mellitus and patient age, except (reason for not received information about DM) which has significant relationship with age sig=0.030 , there is no significant difference between patient knowledge toward control on diabetes mellitus and patient gender, except (do you receive information about DM) which has significant relationship with gender sig=0.04, and (having physical

exercise) which has significant relationship with gender sig=0.003.And there is no significant difference between patient knowledge toward control on diabetes mellitus and patient level of education, except (follow up of DM) which has significant relationship with level of education sig=0.043. Ntaate, (2015) revealed that the concerning attitude, most (82.7%) of the respondents had a good attitude towards the dietary management of DM. Notably one's education status did not show any correlation with how well one scored from the attitude questions. In regard to practice as part of dietary management of DM, all the respondents had poor practices this being more in the females (73.2%) had poor practice scores) and the older(46 years and above) patients(78.6%) had poor practice scores), despite these having scored highest on the knowledge questions.

Conclusion

the present study concluded to

1-More than half of the study sample were male, most of the sample at age 45-64 years old , most of the sample was illiterate, and all of the sample study have >5years experience with diabetes mellitus(DM) .

2-more percent of the receive information about (DM) ,highest percentage of patient know signs and symptoms of(DM),little sample having physical exercise ,most of sample have diet adjustment, little sample know what mean carbohydrate, and more than half of sample know their weight were no appropriate to criteria .

3- study presented that were no significant difference between patient knowledge toward control on diabetes mellitus and patient age, except (reason for not received information about DM), no significant difference between patient knowledge toward control on diabetes mellitus and patient gender, except (do you receive information about DM) and (having physical exercise) ,finally no significant difference between patient knowledge toward control on diabetes mellitus and patient level of education, except (follow up of DM .

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Assessment the Role of Ubiquinone (Coenzyme Q10) against Spermatotoxic Effect of Busulfan in Male Rats

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Abstract

Busulfan is a chemotherapy treatment for cancer patients, and in comparison with the coenzyme ubiquinone is a coenzyme that works to enhance chemical reactions and protect the body from free radicals. It helps in treating cancer and protecting patients undergoing chemotherapy. This study was aimed to investigate the role of ubiquinone against the toxic effect of busulfan on sperm in adult white rats for a period of time, and the experiment was carried out for eight weeks on 24 male adult rats were used, the study was conducted in periods (day 28). Distributed in four groups randomly and equally in each group of 6 male rats. The control group injected with distilled water intraperitoneally , second group were injected with busulfan (chemotherapy) only one dose of 10 mg / kg. while animals of third group injected with single dose of busulfan 10 mg / kg plus coenzyme ubiquinone COQ10) 10 mg / kg) for 4 weeks, while animals in fourth group were injected with the coenzyme ubiquitin coenzyme (COQ10) 10 mg / kg for 4 weeks. At the end of experiment the blood was collected for measuring of blood parameters and hormones levels and sperm analysis has benn done.

Key words : CoQ10; Busulfan, Chemotherapy

Introduction

Busulfan (Busulfex) Chemotherapy are associated with many changes "in the male reproductive system. Busulfan is a drug used in treating "cancer, which "is a white crystalline powder, its" chemical structure is 1,4-butanediol di-methane-sulfonate. Busulfan is an alkylation agent and a "cytotoxic drug back to" the alkyl sulfonate group [(20)]. Busulfan has many side "effects on different body parts such as bladder, liver, skin, nervous system, and" gonadotropic "function, as well as possibly carcinogenic and teratogenic [(47)]. Also, it is "used in "treating of chronic leukemia, cancer of ovary, and before transplantation of bone" marrow in patients with cancer [(20)]. Busulfan has "distorting and carcinogenic properties, It kills the stem cells of sperm and disrupts the connections between Sertoli cells and sperm cells in the base layer [(6)].

Coenzyme Q10 (CoQ10) is a key element in electron transport in oxidative phosphorylation which is occur in mitochondria. [(33,10)], it is found in almost all tissues of the body, and it is a very effective antioxidant [(51)].Coenzyme Q10 is an important electronic shuttle for the electron transport system in mitochondria. Additionally, when quantities are less than CoQ10, there can be inhibition of cellular oxidation of fats, proteins and DNA [(44)]. Several well researched studies have shown that antioxidants are effective. These include vitamins C, E, carnitine and coenzyme Q10, which can be considered the first line of treatment [(11)]. CoQ10 deficiency can result in sperm damage, decreased sperm motility, and sperm count. Studies have shown that the use of supplements containing CoQ10 can improve reproductive outcomes in males who have fertility problems [(2)]. Further more, CoQ10 semen concentrations are positively associated with sperm motility and counting. Accordingly, patients treated with CoQ10 had more sperms and formed better sperms compared to those who did not receive CoQ10 [(7)].

Materials and Methods

The study is perform during the period from November, 2019 to February 2020.Rats kept in animal house for acclimation to the laboratory condition for two

weeks. 24 male adult rats healthy aged (2.5-3) months, weighting 350 -400 gm. Were obtained from the animal house of Science College in University of zakho ,dohuk.

1. GI: 6 male Rats were given normal saline (0.2 mL, daily, intraperitoneally injection) and served as the control.

2. GII 6 male Rats were given single dose of busulfan(10mg/kgIP.)intraperitoneally injection [56]

3. GIII 6 male Rats were given single dose of busulfan (10mg/kgIP.)plus Ubiquinone(10mg/kg, daily BW/IP)for 4 weeks intraperitoneally injection. [57].

4. GIV twelve male Rats were given adose Of the Ubiquinone(10mg/kg, daily, BW/IP) intraperitoneally injection[36].

Blood collection

were collected via cardiac puncture. From each male rat, The blood sample were placed in serum tube and left for 30 minutes. Then the blood sample were drops directly from the heart by using 5 ml disposable syringe the blood putting in the gel tube and then to be centrifuged (3000 rpm for 10 minutes) and kept frozen at -20 °C to obtain .the serum which then were transferred to the Eppendorf tubes. for assessment of reproductive hormones concentrations in serum (FSH, LH, testosterone , adiponectin, inhibin B and activing A).All these of tubes were stored at (-4c) until analyzed. All sample were collected at the morning (8:30 AM – 10:30 AM) in the order to minimize the diurnal variation of the hormone levels.

Results

The results in table (1) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for periods of 28 they showed significant ($p<0.05$) decrease in the hemoglobin level (HB), Red blood cells count (RBC), White blood cells count(WBC), platelets count and packed cell volume (PCV) in Busulfan group and Busulfan +Co10 when compare with control group and co10 alone group.

Table (1) show the statistical analysis for the effect of buselfan, buselfan +co 10 and co 10 alone on some hematological parameters in male rats for 28 days.

Parameter Group	Hb g/dl	RBC ×106cell/mm3	WBC ×103cell/mm3	PLT ×1011cell/mm3	PCV %
Control	12.56 ±2.75A	6.60 ±1.04A	10.02 ±1.14 A	1080.83 ± 214.25A	41.083 ±0 .77 A
Busulfan	7.08 ±.31B	3.81B ±.19	1.69 ±.45C	310.50 ±88.65B	21.25 ±1.32B
Busulfan+ Co10	8.36 ±.80B	4.04 B ± .58	3.58 ±1.61B	435.33 ±30.41B	21.833 ± 1.16B
Co10	13.88 ±1.44A	6.76A ±.72	10.34 ±.70A	960.50 ± 68.48A	42.583 ± 1.90A

The different litters refer to the significant change between groups (($p \leq 0.05$)

Sperms Parameters

The results in table (2)are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for 28 days, they showed significant ($p<0.05$) decrease in the sperm count, sperm motility, sperm viability and sperm abnormality in Busulfan group when compare with control group, busulfan +c10 group and co10 alone group. Also, there is significant increase in busulfan +co10 group when compare with busulfan alone group

Table (2) show the statistical analysis for the effect of buselfan,buselfan +co 10 and co 10 alone on epididymal sperm Analysis in male rats for 28 days.

Parameter Group	Sperm count (106/ml)	Sperm motility (%)	Sperm viability (%)	Sperm abnormality (%)
Control	66.67 ±8.042 A	67.33±13.352 A	73.17±3.312 A	5.83±1.169 C
busulfan	14.83±5.492 C	20.33±5.428 C	19.83±4.446 C	37.67±6.055 A
buselfan+coQ10	28.00±4.733 B	37.83±6.113 B	35.67±2.733 B	12.50±2.074 B
CoQ10	71.83± 14.162 A	71.33±7.916 A	75.50±4.416 A	3.67±1.211 C

The different litters refer to the significant change between groups (($p \leq 0.05$)

Hormonal parameters

The results in table (3) are for the groups of male rats treated with Busulfan, Busulfan +Co10 and Co10 alone for period 28 days, they showed significant ($p<0.05$) decrease in the testosterone level and significant increase ($p<0.05$)in LH &FSH in Busulfan group when compare with control group, busulfan +c10 group and co10 alone group. Also, there is significant increase in busulfan +co10 group when compare with busulfan alone group in testosterone levels and significant increase in busulfan +co10 group when compare with busulfan alone group in FSH &LH.

Table (3) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 and CoQ 10 alone on some Hormonal Level parameters in male rats for 28 days.

Parameter Group	Testosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)	adiponectin(ng/mL)	Activin A (ng/mL)	inhibin B (ng/mL)
Control	2.2667± .79377AB	1.5850 ± .41750C	3.8183 ± 1.10135C	.81167± .046513C	7.14933± .381618A	4.22733± 1.370813A

Cont... **Table (3) show the statistical analysis for the effect of buselfan,buselfan+Co Q10 and CoQ 10 alone on some Hormonal Level parameters in male rats for 28 days.**

busulfan	1.1300± .19990C	3.2900 ± .56749A	8.3050 ± 1.43993A	2.48283± .178924B	1.67433± .638982C	1.29700± .884615C
busulfan+ CoQ10	1.7700± .15388B	2.2133 ± .49168B	5.2183 ± .91729B	4.60067± 1.022271A	2.92600± .544017B	2.70367± .344224B
CoQ10	2.4083±A .58414	1.6400 ± .32760C	3.8350 ± .68731C	3.07467± .248387B	6.92100± .928497A	4.39900± 1.036194A

The different litters refer to the significant change between groups (($p \leq 0.05$)

Discussion

1.Hematological Parameters

From this study, it has been found that some busulfan treated rats has been lost her hair in day 28 treated rats. Hair loos may result either from hair follicle stem cell destruction or from acute damage to the keratinocytes of the lower portion of some follicles. and this is in agreement with [54], who found there is diffuse alopecia characterized by greatly reduced hair density with short, thin hair after busulfan treatment. for the group of male rats treated with the Busulfan only (10mg/kg/BW) and Busulfan +co 10 for 28 days. In table(1) the results of the present study show a significant decrease($p<0.05$) in the rate of hemoglobin (Hb), packed cell volume (PCV), Red blood cells (RBC), white blood cells (WBCs) and platelets count compared with the control group and co10 treated group and this is agreement with [59] which found that the values for RBC, Hb ,WBC and platelets of mice in the i.p. injection group after busulfan treatment were significantly lower than those of mice in the control group. The reason for this is two reasons. The first is that blood cells create a type of cell called “stem cells”. Stem cells are the master cells that divide and mature into different types of blood cells: red cells, white cells, and platelets. The bone marrow in our body acts as a factory, converting stem cells into blood cells. Every new blood cell contains DNA that carries all the instructions the cell needs to grow and function. Once mature, blood cells leave the bone marrow and enter the bloodstream. Busulfan works by interfering with the production of blood cells’ DNA. The drug prevents cells from growing and maturing, which ultimately causes these cells to die. Busulfan is classified as a chemotherapy because it causes some cells in the body to die [28] The fur became dull and dry, there was a reduction in activity and responses, and an abnormal gait and hunched posture developed. The fur became dull and dry, there was a reduction in activity and responses, and an abnormal gait and hunched posture developed. there was no evidence of BU toxicity during or after drug administration. there was pronounced marrow depression as shown by significant decreases in erythrocyte values, all leucocytes, and in platelets, The second reason is low bone marrow caused by busulfan chemotherapy, which may cause aplastic anemia and is low in all blood cells. and this is in agreement with [40].Which found nadir for myelo-suppression after a single dose of busulfan. Also[43] Which found that blood marrow suppression, infection, and bleeding are very common and occur early (day to weeks) after transplantation of hematopoietic stem cells that are given busulfan before bone marrow transplant. Busulfan is an alkyl sulfonate. It is an alkylating agent that forms cross-links within the DNA between the DNA bases guanine and adenine and between guanine and guanine. [25].DNA entanglement prevents DNA replication.

Since the cross-links within DNA cannot be repaired by cellular machines, the cell undergoes apoptosis. [31]. Busulfan is a highly cytotoxic and genotoxic agent [48] that Various adverse effects, both acute and chronic, such as DNA damage and thus activation of apoptosis or aging in a manner dependent on cell type possibly due to oxidative stress[25] can occur in many biological organs such as blood diseases. [3].On day 28, the busulfan + CO10 group showed a slight increase in Hb, RBC, WBC and platelets, but this increase did not reach a significant level when compared with the busulfan group alone, and there was also a slight increase in the same level except for the platelet count when comparing the group CO 10 and the control group and also don't reach much level. This results are agreement with [17] had reported that, CoQ10 can act as an antioxidant in RBCs membrane and has a protective effect on RBCs membrane structure and expansion and cell engineering. Also [33] Which found that erythrocytes previously enriched with exogenous CoQ10 were found to be more resistant to free radical initiator-induced hemolysis. Moreover, CoQ10 was able to protect the isolated enzymes and the RBCs membrane-bound enzymes from the inactivating effect of free radicals, resulting in an increased RBC count and as a result an increase in Hb and PCV. Increase in platelets count is agreement with [41] that found CoQ10 supplementation further increase platelets count (though not statistically significant) after 28 days of supplementation when compare between Busulfan and Busulfan +co10 group. On the other hand, Coenzyme Q10 (CoQ10), is a single compound of antioxidant and structurally bound to vitamin K. It may prevent endogenous CoQ10 from opening up the pores of membrane transmission, as it counteracts many apoptotic events, such as DNA fragmentation and cytochrome release. , And the possibility of depolarizing the membrane.[13]. Previous studies [5] have shown that the nadir of marrow depression after a single BU dose, occurs at day 2.

2.Sperms Parameters.

Among the most important problems in a married couple's life are infertility, its complications, and the causes of some male infertility cases. [4] . Many factors can affect sperm production and the risk of infertility, and among these factors is the use of chemotherapy drugs for cancer, and busulfan is one of these drugs that significantly affect the testicles. [15]. These factors can reduce sperm concentration while generating free radicals and oxidation of germ cells in the testicles [4]. Here, we examined the protective effect of ubiquinone(co10)against busulfan-mediated sperm damage in mice, the time period chosen (4weeks) provides sufficient time to monitor the possible recovery of spermatogenesis in the remaining stem cells In animals treated with busulfan. According to[42], It took approximately 4 weeks after the busulfan treatment for the effect of busulfan to differentiate the cells secreting from the seminal-tubular lumen Our results showed that administration of busulfan in a single dose and daily administration of ubiquinone (Co10) for 4 weeks can significantly reduce busulfan-mediated destruction of the testis. Results showed a significant decrease in busulfan group compared to the other group ($p < 0.05$), (Table 2) in sperm count ,motility, viability and abnormality. Our findings were agreement with [38,27]. Unlike other chemicals that destroy differentiated sperm, Busulfan is an effective agent that kills sperm stem cells of several types. However, at higher doses, the drug may kill contrasting strains of sperm resulting in the depletion of sperm cells and sperm as well. [39]. It has been confirmed that busulfan chemotherapy can induce apoptosis in sperm [12]increase the production of reactive oxygen species and result in sperm death [14]In addition to ensuring the presence of tratospermia, the presence of spermatozoa with abnormal conformation in the semen is another possible outcome of busulfan treatment in mice [46].

These studies have shown that busulfan is involved in stopping spermatogenesis, although some changes are reversible and are dose dependent Busulfan inhibits spermatogenesis, especially by the oxidative damage that increases the level of ck-18, which is a superficial marker on Sertoli cells. The elevation of this marker caused disturbance of spermatogenesis, sterility and reduced sperm motility by reducing the length of the flagellum[35]. Coenzyme Q10 has been used as an attractive intervention approach in a wide variety of diseases or disease disorders, one of which is infertility [26,19]. The successful results of CoQ10 administration in various organ systems prompted us to attempt such a treatment with a model of busulfan-induced testicular disorders. Coenzyme Q10 was first introduced as an ethical drug for patients with heart failure in Japan and

other countries. Coenzyme Q10, which works internally in the electron transport chain in mitochondria, can be ingested to suppress free radicals and contribute to antioxidant defenses in vivo [23]. Both the bioenergy and antioxidant role of CoQ10 indicate potential involvement in sperm biochemistry and male infertility. The amount of CoQ10 can be measured in semen, as its concentration is related to sperm count and motility [1]. It was found that CoQ10 distribution between sperm cells and seminal plasma was altered in varicocele patients, who also exhibited a higher level of oxidative stress and a lower capacity of antioxidants. The redox state of CoQ10 was also determined in semen: an inverse relationship was found between ubiquinone percentage and hydroperoxide levels and between this ratio and the percentage of abnormal sperm shapes.Then, CoQ10 was administered to a group of patients with idiopathic infertility. The treatment significantly increased CoQ10 concentration, in seminal plasma and sperm cells, and improved sperm motility. [10]. The results of the present study indicated that administration of CoQ10 following administration of busulfan could be helpful in improving sperm indices in mice. and this result is agreement with[39]Antioxidants neutralize free radicals and thus the oxidative reactions that cause them. Dietary antioxidants may be helpful in reducing lipid peroxidation and DNA damage in sperms during busulfan therapy [29]. The results showed a positive correlation between the duration of treatment with coenzyme Q10 and the number of sperms, as well as with sperm motility and sperm formation, and this is consistent with our results.

3.Hormonal parameters

In our research as show in (table 3) we found that the LH and FSH levels were significantly ($P \leq 0.05$) increased while testosterone levels decreased in rat treated busulfan as compared with other groups. Busulfan is a drug that significantly affects the testicles. It causes an apparent increase in apoptosis and affects spermatogenesis. Busulfan has toxic effects on the sperm epithelium in rats and causes infertility [15] mainly by killing sperm stem cells. The primary gonadal defect is caused by the testicle. Damage has a deleterious effect on spermatogenesis and / or a dysfunction of Leydig cells [30].Exposure to cytotoxic chemotherapy and radiotherapy leads to associated elevation of FSH levels with decreased sperm count, and thus, the effects of suppressing testosterone and gonadotrophin analogues on spermatozoa. Moreover, chemotherapy is often used with increased testicular impairment and epithelial germs damage. To make it possible to treat male infertility after cancer treatment [48]. On the other hand, free radical accumulation and thus oxidative injury in Leydig cells in the testis by a specific chemical oxidant (such as busulfan) may degrade their response and performance to testosterone synthesis. [9]. Also, there is a significant increase in the testosterone level in Busulfan +co10 group when compare with busulfan group which is agreement with [16, 18,37]. Coenzyme Q10 supplementation has been found to suppress testicular oxidative stress and lipid peroxidation, and restore the antioxidant defense mechanism. [16], Which, in turn, could prevent chemical oxidative injury and preserve the function of Leydig cells to produce testosterone [45,9] also found there is increase in the level of testosterone but not reach to significance level when compare between co10 group and control group and this agreement with our results. As show in table (3)there is a significant decrease($p<0.05$) in the levels of activin and inhibin in busulfan group when compare with other groups of treatment. The activin and inhibin were first described as regulators of follicle-stimulating hormone (FSH) secretion. The activin were originally recognized for their abilities to augment the gonadotropin-releasing hormone (GnRH)-mediated release of FSH,and were named “activins” because their effects were functionally opposite to those of inhibin in this context [32,55]. Inhibin B is the only form of inhibin produced by the testis and is also the major circulating inhibin [24,34]. FSH stimulates inhibin B expression in Sertoli cells, providing negative feedback for pituitary FSH production [52,21], While LH stimulates the production of androgens from rodent Leydig cells, a process that is augmented by inhibin and diluted by Activin [22]. Sertoli cell function is closely related to the biology of Activin and Inhibin. These cells are targeted by the pituitary-derived FSH to regulate their structure, and their levels are the main determinant of male fertility and physiology. .Research has identified specific roles of locally produced activin and ethinbene in testicular development and function. Kate L. Loveland, Mark P. Hedger, in Sertoli Cell Biology (Second Edition, 2015). Treatments with busulfan specifically target germ cells and Sertoli cells respectively (with an efficiency close to 100%), and it is

very likely that copies will be significantly reduced after each treatment regimen specific to germ cells or Sertoli cells.[53]

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Veterinary Medicine and all experiments were carried out in accordance with approved guidelines.

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Effect of Litter Size Manipulation on the Reproductive Performance of BALB/C Mice

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Abstract

Many of previous studies on lactating animals with natural litters did not support the negative link between litter size and pup mass; however, artificial manipulation of litters could be an option to support that. In this study, we manipulated the litters of lactating BALB/C mice with having two groups. Enlarged litter SL (LS=8; N=5) and reduced litters SS (LS=2; N=6) were used in this experiment and also 7 virgin female mice were assigned for control group. Mother's body mass and food intake were daily measured throughout the experiment. Litter size, litter mass, pup mass, mortality rate of pups in the studied groups were also daily measured throughout lactation. The lactating mice in both groups significantly ate more food intake compared to non –reproducing mice. Mice with enlarged litters had significantly greater food intake compared to the mice with reduced litters. Litter masses at weaning of SL mice were significantly higher compared to the SS mice. However, pup mass at weaning was significantly lighter in SL mice compared to the pup mass in the SS mice. Our present study concludes that the litter size of manipulated litters was negatively associated with pup mass at weaning.

Keywords: *Litter size, reproductive efforts, manipulation of litters, lactation*

Introduction

Litter size (LS) is defined as the total number of little pups in a litter. It considers the main vital trait of animal's life history that contributed to the fitness [1] . Species are different in their litters and the litters can also be varied within species [2, 3, 4, 5] . Many of eco-physiological studies have interested in addressing the question about why there are inter and intra variations in LS [6, 7, 8, 9]. In these studies, it was focused on the energetic cost of raising different litter sizes within one species [10, 11, 12, 13, 14, 15, 16] or comparing different species raising the same litter size [17, 18]. Previous studies indicated that are several strategies that animals can follow when rearing larger litters. Firstly, animals can increase their food intake to support the pups. Reducing the available energy for each pup can be another option. In addition, inducing the energy derived from fat store. Animals could also increase the digestive efficiency towards the growth of pups. Finally, lactating animals could kill some of their pups to ensure having an optimize reproductive success. It has been shown that the mother with large litters ate more food compared to animals raising small litters [19, 20, 17, 8, 21, 10, 22, 23, 24, 25]. However, it is not always the case that mother's food intake is positively correlated with number of litters [26, 27]. Generally speaking, pups reared in large litters are lighter than pups reared in small litters [28, 29, 30, 31, 22, 23, 24, 25]. and this was explained that mothers with large litters cannot provide their pups with enough energy. Mothers with large litters could also increase their milk production due to the continuous sucking of litters to the mammy glands [32, 33]. In some cases, mothers faced with large litters could rely on their fat stores to produce more milk during lactation [34]. Another strategy by lactating animals is that the lactating animals may kill some of the raised pups to ensure supporting the rest of the pups [25]. Nevertheless, many of previous studies on lactating animals with natural litters did not support the negative link between litter size and pup mass compared to the negative link between the above traits observed

in the studies with manipulation of litters [35].Therefore, experiments with manipulation litters could expand our understanding on the nature of limits on energetics of reproduction.

Materials and Methods

1. Animals Housing and Mating:

The study included (10) male and (27) female BALB/C mice. Mice were housed in a plastic cage (48"×15"×13") provided with sawdust, cardboard tube, and paper wool for bedding and also with water and food at *ad libitum*. The mice were kept under ambient temperature (21±2°C) with daily 12hrs:12hrs dark and light, respectively.

Twenty female mice were in pairs housed with one male in each cage and 7 female individually housed as a control. The mated mice were provided with standard diet and water as *ad libitun*. The body mass of pairs female were daily monitored for 11 days. If the pregnancy occurred, the male was removed and the pregnant female was then individually housed in a new cage. Daily body mass and food intake of pregnant female were measured until the day of parturition using a balance (± 0.01g, Sartorius). At day of delivery, the pups were counted and weighted and then left with their mother without interruption for 3 days of lactation.

2. Manipulation of Litter Size:

On day 3 of lactation, mother's body mass, litter size, litter mass, and pup mass were measured. On this day, the manipulation of litters was made by adding or removing pups from the lactating female to others which were born on the same day (± 1). Lactating mice were randomly allocated for mice with 2 pups (called reduced litters) or mice with 8 pups (called enlarged litters). On the day of manipulation, lactating mice and control ones were randomly assigned for 3 groups. Group 1 refers to the control (CS; N=7), group 2 refers to the lactating mice with enlarged litter (SL; N=5), and group 3 refers to the lactating mice with reduced litters (SS; N=6). Daily maternal body mass and maternal food intake were measured in lactation period until the day of weaning (day 18 of lactation) as well as in control mice at the same period. Reproductive efforts such as litter size, litter mass, and pup mass were daily measured until the day of lactation. The death of any pup throughout lactation period was also recorded.

3. Statistical analysis:

Prior to perform the statistical analysis, data were checked for normality using shipro test and the transformation was made for non normal-distributed data. Statistical changes in daily measured variables such as maternal body mass, food intake, litter mass, pup mass, and liter size throughout the experiment were tested using repeated measures of General Linear Model (GLM) followed by tukey post-hoc test to establish the changes among days. Differences in the maternal body mass, asymptotic food intake, litter mass, pup mass and litter size between groups were assessed using GLM. Pearson correlation was used to assess the potential relationship between measured variables. The statistical analysis was done using Minitab software 17 [55].

Results and Discussion

1-Maternal body mass:

At the baseline, the body mass was not significantly different in mothers that assigned for enlarged or reduced litters (see table 1). During pregnancy, body mass of pregnant mothers increased significantly throughout the last ten days (RM GLM, day of pregnancy, $F_{9, 100}$= 40.72, P<0.001; see figure1). During lactation, the body mass of mothers were significantly changed throughout the days of lactation with no changes being detected between manipulated mice (RM GLM, day of lactation, $F_{15,150}$= 36.39, P<0.001; group, $F_{1,150}$= 0.54 , P=0.481; see figure3 and table 1). The body mass in lactating mice with enlarged litters was similar to the body mass found in mice with reduced litters. These results were in line with the previous findings. [25]. found that the body mass of lactating mice with manipulated larger litters were not significantly changed compared to the values of mice with manipulated smaller litters. The absence of the changes in mother's body mass during lactation found in the current study could indicate that the lactating mice have not lost their weight, maintained a stable energy balance by not taking out any energy stores in their tissues to support reproduction, and supported their pups by only relying on the energy derived from eaten food [25, 53, 54].

Table 1: Measured physiological traits of mothers raising enlarged and reduced litters. Means that do not share a letter are significantly different

Traits	SL Mice	SS Mice	T value	p value
LS at birth	6.67±1.3	7.33±0.99	-0.42	0.686
LS at day 3	8a±0	2b±0	4.49	<0.001
LS at day 18	7.6a±0.54	2b±0	1.98	<0.001
FI baseline	3.78±0.39	3.77±0.35	-5.8	0.36
Asymp FI 8-13	20.63a±0.64	17.262b±0.39	4.49	0.004
BM at baseline	25±0.95	25.14±0.52	-2.08	0.35
BM at day 3	35.05±0.50	35.405±0.26	-0.64	0.547
BM at day 18	33.96±0.52	34.318±0.23	-0.64	0.55
LM at day 3	22.81a±0.32	6.283b±0.15	46.49	<0.001
LM at day 18	69.43a±1.4	26.717b±0.04	30.45	<0.001
PM at day 3	2.8505a±0.04	3.142b±0.07	-3.5	0.01
PM at day 18	9.152a±0.14	13.358b±0.13	-22.42	<0.001

SL= standard enlarged litters; SS=standard reduced litters; LS= litter size; FI= food intake; Asymp=Asymptotic; BM=body mass; LM= litter mass; PM= pup mass

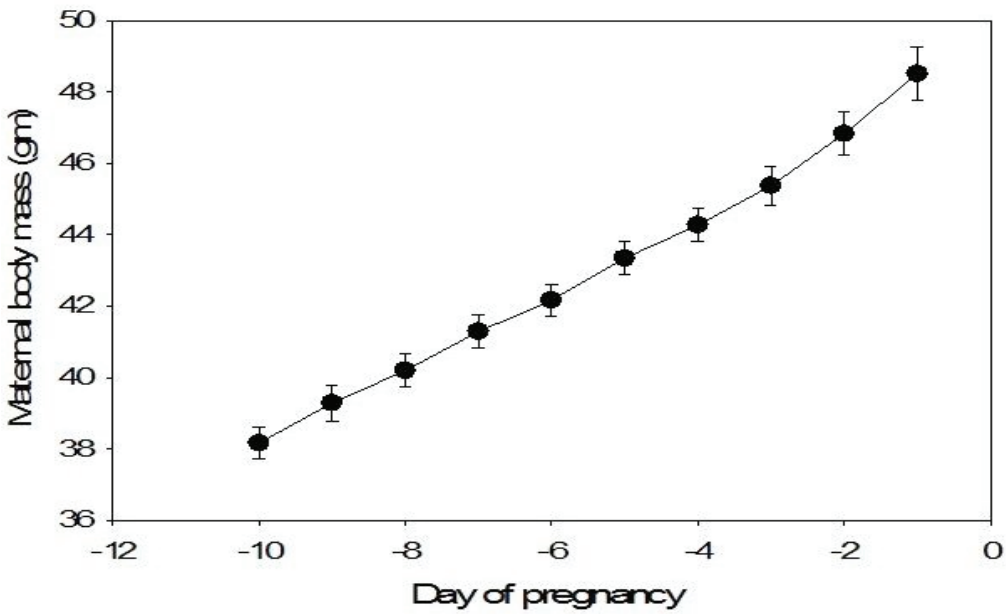

Figure 1: The maternal body mass of pregnant mice throughout pregnancy period

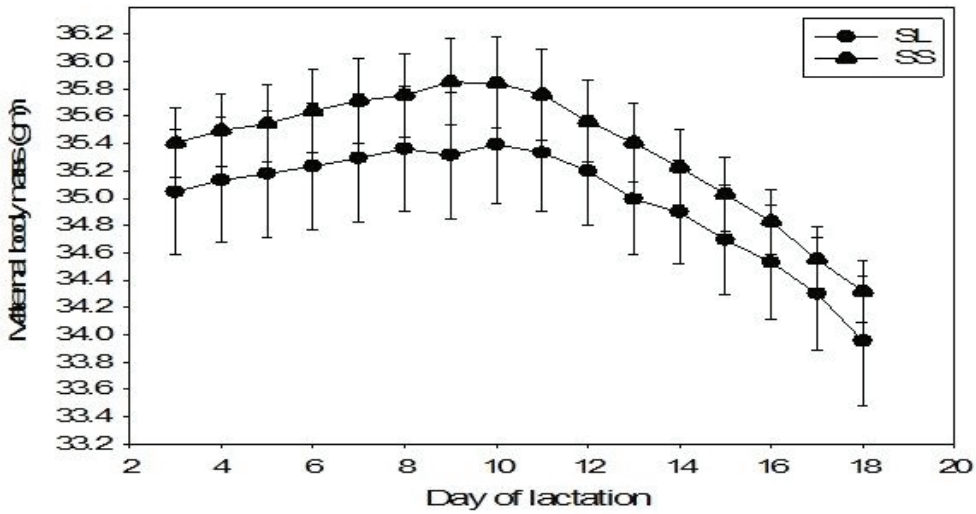

Figure 2: The maternal body mass of lactating enlarged and reduced mice fed standard diet throughout lactation period.

2- Maternal food intake:

At the baseline, the food intake was not significantly different in mothers that assigned for enlarged or reduced litters (see table 1). During pregnancy, food intake of pregnant mothers increased significantly throughout the last ten days (RM GLM, day of pregnancy, $F_{9, 100}$= 40.72, P<0.001; see figure 3). During lactation, the food intake increased significantly across the days of lactation as well as significantly different between mice with enlarged litters and mice with reduced litters (RM GLM, day of lactation, $F_{14, 139}$= 5.63 , P<0.001; group, , $F_{1, 139}$= 22.22 , P=0.00; see figure 4 and table 1). In both groups, the food intake was the highest and not significantly different among the days 8- 13 of lactation and this period considered an asymptotic food intake see table 1.

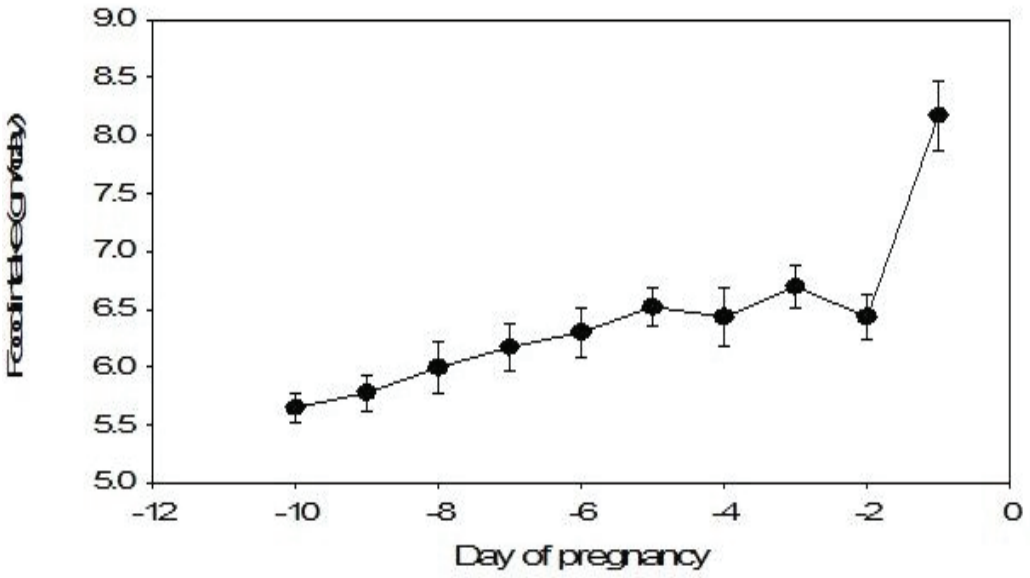

Figure 3: The food intake of pregnant mice throughout pregnancy period.

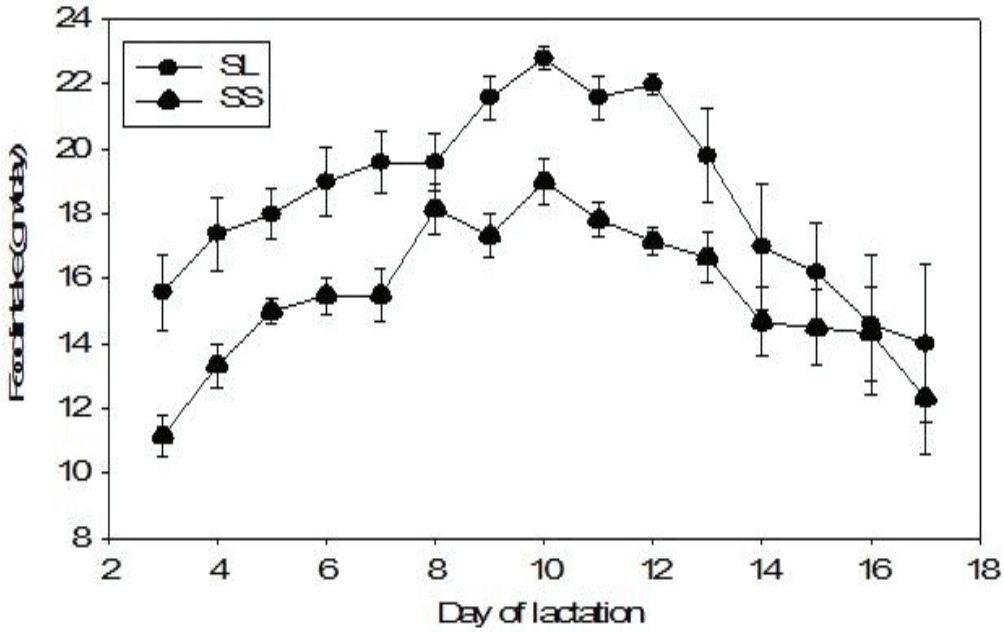

Figure 4: The food intake of lactating enlarged and reduced mice fed standard diet throughout lactation.

3- Reproductive performance

The liter size (LS) at birth (prior manipulation) was not significantly different in mice that assigned for enlarged litters compared to the mice with reduced litters (see table 1). After manipulation, litter size was significantly greater in mice with enlarged litters compared to those in mice with reduced litters throughout lactation but the litter size did not significantly changed cross the days of lactation (RM GLM, group, $F_{1, 135}$= 17160.21 , P<0.001; day of lactation, $F_{15, 135}$= 1.00 , P=0.5; see figure 5 and table 1. The litter and pup masses were significantly different between mice with enlarged and reduced litters. Litter mass (RM GLM, group, F1, 135= 178.26, P<0.001; day of lactation, F15, 135= 7.62, P<0.001) and pup mass, (RM GLM, group, F1, 135= 31.92 , P<0.001; day of lactation, F15, 135= 12.47 , P<0.001). see figure 6 and 7.

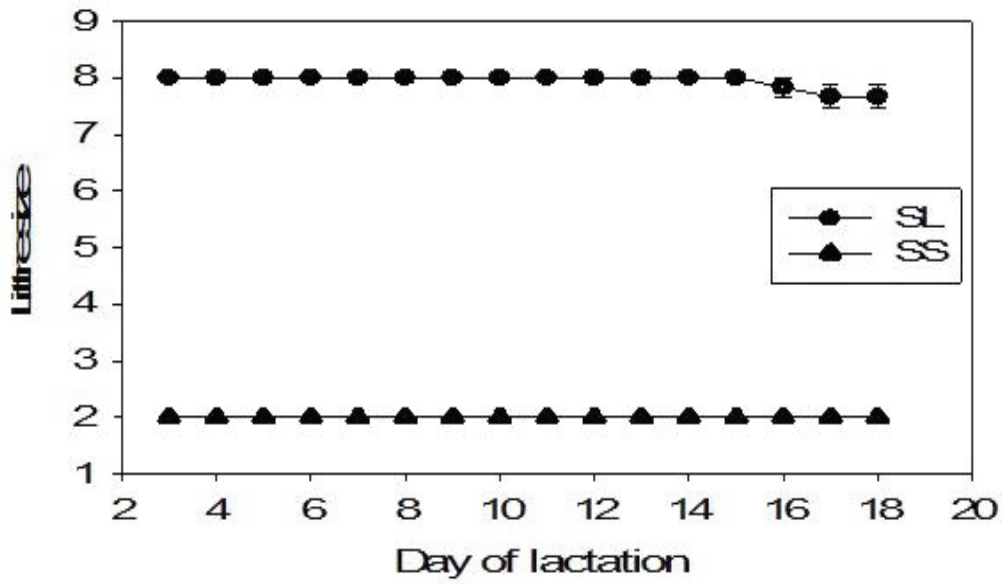

Figure 5: The litter sizes of lactating enlarged and reduced mice throughout lactation period.

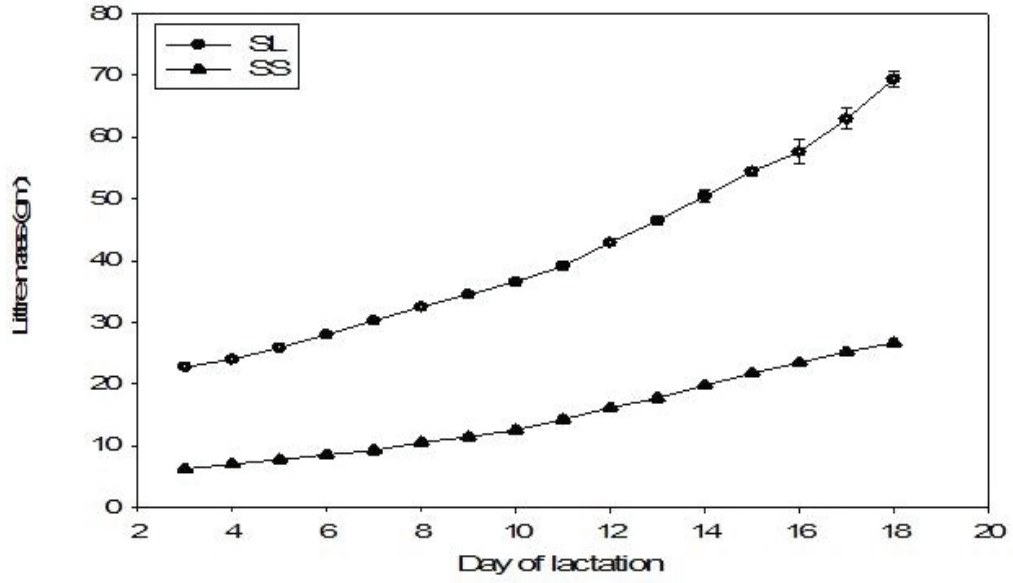

Figure 6: The litter masses of lactating enlarged and reduced mice throughout lactation

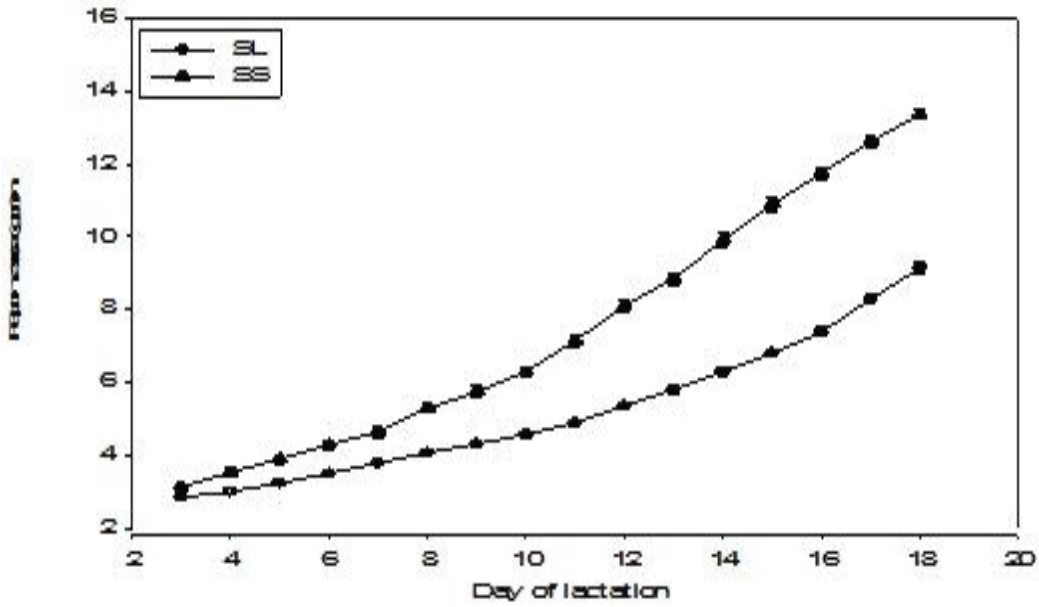

Figure 7: The pup masses of lactating enlarged and reduced mice throughout lactation

During lactation, lactating mice with both enlarged and reduced litters significantly increased the daily food intake over the first 13 days of lactation and reached a maximum of asymptotic food intake (days 8-13) compared to non-reproducing mice. These findings were consistent with many of previous studies conducted on food intake during lactation [12, 36, 37, 38, 39 , 40,41]. Lactating mice with enlarged litters ate significantly more food compared to the lactating mice with reduced litters [42]. This is explained by the fact the mice raised more pups responded by eating more food. Similar to this finding was also found in lactating MF1 mice reared large pups compared to those reared small pups [22, 23, 43, 25]. This means that the lactating mice allocated the energy derived from food intake to support their pups. However, weaned pups raised in large litters were significantly

lighter than in pups raised in small litters. These results were consistent with previous studies conducted on rodents [44, 45, 46, 31, 14,25]. These studies suggested that milk production of small mammals is physiologically limited [47, 12, 16, 48]. Liter size in mothers can be adjusted before or at birth and this adjustment can be different between mothers depending on the mother's resources. One possible way in which the lactating mothers can control their litters after giving birth is by infanticide [49, 50]. In this regard, mothers can kill some of their litters to relive the stress of extra burden [51]. In our current study, mothers with enlarged litters had about 4% mortality rate of their pups compared to zero % in mothers with reduced litters. This result was consistent with previous studied conducted on MF1 mice [25] and also in lactating Syria hamster (Mesocricetusauratus) [52].

Conclusions

Manipulation of larger litters significantly induced mothers to eat more food and weaned lighter pups compared the weaned pup reared in manipulated smaller litters.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Physiology and Pharmacology and all experiments were carried out in accordance with approved guidelines.

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Study the Prevalence and Symptoms of COVID-19 Patients in Kirkuk City

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Abstract

The study was conducted in the city of Kirkuk in July 2020 and included 100 people who visited hospitals and health sectors in the city of Kirkuk, which is located in northern Iraq. As the study included ages that ranged between 12 and 72 years and from both sexes, the study also included 100 people who did not suffer from any symptoms or signs related to the Covid-19 virus. As the people who were included in the study are from the people who reviewed, like what we said in hospitals and sectors, and they were suffering from high temperatures, cough or both, fatigue and fatigue, and those who had direct contact with infected people were taken from them and from a group of nasopharyngeal swabs for detection of Covid-19 virus by PCR. The PCR tests were done by the PCR in the Public Health Laboratory and the Kirkuk General Hospital, and special thanks to the staff. These checks were made for patients by protocols by the Iraqi Ministry of Health and the World Health Organization to investigate the RNA of the virus. The study showed the prevalence of Covid-19 was 66% in suspected patients and 10% in healthy persons. The study demonstrated that, majority of patients with novel corona virus infection were from urban area. The study showed that, majority of patients were aged from 22 to 41 years of old (P. value <0.001). The study showed that 63.64% of COVID-19 patients were males compared with 36.36% females (P<0.05). The study showed that 51.52% of COVID-19 patients were smokers (P>0.05). The study demonstrated that, 45.45% of COVID-19 positive patients were with mild infection, 18.18% were with asymptomatic features, 16.67% were with moderate infection and 4.55% were with severe infection while 80% of healthy person who was +ve to Covid-19 were with asymptomatic infection

Keywords: *Covid-19; Asymptomatic; PCR; Kirkuk; Severe.*

Introduction

The Covid-19 virus is one of the modern viruses that have spread in our societies since the beginning of 2020 and up to this point [(1)]. The spread of this virus in the community is considered one of the most dangerous viruses on the soul of humans, especially in later ages, and for people with chronic diseases, diabetes, hypertension, acute pneumonia, cancer, and chronic kidney inflammation [(2)]. This virus has spread to all countries of the world, including the Middle East, the Republic of Iraq, especially in the city of Kirkuk and Baghdad, and from the south and the cities of Iraqi Kurdistan [(3)]. Among the most common elements and causes of the spread of this disease is the failure to respond to the laws of the World Health Organization and the Ministry of Health in terms of social distancing, wearing masks, sterilizing feet, surfaces and floors, and avoiding touching, kissing and shaking hands [(6,7)]. In this study and in multiple studies, we touched on knowing the level of spread of this disease in people who suffer from high temperatures or shortness of breath and the like. They are exposed to infected persons or direct contact with infected persons in the same house, in the same circle [(8-10)]. Where the study aimed to find and determine the level of spread of the Covid 19 virus in the city of Kirkuk, the elements and the causes associated with it, gender, age, and the level of learning in infected persons, as well as the symptoms appearing on infected persons.

Materials and Methods

The study was conducted in the city of Kirkuk in July 2020 and included 100 people who visited hospitals and health sectors in the city of Kirkuk, which is located in northern Iraq. As the study included ages that ranged between 12 and 72 years and from both sexes, the study also included 100 people who did not suffer from any symptoms or signs related to the Covid 19 virus. As the people who were included in the study are from the people who reviewed, like what we said in hospitals and sectors, and they were suffering from high temperatures, cough or both, fatigue and fatigue, and those who had direct contact with infected people were taken from them and from a group of nasopharyngeal swabs for detection of Covid-19 virus by PCR. The PCR tests were done by the PCR in the Public Health Laboratory and the Kirkuk General Hospital, and special thanks to the staff. These checks were made for patients by protocols by the Iraqi Ministry of Health and the World Health Organization to investigate the RNA of the virus (Sacace Biotecnology, Italy).

Results

The study showed the prevalence of Covid-19 was 66% in suspected patients and 10% in healthy persons, Figure 1.

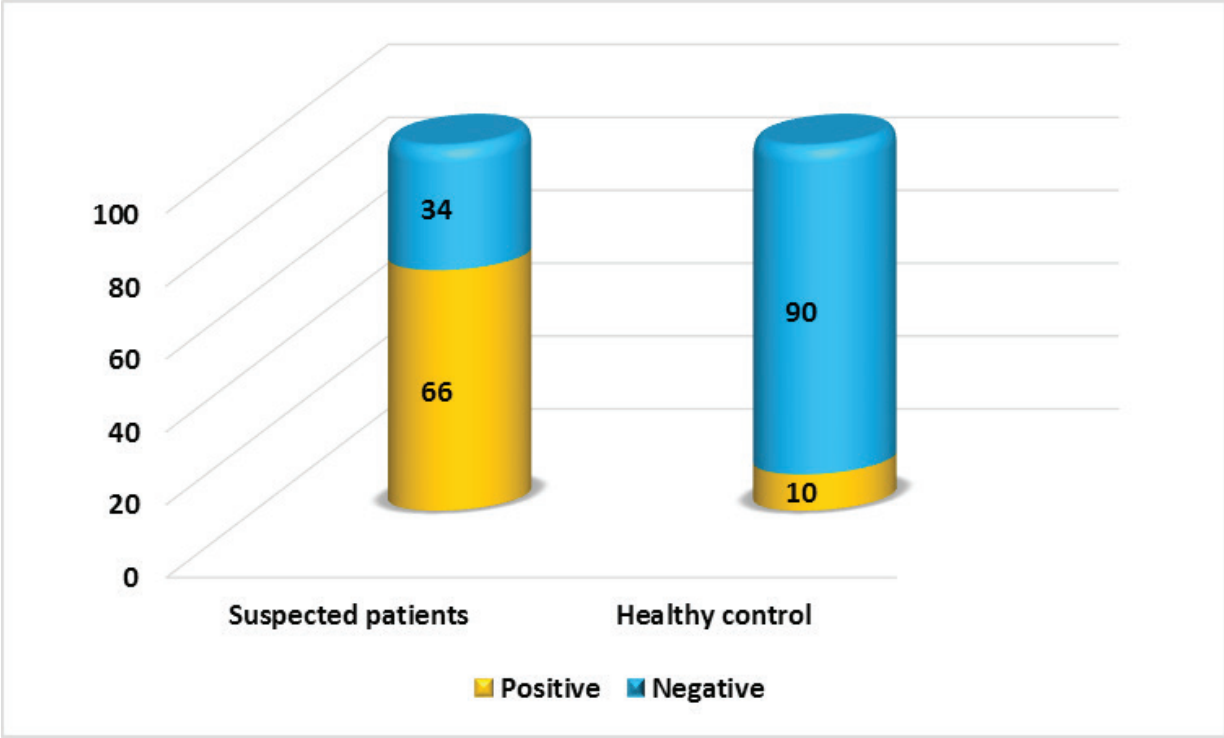

Figure 1: Prevalence of COVID-19

The study demonstrated that, majority of patients with novel corona virus infection were from urban area.

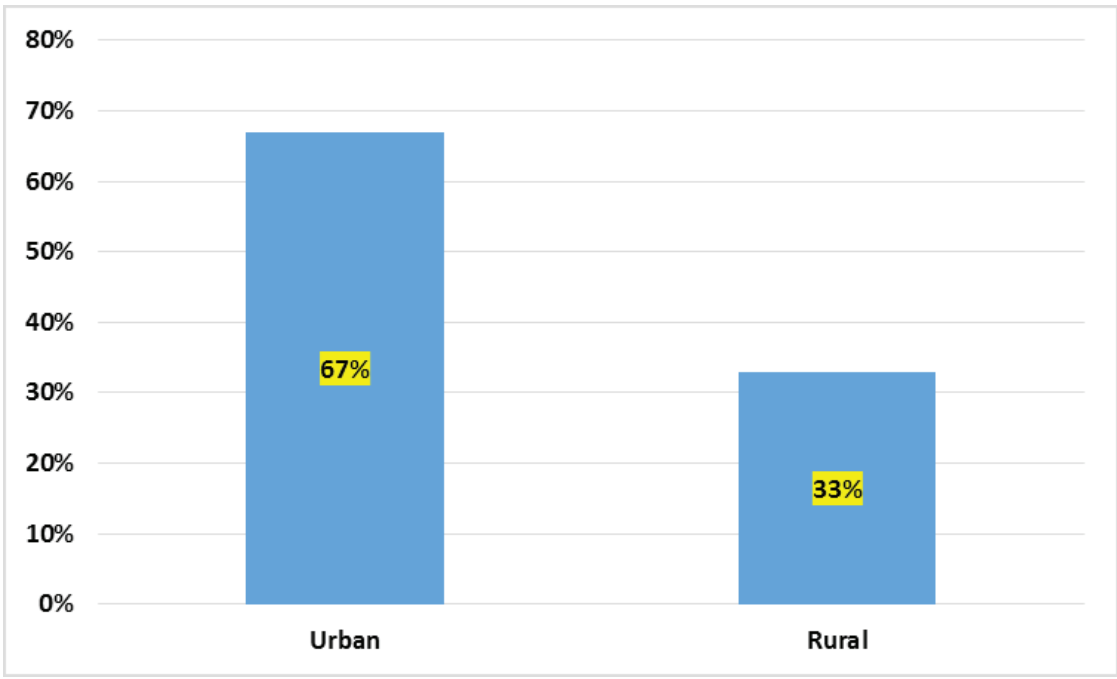

Figure 2: Distribution of COVID-19 patients according to residence

The study showed that, majority of patients were aged from 22 to 41 years of old (P. value <0.001), Table 1.

Table 1: Distribution of studied patients according to characteristics of menstrual cycle

Age (year)	No.	%
12-21	4	6.06
22-31	17	25.76
32-41	21	31.82
42-51	11	16.66
52-61	9	13.64
62-71	4	6.06
total	66	100

P. value <0.001

The study showed that 63.64% of COVID-19 patients were males compared with 36.36% females (P<0.05), Table 2

Table 2: Family history of PCOS

Sex	Positive		P.value
	No.	%	
Male	42	63.64	0.026
Female	24	36.36	
Total	66	100	

The study showed that 51.52% of COVID-19 patients were smokers (P>0.05), Table 3.

Table 3: Family history of PCOS

Smoking	Positive		P.value
	No.	%	
Yes	34	51.52	0.65
No.	32	48.48	
Total	66	100	

The study demonstrated that, 45.45% of COVID-19 positive patients were with mild infection, 18.18% were with asymptomatic features, 16.67% were with moderate infection and 4.55% were with severe infection while 80% of healthy person who was +ve to Covid-19 were with asymptomatic infection, Figure 3.

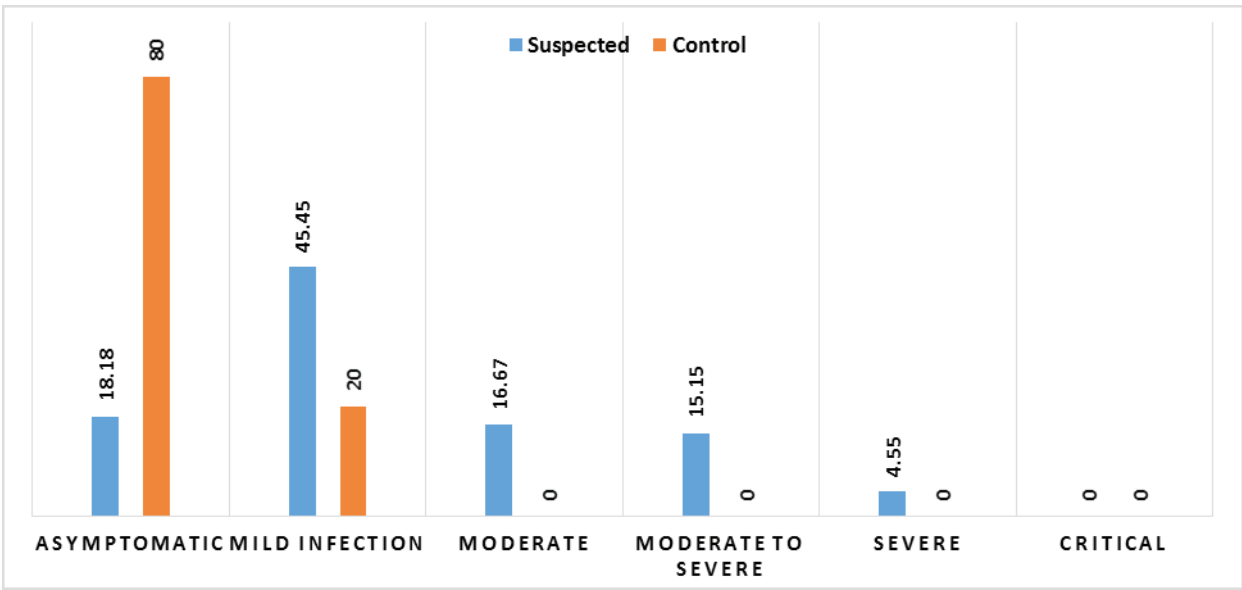

Figure 3: Clinical features of COVID-19 infection

Discussion

Numerous studies have found that the Covid-19 virus is spreading highly in eastern and Middle Eastern countries in Saudi Arabia, Iraq, Iran, Turkey and the Arab Gulf. Covid-19 and those with symptoms similar to those of Covid-19 were actually infected with the virus in this study [6]. And as our study found, the most people infected with the Covid-19 virus are among the people who spend cities, and as such, numerous studies were conducted at the beginning of this year and to the end of this day, as studies indicated that those who say cities are the most vulnerable to infection with covid 19, and there is no doubt [7-10]. The reasons behind this are the population density in the city as well as the narrow space of houses, buildings and apartments, which negatively affect the social distancing program, which provides for distance from each other, which reflects that of the people who live in the villages, including the domestic spaces and the distances between One room and another, between a house and another, due to the building area in the villages [11,12]. This study found that I am the most age group of people who are infected with covid virus 19 are those between the ages of 20 and 40 years. This has led to many studies conducted previously that young people are the most vulnerable to infection due to their frequent interaction with infected people, caring for the sick and suffering from health personnel or Of the people who provide services to the community, all the restaurants, clothes, shops, laboratories, and factories, all of them are young cadres who may be exposed to people infected with covid 19 and they do not know, then they may be infected, then they go to health centers, and then it is discovered that they are infected with Covid virus 19 [13,14]. Women are less likely to be infected with Covid 19 virus, in this it may be the title of a research that can be conducted in any country in the world. Yes, Covid 19 virus affects men more, and other studies have proven that it does not affect it. For many reasons, including that men are the most work and are more exposed to injured people and more going out of the house and more and they are in contact with the whole world, unlike women, especially that in our eastern society in the city of Kirkuk, Iraq, men represent the main element who provide services in society, unlike women [15,16]. With regard to the symptoms and signs associated with infection with covid 19 virus, our studies indicated that most of the people who did not suffer from infection, any of the control group, were people who did not suffer from any symptoms, and that the highest degrees of infection and infection rates were in people who suffer from mild symptoms To an average level, they were similar, to studies conducted previously, as most people infected with Covid 19 virus do not suffer from any symptoms or who suffer from symptoms, and studies have also shown that a small percentage of people infected with Covid 19 virus are present in critical condition [17,18]. Scientific evidence has indicated that the higher the levels of autoimmunity and cellular immunity in healthy or infected people, the symptoms of Covid 19 virus are less severe in the sense that people whose immunity is good have symptoms from few to none, and this indicates and leads us to that adults are the most susceptible Developments [19,20].

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine and all experiments were carried out in accordance with approved guidelines.

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Effectiveness of an Educational Program on Nurses' Practices toward Using of Personal Protective Equipment at Kidney Transplant Center in Baghdad City

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Abstract

The study aims to evaluate nurses' practices toward using personal protective equipment, and to identify the relationships between nurses' demographics including (age, gender, education level, and years of experiences in kidney transplantation units) and their practices toward aseptic techniques. A quasi-experimental study, quantitative design (One-group Pretest - Posttest) was conducted on nurse's practice toward aseptic techniques in kidney transplant units. It was carried out in order to achieve the early stated objectives with the application of pretest and posttest. A non-probability (purposive) sample of (30) nurses who are working in kidney transplant unit, that's selected based on the study criterion, and after obtaining oral consent from them, those nurses were distributed in kidney transplant units at Medical City complex. The study Instrument was composed of two Parts: The First Part is concerned with the socio-demographic characteristics, and the second Part includes Nurses' Practices. Content validity of the instrument was established through a penal of (12) experts. Reliability of the instrument scales determined the internal consistency by using test-retest approach through the computation of Pearson correlation coefficient of the scale which was r=0.83 for nurse' practices. Results have been analyzed through the use of SPSS "Statistical Package for Social Sciences" model 24.0 by using descriptive and inferential statistical methods. Findings of the present study revealed that most of the samples were within the age of (40 - 49) years who were accounted (40%). It has been presented that there were significant differences in the mean of the study sample between the pre and post test procedures, revealing a significant improvement in nurses' practices regarding using of personal protective equipment. Moreover, a high significant difference was also presented among the whole study sample at pre and post-test practices, t-test value (8.462) at p value (.000). The study recommended that nurses should be committed to follow and apply standards of using the personal protective equipment, sharing in training sessions, improve practices through self-learning and training, and establishing future studies to measure nurses' practices toward using personal protective equipment at all healthcare settings in Iraq.

***Key word**: Nurses, practice, personal protective equipment, kidney transplant.*

Introduction

Infections associated with healthcare are infections acquired as a direct or indirect by health workers outcome. International evidence exists to indicate that a major infection burden exists among long-term care patients, but there are few published research on the incidence of infection in Australia [1]. There are about 165,000 HAIs per year in Australian acute health care facilities. This makes HAI the most common complication in hospital patients. As well as causing patients and their families' needless pain and discomfort, these negative effects increased hospital visits and are pricey for the health system [2]. Around Seven per cent of inpatients will receive Hospital acquired infection, the issue does not only impact patients and staff in hospitals. Any person who works in or joins a healthcare facility is at risk. Health-related infection, however, is a potentially preventable adverse occurrence rather than an unexpected risk, and through successful infection prevention and control, the incidence of HAIs can be greatly reduced. [1]

Outbreaks associated with a wide variety of bacterial, fungal and viral infections due to widespread and person-to - person exposures are prevalent in adult and adolescent ICUs, including kidney transplant units (CDC, 2019). Training and encouragement are perhaps the most critical ways in which nurses will impact patients during the entire transplantation process, from first joining the waiting list to the operating phase itself to the follow-up care level, there is a need for detailed nursing feedback. (KDIGO, 2009) [6].

Transplantation procedure more cost-effective than hemodialysis with lower morbidity and better quality of life, transplantation has revolutionized the management of end stage renal disease. For chronic kidney disease, it is the recommended therapy. While effective transplantation is the second type of therapy for (ESRD), it possibly most closely approximates a return to physical pre-ESRD functioning and typically only involves the use of immunosuppression medication [6].

Aseptic technique is a collection of particular activities and procedures that are carried out with the goal of reducing exposure of pathogens, under closely controlled conditions. Aseptic technique is used to maximize and preserve asepsis, the absence of human pathogens in the clinical environment, especially in intensive care units. The purpose of the aseptic procedure is to protect the patient from the spread of infection between them and protect medical materials. In any clinical setting, the theory of asepsis can be applied. Via contact with the environment, staff, or equipment, pathogens can introduce infection to the patient. The atmosphere contains potential hazards that, by movement, contact, or proximity, can spread pathogens. Interventions such as regulating air flow that help reduce environmental impacts by reducing operating theatre flow, separating a patient to protect airborne emissions, or using close to zero-particle garb [2].

Nurses have an important role in helping patients have immunosuppressive regimens during hospitalized periods to minimize exposed to nosocomial infections to survive their lives by using high sterile techniques with medical and nursing care (NHMRC, 2019).

Material and Methods

A quantitative research approach has been used for this study. The quasi-experimental design (pre-test and post-test) was conducted on nurses who work at kidney transplant unit at Baghdad City Complex. It was conducted with application of pre-test and post-test approach to evaluate nurses' practices regarding using the personal protective equipment, in addition to the application of education program. It was carried out in order to achieve the initial stated objectives. The study started from December 7th, 2018 to September 2nd, 2020. Participants were verbally informed about the aims of the study and had been asked to participate voluntarily. They also had been informed that they could refuse to answer a certain question or withdraw from the study at any time. Emphasis was placed on creating a suitable environment during interview using good communication skills with participants.

The present study was conducted in kidney transplant center in medical city complex at Baghdad city. A non – probability (purposive) sample of (30) nurses (males and females), who were working in kidney transplant units, were selected from (55) Nurses in these center. All selected nurses were exposed to the educational program.

The researcher constructed the questionnaire thorough reviewing of previous literature and related studies. The study instrument comprised of two parts; Socio- Demographic Data which was comprised of seven items relative to the gender, age, educational status, years of experience, training of program, and number of training courses; and Nurses' practices Form which was comprised of (45) items that concerned with nurses' practices to assess the nurses practices in kidney transplant center. Content validity for the early developed program and the study instruments were determined using panel of (12) experts. A preliminary copy of the program and questionnaire was designed and presented to (12) experts for the determination of its validity. They were (7) faculty members from College of Nursing – University of Baghdad, (1) faculty member from College of Medicine / university of Kirkuk, (2) faculty member internist (nephrologists) from Disease and kidney transplant Center/ Medical City complex, (1) faculty member surgeon from Disease and kidney transplant Center/ Medical City complex, (1) Nursing specialist in adult nursing from Disease and kidney transplant Center/ Medical City complex.

A purposive sample of (5) nurses was selected from kidney transplant units of working in center to achieve the purpose of pilot study. It was applied on the nurses who had the same criteria of the original study sample and started from December 5th, 2019 to Jan 4th, 2020 to determine the internal consistency of questionnaire related practices of nurses concerning nursing practices in kidney transplant units.

The researcher determines the internal consistency by using test – retest through the computation of Pearson correlation coefficient of the scale which was r= 0.81 for nurse's practices. Data were collected through chick list questionnaire with the study sample by using a constructed questionnaire. Nurses were observed while they were working in their units to provide care concerning nursing intervention by using concealed observational technique. Data were collected from February 2nd2020 to march 6th2020.

The data were analyzed by using SPSS (Statistical Package for Social Sciences) version 24.0 application of the statistical analysis system. The descriptive data analysis includes Frequency (f), Percentage (%), Mean, and Standard Deviation. The inferential data analysis includes Pearson Correlation Coefficient, t-test, and ANOVA Table test with significance of p value ≤ 0.05.

Results

Table (1): Distribution of the Study Samples according to the Demographical Data.

Variable	Groups	Results	
		F.	%
Age Groups	20 – 29	7	23.3
	30 – 39	8	26.7
	40 – 49	12	40
	50 years and over	3	10
	Total	30	100
	$\bar{x} \mp SD$	37.9 + 8.77	
Gender	Male	20	66.7
	Female	10	33.3
	Total	30	100
Education Level	Nursing School Graduate	1	3.3
	Secondary Nursing School Graduate	4	13.3
	Nursing Institute Graduate	20	66.7
	Nursing College Graduate	4	13/3
	Higher Education	1	3.3
	Total	30	100

Cont... **Table (1): Distribution of the Study Samples according to the Demographical Data.**

Years of Experience in Hospital	1 – 5	12	40
	6 – 10	5	16.7
	11 – 15	0	0
	16 and over	13	43.3
	Total	30	100
Years of Experience in Kidney Transplant Unit	1 – 5	7	23.3
	6 – 10	5	16.7
	11 – 15	5	16.7
	16 and over	13	43.3
	Total	30	100
Sharing in training sessions inside Iraq	Yes	25	83.3
	No	5	16.7
	Total	30	100
Sharing in training sessions outside Iraq	Yes	2	6.7
	No	28	93.3
	Total	30	100

It is clear from the table (1) of demographic characteristics for nurses, showed that the majority of the study sample (40 percent) were within age group (40 – 49) years old. Moreover, most of the study group (66.7 percent) were male and the remaining were female. The majority of the study sample (66.7 percent) have nursing institute graduates. In addition, 43.3 percent of the study sample have experience in hospital in about 16 years and over; while, 40 percent of the study sample have one to five years of experience in hospital. Furthermore, approximately 43.3 percent of the study sample have experience in kidney transplant unit in about 16 years and over. The findings also showed that the highest percent of the study sample 83.3 percent were sharing in training sessions inside Iraq; while, 93.3 percent of the study sample were not sharing in training sessions outside Iraq.

Discussion

Results in table (1) presented that The results of the present analysis have shown that age group sample were (40 – 49) years old who accounted for (40%), the age group of less than 39 years old of age accounted for 50 %.

The research findings revealed that the most of the samples were males (66.7%) and the remaining were females.

Concerning to the educational level of nurses, the greater percentage (66.7%) has a diploma in nursing, the remaining (13.3%) graduated from the College of Nursing and (13.3%) graduated from secondary nursing school.

Regarding the years of experience in kidney transplant units, the results indicate that most of the

participating nurses were within years of experience in kidney transplant units (16 years and over) accounted for (43.3%), the remaining (23.3%) of the nurses were within (>5) years and (16.7) of the nurses were within (6-10) and (11-15) years of experience in kidney transplant units. The findings also showed that the highest percent of the study sample 83.3 percent were sharing in training sessions inside Iraq; while, 93.3 percent of the study sample were not sharing in training sessions outside Iraq.

In their report, the findings of Jissir and Hassan, 2015, notice that the majority of nurses are 20-29 years of age. In their report, Haitham, 2016, the investigator agrees with these findings with 75 percent of the sample male gender. In their survey conducted on 77 nurses to assess the nurse's expertise and experience on administering basic measures in intensive care units in Egyptian cancer hospital, Eskander, et al., 2013 concluded that the level of education for most nurses in their survey was nursing college, according to researcher findings, according to the plurality of study sample of nursing graduation diplomas.

Conclusions

Nurses' practices in general were poor before the implementation of an educational program in pre-test, which included their practices. There were significant improvement in Nurses practices after the implementation of an educational program in post-test. The findings of this study indicate that the educational program for nurses was effective after the exposure to such a program, (their practice toward using personal protective equipment). Emphasis should be put on the managers of hospitals, and nursing affairs managers to monitor and follow-up, support and supervise nurses while they are working in their units. Giving the nurses of the patients' knowledge and practices for good preparing before and after any nursing procedure in the kidney transplant units. Emphasis is necessary on hospital managers and administrators on nursing to reward and incentive nursing staffs that are efficient and loyal in their work and to reward the good ones and punish the careless in their duties.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing and all experiments were carried out in accordance with approved guidelines.

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Biocompatibility, Antibacterial Activity and Preparation of Ce doped β-TCP by RF Sputtering Technique to Enhancing Some Properties

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Abstract

Two weight percents of ceria (40 and 80 wt%) were added to tricalcium phosphate to improve some properties for Ce doped β-TCP by RF sputtering method on stainless steel substrate as coating for biomedical applications. The sputtering was done at 150 W and 150ºC. The confirming of doping was achieved by some techniques such as XRD, FESEM/EDS and EDS mapping, and indicated the occurring of incorporation between cerium oxide and TCP as thin film. Biocompatibility and antibacterial test was improved after adding cerium oxide to TCP which candidate the prepared thin film for bio implants. Microhardness also examined for prepared composites to show the increasing microhardness of CeO_2/TCP coatings compared with conventional HA coating.

Keywords: *Ce doping; β-TCP; Biocompatibility; Antibacteria.*

Introduction

Cerium element is a one of lanthanides which has electronegativity and ionic radius is similar to the one of group II element in periodic table which is calcium. Also, Ce has an ability to substitute and accumulate with Ca in its compounds. It is important to know the role of Ce in biomedical process due to its antioxidant property, high thermal-phase stability [1], stimulation of the antimicrobial activity, and regenerative behavior. Cerium ions can be Ce^{4+} and Ce^{3+} in its substitution depending on experimental path. On the other hand, calcium phosphate (TCP) coatings have been achieved due to ability to absorption the proteins to biomaterial surfaces and then followed by healing process at the material interface and bone formation with improving the osseointegration [2].

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Sputtering is a technique that involves the ejection of a material through the transfer of energy from an energetic particle (in the mixture form of ionized atoms or clusters of atoms) to a surface which present as a target of material. Therefore, the coating of Ce doped TCP has an attention to be deposited on stainless steel (SS 316L) in the current work by RF sputtering through prepare two targets (40 wt% CeO_2/TCP and 80 wt% CeO_2/TCP) [3].

Some works were achieved related to Ce doping, M. Ganguly et al. prepared Ce doped barium titanate and they mentioned that the radius of Ce is greater than that of Ti. Therefore, when Ce is doped in barium titanate, distortion is occurred in the structure. The displacement which took place in structure led to generat intermediate states and break some bonds such as (Ce-O), (Ti-O) and (Ba-O), and then form the complex vacancies [1]. N. Sinha et al. synthesized Ce doped ZnO by wet chemical solution route[2]. N. Komuro et al. prepared Ce doped calcium barium phosphate by conventional solid-state synthesis; they found that the doping by Ce^{3+}, Si^{4+} enhanced the luminous intensity due to the

combination to form $Ca_6BaP_4O_{17}$ phase [3]. N. Aisah et al. studied Ce-doped ZnO, and they showed that there is no diffraction peaks of the phases containing Ce were found in the samples which indicated that Ce^{3+} ions substituted or accommodated in ZnO lattice structure [4]. A. Jafari et al. studied the reduction in particle size during the preparation of Ce^{3+} doped TiO_2 by sol-gel method[5].

Experimental Procedure

SS 316 L specimen was used as substrate to apply the coating of Ce substituted TCP, the chemical composition of specimen is (wt%: 0.022 C, 0.407 Si, 1.43 Mn, 0.031 P, 0.004 S, 16.98 Cr, 2.00 Mo, 11.73 Ni, 0.234 Cu and Fe remain). The specimens were cut as square shape with dimension of 2 Í 2 cm and then grained with SiC paper (600, 800, 1000, and 1200 grit size) after that they were polished with alumina paste.

Coating Method

Ce substituted TCP thin film was deposited on SS 316L substrate by RF magnetron sputtering at 150 ºC (Model No. CRC 600, Serial No. MST-CRC-102510-ND2-MS). 40 and 80 wt% CeO_2/TCP target were prepared using commercially available CeO_2 powder (E. Merck. Darmstadt, 99.99 %) and TCP (HIMEDIA, 99.99 %). A pellet of 50 mm diameter and 5 mm thickness was prepared from compacting the powders in a stainless steel pelletizer under 5 tonnes as pressure by hydraulic press, and then this pellet was insert to a furnace for sintering at 1100 ºC for 3 hs.

All experiments were carried out under Ar gas to generate plasma in the sputtering chamber with pressure ($3Í10^{-4}$ mbar), gas flow rate (5 sccm), deposition time (30 min), RF power (150W) and temperature of substrate (150 ºC).

Characterization Methods

The characterization of thin films were done by XRD with CuKa radiation (k = 1.5418 A˚), FESEM/EDS analysis (FEI QUANTA 250, Czech Republic) and AFM exam (SPM 600, Agilent Technologies).

Properties Measurements

Biocompatibility was examined by the microorganisms that suspended in BHI broth at temperature of 37 ºC and shaked at 250 r.p.m. containing the certain concentrations of the (MIC) and (MBC) and DMSO (2 V/V%) in peptone water to obtain the density of cell at ($1–2 \times 10^5$ - 10^7) c.f.u. The contact time recorded at 18 min., after that the centrifuging was done at 10 000 g for 8 min, followed by washing and resuspending in 1 mL sterile PBS. Assaying was done by microdilution broth susceptibility. To enhance the solubility, all experiments were carried out in Mueller–Hinton broth (MHB) supplemented with 2% DMSO. The killing percent was measured by subculture of 20 mL. Each test was repeated at least three times.

Antibacterial test was done by BD Biosciences Calibur. CellQuest Pro Software was used depending on light-scatter and fluorescence signals that result from 15 mW laser illumination at wavelength of 488 nm. These fluorescence signals were collected by FL-1 (BOX) and FL-3 (PI, EB and CTC) band pass filters. All of the acquired data were read, analyzed and performed via Cell Counter.

Microhardness for HA and Ce/TCP coated specimens was performed using HMV- 2, Shimadzu, Kyoto, Japan under a load of 200 mg for 15 sec.. The average of the five readings was recorded for Knoop Hardness number (KHN, kg/mm^2).

Results and Discussion

Fig. (1) shows the XRD analysis of SS 316 L substrate and coated specimens, this analysis indicates the diffraction peaks related with crystalline phase of stainless steel 316 L according to (JCPDS card No. 33-0397) [6]. For 40wt% CeO_2/TCP coated SS, can be seen clearly the 2θ values of SS 316L and peak 2θ value at ≈38.2 is attributed to calcium oxide according to (JCPDS card No. 09.0169). The XRD analysis for 80wt% CeO_2/TCP shows the main peaks of SS and the peak of ceria at 2θ = 28.37, and the other peaks are attributed to presence of α-Ce according to (JCPDS card No. 38-0765) at 2θ values (32.869 and 47.18). Also, the 2θ values at 28.17 and 44.43 may be attributed to intermediate compound ($Ca_3Ce(PO_4)_3$) according to (JCPDS card No. 29-0310).

Fig. (2-a) indicates the FESEM/EDS images of the polished SS 316L surface with little atmospheric corrosion. Also, this image shows a characteristic inclusion observed on the surface. EDS analysis shows

the main elements in SS 316L (Fe, Cr and Ni) in addition to oxygen obtained from iron oxide layer due to the little atmospheric corrosion.

FESEM of 40 wt% CeO_2/TCP coated sample shows spherical crystallites with smooth surface, the cerium begin to incorporate with TCP structure and gave weight percent of Ce in EDS analysis to be (36.4 wt%) which higher than percent of Ca (12 wt%) and P (5.6 wt%) due to clustering the ceria on the top of layer in addition to be doped in structure of TCP. While at higher weight percent (80%CeO_2), the clustering of cerium within coating layer can be formed to gave some particles on the homogeneous layer and reaching Ce wt% to 67.7 % and decreasing in Ca (2%) and P (1.9%) content confirming the doping with covering the film.

EDS mapping of SS 316L is shown in Fig. (3-a), while for the coating is shown in Fig. (3- b & c) revealing the elemental distribution of the metal ions in substrate (**Cr**, **Fe**, **Ni** and **O**) and Ce/TCP coating as calcium (**Ca**) and oxygen (**O**), in addition to cerium (**Ce**) and phosphor (**P**) confirming the doping of cerium in TCP.

The antibacterial results are shown in Fig. (4). The antibacterial responsibility for Ce/TCP coating is more than for uncoated SS 316L, the reason may be the incorporation of mineral cerium which plays a vital role in enhancing the antibacterial activity.

Fig. (5) shows the biocompatibility test for coated specimens by Ce substitution TCP coating compared with the most known ceramic material in bioapplication (HA coated specimen). This figure indicates the biocompatibility of Ce/TCP coatings are more than HA coating. Also the results observe that after 24 h of incubation, the material's surface was completely covered by cell layer, and the coverage increases with increasing the incubation time.

When compared the microhardness of ceria/TCP coatings with HA, can be seen that the CeO_2/TCP coatings have microhardness more than HA coated specimen, this means that the addition of ceria to coating mixture led to increasing microhardness. Addition 40 wt% to TCP slightly increases the microhardness compared with HA. While, the more increasing in microhardness can be seen by adding 80 wt% to TCP.

In the same medium, the microhardness after immersion time (5 days) was decreased which due to form layer like hydroxyapatite by oseointegration or incorporation with tissue contents and the produced layer is regarded more brittle than coating layer. While for the same specimen in different media, there is a variation in the behavior of microhardness which is attributed to the interaction between coating components and the type of medium whereas it is aqueous or non aqueous.

Table (1): Data of microhardness in different media before and after 5 days of immersion.

Type of coating on SS 316L	Water		PBS		Acetone	
	Initial	5 days	Initial	5 days	Initial	5 days
HA	88.1	59.3	89.2	77.0	83.4	68.2
40wt% CeO2/TCP	90.2	78.1	90.8	79.6	92.1	77.3
80wt% CeO2/TCP	121.1	110.7	112.6	109.8	117.6	101.4

Mechanism of Doping

Subhadip and Bikramjit suggested that TCP has a five distinct Ca sites with octahedral coordination suitable for cationic substitution, where monovalent ion substitution prefers substitution at Ca(4) site, while bivalent ion substitution prefers Ca(4) and Ca(5) sites, and trivalent

ion substitution is not simply to substitute at Ca(4) or Ca(5) sites [7]. On the other hand, the largest lanthanides such as (La, Ce, Pr, and Nd) prefer Ca(3) site [8]. In other study, the authors indicated that the substitution of smaller size divalent ions can accommodate at Ca(5) site, whereas larger size ions prefer Ca(4) site [9, 10] because of differences in bond length of these five different sites of calcium. In another work, the X-ray diffraction data illustrated that there are six sites of the Ca atoms in TCP structure to be accommodated as 1, 3, and 5, and 2, 4, and 6, according to equivalent configurations. Theoretically, the uniformly distribution of calcium vacancies must be most stable and every Ca atom in structure has at least one vacancy neighbor associated with defect like electronic states [11].

For the trivalent ions such as cerium, the substitution mechanism may be complex due to size and valence effect as: $3Ca^{2+} \rightarrow 2M^{3+} + \square$, referring to M^{3+} as Ce^{3+} and □ as denotes a vacancy [12]. Depending mean bond length of Ca–O, Ce^{3+} prefers Ca (3). Where for Ca (Atomic radius 197 pm and Covalent radius 176+10 pm) and for Ce (Atomic radius 181.8 pm and Covalent radius 204+9 pm).

From the DFM calculations, the substitution can be done according to energy levels as accommodation. The theoretical calculations for optimized structures were performed in the vacuum medium [13, 14]. The structure of TCP (**Fig. 6**) was calculated the stability by using (UFF/ZDO) method with Gaussian 09 software [15, 16]. The PM6 semi empirical method was used to determining the total electron density (TED) [16, 17]. The red color region indicates the high electronic density area, followed by the orange color region that represents the lower electronic density than it is in the red region, the blue color area represents the low electronic density area. Oxygen and phosphor with high electron density. (**Fig. 7**) presents the electron density above the crystal TCP.

The theoretically discussion for CeO_2/TCP coating, suggests the perfect position of Ce in lattice structure of TCP as follow: Total energy (Et) of TCP without Ce is (5.6807 a.u) and with Ce (6.9299, 13.7252 a.u) as show in (**Fig. 8**) for two positions. According to (Et) values, Ce in structure (***a*** position) is more acceptance than (***b*** position), i.e., Ce ions prefer sites 1, 3 and 6, while Zn^{2+} and Sr^{2+} prefer site (5) and site (4) respectively. The near of Ce from Ca in TCP can be a reason for the stability. The bond length of (Ce–Ca) is in rang of (2.2-3.19 A°) and (Ce–O) is about (1.9-2.9 A°).

In 2016, theoretical study of Mg substituted β-TCP to investigate the improvement of β-TCP biomaterial in bones and teeth replacements has been made by (DFT) [18]. Recently, in 2019 Xianchen et al. replaced calcium atoms by rare earth elements (REEs, La, Ce, Nd and Y) in the lattice structure of fluorapatite ($Ca_{10}F_2(PO_4)_6$) taking into accounts Fermi level of these elements that have 5d, 4f, 4f and 4d level respectively [19].

Conclusion

The doping by cerium in lattice structure of β-TCP enhanced some properties of conventional TCP in biomedical applications. The characterization by XRD, FESEM/EDS and EDS mapping confirmed the doping. Antibacterial test and biocompatibility were improved compared with conventional coating by hydroxyapatite on stainless steel 316 L. Microhardness also increased. The theoretical study of doping by DFT method limited the stable sites for doping in lattice structure.

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The Effectiveness of the Form Model For the Two Hemispheres of the Brain in the Technical Performance of Some Female Badminton Skills

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Abstract

Through the presence of researchers in the field of teaching and training for most activities of racket games in general, and playing badminton in a special way in the College of Physical Education and Sports Sciences - University of Kerbala, they found that most female students in the second stage suffer from difficulty in performing badminton skills, knowing that it is one of the subjects taught at this stage, and through extensive study and investigation about finding solutions to this problem, the researchers decided to rely on the application of a teaching model aimed at investing the energies of the brain and dealing with the tendencies and desires of the learner, a research that the educational curriculum addresses all learners, this is what motivated the researchers to prepare a new educational curriculum with all its contents according to the characteristics of the two hemispheres of the brain using the format model to learn some basic skills for the female students in badminton, and the objective of the research is to prepare an educational curriculum using the (4MAT) model according to the two hemispheres of the brain to learn some skills of the badminton, and to identify the effectiveness of the educational curriculum by using the (4MAT) model according to the two hemispheres of the brain in learning some badminton skills for female students. The researchers used the experimental approach by designing (one group with pre and post- test) in order to suit the nature of the problem and achieve the research objectives. The most important conclusions were that the educational curriculum, using the (4MAT) model according to the two hemispheres of the brain, contributed to learning some basic skills for female students with badminton. The most important recommendations are the necessity of adopting educational curricula according to the (4MAT) model in learning basic skills with badminton.

Keywords: *hemispheres, brain, female, skills, technical performance.*

Introduction

Brain learning is based on the fact that every person has a unique brain, and he is able to learn and acquire if the appropriate conditions are provided for him, and his learning abilities increase [1] by stimulating and activating his neurons by forming the largest number of nerve connections with other neurons, the brain is a complex adaptive system unique is able to handle more than one task in parallel, and brain-based learning confirms that the difference between learners in learning and thinking patterns is due to their reliance on one of the two hemispheres of the brain to receive and process information, and that the learner can learn better when both hemispheres of the brain participate in processing, storing and retrieving information, and that the researcher agrees with this trend in the importance of integrated brain-based learning and not as parts because [2] it works to develop thinking and increase cognitive achievement and this has been confirmed by many studies in the field of application of this type of learning.

Some teachers used to give lectures that were specified and chosen based on the desire and style of the teacher himself, as most teachers believe that the method that he feels is suitable for learning and fixing information is presented to learners because he believes it is suitable for all, forgetting their preferences, desires and preferences in the learning process, that what distinguishes [3] a successful and distinguished teacher is working and

walking according to educational concepts and theories and is keen to understand the aspects and influences of the educational process and applies measures that help learners to consolidate knowledge and provide them with the skills and targeted experiences, and designing educational units and providing appropriate means for his students, not what suits him as a teacher, and among these methods and models is the (4MAT) [(1)].

Badminton is one of the racket games that most of its skills are open-ocean, as each skill has its own movement program, and the main motor program for each skill contains a number of sub-motor programs that differ in (strength, speed, direction) and so there are many responses in the program the single kinesthetic for each of the badminton skills, and the motor program is an important and fundamental goal in kinetic learning to prepare the athlete and form situations similar to competition conditions that are characterized by a variety of speeds, times and distances, and here lies the importance of research in the researchers 'attempt to design an educational approach according to the steps of the (4MAT) model and according to the two hemispheres of the brain to learn some skills of the badminton [4].

Research problem: Through the presence of researchers in the field of teaching and training for most activities of racket games in general and playing badminton in a special way in the College of Physical Education and Sports Sciences - University of Karbala, it was found that most of the students in the second stage suffer from difficulty in performing badminton skills, knowing that it is one of the subjects taught at this stage , and through the extensive study and investigation on finding solutions to this problem, the researchers decided to rely on the application of a teaching model aimed at investing the energies of the brain and dealing with the tendencies and desires of the learner in a search that the educational curriculum addresses all learners, and this is what called the researchers to prepare a new educational curriculum with all its contents according to the characteristics of my two parts the brain using the (4MAT) model to learn some basic skills of badminton for female students.

Research objectives:

- Preparing an educational curriculum using the (4MAT) model according to the two hemispheres of the brain to learn some badminton skills.

- Identify the effectiveness of the educational curriculum using the (4MAT) model according to the two hemispheres of the brain in learning some badminton skills for female students.

Research hypothesis: The educational curriculum using the (4MAT) model according to the two hemispheres of the brain has a positive effect on learning some basic skills of badminton for female students.

Research fields:

The human field: Second stage female students of the College of Physical Education and Sports Sciences - Karbala University for the academic year 2018-2019.

Time field: from 1-11-2018 to 30-1-2019.

Spatial field: The closed hall in the College of Physical Education and Sports Sciences - Karbala University.

Theoretical studies:

The concept of the (4MAT) model [(2)]: This system was developed by Bernice McCarthy in 1987 and its name (4mat) is based on the individual learning style and on the way of thinking according to the left and right hemispheres of the brain. This system was known by a set of definitions, including the following:

- It is a practical system for communicating information in a way that fits all students' learning styles and allows them to practice and make good use of all learning materials during the educational units. The format system encouraged teachers to pay attention to (why) and how the learner learns and not just what he learns.

- It is an educational system that translates the concepts of learning styles into an educational strategy, running in a quadruple learning cycle of successive stages, namely (contemplative observation, conceptual crystallization, active experimentation, and tangible material experiences.

- An educational system based on motivating learners and mastering scientific concepts and their applications, and creative synthesis.

The (4MAT) model was developed by the scientist McCarthy, based on Kolb's classification of learning styles, and the four learning styles are based on different approaches to receiving and processing information. According to the learning cycle that begins with direct experience, then interpretation of experience, formation of special concepts, then acting towards experience, reaching a new experience.

The (4MAT) model and its relationship to the two hemispheres of the brain [3]:

Each style of learning is linked to the two sides of the brain, and the (4MAT) model is designed so that each learner is provided with his favorite task during each lesson, so it can be said that despite the brain being divided into two halves, they work in harmony and complementarity, as each performs its own functions with which it integrates with the other half, one of the assured assumptions according to the principle of individual differences is that learners vary between those who possess higher capabilities in the right hemisphere, and some of them possess higher capabilities in the left hemisphere or those who possess distinct abilities for both halves, and from here the educational curricula must be presented through their goals, scientific content and activities the methods of evaluating them satisfy the two hemispheres of the learner's brain, likewise, the teacher must use teaching methods and strategies that work to develop and refine the capabilities of the spherical hemispheres. The teacher must take into account that the brain is not affected or developed by meaningless information, but rather develops according to an integrated system over time, and that its nature depends on the link between the old and the modern, and that it is genetically characterized as social and cooperative. In light of the foregoing, the term learning and thinking styles appeared, meaning the use of one or both of the spherical hemispheres of the right or left brain, or both (integrated) in the treatment of mental or behavioral processes, after applying the cerebral mastery of the two hemispheres of the brain over its learning role in the (4MAT) model, we have an eight-stage model, as follows:

The (4MAT) form steps [4]:

First Stage : Connect (First Quarter / Right Side):

This step is designed to encourage learners to acquire sensory experiences that lead them to research their previous experiences and knowledge, and in it a collective interactive dialogue takes place that leads to a link between the knowledge and beliefs of the learners and what teachers intend to acquire them from knowledge, and there are no correct answers in this dialogue, the teacher should encourage learners to diversify ideas, dialogue, participation, present concepts from a personal perspective, and attract their attention, depending on solving problems, starting with similar situations among learners, building in light of their previous knowledge and facilitating the work of cooperative teams, and at this stage, encouragement, collective participation and idea generation can be evaluated.

Second stage : Attend (First Quarter / Left Side):

In this stage, the participation and dialogue that took place in the first stage is judged (evaluation), and in it the teacher also encourages the learners to reflect on their knowledge and personal experiences to decide whether they are compatible with the experiences presented to them, and here either the merging or lack thereof between the new knowledge and what they have of Experiences in their cognitive structure.

Third Stage : Image (Second Quarter / Right Side):

This stage aims to expand the representation of meaning among the learners through integration with their personal experiences to understand the concept, and to link the relationship between what the learner knows and what he has reached, using media other than reading and writing to convey knowledge to learners such as literature, music, and movement, and assisting them in shifting to a broader view of the concept, deepening the connection between the concept and its relationship to the lives of the learners, and linking what they already know and what the experience has created, as well as assisting them in the contemplative production that merges between emotion and knowledge, and at this stage the efficiency of the learners is evaluated from their cognitive achievement and meditation.

Fourth Stage : Inform (Second Quarter / Left Side):

That the goal of this step is to integrate learners into

meaningful thinking, and to emphasize the analysis of concepts, facts, generalizations and theories, and that the proposals submitted to teachers towards their learners at this stage are (confirming that the concept is organized and original, and providing information in a sequential order so that continuity occurs, and pushing them towards important and distinctive details and not drowning them with a huge number of facts, and using various methods such as interactive lectures, texts, hosting speakers, films and visual aids whenever available), and at this stage the written or verbal lists that express the understanding of the learners are evaluated.

Fifth Stage : Application (Practice) (Third Quarter / Left Side):

The learner at this stage moves from the stage of acquiring and representing knowledge to applying what he has learned, and the main goal in it is reinforcement and treatment, and the teacher's role towards his learners is to provide them with manual activities that help them to apply and master, and to test their understanding of concepts through relevant materials such as worksheets and exercises and texts that contain problems and written work, and giving them the opportunity to practice new learning through multiple methods such as learning centers and play skills, setting high expectations for skill mastery, and using the concept of learning for mastery to determine if there is a need for re-teaching and how it will be implemented, and at this stage the quality of learners' work is evaluated based on short questions .

Sixth Stage: Extend (Third Quarter / Right Side):

This step confirms John Dewey's ideas about learners as students as scientists, and in this step the learner tests the limits and contradictions of his understanding, and the teacher's role is to encourage learners to develop their practical ideas and their personal levels, and to encourage non-skilled learners to present their ideas and provide multiple experiences for learners so that they can plan Individually for their learning, learners are also encouraged to produce personal applications that are consistent with the learned experiences, and at this stage the behavior and treatment of learners while carrying out the tasks are evaluated in light of their goodness. Finding information that is not ready within the framework of the study - giving an opportunity to the learner to design suggested personal discoveries - giving multiple options to the learner that enable him to be creative - helping the learner to organize his learning in a personal way).

Seventh stage : Refine (Fourth Quarter / Left side):

At this stage, the learner is required to locate new experiences and knowledge from his point of view, and ideas must be refined and contradictions confronted, and the overarching goal of this stage is to evaluate the benefit and application, the proposals presented to teachers are represented in (the necessity to provide feedback and guidance to learners' plans, encourage them and help them to be responsible for their learning and help them to analyze their use of meaningful learning and help them convert mistakes into real learning opportunities), and the evaluation at this stage is through the ability and desire of learners to edit and purify and rework and the extent of analysis and completeness of their work.

Eighth Stage : Perform (Fourth Quarter / Right Side):

The essence of this stage is integration, celebration and closure, and at this stage the learner returns to where he started, and the ultimate goal of learners in this stage is to do things themselves and share what they have done with others, among the proposals presented to teachers are (encouraging them to teach, learn, and participate with others, create a classroom environment that includes various stimuli, give them the opportunity to share new learning, and make learning available to a large number of society by sharing their writings with other classes and displaying their work through the school), and in these the stage assesses the ability of the learners to write reports and present what they have learned and the quality of the final product to them.

Basic skills of badminton:

The main duty of the coach is to learn his players to perform the basic skills of the game from their understanding and awareness of the skills and to reach them to the best technical performance. The following are the basic skills in the game of badminton:

Serve: Serving is the first key to the beginning of the game or after scoring the point, which is a stroke used to place the shuttlecock in play, so that the shuttlecock is sent to the place where it is difficult for the opponent

to force it back or score a point directly from it, and the service is distinguished without the rest of the strokes as the only one that the player can train on. on his own, the player must perform the serve strikes with a great deal of concentration, stability, balance and accuracy. Therefore, the player must avoid tension and nervous tension and relax relatively to his performance[5]. Peter Roper (1995) believes that there are two main types of serve:

- The long high serve.
- The short low serve.

Forehand stroke: It is the basic skill of any of the different racquet games and the most used in training and is performed in many ways, and the front hit is that which is performed on the front face of the racket in response to the shuttlecock coming to the right from the player's body at most (not the left-hand side) and its goal is according to the shape of the front hit[6].

- stroke over the front head.
- The drive front stroke.
- The stroke net front.

Backhand stroke: It is one of the basic skills of any racquet game, as in the front kick, it is performed in many ways and forms according to the positions of the game [7]. And it is a set of strikes that are taken with the racket from the opposite side of the striking arm, so if the player is right-handed then the feathers coming to his left must be played with the back of the racket (the outer surface) and on the left side, and it is considered one of the important and difficult strikes as it takes some time to master it.

- Clear backhand stroke.
- Drive front stroke.
- The drop net stroke back.

Research methodology and field procedures:

Research Methodology:

The researchers used the experimental approach by designing (one group with pre and post- test) in order to suit the nature of the problem and achieve the research objectives.

Community and sample research:

The sample of the research represented the entire research community, they are the 24 students of the second stage in the College of Physical Education and Sports Sciences, this means that the researchers used the method of comprehensive enumeration of all members of society.

Devices, tools and methods used in the research:

Tools and devices used:

- A terrace at a height of 1 m, counting (2) homemade.
- Badminton court, number (2).
- 10 homemade signs.
- Two (2) Chinese-made electronic stopwatch.
- (14) badminton rackets, type (Yonex).
- Badminton balls, count (40) plastic boxes and natural feathers (Yonex).
- 15 hammer strings .
- 10 yonex jumping ropes.
- Plastic columns at heights (170-180 cm cm).
- (2 m) count (2) for each size.
- Homemade ropes of 10 m in length

Skills under study:

Researchers have studied the basic skills of badminton: Front and back stroke skill and front drop skill.

Determination tests used and their description:

A- Clear Forehand stroke test [8] :

Name of the test: Clear forehand stroke.

Test purpose: To measure the accuracy of the clear forehand stroke performance.

Required tools: feather bats, rope, additional legs (244 cm) high, information form, playground test design

scheme.

Performance description:

- After the test is explained to the players, the testers are given a suitable time to warm up and then each player is given (5) experimental attempts.

- The player stands in the area marked (x).

- At the moment the coach sends the shuttlecock, he can move if this move is necessary for the success of the attempt, and he must hit the shuttlecock with a forward sweep (from above the head) to send it over the net and then the rope towards the area specified by degrees.

- The player gives (12) attempts only for the best (10) attempts.

Performance evaluation:

- The player gives (3) points if the shuttlecock falls in the specified area at a distance of (50 cm) after the back line of the court.

- The player gives (5) points in case the shuttlecock falls in the specified area with a distance of (76 cm) between the back line of the court and the beginning of the distant doubles serve line.

- The player gives (4) points if the blade falls in the specified area at a distance of (70 cm) after the distant doubles serve line.

- The player is given two points in case the feather falls in the specified area with a distance of (124 cm) that starts from the end of point (4) and ends with an imaginary line extending down the rope.

- The highest score is given in case the shuttlecock falls on a line between two points, and no point is given for the shuttlecock that falls outside the boundaries of the playing field or gets stuck on the net.

- The upper limit of the points that the player can score is (50) points.

B- Clear back stroke test[9]:

Test name: Clear back stroke.

Test purpose: To measure the accuracy of clear back stroke performance.

Required tools: Badminton court, badminton rackets, tape, tape measure, information form, cone.

Performance description:

- After the test is explained to the players, the testers are given a suitable time to warm up, then each player is given (5) experimental attempts.

- The players stands in the area marked (x).

- The trainer serves so that it reaches the left side of the player (if he is holding his racket with the right arm and vice versa) so that he can hit it with a back stroke.

- The player gives (12) attempts, and the best (10) attempts are calculated for it.

- The player can move to make the attempt successful and can also leave any feather whose response does not result in a successful attempt, and if the trainer believes that its sending is incorrect, he calls (re).

- The maximum number of points that the player can score is (40) points.

Performance evaluation :

- The player gives (1) a point in case the blade falls in the specified area with a distance of (198 cm) extending from the center line of the square under the net to the near serve line.

- The player is given two and three points in case the blade falls in the specified area at a distance of (198 cm), which starts from the near serve line and ends with the distant even line.

- The player gives (4) points in case the feather falls in the specified area with a distance of (76 cm) extending beyond the end line of the arena.

- The player is given two points in case the blade falls in the specified area at a distance of (80 cm) which separates the distant doubles serve line from the remote single serve line.

- The shuttlecock that is attached to the net or outside the boundaries of the playing field is not given

any point.

The drop net stroke back test :

Purpose of the test: To measure the skill of the drop net stroke back.

Required tools: Badminton racket, cone, playground test design diagra.

Description of the performance: After the test is explained to the testers, each player is given (5) experimental attempts to warm up, after which the player stands in the place specified for him (x) and at a distance of 5 m from the net and is holding his racket by holding him in front to receive the shuttlecock serve to him from the opposite court to cross the net trying to drop it in the area The highest grade and listed (3, 2, 1).

Performance evaluation:

- The player makes (12) attempts and gets his (10) best attempts.
- The score is given according to where the shuttlecock.
- The shuttlecock that falls on a line between two regions gives the highest score.
- The highest points a player can get is (30) points.

Exploratory experience:

- Date of the experiment: on 1/11/2019 at ten o'clock morning.
- The place of the experiment: the sports hall at the University of Karbala.
- The sample: The survey sample consisted of the second stage students and the same research sample, which is (6) students

Objectives of the experiment:

- The auxiliary team knows the nature of work and its requirements.
- Avoiding the obstacles that face the researcher during the execution of the tests.
- Knowing the approximate time taken for each test and the time taken to conduct the tests.
- Ensure the scientific parameters of the tests.
- Knowing the appropriate time for the training unit.
- Identify the suitability of exercises for the sample.

Pre-tests:

Researchers conducted pre-tests on 7/11/2019 in the closed hall of the Faculty of Physical Education and Sports Sciences - Karbala University at ten in the morning for the skills studied.

The main experience:

Through the field experience of researchers in the field of badminton, and depending on Arab and foreign sources, an educational curriculum was prepared according to the steps of the (4MAT) model and according to the idea of the two hemispheres of the brain, where the exercises were organized to suit the members of the sample and their educational level, depending on the principle of changing the skill forms of one skill for the purpose of building a general movement program, as the curriculum included 7 weeks every week as an educational unit, and the time of the educational unit was 90 minutes.

Post – tests:

Researchers conducted the post- tests on 29/12/2019 in the closed hall of the Faculty of Physical Education and Sports Sciences - Karbala University at ten in the morning, during which the basic skills of badminton that were the subject of the study were tested.

Statistical means:

The statistical package (spss) [10] was used in the analysis of the research data as follows:

Presentation and analysis of the pre and post test results of the two skill test research groups:

After collecting the pre and post data for the skill tests of the experimental group, and for the purpose of describing the results of the sample members, the researchers processed the data statistically using

measures of central tendency and measures of dispersion, and for the purpose of knowing the significant differences between the pre and post- tests, the researchers used the following methods.

Table (1) Shows the mean, standard deviation, and t-values calculated for the pre and post -test for experimental group.

Skill	Pre-test		Post-test		T test value	Sig level	Sig Type
	Mean	Std. deviation	Mean	Std. deviation			
Clear fronthand stroke	19.3	2.35	31.2	2.2	6.22	0.02	Sig
Clear backhand stroke	16.8	2.25	26.9	3.53	7.30	0.01	Sig
Drop net stroke back	19.1	1.35	24.5	1.43	6.89	0.01	Sig

The degree of freedom = 24 at the level of significance (0.05).

Discussing the results of the pre and the post- test for the research groups in the skill tests:

Researchers attribute the difference between the pre-test and the post-test of the experimental and control group to the exercises used in the educational units and the use of learning methods and training methods that are commensurate with the type of the skill learned, the explanation, presentation, giving guidance, instructions, and feedback had a great impact on the development of the two groups in the post-test, as the correct organization of the exercises used, which took all skill forms and different and different parameters (strength - speed - distance - time) helped the members of the experimental group to form programs varied mobility of one skill, which increases the chances of success of the skill when performing it on the playground, as the teacher or trainer is the designer of the learning environment, as he is the one who creates educational systems, achieves the goals of the educational unit and prepares educational situations[11], the organization of educational units according to the steps of the format model, as it works to provide a fertile learning environment through the use of colored feathers and educational rackets of different weights and different heights for the network, providing safe educational places, as well as providing good ventilation in the workplace of learners in addition to the need to pay attention to feedback and increase stimuli in terms display posters (pictures) of movement parts and music use, as this approach is considered one of the most effective means of highlighting the energies, maintaining the level and achieving the goals. The reason for the development of the experimental group is due to the educational curriculum that was appropriate in terms of the theory of brain-based learning that relied on providing a fertile educational environment, providing suitable devices and tools, using musical tunes and presenting Explanatory films, as well as the use of information processing in linking theoretical and practical aspects, as this theory had a positive effect on progress in learning some basic skills in tennis, and that each student, by virtue of his cognitive style, is able to perform certain tasks and act positively to different stimuli produced by the educational environment.

Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached:

4- The educational curriculum, using the (4MAT) model according to the two hemispheres of the brain, contributed to learning some basic skills of badminton for female students.

5- The educational curriculum, using the (4MAT) model, contributed to activating the energies of the brain, investing most of its energies, and stimulating excitement and pleasure.

6- The educational curriculum, using the (4MAT) model according to the two hemispheres of the brain, contributed to the superiority of the experimental group

in the post-tests, which contributed to learning the studied skills clearly.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

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Effect of Dual-Chamber Pacemakers with Various Atrioventricular Intervals on Systolic Function, Diastolic Function, and Plasma Level of NT-pro BNP in a Sample of Iraqi Patients

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Abstract

Background: The use of dual-chambers (DDD) pacemakers is steadily increasing a year after year and one of the programmable settings for these pacemakers is the duration of the atrioventricular interval (AVI). AVI manipulation is justified for a variety of reasons including better hemodynamic stability and extending the battery life of the pacemaker. Since plasma NT-proBNP level reflects the myocardial wall tension, we hypothesized that different durations of AVI may have an effect on cardiomoycytes stretch and myocardial wall tension and hence an effect on plasma NT-proBNP level. So we tried to use plasma NT-proBNP as a marker for that strain, if present. Also, we tried to explore the effect of DDD pacemakers on both the systolic and the diastolic functions of the heart using conventional echocardiographic parameters.

Methods: 52 patients with permanent dual-chambers pacemaker were recruited. We exclude those with heart failure, renal impairment, and major valvular or coronary vessels disease. All patients had a thorough medical history and examination, ECG, echocardiography (2-D, M-mode, and Doppler) as well as blood sampling for NT-proBNP. Based on the shape of their E-A waves by pulsed-wave Doppler of transmitral flow, they were grouped in 3 categories. Group-A included those with truncated A-wave, group-B comprised those with separated E and A waves, and group-C involved those with fused E-A waves with or without diastolic mitral regurgitation.

Results: there was statistically significant difference in the duration of AVI among the 3 groups ($p < 0.001$) with patients having fused E-A waves recorded the longest AVIs. Plasma NT-proBNP levels were significantly higher when E-A waves were fused ($p < 0.001$). The systolic parameters such as the cardiac output, the stroke volume and the ejection fraction were higher when there was neither A-wave truncation nor E-A wave fusion ($p < 0.001$). All patients showed evidences of diastolic dysfunction.

Conclusion: despite the fact that DDD pacemakers are more physiological mode of pacing, nevertheless they induce some grade of diastolic heart failure. Manipulating the AVI into longer than the device`s default values should be carefully judged since it is associated with higher levels of plasma NT-proBNP levels that reflect cardiac wall tension and strain which in turn may progresses in future into another cardiac problems such as atrial fibrillation.

Keywords: *DDD pacemaker, NT-proBNP, atrioventricular interval (AVI), A-wave truncation.*

Introduction

Sinoatrial nodal dysfunction (SND) and atrioventricular conductivity issues like atrioventricular block (AVB) are regarded the leading causes of bradyarrhythmia worldwide [(1)]. One of the treatment options for such arrhythmia is the use of dual-chamber pacemaker (DDD) as it is regarded as a

more physiological pacing modality that bear distinct resemblance to the normal cardiac electrophysiology [2, 3]. DDD pacemaker is indicated for patients with complete AVB with or without SND and at the same time, they have no significant comorbidities and are free from permanent atrial fibrillation [4, 5]. Owing to the fact that most patients with SND are liable for life-threatening bradycardia, accordingly the use of dual-chamber pacing is increasingly replacing single-chamber pacing [6, 7]. That is why this research is focused on the DDD pacemakers.

The atrioventricular interval (AVI) of a pacemaker is defined as the time taken from the start of sensed or paced atrial event to the start of ventricular sensed or paced event [8]. The duration of the AVI has a profound impact on the hemodynamic status, when optimally programmed, it will allow the maximum achievable time for the left ventricle to fill up with blood prior to contracting [9]. The AVI may set too short as in (figure-1 A) and in this case, the left ventricle may contracts before the left atrium completes its participation to left ventricular filling and consequently the active atrial contribution to ventricular filling may terminate abruptly which is reflected as an A-wave truncation on pulsed-wave Doppler of transmitral flow [10]. On the other hand, the AVI may be too long so that the left ventricle completes its filling while the mitral valve still opens leading to E-A wave fusion with or without some degree of diastolic mitral regurge as in (figure-1 C) [11].

N-terminal pro-brain natriuretic peptide (NT-proBNP) has been established as a biomarker for heart failure according to the European Society of Cardiology (ESC) guidelines since 2005 [12]. NT-proBNP is produced in both the atria and the ventricles where the production is initiated by myocyte stretch with increased wall stress in response to volume expansion or pressure overload. Ischemia also contributes to its release, though it remains unclear to what extent this is caused by the slocal myocardial stunning or ischemia per se [13]. Conventional right ventricular pacing, by inducing a condition known as intraventricular dyssynchrony, may result in altering the phase and magnitude of the mechanical strain of both ventricles, the main stimulus for NT-proBNP production [14].

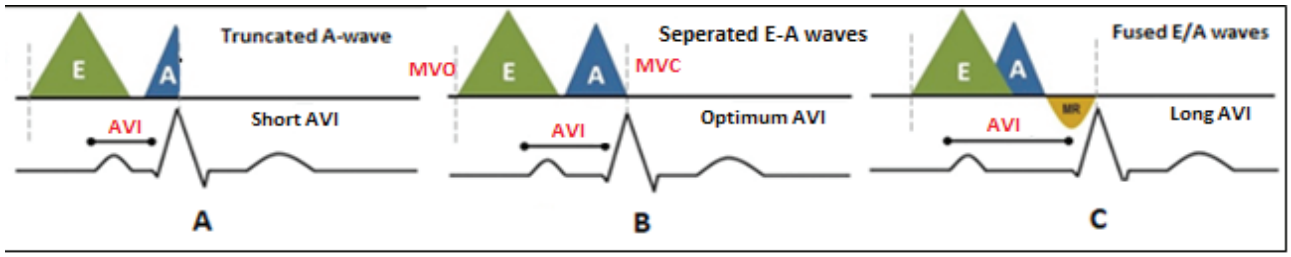

Figure 1: the effect of various durations of atrioventricular interval (AVI) on transmitral pulsed-wave Doppler flow. In (A) the AVI is relatively short so that the mitral valve closes before the completion of atrial contraction resulting in truncation of A-wave. In (B) the AVI is ideal by permitting completion of atrial contraction prior to the closure of the mitral valve. Whereas in (C) the AVI is relatively long resulting in pre-timed atrial contraction so that the A-wave fuses with the preceding E-wave. AV = atrioventricular, MR = mitral regurge, MVO = mitral valve open, MVC = mitral valve close. (Courtesy of ESC).

Aim of the Study

To study the effect of various AVI durations of DDD pacemakers on cardiac wall strain using NT-proBNP as a marker for that strain if present as well as using some recognizable echocardiographic parameters for assessing systolic and diastolic functions.

Methods

Study population

This research was approved by the medical research ethical committee at Karbala directorate of health/Iraq and all participants enrolled in this study gave informed consent after elaborative explanation for their role in the study. A cross-sectional study design was used. The study

was conducted at Karbala center for catheterization and cardiac surgery at Al-Hussain teaching center/Karbala/ Iraq for the period from June 2018 to November 2019. It included 52 participants with dual-chamber pacemakers implanted for different medical indications including SND and complete AVB and had their pacemakers from different manufacturers (Medtronic, Biotronic, and Saint Jude`s).

Inclusion criteria were an age of greater than 18, having a permanently implanted DDD pacemaker, and had no recent changes in their cardiovascular medication. Exclusion criteria were symptoms of overt heart failure or echocardiographic evidence of depressed LV function (LVEF% less than 45 %), documented atrial fibrillation at the time of study, impaired renal function (serum creatinine more than 2 mg/dl), chronic obstructive airway disease, dilated or hypertrophic cardiomyopathy, significant valvular disease, and any patient with complete fusion of early atrial (A-wave) and late atrial (E-wave) where both waves can not be discriminated from each other by pulsed-wave Doppler of transmitral flow, any patient with poor echocardiographic image quality, and any significant acute coronary syndrome within the past 4 weeks documented by angiography to involve more than two vessels or a history of myocardial infarction.

For each participant, a careful medical history and thorough physical examination were conducted with special emphasis on the indication of pacing, comorbidities such as hypertension, diabetes, ischemic heart disease (IHD), current medications, symptoms of pacemaker syndrome which is defined as a constellation of symptoms that build up after the implantation of a permanent pacemaker in which the patient presents with progressive symptoms of congestive heart failure (CHF) that are mainly due to atrioventricular dyssynchrony [15]. At the pacemaker clinic, each patient was routinely examined by the use of a corresponding programmer specified by the manufacturer of the pacemaker, a wand (programmer`s head) is placed over the pacemaker and after allowing time for interrogation between the programmer and the pacemaker, the following parameters were recorded, the atrioventricular interval (AVI) and % ventricular pace.

Each participant was asked to lie down in order to record a baseline 12-lead ECG using (WELCH ALLYN CP 50 ECG unit/ USA) to check for the rate, rhythm and if there were any P-wave changes, R-wave changes, Q-wave changes, PR segment, ST segment changes or bundle branch block and to rule out atrial fibrillation. While still in decubitus or left lateral position, an echocardiographic assessment with concomitant ECG monitoring was done. All echocardiographic measurements were achieved by a single highly skilled and qualified operator to reduce the possibility of inter-personal variation. The available echocardiographic machine was (Vivid 7, GE Medical Systems, Horton, Norway).

By using M-mode, an apical 4-chamber view was obtained to visualize the mitral valve, then by switching to pulsed-wave Doppler (PWD), analysis of the transmitral blood flow was done where the cursor is carefully positioned at the tips of the mitral valve leaflets. The Doppler signal represented the velocity of blood flow during passive left ventricular filling and was displayed as an E-wave (figure-2). A second signal was gained that represented the active atrial participation to the ventricular filling that was displayed as an A-wave (figure-2). The transmitral Doppler measurements were done on at least 3 consecutive cardiac cycles with a sweep rate of 100 mm/second.

The echocardiographic study included the assessment of left ventricular systolic function (LVSF) and left ventricular diastolic function (LVDF). For the LVSF the following parameters were obtained: left ventricular ejection fraction (LVEF%), stroke volume (SV) and cardiac output (CO). Based on the American Society of Echocardiography and the European Association of Cardiovascular Imaging (ASE/EACVI), the diastolic function was assessed using the following parameters: the peak E- wave velocity, the peak A-wave velocity, and the E/A ratio where after obtaining an apical four-chamber view the echocardiography machine ws switched to color flow imaging in order to achieve the maximal allowable alignment of PWD with the direction of blood flow [16]. A sample volume of 2 mm in axial size was taken in PWD between the tips of both mitral valve leaflets. A wall filter of low setting (100–200 MHz) was used with a low signal gain. Obtained waveforms showing neither feathering

nor spikes were accredited for the study. The following values were taken as a reference: normal E/A ratio 0.75-1.5, a peak mitral E-wave velocity of 53-105 cm/sec., and a peak mitral A-wave velocity of 26-73 cm/sec [17].

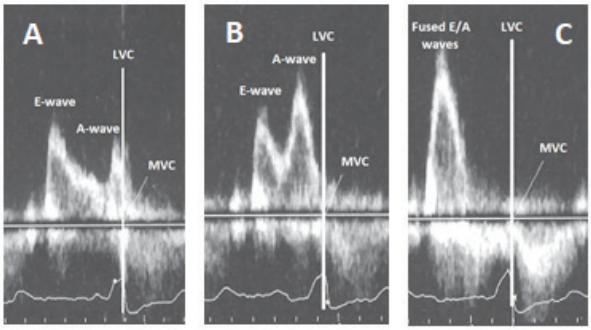

Figure -2: E-wave and A-wave morphology at different AVIs. A) A-wave truncation after short AVI. B) Separated E and A waves with well-timed AVI. C) Fused E and A waves after relatively long AVI. LVC = left ventricular contraction, MVC = mitral valve closure.

Based on the shape of E- wave and A- wave after PWD of transmitral flow, patients were divided into the following three groups:

- **Group-A:** included 17 patients with an A-wave truncation on PWD of transmitral flow as in figure (1-A).

- **Group-B:** involved 19 patients with clearly separated E and A waves on PWD of transmitral flow and with no diastolic mitral regurgitation as in figure (1-B).

- **Group-C:** this involved 16 patients with fused E and A waves on PWD of transmitral flow with or without diastolic mitral regurgitation as in figure (1-C).

Plasma level of NT-pro BNP estimation:

Using commercially available kits (LSBio, Lifespan Biscience Inc., Seatle, USA) NT-proBNP levels in plasma from all patients were measured by enzyme-linked immunosorbent assay. Venous blood sample of 3 ml collected from the antecubital vein at the end of the echocardiographic exam to ensure that at least 30 minutes had passed and the patient is recumbent. Blood samples were collected in tubes containing potassium EDTA. After centrifuging samples at 3000 rpm (15ºC for 10 min) the separated plasma was assayed immediately. A level of less than 125 pg/mL was used as a normal cutoff for patients aged 0-74 years old [18].

Statistical Analysis

Statistical analyses were performed with the IBM® SPSS®/PC software package (version 25.0, SPSS Inc., Chicago, IL, USA). Results are presented as means + SD. A p value < 0.05 was considered to indicate statistical significance. Comparisons were made with the use of one-way ANOVA followed by post-hoc test.

Results

The baseline data for participants are presented in (Table-1). Studied groups had their age ranging from 47-63 years with a mean age of 49.81 ± 12.38. Male participants comprised 68 % (38 male). The results revealed a non-significant difference among groups with respect to age (P= 0.27).

Table 1: baseline features for the participants in all groups.

	Group-A	Group-B	Group-C
Number of patients	17	19	16
Age	53.27 ± 3.4	54.06 ± 2.1	51.52 ± 3.8
Male/ female	9/6	10/5	8/7
Pacemaker type			
- Biotronic	0	5	1
- Medtronic	11	12	5
- St. Jude's	6	2	10

Cont... **Table 1: baseline features for the participants in all groups.**

Hypertension Diabetes IHD	9 3 5	11 2 6	9 3 4
SND	13	7	14
Complete AV block	5	12	2
IHD = ischemic heart disease, SND = sinus node disease, AV = Atrioventricular			

Table 2: results of different parameters for the three groups with among groups and in between groups' statistical significance. All data are expressed as mean and standard deviation with p value level of significance < 0.05.

	Group-A	Group-B	Group-C	Among groups P value	Between groups P value		
					A vs. B	A vs. C	B vs. C
AVI (milliseconds)	134.38 ± 9.67	135.12 ± 9.02	214.68 ± 19.66	< 0.001	0.985	< 0.001	< 0.001
EF%	55.4 ± 5.72	59.08 ± 5.33	58.2 ± 3.2	0. 028	0.028	0.12	0.805
C.O. (L/min)	5.69 ± 0.67	5.84 ± 0.73	5.53 ± 0.11	0.012	0.012	0.416	0.22
S.V. (ml)	55.52 ± 3.6	66.92 ±5.3	53.96 ± 3.6	< 0.001	< 0.001	0.399	< 0.001
% VP	89.99 %	87.73 %	40.21 %	< 0.001	0.631	< 0.001	< 0.001
MV E/A ratio	0.99 ± 0.013	0.57 ± 0.064	0.34 ± 0.072	< 0.001	<0.001	<0.001	<0.001
Peak E-wave velocity (cm/ sec)	0.62 ± 0.11	0.54 ± 0.09	0.45 ± 0.10	< 0.001	<0.001	<0.001	<0.001
Peak A-wave velocity (cm/ sec)	0.70 ± 0.12	0.78 ± 0.12	0.88 ± 0.11	< 0.001	<0.001	<0.001	<0.001
NT pro-BNP (pg/ml)	210.2 ± 44.17	217.48 ± 45.37	335.16 ± 72.33	< 0.001	0.085	<0.001	<0.001
AVI = atrio-ventricular interval, EF% = ejection fraction, CO = cardiac output, SV = stroke volume, %VP = percent of ventricular pace events, MV = mitral valve, (L/min) = liter/ minute, ml = milliliter, (cm/sec) = centimeter/ second, (pg/ml) = picogram/ milliliter.							

tr

AV interval:

A one-way ANOVA was conducted to find out if there is statistically significant difference in the duration of AVI among the three groups. As shown in table-2, there was a significant difference in AVI [F(2,72)=284.15, p <0.001] among the three groups. Post hoc comparisons using the Tukey`s test were carried out. No significant difference in AVI duration was observed between groups A and B but, group C patients have had a significantly higher AVI duration compared to those of group A and group B (p <0.001).

Left ventricular ejection fraction (LVEF %):

Table-2 shows that analysis of variance revealed a marginal difference in LVEF% [F(2,72)=3.76, p = 0. 28] among the three groups. There was a significant difference between groups A and B (p = 0.28). There was no significant difference between group B and C (p = 0.12).

Plasma level of NT-proBNP:

A significant difference in NT-proBNP level [F(2,72)=39.93, p <0.001] among the three groups was found as shown in table-2. There was a significant difference between groups C and A (p <0.001). Also, there was a significant difference between group C and B (p <0.001). There is a moderate positive correlation between AVI duration and plasma NT pro-BNP level, r (34) = 0.34, P-Value < 0.024, as shown in (figure-3).

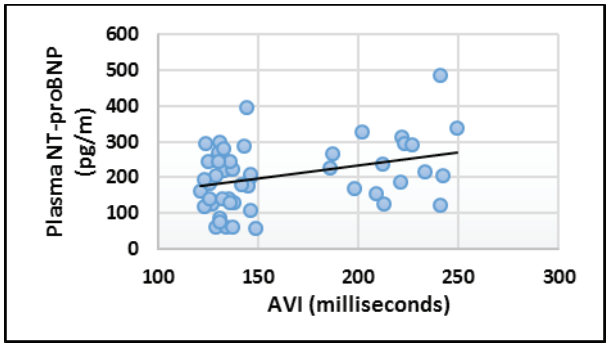

Figure 3: the moderate positive correlation between AVI and the plasma level of NT-proBNP.

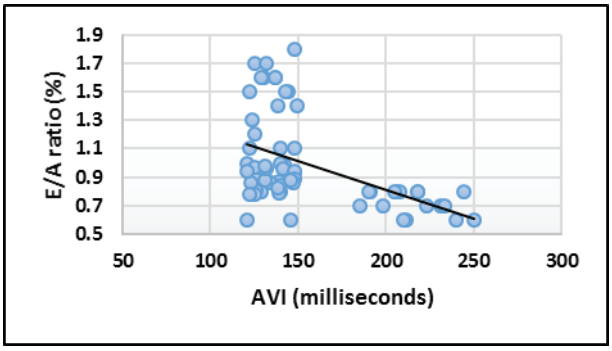

Figure 3: the moderate positive correlation between AVI and the plasma level of NT-proBNP.

Diastolic indices

Table-2 also shows that there is a statistically significant difference in the E/A ratio among the three groups [F(2,72)=150.72, p <0.001]. Post hoc comparisons using the Tukey`s test were carried out. A significant difference in E/A ratio was observed between groups A and B (p <0.001). Also, group-A had significantly higher E/A ratio compared to group-C (p <0.001). Group-B has significantly higher E/A ratio when compared to group-C (p <0.001). The E/A ratio was negatively correlated with the duration of AVI, r(-0.77) = -0.37, p < 0.001 as shown in (figure-4).

Discussion

The results of this study revealed that all patients had their NT-proBNP level above the cutoff point of 125 pg/ml and that plasma NT-proBNP was positively correlated with AVI. Right ventricular pacing (RVP) is known to induce a condition commonly referred to as interventricular dyssynchrony in which the right ventricle contracts before the left ventricle producing a situation similar to that encountered in left bundle branch block (LBBB) [19]. In RVP, there will be an erratic contraction of right ventricular myocardial fibers leading to uneven stretching of different segments of the right ventricle that in turn will affect the closely bound left ventricular function by resulting in stressful contractility [20, 21]. This in part probably explains why such elevated NT-proBNP levels are encountered in a pacemaker patients [22]. Naegeli *et al* had documented such an effect of a pacemaker on plasma NT-proBNP level although the rise was more with single-chamber pacing rather than dual-chamber pacing [23]. Results revealed that patients with longer AVIs had higher peptide levels. One probable explanation is that according to the Frank-Starling principle of the heart, the more preload the more ejection fraction and hence the cardiac output [24]. Since the AVI duration is one of the different factors upon which the preload relies upon, therefore, it seems logical to infer that the longer the interval the more time for the left ventricular diastolic filling and so more stroke volume. Therefore, any increase in the duration of AVI will lead to increased stretching of left ventricular wall and thus increasing the stimulus for NT pro-BNP release [25, 26]. It was found that increasing the AVI duration in hypertensive patients by giving beta-blockers will result in increased NT-proBNP level [27, 28].

A relatively long AVI will make the left ventricular pressure to rise beyond that of the left atrium resulting in a retrograde blood flow across the mitral valve (i. e. diastolic mitral regurgitation) which in turn increases the left atrial filling pressure (LAFP). Such an increase in the LAFP will cause a further volume overload and stretching

on the left atrial wall and by doing so, it increases the stimulus for peptide production and release from the atrial as well as the ventricular myocardium. The latter finding is consistent with that of some researchers where increased atrial stretch secondary to mitral regurge was found to augment NT-proBNP release [29, 30]

AVI

There was no statistically significant difference in AVI duration between patients of group-A and those of group-B, yet, patients of group-A showed truncated A-wave on transmitral PWD (figure 2-A). These patients were most likely suffering from some sort of intra-atrial or inter-atrial conduction delay. The ECG tracing of 14 out of 17 (82.35 %) patients of group-A showed an elements of such atrial conduction issues like a P-wave duration of more than 120 milliseconds, P-wave notching, and shortened PR segment. But in the remaining 3 patients (17.65 %), we weren't able to identify any signs of intra or inter-atrial conduction defects in their surface ECG trace outs. Such inability of detecting intra- or inter-atrial conduction defects was referred to by Chabrah *et al* whom concluded that patients with such conduction blocks may show a perfectly normal surface ECG [31].

Eicher *et al* have found that intra-atrial conduction defects tend to produce short-lived A-wave in transmitral PWD that appear to be truncated and they proposed naming that condition "an atrial dyssynchrony syndrome" [32]. The inter-atrial conduction time (IACT) by definition is the needed time for the electrical impulse arising from the SA-node to reach the left atrium causing atrial depolarization and contraction. An increase in the IACT may explain such truncated A-wave [33]. The left atrium normally depolarizes when the impulse reach it from the SA-node through the Bachmann`s bundle but in the case of DDD pacing, the impulse will not originate from the SA-node and it will not be conducted by the Bachmann`s bundle. Hence, it will take longer time since it will arise from the right atrial auricle and conducts through the atrial myocardial tissue [34]. Therefore, the left atrial systole will be delayed resulting in AV dyssynchrony and a non-physiological contraction of left atrium and ventricle leading into premature closure of the mitral valve and consequently truncated A-wave [35].

On the other hand patients with fused A/E waves had their AVI relatively longer than the nominal value of the device (i.e. 200 milliseconds and above). All of them had some functioning AV-nodal and AV bundle activity therefore, their pacemakers were intentionally programmed with long AV delay in order to reduce unnecessary right ventricular pacing. This was evident by the fact that such patients had a lower percentage of ventricular pace (40.21%) for group-C in comparison to (89.44% and 87.37%) for group-A and B respectively. Such an approach of having a long AV delay has its own virtues of reducing power consumption and extending the battery life of pacemaker [25]. Also to reduce the hemodynamic effects from AV dyssynchrony that arise from right ventricular pacing that is kin to the effect of LBBB [19].

Cardiac out-put (CO), stroke volume (SV), and left ventricular ejection fraction (LVEF%):

The CO, SV, and LVEF% showed a correlation with the duration of AVI but the relationship was curvilinear rather than a linear one where they were reduced in patients of group-A, increased in patients of group-B, then decreased again in patients of group-C. Atrial contribution to left ventricular filling is well documented in various literatures [36, 37]. Any factor that interfere with that atrial contribution will eventually leads to altered cardiac output [37]. One of these factors is the timing of mitral valve closure which in turn depends on the duration of AVI [38]. The synchronic relation between atrial and ventricular contraction is basically a temporal one where the atrium must have completed its contraction at least 0.008-0.2 milliseconds just before the closure of mitral valve [39]. On that base, patients with a relatively short AVIs will have an early mitral valve closure just before the completion of atrial kick which should participate by about 30-40% of diastolic filling of the ventricles [40]. Meisner *et al* reported a drop in CO when AVI of a pacemaker is programmed with values lower than optimum [41]. The same outcome was reported by Nielsen *et al* [42].

Our results were consistent with those of several other studies in which an AVI elongation resulted in reduced CO [30, 43, 44]. Patients with relatively longer AVIs had their mitral valve closed way ahead the onset of left ventricular contraction so that there will

be an excessive ventricular filling time that resulted in an increase in the left ventricular diastolic pressure on the account of left atrial pressure. Thus, some diastolic mitral regurgitation will occur that eventually increases the LAFP resulting in preload reduction and consequent reduced cardiac output.

Diastolic parameters:

Following the results of our study we concluded that active atrial contribution to diastolic ventricular filling increases more and more as the AVI is increased. This was reflected by the decrease in E/A ratio among patients group and progressively increasing height of A-wave. These results are consistent with those of Styliadis *et al* who reported similar findings of filling time and filling pattern after increasing AVI from 100 to 200 milliseconds [45]. D' Andrea et al. also concluded similar outcome by using tissue Doppler imaging (TDI) and Doppler echocardiography [46].

The results also revealed that passive atrial contribution to ventricular filling during diastole as represented by the E-wave is reduced as the interval increases. While, the active atrial participation which is represented by the A-wave increases as the AVI increases. When the interval increases, the E-wave get closer and closer to the subsequent A-wave so that at the end both waves will fuse together, as in (figure-2 C). The A-wave velocity start to increase little by little since it starts from the point where E-wave did not terminate and thus it takes extra momentum of flow and hence its increased velocity [17]. So, our study revealed that DDD pacemakers may inflict a diastolic dysfunction especially of grad-I. Our results are compatible with those of several other studies that reported RVP association with diastolic dysfunction in pacemaker patients with preserved LVEF% [47, 48].

Conclusions

Dual-chamber pacemaker patients with depressed left ventricular function and reduced ejection fraction may benefit from AVI prolongation but those with good left ventricular contractility may not get benefit from such prolongation. Instead they will have an elevated NT-proBNP level that might be associated with increased risk of cardiac morbidity and mortality.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Medicine Faculty of Medicine and all experiments were carried out in accordance with approved guidelines.

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Awareness of Primary Schools Teachers towards Mumps in Basrah City

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Abstract

Objectives: Assessment of primary schools teachers' Awareness towards mumps in Basra city

Methods: A descriptive study was conducted at (9) primary school at Al-Seraghi sectors in BASRA City, from January 14th 2020 to March 12th 2020. A probability stratified random sample of (100) teachers who teach science subject was selected, and had at employment in the teaching field. A questionnaire format was used which was consisted of (2) parts. The overall number of the items included in the questionnaire were (57) items. The first part was related to the demographic data of the teachers, the second part (five sections) was concerned with teachers' knowledge about health promotion. Data were analyzed through the application of descriptive statistical analysis which included; frequency and percentage, and the application of inferential statistical analysis which included; mean of score, and Chi-square test.

Results: The study revealed that teachers have acquired good level of knowledge about mumps disease , medium about method ,symptoms, and control diseases was poor knowledge

Conclusions: the relationship between teachers' knowledge and their demographic characteristics, data analysis has showed that there is significant association between teachers' knowledge mumps except of years experiences.

Recommendations: The study recommends that there is a need for -oriented education program which can be designed, constructed and presented to primary school teachers with great emphasis on all aspects of steps mumps diseases . Furthermore, teacher can be nominated and encouraged to actively participate in education training sessions. Also, the importance of a collaborative work which can be initiated between the health Directorate of Education health promotion and the Directorate of primary teaching to create facilities for the implementation of health promotion oriented education program.

Keywords*: Assessment; Knowledge; Primary School Science Teacher; mumps.*

Introduction

Mumps, is a viral disease caused by the mumps virus, also called epidemic portosis, is a viral disease caused by the mumps virus [(1)]. Initial signs and symptoms often include fever, muscle aches, headache, and a feeling of fatigue, usually followed by painful swelling of one or both parotid salivary glands. Symptoms usually appear 16 to 18 days after exposure and resolve after 7 to 10 days. Symptoms in adults are generally more severe than in children. About one third of people have mild or no symptoms. Complications can include brain cover infections (15%), pancreatitis (4%), permanent deafness, and painful swelling of the testicles that rarely leads to infertility. Women can develop swelling of the ovaries, but this does not increase the risk of infertility [(2)] .

Mumps is primarily contagious and spreads rapidly between people who live in close contact [(1)] .

The virus is transmitted by respiratory droplets or direct contact with an infected person. Only humans get and spread the disease. People are infectious to each other from about seven days before the start of signs to about eight days after. Once an infection has run its

course, a person is typically immune for life. Reinfection is possible but the ensuing infection tends to be mild [3] .

The diagnosis is generally suspected due to parotid inflammation and can be confirmed by isolating the virus from a sample of the parotid duct [1] . Detection of IgM antibodies in blood is simple and can be helpful; however, it can be false negative in those who have been vaccinated.

Objectives of the Study

1-To find out the incidence of mumps disease among primary schools pupils.

2-To assess the teachers knowledge toward mumps disease in Al- Brash city.

3-Association between teachers knowledge and their socio-demographical characteristics such as region, gender, age group, level of education and years of their experience.

Methodology

Design of the study:

Descriptive, design of study was carried out assess the science teachers' knowledge toward mumps disease in primary school at AL-Seraghi sector

Setting of the study :

Setting of the study in primary schools in Basra city AL-Seraghi sector from January 14th 2020 to March 12th 2020 .

Sample of the study:

A stratified random sample of (100) teachers who teach science subjects and who were employed in education was selected.

Sample instrument (questionnaire)

An assessment tools was designed depending on review text books and related lectures and researches, it consisted of two part the first one socio-demographic information such (Age ,gender , education level, years of experience,Source of information and training courses .

The second part about teacher knowledge about mumps disease by 9 main items with 52 sub items , all the items were measured on scale of (30)indicates that the knowledge were persistent (yes). (2) indicates that of the knowledge as (unsure) and (1) indicates the absence of knowledge as (no).

Method of data collection

The data collected started on11th – 2018 through -1st-2019 . the data have been collected through the use of questionnaire and mean of interview teaching and direct observation the teacher knowledge toward mumps disease in primary school at AL- Seraghi sector in Basra.

Statistical data analysis: The data were analyzed by Admitted to the Program (SPSS), version 16 for the interpretation of the results. Descriptive data analysis application

- Frequency and percentage
- Mean & S.D
- Chi-Square
- A Likert scale was used in the questionnaire to Assessment of primary schools science teachers 'Awareness towards mumps .

Results

Table (1): Distribution of the study population according to certain socio-demographic characteristics (N=100) and association with knowledge of study sample

Age groups	**No**	**F %**	**Chi-Square**	**sig**	**P≤0.05**
29-9	6	6	.500	.006	S
30-390	31	31			
40-49	30	30			
≤50	33	33			
total	100	100%			
Mean ±SD=42.59±9.08					
Gander		**F %**	**Chi-Square**	**sig**	**P≤0.05**
Male	17	17	1.000	.000	HS
female	83	83			
Total	100	100%			
Level of education		**Chi-Square**		**sig**	**P≤0.05**
Institute	72	72	0.555	.000	HS
College	23	23			
Secondary	5	5			
Total					
Years of experience		**F %**	**Chi-Square**	**sig**	**P≤0.05**
1-9	21	21	1.000	.357	NS
10-19	14	14			
20-29	28	28			
30-39	37	37			
Source of information		**F %**	**Chi-Square**	**sig**	**P≤0.05**
Reading books	24	24	.134	.000	HS
Reading magazine	45	45			
Inter-Nate	6	6			
Training courses	25	25			

The majority of study sample lied in age (≤50) years old ,and majority of sample study was (female(83%) in institute of level education ,while most of them found in(20-29) years of experience at (28%),them knowledge source of information from reading magazine (45%).

Discussion

The result of table (1) conducted that the majority of sample study lied in age group (≤50)years old with Mean ±SD=42.59±9.08,and the majority of sample of study sample was (80%) female, and (72%) at level of education (institute),while most of them found in (30-39)years of experience at (37%),while about demonstrated the knowledge source for study sample ,the result showed the majority of study sample were (45%)sours Reading magazine. In same table found of distribution of the population studied according to some socio-demographic characteristics and association with knowledge of study sample present all items table were significant relationship by Chi-Square($p \leq 0.005$), except (years of experience) was found no significant.

These findings are consistent with the literature that focuses on enrolling teachers in training sessions to enhance their knowledge, skills, and awareness to keep them up-to-date on communicable disease control. Faraj (4) The researcher offers teachers the opportunity to register for training sessions to improve their knowledge and skills..

The teacher knowledge toward mumps disease was good grand all the items except number (2) was poor (1.8) which contain the name of viruses, so the sample study wanted more education about the general characteristic of virus and these result different from Al-Kafajy (5). The teacher knowledge about the methods of Mumps transfer most of them was Medium referral for grand mean ,therefore it may necessary to give the study sample many information about transmission method of this diseases (6). teacher knowledge about Mumps symptoms was Medium referral for grand mean , thus sample study must increase their information about the side effect was poor as a total ,so it may necessary teacher knowledge must increase about side effect of mumps infection (7). Regarding teacher knowledge about control of disease was Medium. therefore the sample study must increase their information about the control of mumps disease (8). while in study of Hussein et al (9) recommended the needs to teachers that everyone is responsible for prevention of the spread of a communicable disease such as oneself, parents, schools, community members. Apply such educational health programs in all Iraqi governorates primary schools to increase awareness about communicable diseases control among teachers.

Conclusion

1. The most socio-demographic characteristic significant with mean score knowledge except years' experience .

2. The teacher knowledge about meaning mumps disease was good

3. The teacher knowledge about method transfer mumps disease was medium

4. The teacher knowledge about symptoms mumps disease was medium.

5. The teacher knowledge about side effect mumps disease was poor.

6. The teacher knowledge about control this diseases in school was medium.

Recommendations:

1 . Increase the knowledge of sample study toward mumps disease by entering to workshops, education lectures and forums.

2. Increase the knowledge of sample study toward mumps disease especially about side effect and what effect on student in future life, and ways to eradicate the disease in primary schools .

3. Increase the vaccination programs and (allow-up of drop-outs of vaccination) in primary schools in seraghi scoter .

4. Doing further study to assess and evaluate the teacher knowledge in primary school covered all Basra area.

5. Furthermore, the study recommends the need for a focused educational program that can be designed, built and presented to elementary teachers with an emphasis on all aspects of mumps at multiple levels.

Additionally, teachers can be nominated and encouraged to actively participate in teacher training sessions. Likewise, the importance of collaborative work that can be initiated between the Directorate of Health Education and Health Promotion and the Directorate of Primary Education to create means for the implementation of the health promotion oriented educational program..

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing and all experiments were carried out in accordance with approved guidelines.

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Coronavirus (Covid_19) and its Relationship to the Activation of the Healthy Preventive Programs and Health Counseling Social, Theoretical and Analytical Study: in Medical Sociology

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Abstract

Although the deterioration in the health situation inherited from the outbreak of the Coronavirus (Covid_19) in general, the health, social and economic conditions, malnutrition, lack of cleanliness , lack of health protection and health resources, particularly with regard to public health and disease, the importance of health prevention and health counseling health advice has received a lot of attention by some to care for and protect people. Against the risks of spreading chronic epidemics as well as general communicable or non-communicable infectious diseases or potential physical, biological, organic, mental, psychological and other diseases, The Corona virus (Covid_19) is one of the most important coronary viruses, and it is a virus that was first discovered in (1960 AD), and this virus appeared on (December 31, 2019 AD) also in Wuhan Province in Hubei, central China. Due to the development of the virus and its spread in many developed and developing countries. The World Health Organization declared its international importance as a public health crisis, as the country worked to stop all domestic and foreign flights to control the outbreak.

Keywords: *Coronavirus (Covid_19), Preventive Programs, Health Counseling*

Introduction

Health prevention and health consultation are among the most important areas of sociology in general, and medical sociology and health care in particular, and in this regard, Coronavirus (Covid_19) formed an essential [1] material that topped the visual, audio and read media. While social media was One of the most common communication networks; Because this epidemic was known to many countries without differentiation in the level of scientific and technological progress, and it should be really noticed [2] that this epidemic combines the symptoms of influenza and SARS pneumonia, which brought to the whole world a state of utmost anxiety for the concerted scientific and technical efforts to try to limit the spread and development of this epidemic [3], Thus, the most important step that all countries began to take was to provide health prevention programs or health advice through civil society organizations in general. In particular, health care organizations.

First: The research problem:

The research problem is determined by the severity of the Coronavirus pandemic, as well as examining the health and psychological consequences of this disease, as health care institutions and organizations can no longer know or reveal the underlying or unknown causes of this disease, Thus there are many international opinions and reports concerning this disease recognized or identified by the World Health Organization. Coronavirus is regarded as one of the most dangerous diseases and epidemics that affects people with severe, chronic and fatal diseases.

Second: The importance of the research:

The importance of this research lies in the necessity to discover the influence of the spread of this virus and the accompanying steps represented by imposing a curfew and disrupting all issues of social life, as the protection of human being from diseases is one of the most important areas in achieving the interest and safety of the patient who is recovered from diseases and the

problems which he suffered from earlier.

Third: Research objectives:

The current research aims to identify the most important treatment and instruction methods from the Corona virus, as well as to know the most important methods and rules of health prevention and the importance of health counseling in limiting the spread of this epidemic among members of society.

Fourth: Identifying research concepts:

1. Corona virus (Covid-19):

Coronaviruses so named because of the outer edge of the cover proteins that looks like the crown (corona - in Latin) is a family of protein-DNA viruses that are usually pathogenic to mammals and birds and cause respiratory infections.

It is also known as a new strain of coronavirus, and its danger mainly lies in the fact that it infects the human respiratory system so far without final treatment, and it is also a large virus that remains on the surface for a long period of time, and from here it also remains in the air for a period of no more than three hours, and this period is sufficient for the virus to spread from one place to another if we do not follow prevention, proper leadership, activation of the health role or health advice (1).

2. Health prevention programs:

A- The definition of Programs :

Programs are defined as: those organized and dedicated efforts used to help individuals and groups to fulfill their needs through social institutions that facilitate these issues in a stable society [2].

Programs also defined: They are various social services that are provided to individuals and groups through social institutions with the aim of making a change in their personalities, behaviors, and attitudes towards society and the rules that govern it, and then to increase their values, knowledge and professional level[3].

B- Prevention:

The term prevention is taken from the verb "Prevent", meaning (preserving something .. that is, preventing something from happening), and the same meaning gives it the adjective "Preventive", which is intended to prevent something. For example (taking preventive procedures against disease) [4].

Others notice that prevention: It is procedures taken to prevent the emergence of diseases or stop their spread. This is one of the basic principles that must be followed in order to protect the health of the individual, and health departments strive to protect public health [5].

C- Health:

The World Health Organization (W.H.O) defined health: as a full state of physical, psychological, mental and social undertaking for those who are interested in health practices or preventive procedures , and not only free from disease, disability or weakness [6].

Health has also been defined: it is the safety or balance of the physical, mental, psychological or social aspects of the individual personality, where health refers a healthy mind in a healthy body in a normal family and an environment convenient to living and human adaptation [7].

D- Health protection:

The procedures that are taken to protect a person from disease, also known as preventive treatment. They are the opposite of the curative treatment that is tested to a disease and preventive procedures to protect health are needed to stop the spread of the disease [8].

3. Health counseling :

counseling is defined as individual and personal assistance in solving personal, educational, professional and health problems, as it learns all facts related to these problems in terms of their causes, consequences and impact on society and seeks solutions; This is done with the help of specialists and the experience of medical centers or health organizations, as well as through educational interviews, during which the mentor learns how to make his own decisions [9].

BAKAER also defines it as an aspect of procedures used to help a person solve his problems that appear in different aspects of his social life, or is to help a person achieve maximum personal growth to be more beneficial in society [10].

Chapter Two :The historical backgrounds of the emergence of the Corona pandemic (Covid_19), its symptoms and methods of spread

First: The emergence of the Corona virus:

Social life witnessed several times ago the emergence of many diseases that became unable to find a cure for them, as ancient civilizations contributed to discovering ways to treat many diseases and epidemics, such as fever and wounds, as well as the emergence of deadly epidemics ,like the plague and tuberculosis .With the development of life and the modern era, many diseases appeared, such as bird flu, SARS and other diseases, and the Corona virus (Covid_19) is a large family of viruses that cause disease for humans and animals as they affect the human respiratory system and the severity of infection ranges from colds to SARS, which has developed into a new type of Virus, which is a corona virus, was first discovered in the 1960's year. Coronavirus was diagnosed through laboratory tests, but often he did not be affected by the Virus . He did not participate in this test because the infection often causes light symptoms, and this test is only available in a small number of laboratories. These tests, which isolate the virus in cell cultures, determine that virus antibody (PCR) is a technique by which virus DNA is extracted and reproduced in large quantities for more accurate and large-scale analysis [11].

In this regard, a new viral pneumonia originating in China was announced on (December 31, 2019 AD), as this virus appeared in Wuhan, Hubei Province, central China, and due to the development and spread of this virus in many developed and developing societies, the World Health Organization announced International importance to the public health emergency, as the state worked to stop all foreign and domestic flights to limit the spread of this epidemic, and since December of the year (2019 AD) there has been an outbreak of pneumonia for unknown reasons, which was first reported in Wuhan, Hubei Province in China, the first new Corona virus and the (SARS-2) virus The virus that caused the explosion has been identified in China and other parts of the world, the World Health Organization (WHO) was confirmed and on (February 12, 2019 AD) (43103) a pandemic case Among them, (42,708) cases (99,1%) are represented from China. According to health reports and data, China was severely affected by the epidemic, which was a public health disaster, as the century virus is a relative of the SARS virus, which can be transmitted from animals to humans and has been infected with the virus. This virus is also can be found in local seafood and illegally sold wild foods in Wuhan, including bats. If such a catastrophe is not resolved, the destruction of the coronavirus may be similar to the devastation caused by the (SARS) outbreak in (2003 AD), as this outbreak resulted in (800) infections and (800) deaths throughout the year (26) countries. Where it was controlled within eight months (July 2003). Likewise, the effects of the H1N1 virus and the Ebola outbreak have impact on mental, psychological and physical health, including depression and drug addiction, and the population may have some known dangerous factors [12].

As for Iraq, the Coronavirus (Covid_19) pandemic occurred in (2020 AD) in Iraq in Najaf since (February 24, 2020) when an Iranian religious student was investigated, and the result was positive for his infection with Corona - the viral diseases associated with severe acute respiratory syndrome of the type The second (SARS-2 Covid-19), then other cases infected with the (Covid-19) virus were identified, and the total number of confirmed cases in Iraq reached (332,635) cases, including (8,754) deaths until (September 23, 2020 AD) [13].

Second: Coronavirus symptoms:

1. Common symptoms are fever (high body temperature), cough, muscle pain or fatigue.

2. Less common symptoms are sputum production, headache, hemoptysis, and diarrhea. Complications also included acute shortness of breath, acute cardiac injury and secondary bacterial infection.

Third: Corona virus Transmission methods:

1. Transmission by droplets when a person comes into contact with another person suffering from respiratory symptoms (such as coughing or sneezing) in close contact (at a distance of one meter), which may expose the person to the mucous membranes (mouth and nose) or conjunctiva (eyes) to respiratory droplets, potentially contagious.

2. Transmission occurs with polluted tools in the closed areas of an infected person.

3. Transmission happens through contact or shaking hands with an infected person through the mucous membrane or blood transfusion from the patient.

4. Airborne transmission differs from droplet transmission; Because the transmission of infection by air indicates the presence of microbes inside the nuclei of droplets, which are generally considered to be particles with a diameter of (5 micrometers or less), and can remain in the air for long periods and spread from one person to another at distances of more than one meter, as related to the disease (Covid -19), transport by air may be possible in certain circumstances and contexts, where supportive procedures or spray treatments are, for example. Endotracheal tube, bronchoscopy, open aspiration, nebulization therapy, manual ventilation before intubation, placing the patient in the supine position, separating the patient from artificial airways, non-invasive ventilation, positive pressure, tracheostomy, and cardiopulmonary resuscitation [14].

Health Prevention Programs and Health counseling from the Coronavirus (Covid_19)

First: Rules for health prevention programs from the Corona virus:

1. The Rule of Home Isolation: This approach aims to isolate the injured in homes or hospitals and prevent them from mixing with their families or relatives, for a period ranging between (14-24 days) and during the home isolation period or in the hospital, regardless of the location, the patient must follow different methods of Prevention, as well as when excreting saliva or phlegm when coughing, but should follow the right way of treatment, vitamins and foods that help him strengthen the body's immune system and recovery in addition to health protection in regard to protect the health and safety of others and it is necessary to allocate an independent bedroom and bathroom to limit the spread of this disease, because caring for People are the responsibility and mission of medical centers. Thus, those infected with infectious diseases, such as rabies, cholera, bird flu, or coronavirus, are isolated in order to limit or stop the spread and proliferation of these diseases among people [15].

2. Quarantine Rule: This rule aims to separate healthy people suspected of being infected with the Coronavirus from others, and limit their movement and work to keep them in places designated by the specialist authorities until the end of the suspected infection period and it prevents them from mixing with other individuals coming from The country in which confirmed cases of infection with the Coronavirus have been recorded, and whoever crosses the border should be stop and checked for a period of (14 days) as a preventive measure until it is confirmed that he is not infected by the disease.

3. The sanitary isolation rule: separating infected patients from healthy ones and limiting their movements by staying in medical facilities (hospitals for patients and treatment) to get appropriate treatment and care until the patient recovers from illness [16].

Second: Health prevention methods from Corona virus:

There are many features and ways of transmitting Coronavirus (Covid_19) infection that are not yet known, and the ministry is working with the World Health Organization to learn more about it. The Ministry of Health instruct citizens and residents to follow health guidelines to limit the spread of influenza in general, which are:

1. Staying at home: A person with the disease must not leave the house for any reason, exercise at least once a day, stay two meters away from others, and inquire about their needs over the phone or via the Internet or neglect or delay meetings, and visit friends And relatives at home.

2. Social distancing: when coughing or sneezing, small droplets of liquids (droplets) are formed and these droplets may contain the virus that causes the infection.

3. Personal cleanliness: Practicing personal and general cleanliness of the hands, especially washing with soap and water for a period of (at least 20 seconds), especially after returning from a public place or after coughing or sneezing.

4. The role of masks in the prevention of coronary heart disease: The patient must wear protective masks when approaching others and before visiting the doctor, and if they are unable to wear the

masks due to shortness of breath, for example, you must do everything necessary to hide the cough and sneeze in a way that is suitable to the person. As the masked patient being closed and the patient does not come into contact, it is not necessary to wear a mask.

5. Using cleaning materials to prevent corona: cleaning with soap or detergents and water removes bacteria and dirt from surfaces and reduces the number and risk of spreading infection, provided that sterilization and disinfection are followed to completely remove bacteria and viruses, and this process must be done regularly and repeatedly to ensure that there are no infections and pathogens [17].

Third: the importance of health counseling:

The profession of health or counseling is a profession of great value and importance, and is seen as a human experience in a changing world where human relations are clearly weakened, and health scientists assert that counseling allows people to work together to achieve common goals and many opportunities for them and the participation of each other in these interests, as professional counseling can help counselors to show their humanity to each other, and through this profession the importance of counseling has been proven as a primary source to help others solve their problems, identify their abilities and exploit them to the fullest for the individual and society, while summarizing the goals of health counselors or Clinics to help counselors feel comfortable, help them to accept themselves, distinguish between reality and their idealism, and help them think clearly to solve their personal and health problems, and in this regard, health care must pay attention to emotions and influences in thinking. The goal is to help the adviser reach a point where he understands himself not only positively and effective, but more effective and smarter. To understand this, the therapist needs external information to understand himself compared to those around him [18].

Fourth: Objectives of Health counseling:

1. It helps the guided to be able to express his negative psychological feelings and emotions, or what he suffers from physical, mental or psychological diseases.

2. It helps the guided to better understand himself, the situations and problems he faces, and to make important decisions and implement them after helping him to understand his personal and health problems and how to deal with them.

3. The guided is helped to change behavior, increase coping skills and deal with various situations and pressures, improve the decision-making process, improve personal relationships, and develop his creative energies after treating and curing what he was suffering.

4. It helps the guided of using different treatment methods, such as focusing on the person, real-life treatment , psychotherapy, or treatment after coronary heart disease [19].

Fifth: Types of Health counseling :

1. Individual counseling : This type of consultation aims to lead the person to meet face-to-face with guide or consultant every time offers the advice, and it seems that this consultation has dealt with psychological problems and health problems that the person cannot reveal them , so individual counseling aims to deal with these special problems individually and scientifically cautious in order not to adversely affect the individual with the disease.

2. Group counseling: This type of counseling depends on the method of counseling a group of injured people and people in need of care so that this group is similar and close in their mental and health problems, and they are taught and counseled in small groups in order to achieve the guiding goals and treat their health problems [20].

3. Preventive counseling : The aim of this type of counseling to reduce the risk of problems and chronic diseases that are different from the way to see the patient or his awareness of the problems before they arise, and before his illness physical, mental or psychological health and social consequences of falling in, and are practical preventive procedures and precautionary health and guidance through the means of counseling, awareness and advice, including conferences, seminars, seminars, awareness lectures, as well as all preventive means available in public and private health facilities, and placing informational posters on the walls of the department or health institutions.

4. Therapeutic counseling or treatment advice: This type of advice is provided to the patient if he does not benefit from the preventive instructions because it requires that the therapist or caregiver have a full knowledge and understanding of counseling methods and treatment methods to help the patient get rid of problems or stop them before their occurrence and recurrence to improve the patient's health condition. To walk on the path without diseases and health problems that stop or limit the continuous improvement and development of the patient's health [21].

Findings, Recommendations, Sources and References

Results:

1. The study showed that the Coronavirus (Covid_19) is spread through contact, touch, airborne infection, or sneezing.

2. Researches have shown that the coronavirus causes feverish symptoms that are similar to those of seasonal influenza.

3. Researches have shown that the preventive health rules to stop the spread of this virus include home isolation or quarantine.

4. Researches have proved that one of the most important health prevention methods to limit the spread of this disease is staying at home, social distancing, personal cleanliness and other methods of health prevention.

5. Researches have shown that the profession of health care or consulting is a very valuable and important profession, and it is seen as a human experience in a changing world where human relations are clearly weakened, as professional advice can help any teacher to show his humanity to help people with Coronavirus.

6. Health counseling helps to use a variety of treatment methods, such as treatment that focuses on a person, real life, psychological, physical, physical, or mental treatment , or post-coronary heart disease.

Recommendations:

1. At least , it should be a distance of one meter between those infected with the Coronavirus, in addition to avoiding smoking and other activities that weaken the lungs or the body's immune system.

2. The necessity of not walking around to avoid unnecessary trips and avoid large groups of people or crowded .

3. Paying attention to using a piece of cloth when coughing or sneezing, to cover the mouth and nose with it and dispose of it after finishing with an emphasis on the importance of following positive health habits and getting rid of negative habits.

4. The need to keep on other healthy habits such as washing fruits and vegetables before eating, nutritional balance, physical activity, and getting enough sleep as this helps improve the body's immunity.

5. The audiovisual and print media must continue to educate people about the dangers of disease spread, prevention and control.

6. Consider spreading health prevention programs during awareness sessions on coronavirus diseases and forcing people to use them.

7. The need to intensify media communication efforts within the framework of health prevention and awareness programs, and advertisements issued by the health departments responsible for this procedure.

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The Level of Nesfatin -1, Calcium and Vitamin D in Women with Gestational Diabetes Versus Control

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Abstract

Objective: This study was designed to investigate serum level nesfatin-1 and determine the association of nesfatin-1, calcium level and 25-hydroxy vitamin D (25(OH)D) level in women with gestational diabetes mellitus (GDM) versus controls

Methods: A88 pregnant women at 24-28 weeks gestation were included in this study .Forty four women diagnosed with gestational diabetes and 44 women without gestational diabetes both groups were matched in age and body mass index . Then we classify each group depended on 25(OH) D status (sufficient, insufficient and deficient). nesfatin-1 and vitamin D levels were analyzed using ELISA (enzyme link immunosorbent assay) a cut off value for serum calcium were assessed using colorimetric method, cut off value were used according to manufacture instruction and the a comparison was done between above parameters. Furthermore, insufficient and deficient 25(OH)D gestational diabetes group had significantly higher fasting blood sugar compared to matched control group .

Conclusion: Nesfatin-1 levels, 25(OH)D and calcium were not differ between the two groups statistically.

Key words: *Gestational diabetes, nesfatin-1, vitamin D (25(OH)D) , calcium.*

Introduction

(GDM)" is defined as diabetes diagnosed in the second or third trimester of pregnancy in patients with no history of diabetes prior to gestation [(1)] ". It is a popular pregnancy complications[(2)]. After mid pregnancy resistance to insulin evolves due to placental production of anti-insulin hormones such as progesterone, oestrogen ,a human placenta lactogen and cortisol [(3)] .Nesfatin-1 is polypeptide of 82 amino acid (aa) which is obtain from DNA binding protein (NUCB2(and calcium. NUCB2 (nonesterified fatty acid/nucleobinding 2) was found in neuroplasma and plasma membrane. NUCB2 converts to form nesfatin-1, 2 and 3 by the action of Convertase enzymes [(4)] .Nesfatin-1 is secreted by peripheral tissues and central nervous system (CNS). Its expression level has been found 20-folds higher in the stomach oxyntic mucosa than in the brain [(5)]. The significant roles for nesfatin-1 is diminish the ingestion of food, appetite, recurrent hunger results a fullness feelings [(6)]. Metabolic influences of Nesfatin-1 done by increase secretion of insulin through influx of calcium by L-Type Calcium canals which effected by protein kinase A and phospholipase A2. Thus metabolic disorder may be results from any disturbance in regulation of nesfatin-1, specially in DM Type(II). [(7)] Vitamin D, also known as calciferol , is fat soluble vitamin includes two forms, vitamin D2 which used in supplements ,vitamin D3 which can be made in skin of human by a sunlight(when converts the precursor of 7-dehydro-cholesterol.) [(8)]. These are pro-hormones , still inactive till the second hydroxylation occur : the first one in the liver while the second one in kidney) to the active form (1,25-dihydroxyvitamin D). vitamin D is circulated as 25(OH)D of it is bound to binding protein and albumin. It is documented it is good and best indicator for total vitamin D. Homeostasis calcium and phosphate effect bone. [(9)]. Calcium is necessary for processes mediated by insulin in tissues which response to insulin like adipose tissue and skeletal muscle, in narrow range of

calcium intracellulary. Alteration in concentration of calcium in goal tissues for insulin may induce insulin resistance byimpairing transduction of insulin signal leads to reduce the activity of transporter-4 .[10]

Our aim is to investigate the correlation between serum nesfatin-1 level and vitamin D and Ca in pregnant women with gestational diabetes and compare it with match control.

Subjects and Methods

A case control study was performed in AL Yarmouk Teaching Hospital ,Baghdad Teaching Hospital / Ministry of Health and National center for Diabetes research and Treatment / AL-mustansiriya University / Ministry of Higher Education from November to May 2019 this study included 88 pregnant women between 24-28weeks gestation according to their accurate last menstrual period and 1st trimester ultrasound scan ,all women matched in their age and BMI. Informed consent was obtained from all women in this study.

They classified subsequently in to two groups forty four women diagnosed with gestational diabetes and 44 women without gestational diabetes(control) . Exclusion criteria for this study :women with fetal congenital anomaly, placental abnormality, twin pregnancy, pre-existing DM , hyperparathyroidism, liver and renal disease, hypertension , inflammatory disorder ,anti-inflammatory drugs , glucocorticoid of any dose in the previous 3 months.

The diagnosis of GDM is done by the principle of International Association of the Diabetes and Pregnancy (IADPSG) , Fasting blood sugar ≥ 7 mol/l (126mg/dl) or casual blood sugar 11.1mmol/l (200mg/dl) When hyperglycemia in the range of high-risk factors GDM diagnosed by performing OGTT one or more of the glucose concentration must be met or exceeded for a positive diagnosis FBS ≥ 92 after 1hours 180 mg/dl at 2 hours 153mg/dl.

As these test negative so women considered as control .Additional each group was classify by status of 25(OH) D (sufficient, insufficient and deficient)

Method

5ml of blood was performed in the next morning after fasting at least eight hour and centrifuged immediately, then stored at −80ºC till use . Nesfatin-1 and vitamin D levels were performed by kit of ELISA manufactured by ((CUSABIO\ China) (HUMAN / Germany) company respectively. Serum glucose was assayed by an enzymatic colorimetric method. as supplied by Human. Serum calcium was assayed by totally colorimetric method. as supplied by Human . The relationship between nesfatin-1 calcium and vitamin D were evaluated in both groups. All statistical analyses, SPSS software 22 .0.0 (Chicago, IL) was used. Anderson darling test were used for normality of distributions. if follow normal distribution then mean and standard deviation used, if did not follow normal distribution then median and interquartile range will be used to present the data. When comparison between two samples used t-test which analyze the differences in means between two groups (when two sample on normal distribution with no significant outlier). Mann Whitney U test used to analyze the differences in median of two groups (when they do not on normal distribution). Linear regression analysis performed to assess the relationship between different parameters. p value considered when appropriate to be significant if less than 0.05.

Results

Mean maternal age and BMI were higher in gestational diabetic mellitus (GDM) group as compared to normal control but statistically not significance, while Mean gravida, parity, and abortion were significantly higher in group with GDM than normal control, as illustrated in table 1

Table 1: Demographic and maternal characteristics

Variables	Control	GDM	p-value
Number	44	44	-
Maternal age (years), mean ± SD	29.1±6.8	31.3±6.2	0.1161
GA (weeks), mean ± SD	26.6±1.7	26.0±1.9	0.1041
Gravida, mean ± SEM	3.0±0.3	4.6±0.5	0.003 [S]2
Parity, mean ± SEM	1.6±0.2	2.5±0.3	0.016 [S] 2
Abortion, mean ± SEM	0.5±0.2	1.4±0.3	0.001 [S] 2
BMI (kg/m2), mean ± SD	31.9±7.3	33.8±5.3	0.1661

GDM: gestational diabetic mellitus, S: significant, SD: standard deviation, SEM: standard error mean, GA: gestational age
1Independent t - test
2Mann – Whitney U test

P - value ≤ 0.05 is significant

There was no significant difference in mean nesfatin-1 concentration between women with GDM and control. FBS was significantly higher in GDM compared to control .There was no significant difference in serum vitamin D and calcium between GDM and control, as illustrated in table 2

Table 2: Assessment of Nesfatin-1,vitamin D and calcium levels in GDM and controls

Variables	Control	GDM	p-value
Number	44	44	-
Nesfatin-1 (pg/mL), mean ± SD	38.1±7.4	38.8±7.9	0.6761
Vitamin D3 (ng/mL), mean ± SD	16.7±6.9	14.8±4.9	0.1522
<10 ng/Ml	7 (15.9%)	8 (18.2%)	0.5821
10 – 29 ng/mL	34 (77.3%)	35 (79.5%)	
≥30 ng/Ml	3 (6.8%)	1 (2.3%)	
Serum Ca (mg/dL),mean ± SD	7.5±0.2	7.6±0.8	0.7672
FBS (mg/dL), mean ± SD	79.1±8.2	107.7±20.6	<0.001 [S]1

SD: standard deviation, GDM: gestational diabetic mellitus
1Chi square test
2Independent t-test

P- value ≤ 0.05 is significant

There was no significant difference in mean nesfatin-1, calcium, while FBS was significantly higher in GDM compared to control; for subjects with insufficient vitamin D levels

Table 3: Assessment of various biomarkers for GDM for patients with insufficient vitamin D levels

Variables	Control	GDM	p-value
Number	34	35	-
Nesfatin-1 (pg/mL), mean ± SD	37.6±7.9	37.7±7.9	0.9471
FBS (mg/dL), mean ± SD	79.9±8.1	110.7±20.7	<0.001 [S]1
Calcium	7.6±0.2	7.5±0.8	0.9171
SD: standard deviation, IQR: interquartile range, S: significant 1Independent t-test 2Mann – Whitney U test 3Chi square test			

P- value ≤ 0.05 is significant

Mean FBS was significantly higher for GDM patients compared to control, while the rest of the markers show no significant difference between both groups as illustrated in table 6

Table 4: Assessment of various biomarkers for GDM for patients with deficient vitamin D levels

Variables	Control	GDM	p-value
Number	7	8	-
Nesfatin-1 (pg/mL), mean ± SD	42.0±3.0	44.3±5.4	0.3301
FBS (mg/dL), mean ± SD	77.6±9.3	98.4±16.2	0.010 [S]1
Calcium	7.5±0.2	7.7±0.4	0.3601
SD: standard deviation, IQR: interquartile range, S: significant 1Independent t-test 2Mann – Whitney U test 3Chi square test			

P- value ≤ 0.05 is significant

Discussion

In this study we observed that mean age and BMI were not significantly different in both groups .The same observation was shown by Radzisław et al (2019) he noticed that women from both groups were not significantly different in their age [11] .

While study done by (Hedderson (2010) Hana et al.2017)showed that BMI during pregnancy were higher in the GDM group ,as Obesity is the most powerful risk factor for the development of GDM during gestation; high rates of gestational weight gain, especially early in pregnancy, increase a woman's risk of GDM [12,8] .We observed that mean gravida, parity, and abortion was significantly higher in two groups, disagree with Radzisław et al(2018) as he noticed the women from both groups had no significant difference in their gravidity [11] .This study showed that there was no significant difference in mean nesfatin-1 levels between two groups (38.1±7.4 vs 38.8±7.9) pg/mL ,our result in agreement with Aslan et al.2012 who noticed that cord blood nesfatin-1 concentrations were no difference in both groups, and maternal nesfatin-1 concentrations were positively correlated with their special cord blood

levels [13] .

Again our result are in agreement with Zhai et al 2017, seven studies of a meta - analysis of involving 328 patients with type 2 diabetic and 294 subjects without type 2, he observed no significant differences in the levels of nesfatin-1. However, subgroup in Zhai et al 2017 meta-analysis analysis revealed higher levels of nesfatin-1 in patients with newly diagnosed type 2 diabetic and significantly lower nesfatin-1 concentrations in type 2 diabetic patients extradite antihyperglycemic therapy as patients with type 2 diabetes who are extradite antihyperglycemic therapy , circulating nesfatin-1 levels are decreased due to antihyperglycemic therapy goal the deceasing of blood glucose, increased sensitivity of insulin, and reduction food ingestion [14] . This result explained also by J. Dong, H. et al 2013 in his study he observed that nesfatin-1 can catalyze the metabolism of lipid and appear anti-inflammatory effects[15] .

The different results have been published by the group of Zhang et al. 2017 as they demonstrated that maternal serum and cord blood concentrations of nesfatin-1 in GDM were higher compared to control their result might be due to nesfatin-1 is closely related to obesity and insulin resistance in pregnancy [16] .

Radzisław et al.2019 ,Ademoglu et al 2017, Kucukler et al 2016 all observed that in patients with GDM, the level of serum nesfatin-1 remained significantly lower than in control women [8,17,18] .The results of studies assessing nesfatin-1 concentration in patients with T2DM and impaired glucose tolerance (IGT) are similarly equivocal as in GDM group. Algul et al.2016. and Li et al. in his study he proved that fasting nesfatin-1 levels were reduced in T2DM and metabolic syndrome patients, compared to healthy adults and patients with T1DM this result explained by: both GDM and T2DM, and (IGT) share many common pathophysiological mechanisms such as insulin resistance and hyperinsulinemia, and all of these conditions are more often observed in overweight and obese individuals that analysis proposed a possible role of nesfatin-1 in gestational diabetes, and the nesfatin-1 level may be predictor of the development gestational diabetes and a goal for antidiabetic therapy . Although the roles of nesfatin-1 in the pathogenesis of gestational diabetes are currently not good understood, nesfatin-1 therapy may be an efficient future treatment for GDM and obesity [19]. The discrepancy between the results presented by different literatures might be explained by the different study protocols and patient selection processes, including: gestational age when samples were obtained—the second or third trimester, the type of GDM—treated only with diet or with insulin, which could be suggestive of the severity of metabolic disturbances ,the gestational age at the diagnosis of GDM—the first (possible IGT from the pre-pregnancy period) or second trimester (“typical” gestational diabetes. Our study failed to observe any relation between serum vitamin D and serum calcium level between GDM and control .The same was observed by Gashlan et al. 2017, Pleskačová et al. 2015 ,Park et al 2015 Krame et al., 2014) in their study, they do not observed significant differences in 25(OH)D between GDM and control [8,20,21,22,) ,but disagreed with Åse et al 2018 , Hana et al. 2017, ,Alzaim and Wood, 2013studies in which they showed that deficiency of vitamin D were linked to GDM incidence , and 25(OH)D level was significantly lower in women with GDM compared to controls [23,8,24) .

In our study, we showed that there is higher prevalence of 25(OH)D insufficiency and deficiency were noted between GDM and (~79.5%,~18.2 respectively) and control (~77.3% insufficient ,~ 15.9% deficiency),the same observation by Pleskačová et al., 2015study [20] . In 2017 Hana et al.work, was proved that in deficient GDM group, raised FBs was noted when compared with deficiency control. While insufficient GDM group had comparable vitamin D mean to control with normal glucose level . Same direction was observed for mean of FBS , while ameliorate 25(OH)D levels (sufficient group) in women with GDM normalizes FBS [8] .Our study agrees with Amit et al 2018 as he showed an equal prevalence of 25(OH)D defeciency in women with and without GDM [25] . Different observations have been presented in Zuhur et al 2013 which exhibited that GDM is linked with only severe 25(OH)D defeciency but the exposure of sun the differently , the population of study , types of diet , measurement and clarify of 25(OH) D deficiency. [27]

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Comparison of Non-Fasting and Fasting Lipid Profile in Dyslipidemia Patients

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Abstract

Dyslipidemia, a metabolic syndrome characteristic, includes increased flux of free fatty acids, high density lipoprotein cholesterol, low-density lipoprotein, and triglycerides. One of the main topics of screening and management in adult and child populations has been dyslipidemia

The aim of this study was to evaluate non-fasting and fasting when measuring the lipid profile of patients with dyslipidemia.

This study was performed 80 male of dyslipidemia patients, they were monitored and their lipid profile levels measured during non- fasting were collected after 2 hours of meal, and varying hours of fasting periods includes (4 hours, 6 hours, and 8 hours).

In this study we did not found any significant clinical difference between non-fasting and fasting levels of total cholesterol, and low-density lipoprotein and high-density lipoprotein. Thus, we can use the non-fasting blood samples to estimate lipid profile in follow-up the dyslipidemia patients. When comparing the fasting hours, we observed mean triglyceride for all period of fasting were differences, as compared to the non-efforts fasting state significant differences $P< 0.001$

Finally, there is no need to make fasting lipid profile mandatory. This study suggests that should be made to simplify the sampling of blood by replacing the profile of fasting lipids with the profile of non-fasting lipids.

Keywords: *Total cholesterol, triglycerides, high-density lipoprotein, low density lipoprotein, fasting, non-fasting.*

Introduction

Dyslipidemia, a metabolic syndrome characteristic, includes increased flux of free fatty acids, high density lipoprotein cholesterol, low-density lipoprotein, and triglycerides [(1)].

One of the main topics of screening and management in adult and child populations has been dyslipidemia. In 2008, the prevalence of such a problem was as high as 39 percent for men and 40 percent for women in developing countries [(2)].

In addition, with the raising number of people with dyslipidemia discovered due to efficient screening. Recent recommendations have preferred non-fasting lipid profile assessment [(3)]. Raising patient satisfaction by eliminating separate return visits for laboratory drawings and improving hospital and clinic performance are practical benefits of using non-fasting measurements. Moreover, non-fasting triglycerides may improve cardiovascular risk prediction [(4)]. On the other hand, in fasting samples, classification of dyslipidemias has historically been derived, and cohort studies and clinical trials have generally carried out fasting evaluations [(5,6)].

There are particular difficulties in screening for lipid disorders. Prior to a regular doctor's office appointment, most patients would not have fasted. Therefore, most fasting lipid panels must either be scheduled prior to visits or reviewed for corresponding office visits or

additional outpatient phlebotomy center visits [7].

In our study, we will discuss the variance fasting hours among the lipid profile parameters [total cholesterol, low-density lipoprotein and high-density lipoprotein, triglycerides in dyslipidemia patients

Material and subjects

This study was performed 80 male of dyslipidemia patients, they were monitored and their lipid profile levels measured during non- fasting were collected after 2 hours of meal, and varying hours of fasting periods includes (4 hours, 6 hours, and 8 hours).

The following information was submitted to all patients included in the study:

· Personal history was taken with regard to name, age, gender, residence, special medical behaviors such as smoking, diabetes mellitus, systemic arterial hypertension, prior ischemic incidents, and family history as well.

· For all patients, including general examination, vital signs (blood pressure and pulse) and obesity, clinical examination was performed

· The lipid profile parameters were measured using an automated quantitative COBAS INTEGRA® 400 plus test (from Roche, Germany).

Statistical Analysis

The total clinical data of patients was analyzed to identify the number of hours of food interruption. Continuous variables analysis using SPSS 20. Software (SPSS, Inc., Chicago, IL, USA), and expressed as the mean ± standard error. ANOVA was done to evaluate the differences in these variables. $P \leq 0.001$ was measured to indicate a statistically significant difference.

Rustles

The result of lipid profile illustrated in table 1, the statistical analysis showed no any different significant in mean lipid profile, firstly total cholesterol among different period of fasting in non-fasting (284.40 ± 1.05 mg/dl), fasting for 4 hours (282.36 ± 0.51), fasting for 6 hours (282.40 ± 0.24 mg/dl), and fasting for 8 hours (282.60 ± 0.74 mg/dl) figure 1. Also, high density lipid as the mean in non-fasting (41.60 ± 0.92 mg/dl), fasting for 4 hours (42.80 ± 0.86 mg/dl), fasting for 6 hours (42.80 ± 1.46 mg/dl), and fasting for 8 hours (41.60 ± 0.92 mg/dl) figure 2. In addition, low density lipid as the mean in non-fasting (189.60 ± 0.92 mg/dl), fasting for 4 hours (188.60 ± 0.50 mg/dl), fasting for 6 hours (200.80 ± 0.86 mg/dl), and fasting for 8 hours (188.74 ± 0.60 mg/dl) figure 3.

The mean of triglyceride showed the different significant in in dyslipidemia patients among all period of fasting include non-fasting (235.64 ± 0.16 mg/dl), fasting for 4 hours (210.42 ± 0.20 mg/dl), fasting for 6 hours (200.80 ± 0.86 mg/dl), and fasting for 8 hours (192.00 ± 0.70 mg/dl), the p value less than 0.001figure 4.

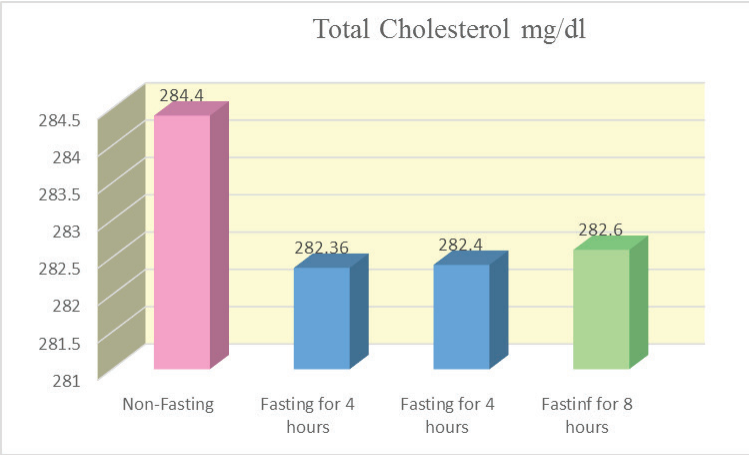

Figure 1 Total cholesterol during the various fasting periods

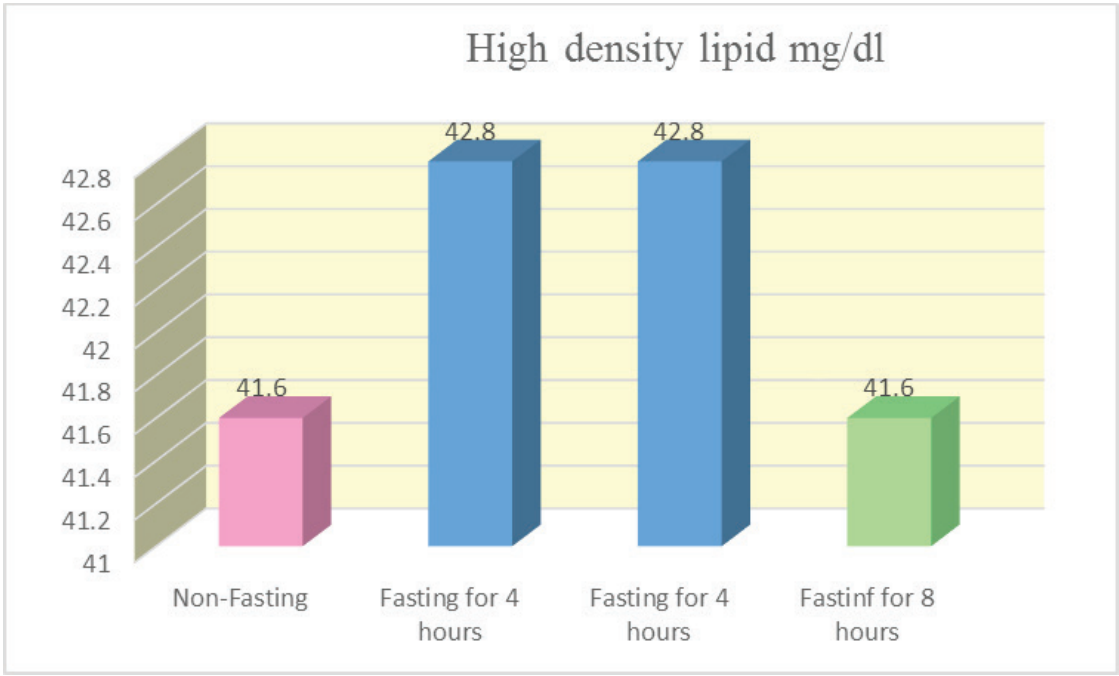

Figure 2 High density lipid during the various fasting periods

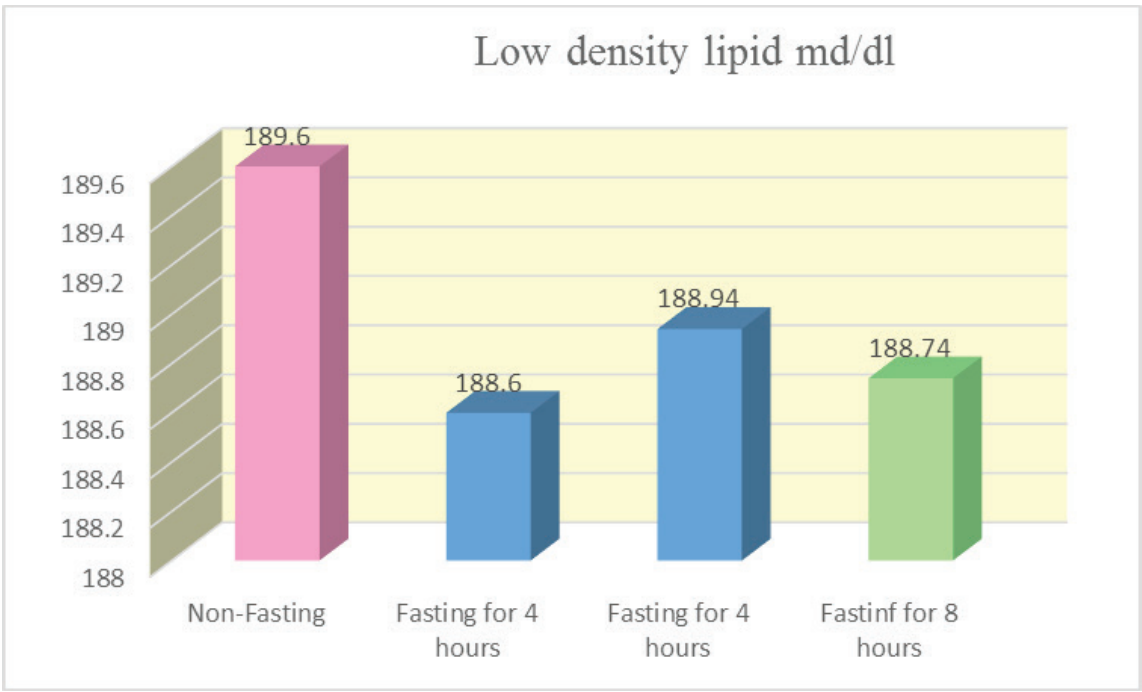

Figure 3 Low density lipid during the various fasting periods

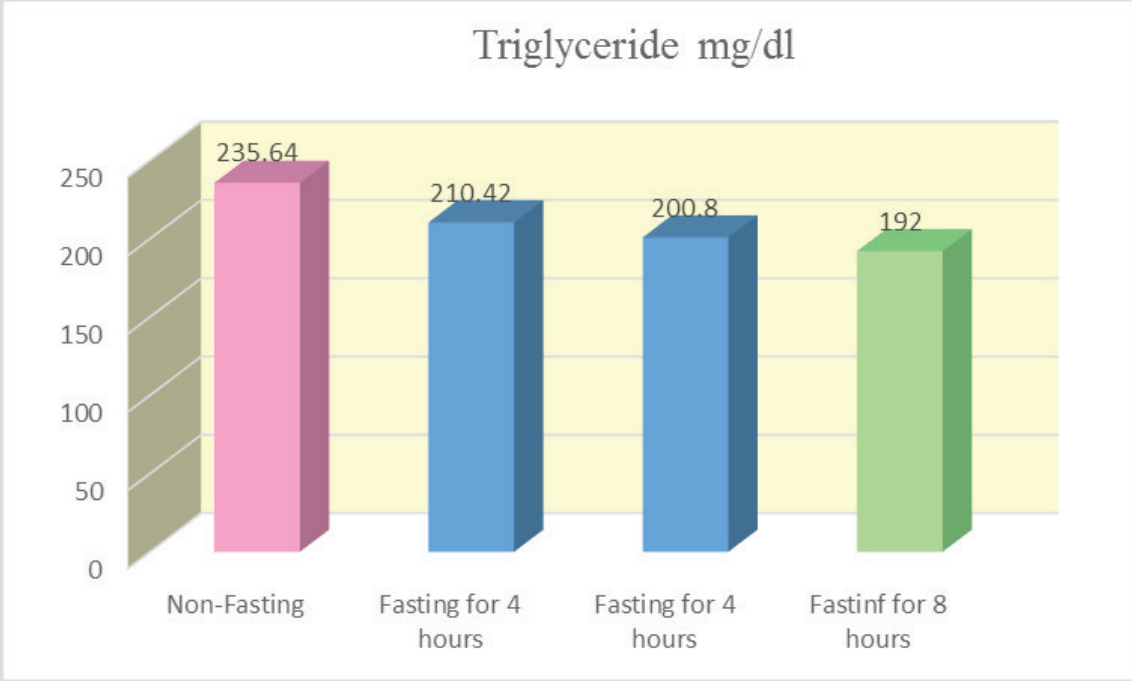

Figure 4 Triglyceride during the various fasting periods

Table. 1 Demonstrating Mean ± Standard error (mg/dl) of different fasting hours versus non fasting in Lipid profile assessment

Type of lipid	Fasting hours		Mean ± SE	Sig
Total C mg/dl	fasting	4	282.36 ± 0.51	N. S
		6	282.40 ± 0.24	
		8	282.60 ± 0.74	
	Non fasting		284.40 ± 1.05	
TG mg/dl	fasting	4	210.42 ± 0.20	Non- fasting vs 4 hours < 0.000 Non- fasting vs 6 hours < 0.000 Non- fasting vs 8 hours < 0.000 4 hours vs 6 hours < 0.000 4 hours vs 8 hours < 0.000 8 hours vs 6 hours < 0.000
		6	200.80 ± 0.86	
		8	192.00 ± 0.70	
	Non fasting		235.64± 0.16	
HDL mg/dl	fasting	4	42.80 ± 0.86	N. S
		6	42.80 ± 1.46	
		8	41.60 ± 0.92	
	Non fasting		41.60 ± 0.92	
LDL mg/dl	fasting	4	188.60 ± 0.50	N. S
		6	188.94 ± 0.3	
		8	188.74 ± 0.60	
	Non fasting		189.60 ± 0.92	

Discussion

In this study we did not found any significant difference between fasting and non-fasting levels of total cholesterol, low and d high density lipoprotein. Thus, we can use the non-fasting blood samples to estimate lipid profile in follow-up the dyslipidemia patients.

Lipid profiles are routinely measured for risk assessment in preventing atherosclerosis disease**.** It is usually done in the fasting blood sample. Researches are in process to replace the fasting blood sampling with that of non-fasting sample to simplify the blood sampling, societies & laboratories adapted non-fasting lipid profiles. Non-fasting lipid testing has been the clinical routine in Denmark since 2009, based on the Danish Society for Clinical Biochemistry's recommendation that all laboratories in Denmark use random non-fasting lipid profiles as a standard, thus giving clinicians the option of re-measuring fasting triglyceride concentrations if non-fasting values are 4 mmol/L (350 mg/dL) [9,10]. In addition, the UK NICE guidelines have allowed non-fasting lipid testing since 2014 in the primary prevention setting [11]. However, the use of non-fasting samples over fasting samples for calculating the lipid profile, however has advantages. [12,13]. Therefore, the change in fasting hours would be much simpler for people worldwide if a lipid profile could be taken after few hours of postprandial [12].

When comparing the fasting hours with non-fasting we observed mean triglyceride showed the differences among a all period of fasting, it increased greatly in non-fasting state as compared to the fasting state significant differences P< 0.001 ([13,14,15]. The removal of fasting hours as a general requirement for triglyceride testing could dramatically improve patient convenience without significantly changing the results of the test [14,15].

They explained that the increased triglycerides were a correction to albumin levels in response to normal food intake and hence a correction to hemodilution due to fluid intake. The fluctuations in these amounts are therefore more likely due to the consumption of food rather than the intake of fluid. Therefore, there was no substantial clinical difference between total cholesterol, HDL, and LDL levels between fasting and non-fasting. These findings are in line with those of Langsted *et al.*[11].,

Since non-fasting can compromise the accuracy of certain types of hyperlipidemia diagnosis, we proposed that laboratories and organizations should also provide clinical-based measurement of fasting triglycerides, as in the case of very high concentrations of non-fasting triglycerides. It was concluded by Cohn *et al*. [14]. & Mihas et al [15]. The reason that preferred fasting lipid profiles is the rise in triglyceride level seen through a fat tolerance assessment. On the other hand, the Fried Ewald equation, which has been assumed to be greatly influenced by food consumption, also measures LDL cholesterol. Therefore, if this equation is used, some underestimation of LDL cholesterol can occur when chylomicrons are present. [16]. In addition to that non-fasting state due to the liberal intake of fluids, plasma LDL cholesterol levels can slightly decline and thus lead to a minor misclassification of cardiovascular risk, as well as errors in initiating or altering lipid-lowering medication, especially for diabetic subjects. [17,18,].

Conclusion

Finally, there is no need to make fasting lipid profile mandatory. This study suggests that efforts should be made to simplify the sampling of blood by replacing the profile of fasting lipids with the profile of non-fasting lipids.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad AlRafidain University College and all experiments were carried out in accordance with approved guidelines.

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Detection Levels of Cytokines (IL-6 and IL-18) and Microbial Parameters in Patients with Renal Failure in Diyala Province

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Abstract

Current study was conducted in Diyala province for kidney failure patients (Ibn Sina Center) for dialysis in Baqubah teaching hospital and started from beginning August 2016 to end April 2017. A total 100 blood sample had from kidney failure patients, has been the number males 61 and females 39 with age range (10 – 88) years and collected 50 of blood sample from oculary healthy individuals act as a control group , has been the number males 25 and females 25 with age range 10 – 88 years . By (ELISA) technique detection immunological parameters (IL-6 , IL-18) , and (SRID) for detection microbial parameters (HBV, CMV , *T. gondii*, HCV) . Result of study expressed that the incidence of disease rate among males 61% more than females 39% with no significant different (*P>0.05)*, also highest rate disease in both age groups (31-50, 51-70) years , while lowest rate disease the age range (10-30 , >70) years recorded with significance different) *P <0.05*(. So, high levels of (IL-18) for patients than to control with significance different between study groups (*P <0.05)*, while (IL-6) was raised in patients compared to control. Also, result showed positivity of (HBV, CMV , *T.* gondii, HCV) in patients was (8% , 13%, 10%, 25%) respectively with high significant different (*P<0.05).* Conclusions of current study showed male more than female, age period >70 and 51-17 year recorded highest percentage infection ,high levels of immunological (IL-6 and IL-18) in renal dialysis patients, as well as to some microbial infected patients with kidney failure. The aim of study is detection levels of cytokines (IL-6 and IL-18) and some microbial infection in patients with renal failure.

Key words*: Renal failure, , microbial infection, and (IL-6 and IL-18)*

Introduction

The kidneys are two important biological organs have several functions, it is unable to keeping fluids, electrolytes, and regulate average of blood pressure [1] . Renal failure is condition that the kidney become unable to remove final metabolic products from blood and regulate fluids, electrolytes, and acidic balance in extracellular fluids [2] . So chronic renal failure (CRF) is the condition that appear when the kidney damage or decrease function it for three months or least (glomerular filtration rate (GFR) <60 ml/min /1.73m2) [3] .

The (CRF) is worldwide distribution disease especially in late years according to mention of (WHO), the mortality rate of (CRF) infection in Iraq approximately (100-130) case for each million person [4] . Showed reports of late years about (110000) of American people treated with kidney transplant (KT) or haemodialysis (HD) in 2007 year [5] , and that number may be increase to arrive 2.2 million at 2030 [6] .

The immune system can be express in two grades; cellular and humeral immunity , cellular immunity composed of (T cell) and (Cytokines) , while the humeral immunity composed of (B cell) which immunoglobulin [7] . (IL-6) is one of the main of acute phase response, which is noted high in patients with end-stage dialysis, and is also is powerful predictor of disease outcome [8] , as well as plays an important role in the relationship between inflammation, malnutrition, and cardiovascular disease (CVD) in patients undergoing hemodialysis (HD) [9] . (IL-18) is produced when the renal tubules damaged [10] , also may be used as a marker for the

diagnosis of dystrophy function loss in renal dysfunction patients [11] .

Artificial kidney patients are more susceptible to viral infection that infect the liver such as (HCV and HBV) because of (HD) process and blood transfusion [12] . Cytomegalovirus (CMV) can be appear frequently in renal failure patients after (KT) [13] . Several studies show the patients with (HD) are related to *T. gondii* parasite, but don't have any evidences about presence *T. gondii* in patients with peritoneal dialysis (PD) .

The aim of current study is detection levels of (IL-6 and IL-18 (and positivity of (HBV, CMV , *T. gondii*, and HCV.) in some patients with renal failure in Baqubah city.

Material and Methods

This study was conducted in diyala province for renal failure patients and started from beginning August 2015 to April 2016. A total 100 blood sample collected from renal failure patients after diagnosis by physical specialist in (Ibn Sina Center) for dialysis in Baquba teaching hospital according to criteria adopted by the WHO for diagnosis the renal failure disease , number of males was 61 and females was 39 with age range (10 – 88) years and collected 50 of blood sample from apparently healthy individuals act as a control group , males was 25 and females was 25 with age range (10 – 88) years. Collected 5 ml of blood and it was left to stand at room temperature 20-25°C to allow it to clot, then the sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20°C till examination. Each aliquot of the serum was used once to avoid freezing and thawing. All sera and reagents were allowed to stand at room temperature before use in the test.

1-Detection levels of (IL-6 and IL-18) in patients with renal failure.

By quantitative sandwich enzyme linked immunosorbent assay technique [14].

2-Detection positivity of (HBV, HCV, CMV) and parasite (*T. gondi*) in patients undergoing dialysis.

By torch rapid cassette test [15].

Statistical analysis

The (Chi-squared and T) tests used to analysis of current data, a level of significance of α=0.05 was applied to test, the statistics software used to process the data analysis were the (SPSS v.22) [16].

Results and Discussion

The current study included 150 sample has been divided into two groups , patients group which included 100 samples, 61 from male and 39 from female . Results of current study showed the percentage of males more than females with non-significant difference between sex of patients *p>0.05* and shown as in table (1).

Table (1) distribution of patients according to sex by using chi-square test.

		Patients
Gender	Male	61 (61%)
	Female	39(39 %)
Total		100(100%)
p value		No Sig. (p>0.05)

Results of current study show that the percentage of males with renal failure more than females and reasons of it may be due largely to the daily effort after exposed then women, large number of protein consume by men than women, also muscle mass in men than women leads to increase concentration of creatinine in men [17]. In addition, result of current study were compatible with results conducted by [18] which express the male with renal failure more than to female.

Age range of renal failure patients were (10-88) years as shown in table (2). Also the results showed that the age periods (10-30 and >70 years) recorded lowest rate of disease , while the age period (31-50 and 51-70) years recorded highest rate of disease. There is significant different between age periods *P<0.05* as shown in table (2).

Table (2) distribution of study groups according to age periods by using chi-square test.

		Patients
Age groups (years)	10-30	11 (11%)
	31 -50	32(32%)
	51-70	47(47%)
	>70	10(10%)
Total		100(100%)
p value		Sig. (p<0.05)

Moreover, results of current study were agree with results that conducted by [19]. Chronic kidney diseases (CKD) are commonly in elderly [20], and this is why the professional organizations to conduct routine tests for older people in health care centre [21]. The individuals have >55 year are more susptible to renal failure disease as compared to young. The age group associate with glomerular filtration rate (GFR), the elderly groups have low (GFR) than young groups [22]. So it will be that, percentage of death for patients with kidney failure patients elderly more than young [23].

Table (3) comparison of (IL-6 and IL-18) between study groups by using student t- test.

Pg/ml		Groups		P value
		Control	Patients	
Serum IL-6	Mean± SD	91.55±37.83	130.82±68.12	No Sig.(p>0.05)
Serum IL-18	Mean± SD	296.95±129.33	442.88±218.27	Sig.(p<0.05)

Results of the present study showed that the level of IL-18 in patients with renal failure was (442.88 ± 218.274 pg / ml) than to control group (296.95 ± 129.337 pg / ml) with significant difference (p <0.05) , also the current study noticed that the level of IL-6 in patients with renal failure was (130.82 ± 98.120 pg / ml) compared to control group (91.55 ± 37.834 pg / ml), but there was no significant difference (p> 0.05) as shown in Table (3).

The results of the present study were comparable with the results of the study conducted by researchers [24, 25, 26]. In the present study, there was an increase in serum IL-6 in patients with renal failure than to healthy patients. IL-6 is a pro-inflammatory cytokines that is produced in response to bacterial infections in the body [27]. IL-6 can be detected in healthy people within normal limits, but it is elevated in most, not all chronic kidney diseases. High levels of IL-6 in a blood stream are thought to be related to disease severity, where high concentration of IL-6 is associated with increased mortality [28]. There are several evidence that IL-6 has an effect on kidney injury, along with viral and genetic infection. Recent studies have confirmed that IL-6 and IL-8 are increasing in patients with UTIs [29]. There are many factors affecting the immune response, such as the accumulation of uremic toxins and dialysis process, so dialysis patients are immunodeficient inhibitors. The increase of IL-6 in the serum of patients with hemodialysis may be stimulated by the production of washing Kidney [24], reduced renal function of hemodialysis patients in the final stage leading to increased production, in addition to the factors prevalent in renal failure such as hypertension, chronic heart failure, Oxidative stress, as all these factors contribute to this increase [25].

The results of the present study are agree with the results of the study expressed by the researchers [10]. In the current study, elevated (IL-18) serum was found in patients with renal failure compared with healthy patients. Il-18 and other biologic factors are

expressed within two hours of kidney injury and then begin to increase [30]. In patients with renal failure, il-18 is significantly higher two days before creatinine elevation in renal failure patients [31]. Therefore, IL-18 is suggested as a predictor factor in renal failure disease [32]. [33] reported that the sensitivity and specificity of IL-18 to diagnosis renal failure is more than 90%, as there are many reports indicating IL-18 in early stages of infection nephropathy.

Dinarello et al., (2013) have noted that IL-18 increases Th1 production and interferon gamma and (IL-18) promotes inflammation through adaptive immunity as well as innate immunity. IL-18 may be due to the activation of T-1 helper cells, thereby stimulating the immune response of the immune cells produced by this interleukin, thereby increasing serum levels compared with the control group. IL-18 also plays an important role in the production of interferon gamma from T cells and natural killer cells [35]. IL-18 and IL-8 may also increase its level due to the increase in monocyte and neutrophils in response to the inflammatory process [36].

Table (4) positivity tests (CMV, HBV, *T.gondii,* HCV) in patients by using chi-square test.

Test	NT.	PN	%	P. Value
T. gondii	100	10	10%	Sig. (p<0.05)
CMV	100	13	13%	Sig. (p<0.05)
HCV	100	25	25%	Sig. (p<0.05)
HBV	100	8	8%	Sig. (p<0.05)

NT= number total PN= positive number

As regard to tests (CMV, HBV, *T.gondii* and HCV) for patients, the results of this study indicated that the positivity was 13%, 8%, 10%, 25% respectively with high significant difference and as shown in table (4).

Results of current study were agree with results of researches [37, 38], who found the percentages of positivity (CMV) among patients with renal failure compared to control. Also [37] showed that (CMV) is associated with end stage of renal failure patients, also showed [39] the patients undergoing (HD) as well as are more susceptible to (CMV). The prevalence of (CMV) in patients with renal failure has widely through frequent blood transfusion or exposure to (CMV) during dialysis procedure [40].

As for (HBV, *T.gondii*, HCV) the results of current study were compatible with results of researches [12], who found that positivity (HBV, *T.gondii,* HCV) in serum patients of renal failure compared to control. Patients undergoing to dialysis are more susceptible to hepatitis virus [41], also [42], showed that kidney patients with liver virus depend on frequent blood transfusion to patients as well as the use of kidney dialysis machine contamination with liver virus . In other hand , the percentage of (HCV) infection in haemodialysis patients was 4%-7% in different countries [43], also [44] showed that the percentage of infection with (HBV) was 13.3%. [12] showed the 10% of patients dialysis has infected with liver hepatitis and infected with (*T.gondii*) that transmitted through blood transfusion and kidney transplantation, in addition this parasite leads to toxoplasmosis in patients with renal failure.

Conclusions

Conclusions of current study showed the patients with renal failure are high in male than female. Renal failure diseases increase with progress of age. So, the pro-inflammatory cytokines (IL-6 and IL-18) in patients are high than control. In addition, there are some microbial infections that indicated in patients with renal failure.

Recommendations

1. Detection levels of (IL-6 and IL-18) and positivity of (HBV, CMV , *T. gondii*, HCV) in patients before and after dialysis process.

2. Use (IL-18) as prognostic factor for renal failure disease.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad AlRafidain University College and all experiments were carried out in accordance with

approved guidelines.

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Mathematical Engineering Model for Studying the Interrelated among Effective Factors in the Sugar Blood Estimation Test

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Abstract

It is a chronic metabolic disease, which is characterized by high blood sugar, as a result of a defect in the pancreas weakens the production of insulin to the body, and spread type 2 diabetes in humans all over the world, where there are more than a million and a half million people with diabetes. The most important factors contributing to the spread of this disease is eating unhealthy, excessive obesity, lack of exercise, psychological tension and genetic factor, so it is the responsibility of the state, as it must provide health education to its population, and urge them to eat healthy food and reduce excess weight, and encourage them on the practice of r Intifada activity, urging them to westernize the marriage, if any diabetes in the family. Therefore, the independents and dependent variables are included using a statistical program to be analyzed and to find the degree of correlation and impact of the variables among them and to analyze and discuss the results.

Keywords: *Effective, Factors, Mathematical Engineering Model, Sugar Blood*

Introduction

Diabetic type I that affects children and young people, and is caused by the loss of insulin in the body as a result of a defect in the pancreas, and makes the person infected with insulin doses for a lifetime. Type II: is the type that affects adults, and is the most prevalent in the world, and is caused by insulin secretion rate is insufficient to the body, or because of the start of other body cells to resist insulin. Other types: gestational diabetes. [1] to [3].

Diabetes symptoms are dehydration of the throat, drinking excessive amounts of water, feeling hungry shortly after eating, frequent urination to urinate, low or excessive weight loss, and vision problems such as poor vision, fatigue, fatigue, itching, Wounds do not heal quickly, cold symptoms such as loss of appetite and body pallor, weakness in gum teeth, short stature, and slow growth if children develop diabetes. For anyone with diabetes, a blood glucose self-test (blood glucose) may be a necessary means of treatment plan and ways to prevent long-term diabetes complications. A blood glucose test can be done at home using a portable electronic device (glucose meter), which measures the level of sugar through a small drop of blood. A blood glucose test - or self-monitoring of your blood glucose - provides useful information for controlling diabetes. It helps the tester to:

• Assess the extent to which the general objectives of treatment are achieved.

• Learn how diet and exercise affect blood sugar levels.

• Identify how other factors, such as illness or stress, affect blood sugar levels.

• Track the impact of diabetes medications on blood sugar levels.

• Determine high or low blood sugar levels.

Methodology of the Tests

However, there are several factors that obviously increase your risk of diabetes, including:

• Age: Age greater than or equal to 45.

Weight: Overweight defined as BMI greater than or equal to 25.

• Genetics: a close relative of a family with diabetes.

• Race: certain ethnic groups known to have a high risk of developing diabetes.

• Physical activity: lack of physical activity.

• Hypertension / hypertension: defined by blood pressure values higher than 90/140 mmHg.

• Hypercholesterolemia: LDL is harmful

• High level of triglycerides in the blood: It is one of the types of fat found in the body. Values higher than 250 mg / dL.

• Polycystic ovary syndrome.

• Vascular diseases: a personal history of these diseases.

• The birth of a child with a large weight: a personal history of women, including the birth of a child with a weight greater than 4.1 kg (the weight of the child immediately after birth).

• Gestational diabetes: a personal history of gestational diabetes.

• Hemoglobin glycosylate values: HBA1C greater or equal to 5.7%.

• Glucose tolerance: Impaired glucose tolerance

• Glucose values: those with glucose impairment in the post-fasting examination impaired fasting glucose

When these factors appear - hypertension, hyperglycemia and hyperlipidemia above the normal level - together with obesity (excess weight) a relationship arises, together, with insulin resistance. Factors of gestational diabetes during pregnancy, the placenta produces hormones that help and support pregnancy. These hormones make cells more resistant to insulin. In the second and third trimesters of the pregnancy, the placenta grows and produces large amounts of these hormones that make insulin work harder and make it more difficult. In normal situations, the pancreas reacts by producing additional insulin to overcome that resistance. Sometimes, however, the pancreas is unable to keep up with the pace, resulting in too little sugar (glucose) reaching the cells, while much of it accumulates in the bloodstream. Thus gestational diabetes (diabetes during pregnancy) is formed. Any pregnant woman may develop gestational diabetes, but there are women who are more likely than others.

Mathematical Analysis of Diabetic blood Test

As mentioned in the previous article, there are many important parameters that the evaluation committee relies on. Constraints of independent parameters (X_1 to X_4) are explained below:

The first parameter is the race type: X_1, Female or male.

The second parameter is age in years of the tested person: X_2, $20 < X_2 < 65$.

The third parameter, life type: X_3, rural or countryside.

The fourth parameter, fasting or none fasting case: X_4, Fasting or none.

The dependent parameter Y represents diabetic degree in the blood. The value of Y is classified into ranges 80 to 600 according to the test of the blood. These ranges have standard description usually mentioned in the graduation manuscript. Table (1) contains the dependent, independents, value ranges of parameters [4].

Table (1): Real results are recorded from the tested persons

S	Race	Age years	Life Type	Fasting or None	Result
	X1	X2	X3	X4	Y
1	Female	35	Rural	None	121
2	male	55	Rural	None	114
3	Female	37	Rural	None	95
4	male	25	Rural	None	94
5	Female	20	Rural	None	104
6	male	27	Rural	None	115
7	Female	43	Rural	Fasting	85
8	male	45	Rural	Fasting	96
9	Female	25	Rural	None	102
10	male	35	Rural	None	84
11	Female	40	Rural	None	101
12	Female	45	countryside	None	105
13	Female	85	Rural	Fasting	90
14	male	40	Rural	Fasting	105
15	male	54	Rural	Fasting	102
16	male	28	Rural	None	106
17	male	60	Rural	Fasting	97
18	male	27	Rural	None	102
19	Female	24	Rural	None	104
20	male	22	Rural	None	91

Results and Discussion

Statistical descriptive Table (2) contains four dependent variables (X_1 to X_4) and one dependent variable (Y). It is clear that the mean of the variables in the following column, the standard deviation in the third column while the fourth column contains number sample elements [5]. Table (3) explains the correlation matrix correlates among the four independent variables. When the two elements are associated with a significant of zero that means the correlation number is less than 5% and then gives a strong correlation.

Table (4) is shown the names of the variables that entered the regression equation did not exclude any variables even if they had a trace effect x_2, x_3, x_4.

Table (5) is a very important to correlate the relation between the dependent variables and independent variables as estimated by R = 0.997 and R^2 = 0.994. Independent variables have about 0.99 of variance in the degree of evaluation of the project. The coefficient values and the coefficient of selection are high indicating that the regression equation or prediction is good [6].

The following Table (7) is called the table of coefficients, it contains:

- Variable coefficients that entered the equation in column B.

- Standard deviation for each Std Error column.

- The coefficients of the independent variables that entered the equation after converting them into the standardization scores in the corresponding Beta column for each variable. In the last two columns of this table, the value of the statistic t and the significance level of the beta value test are shown. If the significant value corresponding to any Beta value is less than 0.05 means that the corresponding variable of these values has a significant statistical significance. In this Table (7).Table (9): Compare them with critical variables. Fig. 1: The repetitive histogram is followed by normality.

Fig. 2: Graphic P-P variables are traceable normality. In the Fig.3, (the propagation plate) between the observed values and the estimation errors, we observe the random propagation pattern. This indicates that the relationship between the two variables is linear and that regression analysis conditions are available, but if other data show that the propagation pattern is similar to quadratic or cubic function, Evidence that the relationship between the variants is nonlinear.

Table (2): Descriptive Statistics

Parameters	Mean	Std. Deviation	N
y	65.8750	23.37543	8
x1	5.1250	1.72689	8
x2	7.4375	3.13320	8
x3	7.4375	3.13320	8
x4	7.4375	3.13320	8

Table (3): Explains the correlation matrix correlates among the four independent variables.

		y	x1	x2	x3	x4
Pearson Correlation	y	1.000	.942	.970	.970	.970
	x1	.942	1.000	.965	.965	.965
	x2	.970	.965	1.000	1.000	1.000
	x3	.970	.965	1.000	1.000	1.000
	x4	.970	.965	1.000	1.000	1.000
Sig. (1-tailed)	y	.	.000	.000	.000	.000
	x1	.000	.	.000	.000	.000
	x2	.000	.000	.	.000	.000
	x3	.000	.000	.000	.	.000
	x4	.000	.000	.000	.000	.
N	y	8	8	8	8	8
	x1	8	8	8	8	8
	x2	8	8	8	8	8
	x3	8	8	8	8	8
	x4	8	8	8	8	8

Table (4): Variables Entered/ Removed b

Model	Variables Entered	Variables Removed	Method
1	x4, x1	.	Enter

a. Tolerance = .000 limits reached.

b. Dependent Variable: y

Table (5): Model Summaryb				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.997a	.994	.977	3.51324

a. Predictors: (Constant), x8, x6, x5, x1, x7
b. Dependent Variable: y

Table (6): ANOVAb						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	3800.189	5	760.038	61.577	.016a
	Residual	24.686	2	12.343		
	Total	3824.875	7			

a. Predictors: (Constant), x8, x6, x5, x1, x7
b. Dependent Variable: y

Table (7): Coefficientsa									
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**	**Correlations**		
		B	**Std. Error**	**Beta**			**Zero-order**	**Partial**	**Part**
1	(Constant)	-54.895	20.496		-2.678	.116			
	x1	-29.114	8.099	-2.151	-3.595	.069	.942	-.931	-.204
	x4	.762	2.799	.102	.272	.811	.970	.189	.015

a. Dependent Variable: y

Table (8): Excluded Variables b						
Model		**Beta In**	**t**	**Sig.**	**Partial Correlation**	**Collinearity Statistics**
						Tolerance
1	x2	.a	.	.	.	.000
	x3	.a	.	.	.	.000s
	x4	.a	.	.	.	.000

a. Predictors in the Model: (Constant), x8, x6, x5, x1, x7
b. Dependent Variable: y

Table (9): Residuals Statisticsa					
Residuals Names	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	29.0000	97.6000	65.8750	23.29988	8
Std. Predicted Value	-1.583	1.362	.000	1.000	8
Standard Error of Predicted Value	1.782	3.513	2.965	.728	8
Adjusted Predicted Value	52.0000	94.0000	79.0192	18.78219	4
Residual	-3.77143	2.40000	.00000	1.87791	8
Std. Residual	-1.073	.683	.000	.535	8
Stud. Residual	-1.246	1.414	.127	.815	8
Deleted Residual	-5.07692	12.00000	2.98077	7.55502	4
Stud. Deleted Residual	-1.859	1.183	-.282	1.524	3
Mahal. Distance	.925	6.125	4.375	2.232	8
Cook’s Distance	.004	1.611	.499	.751	4
Centered Leverage Value	.132	.875	.625	.319	8

a. Dependent Variable: y

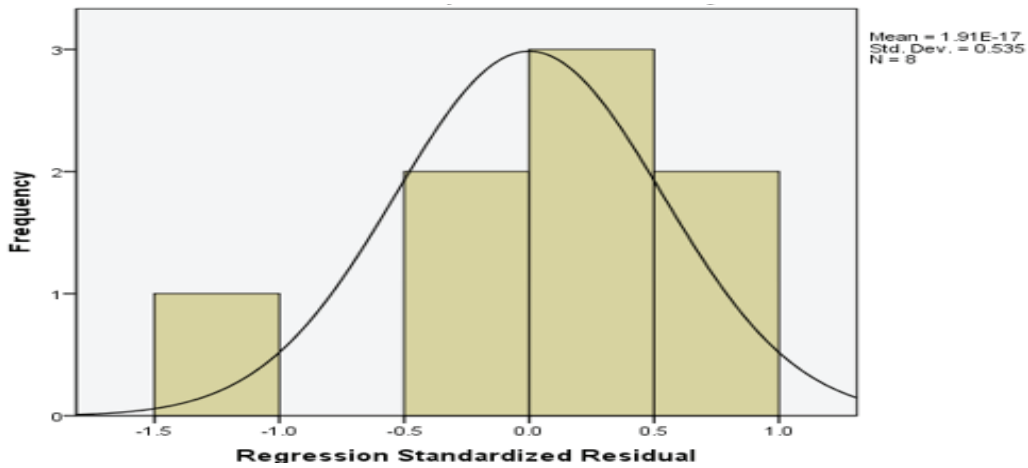

Fig. 1: Histogram of dependent variable y.

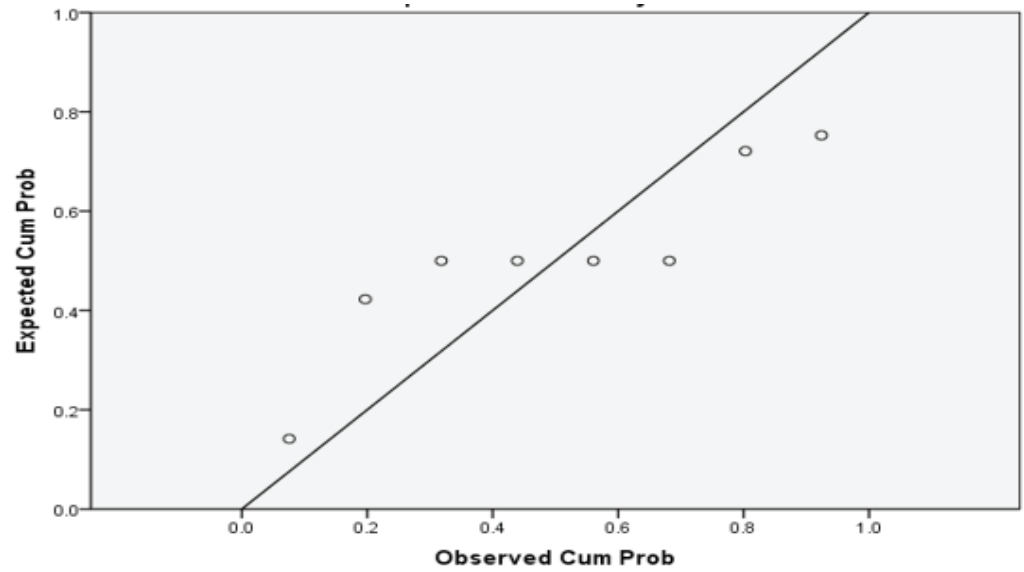

Fig. 2: Normal P-P plot of Regression Standardized Residual of dependent variable y.

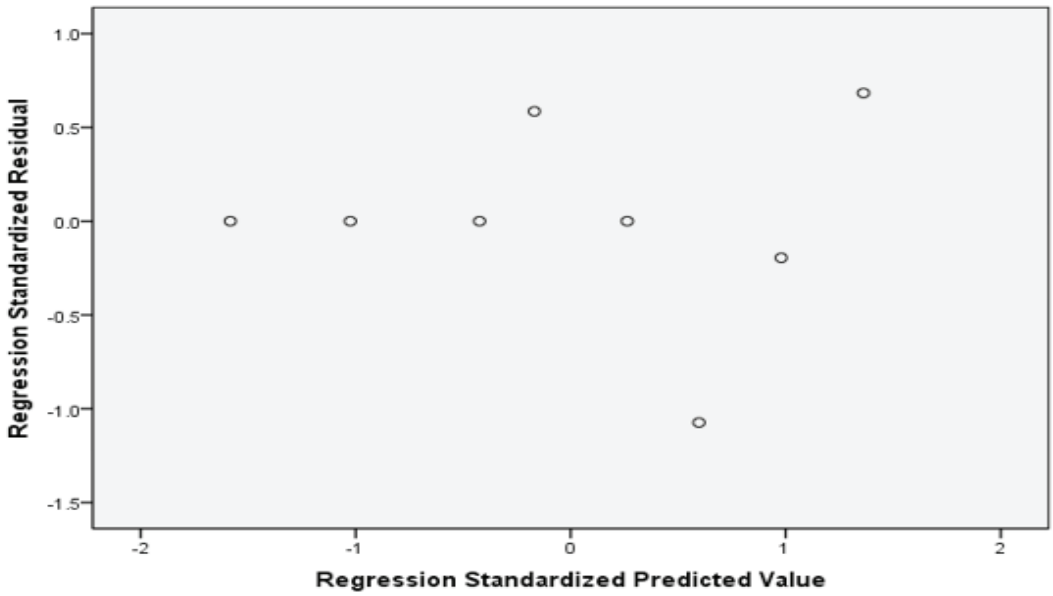

Fig. 3: Scatterplot of dependent variable y.

Conclusion

It is clear from the results presented in the nine tables as well as the four drawings that the independent variables (x_1 to x_4) have varying degrees of impact and effect on the final score value of the tests evaluation. Therefore, we conclude that the variables should be given greater importance in evaluating the test.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Diyala and all experiments were carried out in accordance with approved guidelines.

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Comparison of Oral Isotretinoin vs Azithromycin in the Treatment of Acne Vulgaris

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Abstract

Background: Acne vulgaris is a chronic inflammatory disease of the pilosebaceous follicles, common in adolescents, characterized by comedones, papules, pustules, cysts, nodules, and occasionally scars. This study was designed to compare multi B vitamins, folic acid and homocysteine levels in patients with moderate to severe acne vulgaris with those of the apparently healthy control group and to investigate the effect of isotretinoin and azithromycin treatments on these biochemical parameters and comparison between two drugs.

Methods: This study was conducted on sixty women patients with acne and thirty as a control group. The patients were allocated into two groups, A and B, group A was given 500 mg of azithromycin taken on alternative days for 3 months and group B was given 40mg/day isotretinoin for 3 months.

Results: The study showed significant differences ($p<0.05$) in serum levels of vitamins B_6, B_7, B_{12} and folic acid, while no significant difference in serum homocysteine in patient group when compared to control group. The results showed significant positive correlation between serum vitamins and clinical score and significant difference were detected in both group when compared between pre and post treatment.

Conclusions: The results indicate higher pre-treatment vitamin B_{12} levels, versus the control group and positive correlation between clinical score and vitamin B_{12}, support the assertion that vitamin B_{12} plays a role in the etiopathogenesis of acne vulgaris. Both azithromycin and isotretinoin were effective in acne treatment but azithromycin has less side effects.

Key-words: *Acne; azithromycin; isotretinoin; vitamin B_7; vitamin B_{12}*

Introduction

Acne is one of the chronic inflammatory diseases for pilosebaceous units. It can be characterized by seborrhea, erythematous pustules and papules and in more cases of severe nodules, the closed and open comedones formation, pseudocysts and deep pustules. Inflammatory lesions are followed by scarring [1]. In latest years, therapy of combination has become a vital part of acne treatment. There are different treatment modalities to acne vulgaris including systemic and topical drugs, depending on severity [2].

Isotretinoin (13cis-retinoic acid) Fig. 1-7 is a vitamin A derivative frequently given over a 20-week course (16–24 weeks), with a dosage of 0.5–1 mg/kg/day, depending on clinical response and side effects [3]. It has significant results in sebum production reduction, decreasing in surface and ductal *P. acnes,* influences comedogenesis and shows properties of anti-inflammatory [4]. Azithromycin is one of the antibiotics that have been prescribed, for treatment of acne which is as effective as doxycycline and minocycline [5]. Azithromycin belongs to the azalide group of antibiotics and is closely related structurally to macrolides like erythromycin [6]. This study was undertaken with the main objective of moderate to severe AV treatment,

and to investigate the efficiency and side effects of azithromycin and isotretinoin and compared between them.

Material and Methods

This is a randomized, case-control study that was carried out in the dermatology unit at the Al Imamain Alkadhimain Teaching Medical City/ Baghdad to compare the side effects between 500 mg of azithromycin taken in alternative days and 40mg/ day isotretinoin for 12 weeks of treatment. All patients having moderate to severe acne as categorized by the Global Acne Grading Score (GAGS) were clinically diagnosed and included in the study. The samples were collected within eight months starting from September 2016 until the end of May 2017. Serum samples were collected from 60 Iraqi women patients aged between (15 -35 years old) and were investigated with moderate to severe acne. Control group was consisted of 30 apparently healthy individuals. Cases were excluded if they had any of the following criteria: azithromycin hypersensitivity, renal disease, liver disease, diabetes mellitus, metabolic impairment pregnant women or under multi-vitamin supplementation. The patients were allocated into two groups, A and B, group A was given 500 mg of azithromycin taken on alternate days for 3 months and group B was given 40mg/day isotretinoin for 3 months. Together with the systemic drug, patients were given 5% topical benzoyl peroxide gel to apply to the affected areas of the face and trunk once daily after the skin had been cleansed and dried. Patients were examined at baseline, 6 weeks and 12 weeks, in order to evaluate their clinical improvement and to measure several biochemical parameters. Patients were educated concerning compliance, side effects and constant follow up. The study was accomplished with the institutional review board agreement and written consent was taken from all patients.

Venous blood about 5 ml was drawn from the patients each time before starting treatment, at 6 weeks and at the end of 12 weeks of treatment. The blood was allowed to clot for at least 10-15 min at room temperature, centrifuged for 10 min at 4000xg. Serum was removed and divided into two parts, the first to measure homocysteine by ELISA kit and the other part was deproteinized and stored at - 18 ^{0}C until the time of HPLC assay to measure several vitamin concentrations.

Serum levels of vitaminB_6, vitamin B_7, vitaminB_{12} and folic acid were measured by HPLC method [7]. While serum homocysteine was measured by sandwich enzyme-linked immunosorbent assay (ELISA) kit (Kono Biotech. Co. China).

Results

The results showed that were significant differences ($p<0.05$) in serum levels of vitamins B_6, B_7, B_{12} and folic acid, while no significant difference in serum homocysteine in patient group when compared to control group as listed in table 1. Application of Person correlation test revealed significant positive correlated of vitamin B6 with clinical score in AV patient (r=0.38, P=0.002). There was a significant positive correlation between serum vitamin B12 and clinical score in AV patient (r=0.43, P=<0.001) and also positive correlation between folic acid and clinical score in AV patients (r=0.37, P=0.004) as listed in Table 2.

The results showed there were significant difference- in the two groups- ($p<0.05$) when compared with baseline readings (pre-treatment) of serum folic acid, vitamin B_{12}, vitamin B_7, vitamin B_6, homocysteine and clinical score and those after 12 weeks of treatment (post-treatment) as listed in the table 3 and table 4. Comparison between azithromycin and isotretinoin after 12 weeks of treatment in the two groups (A and B) was listed in table 5.

Discussion

The current results showed a significant decrease in serum folic acid and vitamin B_6 in the patient group compared with the group of control, while there was a considerable elevation in serum vitamin B_7 and vitamin B_{12}. Even a marginal deficiency of vitamins B, B_2, B_{12}, niacin, biotin, pantothenic acid, folic acid, vitamin C, vitamin E, or essential fatty acid that could result in reduced development of skin cells, which manifests itself in the skin which is less elastic, less smooth, more prone, to accelerated aging and prone, to lesions [8]. Elevation in serum vitamin B_{12} in acne patients due to the correlation between the severity of acne and vitamin B_{12} that proved in this study. Also result showed a slight increase in serum homocysteine in patients, but there was no significant difference observed as compared with

control group and these results disagree with Jiang *et al.* that showed significantly elevated serum homocysteine levels in patients with severe and moderate acne compared with the control group [9]. However, it can be reported that different vitamin supplements (the most prominently are vitamin B_{12}), could exacerbate existing acne and/or lead to the development of acneiform eruptions [10]. After administration with isotretinoin, the results showed a reduction in the serum level of folic acid and vitamin B_{12}, while anincrease in the serum homocysteine level after 3 months of treatment. With isotretinoin, this results in agreement with several studies [11, 12, 13], while disagreeing with [14]. Folic acid and vitamin B_{12} (and their interrelated metabolism) are essential vitamins in different metabolic pathways (including the homocysteine pathway). Folic acid and vitamin B_{12} are methyltransferase enzyme cofactors and with their deficiency, hyperhomocysteinemia is noted [12]. In addition, isotretinoin may also affect cystathionine- β-synthase, the enzyme responsible for homocysteine metabolism in the liver and may lead to hyperhomocysteinemia. This hyperhomocysteinemia, as an isotretinoin side effect, might contribute to the missing link between isotretinoin and neuropsychiatric disorders [15]. The previous study concluded that homocysteine level elevation in spite of the normal responsible vitamins values for the amino acid metabolism powerfully suggested that the drug might affect cystathionine-β-synthase, acting as enzyme inhibitor or liver dysfunction which is caused by isotretinoin [13], Karadag *et al.,* are of the opinion that when the treatment with isotretinoin greater cumulative doses lasts longer, it might cause subclinical folic acid and vitamin B_{12} deficiencies that have more effects on the homocysteine level [11]. The exact isotretinoin mechanism action on folic acid reduction is still unknown. Javanbakht *et al.,* suggest that it is probable that isotretinoin interacts with some essential groups in the active site of important proteins or enzymes at folic acid metabolism. This interference might be initiated by the changes in the intestinal absorption of folic acid, different steps of formation tetrahydrofolate from folic acid, induction of enzymes in the liver and finally depletion of folic acid [16], Hilal et al., also propose that using isotretinoin for a long-term may decrease the absorption of vitamin B_{12} and intestinal folic acid [12].

Long-term use of isotretinoin in higher doses affects hair growth and is associated with increased hair loss [17]. The current study showed a decreased level of vitamin B_7 when compared with pre-treatment values, and supports the assertion that isotretinoin leads to hair loss as its side effects.

Increase concentration of vitamin B_6 post-treatment versus the pre-treatment may be due to increasing homocysteine level that leads to increase the conversion of homocysteine to cystathionine in the presence of vitamin B_6 dependent enzyme cystathionine-β-synthase and then cystathionine convert to cysteine and α-ketobutyrate via cystathionine-γ-lyase (vitamin B_6 dependent enzyme) [18].

For our information, the current study is the first one achieving changes in serum vitamin B_7 and vitamin B_6 levels in patients receiving isotretinoin treatment.

After administration with azithromycin, the result showed a significant decrease in serum folic acid and vitamin B_7 may be due to the effects of azithromycin on the absorption of these vitamins, while there was a significant increase in serum vitamin B_{12}. The increase in the serum vitamin B_{12} in the current study may be due to azithromycin effects on one of the mechanisms that lead to increase vitamin B_{12} concentration. This study shows a significant elevation in serum vitamin B_6 which may be due to the relation between vitamin B_6 deficiency and increased facial seborrhea [19] so, the drug may increase the absorption of this vitamin to decrease the facial seborrhea in order to minimize sebum production.

To our knowledge, this is the first study investigating changes in serum vitamin levels in patients with AV that were treated with azithromycin and therefore we could not compare our results with any such studies.

The current study showed a significant increase in serum homocysteine and vitamin B_7 in patients under isotretinoin treatments when compared to those under azithromycin treatment after the same duration (12 weeks), while there was a significant decrease in serum folic acid, vitamin B_{12} and vitamin B_6, this result can indicate wide side effects range of isotretinoin when compared to azithromycin, however the effect of isotretinoin on acne lesion is greater than the effect of azithromycin. In spite of the clinical score of

azithromycin being less than that of isotretinoin because the clinical score in isotretinoin group at the baseline was greater than that of the azithromycin group and also the duration of treatment with isotretinoin will continue 5-6 months so, at the end of treatment the lesion was almost clear.

Conclusions

Higher pre-treatment vitamin B_{12} levels, versus the control group and positive correlation between clinical score and vitamin B_{12}, support the assertion that vitamin B_{12} plays a role in the etiopathogenesis of acne vulgaris. Isotretinoin in dose 40mg/day or azithromycin in dose 500mg on an alternative day was effective in the treatment of patients with moderate to severe acne, but the results indicated that a wide range of side effects -of isotretinoin when compared to azithromycin although, the isotretinoin was more effective and can decrease vitamin B_{12} levels.

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The Association between Serum Nesfatin-1 level and BMI in Iraqi patients with Polycystic Ovary Syndrome (PCOS)

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Abstract

Objective: to evaluate the nesfatin-1 levels in addition to other metabolic components in polycystic ovary syndrome PCOS in a state that to be associated with both functions of ovary and obesity.

Methods: A cross-section observational study was carried on total of forty-five (45) women in the reproductive age with polycystic ovary syndrome (23 obese, 22 non-obese) attending Infertility unit and outpatients clinic of Gynecology and Obstetrics at AL-Yarmouk Teaching Hospital, in addition to forty (40) (30 obese, 10 non-obese) age matched healthy individuals. Serum nesfatin-1, TC, TG, HDL-C, FPG, FPI, E_2, TT, LH, FSH and Prol were measured, in addition to HOMA-IR, BMI and LDL-C was calculated.

Results: FPG (5.1±0.3 mmol/L), HOMA-IR (2.8±1.1) were significantly higher in PCOS group than their results in the control group (4.8±0.2 mmol/L), (2.2±1.3) ($P < 0.05$, 0.01) respectively. Nesfatin-1 level (11.1±3.5 ng/ml), TT (1.7±0.3 pg/ml) and LH (6.5±2.8 mIU/ml) in PCOS group were significantly difference ($P < 0.001$, 0.05) respectively, when compared their concentration in the control group (6.3±3.0 ng/ml), (0.9±o.1 pg/ml), (4.9±1.5 mIU/ml). Among the patients with PCOS, a strong positive correlation were found between nesfatin-1 and WC ($r=0.221$, $P < 0.05$) , BMI ($r=0.267$, $P < 0.001$), TT ($r=0.218$, $P <0.001$), LH ($r=0.263$, $P< 0.001$), IR ($r=0.453$, $P < 0.001$), FBG ($r=0.257$, $P < 0.01$), LDL-C ($r=0.228$, $P < 0.05$) and TG ($r=0.223$, $P < 0.05$). Besides that, a positive association were between HOMA-IR and BMI ($r=0.460$, $P < 0.01$), WC ($r=0.429$, $P <0.001$).

Conclusion: nesfatin-1 gives the impression to play an important action to regulate food intake and energy homeostasis with a strong positive correlation with WC, BMI, IR, LDL-C and TG.

Keywords: *Polycystic Ovary Syndrome (PCOS), Nesfatin-1, BMI, Iraqi patients.*

Introduction

The polycystic ovary syndrome (PCOS) is defined as a hyper androgenic impairment accompanied by chronic oligo-anovulation and polycystic ovarian morphology at ultrasound [1,2,3]. It may be associated with psychological disorders mainly depression and other mode disorders, in addition to metabolic abnormality mainly insulin resistance and compensatory hyperinsulinemia which may be responsible factor for the change of androgen production and metabolism [4]. About 5-10% of young women is affected by PCOS, which represent the usual cause of female infertility. The major number of the women with PCOS are either overweight or obese [4, 5, 6], which may enhance androgen secretion, while diminishing the metabolism reproductive functions with possibly favouring the development of PCOS phenotype [7].

Nesfatin-1 is a neuropeptide derived from the precursor nucleobin-2 ($NUCB_2$) that involve the regulation of feeding and energy metabolism [8,9]. Nesfatin-1 was first discovered by Ch-1 et al 2006 whom described it as a peptide consisting of 82 amino acids[10], consisting of three parts. The first part which is called N-23, starting from the N- terminal end and continues till the 23th amino acids, while the second part is called M 30 starting from the 24th amino acid tells the 53th

amino acid, this part is responsible for the food intake and appetite control [11,12]. Beside that the third part is defined as C29 whom contains the last 29 amino acid from the 53th to the C- terminal end [13].

Several regions of the hypothalamus and peripheral tissues, mainly the adipose tissue, gastric mucosa and pancreatic beta cells are responsible for nestafin-1 expression [12] with half-life of 6 hours and low molecular weight [14].

Patients and Methods

A cross section observational study was done on a total of forty-five (45) women in the reproductive age period with polycystic ovary syndrome with a range age of (25-35 year) participated in this study. They were selected from the patients attended the infertility unite and outpatient clinic of gynecological department of Al-Yarmouk Teaching Hospital, in Baghdad, Iraq, from January 2013 to January 2014.

Diagnosis of PCOS was based on the inclusion criteria: the Rotterdam classification was applied to define PCOS with two or more of three criteria: oligo or anovulation, clinical, and/ or hyperandrogenism beside ultrasound appearance of polycystic ovaries (>12 follicles in each ovary of 2-9mm size)[15].The exclusion criteria: included all the other causes of hyperandrogenism such as Virilising tumor, Congenital adrenal hyperplasia, Cushing syndrome and Prolactinoma.

Those subjects were compared with forty (40) apparently healthy group, selected from the subjects attended the same clinics presenting with non-specific pathology according to the physical examination and laboratory findings, with range age of (25-35 years). Informed consent was taken from each subject from the patients and control groups.

From 10-12 hours fasting subjects' groups, about 10 ml peripheral venous blood was drawn in plastic tube at room temperature, the blood was left to clot, and serum was separated after centrifugation at (3000rpm) to about (30 minutes) and divided in plastic tubes into aliquots and stored at (-20^{0}C) until the time of analysis for: -

· Fasting Plasma Insulin (FPI) chemiluminescent (Diasorine . Italian company).

· Fasting plasma glucose (FPG), done directly after separation.

· Total serum cholesterol (TC, triglycerides TG)

· Serum HDL-C

(FBG, TC, TG and HDL-C) done by auto analyzer Kenza 240 TX, Ranch company.

· LDL-C= TC- (HDL-C+ TG /5) Friedewald et al 1972[16].

· Very low density lipoprotein (VLDL-C)= TG/5. Levels of E_2 (Estradiol), FSH (Follicle- Stimulating Hormone, LH (Luteinizing Hormone), TT (Total Testosterone) was estimated by Minividas (Biomeriuxe French Company).

· Nestafin-1 concentration was estimated by using enzyme-linked- immunosorbent assay (ELISA) method. China.

· Body Mass Index (BMI) was estimated as the weight in kilogram (Kg) per height in meter square(m^2) (National Institute of Health 1998) [17]:

$$BMI\ (Kg/m^2) = Weight\ (Kg)/\ Height\ (m^2)\ .$$

· Homeostatic model assessment as index of insulin resistance (HOMA-IR) was calculated according to the equation of Wallace etal 2004[18]:

$$HOMA\text{-}IR = (FPI \times FPG) / 22.5$$

When FPI is defined as Fasting plasma insulin concentration (μIU/ml) and FPG is fasting plasma glucose (mmol/L).

Results

Table (1) show there were no statistically significant differences between the PCOS and control groups regarding the age (29.3±5.7 yr)(29.5± 5.2 yr), BMI (30.1 ± 4.2 Kg/m^2)(29.3±5.1 Kg/m^2),waist (96.3 ± 12.7 cm) (94.2±15.9 cm), hip (95.9 ± 1.1 cm)(92.1± 3.8 cm) and waist/hip ratio (0.78) (0.77).

The FPG (5.1±0.3 mmol/L), HOMA-IR (2.8±1.1) were significantly higher in PCOS group than their results in the control group (4.8±0.2 mmol/L) (2.2 ± 1.3) ($P < 0.05$, 0.01) respectively, while FPI show no significant different between the PCOS(11.1±1.8 μIU/

ml) and control group(10.9 ±1.7 µIU/ml), table(2).

In addition to that, the concentration of Nesfatin-1 (11.1±3.5 ng/ml), TT (1.7±0.3 pg/ml), and LH (6.5 ±2.8 mIU/ml) for PCOS group show significantly difference(P< 0.001, 0.05,

Table (1): Clinical characteristics for PCOS and control groups

Subjects	PCOS (n=45)	Control(n=40)	P value
Age(years) (Mean±SD)	29.3± 5.7	29.5 ± 5.2	N.S
BMI(Kg/m2) (Mean±SD)	30.1± 4.2	29.3 ± 5.1	N.S
Waist circumference (cm) (Mean±SD)	96.3 ± 12.7	94.2 ± 15.9	N.S
Hip (cm) (Mean±SD)	95.9 ± 1.1	92.1 ± 3.8	N.S
Waist / Hip Ratio	0.78	0.77	N.S
N.S: non significance			

0.05) respectively, when compared their

Table (2): The(Mean±SD) values of fasting plasma glucose (FPG), fasting plasma insulin (FPI),

Subjects	PCOS (n= 45)	Control (n=40)	P value
FPG (mmol/L) (Mean±SD)	5.1 ± 0.3	4.8 ± 0.2	0.05
FPI (µ IU/ml) (Mean±SD)	11.1 ± 1.8	10.9 ± 1.7	N.S
HOMA-IR (Mean±SD)	2.8 ± 1.1	2.2 ± 1.3	0.01

Table (3): Nesfatin-1, Total testosterone (TT), Estradiol(E_2), Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL) for PCOS and control groups.

Subjects	PCOS (n= 45)	Control (n=40)	P value
Nesfatin-1(ng/ ml) (Mean±SD)	11.1± 3.5	6.3 ± 3.0	0.001
TT (pg/ ml) (Mean±SD)	1.7 ± 0.3	0.9 ± 0.1	0.05
E2 (pg / ml) (Mean±SD)	45.2 ± 10.5	46.8 ± 7.2	N.S
FSH(mIU / ml) (Mean±SD)	4.8 ± 1.5	4.6 ± 1.4	N.S
LH(mIU / ml) (Mean±SD)	6.5 ± 2.8	4.9 ± 1.5	0.05
PRL(ng / ml) (Mean±SD)	19.6 ± 5.8	17.9 ±6.2	N.S

concentrations with the control group (6.3±3.0 ng/ml), (0.9±0.1 pg/ml) (4.9 ±1.5 mIU/ml) table(3).

In contrast to that, there were no significant difference between the results of both groups in case of E_2 (45.2±10.5 pg/ml)(46.8±7.2 pg/ml), FSH(4.9±1.5 mIU/ml)(4.6±1.4 mIU/ml) and PRL(19.6±5.8 ng/ml)(17.9±6.2 ng/ml), table (3).

Table (4): Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C), very low density lipoprotein (VLDL-C) and low-density lipoprotein (LDL-C) for PCOS and control groups.

Subjects	PCOS (n= 45)	Control (n=40)	P value
TC(mg / dL) (Mean±SD)	190 .5 ±18.6	183.9 ± 24.1	N.S
TG(mg / dL) (Mean±SD	115.8 ± 25.9	109.1 ± 29.8	N.S
HDL-C(mg / dL) (Mean±SD)	47.7 ± 8.3	48.6 ± 9.1	N.S
VLDL-C(mg / dL) (Mean±SD(	23.1 ± 5.2	21.8 ± 7.2	N.S
LDL-C(mg / dL) (Mean±SD)	112.2 ± 27.1	111.3 ± 25.9	N.S

Table(4) shows no significant differences between the results of lipid profile (TC, TG, HDL-C,VLDL-C and LDL-C) between the PCOS and control groups. (190.5±18.6 , 183.9±24.1 mg/dl), (115.8±25.9 , 109.1±29.8 mg/dl), (47.7±8.3 , 48.6±9.1 mg/dl), (23.1±5.2 , 21.8±7.2 mg/dl) and(112.2±27.1 , 111.3±25.9 mg/dl).

A strong positive correlation were found between nesfatin-1 and WC (r = 0.221, P<0.05), BMI(r = 0.267, P< 0.001), TT (r = 0.218 , P< 0.001), LH (r = 0.263, P < 0.001), IR (r = 0.453, P < 0.001), FPG (r = 0.257, P < 0.01), LDL-C (r = 0.228 , P < 0.05) and TG (r =0.223, P <0.05) , in addition to that, a positive association were found between HOMA-IR and BMI (r =0.460 , P < 0.01), WC (r = 0.429 , P < 0.001) among the patients with PCOS.

Discussion

Both brain and peripheral tissues strictly control the reproductive system and many mediators take part in these communications. One of these messengers are anorexigenic peptides which their levels in subjects with PCOS are not widely known leading to variable results in literatures.

Many researches have been suggested that nesfatin-1 has a direct influence on the obesity, through food intake and glucose metabolism, weight loss and cardiac functions. [19] On a study done by Algual et al, serum nesfatin-1 level was reported to be (0.885±0.01 ng/ml) in subjects with metabolic syndrome, while it's level was (1.09±0.07 ng/ml) in the control group. [20] In another exciting study and due to the exposure of the rates with metabolic syndrome to fructose, nesfatin-1 concentration in both male and female rates is higher than their levels in control group [21], while another research done in two groups of patients suffered from Obstructive Sleep Apnea Syndrome (OSAS), to evaluate the nesfatin-1 levels, the first group with metabolic syndrome it's level was low as (3.97±1.42 pg/ml) in comparison to its level in the second group who were without metabolic syndrome (4.98±1.84 pg/ml), the above data decided that nesfatin-1 had a main action on the metabolic syndrome pathogenesis.

Several studies revealed that hyperglycemia, IR and Hyperlipidemia cause endothelial dysfunction and

leading to atherosclerosis. ([23,24]) This study shows that IR, FPS, LDL and TG, have a strong positive association with an elevated concentration of Nesfatin-1, strictly those with IR and obesity, and the controversial data of this study confirm the above and agree with other researches ([25,26,8]) and disagree with others ([27,5,20])

According to BMI cut-off value of 30 Kg/m^2 the results of subgroups of both PCOS and controls revealed that nesfatin-1 concentration in both obese PCOS (n=23) (11.3±4.2 ng/ml) and non-obese PCOS subgroup (n=22) (8.5±1.2 ng/ml) were higher than their levels in the obese control (n=21) (7.2±2.2 ng/ml) and non-obese control subgroup (n=19)(5.1±2.4 ng/ml).

Besides that, and according to the HOMA-IR cut-off value of 2.7, the results revealed that the nesfatin-1 levels in PCOS subgroup with HOMA-IR < 2.7 (n=25) (8.1±3.6 ng/ml) and those PCOS subgroup with HOMA-IR ≥ 2.7 (n=20)(12.9±1.6 ng/ml) were higher than their values in control subgroup with HOMA-IR < 2.7 (n=30) (6.4±2.6 ng/ml) and control subgroup with HOMA-IR ≥ 2.7 (n=10)(6.7±2.5 ng/ml).Increase production of nesfatin-1 in those obese patients and high IR could be a defensive mechanism against hyperglycemia and obesity.

Conclusion

nesfatin-1 gives the impression to play an important action to regulate food intake and energy homeostasis with a strong positive correlation with WC, BMI, IR, LDL-C and TG.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine and all experiments were carried out in accordance with approved guidelines.

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Detection of Sero-positive Anti- toxoplasmosis Anti-bodes IgG among Women in Baghdad Province

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Abstract

A total of 83 serum samples were randomly collected from different centers in Baghdad province, the results recorded in this study which started in July 2018 until the end of November 2018, by using one technique, method ELISA. The results were showed increased the percentage of seropositive anti- toxoplasmosis IgG antibodies at age groups 20 – 29 years, which were 23.9% among women infected with toxoplasmosis comparative with other age groups. Also showed increase the percentage of sero-positive anti- toxoplasmosis IgG Abs in urban which were 42.9% comparative with rural, which were 8.0%. Also occur increased significantly in percentage IgG Abs in serum of affected women with toxoplasmosis in first and second month from abortion period, which were respectively 17.8% and 16.0%. So increase these antibodies (Abs) in serum of infected women with this disease in 1st trimester period comparative with other months. Therefore so it was necessary to use of early diagnosis for pregnant infected women with toxoplasmosis.

Key word: *Anti-toxoplasmosis antibodies (Abs), Sero-positive, IgG, ELISA, trimester period.*

Introduction

Toxoplasmosis was present in all countries and sero-positivity rates range from less than 10% to over 90% [1]. The causative agent, *Toxoplasma gondii*, has a complex life cycle and an important foodborne pathogen. Human infection can result from the ingestion or handling of undercooked or raw meat contains tissue cysts. Alternatively, infections can result from direct contact with cats or from the consumption of water or food contaminated by oocysts excreted in fasces of infected cats [2]. The infection with the protozoan parasite *Toxoplasma gondii* has a worldwide distribution. This obligate intracellular parasite could infect humans as well as virtually all warm-blooded animals, including mammals and birds. Since its first description in the gondi, a rodent from North Africa, by Nicolle and Manceaux in 1908 [3], the parasite was progressively recognized as the agent of a widespread zoonosis. However, was entire life cycle which definitively understood only in the late 1960s [4], with the discovery of the central role of the cat as a definitive host harboring the sexual parasitic cycle and spreading oocystes through faeces. In the same period of time, it was classified in the coccidian subclass [4], phylum Apicomplexa, and the infectivity in three parasitic stages: chyzoite, cyst and oocystes were well characterized [5]. Also the *Toxoplasma* infection among pregnant women when exposure to infections risk, age and pregnancy-related risk factors [6]. The most common cause of spontaneous abortion during the first trimester is chromosomal abnormalities of the embryo/fetus [7]. But a counting for at least 50% of sampled early pregnancy losses [8]. Other causes include vascular disease (such as lupus), diabetes, other hormonal problems, infection, and abnormalities of the uterus [8]. Advancing maternal age and a patient history of previous spontaneous abortions were leading factors associated with a greater risk of spontaneous abortion [7]. A spontaneous abortion can also be caused by accidental trauma intentional trauma or stress. The cause miscarriage is considered induced abortion or feticide [9]. Therefore the aims of this study to estimate the rate of toxoplasmosis infection among women in Baghdad province to detect specific *T. gondii* Abs IgG, and their effect on the different stages of disease.

Materials and Methods

Form First of July 2018 till the end of November 2018, about 83 total blood samples were collected from females, with age ranging about 16-40 years attended to Baghdad province. Before collection of samples, an information sheet was prepared and designed according to questionnaire which covers all information; 3ml of venous blood was dawned by using disposable Syringes, and then centrifuged at 3000 for 10 minutes to get serum for serological test by ELISA technique to detect IgG. While the calculating the toxoplasmosis IgG of each determination by dividing the mean value of each sample by calibrator value.

Procedure

1- Only remove the required reagents form the refrigerator and allow them to come to room temperature for at least 30 minutes.

2- Use one TXG strip and one TXG SPR for each sample, control or calibrator to be tested. Make sure the storage pouch has been resealed after the required SPR, have been removed.

3- Type or select "TXG "to enter the test code. The calibrator must be identified by "S1" and tested in duplicate. If the positive control is to be tested if should be identified by "C1 "If the negative control need, to be tested, it should be identified by C_2

4- Mix the calibrator, control and samples using a vortex – type mixer.

5- Pipette 100 ml of calibrator, samples or control into the sample well.

6- Insert the SPR, and strips into the instrument. Check to make sure the color tables with the assay code on the SPRs and the Reagent strips match.

7- Initiate the assay as directed in the operation's Manual. All the assay steps are performed automatically by the instrument. The assay will be completed within approximately 40 minutes.

8- After the assay is completed. Remove the SPRs and strips from the instrument.

9- Dispose of the used SPRs and strips into an appropriate recipient.

The Results

In figure (1) the results recorded highly significant difference ($p < 0.001$) of age groups (20-29) years, which were 39 (23.9 %) from 83 examination samples, comparative with other age groups: figure (1).

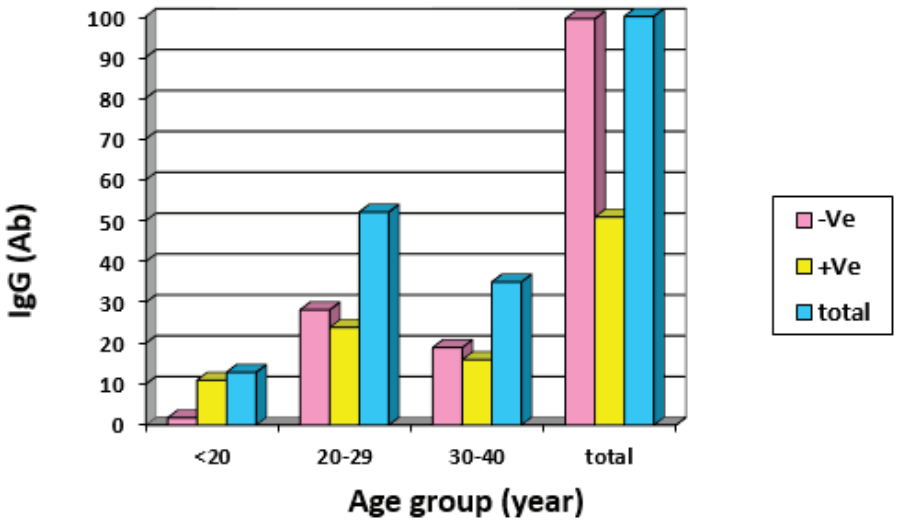

Figure (1): The Relationship between Age groups and IgG antibodies (Abs)

But the relationship between residency and anti-toxoplasmosis IgG Abs was highly significant differences (P> 0.05), because the results in the figure (2) recorded high infection them in urban and rural, which were 70 (42.9%) and 13 (8.0 %) respectively.

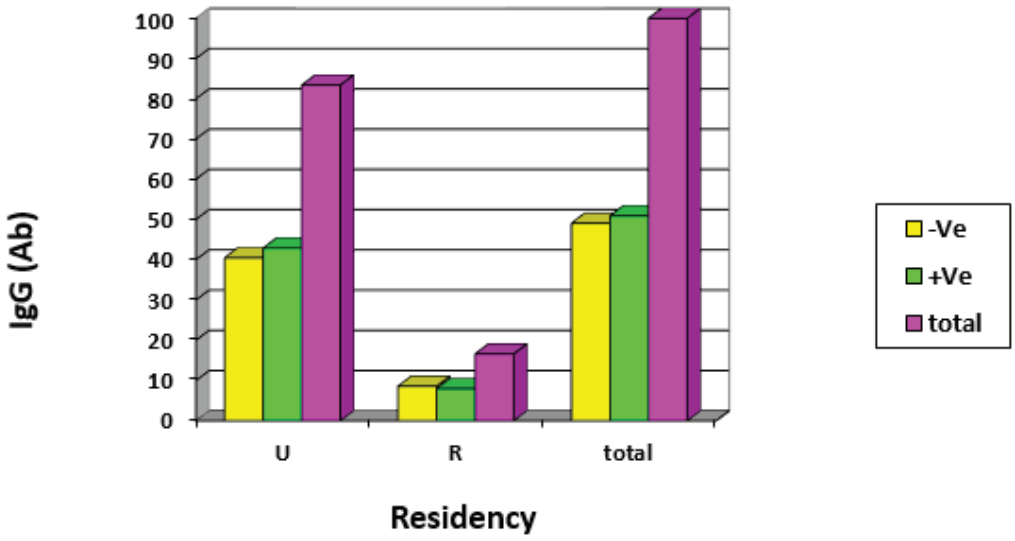

Figure (2): The Relationship between residency and IgG antibodies (Abs)

The figure (3), showed the relationship between gestational period and sero- positive anti toxoplasmosis IgG Abs among normal and abnormal in Baghdad province , this relationship was non–significant difference (P.V.=0.5), but high percentage was recorded for non-pregnant women, which were 75(46.0%) from 83 (total number) as an examination samples.

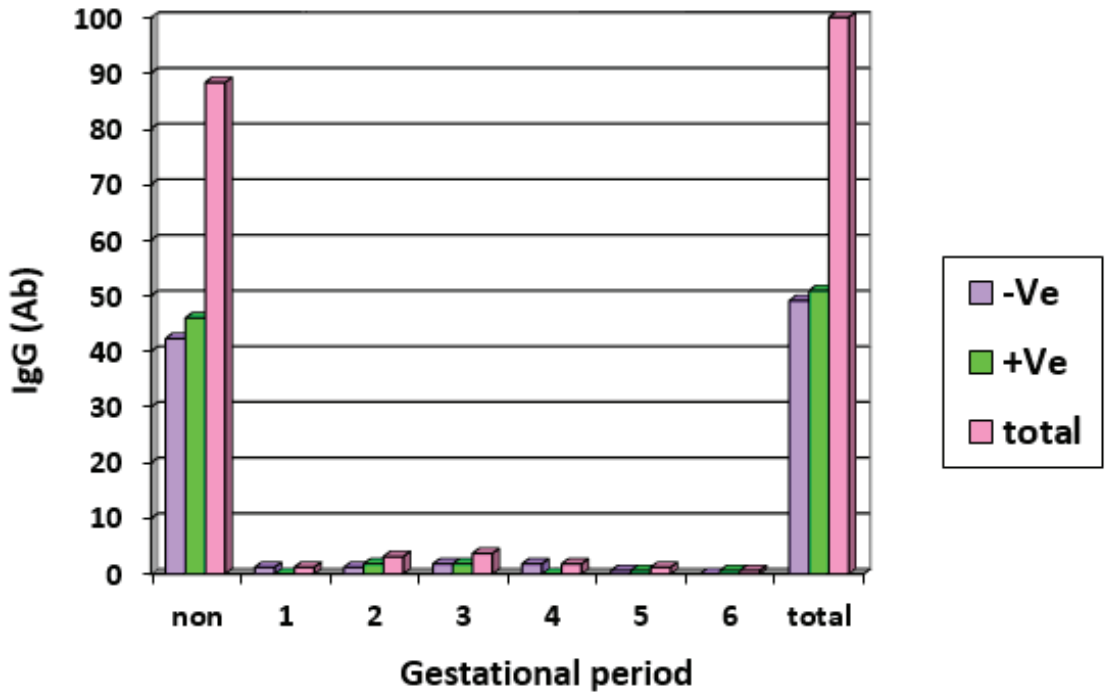

Figure (3): The Relationship between gestational period and IgG antibodies (Abs)

But the figure (4), showed non-significant differences (P.V. =0.5) for relationship between habitual abortion period among normal and abnormal women with sero-positive anti-toxoplasmosis IgG Abs (infection with toxoplasmosis), but the high infection them with toxoplasmosis was recorded 29 (17.8%) and 26(16.0%) respectively for 1st trimester period comparative with other months.

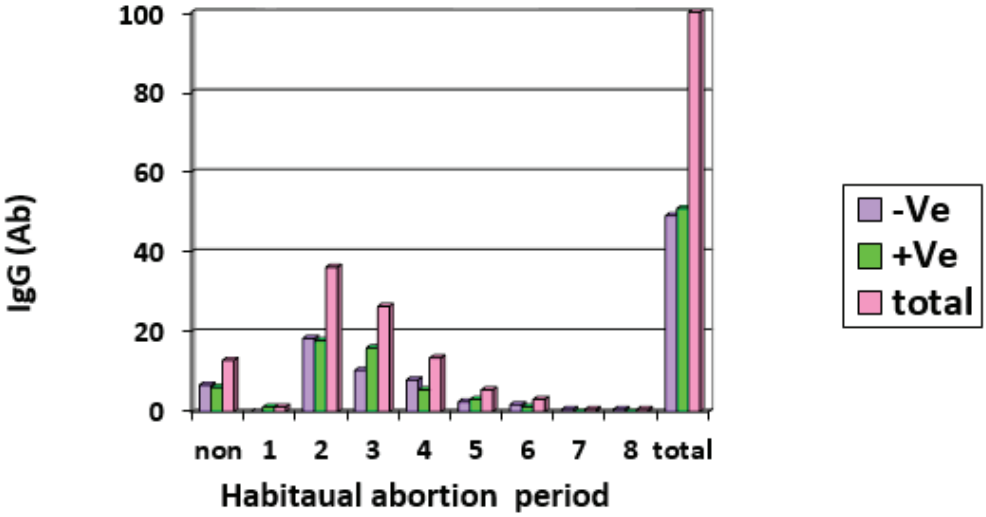

Figure (4): The Relationship between habitual abortion period and IgG antibodies (Abs)

In the other hand this study pointed high significant differences (P.V.=0.5) for relationship between habitual pregnant period and sero-positive anti- toxoplasmosis IgG Abs in infection women, hence recorded high number and percentage for IgG Abs in 1st trimester period, which were in the first month 28 (17.2 %), 2nd month 21 (12.9%) and 3rd month 13 (8.0%). figure (5).

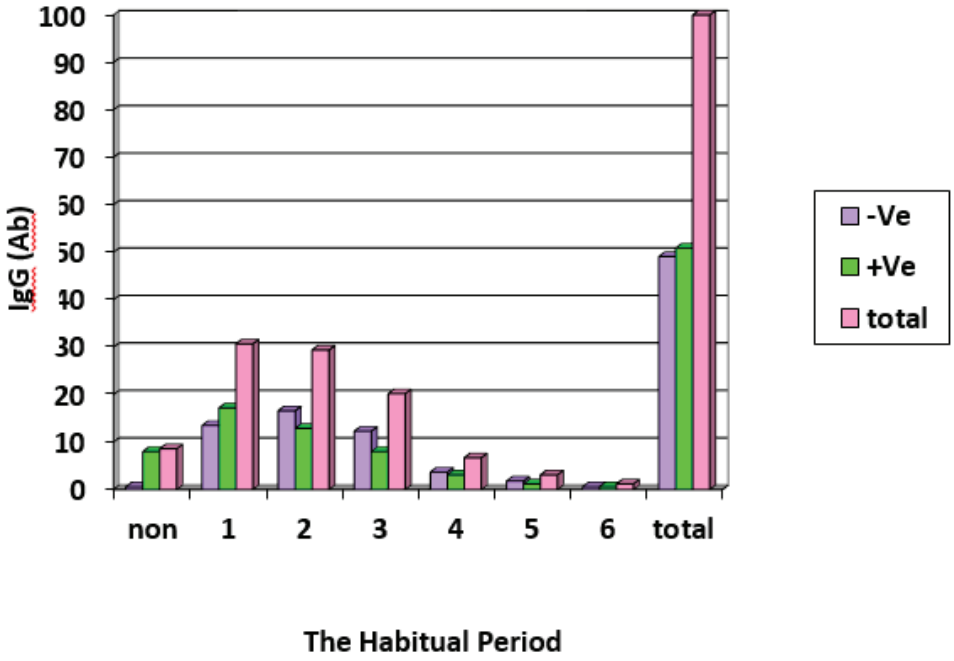

Figure (5): The Relationship between habitual pregnant period and IgG antibodies (Abs)

In figure (6) showed the relationship between previously abortion periods. This study to infection women with this protozoan parasites and IgG Abs was non-significant differences.

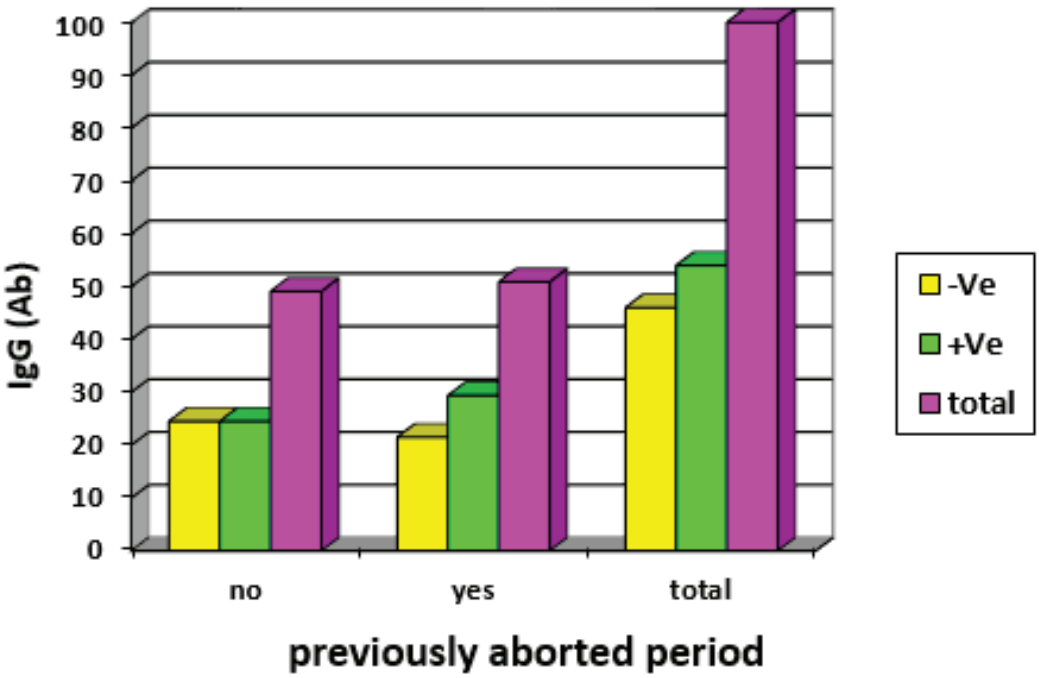

Figure (6): The Relationship between previously aborted period and IgG antibodies (Abs)

Discussion

This study showed the sero- prevalence of Toxoplasmosis among women at age groups (20-29) years with sero- positive anti toxoplasmosis IgG Abs, which high prevalence among women in these age groups in Baghdad province, when comparing with other age groups. This result agreement with the author from Sudan (2009(, about 27 samples [10]. When was found the percentage of studied groups at (25-32) years of age groups was higher than other age ranges. But this result disagreement with the author from Pakistan (2019(, about 54 samples [11]. This result agreement with authors from Iran (2019), about 146 samples [12], when was found that contact with cats is a potential risk factor for acquiring toxoplasmosis but as concerning food habits, which's exhibited that the sero-prevalence rate of toxoplasmosis was higher in blood donors who consumed raw/half-cooked meat than in other blood donors. Therefore, ingesting meat containing tissue cysts seems to be the main route of infection among the other routes. This result disagreement with search of Erbil (2017), about 92 samples [13], which found that rural populations had lower socio-economic levels than urban ones and implementation of more hygienic lifestyle in towns and large cities. This study recorded highly number of sero-positive anti- toxoplasmosis IgG Abs among women in 1st trimester period. This result agreement with authors from United Kingdom (1998), about 13000 samples [14], when found the indicated remarkable decrease of anti – toxoplasmosis Abs (IgG) among abortion / month in 2nd trimester period (4 – 6) month in contrast to 1st trimester period (1-3) month in sudden and previously aborted women. The result in current study differenced with authors from Ghana (2009), about 294 samples [15], who's found that all stages of pregnancy have the same chance of requiring infection. This parasite is also present in contrasting host density environments, but this result disagreement with [16], when they study the IgG antibodies to *Toxoplasma gondii* were detected in, March-April 2004, in 65.8% (95% confidence interval, 60.8-70.8%) of 342 systematically sampled subjects 5-90 years of age (87.5% of the eligible) living in a rural settlement in Amazonia, with a seroconversion rate of 9% over 1 year of follow-up of 99 seronegative subjects.

Multiple logistic regression analysis identified age as the only significant independent predictor of sero-positivity at the baseline. Each additional year of age increases the odds of being seropositive by 6%, and 76.8% of the subjects are expected to be seropositive at 30 years of age. A single high-prevalence spatial cluster, comprising 11.9% of the seropositive subjects, was detected in the area; households in the cluster were less likely to have dogs as pets and their heads had a lower education level, when compared with households located outside the cluster. The challenges for preventing human toxoplasmosis in tropical rural settings are discussed. Also they found dogs as pets and their heads had a lower education level, when compared with households located outside the cluster in rural areas comparison with urban areas.

Moreover this study recorded highly prevalence with toxoplasmosis in province comparison with Baghdad province. These results agreement with [17], who's found the rate of sero prevalence, with toxoplasmosis was 36.67% among 420 infected women in Basra province. And this study recorded highly number of sero- positive anti- toxoplasmosis IgG Abs among women in 1st trimester period. This can explain by the presented of IgG Abs via 2-3 weeks of an infection and there after increased gradually for some time which may cross pound to period of 1st trimester period (1-3) month of patients then starting to decrease and remain constant in low level along the life of women who may related to period of 2nd trimester period (4-6) month of the Patients [18]. This results agreement with [13], when found the indicated remarkable decrease of anti – toxoplasmosis Abs (IgG) among abortion / month in 2nd trimester period (4 – 6) month in contrast to 1st trimester period (1-3) month in sudden and previously aborted women.

For moreover, this study recorded also a high of sero-positive anti toxoplasmosis IgG Abs among an afflicted women by using the enzyme linked immunosorbet assay ElISA technique to detection the increase of this antibodies in serum of an afflicted women, this may be involve in addition of using kits, supplied or purchased from companies with low level of quality control and different laboratory methods. Moreover nutrition, habitat economic state, procedure, in addition to geographical location, plays an important role in difference in the sero- prevalence rate of toxoplasmosis [21]. Also may be due to differences in sensitivity and specificity of the used this technique [22]. This result agreement with [23], when found in Nether land which showed 32%. The Positive IgG Abs by using Elisa method. But this result disagreement with [24], when showed 40– 6% sero rate among aborted women by using latex test [25].

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Health and Medical Technology and all experiments were carried out in accordance with approved guidelines.

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Study Some Kinetic Parameters for Purified Arginase from Two Different Sources: Red Blood Cells and Serum of(Healthy and TypeII Diabetic Patient)

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Abstract

Kinetics parameters for arginase that purified from RBCs, and serum (control, and diabetic patients) revealed that the Km values are (200, 416, and 333mM), Vmax values are (47, 5.5, and 9 U/min),and The values of catalytic constant Kcat are (174, 45.8, and 81.8 min^{-1}). The effect of atrovastatin was also studied on serum arginase, the arginase activity is decreasing by increasing statin concentration, wherein at the lower concentration (0.112 mg/ml) the allosteric regulation percentage is 26% and it is ongoing until reaching its maximum (85%) at the highest concentration (0.9mg/ml). In conclusion affinity of arginase to the L-arginine, and its velocity is more in RBCs, and serum patients compared with serum controls, and statin appears to be an allosteric regulator for arginase.

Key words: *Arginase , diabetes mellitus, kinetic parameters, Atrovastatin, Arginase regulatory .*

Introduction

(L-Arginine amidinohydrolase; E.C.3.5.3.1), Arginase is a trimeric binuclear manganese metalloenzyme, the final enzyme in the urea cycle that is responsible for the detoxification of ammonia in mammalian[1]. Increase the activity of arginase is associated with various diseases by reducing the supply of L-arginine needed by NOS to produce nitric oxide(plays an important role in homeostatic vasodilatation, regulation of blood flow, and also it is thought that it plays an important role in cancer growth as a preventative and therapeutic agent, and by raising production of L-ornithine resulting in vascular structural problems and development of carcinogenesis [2]

In previous study [3], it was found that the arginase activity is increased in diabetes mellitus patients comparing with healthy, but this increasing was less in diabetic with dyslipidemia (who was getting atorvastatin), so the aim of this study is investigating whether the arginase characteristics purified from serum of control differ from that purified from serum of patients and red blood cells by studying arginase characteristics (kinetic parameters).also to find the effect of atorvastatin on arginase.

Materials and Methods

This study is conducted at the Department of Chemistry, College of Science for Women/ University of Baghdad. This study includes 42 healthy subjects, and 92 type II diabetes mellitus patients encountered during their attending the National Diabetes Center for Treatment and Research at Al-Mustansiriya University. This study was approved by the Scientific Committee in the College and a verbal consent form was obtained from each participant enrolled in the study. The chemical reagents that are used: Manganese chloride, Ninhydin,Ornithine, tri-chloro acetic acid, sulfunalic acid, hydrochloric acid, phosphoric acid from BDH/ England, L-arginine monochloride, Sodium barbitone from Rediel dehien/ Germany.

Arginase activity is measured as Zofia and Maria [4]. The color solution was read its absorbance at 515 nm determined. The amount of ornithine is read from the standard curve. Then the arginase activity would be calculated from the following equation:

Arginase units = µg of ornithine (test-control) * 0.505.

Determination of Kinetic Constants of Arginase

Various concentrations of the substrate L-arginine monochlorid solution graduated (100-350mM) are prepared from the stock solution of 350mM. The activity is measured with each concentration of substrate. The values of Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) are calculated by plotting the relation between the reciprocal rate of the reaction velocity (V) and the reciprocal of substrate concentration [S] using Lineweaver- Burk plot, and between (V/[S]) and [S] by using Eadie-Scatchered plot.

Effect of Statin on Serm of Control:

Some of diabetic patients have atorvastatine drug for lowering their lipids and it is noticed those patients have a lower arginase activity so the effect of this drug is studied by determining Km and Vmax values.

A-Solutions:

1- Phosphate buffer of 0.05M (PH 7.4)

2- 0.5% SDS

3- Atrovastatine of 40mg.

B- Procedure

The stock solution of the drug is prepared [5], by grinding 20 tablets of the drug, and dissolving them in 900 ml of phosphate buffer solution containing 0.5% SDS then on magnetic stirrer for 45 minutes at 75 rpm, the supernatant contained the active material of the drug (40mg). The supernatant is dialyzed against the same buffer in order to get the remaining SDS out, if any. Different concentrations of the drug are prepared (0.9, 0.45, 0.225, 0.112) mg/ml. Then two experiments are made, in the first one drug solutions are mixed with the purified enzyme solution with percent of (1:1) for 1hour. in a 37°C, and then the enzyme activity is measured for determining the percentage of inhibition from the following equation:

Regulation percentage=

Activity without statin - Activity with statin

*100%

Activity without statin

In the second one 50 µl of the drug solution of (0.9 mg/ml) is added to the reaction medium with different concentrations of the substrate (100, 150, 200, 250, 300 mM), and the enzyme activity is measured for determining the effect.

Results and Discussion

Kinetic Constants of Arginase

The values of Michalis-Menten constant (Km), maximum velocity (Vmax) as well as catalytic constant (K_{cat}) of purified arginase from RBCs, and serum (of controls and patients) are evaluated utilizing Linweaver- Burk and Eadie- Scatchard to express the relation between velocity of the reaction and substrate concentration (Figure 1, and 2).

The results presented in Table (1) indicate that for RBCs, serum controls, and serum patients the values of Km according to Linweaver- Burk method are (200, 416, and 333mM) respectively, and Vmax values are (47, 5.5, and 9µg/min) respectively (Figure 1 a, b, and c), while according to Eadie- Scatchard method Km values are (107, 138.8, and 126.6 mM) respectively, and Vmax values are (28, 2.5, and 7.6µg/min) (Figure 2 a, b, and c). Michalis-Menten constant refers to the enzyme affinity towards substrate, Vmax occurs when all available enzyme molecules present in the form of enzyme-substrate complex (ES) [6]

From these results it is clear that the affinity of arginase to the L-arginine, and its velocity is more in RBCs, and serum patients compared with serum controls that explain higher activity of arginase in RBCs, and serum patients than in serum controls. It is axiomatic that the high activity in serum patients is in responds to disease case of diabetes [7], while arginase activity is high in RBCs because it is the source for ornithine present in plasma that is important for peripheral tissues such as cartilage and bone, since these tissues have low or no arginase [8].

Table 1: Values of Kinetic Constants of Arginase Enzyme Towards L-arginine Monochloride Obtained from Linweaver- Burk and Eadie- Scatchard Methods.

Method	Purified arginase from	Vmax (µg/min)	Km (mM)	Kcat (min-1)
Linweaver- Burk	RBCs	47	200	204
	Serum controls	5.5	416	55
	Serum patient	9	333	90
Eadie- Scatchard	RBCs	28	107	121
	Serum controls	2.5	138.8	25
	Serum patient	7.6	126.6	76

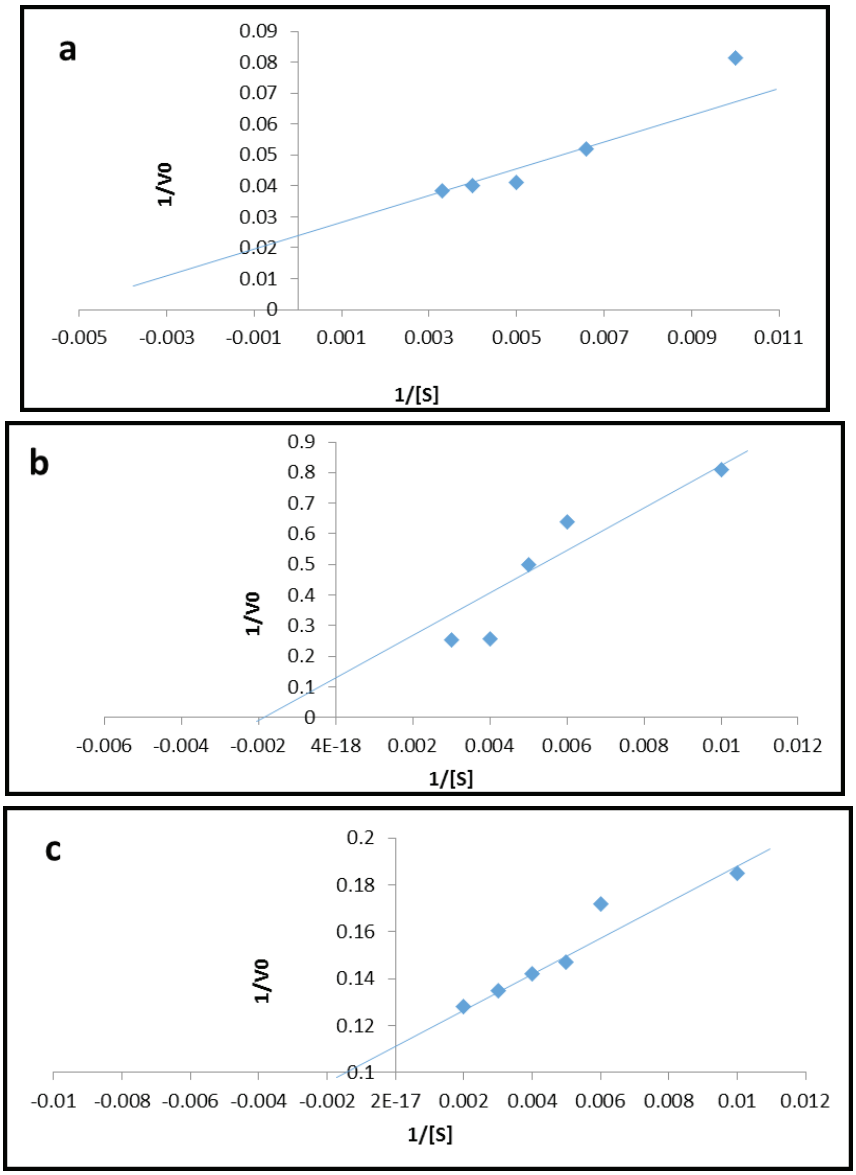

Figure (1): Kinetic Constants of Arginase Purified from (a) RBCs (b) Serum Controls (c)

Serum Patients According to Linweaver- Burk.

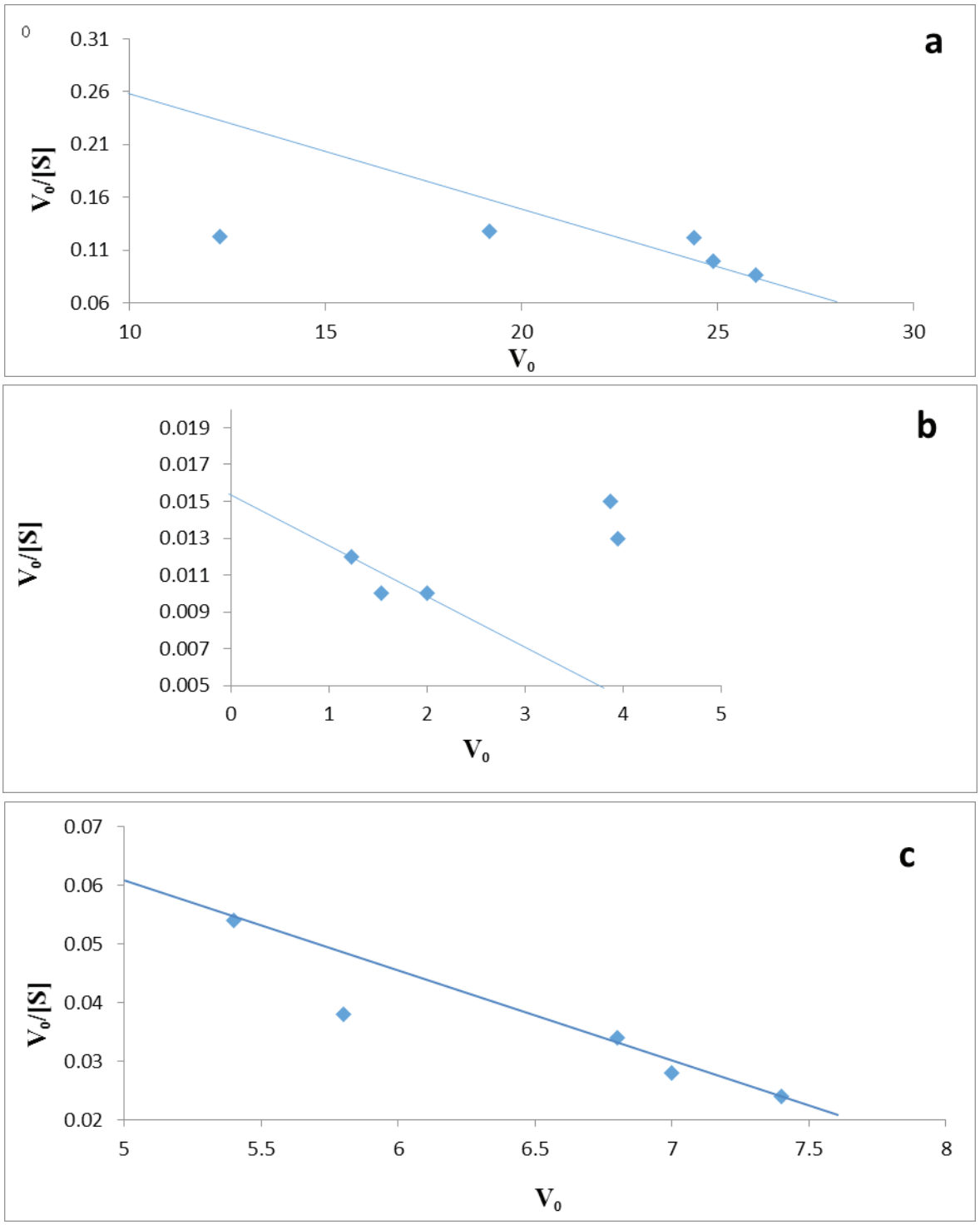

Figure (2): Kinetic Constants of Arginase Purified from (a) RBCs (b) Serum Controls (c) Serum Patients According to Eadie-Scatchard.

It can be noticed that enhancing substrate concentration enhances the rate of the reaction because of increasing the collisions between substrate and enzyme molecules until reaching a certain concentration, after that further increasing in substrate concentration has no effect on the reaction rate because that enzyme becomes saturated and its active site is occupied with substrate concentration become without effect on the reaction

rate, because the enzyme becomes saturated, and the active site is occupied with the substrate [9].

Ferit [10] states that Km value for arginase purified from human vitreous humor is 6 mM to L-arginine, Okonji *et al*. [11] and Okonji, *et al*., [12] report that K_m value to L-arginine monochloride is 250mM, with Vmax of 6.7 µg /min for arginase purified from the gut of grasshopper, and found that the K_m value is 66.7 mM for arginase purified from liver of tortoise. Ezima *et al*., [13] found that the K_m to be 17mM for arginine and Vmax value to be 1.39 µmole ml^{-1} min^{-1} for arginase purified from fruit bat liver. Joseph *et al*. [14] report that the Km for L-arginine of human liver arginase is 10.5mM. The variance in millimolar concentration of arginine may belong to the different methods that used and nonphysiological conditions generally used [15].

The values of catalytic constant K_{cat} according to Linweaver- Burk method are (174, 45.8, and 81.8 min^{-1}), while K_{cat} values according to Eadie- Scatchard method they are (103, 20, and 69 min^{-1}) in each RBCs, serum controls, and serum patients respectively, (Table 1). Kcat is the turnover number that refers to the number of substrate molecules converted to product per unit of time where the enzyme works at its maximum efficiency [16].

The Effect of Statin on Serum Arginase

Clinically, statin appears to affect arginase activity, where arginase activity is decreased in group III of the patients and that may be attributed to statin effect where group III patients are under statin treatment(3). This leads to study the kinetic of arginase in serum where it is treated with statin directly *in vitro*. When arginase is treated with different concentrations of statin the activity is decreased as shown Table (2).

Table 2 : Allosteric Regulatory Percentage of Controls Serum Arginase with Different Concentrations of Statin.

Concentration	Allosteric Regulatory Percentage %
0.112	26
0.225	43
0.45	68
0.625	75
0.9	85

Figure (3) shows that statin in all concentrations regulates arginase, the arginase activity decreases by increasing statin concentration, where at the lower concentration(0.112mg/ml) the allosteric regulatory percentage is 26% and it is ongoing until reaching its maximum (85%) at the highest concentration (0.9mg/ml).

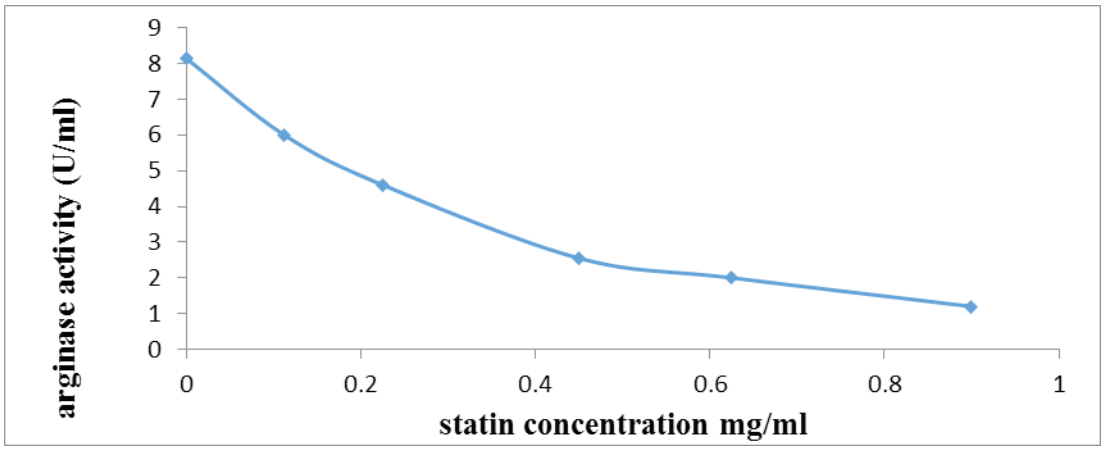

Figure (3) : Effect of Statin at Different Concentrations on Serum Arginase Activity

Different concentrations of substrate (L-arginin monochloride) are used with (0.9mg/ml)of statin in order to study the effect of statin, where it is calculated by using Linweaver- Burk, and Eadie- Scatchard methods as in Figure 4, and 5.

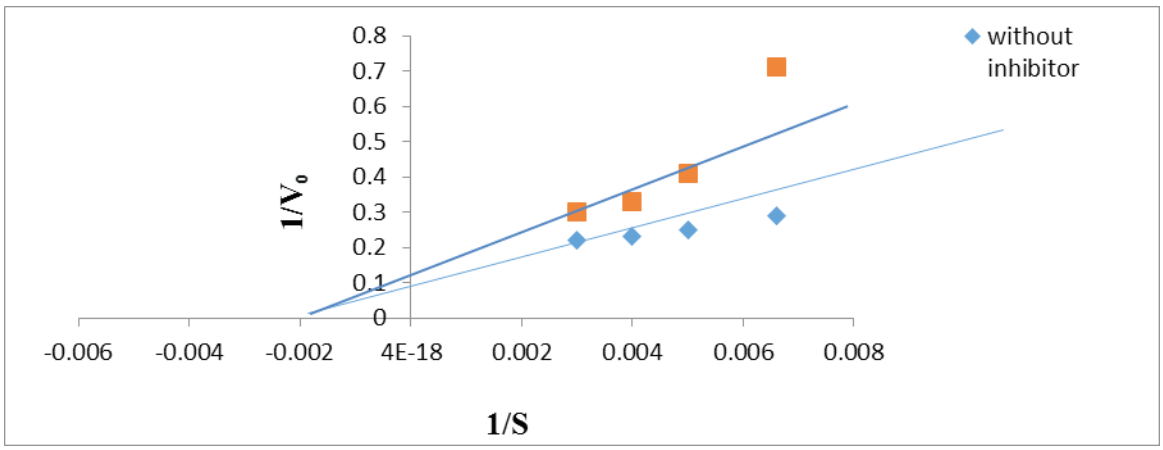

Figure 4: Line Weaver-Burk Plot for The regulation of Serum Arginase by Statin.

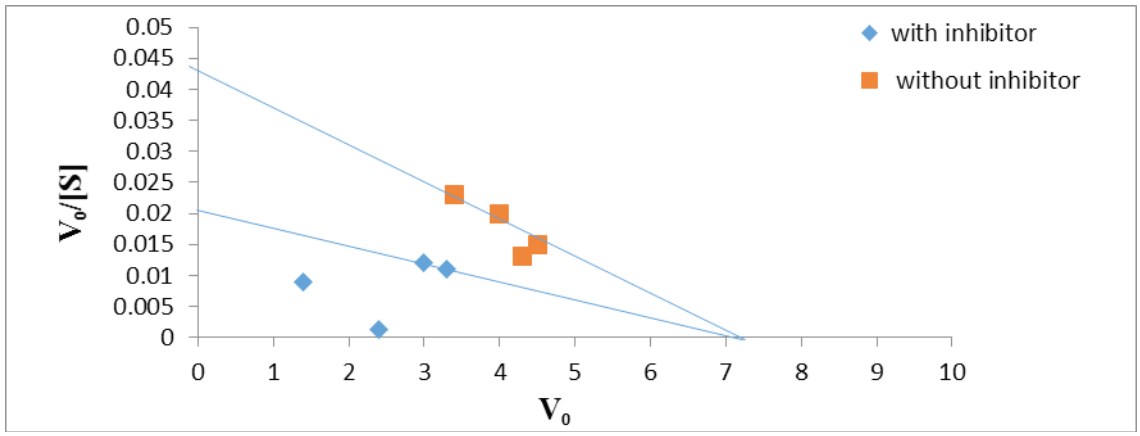

Figure (5) : Eadie- Scatchard Plot for The regulation of Serum Arginase by Statin.

The results show that the effect of statin is allosteric regulation according to that Line Weaver -Burk, and Eadie- Scatchard plots where the Vmax for the arginase is stilled constant (10, and 7.3 µg/min) in the both cases of presence, and absence of the statin while the Km value is increased in the presence of statin (526 mM, and 365mM) compared with case of statin absence (400mM, and 165mM), as in (Table 3).

Table (3): The Kinetic Parameters of Serum Arginase Obtained from Linweaver- Burk, and Eadie-Scatchard in The Presence and Absence of Statin.

Methods	Case	Vmax(µg/min)	Km(mM)
Line Weaver-Burk	Without statin	10	400
	With statin	10	526
Eadie- Scatchard	Without statin	7.3	165
	With statin	7.3	365

Since arginase is an allosteric enzyme as it is regulated by its substrate arginine and its products (ornithine, and urea)[17], so statin considered as a regulator (modulator) molecule that doesn't bind to the active site but it binds to another site (modulator site) and this regulation is characterized by

lowering the enzyme affinity to its substrate with a constant Vmax. That regulation action of statin (lowering of arginase activity) in those diabetic cases can be considered as a good point, where it may lead to enhance NO levels then decrease the chance to get endothelial dysfunction.

Financial disclosure

There is no financial disclosure.

Conflict of interest

None to declare.

Ethical Clearance

All experimental protocols were approved under the Chemistry Dept. and all experiments were carried out in accordance with approved guidelines.

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A Novel Design of Gas System and Develop Culture Medium for the Isolation and Sulfur Reducing Bacteria within Short Time

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Abstract

The current study has developed a proprietary system and a special device for the supply of oxygen-free nitrogen gas with a mixture of carbon dioxide and 80:20% according to the laboratory conditions required for the growth of sulfur-reducing bacteria. To find the best medium that fits the bacteria in those environments with shortening incubation period, the new medium developed is available in the specification of planting rich in energy sources, minerals and vitamins and high reduction ability promotes the early growth of sulfur-reducing bacteria .

Keywords: *Sulfur reducing bacteria, intestinal diseases, colitis, and inflammatory*

Introduction

Worldwide nearly, intestinal and intestinal diseases, colitis, and inflammatory bowel disease (IBD) are a group of chronic diseases that may be genetic, immunological, immunological or bacterial [1,2].

In the 19th century it was believed that the cause of IBD was bacterial and eventually leads to colon disease (CD). At the beginning of the 20th century, there was a link between ulcers Colon (UC) and Bacterial Infection [1].

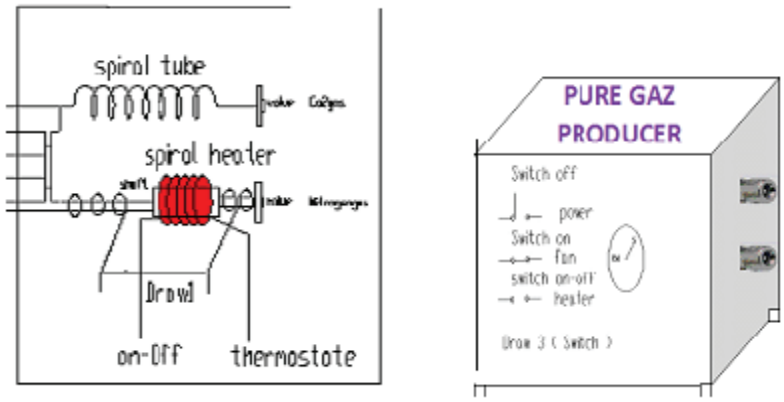
Scientific progress in cultivation-independent technologies and bioinformatics found that patients with colitis and Crohn's disease showed that *Bacteroidetes* and *Lachnospiraceae* were decreasing, while other groups were increasing, such as *Gammaproteobacter* and *Enterobacteriaceae* [3,4]. A significant increase was also observed in sulfate-reducing sulfate- reducing bacteria [5] and due to the activity of the sulfur-reducing bacteria producing the hydrogen sulfide that destroys the normal immune bodies as well as the natural fluorine produced by the vitamins present. Within the mucous membrane and encouraging the increase in the number of harmful bacteria and opportunistic, which causes dysfunction in the lining of the bowel and decrease the amount of mucus, it is called inflammation of the bowel [6].

Since the *Faecalibacterium prausnitzii* was found to be an indicator of the health status of people not infected with IBD because their anti-inflammatory agents secretion [7].

Current study aimed developing isolation diagnosis techniques of pathogenic bacteria and finding the best growth medium for the diagnosis of the laboratory.

Materials and Methods

1 – **Pure gas producer**: The device of the component is made of copper pipes wrapped in several rolls and then a part of a length of 10 centimeters with a cord electric heat around them to provide the required temperature degrees. The heating pipe is larger than the pipe before and after the pipe and are available in copper pipes that work with the help. The heat gained from the electric heater. The gas mixture is then passed through the syringe to a sterile syringe without exposure to contamination. All transplantation, purification and diagnosis are carried out using this method with the addition of the previously mentioned reduced agents. The device is designed to be able to obtain nitrogen gas or carbon dioxide oxide separately or together, as well as electrical control switches and the fan of thermal discharge from within the system manufactured [Figer 1].



Figer (1): The content plan of the gas purification plant

2 - Preparation of converted culture media:

The agricultural communities were used as

As shown in the table (1) below various culture media tested to the best one for faster isolation sulfur reducing bacteria from samples under study

Table (1) various culture media tested for sulfur reducing bacteria

Type of culture media for isolation of sulfur reducing bacteria				
المتركيب الكيميائي Chemical ingredient	Kushkevych, 2013	Postgate&Campbell ,1965	API,1975	Feio,et all.1998
KH2PO4	+	+	+	+
NH4CL	+	+	-	+
Na2so4	+	+	-	+
NaCl	+	+	+	-
C acl2.6H2o	+	+	+	+
MgSo4.H2o	+	+	+	+
Sodium acetate	+	+	-	-
Sodium citrate	+	-	+	+
Casamino-acid	+	-	-	-
Tryptone	+	-	-	-
Thio-glycolic acid	+	-	+	-
FeSo4.7H20	+	-	-	+
Mineral	+	-	-	-
Vitamin	+	+	+	+
Ascorbic acid	+	+	+	-

Cont... **Table (1) various culture media tested for sulfur reducing bacteria**

NH4)2 FeSO4)	+	-	-	-
Cestine	+	-	-	-
Na bicarbonate	+	-	-	-
Yeast extract	–	+	+	+
Ca So4	–	-	-	-
PH	7.5	7-7.5	7-7.2	7-7.5

3- Progression in media by adding one of the following substances per-liter basis and releasing the KM2015 axis: KH_2PO_4 0.3 g, Na_2SO4 0.5 g, K_2HPO_4 0.5g, (NH_4) SO_4 0.2 g, NH_4Cl 1g, $CaCl_2.6H_2O$ 0.06 g , $MgSO_4.7H_2O$ 0.1 g, $C_3H_5O_3Na$ 2ml, Yeast extract 1 g, $FeSO_4.7H_2O$ 0.004 g, Sodium citrate.2 H_2O 0.3 g.

With addition of Mohr's salt solution 1 ml / L, which consisting of the following materials: copper sulphate 0.1 g, boric acid 0.1 g, zinc sulphate 0.1 g, nickel chloride 0.2 g, cobalt 1 g, manganese chloride 1 g, salts of these minerals were present in one liter of distilled water Deodorizer. The following substances were added for each liter: Casamine 2 mg, Vitamin solution 10 ml / l, cestine 3%, Sodium bicarbonate 30 ml from 8%, Sodium thioglycolate 10ml from 1%, NH4) 2Fe (SO4) $2.6H_2O$ 10 ml from 10%, Na_2S $9.H_2O$ 0.05 ml from 1%. [7] . Add 5 grams per liter of acryl to get a semi-solid medium. In the case of steel medium, add 15 grams of salt.

Cultivation of the samples:

Immediately transfer the samples to the refrigerator and store them briefly to speed up the hardening of the food medium. During the incubation period, growth and the observation of developing colonies are observed after the appearance of the colonies that take an alternate shape in the center of growth as shown in Figure 2. The single colony is withdrawn by a pipette Pasteur is transferred to the center of the developer's spindle for the purpose of activation, incubating at a temperature of 37 ° C and for 48 hours. After that, the activated colony was transferred to bottles filled with the center of the steel axis and incubation at the same degree for three days for the purpose of studying their phenotypes and dyeing and conducting biochemical and genetic tests on them. The colonies were isolated in the form with Gram stain and malachite green to separate spore forming isolates from non spore forming in individual colonies.

Results and Discussion

The results show that the best developed medium suitable for bacteria in these environments with shortening the incubation period, the new developed center is available in the form of a rich implantation of energy sources, minerals and vitamins and a high reduction ability promotes the early growth of sulfur reduced bacteria.

Anaerobic conditions preparation machine:

Previous studies have shown the adoption of simplified methods to obtain the gases required for the development of bacteria[8,9] . However, the present study developed a special system for the supply of oxygen-free nitrogen gas with carbon dioxide (80 : 20%), and laboratory conditions required for the growth of sulfur-reducing bacteria. Carbon dioxide and nitrogen are passed from cylinders available in local markets. The device or system enters a copper tube wrapped in several rolls to increase surface area exposed to heat. The copper-assisted copper tubes are available to reduce oxygen and obtain oxygen-free nitrogen gas and facilitate their transfer to the irrigated areas. This is the gateway to the gas supply to the plantations from the other end of the

copper tube. In addition to facilitating the process was of mixing carbon dioxide and required percentage and then passes the mixture of gases to the bottles of the implant by a sterile injection without exposure to pollution, as all the transplantation, purification and diagnosis using this method with the addition of the previously mentioned reduced factors. To ensure the success of the appropriate conditions in the food medium, add drops of blue Rezozarin solution. In the absence of a change in the color of the center towards the blue indicates that the circumstance in the middle is lacking oxygen (conditions of reduction) and vice versa [10]. The system can also be adopted to obtain nitrogen-free gas without mixing with carbon dioxide.

* Development of the growth medium for the growth of the Sulfur reduced bacteria in culture media and the reduction of incubation period :

In the current study, four stem cells were studied, as shown in table (1), and in a preliminary series of experiments to reach the best plant medium for the growth of the sulfur-reducing bacteria in the stomach, intestines and colon environment and shortening the period of the lap, which causes negative effects on human health after vaccination. These circles are characterized by stool, Endoscope and under anaerobic conditions that provide nitrogen gas, carbon dioxide and incubation at a temperature of 37 ° C and for 2 to 3 days. It was found that the center of [12] was the best of the vegetative circles, with the emergence of growth (medium to black color change) as a maximum of three days compared with the rest of the other circles as mean [12,13,14], as shown in Table (2). Preliminary experiments in these circles found that some of them were dedicated to sulfur-reducing bacteria in their natural soil and water environment [11]. No environment was prepared to isolate the bacteria that reduced sulfur from the stomach and intestines and to obtain an intestinal environment closer to the bowel environment. The center was stimulated by carbonic sources such as organic acid [15] and additional energy sources. The incubation period was reduced to 48 hours instead of 72 hours. These results were enhanced by studying bacterial growth density using UV-SPECTROPHOTOMETER, and encouraging the growth of more bacterial species of sulfur-reducing bacteria than other growth-specific species.

Table (2): A better test medium for the development of sulfur-reducing bacteria in stool samples and Endoscope swabs

Culture media	Source of sample	Growth	Number of Healthy	Number of Patient	Incubation time(days)
Kushkevych,2013 (KM2015)developed	Stool Endoscope swab	+ +	15 –	25 10	2-3 2-3
Postgate &Campbell	Stool Endoscope swab	+ +	15 –	25 10	7- 14 7-21
API	Stool Endoscope swab	+ +	–	25 15	7-14 7-21
Feio,et.al.1998	Stool Endoscope swab	+ +	15	25 10	7-21 7-21

The appearance of black color in the growth environment is due to the addition of sulfuric substances to the nutrient medium and their reduction to hydrogen sulfide due to metabolic reactions during the anaerobic respiration process. The reduction of the diastolic condition accelerates the bacterial divisions. [12] This was observed in stool samples and Endoscope scabs under study - Such as sodium thioglycolate and Cystine compounds in the center axis reduced the incubation period to 2-4 days as well as the addition of growth promoters for the same medium of vitamins, minerals of trace minerals and Casamine, and reinforced that assumption as shown in Table (1).

4: Description colony of sulfur reducing bacteria and Cultural Characteristics

The results of the sample implantation on the KM2015 liquid medium showed that the positive growth of the sulfur-reducing bacteria was characterized by homogeneity by the deposition of the iron which exported the sulfite compounds [16]. The members of this group are described as members of the chemo heterotrophic diet. [17] Development and purification of the colonies on the KM2015 semi-steel medium under anaerobic conditions and incubation at a temperature of 37 ° C and for three days, the colonies appear black in the depth of the acre and diameter 1-2 mm, After a week of farm life, it is shrinking, and when exposed to the air, its black color quickly disappears into yellow, meaning cell death [11]. The colonies do not grow except in specialized circles where the optimal conditions for growth are found, in the circles, when developing them in their specialized circles without adding the sources of sulfur to show them growth. These results were reinforced [13], because sulphite compounds are the ultimate future of electrons during aerobic respiration [7]. When the isolates were examined under the microscope, most of them were found to be negative for gram, macrophage, other coliform, staphylococcus and nematode. As for the components of spores a small percentage compared to other groups and groups this is consistent with the novelty of Rey [19]. When examining its ability to produce Catalase, the colonies appeared negative to that test by being a mandatory anaerobic enzyme producing the hydrogenase.

Figure (2) Growth of colonies of sulfur-reducing bacteria on central KM2015 liquid, semi-alkaline and steel

Most of the colonies are produced by hydrogen sulfide gas. This is observed by deposition of iron around the colonies under study and the undesirable odor when opening the implant bottles.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad Alrafidain University College and all experiments were carried out in accordance with approved guidelines.

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Determination the Gene Expression Levels of adhesins and Extracellular Enzymes Genes in *Candida albicans* biofilm producer by Quantitative Real Time PCR Technique (qRT-PCR)

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Abstract

In the present study, all *C. albicans* were biofilm producers with variable strength. Molecular study of virulence genes correlated with biofilm *C. albicans* showed that *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* genes were detected in 100% of *C. albicans* from vaginal and oral infections. In addition to that quantitative real time PCR technique show that *ITS1* was used as the housekeeping gene and was shown to be stably expressed throughout all biofilm conditions and *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* where 100% expressed in all ten tested *C. albicans*. It was appeared that adhesins genes such as *HWP1*, *ALS1* and *ALS3* were highly expressed in HBF and less expressed in LBF, whereas in very low BF producers the fold up-regulation were not particularly high, but they were over expressed in strong biofilm producers in both of vaginal and oral isolates. The average of gene expression folding show that *SAP5* and *PLB1* were highly expressed among vaginal isolates rather than oral ones, in contrast, *ALS1*, *HWP1* and *LIP8* were predominant expressed among oral isolates, whereas *ALS3* was higher in vaginal isolates than oral with little differences.

Gene expression levels by q RT-PCR show that *SAP5* and *PLB1* were up-regulated in both of HBF and LBF producers, and they were highly expressed among the ten tested isolates. The over expressions of *SAP5* and *PLB1* genes were observed in vaginal isolates with high biofilm. The current study found an overexpression of adhesins such as *HWP1*, *ALS1*, *ALS3*, are responsible in strength of biofilm formation in *C. albicans* isolated from oral and vaginal infection with different ages and different clinical states, in addition to upregulated of extracellular hydrolytic enzymes genes belong to *SAP*, *PLB* and *LIP* was important in biofilm formation.

Key words: *Candida albicans, biofilm, ALS1, ALS3, HWP1, SAP5 , PLB1, LIP, gene expression.*

Introduction

In the recent years, the prevalence of serious fungal infections, invasive *Candida* infections particularly, has been increasing due to an increased number of patients receiving immunosuppressive therapy, increased major surgeries and broad-spectrum antibiotherapy, hyperalimentation, prolonged intensive care unit stay for patients with poor health status[1,2].

Candida albicans is responsible for more than 50% of human candidiasis, including two major types of infections, superficial infections (nonlethal), such as oral or vaginal candidiasis; and systemic infections [3,4]. *Candida* spp. is the most causative agents of vulvovaginitis in women and *C. albicans* was the most predominant *Candida* spp.[5].

The medical impact of *C. albicans* (like that of many other microorganisms) depends on its ability to grow up as a biofilm, a closely packed community of cells [6]. Microbial adhesion is considered the first step for biofilm formation. This structure constitutes a protective milieu against environmental stresses and

human host defenses[7]. *C. albicans* has a specialized set of proteins (adhesins) which mediate adherence to other *C. albicans* cells to other microorganisms, to abiotic surfaces and to host cells [8]. *C. albicans* adhesions are the agglutinin-like sequence (*ALS*) proteins which form a family consisting of eight members (*ALS*1–7 and *ALS*9). Another important adhesin of *C. albicans* is *Hwp1*, which is a hypha-associated protein [9]. An essential factor to the virulence of the genus *Candida* is the ability to produce enzymes and this may be crucial in the establishment of fungal infections [10],

There are two major families of histolytic enzymes produced by *Candida* species: the secretory aspartyl proteinases (*SAP*) and phospholipases (*PL*) [11]. The secreted aspartic proteases (Saps) are among the most studied virulence determinants in *C. albicans* [12].

Many biofilm genes encode known or predicted cell wall proteins. These proteins are of special interest because they may play a direct role in cell-substrate or cell-cell adherence, heterologous expression studies indicate that *HWP1, ALS1,* and *ALS3* have such roles [13]. Previous works demonstrated that the expression of *HWP1* and of genes belonging to the *ALS*, *SAP, LIP* and *PLB* gene families is associated with biofilm growth on mucosal surfaces [14], In addition to *SAPs*, *C. albicans* also has two other gene families, namely the lipases (*LIP*) and phospholipases (*PL*) that produce extracellular hydrolytic enzymes that could play roles in candidal adhesion, nutrient acquisition and invasion of epithelial surfaces [15]. Constitutive expression of the *LIP* genes and *PLB* has been demonstrated in *C. albicans* biofilms [16], However, it is known that the expression of *ALS, SAP, LIP and PLB* genes can be influenced by other factors such as the growth medium, temperature and other environmental conditions [17, 18]

Materials and Methods

Samples collection and isolation

High vaginal swabs were collected from 49 patients aged between16-50 years, presented with vulvovaginal candidiasis. Oral swabs were collected from 47 patients with oral candidiasis aged between two days to ten years, during the period from March 2015 to the end of June 2015. Clinical presentations were done by specialized doctors. and were divided in to two smears: one smear was examined immediately under microscope for direct examination; the other usually was cultured on SDA medium.

Identification of Candidal Isolates*:*

the isolates were purified by streaking on sabouraud dextrose agar by using ABC methods then incubated at 37ºC for two days to obtained one isolated pure colony. This isolated colony was transferred to SDA by streaking all the plate, and then incubates at 37 ºC over night

Candida was identified depending on the morphological features on culture medium and germ tube formation conferring the diagnosis by identifying *Candida* Spp By using Vitek 2 system.

Biofilm Formation Assay

In the present study, biofilm formation was determined using pre-sterilized polystyrene 96-well microplates using method described by Melek *et al*. (2012) [20] with modifications. Yeast was inoculated using a loop into tube containing 2 ml of YPD broth incubated at 37°C for 24 h, all tubes were diluted at a ratio of 1:20 by using freshly prepared YPD with 1% glucose, each well of the microplate was filled with 200 µL of this final solution. Microplates were covered with lids and incubated at 37°C for 24 h.

The medium in wells was removed and washed two times with sterile phosphated buffer solution (PBS) and then inverted to blot and let to dry. Microplates were stained by adding 200 µL of 0.1gm/100ml crystal violate to each well incubated for 20 minutes which then washed two times with PBS then inverted to blot and let to dry, finally 200 µL of acetone: ethanol mixture (20:80 v/v) was added to each well, waiting for about 10 min then the results were read at 450nm by an Elisa reader.

Gene Expression Study

Gene expression level for *ALS*1, *ALS*3, *HWP*1, *LIP*8, *SAP*5, and *PLB*1 gene were determined by comparative Ct method to measure the level of gene transcription (mRNA level). The Ct of ITS4 was used as endogenous control for calibrator the Ct values of other genes.

The folding of gene expression was calculated as mentioned bellow:

* Ct= cycle of threshold (number of cycles required for florescent signal

to cross threshold).

* HK= housekeeping gene.

Primers selection and preparation

All primers (table1) were supplied in lyophilized forms. Dissolved in nuclease-free water to give a final concentration of 100 picomol⁄µl as recommended by provider and stored in deep freezer as stock solution until used in PCR amplification. Work solution was prepared by added 90 µl of nuclease free water to 10 µl of stock solution of primer to get 10picomol/ µl concentration.

Isolation of RNA from Yeast:

1) Yeast was cultured on YPD broth for 20 h at 37, the culture was diluted 1:50 and grow until the OD600 is 0.6–1.0. This should only take a few hours.

2) The culture was centrifuged for 2 minutes at 14,000 rpm in a microcentrifuge.

3) The pellet was resuspended in 100µl of the following solution:

· 1M sorbitol

· 0.1M EDTA (pH 7.4)

· Just before use 0.1% β-mercaptoethanol and 50 units of lyticase were added

4) Incubate at 30°C for 15–30 minutes until the solution appears clear.

5) Seventy-five microliter of RNA Lysis Buffer was added, Mix gently.

6) Three hundred-fifty microliter of RNA Dilution Buffer (blue) was added, and Mixed by inversion and centrifuge at maximum speed for 10 minutes in a microcentrifuge.

RNA purification by Centrifugation Method:

1) The cleared cell lysate solution was transferred to a microcentrifuge tube and 200 µl of 95% ethanol were added, then the solution was mixed by pipetting several times.

2) The mixture was transferred to the spin column assembly and centrifuged at 13,000 rpm for 3 minutes.

3) Spin basket was removed from the spin column assembly, and the liquid in the collection tube was discarded; the spin basket was again put back into the collection tube.

4) Six hundred microliters of RNA wash solution was added to the spin column assembly and centrifuged at 13000 rpm for 3 minutes.

5) DNase incubation mix were prepared by combining 40µl yellow core buffer, 5 µl 0.09 M $MnCl_2$ and 5µl of DNase I enzyme per sample in a sterile tube mix by gentle pipetting.

6) Fifty microliters of this freshly prepared DNase incubation mix were applied directly to the membrane inside the Spin Basket and incubated for 15 minutes at 25°C.

7) Two hundred microliters of DNase stop solution were added to the spin basket, and centrifuged at 13000 rpm for 5 minute.

8) Six hundred microliters of RNA wash solution (with ethanol added) were added and centrifuge at 13000 rpm for 5 minute.

9) The collection tube was emptied, added 250µl RNA wash solution (with ethanol added); and centrifuged at 13000 rpm for 5 minutes.

10) Twisting motion was used to remove the cap from the spin basket, and transferred spin basket from the collection tube to the1.5ml elution tube.

11) One hundred microliters of nuclease free water were added to the membrane, and placed the spin basket assemblies in the centrifuge with the lids of the elution tubes facing out, and centrifuged at 13000 rpm for 5 minutes.

12) The spin basket was discard, then capped the elution tube containing the purified RNA and stored at -70°C.

RNA Concentration and purity Determination

Quantus Florometer was used to detect the concentration of extracted RNA in order to detect the

goodness of samples for downstream applications. For 1 µl of RNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temperature, RNA concentration values were detected.

Quantitative Real Time PCR (qRT–PCR)

The expression levels of *ALS1*, *ALS3, HWP1*, *LIP8*, *SAP5*, and *PLB1*, were estimated by One Step qRT-PCR. To confirm the expression of target gene, quantitative real time one step qRT-PCR sybr Green assay was used. Primers sequences for each gene were prepared. The mRNA levels of endogenous control gene ITS4 were amplified and used to normalize the mRNA levels of the up genes.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study.

Results and Discussion

From 49 *Candida* isolates from vaginal swabs; 22(45%) were *Candida albicans* and 27(55%) were non *albicans*. Whereas 22(47%) out of 47 *Candida* isolates from oral swabs were *C. albicans* and 25(53%) were non *albicans*.

In the present study, non *albicans Candida* isolates collectively contributed to more than half (55%) and (53%) of the candidial infections in both of vaginal and oral infection respectively this result agree with Jose *et al*. [24] in a previous study, also observed that the non *albicans Candida* was predominant (70%) as compared to *C. albicans* (30%), which indicate that the non a*lbicans Candida* infections are on the rise.

Similar finding have been reported in the literature by different authors [25], Whereas *C. albicans* was the most dominant species in both of vaginal and oral infections with percentages 45% and 47% respectively. These results are agree with [26] that *C. albicans* and *C. glabrata* were the most common yeast species isolated from patients. Mohammed [5] indicated that *C. albicans* was the predominant species (63.6%) out of 124 HVS, followed by *C. glabrata* (30.9%) and *C. trupicalis* (5.5%), [27] find 63.8% isolates were *C. parapsilosis*20.34% were *C. albicans*.

Biofilm Formation of *C. albicans*

At the present study; all *C. albicans* were biofilm producers with variable strength value depended on the OD value with using crystal violate (fig.1). In general out of 44 BF producers, 18 (40.9%) were weak BF (low biofilm) with significant differences ($P<0.05$) between oral and vaginal specimens, 25 (56.8%) moderate BF (high) and just one isolate from oral was strong (very high) biofilm in percentage about 2.3%. Among vaginal isolates; 10/22 (45.5%) were low BF whereas moderate or high BF were 12/22 (54.5%). In comparable with those; the biofilm among oral isolates was higher than those of vagina, that weak BF were produced by 8/22 (36.4%), moderate BF 13/22 (59.1%) in addition to the appearance of one oral isolates produced strong BF in percentage about 4.5% (table 4).

As any other research these results are agree with some and different with others in some sides. These results show that BF is higher among oral infection than vaginal infection. These results agree with Mahmoudabadi *et al*. [28] indicated that 100% of *C. albicans* isolated from different sources had the ability to produce biofilm *in vitro*. It also agreed with Villar-Vidal *et al*.[29] found all tested isolates of *C. albicans* produced biofilm on polystyrene.

Bruder-Nascimento *et al*. [30] found that total of 198 of 327 (60.6%) *Candida* species isolates were biofilm-positive. Of these, 72 (36.4%) and 126 (63.6%) isolates were low and high biofilm producers, respectively.

Udayalaxmi *et al*. [31] reported that among 40 *C. albicans*, 22(55%) were strong- moderate biofilm producers. which agree with current results. Jose and colleagues [24] found that Among the 100 isolates of *Candida*, 69% were found to be biofilm producers. Among them, 42% were weakly adherent, 23% moderately adherent, and 4% were strongly adherent.

Determination Gene Expression Level by using Quantitative Real Time PCR Technique (qRT-PCR):

Gene expression level is usually used to identifying the important genes involved in *Candida albicans*

biofilm formation. In the present study, gene expression were studied for ten candidal isolates chosen randomly which five isolates from vulvovaginal candidiasis and other five from oral candidiasis according to their biofilm formation. These isolates were termed by numbers from one to five for vulvovaginal candidiasis isolates, from six to ten for oral candidiasis isolates; respectively. The immediately isolated purified RNA from each isolates was used to determine the expression of the goal genes (*ALS1*, *ALS3*, *HWP1*, *SAP5*, *PLB1* and *LIP8*) involve in biofilm formation of *C. albicans*. The expression level for each gene was calibrated and normalized with housekeeping gene of *C. albicans* internal transcription spacer sequence (*ITS*1).The gene expression values of housekeeping gene, tested genes and Delta Ct values. The average of gene expression folding that *SAP5* and *PLB1* were highly expressed among vaginal isolates rather than oral ones, in contrast, *ALS1*, *HWP1* and *LIP8* were predominant expressed among oral isolates, whereas *ALS3* was higher in vaginal isolates than oral with little differences (fig. 2).

Expression Levels of *HWP1* and *ALS* Genes:

C. albicans clinical isolates defined as LBF and HBF were further assessed at a transcriptional level and the expression of genes related to biofilm formation was investigated. *ITS1* was used as the housekeeping gene and was shown to be stably expressed throughout all biofilm conditions.

The present results show that *HWP1*, *ALS1*and *ALS3* genes were expressed in all tested isolates for both of oral and vaginal candidiasis. The expression levels show that these genes were up-regulated in biofilm (BF) formation isolates with highest expression folding was 1.662, 1.176, 0.630 for *ALS1*, *ALS3* and *HWP1* respectively; whereas the lowest expression folding was 0.901, 0.370, 0.210 for *ALS1*, *ALS3* and *HWP1* respectively.

The majority of the genes tested followed a trend up-regulation in HBF compared to LBF. However It was appeared that *HWP1*, *ALS1* and *ALS3* were highly expressed in high biofilm HBFand less expressed in low biofilm, whereas in very low BF producers the fold up-regulation were not particularly high, but they were over expressed in strong (very high) biofilm producers in both of vaginal and oral isolates. In staid of that *HWP1*, *ALS1* and *ALS3* were predominant highly expressed levels among biofilm producers of oral isolates. Microbial adhesion is considered the first step for biofilm formation. This structure constitutes a protective milieu against environmental stresses and human host defenses [7].

these results are agree with Rajendran *et al*. [32] that the expression of biofilm-related genes *HWP1* and *ALS3* in *C. albicans* biofilms was evaluated and it was found that these were up-regulated in isolates with high biofilm. Despite *ALS3* and *HWP1* expression being increased in high BF when compared to low BF. In another recent study of Rajendran *et al*. [33] which improved their previous study; that high BF had hyphal specific gene expression increased *HWP1* and *ALS3*, Similar to the biofilm formation process, there was no difference in the percentage of up-regulated genes associated with cell adhesion and hyphal growth in the LBF and HBF, which in total accounted for only 4% of up-regulated genes in each group.

The expression of *ALS1*, *ALS3* and *HWP1* has already been observed in biofilms associated with abiotic surfaces [34]

Garcia-Sanchez *et al*. [35] found that the *ALS1* gene was clearly up-regulated in biofilms when compared to planktonic cells which can explain the high expression of *ALS1* in LBF (fig.3). The case of *C. albicans*, *ALS3* appears to play a key role in adhesion to oral epithelial cells, and it is also related to the extent of subsequent epithelial damage and induction of epithelial cytokines[36], an overexpression of *ALS3* was observed in initial stages of biofilm formation [34], in study by Nailis *et al*.,reported that overexpression of *ALS3* and *HWP1* were more pronounced in biofilms grown in the *in vivo* model, and their expression levels were higher in the catheter reactor than in the microtiter plate (MTP) and this may explain the up-regulation of *HWP1*and *ALS* genes in some isolates with very low biofilm producers [37].

Hu *et al*. [38] found 16 high biofilm formers from a total of 104 clinical isolates. Although there were two strains showing higher expression of *HWP1* and four different strains showing higher expression of *ALS3*, their up-regulation did not directly correlate with the ability to form abundant biofilms. The present results of

gene expression found that *HWP1* was the most affected gene in HBF fallowed by *ALS3*, whereas *ALS1* have less affect in HBF, that some isolates with LBF show particularly high up-regulation of these genes.

Furthermore, *ALS3* and *HWP1* were highly up-regulated in biofilms grown in the reconstituted human epithelium (RHE) model, Nailis *et al*., [37] established an increase in the percentage of filaments during biofilm formation in this model system. *C. albicans* needs to invade and destroy epithelial cells, in order to grow in the RHE model, and hyphae are known for their increased invasiveness [14] which is not surprising as hyphae are the predominant form in biofilms grown in this model system due to the overexpression of *HWP1* and *ALS* genes [39]

The *ALS* genes aggregate becomes in essence a multivalent adhesin. Thus, even weakly bound ligands are rebound rapidly after they are released [40], which explain the overexpression of adhesins among strong and moderate (HBF) biofilm in this study.

The initial stage of biofilm is adherence, and how is adherence regulated? As mentioned above, many of the major known adhesins are expressed at the highest levels on hyphal cells. Their expression is regulated by transcription factors that also govern hyphal development [41]

It has been suggested that *HWP1* surface protein requires *ALS1/3* association to initiate *in vivo* biofilm formation [42], a complementary role was suggested for *ALS1/3* and *HWP1* genes in *in vivo* and *in vitro* biofilm formation [43]. The present result is consistent with reports showing that the combined expression of the adhesion proteins *HWP1* and *ALS1–ALS3* significantly facilitates biofilm formation. The high frequency of expression of *HWP1* and the *ALS1/3* genes found in this study reveals the capacity of the isolated strains to cause chronic vaginal and oral infections.

Expression Levels of *SAP5, PLB1* and *LIP8* Genes in *C. albicans*:

The present study showed that not only *ALS1, ALS3* and *HWP1*, but *SAP5*, *PLB1* and *LIP8* gene families were also expressed in biofilm; the current results demonstrated that genes encoding adhesins and genes encoding extracellular hydrolases are constitutively expressed in biofilms grown on abiotic surfaces *in vitro*.

qRT-PCR analysis shows that variant expression levels of *SAP5*, *PLB1* and *LIP8* in *C. albicans* high biofilm producers isolates in staid of low biofilm produce, the highest expression folding was 6.727, 2.462, 0.637 for *SAP5*, *PLB1* and *LIP8* respectively; the lowest expression folding was 1.447, 0.724, 0.366 for *SAP5*, *PLB1* and *LIP8* respectively.

In general; gene expression level show that *SAP5* and *PLB1* were up-regulated in both of HBF and LBF producers, and they were highly expressed among the ten tested isolates (fig.3).

C. albicans can generate a number of hydrolytic enzymes with broad substrate activity that can damage host cell structures. Perhaps, the most extensively studied extracellular hydrolytic enzymes of *C. albicans* are the secreted aspartyl proteinases [45] Previous research demonstrated that members of the *SAP* gene family are expressed in biofilms associated with mucosal surfaces [46]. the present results investigated that *SAP5* was also highly expressed in biofilm associated with abiotic surfaces. Mendes *et al*. [47] showed that *C. albicans* biofilms secrete more *SAPs* than do planktonic cells, while Ramage *et al*. [48] showed that an *in vitro C. albicans* biofilm induced *SAP* activity, and that *SAP8* expression within the biofilm correlated with *in vivo* denture stomatitis severity.

Results presented by Monroy-Pérez *et al*. [49] showed that all of the *SAP* genes were expressed in the reconstituted human vaginal epithelium (RHVE), suggesting that the Sap proteins play an important role in the pathogenesis of infection.

Of the 10 *SAP*s, expression of *SAP4, 5* and *6* has been demonstrated in all mucosal and systemic infections by *C. albicans* examined [46]. In addition, *C. albicans* expresses the *SAP4 – 6* ubiquitously in all *Candida* carriers and patients with oral and vaginal candidiasis, regardless of the infection model, suggesting that this proteinase subfamily plays an important role in *C. albicans* colonization and infection [18].

Li and colleagues [50] suggested that *Sap2* and *Sap5* were the most common genes expressed during oral mucosal infection and with *Sap5* and *Sap9* most strongly expressed throughout the course of infection.

Table 1: The primers and their sequences used in conventional PCR technique

Primers	Primers sequences (3`-5`) Forward reverse	Annealing temperature (°C)	Product size (bp)	Reference
ALS1	GAC TAG TGA ACC AAC AAA TAC CAG A CCA GAA GAA ACA GCA GGT GA	52	318	21
ALS3	CCA CTT CAC AAT CCC CAT C CAG CAG TAG TAG TAA CAG TAG TAG TTT CAT C	58	342	22
HWP1	ATG ACT CCA GCT GGT TC TAG ATC AAG AAT GCA GC	52	572	21
LIP8	AGA GTG ATA CAG ACA AAA AAT CAG AAG ACC ATT CAG CAT CAT GGT G	59	521	15
SAP5	AGA ATT TCC CGT CGA TGA GAC TGGT CAA ATT TTG GGA AGT GCG GGA AGA	60	277	23
PLB1	CCT ATT GCC AAA CAA GCA TTG TC CCA AGC TAC TGA TTT CAC CTG CTC C	58	179	23

Table 2: Reaction volume and components of RT qPCR:

Components	Conc.	Volume(µl)\Reaction
GoTaq qPCR master mix	2X	10 µl
RT mix		0.4 µl
Forward Primer	10µM	2 µl
Revers Primer	10µM	2 µl
RNA	1-2ng	4 µl
RNase-free water	-	1.6 µl
Total per reaction	20 µl	

Table 3: Thermal Cycler Programming:

Steps	C°	min:sec	Cycles
cDNA Synthesis	37	15min	1
Initial Denaturation	95 C°	5 min	1
Denaturation	95 C°	30 sec	40
Annealing	60 C°	30 sec	
Extension	72 C°	30 sec	
Melt	65-90 C°		1

Table 4: Biofilm formation by *C. albicans*

Candida albicans source	Biofilm Strength ODc= 0.060 No. (%)		
	Weak BF	Moderate	Strong
VVC (n= 22)	10 (45.5%)	12 (54.5%)	0
OC (n= 22)	8 (36.4%)	13 (59.1%)	1 (4.5%)
Total (n= 44)	18 (40.9%)	25 (56.8%)	1 (2.3%)
Chi-square –χ2	4.367 *	2.159 NS	1.077 NS
P-value	0.0488	0.148	0.375

* (P<0.05), NS= Non-significant.

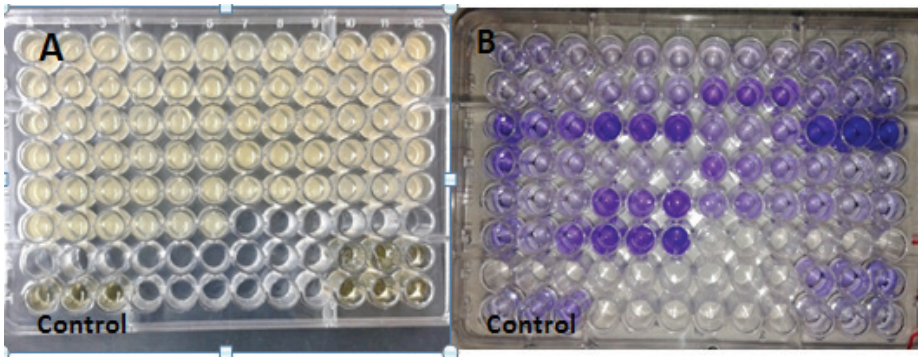

Figure1: Microtiter plate for biofilm formation, *Candida albicans* isolates biofilms after 24h at 37°C cultured on YPD with 2% glucose. A) before staining B) after staining with 0.5 mg/100ml crystal violate.

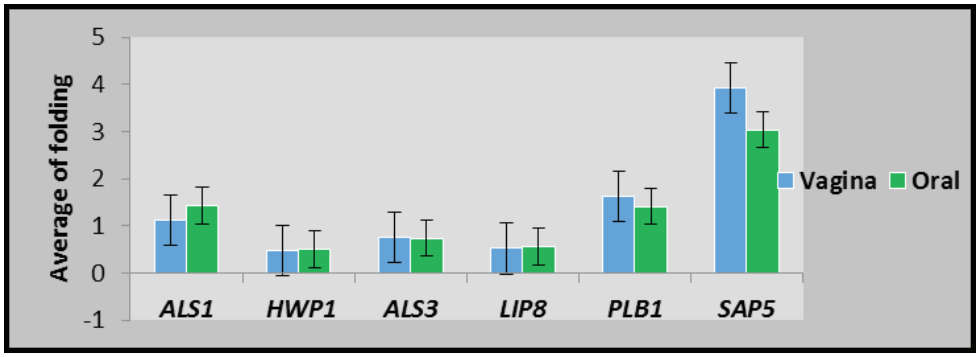

Figure 2: Averages of expression folding of *ALS1*, *ALS3*, *HWP1*, *SAP5*, *LIP8* and *PLB1* of *C. albicans* among oral and vaginal isolates.

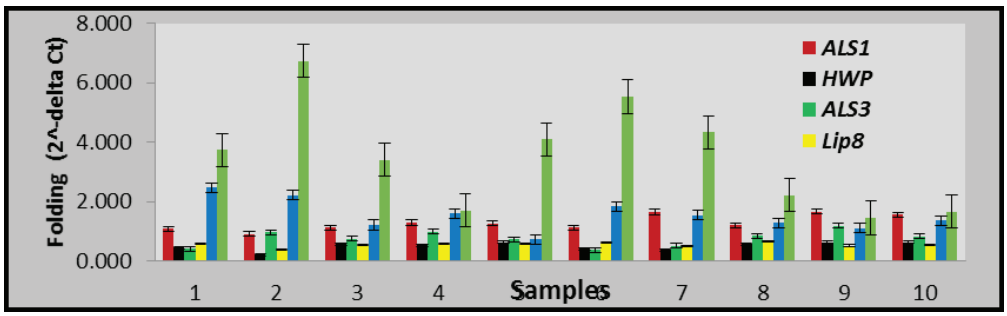

Figure 3: Gene Expression by qRT- PCR Technique of *ALS1*, *ALS3*, *HWP1*, *SAP5*, *PLB1* and *LIP8* Affected in Biofilm Formation of *Candida albicans*, Samples 1-5 Isolated from Vulvovaginal Candidiasis, from 6-10 Isolated from Oral Candidiasis.

Conclusions

1. Quantitative real time PCR technique show that *ITS1* was used as the housekeeping gene and was shown to be stably expressed throughout all biofilm conditions and *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* where 100% expressed in all ten tested *C. albicans*.

2. Adhesins such as *HWP1*, *ALS1* and *ALS3* were highly expressed in HBF and less expressed in LBF, whereas in very low BF producers the fold up-regulation were not particularly high, but they were over expressed in strong biofilm producers in both of vaginal and oral isolates.

3. *SAP5* and *PLB1* were highly expressed among vaginal isolates rather than oral ones; in contrast, *ALS1*, *HWP1* and *LIP8* were predominant expressed among oral isolates, whereas *ALS3* was higher in vaginal isolates than oral with little differences.

4. The overexpression of adhesins such as *HWP1*, *ALS1*, *ALS3*, are responsible in strength of biofilm formation in *C. albicans* isolated from oral and vaginal infection with different ages and different clinical states, in addition to upregulated of extracellular hydrolytic enzymes genes belong to *SAP*, *PLB* and *LIP* were important in biofilm formation.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad AlRafidain University Collage Dyala and all experiments were carried out in accordance with approved guidelines.

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International Responsibility for Hiding (COVID-19) Spread Information

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Abstract

In accordance with the rules of international law, countries that preceded others in exposure to the (COVID-19) have an obligation to inform other countries and the World Health Organization of the outbreak of the said virus as well as to exchange information on laboratory results, source and type of potential risk, number of cases and deaths, the rapid spread of the virus and its medium of spreading between people or through means of transportation such as airplanes, ships, etc., or through goods shipped from them and ways to limit its spread. Then, countries that have suffered damage as a result of infection with the aforementioned virus can claim the necessary compensation towards other countries that have breached their obligations to exchange information on the spread of the virus on their territories in accordance with the principle of international responsibility. Such compensations are to be determined through international arbitration or justice or through consulting international and regional organizations or through referring to other peaceful means that are agreed upon in accordance with Article 33 of the Charter of the United Nations.

Keywords: *COVID-19, Spread Information, International Responsibility*

Introduction

The rules of international law on human rights guarantee several rights that assure an individual's life at a level that guarantees their health and life. As a result of those rules, what is known as the (right to health) is crystallized and considered as one of the basic human rights. Maintaining health requirements is the right of every individual to survive and live without suffering that can be avoided. The research topic raises several important problems, the most important of which is the commitment of countries exposed to infectious diseases to transfer correct and complete health information to other countries and state organizations concerned in the field of health. As well as, it deals with the possibility of countries affected by the spread of the COVID-19 virus to acclaim compensation for damages caused by not taking the necessary measures and precautions because they are not aware from the start of the presence of the virus in the regions of the countries where the virus began to spread.

1. International Obligations of Sharing Infectious Disease Information

To ensure the right to health, there are several obligations on states to ensure its fulfillment, and the obligation to exchange information on infectious diseases is at the forefront of those obligations. The commitment was organized in several international documents, the most important of which is Comment No. 14 of 2000 of the Economic, Social and Cultural Committee of the Economic and Social Council of United Nations as well as the International Health Regulations 2005.

1.1 Countries' Obligations to Exchange Information According to Comment No. (14) Of 2000

The International Committee on Economic, Social and Cultural Rights issued its famous comment No. (14) For the year 2000, which came under the title (The Right to the Enjoyment of the Highest Attainable Level of Health), it mentions creating an appropriate climate to facilitate the fulfillment of the obligations to guarantee the right to health, and considered that their violation constitutes a violation of the said right. Countries must implement them with the utmost speed and effectiveness [(1)]. these obligations can be identified as follows:

A. Violation of the obligation to respect: means the actions of countries and their policies and laws that violate the right to health in its broad sense, and that violation results in physical damage, diseases and deaths that could have been avoided, such as depriving some individuals or groups of health facilities and services, intentional detention and not displaying human health information and its treatment.

Thus, the concealment of information by countries on the spread of epidemics and infectious diseases, as happened in China, Iran and others when they concealed the spread of the virus (COVID-19) in their regions, places them in violation of the obligation to respect, and then they are responsible in accordance with the rules of international law for what this concealment caused in the transmission Virus for other countries.

B- Violation of the obligation to protect: means violations that arise from the failure of the state to take all necessary measures to protect people within its jurisdiction from a violation of their right to health by third parties, such as failure to regulate the activities of individuals, groups or companies to prevent it from violating the right of others to health, and not to stand against traditional medical or cultural practices harmful to human health [3].

C- Violation of the obligation to perform: It means violations by states as a result of not taking the necessary measures to ensure the implementation of the right to health, such as not preparing a public policy that guarantees everyone the right to health without discrimination or failure to implement the prepared policy [4].

1.2 Countries' Obligations to Exchange Information in Accordance with the International Health Regulations of (2005)

The World Health Organization (WHO) is responsible for managing the global system to combat the spread of diseases at the international level. The WHO constitution gives to the World Health Assembly the authority to adopt regulations that are intended to reduce the spread of diseases at the international level. After its approval by the Assembly, it enters into force for all the states parties that do not confirm their withdrawal of their own free will within a specified period.

The regulations clarified the meaning of the phrase a public health emergency that causes international concern as an exceptional event that poses a potential public health risk due to the spread of the disease internationally (Paragraph (1) of Article (1) of the International Health Regulations 2005). It also indicated that each of the states parties and WHO should designate contact points to exchange information at all times (Paragraphs (3,4) of Article (4) of the International Health Regulations 2005). The regulations also outline three ways for states parties to fulfill their obligation to notify WHO and other countries when a specific epidemic occurs:

1. Notification: All countries are responsible for evaluating the events taking place in their territories, and the WHO is notified of all events that constitute a public health emergency causing international concern within (24) hours of the evaluation conducted by the country concerned. These notifications must be followed by detailed continuous information sent to the health organization including identifying the source and type of potential risk and laboratory results, the number of injured and deaths, how the disease spread and measures taken towards it, as well as the difficulties and necessary support that it needs.

2. Consultation: For a state party in which the events do not constitute a public health emergency and do not cause international concern are regularly reported to and consulted by WHO on appropriate health measures.

3. Other Reports: These are reports received from sources other than notifications and consultations. The health organization evaluates them and informs the states parties without preserving the confidentiality of the source except in exceptional cases within which it is justified. The states parties submit to the health organization within (24) hours the existence of imminent public health risks has been identified outside its territory and could potentially cause the spread of a disease on an international scale.

All countries are obligated to check the names of travelers in the regions of the affected countries, review the medical examination evidence, stipulate the vaccination and place the suspect under observation with his isolation when necessary. The country has the right to refuse entry to the suspect and the injured in its region, and it has a procedure for examining those coming from the affected areas, and restrictions on their exit. They shall also comply with the necessary health

measures on all means of transport, inform travelers of the aforementioned measures, and specify transportation methods that are free from sources of infection and bear full responsibility for them. When infected travelers suspected of being infected with the virus arrive, the country must inform the relevant authorities of the information in question.

2. Provisions of International Responsibility for Hiding COVID-19 Virus Spread Information

As a result of the multiplicity and diversity of relations in public international law, international responsibility has become one of the most important results of this multiplicity and diversity, especially in light of the development in the field of science and technology. So, it is assumed that basis of international responsibility and knowledge of its pillars and results in achieving certain effects.

2.1 Basis of International Responsibility for Hiding COVID-19 Virus Spread Information

The basis of international responsibility is the reason for that responsibility being established on the causing country, and two theories have emerged regarding this which can be summarized as follows:

First / Error Theory: International responsibility is not achieved according to this theory unless a country or an international organization commits a mistake that harms other persons of international law. Therefore, it is not sufficient for the emergence of international responsibility to cause harm to a person of international law or its members, but rather, the cause of that harm must be the occurrence of a wrongful act, that is, unlawful according to the rules of international law, whether the error was intentional or unintentional (Sawadi, 2017).

Second / Risk Theory: This theory is based on international responsibility to verify the damage as a result of an act, even if it is legitimate in accordance with the rules of public international law such as nuclear experiments, space and medical research, and others. Then, the damage is the main element for the establishment of international responsibility.

The International Law Committee of the United Nations General Assembly has attempted to broaden the concept of international responsibility by adopting this theory, having included the topic (international liability for injurious consequences arising out of acts not prohibited by international law) during its thirtieth session in 1978. After the determined efforts of the Committee, in 2001 it was able to adopt the draft articles on (Preventing trans boundary harm from hazardous activities). Article (3) of the approved project obliged countries to take all appropriate measures to prevent or limit trans boundary grave harm, and Article 8 obliged courtiers when exposed to risks to send information and evaluate these risks to other countries that are likely to be affected.

To sum up, the basis of international responsibility for hiding information about the spread of COVID-19 virus in its regions is in error theory. As hiding that information is an illegal act in accordance with the rules of international law, in violation of the obligations mentioned in the comment of the United Nations Committee on Social, Economic and Cultural Rights No. (14) for the year 2000 and the International Health Regulations for the year (2005), and the World Health Organization such as notifying other countries at an appropriate time with the real information on the risks of spreading the virus and providing the available information. So that, countries can take the necessary measures to limit its spread in their regions.

2.2 Pillars of the International Responsibility to Hide the Information of the Spread of (COVID-19)

- **Attribution:** It refers to the attribution of the act, whether legitimate or unlawful, to any person of international law, and the act is attributed to the country as long as it has an independent and fully sovereign international personality. States lacking sovereignty are not questioned about their actions. Rather, countries taking the role of protecting, mandate, or trusteeship are questioned on the behalf. The full sovereign states are responsible for the actions of one of their public bodies (legislative, executive, or judicial) regardless of the extent to which these acts conflict with their domestic legislation.

The countries are questioned when legislating any law that contradicts international law or when the necessary legislation is not implemented to international obligations. They are also responsible for the actions of their executive bodies such as the president of the country, prime minister, one of the ministers, or all employees. They are also questioned for the judgments issued by their national courts, or when it does not, contrary to the rules of international law (Hamdi, 2013).

Countries also bear international responsibility for the actions of their nationals. This is because of its breach of its basic obligations to maintain security and public order within its territory (Al-Attiyah, 2008).

- **Harm:** International responsibility is related to the existence and non-existence of the harm as a result of the predicate act of countries or international organizations, and whether that act is legitimate or unlawful in accordance with the rules of international law. Until the harm is achieved for international responsibility, it must be confirmed and not likely, whether the it is physically achieved, such as attacking the borders of the state, its ships or planes, or morally, such as an affront to its dignity or lack of respect for its leaders or its knowledge, as it may affect the nationals of country. The harm may be material to the property of people or their bodies and health, or it may be moral to their dignity and reputation. Both harms may be combined material and moral as a result of one act.

2.3 Effects of International Responsibility to Conceal the Information of the Spread of (COVID-19)

The main effect of international responsibility is the obligation of the country or international organization causing the harm to compensate for the reparation of damages that resulted from the act attributed to them. Several judgments have been confirmed by the international judiciary as a permanent arbitration court ruling in the Chorzow Factory case between Germany and Poland in 1927. It indicated that the breach of international obligations requires adequate compensation, it is one of the principles established in international law (Sawadi, 2017). The harms compensation shall be in several forms:

- **Reconciliation**: When the act attributed to the country or the international organization does not result in any material harm, then the reconciliation is the appropriate compensation for moral harm. It is through the non-approval of the actions issued by its bodies, employees or individuals, and therefore the compensation is in the form of a diplomatic apology or expression of regret or saluting the flag when insulted (Al-Attiyah, 2008).

- **In-Kind Compensation**: In-kind compensation is the natural form of reparation, and is intended to restore matters to what they were before the act that caused international responsibility occurred. In-kind compensation - if possible - is the best image to remove all traces of harm incurred, such as returning money confiscated from foreigners without Right.

- **Financial Compensation**: It means compensation for material or moral harms resulting from the act attributed to the country or the international organization by paying a sum of money similar to the harm without increase or decrease. It includes the losses and profits that the affected country has suffered, and financial compensation is the common image of international responsibility. In kin compensation is not possible in all cases, the Permanent Court of Arbitration referred to that meaning by saying: that all country responsibilities can be settled through financial compensation. We conclude from the above that countries violating the obligation to exchange real information about the spread of the virus in their regions take full international responsibility for compensating the damage caused to others as a result of the inability of the affected countries to take the necessary measures and precautions before spreading the virus in their regions. Certainly, the compensation can only be in a financial form determined through peaceful means to settle international disputes mentioned in the United Nations Charter.

Conclusion

1. Countries that have suffered harm as a result of the spread of the aforementioned virus claim to compensate for those damages from other countries violating the obligation to exchange information in accordance with the rules of international responsibility.

2. Financial compensation is the ideal form of compensation for damages resulting from a violation of the obligation to exchange information on communicable diseases, due to the difficulty of making compensation in kind. The situation cannot be restored to what it was before the violation occurred, and reconciliation alone does not satisfy the reparations.

3. It is noted that the Security Council and the United Nations General Assembly have not exercised their presumed role in maintaining international peace and security to deter and hold accountable countries in violation of the obligation to exchange information, as a result of the threat of the spread of the COVID-19 virus to international peace and security. The health of all peoples is essential to achieving peace and security, as indicated in the 1948 WHO Constitution.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa-Faculty of law and all experiments were carried out in accordance with approved guidelines.

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Psychological Suffering of Families, Children with Mental Retardation at Al- Raja Institute for Mental Retardation in Babylon City

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Abstract

Objectives: To assess psychological suffering of families of children with mental retardation

Methods: Descriptive study conducted among 60 caregivers, they are using self-reported questionnaire to gathering data about the phenomena

Results: (50% & 44%) of families had moderate and severe psychological suffering respectively. The grand mean is (2.15) at moderate level of suffering.

Conclusion: the families have moderate to severe psychological suffering due to caring child with mental retardation. They are need for psychosocial support.

Key words: *Psychological Suffering, Families, Children, Mental Retardation*

Introduction

Mental retardation effects over 120 million people worldwide. It often gives rise to severe life long standing disability and imposes heavy burden on the families and carers.(1)

Mental Retardation (MR) is defined as a disability characterized by remarkably low intellectual functioning (IQ< 70) in conjunction with significant limitations in adaptive functioning. Worldwide prevalence of mental retardation is reported to be as high as 2.3%.(2)

'Burden' may be defined as a multidimensional concept with objective and subjective components. 'Objective burden' is defined as specific happenings and activities related to caregiving, for instance, financial problems or personal activity limitations. 'Subjective burden', on the other hand, encompasses affective responses to the caregiver experience, such as feelings and emotions related to fear, strain and guilt.(3)

The relationship between caring for these children and psychological distress for their parents has been studied but mainly in developed world settings. Some studies have been conducted in Low and Middle Income Countries (LMIC] such as Kenya, Kuwait, Qatar and India which similarly report rates of 47–50% prevalence of psychological disorders amongst these parents. Parenting such children may lead to difficulties with family functioning, parenting stress, and different parenting style compared to parenting normally developing children. The effect of stress on family functioning can also be related to negative couple attribution related to marital satisfaction. It can also lead to divorce and financial problems.(4.5)

Caring for a person with mental illness takes a substantial toll on social relationships, employment and income and psychological well-being. Most families fear for their future health in addition to the stress of caring for their ill family member. Families of people with severe mental illness (SMI) have worse physical health and seek more medical care than those families without SMI. Children of people with SMI have higher risk of developing physical and mental illness for a variety of reasons, including stigma, financial difficulties, the burden of caring for ill parents and genetic vulnerability. The impact on children is long term and affects their

adult health and relationships.[6]

The continuing care of children with mental retardation is often stressful for parents as these children's difficulties inevitably touch their lives. The effects on the family unit can be far-reaching, restrictive, and disruptive, and they may be economic, social, and emotional in nature. Although management of the daily needs of children with mental handicaps has been extensively documented.[7]

Parents of children with mental retardation experiences high level of emotional, financial and physical stress. There are multiple problems of having a mentally retarded child in the family. The problems are mainly related to the social ridicule and social stigma. Feelings of depression are common, particularly when realization of the child's retardation is recent. Mothers of children with disability have the higher the perception of economic situation and income adequacy, parenting social support, and religious practices, and the lower the symptoms of depression and found that minority mothers showed the higher the religious coping, the lower the symptoms of depression.[8]

Family caregivers often feel overloaded with the additional obligations and roles they have to pick up. They find it increasingly burdening to care full-time for the household and provide emotional support for the patient. The family's problems and the way family members regard the disease may be also a result of the family system they are in.[9]

Methods

Descriptive study design was conducted out to assess psychological suffering of families of children with mental retardation at Al- Raja institute for mental retardation. The study carried among 60 caregivers selected by a non-probability (purposive sample).

The study tool was a self-reported questionnaire constructed to measure the variable of psychological suffering of families of children with mental retardation. The questionnaire consists of two parts. 1st part related to the demographic characteristics of the study sample, which include age, gender, level of education, and relative to the child. 2nd part related to psychological suffering of families which is composed of 13 items, rating and scoring according to 3 likert scale answered by 3 key answer always (3), sometimes (2) and never (1), which assessed by cutoff point (0.66) due to scores (1, 2 and 3) respectively. level of assessment: (1-1.66) = low = L, (1.67-2.33) = moderate = M, (2.34-3.00) = severe = S. The data collected after taken the sample agreement to participate in the study The data analysis by using descriptive data analysis

Result

Table 1: Distribution of the Demographic Characteristics of Caregivers

Variables		Frequency	Percent
Age	20-30	11	18.3
	31-40	22	36.7
	40-50	16	26.7
	51 and more	11	18.3
	Total	60	100
Gender	Female	35	58.3
	Male	25	41.7
	Total	60	100

Cont... **Table 1: Distribution of the Demographic Characteristics of Caregivers**

Educational level	Read and write	7	11.7
	Primary school graduate	8	13.3
	Elementary school graduate	12	20
	Secondary school graduate	20	33.3
	Diploma and bachelor graduate	13	21.7
	Total	60	100
Relative person	Father	20	33.3
	Mother	32	53.4
	Grand mother	8	13.3
	Total	60	100

The finding in table 1 indicate that 22 (36.7 %) of them at age 31-40 years old, 35 (58.3%) of them were females, 20 (33.3%) of them were secondary school graduated, and 32 (53.4%) of them were mothers of children.

Table 2: Distribution of the psychological suffering of families of children with Mental Retardation

No	Items	Always	Some times	Never	Mean	Ass.
1	We got insomnia because of our child with disease	28	21	11	2.28	M
2	We feel guilty especially when our child suffers from disease	33	20	7	2.43	S
3	We have a desire to cry because of our child with disease	24	21	15	2.15	M
4	We are scared about the future of our child with disease	22	20	18	2.06	M
5	We are hopeless that our child getting cured	8	17	35	1.55	L
6	We panic when heard about prognoses of our child with disease	14	12	34	1.67	M
7	We are hesitated from having other children because of the disease	45	12	3	2.7	S
8	We feel despair of our child because he could not share playing with his mates	23	21	16	2.12	M
9	We feel incomplete because of disease	37	14	9	2.5	S
10	We feel despair of our child because he has to keep away for long times from his mates	23	22	15	2.13	M
11	We feel pain for his long time of because from school because of his problem	21	19	20	2.02	M
12	We feel pain because of his growth retardation because of disease	21	18	21	2	M
13	We feel pain when we see our child not able to eat all types of food	33	15	12	2.35	S
Grand mean	2.15	Assessment			Moderate	

M= mean, Ass.= assessment, level of assessment: (1-1.66) = low = L, (1.67-2.33) = moderate = M, (2.34-3.00) = severe = S

The finding in table 2 indicate that the families of children with mental retardation have moderate level of suffering at mean score (2.15)

Table 3: Distribution of the Families' Level of Psychological Suffering

Level of suffering	Frequency	Percent
Low level (1-1.66)	6	10
Moderate level (1.67-2.33)	29	48.3
Severe level (2.34-3)	25	41.7
Total	60	100

The finding in table 3 indicate that (48.3% and 41.7%) of families had moderate and severe psychological suffering respectively

Conclusion

The present study concluded that the families of children have moderate to severe psychological suffering related to care of child with mental retardation

Recommendation:

Psychosocial support interventions and decrease the family problem and experience related psychological suffering.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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Assessment of Eating Habits of Adolescents Body Mass Index Percentile at Secondary School in Najaf Governorate

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Abstract

Objective: to assess and find out association between the eating habits and adolescents BMI percentile at secondary school.

Methodology: A cross-sectional study was carried out among 537 adolescents (270 boys and 267 girls) aged 12-15 years selected by means of a multistage stratified random sampling technique.

Results: according to the body mass index; underweight was (6.7), normal weight was (61.5), overweight was (9.5) and obesity was (22.3). (75%) of adolescents have a healthy eating habits while more than two third of adolescents do not consumed fruits and vegetables.

Recommendation: Intervention programs focusing on promoting changes in lifestyles, food habits and increasing physical activity need to be implemented at the earliest stage of children life.

Key words: *Eating Habits, Adolescents, and Body Mass Index.*

Introduction

The term adolescence is transitional period between childhood and adulthood. It is the end of childhood and is characterized by dramatic changes of all aspects of physical, biological, cognitive, social and emotional maturating. Adolescence marks of the end of childhood and is characterized by physical, psychological, social and emotional changes. It is a critical period of great stress. (1) Adolescents are a nutritionally vulnerable group, because they need high requirements of nutrition for growth and maturation. The change in the life of adolescents and they need to achieve their identity, the eating pattern and life style influenced by the environment and the peer group. Inadequate nutrition or unhealthy nutrition of adolescents leads to health problems. (2) Adolescence is an intense anabolic period when requirements for all nutrients increase. During adolescence, 20% of final adult height and 50% of adult weight are attained, bone mass increases of 45% and dramatic bone remodeling occur and soft tissues, organs, and even red blood cell mass increase in size. (3) The term eating habits (or food habits) refers to why and how people eat, which foods they eat, and with whom they eat, as well as the ways people obtain, store, use, and discard food. Individual, social, cultural, religious, economic, environmental, and political factors all influence people's eating habits. (4) Several facets of eating habits are different and more pronounced in adolescents than in other people and each might cause concern in older generation: skipping meals especially breakfast, eating snacks "empty calories", late night snacks, fast food, wow level of some nutrients (calcium and iron), unconventional meals, sweetened beverages and low consumed of milk dairy product and fruits and vegetables. (5)

Methodology

Subjects: The study population included Iraqi nationals, male and female students, aged 12 to 15 years. A representative sample of these adolescents (537 students, 270 boys and 267 girls) was selected from schools in Najaf city by using the proportional stratified sampling. From each school (20%) of the total number of students were randomly selected by interval number. The obese adolescents' number was 120 out of the total

study sample.

Anthropometric measurements:

The weight is measured for each adolescent participant in the study. It is measured without shoes and light clothes as possible. The investigator used weight scale which is highly reliable and borrowed from the Iraqi Nutrition Research Institute made by (Seca Company, Australia), weight scale is a gift from the United Nation Children′s Fund (UNICEF) and has a capacity of (188.8) kg.

Before use the scale, the investigator is checking the scale daily by weight a standard weight. During weighting, the scale was placed on a hard-floor surface, and each participant was stand still in the center of the platform of the scale with the body weight evenly distributed between both feet.

The height of adolescents is measured without shoes by using measuring tape of height two meters (UNICEF tape measure) it is already reliable. The individual should stand on a flat surface with weight distributed evenly on both feet, heals together and the head upward. The arms are hanging freely to the sides, and the head, back, buttocks and heal are against the wall with the knee fully extended and line of vision parallel to floor.[6] According to the Dietary Guidelines for Americans 2010, body mass index is a measure of weight in kilograms (kg) relative to height in meters squared. Body mass index status categories include underweight, healthy weight, overweight, and obese.

Underweight: < 5th percentile of BMI for age

Normal weight: 5th to < 85th percentile of BMI for age

Overweight: 85th to < 95th percentile of BMI for age

Obese: ≥95th percentile of BMI for age. [7]

BMI was calculated by scientific application program (WHO AnthroPlus) which obtained from Iraqi Nutrition Research Institute.

Questionnaire:

The sociodemographic data sheet, consisted of (12) items categorized as general information (adolescents age and gender) and socioeconomic data (parents level of education, parents occupation status, type of family, total number of family, number of rooms, house area, house content and car possession).

Eating habits questionnaire was adopted from food frequency questionnaire to assess the eating habits of obese adolescents. It was composed of (67) items which were grouped and concerned with daily main meals, snack meals, dairy food, eggs and meat, bread cereal and starch, fruits, vegetables, sweet backed food and miscellaneous beverages. [8]

A statistical analysis was performed using the Microsoft office excel 2007 and SPSS package (version 16). Chi- square statistics were used to determine the presence of an association between the variables.

Results

Table 1: Distribution of the Study Sample by their General Information

Variables		No.	%
Gender	Male	270	50.3
	Female	267	49.7
Age (years)	12	79	14.7
	13	183	34.1
	14	145	27
	15	130	24.2
SESS	High	40	7.4
	Middle	416	77.5
	Low	81	15.1

No. = number, % = percentile, SESS = Socio-economic Status Score

Table (1) shows that (50.3%) of the adolescents pupils is male, (34.1%) their age is 13 years old, and (77.5%) of them coming from middle level of socio economic status score.

Table 2: Distribution of the Study Sample by their Overall Measurement through Body Mass Index Percentile Results

BMI percentile	No.	%
Underweight (<5th Percentile)	36	6.7
Normal (5th -84th Percentile)	330	61.5
Overweight (85th -94th Percentile)	51	9.5
Obesity (=>95th Percentile)	120	22.3
Total	537	100%

No. = number, % = percentile

Table (2) shows that (61.5%) is normal weight from the total study sample.

Table 3: Distribution of the Adolescents BMI Eating Habits

Eating Habits		BMI				Total %	Chi Square
		Under Weight %	Normal Weight %	Over Weight %	Obesity %		
meals and Snacks Score	Unhealthy	0.93	6.33	2.42	4.09	32.4	χ^2=11.33 sig=0.01*
	healthy	5.77	55.12	7.07	18.25	57.6	
Fast Food Score	Unhealthy	0.93	11.55	1.49	3.17	17.13	χ^2=1.722 sig=0.632
	healthy	5.77	49,9	8.01	19,18	82.87	
Meat and Eggs Score	Unhealthy	0.56	12.1	2.61	5.77	21.04	χ^2=6.778 sig=0.079
	healthy	6.15	49.35	6.89	18.25	78.96	
Bread, Cereal and Starch Score	Unhealthy	1.3	17.13	3.35	7.82	29.61	χ^2=4.723 sig=0.193
	healthy	5.4	44.32	6.15	14.53	70.39	
Milk Dairy Food Score	Unhealthy	2.79	24.58	5.03	10.43	43	χ^2=3.950 sig=0.267
	healthy	3.91	36.87	4.47	11.92	57	

Cont... **Table 3: Distribution of the Adolescents BMI Eating Habits**

Fruits Score	Unhealthy	3.91	42.64	6.33	16	68.9	χ^2=2.461 sig=0.482
	healthy	2.79	18.81	3.17	6.33	31.1	
Vegetables Score	Unhealthy	4.66	48.23	7.82	16	76.72	χ^2=4.265 sig=0.234
	healthy	2.05	13.22	1.68	6.33	23.28	
Miscellaneous and Candies Score	Unhealthy	2.98	25.14	3.17	7.26	38.55	χ^2=3.743 sig=0.291
	healthy	3.72	36.3	6.33	15.08	61.45	
Funny drinks	Unhealthy	3.17	24.6	3.35	11.36	42.46	χ^2=0.942 sig=0.815
	healthy	3.54	36.88	6.15	10.99	57.54	

Cont. table (3)

Total Score Eating Habits	Unhealthy	0.75	16.01	2.98	5.59	25.33	χ^2=4.933 sig=0.177
	healthy	5.96	45.44	6.52	16.76	74.67	

%= percentage P= probability level, χ^2 = Chi-square, Sig = significant, * = significant at p-value ≤ 0.05

Table (3) shows that adolescents in all weight status have a healthy eating meals and snacks, more of them not eating fast foods, more of them healthy eating meat, eggs, milk dairy food and (Bread, Cereal and Starch) during the week, more than half of adolescents from all weight status have do not consume fruits and vegetables, miscellaneous and candies, more than one third of normal weight and more than half of obese adolescents consumed funny drinks 4 times and more per week and three quarter of the adolescents have a healthy eating habits.

Recommendations

1. The Ministry of Health must be provided a health staff for each school to follow up adolescents' health.

2. Regular visits to schools to detect obesity and its complications.

3. Continue to research the long-term health benefits that result from eating a healthy diet.

4. Research innovative, cost effective ideas to provide nutritious snacks during the school day.

5. Place posters throughout the school showing foods rich in various nutrients.

6. Healthy food tips in the school news letter for parents.

7. Provide facilities and environment for physical exercise in the schools.

8. Educational activities and more orientation about their diet and physical exercise at early ages involving the whole family to control the excess of

weight.

9. Encourage adolescents and their families to read the list of calories on the backed foods.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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Psychological Confusion and Its Relationship to Some Psychological Features of Taekwondo Players

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Abstract

The importance of the research lies in the identification of psychological frequency and some psychological features that players face in taekwondo competitions and to find the degree of correlation between them, and the problem of the research was through the work of researchers in the field of the taekwondo game. One of them has different psychological characteristics from the other, so the researchers decided to study this problem by determining this frequency, determining the psychological characteristics, and then knowing the relationship between them and whether there is an effect of one of them on the other to develop appropriate solutions in the service of Taekwondo players, and the research objectives were to prepare the psychological frequency scale, And the identification of psychological frequency and some psychological features of advanced Taekwondo players, and knowledge of the relationship between psychological frequency and some psychological features of advanced Taekwondo players, the researchers used the descriptive approach on some Taekwondo players in the Najaf Sports Club for the sports season (2019-2020) of (30) A player through the results of the results, the researchers reached the following conclusions. The players of the Iraqi Premier League with Taekwondo appeared as having an amputation. A weak psychological and high self-confidence trait, which increased their motivation for achievement in the competition, the players showed high psychological energy optimal for advanced Taekwondo players, which reduced their psychological reluctance to compete, and the presence of an inverse moral correlation between psychological hesitation and some psychological features (self-confidence - psychological fluency) For players of the Premier League Taekwondo.

Keywords*: psychological frequency, psychological features, taekwondo*

Introduction

The importance of the research lies in the identification of psychological hesitation and some psychological features that players face in taekwondo competitions and to find a degree The connection between them, and the research problem was through the work of researchers in the field of the game of Taekwondo. (1) They noticed that the majority of Taekwondo players suffer from a psychological frequency that differs from one player to another and that No one of them has different psychological characteristics from the other, so the researchers decided to study this problem by determining this frequency and determining the psychological features, and then knowing the relationship between them and whether there is an effect of one of them on the other to develop appropriate solutions in the service of Taekwondo players, and the research objectives were to prepare the psychological frequency scale Knowing the psychological frequency and some psychological features of advanced Taekwondo players, and knowing the relationship between psychological frequency and some psychological characteristics of advanced Taekwondo players(2).

practical part:

The researchers used the descriptive approach on some of the Taekwondo athletes in the Najaf Sports Club for the sports season (2019-2020), which numbered (30), players. To achieve the research objectives in preparing the psychological frequency scale of taekwondo players, the researchers followed the following steps:

Procedures for preparing the psychological frequency scale:

Preparing the initial formula for the psychological frequency scale:

Preparing the initial version of the scale required several procedures, starting with the process of preparing the scale paragraphs, determining the method and foundations for drafting paragraphs, starting to drafting paragraphs, and finally preparing the scale instructions, and the following is a description of those procedures:

Preparation of paragraphs of the psychological frequency scale:

To prepare the appropriate scale paragraphs to measure the concept of psychological frequency according to what is reflected in the theoretical definition of psychological frequency, the researchers conducted a comprehensive review of the relevant previous measures:

Determine the method and principles of drafting paragraphs of the psychological frequency scale:

The developed Likert method was relied on in formulating alternatives to the scale paragraphs, and it is similar to the multiple-choice method, which is considered one of the common methods of measurement and research, as it presents the respondent with a position and asks him to determine his answer by choosing an alternative from several alternatives with different weights.

Perhaps what distinguishes the use of this method is as follows:

1- It is characterized by flexibility due to a large number of alternatives.

2 - It has high reliability and reliability.

3- It reduces guesswork and chance factor. [3]

Formulation of paragraphs of the psychological frequency scale:

By analyzing the responses of the survey sample members to the questionnaire and in light of the theoretical definition and the use of studies and measures related to the psychological frequency scale and its components, (60) paragraphs were formulated distributed into (5) areas, and each paragraph represents one position on the situations that the players face in the form of positive and negative expressions Each position includes three alternatives to the answer and grades are given from (1-3) [4]

Determining the validity of paragraphs of the psychological frequency scale:

After the researchers finished formulating the paragraphs of the scale in its initial form, it was presented to the experts and specialists in educational and psychological sciences and sports psychology to demonstrate their validity and identify the positive and negative paragraphs, as well as if they need to be modified or transferred to a field other than the field to which they belong and the possibility of merging some paragraphs Similar.

To ensure the validity of the adjustments made by the researchers, the scale was presented again to some experts and specialists, to approve the modifications, and it obtained an agreement rate (100%) and accordingly, the psychological frequency scale was fixed according to the opinions and observations of the experts and specialists. [5]

Preparing answer instructions on the psychological frequency scale:

The instructions for the psychological frequency scale were prepared that explain how to answer its paragraphs. It was taken into account in preparing the instructions to be clear and easy to understand. To increase clarity, the instructions included a special model on how to answer the paragraphs of the scale. The important thing is to answer all the scale paragraphs frankly and their answers will be completely confidential It is for scientific research, and the researchers presented in the instruction sheet a template for the answer to facilitate the subject's answer. The answer instructions were placed on the scale without explicitly referring to the scale name or the name of its fields.

Exploratory experience:

To ensure the clarity of the instructions of the psychological frequency scale and the clarity of its paragraphs to the players and to know the time taken for their answers, as well as to identify the conditions

for applying the scale, a survey sample of 10 players from Najaf club was applied randomly, and it became clear from this experience that the scale instructions and phrases are clear The researchers did not face any difficulties with the exploratory sample, and the time it took to answer the psychological frequency scale ranged between (12-16) minutes with an average of (14) minutes, and thus the psychological frequency scale became ready for application to the construction sample.

Basic Experience:

The basic experiment was done by applying the psychological frequency scale to the building sample to conduct a statistical analysis of its paragraphs to select the valid paragraphs and exclude the invalid based on their discriminatory ability, as well as extract the indicators of validity and reliability of the scale. The researchers applied their scale to the building sample of 144 players. They represent some clubs of the Iraqi Premier League with Taekwondo for the sports season (2015-2016) in the period from 12/8/2015 to 29/8/2015, and the researchers stressed the need to read the instructions and paragraphs accurately and answer all the paragraphs honestly and honestly, and more than one opportunity to meet the researchers was invested Construction sample through training and matches.

Correction of the psychological frequency scale:

To find out the players 'answers on the psychological frequency scale, the correction key prepared for this purpose was used, which means (the tool by which the examiner reveals the answers that indicate the existence of the result that is being measured) (1), and the total score was calculated based on the sum of the weights of the answers for the scale and adult paragraphs ((34 paragraphs in which the answer for positive paragraphs (always) was given three degrees, while for the answer (sometimes) two degrees and the answer (never) only one score, and the correct opposite of the negative paragraphs, so the highest score that can be obtained ((102) and the lowest score (34). [6]

Psychometric properties of psychological frequency scale:

Verification of the standard characteristics of the scale is one of the basic requirements for it, and the two attributes of validity and reliability can be one of the most important characteristics, even if honesty is more important than consistency because an honest scale is fixed while the fixed scale may not be honest.

Validated psychological frequency scale:

Honesty is a broad concept that has several meanings that differ according to the use of the test, and truthfulness is intended to measure what the test is designed for. [7]

The researchers adopted two types of validity to ensure the validity of its scale, namely:

Validated content:

It is considered the most important type of honesty in the achievement tests and is related to the answer to the question, and this type of honesty was achieved when the psychological frequency scale was presented to a group of experts and specialists in the field of educational psychology and sports psychology to confirm the validity of the scale paragraphs and the extent to which these paragraphs represent the axes that measure them, and thus The invalid paragraphs were deleted and the valid paragraphs that gained the support of most experts and specialists were preserved, as well as amending and merging some paragraphs.

Stability of the psychological frequency scale:

A static test is a test that has a high degree of accuracy, mastery, consistency and objectivity in what was put to measure, and the stability of the test means that it is not affected by the change of external factors or conditions, which indicates the stability of the response of the individual no matter what circumstances change.

To verify the stability of the psychological frequency scale, the researchers used the following methods:

Halftone segmentation method:

This method relies on splitting the scale after applying it to a certain group into two equal parts, and this method has advantages that are.

1 - The examiner avoided the problem of re-examination or preparation of an equivalent image for the test.

2 - It cancels the effect of changes in the status of the scientific, psychological and health examiners that affect the level of his performance for the test.[8]

The half-segmentation method is one of the most used stability methods, due to its economy in effort and time, and the researchers adopted the data of the individuals of the basic sample of (131) players, as the paragraphs of the scale were divided into two halves, individual and even paragraphs, and the consistency of the degrees of the two halves was verified through Extracting the F-value for them, as their calculated value reached (1.08) degrees and when compared to the tabular value of (P) at two degrees of freedom (130-130) and the level of significance (0.05) amounting to (1.36), its statistical significance was revealed and thus we have verified the homogeneity of the two halves Scales of the scale, and the correlation coefficient between the sum of the scores of the two halves was extracted using the Pearson method by the Statistical Package for Social Sciences (spss), as the correlation coefficient was (0.853), and since the extracted correlation coefficient means stability for only half of the test, and in order to obtain complete stability for the test, the researchers applied the equation Cyberman - Brown, and thus the value of the test reliability coefficient was (0.920), which is a high reliability index of the test.

The scale of some psychological features (self-confidence - psychological fluency):

The researchers used a questionnaire for measuring sports self-confidence on the players of the research sample, a list of the self-confidence trait, designed by Robin Vealy, the Arabization of Muhammad Hassan Allawi, and the psychological fluency of the researcher, Howayda Ismail Ibrahim (2005) Building a scale of psychological fluency for players of excellent class volleyball clubs in Iraq by identifying (9) areas of the scale Through it, (6) areas were identified by experts and specialists and a definition of each field was set in preparation for collecting and preparing the paragraphs, after which (96) paragraphs were initially drafted, and then the initial formula of the scale was presented to a group of experts to judge the validity of the paragraphs in their measurement of psychological fluency. Through this, a section of the paragraphs was deleted and some paragraphs were replaced and corrected. As a result, (41) paragraphs remained distributed over the scale areas.

The exploratory experiment of the two measures:

To know the clarity of the instructions, paragraphs, and alternatives to answering by the research sample, determining the necessary time, and identifying the difficulties that may face the application process and thus avoiding them when applying the two scales in their final form, presenting and applying the scale to a sample consisting of (10) players chosen randomly and by lot, per hour. The second noon on Sunday 5/8/2015 in the places of their training, and it was found from the pilot experiment that the instructions were clear by the research sample and that the time taken for the application was between (17-18) minutes for a questionnaire to estimate the psychological frequency, as for the application of the scale of some features The psychological approach was (17-20) minutes. Either the answer alternatives were appropriate for the individual sample level as well as the paragraphs. Thus, the two scales with its instructions, paragraphs, and answer alternatives became ready for application to the sample.

The final application of the two scales:

After preparing the final form of the research criteria and after ensuring their validity and consistency, the basic sample for the research of (131) players from the Iraqi Premier League Taekwondo was determined. The two scales were applied to the players during matches and their movement between governorates from 4-12 / 10/2015, after which a procedure was conducted. The application of the researchers In addition to the assistance of the assistant staff, the researchers and the assistant staff who work with him explained the objectives of the research and its importance to the members of the sample and emphasized the necessity of answering all questions carefully, accurately and honestly in addition to answering the questions and inquiries they ask about the vocabulary and clarity of the two scales. The two measures were taken by the researchers and the data was filled out by relying on the keys of the questions, thus making all the raw scores as the test results, after which the scores were converted to final scores.

Statistical methods: The researchers used the Statistical Package for the Social Sciences (SPSS)

Results

The relationship of the psychological frequency with psychological characteristics of advanced Taekwondo players: -

Table (1): The presentation of the results of the correlation between psychological frequency and some psychological characteristics of advanced foot players shows

Indications	the value of the correlation	the value (t) of the significance of the calculated correlation coefficient	significant
The psychological frequency - self-confidence	0.44	6.25	moral
Psychological frequency - psychological fluency	0.31	4.16	moral

The tabular correlation coefficient (t) has a significant value of (98.1) at the degree of freedom (129) and the level of significance (05. (0)).

It can be seen from Table (1) that the value of the correlation coefficient computed between psychological frequency and the self-confidence characteristic of advanced Taekwondo players reached (0.44) and upon inferring the significance of the correlation (t t), the significant value of the calculated correlation coefficient of (25.6) appeared, and when compared With the tabular value of (98.1) and at the level of significance (05.0) and the degree of freedom (129) it was found that the computed value is greater than the tabular value, as it indicates that there is an inverse significant correlation between psychological hesitation and self-confidence trait, and this result appears to be logical and this means that The more players are characterized by good self-confidence and mutual relations, they are less prone to psychological hesitation, and the researcher attributes the reason for this relationship to the fact that the advanced players in Taekwondo had knowledge of their good abilities and capabilities. Therefore, the psychological state appeared at its best, which leads to players bearing the pressure of training away from fear and anxiety, if Players who have a high feeling for their clubs push them to exert more effort to achieve the goals set, and you see them merging with the club group in a cooperative social life where such people are seen and the group is an extension of that It matters and that life with them is part of their personalities, which creates a state of courage in making critical and accurate decisions).[9]

Also, the value of the correlation coefficient computed between the psychological frequency and the psychological fluency characteristic of advanced Taekwondo players amounted to (31.0) and upon inferring the significance of the correlation (t t), the significant value of the calculated correlation coefficient of (16.4) appeared, and when compared with the tabular value of (98.1) At the level of significance (05.0) and the degree of freedom (129) it was found that the calculated value is greater than the tabular value, and this confirms that the psychological frequency of the players will gradually decrease as a result of the increase in the optimal psychological energy in training and competitions, and the psychological variables play a role in the player's assimilation and gaining good psychological fluency To move away from competition anxiety and play a good masterful performance through repetition and success experience by following the scientific method that achieves the goals set by coaches, experts and educators to reach the best level of performance.

Conclusion

Through the results of the results, the researchers reached the following conclusions:

1- The players of the Iraqi Premier League appeared with Taekwondo, describing them with weak psychological hesitation and high self-confidence, which increased their motivation for achievement in the competition.

2- The players showed optimal psychological energy high for advanced Taekwondo players, which reduced their psychological reluctance to compete.

3- There is an inverse moral correlation between psychological hesitation and some psychological traits (self-confidence - psychological fluency) of the Taekwondo Premier League players.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of education and all experiments were carried out in accordance with approved guidelines.

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Health Personnel Practices Regarding Prevention of Sharp Injuries in the Delivery Room at the Middle Euphrates Teaching Hospitals

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Abstract

Literatures proved that prevention of sharp injuries are the most important and effective infection prevention and control measures to prevent the spread of microorganisms causing hospital Acquired infections. **Aim of the study**; This study conducted to assess personal protective equipment practices of health care personnel in the delivery room at the Middle Euphrates Teaching Hospitals. **Methods**; A Descriptive research design begin in 20th February to 26th May 2016, Current study sample involve all midwives and physicians in the delivery room (Total coverage.). Questionnaire used for data collection by interview forms and observational checklist was obtain from the extensive review of relevant literature and related studies Data analyzed through utilize (SPSS) software version (16) where, included descriptive analysis and inferential data analysis. The study conducted among 37 physicians and 97 midwives working in the delivery room are females.

Results*:* Overall scores of practices related to prevention of sharp injuries in the four studied hospitals were was good at Karbala (2.77) and Al-Najaf (2.37), while fair at Babylon (1.79) and Diwaniah (2.26). The present study has also indicated that there are a highly significant in health staff practices regarding prevention of sharp injuries and the different studied governorates at p-value less than 0.01.

Conclusion: Based on the finding of present study health care personnel have good practices at Karbala and Al-Najaf, while fair at Babylon and Diwaniah.

Recommendations: Updating practice of health care personnel through continuing in-service educational programs. Regular inspection and follow-up from the Ministry of Health for assurances good practices regarding prevention of sharp injuries, the important of exist motivation system and punishment system to the neglected health care personnel.

Keywords*: prevention, sharp injuries, Health care personnel, Practices.*

Introduction

Sharps and needlestick injuries are wounds caused by needles and other sharp medical instruments (e.g. scalpel, blades and scissors) that accidentally puncture or cut the skin. Sharps and needles may only cause small wounds in the skin, but the effects can be worse. Such instruments come in contact with blood and other body fluids and may carry the risk of infections. More than 20 dangerous bloodborne pathogens including HIV and hepatitis may be transmitted through accidental injuries with contaminated needles and sharps(GOHNET). Sharps include needles, scalpels, stitch cutters, glass ampoules, bone fragments and any sharp instrument. The main hazards of a sharps injury are blood borne viruses such as hepatitis B, hepatitis C and HIV. It is uncommon for the staff to be injured by the unsafe or poor practice of others; for example, cleaners who sustain injuries because of sharps being placed in waste bins [(4)]. Some procedures have a higher than average risk of causing injury. Devices involved in these high-risk procedures include; IV cannula, needles and syringes, winged steel needles (known as butterfly needles),

phlebotomy needles (used in vacuum devices)[6]. Needle stick and other sharps injuries are a serious hazard in any healthcare setting. Contact with contaminated needles, scalpels, broken glass, and other sharps may expose healthcare workers to blood that contains pathogens which pose a grave, potentially lethal risk (CDC, 2012). Factors that can influence the risk of sharps injuries are equipment, design of instruments, working conditions and working practices According to the US Center of Disease Control and Prevention (CDC), sharps injuries often occur in a fast-paced, stressful and understaffed situation. Working under such a demanding situation may result in fatigue, poor concentration, and carelessness, thus increasing the risk of sharps injuries. many Studies show that long working hours and sleep deprivation among medical trainees results in fatigue, which is associated with a 3-fold increase in the risk of sharps injury[3]. Prevention measures to eliminate or reduce sharps injuries can be done in different ways. These measures include: Eliminating the unnecessary use of sharps, Providing medical devices, Incorporating safety-engineered protection mechanisms, Implementing safe systems of work, Implementing safe procedures for using and disposing medical sharps, Banning recapping, Using personal protective equipment, Vaccination, Information and training[1].

Materials and Methods

1. Design of the study: A Descriptive study are utilized to meet the aim of the study.

2. Locale study: -Present study is carried at four Maternal and Child Health teaching hospital in the Middle Euphrates Region /Iraq.

3. Sample of the study: All midwives and doctors in the delivery room (Total coverage.).

4. The study instrument: - For data collection a questionnaire by interview forms and observational checklist was, obtain from the extensive review of relevant literature and related studies.

The study tool consisted from two parts as the following:

A-The first part of questionnaire: Socio-demographic characteristic, which include (6), items as regards to their age, marital status, educational level, years of experience, working experience in delivery room.

B-The second part of the questionnaire: Observational checklist to assess health care personnel practices related to application of prevention of sharp injuries in the delivery room.

5. Method of data collection:

Data are collected through direct interview with physicians and midwives by using constructed questionnaire. However, for health personnel practices date collected through the application of observation technique during they were working in the delivery room.

6. **Validity of the instrument**: Firstly, the questionnaire items are translate from English to Arabia by a professional experienced in translating Health survey questionnaires and then the questionnaires back translated into English in order to check for possible discrepancies and incorporating appropriate changes. Secondly, the content validity of questionnaire was determine by panel of 12 experts in academic and health field to examine the questionnaire content for adequacy and clarity to realize end goal to accomplish the targets of the present study.

7. Pilot study:

A Purposive sample of 10 health personnel was selected from AL-Zahra Teaching Hospital Maternity and pediatric at Al-Najaf during the period from 20th to 29th of February 2016.

This study aimed to:

1- Obtain the clarity and content adequacy of the instrument.

2- Determine the reliability of the instrument.

3- Estimate the average time required for the interview and observation each interview took (15-25) minute and unlimited time for observation.

4- Identify barriers that may encountered during date collection process.

The sample of the pilot study excluded from the original sample of the study.

8. Reliability of the instrument: -

Reliability of an instrument was determine through (Cronbach's coefficient) for the present study. Cronbach's alpha Reliability coefficient for practices of midwives and doctors was (r=0.78), this value of Cronbach's α for the study is considered acceptable.

Table 1 : Reliability of the instrument

Sample	N. Items	Alpha Cronbach's	Acceptable value	Assessment
10	80	0.78	0.71 – 0.91	Pass

Results of Study

Table (1) Distribution of the study subjects according to their demographic data. No=124

This table reveals that, (42.7%) of the sample are 31-40 years old. (64.5%) of them are Secondary midwifery school. 81.5% of them are married. 42.7 % of them have less than 5 years of experiences. Most of them have 47.6 % working experiences in the delivery room.

Table (2) Distribution of the study subjects according to their practices regarding prevention of sharp injuries with the overall evaluation. N=124

Items	Najaf		Karbala		Babylon		Diwaniah	
	M.S	Evaluation	M.S	Evaluation	M.S	Evaluation	M.S	Evaluation
1- Health provider received training in sharps/ splash/ bite injury management	2.4	Good	2.76	Good	2.00	Fair	2.44	Good
2- Sharps containers are available at the point of use	3	Good	2.93	Good	2.03	Fair	2.08	Fair
3- Sharps containers are correctly assembled, labelled with date, locality & signed	1.85	Fair	3	Good	1.18	Poor	1.92	Fair
4- The are no inappropriate items (eg; swabs/ packaging/ gloves) in the sharps container	3	Good	2.98	Good	2.03	Fair	2.96	Good
5- Needles & syringes are disposed of as a single unit	3	Good	2.96	Good	2.97	Good	3	Good
6- Syringes with a residue of prescription medication are disposed of according to current legislation	2.75	Good	2.96	Good	1.97	Fair	2.08	Fair
7- The temporary closure mechanism is used when the bin is not in use	1.45	Poor	1.76	Fair	1.09	Poor	1.44	Poor
8- Sharps containers are visibly clean with no, dust, dirt or debris	1.55	Poor	2.87	Good	1.06	Poor	2.2	Fair

Table (2); This table revealed that health staff practices regarding the items of prevention of sharp injuries at the Middle Euphrates Teaching Hospitals has good score related to the items (1, 2, 4, 5, 6), fair score related to the items (3) and poor score to the items (7 and 8) at Al-Najaf. While, good score related to the items (1-6, 8), and fair score related to the items (7) at Karbala. However, Babylon has good score related to the items (5) fair score to the items (1, 2, 4, 6) and poor score related to the items (3, 7, 8). In Diwaniah health staff practices is good related to the items (1, 4, 5)fair score related to the items(2, 3, 6, 8) and poor score related to the item (7).

Table (3) Distribution of the study subjects according to their overall practices regarding prevention of sharp injuries

Governorates	Levels	Frequency	Percent	M.S	Evaluation
Najaf	Good	14	70 %	2.37	Good
	Fair	6	30 %		
	Total	20	100 %		
Karbala	Good	46	100 %	2.77	Good
	Total	46	100 %		
Babylon	Fair	31	93.9 %	1.79	Fair
	Poor	2	6.1 %		
	Total	33	100 %		
Diwaniah	Good	7	28 %	2.26	Fair
	Fair	18	72 %		
	Total	25	100 %		

The study results indicate that the overall scores for the health staff practices regarding prevention of sharp injuries is good at Karbala and Al-Najaf, while fair at Babylon and Diwaniah.

Table (4); shows the mean difference of the study subjects to overall practices regarding prevention of sharp injuries and governorates

Governorates	N	Mean	Std. Deviation	Std. Error	F	p-value	Significant
Najaf	20	2.38	0.17	0.04	355.78	0.001	High
Karbala	46	2.78	0.13	0.02			
Babylon	33	1.79	0.10	0.02			
Diwaniah	25	2.27	0.14	0.03			
Total	124	2.35	0.41	0.04			

This table show that there is a highly significant in health staff practices regarding prevention of sharp injuries and the different studied governorates at p-value less than 0.01.

Discussion of Results

Part 1: discussion of demographic characteristics

In the current study, nearly 42.7% of the studied samples were aged between 31 to 40 years old. This result agrees with studies conducted in Southeast Nigeria by Polycarp (2015) that reported that most of their study sample are (30-39) years old of HCWs. In addition, this result agrees with a study conducted by Tabatabaei, et al., which indicates that age of HCWs was above 30 years.

The findings of the present study have revealed that the highest percentage of participants were married (81.5%); Fashafsheh, et al., have supported that most of the participants were married (70.5%).

The present study have also revealed that 64.5% of the sample are secondary midwifery school graduates and (24.2%) are physician; this result agrees with a study conducted by Gulilat, et al., which indicates that the majority of his study (68.1%) of midwives and (25%) physician.

Additionally, (42.7%) of the sample have lesser than 5 years of experience. Regarding the years of experience Sharma, et al., support that by their findings and mentioned that more of the participants of the study have lesser than 5 years of work (79.2%).

Part 2: **Health Personnel Practices Regarding Prevention of Sharp Injuries**

The present study indicated that health care personnel received training in sharps/ splash/ bite injury management at the four studied hospitals. Results of this study were better that results of a study conducted in Ethiopia by Gulilat, et al., in 2010 where participants training level in sharp injury management was lower than (39.4%). Where the present study showed that Sharps containers were available at the point of use at four studied hospitals; there were good practices in both Al-Najaf (3) and Karbala (2.93), but fair practices in Diwaniah (2.8) and Babylon (2.3). Such findings agree with those of a study conducted in Iran by Tabatabaei et, .al who found the availability of sharp box at all studied hospitals.

However, three hospitals, viz al-Najaf 1.45, Diwaniah1.44, and Babylon1.09, have poor scoring related to temporary closure mechanism of Sharps containers; only does Karbala have a fair score of 2.76. While, assembled, labelled Sharps containers with date, locality & signed was applied with good score at Karbala 3 and fair practices at both of al-Najaf 1.85 and Diwaniah1.92 but Babylon 1.18 has poor practices regarding labelled of Sharps containers. Sharps containers must be assembled correctly, with date and named person clearly detailed on the label.

On the other hand, this study has found that the visibly clean of Sharps containers were at Karbala 2.87 and Diwaniah 2.2 but al-Najaf1.55 and Babylon1.06. Otherwise, three hospitals have good practices of the absence of inappropriate items, such as swabs, packaging, and gloves, in the sharps container, which are al-Najaf 3, Karbala 2.98, and Diwaniah 2.96 while Babylon 2.03has fair practices. As for needles, syringes, and disposed of single units, the current study has indicated high, score of practices at all the studied hospitals: al-Najaf 3, Karbala 2.96, Diwaniah 3and Babylon 2.97. Furthermore, the study has indicated that two of the studied hospitals, al-Najaf 2.75, Karbala 2.96, have a good scoring related to disposed Syringes according to the current legislation and a fair score at Babylon 1.97 and Diwaniah 2.08.

In 2013, according to the National Health Services (NHS) the recommendations of safe use and disposal of sharp included:

- All sharps must be disposed of safely and properly immediately after use.
- Needles must not be recapped, bent, broken or disassembled before use or disposal. Needle and syringe must be discarded as one unit directly into sharps container.
- Used sharps must be discarded into a sharps container at the point of use by the user. These must not be filled above the mark that indicates that they are full.
- Containers must be assembled correctly according to manufacturer's instructions, i.e. ensuring that the lid is secure.
- Containers should be visible clean and kept in a safe location at the point of use.

• When not in use the temporary closing mechanism on sharps containers must activated.

There are no inappropriate items like swabs, packaging, or gloves, in the sharps container. Overall scoring of HCPs practices regarding prevention of sharp injuries was good at al-Najaf 2.37, Karbala 2.77 while it was fair in Babylon 1.79 and Diwaniah 2.26. CDC, in 2008, have recommended that all healthcare workers should take precautions to prevent injuries caused by needles, scalpels and other sharp instruments or devices during procedures, when cleaning used instruments, during the disposal of used needles, and when handling sharp instruments after procedures. Standard measures to avoid sharps injuries include handling sharp devices in a way that prevents injury to the user and to others who may encounter the device during or after a procedure. After use, single-use syringes and needles, scalpel blades, and other sharp items should be placed in an appropriate container.

This study has indicated that there are high significant differences at p-value 0.001 between health personnel practices regarding prevention of sharp injuries and the four studied hospitals. This agrees with Likis, et,. al (2015) who found that there is significant differences between health care worker practices of sharps injuries and the different studied areas.

Conclusion

Health staff practices regarding prevention of sharp injuries is good at Karbala (2.77) and Al-Najaf (2.37), while fair at Babylon (1.79) and Diwaniah (2.26).

Recommendations

The current study recommends that the Ministry of Health in Iraq: must be improve prevention of sharp injuries program through:

*Updating practice of health care personnel through continuing in-service educational programs.

*Providing training programs for newly health staff about prevention of sharp injuries and at regular intervals.

*Regular inspection and follow-up from the Ministry of Health for assurances good prevention of sharp injuries practices.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the AL-ameed University and all experiments were carried out in accordance with approved guidelines.

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Health Personnel Practices Regarding Waste Management in the Delivery Room at the Middle Euphrates Teaching Hospitals

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Abstract

Literatures proved that waste management is the most important and effective infection prevention and control measure to prevent the spread of microorganisms causing HAIs is considering a vital intervention to promote optimum patient safety in delivery room. Aim of the study; This study conducted to assess **waste management** practices of health care personnel in the delivery room at the middle Euphrates teaching hospitals. Methods; A Descriptive research design begin in 20th February to 26th May 2016, Current study sample involve all midwives and physicians in the delivery room (Total coverage.). Questionnaire used for data collection by interview forms and observational checklist was obtain from the extensive review of relevant literature and related studies Data analyzed through utilize (SPSS) software version (16) where, included descriptive analysis and inferential data analysis. The study conducted among 37 physicians and 97 midwives working in the delivery room are females.

Results: The current study indicate that good overall evaluation for the health staff practices concerning waste management at Karbala while fair overall evaluation at Babylon, Diwaniah and Al-Najaf with highly significant in health staff practices regarding waste management and the different studied governorates at p-value less than 0.01. ***Conclusion:*** based on the finding of present study majority of health care personnel have fair applies related to waste management practices at different studied governorate hospitals. ***Recommendations:*** Updating practice of health care personnel through continuing in-service educational programs. Regular inspection and follow-up from the ministry of health for assurances good waste management, the important of exist motivation system and punishment system to the neglected health care personnel.

Keywords: *waste management, Health care personnel, Practices.*

Background

Hospital waste management is one of the prime concerns of medical authorities as well as concerned government bodies in different countries. Health care wastes are all the accumulated wastes generated in health care establishments, research facilities, laboratories and so forth. Even health care operations conducted in homes can result in generation of hospital wastes [(10)].

Health care waste is a potential reservoir of pathogenic microorganisms, and requires appropriate handling. The only waste, which is clearly a risk for transmission of infection, however, is sharps contaminated with blood, 75% to 90% of this waste is non-risk or "general" health care waste, comparable to domestic waste. This comes from the administrative and housekeeping functions of health care facilities. The remaining 10–25% of health care waste is regarded as hazardous, and may create some health risks [(12)].

Hospital wastes can be of various types and these need to be ascertained in order to determine the kind of disposal means that should be adopted. [(10)].

The first category of hospital wastes consist of infectious wastes. These wastes come from infected wards or from patients infected with drug resistant pathogens like bacteria, fungi, viruses and other forms of microbes. Infectious wastes also formed from cultures of

human fluids, blood, plasters, bandages, dressings, and discarded bio products such as blood serum, medical instruments such as injections, syringes. The second category of hospital wastes is pathological wastes; these are usually comprised of different types of human tissues, organs, placenta, embryo, stored blood and similar items. Pathological wastes are ideally disposed of by cremation or burying. Incineration is a good way of ensuring that the toxic elements in such wastes do not get out into the external environment. (1).

The correct segregation of health-care waste is the responsibility of the person who produces each waste item, whatever their position in the organization.

The health-care facility management is responsible for making sure that there is a suitable segregation, transport and storage system, and that all staff adhere to the correct procedures, segregation should take place in a medical area, at a bedside, in an operating theatre or laboratory by nurses, physicians and technicians. If classification of a waste item is uncertain, as a precaution it should be place into a container, which used for hazardous health-care waste. (15).

The simplest waste-segregation system is to separate all hazardous waste from the larger quantity of nonhazardous general waste. However, to provide a minimum level of safety to staff and patients, the hazardous waste portion commonly separated into two parts: used sharps and potentially infectious items. In the latter, the largest components are typically tubing, bandages, disposable medical items, swabs and tissues. Therefore, the segregation of general non-hazardous waste, potentially infectious waste and used sharps into separate containers often referred to as the "three-bin system". Further types of containers can be used for other categories of wastes, such as chemical and pharmaceutical wastes, or to separate out pathological waste, where it is to be handled and disposed of in different ways from the other portions of the waste flow. (5).

Ideally, the same system of segregation should be in force throughout a country, and many countries have national legislation that prescribes the waste segregation categories to be used and a system of color-coding for waste containers. Where there is no national legislation, a World Health Organization (WHO) scheme is available Color-coding makes it easier for medical staff and hospital workers to put waste items into the correct container, and to maintain segregation of the wastes during transport, storage, treatment and disposal. Color-coding also provides a visual indication of the potential risk posed by the waste in that container. (15).

Labelling of waste containers are used to identify the source, record the type and quantities of waste produced in each area, and allow problems with waste segregation to be traced back to a medical area. A simple approach is to attach a label to each filled container with the details of the medical area, date and time of closure of the container, and the name of the person filling out the label. (8).

The appropriate waste receptacle (bags, bins, sharps boxes) should be available to staff in each medical and other waste-producing area in a health-care facility. This permits staff to segregate and dispose of waste at the point of generation, and reduces the need for staff to carry waste through a medical area. Posters showing the type of waste that should be disposed of in each container should be posted on walls to guide staff and reinforce good habits. (16).

Medical staff should be encouraged to think of waste disposal as part of a patient's treatment, so all aspects of the care process are completed at the bedside or treatment room. If intervention at the bedside is required, a waste container should be taken to the bed. Sharps bins are also sometimes taken to a patient for drug administration or blood sampling. Static bins should be located as close as possible to sinks and washing facilities, because this is where most staff will deposit gloves and aprons after treating patients. If the general waste container is closest to the sink or under a towel dispenser, it will encourage staff to place towels into the non-infectious receptacle. Containers should be similar size to overcome the observed tendency for staff to put waste in the largest receptacle. (8).

Materials and Methods

1. Design of the study: A Descriptive study are utilized to meet the aim of the study.

2. Locale study: -Present study is carried at four Maternal and Child Health teaching hospital in the

Middle Euphrates Region /Iraq.

3. Sample of the study: All midwives and doctors in the delivery room (Total coverage.).

4. The study instrument: - For data collection a questionnaire by interview forms and observational checklist was, obtain from the extensive review of relevant literature and related studies.

The study tool consisted from two parts as the following:

A-The first part of questionnaire: Socio-demographic characteristic, which include (6), items as regards to their age, marital status, educational level, years of experience, working experience in delivery room.

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Data are collected through direct interview with physicians and midwives by using constructed questionnaire. However, for health personnel practices date collected through the application of observation technique during they were working in the delivery room.

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2- Determine the reliability of the instrument.

3- Estimate the average time required for the interview and observation each interview took (15-25) minute and unlimited time for observation.

4- Identify barriers that may encountered during date collection process.

The sample of the pilot study excluded from the original sample of the study.

8. Reliability of the instrument: -

Reliability of an instrument was determine through (Cronbach's coefficient) for the present study. Cronbach's alpha Reliability coefficient for practices of midwives and doctors was (r=0.78), this value of Cronbach's α for the study is considered acceptable.

Results of Study

Table (1) Distribution of the study subjects according to their demographic data. No=124

Demographic Data	Rating and Intervals	Frequency	Percent
Hospital	Al-Najaf	20	16.1
	Al-Diwaniah	25	20.2
	Babylon	33	26.6
	Karbala	46	37.1
Age / years	Less than 20	3	2.4
	20-30	28	22.6
	31-40	53	42.7
	41-50	27	21.8
	More than 50	13	10.5
Levels of education	Physician	30	24.2
	Secondary midwifery school	80	64.5
	Secondary nursing school	9	7.3
	Diploma of nursing	5	4
Marital status	Single	18	14.5
	Married	101	81.5
	Divorced	3	2.4
	Widowed	2	1.6
Years of experience	Less than 5 years	53	42.7
	5-10	26	21
	More than 10	45	36.3
Years of Working in the delivery rooms	Less than 5 years	59	47.6
	5-10	28	22.6
	More than 10	37	29.8

This table reveals that, (42.7%) of the sample are 31-40 years old. (64.5%) of them are Secondary midwifery school. 81.5% of them are married. 42.7 % of them have less than 5 years of experiences. Most of them have 47.6 % working experiences in the delivery room.

Table (2) Distribution of the study subjects according to their practices regarding waste management with the overall evaluation. N= 124

Items	Najaf		Karbala		Babylon		Diwaniah	
	M.S	Evaluation	M.S	Evaluation	M.S	Evaluation	M.S	Evaluation
1- Health provider have attended a training session which includes information about the correct & safe disposal of waste	2.45	Good	2.76	Good	1.94	Fair	2.56	Good
2- The waste storage area is clean & dry	2	Fair	2.04	Fair	2.00	Fair	2.04	Fair
3- All plastic waste sacks are fully enclosed within bins to minimize the risk of injury	2.65	Good	3	Good	2.42	Good	2.96	Good
4- All waste bins used are lidded, foot operated and in good working order	1.1	Poor	1.26	Poor	1.73	Fair	1.32	Poor
5- Waste bags are removed from clinical areas daily	2.8	Good	2.98	Good	2.85	Good	2.88	Good
6- Disposal of Placenta in especial Bucket	1.45	Poor	2.96	Good	1.88	Fair	2.08	Fair
7- Disposal of Sharps in a Sharps Solid Container	3	Good	2.89	Good	1.97	Fair	2.04	Fair
8- Disposal of Gloves in a Red Bag	1.6	Poor	2.09	Fair	2.00	Fair	1.96	Fair
9- Disposal of Swabs and Other Contaminated Materials in a Container with Red Bag	1.6	Poor	2.07	Fair	2.00	Fair	1.92	Fair
10- Disposal of No contaminated Materials in a Black Bag	2.6	Good	3	Good	2.03	Fair	1.92	Fair
11- There is no emptying of clinical waste from one bag to another	2.5	Good	2.96	Good	2.30	Fair	2.32	Fair
12- There are no overfilled bags- bags are no more than 2/3 full	3	Good	2.96	Good	2.42	Good	2.84	Good

This table show that good health personnel practices at al – najaf, Karbala and Diwaniah related to item (1) except Babylon have fair practices. While, at four studied governorate hospitals health personnel practices related to items (3 and 5) was good. On the other hand, health personnel practices related to item (2) was fair score at four studied hospitals. Concerning item (4) health personnel practices was poor at three hospitals which, najaf, Karbala and Diwaniah except Babylon has fair score related to health personnel practices. While, health personnel practices at al-najaf was poor related to items (6, 8, 9) while good health personnel practices related to items (7, 10, 11, 12). While, practices of health personnel at Karbala was good related to items (6, 7, 10, 11, 12) and fair practices of health personnel related to items (8, 9). On the other hand, Babylon and Diwaniah health personnel practices related to items (6, 7, 8, 9, 10, 11) were fair except health personnel practices related to item (12) was good score.

Table (3) Distribution of the study subjects according to their overall practices regarding waste management with the overall evaluation. N= 124

Governorates	Levels	Frequency	Percent	M.S	Evaluation
Najaf	Good	3	15 %	2.22	Fair
	Fair	17	85 %		
	Total	20	100 %		
Karbala	Good	46	100 %	2.57	Good
	Total	46	100 %		
Babylon	Good	2	6.1 %	2.12	Fair
	Fair	31	93.9 %		
	Total	33	100 %		
Diwaniah	Good	4	16 %	2.23	Fair
	Fair	21	84 %		
	Total	25	100 %		

Table (3) show that good overall evaluation for the health staff practices concerning waste management at Karbala while fair overall evaluation at Babylon, Diwaniah and Al-Najaf.

Table (4) shows the mean differences of the study subjects to overall practices regarding waste management related to their governorates.

Governorates	N	Mean	Std. Deviation	Std. Error	F	p-value
Najaf	20	2.14	0.13	0.03	43.123	0.001 HS
Karbala	46	1.89	0.12	0.01		
Babylon	33	1.75	0.08	0.01		
Diwaniah	25	1.84	0.13	0.02		
Total	124	1.88	0.17	0.01		

This table shows that there is a highly significant in health staff practices regarding waste management and the different studied governorates at p-value less than 0.01.

Discussion of Results

Part 1: discussion of demographic characteristics

In the current study, nearly 42.7% of the studied samples were aged between 31 to 40 years old. This result agrees with studies conducted in Southeast Nigeria by Polycarp (2015) that reported that most of their study sample are (30-39) years old of HCWs. In addition, this result agrees with a study conducted by Tabatabaei, et al., which indicates that age of HCWs was above 30 years.

The findings of the present study have revealed that the highest percentage of participants were married (81.5%); Fashafsheh, et al., have supported that most of the participants were married (70.5%).

The present study have also revealed that 64.5% of the sample are secondary midwifery school graduates and (24.2%) are physician; this result agrees with a study conducted by Gulilat, et al., which indicates that the majority of his study (68.1%) of midwives and (25%) physician.

Additionally, (42.7%) of the sample have lesser than 5 years of experience. Regarding the years of experience Sharma, et al., support that by their findings and mentioned that more of the participants of the study have lesser than 5 years of work (79.2%).

Part 2: HCPs practices concerning waste management

The current study has found that health providers had attended a training session, which included information about the correct and safe disposal of waste at the different studied hospitals: al-Najaf 2.45, Karbala 2.76, Diwaniah 2.54 and Babylon1.4). WHO in 2003 recommended that anyone responsible for generating, handling, or transporting contaminated waste must be duly trained .Training should occur at times of employment and be refreshed periodically. However, this study has indicated that all hospitals had fair score related to clean and dryness of waste storage area, as follows: al-Najaf (2), Karbala (2.4), Diwaniah (2) and Babylon (2.4). Further, it has revealed that all plastic waste sacks in the studied hospitals were fully enclosed within bins and removed from the clinical were as every day; all such hospitals had a good score in the due respect. In 2015, NHS has recommended that all waste bags be placed inside bins, sharps bins and bin containers in a clean dry storage were a to avoid contamination prior to use in the clinical were a; the bag or container holding clinical waste should be removed every day. While the study indicated that waste bins in al-Najaf (1.1), Karbala (1.26), and Diwaniah (1.32) were not lidded, foot operated and in bad working order, Karbala (1.73) had a fair score related to this item. USAID and MOH recommend that waste bins must be mechanically closed and foot operated to avoid HCWs contact with waste bins during disposal wastes to minimize infection with microorganisms.

The present study has also indicated that both Babylon (1.88) and Diwaniah (2.08) had fair practices, while Karbala (2.96) has good practice of disposal of placenta in especial bucket score and al-Najaf (1.45) had of poor of this item. These results go in line with Gulilat and Tiruneh's (2014) who found that few health facilities disposed placenta into general waste disposal site not in spatial container.

In [14] recommended that placenta was placed in small clinical waste bag and then into a placenta box.

However, the current study has found that HCPs practices related to disposal of gloves, swabs and other contaminated materials in a container with red bag was fair ranging from (2.09) to (1.96) in Babylon, Diwaniah and Karbala, al-Najaf (1.6) has poor practices. While HCPs practices of disposal of non-contaminated materials in black bags without emptying clinical wastes from one bag to another were good in al-Najaf (2.6), Karbala (3) and fair in Babylon (2.03) and Diwaniah (2.32). According to USIAD and MOH disposal of sharps must be put in a sharps solid container, disposal of swabs and other contaminated materials in a container with red bags, and disposal of non-contaminated materials in black bags.

On the other hand, it has been found that there were no overfilled bags; bags were no more than 2/3 full at almost all the studied hospitals. In 2015, NHS

recommended that bags used for waste disposal should be filled to no more than 2/3 capacity. While being filled, the bags should be hold in an appropriate floor-standing holder. These results showed that the overall evaluation for the health staff practices of waste management was good in Karbala (2.57), but fair in Babylon (2.12), Diwaniah (2.23) and al-Najaf (2.22).

From the viewpoint of the researcher, Lack of awareness of the health hazards related to healthcare waste, inadequate training in proper waste management, absence of waste management and disposal systems, insufficient financial and human resources, and the low priority given to the topic were the most common problems connected with health-care waste. All staffs had a responsibility to dispose healthcare waste in a way that poses minimal hazard to patients, visitors, other healthcare workers, and the community.

It has also been indicated here that there was a high difference in health staff practices concerning waste management and in the p-value in the studied governorates which records lesser than 0.01. This result agreed with that of Kumar (2015) who found that there is a significant association between HCWs practices related to waste management in the Teaching Hospitals of Pakistan

Conclusion

Health staff practices regarding waste management are fair range from (2.12 to 1.57) at Karbala, Al-Najaf, Babylon and Diwaniah. Where, Mean of score (2), cut off point (66.66%), good (mean of scores=> 2.33), fair (mean of scores = 1.67-2.33), poor (mean of scores= 1-1.66).

Recommendations

The current study recommends that the Ministry of Health in Iraq: must be improve hand hygiene program through:

*Updating practice of health care personnel through continuing in-service educational programs.

*Providing training programs for newly health staff about waste management and at regular intervals.

*Regular inspection and follow-up from the ministry of health for assurances good waste management practices.

*The important of exist motivation system and punishment system to the neglected health care personnel.

*Further studies must be done by taking swabs from instruments of the clinical area.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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Molecular Study of Virulence Factors (Cysteine Protease-1 and Amoebapore-C) for *Entamoeba histolytica* in Babylon Province

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Abstract

A molecular study was conducted for the period from December 2019 to May 2020, which included examining (50) positive stool samples by using the direct smear method. that was revealed by the method of polymerase chain reaction (PCR) using the gene (18SrRNA), with a percentage of 82%, distributed 87.8% for males and 70.6% for females. Then for determine the virulence factors (Cysteine proteinase-1) and (Amoebapores-C) by using primers, with (PCR)technique , and then electrophoresis on the agarose gel have been done , the results were positive for Cysteine proteinase-1 (APC-1) at a wavelength (212 bp) and the Amoebapore-C (Amo-C) at a wavelength (536 bp). Five random positive samples of the PCR product in the current study were sent to Macrogen in South Korea for the purpose of determining the DNA sequence of the amoebapore- C gene present in the *Entamoeba histolytica*, and it is the first study in Iraq and Babylon province that is genetically determined by the NCBI These genotypes of the amoebic holes gene are recorded in the gene bank. The results of the study showed that all the transmitted isolates are identical with NCBI isolates with the USA samples registered with the gene bank with accession number AY956434.2, which are the isolated samples belonging to the American samples and diagnosed with the number MS30-1043 of the USA study by the researcher Bhattacharya *et al.* (2005) with the current study samples As it was 100% identical. These samples were recorded in the genebank for the first time for this Amoebapore C gene under Accession No. For samples (1, 2, 3, 4, 5) in sequences are MT951203, MT951204, MT951205, MT951206 and MT951207, respectively.

Key Words : *Entamoeba histolytica, Cysteine Protease-1, Amoebapore-C*

Introduction

E. histolytica is one of the most widespread eukaryotic parasites in the world [1] [and causes amebiasis or ameobic dysentery as it causes widespread tissue destruction [2]. Six species belonging to the genus Entamoeba have been recorded in the human intestine, *E.histolytica*, *E. dispar*, *E. moshkovskii*, *E. coli*, *E.polecki* and *E.hartmanni*. They can be detected in a number of ways, including by direct smear method of faeces or indirectly, such as serological methods such as ELISA or molecular methods such as the polymerase chain reaction (PCR) technique [3,4]. Among the modern diagnostic methods used to detect amoebic dysentery is detection by means of the technique of polymerase chain reaction (PCR), as well as the method of genetic sequencing and the genetic tree Phylogenetic tree, including the study of [5] and the study of both [6,7] . The **aim of the present study** was to use the PCR technique for *E.histolytica* samples and the detection of virulence factors in them, such as the Cysteine protease-1 gene and the Amobapores-C. And the study of genetic sequences and phylogenetic tree of the virulence factor of the Amoebapapor-C gene for *E.histolytica*.

3- Materials and Methods

3-1- kits

Table (1): The kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
1	AccuPrep® stool DNA Extraction Kit	Bioneer	Korea
	Proteinase K		
	Stool Lysis buffer (SL)		
	Binding buffer (ST)		
	Washing buffer 1 (W1)		
	Washing buffer 2 (W2)		
	Elution buffer (E)		
	GD column		
	Collection tube 2ml		
2	Maxime PCR PreMix	iNtRON	Korea
	Tap DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris-HCl pH 9.0, KCl, MgCl2		
	Stabilizer and Tracking dye		

3-2- Primers

The PCR primers were design in this study based on NCBI-Genbank and primer 3 plus design. These primers was provided from Macrogen company, Korea as following table below :

Primer	Sequence (5'-3')		Product Size	Genbank code
18SrRNA gene Entamoeba histolytica-PCR	F	GGCCGTTCTTAGTTGGTGGA	501bp	GQ423748.1
	R	GTGTGTACAAAGGGCAGGGA		
ACP1 gene	F	TGGATGTGAACGAGGACCATC	212bp	S58669.1
	R	TACCAACAGCAACAGGTCCG		
amoebapore C gene	F	TGGTGAAAGAAAAACAAGGAAGT	536bp	X76903.1
	R	TCAACTGCTCCACCAAGGAC		

Methods

Stool samples collection

Form the period from December 2019 to May 2020, which included examining (50) positive stool samples by using the direct smear method. that was revealed by the method of polymerase chain reaction (PCR) using the gene (18SrRNA),

Polymerase chain reaction (PCR)

The PCR technique was performed for detection *E. histolytica*. based 18S ribosomal rRNA gene and some virulence factor genes from stool samples. This method was carried out according to method described by [8] as following steps:

Genomic DNA Extraction

Genomic DNA from feces samples were extracted by using AccuPrep® stool DNA Extraction Kit , Bioneer. Korea, and done according to company instructions as following steps:

1. A 200 mg of the stool sample was transferred to sterile 1.5ml microcentrifuge tube, and then 20µl of proteinase K and 400 µl Stool lysis buffer (SL) were added mixed by vortex. And incubated at 60℃ for 10 minutes.

2. After 10 mins, the tube placed in centrifuge at 12,000rpm for 5 mins.

3. The supernatant was transferred in to a new tube and 200µl Binding buffer was added to each tubes.

4. The tubes were Incubated again for 10 min at 60℃.

5. 100 µl isopropanol was added and the samples mixed by lightly vortex for about 5 seconds, then spin down for 10 seconds to down the liquid clinging to the walls and lid of the tube.

6. DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 8000rpm for 5 minutes. And the 2 ml collection tube containing the flow-through were discarded and placed the column in a new 2 ml collection tube.

7. 500µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube.

8. 500µl W2 Buffer (ethanol) was added to each column. Then centrifuged at 8000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube.

9. All the tubes were centrifuged again for 1 minutes at 12000 rpm to dry the column matrix.

10. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre-heated elution buffer were added to the center of the column matrix.

11. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA.

Genomic DNA estimation

The extracted genomic DNA from stool samples was checked by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).

2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.

3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of DNA was added to measurement.

PCR master mix preparation

PCR master mix was prepared by using (**Maxime PCR PreMix Kit**) and this master mix done according to company instructions as following table:

PCR Master mix	Volume
DNA template 5-50ng	5µL
18SrRNA Forward primer (10pmol)	1µL
18SrRNA Reverse primer (10pmol)	1µL
PCR water	13 µL
Total volume	20µL

After that, these PCR master mix component that mentioned in table above placed in standard **Maxime PCR PreMix** that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler.

PCR Thermocycler Conditions

Table (2): PCR thermocycler conditions by using convential PCR thermocycler system as following:

PCR step	Temp.	Time	repeat
Initial Denaturation	95ºC	5min.	1
Denaturation	95 ºC	30sec.	30 cycle
Annealing	58 ºC	30sec	
Extension	72 ºC	1min.	
Final extension	72 ºC	5min.	1
Hold	4 ºC	Forever	-

PCR product analysis

The PCR products of was analyzed by agarose gel electrophoresis following steps:

1- 1.5% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µl of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5µl of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- PCR products were visualized by using UV Transilluminator.

DNA sequencing method

DNA sequencing method was performed for nucleotide polymorphism genetic analysis some positive

Amoebapore- C gene in Human *E. histolytica* isolates as following step:

1- The PCR product of amoebapore C genes were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system.

2- The DNA sequencing analysis (Phylogenetic tree analysis) was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

3- The amoebapore C gene analysis was done by phylogenetic tree analysis between local Human Entamoeba histolytica isolates and NCBI-Blast *E. histolytica*.

4- Finally identified amoebapore C gene Human *E. histolytica* isolates were submitted into of NCBI-Genbank to get Genbank accession number .

Results and Discussion

The DNA of fifty positive stool samples was extracted by direct examination from the stool of patients suffering from amebic dysentery from patients visiting some hospitals in Babylon province (Babil Hospital for Women and Children, Al Noor Hospital for Children and Marjan Teaching Hospital) for the period from December 2019 to May 2020, in order to diagnose The *E. histolytica* in these samples, its prevalence in males and females, and then studied molecularly .

PCR results of the 18SrRNA gene of the *E. histolytica* parasite

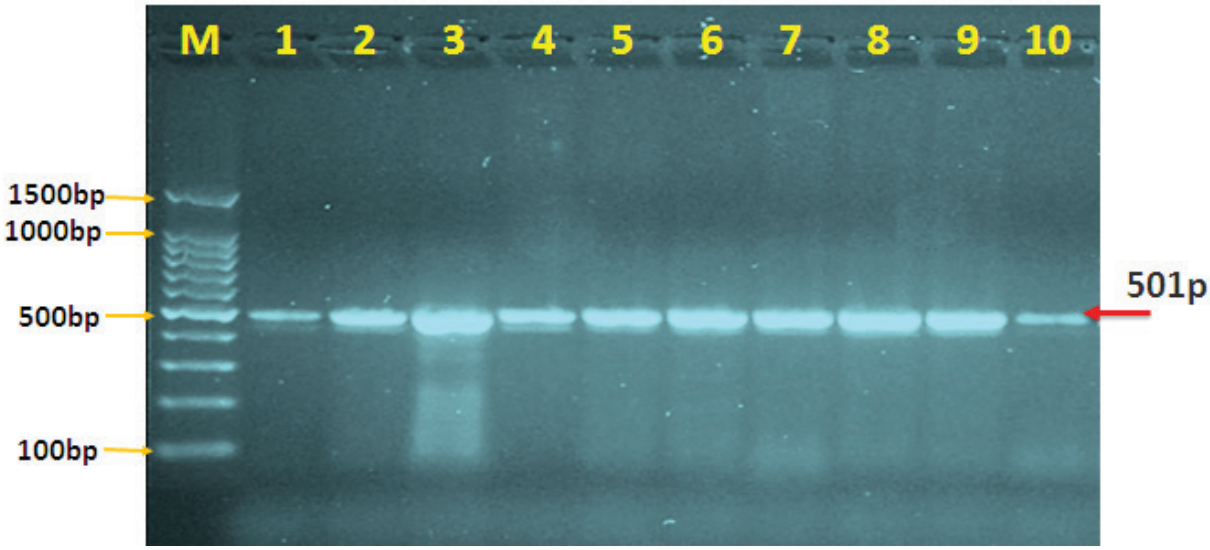
The specific primers for the 18SrRNA gene of *E. histolytica* were used through the technique of polymerase chain reaction (PCR). The results showed the success of all the processes of amplifying the DNA extracted from *E. histolytica* cysts. The amplification results for the target area gave beams of wavelength 501 bp in (41) samples out of the 50 samples examined with a percentage of 82%, distributed 87.8% for males and 70.6% for females according to Table (3) .

Table (3): shows the number of samples from which DNA was extracted and the appearance of positive samples for PCR examination with the percentage of infection.

Gender	Examined No.	Infected No.	(%)
Males	33	29	87.8
Females	17	12	70.6
Total	50	41	82
P= 0.1 ((Non-significant differences according to the Chi-Square test because the p-value is greater than 0.05))			

Table(3) shows the presence of the 18SrRNA gene for the parasite in (41) stool samples out of (50) positive samples that contain *E. histolytica* by examining it with direct smear methods with a percentage of 82% while nine samples(negative) were found free of the 18SrRNA gene and it may be attributed to the presence of these Negative samples indicate the presence of another type of *E. histolytica* , such as other types of amoeba such as *E. dispar, E. moshkoviski*, and *E. hartmani* present in feces as amoeba commensals, or it may be due to the insufficient amounts of the parasite's DNA concentration to show the result. This is in agreement with previous studies, including [9,10], which showed that (10-20)% of samples are negative when examined with the PCR technique, and the results of the current study do not agree with the results study of [11] , which showed that 10% of the samples contain *E.histolytica* amoeba when examined by PCR technique.

The positivity of the test using the PCR technique depends on the quality and quantity of the DNA and the degree of its purity by removing a large variety of inhibitors in the stool sample that must be removed upon extraction, such as bile salts, complex polysaccharides and phenolic compounds. The commercial kits of extractions can reduce these preventions [12].



Figure(1): Agarose gel electrophoresis image that showed the PCR product analysis of *Entamoeba histolytica* 18S ribosomal RNA gene in Human stool samples. Where M: marker (1500-100bp) and some positive 18SrRNA gene at (501bp) PCR product.

After the parasite was diagnosed with the polymerase chain reaction technique, the virulence factors of the parasite were determined, namely the Amoebapores -C , and the Cysteine proteinase-1 enzymes, by determining its genes by the PCR technique. After electrophoresis on the agarose gel, the results were positive for the Cysteine proteinase-1 (APC-1) gene at a product ((212 bp as in picture (2)) and the Amoebapore- C gene (AmoC gene) at product (536) bp as in the picture (3).

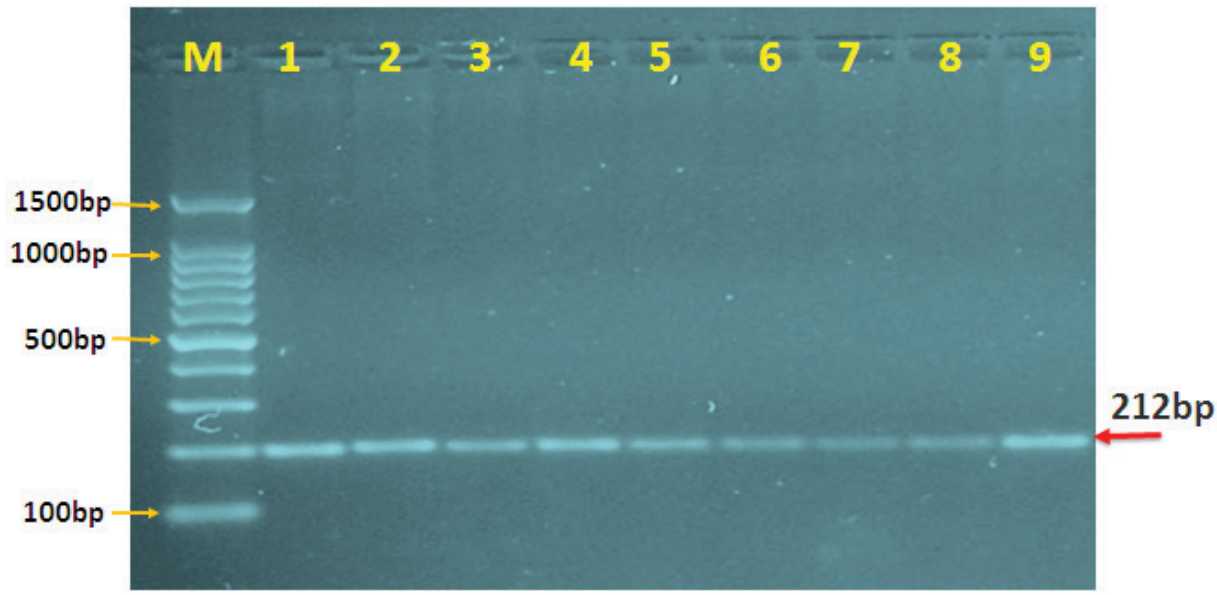


Figure (2): Agarose gel electrophoresis image that showed the PCR product analysis of Entamoeba histolytica cysteine proteinase precursor (ACP1) gene in Human stool samples. Where M: marker (1500-100bp) and some positive ACP1 gene at (212bp) PCR product.

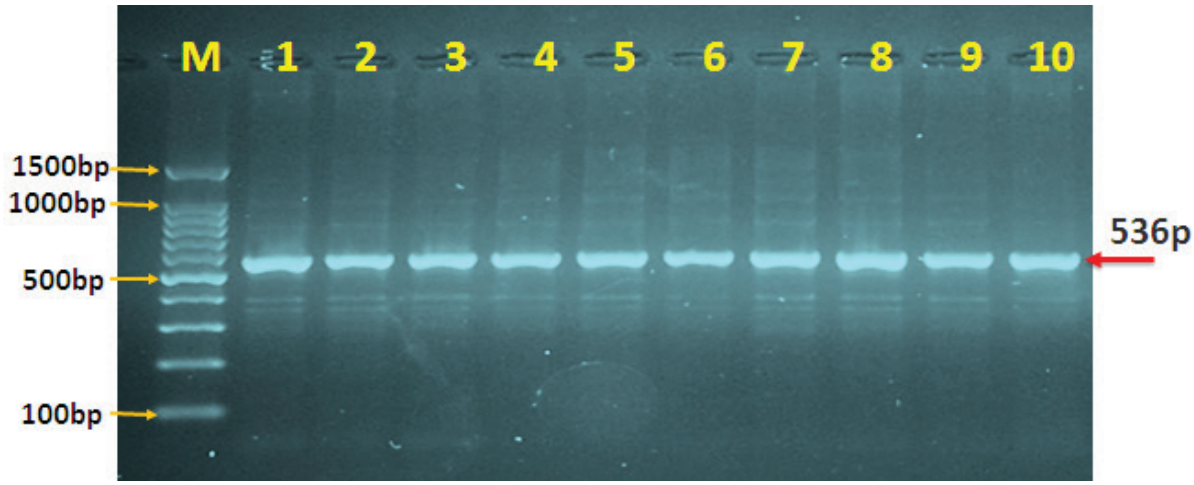


Figure (3): Agarose gel electrophoresis image that showed the PCR product analysis of Entamoeba histolytica amoebapore C gene in Human stool samples. Where M: marker (1500-100bp) and some positive amoC gene at (536bp) PCR product.

Entamoeba histolytica causes intestinal infection by invading the tissues due to its possession of virulence factors such as the enzyme Cysteine proteinases , which enables it to invade the tissues and decay the epithelial tissues lining the large intestine, which facilitates the process of invasion with the help of Amebapores-C protein as well, causing immune disorders in the host, causing Infection, this enzyme encodes for it by a gene called APC1, which is only present in the *E. histolytica* and is not present in other types of amoeba such as *E. dispar*, and thus this gene helps distinguish *E.histolytica* amoeba from other types of Entamoeba[13].

E.histolytica also possesses another virulence factor, Amebapores-C, which is a protein produced by the amoeba that penetrates the host cell membrane causing cytolysis, this protein is responsible for a gene present only in *E.histolytica* and not found in the other type of amoeba is called Amoebapore-C [14].

The results of the current study showed the presence of the cysteine-1 protein enzyme gene (APC1) and the Amoebapore C gene of *E.histolytica* in all samples (41 samples) that were diagnosed using the technique of polymerase chain reaction (PCR) for the 18SrRNA gene, and these results are consistent with the results of [15] On the use of the technique of polymerase chain reaction (PCR) to diagnose the pathogenic factors AP and PLA of *E.histolytica* in the stool of patients with diarrhea in Diwaniyah city, as the study showed the presence of the virulence factor Amoebapore-C in all samples positive for *E.histolytica* . Results of the study by [16] on determining the main virulence factors for *E.histolytica* using the PCR technique, which showed that all samples were positive for CP1 and 93% of the samples were positive for Amoebapore-C .

The results of this study are inconsistent with the results of [17] which included the investigation of virulence factors CP1 and CP5 in Al-Najaf province using PCR technique, which showed that 40% of the samples were positive for cysteine proteinase-1 (CP1) Also, this study does not agree with the study of [18] on diagnosing *E.histolytica* in camel feces by using the technique of PCR to determine the Amoebapore-C gene in Babylon province as the results showed that (78)% of the stool samples containing *E.histolytica* was tested positive for the Amoebapore- C gene.

Results of DNA sequencing analysis of the Amoebapore-C gene and Phylogenetic tree analysis.

Results of the genotyping of *E. histolytica* .

When examining the causative agent of diarrhea in stool samples, it is necessary to determine the molecular characteristics of the genotypes to obtain a better understanding of the prevalence and diversity of *E.histolytica* .

In the current study, five random samples of patients' faeces infected with *E.histolytica* were sent to Macrogen in South Korea for the purpose of determining the DNA sequence of the amoebapore- C gene ,. It is the first study in Babylon province and Iraq that genetically identifies these genotypes of the amoebapore- C gene and records it with the NCBI [19 ,20, 21 ,22, 23].

The results of the study showed that all sent PCR samples were identical with NCBI-BLAST isolates with the USA samples registered with the NCBI with accession number AY956434.2 and the isolated samples belonging to the American samples and diagnosed with the number MS30-1043 of the American study by the researcher [8] with a match ratio of (100) (%) For the current study samples, which are the (4,5)samples, with a match ratio of (99.69)% for sample No. (2) and a match rate of (99.68%) for sample No. (1 and 3) of the samples under study. Iraq and Babylon province under accession No. for samples (1, 2, 3, 4, 5) in sequence are **MT951203, MT951204, MT951205, MT951206 and MT951207** , respectively as that revealed in **Table (4)**

Table (4): NCBI-BLAST Homology Sequence identity (%) of Amoebapore- C gene between local *Entamoeba histolytica* IQB isolates and NCBI-BLAST submitted *Entamoeba histolytica* isolates:

Local isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical isolate	Genbank Accession number	Country	Identity (%)
***E. histolytica* IQB isolate No.1**	MT951203	*Entamoeba histolytica* strain MS30-1047	AY956434.2	USA	99.68%
***E. histolytica* IQB isolate No.2**	MT951204	Entamoeba histolytica strain MS30-1047	AY956434.2	USA	99.69%
***E. histolytica* IQB isolate No.3**	MT951205	*Entamoeba histolytica* strain MS30-1047	AY956434.2	USA	99.68%
***E. histolytica* IQB isolate No.4**	MT951206	*Entamoeba histolytica* strain MS30-1047	AY956434.2	USA	100%
***E. histolytica* IQB isolate No.5**	MT951207	*Entamoeba histolytica* strain MS30-1047	AY956434.2	USA	100%

4-2-2: Mapping the phylogenetic tree for strains

Figure (4) shows the analysis of the phylogenetic tree based on the Amoebopores-C gene series of IQB isolates, which are used in the analysis of genetic relationships. The genetic tree was built using the Maximum Likelihood method . And the Tamura-Nei model tree method, as the Amoebopores-C gene in IQB isolates were genetically identical to the American isolate: NCBI-BLAST *E. histolytica* strain MS30-1047 (AY956434.2) and *E. histolytica* strain DS6-64 (AY956433.2)) With a total genetic difference of 0.00050%.

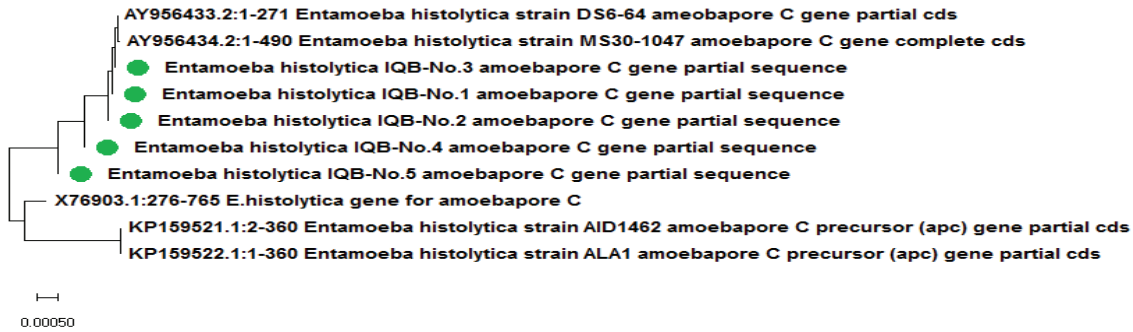


Figure (4): Phylogenetic tree analysis based on amoebapore C gene partial sequence in local *E. histolytica* IQB isolates that used for genetic relationship analysis . The phylogenetic tree was constructed using Maximum Likelihood method and Tamura-Nei model tree method) in (MEGA X version). The Amoebapore-C gene in local *E. histolytica* IQB isolate No.1 to No.5 were showed genetic related to NCBI-BLAST *E. histolytica* strain MS30-1047 (AY956434.2) and *E. histolytica* strain DS6-64 (AY956433.2) at total genetic changes (0.00050%).

Conclusions

Nine negative samples appeared upon examination with the technique of PCR , and these samples may be related to other commensals amoeba races in infected persons such as *E. dispar* .. etc. The molecular examination of the virulence factors Cysteine protease-1 and the amoebopores-C proteins genes proved their presence in all samples under study, which indicates that the examination of samples of amoebic dysentery has been proven. The genetic sequence samples sent to NCBI for amoebapore-C proteins showed a 99% match for samples 1,2,3 for the American study with code MS30-1043, while samples 5,4 gave a match of 100% for the same American study previously recorded.

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Knowledge of Primary School's Teachers about Epilepsy in Al-Basra City Center

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Abstract

Background: Epilepsy is a common disorder that, even for those individuals who frequently work with epileptics, is inefficiently understood. Low comprehension and understanding of the cause for the disorder has been connected with negative attitudes and beliefs, and misconceptions of workplace and school epileptics.

Objective: To evaluate the knowledge of epilepsy among primary school's teachers. And to determine the relationship between primary school teachers' knowledge of epilepsy and their socio-demographic features of age, gender, relative's history, and family history.

Methodology: A cross-sectional descriptive study to evaluate the knowledge of epilepsy among 130 primary school's teachers in the center city of Basra, from February 2020 to July 2020. A probability Simple random sample was done to choice the school's teachers in the schools.

Results: A total of (130) participants gender is approximate; (n=111; 85.4%) for female, and (n=19; 14.6%) for male, their age group less than half of them is within (40-49) years-old (n=42; 32.3%). The results reveals that the majority of participant has a moderate knowledge regarding epilepsy (n=94; 72.3%).

Conclusion: According to results of the current study, the researchers conclude that most participants have a mild understanding of epilepsy.

Key words*: Knowledge, Epilepsy, school's teachers.*

Introduction

Epilepsy is a chronic brain disease characterized by an abnormal nervous system imbalance caused by the sudden, repeated discharge of the total neuron population from the brain. Excessive discharge can cause sensory disturbances, convulsions or mental dysfunction, with or without loss of consciousness [(1)]. Epilepsy is one of the most common diseases that primary school students encounter; however, the disorder often has a social stigma that may contribute to parental reluctance to share knowledge with teachers. In addition, due to the disease itself, antiepileptic drugs (AED) and psychosocial factors, children with epilepsy have much greater learning and behavior difficulties than the public. [(2)]

Epilepsy is a widespread serious neurological disease in the world [(3)], It is often surrounded by prejudice and mythology, but it can overcome huge difficulties [(4)]. It is characterized by Frequent mental disorders. The nervous system is caused by accidental excessive and disorderly charging of brain neurons, resulting in almost instantaneous sensory disturbances, mental function or loss of consciousness, twitching movements or some combination of these [(5,6)].

Teachers are most the top, and the primary parent perform the first line to save primary school students, while the role of the teacher complements the role of the parent. School teachers are the first responder to incidents outside the school hours, injuries arising from

accidents at school. Thus, they must be able to respond correctly to health emergencies affecting school children [7,8,9].

Epilepsy has become a problem for over seventy million population globally [10] and about 80 % of population with epilepsy live in developing country [5,11]. Worldwide prevalence rates, however, range from 2.8 to 19.5 per 1000 of the general population [12]. The prevalence can be as high as 43 per 1,000 people in developing countries (World Health Organization, 2001), and the incapacity caused by epilepsy accounts for around 0.5 percent of the overall burden of disability over the lifetime of the disease [13].

Similarly, epilepsy is one of the most common disorders in pediatric neurology, often found in the first decade of life. 33 million children worldwide are suffering from epilepsy. The serious consequences of epilepsy in school can cause harm to any child, and the teacher's Awareness of first aid is needed to deal with the disease. [15,16,17].

All age groups can have affected by epilepsy, but in children it is more common. The prevalence of epilepsy in developing countries is 5 to 10 per 1,000 persons, according to estimates. The prevalence per thousand individuals is 2.8 to 19.5. [19].

Methodology

A cross-sectional descriptive study to evaluate epilepsy knowledge among 130 primary school teachers in central Basra from February 2020 to July 2020.. Approval was received by school administrations, as well as permission from educators to engage in this questionnaire. The time for completing the questionnaire lasted from 10 to 15 minutes. All teachers were instructed to fill out the questionnaire and questions were explained as needed.

The questionnaire consists from two parts, the first part about demographics characteristic of primary school teachers. And the second part about the knowledge about epilepsy and included eight items. The answers to the knowledge question is binary, that is, "yes" or "no". The correct (positive) reaction score is 1 point, and the wrong (negative) reaction score is 0 points. The questionnaire was modified from a study performed in Central Nepal [1].

A probability (simple random sample) was done to select the teachers in the schools. The researcher obtained permission from the College of Nursing/University of Basra. Additional consent was obtained from directorate of Education in Al-Basra City to the permission for data collection.

All statistical analysis was performed using IBM-SPSS version (20)

Results

Table (1): Distribution by demographic characteristics of Participants

Items	Rating	F.	%
Gender	Male	19	14.6
	Female	111	85.4
	Total	130	100.0
Age	20-29	19	14.6
	30-39	33	25.4
	40-49	42	32.3
	50-59	32	24.6
	60-69	4	3.1
	Total	130	100.0

Cont... **Table (1): Distribution by demographic characteristics of Participants**

Family history of epilepsy	Yes	106	81.5
	No	24	18.5
	Total	130	100.0
Relatives history of epilepsy	Yes	72	55.4
	No	58	44.6
	Total	130	100.0

F.=Frequency; %= Percentage

A total of (130) participants gender is approximate; (n=111; 85.4%) for female, and (n=19; 14.6%) for male, their age group less than half of them is within (40-49) years-old (n=42; 32.3%), the majority of them have family history of epilepsy (n=72; 55.4%).

Table (2): Distribution of the Level of Teachers' Knowledge about Epilepsy

Items	Rating	F.	%
Overall Knowledge	Low	15	11.5
	Moderate	94	72.3
	High	21	16.2
	Total	130	100.0

F.=Frequency; %= Percentage

The results reveals that the majority of participant has a moderate knowledge (n=94; 72.3%).

Table (3): Association between Participants' Knowledge and their Demographics data

Demographics data		Overall Knowledge			Total	Sig.
		Low	Moderate	High		
Gender	Male	2	11	6	19	$\chi2$= 3.937a; df= 2; p-value = .140; P< 0.05 NS
	Female	13	83	15	111	
Total		15	94	21	130	
Age	20-29	0	17	2	19	$\chi2$ = 12.084a; df= 8 p-value = .148;P< 0.05 NS
	30-39	2	25	6	33	
	40-49	9	28	5	42	
	50-59	4	20	8	32	
	60-69	0	4	0	4	
Total		15	94	21	130	
Family history	Yes	13	84	9	106	X2= 24.957a; df= 2 p-value = .000; P> 0.05 HS
	No	2	10	12	24	
Total		15	94	21	130	
Relatives history	Yes	11	55	6	72	X2= 8.437a ; df= 2 p-value = .015 ; P> 0.05 HS
	No	4	39	15	58	
Total		15	94	21	130	

"χ^2." = "chi-square", "df"= "degree of freedom", "p" = "probability value", "P< 0.05= significant", "P >0.05= non-significant"

The table shows that the relationship between teachers' knowledge and their gender is not significant at less than (0.05), and the results indicate that there is a non-significant association between teachers' knowledge and their age at less than (005) (0.05). Finally, the results show that the significant relation between less than (0.05) of teachers' knowledge and their family context is strong, whereas the relationship between less than (0.5) of teachers' knowledge and their families' history is extremely high.

Discussion

The aim of this study is to think about the information of primary school teachers who form an important part of the community, who have recurrent thinking with children have epilepsy. [21] .

(Table 1) This table indicates that the gender of a total of (130) participants is approximate; for females (n=111; 85.4%) and (n=19; 14.6%) for males, their age group is less than half of them (40-49) years of age (n=42; 32.3%). This result corresponds to that obtained by Al-Hashemi and others (2016). 36.9 years was the norm. Most teachers (44.7%) were between 30 and 39 years of age, and 22.5 percent of them were between 40 and 49 years of age.

(Table 2) This table indicates that there was almost a moderate degree of cooperation knowledge (n=94; 72.3%). In contrast to those with less education, the current research is confirmed by Abulhamail and others (2014) is more likely to have good knowledge (moderate or very good on the Likert scale) This result is consistent with the study of (Tuama, 2020), the study results suggest that nearly half of the study subjects were moderate knowledge of epilepsy.

The results of the study underhand depict that there is a non-significant statistical association between the knowledge of teachers and their gender. Kumari& Patlia endorse this finding (2016). For male school teachers, the mean knowledge score was 7.82±4.75, while for female teachers, 9.9±6.12. This outcome is consistent with the research of [23], the results of present study indicate that there are significant association between age and knowledge were moderate.

The difference (t=1.13, p=.261) was found to be non-significant. Therefore, it can be understood that the standard of knowledge for male and female school teachers was equal. A non-significant relation between the expertise of teachers and their age is shown in this table. Abulhamail and others (2014) found a similar result. The age, gender, years of experience, or form of school of the instructor did not correlate significantly with their epilepsy knowledge.

Conclusions

Based on the findings of the study underhand, the researchers conclude that the majority of participants have a moderate level of knowledge. The findings also indicate that there is a highly important association between the experience of the teachers and their family background and the history of their relatives.

Recommendation

The study recommends that, based on the discussion and the study findings, it is important to design epilepsy health education programs to educate school teachers. Workshops/seminars for school teachers on epilepsy are also recommended to be used.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing and all experiments were carried out in accordance with approved guidelines.

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Assessment Risk Factors for Asthma among Children Attending Azadi Teaching Hospital and Pediatric Hospital in Kirkuk City

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Abstract

A descriptive study was conducted from September 2017 to the January 2018. This study was conducted on children with "asthma" and aimed at assessing risk factors for asthma among children attending to Azadi Teaching hospital and pediatric hospital in Kirkuk city. Data were analyzed by applying descriptive statistics (frequencies, percentage). The result of the study showed that the majority of the sample 60 (75%) was attended to pediatric hospital . while 20 (25%) of the sample attended to Azadi Teaching hospital. Also the majority of the sample aged between (1-7) years, approximately 61.4%, most of the participants were male and constituted 63.6%. With regard to the method of feeding, most of the samples were using artificial feeding 52.3%. As for the gestational age, 72.7% were of full duration (9 months). The study explored that the main factor affecting asthma disease was the parents (71.4%) smoke inside the home. The family history 50% was the second state of caution (a genetic predisposition to develop rhinitis and asthma), and as for the recommendation, families should make sure from the child's hereditary tendencies, and after confirming that the child has asthma, parents should not smoke inside the home and during pregnancy, and care must be taken to ensure that the child has sufficient sunlight inside the house

Keywords: *Risk Factors, Asthma, Children, Azadi Teaching Hospital, Kirkuk City*

Introduction

Asthma is one of the most common major non-communicable diseases and for many, has a substantial impact on quality of life. Globally, asthma is ranked 16th among the leading causes of years lived with disability and 28th among the leading causes of burden of disease, as measured by disability-adjusted life years. Around 300 million people have asthma worldwide, and it is likely that by 2025 a further 100 million may be affected There is a large geographical variation in asthma prevalence, severity, and mortality. While asthma prevalence is higher in high income countries, most asthma-related mortality occurs in low-middle income countries. Despite the advances in asthma treatment in recent decades, there are still gains to be made in terms of improving patient education, employing new diagnostic approaches, and implementing personalized case management. A Project submitted A Project submitted healthcare use, and mortality are higher in adults. Interestingly, incidence and prevalence of asthma differs by sex across the lifespan. Pre-pubertal boys have a higher asthma incidence, prevalence, and hospitalization rate than girls of the same age, but this trend reverses during adolescence [(13)].

Females continue to have a higher burden of asthma than males well into the 5th decade of life. However, the female-male gap in asthma burden narrows around the 5th decade. Some even suggest that the sex differential in asthma incidence may reverse again, following a sharp increase in asthma incidence in males around the 4th decade of life [(13)].

The sex reversal in asthma burden around major reproductive events suggests that sex hormones may play a role in the etiology of asthma. The current evidence suggests that asthma is a complex multifactorial

disorder and its etiology is increasingly attributed to interactions between genetic susceptibility, host factors, and environmental exposures. These include environmental factors (air pollution, pollens, mold and other aeroallergens, and weather), host factors (obesity, nutritional factors, infections, allergic sensitization), and genetic factors (asthma susceptibility on genes). Although underlying mechanisms of asthma are not yet fully understood, they may include airway inflammation, control of airway tone and reactivity [10,11].

It is also now recognized that asthma may not be a single disease but a group of heterogeneous phenotypes with different etiologies and prognoses [24].

While phenotyping individuals with asthma has been used to help guide clinical management, defining the entity of "asthma" has been a major challenge encountered in research, especially in epidemiological research, where in-depth data collection needs to be balanced with the large number of study participants necessary for adequate power.

Review of Literature

The International Consensus Report on the Diagnosis and Treatment of Asthma defines asthma as „a chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophil [6,7].

In susceptible individuals, this inflammation causes symptoms that are usually associated with widespread but variable airflow obstruction that is often reversible, either spontaneously or with treatment, and causes an associated increase in airway responsiveness to a variety of stimuli". Asthma typically presents with „wheezing", a high pitched whistling sound heard on expiration, and also on inspiration is the asthma is sever. Asthma also causes shortness of breath and chest tightness, and can cause cough, particularly in children.

About 25 million people in the U.S. have asthma; about 6 million of these are children. The condition affects men and women equally. Asthma causes over 14 million visits to doctors each year and nearly 2 million emergency room visits. Tragically, asthma can kill. Most people who die from asthma are over age 50, but children sometimes die of the condition

Diagnosing asthma clinically may present challenges, as there may be an overlap of symptoms with other chronic diseases. For example, pneumonia causes shortness of breath and sometimes cough; similarly, heart failure (more common in adults) causes shortness of breath and sometimes wheezing (hence the term „cardiac asthma") also, chronic obstructive pulmonary disease (COPD) in adults has symptoms similar to those of asthma. In populations where asthma prevalence is increasing, there may be a lag in its recognition, as occurred in the United Kingdom in the 1970s, when there was increasing concern about under diagnosis of asthma among older children. It is possible that the same phenomenon is occurring in other parts of the world where prevalence has been low and is now increasing. These factors suggest that the current global estimates of asthma may be underestimates.

Asthma is among the top 20 chronic conditions for global ranking of disability-adjusted life years in children; in the midchildhood ages 5–14 years it is among the top 10 causes. Death rates from asthma in children globally range from 0.0 to 0.7 per 100 000. There are striking global variations in the prevalence of asthma symptoms (wheeze in the past 12 months) in children, with up to 13-fold differences between countries. Although asthma symptoms are more common in many high-income countries (HICs), some low- and middle-income countries

(LMICs) also have high levels of asthma symptom prevalence.

Methods

3.1. Design of the Study

A descriptive study was conducted on children attending Pediatric hospital and Azadi Teaching hospital from September / 2017 to the January /2018 in Kirkuk city to assess risk factors for asthma among children aged from (<1–10 years).

3.2. Administrative Arrangement

The administrative permission was obtained from the Ministry of Health / Planning and Development of Resources Office, Kirkuk"s Health Directorate.

3.3. Setting of the Study:-

The study was conducted at Pediatric Hospital and Azadi Teaching hospital in Kirkuk.

3.4. The sample of the study:-

A non-probability (purposive) sample of (80) children attending pediatric hospital and Azadi Teaching hospital in Kirkuk City. These children were assigned for the study according to the following criteria:-

1- Children aged less than 10 years

2- Both sexes

3.5. Method of data collection

Data was obtained by the investigators who interviewed the children‟s families, filling out the structural questionnaire for demographic and cognitive elements. Each interview session

took about (10-20) minutes.

3.6. The Instrument of the study:-

The study instrument was designed and constructed by the investigators to measure the variable underlying the present study by:-

***1- Primary assessment*:**

An open format was administered to (20) children who were suffering from asthma at pediatric hospital and Azadi Teaching hospital in the city of Kirkuk in order to obtain approximate information about the knowledge of asthma in children.

2- The study questionnaire:

An assessment questionnaire was constructed and designed depending on open format information which consisted of two parts after reviewing the related literature and studies.

Part I

This part of the questionnaire included demographic data related to the respondent's characteristics such as :- age, gender, level of education, type of feeding , place of attending ,gestational age .

Part II

This part of the questionnaire consists of (7) structural elements related to the atmosphere in which children live. Items were classified according to three points - like (yes, no ,

uncertain), scale levels are recorded as (2) for the correct answer, (1) for the uncertain answer and (0) for the incorrect answer.

3. Statistical Analysis:

The present study was analyzed by SPSS program v.22.

3.7.Descriptive statistical data analysis:

This approach was applied through the measurement of the

following:-

1. Frequencies (*f*)

2. Percentage (%)

$$\% = \frac{f}{n} \times 100$$

3.8. Limitation of the study:

1- The sample size is not large enough due limit time of the

study.

2- Inadequate literature and no relevant research studies.

Result

Table (1) Demographic characteristics of the study samples (No. =80)

No.	Variables	Frequency	Percentage
Age			
1	less than 1 years	28	35 %
2	1-3 years	30	375 %
3	more than 3 years	22	27.5 %
4	Total	80	100.0%
Sex			
1	Male	22	27.5 %
2	Female	58	72.5 %
Total		80	100.0%
Mother level of education			
1	House Wife	62	77.5 %
2	Teacher	8	10.1 %
3	Officer	2	2.5 %
4	Clerk	3	3.7 %
5	High School Graduate	5	6.2 %
Total		**80**	**100.0%**

This table shows that most of the study sample were aged (1- 3 years) which constitute (37.5 %), while the lower frequency of the samples were (more than 3 years) which constitute (27.5 %).

Regarding the sex, most participants where Female which constitute (72.5%). Most children"s mothers were house wife which constituted (77.5%). Regarding type of feeding, most of the samples were bottle feeding which constitute (52.5%).

Regarding the gestational age most of samples were full terms which constitute (82.5%). Also, most of samples were from urban which constitute (85 %). The most of the participants were attending to Pediatric Hospital (75%) while (25%) of the sample attending from Azadi teaching hospital.

Table 2. the Risk Factors for Asthma

No.	Items	Yes		NO		Uncertain	
		Frq.	%	Frq.	%	Frq.	%
1.	Parent smoking inside the house	52	65 %	25	31.2 %	3	3.7 %
2.	Family history of atopy (genetic tendency to develop allergic rhinitis and asthma)	38	47.5 %	35	43.7 %	7	8.7 %
3.	Presence of pets in the house	30	37.5 %	50	62.5 %	0	0
4.	Heating and cooling system of the Residence	70	87.5 %	8	10 %	2	2.5%
5.	insufficiency sunlight in the house	42	52.5 %	35	43.7 %	3	3.7%
6.	lower respiratory infection during age of than 1-3 years	30	37.5 %	45	56.2%	5	6.2 %
7.	smoking during pregnancy	4	5 %	76	95 %	0	0%

This table demonstrates that 52 of parent (65 %) were smoking inside the house. 47.5 % of cases were from families with history of asthma (genetic tendency to develop allergic rhinitis and asthma). 50 of samples (62.5 %) no presence of pets in the

house. 70 of samples (87.5 %) have heating and cooling system of the residence. 42 of samples (52.5 %) have insufficiency sunlight in the house. 45 of samples (56.2 %) have no lower respiratory infection during age of 1-3 years. 76 of the mothers (95 %) were not smoking during pregnancy.

Discussion

This study aimed to assess the prevalence of asthma risk factors among children (<1–10 years) attending pediatric hospital and Azadi Teaching hospital inKirkuk city. Our study explored that most participants were female which constitute (72.5 %). While male constitute (27.5 %). This is disagree with Abdul Mohsen (2007) who mentioned in his study, more than half of the infected children (56.7%) were males, and that all children living in an urban setting are at increased risk for asthma. Further, most of the children"s mothers were house wife which constituted (77.5 %). In fact, most of the children who admitted to pediatric hospital because of difficulty in breathing then diagnosed as asthmatic patient were bottle feeding (52.5%).

Besides most of samples were from urban which constitute (85%). Also 42 of the sample (52.5%) due to insufficient sunlight This finding is supported by a study from USA which stated that sufficient exposure to sunlight besides street trees were associated with a lower prevalence of early childhood asthma. Asthma has increased during the last decades but seems to have reached a plateau. The burden of asthma is considerable. It influences quality of life; prevention of children from Asthma is one of the most chronic disorders in children.

The study showed that about (65 %) of parents was smoking inside the house. Further, (47.5 %) were having family history (genetic tendency to develop allergic

rhinitis and asthma). 50 of samples (62.5%) declared no presence of pets in the house. 42 of samples (52.5 %) have insufficiency sunlight in the house. 45 of samples (56.2%) no have lower respiratory infection during age of than 1-3 years. 76 of the mothers (95%) no smoking during pregnancy.

Previous studies reported that diagnosis, asthma-like symptoms, and environmental/occupational risk factors among children. Wheeze and asthma were indicated in 9.7% and 8.9% of medical records, respectively. Parents reported that children play in farming fields (21.8%) and feed livestock/animals (10.9%). Additionally, 13.2% and 9.4% of children have a household member who works around livestock or around grain, feed, or dust, respectively more than half of the sample (55.5%) were from infected women. Also, our study showed that most of the children (56.2%) had no lower respiratory infection during age of 1-3 years. 76 of the mothers (95%) were not smoking during pregnancy.

General epidemiologic characteristics including prevalence and severity of asthma and environmental and occupational risk factors have not been extensively evaluated within the pediatric hospital in Kirkuk city. Administering questionnaires allows for the collection of more directed responses concerning asthma-like symptoms as well as detailed exposure information as compared to information generally recorded in medical records. From the results of this hypothesis-generating pilot project, we were able to demonstrate the elevated occurrence of asthma-like symptoms as well as multiple occupational and environmental risk factors in this unique population, which is critical for developing future hypothesis testing research .

Conclusion

Majority of the sample are female, most of them there age ranged between (1-3)years old .More than half of the sample attending to the pediatric hospital .Factors related to environmental influences on a farm such as increased exposure to bacterial compounds in stables where livestock is kept prevent the development of allergic disorders in children. The prevalence of asthma participating may cause school.

Recommendation

1- Teaching about methods of improving housing conditions should be part of the management of asthma. Our results suggest that housing conditions play an important role in asthma among lower income populations.

2- Future studies aiming at providing baseline data to guide policies and planning on healthcare delivery system to children with Asthma in Kirkuk should focus on these issues.

3- Every year flu vaccine is recommended for children. Especially those with asthma. If child with asthma get the flu, they're at risk for flare-ups and developing a more serious illness.

4- A commitment to plant one million trees inside city Given the evidence from previous studies that street trees were associated with a lower prevalence of early childhood asthma. It is clearly important to consider this recommendation by the health policy makers.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of nursing and all experiments were carried out in accordance with approved guidelines.

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Original article

Determinat Factors The Effect Used Personal Protective Equipment Covid-19 For Health Workers in The Hospital

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Abstract

Purpose: to analyze the determinants of the use of Covid-19 PPE (Personal Protective Equipment) on health workers at Salewangang Hospital, Maros Regency.

Methods: The type of research used is the analytical survey method of research conducted without intervention to the research subject. Data collection regarding free and dependent variables was carried out online using Google Form and the results of Google Form data collection were processed using SPSS 20, where a sample of 199 respondents was taken from health workers at Salewangang Maros Regional Hospital in 2020. The analysis was used to explain the influence of the variables - independent variables to the dependent variable partially using Logistic Regression Analysis.

Results: Based on the results shows that knowledge has a 12-fold effect on behavior The use of Covid 19 PPE and the attitude of health workers has a significant effect on the behavior of using the OR value of 16.6, which means that attitudes have a 16.6-fold effect on the behavior of using Covid 19 PPE

Conclusion: Based on this study, it is concluded that the availability of PPE, knowledge and attitudes of health workers have a significant effect on the behavior of using PPE Covid-19 with a significant value of 0.000<0.05.

Keywords: *covid-19, personal protective equipment, health workers, behaviour, hospital*

Introduction

Covid-19 is included in the category of occupational diseases caused by other biological factors in the workplace where there is a direct relationship between exposure to biological factors that arise due to work activities and diseases experienced by workers that can be scientifically proven using appropriate methods. Health and non-health workers in carrying out their work to deal with Covid-19 are at high risk of contracting Covid-19 so that they can cause occupational diseases[1,2]. The incidence of Corona Virus Disease 2019 Covid-19 in hospitals is one of the problems because it can threaten the health of patients, health workers and visitors[3].

Personal protective equipment is a device designed as a barrier against the penetration of substances, solid, liquid, or air particles to protect the wearer from injury or the spread of infection or disease. When used properly, PPE acts as a barrier between infectious material (eg viruses and bacteria) and the skin, mouth, nose, or eyes (mucous membranes) of the health worker and the patient[4,5]. Barriers have the potential to block transmission of contaminants from blood, body fluids, or respiratory secretions. In addition, other infection control practices such as washing hands, using alcohol-based hand sanitizer, and covering the nose and mouth when coughing and sneezing with the inner upper arm or tissue, can minimize the spread of infection from one person to another. Effective use of PPE includes removal and / or proper disposal of contaminated PPE to prevent exposure of wearers and others to infectious materials[6,7].

Based on data from the Covid-19 Patient at Salewangang Maros Regional Hospital in March 2020, 18 people were treated (3 confirmed patients, 5 PDP and 10 ODP patients), 19 people were treated in April 2020 (3 confirmed patients, PDP 6 people and 10 ODP people), in May 2020 as many as 25 people were treated (6 confirmed patients, 14 PDP and 5 ODP people), and 41 people treated in June 2020 (22 confirmed patients, PDP as many as 8 people, ODP as many as 9 people and Suspek as many as 2 people)[8,9]. Personal Protective Equipment (PPE) which came from donations from various parties. In March - June 2020 Salewangang Regional Hospital received 1,739 hazmat suits, 348 googles, 484 N95 masks, 5,220 surgical masks, 629 cloth masks, 412 hand scoons, as many as boots 177 pieces, 264 helmets, 305 hats, 20 raincoats, 65 face shields, 70 dresses, 92 plastic gloves, 114 covel shoes, and as many aprons. 50 pieces and 4 nurse caps / head cove[10].

The Covid-19 PPE at Salewangang Regional Hospital has a very important role to protect workers from Covid-19 Work-related Diseases, but the large benefits of using Covid-19 PPE at work does not guarantee that all workers will wear it. Based on this, the researchers are interested in conducting research on "Analysis of Determinant Factors for the Use of Covid-19 PPE on Health Care workers.

Materials And Methods

The research design was an analytic survey with a cross sectional approach. This research was conducted online using Google Form. The research time was started from April to September 2020. The population in this study were 348 health workers who served in Salewangang Hospital with a total sample size of 199 people. The Data Collection Techniques To collect data in this study using an online questionnaire (Google Form). Before answering / filling in the Google Form, respondents will be asked to read and sign the Informed Consent electronically. Data analysis used logistic regression test, the magnitude of the influence is indicated by the EXP value (B) or also known as the Odds Ratio (OR), if the value is sig. <0.05, H0 is rejected and Ha is accepted, it means that there is a significant (significant) effect, if the sig. > 0.05 H0 is accepted and Ha is rejected, so there is no significant effect (significant).

Result

Table 1: Characteristics Respondent for Health Workers in The Hospital

The Characteristic	n	%
≥ 30 Years	45	22.61
31 – 40 Years	98	49.25
≤ 41 Years	56	28.14
sex Man	61	30.7
Women	138	69.3
Education SMP/SMA	38	19.1
Diploma/S1	156	78.4

Cont... **Table 1: Characteristics Respondent for Health Workers in The Hospital**

S2/S3	5	2.5
length of work <1 – 5 Years	58	29.15
6 – 10 Years	126	63.32
>10 Years	15	7.53
Time work 7 hours	46	23.1
8 hours	137	68.8

Based on the table. 1 characteristic of respondents, it is known that from 199 respondents, most were 31-40 years old (49.25%). 138 people (69.3%) and the least male 61 people (30.7%). The educational level with the most diploma / S1 is 156 people (78.4%) and at least 5 people are S2 / S3 (2.5%). The maximum length of work is 6-10 years with 126 people (63.32%) and at least 10 years (7.5%).

Table 2 Characteristics of Type of Work Profession In the hospital

Type of work	n	%
Docter	2	1
Nurse	52	26
Midwife	21	11
Health Laboratory Institutions	10	5
Pharmacist	15	8
Fisioterapi	8	4
Radiographer	8	4
Medical check up	8	4
Public health ectention	1	0.5
Epidemiology	2	1
Environmental health	10	5
Elektromedis	4	2
Nutrisionist	10	5
K3	2	1
Administrasi/Staf Manajemen	27	14
IPSRS	13	6.5
Ambulance driver	6	3

Based on table 2, it shows that the profession with the most number of nurses is 52 people (26%) and the least number of public health extension personnel is 1 person (0.5%). For service locations, most of them were in IPSRS (Hospital Facility Maintenance Installation) as many as 25 people (13%) and at least 2 people were in polyclinics (1%). The maximum length of work in a day is 8 hours (68.8%) and at least 8 hours (8%).

Table 3: Research Variable Bivariate Test Results

Research variable	The Behavior Of Using Personal Protective Equipment				Sign	OR
	Good		Less			
	n	%	N	%		
availability of personal protective equipment						
Complit	174	92.,1	15	7,9	0,000	11,600
incomplit	10	100	0	0		
Knowledge						
Enough	180	92,3	15	7,7	0,000	12,000
Less	4	100	0	0		
Actitude						
Enough	166	94,3	10	5,7	0,000	16,600
Less	`18	78,3	5	21,7		

Based on Table 3, it shows that respondents who have sufficient knowledge of good behavior in using PPE Covid-19 are 180 (92.3%) and less behavior in using PPE Covid-19 are 15 (7.7%). Respondents who had sufficiently good attitudes in the use of Covid-19 PPE were 166 (94.3%) and 10 (5.7%) had less behavior.

Discussion

Based on the results of statistical tests using logical regression tests in this study, the variable availability of PPE has a significant effect on the behavior of using PPE Covid-19 at Salewangang Maros Regional Hospital with a sig value. 0.000 < 0.05 and the OR (Odds Ratio) value is 11.6. This shows that the availability of PPE has an effect 11.6 times the behavior of using PPE Covid 19. This is in accordance with the data of Covid-19 patients at Salewangang Maros Hospital in March-May 2020, the number of confirmed patients was 2, 3 and 6 people, which tended to be constant, while in June of the year 2020 saw a surge of 22 patients so that the availability of PPE at Salewangang Maros Regional Hospital was limited to meet the needs of health workers. With the limited number of PPE, especially masks, the health workers provide their own[8,11.]

Health workers are a group that is very vulnerable to Covid-19 infection because they are at the forefront of handling cases. Close contact with people who are suspected or confirmed positive for Covid-19 puts him at a high risk of being infected with the virus if handling is not according to standards. One of the health protocols that is very important to note is the availability and use of the correct PPE. Health protocols related to the use of PPE recommended by the Ministry of Health are divided into three service categories[12].

First-level health workers such as doctors, nurses, pharmacists who provide health services are recommended to wear three-layer work clothes, gloves and surgical masks. Second level health workers, namely

doctors, nurses, and analysts in charge of the treatment room, sampling, or virus testing laboratories. Health workers in this category must wear PPE in the form of a gown (surgical gown), head coverings, disposable rubber gloves, three-layer surgical masks, and Google glasses or eye protection. Third level health workers are at high risk, namely doctors and nurses who come in direct contact with patients who are suspected or confirmed positive for Covid-19. Third level health workers are required to wear hazmat suits or coveralls that cover all parts of the body, head covers, sterile surgical gloves, N95 masks, boots and face protection [4,9.13.]

This is in line with the results of research conducted which stated that the results of the logistic regression statistical test partially variable availability of PPE had a significant effect on the compliance of surgical nurses in using PPE with a p-value = 0.003 < 0.05. states that behavior is formed from 3 factors, namely predisposing factors, enabling factors and driving factors. The availability of facilities is included in the enabling factor for the formation of behavior. states that one of the factors that causes someone to behave is the presence of resources in the form of facilities, time, energy[5,10].

Knowledge is a very important domain for the formation of a person's actions, because behavior based on knowledge will be more lasting than behavior that is not based on knowledge. For officers and those who are involved in handling Covid-19, they need to get knowledge about the Covid-19 PPE standard according to the recommendations of the Ministry of Health. Therefore, in Salewangang Regional Hospital, several banners have been posted regarding the Covi-19 health protocol, such as notification of patient visiting restrictions, mandatory wearing of masks, mandatory hand washing, minimum distance limitation of 1-2 meters[13,14.]

The PPE standard recommendation for the level of protection for service locations is divided into 3, the first is PPE that is used in locations or conditions that are relatively less risky. Types of PPE are included in this category, namely various types of masks, work gloves and disposable rubber-based gloves and dresses. One of the officers who are required to wear this PPE is an ambulance driver. They are required to use 3 layers of surgical masks, disposable rubber gloves and gowns when raising and lowering patients with suspected Covid-19[15]. The second level of PPE is used by doctors, nurses, laboratory assistants, radiographers, pharmacies and Covid-19 patient room cleaners. PPE at this level is used when medical personnel, doctors and nurses, in the polyclinic room, examine patients with symptoms of respiratory infection. PPE in the form of 3 layers of surgical masks, gowns, disposable rubber gloves and eye protection. However, PPE for analysts, radiographers, pharmacists and cleaners has different types of PPE used[316]

Medical personnel who use PPE at the third level of protection, namely doctors, nurses and laboratory assistants. The results of this study are in line with research conducted where it is known that there is a very significant relationship between knowledge and the behavior of using PPE among health workers at the Banjarbaru Hospital and the results of the Chi-square statistical test obtainDFDXed a value (p-value = 0, 0001). At Salewangang Maros Hospital in providing services to provide facilities needed for suspected and confirmed Covid-19 patients, always carry out various health protocols when treating Covid-19 patients by protecting themselves as much as possible with PPE according to standards, having sufficient knowledge to handle Covid-19 patients , follow strict requirements to remove official clothes, leave the hospital or meet with family / community, treat with sincerity, even struggle to care for Covid-19 patients.

This research is in line with research conducted) which states that the percentage of good attitudes, namely obedience, is the highest, namely 86.3% and the rest is disobedient. The statistical results of the t test (partial test) of surgical nurses 'attitudes towards surgical nurses' compliance in using obtained a p-value (0.117)> 0.05. Thus it can be concluded that partially the variable attitude of surgical nurses has no significant effect on the compliance of surgical nurses in using PPE[17].

Conclution

The availability of PPE has an 11.6-fold effect on the behavior of using PPE, knowledge has a 12-fold effect on the behavior of using PPE while attitude has a 16.6-fold effect on the behavior of using PPE with Covid 19.

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Ethical Clearance- taken from Comitee ethical Universitas Muslim of Indonesia Makassar

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Original article

Quantitative Helmint Assesment (QHA) on Nutritional Status of Children and Stunting Events For Elementary Students in the City of Makassar

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Abstract

Objective: to analyze the relationship between the Quantitative Helmint Assessment with nutritional status and the incidence of stunting in SD Bangkala III students in Makassar City

Method : This type of research is analytic observational using cross sectional design. Location This research was conducted at SD Bangkala III, Bangkala Village, Manggala District, Makassar City. 63 samples were taken using proportional stratified random sampling technique and simple random sampling. Quantitative examination is needed to determine the intensity of infection or severity of disease by knowing the number of eggs per gram of feces (EPG) in each type of worm.

Results: based on the measurement results of the helmint assessment for the mild category with the incidence of stuting and not stunting kneeling as many as 8 (38.0%), 13 (62.0%), respectively. In the measurement results, the moderate category with the incidence of stuting and non-stunting was 11 (38%), respectively. 13 (62%), while the results of QHA measurements for the severe category of knee stunting were 1 (25%), and 3 (75%), respectively. based on the measurement results of the helmint assessment for the light category with nutritional status of thin, normal and obese knees as many as 5 (23.8%), 13 (62.0%) and 3 (14.2%).

Conclusion: There is a significant relationship between QRA and nutritional status in elementary school students with p value (0.003), there is a significant relationship between QRA and the incidence of stunting with p value (0.022).

Keywords: *QHA, Ascaris, Trichuris, Nutritional Status, Stunting.*

Introduction

Soil-borne worm infection in Indonesia is a major health problem in urban and semi-urban areas with poor environmental sanitation, poor hygiene habits and low socioeconomic status. Environmental sanitation, availability of water sources, availability of latrines at home and poor personal hygiene behavior of the community play a major role in the transmission of worm infection[1,2,3.]

Worms infection that is transmitted through soil develops in the host's body into adult worms and reproduces by laying eggs. The eggs of roundworms and whipworms are excreted along with the feces of the host so that they can contaminate the soil environment. Meanwhile, hookworm eggs will develop into infective filiaform larvae on the ground. Hookworm infection is transmitted to humans by direct contact with soil contaminated by infective filiaform larvae. Meanwhile, ascariasis and trichuriasis infection occurs when the infective worm eggs are ingested by humans[4,5,6.]

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Children are a group of people who suffer from worms the most, especially elementary school children because they often play or come into contact with the soil which is the place where the worms grow and develop. The incidence of worms in elementary school-age children can hinder their growth and physical and cognitive development who are in a very fast and active growth period. At this age children should get a balanced and quality nutritional intake[1,7,8,9]. If left unchecked for a long time, children can suffer from malnutrition, and even become less protein energy (KEP)[6,10].

According to the 2013 RISKESDA, the national prevalence of short is 37.2%, consisting of 18% very short and 19.2% short. This national figure increased from 2010 (35.6%) and 2007 (36.8%). There are 20 provinces with a prevalence above the national level, one of which is South Sulawesi at 23.5% (2013). The incidence of stunting in the PKM Rappokalling work area shows that there are 10 school-age children 6-12 years who have worms. 24.5%[5,7,11].

Based on data from the Puskesmas Bangkala, Makassar city, the incidence of stunting among elementary school students 7-12 years is 13.5% (55 children), this is influenced by poor environmental sanitation, for example there is still a lot of garbage scattered, flood-prone areas and community PHBS behavior that is still low. This is indicated by diarrhea disease which is still relatively high, namely 35.5% of school age children. Therefore, the researcher is interested in examining worm infection with the incidence of stunting in elementary school students in Makassar City.

Materials and Methods

This type of research is analytic observational using cross sectional design. Location This research was conducted at SD Bangkala III, Bangkala Village, Manggala District, Makassar City. Samples were taken as many as 63 with proportional stratified random sampling technique and simple random samplingPemeriksaan sampel.

Quantitative examination is needed to determine the intensity of infection or severity of disease by knowing the number of eggs per gram of feces (EPG) in each type of worm.

(1) **How to Make Preparations**

a) Filter the stool using a filter wire.Place the cardboard with the hole on the slide and then insert the filtered stool into the hole.

b) Take the cardboard with a hole and cover the stool with cellophane soaked in Kato solution.

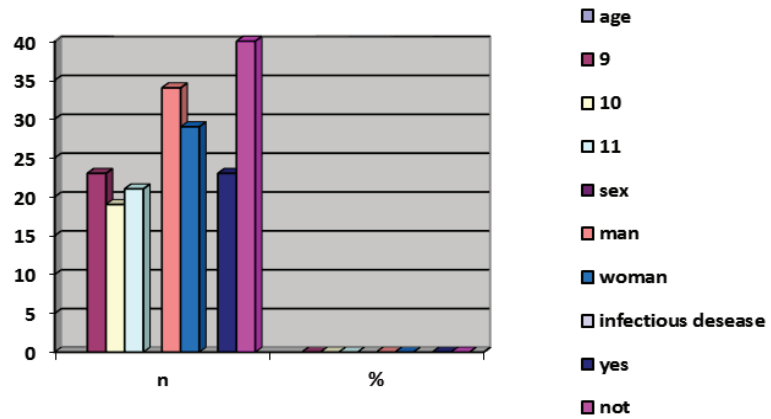
c) Flatten with a rubber bottle cap until evenly distributed. Let the preparation stand for 20-30 minutes.

d) Examine it under a microscope and count the number of eggs in the preparation.

(2) **How to Count Eggs**

The result of quantitative stool examination is the intensity of infection, namely the number of eggs per gram of feces (Egg Per Gram / EPG) of each type of worm.

Result



Graph 1 Characteristics of Respondents in SD Bangkala III students

Table 1: Distribution of Respondents About the Types of Worms Infection in SD Bangkala III Students in Makassar City

Type of worm	Quantitative Helminth Assessment (QHA)							
	Mild		moderate		Severage		Total	
	n	%	n	%	n	%	N	%
Ascaris	14	82,3	3	17,7	0	0	17	100
Trichuris	3	27,3	8	72,7	0	0	11	100
Ascaris &Trichuris	4	25	8	50	4	25	16	100
Trichuris dan entero	0	0	1	100	0	0	1	100
Total	0	0	20	44.5	4	8,8	45	100

Based on table 1, it shows that the types of worms that infects the most are Ascaris worms, which are included in the mild category as many as 14 (82.3%), and 17.7% moderate. In this type of trichuris worm, the number of mild worm infections (27.3%) was moderate as much as 72.7%.

Table 2: Distribution Of Respondents About PHBS Behavior Towards Worm Infection At SD Bangkala III Makassar City

Reseach Variable	Quantitative Helminth Assessment (QHA)						Total	
	Mild		Moderate		Severage			
	n	%	n	%	n	%	N	%
Hand washing								
Routine	4	57,1	2	28,6	1	14,3	7	100
Not routine	17	44,7	18	47,7	3	7,9	38	100
cutting nails								
Routine	2	66,7	0	0	1	33,3	3	100
Not routine	19	45,2	20	47,6	3	7,2	42	100
consume deworming medicine								
Routine	1	50	0	0	1	50	2	100
Not routine	20	46,5	2 0	46,5	3	7,0	43	100
Wearing footwear								
Routine	17	85,0	2	10.0	1	5,0	20	100
Not routine	4	16,0	18	72,0	3	12,0	25	100

Based on the table 2 shows that the habit of washing hands routinely and in the QHA (mild) category is 4 (57.1%), 17 (44.7%) is not routine, the habit of routine nail cutting is in the QHA (mild) category as many as 2 (66.7%) and 19 (45.2%) who were not routine.

Table 3: Distribution of Respondents Based on Quantitative Helmint Assessment (QHA) with Nutritional Status of Bangkala III Elementary School Students in Makassar City

Quantitative helmint Assement	Nutritiona status						Total		p
	skinny		Normal		Fat				
	n	%	n	%	n	%	N	%	
Mild	5	23,8	13	62,0	3	14,2	21	100	0,003
Moderate	7	35	12	60	1	5	20	100	
Severage	1	25	2	50	1	25	4	100	
Total	13	28,9	27	60	5	11,1	45	100	

Based on table 3, The results of the analysis show that there is a significant relationship with the p value (0.003).

Table 4: Distribution of Respondents Based on Quantitative Helmint Assessment (QHA) with Nutritional Status of Bangkala III Elementary School Students in Makassar City

Quantitative helmint Assement	incidence stunting				Total		p
	Stunting		Not stunting				
	n	%	N	%	N	%	
Mild	8	38,0	13	62,0	21	100	**0,022**
Moderate	11	55	9	45	20	100	
Severage	1	25,0	3	75,0	4	100	
Total	20	44,4	25	55,6	45	100	

Based on table 4nThe analysis showed that there was no significant relationship with the p value (0.022).

Discussion

In theory, the chances of worm infection are more common in boys because they play more outside the home and interact with soil media and work in the garden. However, in reality, the observations of boys and girls have almost the same playing habits. Although the types of games are different, their activities are mostly carried out on the ground. Boys usually play ball[5,9,12.]

Body Mass Index (BMI) can be used to evaluate the nutritional status of children aged 5 years to adolescence. The distribution of body mass index was obtained by children with a low BMI of 79.5% and children with a normal BMI of 46%. The number of students with a low BMI. 30.3% were infected with worms and 28.3% of normal students were infected. The existence of ascariasis infection causes malnutrition in elementary school students to be higher, which will cause weight loss and inhibit the child's growth[10,13,14,.]

The results of research conducted in Kediri showed that there were 7 students infected with worms and anemia (25%). The results of the bivariate test did not provide significant results between the worm infection variable and the anemia status variable. This is because the respondents who were infected with trichuriasis and hookworm were very few and the intensity of the infection was mild so that it had little effect on reducing Hb levels.[4,7,9,15.]

In this study, the highest number of infections was a single T. trichiura infection (79.3% of the total infection) followed by a single A. lumbricoides infection (20.7%), so it can be said that in this study the STH infection was dominated by T. trichiura and A. lumbricoides. Another trigger factor for worm infections is the weather factor. This research was conducted from September to October in Makassar City when it is dry and rainy season which has a temperature of ± 30 ° C which is the optimum temperature for the development of T. trichiura eggs. The soil in this research location looks dry because the location of this research is experiencing a dry season. Because it is in the dry season, worm eggs can fly through the air[5,10,16,17.]

In this study, STH infection was most prevalent in children aged 8 years, amounting to 24.1%. The results of this study are similar to research conducted by Annisa et al. (2017) who stated that the prevalence of STH infection is higher in children aged 612 years, and Eryani et al. (2015) which states that the highest prevalence of STH infection is found in children aged 6-8 years.

From 63 samples, it was found that the proportion of malnutrition status was high, namely 43.9%. These results are similar to research conducted on elementary school students in the Riau area, it was found that 58.16% of students had good nutritional status and 41.84% of students were malnourished 35. This is due to the economic level of the parents in the school including middle and lower levels.

The results of statistical tests using the Chi-square test the relationship between STH infection and nutritional status obtained p value = 0.037 and OR = 2.765 (95% CI: 1.147-6.662). These results indicate that there is a statistically significant relationship between STH infection and nutritional status. The results of this study correlate with several previous studies, including the results of research conducted by Simarmata et al. (2015) at 3 primary schools in Simpang Empat and Kabanjahe Districts, Karo District, North Sumatra which stated that there was a significant relationship between STH infection and nutritional status (p = 0.001) Similar to the research of which stated that STH infection had a significant relationship with nutritional status with p value = 0.025 and OR = 2.05 (95% CI: 1.08-3.87)[18,19.]

However, this result research which states that there is no significant relationship between STH infection and nutritional status in students of SDN 126 Sematang Borang District, Palembang City with a value of p = 0.065 (p> 0.05) and OR = 2.669 (95% CI: 1,439-15,192), Several studies have been conducted to find an association between nutritional status and STH infection. Relationships are complex and may depend on environmental, social and economic influences. The interplay between malnutrition and infectious disease makes it difficult to identify which of the two conditions came first.infectious diseases such as worms that attack children can interfere with the child's nutritional condition due to several things, including decreased appetite for children due to discomfort experienced so that the intake of nutrients is reduced, even though children need more nutrients, especially to replace their body tissues. damaged by the disease[5,9,20,]

Conclution

There is a significant relationship between QRA and nutritional status in elementary students with p value (0.003), there is a significant relationship between QRA and the incidence of stunting with p value (0.022).

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Conflict of Interest- None of the authors has competing interests

Ethical Clearance- from cometeee etical clearance University Muslim Indonesia.

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Original article

Subjective Complaints of Respiratory Disorders Due to Silica Dust Exposure to Workers at PT Bumi Saran Beton Kalla Block

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Abstract

Purpose: The aims to analyze the effect of exposure to silica dust and individual characteristics on subjective complaints of workers'

Methods: The study design used a cross sectional approach. This research was conducted at PT. Bumi Sarana Beton Kalla Block, samples in this study were workers in the ballmill, mixing and packing as many as 27 people using the total sampling method. Data analysis using chi-square test and multiple regression test.

Results: the results showed that the effect of age on subjective complaints of respiratory disorders was p 0.904> 0.05, the effect of smoking on subjective complaints of respiratory disorders p 0.485> 0.05, the effect of working tenure on subjective complaints of respiratory disorders p 0.728> 0.05, the effect of The use of respiratory protective equipment against subjective complaints of respiratory disorders p 0.820> 0.05, the effect of work history on subjective complaints of respiratory disorders p 0.647> 0.05, the effect of exposure to silica dust on subjective complaints of respiratory disorders p 0.529> 0.05.

Conclusion: exposure to silica dust and individual characteristic factors such as age, smoking habits, years of service, use of respiratory protective equipment, and work history are not significant to the subjective complaints of respiratory

Keywords: *Silica Dust, Subjective Complaints, Respiratory Disorders.*

Introduction

Health problems can arise due to various factors in the work environment, such as: physical, chemical, biological, physiological, and psychological factors[1]. The work environment is very easily exposed to chemical factors such as dust, vapors and gases. Dust under certain conditions is a chemical agent that can reduce work comfort, visual disturbances, pulmonary function disorders, and can even cause general poisoning. Continuously inhaled dust can cause lung damage and fibrosis. Dust with a smaller size has a greater potential to cause lung function problems because dust with a size of less than 1 μ can enter the alveoli, while dust particles <0.1 μ move in and out of the alveoli and do not settle on the alveoli surface[2]

Data from the ILO suggests that among all occupational diseases, 10% to 30% are lung diseases. It is detected that about 40,000 new cases of pneumoconiasis occur worldwide each year (ILO, 2010). Based on the results of a survey by the Directorate General of PPM & PL in Indonesia, chronic obstructive pulmonary disease ranks first as a contributor to morbidity (35%), followed by bronchial asthma (33%), lung cancer (30%), and 2% others[3,4,5].

One of the health problems caused by the work environment is the disruption of lung function for workers and communities around industrial areas. Various substances can pollute the air such as dust, cement, cotton, asbestos, chemicals, poisonous gases. The effect of dust exposure on labor can cause disturbances, including enjoyment of work, irritation to both the eyes and the respiratory tract and impaired lung function[1,6,7]. The accumulation of dust in the lungs can reduce the ability of the lungs to inhale and exhale air so that the volume of air inhaled in the lungs is reduced[4, 8,9].

One of the products of PT. Bumi Sarana Beton is a lightweight brick, where the materials used in the manufacturing process are silica sand, cement, lime, a little gypsum, water, and aluminum paste as a building material[10,11]. Where these materials are one of the factors causing the disruption of lung function when continuously exposing workers to certain concentrations, one of which is silica.[5,12,13.] According to the International Agency for Research on Cancer (IARC), silica belongs to group 1 substances that are carcinogenic in humans. Silica is usually found in crystalline form and rarely in an amorphous state[5].Inhaled silica crystals cause decreased lung function, acute pneumonia, autoimmune disorders, and even lung cancer[10,14,15]. Silica crystals that settle in the lungs, will oxidize the alveoli walls causing fibrosis. The more silica crystals that settle in the lungs, the fibrosis that occurs in the alveoli will get worse and cause a disease known as pneumoconiosis silicosis.

The result of the preliminary survey that has been conducted is by interviewing at PT. Bumi Sarana Beton Kalla Block, the total workforce in the three parts of the production process is 27 people. There are several workers who are at risk of direct exposure to dust including complaints from some workers who experience shortness of breath, coughing and sneezing. The workers who are dominant work 12 hours mand it is assumed that the majority of workers breathe dust from the grinding and finishing process. Observations that have been made by researchers are in fact most workers still do not realize the importance of using masks while working.

Materials and Methods

This research is a quantitative study using a cross sectional approach. This research was conducted at PT. Bumi Sarana Beton Kalla Block. The population in this study were workers from the ballmill, mixing, and packing sections. The number of samples in this study were 27 people. Data analysis used chi square test and multiple regression test. The sampling technique used total sampling. The instrument used to measure humidity and air temperature is a thermo / hygrometer, while the wind speed and direction is an anemometer.

Result

Table 1. Distribution of Respondents Based on Individual Characteristics Variables

Variabel	Total	
	n (27)	%
Age		
< 40 years	23	82,5
≥ 40 years	4	14,8
Masa Kerja		
< 3 years	5	18,5
≥ 3 years	22	81,5

Cont... **Table 1. Distribution of Respondents Based on Individual Characteristics Variables**

Smoking habit		
Smoking	5	18,5
Never smoking	16	59,3
No smoking	6	22,22
Wearing APP		
Always	5	18,5
Seldom	11	40,7
Never wear	11	40,7
Job experience		
dusty	6	22,2
no dusty	15	55,6
no history	6	22,2

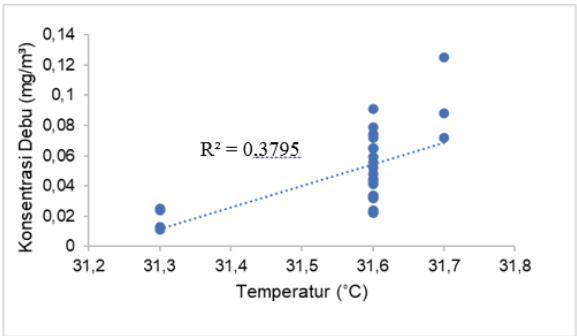
Based on table 1 shows that the majority of workers are <40 years old as many as 23 people (82.5%), the most working tenure is ≥ 3 years as many as 22 people (81.5%), the highest result of the respondents' smoking habit is in the category 16 people (59.3%) have ever smoked, the frequency of using APP by workers in the infrequent and non-using categories respectively has the same number, namely 11 people (40.7%), and the highest result of the respondent's work history lies in a not related to dust a number of 15 people (55.6%).

Table 2. Measurement Results of Environmental Physics Parameters

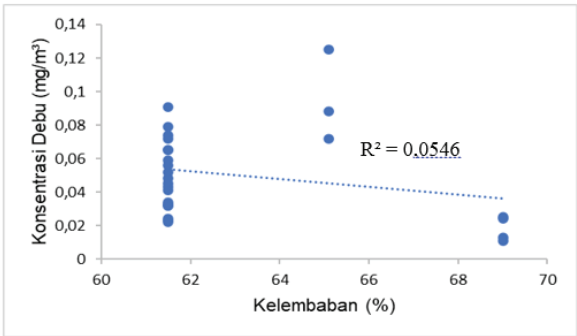
Location	**Result assessment**		
	Ta (°C)	**RH %**	**Wind velocity (m/s)**
Ballmill	31,7	65,1	0,21
Mixing	31,3	69	0,19
Packing	31,6	61,5	0,45

Table 3. Measurement Results of Silica Dust Exposure

Responden	Result assessment
	mg/m³
Packing1	
Packing2	0,041
	0,079
Packing3	0,091
Packing4	0,074
Packing5	0,032
Packing6	0,065
Packing7	0,056
Packing8	0,023
Packing9	0,034
Packing10	0,024
Packing11	0,059
Packing12	0,048
Packing13	0,072
Packing14	0,033
Packing15	0,043
Packing16	0,032
Packing17	0,045
Packing18	0,065
Packing19	0,022
Packing20	0,052
Ballmill21	0,125
Ballmill22	0,088
Ballmill23	0,072
Mixing24	0,013
Mixing25	0,024
Mixing26	0,011
Mixing27	0,025



(a) Temperature – consentration



(b) humidity - consentration

Figure 1. Effect of Work Environment Physical Parameters on Silica Dust Concentration

In Figure 1a, it can be seen that the effect on dust concentration has a fairly high R^2 value of 0.3795, so it can be said that temperature has a sufficient effect on the concentration of silica dust. In Figure 1b, it can be seen that the effect of humidity on concentration has a very small R2 value, namely 0.0546, so it can be said that humidity does not have a major effect on the concentration of silica dust.

Table 4 Analysis of the Effect of Variables on Subjective Complaints of Respiratory Disorders

Variable	Subjective Complaints %		p-value
	Yes	No	
Age			
< 40 years	52,2	47,8	0,904
≥ 40 years	75,0	25,0	
Work period			
< 3 years	40,0	60,0	0,728
≥ 3 years	59,1	40,9	
Smoking habit			
Smoking	80,0	20,0	
Never smoking	50,0	50,0	0,485
No smoking	50,0	50,0	
Wearing APP			
Always	40,0	60,0	
Seldom	72,7	27,3	0,820
Never wear	45,5	54,5	
Job experience			
Dusty	66,7	33,3	
No dusty	52,4	47,6	0,647
No history	50,0	50,0	
Silica expusore			
qualify	53,8	46,2	0,529
not qualify	100,0	0,0	

Based on table 4 it can be concluded that the variables age, years of service, smoking habits, use of APP, work history, and exposure to silica dust have no effect on subjective complaints of respiratory disorders in workers.

Discussion

The older a person, the more susceptible to dust exposure. The elasticity of the lungs does not change at the age of 7-39 years, but there is a tendency to decrease at the age of 25 years, and this decrease is more evident after the age of 30 years, so that breathing power

decreases as a result of which the volume of air during breathing will be less. the age variable is significant with respiratory complaints, an increase in age in workers is followed by an increase in the percentage of workers who experience respiratory complaints[4,8.16,17.]

Smoking habits will accelerate the decline in lung function. The effect of smoke can be greater than the effect of dust which is only one third of the bad effects of smoking. The relationship between smoking and impaired lung function is a dose response relationship. The purpose of the dose response relationship is that the more cigarettes smoked each day and the longer the smoking habit, the risk of developing several respiratory complaints and disease is greater[9,13,18,19.]

Toxins in cigarettes will accumulate in number in the body, especially in the lungs. The presence of this toxin will inhibit the process of exchanging O2 gas with CO2 in the alveoli[17,20,21.] This condition will worsen as the number of cigarettes smoked increases, alveolar damage is also very likely due to smoking. This will certainly reduce the number of functional alveoli that play a role in the respiration process[7,17,20]. As a result, there will be a decrease in the function of the lung organs, causing several complaints about breathing such as coughing, phlegm, shortness of breath, and sputum [8,12,17].

This is because the workers who actively smoke are only 5 people (18.5%), while the others have ever smoked or have quit smoking, and never smoked at all. In addition, based on the results of interviews, respondents said that the company prohibited workers from smoking while in the work environment so that the smoking habit did not affect the complaints of respiratory problems by workers during their work[5,10,22,23.]

Some of the workers also switch to e-cigarettes or what is commonly known as vape, especially younger workers. The use of e-cigarettes does not pose a serious health risk compared to regular cigarettes, which are consumed by burning. vaping does not cause lung problems[10, 22.] This means that vape is considered safe to use[5,8,13]. Even consumers who use e-cigarettes on a daily basis are not affected by their lung health. These findings were obtained from physiological, clinical, or inflammatory effects analysis. This study report was analyzed among e-cigarette users aged 23-35 years. It also targets another group of non-smokers of the same age range6,18,13

But the results of this study contradict previous studies that smoking is a trigger for subjective respiratory complaints. Likewise with the results of the study which states that workers who have a smoking habit have the opportunity to experience complaints and pulmonary function disorders. Workers who smoke and are in a dusty environment are more likely to experience respiratory problems than workers who are in the same environment but do not smoke.

The longer a person works, the more he or she has been exposed to the dangers posed by the work environment. Based on the results of research which states that tenure in a company that contains a lot of dust has a high risk of silicosis. Workers who are in an environment with high dust levels for a long time have a high risk of developing obstructive pulmonary disease. In this process, dust pollution in the air is quite high, especially if it is supported by conditions of low room temperature and humidity, which can increase the dust concentration. Workers in this section have worked ≥ 3 years with a length of work ≥ 8 hours per day and based on the results of measurements of respirable dust, the average worker almost exceeds the threshold value, with one respondent who has exceeded the threshold value with complaints of shortness of breath and coughing at the time do its job.

The onset of respiratory problems in industrial workers can greatly depend on the length of exposure and the dose of exposure received. Long exposure to low levels may not immediately indicate respiratory distress. The relationship between exposure and this effect is highly dependent on three things, namely the level of dust in the air, the cumulative exposure dose (the sum of the levels in the air and the duration of exposure) and the residence time or duration of dust in the lungs. Long-term exposure to low levels causes less severe disease than short-term exposure to high levels[3,7,14.]

Based on the measurement results of respirable dust exposure, it was found that there was only one worker who exceeded the threshold value, namely workers who operated in the ballmill section, the other three people almost exceeded the threshold value. Some of the complaints also came from the ballmil and packing section where the dust came from the manufactured

goods[14,18]. In a day there are approximately 1000 cubic cubic meters of light bricks produced by the company, the dust produced from the lightweight bricks spreads into the air when workers in the ballmill grind the raw materials, namely silica sand and cement and spill it into containers, as well as in the packing department. who organize and move the production.

Conclution

exposure to silica dust and individual characteristics such as age, smoking habits, years of service, use of APP, and work history have no effect on subjective complaints of respiratory disorders for workers

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Effectiveness of Giving Secang Wood Extract (Caesalpinia Sappan L) Against IL-6 And IL-10 Levels in Balb / C Mice with Vulvovaginalis Candidiasis

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Abstract

The aim of this study was to test the effectiveness of secang wood extract *(Caesalpinia* sappan L) on IL-6 and IL-10 expression in Balb / c mice with vulvovaginalis candidiasis. This type of research is an experimental laboratory, with a control group design using experimental animals as research objects. The research subjects were BALB / c mice weighing 35-40 grams, aged 10-12 weeks, the number of experimental animals needed in this study was 18 for 3 random groups, each group being 6. Analysis and data processing using the excel program SPSS Repeated ANOVA Test was used to measure differences in cytokine levels. IL-6 levels in group 1 (control) where the value of IL-6 expression after infection (H1) was 4309.40 pg /mL (IQR value 3803.8-4963.5) after treatment (H7) in the control group increased by 5578.12 pq / mL (IQR 5330.3 - 6162.9). In group 2 that were given fluconazole and secang wood extract, IL-6 levels tended to decrease by 3516.4 pq / mL (IQR: 3070.4-4061.6) Secang wood after infection decreased from 3417.34 pq / mL to 2782.97 pq / mL after treatment. In Group 2 that was given fluconazole and secang wood extract, IL-10 levels increased by 1414.8 pq / ml (IQR: 1061.7- 3158) after infection, to 3268.35 pq / ml (IQR value: 1789.9-3709.6) after treatment. given secang wood after infection increased from 2032.67 pq / ml to 3091.83 pq / mL after treatment. The p value is 0.008. Obtained the effect of secang wood extract in reducing levels of Interleukin 6 and can increase the value of IL-10 levels after administration of secang wood extract on candidiasis vulvovaginalis

Keywords: *Secang wood, IL-6, IL-10, vulvovaginalis candidiasis*

Introduction

According the Research shows that 75% of women have had one episode of vulvovaginal candidiasis (CVV) and another 40-45% have two or more episodes of vulvovaginal candidiasis (KVV) in their lifetime,[1,2.] Vulvovaginal candidiasis (KKV) is commonly known as vaginal discharge. by the community and is a quite disturbing problem. Chronic vulvovaginal candidiasis is also known to be a triggering factor for vulvovaginalis.[3,4] The World Health Organization (WHO) is a problem that should be regularly investigated because it reduces the quality of life for women and their partners[5,6].

Common symptoms are pain in the vaginal area, irritation, burning sensation, dyspareunia, and pain when urinating, which begins with acute pruritus and vaginal discharge (fluor albus). Clinical manifestations of candidiasis vulvovaginalis are the interactions between the pathogenicity of Candida species and the host defense mechanism which is related and influenced by several predisposing factors. Treatment

of conventional KKV vulvovaginal candidiasis (candidiasis vulvovaginalis) with systemic and topical drugs. Generally, Systemic therapy uses a single dose of 1x150mg flukoazole. While topical uses Ketocenazol by rubbing it on the lesion[7,8,9.]

Interleukin 6 (IL-6) is mostly considered a pro-inflammatory cytokine, but it also has regenerative and anti-inflammatory activity. While IL-10 is a cytokine that has the main function of limiting and terminating the immune response (anti-inflammatory). IL-10 cytokines, which are anti-inflammatory cytokines, during this candida infection condition it can increase phagocytosis and neutrophil recruitment so as to mediate inflammation [1,2,3] . Cytokines inhibit the activity of Th2 cells, NK cells and magrophages. When pathogens are able to withstand the destruction of normal immune mechanisms. If an IL-10 infection occurs, it will be produced to reduce inflammation which will minimize pathological conditions due to excessive inflammation[4,5,6].

The use of herbal ingredients as alternative medicines in healing diseases is increasing. This is because the therapeutic effects of herbal ingredients are constructive, the side effects that are caused are very small so that herbal ingredients are relatively safer from chemicals[3,8,9.] Currently, there are known treatments with Chinese herbal medicines which include Syngonanthusnitens, Euphorbia hirta L, Centellaasiatica, Cymbopogoncitratus (DC) Stapf (Gramineae), Areca Cathechu, L. Piper Betle L., Terminaliacatappa shows a deep[3,10,11.]

Secang wood has an anti-fungal effect against the fungus Candida Albicans allegedly due to the active substances in secang wood that are soluble in ethanol[5,9,12] The main active substances contained in secang wood include polyphenolic compounds, namely tannins and brazilins The extract of secang wood (Caesalpinia sappan) showed the presence of tannins and alkaloids. Among gram-positive and gram-negative bacteria, gram-positive strains of bacteria were more susceptible to extracts when compared to gram-negative bacteria. This may be due to the fact that these two groups differ in the structure of their cell wall components. The ability of tannin compounds to cause bacterial colonies to disintegrate, most likely due to their interference with the bacterial cell wall; thus inhibiting microbial growth .

Materal and Methods

The type of research used is pure experimental, namely laboratory experiments, pretest - posttest control group design using experimental animals as research objects. The research subjects were BALB / c mice weighing 35-40 grams, aged 10-12 weeks. healthy and fulfilling the inclusion criteria. The sample size was divided into 3 groups randomly in each group at least 5 (n = 5) and added by 1 animal for each group as a reserve so that the number of experimental animals needed in this study was 18 animals for 3 random groups, each group being 6.

a. Group (negative control) the group given distilled water and not given secang wood extract and intravaginal stimulation with candida albicans

b. Group 2 (positive control): the group that was given anti-candida drug (Fluconazol) at a dose of 19.5 mg / kgb was stimulated by intravaginal candida albicans

c. Group 3 (intervention): Given the extract of secang wood (Caesalpinia sappan L) at a dose of 510 mg / kg body weight and stimulated with candida albicans intravagina.

The Analysis and data processing using The excel program SPSS Repeated ANOVA Test was used to measure differences in cytokine levels in repeated measurements of each group. The results of the study were considered significant if the p value was <0.05. Research data will be presented in tables and graphs

Result

Table 1 : IL-6 Levels After Being Infected (H1) Given Candida Albicans And After 7 Days Of Treatment

Group	Post Infection (H1) Median (IQR) (Fc)	Post Treatment (H7) Median (IQR (Fc)	Mean	p
Group 1 (Negatif Control)	4309.40 (3803,8 – 4963.5)	5578.12 (5330.3 –6162.9)	1268.72	0.003
Group 2 (Positive Control) Flukonazole 19.5 mg/kgBB	3516.46 (3070.4 – 4061.6)	1890.91 (1464.6 – 2346.8)	-1942.73	
Group 3 (Intervensi) Secang wood	3417.34 (3040.6 – 3952.5)	2782.97 (2584.7 – 3030.7)	-317.18	

According table 1 The results showed that group 1 (control) post infection (H1) obtained IL-6 levels: 4309.40 pg / mL (IQR values 3803.8 - 4963.5) post treatment (H7) IL-6 levels in the control group increased by 5578.12 pg / mL (IQR values 5330.3 - 6162.9) with an average value (mean) of 1268.72 Furthermore, in the group given secang wood and fluconazole a dose of 19.5 mg / Kgbb post infection, the IL-6 level value of 3516.46 pg / mL (IQR 3070.4 – 4061.6) decreased to 1890.91 pg / mL (IQR 1464.6-2346.8) post treatment. Furthermore, group 3 obtained the results of IL-6 levels of 3417.3 pg / mL (IQR 3040.6 – 3952.5) decreased to 2782.9 pg / mL (IQR 2584.7 – 3030.7)

Table 2: IL-10 levels, after being infected (H1) given candida albicans and after 7 days of treatment

Group	Post Infection (H1) Median (IQR) (Fc)	Post Treatment (H7) Median (IQR (Fc)	Mean	p
Group 1 (Negatif Control)	4018.60 (3268.3 – 5828.0)	2871.17 (1988.5 – 3775.8)	-1368.09	0.008
Group 2 (Positif Control) Flukonazole 19.5 mg/ kgBB	1414.82 (1061.7 – 3158.0)	3268.35 (1789.9 – 3709.6)	529.58	
Group 3 (Intervensi) Secang wood	2032.67 (819.03 – 2694.6)	3091.83 (2429.8 – 3709.6)	1279.83	

According table 2 The result showed that group 1 (control) post infection (H1) obtained IL-10 levels of 4018.60 pq / ml (IQR values 3268.3 - 5828.0) post treatment (H7) IL-10 levels in the control group decreased by 2871.17 pq / ml (IQR values 1988.5 - 3775.8) with an average value (mean) of -1368.09. Furthermore, in group 2 who were given secang wood and fluconazole at a dose of 19.5 mg / KgBW after infection (H1) the IL-10 level of 1414.82 pq / ml (IQR 1061.7-3158.0) increased to 3268.35 pq / ml (IQR (1789.9 - 3709.6) after treatment (H7) .

Discussion

The high content of flavonoids in secang wood extract (Caesalpinia sappan L.) of 6.02% influenced the strong anti-fungal activity. Secang wood extract (Caesalpinia sappan L.) also contains 2.43% anthocyanins. Apart from being good antioxidants, anthocyanins can also act as antimicrobials. The factor which also influenced the increase in inhibition diameter was due to the presence of the concentration of antimicrobial substances which increased with each concentration. In addition, the ability of the antifungal activity of secang wood extract (Caesalpinia sappan L.) is due to the fact that secang wood positively contains other secondary metabolites compounds that also act as antifungal activity. According research the ethanol extract of secang wood (Caesalpinia sappan L.) is positive for flavonoids, saponins, alkaloids, tannins, phenolics, triterpenoids, steroids and glycosides. These metabolite compounds are able to act as good antifungals[5,7,13,14].

The mechanism of action of flavonoids as antifungal compounds is divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism In inhibiting the synthesis of nucleic acids, the A and B rings of flavonoid compounds play an important role in the intercellation process or hydrogen bonding, namely by accumulating nucleic acid bases so that they inhibit the formation of DNA and RNA[3,7,915]. The results of flavonoid interactions will also cause damage to cell wall permeability. In inhibiting the function of the cell membrane flavonoids will form complex compounds from extracellular and dissolved proteins so that the cell membrane will be damaged and intracellular compounds will come out. Meanwhile, in inhibiting energy metabolism by inhibiting the use of oxygen by bacteria, namely by preventing the formation of energy in the cytoplasmic membrane and inhibiting the motility of bacteria that play a role in antimicrobial activity and extracellular proteins[2,16..]

Inflammation is a response to injury, where there is accumulation of leukocytes, inflammatory mediators such as cytokines. Inflamation occurs in acute and subacute / chronic stages. In the inflammatory stage, proinflammatory cytokines including IL-6 are released. In the early stages, the infection takes the form of Candida. Planktonic albicans occurs by introduction of yeast immunity to mice. Introduction of the immune system through PAMPs molecules derived from, which bind C. albicans to the receptors (PRRs) on the surface of polymorphonuclear (PMN) cells in the intestinal mucosa[4,8,10.]

The cell surface contains β-1,3- Candida glucan sugar groups which act as PAMPs (17). Levels of Cytokine nterleukin-6 (IL-6) Serum I The process of introducing PAMPs and PRRS, namely in the early phase of infection, occurred on days 7 and 14 of candida abicans after

inoculation which was marked by no C. albicans discovery of secretion of the cytokine IL-6 (0pg / ml). The next step occurs when the bond between the sugar group and the receptor on PMN cells initiates the release of cytokines including IL-6[.5,8,17]

This study is similar to the study conducted by Masfufatun's, putu which states that inflammation is a response to injury, where there is an accumulation of leukocytes, inflammatory mediators such as cytokines. Inflammation occurs in acute and subacute / chronic stages. In the inflammatory stage, proinflammatory cytokines including IL-6 are released. In the early stages, the infection takes the form of Candida. Planktonic albicans occurs by introduction of yeast immunity to mice. Levels of Cytokine nterleukin-6 (IL-6) Serum I The process of introducing PAMPs and PRRS, namely in the early phase of infection, occurred on the 7th and 14th day of candida abicans after inoculation which was marked by no C. albicans discovery of secretion of the cytokine IL-6 (0pg / ml). The next step occurs when the bond between the sugar group and the receptor on PMN cells initiates the release of cytokines including IL-6.

In this study, the value of IL-6 levels increased in group 2 which was given fluconazole and secang wood extract. and group 3 who were given only secang wood. According to Couper KN states that serum levels of anti-inflammatory cytokines (IL-10) In infectious conditions, very candida pro-inflammatory cytokines play a role in increasing phagocytosis, neutrophil recruitment, thus mediating inflammation.[18,21,22,23] The IL-10 cytokine is an anti-inflammatory cytokine. During infection, these cytokines will inhibit the activity of Th2 cells, NK cells and marophages. When the pathogen is still able to withstand destruction through normal

immune mechanisms, IL-10 will be produced to reduce inflammation which in turn minimizes pathological conditions due to excessive inflammation.[11,24,25]

Conclution

obtained the effect of secang wood extract can reduce levels of interleukin IL-6. The addition of secang wood extract with fluconazole provided a better reduction effect which could be observed in this study. It was also found that there was an effect of secang wood extract on increasing IL-10 levels.

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Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University.

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Expectant Management in Tubal Ectopic Pregnancy in Thi-Qar Province: Interventional Comparative Trial

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Abstract

Background: Ectopic pregnancy one of the life threatening condition, that can't be prevented but can managed safely. **Rationale:** rolling out of surgical intervention to medical management is hope of all, even though surgeon. **Aim of the study:** to compare between the expectant and surgical management of tubal ectopic pregnancy. **Patients and Methods:** Single blinded, controlled and interventional study conducted in Bint Al-Huda teaching hospital in Thi-Qar-Iraq, extended all over the 2019 since January till last week of December . Totally (74) patients enrolled in the study, all of them were complained from tubal ectopic pregnancy, questionnaire, B-HCG, Trans- vaginal US were the main tools of follow up, **Results:** The target patients for our study was 116, 34 of them were excluded, 16 was with positive fetal heart, 20 were had hemoperitonium extend beyond the pelvis at the time of presentation and 8 patients were patients refuse fallow up (expected management). Most of them(40%) were older than 34 years, followed by (32.5%) with in age of 32.5%, while the youngest age group occupying minimal proportion (27%), According to B-HCG concentration most of them (58%) were with <1500 mlU/ml concentration. **Conclusion:** Highly proportion of tubal pregnancy managed by follow up without surgical intervention. β-HCG titer and trans- vaginal US are the golden keys of management

Key words- *B-HCG, TVUS, tubal ectopic pregnancy, Thi-Qar*

Introduction

Ectopic pregnancy is defined as implantation of the fertilized ova outside the female uterine cavity, finally result in death of embryo and affects mother life. It's an Emergency condition that threaten the mother life[1].

More than95.5 % of implantation in fallopian tube[2,3], In rare cases the concepts implant at other site including cervical canal, abdominal cavity, ovaries and previous operation site of caesarean section[4].

The prevalence of ectopic vary among different region because its affect by the diagnostic tools and its difficult to estimated. world wide prevalence about 1-2% of all reported pregnancy book[5]. The prevalence was higher among developing region[6].

Annually, UK diagnosed 12000 women with ectopic pregnancy and 1.5% admission to the hospital due to EP. The maternal mortality rate attributed to ectopic pregnancy in developed country was 0.02% which is lower rate as compared to developing country (1-3%)[5].

Abnormal lesions of the fallopian tube are attributed to the EP such as; previous ectopic or pelvic surgery, pelvic infection specially by *Chlamydia trachomatis*, smoking, contraception using intrauterine contraceptive device and multiple sex partner[7].

The high clinical suspension is recommended to diagnosis an ectopic pregnancy in women at child bearing age. Women with an EP presented with different sign and symptom; shock, low blood pressure, increasing heart rate, abdominal pain, frequent bowel motion, vomiting with or without vaginal bleeding.so these women should have β-HCG test which highly sensitive and specific available test[8].

The diagnosis of EP by vaginal & abdominal and examination with limited value[9].

Trans-vaginal ultrasonography by well hand good experience doctor and well equipped machine is the method of choice for detection of an EP with 90% sensitivity based on detection of an ectopic mass rather than non-detection of intrauterine pregnancy[10].

There is no way to prevent the EP but it can prevent the serious sequels of EP by early diagnosis of women with risk factors [3].

EP can be treated either medically, surgically and by expectant [11,12]:

1-Surgical treatment: the common surgical treatment by laparotomy specially for hemodynamically stable patient with no mortality [13]. While laparoscopic procedure is indicated in hemodynamically stable patient with less minimal bleeding at operation, less pain and early discharge from hospitals [14,15].

2-Medical (local or systemic route): its indicated for haemodynamically stable patient with un-rupture ectopic by using methotrexate [16].

3-Expectant management: Some ectopic regress or aborted through the tube without effect on the patient health. This conservative treatment including observation of the conceptus weather spontaneously resolve without medical or surgical intervention [17].

Aim of the study: to compare between the expectant and surgical management of ectopic pregnancy.

Patients & Methods

Single blinded, controlled and interventional study conducted in Bint Al-Huda teaching hospital in Thi-Qar-Iraq, extended all over the 2019 since January till last week of December . Totally (74) patients enrolled in the study, all of them were complained from tubal ectopic pregnancy, diagnosed to have this obstetric problem as an early pregnancy (positive serum B-HCG) with amenorrhea at the studied month only.

Sources of the cases were:

1-Outpatients clinic.

2-Referal from private clinic

3-Directly consulting the emergency department.

Inclusion criteria

All delayed menstrual cycle, whatever their reproductive age and positive serum B-HCG titer diagnosed as ectopic pregnancy and hemodynamiclly stable

Target population of our study

1-pregnancy after IVF

2- moderate to severe risk EP woman [History of EP, Ovulatory ion inductive females (controlled ovarian hyper-stimulation) , P ID, Infertile, Assisted Reproductive Technology and Cigarette smoking**]**

Exclusion criteria

1-Extra tubal ectopic pregnancy, positive fetal heart (16) patients.

2- Hemoperitonium extend beyond the pelvis at the time of presentation, they were 20 patients.

3-Refuse fallow up (expected management)they were 8 patients

Tools of the study

1-Well-constructed studied (by Obstetrician and community physician) questionnaire including bio-demography including race, personal character (residence, education and occupation), medical history (past and current) and full obstetric and gynecological history. Obstetric history regarding the LMP, were subdivided into less than 7 weeks or more than 7 weeks.

2- Serum beta B-HCG titer: Diagnosis of EP by detection of Fetal beat any were or embryonic sac outside the uterus, or if the US was negative (can't detect the fetal heart), B-HCG titer is measured if was >1500 mlU/ml the EP is highly suspected

If the US negative & B-HCG elevated but the level don't reach 1500-2000mlunite/ml the suspicion of EP is stay in addition to an early intrauterine pregnancy, in this situation the EP proved by follow up elevation of B-HCG It is also useful in monitoring of the pregnancy progress.

Raised median slop in concentration about 124% within 2days follow up highly indicative of EP (18). A two measured levels in 48 hours apart , may aiding EP diagnosis. While 66% of B-B-HCG rising level is highly suggestive of a pregnancy (viable intrauterine) , A 50% indicate the ruled out of an EP possibility & a viable intrauterine pregnancy where the (ratio of likelihood is zero) (19,20).

3-Transvaginal Ultra Sound:

clearer visualization of all pelvic organ including uterus is allowed after US transducer insertion. It can generally detect intrauterine pregnancies that are 5 to 6 weeks along.

US is most sensitive tool in detection and diagnosing the pregnancy and its site even-though it diagnostic criteria might be later than Beta B-HCG level it has a false negative value at early pregnancy (negative US doesn't exclude the EP)

4-Color Doppler TVUS: for vascularity detection, which sub classified into: grade 0(avascular), grade 1 (<1/3of mas vascular), grade 2(<2/3 vascular) and grade 3(>2/3 vascular)

Works in field: All patients' related data were collected by filling individual forms and later described in terms of mean and standard deviation or percentages. To have a baseline and follow-up objective severity assessment, we define each by B-B-HCG , where Initial B-B-HCG had registered for each patient, strict 48 hours fallow up by 15% reduction, or increment was the main cut off point to make the decision, even though some of them had steady level of B-B-HCG .

A trans V.US, which done daily at the first week, then weekly for the next three weeks. The cut of value for the solid extra overian adnexal mass (<35mm or > 35mm). We firstly provide each patient with informed consent to sign then allocate her into one of two groups either on surgical intervention or follow-up without any medical treatment. Our targeted duration of study participant for each patient was at maximum 28 days. The surgical intervention was done in Bint Al-Huda teaching hospital for those who develop Sever pain, ≥35 mm adnexal mass, visible Fetal H. beat by US Serum and ≥5000 IU/liter B- B-HCG . Lastly if doubtful medical treatment Compliance , while second group follow a strict follow up. Each pregnant informed to phone us at any time when develop any of alarming signs. Surgery either by laparoscopic approach (inserted instruments to the abdomen through a few small incisions) or through an abdominal incision , laparoscopy experience less pain and faster recovery.

Statistical Analysis

Frequency and percentages with estimation of Chi square and ANOVA test to detect P value at the level of 0.05 by SPSS version 24

Results

The target patients for our study was 116, 34 of them were excluded, 16 was with positive fetal heart, 20 were had hemoperitonium extend beyond the pelvis at the time of presentation and 8 patients were patients refuse fallow up (expected management). Most of them(40%) were older than 34 years, followed by (32.5%) with in age of 32.5%, while the youngest age group occupying minimal proportion (27%), According to B-HCG concentration most of them (58%) were with <1500 mlU/ml concentration. As shown in figure 2.

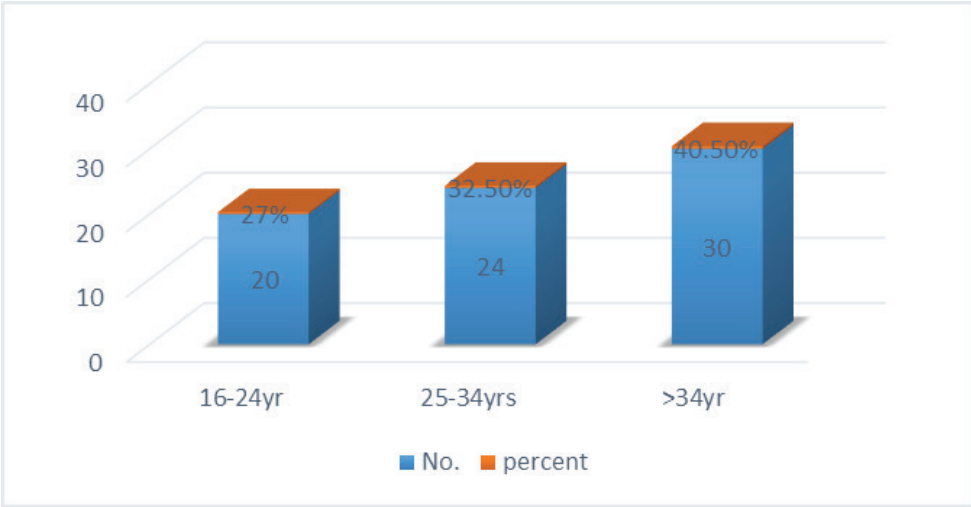

Figure 1: Age distribution of studied population

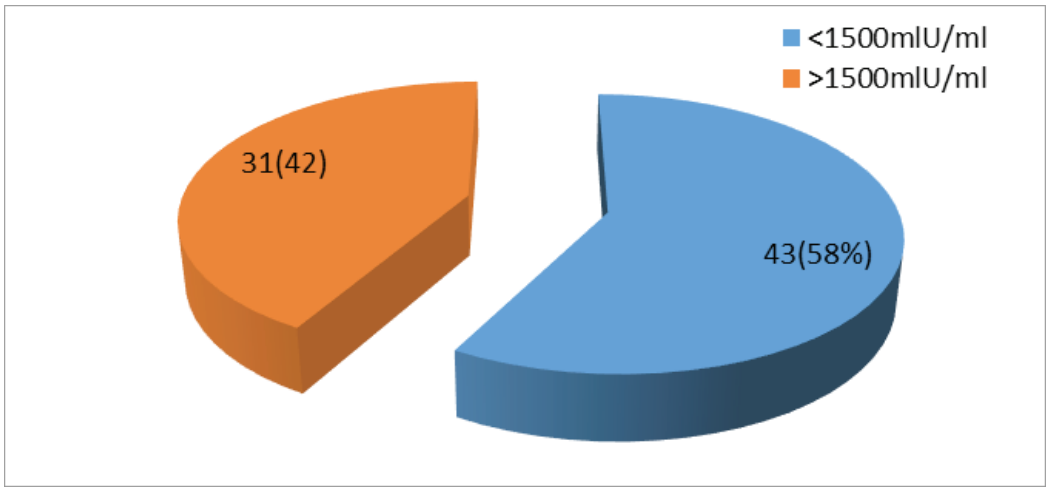

Figure 2: β-HCG distribution of studied population

More than half of tubal E. Pregnancy were decrease the B-HCG during follow up and only 13.5% had rising the B-HCG level.

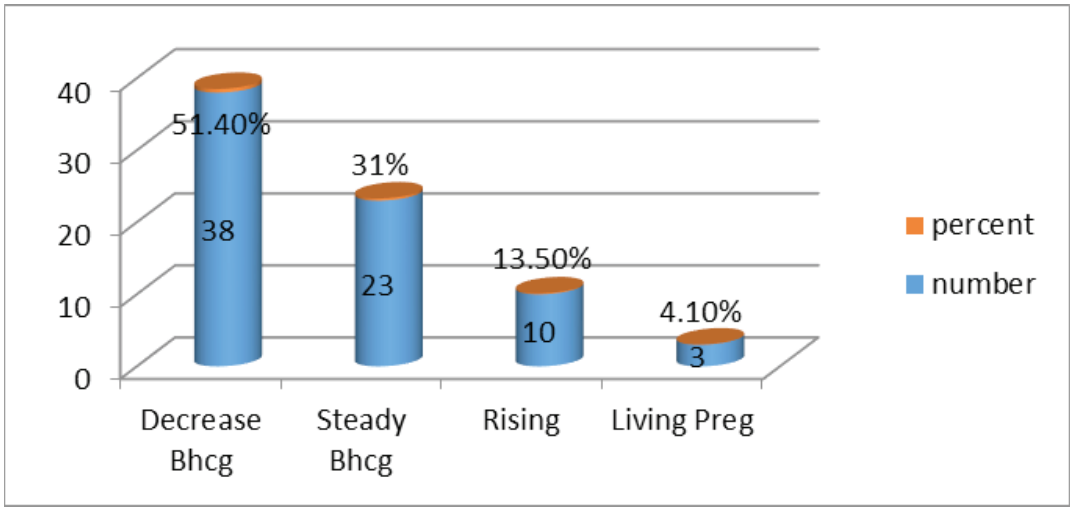

Figure 3: β-HCG follows up

Table 1: Statistical differences between studied group according to Socio-demography and obstetric parameters.

	Follow-up group	Surgery group	Total	P value
Mean age	26±10.4	34±8.8	31±10.7	0.431
Address				
Rural	44(64.7%)	4 (66.7%)	47(63.5%)	0.082
Urban	24(35.3%)	2(33.3%)	26(35.1%)	
Education				
<12 yrs	31(45.5%)	2(33.3%)	33(44.5%)	0.063

Cont... **Table 1: Statistical differences between studied group according to Socio-demography and obstetric parameters.**

>12yrs	37(54.5%)	4(66.7%)	41(55.5%)	
Occupation				
Employed	21(30.8%)	5(83.3%)	26(35.1%)	0.043
None	47(69.2%)	1(16.7%)	47(63.5%)	
Parity				
Primi	23(33.8%)	0(0%)	23(31.1%)	0.001
grand	11(16.2%)	0(0%)	11(14.8%)	
Multi	34(50%)	6(100%)	40(54.1%)	
History of IVF				
Yes	2(3%)	0(0%)	2(2.7%)	0.001
No	66(97%)	6(100%)	72(97.2%)	
Have risk factor				
Yes	35(51.4%)	5(83.3%)	38(51.3%)	
No	33(48.6%)	1(16.7%)	34(48.7%)	
B-HCG				
<1500	43(63.2%)	0(0%)	43(58.1%)	0.03
1500-2000	22(32.3%)	3(50%)	25(33.7%)	
>2000	3(4.4%)	3(50%)	6(8.1%)	
Total	68	6(100%)	74(100%	

Significant statistical difference had been noticed in relating the studied group according employment history, parity, history of IVF, had risk factors and B-HCG levels, where p value <0.05.

Table 2: Statistical differences between duration of LMP groups according to demography and obstetric parameters

	< 7week from LMP	**>7week from LMP**	**Total**	**P**
Mean age	29±11.4	34±9.8	31±10.7	0.431
Residence				
Rural	15(31.2%)	33(68.8%)	48	0.001

Cont... **Table 2: Statistical differences between duration of LMP groups according to demography and obstetric parameters**

Urban	24(92.3%)	2(7.7%)	26	
State of β-HCG				
↓↓ titer	14(36.8%)	24(63.2%)	38	0.04
Steady or ↑↑ titer	25(69.4%)	11(30.6%)	36	
Mass size				
16-34mm3	38(86.4%)	6(13.6%)	44	0.001
35-44mm3	9(30%)	21(70%)	30	

Significant statistical difference had been noticed in studying of the duration of LMP groups according residence, state of β-HCG levels, and mass size, where p value <0.05.

Table 3: Relationship between pattern of Beta B-HCG level and demography and obstetric parameters

	↓↓ β-HCG titer	Steady or ↑↑ β-HCG titer	Total	P value
Mean age	21±7.6	32±11	31±10.7	0.045
Risk factors				
Positive	24(63.2%)	16(44.4%)	40(54.1%)	0.076
Negative	14(37.8%)	20(55.6%)	34(45.9%)	
Adnexal mass size				
<35	28(73.7%)	11(30.5%)	39((52.7%)	0.042
>35	10(26.3%)	25(69.5%)	35(47.3%)	
Color Doppler TVUS				
Vascular	28(73.7%)	12(333%)	40(54.1%)	0.039
Avascular	10(26.3%)	24(66.6%)	34(45.9%)	
Initial level				
<1500	24(72.7%)	19(46.3%)	43(58.1%)	0.082
>1500	9(27.3%)	22(53.7%)	31(41.9%)	

Significant statistical differences had been noticed in relation of the B-HCG titer difference according to the mean age, adnexal mass size, color Doppler TVUS, where P values (0.045, 0.042 and 0.039) respectively.

Discussion

Patient characteristic were similar in both group regarding age ,address and education status and it was not significantly associated with the type of management . Sharon et.al [21] reported that age of the patient not affect the type of treatment ,while Nizam et .al [22] reported that maternal age and parity are risk factors for rupture of ectopic pregnancy, he concluded that, identification of these risk factors will affect the choice of type of treatment either medical or surgical treatment (increase mother age and parity will decrease the chance of EP).

It found that , occupation ,I.V.F and initial β-HCG level will affect the choosing the type of management. Its consistent with Sharon study [21] regarding the β-HCG level .several other studies are reported that ,the expectant management was indicated for asymptomatic women with <2000 IU/l and decreasing β-HCG level [23-26].

Regarding the time of presentation ,this study concluded that:

1-patient of urban area present earlier(<7 week) than patient from rural area this could be explained by the patient of urban area were consult earlier.

2-the decreasing β-HCG titer in the fallow up period was more common among patient present later, however steady or increasing titer was more common among patient present earlier. This mean that patient presented before 7 week of pregnancy most likely need surgical management and this need further study to improve and compare these result.

3-the patient with small mass size(16-34mm3) presented early while the patient with large size mass (35-44mm3) present later. This mean the presentation of small mass size was before 7weeks is go with expectant management while large mass that presented after 7 weeks is go with surgical treatment this need further study to improve and compare these result.

Regarding the β-HCG level in the fallow up period :

1-It decrease with younger age group and remain steady or increase with increasing age. this mean younger age women are more favorable expectant management this agreed with Nizam et al. [22].

2-It decrease with small mass size and remain steady or increase with increase mass size. This explain why small mass size is an indication of surgery and it is consistent with Fernadez study [27].

3- It decrease with vascular feature and remain steady or increase with avascular feature.

These result agreed with the predictive score for the management of ectopic pregnancy by Fernadez [27], k.Ramakrishan who put some recommendation point for the clinical practice as following:

-Expectant management is needed for women with small size adnexal mass ≤ 3cm,low β-HCG level (<1000mlIU/ml)and falling level in fallow up period for the women that agree with this type of treatment.

Surgical treatment is needed for women with adnexal mass >3.5-4 cm on US and β-HCG level (>5000mlIU/ml) [28].

4-there was no significant association between the initial Beta B-HCG level and its level in the fallow up period.

Our study finding differ from other study in clearance time where considerable variation of clearance time showed in D. Mavrelos study that ranged from few days-7 weeks. Where > 90 % of ectopic pregnancies were resolved at 7th week -follow up [5]

Conclusion

Highly proportion of tubal pregnancy can be managed by follow up without surgical intervention.

Recommendations: β-HCG titer and trans- vaginal US are the golden keys of management

Ethical Clearance: The ethical approval was acquired from the Ethics Committee of Department of Obstetrics and Gynecology, College of Medicine, University of Thi-Qar, Iraq.

Source of Funding: there is no founding.

Conflict of Interest: there is no conflict of interest.

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COVID 19 Patients /Southern of Iraq: Character and Dermatologic Manifestations

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Abstract

Background: the spread of coronavirus diseases (COVID-19) in China and then around the world where the current study explores the link between skin manifestations and COVID-19. **Methods:** A cross sectional study including all diagnosed cases of COVID-19 in Thi-Qar and Al-Muthana governorates, where started by the diagnosis of the 1st case in Thi-Qar province, and ended with the 21th of April 2020, the cases were distributed as 62 cases from Thi-Qar and 54 cases from Al-Muthana, information had been gathered from COVID -19 isolation unites in the two main hospitals of isolation, Al-Husain teaching Hospital and Al-Muthana teaching hospital. **Results:** The result showed that there are 116 cases of SARS-COV2 infection was recorded in Thi-Qar and Al-Muthanna, the high percentage of infection was detects in the middle adult 80(69%) in the age group over 60 years old**.** The distribution of infection according to the gender revealed significant differences where the males comprise (87.75%) of cases**.** The direct contact route of transmission was predominant among patients 101 (87.1%)**.** Also, the highest percentage was live 110(94.8%)**.** According to the results there was a negative relationship between the two variables was detected, there is no correlation. So, there is no correlation in skin manifestations according to the system involvement among COVID-19 infected cases with the pearson correlation (0.327)**.** The results show non-statistical significance in skin manifestation according to biography among only who had dermatological lesions at p value (0.248)**.** Also, there are non-statistical significant in source of infection according to skin manifestation and no correlation between skin manifestations and death with corona virus. **Conclusions:** It can be concluded from the current study that there is no close correlation between skin manifestations and COVID-19 infection, a very weak direct relationship because some patients may not show any symptoms and these are considered negative signs, meanwhile the unique skin lesion are zinc like deficiency(acrodematitis enteropathica)not reported in any other areas.

Key word: *COVID 19, southern of Iraq, acrodematitis enteropathica*

Introduction

There are two types of coronavirus before the emergence of SARS: OC43 and 229E, both of which cause acute respiratory diseases. Also, there were many expectations that coronaviruses that infect humans have a close association with hepatitis, intestinal diseases, multiple sclerosis, skin inflammation or other diseases but so far These links have not been established [1]. In 2003, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread [2]. Also, in 2019 Wuhan, a group of pneumonia infections has spread, where an unknown origin has been announced, as these cases have been confirmed linked to coronaviruses by Chinese health authorities. The cause of respiratory syndrome cases that have spread in China is a virus that the disease is called coronavirus, as this disease is closely related to bats [3]. There is a group of coronaviruses that exist in animals that can convert into human coronaviruses that cause acute respiratory disease and that it spreads from

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one person to another and that causes the disease known as COVID-19.Symptoms of coronavirus may include fever, cough, difficulty breathing, chills,, muscle pain, headache, repeated shaking with chills sore throat, or new loss of taste [4]. Perhaps, sick people are asymptomatic and able to transmit the disease to others where the most susceptible group to the virus raged ≥80 years of age [5]. As of 10 February 2020, WHO has reported a total of 40,554 confirmed cases of laboratory-confirmed COVID-19 infection in the world [6]. The disease can be diagnosed using a real-time polymerase chain reaction (RT-PCR)[7]. As there are many studies and experiments carried out for the purpose of discovering a treatment for Coronavirus, Chinese experts recommend that patients with COVID-19 be given 500 mg of chloroquine twice a day for ten days twice a day [8]. Whereas, antimicrobials did not work in treating patients with human coronavirus [9]. Where there is a certain category of people suffering from acne or rosacea due to wearing masks during the epidemic period to prevent infection with corona virus also repeated use and many disinfectants and sterilizers as it led to the occurrence of contact dermatitis both in Corona virus patients and healthy people, where a study indicated that people with psoriasis are more susceptible to heart disease, depression, and others from health cases that are considered to be the most vulnerable group they are most susceptible to infection with corona virus [10]. There is a study that indicated that 88 patients are infected with Corona virus, where they suffer from skin symptoms, itching and redness. The study indicated that there is no correlation with the severity of the disease [11]. COVID-19 virus may weaken a person's immunity in the early stage of the disease as symptoms appear within 14 days as there is no strategy to treat the disease. All scientists are in a race to find a cure to kill the virus [12]. This study aimed to determine the relationships between the skin manifestations and COVID 19 infection.

Material and Methods

A cross sectional study including all diagnosed cases of COVID-19 in Thi-Qar and Al-Muthana governorates, where started by the diagnosis of the 1st case in Thi-Qar province, and ended with the 21th of April 2020, the cases were distributed as 62 for Thi-Qar and 54 for Al-Muthana, information had been gathered from COVID -19 isolation unites in the two main hospitals of isolation, Al-Husain teaching Hospital and Al-Muthana teaching hospital, where the admission of suspected COVID 19 cases whatever their severity, and quarantine of the contact, we dealt with the only positive cases that proved as COVID -19 positive nasopharyngeal swab, ethical consideration had been attained from the whole responsible for this critical situations, verbal consents from all patients had been gained, full identity of patients were registered and coded and involvement of other system as diagnosed by first line clinician doctors who dealt with the COVID19 in the hospitals and important dermatological symptoms and signs that mentioned by patient and the final dermatological lesion as diagnosed y dermatologist , SPSS version 25 used for statistical analysis to find the association, relationship and differences of our outcome with specific determinants of interest, where p value <0.05 consider as significance, excel sheet also had been used for graphing.

Result

A cross sectional analytical study recruit (116) COVID-19 patients with mean of (39.9828 ±11.90395 years) ,

The result showed that there are total number of samples in Thi-Qar and Al-Muthanna is 116 cases with corona virus, the results showed that there was a high percentage in the middle adult 80(69%) in the age group over 60 years old while the lowest percentage was in children 3 (2.6%) as shown in the Figure (1).

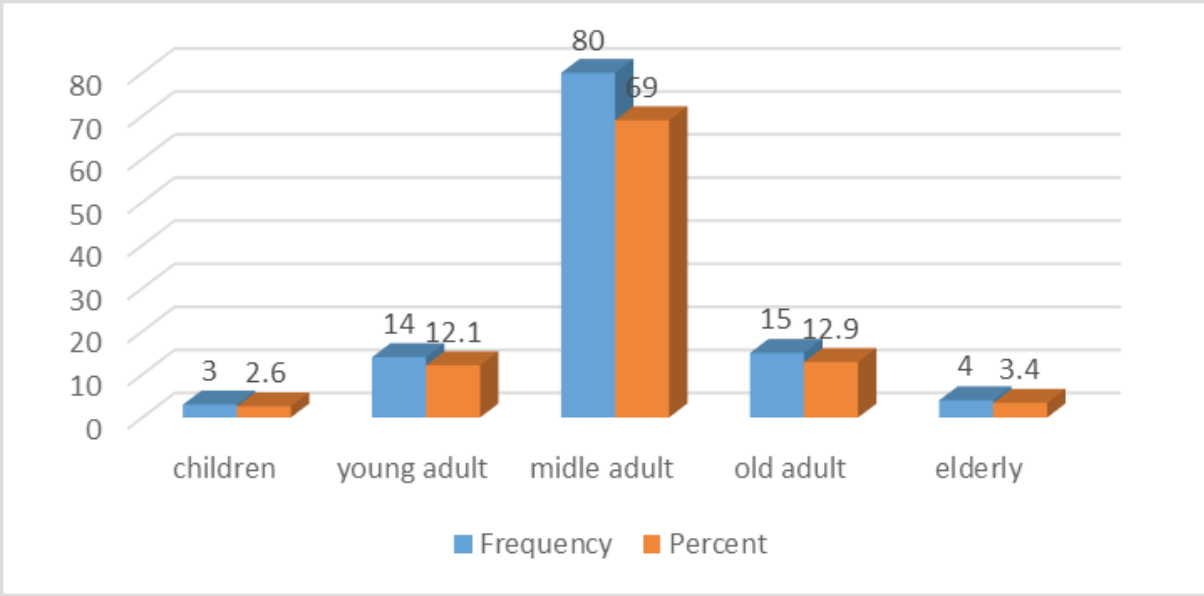

Figure 1: Age distribution of COVID-19 patients

The results show the gender distribution of COVID-19 among the studied population with highest percentage of infections in males (87 .75%), while the lowest percentage is among females, (29.25%).

The results of the current study in Figure (2) revealed the ratios of the infections with COVID 19 virus according to the source of infection. The highest percentage was through direct contact 101 (87.1%). while the lowest percentage of infection source by travel 15 (12.9%).

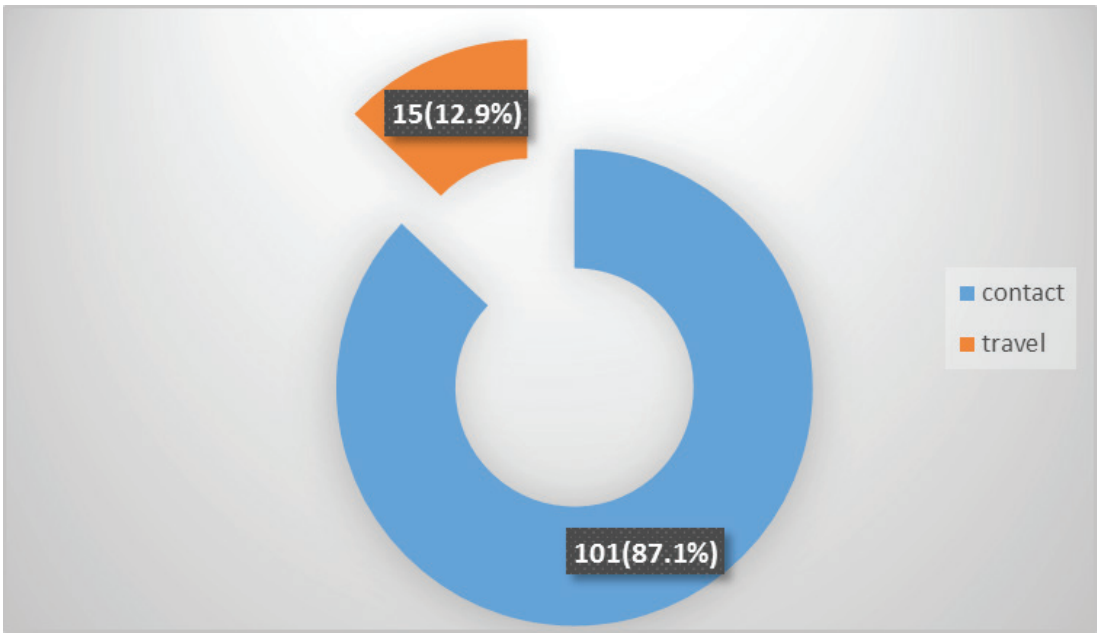

Figure 2: Distribution according to source of infection

The current study showed in the distribution of coronavirus infection according to systematic manifestation other than dermatology in COVID-19 where the high percentage of patients showed no any system involvement 70(60.3%), while the lower percentage shown in cardiac patient 1(0.9%).

The above figure shows results according to skin manifestation of COVID-19 where the highest percentage in no skin manifestation 75(64.77%), while in urticaria 18(15.5%), pruritis 13(11.2%), acrodematitis enteropathica 7(6%) and chickenpox like 3(2.6%) .

The results show distribution according to final outcome where the highest percentage was live110(94.8%), while the lowest percentage was death 6(5.2%).

The table below shows the skin manifestations according to biography among all COVID-19 patients where the highest percentage urticarial detects in middle adult 53 (66.3%), while the lower percentage in child 1(33.3%). Also, the high percentage in male 63 (69.9%). The result show in pruritus highest percentage in middle adult 10(12.5%), while in chicken pox like highest percentage also in middle adult and in acrodermatitis enteropathica highest percentage also in middle adult. The following results also showed that there was a negative relationship between the two variables, that is, there is no correlation at spearman correlation (-0.117) and the results show non-statistical significance in p. value ($P \leq 0.05$) as shown in table (1).

Table 1: Skin manifestation according to biography among all COVID-19

Age group		Skin manifestation					Total	F.E
		No	Urticarial	Pruritus	chicken pox like	Acrodermatitis enteropathica		P value
	child	No.	1	2	0	0	0	3	17.808 0.217
		%	33.3%	66.7%	0.0%	0.0%	0.0%	100.0%	
	Young adult	No.	7	4	1	1	1	14	
		%	50.0%	28.6%	7.1%	7.1%	7.1%	100.0%	
	Middle adult	No.	53	10	11	1	5	80	
		%	66.3%	12.5%	13.8%	1.3%	6.3%	100.0%	Spearman correlation= -0.117
	Old adult	No.	12	1	1	1	0	15	
		%	80.0%	6.7%	6.7%	6.7%	0.0%	100.0%	
	elderly	No.	2	1	0	0	1	4	
		%	50.0%	25.0%	0.0%	0.0%	25.0%	100.0%	
Female		No.	22	1	3	1	2	29	5.264 0.224
		%	75.9%	3.4%	10.3%	3.4%	6.9%	100.0%	
Male		No.	53	17	10	2	5	87	
		%	60.9%	19.5%	11.5%	2.3%	5.7%	100.0%	
Total		No.	75	18	13	3	7	116	
		%	64.7%	15.5%	11.2%	2.6%	6.0%	100.0%	

The table below shows the skin manifestation according to system involvement among all COVID-19 where the highest percentage Flue like in Urticarial was 10(27.0%), while the lower percentage in renal and cardiac1. The result show in pruritus highest percentage in no any other system invol. 7(10.0 %), while in chicken pox like highest percentage also in Flue like 2(5.4%) and in acrodermatitis enteropathica highest percentage also in Flue like 3(8.1%), also showed pearson correlation(0.327). The following results also showed that there was a complete negative linear relationship between the two variables,there is no correlation in skin manifestation according to system involvement among COVID-19 showed pearson correlation(0.327) and at p. value ($P \leq 0.05$) as shown in table (2).

Table 2: Skin manifestation according to system involvement among COVID-19

Other system	skin					Total	F.E
	No	Urticarial	Pruritus	chicken pox like	Acrodermatitis enteropathica		P value
No any other system invol.	54	7	7	1	1	70	32.291, 0.001 Pearson Correlation= 0.327
	77.1%	10.0%	10.0%	1.4%	1.4%	100.0%	
Flue like	17	10	5	2	3	37	
	45.9%	27.0%	13.5%	5.4%	8.1%	100.0%	
Respiratory	3	1	1	0	2	7	
	42.9%	14.3%	14.3%	0.0%	28.6%	100.0%	
Renal	0	0	0	0	1	1	
	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%	
Cardiac	1	0	0	0	0	1	
	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%	
Total	75	18	13	3	7	116	
	64.7%	15.5%	11.2%	2.6%	6.0%	100.0%	

The following figure shows results according to contact count of COVID-19 where the highest percentage 67, while in urticaria14, pruritis 12, acrodematitis enteropathica 5and chickenpox like 3as shown in Fig (3).

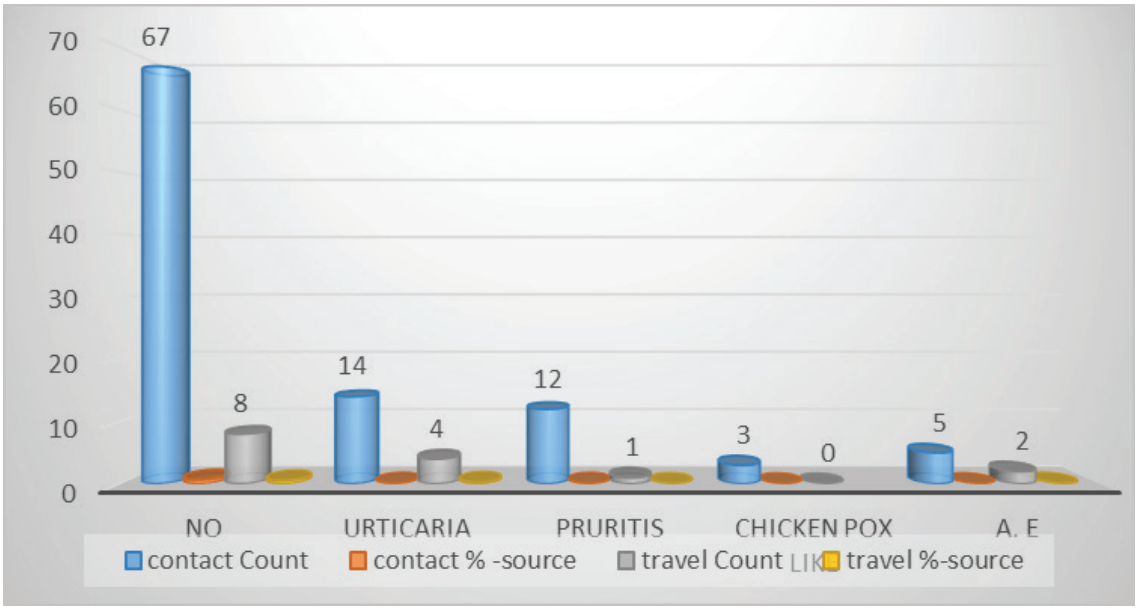

Figure 3: shows results according to contact count of COVID-19

The outcome of the study also shows the skin manifestation according to biography among only who had dermatological lesions where the highest percentage in middle adult Urticarial was 10(37.0%) ,also in high percentage in male 17(50.0%). The result show in pruritus highest percentage in middle adult 11 (40.7 %), while in chicken pox like highest percentage also in young adult 1(14.3%) and in acrodermatitis enteropathica highest percentage also in middle adult 5 (18.5%). The results show non-statistical significance in skin manifestation according to biography among only who had dermatological lesions at p. value ($P \leq 0.05$). The following results also showed a weak association between the two variables spearman correlation (0.194).

The table below shows source of infection according to skin manifestation where the highest percentage by travel urticarial was 4(57.1 %).The result show in pruritus highest percentage by contact 12 (35.5 %), while in chicken pox like highest percentage also by contact 3(8.8%) and in acrodermatitis enteropathica highest percentage also by contact 5 (14.7 %). The results show non-statistical significant in source of infection according to skin manifestation at p. value ($P \leq 0.05$) as shown in table (3).

Table3: Source of infection according to skin manifestation

Crosstab						
source	skin				Total	F.E
	Urticarial	Pruritus	chicken pox like	Acrodermatitis enteropathica		P Value
contact	14	12	3	5	34	2.150
	41.2%	35.3%	8.8%	14.7%	100.0%	.622b
travel	4	1	0	2	7	
	57.1%	14.3%	0.0%	28.6%	100.0%	
Total	18	13	3	7	41	
	43.9%	31.7%	7.3%	17.1%	100.0%	

This can be observed that the skin manifestation according to other system involvement, where the highest percentage in flue like urticarial was 10(50.0%). The result show in pruritus highest percentage in no system involvement 7 (43.8 %), while in chicken pox like highest percentage also in flue like 2(10.0%) and in acrodermatitis enteropathica highest percentage 3(15.0%) flue like. The results show non-statistical significant at p. value ($P \leq 0.05$). The following results also showed that there was a complete negative linear relationship between the two variables, there is no correlation in skin manifestation according to other system involvement showed pearson's (0.331).

The table below shows the skin manifestation according to death, where the highest percentage in urticarial was17(44.7%). The result show in pruritus highest percentage in 12(31.6%), while in chicken pox like highest percentage 2(7.9%) and in acrodermatitis enteropathica highest percentage 6(15.8 %) , the results show non-statistical significant in Skin manifestation according to death .The results show non-statistical significant in skin manifestation according to death at p. value ($P \leq 0.05$) as shown in table (4).

Table 4: Skin manifestation according to death

Death		skin				Total	F.E
		Urticaria	Pruritis	chicken pox like	Acrodermatitis enteropathica		P
No		17	12	3	6	38	1.519
		44.7%	31.6%	7.9%	15.8%	100.0%	0.808
Yes		1	1	0	1	3	
		33.3%	33.3%	0.0%	33.3%	100.0%	
Total		18	13	3	7	41	
		43.9%	31.7%	7.3%	17.1%	100.0%	

Another striking point is that the skin manifestation according to death among all COVID-19 patients, where the highest percentage in no skin manifestation was 72(65.5%). also the highest percentage in urticarial no death was17(15.5. %). The result show in pruritus highest percentage in 12(10.9 %), while in chicken pox like highest percentage 3(2.7 %) and in acrodermatitis enteropathica highest percentage 6(5.5%), the results show non-statistical significant in Skin manifestation according to death among all COVID-19 patients. The results show non-statistical significant in skin manifestation according to death among all COVID-19 patients at p. value ($P \leq 0.05$).

Discussion

After the spread of Corona virus around the world and caused anxiety in countries and cities, this led to the many studies and coronavirus correlation with others diseases cases [13]. Since the first case of coronavirus was confirmed in Iraq on February 25, the Iraqi government immediately launched a quarantine procedure on February 28 [14]. The global epidemic has become more than a million people infected by the severe acute respiratory syndrome coronavirus 2 SARS-CoV-2 , It led to an increase in the death rate due to the absence of a specific treatment for the disease [15]. In a previous study conducted by Surveillances, (2020) in China, Where

they found the high rate of repeated infections with Corona virus in the age group ≥80 years and this percentage agree to the current study. Older people may be more susceptible to infection due to coronavirus due to their weak immune response, this study also confirmed that the incidence of coronavirus in males (51.4%) is higher than that of females (48.6%), and this percentage corresponds to the current study(87.75%) in males while in females(29.25%). The current study also corresponds to a previous study done by Jebril, (2020) in Iraq , They found the age structure of the population correlated with higher mortality rates with COVID-19 in older ages. There is a study conducted by Shi *et al*., (2020), where they found the infection rate of coronavirus in the age group over 50 years and this is consistent with the current study and also they found the infection rate of coronavirus in males (52%)higher than females (48%)and this is consistent with the current study in males. It is believed, according to previous studies, that aging and male sex, as well as diseases associated with people suspected of being infected with Coronavirus, may be risk factors for poor diagnosis in patients [16]. A previous study showed by Hellewell *et al*., (2020), that the percentage of infection with COVID-19 through direct contact is more than infection by movement and this study corresponds to the current study. As there is a previous study that showed that the transmission of the virus from one person to another through direct contact is considered more dangerous and also the results of the previous study indicated that people carrying symptoms or without symptoms will transfer the virus from one person to another through direct contact [18]. In this current study, no high incidence of pathological conditions associated with corona virus, such as flu, heart disease, kidney failure and other pathological cases, appeared, and this study is agree with the previous study conducted by S. Shi *et al*., (2020),they found the relationship between cardiac infections and death in patients with COVID-19 (19.7%). The risk of death may be greater in people with heart disease and coronavirus because they suffer from more severe disease and high creatinine level and lead to weak immune response, and therefore the risk rate may be greater than those without cardiac diseases [19]. Where the current study by Elston, (2020) showed the extent of the impact of skin infections and its correlation with corona virus, where a previous study was conducted by, they found it difficult to wear protection masks for people who always wear them because of the cause of skin allergies and their complications on the skin. The prevalence of skin damage associated with improved prevention measures was 97.0% among care workers Health care for people living withCOVID-19, as it included skin lesions that in turn affect the bridge of the nose, forehead, cheek, and hands. Where there is a previous study by Fernandez Nieto *et al*., 2020) is considered the first study on the skin manifestations associated with corona virus, where specialist dermatologists evaluated a 18 skin infection out of 88 involving widespread urticarial (n = 3) and chickenpox-like vesicles (n=1) namely erythematous rash (n=14), this study agree with the current study where they found in this study that the highest percentage for Urticarial category middle adult is 12.5%.Skin manifestations by type COVID-19 the highest percentage was Male in urticarial 19.5%. A previous study by Zhang *et al*., (2020)was conducted by the examination procedure was the patients suffering from skin lesions despite the symptoms of skin lesions are fever and symptoms of corona virus fever also were stone infected with skin lesions where through these expectations the Chinese Health Society for Dermatology organized experts to take the necessary and necessary measures to prevent the outbreak of the epidemic. It is a good idea to introduce a new clinical tool that is the appearance of the skin with COVID-19 is an excellent step to deal with the challenge we face against the Corona virus epidemic, and it is better to do many studies on skin manifestations and this epidemic because skin diseases are considered to be wide range [23]. In a previous study in Iraq, it showed the number of deaths of people infected with coronavirus 36 in 27 March 2020, and this is contrary to the current study, as it found the number of deaths during the study period and it is certainly a good indicator of the low number of deaths [24]. n a previous study in Italy, it confirmed the presence of several cases ofCOVID-19 infection and they had a rash in spite of their peace before they were infected with the virus, as most of the patients were suffering from foot lesions. This corresponds to the current study, that is, corona virus correlation with skin lesions. In the current study, the highest percentage in middle adult urticarial was 53 (66.3%) and chicken pox like, and this contradicts a previous study, as Shinkai found the rash associated with the virus appears more frequently in children. It is

surprising to see many reports of rashes in adult patients with COVID-19 as it is a new study to identify the association between skin infections and COVID-19. As the results of the current study showed a negative relationship, there is no correlation between the two variables, so the extent of the rash with the virus is still not completely clear, as well as a previous study in China confirmed to more than a thousand patients with coronavirus, the proportion of those with skin infections was 0.2%, and this contradicts the current study, which may be the reason in the number Cases of infected patients or it could be a disease completely isolated from the virus. The study also showed that the common source of infection is through direct contact and the lowest percentage is through travel despite the spread of the virus throughout the world and this corresponds to a previous study [24]. the highest percentage in flue like urticarial was 10 (50.0%) and this result is consistent with many studies that may be due to immunity and response. Also in the description of many patient reports on April 30 in JAMA Dermatology a man has put "freckles" or small red dots on the skin living in Spain. Also patient who developed larger lesions known as a "digitate papulosquamous eruption in France, it is clear that the appearance of symptoms of skin manifestations with COVID-19 varies greatly in cases where the rash appears a few days after the disease or does not appear until late in the course of the infection. Or other conditions that precede the rash, symptoms such as fever. It can be concluded from the current study that there is no close correlation between skin manifestations and COVID-19, a very weak direct relationship because some patients may not show any symptoms on them and these are considered negative signs [25]. Acrodermatitis enteropathica (AE) is an autosomal recessive disease with rare incidence associated with the error in zinc uptake (deficiency). Its characterized mainly by the skin inflammation (dermatitis) around the body, the tips of fingers and toes, alopecia and diarrhea. The most accurate method for diagnosis of AE is the detection of zinc plasma levels [26]. The AE is first records in the patients with COVID 19 in this study which may belong to changes in nutritional and immunological states of patients that occur due to Psychiatric disorders.

Conclusions

It can be concluded from the current study that there is no close correlation between skin manifestations and COVID-19 infection, a very weak direct relationship because some patients may not show any symptoms and these are considered negative signs, meanwhile the unique skin lesion are zinc like deficiency (acrodematitis enteropathica)not reported in any other areas.

Ethical Clearance: The ethical clearance was obtained from the Ethics Committee of Community Medicine Family and Community Medicine department, College of Medicine, University of Thi-Qar, Iraq.

Source of Funding: self- founding.

Conflict of Interest: the authors declare that there is no conflict of interest.

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Retrograde Intra-Renal Surgery Versus Percutaneous Nephrolithotomy in Management of Renal Stone 10-20 Millimeters, A Comparative Clinical Study

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Abstract

Purpose: comparison the efficacy and safety of percutaneous nephrolithotomy (PNL) and retrograde intra-renal surgery (RIRS) in treatment of renal stones. **Patients and Methods**: Records of patients who underwent surgery for renal stone were retrospectively reviewed, for two years from the beginning of 2017 till the end of 2019. Patients who had renal stones between 10 - 20 Millimeter and located in different renal calices were included, patients who underwent percutaneous nephron-lithotomy PNL and retrograde intra-renal surgery RIRS were defined as Group I and Group II, respectively. Patient criteria (age, sex, BMI); the stone characteristics; time of procedure, fluoroscopy and hospitalization; stone-free and complications (bleeding, pain, infection, visceral injury, conversion to open surgery, and need for blood transfusion) were evaluated between the treatment groups. **Result:** There were no significant differences in terms of age, gender, BMI, laterality, number of stones, Hounsfield units and surface area characteristics of the stone between the PNL (n 25) and RIRS (n = 35) groups (P = .558, P = .278, P = .375, P = 0.051, P = .053, P = .064, P = .642, P = .080, respectively). Stone free rate was 59.6% (n=28) in PNL, and 88.6% (n=31) in RIRS (P=.004). 1st or 2nd degree complications according to Modified Clavien Classification developed in 10 patients (21.3%) in Group I and 1 patient (2.9%) in Group II (P = .015). The 3A or 3B complications were similar in groups (P = .077). Time of procedure, fluoroscopy and hospitalization were significantly lower in Group II (P < .001, P < .001 and P < .001, respectively). **Conclusion**: RIRS is more effective and more reliable procedure than PNL with higher stone-free and lower complication rates in treatment of single renal stone (10 t0 20 mm).

Keywords - *Retrograde Intra-renal Surgery, Percutaneous Nephrolithotomy, treatment of renal stones.*

Introduction

Minimally invasive procedures have almost Completely replaced open surgery in patients with kidney Stones over the past two decades technological development of surgical instrumentation used in treatment of renal stones and research into less invasive and safer techniques are major topics in endo-urology today [1,2,3].

After technical revolution in European countries Percutaneous nephrolithotomy (PNL) and retrograde intrarenal surgery (RIRS) are the primary technical choices in treatment of moderate- to large-sized renal stones that are not compatible with shock wave lithotripsy (SWL), and these procedures have been compared in many studies of various conditions [1,4,5].

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Meta-analyses have shown that PNL and RIRS methods are effective with high stone-free rate, are safe for medium and large size stones and are alternative procedures to each other [6,7].

It has been reported that localization of stones affects the success of the treatment option as much as stone sizes and therefore efficacy and safety could be changed [8,11].

It is known that in cases with 10-20 stones there is a decrease in stone-free rates after procedures and that treatment of solitary stones is more effective than treatment of multiple stones with similar stone burden in RIRS [12,13].

Although PNL and RIRS have been compared with each other in terms of size and localization of stones, the knowledge relating to multiple stones in the same renal unit is limited.

Aim of the Study

In this study, we aimed to compare efficiency and safety of RIRS and PNL in treatment of single and size between 10 and 20 mms. In the current literature, no previous study has examined RIRS and PNL for these specific conditions.

Patients & Methods

Between 2017and2019, records of patients who underwent surgery for renal stone were retrospectively reviewed after approval by the local ethics committee. The renal stones were classified by localization as lower pole, middle pole, upper pole and pelvic.

Patients who had single stone (10 to 20 mm) were included in the present study. Patients with congenital renal anomaly, coagulopathy, obesity (BMI of 30 kg/m2 and more), skeletal deformity, previous renal surgery and untreated urinary tract infection were excluded from the present study.

Patients who were included in the study and underwent PNL were referred to as Group I, and underwent RIRS were referred to as Group II.

For PNL, patients were placed in the prone position. Using multidirectional C-arm fluoroscopic guidance (Ziehm Vision R C-arm, Orlando, USA), a calycial puncture at the appropriate calyx was performed with an 18-gauge Skinny Needle (coloplast).

A flexible 0.035-inch Terumo guidewire (Boston Scientific Corp., Miami, FL, USA) was inserted into the ureter or an upper-pole calyx through the renal pelvis. The skin and fascia were incised and a 24 to 30-Fr amplatz renal dilatator set (Boston Scientific Corp. USA) was used.

A 26-Fr Nephroscope Karl storz, Germany) andregide (nephroscope karl storz, Germany) were inserted inside the sheath and the renal stones were fragmented by pneumatic lithotripter. Stone fragments were retrieved using 5-Fr grasping forceps. After the stone fragments were removed, a percutaneous nephrostomy tube was inserted if necessary after JJ stent inserted. All PNL operations were performed with single access.

For RIRS, patients were positioned in the dorsal lithotomy position. Before RIRS, diagnostic rigid ureterorenoscopy (URS) (6.5/8.5 Fr) (karl storz, Germany) was done with the same procedure routinely used for passive dilatation.

After that a 9.5- or 11.5-Fr ureteral access sheath (Cook Medical) was placed in position. A 7.5-Fr Flex-X2 flexible ureteroscope (Karl Storz) was inserted through the access sheath. A 272-µm laser fiber was used for treatment of the stones. Holmium laser power was set to 10 W. Fragmented stones were removed with stone basket.

Following completion of fragmentation, ureter was visualized all along its length to see any ureteral injury. JJ stent was routinely inserted for safety after the procedure specially when there was mucosal edema, injury or the duration of the procedure was long. The ureteral JJ stent was usually removed within 4 t0 6 weeks postoperatively.

The patients' criteria (age, sex, BMI), the stone characteristics (side and number of stones, number of localizations, Hounsfield units, stone surface area), and procedure time, fluoroscopy time, and hospitalization time were evaluated between the treatment groups. Stone-free status and complications in the groups were also compared to determine efficiency and safety of

RIRS and PNL.

Low-dose non-contrast computed tomography (CT) and intravenous urography (IVU) were performed before the operation to determine the number, localizations, Hounsfield units (HU) and surface area of stones. "Stone surface area = length x width x 0.25 x ≤" formula was used to calculate the surface area of stones from CT [1,14]. The stone-free status was evaluated one month after PNL or RIRS by non-contrast CT.

The Absence of a stone at any size was considered as the stone-free status. Complications were defined and graded according to the modified Clavien classification [15]. Statistical analysis The data analysis was performed by using SPSS for Windows, version 22 (SPSS Inc., Chicago, IL, United States). The normality of the distribution was tested with Shapiro-Wilk and Kolmogorov-Smirnov tests.

Descriptive statistics for variables with a non-normal variable, normal variables and nominal variables were shown as median (min - max), mean ± standard deviation and number of cases and (%), respectively. The differences between independent groups regarding continuous variables were evaluated using the Mann-Whitney U test and Student t-test. For categorical comparisons, Chi-square or Fisher's exact test were used whenever convenient. $P < 0.05$ was regarded as statistically significant.

Results

The characteristics of the patients including age, gender, BMI, and the laterality of the stones were similar in Group I (n=60), and in Group II (n=30) (P = 0.08, P = 0.35, P = 0.125 P = 0.06, respectively) (Table 1).

There were also no differences in mean HU of stones (P=0.052) (Table 1). Mean stone surface area was 265.15 ± 89.9 mm2 in Group I and 244.38 ± 101.65 mm2 in Group II.

These outcomes were also similar (P = 0.12) (Table 1). Of 60 patients who underwent PNL 40 were stone-free and of 30 patients who underwent RIRS 25 were stone free. RIRS effectuated higher stone-free rates than PNL (83.3%, 66.6%, respectively) and this difference was statistically significant (P = 0.001).

The mean residual stone sizes were also statistically different (P = 0.02) (Table 2). When the complications were compared, it was seen that 1st or 2nd degree complications according to Modified Clavien Classification developed in 20 patients (33.3%) in Group I and 8 patients (26.6%) in Group II. This was also statistically significant (P = 0.03) (Table 2).

Complications of 3A degree developed in 12 patients (16.6%) in Group I and 4 patients (13.3%) in Group II. The difference was significant between groups (P = 0.005). Complications of 3B degree developed in 6 patients (10%) in Group I and 0 patients (0%) in Group II (Table 2), no significant statistical difference between groups (P = 0.07). No 4th and 5th degree complications were seen in any patient (Table 2).

When the operation data were evaluated, procedure time and fluoroscopy time in Group II were significantly lower (P < 0.005 and P < 0.002) (Table 2). The median hospitalization time for RIRS was 1 (1-2) day, while it was 2 (1-7) days for PNL. This value was statistically significant (P < 0.001) (Table 2).

Table 1: Shows The demographic data and stone characteristics

	Group I	Group II	p-value
Patient number	60	30	0.08
Age	45.3 ± 15.2	43.1 ± 13.6	0.35
BMI	28.5 ± 3.8	26.2 ± 4.1	0.125
Gender (male/female)	42/18	18/12	0.06
Stone laterality (right/left)	22/38	10/20	0.2
Mean stone size (mm2)	265.15 ± 89.9	244.38 ± 101.65	0.12
Density (HU)	715.2±186.2	798.5±172.8	0.052

Table 2: shows comparison of operative and postoperative data

	Group I	Group II	p-value
Stone free rate (%)	40 (66.6%)	25 (83.3%)	0.001
Mean residual stone size (mm2)	127.75 ± 78.25	53.25 ± 38.2	0.02
Complication, clavien I-2 (%)	20 (33.3%)	8 (26.6%)	0.03
Complication, clavien 3A (%)	12 (16.6%)	4 (13.3%)	0.005
Complication, clavien 3B (%)	6 (10%)	0	0.07
Median fluoroscopy time (s)	160 (120-445)	32 (15-380)	0.002
Mean procedure time (min)	92.66 ± 30.07	67.7 ± 20.5	0.005
Median hospitalization time (day)	1 (1-2)	2 (1-7)	0.001

Discussion

In our ward recent use of minimal invasive techniques have replaced open surgical methods in renal stone treatment; however, they try to discuss the efficient and safest in endourology technique. PNL and RIRS are the most important techniques in this field and their success rates have been frequently compared in the literature [1,7].

After definition of percutaneous stone extraction [16], the PNL procedure has replaced open surgery in treatment of moderate- to large-sized stones and its efficacy has been researched in many studies [17,18]. Since it was first performed by Huffman et al. [19], RIRS has become an important treatment modality for urinary stone disease using flexible devices and holmium laser [20].

Though PNL technique results in high stone-free rates for moderate- to large-sized renal stones, there has been a search for alternative treatment methods due to its morbidity and mortality rates [21,22].

Increased experience with the RIRS technique revealed that it has high stone-free rates even for large-sized stones and lower morbidity rates when compared with PNL [6,7]. When two meta-analysis studies comparing PNL and RIRS are taken into account, Shuba De et al. found that PNL has higher stone-free rates, complication rates and blood loss [7].

Another meta-analysis published in 2014 proposed RIRS as an alternative to PNL since RIRS has similar stone-free rates when compared to PNL even for stones larger than 2 cm along with lower complication rates and shorter hospital stay periods [6].

When localization of stones is taken into account, lower pole stones result in different stone-free rates and the PNL procedure was found to be more successful than RIRS [23,24].

A meta-analysis study, published in 2015, reviewed 6 randomized and 8 non-randomized studies comparing PNL, RIRS and SWL techniques for lower pole stones and found that PNL results in higher stone free rates when compared to RIRS and SWL [25].

It is known that presence of more than one stone decreases the success rates of treatment in kidney stone disease. Cass et al. [26] reviewed 13,864 SWL cases and found that the stone-free rate was 69.5-72.1% in single stone cases, whereas it was lower than 50% in multiple

stone cases for same renal unit [32].

A study of pediatric cases and SWL showed that average stone number of cases was 1.87 for patients with stone-free treatment, whereas it was 2.81 for cases where treatment could not provide stone-free state in the same renal unit and they concluded that stone number influences success of SWL [27].

Meanwhile, Ozgor et al [13] reported that RIRS technique resulted in lower stone-free rates in patients with multiple renal stones when (located in minimum two calyces) was reported to be 53.9% in a recent study [29].

A United Kingdom-based prospective study of 1000 renal units reported that stone-free rate was seen to be 68% for PNL [30]. Despite there seems to be a contradiction between recruitment of flexible devices in our PNL operations and resultant low stone-free rates, we think that was due to degree of flexion of flexible devices moving to another calyx as calyceal access via flexible equipment is more difficult than reaching a calyx with pelvic access.

Moreover, it is known that a flexible nephroscope has limitations with respect to field of view due to bleeding complications in PNL [31]. From this point of view, we think that RIRS is more advantageous than PNL. When each group is examined according to data of during operation; RIRS was found to be superior to PNL with respect to procedure time, fluoroscopy time and hospitalization time ($P < .001$, $P < .001$, $P < .001$, respectively).

These findings were also compatible with the previous literature [7,24]. When limitations of our study are considered, first of all it is retrospectively designed. Another limitation is that although multicalyceal stones were operated in our study, only one access was used for PNL. However, based on previously reported high complication rates in PNL operations performed with multiple access [13,28], we prefer single access in our department.

To the best of our knowledge our study is the first to compare efficacy and reliability of PNL and RIRS techniques in this specific patient group that poses difficulties for treatment. compared to patients with solitary renal stone even though both groups have similar stone burden (83.8% and 89.2%, respectively). When PNL is performed for multiple renal stones located in more than one calyx, more than one access may be required and it is known that multiple access may cause serious bleeding complications and loss of kidney function from previous studies [13,28].

With regard to previous studies in the literature, we aimed to compare efficiency and reliability of RIRS and PNL for treatment of moderate- to large-sized multicalyceal and multiple renal stones in the same renal unit. To the best of our knowledge this is the first such study in the literature.

Postoperative stone-free rate, which is considered as the most important parameter for evaluating efficacy, was 88.6% for RIRS and 59.6% for PNL and this difference was statistically significant ($p = .004$). Complications were defined and graded according to the modified Clavien classification.

We found that grade 1 or 2 complications were encountered more frequently with the PNL technique and this finding was statistically significant; however, there was no statistically significant difference between the two techniques with regard to major complications such as grade 3A or 3B ($P = .015$ and $P = .077$, respectively). Higher complication rates for the PNL technique is compatible with previous studies [6,7].

It was noteworthy that stone free rates for this special patient group, performed PNL, were lower than previous literature data. However, stone-free rate for staghorn and partial staghorn

Conclusion

In conclusion, RIRS is superior to PNL with respect to both efficacy and reliability for single renal stone. Moreover, RIRS is advantageous when fluoroscopy time, complications and hospitalization time periods are taken in consideration. Therefore, we think that RIRS should be the first choice of treatment in this specific patient group when their stone burden is considered.

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Radiographic Assessment of Post-Endodontic Pain

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Abstract

Aim:To radiographically assess and identify the factors that influence post-endodontic pain (PEP) in patients received nonsurgical root canal treatment. **Study Design:** a longitudinal study. **Place and Duration of Study:** the study performed on 375 patients (200 male, 175 female), aged 15–65 years randomly attend Dr. Sameer Hatem private clinic in Karbala between 2014- 2019 Method. the complete history of patients was taken, clinically examined and then radiographically examined by CSN x-ray machine and Xpod digital sensor to identify the possible factors and assess the status of previously treated teeth, the patients divided into groups according to the age, causative factors, and the onset of pain from patients' chief complain. **Results:**(Table.2) shows the differences in numbers and percentages of overall patients according to the onset and the causative factors of pain after root canal treatment, the high percentage of the pain of 102/(27.2%) of patients was due to overextension of filling material, (most of these patients were suffering from spontaneous pain 47.05%). Followed by 78/(20.8%) which were due to failure of treatment due to under-filled root canal (most of them 34.61% have intermittent pain). **Conclusion:** Periapical x-ray radiography is a valuable technique to identify the causative factors of post endodontic pain and evaluate the success of RCT and the status of endo treated tooth and periapical tissues. All procedural errors can not be depicted on radiographs.

Keywords*: Nonsurgical root canal treatment, Post-endodontic pain (PEP), Radiographic, Radiographic assessment, longitudinal study.*

Introduction

In the past, root canals endodontic procedures were extremely painful. This is one reason of many reasons why people sometimes avoided such procedures. Dentists now have pain-relieving measures that can be used to reduce the amount of pain experienced during the procedure. Prevention and management of post-endodontic pain (PEP) is an integral part of endodontic treatment. Informing patients about expected post-endodontic pain (PEP) and prescribing medications to manage it can increase patient confidence in their dentists, increase patient's pain tolerance, and improve their attitude toward future dental treatments [1, 2]. According to recently published data, pulp therapy and root canal treatment (RCT) causes more frequent and more severe postoperative pain compared to other dental procedures [3, 4]. In this published literature, reported frequencies of PEP range from 1.5 [5] to 53% [3]. But less than 12% of patients experienced severe pain [6]. Pain after endodontic procedure is one of the most commonly known complications of endodontic treatment. It can be caused due to many preoperative factors like acute exacerbation of chronic lesion, non-vital tooth, previously opened canal, the extension of either the filling material and/or the instrument beyond the tooth apex during the procedure, and any leakage in temporary or permanent filling done after endodontic treatment. Various factors responsible for post endodontic pain are the position of the apical foramen, pulp tissue between two canals which cannot be easily instrumented without proper care, presence of fourth accessory canal in case of maxillary first molars which may be left out without instrumentation, inaccurate determination of working length can lead to over instrumentation, extrusion of root canal debris beyond the apex during instrumentation, irrigants used like sodium hypochlorite and hydrogen peroxide may cause periapical discomfort, an obturating technique like lateral condensation causes immediate postoperative pain than single cone obturation technique [7]. Mechanical factors, including over instrumentation or extrusion of root-filling materials, have been associated

with the presence of postoperative pain [8,9], suggesting that root canal instrumentation and obturation techniques may influence postoperative pain. In fact, several studies have found a correlation between the root canal instrumentation technique and postoperative pain [10,11]. Another overlooked factor that may play a role in the higher frequency of PEP in the current study is that the whole root canal treatment was performed at a single visit. The single-visit treatment has been shown to result in a higher frequency of PEP, and consequently, higher consumption of analgesics [13, 14, 15,16,17]. Nevertheless, the main advantages of single visit treatment are the reduced time and added convenience for both patient and dentist, without increasing short or long complications [16] Radiographic examination is an essential component of endodontic management [34] the principal aspects of diagnosing, treatment planning, intra-operative control and procedural outcome assessments is the intra-oral periapical radiographs which are still most commonly exposed during endodontic procedures [35], providing usefulinformation for the presence and location of periradicular lesions, root canal anatomy, and the proxymity of adjacent anatomical structures. Understanding the etiology of postoperative pain after obturation may greatly help clinicians to adopt strategies to prevent such a highly distressing phenomenon [12].

The purpose of this study was to radiographically assess and identify the factors that influence post-endodontic pain (PEP) in patients who received nonsurgical root canal treatment.

Methods

This is a longitudinal study performed on 375 patients (200 male, 175 female), aged 15–65 years randomly attend Dr. Sameer Hatem private clinic between 2014-2019 presenting with post endodontic pain with a different intensity that not resolved after root canal treatment, with onset ranged from 1 day to 60 days post-operatively, the complete history of patients were taken, clinically examined and then radiographically examined by CSN x-ray machine and Xpod digital sensor to identify the possible factors and assess the status of previously treated teeth, the periapical radiographs show that the factors were different, so the patients divided into groups according to the age, causative factors and the onset of pain from patients chief complain.

Table 1: The distribution of patients according to the age group is presented.

Age (Pt. No.)	Factors/ Pt. No./ (%)	Spontaneous pain /(%)	Intermittent /(%)	After 3-7 days /(%)	After 2 weeks or more /(%)	Chi & p.value
15-24 N=125	Under-filling/ 24/(19.2%)	5/(20.83%)	9/(37.5%)	3/(12.5%)	7/(29.16%)	3.3 & 0.34
	Over-filling/ 42/(33.6%)	17/(40.47%)	8/(19.04%)	6/(14.28%)	11/(26.19%)	6.57 & 0.09
	Broken instrument/ 9/(7.2%)	5/(55.55%)	2/(22.22%)	1/(11.11%)	1/(11.11%)	4.78 & 0.19
	Unhealed periapical lesion/18/(14.4%)	9/(50%)	3/(16.66%)	2/(11.11%)	4/(22.22%)	6.44 & 0.092
	Perforation/ 6/(4.8%)	3/(50%)	2/(33.33%)	1/(16.66%)	0/(0.0%)	1.0 & 0.61
	Missed canal/ 7/(5.6%)	2/(28.57%)	2/(28.57%)	1/(14.28%)	2/(28.57%)	0.43 & 0.934
	Unknown factors/ 19/(15.2%)	10/(52.63%)	2/(10.52%)	5/(26.31%)	2/(10.52%)	9.0 & 0.029
Chi & p.value	53.7 & 0.00	22.157 & 0.001	14.5 & 0.025	9.37 & 0.154	16.33 & 0.005	
25-34 N=70	Under-filling/ 16/(22.85%)	6/(37.5%)	3/(18.75%)	2/(12.5%)	5/(31.25%)	2.5 & 0.48
	Over-filling/ 21/(30%)	8/(38.09%)	4/(19.04%)	3/(14.28%)	6/(28.57%)	2.81 & 0.42
	Broken instrument/ 6/ (8.57%)	1/(16.66%)	2/(33.33%)	1/(16.66%)	2/(33.33%)	0.67 & 0.88
	Unhealed periapical lesion/9/(12.85%)	2/(22.22%)	1/(11.11%)	2/(22.22%)	4/(44.44%)	2.11 & 0.55
	Perforation/3/(4.28%)	2/(66.66%)	1/(33.33%)	0/(0.0%)	0/(0.0%)	0.33 & 0.56
	Missed canal/ 5/(7.14%)	2/(40%)	1/(20%)	1/(20%)	1/(20%)	0.6 & 0.9
	Unknown factors/ 10/(14.28%)	5/(50%)	3/(30%)	1/(10%)	1/(10%)	4.4 & 0.22
Chi & p.value	24.8 & 0.00	11.15 & 0.08	4.13 &0.66	2.0 & 0.85	7.21 & 0.21	
35-44 =62	Under-filling/ 13/(23.63%)	3/(23.07%)	6/(46.15%)	2/(15.38%)	2/(15.38%)	3.31 & 0.35
	Over-filling/ 18/(32.72%)	12/(66.66%)	1/(5.55%)	2/(11.11%)	3/(16.66%)	17.11 & 0.001
	Broken instrument/ 4/(7.27%)	2/(50%)	1/(25%)	1/(25%)	0	0.5 & 0.78
	Unhealed periapical lesion/7/(12.72%)	3/(42.85%)	1/(14.28%)	1/(14.28%)	2/(28.57%)	1.57 & 0.67
	Perforation/ 5/(9.09%)	3/(60%)	0/(0.0%)	2/(40%)	0/(0.0%)	0.2 & 0.66
	Missed canal/ 4/(7.27%)	1/(25%)	1/(25%)	1/(25%)	1/(25%)	0.00 & 1.0
	Unknown factors/ 11/(20%)	7/(63.63%)	1/(9.09%)	2/(18.18%)	1/(9.09%)	9.0 & 0.029
Chi & p.value	19.29 & 0.002	19.81 & 0.002	11.36 & 0.045	1.09 & 0.98	1.56 & 0.82	

Table 2: Total numbers of examined cases

Factors/ Total Pt. No.	Spontaneous pain /(%)	Intermittent /(%)	After 3-7 days /(%)	After 2 weeks or more /(%)	total	Chi & p.value
Under-filling/ 78/(20.8%)	19/(24.35%)	27/(34.61%)	10/(12.82%)	22/(28.2%)	78	
Over-filling/ 102/(27.2%)	48/(47.05%)	16/(15.68%)	14/(13.72%)	24/(23.52%)	102	
Broken instrument/ 35/(9.33%)	13/(37.14%)	11/(31.42%)	4/(11.42%)	7/(20%)	35	105.05 &
Unhealed periapical lesion/59/(15.73%)	23/(38.98%)	9/(15.25%)	6/(10.16%)	21/(35.59%)	59	0.00
Perforation/ 21/(5.6%)	12/(57.14%)	4/(19.04%)	4/(19.04%)	1/(4.76%)	21	
Missed canal/ 19/(5.06%)	6/(31.57%)	6/(31.57%)	3/(15.78%)	4/(21.05%)	19	
Unknown factors/ 61/(16.26%)	34/(55.73%)	9/(14.75%)	11/(18.03%)	7/(11.47%)	61	
total	155	82	52	86		
Chi & p.value	80.72 & 0.00					

Results

During the study period, 375 patients were examined and statistically analyzed. In (Table.2) we notice the differences in numbers and percentages of overall patients according to the onset and the causative factors of pain after root canal treatment, the high percentage of the pain of 102/(27.2%) of patients was due to overextension of filling material, (most of these patients were suffering from spontaneous pain 47.05%). Followed by 78/(20.8%), which were due to failure of treatment due to the under-filled root canal (most of them 34.61% have intermittent pain). The least affecting factor was missed untreated canal, which mostly causes spontaneous and intermittent pain (31.57%). The unknown factors including (over-instrumentation, NaOCl extrusion) which represent a (16.26%) of patients and mostly cause spontaneous pain (55.73%)

The high incidence of post endodontic pain present in (15-24), (25-34) & (35-44) age groups was due to over-filled canal (33.6%), (30%) & (32.72%), most of them have spontaneous pain, with variable percentages of other factors as present in table.1, while (45-54) age group show a high percentage of unknown factors (21.33%) (discussed in the discussion) followed by (18.66%) over-filled canal.

In (55-65) age group, the causative factors were mostly (28%) under-filled canal with (35.71%) have intermittent pain, and (26%) due to Unhealed periapical lesion with (53.84%) start suffering from pain after two weeks or more post-operatively.

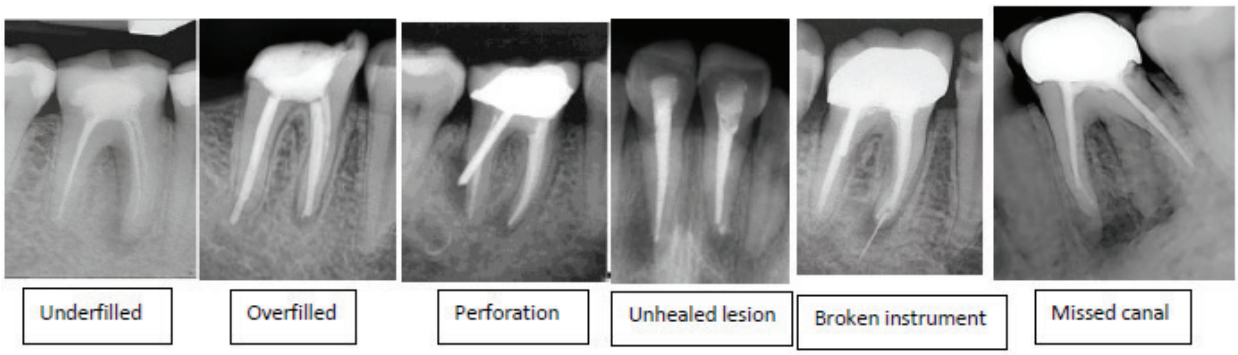

Figure1: Showes the effect of teeth

Discussion

Dental patients have become increasingly less tolerant of any dentist or dental procedure that causes pain. In endodontics, offering adequate local anesthesia is essential for successful patient management and represents a practice-building strategy that increases both patient loyalty and treatment acceptance. Endodontic pain management must encompass all aspects of treatment: preoperative pain control encompasses accurate diagnosis, anxiety reduction, intraoperative pain control revolves around effective local anesthetic and operative techniques, and postoperative pain management can involve a variety of pharmacologic agents [18]. In this longitudinal study, the post-endodontic pain in 375 atients received root canal treatment that has been radiographically analyzed according to age, causative factors, and its relation with the onset of pain post-endodontic intervention that describes from patients chief complain. The results revealed that the most common factor was over-filled root canal (extrusion of filling material) (27.2%) in most age groups, as shown in Table.1, and the pain was spontaneous in most cases.

It has been well established that endodontic overfilling should be avoided during obturation. its only indicated when it would be followed by apicoectomy when the foreign material has been removed. Accidental overfilling may still occur with soft materials (for example, certain, pastes, and cement) or with hard materials (like gutta-percha or silver cones). Such overfilling might cause an unnecessary mechanical and chemical irritation, which hinders the healing of the periapical tissue and thus reduce the probability of a successful outcome. The patient may experience localized, sharp pain after the procedure, which gradually fades down.if the mandibular nerve is injured during the treatment, the pain will be located in the immediate periapical area with radiation to the whole area innervated by the injured nerve [19].

Initial radiographs taken with the files in position will not only ensure the correct working length but also prevent canal perforation and possible subsequent damage to the inferior alveolar nerve resulting from endodontic treatment. Diagnosis of complications caused by accidental overextension of endodontic filling material will require an accurate dental history to determine the onset of altered sensation and its evolution. When complications are suspected from the patient's complaints, history, and clinical examinations, overextension of filling material should be confirmed via radiography to verify any overextension of materials and to determine the specific location of the overfilled materials [23]. The second most common factor of PEP was under-filled root canal in (20.8%) of cases that were mostly suffering from intermittent pain (34.61%) every now and then. Underfilling was determined when root canal obturation was shorter than the radiographic apex by ≤2 mm resulted from inaccuracies in working length determination and mechanical preparation, which are mostly seen in the old age group due to pulp calcification and narrowing of canals with age. The endodontic treatment failures most commonly occurred in underfilled root canals, followed by poorly filled root canals and were associated with periapical radiolucencies [20].

The unhealed periapical lesion was reported in (15.73%) of total cases with high incidence in older age than in younger age patients, especially in diabetic patients. (38.98%) have spontaneous PEP, and (35.59%) have PEP started after two weeks or more. Periapical lesions develop as sequelae to pulp disease. They often occur without any episode of acute pain and are discovered on routine radiographic examination. The incidence of cysts within periapical lesions varies between 6 and 55%. The occurrence of periapical granulomas ranges between 9.3 and 87.1%, and of abscesses between 28.7 and 70.07%. It is accepted that all inflammatory periapical lesions should be initially treated with conservative nonsurgical procedures [21]. On the basis of the average healing rate of approximately 3 mm2/mo, a 30 mm2 lesion will require ten months for complete resolution. If the lesion becomes larger, remains the same size, or demonstrates a below-average rate of healing, then surgical intervention must be considered, and there is always the possibility that quiescent epithelial cells may be stimulated by instrumentation in the apical region, with resultant proliferation and cyst formation [22].

Broken instruments presented in (9.33%) of total cases and increase with aging, (37.14%) of them have spontaneous pain. Rotary instruments tend to fracture inside the canals when either law of access

cavity preparation are not adhered to, or when rotary instruments are not used according to guidlines. As a result of instrument fracture, the access to the apical portion of the root canal becomes limited, which can cause deleterious effect on canal disinfection distal to the fractured instrument, and later on, on obturation. studies conducted on the effect of fractured instruments have demonstrated a minimal influence on the success rate of the treatment [25, 26, 27]. The stage of instrumentation at which the instrument breaks can affect the prognosis. The disinfection and obturation of the canal distal to the fractured instrument become difficult, leading to a persistent infection in that area [27]. However, the fractured instrument itself has less to do with failure because most of the time, the success is only affected when a concomitant infection is present [28]. Root perforation presented in (5.6%) of cases, (57.14%) have spontaneous PEP. Root perforations can occur pathologically as a result of resorption and caries or iatrogenically during root canal treatment [29]. Such perforations might compromise the treatment outcome and persist as a significant complication if not repaired. Perforation might occur during the preparation of access cavities, post space, or may occur as a result of the extension of internal resorption into periradicular tissues [30].

Missed canal reported in (5.06%) of total examined cases, with (31.57%) have spontaneous pain and the same percentage presented with intermittent pain. It's not an uncommon practice to miss a canal during an endodontic treatment, especially in molar teeth where one root, one canal formula, is frequently overruled by the fact that the number of canals is more than the number of roots. Moreover, a less than adequate access opening makes it difficult for the primary dentist to locate the supplemental canals. The inability to treat all the canals is one of the causes leading to endodontic failure. Bacteria residing in these canals lead to the persistence of symptoms. The results of one study carried out on 5616 molars, which were retreated, showed that failure to locate the MB2 canal had resulted in a significant decrease in the long-term prognosis of those teeth [24]. The Unknown factors that could not be detected by radiograph mainly include the complications of over-instrumentation and Sodium Hypochlorite Leakage during the irrigation of the root canal. These factors consist (16.26%) of total examined cases and presented with (55.73%) spontaneous PEP. Inadvertent injection of NaOCl beyond the apical foramen is uncommon and is infrequently reported in the literature. This complication occurs in teeth with wide, apical foramina or when the apical constriction is destroyed during root canal preparation. In addition, extreme pressure during irrigation may result in contact with large volumes of the irrigant with the apical tissues. If this occurs, the excellent tissue-dissolving capability of NaOCl will lead to tissue necrosis [31]. The sequence of signs and symptoms that occurs after the extrusion of NaOCl into the periapical tissues seems to follow a typical pattern. According to Hulsmann's criteria, the diagnosis of NaOCl accident includes the following: (1) acute pain, swelling, and redness; (2) bruising; (3) progressive swelling involving the infraorbital area or mouth angle depending on the site of NaOCl injection; (4) profuse hemorrhage often manifesting intraorally from the orifice of the tooth; (5) numbness or weakness of the facial nerve; and (6) secondary infection, sinusitis, and cellulitis [31,32]. Iatrogenic over instrumentation promotes the enlargement of apical foramen and irritate the apical tissue, which may allow an increased influx of exudate and blood into the root canal [33]. This will enhance the nutrition supply to the remaining bacteria inside the root canal, which can then proliferate and cause exacerbation of a chronic periradicular lesion and pain.

Conclusion

There are many factors that influence post-endodontic pain (PEP); these factors cause a variable onset of pain and other complications that eventually lead to the failure of root canal treatment. Periapical x-ray radiography is a valuable technique to identify the causative factors of post-endodontic pain and evaluate the success of RCT and the status of endo treated tooth and periapical tissues. All procedural errors cannot be depicted on radiographs. Over-instrumentation, for example, may push the pulp remnants and microbes beyond the apex of the tooth, causing acute apical periodontitis, which can be demonstrated by using radiographs only, which is followed by extrusion of the filling materials but not during previous stages of RCT.

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Evaluation of Hinge Position on Corneal Sensation and dry Eye after Laser in Situ Keratomileusis (LASIK) in Thi-Qar Governorate

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Abstract

Purpose: This investigation planned to assess the impacts of Laser In Situ Keratomileusis (LASIK) with prevalent and nasal pivot areas on corneal sensation and dry eye condition. Planned randomized similar investigation was completed from February 2018 to December 2019. The subsequent term were preoperatively evaluated just as 1week and 1,3,6 months postoperatively. This examination included 600 eyes of 420 patients (180 guys and 240 females), all patients went through LASIK medical procedure for rectification of nearsightedness as well as nearsighted astigmatism utilizing MEL 90 Excimer laser for stromal removal , Moria M2, SBK microkeratomes were utilized to make a prevalent and nasal pivoted folds. At the point when patients chose to have medical procedure, they were randomized to get nasal or predominant pivot medical procedure based on the randomization succession. Focal Cochet-Hat esthesiometry, Schirmer fundamental tear discharge test, tear film separation time (TBUT) were assessed preoperatively and at multi week , 1, 3, and a half year postoperatively. After medical procedure, the corneal sensation diminished essentially in both nasal-pivot and prevalent pivot folds at multi week however got back to gauge by half year. The Schirmer essential emission test esteems were not altogether changed postoperatively in the nasal pivoted and predominant pivoted eyes. TBUT diminished inconsequential at multi week and multi month postoperatively and recouped by 3 months after medical procedure in the two gatherings. There were no huge contrasts in all the abstract and target boundaries tried between nasal-pivoted and unrivaled pivoted folds whenever point. There is no intraoperative and postoperative inconveniences in everyone's eyes. There was a huge decrease in corneal sensation following LASIK in nasal and predominant gatherings. Sensation improved at untouched stretches and re-visitations of nearly, preoperative levels around a half year postoperatively. Related with the corneal denervation is a prompt postoperative increment in dry-eye as estimated by TBUT and schirmer test that improved utilizing oils and with ensuing visits.

Key words - *cornea, corneal sensitivity, Schirmer test, dry eye disease, LASIK*

Introduction

Dry eye sickness (DED) is an ordinarily experienced finding in ophthalmology, yet is ineffectively perceived, hard to characterize, and comes up short on a remarkable analytic test. The Worldwide Dry Eye Work Shop (DEWS) as of late characterized DED as "a multifactorial illness of the tears and visual surface that outcomes in manifestations of distress, visual aggravation, and tear film unsteadiness with expected harm to the visual surface. It is joined by expanded osmolarity of the tear film and aggravation of the visual surface [1]. Laser-aided situ keratomileusis (LASIK) is a protected and powerful careful choice for treatment of refractive blunders [2], notwithstanding, dry eyes are a surprisingly successive result of LASIK medical procedure, with up to 95% of patients encountering indications of dry eyes after corneal refractive medical procedure [3]. Dry eye indications are genuinely regular in patients before LASIK. The pervasiveness of dry eye side effects preceding going through LASIK is assessed to be somewhere in the range of 38 and 75% [4,5]. Post-LASIK, it has been broadly indicated that a dominant part of patients whines of dry eye manifestations, particularly in the early postoperative period [3,6,8]. Following LASIK, 95% of patients report some dry eye indications [3]. Dry eye manifestations are accounted for in as high as 60% of patients multi month

after LASIK [3,7]. Post-LASIK dry eye typically tops in the initial barely any months after medical procedure, and afterward side effects start to improve in by far most of patients at 6 a year after medical procedure. Corneal refractive specialists additionally report dry eyes as the most widely recognized intricacy of LASIK [9]. Keratorefractive methods, for example, outspread keratotomy, photorefractive keratectomy and LASIK produce restricted harm of stromal nerves and the corneal sub-basal plexus prompting transient gentle to serious epithelial modifications related dry eye and additionally neurotrophic wonders [10]. There are various hypotheses concerning how LASIK adds to the pathophysiology of dry eyes. The principle proposed cause is iatrogenic corneal nerve harm. LASIK disturbs both the thick sub-basal nerve plexus and stromal corneal nerves in the production of the foremost stromal fold and excimer laser removal of the cornea. Microkeratome is the fundamental instrument needed to make a uniform and a homogeneous corneal fold by cutting over the stromal corneal lamellae. Loss of conjunctival flagon cells has likewise been recognized after LASIK, likely because of direct harm from the pull gadget utilized during making of the LASIK fold [11]. Harm to the challis cells happens with both microkeratome-and femtosecond laser-made folds [12–13]. Postoperative fiery changes may likewise add to post-LASIK dry eyes [14]. LASIK-initiated change fit as a fiddle may influence the connection between the eyelids and visual surface and lead to anomalous tear appropriation during squinting [3,15]. Diminished corneal sensation can add to the advancement of LASIK actuated dry eye by influencing the useful unit created by the visual surface, trigeminal nerve, mind stem-facial nerve-lacrimal organ hub liable for diminished tear creation [10,14]. In view of these perceptions, corneal affectability can possibly fluctuate as per the area and width of the LASIK fold pivot. A few investigations feature that LASIK can cause supported brokenness of the coordinated visual surface/lacrimal organ useful unit, bringing about incessant dry eye [14]. In 2001, 2003 and 2004 studies of individuals from the American Culture of Waterfall and Refractive Specialists, they all found that the most widely recognized entanglement of LASIK was dry eye [9,16,17]. LASIK isn't the main corneal methodology that may influence tear creation, as in 2002, Kessler et al found that there was transient dry eye following Intact position, however the tear film quality was reestablished inside multi week of medical procedure [18,43].

Materials & Methods

In this planned, randomized, similar investigation, LASIK was performed to 600 eyes of 420 patients (180 guys and 420 females). At the point when patients chose to have medical procedure, they were randomized to get nasal-or predominant pivot medical procedure based on the randomization succession.

This investigation was performed at Nasiriya specific Eye place, Branch of Ophthalmology, in Thi-qar governorate in the period between February 2018 to December 2019. LASIK systems were performed by one specialist. The examination up-and-comers were chosen from the people that were going to requesting careful remedy of vision. Result measures were assessed at multi week, multi month, 3 months, and a half year after the medical procedure.

Incorporation models:

- Age: over 18 years of age.
- Myopia: under 9.0 D and astigmatism of under 2.0 D.
- Scotopic understudy size not in excess of 6 mm.
- Corneal thickness more than 500 um estimated by ultrasonic pachymetry.
- Normal visual status checked by cut light assessment and fundus assessment.
- Basic Schirmer test with => 15 mm.
- Corneal sensation run between 50-60 mm estimated by Cochet–Hood esthesiometer.
- Intraocular pressure (IOP) <20 mmHg

The patients were arranged haphazardly into two gatherings

Gathering 1 (nasal pivots):

It included 300 eyes of 220 patients (respective in 80 cases and one eye 140 cases) for whom a nasal pivoted fold LASIK was performed

Gathering 2 (prevalent pivots):

It included 300 eyes of 200 patients (respective in 100 cases and one eye 100 cases) for whom a predominant pivoted fold LASIK was performed

Preoperative assessment:

- Measurement of the BCVA, communicated in the terms of Snellen graphs in

the type of decimal sharpness.

- Slit light assessment.

- IOP estimation utilizing Goldmann applanation tonometry.

- Fundus assessment

- Corneal sensation: Corneal sensation was estimated at the focal cornea utilizing Cochet Hood esthesiometer.

- Schirmer test: A Schirmer test (Essential emission test) was performed by putting Schirmer test strips estimating 35 mm x 5 mm size after establishment of a drop of effective sedation over the lower cover edge at the intersection of the parallel and center thirds, for 5 minutes

- Tear film separation time (TBUT): The TBUT was assessed 1 moment after the infero fleeting bulbar conjunctiva was contacted with a sodium fluorescein strip.

LASIK was performed utilizing the MEL 90 Excimer laser was utilized for stromal removal. The laser in situ keratomileusis (LASIK) technique included mechanical fold readiness utilizing a microkeratome, either a direct kind with a solitary utilize 90 μm head to make a nasal pivot or a turning type with a solitary utilize 90 μm head to make a predominant pivot.. All patients were inspected an hour postoperatively to check fold adherence. All patients were analyzed at the first postoperative day and the first week to avoid the chance of early postoperative intricacies. At that point, customary follows up visits were done at 1, 3 and a half year postoperatively. In each subsequent visit, the corneal sensation, TBUT and Schirmer trial of the patients eyes were estimated. The preoperative and postoperative information of each gathering were investigated independently. At that point, information of the 2 gatherings were contrasted and one another.

Results

A forthcoming, similar investigation was performed on 600 eyes of 420 patients, 180 guys and 420 females. The mean age for the patients was 25.50± 4.67 years old (rang 19-38 years of age). No intraoperative inconveniences happened in our patients. We isolated the patients into 2 separate gatherings as indicated by pivot fold position (Nasal and Unrivaled):

Consequence of gathering 1 (Nasal Pivot)

This gathering included 300 eyes of 220 patients (100 guys and 120 females). The mean age for these patients at the hour of medical procedure was 26.01± 4.96 years old (range19-38 years of age). The underlying examination was with a critical measurable contrasts of the corneal sensation, Schirmer test that will in general be non-noteworthy with term of follow-up headway, while the examination of Yet at pre-usable evaluation and (first week, first month, third month and sixth month) as an events of correlation were non-huge, where the connection were of frail kinds with addition of follow up an ideal opportunity for the three markers of intrigue (corneal sensation, TBUT and Schirmer test) (Table1)

Table 1: The differences in parameters before operation and 1week, 1, 3, and 6 months after operation of nasal group.

		Mean	Range	N	Std. Deviation	Correlation	Significance	Paired t test	P value*
corneal sensation	Preoperative-nasal After 1 week-nasal	55.07 30.87	50-60 20-40	300 300	3.162 4.864	.068	.240	24.838	.0001
	preoperative-nasal After 1 month-nasal	55.07 30.32	25-35	300 300	3.162 3.655	-.037-	.528	25.309	.0001
	preoperative-nasal After 3 month-nasal	55.07 41.05	35-45	300 300	3.162 3.562	-.101-	.080	14.584	.0001
	preoperative-nasal After 6 month-nasal	55.07 49.82	40-55	300 300	3.162 4.034	-.074-	.203	5.853	.045
T BUT	preoperative-nasal After 1 week-nasal	7.88 6.31	7-10 4-8	300 300	.823 1.225	-.011-	.845	1.732	.065
	preoperative-nasal After 1 month-nasal	7.88 7.14	6-9	300 300	.823 .896	.014	.804	.874	.465
	preoperative-nasal After 3 month-nasal	7.88 7.57	7-9	300 300	.823 .708	.052	.374	.430	.544
	preoperative-nasal After 6 month-nasal	18.92 8.05	7-10	300 300	1.419 .984	-.042-	.464	-.021-	.025
Schirmer	preoperative-nasal After 1 week-nasal	18.92 13.43	17-21 12-16	300 300	1.419 .980	-.021-	.716	5.691	.001
	preoperative-nasal After 1 month-nasal	18.92 15.61	13-19	300 300	1.419 1.800	.117	.042	3.552	.032
	preoperative-nasal After 3 month-nasal	18.92 16.33	15-18	300 300	1.419 .957	-.050-	.392	2.792	.042
	preoperative-nasal After 6 month-nasal	18.92 17.67	15-20	300 300	1.419 1.420	.093	.108	1.471	.321

*p Value <0.05 was statistically significant. TBUT: tear film break up time

Corneal Sensation

The mean preoperative Corneal Sensation in this gathering was 55.07 mm ± 3.16. Decrease of the sensation happened at the postoperative period to be 30.87 ± 4.86 at the finish of the primary week, 30.32 ± 3.65 toward the finish of the principal month, 41.05 ± 3.56 at the end of the third month, lastly 49.82 ± 4.03 at sixth month follow-up(Fig.1). The adjustments in the mean corneal sensation during the subsequent period were factually critical (p< 0.001 at the 1week, 1and 3 months and 0.045 at a half year). The most extreme increase of corneal sensation was seen toward the finish

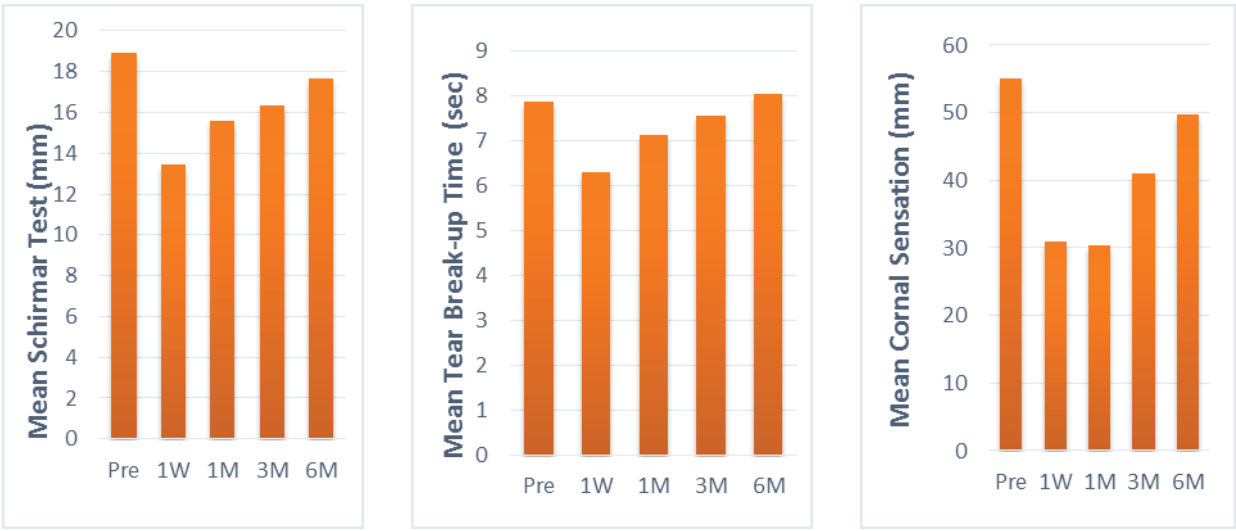
of the subsequent period. This was because of mending of the nerve fiber (Tab. 1).

Tear Break-Up Time (TBUT)

The mean preoperative BUT in this group was 7.88 seconds ± 0.82. At the end of the 1st postoperative week the mean BUT reduced to be 6.31 ±1.22, at the end of the 1st postoperative month the mean BUT was 7.14 ± 0.89, at the 3rd month was 7.57 ±0.70. Finally at the end of the 6th postoperative month the mean TBUT improved to 8.05± 0.98(Fig.1). The changes in the mean BUT were statistically non-significant at 1week, 1 and 3 months (p= 0.065, 0.465, 0.544 respectively) and statistically significant at the end of the 6th month (p=0.025) (Tab.1)

Schirmer Test

Measurably critical decay in the mean Schirmer test happened at the all postoperative period. The mean preoperative Schirmer test in this gathering was 18.92 mm ±1.41 Toward the finish of the first postoperative month the mean Schirmer test diminished to 13.43± 0.98 At the first month was 15.61 ±1.80. Toward the finish of the third postoperative month, the mean Schirmer test became16.33 ± 0.95. At last, toward the finish of the sixth month the mean Schirmer test was 17.67 ± 1.41 (Fig. 1). All the adjustments in the mean Schirmer test were factually noteworthy (p=0.001 ,0.032 ,0.042 at first week 1,3 months individually) and non-critical 0.321 at half year (Tab. 1).Statistically critical weakening in the mean Schirmer test happened at the all postoperative period. The mean preoperative Schirmer test in this gathering was 18.92 mm ±1.41 Toward the finish of the first postoperative month the mean Schirmer test diminished to 13.43± 0.98 At the first month was 15.61 ±1.80. Toward the finish of the third postoperative month, the mean Schirmer test became16.33 ± 0.95. At last, toward the finish of the sixth month the mean Schirmer test was 17.67 ± 1.41 (Fig. 1). All the adjustments in the mean Schirmer test were measurably noteworthy (p=0.001 ,0.032 ,0.042 at first week 1,3 months separately) and non-critical 0.321 at half year (Figure1).



A B C

Figure 1: Change in A. mean corneal sensation, B. mean tear film break up Time, and C. mean Schirmer Test with time in the nasal Group

Superior Hinge

This gathering included 300 eyes of 200 patients (80 guys and 120 females). The mean age for these patients at the hour of medical procedure was 26.01± 4.96 years old (range:19-38 years of age). There was huge factual contrasts for the examination of the corneal sensation, TBUT and Schirmer test at pre-employable evaluation and (first week, first month, third month and sixth month) as an events of correlation, where the connection were of feeble sorts with addition of follow up an ideal opportunity for the three pointers of intrigue (corneal

sensation, TBUT and Schirmer test) (Table 2)

Table 2: The differences in parameters before operation and 1week, 1, 3, and 6 months after operation of superior group

		Mean	Range	N	Std. Deviation	correlation	significant	Paired t test	P Value*
Corneal sensation	preoperative-superior After 1 week-superior	54.67 25.33	50-60 10-35	300 300	3.865 7.192	.010	.866	29.99	.001
	preoperative-superior After 1 month-superior	54.67 30.67	25-35	300 300	3.865 3.596	-.020-	.729	77.965	.001
	preoperative-superior After 3 month-superior	54.67 41.33	35-45	300 300	3.865 3.405	-.023-	.688	44.325	.001
	preoperative-superior After 6 month-superior	54.67 46.17	40-55	300 300	3.865 3.584	-.099-	.088	26.650	.001
T BUT	preoperative-superior After 1 week-superior	8.60 6.33	7-10 5-8	300 300	.954 1.077	.003	.955	27.337	.001
	preoperative-superior After 1 month-superior	8.60 7.33	6-9	300 300	.954 .871	.028	.627	20.190	.001
	preoperative-superior After 3 month-superior	8.60 7.93	7-9	300 300	.954 .773	-.058-	.318	20.113	.001
	preoperative-superior After 6 month-superior	8.60 7.87	7-9	300 300	.954 .807	.056	.329	10.463	.001
Schirmer	preoperative-superior After 1 week-superior	18.47 13.87	16-21 12-16	300 300	1.457 1.206	-.124-	.031	39.771	.001
	preoperative-superior After 1 month-superior	18.47 15.20	13-20	300 300	1.457 1.603	.032	.587	26.543	.001
	preoperative-superior After 3 month-superior	18.47 16.73	16-18	300 300	1.457 .574	.061	.290	19.584	.001
	preoperative-superior After 6 month-superior	18.47 17.47	16-19	300 300	1.457 .719	-.023-	.686	10.563	.001

p Value <0.05 was statistically significant. TBUT: tear film break time*

Corneal Sensation

The mean preoperative Corneal Sensation in this gathering was 54.67 mm ±3.86. Decrease of the sensation happened at the postoperative period to be 25.33 ±7.19 toward the finish of the principal week, 30.67 ±3.59 toward the finish of the primary month, 41.33 ± 3.40 toward the finish of the third month, lastly 46.17 ± 3.58 at sixth month follow-up.(Fig. 2) The adjustments in the mean corneal sensation during the subsequent period were factually noteworthy (p=0.001, 0,001, 0.001, 0.001 at the 1week and 1month, 3 months and a half year individually). The greatest increase of corneal sensation was seen toward the finish of the subsequent period. This was because of mending of the nerve strands (Table2)

Tear Break-Up Time (TBUT)

The mean preoperative TBUT in this predominant gathering was 8.60 ± 0.95 . Toward the finish of the first postoperative week the mean Yet decreased to be 6.33 ±1.07 , toward the finish of the first postoperative month the mean Yet was 7.33 ± 0.87, at the third month was 7.93 ±0.77. At last, toward the finish of the sixth postoperative month, the mean TBUT improved to 7.87 ± 0.809(Fig.2) . The adjustments in the mean TBUT were factually critical at 1week, 1,3 and a half year (p=0.001 , 0.001 ,0.001,0.001 respectively)(Table 2)

Schirmer Test

The mean preoperative Schirmer test in this gathering was 18.46 mm ±1.45 . Toward the finish of the first postoperative week the mean Schirmer test diminished to be13.86 ±1.20. At the 1stmonth it was 15.20 ±1.60 . Toward the finish of the third postoperative month, the mean Schirmer test got 16.73 ± 0.57. At last, toward the finish of the sixth month the mean Schirmer test was 17.46 ± 0.71(Fig.2). All the adjustments in the mean Schirmer test were measurably huge (p=0.001 ,0.001 , 0.001 and 0.001 at multi week, 1,3 and a half year separately) (Figure2)

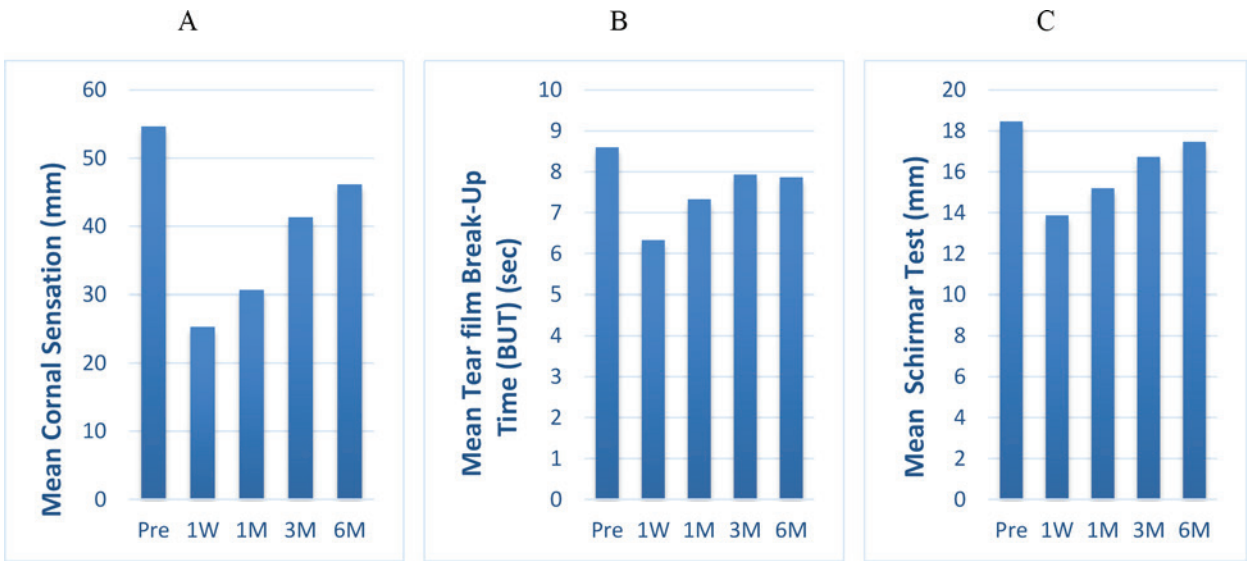


Figure 2: Change in A. mean of corneal sensation, B. mean tear film break up time, C. mean Schirmer test with time in the superior Group

Comparison between the results of the 2 groups

The two gatherings had been looked at with respect to the accomplished improvement in the corneal sensation, tear film separation time lastly the Schirmer test. There was no noteworthy measurable contrasts for the examination of the two gatherings at preoperative time, the corneal sensation show distinction at first week and sixth month, TBUT and Schirmer test at evaluation of the first month, and third month show huge contrasts while sixth month follow up show no huge distinction, where the relationship were of powerless sorts with addition of follow up an ideal opportunity for the three markers of intrigue (corneal sensation, Yet and Schirmer

test) (Figure3)

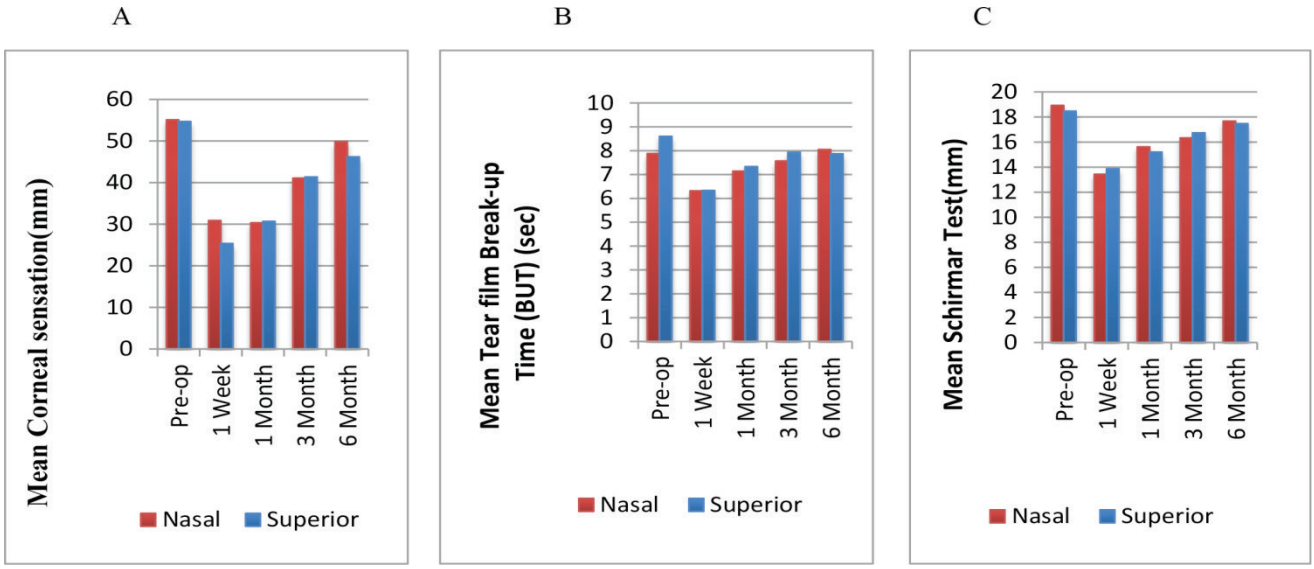


Figure 3: Change in A. mean of corneal sensation, B. mean Tear film Break up time, C. mean Schirmer test with time in both nasal and superior groups

All eyes had no intraoperative and postoperative flap complications.

Discussion

Laser-aided situ keratomileusis (LASIK) is one of the most generally performed refractive methodology with superb visual results. Dry eye disorder is one of the most regularly observed inconveniences after LASIK, with most patients creating probably some mellow dry eye indications postoperatively [19]. In LASIK medical procedure, a corneal fold is made, interfacing a corneal tissue pivot with the remainder of the cornea, at that point just the corneal stroma is removed utilizing the excimer laser. A microkeratome is utilized to make a corneal fold in LASIK. One of the major perceived inconveniences of this medical procedure is dry eye. LASIK has been accounted for decline in corneal affectability, tear film flimsiness, diminished watery tear creation and epitheliopathy of the cornea and conjunctiva [8,14,15,20-23]. Lasik methods went smooth and unremarkable. Descending uprooting of the fold was appeared in seven instances of the nasal gathering, which was overseen promptly with amazing visual result toward the finish of our subsequent period. Unfamiliar body and consuming sensation were appeared in the early postoperative period in 100 patients in the nasal and 120 patients in unrivaled gatherings which vanished later in all patients, no other postoperative difficulties were accounted for. No intraoperative troubles or entanglements were accounted for in the two gatherings as all. Our examination showed stamped decline in corneal sensation toward the finish of the first week postoperative with $p = 0.001$ which is going to arrive at factual hugeness level-between the two gatherings. Corneal sensation continuously improved through first and third months to reach practically approach the preoperative levels toward the finish of the sixth month in the two gatherings. Corneal sensation was at a higher clinical level everywhere on over the subsequent period in the nasal pivot bunch contrasted with the predominant pivot gathering despite the fact that, there was no noteworthy measurable contrasts for the correlation of the two gatherings at preoperative time, the corneal sensation show distinction at first week and sixth month, TBUT and Schirmer test at evaluation of the first month, and third month show critical contrasts while sixth month follow up show no huge contrast. Throw et al found that corneal sensation has gotten back to approach preoperative levels by 3 weeks after LASIK. This degree of return of corneal sensation is likely a helpful degree of recuperation to permit corneal security by the sensation-instigated squint and tearing reflexes [20]. This watched time is generously shorter than the recently announced time 9.3 months [24]. Donnenfeld et al showed a huge decrease in corneal

impression that didn't re-visitation of preoperative levels even by a half year in eyes with prevalent pivot folds, though there was full recuperation at a half year in the nasal pivot gathering ,additionally they detailed that mean corneal sensation was more noteworthy in corneas with a nasal-pivot fold contrasted and corneas with an unrivaled pivot fold at all postoperative visits ($P < 0.001$). The loss of sensation was most prominent at multi week and indicated improvement at each resulting time stretch as long as a half year. By and large, dry eye signs and indications were most noteworthy during the quick postoperative period and improved at all ensuing time stretches [25]. Kumano et al demonstrated that the diminishing in corneal affectability in patients with a nasal pivot was fundamentally more prominent than in those with a prevalent pivot at 1 and 3 months postoperatively despite the fact that there was no critical distinction between the gatherings at 6, 9,and a year after surgery [26]. In 2005, Vroman et al found a noteworthy distinction in nasal corneal affectability just at multi month, with better sensation in the nasal pivot group [27]. Nassaralla et al have arrived at a resolution that pivot area and fold thickness seem to assume a part in the pace of recuperation of corneal affectability after LASIK, with quicker recuperation related with more slender folds and a nasally found hinge [28]. Notwithstanding, in our examination we exhibited that the pace of recuperation was comparative in the two gatherings in spite of the fact that we didn't utilize the fold thickness as a looking at point as we made the fold thickness consistent in all instances of the two gatherings. The microkeratome cuts off a large portion of the nerves that course from the limbus to innervate the stroma and epithelium in the focal cornea. This may create a neurotrophic epitheliopathy that could cause diminished tear [14,23, 25,26,29, 30]. Ordinary tear work is fundamental for keeping up corneal capacity and structure. Dry eye has become an undeniably very much reported inconvenience following LASIK. Various potential etiologies clarifying the presence of this complexity have been proposed, including harm of the challis cells by the weight produced by the attractions ring, modification of the corneal ebb and flow influencing tear strength, and prescriptions that can actuate transient dry-eye symptoms [10]. In our investigation, we performed TBUT and Schirmer tests to equitably decide the distinction in tear-film security in both pivot gatherings. Despite the fact that, the mean preoperative Yet was 7.87 sec. what's more, 8.60 sec. in the nasal and unrivaled gatherings separately which is moderately low scores, there was no any clinical indications or indications of dry eye preoperatively. The outcomes indicated that in spite of the fact that tear creation and capacity seemed to diminish in the two gatherings, there was no factual essentialness contrast was seen between both nasal and prevalent pivot gatherings. Lee and Joo additionally looked at pivot area and dry-eye signs. They found a noteworthy. decline in TBUT at 2 months in the predominant pivot bunch contrasted and the nasal-pivot group [31].

Conclusion

There was a huge decrease in corneal sensation following LASIK in nasal and predominant gatherings. Sensation improved at untouched stretches and re-visitations of nearly, preoperative levels around a half year postoperatively. Related with the corneal denervation is a prompt postoperative increment in dry-eye as estimated by TBUT and Schirmer test that improved utilizing oils and with ensuing visits.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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Phenotypic and Molecular Detection of Pyocin from Multidrug-Resistant *Pseudomonas aeruginosa* Isolated from Various Pathogenic

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Abstract

This study included collecting (114) pure isolates of *Pseudomonas aeruginosa*, distributed over wounds (39) isolates, burns (31) isolates, urinary tract infections (23) isolations, ear infections (12) isolations, and respiratory infections (9) Isolates. The test for the sensitivity of the bacteria to (14) types of antibiotics showed a clear variation in their resistance to antibiotics, as all isolates showed 100% resistance to Ampicillin and Novabocin, Nalidixic acid by 93.8%, and Bacitracin by 87.7%, while they had low resistance to Ciprofloxacin, 21%, Cefotaxime 19.2%, and Chloramphenicol 18.4%. While the results of the phenotypic detection of pyocin showed the presence of (85 isolates, at a rate of 74.5%), producing Pyocin, while (29 isolates, at a rate of 25.4%), it showed its inability to produce Pyosin. As for the molecular detection of the *PrtN* gene, which is one of the most important genes responsible for the production of Pyocin, It was shown that (17 isolates, 77.2%) possess this gene, while (5 isolates, at a rate of 22.7%), they did not possess this gene.

key words**:** *Pseudomonas aeruginosa, Pyocin , PrtN.*

Introduction

Bacteriocins produced by *Pseudomonas aeruginosa* are called Pyocins, and many types of these strong bactericidal protein molecules can also be described [1,2], and produce them to compete and eliminate bacterial strains of the same species or related races, Polypeptides are highly toxic antibiotics known to transport across the outer membrane of many Gram-negative bacteria [3], These antibacterial proteins differ from conventional antibacterial agents, as they are derived from secondary metabolism. These toxins are generally produced under conditions of stress, such as nutrient depletion, overcrowding, and DNA damage, and induce expression and release of bacteriocins from decaying cells [4]. Its current development as an antimicrobial against multi-drug resistance of bacteria [5]. It can take the form of DNase or tRNase or it can inhibit peptidoglycan synthesis, and parasitize the nutrient uptake pathways of *P. aeruginosa* to achieve active trans-membrane transport. These extremely potent proteins are excellent tools for developing antibiotics [6].

There are three types of biosynthesis, types F, R, and S [7]. Pyocin synthesis begins when mutations increase the expression of the *recA* gene and activate the *recA* protein, which cleaves the inhibitor or inhibitor *PrtR*, and liberates protein expression leading to gene activation. *prtN* geneR Pyocinat R and F-Pyocins are derived from the ancestral gene, with similarities to the phage family and the phage family, respectively [2], It was found that the *prtN* gene, which is mainly responsible for Pyocin production, and the production of Pyocin is enhanced as a response to SOS, which induces DNAdamaging

under stress conditions [8]. In this study, we will test the ability of *P.aeruginosa* to produce Pyocin and its role in antibiotic resistance, as we will investigate Molecularly about how widespread the *PrtN* gene is.

Materials and Methods

Isolation and diagnosis

Our study collected (523) samples from August 15, 2019, to March 1, 2020, collected (114) included various clinical infections, including (Wounds, Burns, Urinary tract infections, Ear infections, Respiratory system infections), which were collected from Sharqat General Hospital In the district of Sharqat, Governorate-Salah al-Din-Iraq, these samples were grown on Nutrient agar, Macconky agar, Blood agar, and finally on cetrimide agar selective for *P.aeruginosa* to obtain more pure isolates.

The phenotypic and cultural characteristics were studied according to what was mentioned in [9], then the isolates were subjected to microscopic diagnosis according to what he described [10], then biochemical tests were performed which included Oxidase tests, Catalase, and IMVC tests (Indol, Methyl red, and Vogase-Proskauer and Simmon citrate), the H2S formation test, the movement test, the gelatin degradation test, the urease test, and the growth test at 45 ° C and 4 ° C, and these tests were conducted as stipulated [11], and the diagnostic tests were completed by confirmatory testing with the Vitic2 compact system.

Susceptibility test

The test was carried out according to the Kirby-Bauer Disk diffusion technique described in [12], and using (14) antibiotics supplied by the company (Bioanalyse_ Turkey) namely Ampicillin (25), Novabocin (30), Nalidixic acid (30), Cefotaxime (30), Bacitracin (10U), Ciprofloxacin (10), Aztreonam (30), Tetracycline (10), Trimethoprim (10), Vancomycin (30), Gentamycin (10), Rifampin (5), Chloramphenicol (30), Imipenem (10). The damping diameters were measured based on [13].

Phenotypic detection of Pyocin production

The test was carried out without the use of catalysts as prescribed by [23], where the Tryptcase broth soy medium was inoculated with one of the isolates with multiple antibiotic resistance and incubated in the incubator for 24 hours at a temperature of 37 C°, then the tubes were placed in a Centrifuge apparatus at 4000 rpm. For a period of 10 minutes, during this period, the medium of Muller Hinton agar was brought in glass dishes, and two holes were made in each dish and were applied to the activated bacteria *Staphylococcus aureus*, at a lifetime of 24 hours, with a dilution (10x1 cells/ milliliter). It was taken from the filtrate (100 µl) and placed in one of the pits and (100 µl) from the sediment and placed in the second hole by a micropipette, then the plates were incubated for 24 hours at a temperature of 37 C°, in the case of a suppressive diameter around the pits, these bacteria produce Pyocin and in If there is no inhibitory diameter, this means that the bacteria are not producing Pyocin.

Molecular study

Was selected (22) isolates from *Pseudomonas aeruginosa* isolates with multiple antibiotic resistance for molecular study, and DNA extraction was carried out according to the practical methods described in [15], while electrophoresis was carried out according to [16]. The polymerase chain reaction was conducted in laboratory conditions. Sterilized by using primers supplied by (Bioneer-Koria) for the diagnostic gene (Bioneer-Koria) for the 16 SrRNA diagnostic genes with a molecular size of 556bp and its gene sequence (F_GGAGCTTGCTCCTGGATTC, R_ GATGCAGTTCCCAGGTTGAG) and taking this gene from [17], and The Pyocin gene *PrtN* with a molecular size of 197bp and its genetic sequence (F_CCCTAGCACTCCGATTCCAC, R_ ACTGCATGGCCTTGTGACTA) which was designed for this study. Designed for this study, while the interaction program for each gene was approved as shown in Table (1(.

Table (1) PCR device program

Steps		The operation
1-		One cycle 5-minute cycle at 95 C° for initial template DNA mutilation
2-		35 cycles included: -
	A	30 seconds at 95 C° to scan template DNA
	B	30 seconds at 59 C° to attach the primers to the mold
	C	30 seconds at 72 C° to elongate bound primers
3-		One cycle 5-minute cycle at 72 C° for final elongation of the replicated DNA strand.

Results and discussion

Isolation and Identification

(523)bacterial samples were collected from different clinical sources, and after isolation and diagnosis, (114) pure isolates of *P.aeruginosa* were obtained, as in Table (6). These isolates were distributed among infections in wounds (39), burns (31), and urinary tract infections. (23), ear infections (12) and respiratory infections (9) note chart (1).

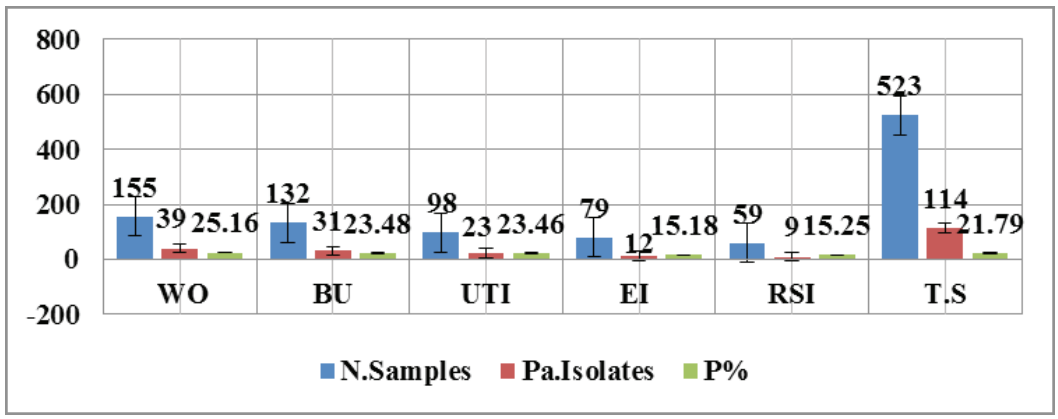


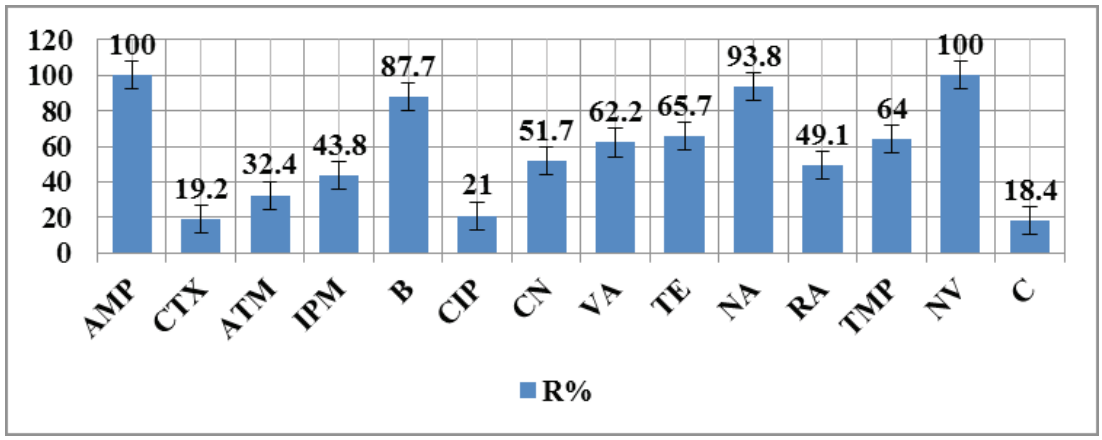
Chart (1) The ratios and sources of sample collection. Abbreviations_ WO: Wound isolates, BU: Burn isolates, UTI: Urinary Tract Infection isolates, EI: Ear Infection isolates, RSI: Respiratory tract infections isolates. N.Samples: Number of samples, Pa.Isolates: *P.aeruginosa* isolates, P%: percentage, X2 = 4.9150, P.value296 ns,

This result was consistent with that obtained by Shamaa [18], and this high percentage of *P.aeruginosa* may be attributed to what Lila *et al* [19] that the infection acquired in hospital contributes to the high rate of infection, and the main factor remains the lack of necessary support and weak implementation of prevention policies.And control and protocols related to this issue, limited human capacity and limited financial resources to combat this infection. Our study showed that the highest infections with *P.aeruginosa* are wound infections and then burns, and this is consistent with Al-Dulaimi's findings [17] and this is due to what Murali and his group confirmed (2014) [20] That this opportunistic pathogen is frequently present in wounds and burns infections. The reason for this may also be due to the fact that these bacteria are opportunistic pathogens that take advantage of a general or local defect in one of the body's mechanical or immune defenses, or both, to cause injury [21]. The reason may also be due to the contamination of burn wounds with these bacteria as a result of pollution

It may occur in the hospital environment, the tools used, and the hands of hospital workers. This contamination occurred because the burn wounds are vulnerable to infection due to the loss of the protective covering and the presence of a highly nutritious serum [22[.

Antibiotic resistance

The results of the Pseudomonas aeruginosa isolates under study showed a clear variation in their resistance to antibiotics, as all isolates showed 100% resistance to Ampicillin and Novabocin, and their resistance was high to Nalidixic acid by 93.8% and Bacitracin by 87.7%, while they had low resistance Against Ciprofloxacin by 21%, Cefotaxime by 19.2% and Chloramphincol by 18.4%, and all isolates showed multiple resistance to MDR antagonists, as noted in Scheme (2).

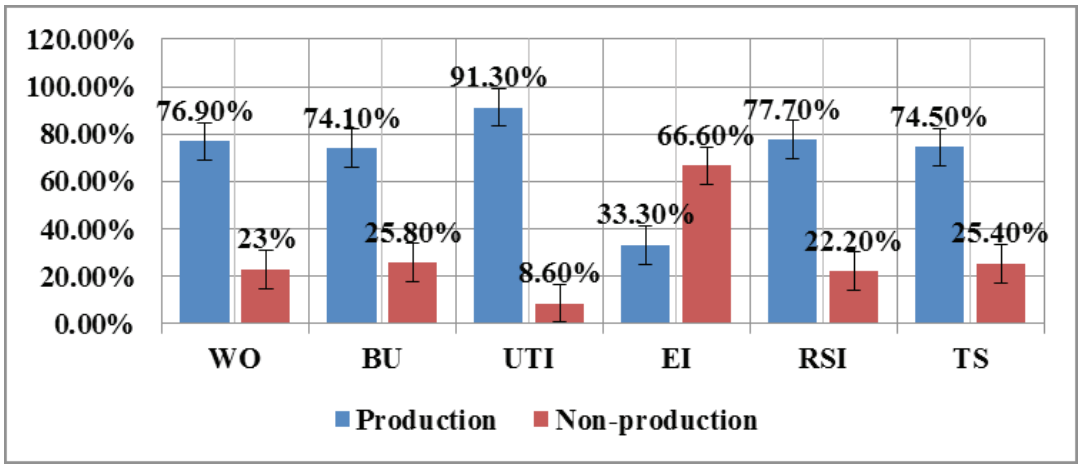


Scheme (2): Antibiotic resistance ratio of *P.aeruginosa* isolates. Abbreviations _ (CIP) Ciprofloxacin. (ATM) Aztreonam. (TMP) Trimethoprim. (CTX) Cefotaxime. (AMP) Ampicillin. (CN) Gentamycin. (TE) Tetracycline. (NA) Nalidixic acid. (RA) Rifampin. (IPM) Imipenem. (C) Chloramphenicol. (NV) Novobiocin. (VA) Vancomycin. (B) Bacitracin. S_Sensitive, I_Intermediate, R_Resistant X2 = 727.844 P-Value = 0.000008 **

The antibiotic resistance shown by *P.aeruginosa* is attributable to several mechanisms that this bacterium possesses, including the ability to change the permeability of the outer membrane, its production of ESBLs, the systems of flow pumps, the formation of biofilms, as well as its possession of resistance plasmids. [23], and the reason for the high resistance may be due to its frequent use, and this incomplete use of frequent and incorrect dose, which helps the bacteria to develop different resistance mechanisms towards these antibiotics [24].The difference in antibiotic resistance among Pseudomonas aeruginosa isolates indicates that these bacteria may use multiple resistance mechanisms simultaneously and that not all isolates necessarily use the same mechanisms to resist a particular class of antibiotics [25]. PAMDR can be acquired by the process of transmitting multiple resistance genes or plasmids from one bacterial cell to another [26], As well as the result of the combination of many resistance mechanisms, such as acquiring targeted mutations, enhancing multiple drug flow systems, and producing modified enzymes [27]. Several studies have demonstrated a relationship between Pyocin and multiple antibiotic resistance, as Pyocin induces alterations in LPS. This hinders the permeability of the outer membrane to antibiotics [28[.

Phenotypic detection of Pyocin production

The test results showed the presence of (85 isolates or 74.5%) producing Pyocin, while (29 isolates or 25.4%) showed their inability to produce Pyocin, and the highest isolates producing biocene were urinary tract infections (21 isolates or 91.3%), while The least productive isolates were ear infections (4 isolates, at a rate of 33.3%), as can be seen in Scheme (3).



Scheme (3) Pyocin production detection test for *P.aeruginosa* isolates under study. Abbreviations_ WO: wounds, BU: burns, UTI: ear infections, RSI: respiratory system infections, TS: total, X2 = 34.175 P-Value = 0.0008 **.

The inhibitory effect of Pyocin from sediment and filtrate on the growth of *S.aureus* bacteria indicates the presence of two or more types of Pyocin produced by *P.aeruginosa* isolates under study [29]. As noted in Figure (1). This result approximated the findings of Iwalokun *et al* [14] who obtained 81.5% of their isolates producing Pyocin. While Sharp *et al* [30] confirmed that Pyocin is produced by more than 85% of the *P.aeruginosa* strains. Our study demonstrated this productivity. It is high for Pyocin for antibiotic-resistant *P.aeruginosa* isolates. The inhibitory diameters ranged between (5-880 mm) indicating its great importance in protecting, virulence, and resistance of *P.aeruginosa* to antibiotics**.** Fan *et al* [31] recently revealed the role of Pyocin in antimicrobial properties and a contribution to the main antagonist resistance to ciprofloxacin. The characteristic of Pyocin production is genetically determined on chromosomes [32], and the decrease in production may be due to the presence of genetic mutations that lead to a change in the production trait. Or the reason may be due to the lack of use of stimulants in production [29]. Godard *et al* [33] indicated that Pyocin has an increasingly prominent role in a variety of serious infections in hospitalized patients and is the main cause of pneumonia and cystic fibrosis, and confirmed So [34], Govan [35] reported that Pyocin is more frequent in the pus of clinical isolates than in the environment.

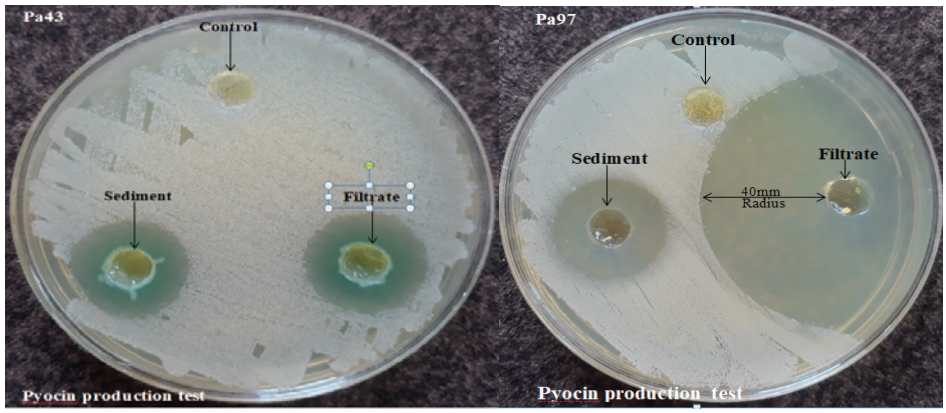


Figure (1) The inhibitory diameter in the pyocin production test.

Molecular diagnosis of *P.aeruginosa* and detection of the *PrtN* Pyocin gene

The final genetic diagnosis of these isolates was made by using the 16S rRNA gene and by using a polymerase chain reaction (PCR) device. It confirmed that the isolates of P. aeruginosa were observed in Figure (2).

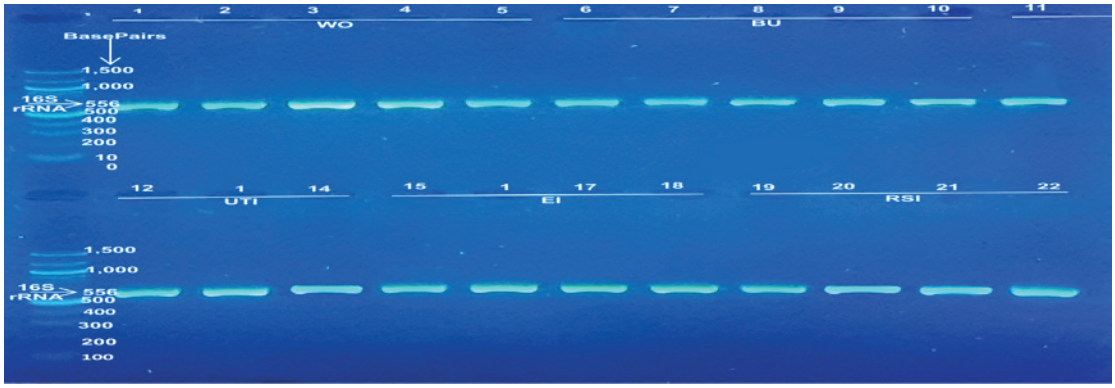
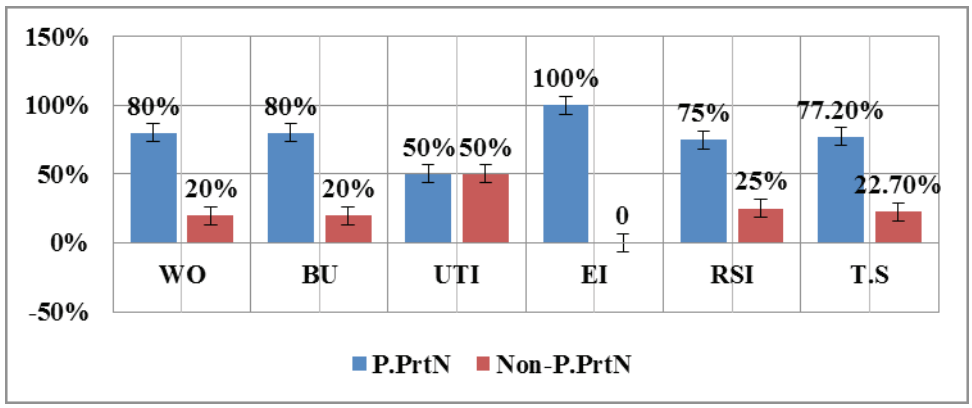


Figure (3) Electrophoresis of DNA polymerase chain reaction results using a *16srRNA* primer on mucinous gel medium at a concentration of 1.5% M: volumetric index 100 bp. Abbreviations_WO: wound isolates (1-5), BU: burn isolates (6-10), UTI: isolates Urinary tract infections (14-11), EI: ear infections isolates (15-18), RSI: respiratory tract infections isolates (22-19).

Diagnosis using the *16S rRNA* gene is one of the accurate diagnostic genes for *P.aeruginosa* and other species. It has a stable sequence for each bacterial species and has a very important role in both molecular diagnosis and classification [62[.

While the results of the screening for the *PrtN* gene revealed that (17 isolates, at a rate of 77.2%), possess this gene, while (5 isolates, at a rate of 22.7%), they showed that they do not possess this gene, and the most isolates possessing this gene were the ear infections isolates for all isolates by 100%, while it was The least possessing this gene are urinary tract infections isolates for two isolates, at a rate of 50%, as noted in the Scheme (4).



Scheme (4) The result of Detection for the *PrtN* gene in *P. aeruginosa* isolates under study. Abbreviations_ WO: Wound isolates, BU: Burns isolates, UTI: Urinary Tract isolates, EI: Ear Infection isolates, RSI: Respiratory infections isolates, TS: Total, P.*PrtN*: Possesses the *PrtN* gene, Non-P.*PrtN*: Has Biosyn gene *PrtN*

Our results did not agree with what Ghequire and De Mot [37-42-43] reported that biocin is a bacteriocin synthesized in 90% of *P. aeruginosa* strains. Another independent regulatory Pyocin that has been added to the *PrtN* gene includes the Quorum Sensing system. Matsui *et al* [39] indicated that many other genes regulate Pyocin production, namely *PrtR*, *PrtV*, *PrtH*, *RecA*, and *IexA*. It was found that the *PrtN* gene is responsible for the most common types of biocenes, which are R2, S1, S2, and F2, but it was found that Deleting the *PrtN* gene

does not stop the production of Pyocin. It was also found that mutations in the Pyocin genes lead to an increase in antibiotic resistance, especially fluoroquinolone and quinolone [40], and the production of Pyocin is subject to strict regulatory control controlled by the *PrtN* gene, which is a transcription activator that directly controls Expression of biosynthesis [41[.

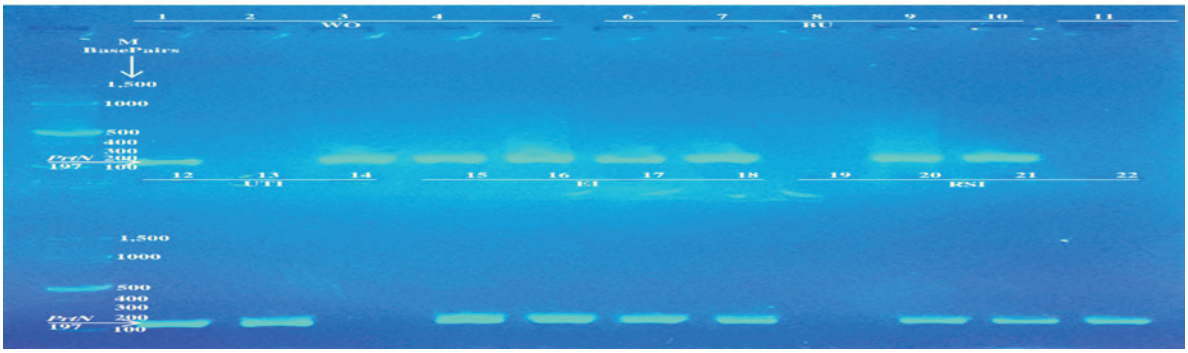


Figure (3) Electrophoresis of DNA polymerase chain reaction results using PrtN primer on a 1.5% M agarose gel medium: 100 bp volumetric index. Abbreviations_WO: Wound Isolates (1-5), BU: Burn Isolates (10-6), UTI: Urinary Tract Infection Isolates (14-11), EI: Ear Infection Isolates (15-18), RSI: Respiratory Infections Isolates (22-19).

Conclusions

Our study revealed the efficacy of Ciprofloxacin, Cefotaxime, and Chloramphincol in inhibiting *P.aeruginosa*, and the isolates also showed great susceptibility and attention to Pyocin production, which indicates its role in protecting these bacteria and their resistance to antibiotics. Isolates and their major role in the expression of Pyocin production.

Ethical Considerations:

Funding: The work was supported by the researchers themselves.

Conflict of Interest: The author declare no conflict of interest regarding this research.

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Evaluation of Lipocalin-2 and Visfatin, and Vitamin (D,C, and E) in Serum of Diabetic Patients with Chronic Periodontitis

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Abstract

Objective: The aim of the present study is to verify the hypothesis that salivary and serum lipocalin-2 and visfatin, and vitamin (D,C, and E) used as indicators for chronic periodontitis among DMT2 patients. Subjects and methods: We enrolled 41 type 2 diabetic patients with chronic periodontitis -18 males and 23 females, aged 60± 14 years and 49 controls- 26 males and 23 females, aged 61.8 ± 12.5 years. Blood samples were collected after an overnight fast and routine biochemical parameters such as glucose, lipocalin-2 and visfatin, and vitamin (D,C, and E)were determined in all samples. Data were considered significant at a level of $p < 0.05$.Periodontitis status was established using classic clinical parameters, Plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL). Results: 1) The mean serum of blood glucose, Visfatin, and LCN2 were higher in patients group than in those with normal control group ($P<0.001$ for all comparisons) ; 2) patients with had a lower Vitamin D,C, and E than those with a normal group ($P < 0.001$ for all comparisons).Conclusions: The activity of lipocalin-2 and visfatin in saliva and serum can be useful in diagnosis, monitorization and treatment of this disease. Further studies are needed to elucidate the role of lipocalin-2 and visfatin in periodontitis and systemic disease interactions.

Key words: *Type2 diabetes; Visfatin; Lipocalin-2;Serum.*

Introduction

The oral cavity is constantly being exposed to bacterial, viral, and fungal activity, and is dependent on a symbiosis between a strong resident microbiome and the innate immune responses for the maintenance of health. Human saliva is a biological fluid with myriad of biological functions important for the maintenance of oral and general health, it is considered as the gold standard in biochemical assays and analysis[(1,2)].

Periodontitis is a chronic inflammatory disorder mediated by specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone, with periodontal pocket formation, gingival recession, or both[(3,4)]. The condition is started by pathogenic microbes in dental plaque attached to the tooth surface and harbors a complex microbiological community by anaerobic bacteria (*Porphyromonas gingivalis*, *Treponema denticola*, *Prevotella intermedia*, *Prevotella nigrescens*, *Eikenella corrodens*, *Aggregatibacter actinomycetemcomitans*, among others) [(5)] , which increase the level of fructose in the body, causing insulin resistance to worsen[(6)].

In the same context, periodontitis can provide risk factor for diabetes, as chronic inflammation in periodontitis causes a systemic response to bacteria causing insulin resistance predisposing to DM or aggravating glycemic control and increasing the hazard of diabetic complications[(7)].

The adipose tissue is a complex, essential, and highly active metabolic and endocrine organ. that releases a large number of bioactive mediators such as adipokines, hepatokines and myokines, involved in inflammatory processes, pointing toward comparable pathways involved in the pathophysiology of DM, periodontitis, and related inflammatory diseases among these mediators lipocalin and visfatin[(8-10)].

The present study was undertaken to evaluate the levels of lipocalin and visfatin, and vitamin (D,C, and E) in patients with both the chronic diseases, i.e., type 2

diabetes and chronic periodontitis.

Materials and Methods

Forty-one type 2 diabetic patients with chronic periodontitis -18 males and 23 females, aged 60± 14 years and 49 non-diabetic individuals 26 males and 23 females, aged 61.8 ± 12.5 years were selected from the population referred to the Periodontal Clinic of Tikrit University, from December 2019 to February 2020.The data were collected through a standard questionnaire. All subjects were interviewed regarding a full medical history that included age, sex, occupation, duration, and family history of diabetes mellitus. The biochemical parameters such as glucose, lipocalin-2 and visfatin, and vitamin (D,C, and E) were measured. The material for the study was the peripheral venous blood. Samples were drawn after an 8 to 12 hour of overnight fast. All the tubes were subjected to centrifugation at 3000 rpm for 10 minutes followed by storage at -40ºC until assayed.

Clinical periodontal parameters, including plaque index (PI), and bleeding on probing (PPD) were measured. PD and CAL were measured at six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual), except for third molars[11]. All clinical examinations were carried out by a single examiner, who was trained, calibrated, and masked to the systemic condition of the patient.

Statistical analysis was executed by SPSS 20 (SPSS inc., Chicago, IL) program. The independent *t*-test was used for comparison of means of quantitative variables. *P*<.05 was considered statistically significant.

Results

The mean age of patients in the patient group was 60± 14 years, whereas the mean age of participants in the control group was 61.8 ± 12.5 years, there was no significant difference between the groups as regards age (p>0.05). Among the 41 patients in the diabetic with CP group, 18 (44%) were male and 23 (56%) were female. Among the 49 participants in the control group, 26 (53%) were male and 23 (47%) were female.

Table No. 1 Basic characteristics of study groups:

Parameters	Diabetic subjects with CP N = (41)	Control subjects N = (49)
No. of subjects	41	49
Sex (M/F)	18 (44%) /23 (56%)	26 (53%) /23 (47%)
Age (years)	60± 14	61.8 ± 12.5

As expected, the mean values of PI(1.5±±0.089 vs 0.37±0.178) GI (1.63±0.08 vs. 0.00), PPD (1.88±0.121 vs. 3.74±0.34), and CAL (2.9±0.38 vs. 0.00) between the study group and control group were statistically highly significant (*P*<0.001) [Table 2].

Table No. 2 Comparison of mean periodontal parameters PI, PPD and CAL between the both groups.

	Group 1 (n=50) Patients	Group 2 (n=50)
Plaque index	1.5±±0.089	0.37±0.178*
Gingival index	1.63±0.08	0.00
Pocket probing depth (mm)	1.88±0.121	3.74±0.34*
CAL (mm)	2.9±0.38	0.000

Table No. 3: Comparison of study biochemical parameters in PCOS and control subjects.

Parameters	Group A (Control)	Group B (Patients)	P value
Fasting blood glucose (mg/dl)	101.2 ± 7.18	184 ± 10.2	<0.0001
Visfatin (pg/ml)	16.17 ± 0.93	23.59 ± 1.13	P<0.001*
Vitamin D (ng/ml)	33.1 ± 14	20.71 ± 10.03	P < 0.05
vitamin C (mg/dL)	1.18±0.182	0.71±0.151	P < 0.001
Vitamin E (mg/L)	13.36±1.432	5.62±0.811	<0.001*

The mean Vitamin D level in case group was 18.726 ± 6.259 ng/ml, while in control group, it was 42.851 ± 14.516 ng/ml with $P < 0.05$ which is highly significant.

Discussion

Type 2 diabetes mellitus (T2D), a subclass of diabetes mellitus that is not insulin responsive or dependent, is a metabolic disorder that is categorized by chronic hyperglycemia caused by increased production of glucose in the liver and increased peripheral insulin resistance, which might eventually lead to a reduction in insulin secretion[12-14].

Visfatin,also known as nicotinamide phosphoribosyl transferase a 52 kDa molecule , is one of the most recently identified adipokine that is highly enriched in the visceral fat, It is a pleiotropic adipocytokine, which acts as a cytokine, a growth factor and an enzyme[15], with a potential glucose-lowering effect due to its nicotinamide phosphoribosyltransferase (NAMPT) activity[16].

Insulin bind to different sites of visfatin non-competitively, leading to the insulin signaling cascade, increasing glucose uptake, and inhibiting glucose release[17]. The reason for the higher level of serum visfatin in these patients may be due to the following[18-21] :

I. Visfatin may be related to the aging-dependent circadian cycle, which lead to the decline of pancreatic cell function, insulin mimetic effects, increased plasma visfatin levels could be a compensatory mechanism in response to hyperglycemia that ameliorates the functional consequences of insulin resistance.

II. impairment in visfatin signaling in target tissues or dysregulation of its biosynthesis in response to hyperglycemia, hyperinsulinemia, or adipocytokines in the condition of diabetes.

III. visfatin mediated nicotinamide adenine dinucleotide biosynthesis that regulates glucose-stimulated insulin secretion

IV. It has more potent destructive and pro-inflammatory properties and has a crucial role in the diligence of inflammation through reticence of apoptosis and neutrophils

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kD , recently identified adipokine. which mainly released from granules of activated neutrophils. It is expressed in several tissues including adipocytes, neutrophils, salivary gland, stomach, liver, lung, kidney endothelial cells, macrophages, and vascular smooth muscle cells which induced by many pro- and anti-inflammatory cytokines , factors like lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-7, or IL-17, and anti-oxidant enzymes reducing free radicals in a variety of cell types[22-23]. It is an acute phase protein plays a pivotal role in glucose homeostasis and insulin sensitivity vascular remodeling, plaque instability specifically LCN2 expressed in macrophages[24].

Lipocalin-2 as a novel autocrine and paracrine adipokine, acts as an antagonist to the effect of inflammatory molecules on inflammation and secretion

of adipokines[25]. LCN2 was significantly elevated in patients with diabetes compared with controls.LCN-2 might be an indicator for β cell dysfunction, it is consider as iron delivery protein. Increased LCN-2 levels will increase cytoplasmic iron which may result in β cell oxidative stress and impaired insulin secretory capacity and activation of ferritin[26].

Vitamin D refers to a group of fat-soluble a fat-soluble biomolecule, which was first discovered in 1919–1924 discovered in 1922 by McCollum as an antirachitic agent[27] synthesized in the skin by the action of ultraviolet irradiation from the sun related to bone metabolism and skeletal integrity as well. Currently, vitamin D, and especially its most reactive metabolite, 1,25(OH)2D3 (calcitriol), is considered a hormone involved in complex endocrine systems and modulating growth and differentiation of cells from various lines, [28]. As well its best known for its role in regulating calcium and phosphate metabolism[29]. The synthesis and secretion of insulin is effected by existence of the vitamin D response element (VDRE) in the human insulin gene promoter and transcriptional activation of the human insulin gene triggered by 1,25(OH)2D[30].

Because chronic periodontitis is characterized by bone loss triggered by a host immune response reaction to bacterial plaque[31]. Vitamin D is believed to improve the body's sensitivity to insulin and thus reduce the risk of insulin resistance. It can also regulate the production of insulin in the pancreas through control of the insulin receptor gene[32]. Vitamin D deficiency could negatively affect the periodontal health. Its anti-inflammatory effect is mediated through inhibition of cytokine production, stimulation of monocytes and macrophages, and secretion of peptides with potent antibiotic activity. Recently, its effect on periodontitis through its anti-inflammatory effect[33].

Although evidence for the relationship between vitamin D status and DM is sparse, The possible mechanisms assumed that role of VDD in DM systemic inflammation by the formation and the effects of modulating cytokines, which lead to enhanced insulin sensitivity and promote beta-cell survival, and insulin resistance through induces the expression of the insulin receptor, thus increasing insulin sensitivity during transportation of glucose and pancreatic beta cell malfunction via a change of calcium flux, which can adversely affect beta cell function[34].

Vitamin C (ascorbic acid), a water-soluble vitamin, is an essential nutrient, that exerts a reducing and anti-oxidant effect, which scavenges free radicals and protect cells from oxidative stress, acts as an as an essential co-factor for the hydroxylation of proline and lysine which is vital to collagen biosynthesis in connective tissue, and probably reduce insulin resistance by improved endothelial function and lowering oxidative stress[35-37]. It is structurally similar to glucose and can replace it in many chemical reactions and thus is effective for prevention of nonenzymatic glycosylation of protein[38-45].

In DM subjects with CP, serum vitamin C was significantly lower compared with healthy individuals. Possible mechanisms for reduced vitamin C levels in diabetes assumed that the elevated free radicals caused by hyperglycemia reduce vitamin C, renal reabsorption of vitamin C is reduced and increase in clearance, increased turnover of vitamin C, due to the increased oxidation of ascorbate to dehydro-ascorbic acid in tissue mitochondria[38-40]. Deficiency of vitamin C increases susceptibility to infection, impair the function of neutrophils and macrophages, reduces antibody -mediated, cell- mediated, phagocytic and delayed type of hypersensitivity reactions and depletion of antioxidants[41].

Vitamin E is the most important lipophilic membrane radical-scavenging antioxidant antioxidant and that protect the cells from reactive oxygen species prevent damage of lipids particularly of polyunsaturated fatty acids. The decreased level of vitamin E results in damage to internal structures by enhanced free radical production[42,43.44].

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way and the samples were collected according to the guidelines of blood sample collection guidelines provided by WHO.

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In Vivo Treatment of *Giardia lamblia* by *Plantago lanceolata L.* extract and Investigation some Serological Parameters and Histopathological Changes

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Abstract

Giardia lamblia samples were taken from patients at Salah al-Din General Hospital /Tikrit, suffering from Giardiasis from October 2018 until March 2019. cysts of *G. lamblia* was isolated and infected in laboratory mices Balb/c and then therapeutic attempt was made with water extract of *Plantago lanceolata* in three different concentrations .and some biochemical parameters have been studied through rate of changes in Cholesterol, Triglycerids, Total protein and some of liver enzymes (AST) Alanine transaminase, (ALT) Aspartate transaminase, and response of infected mice for treatment with water extracts.

A significant decrease was observed in concentration of lipids in general. The decrease was significant in concentration of Cholesterol and Triglycerides and total protein in laboratory mice. The lowest concentration was achieved when using extract with 0.5 ml, 121, 31, and 4.311 mg/dl Respectively, The highst concentration when using extract was with 2 ml, 180, 36, and 5.220 mg/dl Respectively, compared to control group 184, 37 and 5.040 mg/dl Respectively, Significant changes were also observed.

As for concentration of both AST/ ALT, the results showed significant changes in their concentration in infected mice. level of decrease was significant in their concentration. The lowest concentration of AST was at 0.5 mL, 18 IU/L, and ALT 15 IU/L at 0.5 mL of extract, The highest concentration of AST was at 2 mL, 30 IU/L, and ALT 29 IU/L at 2 mL of extract compared to control group of 28 IU/L. Significant changes were observed in concentration of AST and ALT after treatment with therapeutic extract Above.

The microscopic examination of tissue sections of infected mice showed presence of a detachment of some lining cells of colon. presence of leukocyte infiltration at base of mucous glands was also observed. This infiltration was extended to smooth muscle layer forming colon wall. colon cavity consisted of large masses of mucus. In liver, there was an enlargement of hepatic cells and presence of degeneration of nuclei of these cells, and found small concentrations of inflammatory cells between hepatocytes, some central veins in liver, and observed lymphocytic infiltration.

Key words: *Giardia lamblia*, *Plantago lanceolata*, histopathology, biochemistry tests.

Introduction

G. lamblia is a protozoan causes Giardiasis and belongs to Retortamonada phylum and Diplomonadea class Diplomonadida oeder[1]. The parasite include through two stages, traphozoite and cyst, and infection occurs by eating food and drink contaminated Cyst in unhealthy conditions, and traphozoite live in the duodenum, jejunum and upper ileum of humans and feeds on epithelial cell lining the host's intestines, causing Giardiasis [2,3].

P. lanceolata plant is the used part of leaves, the whole plant and the juice of plant, and its chemical components are mucilages by 2-6%, including rhamnogalacturonane, abinogalactane, glucomannans and iridoide monoterpenes by 2-3%, the most important are catalpol, aucubin and flavonoids, the most important are catalpol, aucubin and flavonoids. Compounds of caffeic acid esters, the most important of which are acteoside, neochlorogenic a, chlorogenic acid, and tannins 6%, and a quantity of soap compounds, silicic

acid 1%, aesculetin and minerals, the most important are zinc and potassium [4,5]. The juice of green plants has anti-bacterial properties and aucubigenin resulting from the breakdown and hydration of aucubin and the soapy compounds contribute to these properties, and the leaf juice speeds up the process of blood clotting and wound healing, and is used to treat coughs and bronchitis, and as a urinary tract and in the treatment of cystitis, urinary incontinence, gastric colic and ulcers. Infectious, liver diseases, respiratory tracts and diarrhea, and is used topically in the treatment of mouth and pharyngitis, skin and wound infections, and conjunctivitis of the eyelids[6].

Aims study to study effect of different concentrations of *P. lanceolata* extracts in experimental treatment of Giardiasis and its effect on some biochemical variables such as cholesterol, triglyceride, total protein, some liver enzymes AST and ALT, and its histopathological effects.

Materials and Methods

1- collection of parasite samples: *G. lamblia* samples were taken from patients at Salah al-Din General Hospital / Tikrit, who suffer from severe diarrhea to average and most often with blood fat diarrhea, during period from October 2018 to March 2019 ,The samples were isolated by Sedimentation and Floatation method and preserved [7,8].

2-Preparation of water extract : leaves of *P. lanceolata* were taken from local markets. They were washed and cleaned from dirt and suspended soil. They were dried by oven and at room temperature until drying to ensure that the leaves did not rot and were infected with fungus. dry leaves were kept in tightly sealed containers under humidity-free conditions.

Preparation of water extract doses (0.5,1,2) for *P. lanceolata* by bringing the plants in oven at 25C° for 45 minutes and then grinded with a powder-shaped electric mill. Add to warm water 40° until powder is dissolved in water and then filtered the solution and take the leachate and leave precipitate [9,10] and put vegetative suspension on the rotary evaporator device for two hours, after which suspension was filterted.

3-Experimental animals: 60 mice Balb-C, aged 6-8 weeks and weight 10-20 g, were taken from Animal House of the Faculty of Veterinary Medicine/ Tikrit University. The experiment was conducted at Animal house in Biology Department / College of Education for women / Tikrit University / Iraq.

4- experiment Design: laboratory mice weights ranged between 28.6-34.7gr, averaged 31.65gr, and randomly evaluated into 5 groups:

Group 1 (negative control group): 10 mice were injected with Normal Saline solution.

Group 2 (positive control group): 20 mice were injected orally with *G. lamblia*.

Group 3: 10 mice were treated with *P. lanceolata* extract and 0.5 ml/L (0.015gr/gr body weight) daily for 10 days after infected with *G. lamblia*.

Group 4: 10 mice were treated with *P. lanceolata* extract and at 1 ml/L (0.031gr/gr body weight) daily for 10 days after infected with *G. lamblia.*

Group 5: 10 mice were treated with *P. lanceolata* extract and at 2 ml/L (0.063gr/gr body weight) daily for 10 days after infected with *G. lamblia*.

5 - Administration of laboratory mices: The laboratory mices were injected orally with *G. lamblia* (1000 systs) orally using a modified syringe. In same way, negative control animals were injected with a Normal Saline solution. For two weeks after infection, cysts were examined in infected mice faeces to confirm infection. Mice were removed after 10 days of treatment with extracts, blood samples were withdrawn from heart and placed in test tubes free of anticoagulant left approximately 15 minutes at 37C°. Serum was then obtained by Centrifuge at 3000 rpm For 15 minutes, and kept at -20C° in new plastic tubes and clean until biochemical tests.

4- Determination of serum triglycerides, cholesterol, and total protein concentrate: Its concentration was estimated using BIOLABO SA-France kit.

5 - Determination of Aspartate transaminase (AST) in serum: It was measured using several special analysis processed by Randox English Company (NO.147) kite.

6. Determination of Alanine transferrase ALT in serum: Its concentration was determined using several

special analyzes processed by Randox English (NO.146) kite.

7- Preparation of Histopatholohical sections: histological section was prepared for liver, intestine and kidney attended by [11,12].

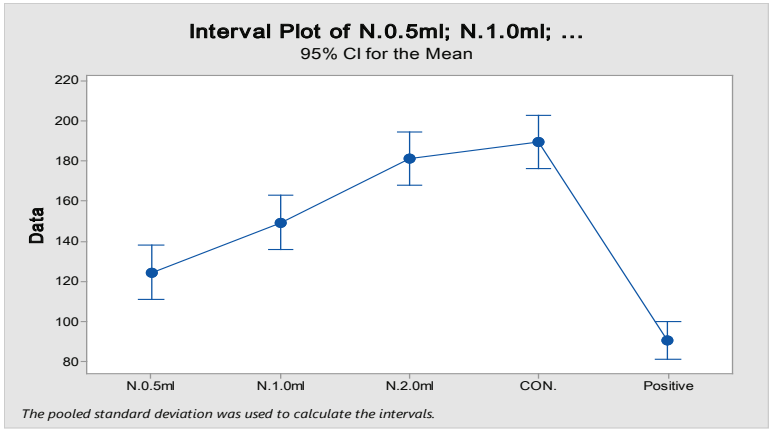
8-Statistical Analysis: The statistical analysis system (SAS) (2010) used to study the effect of different factors in the studied traits. The differences between mean were compared with the least significant difference (LSD). The percentages were compared with Chi-square test.

Results and Discussion

The results showed a difference in concentration of lipids and total proteins in laboratory mice with Giardiasis and treatment with different concentrations of the plant extract of *P. lanceolata* and using three concentrations (0.5, 1 , 2) ml. It was noted that cholesterol decreased its concentration when using extract with 0.5 ml concentration Was 121 mg/dL, while its highest concentration was 2 ml at 180 mg/dL compared with control group of 184 mg/dL (Fig. 1).

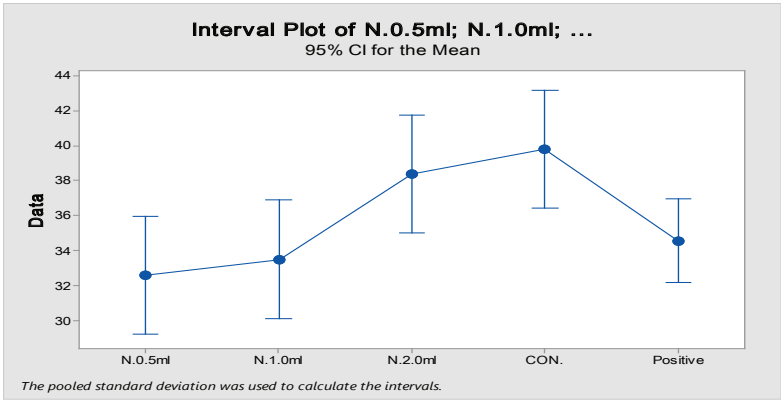
It was also observed that triglycerides were affected by use a minimum concentration of water extract 31 mg/dl at 0.5 mL concentration, while the highest concentration of triglycerides was 36 mg/dl at 2 ml of plant extract compared with control group of 37 mg / Deciliter (Fig. 2).

The total protein was 5.220 mg / dl when using 2 ml while the lowest rate was 4.311 mg/dl when the dose was 0.5 ml compared with control group of 5.040 mg/dl. The statistical analysis showed significant differences between the three concentrations of water extract of plant where the highest effect of was used at 2 ml at 5.220 mg / dl (Fig. 3).



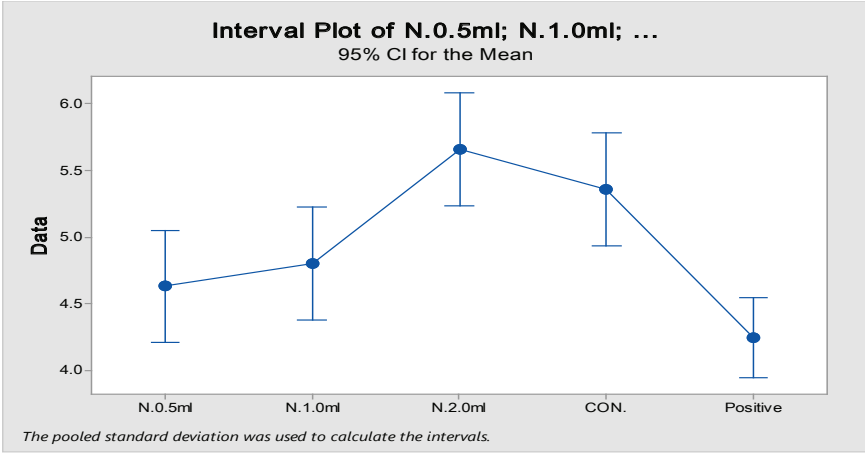
Chol. Con.mg/dl

Figure (1): Effect of *P. lanceolata* extract to the level of serum cholesterol in infected mice by *G. lamblia*



trigly. Con.mg/dl

Figure (2): Effect of *P. lanceolata* extract to the level of serum triglycerides in infected mice by *G. lamblia*



Total protin. Con.mg/dl

Figure (3): Effect of *P. lanceolata* extract to the level of serum total protein in infected mice by *G. lamblia*

The results of study agree to the findings of Bansal and Khaleel [12,13,18] , which recorded a decrease in lipid profil concentration level in general, they pointed out that parasites use fat during growth within host body, that cause of change in fat level occurs in acute cases In which host contains large numbers of *E.histolytica* where parasite casing proteins metabolize fats and cholesterol in acute cases where the intestinal fat is absorbed. As for how cholesterol enters parasite, the studies hypothesized that the parasite takes the fat needed by lipoprotein molecules that transport the free cholesterol found in the intestines and tissues of host, While this study did not correspond to what was reported [14], which indicated high cholesterol for people with parasites *E.histlytica* and *G.lamblia*.

The reason for the high cholesterol, triglycerides and total protein at 2 ml of plant extract is that many studies have shown that the mint is used treatment of various infections because it contains anti-inflammatory substances, and gall bladder secretion and liver stimulator and remove the toxic effect of cells [15,16].

The results showed that liver enzyme AST in infected white mice with Giardiasis and treatment with different dose of *P. lanceolata* was affected by dose amount. The lowest concentration was at 0.5 ml and U/L was 18 IU/L while highest concentration was at 2 ml Reaching 30 IU/L compared to control group of 30.00 IU/L.

liver enzyme ALT was affected by dose of water extract at 15 IU/L at a dose of 0.5 ml while the highest concentration of enzyme was at dose of 2 ml at 29 IU/L compared to control group which amounted to 28 IU/L On effect of water extract of the plant on enzyme very clearly, where statistical analysis showed that there are significant differences in liver enzyme ALT was more affected than enzyme AST (Table 1).

The results of current study showed a decrease in the concentration of liver enzyme AST/ ALT in infected mice with Giardiasis, possibly because the parasite caused poor absorption and diarrhea, leading to a disruption of certain liver functions [17].

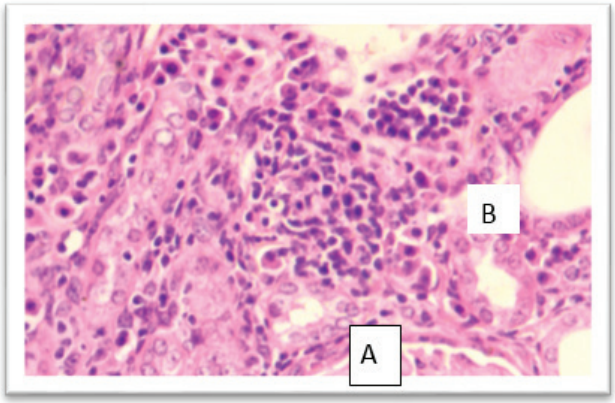
Table (1): Effect of *P. lanceolata* extract to liver enzymes (AST/ALT) activety in sera of mice infected with *G. lamblia*

activety	ALT U/L		AST U/L	
	Mean	St Dev	Mean	St Dev
0.5ml(0.015gr/gr)	15	4.31	18	5.29
1ml(0.031gr/gr)	21	6.82	24	4.25
2ml(0.063gr/gr)	29	8.94	30	3.90
Positive	14	4.05	17	5.96
Control	28	4.73	30.00	2.906
	F-Value = 21.09 ** P-Value =0.0007		F-Value =20.55** P-Value = 0.0006	

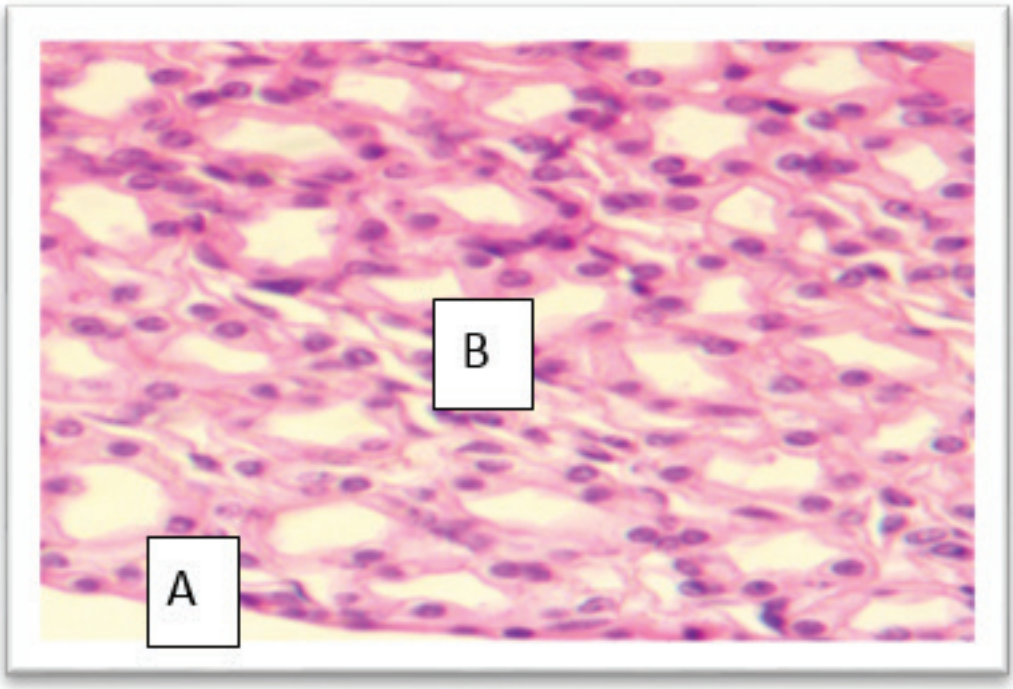
The microscopic examination of tissue sections from control group (negative group) showed presence of renal glomeruli in cortical tissue that filled each Bowman purse and on surface of each glomerulus of lymphocytes, which were found around Bowman's portfolio as well. All the proximal and distal tubules had no satisfactory (Fig. 1). The presence of renal leukocytes is surrounded by a number of inflammatory cells, especially lymphocytes, and largest number of renal tubules have appeared correctly (Fig. 2.3)

In mucous layer of intestine, the long, finger-shaped villi are lined with simple vertical epithelial cells arranged regularly with the emergence of capillary cells among these cells. The pulp of villi contains several lymphocytes that are continuously leaking from the underlying page under those vesicles with mucous glands, And short in villains core (Figure 4).

The hepatocellular of liver contains multiple forms and sizes of hepatocytes, where most of cells near the capsule appear enlarged and free of cytoplasm with other hepatocytes, which are small in size with presence of nuclei in some cells. The central vein in middle of the liver is a blood clot with leakage Some lymphocytes with covert cells are present in some blood cells (Figure 5).



Figure(1): cortex of kidney (control group-): renal tubules (A), distant tubules (B) (H & E, 200x)



Figure(2) :kidney cortex core (control group-): renal tubules (A), (H & E, 200x)

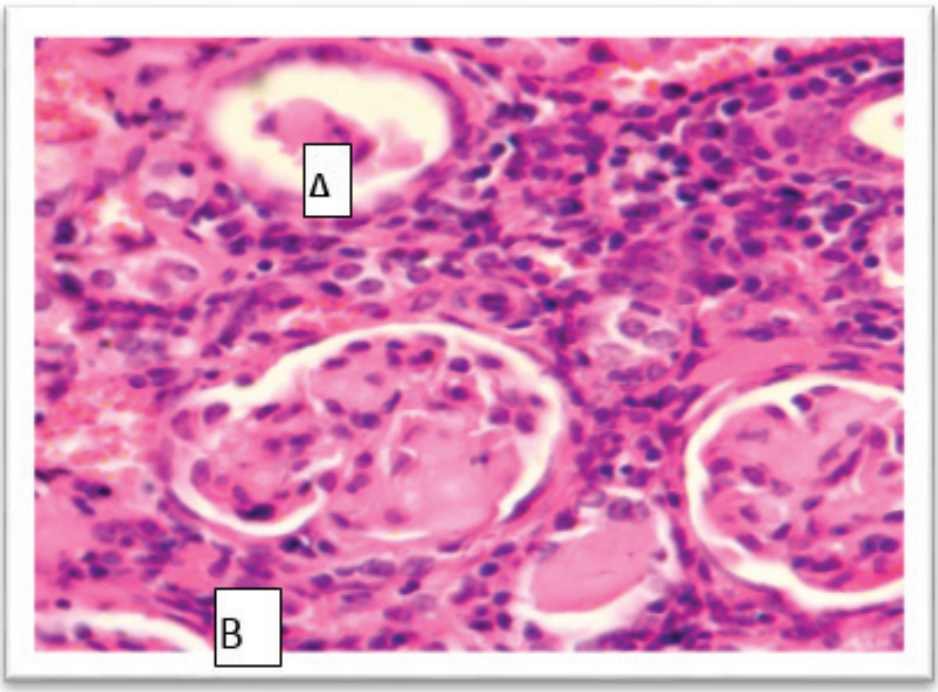


Figure:(3) 0.5ml concentrate(+group treated), kidney core: acute blood congestion (A), lymphatic nodules about renal tubules (B) (H & E, 200x)

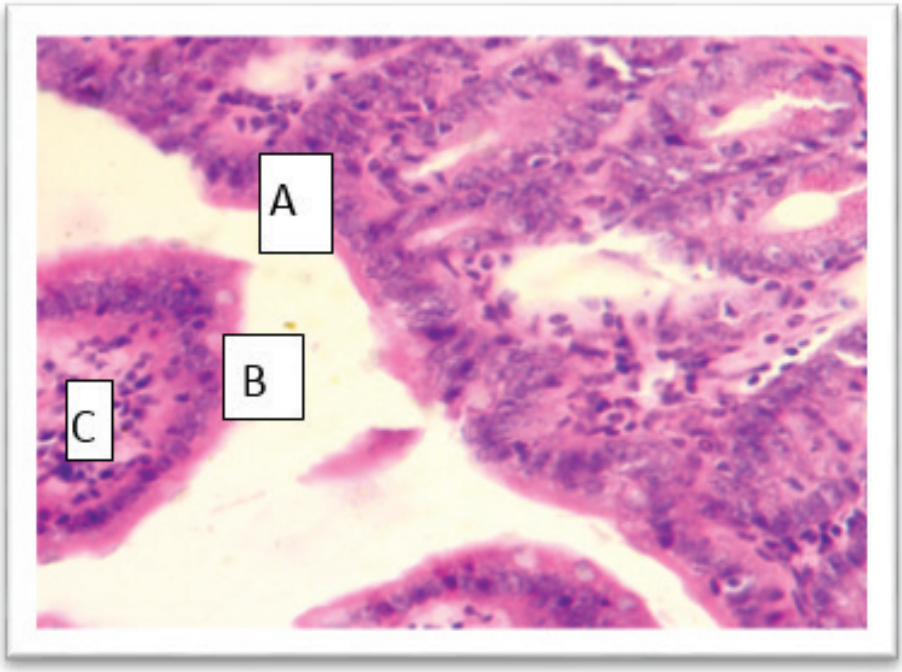
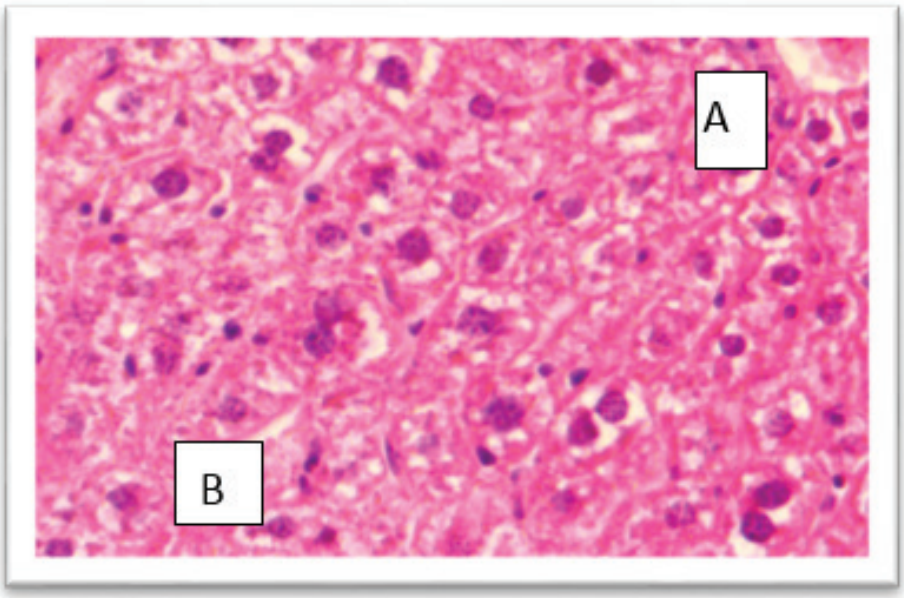


Figure (4): infected intestines(+group): intestinal villi (A), clotting in some vertical cells (B), lymphocytes in core of vesicles (C) (H&E, 200x)



Figure(5) :Liver (+group): central vein has a blood clot in hepatocyte (A), Coffer cells (B) (H & E, 200x(

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way and the samples were collected according to the stool collection guidelines.

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The Caries Experience in the Smoker and Non-Smoker among College Student

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Abstract

Dental caries is the scientific term for tooth decay or cavities. It is caused by specific types of bacteria. They produce acid that destroys the tooth's enamel and the layer under it, the dentin. The bacteria turn sugar and carbohydrates (starches) in the foods we eat into acids. Smoking is also a global and national health problem and is one of the most significant health risks that impact all age groups.

The Study Aims to investigate the effects of different tobacco smoking methods and their relation to dental caries in comparison to non-smoker students.

Material and Method: A random sample of 336 students from Tikrit university aged 19-24 years, the sample grouped to cigar, shisha, cigar and shisha together and non-smoker groups. Dental caries was recorded according to WHO criteria (1987) were decayed, missing and filled with permanent teeth (DMFS). The data were analyzed as a descriptive statistic includes frequency, percentage, mean, and for statistical analysis of the data using T- test and one- way ANOVA test to detect the significance of the relation between various variable.

Result: the result of this study showed that the severity of dental caries higher in smoking cigar and smoking shisha than in the non-smoking group with statistically highly significant.

Conclusion Nicotine may be increasing the incidence and severity of dental caries. So that, nicotine might be considered as a risk factor for inducing of dental caries.

***Key words**: dental caries, cigar, shisha, non-smoker*

Introduction

Dental caries is a well-known infectious disease that affects all ages [(1)]. Caries results from the interplays between enamel and dentin tooth structurers, diet, and cariogenic bacteria. These interactions make the bacteria to release acidic byproducts resulting in degrading the hard tissues of the teeth [(2)]. Without treatment, caries lesion has the potential to advance and reach the pulp, which can result in severe pain [(3)]. Cariogenic bacteria such as Streptococcus mutans that colonize on the firm surfaces of the oral cavity performs a vital role in plaque development and dental caries formation. Streptococcus mutans competes with other bacteria to attach to the tooth surface. S. mutans is remarkably aciduric and acidogenic. It produces organic acids, mainly lactic acid, which damages the enamel by demineralization [(4)].

Smoking dates to 5000–3000 BC, when the farm product began to be cultivated in South America and Mesoamerica; consumption later developed into burning the plant material either by chance or with the intent of exploring other ways of consumption [(5)]. Tobacco was first introduced in France in 1560 by a Frenchman named Jean Nicot (from whose name the word nicotine is derived). Tobacco then spread from France to England [(6)]. Tobacco products are extensively used by cigarettes, which are often smoked widely [(7)]. about 1.1 billion adults (29% of the adult population) are cigarette smokers throughout the world [(8)]. Tobacco use forces a huge and increasing burden on public health worldwide. Approximately 5 million death annually is attributed

to tobacco use. According to the current trends, it is expected that by the year 2030, this number will increase to 10 million, with about 70% of these deaths occurring in low- and middle-income nations [9].

The main consistency of tobacco smoke is the nicotine, which is an alkaloid from the oxygen-free alkaloid cluster [10]. There is no clear explanation of why smoking increases the risk of dental caries. The relationship between dental caries and smoking has been the subject of debate. A common belief in the earlier literature was that smoking helps to reduce dental caries [11, 12, 13]. Schmidt, in 1951, held this belief when he proclaimed that an increase in tobacco smoking was succeeded to decrease caries rate [14]. Thiocyanate concentration, a constituent of tobacco smoke and in normal saliva, was found a higher in the saliva of the smokers, thiocyanate caries inhibiting effect on dental caries [15]. So, one might predict fewer dental caries in smokers. However, the reduced buffering effect and probable lower pH of the smoker's saliva and the more significant number of Lactobacilli and S. mutans may indicate a higher susceptibility to caries [15, 16]. Several types of research had recorded tobacco smoking as a risk factor. In the UK, there was a considerable increase in root and coronal caries due to exposure tobacco products for years [17]. also, there was a dose-dependent relation between root caries and tobacco in the USA [12] other studies significantly showed the effect of smoking can lead to dental caries, tooth discoloration and gum recession which may lead to uneven margins on the crown and other restoration [18].

There is limited information concerning the consequences of nicotine on oral bacteria and S. mutans, and important microbe in the etiology of dental caries. Nicotine has been described within the saliva of smokers and can stimulate the growth of S. mutans and likely place smokers at increased chance for dental caries [19,20].

Material and Method

Study participants were constituting of a random sample of 336 students from Tikrit university aged 19-24 years, the student in the sample was from the different colleges of the university, the duration of data collection was from October 2018 to May 2019. Smoking status was obtained through a questionnaire. The students with a history of systemic diseases which may affect the result were excluded. The tooth was diagnosed to be caries according to the criteria suggested by WHO, 1987 [21]. were decayed, missing, and filled with permanent teeth (DMFS). The examination of dental caries was conducted by using plane mouth mirrors and bland explorers. Radiographic studies were not used. The students were categorized into three groups as follows: non-smoker 80 students, smoker cigarette only 156, smoker shish 100 students and the duration of smoking range between (1-3) years, all the students are male-only. Data analyzed using a statistical program (SPSS - 10); were descriptive statistic includes frequency, percentage, mean, standard deviation, and standard error and statistical analysis of data using T-test and ANOVA test used to detect the significance of the relation between the variable. P-value <0.05 was considered as statistically significant while P-value >0.05 was considered as statistically not significant and P-value < 0.01 was considered as highly significant.

The Results

A total of 336 students were included in this study. The age of study participants was between 19 and 24 years old. The distributions of the total sample by types of smoking are seen in Table (1).

Tables (2) illustrates the mean values, standard deviation and standard errors of caries severity for permanent dentition in the non-smoker and smoking cigar, DMFS mean value for the smoking cigar students was found to be higher (19.53 ± 0.50) than in non-smoker students (9.20 ± 0.45). The differences were found to be statistically highly significant (T-test value =15.39, P-value = 0.001).

The mean values of caries experience for permanent dentition (DMFS), were found to be higher in the students smoking shisha (12.50 ± 0.47) than non-smoking students, statistically, there were highly significant differences founded between them (T-test = 5.05, P-value = 0.015) as seen in table (3), while the mean value of DMFS was higher in the students were smoking cigar than in students who smoking shisha. The difference was reported to be statistically highly significant (T-test = 10.25, P-value = 0.002) as shown in table (4). Analysis using One-way ANOVA between three groups showed statistically highly significant F-value = 123.91, P-value = 0.00006 (smoking cigar

19.53 a, smoking shisha 12.50 b and non-smoking 9.20 c) this illustrated by table (5) and diagram (1).

Table (1) distribution of total sample according to the types of smoking.

Type of smoking	number	Percentage %
Non-smoker	80	23.81
Smoking cigar	156	46.43
Smoking shisha	100	29.76
total	336	100

Table (2) Caries experience of permanent dentition (mean, standard deviation and standard error of DMFS index) among non-smoking group and smoking cigar.

Types of smoking	mean	SD	± SE
Non -smoker	9.20	0.78	0.45
Smoking cigar	19.53	0.86	0.50

T-test value = 15.39, P-value = 0.001

Table (3) Caries severity of permanent dentition DMF index (mean, standard deviation and standard error) among non-smoking group and smoking shisha.

Types of smoking	mean	SD	± SE
Non -smoker	9.20	0.78	0.45
Smoking shisha	12.50	0.82	0.47

T-test value = 5.05, P-value = 0.015

Table (4) Caries severity of permanent dentition DMF index (mean, standard deviation and standard error) among smoking cigar group and smoking shisha.

Types of smoking	mean	SD	± SE
Smoking cigar	19.53	0.86	0.50
Smoking shisha	12.50	0.82	0.47

T-test value = 10.25, P-value = 0.002

Table (5) the differences between the total sample (smoking cigar, smoking shisha and non-smoking) by using One-way ANOVA (Analysis of Variances).

Types of smoking	numbers	Mean	SD
Smoking cigar	3	19.53 a	0.86
Smoking shisha	3	12.50 b	0.82
Non-smoking	3	9.20 c	0.78

F-value = 123.91, P-value = 0.00006

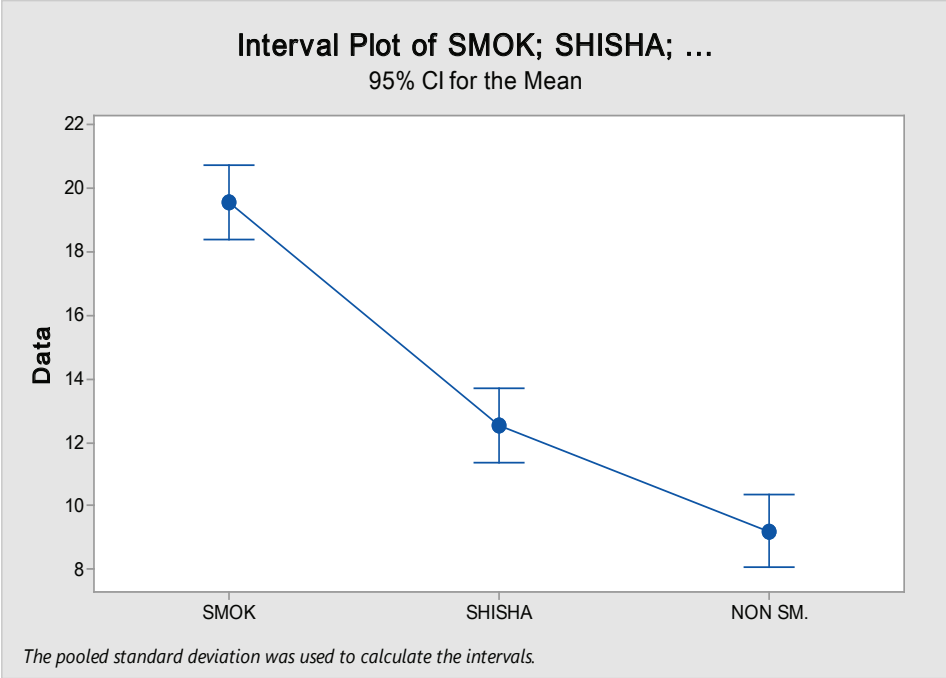

Diagram (1) the caries experience for the three groups

Discussion

The present study is the first Iraqi study that deals with the relation between different types of smoking and dental caries in Tikrit city. The caries experience (DMFS) in this study was found to be higher in the smoker students (cigarette and shisha) than in the non-smoker students which agrees to the other studies [22.23.24]. Also, this study agrees with the study was conducted in Japan 2013, where clinical data of smokers and non-smokers from 753 clinics concluded that patients who smoked had significantly more treatment procedures than non-smokers [25]. This is may be due to that nicotine stimulates biofilm of metabolic activity of oral pathogens such as Streptococcus mutans, a Gram-positive oral bacterium; as a result, this stimulation increases S. mutans biofilm formation and metabolism of sucrose into lactic acid, consequently contributing to dental caries [26] in addition, various sugars and sweeteners are added deliberately during tobacco production method up to 4%wt or can be up to 13%wt of sugars. Sugars used as a cigarette additive include glucose, fructose, invert sugar (glucose/fructose mixture) and sucrose. Also, many tobacco additives contain a high amount of sugars, for example, fruit juices, honey, molasses extracts, cones, and maple syrup and caramel. The added sugars are usually reported to serve as flavor/casing and humectants. However, sugars also encourage tobacco smoking, because they create acids that neutralize the sharp taste and throat impact of tobacco smoke. furthermore, the sweet taste and the pleasant smell of caramelized sugar flavors are appreciated by starting adolescent smokers [27]. Smokers usually had bad oral hygiene and less primitive outlook on health, besides, they usually have different eating habits, presumably consuming a high number of sugar-containing products like soft drinks and snacks [22,28]. Daily smoking has been reported to be associated with an increase in the use of sugar in coffee or in tea, and with more frequent alcohol consumption [29.34,36].

The current study added to multiple other studies to confirm the harmful effects of smoking and disagree with early studies reported reduce dental caries in individuals with a smoking habit, which reported that smoking reduces dental caries [12,14, 30]. In contrast is the case reported by Zitterbart et al., which did not find any relation between dental caries and nicotine [31.32]. Some researchers estimated the percentage of the highest amount of caries-promoting such as sugar and caries-inhibiting chemicals such as fluoride in commercially

available [33.35].

Conclusion

Our Study conducted that dental caries higher in the smoker than in the non-smoker student and dental caries showed higher prevalence in the student that smoke cigar than smoking shisha. Smoking with cigar or shisha may be increased the incidence and severity of dental caries. So, nicotine might be a risk factor for smoking-induced caries.

Ethical Considerations:

All Research participants haven't been subjected to any kind of harm in any way and the dental caries was recorded according to WHO criteria (1987) were decayed, missing and filled with permanent teeth (DMFS).

Funding: The work was supported by the researchers themselves.

Conflict of Interest: The author declare no conflict of interest regarding this research.

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Comparison of the Nutrition on Different Fats on Lipid Profile, and Some Biochemical Parameters and their Relationship with Liver and Kidney Function in Male Rats

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Abstract

This study was conducted on forty-nine healthy male albino males rats, from 1/11/2019 to 12/30/2019 for two months in the animal house of the College of Veterinary Medicine - University of Tikrit, their ages ranged from 3-4 months and weights of 250-350 g current study aimed to determine the effect of nutrition on the different types of fat in the levels of blood lipids, kidney function, and liver enzymes. The results showed a significant increase with a level of 0.05 (p≤) in blood sugar concentration in the two groups of animal fat and vegetable oil group with a concentration of 8% g / kg of diet, and the two olive oil groups did not show significant differences compared to the control group. Levels of cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins in a group that was given fat animal by 8% g / kg of diet and a significant decrease in the group of olive oil by 4% g / kg of diet, significant increase (p≤ 0.05) in the concentration of high-density lipoproteins in the two groups that have been treated with Olives oil by: 4% and 8% g / kg, respectively. There were no significant differences in the rest of the other study treatments compared to the control group, and a significant increase of (p≤0.05) in the concentrations of urea, creatinine and uric acid in the animal fat group by 8% g/kg of the diet, and a significant decrease in the group that was treated with olive oil. As for the liver enzymes activity ALT, AST, and ALP there was a significant increase (p≤ 0.05) in the group that was treated as animal fat by 4% g / kg of diet, while we did not observe significant differences in the group that was treated with olive oil by 4% g / Kg of diet compared to a control group.

Keywords: *Biochemical markers, Liver function, kidney function.*

Introduction

Fats are one of the essential and main substances in food, as well as fats are one of the most important nutrients for humans, as metabolism generates many biologically active molecules [1] Fats are of great importance in our daily diet because they are an important source of energy stored in the body because they are play main role in the synthesis of cellular membranes as well as in the manufacture and synthesis of steroid hormones, and has a role in the manufacture of fat-soluble vitamins, and in the regulation of vital body functions, as well as in the manufacture and formation of bile acids important in the emulsification of fats [2] Whereas one gram of fat produce 9 kilograms / caloric energy twice as much as protein and carbohydrates, as the excess of fat is stored in fatty tissue and in the liver until it is needed [3], humanity faces many health problems regarding the fat content of food due to its association with common diseases as a result of this relationship, the cholesterol content of food has become an important topic after the recommendations made by many studies to reduce fat consumption in food [4], Fats differ in some characteristics depending on the source of the fat, and some physical characteristics of fats are an important factor in distinguishing between one fat and another depending on its composition and content in fatty acids [5]. Fats are classified according to their source to animal fats, which is the food supply obtained from animal sources and includes Cow and sheep fat, and milk fat from cows and sheep, are all saturated fats that are solid at room temperature [6]. Free beef fat is considered a complex fat consisting of saturated fatty acids such as short, medium and long chain fatty acids [7]. Free fat is considered a healthy source of edible fats with many beneficial properties. It is also considered a

powerful antioxidant because it contains carotenoids and some vitamins dissolved in fats such as A, E, and D, in addition to that they contain minerals such as calcium and magnesium all give high oxidative efficacy against many diseases [8].

Any oil product extracted from plants is called Vegetable fat, and possesses many fruits, whether seeds or pulp, on different amounts of oil, and the most important plants are sunflower, soybeans, peanuts, olive oil, palm oil and other plants, and vegetable oils are one of the most important plant ingredients. Which are mainly included in food [9], as well as vegetable oils are a good source of energy and of great economic importance in addition to being consumed largely in the prevailing diet [10], It is characterized by its containment of unsaturated fatty acids that the body cannot synthesis. Among the most important vegetable oils, olive oil is one of the healthiest nutritional oils [11]. Those who are deprived of the best types of oils, and the health-promoting component of a healthy diet, and he noted its effects on the pathogenic genes involved in diseases such as heart disease, cancer and neuropathy through the chemical components of olive oil Such as biophenol and oleic acid, and vitamin E, as well as containing phenolic compounds and monounsaturated fatty acid and unsaturated fatty acids, which act as antioxidants [12]. In addition to the above, many recent studies have confirmed the ability of monounsaturated fatty acids to limit the cardiovascular system through their ability to reduce lipid profiles, inhibit the oxidation of low-density lipoprotein LDL-C and activate antioxidants in the body [13], and sunflower oil is considered one of the most important oilseed crops in the world [14] It is also considered one of the important oils in human nutrition and is yellow in color and light in density [15] and contains polyunsaturated fatty acids, which is linoleic acid (48-74%) and monounsaturated fatty acids, which are oleic acid (14-40)., %) And linolenic acid and contains saturated fatty acids in a small percentage represented by palmitic acid and stearic acid, and that the consumption of these fats is beneficial to health and thus reduces the risk of developing atherosclerosis and heart disease by lowering the level of cholesterol in the blood [16].

Materials and Method:

Experimental animals:

Albino males rats were used in this study. The ages of the animals ranged from (3-4) months and their weights ranged from (250-300) grams. The animals were obtained from animal house of the College of Veterinary Medicine at the University of Tikrit, and placed in plastic cages with mesh metal covers prepared for this purpose. The animals were subjected to laboratory conditions of light, temperature of 25 C°, and the degree of humidity ranged between 10-30% throughout the study period. the animals were subjected to a two-week introductory period for the purpose of acclimatization to the place and leeches before starting the treatment after ensuring her safety from diseases.

Experiment design

In this study, 49 white male rats were used, and they were divided randomly into seven groups, and each group consisted of seven animals of similar weights as follows:

* The first group (C), the control group, was given throughout the trial period only a standard diet.

* The second group, A1, this group was given the feed treated with animal fat (4% g / kg) of the diet throughout the experiment period.

The third group, A2, is the group of animals that were given animal fat (8% g / kg) from the diet and water throughout the experiment period.

* The fourth group, P1, a group of animals that were given vegetable oil (4% g / kg) of the diet throughout the experiment period.

* Fifth total, P2, the group of animals that were given vegetable oil at a ratio of (8% g / kg) throughout the experiment period.

*The sixth group, O1, is a group of animals that were given olive oil (4% g / kg) of the diet throughout the experiment period.

*The seventh group, O2, the group of animals that were given olive oil (8% g / kg) throughout the experiment period.

Preparing of food processing:

The components of the concentrated feed mixture were obtained from the College of Veterinary Medicine, University of Tikrit, and its components and the chemical composition of the diet used in feeding rats were in accordance with the nutritional and physiological requirements established by it [17] Seven types of diets were used in this study, which include the standard diet especially for the control group and three types of diets. Added to it 4% for each kilogram of a diet, animal fat, vegetable oil, olive oil, and the other three diets add 8% to each kilogram of rations from animal fat, vegetable oil and olive oil, respectively.

The feed mixture for rats was modified by adding wheat flour to it to facilitate the formation of the food pieces, then the food used in feeding rats was made by adding the required weight of fat to the flour, then adding the flour and mixing it well, then it was added to the other ingredients and distributed well to obtain a uniform distribution of the fat, Then forming the food after mixing it with a little distilled water and placing it in the feed machine for the purpose of obtaining food in the form of fingers with a diameter of 1.5 cm and a length of 1-2 cm, then drying the food at room temperature for two days away from sunlight to avoid dust and insects, and then Complete the drying in an electric oven at a temperature of 50 ° C and store the dried food in a cool dry place in nylon bags, and number the bags according to each group [18].

Blood sample collecting:

After the end of the 60-day trial period, blood was drawn once every 14 days, with four withdrawals, as blood was drawn from the vein located in the eyehole according to [19] and blood samples were collected in plastic tubes with tight covers and left for a period of 20 minutes at laboratory temperature, after that, the serum is separated from the blood plasma by a centrifuge for a period of ten minutes at a speed of 3000 rpm, after which the serum is kept in freezing at a temperature of -20 ° C until the laboratory tests are carried out.

Biochemical tests

1- Determination of serum glucose concentration: The concentration of glucose in the blood serum was determined using the France Biolabo Analysis Kit, an enzymatic method in which glucose is oxidized.

2- Determination of serum cholesterol concentration: The determination of serum cholesterol was carried out using a ready-made kit from the Spanish company Bio Systems [21].

3- Triglyceride concentration measurement: The measurement of TG concentration in rat blood serum was determined by the enzymatic method by using a ready-made kit from the Spanish company (Bio Systems) [22].

4- Determination of the concentration of high-density lipoproteins of cholesterol in serum: The concentration of high-density lipoprotein (HDL-C) for cholesterol in the blood serum of rats was determined by using a ready-made kit from the Spanish company Bio System [23].

5- 5- Determination of the concentration of low-density lipoproteins of cholesterol: The concentration of C-LDL has been estimated in light of the following equation:

LDL-c(mg/dl)=Total cholesterol-(HDL-C +VLDL-C

6- determination the concentration of very low-density lipoproteins for cholesterol: The value of VLDL-C was calculated according to the following method:

VLDL-C concentration (mg/dl)=(Triglycerides/5) according to [22].

7. Urea-Creatinine Concentration Measurement: The concentration of creatinine and urea in the blood serum of the experimental animals was measured using the Analysis Kit manufactured by France, BIolabo [25].

8. Determination of uric acid concentration: The concentration of uric acid in the blood serum was measured using the BIOLABO SA, France test kit.

9. Determination of the activity of the enzyme alanine amino transferase transporter enzyme activity and aspartate amine transporter enzyme: the enzymatic methods were used to measure the activity of AST and ALT in the blood serum by using of a ready-made analysis kit from the French company BIOLABO.

10. Determination of the effectiveness of alkaline phosphatase enzyme: it was measured according to the colorimetric method shown in the analysis kit of the French-made Bio Merieux company

- Statistical analysis: The results were statistically analyzed using the One-way ANOVA system to extract the significant differences between more than two averages from the experiment groups with emphasis on these differences by measurement the Standard Error (SE), where the statistical analyzes were performed with significant differences (P≤0.01), P≤0.05) [26].

Results and Discussion

Comparison of the effect of nutrition on different lipids in blood sugar, cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C concentrations:

Table (1) shows a comparison of the effect of nutrition on different types of fats in the concentrations of blood sugar, cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C for the study treatments, where the highest concentration of blood sugar level was recorded in group A2 that was treated with animal fat at (8% g / kg of diet). The reason is that following a diet containing saturated fats can reduce the activity of intracellular enzymes associated with fatty acid synthesis and thus reduce the ability Intracellular use of insulin [27]. The O2 group that was treated with olive oil at a rate (4% g / kg of the diet) recorded a significant decrease with the level of P (≤ 0.05). The reason is due to the nature of the active ingredients and their concentrations in olive oil, the oil contains high concentrations of polyphenols, especially compounds Olirubin, in addition to that, monounsaturated fatty acid, especially Oleic acid, works to improve blood sugar concentrations by reducing insulin resistance in cells and preventing blood lipid disorders that accompany a decrease in the concentration of glucose in the blood. Also, a significant increase was observed, with a P-value of 0.05 level, in the concentrations of cholesterol and triglycerides, LDL-C and VLDL-C, in the A2 group that were treated with animal fat by (8% g / kg of diet).

Nutrition on saturated fats raises the level of cholesterol and triglycerides, and since animal fat contains a high percentage of saturated fatty acids and these acids are Lauric Palmitic and Meristic, therefore their consumption led to an increase in harmful fats in the serum, as an increase in the proportion of fats leads to an increase Chylomicron production in the intestine and when it degrades causes the release of fatty acids. Therefore, liver cells will receive large quantities of fatty acids, causing an increase in the liberation of triglycerides, as the increase in TG is caused by an increase in VLDL and chylomicron, which leads to hypertriglyceridemia resulting from increased LDL. C and low HDL-C[28,29]. The time for feeding on olive oil leads to a reduction in the lipid profile because it contains squalene, which works to remove free radicals and thus reduces LDL oxidation, or it may be due to its containment of phenolic compounds that reduce or inhibit the reactions of the lipid peroxidation chain reaction.

The reason is due to the inhibition of the Triglyceride Lipase enzyme responsible for the fragmentation of triglycerides, and consequently, an increase in the metabolism of fats, and that nutrition on olive oil leads to an increase in high-density lipoproteins. Its antioxidant activity increases the activity of Catalase and Superoxide dismutase (SOD) in the liver [30].

Table (1) Comparison of the effect of nutrition on different lipid types in blood sugar concentrations and lipid profiles throughout the study period.

Treatments/ features	control	Animal fat4%	Animal fat8%	Vegetable oil P1	Vegetable oil P2	Olive oil O1	Olive oil
Glucose	87.45±17.7 B	103.70 ±17.94 a	108.00±23.44 a	97.65 ±14.23 ab	106.77 ±12.93 a	90.37±5.19 b	91.24 ±7.58 b
Cholesterol	96.93± 3.11 Cd	107.05 ±20.59 bc	122.30 ±20.98 a	101.90 ±18.10 bc	111.09 17.20± B	89.16 ±7.60 d	98.48±12.04 c

Cont... **Table (1) Comparison of the effect of nutrition on different lipid types in blood sugar concentrations and lipid profiles throughout the study period.**

Triglyceride	69.05±5.75 c	83.65±5.75 b	103.55±34.40 a	82.00 ±14.11 b	84.41 ±18.14 b	78.11±7.37 bc	80.38 ±9.32 b
HDL-C	34.500 ±2.94 B	34.650 ±4.24 B	35.850 ±7.10 b	34.500±2.81 b	37.182 2.15 ± ab	38.316 2.49± a	39.810 ±3.65 a
LDL-C	52.17±4.73 B	54.26 ±15.25 B	65.57±10.11 a	51.53 ±15.14 b	56.98 ±14.48 B	35.31±7.00 d	42.70 ±10.69 C
VLDL-C	13.790 ±0.99 C	16.640 ±2.83 B	20.520 ±6.80 a	16.32 ±2.72 b	16.65 ±3.44 B	15.537 ±1.37 bc	16.162 ±1.85 B

A comparison of the effect of nutrition on different types of fats in the concentrations of urea, creatinine and uric acid:

Table (2) shows the effect of nutrition on different types of fats in the concentrations of urea, creatinine and uric acid. The results of current study showed a significant increase in the levels of urea, creatinine and uric acid in a group that was fed on animal fat by (8% g / kg of the diet) and a significant decrease in the group that were given olive oil by (4% g / kg of the diet). The excretion of urea is a result of the metabolism of proteins and amino acids, and that the estimation of the concentration of urea is an important and diagnostic function of disorders and diseases of the kidneys such as urinary tract infections, stones in the kidneys and deficiencies in their functions [31] The results of our current study agree with [32] when feeding on saturated fats leads to raising the level of cholesterol and triglycerides, thus leading to oxidative stress, free radical production, and oxidation of proteins and amino acids. This process results in an increase in the production of urea, and that feeding on unsaturated fats, especially olive oil, reduces the level of urea because it contains polyphenols and maintains its normal level in the body, as well as the effectiveness of antioxidants and vitamin E that prevent oxidative effect to renal cells and glomeruli. Creatinine is one of the least variable compounds in concentration levels as it is one of the best and most accurate tests for indicating the safety of the kidneys is to estimate the concentration of creatinine in the blood serum. The results of our current study agree with [33], the reason of the decrease in the concentration of the Cyclooxygenase-2 enzyme, which leads to the production of Prostaglandins, and this leads to a decrease in the renal blood flow, impair the kidney function and increase the levels of creatinine in the blood.

Uric acid is the final product of the purine metabolism processes in the body as well as acts as an antioxidant in some liquids. It is an inhibitor of lipid peroxidation and converts some metal ions such as copper and iron into weakly reactive forms capable of capturing hydroxyl radicals [34]. Excessive uric acid concentration is due to an increase oxidative stress from cardiovascular disease.

Table (2) shows the effect of nutrition on different types of fats in the concentrations of kidney function.

Treatments/ features	control	Animal fat4%	Animal fat8%	Vegetable oil P1	Vegetable oil P2	Olive oil O1	Olive oil
urea	33.65 ± 5.35 Ab	32.65 ± 5.59 B	37.75± 4.55 a	34.10± 9.21 ab	30.14 ± 4.71 B	31.84± 2.71 B	33.91± 3.49 Ab
Creatinine	0.67± 0.08 C	0.79± 0.15 B	0.85± 0.17 a	0.77± 0.07 B	0.78± 0.09 B	0.67± 0.08 C	0.66± 0.09 C
Uric acid	1.57± 0.16 D	1.95± 0.31 C	2.71±0.57 a	2.13± 0.23 Bc	2.21± 0.26 B	1.96± 0.23 C	2.11± 0.27 Bc

The effect of nutrition on different types of fats in the concentrations of ALT, AST, and ALP liver enzymes:

Table (3) shows the effect of nutrition on different types of fats in the concentrations of liver enzymes. The results of our current study showed a significant increase in the level of AST and ALP concentrations in a group that was given animal fat by 8% g / kg of diet and a significant decrease in the group that were given olive oil by 4% g / kg of diet compared to the other treatments groups of this study.

No differences in ALT concentration were observed in all study treatments. The liver plays an important role in the metabolism of foodstuffs such as fats, carbohydrates and proteins and works on producing bile salts that are important in the metabolism of fats [36] that feeding on olive oil works to reduce the concentration of ALP and AST because it contains antioxidants that work to sweep free radicals, or the reason may be attributed to the activation of neurotransmitters, especially acetylcholine which is located in the meeting areas between nerve cells - nerve cells or nerve cells - muscle, so all vital activities in the body are activated. And that feeding on saturated fats leads to an increase in the concentration of AST. The reason is that the continuation of feeding on saturated fats leads to atherosclerosis and thus increases the concentration of AST in the blood serum as a result of the occurrence of necrosis in the body tissues and damage to the liver causing the release of liver enzymes into the bloodstream through the hepatic channels. Or the reason is due to an increase in the base enzyme phosphatase that occurs in the case of hepatic obstruction, as this enzyme is secreted in the liver, and with all of it, the change in the concentration of enzymes in the serum is attributed to a weakness in liver function. These enzymes appear in the serum as a result of their release in the liver when hepatic tissue is destroyed when the liver suffers from chronic diseases as in acute hepatitis.

The activity of liver enzymes in the tissues is greater than their activity in the blood serum. Or the reason is the increase in the concentration of the levels of ALP and AST, which is due to the cumulative over-nutrition of the fats and vegetable oils in the expression of the enzyme Glucose-6-phosphate dehydrogenase which increases the level of oxidative stress in the liver and thus causes damage. Animal also contains many alkaline components such as phospholipids that activate the saponification process, which affects the increase of liver enzymes [37,38].

Table (3) the effect of nutrition on different types of fats in liver enzyme concentrations.

Treatments/ features	control	Animal fat4%	Animal fat8%	Vegetable oil P1	Vegetable oil P2	Olive oil O1	Olive oil
AST	26.70 ±3.51 d	31.40 ±4.46 Cd	45.55 ±6.55 a	35.70 ± 10.24 bc	43.73 3.990 ± a	27.74 ±6.90 d	37.19 ±13.35 B
ALT	19.35 ±3.32 A	21.40 ±10.26 A	23.35±11.98 a	24.45±7.69 a	22.95±5.93 a	21.53±7.28 a	26.10±8.75 a
ALP	70.25 ±6.53 c	111.55 ±27.63 A	113.15 ±14.94 a	90.95 ± 15.17 b	105.18 ± 23.16 Ab	77.89 ± 7.29 c	100.43 ± 27.57 Ab

Ethical Considerations:

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Original Research Paper

A Prospective Study of HIV Positive Autopsies Conducted at Aniims, Port Blair, Andaman & Nicobar Islands

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Abstract

Medical and Medico Legal deaths claim a substantial number of lives in Andaman & Nicobar Islands. During the 3-year-period from January 2016 to December 2018, 6 antibody HIV positive cases were autopsied at the Department of Forensic Medicine & Toxicology in ANIIMS, Port Blair, Andaman and Nicobar Islands. The purposes of this study were to report the autopsy findings of anti-HIV positive individuals, and to identify the prevalence of specific factors such as gender, age, postmortem findings, cause and manner of death. The 61 anti-HIV positive individuals who died at G B Pant Hospital, Port Blair consisted of 35 males and 26 females. Here, 55 cases died from natural cause, 6 cases due to unnatural causes termed as medico legal death, which included 2 cases of accidents and 4 cases of suicide. All Medico legal cases underwent full autopsy with universal precautions.

The prevalence of anti-HIV positive underlined the importance of awareness of HIV transmission in forensic casework. In conclusion, full autopsies should be performed regularly on HIV infected patients with due protective measures not only for medical research, but also for forensic study.

Key words: *Autopsy, Cause of death, Manner of death, Anti-HIV positive medico legal deaths.*

Introduction

There are 572 Islands in the Andaman & Nicobar group with an area measuring 8249 Sq. KM., among them only 32 islands are inhabited, Port Blair, being its capital city. Medical and Medico legal deaths claim a substantial number of lives in Andaman & Nicobar Islands. Autopsies serve a variety of useful and important roles not only for forensic medicine purpose but also for medical researches [1, 2]. Since the first case of AIDS have been reported, there were many autopsy studies of HIV infected person in many countries around the world [3, 4, 5.6, 7, 8]. In developed countries, the range of opportunistic infections and their contributions to morbidity and mortality in HIV -infected persons are well document. There are many cases of autopsies done on anti-HIV positive individuals, which are medico legal cases, though the death in substantial number of the HIV infected individuals are natural due to opportunistic infections in developing countries. The prevalence of anti-HIV positive cases at medico legal autopsies in a developing country like India will reflect the incidence of these cases. Great care should be administered and universal precautions should be adopted while conducting the autopsy.

Therefore, a Prospective study of anti-HIV Positive autopsy cases analyzed by statistical method to create useful database in this region was undertaken. This study was pinpointed into the examination and description of the cause and manner of death, postmortem findings and the association between cause and manner of death, and specific factors in HIV positive cases which has not been

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reported before.

Material and Methods

This study, conducted for 3 year period from January 2016 to December 2018, which included 61 anti-HIV positive inpatient deaths at G B Pant hospital, Port Blair, Andaman and Nicobar Islands., out of which 55 were natural deaths and 6 were Anti-HIV positive medico legal deaths.

These 6 cases were autopsied at the Department of Forensic Medicine & Toxicology, ANIIMS Port Blair during the year January 2016 to December 2018.

The data was collected regarding the age and sex wise distribution of cases, year wise distribution of cases, marital status and occupation of the individuals, manner and cause of death.

The data thus obtained was statically analyzed and depicted in the results.

Results

This study showed that out of 6 anti-HIV positive medico legal cases autopsied there were 3 cases of anti-HIV positive medico legal deaths that were autopsied in 2016 and 2 cases in 2017 and 1case in 2018. (Table 1)

Out of 55 numbers of deaths due to natural disease 18 cases in 2016, 21cases in 2017 and 16 cases in 2018 at G B Pant hospital, Port Blair. (Table-1)

In our study out of 6 cases there were 4 males (66.66%) and 2 females (33.33%). Out of 4 males 2 were in the age group of 41-50 years and 1 each in the age group of 21-30 years and 31-40 years. Among the 2 female cases 1 each were in the age group of 21-30 years and 31-40 years. (Table 2)

Our study which included 6 anti-HIV positive medico legal cases were all married. (Chart -1)

The occupations of the deceased were Drivers 2(33.33%), Farmer 1(16.66%), Housewife 2(33.33%) and Unemployed 1(16.66%). (Chart 2)

In our study, out of total 61 cases 55 cases (90.16%) died from natural disease at our hospital, 4 cases (6.55%) from committing suicide, 2 cases (3.27%) from accident. There were no Homicidal deaths. (Table-3)

In our study, among the 4 suicidal deaths 2 cases died due to consumption of poison and other 2 cases died due to hanging. The 2 accidental deaths were due to road traffic accidents and the deceased were drivers of heavy vehicles. (Table-4)

Table 1:- Year wise distribution of anti HIV positive Hospital deaths.

YEAR	2016	2017	2018	Total	Percentage
TOTAL NO. OF DEATH DUE TO NATURAL DISEASE	18	21	16	55	90.16%
TOTAL NO. OF UN-NATURAL DEATHS (MEDICOLEGAL CASES AUTOPSIED)	03	02	01	06	9.83%
TOTAL NO. OF DEATHS	21	23	17	61	100%

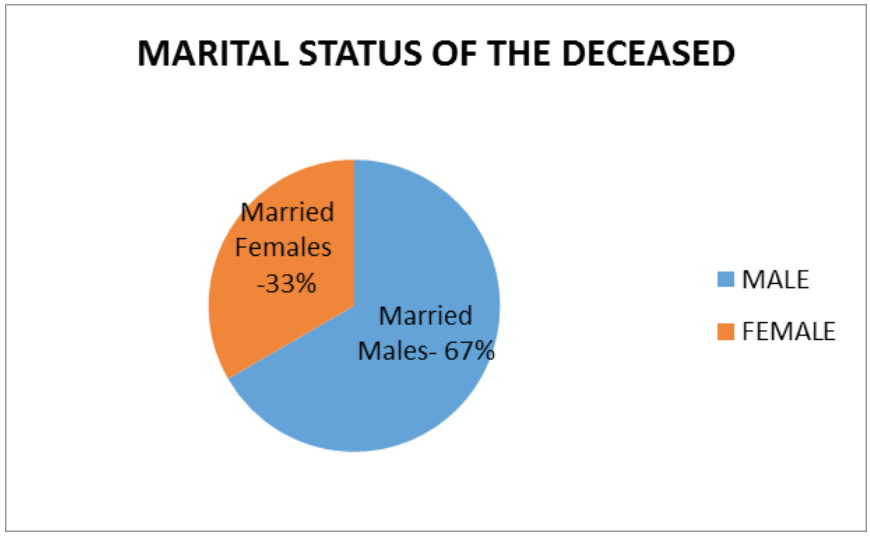
Table 2 :- Age and sex wise distribution of cases				
GENDER / AGE	MALE	FEMALE	TOTAL	PERCENTAGE
<11 YEARS	0	0	0	0
11 TO 20 YEARS	0	0	0	0
21 T0 30 YEARS	1	1	2	33..33
31 TO 40 YEARS	1	1	2	33.33
41 TO 50 YEARS	2	0	2	33.33
>50 YEARS	0	0	0	0
TOTAL	4 (66.66%)	2 (33.33%)	6 (100%)	100 %

Table 3:- Manner of death among anti HIV positive cases.

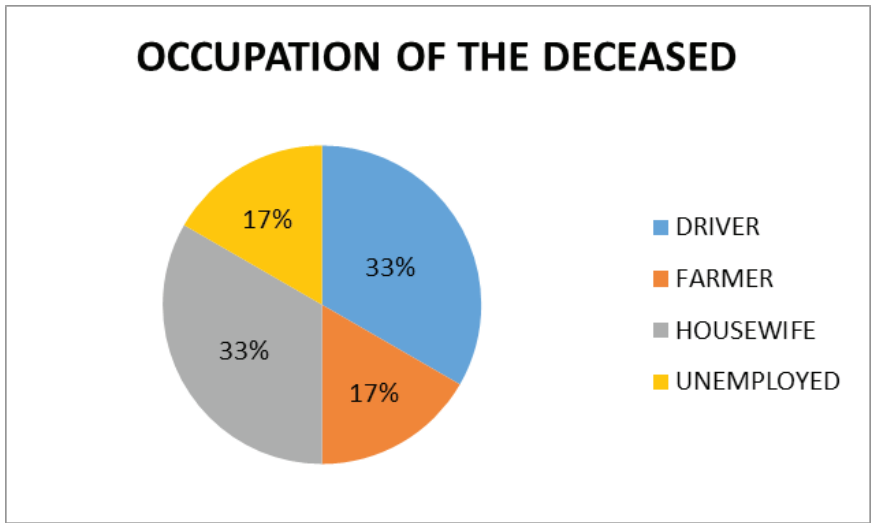
MANNER OF DEATH	NO. OF CASES	PERCENTAGE
1) NATURAL	55	90.16%
2) ACCIDENTAL	2	3%
3) SUICIDAL	4	6.55%
4) HOMICIDAL	0	0%
TOTAL	61	100%

Table 4:- Cause of death among the anti- HIV positive cases.

CAUSE OF DEATH	NO. OF CASES	PERCENTAGE
1) POISONING	2	33.33%
2) RTA	2	33%
3) HANGING	2	33.33%
TOTAL	6	100%



PIE CHART - 1



PIE CHART - 2

Discussion

All violent and unnatural deaths are subjected to forensic autopsy to know the cause and manner of death. Most of the cases of anti-HIV positive individuals will die due to many opportunistic infections and are classified as a natural death. In our study out of 61 cases of hospital deaths, 55 cases (90.16%) died due to natural disease and 6 cases (9.83%) died due to unnatural causes which were autopsied. Our study is quite similar to the study conducted at Ramathibodi hospital [9] where 76.5% died due to natural disease and 22% were medico legal deaths.

In our study, the age group of 21-30 years, 31-40years and 41-50 years all involved 2 cases each and male predominance was seen.

But by and large the age group of 21- 30 years are more commonly involved as per the study conducted at the morgue of the city of Sao Paulo [10] and males were commonly involved in all the age groups. This may be attributed to the movement of male populations from place to place in search for jobs and other needs and more particularly the age group of 21-30 years being young are exposed more to the unprotected sexual intercourse.

Our study showed that all the cases were married (100%) and driver (33.33%) and housewife (33.33%) were more commonly involved. This proves that the drivers of heavy vehicle are at more risk as they stay away from the home for weeks or months and are exposed to unprotected sex, on the other hand house wives being the recipient and contract this deadly disease through

their husband.

On considering the manner of death. In the current study out of 61 deaths at our hospital, 55 cases (90.16%) died due to natural disease, 4 cases (6.55%) died due to suicide and 2 cases (3.29%) died due to accidents. There were no homicidal deaths in our study. The most common method adopted for committing suicide were poisoning (33.33%) and hanging (33.33%). The 2 cases (33.33%) of road traffic accidents, the deceased were drivers of heavy vehicles.

The studies conducted by Maccario,M , Scharre, D.W [11]and Reichert,C.M., O'leary, T.J., Levens, D.L., Simrell, C.R. & Macher, A.M.[12] prove that suicide is not related to the presence of encephalopathy or Psychosis, which develop later in the course of the syndrome.

Conclusion

This study reflects the magnitude of anti-HIV positive medico legal deaths in Port Blair region. Performing a full medico legal autopsy on these anti-HIV positive medico legal cases requires greater precautions especially universal work precautions has to be adopted in each and every of these cases to prevent risk of HIV transmission among the forensic workers and technicians. In our study, the suicidal rates are more common in the anti-HIV positive individuals could be due to Psychological trauma, Depression, social stigma associated with HIV/AIDS and many more. There is a need for further study to look into why the suicidal rates are more common in the anti-HIV positive individuals.

To conclude there is a very little information regarding the number of autopsies of anti-HIV positive medico legal deaths in developing country like ours. This suggests that each & every centers conducting autopsies should come up with such studies to reflect the magnitude of occurrence of these deaths.

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Effect of *Crataegus Azarolus* Extracted in Treatment of *Giardia Lamblia* Infection

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Abstract

The infection of *Giardia lamblia* has a universal distribution and it is a major cause of diarrhea condition in adults and children and is transmitted by route of fecal-oral through direct or indirect cysts ingestion. 20 adult male rats were used and distributed to the following groups (5 rats in each group); negative group: rats received distill water, positive group: infected rats with *G. lamblia* at dose 10^3 cyst/ml. third group infected with *G. lamblia* and treated by 150mg/kg of C. azarolus extract for six weeks. Fourth group: infected with *G. lamblia* and treated by 250mg/kg of *C. azarolus* extract for six weeks. The results demonstrated significant ($P < 0.05$) increased in levels of malondialdehyde (MDA) immunoglobulin (Ig) A, IgG and IgM with significant ($P < 0.05$) decreased in levels of glutathione (GSH) in an infected group compared with control group. The findings of treated groups demonstrated non-significant ($P < 0.05$) difference in all studied parameters compare with control group when using *C. azarolus* extract. It was concluded that C. azarolus extract has been role against the *G. lamblia*.

Keywords: *G. lamblia*, *C. azarolus* extract; *Immunoglobulin*

Introduction

The agent of Giardiasis diasease, parasite called *G. lamblia* is one of the most infection of intestinal [1-2]. Infections of G. lamblia are most common all over the world and mostly causes of diarrhea disease in industrialized countries [3-4]. The infection range in children without any symptoms has been reported from 8% to 30% in developing regions and 1-8% in industrialized regions and countries [5]. The happen of giardiasis disease is possible higher in person with diarrhea [6]. *Giardia lamblia* possess two stages in their life cycle. Host cells infection is begin when the cyst stage is ingested by human with water, polluted food or by direct contact of fecal-oral. The cyst stage is inert stage, allowing long period survival at different conditions. After the cyst reach to the acidic condition of stomach, cysts convert to trophozoites in the doudenum [7]. The native of *Crataegus azarolus* plant is Mediterranean Basin. *C. azarolus* is a low and spiny tree with an inflorescence up to 6 m tall and with fruits colored orange [8-9]. pharmacological features of *C. azarolus*, including antioxidant, antiinflammatory activity, anticardiac remodeling activity and vasodilating effects, also decreasing of migration of smooth muscle and protection, antiarrhythmic, lipid lowering and decreasing effects of pressure of arterial blood [10-11]. So, and because the studies showed many of the medicinal properties of a plant and there was no study showing its role as an anti-parasitic in general and against a parasite in particular, The current study was aimed to detect the effect of *C. azarolus* extract in treatment of *G. lamblia* infection in adult male rats.

Materials & Methods

Samples Collection

Fecal samples were collected from patients (5-12 years children) suffering diarrhea from Health Centers and Private Laboratories during a period between (March. 2019 to October 2019). All collected samples preserved in a chilled sucrose until reaching to the laboratory for examination.

Stool samples examination

The samples were examined with direct microscopical observation by using physiological

normal saline to identify *G. lamblia* trophozoites and cysts.

Cysts purification and enumeration

Cystic stage of *G. lamblia* was purified with some modifications [12] with some modifications. Cysts enumerate by using neubaure chamber under oil objective lens. The sample was diluted with normal saline to (10^3) cyst for each rat.

Plant extraction

l0 g of *C. azarolus* fruits (collected from Sulaymaniyah city, Iraq, during a period between March. 2019 to October 2019) extracted for 30 min. The mixture was filtered by using three layers of gauze, and remain part was then extracted by two additional parts of the similar solution. The final solution was centrifuged at 5000 rpm for 15 min. Ethanol in filtrate was removed by using rotary evaporator device under optimum condition.

Animal model

In current work 20 rats, (wt 200-250 gm with age4-6 month) obtained from Science College/ Tikrit University. The study was done in laboratories of University of Samarra, Iraq.

Experimental design

20 rats (male) were used in current study and then distributed as follow (six rats in each group):

A. Control group: male rats received standard diet only for seven days and then killed.

B. Infected group: administrated with *G. lamblia* at dose 10^3 cell/ ml.

C. Treated group: administrated with *G. lamblia* and treated with 150mg/kg of *C. azarolus* extract for six weeks.

D. Treated group: administrated with *G. lamblia* and treated with 250mg/kg of *C. azarolus* extract for six weeks.

Malonedialdehyied (MDA) and Glutathione (GSH)

MDA was measured according to reaction of colorimetric with thiobarbituric acid (TBA) using spectrophotometer device [18]. GSH measured by mixed buffer (2.3 ml) with of serum (0.2ml) and then added 0.5ml of compound called 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture of compounds and serum was analyzed by device of spectrophotometer [19].

Single Radial Immunodiffusion Assay

This test was used to determine the concentration of IgA, IgG and IgM by commercially available kit (Demeditec Diagnostics GmbH/ Germany) according to [14].

Statistical Analysis

Current data were analyzed by using program known as Minitab (statistical program). A statistical change between the groups means were analyzed using one-way analysis of variance.

Results

MDA & GSH

The levels of MDA show significant increased ($P<0.05$) in an infected group (2.21±0.035) compare with control group (1.58±0.049). After treatment, third and fourth groups (1.59±0.09; 1.52±0.042 respectively) show non-significant changes compare with control group as shown in figure (1). The levels of GSH show significant decreased ($P<0.05$) in an infected group (0.264±0.012) compare with control group (0.39±0.012). After treatment, third and fourth groups (0.386±0.005; 0.405±0.008 respectively) show non-significant changes compare with control group as shown in figure (2).

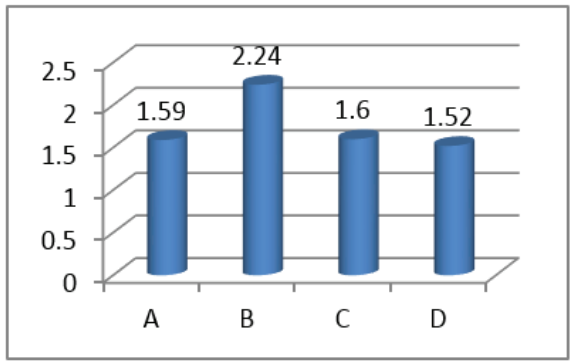

Figure (1): MDA (nm/L) levels in all groups

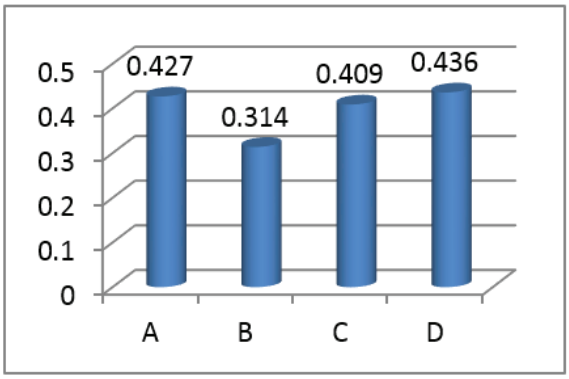

Figure (2): GSH (nm/L) levels in all groups

Immunoglobulin

The levels of IgA, IgG and IgM show significant increased (P<0.05) in an infected group (349.1±10.04; 2758.5±34.6; 66.55±6.29 respectively) compare with control group (271.05±14.07; 1822.0±29.7; 95.3±5.3 respectively). After treatment, third (268.5±3.54; 1833.5±98.3; 191.3±4.1 respectively) and fourth (291.0±2.83; 1873.0±28.3; 100.3±5.23 respectively) groups show non-significant changes compare with control group as shown in figure (3-5).

Discussion

Giardiasis diasese has a univerasl spread and it is affect in all ages (children and adults). The diagnosis of Giardia infection is more in developing countries compare with undeveloping countries. More than 200 million pateients with giardiasis are prognosed worldwide [15]. In current study, results of MDA show significant elevate and significant reduce in GSH levels in an infected group compare with control group, because reactive oxygen species (ROS) generation and produced inside cells by infection with Giardia spp [16], that demonstrate the effect of Giardia to elevate MDA levels in current study. About immunoglobulin concentration in current work, the results demonstrate significant elevate in IgA, IgG and IgM concentrations in an infected group compare with control group. These findings similar the results of AL-Khayat et al. [17] who inducated that the higher concentrations of IgM, IgG, and IgA were appeared in patients aged 2-12 years old in compare with other age category. In another study, the results demonstarte that serum concentration of IgM, IgG, IgA elevated in infected persons compare with the control subject and suggest that concentration of IgM may reflect exposure to the parasite early in life, concentration of IgA may reflect recurrent exposure to G. lamblia [18-24]. Otherwise, the results of current work demonstrate high activity role of C. azarolus against the toxicity of G. lamblia. The elevate in activity of GSH observed after the treatment with extract of *C. azarolus* may be back to high levels of agents of exogenous antioxidant as polyphenols in plant extract. Similar studies demonstrate a positive effect of various polyphenols classes on activities of enzymes of antioxidant (super peroxidase, catalase and GPx) in animal and human studies [19-22]. Also, the results odemonstrate high activity role of C. azarolus as anti-inflammatory features, the extract of flavonoid from C. pinnatifida fruits reduced the production of prostaglandin E2 (PGE2) and NO induced by agent called lipopolysaccharide (LPS) in macrophage cells in vitro [23-25-26].

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way and the samples were collected according to the stool collection guidelines.

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Conflict of Interest: The author declare no conflict of interest regarding this research.

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Teledentistry: The Need of An Hour During Pandemic COVID-19

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Abstract

The epidemic of coronavirus disease 2019 (COVID-19), originating in Wuhan, China, has become a major public health challenge. According to WHO, "It is possible to halt the virus spread, by adopting measures to detect disease early, isolate and treat the cases, tracing the people who came in contact with those who are infected, wearing gloves and masks, limitation of people going outside their home and promote social distancing measures." Due to the characteristics of dental settings, the risk of cross infection can be high between patients and dental practitioners and can spread from patient to patient.As part of its efforts to mitigate the spread of the COVID-19 pandemicthe American Dental Association (ADA) provided essential guidance on (i) emergency vs. nonemergency dental care and (ii) the use of teledentistry. In response to the current health crisis, teledentistry is emerging as a viable care option in an effort to help "flatten the curve" of the COVID-19 pandemic.During the COVID-19 outbreak, teledentistry is an effective way to triage patients and conduct problem-focused evaluations in order to limit office visits to patients needing urgent or emergency care. This article, based on relevant guidelines and research, introduces the essential knowledge about COVID-19, and focuses on how teledentistry can be helpful in preventing dental emergencies by providing effective dental care to patients during quarantine, and also educate and motivate people to opt necessary dental hygiene measures.

Keywords: *COVID-19, Teledentistry, Cross infection, Social distancing.*

Introduction

On January 8, 2020, a novel coronavirus was officially announced as the causative pathogen of COVID-19 by the Chinese Center for Disease Control and Prevention (Li et al. 2020).[1] The epidemics of coronavirus disease 2019 (COVID- 19) started from Wuhan, China, last December and hasbecome a major challenging public health problem for not only China but also countries around the world. OnJanuary 30, 2020, the World Health Organization (WHO) announced that this outbreak had constituted a public health emergency of international concern (Mahase 2020).[2]

The New York Times published an article on 15 March 2020 entitled "TheWorkers Who Face the Greatest Coronavirus Risk", where figures described that dentists are the workers who are most likely to exposed to the risk of being affected by COVID-19, much a more than nurses and general physicians.[3] Various measures are taken to combat this pandemic and highly contagious disease by the limitation of people circulating outside their home,forced quarantines on an extraordinary scale, social distancing, the cessation of almost all working activities and the request to the population to use protective masks and gloves, all have the aim of minimizing the likelihood that people who are not

infected come into contact with others who are already infected and probably still asymptomatic.[4] As part of its efforts to mitigate the spread of the coronavirus disease 2019 (COVID-19) pandemic, on March 18, 2020, the American Dental Association (ADA) provided essential guidance on (i) emergency vs. nonemergency dental care and (ii) the use of teledentistry. In response to the current health crisis, teledentistry is emerging as a viable care option in an effort to help "flatten the curve" of the COVID-19 pandemic.[5]

Teledentistry is the use of information technology andtelecommunicationsfor dental care,consultation, education and publicawareness in the same manner as telehealth and telemedicine.[6] The ADA defines teledentistry as "the use of telehealth systems and methodologies in dentistry," which includes "a broad variety of technologies to deliver virtual medical, health and education services."[5]Such technologies can take placethe following ways – "real-time consultation" and "store-and-forward method." Real-time consultation includes a video conferencing between dental professionals and their patients, at different locations, they may see, hear, and communicate with one another. Store and forward method is the method in which clinical information and static images collected and stored by the dental practitioner are stored first and later theyforward them for consultation and treatment planning to teleconsultants. The third method has also been described, known as "remote monitoring method," in which patients aremonitored at a distance and can either be hospital-based or home-based.[7]

Inspite oflarge-scale transmission of COVID-19 in China during the epidemic; demand for urgent dental treatment decreased by only 38% (Guo et al., 2020).[8] This impressive figure showsthat the public need for urgent dental care even during this pandemic will always be essential. Dental associations responses and actions around the world varied from advising practitioners to close their practices in the UK (Scottish Government, 2020)[9]; to reducing the number of routine check-ups inCalifornia, USA (CDA, 2020)[10]; to no advice at all from several dental associations around the world. These all steps were taken in order to halt disease spread.

A fundamental concept behind transmission of the virus is mainly through inhalation, ingestion, direct mucous contact with saliva droplets; it is also critical to remember that the

virus can live on hands, objects or surfaces that were infected saliva with in the previous nine days. The most recommended guidelines indicate that dentists should avoid the scheduling of any patient: only urgent dental diseases can be considered during the COVID-19 outbreak. This action will drastically limit the interpersonal contact, the waiting time of patients in dental cabinets and, in general, the conditions predisposing patients to be infected.[3]

The use of communication and information technologies to provide dental care (teledentistry) can help dentists to sort out emergency need and urgent dental care, and provide non-essential services while avoiding close contact with patients.

By telecommunication patients can avoid unnecessary dental visits and thus prevent themselves and clinicians from exposure to COVID-19. Rather than directly visiting the dental clinics patient can use any of the above methods to consult the dental surgeon and can get advice on self help care.[11] By listening to patient's chief complaint, dental and medical history treating doctor can determine whether the condition falls under emergency dental care or non emergency dental procedures, and subsequently decides whether there is a need for patient to visit dental clinic or not.

Non-essential dental work should be delayed until further notice during the pandemic according to the American Dental Association. Orthodontic concerns can also be reviewed by teledentistry. "A pencil eraser could be used to push in poking wires or wax could be used." Viewing photos or face-timing with patients can provide consultation and professional advice for parents when their children have dental issues. Sometimes after a phone consult, a patient doesn't have to come into the office immediately and may get relief in his pain by the prescribed antibiotic.[12]

Apart from the consultation, diagnosis and treatment planning teledentistry also plays role in educating the patients by using"Web-based self-instruction educational system," which contains the information that has been developed and stored. Teledentistry could also be used to increase the awareness regarding oral hygiene, dental,

and oral diseases, counselling, assessment or evaluation (e.g., for new or existing conditions or lesions, and for the fit of appliances); monitoring or follow-up (e.g., for existing ongoing treatment, new devices, or following recent treatment) in general population during such kind of adversities when people are under strict quarantine and are not allowed to visit dental clinics.[13]

Conclusion

Teledentistry is a relatively new and exciting field that has vast potential. It is useful in long-distance consultations, screening, treatment planning and dentist laboratory communication. Due to pandemic COVID-19 the dentists and their patients are facing unprecedented and extraordinary circumstances. WHO, ADA and various other organizations across the world have given their guidelines to halt transmission while also supporting emergency care for patients. ADA recognizes that during this time patients would be best served when telecommunication technology is added to support dental care. The use of information and communication technologies to provide dental care remotely (teledentistry) can enable dentists to sort out emergency and urgent dental care and provide non-essential services while avoiding close contact with patients during these unfavourable conditions when patients are forced quarantined with limited access to dental clinics.

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Ethical approval: The study protocol was reviewed by the Ethical Committee and was granted ethical clearance.

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Comparative Study in Surgical Management of the Pilonidal Sinus Disease

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Abstract

The objective: the current study conducted to assessment the recurrence rate of Pilonidal sinus.

Materials & Methods: This retrospective study that shows all the cases of Pilonidal sinus treated in the Khanaqin General Hospital and Jalawla General Hospital at period between April 2016 to September of 2019. The number of patients is 200, including 48 women and 152 men, with ages ranging from 19 to 50 years.

Results: 200 Pilonidal sinus patients were participated in this current study. The recurrence rate after surgery reached 13(6.5%) from all patients. The patients who used in present study include male 152(76%) and female 48(24%). According to Chronicity, the present results demonstrate 73 (36.5%) cases as acute disease and 127 (63.5%) as chronic disease. 79 patients who suffering from acute disease were lay open and collection drainage. On the other hand, 121 patients who were suffering from chronic disease treated with sinus excisional procedures and primary midline closure.

Keywords*: Recurrence rate; Pilonidal sinus; Primary midline closure; Lay open.*

Introduction

The pilonidal sinus PS is a disease that effect generally of young males [1-2]. Although pilonidal sinus is harmless in most cases, pilonidal sinus may strictly weak the ability of patient to perform his work and life quality, especially if the process of wound healing was delayed. Furthermore, the disease recurrence was noted and reported in about 30% of patients [3-4]. The disease of pilonidal sinus include loose of hair and perineal flora. The major risk factors of disease of pilonidal sinus involve male sex (Pilonidal sinus appear more in young men), the occupations that needed sitting for long times, deep natal cleft existence and hair presence inside natal cleft. The family history is noted in 38% of patients [5-6]. It is commonly located in sacrococcygeal region, but also located in other positions like the umbilicus, axilla, on foot sole, penis, and clitoris and in anal canal [7]. Pilonidal sinus treatment depends on giagnosis. Intervention could extend from incision toexcision with reconstructive procedures. The treatment of pilonidal sinus is generally surgical [8-9]. Various surgical treatments were described to pilonidal sinus, inclusive flap surgery [10-11]. The perfect operation to this disorder must be successfully eradicate the pilonidal sinus, minimize recurrence risk and carry a low morbidity rate [12].

Materials & Methods

This retrospective study shows all rudimentary cases of pilonidal sinus that were treated in the Khanaqin General Hospital and Jalawla General Hospital at period between April 2016 to September of 2019. The number of patients is 200, including 48 women and 152 men, with ages ranging from 19 to 50 years. The data of present work include some socio-demographic properties of patients, recurrence rate, clinical presentation, and clinical management. The pilonidal sinus patients were diagnosed with recurrence via clinic follow-up for patients if the following feature developed after surgery that including: complete wound healing process after surgery, no trauma development during the healing of wound, present one tough criterion (surgical re-intervention, new sinus forming, pus discharge and hair

developed in the sinus and) or two soft criteria (pain, redness the wound, swelling). The technique of surgery as report could be either by lay open technique or by the primary midline closure technique. Technique called Lay open is vast pilonidal sinus excision and secondary intention was occur to wound healing. The second technique was primary midline closure of pilonidal sinus excision. The analysis of outcomes was done by using statistic program known as SPSS (version 22). The data of current study were expressed as frequencies and percentages with several of results presented in tables; Chi-Square test was utilized to measure statistical significance differences. Cross-tabulations were done to compare the relationship between recurrence and other variables.

Results

200 Pilonidal sinus patients were participated in this current study. The recurrence rate after surgery reached 13(6.5%) from all patients. The patients who used in present study include male 152(76%) and female 48(24%). According to Chronicity, the present results demonstrate 73 (36.5%) cases as acute disease and 127 (63.5%) as chronic disease, table (1).

Table (1): demographic and social characteristics of patients

Characteristics		Non-recurrent	Recurrent	Patient total
Age		37.17±15.14	32.00±13.3	200
Gender	Male	142 (93.4%)	10 (6.6%)	152
	Female	45 (93.75%)	3 (6.25%)	48
Education Level	Read & write	16 (94.1%)	1 (5.9%)	17
	Primary	26(96.3%)	1 (3.7%)	27
	Intermediate	31 (91.2%)	3 (8.8%)	34
	Secondary	54 (93.1%)	4 (6.9%)	58
	Institute and college graduate	60 (93.75%)	4 (6.25%)	64
Marital status	Single	69 (94.5%)	4 (5.5%)	73
	Married	118 (92.9%)	9 (7.1%)	127
Chronicity	Acute	74 (93.7%)	5 (6.3%)	79
	Chronic	113 (93.4%)	8 (6.6%)	121
Residential Area	Urban	106 (92.2%)	9 (7.8%)	115
	Rural	81 (97.6%)	2 (2.4%)	83

79 patients who suffering from acute disease were lay open and collection drainage. On the other hand, 121 patients who suffering from chronic disease were treated with sinus excision and primary midline closure, table (2).

Table (2): Management of acute and chronic patients

Characteristics		Acute Patient n=79	Chronic Patient n=121	Total	P-value
Anesthesia	General	52 (41.3%)	74 (58.7%)	126 (63%)	<0.05
	Spinal	27 (36.5%)	47 (63.5%)	74 (37%)	
Surgery	Lay open	76 (66.7%)	38 (33.3%)	114 (57%)	<0.05
	Primary midline closure	3 (3.5%)	83 (96.5%)	86 (43%)	

Discussion

Patients with pilonidal sinus are suffering from pain and various complications like formation of abscess or sinus related drainage that lead to low quality of life for patients [13-14]. Recurrence condition is usually a result of the deletion of any aria during the first operation or wound infection, abscess formation leads to forming new sinus tract within cicatrizing wound. Cumulating of dead tissues, poor hygiene of wound, polluted the wound with sweating, the friction are factors that causes recurrence [15-16]. Also, Family medical history is considering a risk of the non-modifiable factors of the formation of pilonidal sinus recurrence [17-18]. Various points must be noted when choosing between the two techniques primary closure and Lay-open after sinus excision [19]. The infected or injured tissue excision may be its incomplete process and the site of operation is kept open with draining for a couple of weeks and the recurrence rate is about 20%. For that, open technique that needed more and prolonged care with regular curettage and remove of the granulating tissue in order to avoid premature closure or skin edges binding before incomplete healing process of cavity [20-22]. Otherwise, Meta-analytic works and studies demonstrates rate of recurrence was lower for wide excision which leaving the site of operation open than primary closure technique but they referred that primary closure technique lead to heals faster [23-24-26]. Different factors showed relationship with Pilonidal sinus. Family history, prolong sitting with less baths number weekly considered to be a risk for Pilonidal sinus and overweight of patient has relationship with Pilonidal sinus [17-18].

Conclusion

The recurrence rate of Pilonidal sinus in current study is higher in the closed methods than in the opened methods, also, the same result appears in the other study, so, the open methods is regarded as the best methods in the treatment of pilonidal sinus diseases.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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The Effectiveness of Zeolite in Treating Some Physical and Chemical Properties of Wastewater Discharged from General Sharqat Hospital

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Abstract

The current study where done to evaluation of sewage water and processing it in General Sharqat hospital / Salah El Din governorate –Tikrit –Iraq on 2018-2019. The study include analysis and measurement some physical and chemical properties to processing with natural, cheap and have no side effect to reduced harmful of water pollution of Tigris river .The results of present study showed conductivity and turbidity of sewage water was decreases from (1596) µc/cm to (727) µc/cm from (343) to (19) Nut , (633) to (491) ml/L respectively , and has risen concentration of dissolved oxygen (BOD_5) (T.S.S) (T.D.S) and nitrates preprocessing (104 , 0.53 , 966 and 25.2) which varied significantly with results post processing (52, 0.03 , 361 and 12.0) respectively . The results showed efficient processing of sewage water in station when treated with zeolite rocks powder. Sharqat hospital . discharged.

Key words : *Wastewater, Treatment, Zeolite*

Introduction

The process of collecting and disposing of liquid waste from the sources of its production of sewage water and pollutants of hospitals and factories and dumping it into the Tigris River has a great impact on the general health of the human being and a great impact on the rest of the neighborhoods[(1)]. Hospitals produce relatively large quantities of wastewater which may contain various hazardous materials [(2)] It affects all characteristics and the concentration of hydrogen ions present in any medium with pH, which is considered an indicator of the nature of the medium, whether basic or acidic [(3)]. As well as dissolved materials TDS and TSS as the water leaving hospital departments contain these types of material, Likewise, its quantity is one of the main pollutants of this water, and some of it may contain highly toxic substances that are, in turn, discarded from the hospital to the Tigris River in relation to the current study site. The values of this study after treatment came less than [(4)]. in their study of wastewater in Halab city, The reason for this is the efficiency of these processors, As for nitrates, they are formed in water when dissolved oxygen is available, which leads to the oxidation of nitrates into nitrates [(5)]. And because the nitrite ion is unstable, it turns into the more stable form which is nitrate [(6)]. The low values of nitrite are a result of the decreased reduction of nitrate to nitrite in winter, and the presence of nitrite increases with the decrease in dissolved oxygen [(7)]. Hospital wastewater is one of the most important sources of drug waste in all wastewater treatment plants and its ineffectiveness in removing these pollutants permanently. Chemical pollutants, heavy materials, disinfectants and specific detergents resulting from diagnosis, laboratory, research activities and drug excretion by patients makes wastewater in hospitals Negative impact on human health and the environment [(8)]. Mineral absorption in plants presents a great opportunity to use suitable plant species to clean the environment. Although increasing studies are now examining this issue, few studies have reported the simultaneous removal of nitrogen and phosphorous in real wastewater using nanomaterials [(9)].

Objectives of the Study

1. Shedding light on the current situation and making use of wastewater in various uses, whether agricultural or for human uses in conditions of water scarcity that our beloved country is going through.

2. Finding natural and environmentally friendly alternatives in water purification at low economic costs.

Materials & Methods

Sample collection

The experiment samples included zeolite powder, Zeolite was obtained from Amman Company - Tla 'Al-Ali / Wasfi Al-Tal Street / Amman ,Then it was milled with a laboratory electric grinder until a fine powder was obtained, and then the powder was passed through a sieve with a hole diameter of 0.5 mm. Then the powder was placed in sealed plastic bags and kept until use.

How to use the hydrotherapy

Specific proportions (2,4,8) of zeolite rock powder were used per 100 ml of contaminated water and then the most efficient concentration was chosen as a result of the treatment.

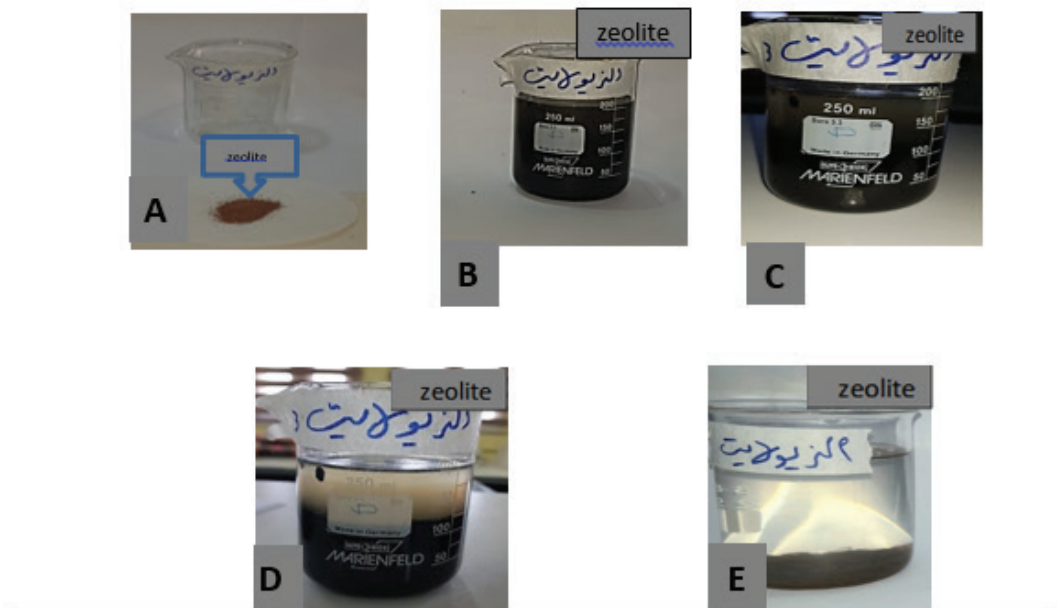

Figure (1) shows the stages from A to E of wastewater response to zeolite rock powder

Electrical Conductivity

The Multi parameter analyzer (Lovibond) model (con200) was used to measure the electrical conductivity of the samples after calibrating the device, and the results were expressed in (Microsiemens / cm) µc / cm.

pH

Use a pH meter (C830)type Consort after calibrating the device with Buffer solutions with a pH of (4, 7, 9).

Biological Oxygen Demand

The same method of measuring dissolved oxygen was used, and the BOD bottles were filled with a volume of (250) ml of the sample, then transferred to the laboratory, and kept for five days in a water bath at a temperature of (25 ° C), and the results were expressed in milligrams / liters depending on [10].

Turbidity

Water turbidity was measured by a HANNA-LP2000 Turbidity meter, as the device expresses standard solutions in (N.T.U) Naphthalene unit as the brownish unit, after the device is zeroed.

Total dissolved solid (T.D.S)

The dissolved materials were measured according to the method mentioned in [11].by filtering (100) ml of the sample on a filter paper (0.45) micrometer and collecting the filtrate in a vessel of known weight (B), then evaporating the filtrate in an oven at a temperature of (103-105). M) for a period of (24) hours, after which we were weighed (A):

Total solid suspended (T.S.S)

The suspended solids were measured by filtering (100) ml of sample on a filter paper (0.45) micrometer with a given weight (B), then evaporating the filtrate in an oven at a temperature of (103-105) m for a period of (24) hours, after which it was weighed (A).

$$T.S.S(mg/L) = \frac{(A-B)\times 10^3}{Volume\ of\ Sample\ (ml)}$$

Nitrate

Nitrate was measured using Ultraviolet Spectrophotometer Screening Method [12].using the Ultraviolet Visible Biochromic, LKB Spectrophotometer, as the absorbance was measured for each sample at the wavelengths of 220 nm and 275nm, and through standard solutions, the nitrate concentration was found from the equation for each curve. It is expressed in mg / L.

Choose a zeolite treatment

Zeolite is a 100% natural product that was created as a result of geological processes millions of years ago by the decomposition of volcanic glass and its interaction with alkaline water [13].Zeolites are mainly composed of aluminum silicate minerals and are distinguished by numerous qualities due to their unique structural composition [14].They are among some of the most popular and widely available natural ion exchanger, which consist of an aluminosilic molecular structure with weak positive bonding sites [15].

Zeolite was used as a major step in this type of treatment in getting rid of and reducing some of the physical and chemical characteristics that cause pollution. The zeolite was milled and used on samples taken from the wastewater plants of the Sharqat General Hospital and left for a period of time to reach the maximum treatment.

Figure (2) shows the outward appearance of the zeolite

Results and Discussion

pH

The results of the study shown in Table (1) for the pH characteristic indicated that there are significant differences in the values of this characteristic with regard to the water wastes discarded from the hospital under study, as it is noticed that the type of treatment had an effect on reducing PH, and [16] agreed with this study in Zeolite efficiency as the maximum removal efficiency has been found up to 85% pH.

EC

The electrical conductivity represents the positive and negative ions present in the discarded water from the sites covered by the study. It is noted from the results of the current study that the discarded water from the different sites under study differed in the values of electrical conductivity in a high significant manner, Table (1). The value of electrical conductivity in the water discharged from the hospital increased and it gave a value of 1596 microsmins / cm, while the value of this characteristic in the liquefaction water, which recorded 276 microsmins / cm.

The reason for this rise is due to the fact that the water leaving hospital departments contains various dissolved substances as a result of the effectiveness of work in these sites, and the presence of these dissolved materials leads to an increase in the solution degree and

thus increase the conductivity values. As for the effect of treatment methods, the studied results in Table (1) indicated the effectiveness of treatment with zeolite powder in reducing these values, as the conductivity value in this method reached 727 microsiemens / cm for hospital water and liquefaction water, which gave a value of 168 microsiemens / cm.

Total dissolved solid(T.D.S)

Total dissolved materials are one of the main components of the water discharged from hospital departments. As it represents the waste material of the raw materials during the conduct of operations, laboratory analyzes, or patient lounges. As the results of this characteristic shown in Table (1) indicated that there are differences in the values of total dissolved substances at the study site, Sharqat General Hospital. If it was found that the highest value in total soluble substances was in the wastewater from the general Sharqat, it was 966 mg / liter, and after treatment it was 361 mg / liter, While the value of these materials in the water sample of the liquefaction plant was 182 mg / l. The high concentration of dissolved substances may be caused by the large number of wastes produced during the conduct of the operations, as well as the waste after the end of the operations and the cleaning of the polluted hall. As for the effect of treatment methods for these materials, the results are shown in a table. (1) The water treatment with zeolite powder achieved positive results in reducing the concentration of total solids. The concentration of these substances decreased to 361 mg / liter for zeolite powder. The concentration of dissolved salts in the water of the present study treated sites is in agreement with [17]. It is similar to the results obtained from US Environmental Protection Agency [18].

Total solid suspended (T.S.S)

This characteristic in environmental studies represents the amount of solid plankton in the water, whether it is drinking water or wastewater, and the presence of these plankton in a large proportion in the water leads to it being unfit for human uses if its increase is due to the frequent use of formaldehyde as it is considered the most dangerous pollutant of wastewater by virtue of Its use in pathological laboratories and surgical departments for preserving samples and sterilizing medical devices and tools

Therefore, environmental research has been concerned with measuring the concentration of these substances and how to treat them to get rid of them or reduce them in the water. From the waste water, treatment with zeolite powder showed high efficiency in reducing the amount of these substances present in water outside the hospital. It became clear that the treatment method with this substance reduced its concentration from 0.53 mg / liter to 0.03 mg / liter. Reducing the concentration of these substances from the water leaving the hospital that is destined for the waters of the Tigris River will lead to contamination of that water and the arrival of suspended materials to the water liquefaction stations. Water is pumped to vital homes and facilities. Reducing them means preventing these materials from reaching drinking water.

Biological Oxygen Demand (BOD_5)

The results shown in Table (1) showed that the highest concentration of bio-oxygen in hospital wastewater was 104 mg / liter, while its concentration after treatment was 52 mg / liter. The reason for this increase in the vital oxygen requirement may be due to the increase in organic excreta due to the vital activities of the patients. The increase in the BOD value in the wastewater discharged water is an indication of pollution (1). As for the treatment parameters for water, Table (1) showed that treatment with zeolite powder achieved the best results in reducing this requirement. The concentration of the oxygen vital requirement after treatment for liquefied water was 52 mg / liter compared to the water before treatment. The reason for this decrease may be due to the chemicals in it that have the ability to bind with biological oxygen, which leads to a decrease in its concentration in the aqueous medium ,Or because of the increase in the percentage of solvents used by hospitals and medical laboratories, as well as the increase in medical waste mercury, which is highly toxic, which is used in medical clinics and is discharged into the sewage network. The results of the BOD of the treated samples matched [19]. and also did not match [17].

Total Hardness

The total hardness values shown in Table (1) indicate that it was the highest value in the hospital water, which gave values of 633 mg / liter. While the value of harshness decreased after treatment to 491 mg / L. The

cause of the higher hardness may be due to the nature of the chemicals introduced in these waters and used in the surgical procedures. The reason is due to the waste added to the river [20].It also agrees with many studies that have indicated a high total hardness in Iraqi waters [21]. This water, after treatment, is suitable for human use according to the Iraqi Standard Specifications No. (417) as well as the specifications. World Health Organization [22].

Turbidity

Wastewater is known to be very cloudy because it contains large quantities of suspended matter, which reduce the temperature of water by dispersing and absorbing light [23].However, the treatment methods of the discarded water had a significant effectiveness in reducing the brownish values. It is noted from the results table (1) for this characteristic that the zeolite powder had a strong effect in reducing the brownishness significantly, as it gave a value of 19 NTU for the zeolite, while the value before treatment was 343 NTU. The ability of zeolite powder to reduce brownish values is due to the containment of this powder, which consists of grains of limestone and limestone, and this has the ability to catch plankton and raise its molecular weights, which leads to its sedimentation at the bottom and making the water clear. The results of the brownish values were in agreement with a study [24].

Nitrite (No_3)

The results of this characteristic shown in the table indicate that the nitrate values under study before treatment with zeolite powder were 25.2 mg / l. They differed significantly from all results after treatment with zeolite powder, which was 12 mg / L. As the high nitrate in the waste water may be due to the tests and analyzes that take place in hospitals, in which the main component of nitrogen is nitrogen, and this element when combined with dissolved oxygen in water turns into nitrates. As for the type of treatment and its effect on nitrate concentration, it is caused by the zeolite chemical powder components [25.26].

Table (1) shows the changes in the estimation of some physical and chemical characteristics for wastewater of Sharqat General Hospital

Processing type	ZEOLITE		Liquefaction plant water before and after zeolite treatment		Measurements of the Tigris River in the district of Sharqat	Allowed limits
Measurements	Before Tr	After Tr	Before Tr	After Tr		
pH	7.5	8.5	8	7.5	7.8	6-9.5
EC µc/cm	1596	727	276	168	1687	1600
TSS ml/L	0.53	0.03	0.06	0.03	68	60
TDS ml/ L	966	361	182	168	595	1000
BOD5 ml/L	104	52	10	5	140	40
Turbidity NUT	343	19	325	8	172	-
Total Hardness ml/L	633	491	138	121	540	250
No3 ml/L	25.2	12	10.2	6.3	21.5	50

Conclusions

1- The low level of contamination of the traits in the treated water, where the material used in the treatment showed high efficiency.

2- The results showed that the zeolite rock powder has achieved clear results in removing pollutants from the hospital water.

3- The study showed alternatives to traditional treatment methods with low-cost and highly effective methods of treating polluted water.

Recommendations

1- Hospital waste management should be concerned with dealing with the large quantities of liquid and solid medical waste it discards daily, coinciding with the increasing number of patients and the lack of means of treating waste.

2- Recommending the possibility of treating hospital discarded water by the method that has proven its high ability to be treated, such as zeolite rocks powder.

3- Conducting studies on finding efficient and cheap sources in treating the wasted water from hospitals and laboratories in an environmentally friendly manner without changing the properties of water and soil instead of adopting traditional techniques and chemicals.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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Conflict of Interest: The author declare no conflict of interest regarding this research.

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Effect of Sex and Age Differences on Premature Ventricular Ectopic Beats in Apparently Normal Hearts

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Abstract

Ventricular ectopic beat (VEB) is a common cause in clinical practice. sometimes this case is asymptomatic but can cause uncomfortable symptoms in patients. There are some cases where the VEBs presence indicates a risk factor towards severe arrhythmias, especially when associated with normal hart. Normal cases that usually encounter episodes of ectopic beats were collected (58 individuals 23 were females and the rest (34) were males) from the private consultative clinic or from the Sherqat general Hospital attendance in Salah al-Din Governorate whose ages ranged between 38-66. These cases were followed up closely for two years with a clinical examination every 3 months with electrocardiogram Heart, chest x-ray, echocardiography. Regarding the management strategies (Table 2), 36 patients (19 males and 11 females) (49%) were satisfied by B-Blocker 19 of them (33%) were less than 60 years and 9 were equal or above the 60. Thirteen of the participants (23%) were given reassurance 3 of them (5%) were less than 60 years and 10 were equal or above 60. Nine individuals (16%) cases responded to simple sedativezers) 5 of them (9%) were less than 60 years and 9 were equal or above 60. 7 (12%) were treated by a combination of sedative and prokinetics 6 of the cases (9%) were less than 60 years and 9 were equal or above 60. Electrocardiographic and Cardiac Electrophysiological Properties of the study (Table 3) shows that 33 individuals (40%) were unifocal) were unifocal premature Ventricular ectopic beats their age were under 60 for all of them. The rest (24) were multifocal type. The QRS were significantly longer in male than in female and in the =60 more than that of the <60. In this regard, the result demonstrates that predominant S wave in 15 male participants versus 12 females. While there was 19 male having dominant R wave. Sex and age have a significant impact on cardiac electrophysiological characteristics of Premature Ventricular ectopic beats.

The males are more affected with Premature Ventricular ectopic beats than females regarding this effect.

Keywords*: : Ventricular ectopic beat, VEB, sex differences, age differences.*

Introduction

The first recorded description for VEB was by the Chinese physician Pien Ts'Io in the sixth century. he described it as an intermittent pertu-rbbation interrupting the regular pulse. He indicated that these irregular patterns did not affect the normal lifestyle when they were transverse but a bad prognosis was implicit if that were frequent. This appears to have been in the presence of myocardial infarct patients had a more likely to sudden death if they had frequent ventricular ectopics beats [1]. The studies reveal that VEBs were diagnosed by standard ECG at a rate one percent of clinically normal individuals while, about 40% to 75% of apparently healthy individuals were detected using by (Holter) ECG recording. On the other hand, some studies had been criticized because they did not investigate the presence of heart disease with echocardiography, stress testing, or invasive tests. Kostis et al [2] conducted a study over a 24-hour period of time in subjects without any recognizable heart disease, such as coronary arteriography and right- and left-heart catheterization, and revealed that 39 out of the 101 subjects had at least one VEB and four subjects had. 10 every 24 hours, five having 3 VEB every hour, and 4 having multiform VEBs [3]. Clinical studies have been shown that VEBs is frequently associated with hypertension. In a study with a 10 000 men population cohort aged between 35–57 years, the systolic blood pressure

level was associated with the VEBs prevalence. these findings were extended in the Atherosc-lerosis Risk in Communities (ARIC) recent data of studying more than 15 000 white and African American males and females and revealed that frequent or complex VEBs are associated with hypertension [4]. The VEBs frequency and incid-ence increase with age and yet several studies did not succeed to establish a clear relation between arrhythmic death and VEBs in aged individuals [3].

Both the high heart disease incidence and VEBs increased prevalence are contribute to the low VEBs predictive value for arrhythmic death specificity in the population. Furthermore, the Cardiac Arrhythmia Suppression Trial (CAST) results published in 1989 questioned the VEBs importance in risk increasing and revolutionized the necessary thinking to suppress VEBs as well as revolutionized therapeutic thinking through challenging the old concept of using antiar-rhythmic drugs to suppress ventricular ectopics, and focused on the proarrhythmic potential of the Ic class of antiarrhythmic drugs in heart disease patients with [5, 6]. Some studies [6] observed that females resting heart rate are higher than males and this was confirmed later through many other researchers. In a study with a large popu-lation of 5116 individual, the females average heart rate was 3–5 beats/minute faster than that of males [7].

This can be explained partly by the exercise tolerance and body habitus differences, as well as the role played by the different essential electrophysiological charact-eristics of the autonomic nervous system and sinus node [8] as the females sinus node recovery time is shorter and the tachycardia is occurring more frequently in females [9]. The aim of the current study is to evaluate the effect of sex and age differences on Premature Ventricular ectopic beats in apparently normal hearts.

Materials and Methods

The study was carried out during the period from January 2016 to June 2018. Cases who suffer from ventricular ectopic beats (VEB) were collected from the private consultative clinic or from the Sherqat General Hospital attendance in Salah al-Din Governorate. The history of each case, and of heart disease, was taken, drugs that affects the heart was considered and the regarded case was excluded.

An electrocardiogram (ECG) was perfo-rmed, apart from the ventricular ectopic, any abnormalities such as a T inversion or a Q wave excluded the patient. An x-ray of the chest was done to rule out any patient with an enlarged heart or pulmonary congestion. After these procedures, the number of normal cases that facing episodes of ectopic beats were 58 individuals 23 were females and the rest (34) were males whose ages ranged between 38-66 The study ethical approval was obtained from Salah Aldine ethics committee, the participants were informed of the study details, and written approval was obtained from all participants separately**.**

2.1. Data Analysis

Statistical analyses were done using SPSS software. The data of this study were presented as mean ±SD. Two-way analysis of variance was used to analyze the sex and aging differences effect on electrophysiological properties of the patients. A 0.05 P. value was considered statistically significant.

Results and Discussion

The males and females' total numbers enrolled in the study is presented in Table 1. The mean age of the participant's\was 52±14 years. The average percentage of ventricular ectopic beats per 2-hour was 2.4±2.1 for all the study individuals and differed significantly between mal-es and females (2.1±1.9 vs 2.7±2.4, respectively, P=0.033).

Table 1. Baseline characteristics of study participants

characteristic		Value
Males %(n)		60% (34)
Females %(n)		40% (23)
Age (years, mean ±SD)		52±14
BMI (kg/m2, mean ±SD)		27±3
Out-patients %(n)		90% (51)
ventricular ectopic beats percentage for participants per 2-hour	Males	2.1±1.9
	Females	2.7±2.4

Regarding the management strategies (Table 2), 36 patients (19 males and 11 females) (49%) were satisfied by B-Blocker 19 of them (33%) were less than 60 years and 9 were equal or above the 60. Thirteen of the participants (23%) were given reassurance 3 of them (5%) were less than 60 years and 10 were equal or above 60. Nine individuals (16%) cases responded to simple sedativezers) 5 of them (9%) were less than 60 years and 9 were equal or above 60. 7 (12%) were treated by a combination sedative and prokinetics (drugs enhancing gastrointestinal motility, and decrease gastric distension) 6 of them (9%) were less than 60 years and 9 were equal or above 60.

Table 2: Participants management strategies.

Management strategies	Gender n (%)		Age n (%)	
	Males	Females	<60 years	≥60 years
B- B-blockers	17(30)	11(19)	9(16)	19(33)
Reassurance	8(14)	5(9)	3(5)	10(18)
Sedative	5(9)	4(7)	5(9)	4(7)
edative and prokinetic	4(7)	3(5)	6(10)	1 (2)
Total	57(100%)		57(100)	

Electrocardiographic and Cardiac Electrophysiological Properties of the study (Table 3) shows that 33 individuals (40%) were unifocal premature Ventricular ectopic beats their age were under 60 for all of them. The rest (24) were multifocal type.The ECG related to premature ventricular ectopic peat revealed a significant difference in the PR duration between age groups (<60 and ≥60) and yet these changes were in the normal range. The QRS were significantly longer in male than in female and in the ≥60 more than that of the <60. In this regard, the result demonstrates that dominant S wave in 15 male participants versus 12 females. while there was 19 male having dominant R wave. This study contributes to the underst-anding of sex and age differences as a factor associated with VEP occurrence and showing the influence of sex and age differences on ventricular ectopic beats. The result revealed that the majority of the participant are taking B-Blocker, more than other medicine, the majority of them were the males and the older individuals. B -blockers protective effect toward ventricular ectopic beats were observed in males and the older participants have been monetarized in other studies. Èuliæ et al. [10] stated that blockers of the calcium channel did not affect the ventricular tachycardia circadian pattern but they influence the morning excess in ventricular ectopic beats frequency. This can result from the absence of sympathetic arousal suppression in the morning, which could react to because of the coupling of parasympathetic predomination at nighttime and a decrease in blood pressure and heart rate related to calcium channel blocker.

Table 3. Gender and age differences in Electrocardiographic and Cardiac Electrophysiological Properties related to Premature Ventricular ectopic beats.

PR		Gender		Age (years)	
		Males	Females	<60	≥60
		161 ± 23	156 ±36	156 ± 24**	180 ± 53*
QRS duration Mean±SD in ms.		154 ± 17**	122 ± 15	136 ± 24	141 ± 19**
QT		375 ± 40	376 ± 39	371 ± 39	395 ± 36*
QRS morphology n (%)	dominant S wave	15 (26.2) **	12 (21.3)	15(26)	12 (21)
	dominant R wave	19(33.3) **	11(19.2)	16 (28)	14 (25)
Unifocal n (%)		23 (40) *	10 (18)	33(58) **	0(0) **
Multifocal n (%)		11 (19)	13 (23)	9(16)	15(26)

The present study revealed that age and sex of the patient influence cardiac electrophy-siological properties and type of Premature Ventricular ectopic beats. The findings revealed that QRS duration are longer in males. Many stu-dies suggested that cardiac electrophy-siological properties may be altered by sex horm-ones. The dihydrotestosterone and estradiol prol-onged QT intervals significantly in cardiac ventricular tissue in ovariectomized rabbits throu-ghhout reducing the delayed rectifier potassium channel mRNAs levels whereas atrial ERPs was prolonged by 17b-estradiol in dogs [11,12].

Sexual differences have also been noted in electrophysiological properties as well as in electrocardiographic in human studies. SCL wave differences are known perfectly as the resting heart rate is higher in females than males [7,13] These persist differences after the blockade of the double autonomic, propose that the heart rate difference is of intrinsic origin and not related to the differences in autonomic tone [13,14]. In a study conducted on 185 healthy volunteers there, ages ranged between 20–83 years and normal ECGs. the result revealed that the PR interval was longer significantly in males than females as well as the PH interval (P 5 0.05) [15].

A study by MacFarlane et al.[16] reported that the male's QRS duration was longer than that of the females in all age groups. on the other hand, in a cross-sectional study from the Framingham Heart study of individuals between the 28 and 62 years, Levy et al.[17] found that with age advancing there was a narrowing of the QRS in males while there was no alteration in females. The difference in the QRS duration can be justified due to the larger area of the body surface in males more than females [18]. Other studies suggest that the presence of Particular pathophysiological diversity is the main reason for the existing of such differences and these changes can be attributed to such a pathophysiological diversity [19,20].

Observations were recorded on the effects of emotional stress and physical activity on VEPs. And despite the fact that these two factors increase sympathetic activation [19, 21], different cardiovascular responses can be produced by them. Case-crossover studies in implantable cardioverter-defibrillators patients proofed that physical activity and emotional stress can be the cause of ventricular arrhythmias [22,23].

Conclusions

Sex and age affect the electrophys- iological properties of Premature Ventricular ectopic beats of the apparently healthy indiv-iduals. The males are more

affected with Premature Ventricular ectopic beats than females regarding this effect.

Conflict of Interest: We declare that there is conflict of interest

Ethical Approval: The research approved by scientific and ethical committee at our department

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Review Article

Recent Advances of Therapeutic Strategies Based on the Molecular Signature in Non-Syndromic Congenital Neutropenia

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Abstract

Neutropenia has been defined to be classified into transient and chronic forms based upon duration and acquired or congenital forms. Chronic neutropenia is considered as congenital, cyclic, autoimmune and idiopathic categorizes, where congenital and cyclic categories are hereditary forms of neutropenia, but the idiopathic and autoimmune are known as acquired neutropenia. In the present review, we focus highly on the genetic subtypes of non-syndromic congenital neutropenia and therapeutic strategies regarding to recent evidence. All aspects of molecular pathogenesis and biology have not been clarified so far; therefore, further in-depth understanding of the molecular mechanisms is needed to reveal roles of molecular mechanisms, and pathways involved in the development of congenital neutropenia and to provide a basis for achieving novel therapeutic strategies.

Keywords: *Neutropenia, Molecular mechanisms, Therapeutic strategies, Mutations*

Background

Definition of Neutropenia

Neutropenia is considered as a blood neutrophil count $<1{\cdot}5 \times 10^9$/l, that is commonly detected in 6–8% of all newborns during their neonatal intensive care unit (NICU) stay [1]. Neutropenia is considered as a reduction in the number of circulating neutrophils. The presence of neutropenia can be associated with an underlying systemic or hematological disease. Neutropenia is labelled as mild, moderate- and severe neutropenia [2-4]

In an asymptomatic neutropenic neonate, the association between low ANC and the risk of infection will be considered speculative, and children's neutropenia can be involved in Kostmann syndrome or chemotherapy. It has been defined that an ANC <500/μL may be potentially associated with an increased risk of secondary infections, especially when neutropenia continues for more than a few days [5, 6]

A number of factors have been defined to be capable of influencing the susceptibility of pyogenic bacteria, enteric bacteria, and some fungi in patients suffering from neutropenia including severity of neutropenia, bone marrow myeloid reserves, speed of onset and duration of the neutropenia, absolute monocyte count (AMC), and

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the functional situation of phagocytes [7, 8]. Overall, many etiologies are involved in pediatric neutropenia [7, 8]. The duration of neutropenia is of particular importance and there is needed for further assessment of neonates suffering from mild-moderate neutropenia for >7–10 days [6, 9]

Moreover, the risk of life threatening bacterial can be markedly increased, when the neutrophil count is less than 1.0×10^9/L [2-4]. The appropriate reference range plays a key role in preventing the mistake of detecting a child with neutropenia. The appropriate reference range plays a key role in preventing the mistake of detecting a child with neutropenia. Based on the available date, Caucasian white people show a higher neutrophil count compared to African, Arabic-Middle Eastern origin and Afro-Caribbean. It has been shown that 25% of African people have shown neutrophil counts of less than 1.0× 10 / liter, where this condition called "ethnic neutropenia" and exhibiting little clinical consequence [10]. It has been indicated that neutropenia is capable of affecting up to 50% of very low birth weight infants as compared term newborns, leading to an increased risk of sepsis and mortality, as well as an enhanced risk of infection secondary to neutropenia [9,11,12]

It is noteworthy that ethnic neutropenia has been found to be persistently seen until adulthood. Certain types of neutropenia (e.g., benign neutropenia of childhood) can present with profound neutropenia yet have little clinical consequence. Some types of neutropenia (i.e., benign neutropenia of infancy/ childhood) can be associated with profound neutropenia (ANC below 100/uL), but exhibit few clinical outcomes [13]. On the other hand, 6% of premature or small for gestational age newborns are likely to exhibit ANC lower than 1·0 × 109/l [14,15]

Neutropenia is classified into acute (or transient) and chronic forms based upon duration and acquired or congenital form [16]. Chronic neutropenia is considered as congenital, cyclic, autoimmune and idiopathic categorizes, where congenital and cyclic categories are hereditary neutropenia, but the idiopathic and autoimmune are known as acquired neutropenia [8,17]

A heterogeneous group of diseases can potentially lead to the occurrence of neutropenia such as benign transient post-viral suppression, and overwhelming systemic disease and the clinical pattern of causes and outcome are different at different ages for children and adults [10]. Neutropenia arises as an adverse reaction with certain drugs or chemotherapy. It is important to note that recognizing the causes of neutropenia may be difficult; therefore, the cause of the illness plays a key role in its management and prognosis.. It is noteworthy that the mechanisms of isoimmune vs alloimmune are involved in the onset of neutropenia at birth and for the first few months, where the risk of infection with severe bacteria may increase [15]. Neutropenia in children may be diagnosed accidentally when minor symptoms are recognized, but some issues are of particular importance, including the discrimination of acute or benign causes and severe congenital neutropenia as a rare condition, as well as neutropenia linked to hematological disorder [10]

Hereditary neutropenia (congenital neutropenia)

Congenital neutropenia is considered to be a rare primary immunodeficiency disease and is characterized by chronic neutropenia linked to genetically heterogeneous phenotypic traits. It occurs in the first year of life as a recurrent fever, which usually depends on the type of infection. However, it should be noted that there are difficulties in identifying idiopathic, autoimmune or congenital neutropenic conditions [15]. Therefore, the genotype plays a key role in distinguishing neutropenic types, but cannot be easily applied during primary evaluation [18]. However, it is important to note that severe and recurrent infections are commonly seen in congenital disorders [19]. Furthermore, some parts of an organ involved in the diseases may not be visible in the initial stage [18]. It has been documented that different distinctive syndromes are involved in congenital neutropenia in children. Family history, physical examination, and laboratory testing may lead the physician to a conformational genetic test [20]. Importantly, the genetic diagnosis has been described as an important criterion for categorizing patients. It has been proven that mutations in ELANE, HAX1, GFI1, or WAS are related to non-syndromic variants of congenital neutropenia. On the other hand, congenital neutropenia syndromes are involved in different gene defects and mutations including encoding genes of mitochondrial proteins (Gene defects: AK2, TAZ), encoding genes of ribosomal proteins (Gene defects: Shwachman-Bodian-Diamond syndrome (SBDS), RMRP (RNA component

of mitochondrial RNA processing endoribonuclease)), and controlling genes of glucose metabolism (Mutations: SLC37A4 [G6PT1], G6PC3) or controlling genes of lysosomal function (Mutations: LYST (Lysosomal Trafficking Regulator), AP3B1, RAB27A, VPS13B, ROBLD3/p14), [20,21]. Therefore, we have described the genetic cause involved in the non-syndromic congenital neutropenia that is characterized by maturation or deficiency.

Non-Syndromic Congenital Neutropenia

Severe congenital neutropenia (SCN) and cyclic neutropenia (CYN)

Multiple hereditary syndromes play a key role in the development of SCN, for which there is strong evidence, and the similar role has been defined for the underlying genetic defects, however, its etiology is unknown in many patients [22,23]

Congenital neutropenia is known as a rare disease, which its prevalence ranged from 3 to 8.5 cases per million [24]. The autosomal dominant disorders are widespread throughout the world, while an increasing body of evidence suggests that recessive disorders are commonly diagnosed in consanguineous populations. It is noteworthy that recurrent bacterial infections are predominantly appeared in mucous membranes, skin and oral cavity of patients suffering from SCN and disease accompanied with aphthous stomatitis, periodontitis, and abscesses and damaged teeth associated with frequent gingivitis [25,26]

HAX1 mutations-related SCN has been estimated to be high due to due to consanguineous marriages of Turkish and Arabic families. Confirmation of the diagnosis of SCN by determining the ELANE mutation should be first taken into consideration unless other evidence is achieved including family history and clinical clues obtained from the physical assessment and laboratory findings. Regarding the hereditary factors, it has been revealed that Caucasian white people show a higher neutrophil count compared to African, Arabic-Middle Eastern origin and Afro-Caribbean. In comparison, African heritage a lower neutrophil count, when compared with people white European or Asian origin. Moreover, polymorphism in the Duffy antigen/ chemokine receptor has been indicated as a key factor associated with neutropenia in African heritage [27, 28]. Overall, mutations and deficiency in many genes have been revealed to be involved in SCN. These include the ELA2, HAX1, GFI1, WASP, VPS45, JAGN, SLC37A4, G6PC3 and CSF3R. We focus highly on the genetic subtypes of non-syndromic congenital neutropenia that are characterized by maturation or deficiency.

Autosomal Dominant

CYN has been defined as rear and autosomal dominantly inherited disorder that is characterized by oscillations in neutrophils and recurrent episodes of severe neutropenia with an average 21-day interval. It can be clinically exhibited recurrent acute stomatologic disorders (e.g., mainly aphthae), fever, painful oral ulcers, recurrent oropharyngeal infections [25, 29]. ELANE□CYN is attributable to an increased risk of serious infections, especially for children [18]. Genetic findings have attributed a role to mutation of neutrophil elastase gene, ELANE or ELA2 in developing CYN [30,31]. ELANE mutations are responsible for CYN and SCN, which are considered as bone marrow failure syndromes. The mutation has been estimated to be found in 80- 100% of subjects of CN and in 63-63% of SCN cases [32,33]. To best of our knowledge, more than 50 mutations have been described for of ELANE, where some of them are attributable to both SCN and CYN patients and some are responsible for SCN cases [34,35]. Emerging evidence has indicated that different functional perturbations in the ELANE protein are likely responsible for the maturation arrest in granulopoiesis [36]

Other study supporting the existence of unfolded protein response model for the pathogenesis of SCN linked to ELA2 mutations [35], the accumulation of misfolded proteins in the endoplasmic reticulum has been indicated to trigger the UPR pathway as a cellular stress response. The UPR pathway plays its role by triggering protein kinase RNA-like endoplasmic reticulum kinase (PERK), transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1). Activation of these key regulators reduces misfolded proteins via three mechanistically distinct arms including attenuated global protein synthesis, transcription of several ER-resident proteins (I.e., ER chaperone proteins) as well as via triggering ER-associated protein degradation pathway of misfolded proteins [37,38]. ATF6 has been shown to

participate in the regulation of master-chaperone (heat shock protein 5 (HSPA5), [39]

The fundamental question is how similar mutations can lead to distinct phenotypes, where are related to SCN and CYN. As a matter of fact, the same ELANE mutants can lead to the UPR in SCN patients, while not in patients suffering from CYN [39, 40]. Several mutations have been defined for SCN and CYN, where an increasing body of evidence suggests that there is no definite genotype-phenotype relationship [25,31]. A previous study indicated that SCN and CYN in patients with a shared ELANE mutation and paternal haplotype can play a role for determining congenital neutropenia phenotypes via modification of genes, and categorizing CYN and SCN in a spectrum of phenotypes, indicating the different degrees of the same disease processes [41]

Additionally, siblings sharing a similar mutation of ELANE can be capable of developing various phenotypes. Regarding tow aforementioned evidence, increasing evidence suggests that some scholars regard SCN and CYN as two sides of a coin [41, 42]

Growth factor independence-1 (GFI1) plays a key role in granulopoiesis in both mice and humans. Knockout (KO) murine GFI1 has been demonstrated to be crucial for neutrophil differentiation [43], which its knockout model showed myeloid differentiation arrest, leading to SCN [43, 44]. GFI1 has six C_2H_2-type zinc fingers and a SNAG domain that are involved in its repressor activity by binding DNA. The mutated protein loses the ability to bind to DNA, and consequently leads to stopping repressor activity on target genes.

This mutation has been found in patients who suffered from an inherited dominant bleeding disorder with anisopoikilocytosis and moderate congenital macrothrombocytopenia (CMTP), [45]

Whilst there is often experimental evidence that mice exhibited severe neutropenia and defects in the HSC fraction and in B cells and T helper cells due to a lack of truly Gfi1 [46-48]. GFI1mutantatin leads to elevated numbers of monocytes and decreased numbers of CD4-T and B cells. Overall, Gfi1 role in controlling the function of myeloid and lymphoid cells has been confirmed [48, 49]. Heterozygous mutations of GFI1 have been demonstrated in patinas suffering from SCN and CYN [50, 51], which SCN patients exhibit abnormal promyelocytes accumulate. Heterozygous human mutations in GFI1 are predominantly linked to the dominant negative p.N382S and p.K403R substitutions and a reduced level of neutrophil elastase result in GFI1 deficiency consequently has accompanied by additional defects of lymphocyte proliferation [45,52]. The GFI1N N382S mutation has been found to contribute to the inhibition of GFI1 DNA binding, leading to a dominant-negative block to murine granulopoiesis via colony stimulating factor-1 (CSF1), [52]

An increasing body of evidence indicates the interaction of NEDD4-binding protein 2-like 2 with neutrophil elastase and GFI1, and potentially leading to GFI1 mutations and decrease in ELANE expression in GFI1 deficiency [45,53]. It has been previously demonstrated that Gfi1 is capable of regulating the G-CSF signaling and development of neutrophil via the Ras activator Ras guanine-releasing protein 1 (RasGRP1), [54]

Autosomal recessive severe congenital neutropenia

Kostmann syndrome (HAX1-neutropenia)

Kostmann syndrome is defined as an autosomal recessive disorder, which is now known as SCN has been characterized by severe neutropenia and onset of severe bacterial infections.

As mentioned, patients who suffer from autosomal-dominant SCN exhibited the ELANE gene. Mutations arrest at the promyelocyte stage of neutrophil development has been described to be the result of this mutation and consequently result in ineffective neutrophil production, as well as cognitive impairments, delays, and epilepsy [55,56]. Biallelic mutations in the HAX1 gene have been proved to develop autosomal SCN. Maturation arrest' at the promyelocyte stage of neutrophil development is very similar to those seen in patients with mutations in ELANE.

It has been defined that deficiencies in HAX-1 expression are responsible to a genetic alteration contributing to the etiology and pathophysiology of certain clinical situations including SCN and/ or Kostmann syndrome (HAX1-neutropenia), and central nervous system or neurodevelopmental delays, as well

as abnormalities [57]

HAX1 deficiency, as other genetic subgroups of SCN, has been found to be capable of increasing the myeloid cell apoptosis [58-60]. It seems that there are other functions be associated with the cause of SCN; different HAX1 protein isoforms may be involved in its functions [61]. It is noteworthy that a more previous data has suggested a role for HAX-1 in the regulation of apoptosis, where apoptosis is capable of controlling neutrophil development [61]

Genotype-phenotype correlations have been identified (allelic heterogeneity) to be capable of affecting HAX1 transcript variants I, leading to neutropenia [31, 61, 62]. Additionally, the pathological mutation has been found to be removed from variant II, because of splicing out of the 5′end of exon 2 [61]

Accumulating evidence revealed that two phenotypes of HAX-1 deficiency are implicated in the presence of two isoforms of the HAX-1 protein. HAX-1 variant 001 and 004 (as mutation) are considered to be corresponding for the severe course of disease with SCN. Mutations in isoform 001 can lead to the development of the milder course of disease with isolated SCN because of splicing out of the 004 [57]. HAX1 mutations have been identified to be linked to the ethnic composition, where most subjects with Kostmann syndrome belonged to consanguineous marriages from Middle-Eastern descent [31, 63]

Future investigations can lead to an increased understanding of the complexity of multifunctional roles of HAX-1 in different cellular processes and human diseases (e.g., hematopoietic and malignancies disease, severe congenital neutropenia).

The iPSC differentiation system is suitable for the study of the clinical phenotype of Kostmann SCN and to develop new therapeutic options.

The patient-derived HAX1W44X-iPSC has been indicated to be capable of recapitulating the Kostmann syndrome phenotype in vitro, where an increasing body of evidence exhibited HAX1 mutations as a monogenic lesion of the disease. As a matter of fact, targeting the genetic correction of the HAX1 gene has also been considered as a means of repairing patient-derived iPSCs, where was capable to induce pluripotent stem cells. HAX1 and HCLS1 have been demonstrated to play their key roles in a large dysregulated genetic network and highlighted HAX1 involvement in other cellular signaling pathways [64]. A recent study demonstrated that HAX-1 play a fundamental role in the regulation of oxidative.

Stress and SERCA2a degradation, where it is involved, not only in calcium homeostasis, but also in the cell survival pathways and can play a key role in cardioprotection by regulating SERCA2a levels. A increasing body of evidence suggests that HAX-1 are not only responsible for the SR Ca2+ transport but also play an important role in multiple tissues and diseases, indicating the important nature of NOX4 derived ROS [65]

One of the questions that remain about the autosomal recessive of SCN is why the clinical manifestations in these patients are markedly restricted to the brain and the bone marrow [61], while ubiquitous expression of HAX1 has been revealed across various human tissues? [61, 62, 66]. An increasing body of evidence highlighted HAX-1 as a multifunctional protein. Aforementioned questions can be likely associated to different splice variants, by which isoforms may be encoded with several tissues' distribution and/or *many* functions and specific expression of the different HAX-1-interaction can potentially lead to subcellular localization and cellular function of HAX-1 in certain tissues [61]. Therefore, further in-depth understanding of the molecular mechanisms is needed to reveal roles of HAX-1 in various cellular processes and different tissues.

Conclusion

It should be taken into consideration that all aspects of molecular pathogenesis and molecular biology have not been clarified so far, decision making on treatment are currently sophisticated and limited to G-CSF and *HSCT*. Further in-depth understanding of the molecular mechanisms is needed to reveal roles of HAX-1 in various cellular processes and different tissues, where an increasing body of evidence highlighted HAX-1 as multifunctional protein, specific expression of the different HAX-1-interaction can potentially lead to subcellular localization and cellular function of HAX-1 in certain tissues. An increasing body of knowledge is not only relevant for individualized diagnostic and

therapeutic advice for congenital neutropenia, but also for the scientific community in general. Nevertheless, many questions remain unanswered,

A variety of diagnostic and therapeutic approaches to preventing non-syndromic congenital neutropenia are currently being achieved, however, all aspects of molecular pathogenesis and molecular biology have not been deeply understood, further advances are needed for providing both a better understanding of the involvement of molecular mechanisms, and pathways involved in the development of congenital neutropenia and a basis for providing novel therapeutic strategies.

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Effect of Lead on Some Phenotypic Characteristics, Protein Changes and Productivity of *Beta vulgaris var. cicle*

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Abstract

This study was conducted at the College of Education for women, Tikrit University / department of biology, and aims to find out the effect of lead in different concentrations (125, 250 and 500) mg / kg on the phenotypic, physiological and genetic characteristics (amino acids) of chard *Beta vulgaris var. cicle*.

The results showed that soil pollution with lead leads to inhibition of vegetative growth through its effect on the processes of division, cellular expansion and hormonal regulation, as well as negatively affects various vital activities and different metabolic processes (photosynthesis, respiration, building of nucleic acids, proteins, enzymatic activities, and other vital activities necessary for plant growth and reduces productivity).

Key words*: lead, amino acids, chard plant, genetic characteristics, phenotypes.*

Introduction

In recent times, environmental and genetic studies are of interest to many researchers, especially after the high rate of environmental pollution (soil, water and air) in the world with heavy metals, and the emergence of many problems resulting from high concentrations of heavy metals as a result of their wrong uses in industry, agriculture and energy, in addition to the high local waste [(1,2)]. Heavy metals have the ability to transmit to humans and animals fed on edible plants through their accumulation in their tissues and at high levels that lead to toxicity [(3)]. One organic complex to another, and thus this environmental problem raised concerns and worries of many researchers [(4)]. The two elements of lead and cadmium are among the most heavy elements affecting human life even when they are present in a small percentage and although their physiological role is not known, they do not pose a danger to humans [(5)]. The human ingestion of plants contaminated with cadmium or lead leads to damage to the lung, kidney and nervous system and great damage to it [(6)(31)]. The presence of heavy metals such as lead, cadmium and nickel also affects the plant's growth and physiological activity, and its high concentrations reduce the expansion and division of cells and thus affects plant growth and productivity [(7)]. Chard plant Beta vulgaris var. cicle is a species of the Beta genus that belongs to the Betaea clan of the Chenopodiaceae family, this plant belongs to the field crops cultivated in winter and from the leaves have large bright leaves that are used as food and fodder in Europe, Asia, Iraq, Jordan, Lebanon and Syria [(8)].

The plant of the Rumatica family contains a high percentage of vitamins such as A, B, and C, and from here it acquires its nutritional value, and the difference in amino acids content is observed in the types of plants of this family. Amino acids gain their importance from their ability to help the plant to withstand the stresses resulting from environmental conditions and also help in the synthesis process, it is a bio-enzymatic stimulant.

There are many studies that have confirmed the toxicity of these minerals and their negative impact on productivity and human health, including the study [(9-11)] studied the potential of halophytic plants to eliminate the influence of heavy metals like lead and nickel in the soil. While Yongsheng *et al*. [(12)] studied the effect of lead accumulation on the components and growth of the tea plant, and noted its negative effect, and the decrease in the yield and quality of the tea plant. Alamari *et al*. [(13)] studied the efficacy of ascorbic acid in improving the

tolerance of wheat plants to toxicity to lead. Despite the presence of many recent studies of the effect of heavy metals on the growth and effectiveness of plants, there is a dearth of knowledge of the effect of these metals on the protein activity of the various plants spread in the Iraqi lands. That is why the current study aims to know the extent to which genetic factors (amino acids) are affected by soil pollution with different concentrations of lead and its reflection on some phenotypic characteristics and the concentration of amino acids of the chard plant for different growth stages, and to shed light on the most important protein variables as a result of high pollution in Iraqi lands.

Materials and Methods

Initialization and examination of samples

The current study was conducted in the laboratories of the department of Biology/ College of Education for women/ University of Tikrit - Iraq for a period between 2-5 months of 2020 to find out the effect of lead on the phenotypic and genetic characteristics of the chard plant and its reflection on the amino content of plant leaves in the vegetative growth and harvest stages and seeds.

After developing the plant by following the scientific environmental conditions (preparing soil, determining the type of seeds, analyzing the soil, estimating the field capacity of the soil and preparing the pots used for planting the plant) according to the method of Black and Hartge [14] and determining the treatments for lead by three replicates and at three concentrations (125, 250 and 500 mg / kg).) And estimating the percentage of germination, method of cultivation and method of irrigation, and after 45 days of planting, three replications were taken (treatment) and the phenotype of the plant was measured and observed with the control plant, where the phenotypes of the plant height, the length of the root system, and the leaf area were recorded and the relative water content in the leaves was estimated using the equation below.

$$\text{Relative water content (\%)} = \frac{\text{Soft Weight - Dry Weight}}{\text{Bulging Weight - Dry Weight}} \times 100$$

The degree of damage (the degree of stability of the membranes) according to the method [15]. The root and vegetative total were weighed and the dry weight was measured. Chlorophyll [16] and carbohydrates were estimated. After 125 days of planting, the mature plants were uprooted in the final growth stage, washed, and performed some chemical and phenotypic tests again, such as plant height, root system length, dry weight, shoot weight, number of branches, number of heads, number of seeds, seed weight, and the amount of the concentration of nutrients in the plant in the dried parts. For each of the shoots, the root system and the seeds, as samples were taken after being dried and milled with a special mill, 0.5 gm of each sample was taken and digested by the method of wet digestion [17] and the nutrients potassium, sodium, chloride, calcium and magnesium were estimated to see the extent to which proteins in the plant and affected cell membranes were affected. Amino acids affected in turn by the heavy metal lead then the heavy metals were estimated using the Atomic Absorption device and the lead element was estimated in the root and vegetable group in the vegetative growth and seed stages by the method [18], taking 0.5 g of the powder of the previously digested dried plant samples using sulfuric acid and perchloric acid at a ratio of 3: 1. [18]. After that, the last and basic step of the research was done in the detection of the type and concentration of amino acids in plant leaves and seeds.

Detection of type and concentration of amino acids in plant leaves and seeds:

The leaves and seeds of the chard plant were dried in the vegetative and reproductive growth stages in order to detect the type and concentration of amino acids. Leaves and dried seeds were ground and 10 g was taken from them, 100 ml of ethyl alcohol, 80% concentration, were added to them and left in the refrigerator for 5 minutes. Then the sample was placed in a Sixhlet for an hour and 100 ml of ethyl alcohol was added to it. The temperature of 50 ° C and the dry residue at the bottom of the flask were allowed to dissolve with 2 ml of methanol, after which the sample was mixed with the Orthophthalaldehyde (OPA) amino acid derivative, and injected with an HPLC device.

According to the method Carpena *et al*. [19].

Statistical Analysis

The experiments were designed and analyzed statistically using a Completely Randomized Design [20]. The significant differences between the transaction rates were compared using the lowest significant difference at the 5% probability level according to Test Duncan New Multiple Round.

Results and Discussion

The results of soil treatment with lead in three concentrations (125, 250 and 500 mg / g) showed that there was a significant decrease in plant height compared to the control sample that reached 30 cm, while the lowest plant height was 17 cm at a concentration of 500 mg / g, and a significant decrease was observed at the two concentrations (125 and 250 mg / g). It reached 18 and 20 cm (Table 1). The high concentration of lead has a toxic effect, which in turn reduced the plant height and the occurrence of significant reduction in vegetative characteristics as a result of inhibiting the hormonal regulation of plant growth or inhibiting the process of cell division and expansion and thus the accumulation of lead leads to inhibit the processes and metabolic activities such as photosynthesis, respiration, amino acid synthesis, and enzyme activity [7, 21,22].

The current study showed a significant increase in the length of the root group when increasing the concentration of the element of lead compared to the control sample, so the length of the root group reached 19 cm at the concentration of 125 and 250 and reached 20 cm at the concentration of 500 mg compared to the control treatment and the presence of this significant difference may be due to the fact that heavy elements increase the radical secretions and reduce the pH Soil and the increase in the readiness of heavy elements for absorption into the soil by the root system. It was also found that there was a significant decrease in the leaf area of the chard plant when treated with different concentrations of lead, the highest decrease was 84 cm at a concentration of 500 mg / g compared to the control treatment as in Table 1. The reason is the decrease in the leaf area leads to the accumulation of lead and its toxicity, which inhibit cell division and reduce the paper space. It was observed that there was a significant decrease in the relative water content of the chard plant when it was treated with different concentrations of lead, indicating a significant decrease at the concentration (500 mg / g) compared to the control sample in which the percentage of water content reached 92%, and a significant increase was found at the concentrations of 125 and 250 mg / g for the element. Lead reached 92.5% and 92.5%. The reason for the difference is due to the change in the movement of water inside the plant through the lack of transport vessels (their area and number). Thus, the results of the current study are in agreement with the results of the study of Vassileu *et al*.[23] and Maruthi *et al*.[24]. While the degree of stability of the membranes was affected. Cytokinesis and the percentage of damage (MSI) according to different concentrations of lead, and it was found that there was a significant increase compared to the control sample, so the damage percentage reached (33, 32 and 45), respectively, Table 1. Here it should be noted that the cause of the damage is due to the occurrence of changes in the composition and functions of cellular membranes, and that the increased damage occurred as a result of the reduction of the ATPase enzyme present in the cytoplasmic membranes, and thus the results of the study agree with the results of the study of Bnavide *et al*.[25]. It was also found that there was a significant decrease in the dry and soft weight of the shoots and the percentage of carbohydrates when treating the chard plant with different concentrations of lead compared to the control sample, while there was no significant decrease in the dry and fresh weight of the root total of the chard plant treated with different concentrations of lead compared to the control sample, as shown in Table 1. These results are consistent with the results of the study [7].

The current study showed its agreement with the study of Zhou [26], which emphasized the existence of a relationship between the stability of the membranes and the relative water content. The more proline accumulation, the higher the relative water content, and this leads to a water stress and a reaction in the plant cell and an increase in the accumulation of proline and thus it is an opposite relationship or negative.

Table 1. The effect of lead on the general characteristics of chard plants during the vegetative growth stage.

Treatment / Plant Parts	Control	Pb mg/kg		
		500	**250**	**125**
Plant length (cm)		17c	18c	20c
Root total length (cm)	30a	20b	19b	19b
Leaf space (cm2)	16c	84f	93d	216b
Relative water content %	273a	88b	92.5a	93a
Degree of stability of the films (damage ratio)	92a	45b	32c	33c
Vegetable dry weight (g)	22e	0.44b	0.65b	0.32b
Root dry weight (g)	5.1a	0.25a	0.46a	0.41a
Fresh weight of vegetative total (g)	0.9a	2.2.4c	4.5bc	2.1c
Fresh Weight of Root total (g)	26.1a	1.6b	1.9b	1.4b
Carbohydrate percentage (mg / g)	3.0a	4.3cd	6.1b	6.9b
Percentage of chlorophyll a(mg/g)	8.7a	19.2a	10.5b	4.8c
Percentage of chlorophyll b(mg/g)	11.96a	9.9a	1.2b	3.7d
Percentage of chlorophyll a+b(mg/g)	8.1cd	9d	6.7a	8.5d

* Similar letters indicate no significant differences

The results of the current study showed that there was a significant decrease in plant height in the harvest phase compared to the control sample, as the highest decrease in the concentration of 250 mg / g was found, while it was found that there was a significant increase in the length of the root system in the harvest phase compared to the control sample and the vegetative growth phase at the concentration of 125 mg / g while it was not recorded. The concentration is 500 mg / g, no significant difference compared to the control sample, and there was a significant difference in the number of branches and fruiting heads and the number of seeds, so 500 mg / g was one of the most effective concentrations on the number of fruiting heads and the number of seeds, while the concentration of 500 mg / g was more influential on the decrease in the number of branches as in Table 2. The results of the present study are in agreement with the findings of the Alamri study [13] which indicated a decrease in growth in roots and stems, decrease in fresh and dry weight, lack of relative water content, deterioration of chlorophyll, and low nutrients in wheat plants treated with lead concentrations.

Table 2. The effect of lead on the general characteristics of chard plants during the harvest stage.

Treatment / Plant Parts	Control	Pb mg/kg		
		500	250	125
Plant length (cm)	104 a	80b	71c	100a
Root total length (cm)	35 b	33b	22e	45a
Vegetable dry weight (g)	15 a	10.4 c	7.1d	13.7b
Root dry weight (g)	2.60 b	2.4b	2.3b	4.0a
Number of branches plants	12 a	11a	7c	9bc
Number heads fruity	207 a	67b	79b	77b
Number of seeds	355 a	102e	200c	250f

* Similar letters indicate no significant differences.

The results of the current study (Table 3) showed a significant decrease in the concentrations of all the nutrients that were measured, such as potassium (K^+) and sodium in the leaves and roots of the chard plant. The higher the concentration of lead, the greater the decrease in the absorption of potassium from the soil, and the reason for the lack of absorption of potassium and sodium and chloride (Cl^-) by the roots compared to the control sample. The cause of the disturbance of nutrients in plant tissues may be due to the imbalance caused by heavy metals and their negative effect on the osmotic pressure as well as lead to an imbalance in the ionic balance.

It was also found that there was a significant increase in the concentration of magnesium in the roots of the chard plant, whenever the concentration of lead was high in the soil compared to the control sample. The reason for this is due to an imbalance in the ionic balance, and thus the results of the present study agree with the results of Vassilev[23].

Table. 3 The effect of lead on the percentage of nutrients in the leaves and roots of the chard plant, measured in mg / kg

Treatment / Nutrients	**Control**		**Pb mg/kg**					
	leaves	**roots**	**500**		**250**		**125**	
			leaves	**roots**	**leaves**	**roots**	**leaves**	**roots**
Potassium	20a	13a	1.1e	2.5ef	1.8e	2f	1.2e	3e
Sodium	80a	9a	3.6e	3d	2.5e	4.2c	1.7e	4c
Magnesium	30a	7c	8.8d	8.8a	6.3d	9a	1.6e	10a
Calcium	18a	3c	3d	2d	3.2d	2.5d	3.2d	4b
Chloride	10c	25a	8d	7d	4e	8.6bc	9.3cd	9d

* Similar letters indicate no significant differences.

The results of the current study (Table 4) showed that the treatment of soil with different concentrations of the element lead led to the emergence of a significant increase in the concentration of the element lead in the roots, reaching (3.6, 6.8 and 8 mg / kg), respectively, compared to the control sample as it reached 5.1, 8.1 and 8.8 mg / kg. In leaves, compared to control sample, which reached 2 mg / kg, while the concentration was 12, 12.9 and 13.1 mg / kg of seeds compared to control sample.

The accumulation of heavy metals in the different plant parts has a negative effect on human and animal health due to its link with the food chain, and the accumulation of lead depends on the efficiency of the plant in absorbing the metal from the soil, the type of plant and its ability to neutralize the metal in different parts of its body. It was found that the chard plant has the ability to absorb the lead element from the soil in very low concentrations, and this indicates the danger of growing it in soils contaminated with lead. The biological assembly of the element was observed to increase with increasing concentration, and this is consistent with the results of Saeed [27], who indicated that the concentration of lead in the wheat plant increased when its concentration increased in the soil. The results of the current study are also consistent with the study of Siddhu [28], which confirms that despite the high salts in the soil, its negative effect on plants may be related to many factors, including the acidic function and the solubility of salts and carbonates in the soil.

Table 4. Concentration of the element lead in the different parts of the chard plant under study.

Treatment / Parts	Control	Pb mg/kg		
		500	250	125
Roots	3.3d	8.0a	6.8b	3.6c
Leaves	2.0a	8.8b	8.1b	5.1c
Seeds	1.0d	13.1a	12.9b	12c

* The values in the table represent ranges.

The effect of lead on amino acids: It was found through the results of the current study that the accumulation of lead occurs as a decrease of aspartic acid in the vegetative growth stage of all concentrations, and the accumulation of lead also affects the accumulation of amino acid in the roots, which led to the appearance of an increase in the proportion of amino acid than the control sample and its increase was not confirmed in the seeds compared to the control sample at the concentration of 125,500 mg / kg, however, an increase in the concentration of histidine was observed at the concentration of 250 mg / kg of lead, while the glutamic amino acid decreased compared to the control sample with regard to the concentration of 125 mg / kg and it increased at the concentration of 250 mg / kg. Kg and the percentage varied at the concentration 500 mg / kg. Also, a clear decrease in serine acid was observed compared to the control sample in the two concentrations 125 and 250 mg / kg for vegetative and root growth, while the results showed a clear rise in amino acid (serine) for all concentrations and for all plant parts, and it was found that there was a decrease in the values of the amino acid glutamine. In vegetative and root growth for all concentrations except for the concentration of 500 mg / kg, an increase was observed in the vegetative growth stage, and the values of this acid increased for all concentrations in seeds. While a decrease in the concentration of the amino acid threonine was observed in the stage of vegetative and root growth and for all the studied concentrations except for the concentration of 500 mg / kg for vegetative growth, it was also found that there was an increase in the seeds. It was observed

through the study that there was a significant decrease, not a predominance of amino acids, in the stage of vegetative growth and sometimes the root whereas, a significant increase was observed at the concentration of 500 mg / kg for glycine, histidine, alanine, tyrosine, arginine, methionine, valine, tryptophan, phenylalanine, iso-lysine and eosin, while it was found that there was a significant increase in amino acid concentrations for seeds and for all concentrations. The results of the study agree with the results of the study of Gioseffi *et al*. [29], which indicated the importance and effect of the element of nitrogen on amino acids such as glycine and glutamine in the roots of the wheat plant. and this study dot agree with [30].

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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Computer-Aided Measurement of Total Occlusal Convergence of Teeth Preparations for All-Ceramic Crowns by Dental Students

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Abstract

Objective: This study was based on the evaluation the degree of total occlusal convergence of teeth prepared for all-ceramic crowns which is the main factor that retention and resistance form of indirect restorations depended on. Teeth preparation done by 3rd year dental students at Al-Esraa university collage, Iraq in 2019.

Materials and Method: Eighty typodont teeth, maxillary central incisor(n=40) and first premolar teeth (n=40) were prepared for all-ceramic crowns by preclinical dental students under examination condition. The prepared teeth were scanned and digitized using the laser scanner 3Shape D710 (SculptCAD, Texas, USA). The datasets were evaluated using inspection software (shaper 3D zrt. Hungary, 2020) that used for the assessment of the total occlusal convergence. Each tooth was virtually sectioned by four vertical planes: buccopalatal, mesiodistal, mesiobuccal–distopalatal and distobuccal– mesiopalatal and for each plane the total occlusal convergence angle (degree) was measured by drawing lines along the two opposing axial walls from the finish line extended coronally. The data were analyzed with appropriate statistical tests using analysis of variance (ANOVA) and unpaired t-tests. Differences between groups were tested for significance at 95% confidence interval.

Results: The results revealed that the students prepared the central incisor teeth with average TOC values of 11.46° ± 6.81°, 9.91° ± 4.92°, 10.54° ± 7.05° and 12.06° ± 6.71° respectively, from the buccopalatal, mesiodistal, mesiobuccal–distopalatal and distobuccal– mesiopalatal planes. Concerning first premolar teeth, students prepared them with average TOC values of 12.03° ± 6.63°,11.57° ± 6.34°, 14.3° ± 6.94° and 12.55° ± 6.6° respectively.

The findings revealed that the approximated average TOC values for all the measured planes were greater than the optimal TOC range 4 to 6 ° but stayed within the TOC acceptable range 4 to 14 °.

Ordinary one-way ANOVA test showed no significant (P >0.05) differences in the average TOC between the different four planes within each tested group of teeth. The average value of overall total occlusal convergence (TOCs) in the first premolar teeth= 12.61°± 6.63°was higher than for the central incisor teeth= 10.99°±6.4°. Unpaired t-test for the two tested groups revealed significant ($p < 0.05$) differences.

Conclusions: The presented study found that about 85%, 65.83% respectively of upper central incisor and first premolar teeth prepared for all-ceramic crowns by pre-clinical dental students with acceptable recommended convergence angle range 4-14°.

***Keywords*:** *Total occlusal convergence, teeth preparation, All-ceramic crowns.*

Introduction

Preparation of teeth for full coverage crowns play a crucial role in preclinical training and subsequently enhance the dental treatment. One of the fundamental principles of tooth preparations for indirect restoration is the retention and resistance form. Number of factors that affect the retention and resistance of a full coverage

crown involve the degree of taper, surface area, height of the preparation, diameter of the tooth and type of cement used [1].

Taper or more precisely known as total occlusal convergence (TOC) is the most challenging part of the preparation to assess by inspection only. TOC has been defined as the angle of convergence formed by the two opposing prepared axial walls in each plane [2,3,4]. It is one of the factors which determine the amount of axial and non-axial forces which can be tolerated without degrading retention and loosening of the crown [5].

Studies by Jorgensen [6] and Kaufmann et al. [7] have found an inversely proportional between (TOC) and the retention and stability of complete coverage crowns on the prepared teeth; increasing the convergence angle from 5° to 10° reduces the retentive force by as much as 40-50%. They concluded that maximum retention with a 5° taper angle. Another study by Wilson and Chan in 1994 [8] recommended that maximal tensile retention occurred between 6° and 12°degrees (TOC). Further researchers found that by increasing the angle of convergence from 0 to 6 or 12°, no major change was observed in crown retention. They concluded that increasing this angle from 18 to 24° reduced retention significantly [9]. Another study found that 12° total occlusal convergence angle in teeth prepared for zirconia copings resulted in the best precision of fit and had no influence on marginal adaptation compared to 4° or 8° axial walls tapers [10]. Textbooks of crown and bridge prosthodontics advocate that in full crown preparations axial walls should be as parallel as possible, often recommend a convergence angle of approximately 5° (4-6°) as the ideal, but with a range of (4-14°) as acceptable [2,4,11].

Earlier, many researches have been directed to ascertain the optimal TOC value. later, studies were done to evaluate whether operators could achieve these values. Visual preclinical assessment of (TOC) have traditionally been accomplished but are of limited value for determining the actual convergence angle formed [12,13]. The increasing spread of digital technology in dentistry, specifically direct digital approach for obtaining dental impressions that are capable of capturing and storing high quality 3-dimensional (3D) virtual images of tooth preparations or dental casts, it is become easier to assess the prepared tooth. and to improve undergraduate compliance to the recommended degree of taper. Various studies have contrasted the (TOC) of the tooth preparation in both the Faciolingual and Mesiodistal plane. While, the oblique planes have not been included. Measurement of the TOCs in the oblique planes is important due to the lateral forces during excursive movements which might influence the retention of the indirect restoration [14].

This study was done to evaluate the (TOC) of the prepared teeth by dental students wouldn't vary from the recommended optimal range to provide maximum retention of the crown by measuring TOC in 4 different planes for each tooth, (1) buccopalatal (BP), (2) mesiodistal (MD), (3) mesiobuccal–distopalatal (MB-DP) and (4) distobuccal– mesiopalatinal (DB-MP). Also, to assess the effect of the tooth position (maxillary central incisor and maxillary first premolar) on the degrees of Convergence angle of the prepared teeth.

Materials and Methods

A total of 80 typodont teeth (40 samples for maxillary central incisor and 40 samples for maxillary first premolar) were prepared for all-ceramic crown by the 3rd year preclinical dental students under examination environment. Manikins (Nissin Dental Products) were mounted onto the phantom head to simulate real clinical situation. All the students were given equal and enough time to accomplish each tooth preparation. students were instructed to the ideal preparation considerations including a smooth, 1.0 mm shoulder finishing line that follows the level of gingival margin using tapered diamond burs with flat end. The samples were randomly selected then divided into two groups of teeth. Special plastic squares were fabricated and used as a bases to position the typodont teeth vertically with its long axis.

The typodont teeth were scanned using laser scanner of the CAD/CAM digital impression system and digitized with the computer-aided design software (3Shape D710; SculptCAD, Texas, USA) according to the manufacturing instruction of full crowns by CAD/CAM system. The STL datasets for the prepared teeth were measured by the inspection software (shaper 3D zrt. Hungary, 2020). Each tooth was virtually sectioned by four vertical planes from the: buccal view the mesiodistal (MD) plane, proximal view the buccopalatal (BP) plane, lateral view the mesiobuccal–distopalatal (MB-DP) and

(distobuccal– mesiopalatal (DB-MP) planes. All these planes crossed one virtually central long axis (Figure 1).

For each tooth, the four planes were imaged and filed and for each plane the TOC angle was measured and recorded separately by drawing lines along the two opposing axial walls from the finish line extended coronally (Figure 2).

The data subjected to statistical analysis using GraphPad Prism 8.0.2 software. The relationships and differences of total occlusal convergence angle (degrees) among four different cross-sectional planes for each group of teeth type (upper central incisor and first premolar) Also, the differences among upper central incisor and upper first premolar teeth were tested for significance. One-way, two-way analysis of variance (ANOVA) with Sidak's multiple comparisons test and unpaired t test were used to indicate the significant differences. Data have been shown as Means ± Standard Deviation (SD). Differences between groups were tested for statistical significance at a 95% confidence interval.

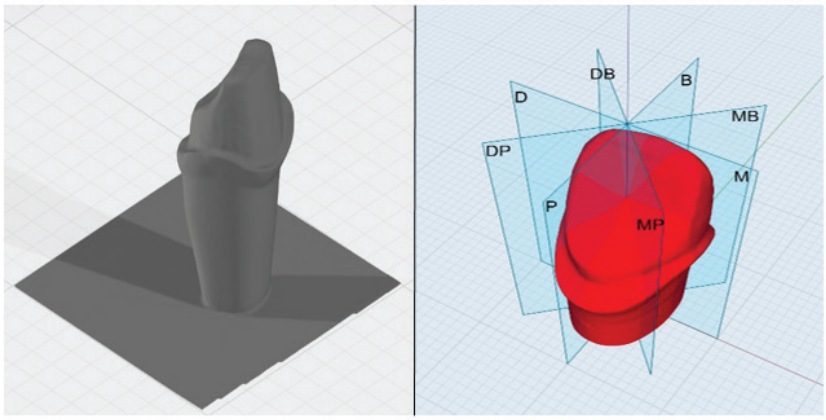

Fig. 1 Sectioning of STL dataset for each tooth into four vertical planes: buccal (B), palatal (P), mesial (M), distal (D), mesiobuccal (MB), distopalatal (DP), distobuccal (DB), mesiopalatal (MP).

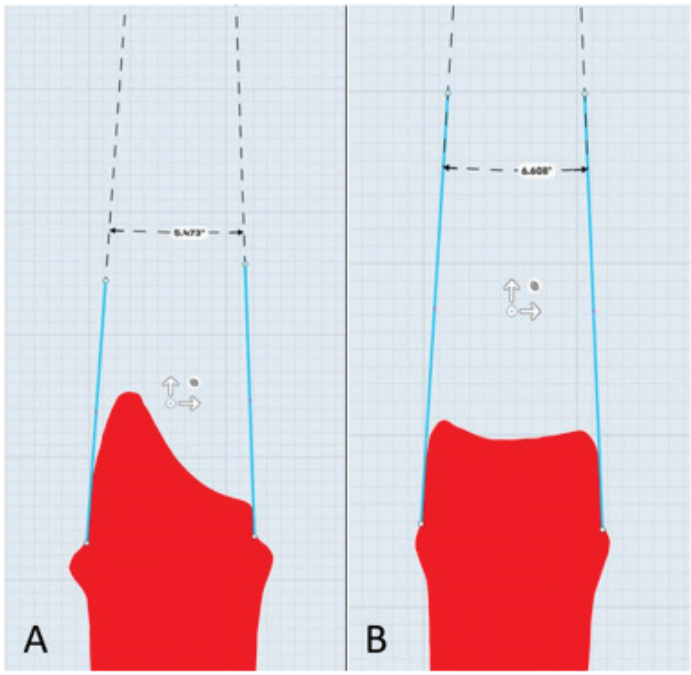

Fig. 2 Measurement of total occlusal convergence in A-BP plane (5.47°), B- MD plane (6.60°).

Results

Table 1 shows the distribution of average TOC (degrees) of different four planes (BP, MD, MB-DP, and DB-MP) for each group of teeth prepared for all-ceramic crowns , upper central incisor (n=40) and upper first premolar teeth (n=40). The highest mean TOC values were observed with first premolar for MB-DP plane (14.3°±6.94°). The mean TOC values for MD plane of upper central incisor were consistently the lowest for all parameters of convergence angle degrees (9.91°±4.92°).

The results of one-way ANOVA test with Sidak's multiple comparisons test showed statistically non-significant ($P > 0.05$) differences with the mean TOC (degrees) between different four planes for each group [Table 2]. Two-way ANOVA test with Sidak's multiple comparisons analysis of average TOC (degrees) of different four planes between two groups. The data has showed there was no significant differences among groups except for MB-DP plane, there was significant ($P = 0.037$) differences between upper central incisor tooth and first premolar tooth [Table 3].

Furthermore, Unpaired t test was used to make comparison of Average TOCs (degrees) between teeth preparation for crowns of upper central incisor and upper first premolar. The results showed that the mean TOCs degrees for first premolar was statistically higher as compared with central incisor teeth ($P < 0.05$) [Table 4].

Table 1: Summary statistics of TOC (degrees) of different four planes for two tested groups of teeth prepared for all-ceramic crowns.

Planes (TOC)	Upper Central incisor				Upper First premolar			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
BP	11.46	6.81	4.16	26.58	12.03	6.63	4.89	26.31
MD	9.91	4.92	2.57	22.45	11.57	6.34	5.8	25.57
MB-BP	10.54	7.05	4.85	28.93	14.3	6.94	6.94	28.67
DB-MP	12.06	6.71	6.3	29.41	12.55	6.6	5.92	26.26

BP:buccopalatal; MD: mesiodistal; MB-DP: mesiobuccal–distopalatal; DB-MP: distobuccal– mesiopalatal; TOC: total occlusal convergence; SD: Standard deviation; min: minimum value; max: maximum value.

Table 2: Comparison of mean TOC (degrees) between different four planes for each tested group of teeth prepared for all-ceramic crowns by one-way ANOVA test.

Planes (TOC)	Central Incisor		First Premolar	
	Mean diff.	P-value	Mean diff.	P-value
BP vs. MD	-1.549	0.8639 ns	0.4565	0.9998 ns
MB-DP vs. DB-MP	1.525	0.8723 ns	-1.747	0.8077 ns

BP: buccopalatal; MD: mesiodistal; MB-DP: mesiobuccal–distopalatal; DB-MP: distobuccal– mesiopalatal; TOC: total occlusal convergence; mean diff: mean differences; ns: non-significant differences (P>0.05).

Table 3: Comparison of mean TOC (degrees) of different four planes between two tested groups of teeth prepared for all-ceramic crowns by two-way ANOVA test.

Central incisor vs. First premolar	Mean diff.	P-value
BP Plane	-2.114	0.4620 ns
MD Plane	-0.1083	>0.9999 ns
MB-DP Plane	-3.763	*0.0374
DB-MP Plane	-0.4905	0.9950 ns

BP: buccopalatal; MD: mesiodistal; MB-DP: mesiobuccal–distopalatal; DB-MP: distobuccal– mesiopalatal; TOC: total occlusal convergence; mean diff: mean differences; ns: non-significant differences (P>0.05); *: significant differences (P<0.05).

Table 4: Comparison of mean TOCs between two tested groups of teeth prepared for all-ceramic crowns by Unpaired t test.

Tooth type	Mean (TOCs)	SD	Mean Diff. ± SEM	P - Value
Central incisor	10.99	6.4	1.619 ± 0.7307	*0.0274
First premolar	12.61	6.63		

(TOCs): overall convergence angles; SD: Standard deviation; mean diff: mean differences; SEM: standard error of mean; *: significant differences (P<0.05).

Discussion

In the presented study about 85 % of the upper central incisor teeth preparations and 65% of the upper first premolar teeth prepared by 3rd year pre-clinical dental students revealed a convergence angle between acceptable TOC range as mentioned in fixed prosthodontics textbooks 4° to 14°. The average TOC values of all assessed planes exceeded the optimal 4° to 6° TOC range. This means that the optimal tapers and Convergence angle degrees mentioned in fixed prosthodontics textbooks and the dental literatures are hard to accomplish by the dental students. The findings of this study were agreed with study by Kirov et al. [15] who found that preclinical students could not finish crown preparations within the 4-6° criteria.

They suggested that a 12º TOC degrees is more realistic and easier to achieve than 6º criterion for full coverage crown preparations. These recommendations balanced with Mack's investigation that a minimum taper of 12° was needed to be visually certain of avoiding undercuts and allow more satisfactory placing of restorations along path of insertion [16].

Patel et al. [17] asked 63 dental students to create convergence angles of 4 to 10°. Only eight students (12%) were able to do so, and the average of the convergence angle was 19°. Although the mean convergence angle found in this study among the two groups was higher than the optimal value, it was still within the recommended acceptable limits. This might be due to the newly picked knowledge by the students of how the ideal preparation is necessary to be performed and the continuously checking and correction by teachers to their students. Also, the students were under final exam's stress and they tried to do the best.

The results of this study showed that was no significant differences after the comparison the mean TOC (degrees) for all planes within each tooth type prepared for all-ceramic crowns evaluated. MB-DP plane in first premolar presented a highest TOC degree (14.3°±6.94°) of all the planes for the two teeth types. It could be supposed that dental students have trouble estimating the right taper of the axial walls in lateral view during preparation, especially in the mesiobuccal to distopalatal directions. Inversely, the MD plane of central incisor recorded the lowest TOC degree (9.91°±4.92°). This could be ascribed to the students who use direct vision and instruments control while preparing the proximal walls of central incisor. Moreover, as compared to other teeth, central incisors have straighter and parallel interproximal walls with a long axis. This finding coincided with the findings of Yoon et al.'s analysis [14] found that the central incisor tooth's MD plane showed at 6.3 degree the least TOC value of all planes for three teeth types.

The results of this study showed that the mean BP Convergence Angle was greater than the mean MD Convergence Angle in the two groups of teeth. This might be attributed to the difficulty to assess the buccal and palatal axial walls of tooth for undercuts and the existence of relatively short palatal surface on the cingulum of central incisor tooth was the reason for increasing taper in the bucco-palatal aspect. Results from studies by Makker et al. [18] and Tiu et al. [19] confirm this inspection with exception the investigation of Convergence angle achieved in clinical settings, displaying that all maxillary anterior samples, premolars and molars had an average faciolingual cross-section with higher TOC values relative to the average mesiodistal cross-sectional TOC values.

Our survey results showed that the average over all convergence for upper teeth preparation done by the third-year dental students were 12.61°±6.63° for first premolars and 10.99°±6.4° for central incisor. After the comparison analysis, the results showed that the mean TOCs for the upper first premolar preparation was significantly higher than the mean TOCs for the upper central incisor tooth preparations ($p<0.05$). The difference in the convergence angle between the two groups that were evaluated could be due to the highest mean TOC (degrees) value of MB-DP plane of first premolar that showed significant higher differences after the comparison with MB-DP plane of central incisor tooth. Also, the difference among upper central incisor and first premolar could be strongly correlated to the difference in the anatomical shape and student's ability to directly visualize the anterior teeth. these reasons could be resulted in better accessibility for one group than for other. This was contrary to expectations as premolars (bicuspids) had the lowest convergence values compared to all the other teeth all of which had similar convergence angles [20]. Many studies explained the difference in convergence angle from tooth to tooth might be correlated to overall variations in anatomy, location of the tooth, visual access and positioning the bur next to the teeth [14,21,22].

The utilization of Digital method to assess students' performance may be a valuable resource for dental education and provide a fair assessment of preparations. Since the STL data type is the root of most computer-assisted

design/computer-assisted manufacturing CAD/CAM systems, thus promote the utilizations of digital methods for the preclinical evaluation of teeth preparations features for proper taper or total occlusal convergence, adequate reduction, marginal design and surface texture. Digital method could give direct feedback to the teaching stuff and dental students on the quality of preparations and possible errors and this direct feedback could be enhance the educational quality and student's skill particularly at a pre-clinical level. Studies by Güth et al. [23] and Mays et al. [13] have established that visual measuring the degree of TOC can be a challenge in clinical assessment while digital approach can determine a numeric value that represents definitely the degrees of taper.

Conclusion

Within the limitations of this study, about 85%, 65.83% respectively of upper central incisor and first premolar teeth prepared for all-ceramic crowns by pre-clinical dental students with acceptable recommended convergence angle range 4-14°. The digital approach presented in this study could be a viable tool to assess the degree of total occlusal convergence for the prepared tooth.

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Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: taken form dental laboratories and patient

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Using of Procalcitonin Level in Differentiation between Bacterial Vaginitis Due to Pathogenic Bacteria and Normal Flora

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Abstract

This study conducted to view the role of PCT level in initial finding of bacterial vaginitis in pregnant women. A total of 150 deep vaginal swabs (100 pregnant women and 50 pregnant women as control group) were collected from the beginning of February 2019 to the end of May 2019 from inpatients and outpatients women attended to Al-Fallujah teaching hospital. Media were organized & purified according to producer's guidance. The ready media were utilized for segregation , definition of the viable enumeration , identifying & sensibility workout these media were achieved in the wake of being solidified. Swabs was inoculated bacteriological agars for isolation of causative bacterial types . Blood samples collected by vein puncture for determination of PCT by using ELISA technique. The study showed that that 57% of pregnant women have vaginal swab for culture as compared with 48 of the control group (non- pregnant women). The results demonstrated that, 54.39% of pathogenic bacteria isolated from vagina of pregnant women was G-ve bacteria and 45.61% was G+ve bacteria. *S. aureus* represented the highest rate of pathogenic isolates 45.61% in cases (43.86%) while 29.17 of the control group were infected equally by *E coli* and *K. pneumonia*.The highest rate of antibiotic sensitivity toward *S. aureus* isolates was occurred in levofloxacin (88.46%) while it was completely resistant to ampicillin and lincomycin, 94.11% of *E. coli* isolates was sensitive to each of levofloxacin and cefotaxime and 100% resistant to lincomycin, 92.85% of *Klebsiella* isolates was sensitive to amoxicalve while it was 100% resistant to ampicillin, erythromycin and lincomycin. Procalcitonin level was highly elevated among pregnant ones followed by healthy ones (26.79±9.4 *v.s* 9.236±0.6 pg/ml) at P value <0.01. The highest mean of Procalcitoninlevel is found among pregnant women with pathogenic bacteria isolates of HVS culture (30.15±9.84 pg/ml) and the lowest mean is recorded among pregnant women with normal flora isolates (23.72±7.25 pg/ml).**Conclusions:** Serum PCT was highly elevated in pregnant women with vaginitis especially who diagnosed with pathogenic bacteria.

Keywords: *Procalcitonin; PCT; Pregnancy; Vaginitis; HVS*

Introduction

Not long following birth, lively lacto-bacilli occur inside vaginal duct & proceed so long PH of vagina remains low (a short time). But vaginal pH gets impartial (lasting so till pubescence), a blended microbiota of bacilli in addition to bacilli is present [(1).] If lacto-bacilli are smothered by association of anti-microbial meds, increased the numbers of yeasts and different organisms will occur lead to disturbance & bothering (vaginitis) [(2).] Bacterial vaginosis is a condition separate by hair-raising developments in the sorts and relative degrees of the vaginal microbiota as the vaginal natural framework changes from a sound state, depicted by the presence of lactobacilli, to a wiped out state depicted by the presence

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of animals having a spot with phylotaxa, for instance, Actinobacteria and Bacteroidetes species[1]. Declining in the number of lacto-bacilli will happen again next menopause, leading to a mixed microbiota returning. In about 25 percent of women of childbearing time have B strepto- cocci in their vagina as a normal microbiota[3,4]. at the time of birth cycle, a newborn child may get bundle B strepto-cocci, , thusly might cause meningitis &sepsis for neonate. Common microbiota of vagina routinely as well including pepto-streptococci ; Gardnerella vaginalis ; clostridia;α-hemolytic streptococci; Prevotella species; Ureaplasma urealyticum & sometimes Mobiluncus species or Listeria[5]. mucosal secretion of cervix has anti-bacterial action & has lysozyme. Introitus of vagina in several dames have dense microbiota similar to microbiota of peri-anal zone & perineum. This could be a predisposing agent in repeated infection of urinary system . living creatures of vagina existing at the confinement period might taint baby (for example B strepto-cocci) [6]. Assessment of clinical and lab revelations sets up an ideal technique for diagnosing illness. Improvement of a powerful administrator through refined gives the most critical lab evidence. In any case, this methodology is dreary. While envisioning the results from refined, other definite examination place limits, for instance, the degrees of procalcitonin (PCT) [7]. Over progressing years, serum PCT has been found to be a critical and promising biomarker for making the basic investigation of sullying[8,9]. The aim of this study was to evaluate the role of PCT level in initial diagnosis of bacterial vaginitis in pregnant women.

Materials and Methods

A total of 150 deep vaginal swabs (100 pregnant women and 50 pregnant women as control group) were collected from the beginning of February 2019 to the end of May 2019 from inpatients and outpatients women attended to Al-Fallujah teaching hospital collection of vaginal swabs included:

1-The swab group was to some degree opened.

2-Carefully the swab was implanted into vagina around 2 inches (5 cm) past the introitus and delicately turned for 10 to 30 seconds.

3-When the swab reached the vagina dividers and sogginess and absorbed the soddenness, it was pulled back without reaching the skin.

4-Swabs was passed on to the examination office inside 1 hour of grouping.

5- culturing of swabs in agar of blood & Mac-Conkey Agar for twenty-four hour.

Media were organized & purified according to producer's guidance. The ready media were utilized for segregation , definition of the viable enumeration , identifying & sensibility workout these media were achieved in the wake of being solidified. Upon Mac-Conkey, supplement & agars of blood inoculation of swabs was done . thereafter incubation of vaccinated tray at thirty seven °C for twenty four hr .Substance used for inoculation from the examined bacterium was ready. dipping into the inoculum by the disinfected cotton - swab & thereafter swabbed equally upon face of Muller-Hinton agar dish, later on inoculation for fifteen minutes , the anti-microbial included drum are opposed to the agar by using a forceps compressed promptly to guarantee touch with agar & thereafter dish puts upside down and incubated at three seven C for eighteen hr.

Three ml of blood was assembled by vein cut using needle from each woman got together with this examination. Blood tests were set into sterile test tubes, centrifuged at 3000 rpm for 15 minutes, the gained sera were suctioned using customized micropipette and moved to Eppendorf cylinders and set aside in significant freeze at - 20°C for affirmation of PCT by using ELISA technique.

Results

Table 1 shows that 57% of pregnant women have vaginal swab for culture as compared with 48 of the control group (non- pregnant women). The results demonstrated that, 54.39% of pathogenic bacteria isolated from vagina of pregnant women was G-ve bacteria and 45.61% was G+ve bacteria, Table 2.

Table 1: Result of vaginal swab for culture

Culture finding	Study groups	
	pregnant women (n:100)	Control group (n:100)
	No.(%)	No.(%)
Pathogenic bacteria	57(75)	24(24)
Normal flora	43(43)	26(26)

P. value 0.22

Table 2: Result of pathogenic isolates according to Gram stain.

Bacteria of vaginal isolates	Studied groups	
	pregnant women (n:57)	control group (n:24)
	No.(%)	No.(%)
G+ve bacteria	26(45.61)	4
G-ve bacteria	31(54.39)	20

P <0.05

Table 3 shows that *S. aureus* represented the highest rate of pathogenic isolates 45.61% in cases (43.86%) while 29.17 of the control group were infected equally by *E coli* and *K. pneumonia*.

Table 3: bacterial isolates (species) from the studied groups.

Bbacterial isolates	Positive HVS cases			
	Pregnant women		Non-Pregnant women (control)	
	No	%	No	%
Escherichia coli	17	29.82	7	29.17
Staphylococcus aureus	25	43.86%	4	16.67
Klebsiella pneumoniae	15	26.32	7	29.17
Proteus	0	0	6	25.0
Total	57	100	24	100

P<0.01

Table 4 shows that the highest rate of antibiotic sensitivity toward *S. aureus* isolates was occurred in levofloxacin (88.46%) while it was completely resistant to ampicillin and lincomycin, 94.11% of *E. coli* isolates was sensitive to each of levofloxacin and cefotaxime and 100% resistant to lincomycin, 92.85% of *Klebsiella* isolates was sensitive to amoxicalve while it was 100% resistant to ampicillin, erythromycin and lincomycin.

Table 4: Distribution of antibiotics sensitivity toward pathogenic bacteria isolated from vagina of pregnant women.

Type of antibiotics	Staphylococcus aureus (n:26)		E coli (n:17)		Klebsiella (n:14)	
	No.	%	No.	%	No.	%
Ciprofloxacin	20	76.29	14	82.35	8	57.14
Levofloxacin	23	88.46	16	94.11	10	71.43
Ampicillin	0	0	3	17.64	0	0
Erythromycin	17	65.38	4	23.52	0	0
Gentamicin	20	76.92	7	41.17	5	35.71
Streptomycin	18	69.23	6	35.29	4	28.57
Trimethoprim	19	73.07	4	23.52	6	42.86
Lincomycin	0	0	0	0	0	0
Ceftazidim	14	53.84	8	47.06	5	35.71
Amoxicillin	3	11.54	3	35.64	4	28.57
Amikacin	1	3.85	14	82.35	4	28.57
Amoxiclave	10	38.5	15	88.23	13	92.85
Cefotaxime	12	46.15	16	94.11	12	85.71

Table 5 shows that, Procalcitonin level was highly elevated among pregnant ones followed by healthy ones (26.79±9.4 *v.s* 9.236±0.6 pg/ml) at P value <0.01.

Table 5: Estimation of Procalcitonin level in the studied group

PCT	Study groups		P. value
	Recurrent abortion women	Control group	
No.	100	50	0.0001 HS
Mean	26.79	9.236	
SD	9.4	0.6	

Table 6 shows that the highest mean of Procalcitonin level is found among pregnant women with pathogenic bacteria isolates of HVS culture (30.15±9.84 pg/ml) and the lowest mean is recorded among pregnant women with normal flora isolates (23.72±7.25 pg/ml).

Table 6: Relation of Procalcitonin level with bacterial isolates in pregnant women.

Procalcitonin level (pg/ml)	Positive HVS culture			P. value
	pathogenic	Normal flora	Mixed of pathogenic with normal flora	
No.	46	43	11	0.007
Mean	30.15	23.72	25.56	
SD	9.84	7.25	8.24	

Discussion

In present study, the overall prevalence of vaginal infections (57%) was coherent with several studies done earlier. A study done in Kirkuk found that 33.2% of women with recurrent abortion have positive HVS culture [5]. Martin *et al* [2] displayed that 34.7 % of pregnant women in India had positive HVS culture. Krishna *et al* [3] revealed that, the rate of cervical infection 49.5 %. Zevin *et al* [4] found that HVS were positive in 27.6% pregnant women. This variation may do approach uniqueness in disengagement & distinguishing evidence of vaginal contaminations etiologies. Additionally, ecological components & uniqueness on the authentic investigation members may as well clarify disparity above . Our result was also agreed with [6] when indicated that multiple causes may also responsible for vaginitis.

The clinical criticalness of serum PCT in isolating amidest bacterial defilements and non-bacterial sicknesses, for instance, central inflammatory- response condition (SIRS), has been differentiated and these ones of various markers included endo-toxin, interleukin 6 (IL), β-D-glucan , C-interactive protein (CRP), & white – cell of blood (WBC) counting[9]. A couple of examinations were represented that, levels of serum PCT in women have vaginal bacterial defilements were basically greater than these ones in patients with non- bacterial ailments or non- infectious diseases(10-12). Additionally, PCT-guided counter-agent poison treatment for respiratory plot maladies has been appeared to through and through lessen counter-agent poison introduction with resulting decline in against disease related responses and counter-agent poison impediment. PCT is moreover a fruitful device for finding and peril depiction of upper urinary parcel maladies in pregnant women[13].

In synchronization with the stream exposures[14]. amazingly inspected 166 crisis unit septic patients with circulatory structure infection (BSI), basically higher PCT levels were seen with Gram-negative microorganisms than with Gram-positive living creatures.Likely the most grounded inducers of PCT fuse red hot cytokines and bacterial endotoxins and exotoxins [15]. Pro- calcitonin is seen as an unequivocal marker of genuine bacterial infection in the patients with questionable sepsis/bacteremia[16]. Differentiating and for the most part used exploration community limits, PCT has higher logical exactness[11]. Growing in plasma PCT levels occurs inside post-tainting 2-4 hours and continues until reasonable treatment is begun or the illness is taken leveled out. Half-presence of plasma PCT is approximately 24 hours[2].According to the flow results, Oussalah *et al*.,[17].showed that procalcitonin level was through and through brought up in bacteremic patients and could be used for predicting blood culture achieves patients with suspected circulatory framework illness. A couple of various assessments moreover exhibited that procalcitonin level was extended through and through in patients with bacteremia than in control gathering[12,14]. A development in serum PCT is conspicuous four hour following endo-toxaemia. Serum PCT tops six hr. following endo - toxaemia & has half-life in the serum about 25–30 hr. Late disclosures show that PCT could be a cytokine-like inflammatory mediator. PCT expressed by mono - nuclear cells yet its exact patho - physiological work has not been settled till now[18].

Conclusions

Serum PCT was highly elevated in pregnant women with vaginitis especially who diagnosed with pathogenic bacteria.

Conflict of Interest: non

Source of Findings: non

Ethical Clearance: non

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Assessing Patient Preoperatively and Role in Decreasing Risks on Patients and Preventing Post-Operative Complications for Cholecystectomy

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Abstract

Background: Laparoscopic cholecystectomy has many difficulties which include port Insertion, Dissection of the Calot's Triangle , Grasping of the Gallbladder , Wall thickness, Adhesion and extraction of the Gallbladder. **Aim of the Study:** To predict how difficult cholecystectomy will be from assessing the patient preoperatively which, in turn, help in decreasing the risks on the patients and preventing post-operative complications. **Patients and Methods:** A prospective study conducted in the department of General Surgery at Al-Ramadi Teaching Hospital for the period of nine months from 15th of May 2018 till the 15th of February 2019. It included 60 patients, all of them were undergone laparoscopic cholecystectomy for Gallstone. Patients with common bile duct calculus, dilated common bile duct, current attack of acute cholecystitis, those with absolute contraindications to laparoscopic cholecystectomy, The data were collected prospectively to predict the significance of association with patients' characteristics. The time of operation was calculated from the first port site incision until the last port closure. All the intra operative events were recorded. Conversions to open cholecystectomy were done by median or subcostal laparotomy according to the surgeon's decision and each patient's condition. **Results:** In this study, the highest proportion of operations was categorized as easy (58.3%), 28.3% of operations were considered difficult and 13.3% of them were very difficult. A significant association ($P < 0.05$) between operation difficulty and all of the following characteristics: Aging, obesity, history of previous biliary hospitalization, thickened bladder wall, pericholecystic collection, and large stones. **Conclusions:** The difficult laparoscopic cholecystectomy can be predicted preoperatively based on number of factors, like: obesity, previous biliary admission, gall bladder wall thickness, ultrasound findings (stone size and pericholecystic fluid) and aging process, evaluating of such factors minimize the complications of laparoscopic cholecystectomy and conversion to the open procedure.

Keywords: *Laparoscopic cholecystectomy, difficulty, conversion, Iraq*

Introduction

Laparoscopic cholecystectomy though safe and effective, yet can be difficult at times. Various problems faced are difficulty in creating pneumoperitoneum, accessing peritoneal cavity (difficult port insertion), garsping of the Gallblader, releasing adhesions, identifying anatomy of Calot's triangle, anatomical variation and extracting the gall bladder. Many advantages had been reported in past that includes cosmesis, shorter hospital stay, less pain and postoperative morbidity. Despites these advantages there are several cases where its lethal complications had been reported in cases where anatomy of Calot's triangle is difficult to identify or distorted even in experience hands [(1)].Geography and ethnicity has an enormous role in the prevalence of GSD and also the type of stone that forms: cholesterol gallstones prevalent in the developed countries of the

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Western world; brown pigment stones in the bile ducts are more common in Asia [2]. female gender has an obvious association with GSD, especially during the fertile years. They are almost twice as likely as men to form stones; parity, oral contraceptive use and estrogen replacement therapy are an established risk factors for cholesterol gallstone formation [3].particularly abdominal or centripetal obesity, is a well-established risk factor for GSD [4]. At least 25% of morbidly obese individuals have evidence of GSD. Obesity in the late teenage years carries the greatest risk, whereas thinness protects against GSD [5].Patients with biliary colic typically have normal laboratory test results. blood tests should include a complete blood count with differential, liver function, amylase and lipase [6].Ultrasonography has a specificity and sensitivity of 90-95%. The sonographic features of acute cholecystitis include gallbladder wall thickening (>5 mm), pericholecystic fluid, gallbladder distention (>5 cm), and a sonographic Murphy sign. Also has the efficacy in identifying the anatomy of the pancreas and common bile duct diameter . Gallstones appear as echogenic foci in gallbladder which move freely with positional changes with an acoustic shadow [7].

Aim of study: To predict how difficult cholecystectomy will be from assessing the patient preoperatively which, in turn, help in decreasing the risks on the patients and preventing post-operative complications.

Patients and methods: A prospective study conducted in the department of General Surgery at Al-Ramadi Teaching Hospital. The data collection was completed during the period of nine months from 15th of May 2018 till the 15th of February 2019. This study included 60 patients, all of them were undergone laparoscopic cholecystectomy for Gallstone. Diagnosis of cholelithiasis was confirmed by an abdominal ultrasonography (U/S) in patients presenting with upper abdominal pain, or vomiting or dyspepsia or jaundice.

Preoperative characteristics, intraoperative details, and postoperative outcomes were further analyzed. The data were collected prospectively to predict the significance of association with patients' characteristics.

Statistical Analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Chi square test was used to show the association between LAP difficulty and categorical variables. A level of P – value less than 0.05 was considered significant.

Results

The total number of study patients was 60. All of them undergone laparoscopic cholecystectomy for Gallstone.

The distribution of study patients by general characteristics is shown in table (3.1). Study patients age was ranging from 17 to 65 years with a mean of 39.05 years and a standard deviation of ± 12.11 years. The highest proportion of study patients was aged between 30 – 49 years (53.3%). More than three quarters of study patients were females (76.7%) with a male to female ratio of 1: 3.28.

About BMI level, 51.7% of study patients were overweighed.

Table 1: Distribution of study patients by general characteristics

Variable	No. (n= 60)	Percentage (%)
Age (Years)		
< 30	12	20.0
30 – 49	32	53.3
≥ 50	16	26.7

Cont... **Table 1: Distribution of study patients by general characteristics**

Gender		
Male	14	23.3
Female	46	76.7
BMI level		
Normal	13	21.6
Overweight	31	51.7
Obese	16	26.7

Table (2) shows the distribution of study patients by clinical information. We noticed that 16.7% of patients had a history of biliary hospitalization and 33.3% of them undergone caesarean section before. Gall bladder was not palpable in all patients (100%).Regarding comorbidity, 18.3% of patients were hypertensive and 10% were diabetics. Liver function test and Pancreatic enzymes (ALP , TSB , SGOT , SGPT , s.amylase) was normal in all patients.

Table (2) : Distribution of study patients by clinical information

Variable	No. (n= 60)	Percentage (%)
History of biliary hospitalization		
Yes	10	16.7
No	50	83.3
Previous Surgery		
No	34	56.7
Caesarean Section	20	33.3
Appendectomy	4	6.7
Abdominal hysterectomy	2	3.3
Comorbidity		
Hypertension	11	18.3
Diabetes Mellitus	6	10.0
Hypertension + Diabetes	4	6.7
No	39	65.0
Liver Function Test and Pancreatic enzymes		
Normal	60	100.0
Elevated	0	0

The distribution of study patients by ultrasound finding is shown in table (3). In this study, the wall of gall bladder was thin in 81.7% of patients, pericholecystic collection was presented in 10%, impacted stone was detected in the neck of Gall bladder in 5% and the content of gall bladder was stone in 81.7% of cases. Most of gall stone were ≤ 1 cm in size (65%).

Table (3): Distribution of study patients by ultrasound finding

Variable	No. (n= 60)	Percentage (%)
Wall Thickness		
Thin (≤ 3 mm)	49	81.7
Thick (> 3 mm)	11	18.3
Pericholecystic collection		
Yes	6	10.0
No	54	90.0
Impacted Stone (In the neck)		
Yes	3	5.0
No	57	95.0
Size of stone (cm)		
≤ 1	39	65.0
> 1	21	35.0
Gall Bladder Content		
Stone	49	81.7
Polyp	8	13.3
Sludge	3	5.0

Table (4) shows the distribution of study patients by intraoperative information. It was obvious that 58.3% of operations were lasted for < 60 minutes. Bile leak was occurred in 18.3% of cases, bleeding occurred in 6.7%, and stone spillage in 20%.

Conversion to open cholecystectomy was occurred in 13.3% of cases.

Table (4): Distribution of study patients by intraoperative information

Variable	No. (n= 60)	Percentage (%)
Time of operation (Minutes)		
< 60	35	58.3
60 – 120	17	28.3
> 120	8	13.3
Bile Leak		
Yes	11	18.3
No	49	81.7

Cont... **Table (4): Distribution of study patients by intraoperative information**

Bleeding		
Yes	4	6.7
No	56	93.3
Stone Spillage		
Yes	12	20.0
No	48	80.0
Conversion to open		
Yes	8	13.3
No	52	86.7

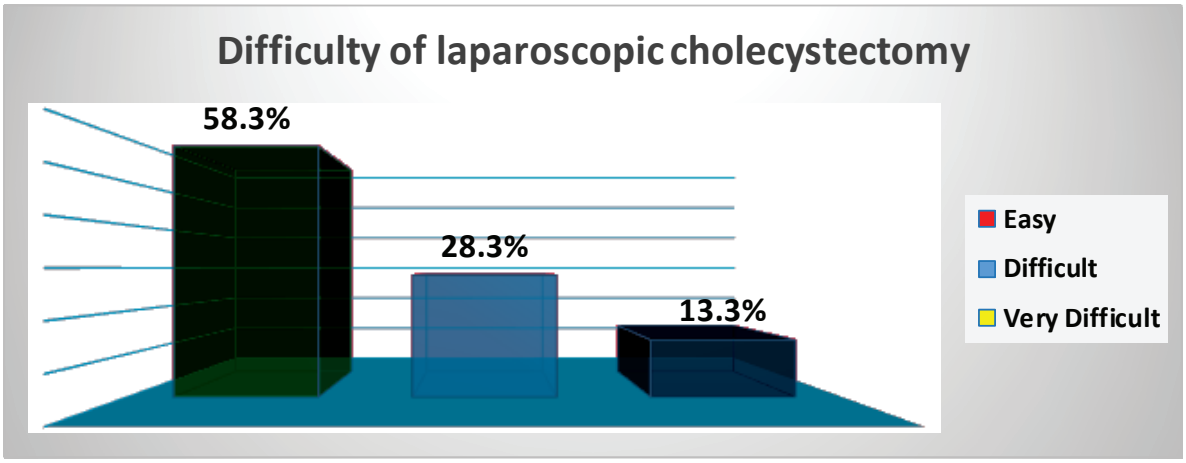

Figure (1): Distribution of study patients by difficulty of laparoscopic cholecystectomy

Figure (1) shows the distribution of study patients by difficulty of laparoscopic cholecystectomy. In this study, the highest proportion of operations was categorized as easy (58.3%), 28.3% of operations were considered difficult and 13.3% of them were very difficult

The association between operation difficulty and demographic and clinical information is shown in table (5). In this study, the highest prevalence of difficult and very difficult operation was seen in patients aged ≥ 50 (68.8%) with a significant association (P= 0.036) between operation difficulty and age. Regarding history of biliary hospitalization, 80% of patients who had a previous biliary hospitalization were suffering from difficult and very difficult operation with a significant association (P= 0.007) between operation difficulty and history of biliary hospitalization.About BMI level, we noticed that 62.5% of obese patients were suffering from difficult and very difficult operation with a significant association (P= 0.037) between operation difficulty and BMI level.No statistical significant association (P ≥ 0.05) between operation difficulty with gender, previous surgery and comorbidities.

Table (5): Association between operation difficulty with demographic and clinical information

Variable	LAP Difficulty		Total (%) n= 60	P - Value
	Difficult and Very Difficult (%) n= 25	Easy (%) n= 35		
Age (Years)				
< 30	4 (33.3)	8 (66.7)	12 (20.0)	0.036
30 – 49	10 (31.3)	22 (68.7)	32 (53.3)	
≥ 50	11 (68.8)	5 (31.2)	16 (26.7)	
Gender				
Male	7 (50.0)	7 (50.0)	14 (23.3)	0.47
Female	18 (39.1)	28 (60.9)	46 (76.7)	
BMI level				
Normal	2 (15.4)	11 (84.6)	13 (21.6)	0.037
Overweight	13 (41.9)	18 (58.1)	31 (51.7)	
Obese	10 (62.5)	6 (37.5)	16 (26.7)	
History of biliary hospitalization				
Yes	8 (80.0)	2 (20.0)	10 (16.7)	0.007
No	17 (34.0)	33 (66.0)	50 (83.3)	
Previous surgery				
Yes	11 (42.3)	15 (57.7)	26 (43.3)	0.376
No	14 (31.8)	30 (68.2)	34 (56.7)	
Comorbidity				
Yes	10 (47.6)	11 (52.4)	21 (35.0)	0.492
No	15 (38.5)	24 (61.5)	39 (65.0)	

Table (6) shows the association between operation difficulty and ultrasound finding. The highest prevalence of difficult and very difficult operation was found in patients with thick wall gall bladder (81.8%) with a significant association (P= 0.002) between operation difficulty and wall thickness. Regarding pericholecystic collection, 83.3% of patients who had pericholecystic collection were suffering from difficult and very difficult operation with a significant association (P= 0.029) between operation difficulty and pericholecystic collection.Concerning size of stone, we noticed that 66.7% of patients with stone > 1 cm were suffering from difficult and very difficult operation with a significant association (P= 0.003) between operation difficulty and size of stone.No statistical significant association (P ≥ 0.05) between operation difficulty with impacted stone in the neck , and gall bladder content.

Table (6) : Association between operation difficulty and ultrasound finding

U/S Finding	LAP Difficulty		Total (%) n= 60	P - Value
	Difficult and Very Difficult (%) n= 25	Easy (%) n= 35		
Wall Thickness				
Thin (≤ 3 mm)	16 (32.7)	33 (67.3)	49 (81.7)	0.002
Thick (> 3 mm)	9 (81.8)	2 (18.2)	11 (18.3)	
Pericholecystic collection				
Yes	5 (83.3)	1 (16.7)	6 (10.0)	0.029
No	20 (37.0)	34 (63.0)	54 (90.0)	
Impacted Stone in the neck				
Yes	2 (66.7)	1 (33.3)	3 (5.0)	0.367
No	23 (40.4)	34 (59.6)	57 (95.0)	
Size of stone (cm)				
≤ 1	11 (28.2)	28 (71.8)	39 (65.0)	0.003
> 1	14 (66.7)	7 (33.3)	21 (35.0)	
Gall Bladder Content				
Stone	20 (40.8)	29 (59.2)	49 (81.7)	0.656
Polyp	3 (37.5)	5 (62.5)	8 (13.3)	
Sludge	2 (66.7)	1 (33.3)	3 (5.0)	

Discussion

Gallstone disease is one of the most common problems affecting the digestive tract. The prevalence of gallstones is related to factors like age, gender, and ethnic background. At present, laparoscopic cholecystectomy (LC) is considered the treatment of choice for symptomatic cholelithiasis. It has many advantages over open cholecystectomy in terms of minimal postoperative pain, shorter hospital stay, better cosmetics and early recovery [8]. In this study, easy operation represented the highest proportion of operations as constituted (58.3%), while, 28.3% of operations were considered difficult and 13.3% of them were very difficult. A comparable result observed in Agrawal et al study in 2015, in which 30 patients involved in their study, they found that seventeen patients were scored easy (56.7%) and 13 (43.3%) were difficult and nil in very difficult group [9].

In the current study, difficult and very difficult operation was seen in more than two third of patients aged ≥ 50 (68.8%) with a significant association (P= 0.036) between operation difficulty and age.In contrast to the current results, Agrawal and colleagues in their study in 2015, the majority of patients were in the age group of ≤50 years (83.3%) and only (16.7%) were >50 years, in which no significant correlation between age and the difficult level of surgery (P>0.05) [9]. Increasing age

is associated with an increased probability of multiple attacks of cholecystitis and also increased frequency of upper abdominal surgeries [10].In the present study, a previous biliary hospitalization was significantly observed in 80% of patients suffering from difficult and very difficult operation (P= 0.007).

Similarly, Agrawal et al study that conducted in 2015, found in their univariate and multivariate analysis that previous hospitalization was statistically significant in predicting difficult LC [71].Another agreement observed in Randhawa et al study in 2009, in which found that previous hospitalization was significantly associated with difficult LC (P<0.05) [10].

In concern to BMI level in this study, nearly two third of obese patients were suffering from difficult and very difficult operation with a significant association (62.5%, P= 0.037) between operation difficulty and BMI level.

An agreement observed in Dhanke *et al*., study in 2014, in which discover that difficult LC was significantly higher observed in patients with BMI >27.5 kg/m^2, as found in 80% of those had a difficult LC (P<0.001) [11].

In the current study, no statistically significant association (P ≥ 0.05) between operation difficulty with gender, previous surgery and comorbidities.

Comparable results observed in a study conducted by Dhanke and colleagues in 2014, in which noticed that no significant association observed between difficult LC and gender of the participants and the previous surgery performed (P=0.596 for both) [11].Male with symptomatic gall bladder are more prone to inflammation and fibrosis with the same disease intensity thus leading to difficulty in dissection.

In the current study, the difficult and very difficult operation was found in patients with thick wall gall bladder (81.8%), 83.3% of patients who had pericholecystic collection and 66.7% of patients with stone > 1 cm with a significant association between operation difficulty and wall thickness, pericholecystic collection and size of stone (P< 0.05), while no statistical significant association (P ≥ 0.05) between operation difficulty with impacted stone, common bile duct diameter, and gall bladder content.

In contrary, a higher conversion rate was observed in Bat O study in 2015, in which the rate of conversion to open cholecystectomy observed was 67.1%, in which found that operation time was significantly prolonged in conversion group (P<0.0001) [77].

Furthermore, a lower result observed in Bourgouin et al study in 2016, as found that 4.3% of cases operated laparoscopically required conversion to open cholecystectomy, in which the major causes for conversion include; tight adhesions, severe inflammation and uncontrollable bleeding [12].

Another lower result observed in Ahmed et al study in 2018, in which noticed that conversion rate to open surgery was 6.7% [13]. In this study, the wall of gall bladder was thin in 81.7% of patients, pericholecystic collection found in 10%, impacted stone was detected in 5% and the content of gall bladder was stone in 81.7% of cases. Most of gall stone were ≤ 1 cm in size (65%).

Another different result observed in Atta et al study in 2017, in which U/S findings showed that thickened gallbladder wall in difficult group represented 5.8% of the study patients [14].

In the current study, a mean and a standard deviation of age was 39.05 ± 12.11 years (ranging from 17 to 65 year). The highest proportion of study patients was aged between 30 – 49 years (53.3%). Females predominance exist as it constituted 76.7% of patients with a male to female ratio of 1: 3.28. About BMI level, 51.7% of study patients were overweighed.

Comparable results observed in Agrawal et al study in 2015, in which the mean and SD of the participant's age was 39.47 ± 12.008 years with the minimum age being 18 years and the maximum being 64 years. The majority of patients were in the age group of ≤50 years. Female were the predominant in the study, as formed the majority of the study patients (80%), with female to male ratio was 4:1 [9].

Conclusion

The difficult laparoscopic cholecystectomy can be predicted preoperatively based on number of factors, like: obesity, previous biliary admission, gall bladder wall thickness, ultrasound findings (stone size and pericholecystic fluid) and ageing process, evaluating

of such factors minimize the complications of LC and conversion to the open procedure.

Conflict of Interest: Non

Source of Findings: Self-findings.

Ethical Clearance: Taken from hospital and patients

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Clinical Characteristics of Coronavirus Disease 2019 in a Cohort of Iranian Patients: Olfactory Dysfunction as a Frequent Clinical Presentation

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Abstract

Background: Since its outbreak, the 2019 novel coronavirus (SARS-CoV-2) has been associated with coronavirus disease 2019 (COVID-19). The full spectrum of disease severity of COVID-19 is still unknown. In this study which is the first report from Iran, we showed that olfactory dysfunction is a frequent clinical manifestation among COVID-19 patients.

Methods: All patients with suspected SARS-CoV-2 infection were admitted to Arak hospitals in Iran. We retrospectively collected and analyzed data on patients with either laboratory-confirmed SARS-CoV-2 infection by real-time RT-PCR and/or chest CT

Results: Most patients in our cohort had mild COVID-19 (104 [60.4%] of 172) . Common symptoms at the onset of disease included (subjective) fever (131 [76%] of 172 patients), cough (130 [75.5%]), myalgia (107 [62%]) dyspnea (92 [53.5%]), chills (96 [55.8%]), and headache (94 [54.6%]). Olfactory dysfunction (OD), including anosmia, hyposmia, hyperosmia, and dysosmia, were reported by 61 ([36.5%] of 167) patients. The most common concurrent symptoms beside the olfactory dysfunctions, were fever (39 [69.6%] of 56 patients) and chills (26 [46.4%]), respectively. For the most part, the patient's condition worsened after the notice of anosmia/dysgeusia (48 [85.7%] of 56 patients). The majority of anosmic patients were recovered from COVID-19 (50 [89.3%] of 56 patients). The anosmia/dysgeusia was resolved in 42 [75%] of 56 patients after two weeks.

Conclusion: Taken together, this study reports the disease spectrum of SARS-CoV-2 infection in a cohort of 172 Iranian adult patients. To the best of our knowledge, this is the first report from Iran.

Keywords: *COVID-19, Iran, SARS-CoV-2, Olfactory dysfunction;*

Introduction

Since its outbreak, 2019 novel coronavirus (SARS-CoV-2) has been associated with a spectrum of a disease, known as coronavirus disease 2019 (COVID-19), ranging from asymptomatic to conditions required intensive care support and invasive mechanical ventilation, to multiple organ dysfunction syndromes. The full picture of the spectrum of COVID-19 disease severity is not yet complete. Common symptoms include fever, cough, and myalgia [1-3]. This is the first study from Iran, which has the fourth-highest number of COVID-19 deaths after

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Italy, China, and Spain, and the sixth-highest number of SARS-CoV-2 cases in the world [4].

In the present study, clinical characteristics of 172 COVID-19 patients have been collected. All patients were admitted between February 7, 2020 to March 28, 2020, to designated hospitals (which co-authors are affiliated with them) in Iran. Their clinical manifestation and olfactory dysfunction have been evaluated.

Material and Methods

Patients

All patients with suspected SARS-CoV-2 infection were admitted to designated hospitals (which co-authors are affiliated with them) in Iran. We retrospectively collected and analyzed data on patients with either laboratory-confirmed SARS-CoV-2 infection by real-time RT-PCR and/or chest CT. Demographic and clinical data were collected with standardized data collection forms developed by The Centers for Disease Control and Prevention [5].

Evaluation of olfactory and gustatory dysfunctions

For evaluations of olfactory and gustatory dysfunctions, we utilized the Anosmia Reporting Tool for Clinicians for COVID-19, which was developed by the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) [6]. The authors also interviewed patients or their families to ascertain symptom data. Furthermore, the Rapid Smell Test (RST) was utilized for validation of patient self-report of smell perception change following SARS-CoV-2 infection [7].

Results

Clinical findings

From February 7, 2020 to March 28, 2020, 172 patients with COVID-19 diagnosis based on either laboratory- or CT-based diagnosis of SARS-CoV-2 infection were included in this study. The median age of COVID-19 patients in this study was 51 years (IQR 39·0□61·8). Gender distribution was symmetrical; about half had underlying diseases (82 [48%] of 172 patients), like diabetes (44 [25%]) and hypertension (36 [20%]). We classified our patients based on disease severity according published guidelines; most patients in our cohort had mild COVID-19 (104 [60.4%] of 172) [8]. Common symptoms at the onset of disease included (subjective) fever (131 [76%] of 172 patients), cough (130 [75.5%]), myalgia (107 [62%]) dyspnea (92 [53.5%]), chills (96 [55.8%]), and headache (94 [54.6%]). Olfactory dysfunction (OD), including anosmia, hyposmia, hyperosmia, and dysosmia, were reported by 61 ([36.5%] of 167) patients; five patients reported non-COVID-19-induced anosmia which were excluded from the total cases; a before and after study [viral exposure] design was used for evaluation of OD). There was a reverse correlation between the severity of COVID-19 and olfactory problems [odds ratio (OR): 0.37, 95% confidence interval (CI): 0.19 - 0.75]. The proportion of patients with mild COVID-19 was higher in individuals with olfactory dysfunction in comparison to those without olfactory problem. In other words, the anosmia was reported more frequently in COVID-19 patients with less severe disease. However, this conclusion can be disputable on the ground that geriatric patients tend to have more severe/critical COVID-19 and about half of individuals over the age of 60 years have OD attributable to different etiologies, such as alcoholism, cigarette smoking, diabetes, neoplasms, Parkinson's disease, and trauma. Therefore, they are less sensitive to decline in olfactory ability. Hypogeusia, hypergeusia, and dysgeusia was reported by 46 [26.5%] of 167 patients.

Evaluation of olfactory dysfunction among the COVID-19 patients

With rapidly accumulating anecdotal evidence surfacing from multiple countries about the association of anosmia with the COVID-19 pandemic, we evaluated OD in our cohort via the Anosmia Reporting Tool for Clinicians for COVID-19. From a total of 61 COVID-19-induced patients with OD, 56 patients participated in this evaluation. Most patients (45 [80.3%] of 56) had any other symptoms before the development of anosmia /dysgeusia. Therefore, in 11 [19.6%] of 56 patients, olfactory and resultant gustatory dysfunction was the first sole manifestation of COVID-19. The most common concurrent symptoms beside the olfactory dysfunctions, were fever (39 [69.6%] of 56 patients) and chills (26 [46.4%]), respectively. For the most part, the patient's condition worsened after the notice of anosmia/ dysgeusia (48 [85.7%] of 56 patients). The majority of

anosmic patients were recovered from COVID-19 (50 [89.3%] of 56 patients). The anosmia/dysgeusia was resolved in 42 [75%] of 56 patients after two weeks.

Furthermore, we used RST to confirm and semiquantify the OD in a subgroup of our cohort of COVID-19 patients with self-reported OD (n=19). RST is a fast test for assessment of the olfactory function, which was modified, validated, and adopted for Iranian culture. This test utilizes only five high familiar smells (coffee, smoke, cinnamon, garlic, and banana). Eighteen patients out of 19 scored less than 5 (abnormal < 5) with mean ± SD of 2.39 ± 0.978. Therefore, SARS-CoV-2 infection impairs olfactory function.

Discussion

In sum, this study reports the disease spectrum of SARS-CoV-2 infection in a cohort of 172 Iranian adult patients. To the best of our knowledge, this is the first report from Iran. Besides, we expanded the clinical spectrum of COVID-19 and highlighted OD as a frequent clinical symptom in one-third of patients. In human infections with upper respiratory involvement like coronavirus, rhinovirus, picornavirus, parainfluenza virus type 2, and Epstein-Barr virus, OD is common [9]. Our data show that anosmia was resolved spontaneously in the two-third of patients after two weeks. The OD among patients with SARS-CoV-2 infections are more prevalent, severe, and long-lasting than in patients with influenza, or rhinoviral infections [10]

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Ethical Clearance: The study protocol was approved by the Institutional Review Board of the Arak University of Medical Sciences, Arak, Iran (approval no. IR.ARAKMU.REC.1399.001). The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Conflict of Interest: Non

Funding: Self-funding

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***Original Research**:*

A Retrospective Study of Pattern of Cranio-Cerebral Injuries Due to Road Traffic Accident in a Tertiary Care Hospital

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Abstract

Head injury is defined as sudden injury due to an external force that affects the functioning of brain. It may be either caused by sudden blow or impact (closed head injury) or by an object penetrating the skull (Penetrating injury). The common causes of head injury include road traffic accidents, fall from height and assault. The mortality from head injury is reported to be between 1-2% of death from all the causes. Not only it is one of the major causes of death in young adults but also may cause permanent disability in survivors. The importance of head injury as a public health problem cannot be overstated. In the studies of Road Traffic accidents provide valuable data for implementing effective emergency services to reduce the trauma related mortality and strengthening legal measures in peak hours of fatal accidents. We aimed to study, pattern of injuries especially cranio- cerebral injuries occurring in vehicular accidents. Patients case records with history of head injury and MLC records of victims of road traffic accident reported to Saveetha hospital, Chennai from 1st Jan 2019 to 31st Dec 2019 period were analysed retrospectively.

Out of total 1846 Road Traffic Accidents cases reported to Emergency Medicine department, Saveetha Medical college hospital, Chennai, 10.40% cases had history of head injury. The male/female ratio was 9:1. Commonest age group affected was between 21-40 years involving 126 (65.62%n=192) cases. Fatal traumatic brain injuries were seen in 178 (92.70%, n=192) cases. Amongst 192 head injury cases, scalp injury noted in 167 (86.97%) cases, most common bone fractured was temporal bone 94(48.95%), followed by other bone parts. The commonest variety of intracranial haemorrhage was subdural haemorrhage 173 (n=192, 90.10%) followed by Subarachnoid haemorrhage and Extradural haemorrhage. In 26 (13.54%) patients of head injury, craniotomy surgery was done. The timings between 3PM to 6PM showed the highest number of cases accounting to 61.7% of the cases followed by 26.0% of cases between 6PM to 9PM. The two-wheeler accidents accounted for 69.9% of the head injury cases followed by that due to other vehicles (16.7%)

***Keywords**: Road Traffic Accidents, Head injury, Skull fracture, Intracranial haemorrhages.*

Introduction

Head injury is regarded as a main health problem that is a common cause of morbidities and mortalities and makes great demand to control and prevent it.[1] Traumatic Head Injury has been called the "Silent Epidemic". It is defined as sudden injury due to an external force that affects the functioning of brain. The total or subtle structural changes in the scalp and the contents of the skull. It may be either caused by sudden blow or impact (closed head injury) or by an object penetrating the skull (Penetrating injury). [2,3] Most of traumatic head injuries are the result of blunt force, either local or general. Head injuries are most common and account for about one fourth of all deaths due to

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violence and are responsible for 60% of all fatal road accidents. [4] The common causes of traumatic brain injury include road traffic accidents, fall from height and assault. [5]

Patients with head injury constitute a major health problem throughout the world. Of all the traumatic death one third to one-half are due to head injury.[6] Out of those who survive, the majority are left with important disabilities, including 3% in a persistent vegetative state. Only about 30% make a good recovery. More than 50% of all cases of death encountered at forensic autopsy are associated with primary or secondary involvement of the Nervous System, especially of the brain.[7]

The statistics of National Crime Records Bureau of India (NCRBI) are staggering. A total of 4,81,805 traffic accidents comprising of 4,50,898 road accidents, 28,360 railway accidents and 2,547 railway crossing accidents were reported, these accidents caused 1,41,526, 25,006 and 2,575 deaths respectively during 2014. There was an increase in traffic accidents by 1.3% during 2014 compared to 2013. State of Uttar Pradesh followed by Maharashtra and Tamil Nadu have reported maximum fatalities in traffic accidents in the country, these 3 States accounted for 12.2%, 11.0% and 10.1% of total deaths in traffic accidents during 2014. It also reported a rising trend in absolute number of deaths in 'Traffic Accidents' during the last five years.[8]

Road traffic accidents (RTAs) are a rising problem worldwide accounting for around 1.2 million deaths and over 50 million injuries annually. About two third of these RTAs are in the third world countries. It is expected that by the year 2020 they will rank third in the Global Burden of Diseases. India has just 1% of the total vehicles in the world but it contributes to 6% of the global RTAs. Estimates suggest that Delhi has the highest number of road crash fatalities in India. This lack of attention to road safety issues further adds to the load of problems of road traffic injuries and need public health concerns. Thus, reducing the epidemic of accidents.[9]

Despite the stringent laws being made to decrease the incidence of head injuries secondary to Road Traffic Accidents there is tremendous increase in traumatic brain injuries due to motor vehicle accidents. It is one of the leading causes of morbidity and mortality in young adults in developed as well as developing world [10]. In context of India it is important to know that World Health Organization (WHO) in its first ever Global Status Report on Road Safety stated that more people die in road accidents in India than anywhere else in the world. It moreover warned that by 2030 road fatalities will become 5th biggest killer.[11] This study was done with an objective to determine the pattern and distribution of head injuries in blunt trauma cases of this region which were brought to the Emergency medicine department at Saveetha Medical College.

Materials and Methodology

The present study is a retrospective cross-sectional study. The data was collected from the case records from Medical Record Department and Accident/MLC register available at the department of Emergency Medicine, Saveetha Medical College, Thandalam, Chennai. The study period was for one year from January 1st, 2019 to December 31st, 2019. Out of 1846 Road Traffic Accidents cases reported to Emergency Medicine department, 192 cases had history of head injury. These 192 cases were scrutinized for Sociodemographic and pattern of head injury. The data compiled and then analysed with appropriate statistical tests using Statistical Package for Social Sciences (SPSS) version 16.0 for descriptive analysis and to calculate frequency distribution.

Aims & Objectives

- To study the pattern of cranio-cerebral injuries due to road traffic accident in a tertiary care hospital.
- To study the duration of survival, gender preponderance, age group involved and probable accident occurrence timings.

Ethical Consideration:

Permission to retrieve the case records was obtained from the institution authority. Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai, prior to the study. Confidentiality was maintained.

Observations and Results

Out of total 1846 Road Traffic Accidents cases reported to Emergency Medicine department, Saveetha Medical College hospital, Chennai, 192 (10.40%,

n=1846) cases had history of head injury. (Chart 1) Total number of male and female victims were 173 & 19 respectively. The male/female ratio was 9:1. (Chart 2) The commonest age group affected was between 21-40 years involving 126 (65.62% n=192) cases. (Table-1) Fatal traumatic brain injuries were seen in 178 (92.70%, n=192) cases. Out of 192 head injury cases, scalp injury noted in 167 (86.97%) cases, skull fractures were found in 178 (92.70% n=192) cases, most common bone fractured was temporal bone noted in 94(48.95%) cases, followed by parietal bone 85 (44.27 %), frontal bone 65 (33.85%)and occipital bone 54 (28.12 %). Fracture of base of skull noted in 68 (35.41 %) cases. (Table 2) The commonest variety of intracranial haemorrhage was subdural haemorrhage 173 (n=192, 90.10%) followed by Subarachnoid haemorrhage 154 (n=192, 80.20 %) cases. Extradural haemorrhage was present in 47 cases (24.47%). Brain contusion & laceration was noted in 27 (14.06%) cases. (Chart -3) The current study shows that the majority of the victims are two-wheeler rider and pedestrians i.e., 69.79% & 13.54 % respectively followed by three-wheeler (9.89 %) and four-wheeler (6.77 %). (Table 3).

Out of 192 Head injury cases, 85 (44.275%) number of patients died. Among all deaths, 13 (15.29% n=85) victims were brought dead to the hospital. In 26 patients of head injury, craniotomy surgery was done and clots were drained out to release the pressure effects, among them 21 (24.70%, n=85) of died within 48 hrs. Maximum mortality, i.e., 51 victims (60 %, n=85) died within 4-14 days which indicates the severity of the head injury. (Table 4) The timings between 3PM to 6PM showed the highest number of cases accounting to 61.7% of the cases followed by 26.0% of cases between 6PM to 9PM.

Table 1: Age and Sex wise distribution of head injury cases

Age in years	Male	Female	Total no. of cases	Percentage
1-20	10	06	16	8.33 %
21-40	118	08	126	65.62 %
41-60	26	05	31	16.14 %
61-80	19	00	19	9.89 %
Total	173	19	192	100 %

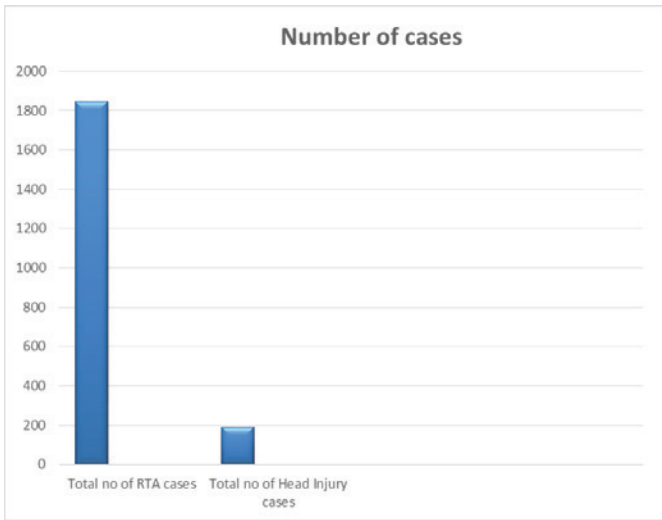

Chart 1: Distribution of Cases

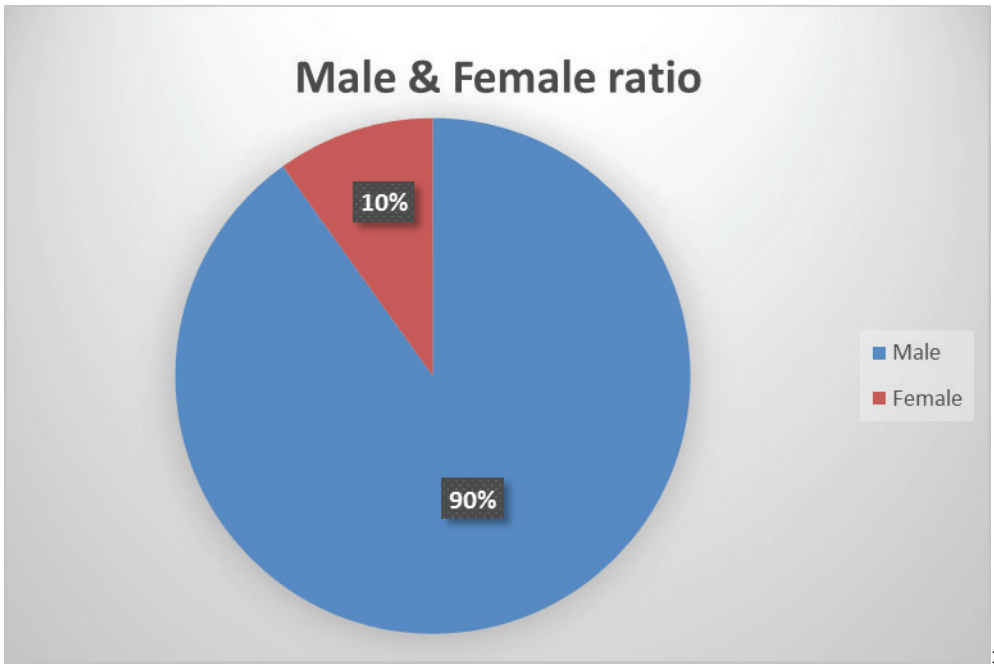

Chart 2: Sex Wise Distribution of cases

Table 2: Incidence of skull fracture in total head injury cases (n=192) due to road traffic accidents

Sl. no	Bone Involved in skull fracture	No of cases	Percentage
1	Temporal	94	48.95%
2	Parietal	85	44.27 %
3	Frontal	65	33.85%
4	Occipital	54	28.12 %
5	Base of skull	68	35.41 %

Table 3: Victims involved in Accidents

Category	Victims	Percentage
Pedestrian	26	13.54
Two-Wheeler vehicle	134	69.79
Three-wheeler Vehicle	19	9.89
Four-wheeler & lorry	13	6.77
Total	192	100.0

Chart 3: Distribution of Intra Cranial Hemorrhage & cerebral injury

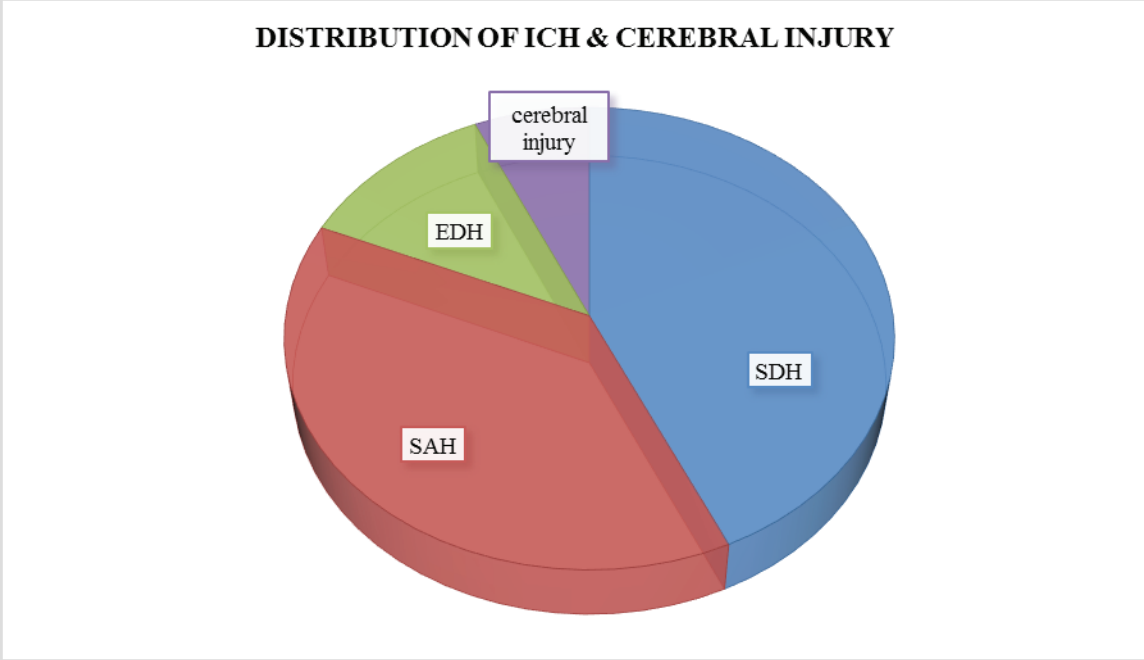

Table 4: Survival time of Head injury victims

Duration	Number of Deaths
Brought dead	13
< 24 Hrs.	09
< 48 Hrs	12
3-5 days	18
5-7 days	19
7-14 days	14
Total	85

Discussion

In the current study, out of total 1846 Road Traffic Accidents cases reported to Emergency Medicine department, Saveetha Medical College hospital, Chennai, 192 (10.40%, n=1846) cases had history of head injury.

In the present study, out of total 192 cases of Traumatic Head Injury due to blunt force trauma, Males comprised a majority and constituted 173 (90%) cases compared to females who were only 19 (10%) of the total cases. It is due to greater male exposure on urban streets and similar higher incidence of traffic accidents among males has been found by many other researchers.[12-14] The reason for the male majority is their participation in occupational and leisure activities which puts them at certain higher risk than their female counterpart.

The most common age group affected in the study was between 21-40 years (65.62%) and is consistent with the studies available from India and other countries. [12,13,15-18] The age group 20-40 years is the most active phase of life, physically and socially, and hence outnumbers the other road users. They, therefore account for the maximum number of accidental deaths.

Considering the maximum involvement of individuals in the economically productive years, vehicular collision fatalities may have an important economic impact. The children and older people which form the group in extremes of age are usually indoors and the individuals in the age group of 21-40 years are mostly injured outdoors due to various occupational, educational, sports activities which involves frequent travelling, thus making them more prone to injuries. A second peak of head injury incidence in the present results was seen in the elderly (25.7%), namely those nearing and above 60 years of age. This is in keeping with the findings of a large number of previous studies in this field. Most of the episodes of TBI in this older age group are attributed to falls and motor vehicle accidents. Another possible explanation for this might be the medical comorbidities and frailty associate with elderly people attributed to a combination of sensory and motor decline, de-conditioning and cognitive impairments as described.

The current study shows that the majority of the victims are two-wheeler rider and pedestrians i.e., 69.79% & 13.54 % respectively followed by three-wheeler (9.89 %) and four-wheeler (6.77 %). Similar findings were observed in other studies also.[1,7,17] Pedestrians and two-wheeler riders & pillion riders were injured by the direct contact with the vehicle and is forcibly thrown on the ground. These injuries are mostly due to blunt trauma.

In our study out of 192 head injury cases, scalp injury noted in 167 (86.97%) cases, skull fractures were found in 178 (92.70% n=192) cases, most common bone fractured was temporal bone 94(48.95%), followed by parietal bone 85 (44.27 %), frontal bone 65 (33.85%) and occipital bone 54 (28.12 %). Fracture of base of skull noted in 68 (35.41 %) cases. Similar findings were observed in the studies conducted by Arvind Kumar MD et al, V V Pillay and Menon A et al studies.[17-19] This ranking of the skull bones fractured was also noted in the observations collected by Bose T.K, Karmakar R and Basu R.[7]

In the present study, the commonest variety of intracranial haemorrhage was subdural haemorrhage 173 (n=192, 90.10%) followed by Subarachnoid haemorrhage 154 (n=192, 80.20 %) cases. Extradural haemorrhage was present in 47 cases (24.47%). Brain contusion & laceration were noted in 27 (14.06%) cases. The findings are in consistent with the findings by other researchers.[18] But in contrast with other studies conducted by Puttuswamy & Sachin Chourasia [1,4] in which researchers are observed Subarachnoid haemorrhage as most common than other haemorrhages.

Out of 192 Head injury cases, 85 (44.275%) number of patients died. Among all deaths, 13 (15.29% n=85) victims were brought dead to the hospital (Pre-hospital mortality). the rest 72 deaths have occurred in hospital, out of 72 cases 21 (24.70%, n=85) died within 48 hrs and maximum mortality, i.e., 51 victims (60 %, n=85) died within 4-14 days of hospital stay which indicates the severity of the head injury. Our study observations are in consistent with the other studies.[15-19]

Conclusion

In the present study various age groups, sex wise distribution, types of skull fractures, intra cranial lesions due to head injury have been critically analyzed. In our study the majority of the victims of fatal craniocerebral were in the age group of 21-40 years and of male sex. Road Traffic Accident comprised a majority of cases of head injury were Two-wheeler rider, pillion rider and pedestrians. Among the intracranial injuries, Sub Dural haemorrhage was the commonest. Young Males seemed to be the ones who were most commonly affected and the vehicle involved were mainly two wheelers. This study highlights the need for taking urgent steps for establishing good pre-hospital care and provision of trauma services at site in India. A nationwide computerized trauma registry is urgent required to bring out the risk factors, circumstances, chain of events leading to the accidents and will be extremely helpful in policy making and health management at the national level in India. A collective effort from good governing bodies, NGO's, Schools and community education with better implementation of traffic policies (wearing helmet by rider & pillion rider, wearing seat belt, following traffic rules) and good roads would help in curbing down this menacing issue worldwide.

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Conflict of Interest: Nil

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Original Research:

Awareness, Knowledge and Attitude Towards Medical Ethics among Medical Students - A Questionnaire based Study

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Abstract

Introduction: Ethics is concerned with moral principles for the members of the medical profession in their dealings with each other, their patients and the State. A physician must uphold dignity and honour of his profession. He must render service to humanity and should maintain good medical practices. A physician must provide highest quality assurance in patient care. Duties of physician towards sick include obligation to sick, must be patient while treating them. Its an era of litigation against medical professionals. Awareness, Knowledge of medical ethics during MBBS phase is need of the hour to prevent these.

Aims & Objectives: • The study aims to assess Awareness, Knowledge and Attitude towards medical ethics among medical students in a teaching hospital.

• To suggest and implement necessary changes in the medical curriculum like AETCOM module to train them & to focus on ethical practices.

Materials and Methods: A Prospective, cross sectional questionnaire-based study was carried out among medical students of Saveetha Medical College hospital, Chennai. The Medical Students from Phase II & Phase III MBBS were approached and the questionnaires were sent to about 250 medical students, out of which 216 responses were recorded & included in the study. Received responses/results were tabulated and statistically analysed, depicted in observations.

Conclusion: There are no potential risks in the study. Study helps to identify the amount of awareness of medical ethics among medical students and also to stress on the need for awareness programs on medical ethics. The medical students may not have sufficient knowledge towards medical ethics, need much training among medical students through Value added courses on Ethics and AETCOM module of CBME curriculum in future throughout the curriculum.

Key Words: *Medical Ethics, Medical Students, CBME curriculum, AETCOM*

Introduction

Ethics is concerned with moral principles for the members of the medical profession in their dealings with each other, their patients and the State. It is a self-imposed code of conduct assumed voluntarily by medical professionals.[1,2]

A physician must uphold dignity and honour of his profession. He must render service to humanity and should maintain good medical practices. A physician must provide highest quality assurance in patient care. Duties of physician towards sick include obligation to sick, must be patient while treating them. All consultation must be carried out for patient benefit, the

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physician must be punctual and available for it and good treatment must be provided after consultation. Physician must display fees for consultation and treatment in the hospital and prescription letter head must include name, designation and registration. Physicians should maintain good conduct during consultation without engaging in rivalry with other physicians. If a physician is called for consultation, the consultant must not take charge of the case on the solicitations of patient or friends. A substitute physician can attend patients in other physician's absence only when he has capacity to discharge additional responsibility along with his own duties.[3]

Unethical acts which should not be performed by a doctor include using his/her name as subject in any form of advertising, running an open shop for dispensing prescriptions prescribed by other doctors other than himself or which causes exploitation of patient, patented surgical instruments or appliances are not made available in situations where interest of large population is involved.A physician must never receive or give commission in any form. He must never give secret remedies or medicines to patients of which he is unaware of the composition, mode of action and adverse effects. A doctor must preserve human rights and should never help in euthanasia.[3,4]

There has been growing public awareness regarding the ethical conduct of medical practitioners and complaints against physicians appear to be escalating. This may reflect an increase in unethical practices by doctors or increasing public awareness of such unethical practices. [5]

Doctors are called to court to give expert witness regarding assault cases, poisoning cases etc. Due to lack of proper medico- legal knowledge, most of the doctors are very badly exposed in courts. Every doctor should realise that irrespective of the post graduate speciality they choose, throughout their career they have to deal with medico-legal cases. Hence, they should have sufficient knowledge about medico-legal aspects whether they work in private clinics or government hospitals.[6,7] Doctors have several ethical, moral and legal obligations in their duties. It is therefore very important that every doctor understands the nature of these obligations and fulfil these obligations to the best of their ability. [8]

Keeping the above facts in mind, a study was done to assess medical students about the knowledge of medical ethics and attitude towards it and also to make them aware of this important subject so that they can effectively handle medico-legal cases.

Aims & Objectives

- The study aims to assess Awareness, Knowledge and Attitude towards medical ethics among medical students in a teaching hospital.

- To suggest and implement necessary changes in the medical curriculum like AETCOM module to train them & to focus on ethical practices.

Materials and Methods

A Prospective, cross sectional questionnaire-based study was carried out among medical students of Saveetha Medical College hospital, Chennai. The Medical Students from Phase II & Phase III MBBS were approached and total 216 medical students' responses were included in the study. Those who were refused to participate and those who were not accessed our google doc questionnaire were excluded from the study. Willingness for participating in the survey is recorded before initiating the questionnaire in the google doc document. They were informed that the questionnaire is the part of study and they are free to accept or deny completing it. A self-administered, structured questionnaire written in English which covered various aspects of Ethics in the Medical practice was given for all participants to assess awareness, knowledge and attitude towards medical ethics. The questionnaire was pretested on a group of 14 medical students, who were excluded from the study and questionnaire was modified accordingly. The questionnaire was asked through online google doc format. The questionnaires were sent to about 250 medical students of Phase II & III MBBS out of which 216 responses we got & included in the study. Received responses were tabulated and statistically analysed. Results are tabulated, analysed using SPSS softwareversion-16.0. The student's t-test, ANOVA test were used as tests of significance for statistical evaluation. The level of significance was set at $P \leq 0.05$.

Results and Observations

Total 216 medical students participated in the

questionnaire-based study on awareness, knowledge and attitude towards medical ethics among medical students. The results of the study shown that out of 216 students, 194 (89.8%) medical students felt that service to humanity is a doctor's primary objective and 20 (9.2%) students felt that it may be a doctor's primary objective. Amongst all participants, 208 (96.2%) students had opinion that it is important for a doctor to be punctual during consultation, 8 students felt it may be important. Majority (97.2 %) of students gave answer that a physician must protect patient confidentiality, 2 students felt it is not important for a physician to protect it and 4 students felt it may be important for a physician to protect it. Out of 216 students, 160 (74%) felt a doctor must not include his/her name, 42(19.4%) students felt maybe a doctor can include his/her name, 6 students didn't know whether a doctor can include his/her name as subject in advertising and 8 students felt a doctor must include his/her name as a subject in any form of advertising. Most (87.9%) of the students felt a physician cannot give a particular drug to patients without being sure of the uses, adverse effects and composition of drug, 10 (4.6%) students felt a physician can give and14 (6.4%) students felt maybe a physician can give such a drug. Majority of students (95.3%) felt that, a doctor should not criticize another doctor, 8 students felt maybe a doctor can criticize another doctor. Amongst 216 participants, 144 (66.6%) students felt it is necessary for a physician to involve the patient while making decisions regarding management of patient, 50 (23.1%) students felt it is maybe necessary to involve patient and 22(10.1%) students felt it is not necessary to involve patient. Most of the (94.4%) students had opinion that it is important for a physician to listen and respect patient's views while treating patients, 12 (5.5%) students felt it is maybe important to listen and respect patient's views. The highest number, i.e., 214 (99%) students felt that it is not advisable for a doctor to associate with pharmaceutical company or with touts or with another doctor for benefits. Most of the students (89.3%) felt it is mandatory to provide pre and post-operative instructions to patients, 21 (9.7%) students felt it is maybe mandatory to provide pre and post-operative instructions. Majority of (97.2%) students felt that, it is unethical for a doctor to issue false certificate to his/her friend or relative and 6 students felt it is maybe unethical to issue such a certificate. Almost all of the students (99.5 %) felt it is unethical for a doctor to leave a patient during emergency on the basis of gender, religion & socio-economic status. Majority of participants (216) opined in 'Any other comments' section that they need more training in medical ethics & law for their future clinical practice.

Questionnaire Table:

Sl.no	Question	Yes	No	Maybe	Don't Know
1.	Do you feel service to humanity is a doctor's primary objective?	194	2	20	0
2.	Is it important for a doctor to be punctual during consultation?	208	0	8	0
3.	Do you feel a physician must protect patient privacy & confidentiality? (Including patient's personal information and treatment details)	210	2	4	0
4.	Can a doctor include his/her name as subject in any form of advertising?	8	160	42	6
5.	Can a physician give a particular medical drug to patients when he/she is not sure of uses, adverse effects and composition of drug?	10	190	14	2

***Cont...* Questionnaire Table:**

6.	Is it okay for a doctor to criticize another doctor in front of his patients?	2	206	8	0
7.	Is it necessary for a physician to take informed consent & involve the patient while making decisions regarding management of patient?	144	22	50	0
8.	Is listening and respecting patient's views important while treating patients?	204	0	12	0
9	Is it advisable for a doctor to associate with pharmaceutical company or with touts or with another doctor for benefits?	0	214	0	2
10	Is it mandatory to provide pre & post-operative instructions to the patient?	193	2	21	0
11	Do you feel Issuing false certificate to his/her friend/ relative is unethical?	210	0	6	0
12	Do you feel Leaving the patient during emergency on the basis of gender/religion/SE status by a doctor is unethical?	215	0	1	0

Discussion

More awareness and knowledge about medical ethics among medical students is brought by teaching it in medical colleges and also by clinical exposure in hospitals. This study gives us an insight into various aspects of medical ethics. The current study showed us about the degree of awareness, knowledge and attitude towards Medical Ethics among medical students.

Most of the medical students (95.3%) felt that a doctor cannot criticize another doctor in front of his patients which is similar to study done by Jatna SK in a medical college in Malaysia in 2018.9 A majority of medical students (87.9%) in the study felt that a physician cannot prescribe a drug without being sure of composition of drug which is similar to the results obtained in a study by Arun Babu T in medical college in Pondicherry10. Majority of participants in this study (97.2%) felt that a physician must protect patient privacy and confidentiality which is in accordance with the study conducted by Ramesh P Aacharya in a medical college in Kathmandu [11].

Most of the students (66.6%) felt that it is necessary for a doctor to involve patient while making decisions regarding patient management which is similar to the study conducted by Jatna SK in a medical college in Malaysia.[9] Almost all the medical students(99.5%) felt that leaving a patient during emergency on the basis of gender/religion/SE status by doctor is unethical which is similar to study done by Jatna SK in medical college in Malaysia. [9]

Conclusion

There are no potential risks in the study. Study helps to identify the amount of awareness of medical ethics among medical students and also to stress on the need for awareness programs on medical ethics. The medical students may not have sufficient knowledge towards medical ethics, need much training among medical students through Value added courses on Ethics and AETCOM module of CBME curriculum in future throughout the curriculum.

Ethical Clearance: Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai prior to the study. Consent was taken from each respondent. Confidentiality was maintained.

Source of Funding: Self

Conflict of Interest: Nil

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Original Research

A Study on Dactylographic Pattern in Relation to Abo - Blood Group and Iris Colour

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Abstract

Dactylography or Fingerprint is one of the oldest reliable biometric indicators. Dermatoglyphics (fingerprint/ dactylography) is derived from the Greek word "Derma = Skin, Glyphe = Carve." Fingerprint patterns are unique in each individual and the chance of two persons having identical fingerprints is about one in 64 thousand million. Fingerprints represent the impressions left by the friction ridges of our finger.

Out of many blood grouping systems available, ABO and Rh systems are the most important and are considered for the present study. Due to the immense potential of fingerprints as an effective method of identification an attempt has been made in the present work to analyse their correlation with blood group and Iris colour of an individual. This study was carried out on 200 medical students (100 male and 100 female) having different ABO blood groups and belonging to different age groups. All the 10 fingers fingerprints were taken and divided into loops, whorls, arches and composite. Iris colour recorded by direct illumination by Slit lamp examination. Results shown that loops pattern being most common followed by whorls, arches and composite. Brown colored iris being more predominant in both the genders than green and blue colored iris.

Loops are predominant in males whereas whorls and arches are seen more in females. Composites being in same proportion in both sexes. Most of the subjects with loops pattern were belongs to O positive blood group and having brown iris colour followed by B positive. Among loops ulnar loops were more predominant than radial loop.

Among all subjects, green colored iris recorded in 5 subjects, out of 5, 4 had loop fingerprint patter & another one subject had arch pattern. Blue colored iris recorded in two subjects with loop pattern.

Keywords: *Dactylographic pattern, Blood group, Iris colour*

Introduction

Dermatoglyphics (fingerprint/dactylography) is derived from the Greek word "Derma = Skin, Glyphe = Carve." Dermatoglyphics is defined as the scientific study of naturally occurring epidermal ridges and their configuration on the digits. Dactylography or Fingerprint is one of the oldest reliable biometric indicators. Fingerprints represent the impressions left by the friction ridges of our finger. It is formed due to unequivocal pressure by amniotic fluid over finger pads during in-utero development of foetus. A finger print is an impression of the friction ridges on all parts. The dermal carvings or finger prints the first time on the human fingers, palm, soles and toes from 12th to 16th week of embryonic development and their formation gets completed by the 14th week i.e. about the 6th foetal month. The ridges thus, formed during the foetal period

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do not change their course or alignment throughout the life of an individual, until destroyed by decomposition of the skin after death.[1,2] There are four common patterns of fingerprints: loop, whorl arch & composite. There are four common patterns of fingerprints: loop, whorl arch & composite. It has been reported that the characteristic patterns of epidermal ridges are differentiated in their definitive forms during the third and fourth months of intrauterine life. Fingerprint patterns are genotypically determined and remain unchanged from birth until the person dies and decomposes.[3]

Fingerprints are constant and individualistic and form the most reliable criteria for identification. Finger prints follow the Locard's Principle of Exchange. The secretions in the fingerprints contain residues various chemicals and their metabolites which can be detected and used for the forensic purposes.[4] Human finger prints fall into three main groups: loops, whorls and arches. Loops are the most common type, accounting for approximately 65% of all fingerprints. They are formed by ridge lines that flow in from one side of the print, sweep up in the center like a tented arch and then curve back around and flow out on the side where they entered. Loops are designated as being either radial or ulnar, depending on which side of the finger the lines enter. Whorls account for approximately 30%. They consist of four different patterns; the plain whorl, central pocket loop, the double loop and accidental whorl. Their common features are that they have at least two deltas and one or more ridge line curves around the core to form a circle or spiral. The accidental whorl can be any pattern or combination of patterns that do not fit into any of the above classifications. Arches are the simple pattern but rare (about 5%). They are of two types; plain and tented arches. In both types the ridge lines flow into the print from one side, rise in the middle of the pattern and flow out to the other side of the print. Despite these broad patterns, each individual has a unique set of prints which can be used as a means of personal identification.[5]

Blood group system was discovered way back in 1901 by Karl Landsteiner. It consists of mainly the 'ABO' and 'Rhesus' groups. The 'ABO' system is further classified as A, B, AB and O blood group types according to presence of corresponding antigen in plasma. 'Rhesus' system is classified into Rhesus Positive (Rh +ve) and Rhesus Negative (Rh –ve) according to the presence or absence of 'D' antigen.[6]

Eye colour is a polygenic phenotypic character determined by two distinct factors: the pigmentation of the eye's iris and the frequency dependence of the scattering of light by the turbid medium in the stroma of the iris.[7] In humans, the pigmentation of the iris varies from light brown to black, blue & green depending on the concentration of melanin in the iris pigment epithelium (located on the back of the iris), the melanin content within the iris stroma (located at the front of the iris), and the cellular density of the stroma.[8] Eye colour is an inherited trait influenced by more than one gene.[9] A study in Rotterdam (2009) found that it was possible to predict eye colour with more than 90% accuracy for brown and blue using just six single-nucleotide polymorphisms. There is evidence that as many as 16 different genes could be responsible for eye colour in humans.[10] Because of uniqueness of fingerprint among general population and being effective method of identification, In the current study, we aimed at analysing and correlating dactylographic patterns in relation to blood group and iris colour.

Need for Study

To understand the importance of dactylographic patterns in relation with blood groups & Iris colour and its use in forensic as a tool for medico-legal identification.

Aims & Objectives

· To determine the relationship between dactylographic patterns, blood group and iris color.

· To study the statistical significant relationship between the fingerprint pattern, blood group and iris color in an individual and the extent to which they can be used for medico-legal identification purposes.

Materials & Methods

Study Population:

A cross sectional descriptive randomized study was conducted among 200 Medical students studying at Saveetha Medical College and Hospital, Thandalam, Chennai, India. A total of 200 students between the age group 18-24 years participated in our study. All the study subjects were healthy and the subjects having hand or finger deformities and blood group, eye diseases were

excluded.

A proforma was prepared on a durable white paper, rubber stamp ink pads were used for smearing each finger, imprints were taken, and each pattern of fingerprint was observed by powerful hand lens and recorded. Fingerprints were taken using the INK method as illustrated by Cummins and Mildo in 1961. The materials used for this study were Faber-Castell blue color INK pad, A4-size white paper, cardboard, gauze pads, magnifying lens, pencil, and pen. Each white paper was filled with basic details of subjects such as name, sex, age, date, blood group, and fingerprints of right- and left-hand fingers recorded.

Methods

Each subject was asked to wash their hands and dry them with the help of a towel. After that, pressed each right- and left-hand fingertips separately in the stamp pad. Prints were taken on a white A4-size paper. Finally, fingerprint patterns (loops, whorls, and arches) were analysed and identified as loop, whorls and arches, based on the appearance of the ridge lines with the help of a magnifying lens and blood group data of each subject were collected.

Iris colour recorded by direct illumination by Slit lamp examination in Ophthalmology department by taking assistance of an Ophthalmologist.

Statistical Analysis

The data collected were entered and analysed using SPSS, version 16.0. Data were expressed as numbers and percentages, and analysed by X2. Level of significance was set as P value < 0.05. Chi-square test revealed that the relation between fingerprint and blood group is statistically significant at $P < 0.05$.

Results

The results of the Distribution of Dactylographic Pattern of all fingers are showed in **Table 1.** Majority of the subjects have loop pattern 112 (56 %), followed by whorl 63 (31.5%), arch 19 (9.5%) and composite 6 (3%).

TABLE 1: Distribution of Dactylographic Pattern of all fingers

Predominant Finger print pattern	Total No of Subjects	Percentage
Loop	112	56 %
Whorl	63	31.5 %
Arch	19	9.5 %
Composite	6	3 %
Total	200	100 %

The Distribution of fingerprints in relation to gender are shown in **Table 2.** It depicts that males have the highest number of loop (31.00 %) and whorl pattern (17.00 %) which are 62 and 34 are in number respectively. Females (8.50%) have the highest number of arch patterns.

TABLE 2: Distribution of fingerprints in relation to gender

Predominant Finger print pattern	Males	Percentage	Females	Percentage	Total
Loops	62	31.00 %	50	25.00 %	112
Whorls	34	17.00 %	29	14.50 %	63
Arches	8	4.00 %	17	8.50 %	25

Table 3 shows the Distribution of Patterns of Fingerprint in finger of both hands in relation to different blood groups. Majority (56.5%) of the subjects belong to group 'O' in total 113 out of 200 followed by group B 38 (19%), group A 31 (15.5%) and group AB 18 (9%). Blood group O had the highest number of loop 60 (30%), whorl 36 (18%) and arch pattern 15(7.5%). Blood group O and B had the highest number of composite patterns 2 (1%).

TABLE 3: Distribution of Patterns of Fingerprint in finger of Both Hands in Relation to Different Blood Groups

Predominant Finger print pattern	Blood Group A	Blood Group B	Blood Group AB	Blood Group O	Total
Loops	20 (10%)	23 (11.5%)	9 (4.5%)	60 (30%)	112
Whorls	9 (4.5%)	11 (5.5%)	7 (3.5%)	36 (18%)	63
Arches	1 (0.5%)	2 (1%)	1 (0.5%)	15 (7.5%)	19
Composite	1 (0.5%)	2 (1%)	1 (0.5%)	2(1%)	6
Total & Percentage	31 (15.5 %)	38 (19 %)	18 (9%)	113 (56.5%)	200 (100%)

Table 4 shows distribution of fingerprints in relation to Iris Colour. Iris colour recorded by direct illumination by slit lamp examination by taking Ophthalmologist assistance. Results shown that 193 (96.5%) subjects had brown colored iris, which is being more predominant in both the genders than green and blue colored iris.

Among 200 subjects, green colored iris recorded in 5 (2.5%) subjects, out of which 3 are female, 2 are male and 4 showed loop fingerprint pattern & another one subject had arch pattern. Blue colored iris recorded only in two (1%) subjects who showed loop pattern.

TABLE 4: Distribution of fingerprints in relation to iris colour

Iris colour	Loops	Whorls	Arches	Total Number	Percentage
Brown	106	63	24	193	96.5%
Green	4	0	1	5	2.5%
Blue	2	0	0	2	1%

Discussion

This study was an approach to associate finger prints, gender, blood groups and Iris colour. In the present study, Majority of the subjects have loop pattern 112 (56 %), followed by whorl 63 (31.5%), arch 19 (9.5%) and composite 6 (3%). This is in accordance with the studies conducted by various authors.[11-14]

Frequency of loops & whorls were found to be higher in males and arches were found to be higher in females as compared to males. This is in accordance with the study done by Margi et al.[15]

In our study, the distribution of primary finger prints among the subjects was high frequency of loops moderate of whorls and low of arches. In the present study, majority (56.5%) of the subjects belong to group 'O' in total 113 out of 200 followed by group B 38 (19%), group A 31 (15.5%) and group AB 18 (9%). Blood group O had the highest number of loops 60 (30%), whorl 36 (18%) and arch pattern 15(7.5%). Blood group O and B had the highest number of composite patterns 2 (1%). This is in accordance with the study done by Bhardwaja A[4] and Patel et al.[12] Bharadwaja et al conducted a study during 2000-2001 on 300 medical students with different ABO blood groups in Rajasthan which revealed that individuals with blood group A have more of loops, while that of blood group AB had more of whorls.[13]

In another study done by, Dr. Prateek. Rastogi and Ms. Keerti R Pillai among 200 medical students of Kasturba Medical College, Mangalore which showed that blood group A had higher frequency of Loops and blood group O had higher frequency of whorls and arches were found to be high in blood group A.[16] Results of our study differ comparatively with this study.

In the present study, a greater number of loops were found in blood group 'O' followed by blood group 'A' and 'B'. Incidence of whorls were found to be predominant in 'O' followed 'A' and 'B' blood groups. Arches & composite were least commonly found pattern in all the blood groups which is supported by the study done by Roy et al [17] and Wang et al.[18]

In this study, brown colour iris has the highest frequency of all finger prints while blue iris had the least. In our study population, almost no variation was observed in iris colour other than dark brown to black, green & blue. Out of 200 subjects, 193 have dark brown to black iris (Martin-Schultz scale 14-16), only 5 subjects have green iris (Martin-Schultz scale 10) & 2 had blue coloured iris. Almost all the study population belong to same ethnicity may be the reason behind this observation. A thorough search is made to find out about availability of research work and literature done on relationship between fingerprint pattern & colour of iris, but we hardly got very minimum information on this aspect.

Conclusion

To conclude, the present study was conducted to analyse and correlate fingerprint patterns with gender blood group and iris colour of an individual. It is known fact that fingerprints are unique for every individual and never change from birth till death. This study was an attempt to correlate fingerprint patterns with gender and blood group, so that it may increase the authenticity of fingerprints in identification of individuals. As observed in the study, it is may be concluded that prediction of Rhesus blood group of an individual is not achievable depending on the fingerprint pattern available. It is probably due to the fact that the origin of finger-print patterns is largely due to a complex interplay of genetics and developmental factors. Blood groups, on the other hand, originate due to interaction of the paternal and maternal genes regulating their expression However, the prediction of ABO group and ABO-Rhesus blood group together of someone is possible based on the finger ridge pattern of the person. Therefore, fingerprints, Rhesus blood groups & iris colour can only be used separately to identify a person. Further research in this domain can throw more light upon this intriguing aspect of forensic identification.

Ethical Clearance: Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai prior to the study. Informed consent was taken from each respondent. Confidentiality was maintained.

Source of Funding: Self

Conflict of Interest: Nil

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Knowledge and Attitude of Medical Students Towards Medico Legal Autopsy- A Questionnaire Based Study

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Abstract

Introduction: Autopsy is a research tool which has been used for centuries. Autopsy is a scientific study of a dead body and is categorized into clinical and medico-legal autopsies. A Medicolegal autopsy or post-mortem examination is a scientific examination of dead body carried out under the law of the state mainly to assist the identification and prosecution of the guilty in cases of unnatural deaths. This study is done to assess the knowledge, Perception and Attitude of medical students towards medico legal autopsy.

Materials and Methods: A questionnaire-based study was conducted among the students of Saveetha medical college, questionnaires related to autopsy were distributed and students were asked to fill them. According to the data obtained was analyzed to know the Knowledge and Attitude of Medical Students Towards Medico Legal Autopsy

Results: Majority of the students (92.3%) thought that autopsy is necessary for all unnatural deaths. 84.6% said that inquest report, requisition letter is needed for autopsies. 80.8% knew autopsy is done to know the cause of death and reach the culprit of the crime and was a legal formality in unnatural deaths. 96.2% of students have not felt performing autopsy is disrespectful to the body. 94.2% of participants had a opinion that the autopsies they witnessed were not enough. 96.9% agreed that autopsy is necessary in medical education. Almost half (49.9%) of the students felt uncomfortable while witnessing autopsy, 29.6% were Comfortable and 20.5% of students have not witnessed autopsy yet. Only 67.3% were comfortable with live autopsies, rest 32.7% preferred virtual autopsy. 96.2% knew that preserving viscera is necessary during autopsy on suspected case of poisoning. Majority of students (98.1%) felt that medical students should actively participate in autopsies. Most of them (98.1%) knew that both external and internal examinations are done in autopsies and 98.5% felt that ED posting specifically for Forensic Medicine for trauma, poisoning and other medico-legal cases is necessary during CRRI.

Conclusion: Overall this study shows that students have some knowledge of autopsies but think that the autopsies that they have witnessed were not enough and encourages that medical students should witness and actively participate in more autopsies. We also suggest to revise the Forensic Medicine curriculum by increasing the number of teaching and practical hours thereby the students will be able to make observations and come to conclusions in criminal matters or Medico-legal cases.

***Keywords**: Medico Legal autopsy, Knowledge, Attitude, Medical Students*

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Introduction

Autopsy is a research tool which has been used for centuries. Autopsy is a scientific study of a dead body and is categorized into clinical and medico-legal autopsies. A Medicolegal autopsy or Post-mortem examination (Necros = dead; opis = view; Post = after;

Mortem = death) is a scientific examination of dead body carried out under the law of the state mainly to assist the identification and prosecution of the guilty in cases of unnatural deaths.[1,2] The autopsy is beneficial in determining the cause of death, the time of death, validity of therapeutic modalities, potential medico legal issues surrounding death and providing data on disease and injury. The autopsy sessions provide opportunities to discuss subjects like death certification, forensic pathology, appropriate attitudes towards deaths and communication skills essential for giving bad news or seeking consent for autopsy. The main cause of low autopsy rate seems to be changes in system of medical education and attitude of medical profession. [3]

Recently, there has been a marked reduction in the number of hospital autopsies being requested. Besides curriculum reform, technological alternatives such as videos and CD ROMS, the general decline in hospital autopsy rate prevailing legislation and legal bureaucratic, financial, political, religious reasons have all contributed to declining use of autopsy as a teaching tool. Despite the fact that the literature indicates that forensic autopsy rates worldwide remain fairly constant there is little mention of their use in undergraduate medical training. The use of autopsy as an educational tool is in decline and there is evidence that many recent undergraduates and junior doctors have never attended an autopsy. [3]

In India according to the curriculum of the medical council of India, a medical student should witness medico legal autopsies in the second year of MBBS so that they can observe and interpret the various changed in organs and tissues.[4] It is only when students attend and autopsy section that they can appreciate the large number of pathological conditions in different patients. An intern is supposed to acquire the skills of doing a medico legal autopsy. Thus, every medical graduate is presumed to be capable of doing a medico legal autopsy after registering as a medical practitioner. Various reasons given for the decline in autopsy-based teaching include insufficient hospital autopsies, competing departmental duties and limited curriculum time. It is obvious that knowledge of autopsy will broaden the students mind and enhance a more accurate diagnosis during the subsequent years of their clinical practice as doctors. In undergraduate medical education the training of post-mortem examination on dead bodies might evoke strong emotional reactions in medical students that could counteract the intended learning goals. [5,6]

Very few studies have been conducted on attitudes of medical students to clinical autopsy/medico legal autopsy. This study is carried out to assess the knowledge, Perception and Attitude of medical students towards medico legal autopsy.

Aims & Objectives

- The study aims to assess knowledge and perception of medical students towards medico legal autopsy in a teaching hospital.
- To study the attitude of undergraduate medical students towards medico-legal autopsies.

Materials and Methods

A Prospective, cross sectional questionnaire-based study was carried out among medical students of Saveetha Medical College hospital, Chennai. The medical students from Phase II & Phase III MBBS were approached and total 200 medical students' responses were included in the study. Those who were refused to participate and those who were not accessed our google doc questionnaire were excluded from the study. Willingness for participating in the survey is recorded before initiating the questionnaire in the google doc document. They were informed that the questionnaire is the part of study and they are free to accept or deny completing it. A self-administered, structured questionnaire written in English related to Medico Legal autopsy was given for all participants and they were asked to respond anonymously to a set of questions on Medico Legal autopsy. The questionnaire was pretested on a group of 12 medical students, who were excluded from the study and questionnaire was modified accordingly. The questionnaire was asked through online google doc format. Students were asked to respond to preset questionnaires based on their response to the questionnaire their knowledge and attitude towards medical autopsy were assessed. The questionnaires were sent to about 250 medical students of Phase II & III MBBS out of which 200 responses were included in the study. Received responses were tabulated and statistically analysed. Results are tabulated, analysed using SPSS softwareversion-16.0.

Observations & Results

Questionnaire table:

Out of 200 responses from medical students of Phase II & III MBBS:

v 92.3% thought that autopsy was necessary for all unnatural deaths.

v 84.6% say that consent of relatives, inquest report, requisition letter was needed for autopsies.

v 80.8% knew autopsy can be done to know the cause of death and reach the culprit of the crime and was a legal formality in unnatural deaths.

v 96.2% don't think doing autopsy is disrespectful to the body.

v 94.2% think that the autopsies, that they have witnessed were not enough.

v 96.9% agreed that autopsy is necessary in medical education

v 49.9% felt uncomfortable while witnessing, only 29.6% were comfortable during their 1st autopsy & 20.5% haven't seen any autopsy yet.

v Only 67.3% were comfortable with live autopsies, rest 32.7% preferred virtual autopsy.

v 96.2% knew that preserving viscera is necessary during autopsy on suspected case of poisoning.

v Majority of students (98.1%) felt that medical students should actively participate in autopsies.

v 98.1% knew that both external and internal examinations are done in autopsies.

v 98.5% felt that ED posting specifically for Forensic Medicine for trauma, poisoning and other medico-legal cases is necessary during CRRI.

Discussion

The knowledge of medico-legal autopsy will broaden the student's perceptive and will help the legal system in delivering quick justice in unnatural deaths. In the current study, most of the students knew that autopsy is required in all cases of unnatural death. However, several students have raised objections to autopsies on the victims of road traffic accident in 'any other comments' section, on the grounds that the cause of death was already known in these cases. This compares favorably with the study of E W Benbow. [7]

The student's emotional reactions are important, but unfortunately neglected aspect of medical training. The dissection of a dead body is not simply a technical exercise, it also raises emotional feelings with regard to human mortality and dignity. Most of the students felt they were uncomfortable at the first sight of autopsy findings are in accordance with other studies done in the past. [8,9]

In present study, majority of the students agreed that autopsy is necessary in medical education. This compares favorably with the study of Botega et al [6] in Brazil and a study by Conran et al[10] in Ohio, USA, where 85% of the students also said that autopsy should be mandatory for all medical students. Majority of the respondents were of the opinion that the number of autopsies witnessed were enough while a few recommended for more numbers of autopsies to be witnessed by medical students. This is in contrast to the study by Ekanem and Akhigbe [11], where the figure was 57% and 74% respectively. Majority of the students opined that autopsy is necessary and they learned something from it which compares favorably with the studies of other authors in the past.[6,8,10,11] However, most of the mortuaries in India are in a primitive state and do not encourage the student to become more interested in observing autopsies. Majority of students (96.2%) knew preserving viscera necessary during autopsy on suspected case of poisoning. Most of students (98.1%) felt that medical students should actively participate in autopsies & knew that both external and internal examinations are done in autopsies, findings are in accordance & favorable with the studies of other authors in the past.[8,10,12] Most of the students were of the opinion of having 98.5% felt that ED posting specifically for Forensic Medicine for trauma, poisoning and other medico-legal cases is necessary during CRRI, which is similar to the study by Ahmad N et al. [9]

Conclusion

Overall, our questionnaire-based survey findings showed that medical students have a reasonable awareness and positive attitude towards Medico Legal

Autopsy procedure and Forensic Medicine speciality, but they feel the autopsies that they have witnessed were not enough. So, this study is to encourage that medical students should witness more autopsies and actively participate in autopsies, which will help them in finding the cause of death or to lead to the culprit of the crime when they become professionals. This study supports those studies conducted with a quantitative methodology, which indicate that the autopsy is of greater value in medical education.

We suggest for increased training in the autopsy procedure as well as improving the facilities. We also suggest to revise the forensic curriculum by increasing the number of teaching and practical hours thereby the students will be able to make observations and come to conclusions in criminal matters or Medico-legal cases.

Ethical Clearance: Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai prior to the study. Consent was taken from each respondent. Confidentiality was maintained.

Source of Funding: Self

Conflict of Interest: Nil

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Assessment of Knowledge and Awareness about Consent among Medical Practitioners in a Tertiary Care Hospital – A Questionnaire based Study

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Abstract

Introduction: The medicine being a moral profession, the relationship of doctor and patient entails the highest conceivable degree of trust and fidelity. Good bedside manner, technical competency and communication skills are the physician's behaviors most strongly associated with patient trust. The changing medical practice of the 20th century has given place to patient's autonomy and informed consent. Consent is not mere submission of the patient to a particular treatment, but a process of communication requiring the fulfilment of certain established elements like competence, sufficient disclosure, understanding and volunteering**.** However, less attention has been paid to the implications of obtaining informed consent, from the physicians' perspective. Considering the above fact, the study was undertaken to assess the knowledge and awareness about informed consent among medical practitioners in a tertiary care hospital.

Materials and Methods: A prospective, cross-sectional, questionnaire-based study was undertaken in 100 medical practitioners during May 2020 to July 2020.

Results: It was observed that most of the participants were aware and had sufficient knowledge about informed consent, but the law related to consent and other inner details of informed consent in medical practice were lacking.

Conclusion: We concluded that medical institutions, various medical associations and medico-legal bodies must conduct regular medico legal training programmes, continuum medical education programmes, seminars and workshops and must be made mandatory to participate actively for all medical practitioners to update their knowledge related to consent in medical practice and law related to medical practice.

Keywords: *Knowledge, Assessment, Informed Consent, Medical practitioner,*

Introduction

The medicine being a moral profession, the relationship of doctor and patient entails the highest conceivable degree of trust and fidelity. Good bedside manner, technical competency and communication skills are the physician's behaviors most strongly associated with patient trust. The changing medical practice of the 20th century has given place to patient's autonomy and informed consent.[1] As per Section 13 of Indian Contract Act, two are more persons are said to consent when they agree upon same thing in same senses**.** The word consent means to agree to something or to allow something to happen. Consent in medical practice means the agreement to undergo any medical treatment or to participate in any medical research. [2]

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Thus, it is clearly observed that informed consent is a vital component of medical practice.[3] Informed consent is believed to be an effective approach to building mutual trust between clinical practitioners and patients. The patient's moral right to self-determination and the corresponding duty of health care professional to "do no harm" create a strong moral basis for gaining a patient's informed consent.[4]

The ethics of clinical practice and scientific research supporting a patient-centered approach informed consent empowers patients to establish dialogue with doctors in clinical decision making and make autonomous decisions.[5] Under this paradigm, doctor's obligations are to provide proper and sufficient medical information about a patient's condition and available treatment options.[6]

The implications of obtaining informed consent have been widely reviewed from the ethical, legal, and moral viewpoints, and from both individual and societal perspectives. In particular, the impact of this process on patients has been examined with regard to patients' rights, and the patients' ability to retain the full meaning of the document. Nowadays, more and more patients are demanding detailed information about their disease, various diagnostic methods available and options available for treatment. Consent is not mere submission of the patient to a particular treatment, but a process of communication requiring the fulfilment of certain established elements like competence, sufficient disclosure, understanding and volunteering.[7] However, less attention has been paid to the implications of obtaining informed consent, from the physicians' perspective. Considering the above fact, the study was undertaken to assess the knowledge and awareness about informed consent among medical practitioners in a tertiary care hospital.

Need for the Study

The process of obtaining a written informed consent is one mechanism for protecting a patient's dignity in the health-care environment and also future allegations against medical practitioners. Now, it is the era of litigations against medical practitioners, proper informed consent and documentation, proper communication with patient and/or patient relatives may help in decreasing these patients – doctor conflicts. Considering the above facts, the study was undertaken to be undertaken to assess the knowledge and awareness about informed consent among medical practitioners in a tertiary care hospital.

Aims and Objectives

- To assess the knowledge and awareness about informed consent among medical practitioners in a tertiary care hospital.
- To know the facts, level of satisfaction, knowledge about informed consent among medical practitioners.

Materials and Methods

A Prospective, cross sectional questionnaire-based study was carried out among medical practitioners at Saveetha Medical College hospital, Chennai during May 2020 to July 2020. The doctors of the various departments were approached and total 100 medical practitioners are included in the study. Those who were on leave, refused to participate, those who were not accessed our google doc questionnaire and non-clinicians were excluded from the study. Willingness for participating in the survey is recorded before initiating the questionnaire in the google doc document. They were informed that this questionnaire is the part of study and they are free to accept or deny completing it. A self-administered, structured questionnaire written in English which covered various aspects of consent, commonly encountered in the medical practice was given for all participants to check their knowledge about consent in medical practice. The questionnaire was pretested on a group of 10 medical practitioners, who were excluded from the study and questionnaire was modified accordingly. The questionnaire was asked through online google doc format. Total 116 questionnaires were distributed among medical practitioners of various specialties out of which 100 responses were taken for the study. Received responses were tabulated and statistically analyzed using SPSS softwareversion-16.0. The student's t-test, ANOVA test were used as tests of significance for statistical evaluation.

Results & Observations

The current study consists a total of 100 doctor's responses from various clinical specialties. It was found

that only 21% of the doctors knew the various types of consents in medical practice and whose consent is valuable, 63% were not aware and 16% responded that it depends on circumstances. It was observed that 91% doctors were satisfied with their knowledge about consent in medical practice, 6% were not satisfied and 3% responded that it depends on circumstances. When asked the question, are you taking Written Informed consent before any examination or procedure? Most of the doctors (72%) responded that they take informed consent before any examination or procedure, 16 responded no and 12% take depending on circumstances. Among study participants, 88% doctors believe informed consent and documentation can prevent a charge of negligence, 1 felt no and 11% of physicians responded that it depends on circumstances. 79% of doctors believe doctors are the best person to obtain consent, 8% responded no and 13% responded that it depends on circumstances. Most (88%) of doctors believe written consent is the best type of consent in medical practice, 5 responded no and 7% responded that it depends on circumstances. Majority (89%) of doctors believe that treatment can be started to a patient without consent in emergency lifesaving situation, 3% responded no and 7% responded that it depends on circumstances. It observed that only 6% of doctors knew the sections of the Indian Penal Code applicable to consent, its medico-legal importance and laws in relation to consent, 88% responded no and 6% responded that they knew sometimes. Most of the participants (87%) believe only a person above 18 years of age with sound mind can give valid consent for general physical examination and procedures, 2% responded no and 11% responded that it depends on circumstances. 54% of doctors believe a child above 12 years of age with sound mind can give valid consent for simple, general and physical examination, 2 responded no and 11% responded that it depends on the circumstances. Among study participants, 82% doctors responded that informed refusal should be agreed in a case of established doctor – patient relationship, 5 responded no and 13% responded that it depends on the circumstances. Among study participants, Majority of the doctors (93%) felt that CME in medical ethics, consent in medical practice, medical negligence and laws related to medical practice should be conducted in regular interval, 3% of them responded 'no' and 4% responded that it should be conducted sometimes.

Questionnaire Table

Sl. No	Question	Yes	No	Sometimes/ Depending on Circumstances
1.	Do you know the various types of consent in medical practice & whose consent is valid?	21%	63%	16%
2.	Are you satisfied with your knowledge about consent in medical practice?	91%	06%	03%
3.	Are you taking Written Informed consent before any examination or procedure?	72%	16%	12%
4.	Do you think Informed consent and documentation can prevent a charge of negligence	88%	01%	11%
5.	Do you think doctor is the best person to obtain consent?	79%	08%	13%
6.	Do you think written consent is the best type of consent in medical practice?	88%	05%	07%
7.	Do you think treatment can be started to a patient without consent in emergency lifesaving situation?	89%	03%	07%
8.	Do you know the sections of Indian Penal Code applicable to consent, its Medico-legal importance and laws in relation to consent?	06%	88%	06%

Questionnaire Table

9.	Is only a person above 18 years of age with sound mind can give valid consent for general physical examination and procedures?	87%	02%	11%
10.	Do you think a child above 12 years of age with sound mind can give valid consent for simple, general & physical examination?	54%	32%	24%
11	Informed refusal should be agreed in a case of an established Doctor-Patient relationship.	82%	05%	13%
12	Do you feel CME in Medical Ethics, Consent in medical practice, Medical Negligence and laws related to medical practice shall be conducted in regular interval	93%	03%	04%

Discussion

It is of paramount importance that all physicians must know the actual meaning of valid consent. The valid consent is one which is informed, given voluntarily without any fear or misconception in mind by a person who is eligible for it. The Supreme Court of India in a recent Judgment, Samira Kohli vs. Dr. Prabha Manchanda & ANR, Appeal (civil) 1949 of 2004 (SC) has elaborated various aspects of consent taking. It has further laid down certain guidelines for taking a real or valid consent. As such it is an attempt to stream line consent process in India.[8]

In the present study, majority of the doctors (91%) were satisfied with their knowledge about consent in medical practice and 90 % respondents were aware of informed consent, their main source of knowledge on healthcare ethics was during training, and such experiences should be used to reinforce ethical knowledge and practice. This is in accordance with the study done by Dash S.K.in 2010 [9] and in a study by Rai JJ et al.[10].

Most of the doctors (72%) responded that they take informed consent before any examination or procedure, 88% doctors believe informed consent and documentation can prevent a charge of negligence. Among study participants, 79% of doctors believe doctors are the best person to obtain consent, the similar observations are recorded in the studies carried out by Dash S.K. Rai JJ et al and Heywood R. in 2007.[9-11]

In the present study, it was found that 88% of doctors believe written consent is the best type of consent in medical practice and 89% of doctors believe that treatment can be started to a patient without consent in emergency lifesaving situation. It can be inferred from section 92 of Indian penal code that act done in good faith for benefit of a person without consent is not an offence if the circumstances are such that it is impossible for that person to signify consent, or if that person is incapable of giving consent, and has no guardian or other person in lawful charge of him from whom it is possible to obtain consent in time for the thing to be done with benefit.[12]

So, it is obvious that if relatives or legal heir are denying for consent in the situation where major patient is in unconscious state, it is not advisable to perform surgery against their refusal. As mentioned by Christian P Selinger [13] that there are several legal exceptions to the right of consent concerning minor, patient with mental illness, patient suffering from communicable disease and incapacitated patient.[14]

In the current study, it was observed that only 6% of doctors knew the sections of the Indian Penal Code sections applicable to consent, its medico-legal importance and laws in relation to consent, 88% responded don't know, 87% of doctors believe only a person above 18 years of age with sound mind can give valid consent for general physical examination and procedures, 2 and 11% responded that it depends on circumstances. Among participants, 54% of doctors believe a child above 12 years of age with sound mind can give valid consent for simple, general and physical examination and 11% responded that it depends on the circumstances. Similar findings were noted by Gupta S et al., Taylor MK and others.[15,16] It can be clearly

inferred from section 87-89 of Indian penal code that a child above 12 years of age can give consent for simple, general or physical examination which is not associated with any harm to his/her life. In cases where a procedure is associated with harm or may lead to grievous hurt or death, consent of a person above 18 years is mandatory if he or she is in position to give.[14,17] Among study participants, most of the doctors (82%) responded that informed refusal should be agreed in a case of established doctor – patient relationship and 13% responded that it depends on the circumstances. Our observations are in consistent with other studies.[1,9,15,16]

In our study, among study participants, majority of the doctors (93%) felt that CME in medical ethics, consent in medical practice, medical negligence and laws related to medical practice should be conducted in regular interval. This observation is in accordance with other studies.[18,19]

The better knowledge about informed consent in medical practice among the medical practitioners comes from the clinical exposure in hospital setting. This study has given further insight into the domains of perceptions & apprehensions of the doctor about informed consent and to deliver better patient care and protect them.

Conclusion

The guiding principal is that informed consent should be an important means of facilitating communication between doctor and patient. As the profession of physical therapy continues to explore its full potential, the proper place of a formalized informed consent procedure for all patient care will be more obvious. This not only builds a healthy doctor – patient relationship, but also helps doctors save themselves from this era of litigation. Many doctors unfortunately get involved in medical negligence case in their career due to lack of documentation, lack of informed consent and negligent supervision. Lack of time to update their knowledge on law related medical practice is probably the reason for this. The medical ethics and such acts related to medical practice should be emphasized in the undergraduate and post graduate syllabus and examinations. Regular medico legal training programmes, continuum medical education programmes, seminars and workshops must be conducted in every institution and must be made mandatory to participate actively for all medical practitioners to update their knowledge related to consent in medical practice and law related to medical practice.

Limitations

The limitation of our study is that it was conducted in our institute only, even though the hospital consists of a diverse group of healthcare providers coming from different and almost all parts of the district, it cannot predict the overall situation in the country.

Ethical Clearance: Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai prior to the study. Consent was taken from each respondent. Confidentiality was maintained.

Source of Funding: Self

Conflict of Interest: Nil

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Original Research

A Retrospective Study of Socio-Demographic Profile and Pattern of Poisoning Cases at Tertiary Care Hospital

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Abstract

Death due to poisoning has been known since time immemorial. Poisoning is a major problem all over the world, although its type and the associated morbidity and mortality vary from country to country. According to the WHO, more than three million poisoning cases with 251,881 deaths occur worldwide annually, of which 99% of fatal poisonings occur in developing countries, particularly among agricultural workers. Pattern of poisoning in any region depends on availability of poisons, socio economic status of the population, religious and cultural influences, occupation prevalent in the region and likewise. Organophosphorus poisoning occurs very commonly in southern India, where farmers form a significant proportion of the population who commonly use organophosphorus compounds like parathion as insecticides. A retrospective analysis of all poisoning cases admitted to Saveetha Medical College Hospital, Chennai from Jan 2019 to Dec 2019 was done to study the pattern of poisoning reported. Acute poisoning is a common medical emergency and one of the important causes of morbidity and mortality in developing countries due to easy availability of poisonous substances and its low cost. So, it was necessary to know the demo-graphic profile & pattern of poisoning cases at Saveetha Medical College Hospital, Chennai. Data was collected using a pretested proforma from case records, MRD department & Emergency Medicine department and the data was analyzed and presented.

Key Words: *Poisoning, organophosphorus compounds, Suicide*

Introduction

A poison is a substance that may produce death, serious illness, or harmful effects when introduced into the body/come in contact with the body in a relatively small quantity.[1] Death due to poisoning has been known since time immemorial. Poisoning is a major problem all over the world, although its type and the associated morbidity and mortality vary from country to country. According to World Health Organization, (WHO) poisoning occurs when people drink, eat, breathe, inject, or touch enough of a hazardous substance (poison) to cause illness or death.[2] Poisoning is a medical emergency and a patient is always invariably rushed to the hospital at the earliest possible moment, irrespective of the amount and nature of poison ingested.[3] According to the WHO, more than three million poisoning cases with 251,881 deaths occur worldwide annually, of which 99% of fatal poisonings occur in developing countries, particularly among agricultural workers. Pattern of poisoning in any region depends on availability of poisons, socio economic status of the population, religious and cultural influences, occupation prevalent in the region and likewise.[4] Organophosphorus poisoning occurs very commonly in southern India, where farmers form a significant proportion of the population who commonly use organophosphorus compounds like parathion as

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insecticides. Thus, due to the easy accessibility of these compounds, a large number of suicidal cases are encountered in this region.[5] In addition to that, snakebite is a common acute medical emergency faced by rural populations in tropical and subtropical countries with heavy rainfall and humid climate.[6] Some 35,000–50,000 people die each year from snakebite, which is a common cause of morbidity and mortality in India.[7] A comparative data revealed that in developed countries, the mortality rate due to poisoning is only 1% to 2%, but in developing countries like India it varies between 15% to 30% 13 and is the fourth most common cause of mortality especially in rural India.[5]

India accounts for one third of pesticide poisoning cases in the third world and the worst affected are the farm workers who contribute nearly three quarters of the labour force. In general, accidental poisoning is more common in children whereas suicidal poisoning is more common young adults. Most of the fatality rate is of intentional poisoning by Organo-phosphorous compounds, which has been reported in southern India. It is important to know the nature and severity of poisoning in order to take appropriate preventive measures. Studies of this nature will be a useful tool in planning and management of critically ill acute poisoning cases.

Considering the cost and outcomes of poisoning cases reported to hospitals it is found necessary to establish a poison information center which should be network with other PICs information centers in India and other countries by which identifying the poisons and managing cases will become more efficient. The present study was conducted with the objective of determining the socio-demographic profile and assessing the pattern and outcome of poisoning cases admitted at a tertiary care hospital, over a period of one year.

Materials and Methodology

The present study is a retrospective cross-sectional study conducted in a Saveetha Medical College hospital, a tertiary care hospital. The data was collected from the case records from Medical Record Department and Accident/MLC register available at the department of Emergency Medicine, Saveetha Medical College, Thandalam, Chennai. The study period was for one year from January 1st, 2019 to December 31st, 2019. Permission was obtained from the Medical Superintend of the hospital to allow us to access the information from the patients' case notes in the record section, strictly for purpose of this research. The study included 228 cases of various poisonings due to drugs and chemicals from January 1st, 2019 to December 31st, 2019. Cases of food poisoning, alcohol intoxication, toxic or allergic reactions to drugs, scorpion sting, bee sting cases and cases discharged against Medical Advice (DAMA) were excluded in the study. Demo graphic details such as age, gender, occupation, residence, time elapsed after intake, type of poison, manner and route of poisoning, duration of hospitalization and outcome was collected in a pre-structured proforma. The data compiled and then analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 using standard statistical methods.

Aims & Objectives:

- To find prevalence of poisoning cases in and around Chennai.
- To determine the socio-demographic profile of poisoning cases and assessing the pattern and outcome of poisoning cases admitted at a tertiary care hospital.

ETHICAL CONSIDERATION:

Permission to retrieve the case records was obtained from the institution authority. Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai, prior to the study. Confidentiality was maintained.

Results & Observations

Table 1 shows that out of 228 poisoning cases recorded, 136 were Males & 92 were Females (M = 59.64%, F = 40.35%). Out of 136 males, 12 succumbed to death and out of 94 females 7 died due to poisoning.

Table 1: Gender distribution of poisoning cases.

Gender	Number of cases	Total no deaths
Male	136 (59.64%)	12 (8.8%)
Female	92 (40.35%)	07 (7.60%)
Total	228	19

We observed that out 136 males came with poisoning, 55 % patients were married and 45% patients were unmarried. Out of **92** females, who admitted for poisoning 65% patients were married and 35% were unmarried.

Table 2 shows that most of the cases reported were observed in the age group of 21-30 yrs. (94 cases, 41.22%) in both genders followed by the age group of 11- 20 yrs. (36 cases, 15.78 %) and least in the age group of more than 60 yrs. 14 cases, 6.14%). The male preponderance was seen in all age groups.

Table 2: Age-wise frequency distribution of poisoning cases in both genders.

Age in yrs.	Male	Female	Total
0-10	09	08	17 (7.4%)
11-20	23	13	36 (15.78%)
21-30	55	39	94 (41.22%)
31-40	15	09	24 (10.52%)
41-50	15	11	26 (11.40%)
51-60	11	06	17 (7.4%)
>60	08	06	14 (6.14%)
Total	136	92	228

Chart 1 shows that maximum cases were from rural areas 161(71%) and 67 (29%) cases reported from urban areas.

Chart 1: Region wise distribution of poisoning cases

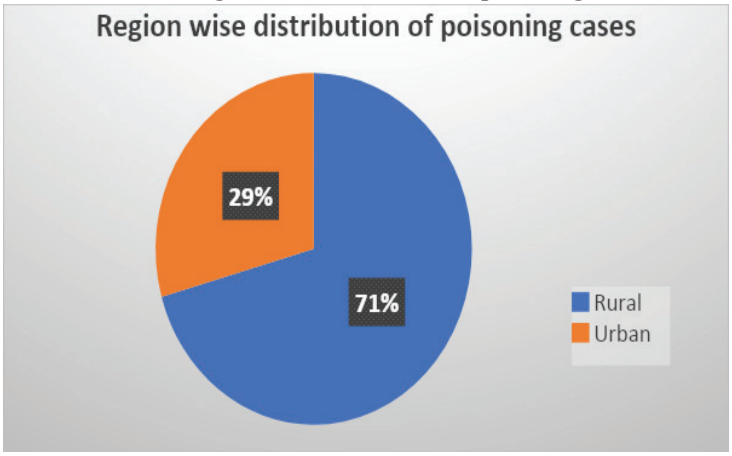

Table 3 shows that most of the poisoning cases were due to organophosphorus insecticide consumption (30.26%), followed by miscellaneous & unknown poisons (15.78 %), Organo-chlorines (10.08 %), Kerosene (8.33%), Tablet over dose (7.89%), and Carbamates (6.6%). Other cases of poisoning were due to corrosives, rat poison, plant products. Miscellaneous includes Naphthalene balls, mosquito repellants, baygon etc.,

Amongst 228 total poisoning cases, 29 had consumed alcohol along with the poison. Out of 19 deaths 15 people had consumed OP compound. Most of these patients who died due to OP compounds had respiratory arrest, pneumonia and ARDS. (Table 4)

Table 3: Type of poisons & distribution of cases

Type of Poison	Number of Cases	Percentage
Organo-phosphorus	69	30.26
Organo-chlorines	23	10.08
Carbamates	16	7.01
Corrosives	08	3.50
Kerosene	19	8.33
Rat poison	13	5.70
Snake bite	17	7.45
Plant products	09	3.94
Tablet Overdose	18	7.89
Unknown & Miscellaneous	36	15.78
Total	228	100 %

Table 4shows the seasonal variation in occurrence of poisoning cases. Results indicate Maximum incidence of poisonings cases in the months from Aug-Nov (37.71%).

Table 4: Seasonal variation in poisoning cases

Month	No of cases	Percentage (%)
Dec – Mar	73	32.01
Apr – Jul	69	30.26
Aug – Nov	86	37.71
Total	228	100

The study results showed that 164 (71.92 %) of cases were due to suicidal manner of poisoning (M=69.85%, F=75 %) followed by Accidental (26.75 %) and Homicidal (1.31%). (Table 5)

Table 5: Manner of poisoning

Manner	No of cases (Male)	No of cases (Female)	Total no of cases	Percentage
Accidental	39 (28.67%)	22 (23.91%)	61	26.75%
Suicidal	95 (69.85 %)	69 (75%)	164	71.92%
Homicidal	02 (1.47%)	01 (1.08%)	03	1.31%
Total no of cases	136	92	228	100%

About time lapse observation depicts that the majority of patients (63.59%) were reported and admitted to the hospital after a delay of >2 hrs following the exposure to the poison and most of the deaths (73.68 %) occurred when the time lapse was >2 hrs. Amongst 228 cases 83 (36.40%) patients reported < 2 hrs of exposure, 5 deaths (26.31 %) occurred in cases with the time lapse of < 2 hrs . Time lapse had a significant role in mortality in case of acute poisoning (c2 =5.22, p< 0.05).

Discussion

Poisoning is a common medico social problem nowadays all over the world, factors like degree of the toxicity and speed at which the patient needs treatment have been studied. In the present study out of 228 poisoning cases, 136 were males and 92 were females. These findings are in consistent with other studies. [8-10] The high incidence may be because males are more exposed to stress, strain and occupational hazards compared to females.[11]

In the present study the most common age group involved was between 21-30 years followed by the age group between 11- 20 years. Thus, adolescent and young adults are at more risk compared to other groups. Similar observations were reported by other studies conducted by authors.[3, 8-10, 12]

In this study, results showed that 164 (71.92 %) of cases were due to suicidal manner of poisoning (M=69.85%, F=75 %) followed by Accidental (26.75 %) and Homicidal (1.31%). Most of the patients were given psychiatric counseling and drug therapy. Reactive depression was also seen in majority of patients secondary to failure in academic, unemployment, financial crunch, domestic violence & problems in interpersonal adjustment. Other contributory factors were chronic alcoholism, financial stress, psychotic disorder, impulsive disorder and anxiety. As increasing trend of suicidal mode in males as compared to females was noticed, as reported in other studies. Low socio-economic status, rapid urbanization and other frustrations could probably be contributing factors for higher suicide rates among men. The study results are in consistent with the various studies published in India.[8-10,13] Pesticides have also previously been implicated as possible poisoning agents in India. Ignorance about proper storage in households and easy availability of pesticides could be mainly responsible for both suicidal and accidental poisoning.[8]

In the present study 69 cases (30.26.%) were reported to the hospital with the history of consumption of organophosphorus poisons, which were the most commonly responsible agents for toxicity in poisoning cases. Similar observations were recorded in the studies conducted by the authors.[8-10,13-14]

In our study, we observed that married person more often become victim of poisoning which was found similar with other studies.[3,8,10,15] The reason of fact could be that the amount of stress carried by the married people on their day to day life is more than the single males or females which makes them more vulnerable.[11]

In seasonal variation in occurrence of poisoning cases. Results indicate Maximum incidence of poisonings cases in the months from Aug-Nov (37.71%)

easy availability of insecticides during this season. The similar observations were recorded in the studies conducted by the authors.[8-10,13-14]

In the current study, the majority of patients (63.59%) were reported and admitted to the hospital after a delay of >2 hrs following the exposure to the poison and most of the deaths (73.68 %) occurred when the time lapse was >2 hrs. Amongst 228 cases 83 (36.40%) patients reported < 2 hrs of exposure, 5 deaths (26.31 %) occurred in cases with the time lapse of < 2 hrs . Time lapse had a significant role in mortality in case of acute poisoning, the similar results noted in the various studies.[8-10,15,16]

We observed that most of the poisoning cases were reported from rural areas (71%) possibly due to illiteracy and poverty of the agricultural farmers residing in these parts. They solely depend on agricultural income for their livelihood. Pesticides are easily accessible to them as they store those in households. Ignorance about proper storage in households and easy availability of pesticides could be the reason for both suicidal and accidental poisoning. The similar observations are noted in the other studies. [3,8,10,16]

Conclusion

The present study highlights the profile of poisoning cases admitted to Saveetha Medical College Hospital, South Chennai over a period of one year (Jan 2019- Dec 2019) , showing that males of 21-30 yrs. age group are the major victims followed closely by females in the same age group. It also points towards the commonest poison used i.e. OP compounds with suicidal intention being the most common manner of poisoning.

The incidence, trends of poisoning, the morbidity and mortality due to poisoning can be possibly curtailed by strict vigilance over the sale and distribution of insecticides/pesticides, educating the users regarding the safety measures, good treatment facilities (i.e. antidotes etc.) at rural areas like PHC's and CHC's. Establishing poison information centers at almost all tertiary care centers and proper and correct implementation of social and economic projects aimed for upliftment of the rural population and low socio-economic group of population.

Ethical Clearance: Ethical Clearance was obtained from Institutional Ethics Committee, Saveetha Medical College, Chennai prior to the study. Consent was taken from each respondent. Confidentiality was maintained.

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Association Between CT Scan Findings and Mortality in Covid-19 Cases

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Abstract

Background: Covid-19 epidemia is a worldwide infectious viral disease initiating from December 2019 from Wuhan, China. Recognition of the prognostic role of imaging findings is important to improve the outcomes in patients. Accordingly, regarding the lack of studies in this era among Iranian patients, this study was done to determine the association between CT scan findings and mortality in Covid-19 cases.

Methods: In this prognostic prospective cohort study, 192 consecutive patients with established Covid-19 disease by clinical findings, and laboratory results in Besat Hospital, Tehran, Iran in March and April 2020 were enrolled. The findings in CT scan were involved zone and lobe, ground glass, consolidation, ground glass with reticular, reverse hallo sign, microvascular dilation sign, fibrotic streaks, sub pleural line, air bronchogram, Bronchus distortion, broncheiectasis, pleural thickening, pleural reaction, and pleural effusion. According to involvement degree the points in CT scan were 0, 1, and 2 for lack of lung parenchyma involvement, less than 50 percent involvement, and more than 50 percent involvement.

Results: The involved zones were significantly differed between survived and dead cases (P=0.001). As shown in Table 4, the scores of CT scan for lung involvement and also number of involved lobes were differed across the alive and dead cases (P=0.001) the mean hospital stay was 7.1 (5.6) and 5.7 (2.5) days that was same across the groups (P=0.325). There were significant differences between groups for consolidation, ground glass plus reticular pattern, fibrotic streaks, sub-pleural line, air bronchogram, bronchiectasis, pleural thickening, pleural effusion, and pleural reaction ($P < 0.05$).

Conclusion: Totally, according to the obtained results it may be concluded that CT scan is useful prognostic modality to predict the mortality among patients with Covid-19 disease. Consolidation, ground glass plus reticular pattern, fibrotic streaks, sub-pleural line, air bronchogram, bronchiectasis, pleural thickening, pleural effusion, and pleural reaction are the man CT scan findings predicting higher mortality rate in these patients.

Keywords: *Covid-19, Imaging, Prognosis, Mortality*

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Introduction

Covid-19 epidemia is a worldwide infectious viral disease initiating from December 2019 from Wuhan, China [1, 2]. Viruses in corona-virus family are usually affecting the animals but mutant species can infect humans [3-5]. Diagnosis of viral infections is done by PCR and similar microbiological methods 6. However

expensive status and non-feasibility of these tests usually leads to use of serological tests that the main limitation of them is false positive and negative results [7-9].

Imaging methods especially computerized tomography (CT) scans may be used as feasible appropriate techniques for screening of suspected cases 10, 11. Familiarity with CT scan findings as the main imaging diagnostic method may help to better control of Covid-19 disease and improvement of final prognosis in patients [12, 13]. The CT scan criteria include two groups of highly suggestive (ground glass opacity, peripheral distribution, and peri-bronchovascular involvement) and inconsistent (centriloboular nodules, tree-in-bud opacity, reticular opacity, nodular opacity, cavity, lymphadenopathy, and pleural effusion) [14]. Recognition of the prognostic role of imaging findings is important to improve the outcomes in patients. Accordingly, regarding the lack of studies in this era among Iranian patients, this study was done to determine the association between CT scan findings and mortality in Covid-19 cases.

Materials and Methods

In this prognostic prospective cohort study, 192 consecutive patients with established Covid-19 disease by clinical findings, and laboratory results in Besat Hospital, Tehran, Iran in March and April 2020 were enrolled by census manner. The diagnosis was made by two radiologists, one infectious disease specialist, and one emergency medicine specialist. For reduction of selection bias dead and survived cases were selected from single center. The exclusion criterion was death due to causes other than Covid-19 disease. CT scan was done in Besat Hospital for all cases at first admission day. The study was approved by ethical committee in AJA University of Medical Sciences.

Data were gathered by checklist including age, sex, outcome, involvement type (peripheral/central, unilateral/ bilateral), simultaneous findings (including cardiomegaly, aortic calcification, hiatal hernia, and pneumonia), background diseases (renal insufficiency, obstructive pulmonary disease, hypertension, asthma, and coronary artery disease), involvement severity, hospital stay, and CT scan findings [14]. The findings in CT scan were involved zone and lobe, ground glass, consolidation, ground glass with reticular, reverse hallo sign, microvascular dilation sign, fibrotic streaks, sub pleural line, air bronchogram, Bronchus distortion, broncheiectasis, pleural thickening, pleural reaction, and pleural effusion. According to involvement degree the points in CT scan were 0, 1, and 2 for lack of lung parenchyma involvement, less than 50 percent involvement, and more than 50 percent involvement.

Data analysis was done by SPSS version 20.0 software. The utilized tests were Kolmogorov-Smirnov, Chi-Square, Fisher, Mann-Whitney, and Independent-Sample-T and P values less than 0.05 were considered statistically significant.

Results

There were 64 dead and 128 survived cases. Totally 68.9% and 63.0% among male and female cases were died (P=0.400). The mean (standard deviation) ages in dead and survived cases were 65.7 (13.5) and 49.6 (13.8) years (P=0.001). None of the cases had pneumonia. The frequency distribution of background disease is shown in Table 1.

Table 1- Frequency distribution of background disease

Outcome					
	Dead		Alive		
	Count	Column N%	Count	Column N%	P-value
Hiatal Hernia	1	1.6%	2	1.6%	1.0
Cardiomegaly	5	7.8%	8	6.3%	0.763
Aortic Calcification	18	28.1%	17	13.3%	0.012

Cont... **Table 1- Frequency distribution of background disease**

Diabetes Mellitus	21	32.8%	18	14.1%	0.002
COPD	2	3.1%	0	0.0%	0.11
HTN	23	35.9%	15	11.7%	0.001
Asthma	1	1.6%	1	0.8%	1.0
Ischemic Heart Disease	13	20.3%	10	7.8%	0.012
CRF	1	1.6%	0	0.0%	0.333

As shown in Table 2, the lung conflict was significantly differed across the groups (P=0.001). According to Table 3, the involved zones were significantly differed between survived and dead cases (P=0.001). As shown in Table 4, the scores of CT scan for lung involvement and also number of involved lobes were differed across the alive and dead cases (P=0.001) the mean hospital stay was 7.1 (5.6) and 5.7 (2.5) days that was same across the groups (P=0.325).

Table 2- Frequency distribution of lung conflict

	Long Conflict			
	1	2	3	Total
Outcome Dead	37	0	27	64
	23.9%	0.0%	75%	33.3%
Alive	118	1	9	128
	76.1%	100%	25%	66.7%
Total	155	1	36	192
	100%	100%	100%	100%

Table 3- Frequency distribution of lung zones

Outcome						
Dead		Alive				
		Count	Column N %	Count	Column N %	P-value
Anterior upper zone of right lung	0	22	34.4%	80	62.5%	0.001
	1	33	51.6%	44	34.4%	
	2	9	14.1%	4	3.1%	
Posterior upper zone of right lung	0	24	37.5%	78	60.9%	0.001
	1	23	35.9%	47	36.7%	
	2	17	26.6%	3	2.3%	
Anterior middle zone of right lung	0	12	18.8%	49	38.3%	0.001
	1	33	51.6%	75	58.6%	
	2	19	29.7%	4	3.1%	

Cont... **Table 3- Frequency distribution of lung zones**

Posterior middle zone of right lung	0	9	14.1%	34	26.6%	0.001
	1	32	50%	80	62.5%	
	2	23	35..9%	14	10.9%	
Anterior lower zone of right lung	0	10	15.6%	47	36.7%	0.001
	1	46	71.9%	80	62.5%	
	2	8	12.5%	1	0.8%	
Posterior lower zone of right lung	0	2	3.1%	17	13.3%	0.001
	1	19	29.7%	69	53.9%	
	2	43	67.2%	42	32.8%	
Anterior upper zone of left lung	0	21	32.8%	76	59.4%	0.001
	1	30	46.9%	50	39.1%	
	2	13	20.3%	2	1.6%	
Posterior upper zone of left lung	0	29	45.3%	85	66.4%	0.001
	1	22	34.4%	41	32.0%	
	2	13	20.3%	2	1.6%	
Anterior middle zone of left lung	0	18	28.1%	46	35.9%	0.001
	1	30	46.9%	80	62.5%	
	2	16	25%	2	1.6%	
Posterior middle zone of left lung	0	12	18.8%	53	41.4%	0.001
	1	22	34.4%	66	51.6%	
	2	30	46.9%	9	7.0%	
Anterior lower zone of left lung	0	14	21.9%	46	35.9%	0.001
	1	35	54.7%	79	61.7%	
	2	15	23.4%	3	2.3%	
Posterior lower zone of left lung	0	2	3.1%	21	16.4%	0.001
	1	20	31.3%	73	57%	
	2	42	65.6%	34	26.6%	

Table 4- Frequency distribution of lung involvement scores and number

	Outcome	Mean	Std. Deviation	P-value
Score of right lung	Dead	6.66	2.43	0.001
	Alive	4.15	2.0	
Score of left lung	Dead	6.52	2.74	0.001
	Alive	3.86	1.98	
Total score of both lungs	Dead	13.19	4.79	0.001
	Alive	8.02	3.55	
Number of Involved Lobes	Dead	5.09	1.26	0.001
	Alive	4.32	1.64	

Table 5- Frequency distribution of lung involved lobe

	Outcome				
	Dead		Alive		
	Count	Column N %	Count	Column N %	P-value
Right upper lobe	49	76.6%	73	57%	0.008
Right middle lobe	59	92.2%	94	73.4%	0.002
Right lower lobe	63	98.4%	116	90.6%	0.064
Left upper lobe	49	76.6%	81	63.3%	0.064
Left lingual	53	82.8%	79	61.7%	0.003
Left lower lobe	54	84.4%	113	88.3%	0.448

The lung involved zoned are shown in Table 5. There were no cases with ground glass opacities alone in groups. There were significant differences between groups for consolidation, ground glass plus reticular pattern, fibrotic streaks, sub-pleural line, air bronchogram, bronchiectasis, pleural thickening, pleural effusion, and pleural reaction (Table 6).

Table 6- Frequency distribution of other CT scan findings

	Outcome				
	Dead		Alive		
	Count	Column N %	Count	Column N %	P-value
Consolidation	50	78.1%	67	52.3%	0.001
Ground glass with reticular	51	79.7%	57	44.5%	0.001
Reverse hallo sign	23	35.9%	59	46.1%	0.18
Microvascular. Dilation. sign	49	76.6%	105	82.7%	0.313
Fibrotic streks	39	60.9%	53	41.4%	0.011
Subpleural line	35	54.7%	32	25%	0.001
Bronchdistortion	3	4.7%5	5	3.9%	1.0
Air bronchogram	49	76.6%	63	49.2%	0.001
Bronchiectasis	5	7.8%	2	1.6%	0.042
Pleural thickening	31	48.4%	21	16.4%	0.001
Pleural Reaction	47	73.4%	67	52.3%	0.005
Pleural Effusion	11	17.2%	5	3.9%	0.002
Bilateral Confict	62	96.9%	114	89.1%	0.065

Discussion

In this cohort, various CT scan findings were compared between alive and dead cases with Covid-19 disease. We found significant differences between two groups for both distribution and extension of lung involvement across the groups according to CT scan. However, the age was also higher among dead cases but the sex was not differed across the groups. Aortic calcification, hypertension, ischemic heart disease, and diabetes mellitus were the main risk factors affecting the mortality among Covid-19 diseases. It was seen that involvement of right and lingual lobes were higher among dead cases but left lobes were same between alive and dead patients. Consolidation, ground glass plus reticular pattern, fibrotic streaks, sub-pleural line, air bronchogram, bronchiectasis, pleural thickening, pleural effusion, and pleural reaction were all more commonly seen in non-survived cases. These were among both groups of highly suggestive and inconsistent CT scan criteria as mentioned in Introduction portion.

The review study by Wang et al [15] declaimed that use of CT scan should be cautioned for dose and times to expose lower radiation to the patients but it can predict the mortality. The article was in line with our study. The retrospective study by Li et al [16] demonstrated that majority of cases had multiple involved lobes especially with bilateral pattern. In their study it was found that consolidation and bilateral involvement were related to higher mortality rate as same as our study.

Majidi et al [17] in their review study reported that consolidation, bilateral involvement, and multi-focal involvement were among main CT scan findings in Covid-19 cases and also declaimed that further extension was related to higher mortality as seen in our study. Also, Ostad et al [18] demonstrated that patchy consolidation and ground-glass involvement were main contributing factors for mortality in Covid-19 diseases. It is in line with our findings. Another study by Ye et al [19] showed that ground glass pattern and consolidation are main CT scan findings that were related to mortality among patients with Covid-19 disease. Similar findings were reported by other studies [20, 21].

Totally, according to the obtained results it may be concluded that CT scan is useful prognostic modality to predict the mortality among patients with Covid-19 disease. Consolidation, ground glass plus reticular pattern, fibrotic streaks, sub-pleural line, air bronchogram, bronchiectasis, pleural thickening, pleural effusion, and pleural reaction are the man CT scan findings predicting higher mortality rate in these patients. However further studies with comparison of the results with other diagnostic imaging methods can develop further evidence for better control of the disease and reduction of mortality in affected cases.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of AJA University of Medical Sciences

Conflict of Interest: Non

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Prevalence and Incidence of Dermatophytosis in Al-Diwaniya City, Iraq

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Abstract

The present study carried out in Al-Diwaniya city, 71clinical specimens were taken from the patients with dermatophytosis. The age of patients were between (1-50) years, real positive cultural examinations were 49(69%), whereas negative cultural examinations were 22 (31 %) . The occurrence of dermatophyte fungi were higher in females than males, depending on the gender, as for the age groups, the infection was higher in the age group 31-40 and 41-50 years followed by the age group ≤ 11 years, whereas the lowest infection was among the age group 21-30 and 12-20 years. Also, the result of this study showed that 37(52.11%) patients were housewives, 20 (28.16%) students, 7(9.85%) office employees, 7(9.85%) hospital workers. Out of 49 patients 25(51.0 %) cases were Tinea pedis, followed by Tinea cruris15(30.6%), Tinea capitis 5(10.2%), and 4(8.2%) Tinea unguium. The result of this study also showed that 31(63.3%) isolates were *T. rubrum*, 11(22.4%) *T. mentagrophytes* , *E. floccosum* and *M. canis* isolates were found 2(4.1%) and 5(10.2%) respectively.

Keywords : *Dermatophytosis, Tinea unguium, T. rubrum, KOH* .

Introduction

Dermatophytes are a group of fungi that cause a superficial infection (dermatophytosis). Dermatophytes are keratinophilic fungi , infecting the epidermis and keratinized structure such as hairs and nails for both humans and animals [(1)].There is a wide range of hosts that are preferred by dermatophytes such as ; carnivores , rodents , rabbits and people [(2)]. The infection is usually transmitted by direct contact from human to human or infected animals or by fomites [(3)]. Dermatophytes are included anthropophiles , zoophiles and geophiles according to natural habitat , where the first is human associated while the second is animal associated but the latter soil dwelling [(4)] . It is important to note that the infection of dermatophyte fungi was not invasive and easy to recover, but it is widespread and treatment costs are one of the major health problems and cause serious damage to the economic situation of countries such as Iraq[(5)] . This study aims to isolation and identification of local dermatophyte species from patients with both sexes and different ages admitted to department of dermatology at Al-Diwaniya Teaching Hospital, Iraq.

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Materials and Methods

Collection of specimens : Seventy-one samples were collected from patients with both sexes and different ages admitted to Department of Dermatology at Al-Diwaniya Teaching Hospital during period from February 2018 to January 2019. The samples included : hair, nail, and skin collected by using sterilized forceps, fine scissors and blunt scalpel. In addition to, the skin scales were collected after sterilization of the affected area with alcohol 70% and then edge scrape with a sterile blunt scalpel, whereas infected hair were collected by using sterile forceps . The nail samples was taken by using sterile fine scissors, as well as all samples

were divided into two portions ; one portion was tested directly under light microscope but the other portion considered for cultivation [6].

Direct microscopy :

The hair, scales, and nail specimens were put on new slide with drops of KOH 10% and heated gently for 5-10 min., subsequently the slide left cooled and placed the cover slip and tested under the microscope (40x) to observe septate hyphae and arthroconidia [7] .

Culture :

The clinical samples were primary cultured on sabouraud's dextrose agar containing of cyclohexamide 0.5gm to suppress the growth of non-dermatophytic fungi and permit only the growth of dermatophyte fungi , as well as added to media chloramphenicol (0.05gm) to inhibit the growth of bacteria. The plates were inoculated with samples were duplicated and incubated at 28°C for at least 3–4weeks.

Lacto phenol cotton blue method:

The isolates were tested microscopically by separating a portion of aerial mycelium with needle, as well as the material was placed on slide with a drop of (Lacto phenol cotton blue) and covered by thin slip , also excess of stain removed with blotting paper and examined under (100 and 400 magnifications).

Results and Discussion

Seventy one specimens were collected from patients with symptoms of dermatophytosis . Real positive cultural examinations were 49(69%), whereas negative cultural examinations were 22 (31 %) by examination of KOH 10% and culture on Sabouraud dextrose agar with cyclohexmaide (Table 1).

Table (1): Numbers and percentages of clinical samples with dermatomycosis infections.

Results	N	%
Positive	49	69
Negative	22	31
Total	71	100
P value	0.001*	

*** represents a significant difference at p<0.05.**

This result is similar to that found by Ismael,[8] who recorded the positive cultural results (79.31%) which was higher than negative cultural results . Table (2) shows the occurrence of dermatophyte fungi were higher in females than males, depending on the gender.

Table (2): Distribution of dermatophyte infections according to age and gender.

Gender	Age Groups No. (%)					Total	P value
	≤ 11	12_20	21-30	31-40	41-50		
Female	6(66.7)	3(75)	2(66.7)	10(52.6)	8(57.1)	29(59.2)	a- 0.901
Male	3(33.3)	1 (25)	1(33.3)	9 (47.4)	6(42.9)	20(40.8)	b- 0.199
Total	9(18.4)	4(8.2)	3 (6.1)	19(38.8)	14(28.6)	49 (100)	c- 0.001*

*** represents a significant difference at p<0.05. letters (a, b and c) represent the following statistical analysis in sample distribution: a- between gender and age groups, b- between male and female, c- among age groups.**

This result was matching with Lafta, [9] ; Maluki and Al-Hulli [10] and Abed Ali *et. al*. [11] revealed the occurrence of Onychomycosis in females were higher than males. As well as , Bassiri-Jahromi *et. al*[12] showed that the infection with dermatophyte was more predominant in females 72.3% than males 27.7% . This may be due to the females are more in contact with water in their home works, hence they may be more exposed to moisture which preferred by dermatophytes fungi . Other causes such as ; Quality of lifestyle , hormonal differences and physiological functions for both males and females [13] .

As for the age groups, the infection was higher in the age group 31-40 and 41-50 years followed by the age group ≤ 11 years, whereas the lowest infection was among the age group 21-30 and 12-20 years . This result is an approach with that found by Ismael, [14] who found that the dermatophyte infections were most predominant among the age group 41-50 years 38.8% followed by age group 41-50 years which was recorded 20.9% and then age group 21-30 which was 14.92% , as well as the age group 1-10 and 11-20 years each both was 7.46% then the age group 51-60 years was 5.98%, also age group 61-70 years which was 2.98%, but the rate of the lowest infection was 1.5%, among the age group 61-70 years . Table (3) showed that out of 71 patients, 37(52.11%) patients were housewives, 20 (28.16%) patients were students, 7(9.85%) patients were working as office employees, 7(9.85%) patients were a hospital workers .

Table (3): Distribution of patients according to Occupational status:

Results	N	%
Housewives	37	52.1
Students	20	28.2
Office employees	7	9.9
Hospital worker	7	9.9
Total	71	100
P value 0.002*		

*** represents a significant difference at p<0.05.**

This result is identical to Abed Ali *et al* . [11] who revealed that the patients (20%) were students whereas (32.5%) were housewives. This can be explained by many reasons, including ; exposure to many different environmental factors such as; trauma, sport events, abnormal lifestyles, tight shoes, using of cosmetics, exposure to stressful conditions, and using of the strong detergent which affecting on the nails and constant contact with animals, also lack of personal care or the use of personal objects which may be contaminated [15] .

Table (4) showed that out of 49 patients 25(51.0 %) cases were Tinea pedis, followed by Tinea cruris which was represent 15(30.6%), Tinea capitis which was represent 5(10.2%), and 4(8.2%) cases were Tinea unguium.

Table (4): Distribution of clinical types among the gender of patients.

Types	Gender No. (%)		Total (%)
	Male	Female	
T. pedis	11 (50)	14 (51,9)	25 (51.0)
T. cruris	6 (27.3)	9 (33.3)	15 (30.6)
T. capitis	3 (13.6)	2 (7.4)	5 (10.2)
T. unguium	2 (9.1)	2 (7.4)	4 (8.2)
Total	22 (100)	27 (100)	49 (100)
P value	0.031*	0.062	<0.0001*

*** represents a significant difference at p<0.05.**

The result of this study is inconsistent with Tan [16] who revealed the most important infection in Singapore was T. pedis 27.3%. T. pedis is the most prevalent type among the dermatophytosis; it is reaches about 70% of the total infection [17]. As well as the result of this study showed that 31(63.3%) isolates out of the 49 culture positive isolates were *T. rubrum*, also it is regarded the most frequent etiological agent followed by11(22.4%) *T. mentagrophytes* whereas *E. floccosum* and *M. canis* isolates were found 2(4.1%) and 5(10.2%) respectively (Table 5) .

Table (5): Distribution of fungal species among positive cultures.

Types	No. (%)
T. rubrum	31(63.3)
T. mentagrophytes	11(22.4)
M. canis	5(10.2)
E. floccosum	2(4.1)
Total	49 (100)
P value	<0.0001*

*** represents a significant difference at p<0.05.**

This result is in agreement with that found by Tan, [16] who found that the *Candida sp*. were most prevalent , also this is in consistence with Di Silverio et. al. [18] who found that the dermatophytosis infection was (34.6%) whereas Candidiasis was (10.8%) .

Conclusion

We conclude from the above that the dermatophyte infection is widespread in a worldwide and occurs in increasing proportions, especially in tropical countries such as Iraq. Also, there are several factors that control on increased infection rates such as poor hygiene, illiteracy, age, sex, and social economy . In the present study we

also concluded *T. pedis* was the major clinical infection and *T. rubrum* was the most frequent etiological agent . This has been proven by our current study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Interleukin-8 Concentration Correlation with the Size of Hydatid Cyst in Patients with Hydatidosis and Hepatitis C Mixed Infection (Case Control Study)

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Abstract

Background: Hydatid cyst is a disease that is transmitted from the lactating animals that come into contact with a person, and its danger is to generate hydatid cysts that may put pressure on some vital organs in the human body. Here comes the role of joint infection with other pathogens such as the hepatitis C virus, especially in the same organs that are infected with the parasite, which may result from these mixed infections serious disease complications

Aim of study: The present study aimed to examine the possibility of a relationship between interleukin-8 concentration level with the size of Hydatid cyst patients with mixed infection of hydatidosis and hepatitis C virus.

Patients and Methods: The current research was run for the period January 2019 to January 2020. The patients were referred to AL-Furat al-awsat Hospital , Al-Sadder medical city, Public Health Laboratory and Al-Hakeem Hospital in Al- Najaf governorate, Iraq. Eighty samples were divided into two groups. This first group was comprised of 40 patients with hydatidosis, the second group comprised of 40 patients with hydatidosis and hepatitis c virus mixed infection. The patients were aged 10 to 60 years old.

Result: A higher interleukin-8 concentration was observed in patients with hydatidosis compared to the healthy group, as the interleukin-8 concentration was (89.36) While the concentration of interleukin- 8 reached in the healthy group (30 .14) ,while A high concentration of interleukin 8 is observed in combined infection of hydatidosis and hepatitis C virus compaer with healthy group , As the concentration of interleukin 8 in the combined infection of hydatidosis and hepatitis C virus (108.73) while in the healthy group (30.14).

Conclusion: The presence of a high level of interleukin-8 in mixed-infection patients is higher than in single-infection patients, which may lead to exacerbation and complications of hydatidosis disease.

Keyword: *interleukin-8, hydatidosis, hepatitis C virus, Hydatid cyst.*

Introduction

Hydatidosis or Hydatid cyst (HC), is a, zoonotic disease (helminthic), that results, from infection with, ,*Echinococcus granulosus* cyst stage, [1-2]. This disease is a chronic, and highly endemic in most countries of the world including Middle east [3]. HC is regarded, as one of the world's major zoonosis affecting both man and domestic animals, with cyst (larva) while, dogs affected by adult stage [4]. Globally many studies have been carried out, on different aspects, such as prevalence, epidemiology, immunology, diagnosis and treatment, [5-8]. In Iraq, also many studies have been conducted on various aspects of hydatidosis [9-11]. Hydatidosis in Iraq is, still a major economic ,and public health ,problem because there is no national, control program [9].The surveillance and, epidemiology are essential,

for knowledge of the transmission ,cycles and control, measures [12]. Hydatid cyst, develop in various organs,, the commonest ,site of infection, is the liver, the lung, ,followed by other organs such as kidneys,, spleen, heart, and the ,brain, may also be infected Infection may, even take place in, the bone marrow, cavities, but here the cysts are, generally sterile and differ in, their structures [13].

Hepatitis C virus (HCV) is a member Flaviviridae family, Hepacivirus genus, frequently related, to most hepatic disorders, involving chronic hepatitis that may approximately10–20% ,of cases develop into cirrhosis in and then in 1–5%to hepatocellular carcinoma (HCC) of cirrhotic [14]., HCV is the most common cause, of parentally ,transmitted hepatitis. The majority of, HCV infections are, transmitted by blood transfusion and other parenteral ways as sharing of needles, occupational exposure, to blood and, hemodialysis [15]. Prenatal transmission and sexual transmission are, relatively rare. However, the route of transmission is, mysterious in about 50%, of individuals with HCV infection [16]. ,The appearance of HCV,-specific T cells can, be identified according, to some studies of experimentally infected, chimpanzees, the following, picture of early events, in acute HCV infection ,emerges. After virus entry, hepatocytes ,are stimulated to ,produce IFN-α and ,IFN-β, which induce, Kupffer cells to produce ,macrophage inflammatory protein–1a, that recruits natural, killer (NK) cells, so, in turn, ,secrete IFN-ɤ this interferon then, up-regulates chemokine that, direct liver-infiltrating lymphocytes, into the hepatic parenchyma. These effector cells recognize, major histocompatibility complex class I–HCV peptide complexes on, the surface of infected hepatocytes and bring about their clearance by apoptosis [17].

If the cysts hit the liver, they will destroy and destroy its cells by pressing them and

lead to a decrease in the capacity of the liver and its failure to perform its tasks. Cyst burst, and this leads to the spread of small fries inside the cyst to the rest of the organs. If it bursts into the abdominal cavity, the larvae will settle on the peritoneum and each larva will form a new cyst. Blast of the cyst can lead to anaphylactic shock, which leads to fainting and severe hypotension, and can lead to death. A cyst may develop inflammation and turn into an abscess of the liver or lung.

Here comes the role of joint infection with other pathogens, such as the liver virus, especially in the same organs that are infected with the parasite, which may result in serious complications from these joint infections.

There is no research and study into the common infection between this parasite and the virus and the nature and complications of the disease. There are no findings and conclusions on this in the world; therefore, this study was an attempt to identify some aspects of this common infection.

Materials and Methods

Patients

In This case control study ,first group was comprised, of 40 patients with hydatidosis (16 males and 24 females), the second group, comprised of 40 patients with hydatidosis and hepatitis c virus (21 males and 19 females).

Clinical diagnosis: Hydatidosis cases were diagnosed physically and clinically including radiologic, ecographic, or tomographic images compatible Plain abdominal x-rays, ultrasonography were, done for all patients, CT-Scan were used as imaging techniques for, selected patients.

Laboratory diagnosis:

Serological techniques were done in order for viral infection, liver function and IL8 detection by using commercial kits.

Statistical analysis: Chi Square satisfice and t-test were used for testing relationships between categorical variables

Result and Discussion

1- Interleukin-8 concentration in patients with hydatidosis infection

A higher interleukin-8 concentration was observed in patients with hydatidosis compared to the healthy group, as the interleukin-8 concentration was (89.36) While the concentration of interleukin- 8 reached in the healthy group (30 .14) , [18] indicate that infection with

E. granulosus virus is associated with high levels of circulation of IL-12 and IL-8. Emphasize the permeability of the sac wall of the host's soluble immune system. The relationship between cyst fertility and cytokine infiltration indicates a strong interaction between parasites and the host.

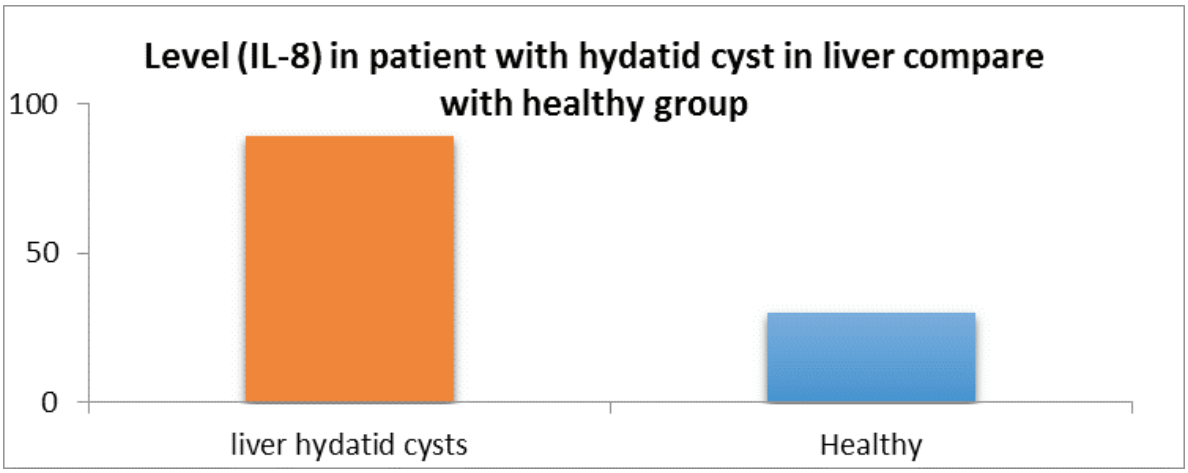

Figure (1) Interleukin-8 concentration in patients with hydatidosis infection compare with healthy group

2- **Interleukin-8 concentration of in patients with hydatidosis and hepatitis C virus mixed infection**

A high concentration of interleukin 8 is observed in combined infection of hydatidosis and hepatitis C virus compare with healthy group , As the concentration of interleukin 8 in the combined infection of hydatidosis and hepatitis C virus (108.73) while in the healthy group (30.14). In fact, studies and research that examine the relationship between this virus and the parasite are very few, as well as studying the complications that result from this common infection and the number of cases is not available. [19] Justified that Hepatitis C virus (HCV), a main cause of liver disease worldwide, is frequently resistant to the antiviral alpha interferon (IFN). Some studies have revealed that HCV NS5A protein induces expression of the pro-inflammatory interleukin-8 (IL-8).

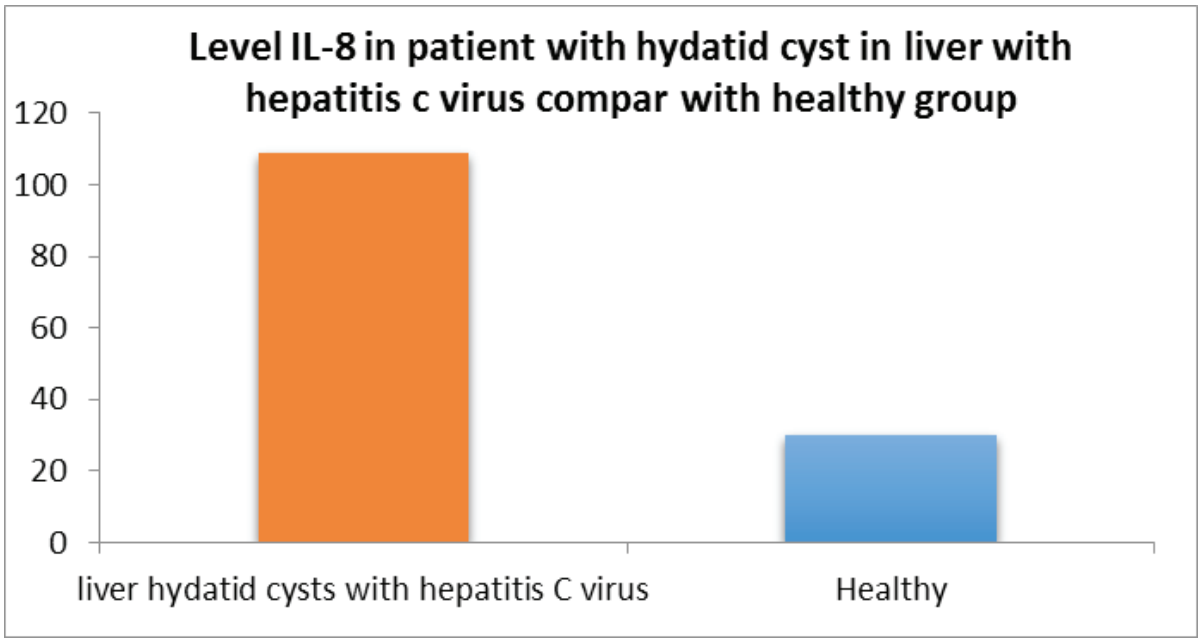

Figure (2) Level of concentration of interleukin 8 in patient with hepatic hydatid cyst with hepatitis c virus compare with healthy group

3- Interleukin 8 concentration in-patient with hepatic hydatid cyst

A high concentration of interleukin 8 is observed in mixed infection of hydatidosis and hepatitis C virus compare with infection of hydatidosis, As the concentration of interleuki 8 in the combined infection of hydatidosis and hepatitis C virus (108.73) while in the infection of hydatidosis only (89.36) . [19] Explained Interleukin-8 (IL8) is a pro-inflammatory cytokine included in the inflammation cellular response, Being a strong chemical attractant to neutrophils. Wide variety of cell types were produce IL-8, including endothelial cells, fibroblasts, monocytes and neutrophils. It serves as a chemical signal that attracts neutrophils at inflammation site, Thus it is also known as neutrophil chemotactic factor. Interleukin-8 (IL8) is a cytokine included in the cellular response to inflammation, being a powerful chemo-attractant for neutrophils. Our objective was to estimate interleukin 8 level in patient's serum with chronic liver disease due to hepatitis C virus infection.

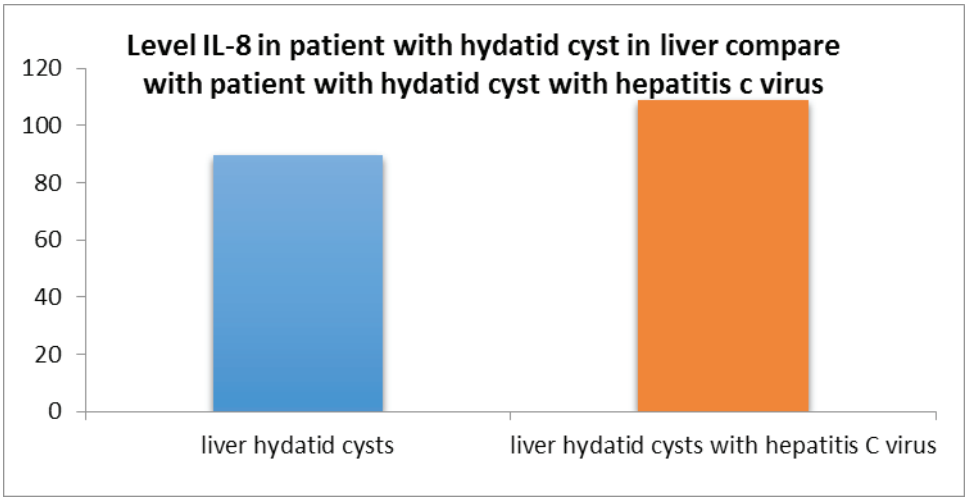

Figure (3) Interleukin 8 concentration of in patient with hepatic hydatid cyst compare with hepatitis c virus with hepatic hydatid cyst

4- The relation between of interleukin 8 concentration and size of hepatic hydatid cyst

It was observed that there was a direct correlation between size of hepatic hydatid cyst and the concentration of interleukin 8 in the patient with hepatic hydatid cyst , Where the coefficient of correlation (R=0.823)

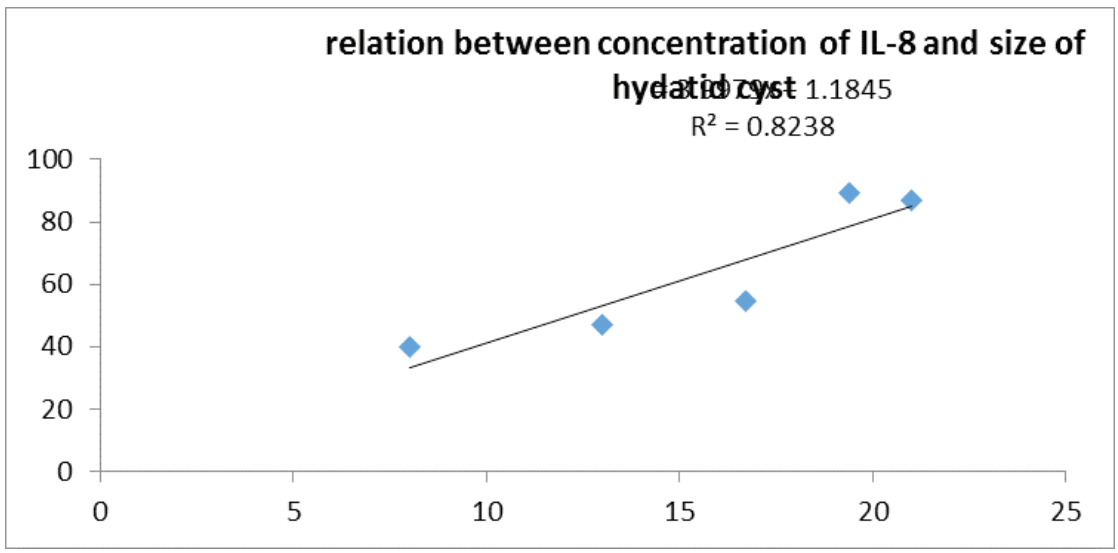

Figure (4) relation between concentration of interleukin 8 and size of hepatic hydatid cyst

5- Relation between interleukin 8 concentration and size of hepatic hydatid in mixed infection.

It was observed that there was a direct correlation between size of hepatic hydatid cyst and the concentration of interleukin 8 in the patient combined infection of hydatidosis and hepatitis C virus , Where the coefficient of correlation (R=0.7005)

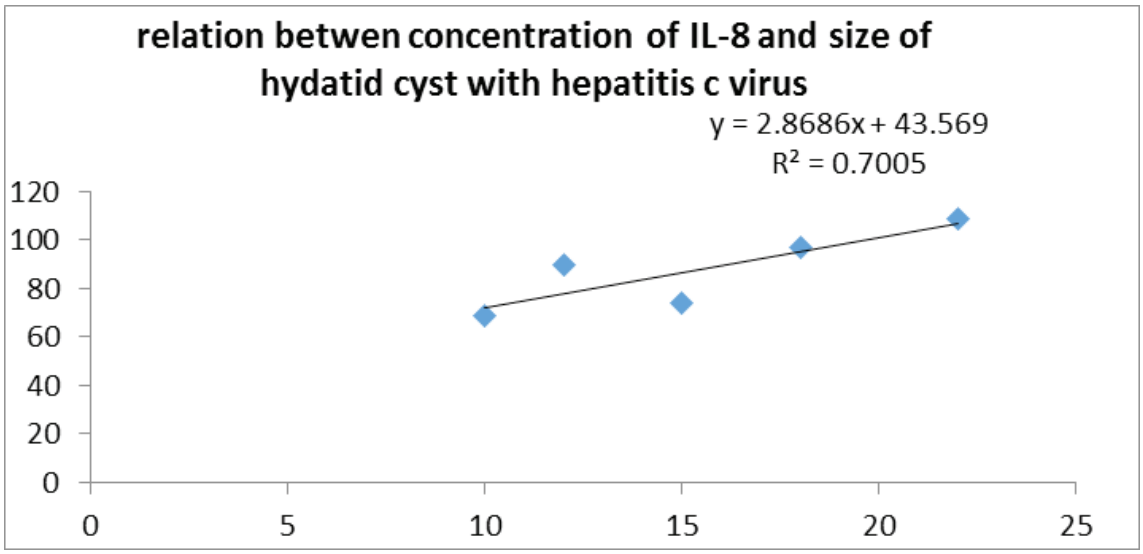

Figure (5) relation between concentration of interleukin 8 and size of hepatic hydatid cyst and size of hepatic hydatid with hepatitis c virus

Conclusions

The presence of a high level of interleukin-8 in patients with mixed infection more than patients with single infection, which may lead to the exacerbation of the disease with hydatidosis disease and its complications.Cyst burst, and this leads to the spread of small fries inside the cyst to the rest of the organs. If it bursts into the abdominal cavity, the larvae will settle on the peritoneum and each larva will form a new cyst. If it bursts into the abdominal cavity, the larvae will settle on the peritoneum, and each larva will form a new cyst. Mixed infection with HCV may give rise to the possibility that echinococcosis may play a role in hepatocellular carcinoma development. HCV in hydatid cyst pationts. This study further explores the possibility that echinococcosis could play a role in the development and metastases of HCC.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Cephalometric Assessment of Iraqi Sample from Basrah City for Orthodontic and Surgical Treatment Planning

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Abstract

Objectives: The aims of the present study were to establish the cephalometric norms for Iraqi sample from Basrah city and to study the sexual dimorphism.

Materials and Methods: Fifty dental students having normal occlusion were recruited and examined clinically then digital true lateral cephalometric X-rays were taken and analyzed using Smile stream software. Mean values and standard deviations were obtained and unpaired t-test was applied to assess the genders difference.

Results: Most of the skeleto-dental measurements lie within the normal range with almost proclined mandibular incisors and no significant gender differences.

Conclusions: Assessing the skeleto-dental relationships is far important to arrange accurate diagnosis and treatment planning for orthodontic and orthognathic cases.

Key words: *Orthodontics, orthognathic surgery, treatment planning.*

Introduction

The sagittal relation of maxilla to mandible is considered as the most important diagnostic criterion even prior to the introduction of Angle's classification of malocclusion at the beginning of previous century. To some degree, this relationship could be assessed by clinical examination, but more accurately it must be evaluated using lateral cephalometric radiograph [(1)].

Broadbent's [(2)] in 1931 introduced his cephalometer to the profession and made such films obtainable despite their primary use was for research and growth studies till the end 1940's. Between 1940 and 1993, a number of different analyses have been devised such as that of Downs [(3-5)], Steiner [(6-8)], Tweed [(9,10)], Ricketts [(11-13)], Wits [(14)], Jarabak and Fizzel [(15)], Burstone *et al.* [(16)], McNamara [(17)] and Arnett and Bergman [(18,19)].

Studies world-wide have remunerated attention to the ethnic factor and attempted to institute cephalometric values for different groups to help planning the right treatment for orthodontic and orthognathic cases [(20)].

In order to analyze dentofacial and craniofacial morphology accurately, a combination of various cephalometric parameters had been made and the majority of these analyses are relied on established norms derived statistically from population samples. Their prime exploit is to give resources for comparison an individual's dentofacial characteristics with a population average so as to recognize areas of considerable deviation, in addition to express the spatial relationship among different craniofacial structures' components [(21)].

In Iraq, many studies have been conducted to establish the cephalometric norms using different analyses and at different ages using sample from Baghdad and Mosul only [(22-30)], so this study aimed to establish the cephalometric norms for Iraqi sample from Basrah city and to verify the existence of sexual dimorphism.

Materials and Methods

Sample

The samples of this cross-sectional prospective cephalometric study included dental students at Basrah University collected between January and September 2020. The inclusion criteria for sample selection included the followings:

1. All were Iraqi Arab white from Basrah city.
2. The age ranged from 19-23 years old.
3. All have complete permanent dentition.
4. No history of previous orthodontic treatment, bad oral habits, facial trauma or craniofacial disorders.
5. All have Class I dental relationships (molars, canines, incisors) with normal overjet and overbite (2-4 mm).
6. Class I skeletal relationship verified clinically by the two fingers method.
7. No spacing/ crowding or it may minor of less than 3 mm.

Methods

After explaining the aims of study, about 200 students agreed to participate then examined clinically to ensure their fulfillment of the inclusion criteria, finally only 50 students (25 males and 25 females) were included. Digital lateral cephalometric radiographs were taken using PaX-i Insight™ machine (Vatech Co. Ltd., Gyeonggi-do, South Korea) with subjects' Frankfort plane parallel to the floor. These radiographs were analyzed after correcting the magnification using Smile stream software (USA). The following points, planes and measurements were determined [21,31]:

Cephalometric points

1. Point S (Sella): The midpoint of the hypophysial fossa.
2. Point N (Nasion): The most anterior point on the nasofrontal suture in the median plane.
3. Point Or (Orbitale): The lowest point on the inferior rim of the orbit.
4. Point Po (Porion): The most superiorly positioned point of the external auditory meatus.
5. Point ANS (Anterior Nasal Spine): It is the tip of the bony anterior nasal spine in the median plane.
6. Point PNS (Posterior Nasal Spine): This is a constructed radiological point, the intersection of a continuation of the anterior wall of the pterygopalatine fossa and the floor of the nose. It marks the dorsal limit of the maxilla.
7. Point A (Subspinale): The deepest midline point on the premaxilla between the Anterior Nasal Spine and Prosthion.
8. Point B (Supramentale): The deepest midline point on the mandible between Infradentale and Pogonion.
9. Point Is (Incisor superius): The tip of the crown of the most anterior maxillary central incisor.
10. Point Ap 1 (Apicale 1): Root apex of the most anterior maxillary central incisor.
11. Point Ii (Incisor inferius): The tip of the crown of the most anterior mandibular central incisor.
12. Point Ap 1 (Apicale 1): Root apex of the most anterior mandibular central incisor.
13. Point Pog (Pognoion): The most anterior point on the symphysis of the mandible.
14. Point Gn (Gnathion): The midpoint between Pogonion and Menton points of the bony chin.
15. Point Me (Menton): The lowest point on the symphyseal shadow of the mandible.
16. Point Go (Gonion): A point on the curvature of the angle of the mandible located by bisecting the angle formed by the lines tangent to the posterior ramus and inferior border of the mandible.
17. AO: Point of intersection of the perpendicular line drawn from point A on to the functional occlusal plane.
18. BO: Point of intersection of the perpendicular line drawn from point B on to the functional occlusal

plane.

19. Point Sn' (subnasale): It is the point where the lower border of the nose meets the outer contour of the upper lip.

20. Point Pog' (soft tissue pogonion): It is the most prominent point on the soft tissue contour of the chin.

21. Point Pn (pronasale): The most prominent point of the nose.

22. Point Li (labrale inferius): It is the median point in the lower margin of the lower membranous lip.

23. Point Ls (labrale superius): It is the median point in the upper margin of the upper membranous lip.

Cephalometric planes

1. Sella-Nasion (S-N) plane: It is the anteroposterior extent of anterior cranial base.

2. Frankfort plane: A line passing through the points Porion and Orbitale.

3. N-A line: Formed by a line joining Nasion and point A.

4. N-B line: Formed by a line joining Nasion and point B.

5. Palatal plane (PP): A plane joining between anterior nasal spine and posterior nasal spine.

6. Mandibular plane (MP): Formed by a line joining Gonion and Menton.

7. A-Pog line: A line from point A to Pogonion.

8. Nasion perpendicular (NP) line: A vertical line drawn from Nasion perpendicular to Frankfort horizontal.

9. A vertical line: A vertical line drawn through point A parallel to the Nasion perpendicular.

10. Long axis of the upper incisor (U1): A line connecting Is and Ap 1.

11. Long axis of the lower incisor (L1): A line connecting Ii and Ap 1.

12. Functional occlusal plane: A line drawn through the region of the overlapping cusps of the first premolars and first molars.

13. Y-axis plane: A line extending between Gn and S.

14. E-line: A line extending from the tip of the nose to the soft tissue pogonion.

Cephalometric measurements

1. SNA: The angle between lines S-N and N-A. It represents the angular anteroposterior position of the maxilla to the cranial base.

2. N Per A: The perpendicular distance between Nasion perpendicular and point A. An anterior position of point A is a positive, and a posterior position is a negative value.

3. SNB: The angle between lines S-N and N-B. It represents the angular anteroposterior position of the mandible to the cranial base.

4. ANB: The angle between lines N-A and N-B. It represents the difference between SNA and SNB angles or it may be measured directly as the angle ANB.

5. Wits appraisal: The distance between AO and BO

6. N Per Pog: The perpendicular distance between Pogonion and Nasion perpendicular. An anterior position of Pogonion is a positive value and posterior position is negative value.

7. Y-axis angle: It is the angle between S-N and S-Gn planes anteriorly. It determines the position of the mandible relative to the cranial base.

8. FMA: The angle formed between the mandibular and Frankfort planes.

9. PP-MP: This defines the angle of inclination of the mandible to the maxillary base.

10. LAFH: The vertical distance between ANS and Me.

11. U1-A vertical: The perpendicular distance between the facial surface of the upper incisor and the point A- vertical plane.

12. U1-Apog: The perpendicular distance between the facial surface of the upper incisor and the point A-Pogonion plane.
13. U1-SN: The angle between long axis of upper incisor and S-N plane, posteriorly.
14. L1-Apog: The perpendicular distance between the facial surface of the lower incisor and the point A-Pogonion plane.
15. L1-NB: The perpendicular distance between the crown tip of the most proclined lower incisor and the NB plane.
16. L1-MP: The angle formed by the long axis of the most labial mandibular incisor to the mandibular plane, posteriorly.
17. U1-L1: The angle formed by the intersection of the lines representing the long axes of the most labial maxillary and mandibular incisors, posteriorly
18. Nasolabial angle (NLA): The angle defined by the columellar point-to-subnasale line intersecting with the subnasale to- labrale superior line.
19. Ls-E: The shortest (perpendicular) distance between Ls and E line.
20. Li-E: The shortest (perpendicular) distance between Li and E line.

Statistical Analysis

Data were analyzed using SPSS version 25 computer program. Means, standard deviations (SD) and independent samples t-test were obtained. Intra-class correlation coefficient test was used to test the intra- and inter-examiner reliabilities. The probability value was set at 5%.

Results

After good training on the software, 10 radiographs were measured by well-trained operator and the results were compared with professional radiologist to test the inter-examiner reliability. On the other hand, the same 10 radiographs were re-measured by the same operator after 2 weeks to verify the intra-examiner reliability. In both situations, the intra-class correlation coefficient test revealed excellent reliability (0.92 for inter- and 0.95 for intra- examiner reliability).

Table 1 showed the descriptive statistics and gender difference of all variables measured. Regarding the position of maxilla relative to the cranial base (SNA angle), there was no significant gender difference with nearly similar mean values. The position of the maxilla relative to N perpendicular showed significant gender difference with higher mean value in males.

Like the maxillary base, the position of the mandible, the difference between the maxillary and mandibular bases represented by ANB angle, Wits appraisal and the position of the chin relative to nasion perpendicular line did not show significant gender difference.

Regarding the vertical jaw relationship, the angular measurements represented by FMA, PP-MP and Y axis angles did not show any statistical gender difference in contrary to the lower facial height measurement that showed significant difference being higher in males.

Assessment of the position and inclination of the maxillary and mandibular incisors were performed using different angular and linear measurements. In general, no significant gender differences were reported in all measurements except the position of maxillary incisors to the A-Pog line and the position of mandibular incisor to the N-B line.

Nasolabial angle and the position of the upper and lower lips relative to the esthetic line showed non-significant gender differences too.

Table 1: Means ± SD and gender difference of different measurements

Measurements		Descriptive statistics				Gender difference	
	Males (N=25)		Females (N=25)				
	Mean	S.D.	Mean	S.D.	t-test	p-value	
Sagittal jaw relations	**SNA(º)**	82.050	2.336	82.376	3.814	-0.365	0.716
	N Per A (mm)	3.063	1.354	1.830	2.550	2.136	0.038
	SNB (º)	81.201	3.600	79.388	3.840	1.722	0.091
	N Per Pog (mm)	-1.296	2.856	-0.264	3.136	-1.218	0.229
	ANB (º)	3.638	0.749	3.232	1.119	1.507	0.138
	Wits (mm)	0.586	1.794	0.670	1.952	-0.158	0.875
Vertical jaw relations	**Y-axis (º)**	59.119	3.073	59.936	3.288	-0.908	0.369
	FMA (º)	23.813	3.754	23.560	4.640	0.212	0.833
	PP-MP (º)	26.502	3.273	27.870	4.365	-1.253	0.216
	LAFH (mm)	76.416	6.530	69.600	6.459	3.711	0.001
Dental relations	**U1-A vertical (mm)**	5.463	1.625	4.744	1.308	1.725	0.091
	U1-Apog (mm)	6.875	1.163	5.856	1.708	2.466	0.017
	U1-SN (º)	102.743	2.952	102.464	3.689	0.295	0.769
	L1-Apog (mm)	3.644	1.579	3.251	1.332	0.951	0.346
	L1-NB (mm)	5.812	1.432	4.833	1.814	2.118	0.039
	L1-MP (º)	96.872	4.834	97.232	5.671	-0.241	0.810
	U1-L1(º)	126.130	4.149	127.239	4.748	-0.880	0.383
Soft tissue relations	**Nasolabial angle (º)**	108.501	5.148	109.292	4.441	-0.582	0.564
	Ls-E line (mm)	-3.528	2.112	-3.344	2.042	-0.312	0.756
	Li-E line (mm)	-1.770	2.517	-0.896	2.497	-1.232	0.224

Discussion

Determination the normal values of cephalometric measurements becomes mandatory for each ethnic group and gender to get a prefect result. In this study, a group of dental students were selected carefully with specific criteria to study the skeleto-dental and soft tissue measurements relevant to the orthodontic and orthognathic cases.

To exclude the effect of growth, all of the participants were aged above 18 years old with normal dental relation and acceptable facial profile. Measurements from different analyses were taken to determine the cephalometric norms of the selected sample.

The first component evaluated was the position of the maxillary base relative to the cranial base represented by SNA angle and the position of point A relative to nasion perpendicular line of McNamara [17]. Regarding SNA angle, the mean value was slightly higher in females with no significant difference; additionally it was near to that of Steiner [6-8], McNamara [17] and other Iraqi studies [25-27]. On the other hand, the position of maxilla in relation to N perpendicular line indicated that males apparently had significant prognathic maxilla. This may be related to the position of point Nasion as reported by Al-Sahaf [22] where the position of point N is more anterior in males so reducing their SNA angle and increasing the distance between point A and Nasion perpendicular line.

Regarding the position of the mandible relative to the cranial base and N perpendicular line, the findings of SNB angle indicated that males had statistically insignificant protrusive mandible on contrary to the position of point pogonion relative to the N perpendicular line where females had protrusive chin; this can be supported by the finding of Kadhom and Al-Janabi [28] who found a more anterior position of point pogonion in females in comparison with males. The two measurements are different as Pogonion is the most anterior point of the chin and it may be differed according to its prominence that may be affected by the facial type and the genetic factor. Moreover, the position of point Nasion may vary between genders.

The vertical jaw relationships were assessed by three angles and one linear measurement. Basically in the literature, the linear measurements in males were reported to be significantly higher than females; this is in accordance with the finding of the present study, while the angular measurements (FMA, PP-MP and Y axis angles) showed non-significant differences and were near to the normal mean values reported in the previous studies [9,29].

Regarding the inclinations and positions of maxillary and mandibular incisors, these teeth were related to many planes. Concerning maxillary incisors position in relation to A-vertical line, it was comparable to that of McNamara [17] and appeared to be more protrusive than Downs [3,4] and Ricketts [11-13] with significantly higher mean values in males in relation to A-Pog line. On the other hand, the inclinations of these teeth were the same as reported by Jarabak and Fizzel [15]. The difference belongs to the point of measurement like the facial surface of the central incisors or the incisal edge and the posterior position of Pognion in males.

The mandibular incisors were related to A-Pog and N-B lines and the findings indicated that the results were comparable to that of Steiner [6-8] and Ricketts [11-13], moreover the mandibular incisors appeared to be proclined in relation to the mandibular plane and this makes the inter-incisal angle slightly lower than reported by Steiner [6-8]. Previous Iraqi studies found the same findings i.e. proclined mandibular incisors [25,29].

The naso-labial angle and lips position in relation to esthetic line of Ricketts determined the soft tissue relationships in the present study. In both genders, naso-labial angle was lie within the normal range of Burstone [16] and slightly protrusive lips that reported by Ricketts [11-13] but within the normal range.

Normal cephalometric values for Basrah population were determined for the first time and appeared to be near to that of Baghdadis and Mosulian people. Further studies are needed to establish the normal values at different ages and Negroid population in Basrah city.

Conclusions

Assessing the skeleto-dental relationships is far important to arrange accurate diagnosis and treatment planning for orthodontic and orthognathic cases.

Ethical Clearance: Approval for this study was taken from the scientific and ethical committees in the

College of Dentistry, Basrah University with reference no. 5 in January 2020. Ps The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Pathological Evaluation of Canine Hepatic Cirrhosis and Necro-inflammatory Activity

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Abstract

Background: Hepatic fibrosis is a common pathological result of chronic liver diseases, which is a dynamic process of liver decomposition mediated by cellular mediators in response to injury.

Objective: The present study aims to evaluate the histopathological features of dog-liver fibrosis and / or cirrhosis.

Materials and Methods: 40 liver samples were collected; 12 liver fibrosis suspected animals were performed the histopathology to determine the main features of canine liver fibrosis.

Results: The histopathology revealed chronic hepatitis with liver islands separated by strips of fibroblasts and collagen, moderate numbers of macrophages, lymphocytes and plasma cells. Also in fibrosis between chronic hepatitis/cirrhosis had a wide range of severity and inflammation was observed primarily around fibrous tissue or was surrounded by fibrous tissue but extended to the liver parenchyma. The histochemical study revealed to a deposition of collagen fibers in the portal, subscapular and interlobular regions.

Conclusion: The histopathology revealed a range of lesions vary from mild hepatic degenerative changes to severe fibrotic features associated with canine hepatic fibrosis and early cirrhosis.

Key words: *Pathological, Hepatic, Fibrosis, Canine.*

Introduction

Cirrhosis/ liver fibrosis is a common pathological result of chronic liver disease, cirrhosis is a dynamic process of liver decomposition mediated by many cellular mediators in response to an inflammatory process [1]. The infected animals with liver chronic diseases can cause a harm affection in either health status and economic as a results to the inflammatory changes related to this disease [2].

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Hepatic star cells (HSCs) are a major component of cirrhosis, in the fibrous liver, HSCs quiet across different to reproductive, migratory and perxing myofibroblasts, show transcription properties and pro-fibrous secretions so-called cell activation and the secretion of ECM molecules that accumulate and form the deterrent tissues in space from the diss that leads to inflammation[3]. Liver diseases, especially chronic hepatitis and copper-related hepatitis, are common in dogs, with varying amounts of fibrosis, necrotizing, and inflammation among individual patients [4].

Fibrosis is an integral part of chronic liver disease, hepatitis caused by various detogenal sedation raises fibrous tissue deposition in parenchyma, which replaces normal functional liver cells, reshapes blood vessels in the system, waives liver functions[5]. Cirrhosis is characterized by the gradual accumulation of

extracellular matrix components (ECM) in the liver with persistent inflammation, the collagen profile of the liver changes, with increased relative amounts of first and third collagen types accompanied by modification and binding of ECM components [6]. Histologically cirrhosis is well estimated on the sections of the hematosis-eosin or with histochemical spots (titular mason or red sirius), which show collagen deposition with varying degrees of architectural distortion [7]. The current study concluded that the canine hepatic fibrosis was associated with a serious inflammatory events represented by multiple lesions vary in it severity in which the histopathology revealed a range of lesions vary from mild hepatic degenerative changes to severe fibrotic features associated with canine hepatic fibrosis and early cirrhosis.

Materials and Methods

The 12 dogs with clinical evidence of acute or chronic liver disease from 40 liver samples were collected, which were presented to liver biopsies to the faculty of veterinary medicine at the University of Basrah. The samples of dog fibrosis / cirrhosis were a medical procedure performed to obtain a small piece of liver tissue to diagnose liver fibrosis during the period from August 2019 to February 2020.

The livers were collected and thoroughly examined, after a clinical evidence of liver disease, the liver histopathology were detected from the diseased and control Dogs. All dog owners provided informed consent prior to enrolling in the study. Subsequently, the histological steps was conducted according to [8] in which the specimens were washed in water for removal of the blood then placed it in a plastic container, which contains formalin a concentration of 10%. These specimens were washed with tap water to remove excess fixation. The obtained specimens used for routine hematoxylin and eosin stains and Masson's trichrome staining, the stigma of these special stain used allows for the identification of more clinically important information than is available on the slicks of the hicnosis and eosin alone, and important for establishing a specific diagnosis. This method yields specimens for histological examination to study the microscopic changes in the tissue of the liver fibrosis.

Results

The histopathological results (H&E stain) revealed that the liver showed islands of liver cells separated by strips of fibrous and collagen cells, moderate numbers of macrophages, lymphocytes and plasma cells, besides, there was a dilation of center vein and filled with inflammatory cells infiltration mainly polymorphic inflammatory cells, in addition marked area of degenerative changes as form of vaculation of hepatocyte particular in the pericentral vein region (figure 1). The Fibrosis among the chronic hepatitis group/cirrhosis was a wide range of severity and inflammation was observed primarily around fibrous tissue or was surrounded by fibrous tissue but extended to the liver parenchyma (most inflammatory cells were lymphocytes, with slight infiltration by macrophages and justices) (figure 2).

The histochemical results showed a sever deposition of collagen fibers in the portal trait to demonstrate the portal fibrosis associated and liver cirrhosis which appeared surrounded the bile ducts, hepatic artery and portal vein (figure 3). Moreover, it showed a deposition of collagen fibers in the subscapular region and interlobular regions referred to periportal fibrosis (figure 4).

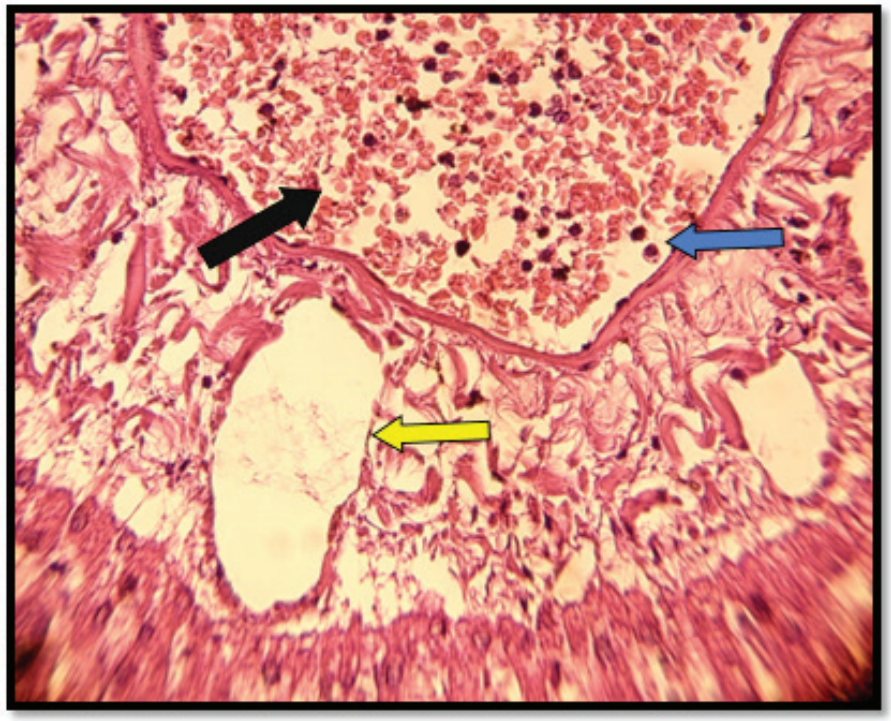

Figure (1) Histopathological section of liver fibrosed animals showed dilation of center vein (black arrow), filled in mononuclear inflammatory cells (blue arrow), in addition there are area of degenerative changes in the form of cystic dilation of hepatocyte particular in the pericentral vein region (yellow arrow) (H&E stain, 400X).

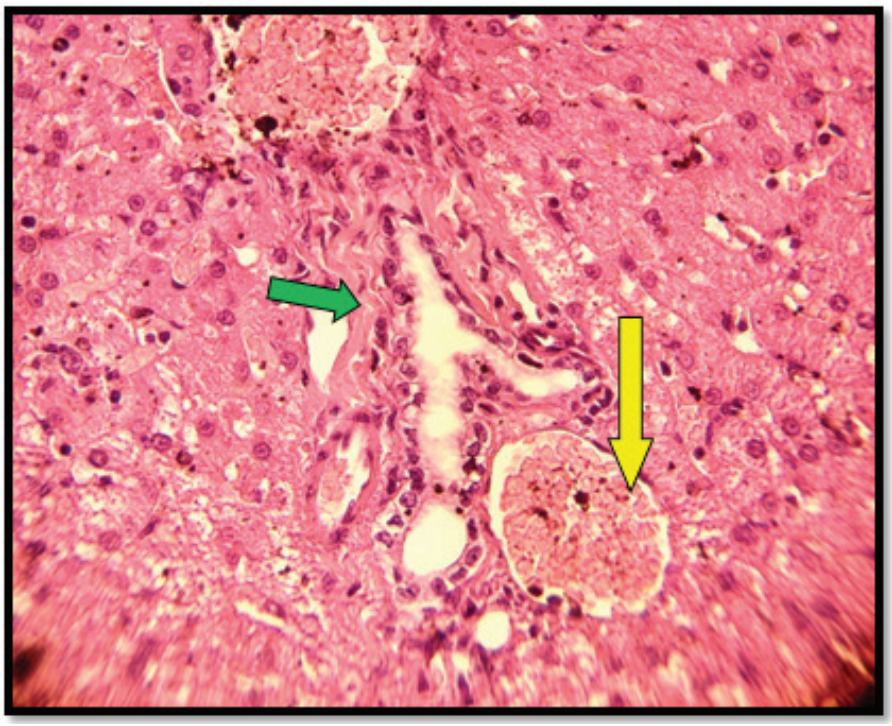

Figure (2) Histopathological section of liver fibrosed animals showed marked fibrosis present within portal area (green arrow) and the inflammation was observed primarily around the fibrous tissue, with portal vein congestion (yellow arrow). (H&E stain, 400X).

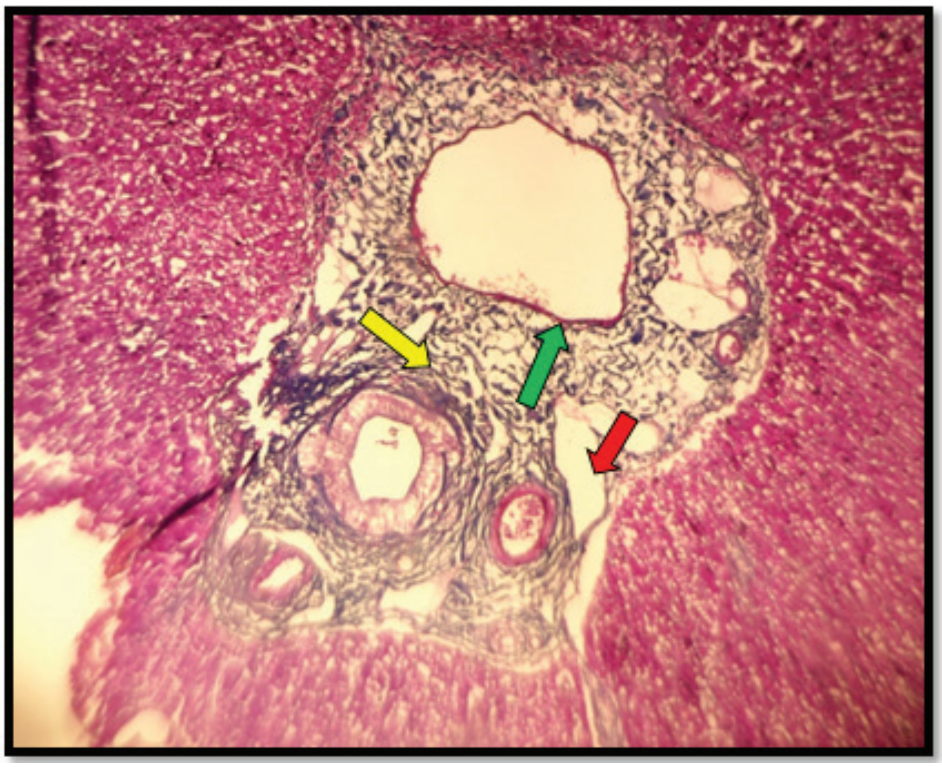

Figure (3): Histochemical section of liver of liver fibrosed animals show sever deposition of collagen fibers stained blue in the portal trait to demonstrate the portal fibrosis associated with liver cirrhosis appeared surrounded the bile ducts (yellow arrow), hepatic artery (red arrow) and portal vein (green arrow). (Masson's trichrome, 100X).

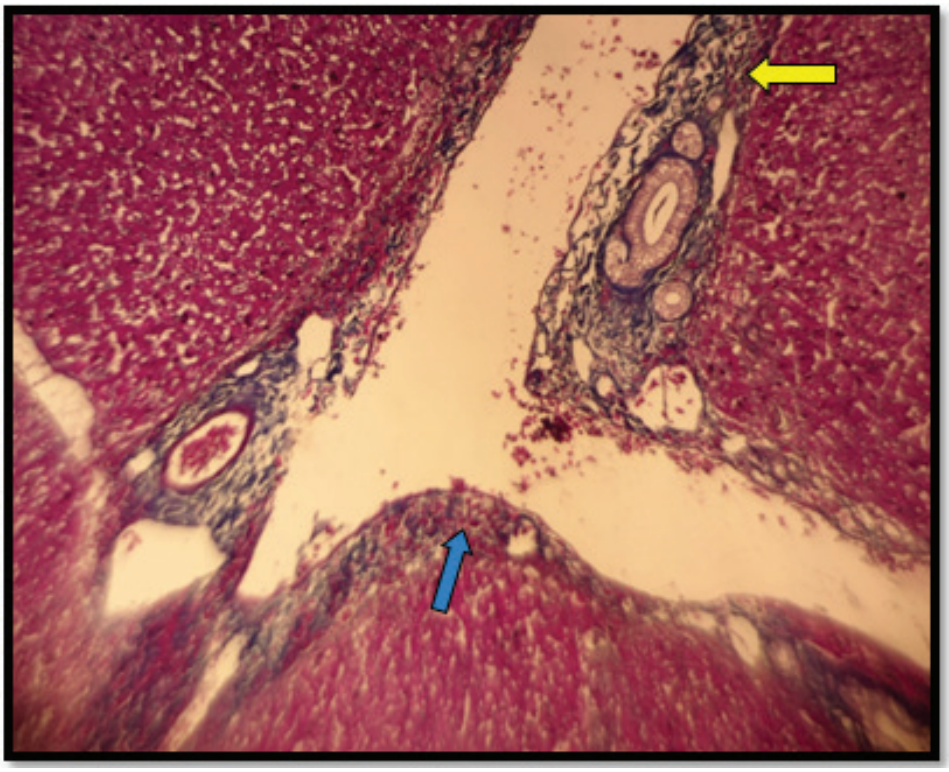

Figure (4): Histochemical section of liver of liver fibrosed animals show deposition of collagen fibers in the subscapular and interlobular regions (blue arrow) referring to periportal fibrosis (yellow arrow) (Masson's trichrome ,400X).

Discussion

Cirrhosis is the final stage of chronic hepatitis and is defined as a diffuse distribution characterized by cirrhosis of the liver and the conversion of the normal liver structure into structurally abnormal nodules, partial or total nodules, and is considered irreversible [9].

The microscopical results showed vary degrees of severity extend from inflammatory cells infiltration to the fibrous tissue lesions in the liver fibrosis animals, therefore, the histopathological result revealed dilation of center vein, also the central vein filled in inflammatory cells infiltration mainly mononuclear inflammatory cells, marked area of degenerative changes as form of vacuolation of hepatocyte particular in the pericentral vein region.

In other sections, there was Fibrosis between the chronic hepatitis group/cirrhosis was a wide range of severity and inflammation was observed primarily around fibrous tissues or was surrounded by fibroblast tissue but extended to the liver parenchyma (most of the inflammatory cells were lymphocytes, with a slight infiltration of macrophages and niatrovia), and in another section the livers deteriorated or necrotic, and the limit plates were damaged with some, Similar results were also recorded by [10] , which these findings in consistence with , who mentioned that liver fibrosed animals extent of hepatic fibrosis, inflammatory cells, cellular debris and fibrosis connective tissue.

Besides, there is fibrous connective tissue with the formation of two sublobules, fibrous connective tissue prominent in gate areas and thin bands tend to encircle small groups of liver cells. In addition, it noticed degradation with balloons and the presence of mild lymphatic inflammatory infiltration, associated with moderate disease, as well as the proliferation of kupffer cells.

The histochemical studies were good diagnostic tools for evaluation of the many hidden aspects of inflammation and repair [11] ; therefore, the current study used several special stains to estimate the role of the tissue against the invaders and the pathological changes.

The histochemical study of the liver fibrosed animals revealed to deposition of collagen fibers in the portal region of the liver, which stained blue to demonstrate the collagen deposition referring to moderate fibrosis in masson's trichrome, stain; also nuclei stained black and cytoplasm of hepatocytes and erythrocytes stained red. On other hands, the other histochemical section of the liver of liver fibrosed animals show severs deposition of collagen fibers stained blue in the portal trait to demonstrate the portal fibrosis associated with a liver abscess in masson's trichrome stain which appeared particularly in the surrounded area of bile ducts, hepatic artery, and portal vein. Also, the other histochemical section of the liver of the liver fibrosed animals showed pericentral vein deposition of collagen fibers stained blue to demonstrate the pericentral vein fibrosis in masson's trichrome stain.

Moreover, the histochemical section of the liver of the liver fibrosed animals which displayed the severe elastic collagen fibers deposition that stained blue around of portal vein revealed to perivascular fibrosis. Nearly similar results clarified by [12] who reported that the collagen fibers which stained blue moreover, the cytoplasm of hepatocytes are stained red with this histochemical stain. The above results were consistent with our microscopical results, which showed severe area of fibrosis particularly in the pericentral vein region as well to the portal area of the affected liver.

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Ethical Clearance: The ethical committee, college of veterinary medicine, university of Basrah, Iraq, approved the study.

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Genotoxicity and Clastogenicity of Bisphenol A

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Abstract

Objectives: This review represents a critical and constructive analysis of literature in the content of genotoxicity and clastogenicity of Bisphenol A. The review is generated through summary, classification, analysis and comparison of already existing material and researches on field.

Methods: Databases as Scopus, PubMed, Medline and Web of Science were used to extract data for the review. Search terms like "Bisphenol A", "endocrine disruptors","clastogenicity" and "genotoxicity" were inquired. Out of 350 research articles screened, 60 most relevant studies are included in this review.

Conclusion : This review highlights the endocrine disrupting potential of Bisphenol A, thus leading to genotoxic and clastogenic events, especially impacting fragile categories like pregnant women, infants and children. BPA induces oxidative stress, inflammatory response, DNA strand break, chromosome aberrations and epigenetic changes, affecting neuroendocrine and reproductive system, metabolism, immunity, liver function, and increases the incidence of thyroid, liver, breast, uterine and ovary cancer.

We can conclude that human exposure to BPA, as one of the leading environmental contaminants, represents a major global issue. BPA by its genotoxic and clastogenic potential can be debilitating for human health. Further research on field considering BPA distribution, varying exposure rates, racial disparities and inter-species differences, should be conducted. Avoiding exposure to BPA and finding safer alternatives to refine, reduce or replace BPA in market should remain a priority.

Keywords: *BPA; endocrine disruptors; clastogenicity; genotoxicity;*

Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl) propane (CAS No. 80-05-7), is a synthetic chemical excessively produced worldwide.[1] BPA commercial production began in the United States in 1957, followed by Europe one year later. Its global production grows consistently, varying between 0% and 5% annually, with the strongest growth occurring in China.[2] A yearly increase of 4.6% in production of BPA is envisioned to happen from 2013 to 2019.[3]

Bisphenol A is synthetized by the condensation of phenol with acetone in the presence of a catalyst, a strongly acidic ion-exchange resin. BPA consists of a central tetrahedral carbon atom with two methyl and two phenol groups. BPA is relatively water-soluble (120-300 mg/L) [4], dissociates in alkaline environment, has a moderate bioaccumulation rate, low vapor pressure, high melting point, and low half-life in air (0.2 days).[5,6]

Bisphenol A is used to manufacture polycarbonate plastics and epoxy resins.[7]Out of the total production of BPA, 65% is dedicated to polycarbonate synthesis, 25% for epoxy resins and the rest 10% for our daily use products such as food storage containers, food antioxidants, metal food cans, baby bottles, thermal papers, dental seals, medical devices, personal care products, safety helmets, sunglasses, lenses, cosmetics, infant incubators, CD/DVDs, hair dryers, fridges, computers and smartphones.[8]

The United States Environmental Protection Agency (EPA) reported that more than 400,000 kilograms of

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BPA leach into environment every year.[9] BPA leaches into environment and can impact human health during its production, processing or waste disposal.[10] BPA leaches also when polycarbonate and epoxy resin-containing products are exposed to heat, are re-used or their pH changes.[11] BPA products in contact with heat, acidic or basic conditions, accelerate the hydrolysis of the ester bonds between BPA molecules, thus exposing humans to its harmful metabolites. This happens when people heat cans to sterilize food, place acidic or basic food in cans or polycarbonate plastic, and keep heating or washing these products regularly.[12,13] Even though ingestion of contaminated food is the most common way how human get exposed to BPA, inhalation and skin absorption are considered to be of great importance too.[14,15] In addition, moderate levels of active unconjugated BPA have been detected in human serum, adipose tissue, breast milk, placenta, maternal and fetal plasma, indicating that BPA can accumulate in human body.[16,17,18]

Bisphenol A mechanisms of action and its endocrine disrupting potential

BPA is qualified as a xeno-estrogen that disturbs synthesis, transport, activity and metabolism of endogenous estrogens, consequently affecting the development, growth and reproduction of organisms.[19,20]

BPA can mimic or antagonize endogenous hormones, subsequently perturbing endocrine function, by binding weakly to several steroid receptors including the estrogen receptors (ER α and β) and thyroid hormone receptor.[21,22,23] As well, BPA strongly binds to transmembrane endoplasmic reticulum, G protein-coupled receptor 30 (GPR30) and estrogen-related receptor gamma (ERRγ).[24,25] BPA can activate peroxisome X receptor (PXR) and the aryl hydrocarbon receptor (AhR), often involved in cross-talk with steroid receptors.[26,27] Many of these receptors play an important role in gene regulation, suggesting that BPA may influence normal differentiation and maturation processes especially during embryonic and fetal development.

The United States Environmental Protection Agency established a reference dose (RfD) for humans of 50 µg BPA/kg body weight (BW) day^{-1}, based on a thousand□ fold reduction of the lowest observed adverse effect level (LOAEL) of 50 mg kg^{-1}BW day^{-1}.[28,29] Studies indicate that daily human intake of BPA is less than 1 µg kg^{-1} BW day^{-1}, rendering the reference dose to be considered safe to humans.[30]

However, studies have shown that administration of low-dose BPA as 0.2 µg kg^{-1} BW day^{-1} can reduce fertility and sperm production in male animals,[31,32] or at doses of 0.23–23 ng kg^{-1} BPA the calcium ion signaling in pancreatic cells can be suppressed leading to diabetes mellitus.[33,34,35]

Genotoxic and clastogenic potential of Bisphenol A

Several studies have revealed that the main course of DNA damage caused by BPA is in single strand-breaks (SSBs), especially recognized in peripheral blood mononuclear cells (PBMCs). BPA also induces DNA damage in human epithelial type 2 cells (HEp-2) and human lung fibroblasts (MRC-5), that can be evaluated by comet assay and micronuclei induction. Though, BPA in submicromolar levels can prompt DNA synthesis and elevate cell cycle protein expression in human hepatocyte line.[36]

In addition, BPA can impair chromosome synapses and disrupt meiotic double strand break repair (DSBR) through DNA dependent protein kinase (DNA-PK) activity. BPA can disturb normal spindle assembly, chromosome alignment and kinetochore microtubule attachment, affecting first polar body extrusion and can cause DNA damage through inhibiting telomerase activity in a non-monotonic manner mediated by activation of ER/GPR 30-ERK transduction pathway. BPA can also induce cell apoptosis through mitochondrial damage, energy depletion and increased levels of oxidative stress.[36]

In recent studies, even submicromolar doses of BPA are thought to elicit aneugenic effects by interfering with microtubule assembly, spindle apparatus function, chromosome segregation during mitosis [37,38], and disturbing DNA damage signaling pathways [39], thus affecting DNA stability and potentially leading to carcinogenesis.

Epigenetic modifications such as DNA methylation, histone modification and non-coding RNA regulation were reported to influence BPA genotoxicity potential as well. BPA can induce DNA hypermethylation through up-regulation of Dnmt1, decreasing MMPs and WNT2/b-catenin, glutamate transporter Slc1a1 or downregulation of TET enzymes, thus resulting on preeclampsia, changes on placenta formation or impaired neurobehavioral development.

In addition, BPA can lower histone H3 and H3K14 acetylation levels, thus inhibiting testosterone synthesis and testicular development. Or BPA decreases histone3 lysine 4 trimethylation abundance in Gnhr promoter, thus altering neuroendocrine control.

Furthermore, BPA can increase the oncogenic miR-19a and miR-19b expressions and modifies expression of phosphatase, PTEN, p-AKT, p-MDM2, p53 and PCNA.[36]

A comparison of various studies assessing genotoxic and clastogenic potential of BPA

Numerous studies investigating BPA's genotoxic and clastogenic potential, present controversial results.

A genotoxic study revealed that BPA induces an increase of DNA damage in the Hep-2 cell line and an oxidative damage in the MRC-5 cell line, while cytotoxicity analysis showed that BPA did not affect cellular viability.[40] In contrast, another study potentiates BPA cytotoxic effect on RAW264.7 macrophages, by observing parallel trends on cytotoxicity, apoptosis, genotoxicity, p53 phosphorylation, BCL2 family expression exchange, caspase-dependent/independent apoptotic pathways and ROS generation.[41] Further, assessing BPA's activity in CHO cells revealed significant increases in cytotoxicity and DNA damage, significant increases in micronucleus frequency and conventional chromosome aberrations as breaks, gaps and fragments.[42]

Micronucleus, chromosome aberration and single cell gel electrophoresis (SCGE) assays revealed that BPA exposure causes significant increase in the frequency of micronucleus (MN) in polychromatic erythrocytes (PCEs), structural chromosome aberrations in bone marrow cells and DNA damage in blood lymphocytes and as well decreased glutathione activity in liver of rats.[43]

Moreover, increased reactive oxygen species (ROS), lipid peroxidation (LPO), depletion of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione-s-transferase (GST) antioxidant activities were observed in Drosophila melanogaster BPA treated group in comparison to the control. Suggesting that BPA exposure induces oxidative stress, which could be the possible mechanism for induction of genotoxicity.[44] In addition, assessing the potential adverse effects of BPA on dam rats and their first generation females in a comparative toxicological study revealed that long term exposure to BPA depicted total genomic damage, significant alterations in liver enzymes, lipid profile, antioxidant enzymes and reproductive hormones with up-regulation in the expression of ERβ.[45]

Studies assessing the effect of BPA, bisphenol S, bisphenol F and bisphenol AF on DNA bases oxidation, found an oxidative damage to DNA pyrimidines and even more strongly to purines in human peripheral blood mononuclear cells.[46] In contrast, in another study none of the eight BPA structural analogues (BPF, BPAF, BPZ, BPS, DMBPA, DMBPS, BP-1, and BP-2) showed a mutagenic effect in Salmonella typhimurium, whereas potential genotoxicity was determined in the human hepatoma cell line (HepG2) at non-cytotoxic concentrations after 24-hour exposure.[47]

BPA's ability to alter multiple molecular pathways in cells, namely G protein-coupled receptor (GPER), estrogen-related receptor gamma (ERRγ), HOXB9 (homeobox-containing gene), bone morphogenetic protein 2 (BMP2) and 4 (BMP4), immune-regulatory cytokine disturbance in the mammary gland, EGFR-STAT3 pathway, FOXA1, enhance zeste homolog 2 (EZH2) and epigenetic changes [48], may increase the incidence of multiple cancers like breast [49,50,51], ovarian [52,53] and uterine cancer [54].

Furthermore, a study assessing the sensitivity threshold of DNA adduct detection by 32P-postlabelling in both liver and mammary cells of female CD-1 mice receiving BPA, resulted in a dose-dependent formation of multiple DNA adducts in liver (3.4-fold higher levels than in controls) and in target mammary cells (4.7-fold higher than in controls). Although DNA adducts do not necessarily evolve into tumors, the formation of these molecular lesions in target mammary and liver cells may suggest for BPA's potential involvement in breast and liver carcinogenesis [55].

Additionally, a ten year study comparing BPA levels in amniotic fluid between pregnant mothers with normal and abnormal karyotype fetuses highlighted that mothers with abnormal karyotype fetuses had higher levels of BPA concentrations in amniotic fluid, in comparison to pregnant women with normal karyotype fetuses. Therefore, these findings highlight the distorting potential BPA has in DNA stability and carcinogenesis induction [56].

Nonetheless, another study suggests that BPA can be included in the group of substances with dual effects, involving genotoxic and DNA-protective activity. While rising concentrations of BPA increase the risk of DNA double-strand breaks and modified purines in human lymphocytes through ROS, in contrast BPA can manifest a protective ability toward plasmid DNA from the damage of iron ions in cell-free system [57].

Conclusions and Perspectives

As stated previously, Bisphenol A can induce oxidative stress, inflammatory response, DNA strand break, oxidative DNA damage, chromosome mutations and epigenetic changes, thus affecting neuroendocrine and reproductive system, distorting metabolism, immunity, liver function, and potentially increasing the incidence of thyroid, liver, breast, uterine and ovary cancer.

To date, studies investigating BPA's genotoxicity and clastogenicity potential had different population sample size, sample sources, sample storage and detection, which resulted in inconsistent conclusions in population-based studies. Thus, to further understand BPA toxicity we should consider studying exosomes, ncRNA (circRNA and long non coding RNA), gene chips and monitor BPA metabolites.

Additionally, more complex, systematic and diverse studies analyzing BPA's impact on endoderm derived organ's transcriptomes, by using in-vitro toxicogenomics, would serve as an ideal tool for identification of new BPA toxicity mechanisms and would help us on having a clearer picture of its long-term adverse health outcomes [58].

Replacing Bisphenol A with its analogues and derivatives, proved to be not a good choice, as some of these compounds revealed even higher levels of toxicity in comparison to BPA [59,60].

Therefore, exposure to endocrine disrupting chemicals as BPA must be carefully regulated by authorities, especially for vulnerable population categories, in order to avoid BPA's potential at impairment of growth and neurological development, disturbance of metabolism or increased risk of cancer [61]. Standardized procedures and statistical tests should be used as the base for developing a protocol to assess human exposure rates to EDCs, to build strategies and health policies which would help on reducing human exposure to EDCs, thus mitigating and avoiding EDCs debilitating life-time consequences [62].

To conclude, implementing prevention and control measurements is highly important on reducing long life term genotoxic potential of Bisphenol A. These measurements must include: elimination and substitution of BPA, meeting legal requirements and policies regarding BPA's management, offering engineering control of local BPA exhaust through regular ventilation of working spaces, implementing safe working procedures by using personal protective equipment, maintaining high personal hygiene and potentially eliminating or reducing time to BPA exposure through alternating work tasks. The last, but the most important is to train employers on how to deal with emergency cases of excessive BPA exposure and continuously raise their awareness in BPA's genotoxic potential that can accur only after long time of exposure [63].

Ethical Clearance- Taken from PhD studies committee

Source of Funding- Self funding

Conflict of Interest - Nil

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The Association between Teeth Loss and Oral Health Problems

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Abstract

Background: The teeth play various functional roles, from the most basic functions to more subtle functions. One of the most tragic events that can happen to the teeth are loss it, as a result trauma or dental diseases. Tooth loss has esthetic, functional, positional and psychological impacts on the life of individuals.

Objectives: To investigate the association between tooth loss and oral health problems among partially and completely edentulous patients. Method: (Questionnaire and Clinical Examination). Self-administered questionnaire was distributed to the patients to collect information relating to demography and cause of tooth loss, in addition to mastication and speech state after tooth loss. Four clinical conditions including supra-eruption, drifting teeth, temporomandibular disorders and bone resorption were used to recognize the effects of missing teeth. Patients seeking dental treatment at General Hospital, Morzuk, Libya were recruited for the study. Criteria included age of 16 years and above with one or more missing teeth except for third molars. Results: altogether 58 participants, 31 (53.4 %) were males while 27 (46.6%) were females. The participants were aged 16–>45 years, among them, 44 patients (75.9%) are partially edentulous, and 14 patients (24.1%) were completely edentulous. The patients with complete teeth loss and the patients with missing both the anterior and posterior teeth are most groups suffer problems among patients who involved in the study. patients who loss posterior teeth are the lowest group had, suffer of health problems due to loss of teeth among all of them.

The general relationship between tooth loss and the oral health problems was positive and strong. Conclusions: there are statistically significant relationship between tooth loss and oral health problems, where the strong value of this relationship (0.614) and significance within less than (0.05).

Key words: *Tooth loss, consequences of tooth loss, causes of tooth loss.*

Introduction

The teeth play various functional and aesthetic roles as essential daily activities from the most basic functions like eating and speaking to subtler functions related to good appearance[1].

Teeth are anchored in the jaws by the periodontal ligament. This ligament connects the cervix (neck) of the tooth, at the junction between the crown and root, to the gingiva. Below that, the ligament connects the outer layer of the tooth root (cementum), to the adjacent bone (jawbone). The jawbone of the maxilla and mandible that support the teeth is the alveolar bone[2]. One of the more dramatic discoveries in the medical sciences in the twentieth century has been the realization that tooth loss is not an inevitable consequence of aging, but the result of trauma or dental diseases[3].

Tooth loss continues to be a major problem in clinical dentistry and has received significant attention in everyday dental practice[4].

In most developing countries, the main reason for people to seek for dental care is pain that has become intolerable after a long period of "wait-and-see"[5-6]. Because of the delay in seeking treatment, the patients present with destroyed teeth

that is impossible to treat by the conventional restorative procedures. Likewise, insufficient finances limit restorative and rehabilitative dental treatment as

well as shortage of professionals and dental materials. Therefore, we can assume that, many patients have a large number of missing teeth[7]. The absence of one or more natural teeth often results in disability. This can trigger several positional and functional changes such as impairment in speech, mastication ability and integrity of dental arch.

Studies have shown that, tooth loss can have a substantial effect on the emotions as well as oral health and function[8-9].

In the past, many patients felt that tooth loss was inevitable and were, to a certain extent, prepared for that eventuality.

Today, patients' expectations have changed and many see tooth loss as a negative event. Patients may suffer real or perceived detrimental effects following the loss of one or more teeth[10].

Problem of Research

Recent studies have confirmed the findings of many researchers that, both functional and psychological compromises as well as positional changes are following tooth loss if unrestored[11].

These positional changes may be of sufficient magnitude to complicate restorative treatment. Therefore, the desire of patients to have an excellent prosthesis for their missing teeth may pose dental technician with many challenges to achieve ideal function and best esthetics.

Many studies are available exhibiting the causes of tooth loss, but comparatively there are fewer studies documenting the effects or consequences of tooth loss, literature is scarce about the consequences of tooth loss in our country.

There is however, paucity of information on whether patients or people with missing teeth are aware of the side effects of tooth loss on them or on the remaining teeth.

This study will help :

· To understand the causes and consequences of tooth loss among

our patients.

· Demonstration about the

relationship between tooth loss and oral health problems.

· Encourage our patients for replacing their lost teeth.

Objectives

· To recognize the causes and effects of tooth loss.

· To investigate the association between loss teeth and oral health problem.

· To determine functional and positional changes that following tooth loss.

· To prioritize restoration and replacement of missing teeth to meet functional

demands, and ensure quality dental services for all citizens

(Questionnaire and Clinical Examination)

Self-administered questionnaire was distributed to the patients to collect information relating to demography and cause of tooth loss, in addition to mastication and speech state after tooth loss.

Four clinical conditions including supra-eruption, drifting teeth, temporomandibular disorders and bone resorption were used to recognize the effects of missing teeth.

Patients seeking dental treatment at General Hospital, Morzuk, Libya were recruited for the study.

Criteria included age of 16 years and above with one or more missing teeth except for third molar.

A. Study Design.

A short-term study design

B.study Area.

This study conducted in the prosthetic clinic at the General Hospital of Morzuk, Libya. The location of this hospital is in the city center beside the modern mosque along the main road.

B. STUDY SAMPLE.

The study was conducted among the partially and completely edentulous patients who attended dental clinic at the hospital for refers or received various oral health services.

C. Inclusion Criteria.

· The people who have missing teeth, either partially or completely edentulous.

· Age is 16 years old and above.

D. Exclusion Criteria

· Any subject who have loss of third molars alone (considered as complete dentition).

· The patients who have teeth extraction for orthodontic purposes (treatment objectives).

E. Ethical Considerations

Ethical clearance for this study was obtained from Dental Prosthetic Department of the Medical Technology College in Sabha University.

The study also was approved by the administration protocol of General Hospital of Morzuk, Libya. In addition, every 23 participant was informed of his or her right to refuse participation or to withdraw from the study at any moment.

TOOLS AND TECHNIQUE OF COLLECTION OF DATA.

1. QUESTIONNAIRE (OPEN-CLOSE):

- Self-administered open-close questionnaire used to collect information from the patients. The questionnaire contained 13 questions, one was dichotomous, and one question had space for the participant to write the answer.

The questions in the beginning enquired about the personal information

such as age, gender, economic and education status. Thereafter, the subjects questioned about causes and duration of tooth loss, in addition to some effects that happened after tooth loss.

The subjects were of four age groups ascending from 16 to more than 45 years of age (16-25years, 26-35 years, 36-45 years, and >45 years).

2. Clinical Examination:

- Clinical examination conducted by using examination tools: dental chair, mouth mirror, tweezers and periodontal probe.

The examination included clinical variables such as type of edentulousness (partial or complete), position and type of missing teeth. In addition to the consequences of tooth loss on patients' wellbeing, such as temporo-mandibular disorder, drifting of remaining teeth, over-eruption of unopposed teeth and bone loss (resorption).

To determine the position and type of missing teeth, we used graphical representation to represent the upper and lower teeth. In addition, we have also used it to determine whether the teeth have drifting (inclining) or overeruption.

Results

Altogether 58 participants, 31 (53.4 %) were males while 27 (46.6%) were females. The participants were aged 16 – >45 years, among them, 44 patients (75.9%) are partially edentulous, and 14 patients (24.1%) were completely edentulous. Among the study group, the patients with complete tooth loss, patients with missing both anterior and posterior teeth were suffering with more problems. The least suffering was those who lost posterior teeth.

Many oral health problems had shown high average occurrence on T-Test and strong relationship on Pearson correlation. The general relationship between tooth loss and the oral health problems was positive and strong.

Conclusions

There is a statistically significant relationship between tooth loss and oral health

problems, where the strong value of this relationship was (0.614), and the level of significance was less than (0.05).

F. Study Design.

A short-term study design

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Data Analysis

All Data was analyzed by using Statistical package for Social Science SPSS (Version 18)

. Analysis included one sample T-Test and Pearson Correlation to find the correlation between the tooth loss and oral health problems. In the analysis, the tooth loss category was used as independent variable; it is divided into four groups:

· Missing anterior teeth only.

· Missing posterior teeth only.

· Missing both anterior and posterior teeth.

· Complete teeth loss.

The dependent variable was that problems who followed the tooth loss: positional changes (overeruption and drifting teeth), functional changes (low mastication, poor aesthetic and speech distortion), bone loss (resorption) and emotional effects of tooth loss..34 significance (0.000). Similarly, increase in tooth loss led to increase in low mastication or low chewing ability.

As for the relationship between tooth loss and speech distortion was positive with medium-strength, the level of significance reached (0.002).

With increase of teeth loss, the distortion of speech also increased.

The relationship between tooth loss and aesthetic problems (poor appearance) was positive with slight-strength correlation (1.261), the level of significance less than (0.05).

With increase of teeth loss, the aesthetic problems

also increases. The problem of drifting teeth showed a negative low correlation (reverse relationship) the level of significance was (0.037).

Increase in tooth loss led to a decrease in drifted teeth.

As for the problems of the negative emotional effects and bone loss (resorption) are showed positive correlation but low strength (weak), but indicative of the level of significance higher than (0.05), that correlation is not significance in both problems. While, the overeruption of unopposed teeth is showed a negative correlation (reverse relationship), but the significant is higher than (0.05). Therefore, the correlation is not significant.

The general relationship between tooth loss and the oral health problems (all previous problems), the correlation value was (0.614), and the level of significance was (0.000), which is less than (0.05). It is a positive and strong correlation, and with increasing teeth loss, there is also an increase in oral health problems.

Result

Fifty-eight patients consisting of 31 male (53.4 %) and 27 (46.6%) female participated in the study. The ages ranged from 16 to more than 45 years. The majority of participants were within the age group 26-35 years (29.3%). About half of the respondents (48.3%) had academic education or more.

About 18 (31%) of participants were their answers about first experience with tooth loss are (5-10 years ago).

Among the participants, 44 patients (75.9%) are partially edentulous, and 14 patients (24.1%) were completely edentulous. Of those with partially missing teeth some of them had, missing teeth in anterior only 16 (27.6%), and some had missing teeth only in the posterior region 14 (24.1%), while others had missing teeth in both anterior and posterior regions 14 (24.1%).

Causes of tooth loss

The most common cause of tooth loss in this study was dental caries 27 (46.6%), followed by Periodontal disease 20 (34.5%), trauma was responsible for 9 (15.5%) cases of tooth loss, and lastly, just two patients (3.4%) had congenitally missing teeth.

TABLE 1: CAUSES OF TOOTH LOSS

The causes		Frequency	Percentage	Valid Percent	Cumulative Percent
Valid	Dental caries	27	46.6	46.6	46.6
	Periodontal disease	20	34.5	34.5	81.0
	Congenitally	2	3.4	3.4	84.5
	Trauma	9	15.5	15.5	100.0
	Total	58	100	100	

The results of T-test about the consequences of tooth loss.

I. PATIENTS WITH MISSING ANTERIOR TEETH ONLY

Table 2: The health problems which suffers of it patients with missing anterior teeth

N	Oral health problems	No	Yes	Mean	S.D	P-value
		N (%)	N (%)			
1	Temporomandibular disorder (TMD)	16 (100%)	0 (0%)	1	0.000	----
2	Overeruption	16 (100%)	0 (0%)	1	0.000	----
3	Drifting teeth	12 (75%)	4 (25%)	1.25	0.447	0.041
4	Low chewing ability	15 (93.8%)	1 (6.2%)	1.063	0.25	0.000
5	Poor aesthetical effects	1 (6.2%)	15 (93.8%)	1.938	0.25	0.000
6	Speech distortion	3 (18.8%)	13 (81.2%)	1.813	0.403	0.007
7	Bone loss (resorption)	1 (6.2%)	15 (93.8%)	1.938	0.25	0.000
8	Negative emotional effects	6 (37.4%)	10 (62.5%)	1.313	0.946	0.44

From previous table, it is clear that, these patients were exposure significantly to aesthetic problems (poor appearance) and bone loss (resorption) due to loss of anterior teeth, where the mean was in both of them (1.938) and standard deviation was (0.25), the level of significance is less than (0.05).

As well as they had clearly speech distortion, where the average (1.813) and standard deviation (0.403) the connotation level of significance less than (0.05).

As for the problem of temporomandibular disorder (TMD) and overeruption of unopposed tooth, did not founded ever among them, where the mean was in both of them (1) and standard deviation was (0.000), the level of significance is less than (0.05).

II. Patients with missing posterior teeth only

Table 3: The health problems that suffers of it patients with missing posterior teeth

N	Oral health problems	No	Yes	Mean	S.D	P-value
		N (%)	N (%)			
1	Temporomandibular disorder (TMD)	13 (92.9%)	1 (7.1%)	1.071	0.267	0.000
2	Overeruption	6 (42.9%)	8 (57.1%)	1.571	0.514	0.612
3	Drifting teeth	4 (28.6%)	10 (71.4%)	1.714	0.469	0.111
4	Low chewing ability	11 (78.6%)	3 (21.4%)	1.214	0.426	0.026
5	Poor aesthetical effects	11 (78.6%)	3 (21.4%)	1.214	0.426	0.026
6	Speech distortion	14 (100%)	0 (0%)	1	0.000	----
7	Bone loss (resorption)	4 (28.6%)	10 (71.4%)	1.7143	0.469	0.111
8	Negative emotional effects	10 (71.4%)	4 (28.6%)	1.143	0.663	0.065

The previous table shown that, A few of these patients complain of low chewing ability and aesthetic problems (poor appearance) due to the loss of posterior teeth, where the average of answers was (1.214), the standard deviation (0.426) and the moral level of significance less than (0.05).

The temporomandibular disorders are very slightly among them, where the average was (1.071) and standard deviation was (0.267), and within less significance of (0.05).

As for the problems overeruption and the drifting teeth and the negative emotional effects and the bone loss (resorption) the indicative of significance level of these problems was higher than (0.05), so can consider that the problem does not exist among them.

III. PATIENTS WITH MISSING BOTH ANTERIOR AND POSTERIOR TEETH

Table 4: The health problems which suffers of it patients with missing both anterior and posterior teeth

N	Oral health problems	No	Yes	Mean	S.D	P-value
		N (%)	N (%)			
1	Temporomandibular disorder (TMD)	13 (92.9%)	1 (7.1%)	1.071	0.267	0.000
2	Overeruption	3 (21.4%)	11 (78.6%)	1.786	0.426	0.026
3	Drifting teeth	1 (7.1%)	13 (92.9%)	1.929	0.267	0.000
4	Low chewing ability	5 (35.7%)	9 (64.3%)	1.643	0.497	0.302
5	Poor aesthetical effects	2 (14.3%)	12 (85.7%)	1.857	0.363	0.003
6	Speech distortion	10 (71.4%)	4 (28.6%)	1.286	0.469	0.111
7	Bone loss (resorption)	0 (0%)	14 (100%)	2	0.000	----
8	Negative emotional effects	5 (35.7%)	9 (64.3%)	1.427	0.852	0.759

The previous table shown that, the exposure to bone loss (resorption) is very high among these patients, and they suffering from it clearly, where the average (2) standard deviation (0.000) and the level of significance less than (0.05).

Furthermore, the degree of exposure of these patients to drifting teeth, aesthetic problems (poor appearance) and overeruption of unopposed teeth was high, within significance less than (0.05).

While the degree of exposure of these patients to temporomandibular disorder (TMD) is very low, and the level of significance is less than (0.05). Moreover, about other problems in the table, it have the highest significance of (0.05).

IV. PATIENTS WITH COMPLETE EDENTALOUS (COMPLETE TEETH LOSS)

Table 5: The health problems which suffers of it patients with complete teeth loss.

N	Oral health problems	No	Yes	Mean	S.D	P-value
		N (%)	N (%)			
1	Temporomandibular disorder (TMD)	0 (0%)	14 (100%)	2	0.000	----
2	Overeruption	14 (100%)	0 (0%)	1	0.000	----
3	Drifting teeth	14 (100%)	0 (0%)	1	0.000	----
4	Low chewing ability	0 (0%)	14 (100%)	2	0.000	----
5	Esthetical effect	2 (14.3%)	12 (85.7%)	1.857	0.363	0.829
6	Speech distortion	0 (0%)	14 (100%)	2	0.000	----
7	Bone loss (resorption)	1 (7.1%)	13 (92.9%)	1.929	0.267	0.000
8	Emotional effect	5 (35.7%)	9 (64.3%)	1.357	0.929	0.175

the previous table shows that, All patients with complete teeth loss are complains about low chewing ability and speech distortion, moreover they are very clearly suffer from temporomandibular disorder (TMD), where the occurrence average of these problems were (2), while standard deviation (0.000) and the level of significance is less than (0.05).

In addition, a lot of them are suffer the problem of bone loss, where an average of this problem is (1.929), the standard deviation (0.267), and the level of significance less than (0.05).

As for the aesthetic problems (poor appearance) and negative emotional effects (psychological effects) there are no statistically significant prove the occurrence of this problems between these patients, because the level of significance of these problems was higher than (0.05).

Because the patients with complete teeth less do not have remaining teeth, considered their answers about the problems of drifting teeth and over-eruption are (NO).

The Summary of T-Test Results

The patients with complete teeth loss and the patients with missing both the anterior and posterior teeth are most groups that clearly suffer problems among patients who involved in the study.

While those who loss posterior teeth only are less group suffer health problems due to loss of teeth among all of them.

The results of Pearson Correlation

The following results shows the general relationship between tooth loss and problems of oral health on the one hand, and then shows the relationship between tooth loss and every problem separately.

The strongest relationship was between tooth loss and temporomandibular disorder (TMD) (0.813). It is a strong positive correlation, and the level of significance (0.000), which is less than (0.05). Whenever increasing the teeth loss, increasing problem of TMD.

Then followed that, the relationship between tooth loss and low chewing ability. It is also a positive and strong correlation (0.730) and was indicative of the level of significance (0.000). Whichever, with increase of teeth loss, the problem of low mastication increasing.

As for the relationship between tooth loss and speech distortion was positive medium-strength, the level of significance reached (0.002). Whichever, with increase

of teeth loss, the distortion of speech well increasing.

The relationship between tooth loss and aesthetic problems (poor appearance) was positive slight-strength correlation (1.261), the level of significance less than (0.05). Whichever, with increase of teeth loss, the aesthetic problems will be increasing.

As for the problem of drifting teeth, showed a negative low correlation (reverse relationship) the level of significance was (-0.037). With increase of teeth loss, will decreasing the drifted teeth.

As for the problems of the negative emotional effects and bone loss (resorption) are showed positive correlation low strength (weak), but indicative of the level of significance higher than (0.05), that correlation is not significance in both problems.

While, the overeruption of unopposed teeth is showed a negative correlation (reverse relationship), but the significant is higher than (0.05). Therefore, the correlation is not significance.

The general relationship between tooth loss and the oral health problems (all previous problems), the correlation value was (0.614), and the level of significance was (0.000), which is less than (0.05). It is a positive and strong correlation, whichever, with increasing of teeth loss, the oral health problems will be increasing.

Discussion

Studies concerning the epidemiology in dentistry have showed that dental caries and periodontal diseases are the most prevalent pathologies that affect the oral cavity. Previous studies performed by American researchers had suggested that dental caries was the main reason for teeth extraction, and other studies accomplished in New Zealand, Sweden, and even in Brazil confirmed that caries may lead to tooth mortality[12-13].

The association between positional changes and tooth loss.

· Temporomandibular disorder (TMD)

The TMD has been very clear in patients with complete teeth loss, because without teeth, the occlusion plane changed in these patients, therefore disorders such as excessive closures and other problems are exposed.

While, the remaining teeth in patients with partial teeth loss maintained at the occlusion plane in the proper place.

Therefore, the TMD is commonly in complete edentulous patients than partial edentulous patients.

This study concluded that, the correlation is strong between tooth loss and TMD, unlike that study conducted by Ciancaglini and his colleagues[14] who have concluded that tooth loss is of little relevance in the etiology of temporomandibular disorders.

· Over-eruption

The overerupted teeth emerged in patients with missing both anterior and posterior teeth only, maybe because this problem needing too many gaps and teeth without opposite in the jaws. The relationship between tooth loss and this problem was negative, because with increase of teeth loss will not be teeth there to occur overeruption on it after that.

· Drifting teeth

The drifting teeth common between patients with loss anterior and posterior teeth clearly, which also for occurs is required many gaps and remaining teeth in the jaws, so the proportion of occurrence it slight. Like previous problem, the correlation between tooth loss and this problem was negative, because with increase of teeth loss will not be teeth there to occur overeruption on it after that.

THE ASSOCIATION BETWEEN FUNCTIONAL CHANGES AND TOOTH LOSS.

· Low chewing ability

The patients who suffered of low mastication on extensively were patients with complete teeth loss, as a known without teeth the chewing ability is absent.

The partial teeth loss patients who suffered of this problem were very simple; because good chewing ability is assured even with remain just (20 well-distributed teeth). Similarly, (Sarita et al., 2003)[15] he has found these previous results.

· Poor appearance

The aesthetic problem due to tooth loss is clearly in patients who have lost their anterior teeth. These patients have complained about their appearance after the loss

of these teeth, because the anterior teeth visible and complementary to the beauty of the face.

Because this problem is restricted by spatial conditions (place of the missing tooth), thus not experienced by all patients with tooth loss. Therefore. The correlation was positive but weak.

· Speech distortion

The patients with missing anterior teeth and with complete teeth loss are suffering from this problem significantly. Because the anterior teeth have an important role in the process of speech and produce pure sound.

Thus, the loss of the anterior or all teeth, the pronunciation and aesthetic would affected.

BONE LOSS (RESORPTION)

The majority of patients' categories had loss (resorption) in their alveolar bone clearly. Because after tooth loss the bone begins to resorb immediately.

The bone loss among patients with missing posterior teeth were somewhat low. Because the bone in the posterior wider than anywhere, so it needs longer time to be absorbed.

On reverse a study by (Bhaskar)[2] who concluded that, bone loss is more pronounced posteriorly than anteriorly.

THE NEGATIVE EMOTIONAL EFFECTS OF TOOTH LOSS

The negative emotional effects due to tooth loss are not frequently occurring among patients, because with passage of time getting used to it and accept the event, after it was initially difficult admission.

Moreover, a lot of them not care about teeth loss and did not lend attention to this issue originally. Therefore, the correlation was not significant.

The association between tooth loss and oral health problems

Most of previous problems had shown high average occurrence on T-Test and strong relationship on Pearson correlation. Therefore, the general relationship between tooth loss and the oral health problems was positive and strong.

CONCLUSIONS

This study demonstrated that tooth loss causes several oral health problems. There is a statistically significant relationship between tooth loss and oral health problems, where the strong value of this relationship was (0.614), and the significance was less than (0.05).

Recommendations

Based on the findings of the study, the following recommendations are made:

· Dental personnel should make an effort to identify individuals with risk of tooth loss on oral health in order to prevent tooth loss.

· The patients with tooth loss should replace their missing teeth to restore function and protect the remaining teeth.

· Improvement of dental laboratories to provide quality replacement of missing teeth at affordable costs.

· Further long-term multicenter studies to evaluate the consequence of tooth loss and assist in giving a more accurate projection of the patients' needs across the nation is mandatory.

Ethical Clearance: Ethical approval obtained from local Ethic Committee of Sebha University (South Libya).

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Antiretroviral Medicine Ethical Execution Policy for HIV / AIDS Survivors in Indonesia

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Abstract

Introduction: One of the health problems in Indonesia that has not been resolved is the HIV pandemic or Human Immunodeficiency Virus or a virus that attacks/infects white blood cells which causes a decrease in human immunity. To combat HIV/AIDS, treatment is performed through antiretroviral drugs. But in fact, antiretroviral drugs are often scarce which causes a decrease in the welfare of people with HIV / AIDS in Indonesia.

Research Objectives: This study aims to discover and analyze some policies on administering antiretroviral drugs that are fair for people with HIV/AIDS in Indonesia.

Methods: This study uses a qualitative method with a normative juridical approach. The data used are secondary data consisting of primary and secondary legal materials. The data was collected through a literature study and analyzed qualitatively.

Conclusion: In making policies, the implementation of antiretroviral drugs must be centered on PLWHA and use the PLWHA as policy actors. The policy of delivering ARV drugs that are centered on PLHIV is expected to produce fair policy outputs and then have implications for the high welfare of PLWHA in Indonesia, this is in line with the formation of a policy based on the study of responsive legal theory initiated by Nonet and Selznick. Responsive legal theory requires that the law that has implications for PLWHA, should always be sensitive to the needs of PLWHA, especially regarding the availability of ARV drugs.

Keywords: *Policy; Antiretroviral Drugs; HIV/AIDS*

Introduction

One of the health problems in Indonesia that has not yet been solved is the HIV pandemic or Human Immunodeficiency Virus, or a virus that attacks/infects white blood cells which causes a decrease in human immunity. One of the diseases caused by HIV is AIDS or Acquired Immune Deficiency Syndrome, which is a group of symptoms that arise due to the decreased immunity caused by HIV[1]. There are currently 35 million people living with HIV worldwide, which includes. 1 million women and 3.2 million aged <15 years. The number of new HIV infections was 2.1 million consisting of 1.9 million adults and 240,000 children aged <15 years[2]. The number of deaths due to AIDS was 1.5 million, consisting of 1.3 million adults and 190,000 children aged <15 years[3].

In Indonesia, the first HIV / AIDS case was found in Bali Province in 1987. Until now, HIV / AIDS has spread in 386 districts/cities in all provinces in Indonesia. Between 2000-2018, HIV / AIDS infections declined by 37% and HIV / AIDS-related deaths by 45%, and as many as 13.6 million people survived due to the consumption of antiretrovirals (ARVs)[4]. This achievement is the result of the major efforts of a national HIV program supported by communities and international development partners. However, currently, 23% of people living with HIV /

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AIDS (PLWHA) have dropped out of ARV treatment, even though the drug can be used free of charge in hospitals and health centers in 34 provinces and 296 districts/cities throughout Indonesia. The Ministry of Health's strategy to stop the HIV / ADIS pandemic is by implementing Suluh Find Treat and Maintain (STOP), which is one of the strategies to implement Regulation of the Minister of Health of the Republic of Indonesia No. 21 of 2013 concerning HIV and AIDS Control.

Based on a report from The Joint United Nations Program on HIV and AIDS (UNAIDS), the situation for handling HIV in Indonesia is still far from qualified, it is even one of the worst in Asia-Pacific. Although the government already has a special HIV prevention program, the annual rate of infection is still quite large. One of the reasons why handling HIV in Indonesia is still far from qualified is related to the scarcity of ARV drugs. ARV drugs are scarce in 40 districts and cities in Indonesia[5].

Based on records from the NGO Indonesia AIDS Coalition (IAC), this crisis of drug stocks has occurred several times in the last 2 years without any concrete solution from the Ministry of Health. The State Revenue and Expenditure Budget (APBN) which has been allocated for the purchase of ARV drugs, cannot be executed because the system and mechanism for drug procurement is inefficient, this is because the process of procurement of ARV drugs for Fixed-Dose Combination types of TLE was declared failed in 2018, so the allocation of APBN funds cannot be channeled to buy these drugs. The drug tender failed because PT Kimia Farma did not agree with the price offered by the Ministry of Health (Kemenkes). A limited auction process with participants from PT Kimia Farma and PT Indofarma Global Medika was then carried out, but it did not bring out any winners.

The failure of the tender process for ARV drugs resulted in the TLE type of Fixed-Dose Combination being increasingly difficult to find in several cities in Indonesia. Based on data compiled by IAC, the relatively safe drug availability status is only found at Ananda Bekasi Hospital and Elisabeth Bekasi Hospital. Meanwhile, in several other hospitals in Jabodetabek, Central Java, Deli Serdang, and Makassar, Palembang and Medan majorly were recorded as empty. Besides that, there are still several hospitals that are noted to have limited availability, or only provide in splits. This situation endangers the health of people living with HIV as well as undermines efforts to stop the epidemic and discredits efforts to optimize the process for procuring essential medicines, especially ARVs. From previous problems, this study aims to identify and analyze policies on administering antiretroviral drugs that are fair for people with HIV / AIDS in Indonesia.

Research Methodology

This study uses a qualitative research method with a normative juridical approach. The normative juridical approach method is a study of the principles of positive law written in the constitution and conceptualizing law as a written rule. This research is focused on studying and researching legal material, namely the policy of administering antiretroviral drugs to later criticize it to achieve a model for administering fair antiretroviral drugs for people with HIV / AIDS in Indonesia. The data used in this research is secondary data. The secondary data consists of primary legal materials and secondary legal materials[6]. The primary legal materials referred to are all laws and regulations related to the administration of antiretroviral drugs, particularly Law Number 36 of 2009 concerning Health. The secondary legal materials used are journals, books, or research related to the title of this research. Secondary data is collected through literature study and will be analyzed qualitatively.

Discussion

Article 1 Number 8 of Law 36 of 2009 concerning health defines medicine as a material or material guide, including biological products, used to influence or investigate physiological systems or pathological conditions in the context of determining a diagnosis of prevention, cure, recovery, health improvement and contraception for humans. Article 8 stipulates that every person has the right to obtain information about his / her health data, including the actions and treatments that he has or will receive from health workers. Based on Article 36, the government guarantees the availability, equity, and affordability of medical supplies, especially essential medicines. In ensuring the availability of these emergency medicines, the government can implement special policies for the procurement and utilization of drugs and medicinal substances.

One of the drugs in Indonesia that is quite important today is antiretroviral drugs (ARV). ARVs work directly to block HIV replication. ARV therapy aims to reduce the amount of virus in the blood to low or below detectable levels for a long period. Currently, there are three classes

of ARVs available in Indonesia, including[7]:

1. Nucleoside Reverse Transcriptase Inhibitor (NRTI): this drug is a nucleoside analog that blocks the process of converting viral RNA into DNA. Included in this class of drugs are zidovudine (ZDN. AZT), lamivudine (3TC), didanosine (ddl), zalcitabine (ddC), stavudine (d4T), and abacavir (ABC);

2. Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI): including this class of drugs are nevirapine (NVP), and delavirdine (DNL); and

3. Pretease Inhibitor (PI): works to inhibit the protease enzyme which cuts long chains of amino acids into smaller proteins. These include indinavir (IDV), nelfinavir (NFV), saquinavir (SQV), ritonavir (RTV), amprenavir (APV), and lopinavir/ritonavir (LPV).

The provision of ARVs in Indonesia is based on the Regulation of the Minister of Health of the Republic of Indonesia Number 87 of 2004 concerning Guidelines for Antiretroviral Treatment. Antiretroviral treatment based on Article 1 of this ministerial regulation is part of HIV / AIDS treatment to reduce the risk of HIV transmission, inhibit the worsening of opportunistic infections, improve the quality of life of HIV sufferers, and reduce the amount of viral load in the blood until it is undetectable.

Antiretroviral treatment is given to HIV patients aged 5 (five) years and over who have shown clinical stage 3 or 4 or CD4 T lymphocyte counts are less than or equal to 350 cells / mm3; pregnant women with HIV; babies born to mothers with HIV; HIV sufferers, infants or children less than 5 (five) years old; HIV sufferers with tuberculosis; HIV sufferers with hepatitis B and hepatitis C; people with HIV in key populations; HIV sufferers whose partners are negative; and HIV sufferers in the general population living in areas of the HIV epidemic.

Antiretroviral treatment can be given comprehensively with the treatment of opportunistic infections and comorbidities and other supportive treatment as needed. Antiretroviral treatment starts from a hospital that is at least class C and can be continued at a health center or other health care facility that can treat antiretroviral drugs. In blood with widespread and concentrated HIV epidemic levels, antiretroviral treatment can be started at a health center or other health care facility that has antiretroviral treatment capability and is excluded from antiretroviral treatment given to infants and children less than five years of age.

Guidance and supervision of the implementation of this Ministerial Regulation is carried out by the Minister, provincial government, and district/city government by involving professional organizations by their respective duties and authorities.

The Ministry of Health through the Directorate General of Disease Control and Environmental Health has launched the Care, Support, and Treatment (CST) Program for people living with HIV-AIDS (PLWHA) starting in 2004 with training for 25 Antiretroviral Referral Hospitals, which is also a referral hospital for the treatment of PLWHA. The determination of the ARV Referral Hospital was through the Decree of the Minister of Health. With the increasing number of cases, until 2011, 278 ARV Referral Hospitals have been established, by Decree of the Minister of Health Number 782 / Menkes / SK / IV / 2011.

The procurement of ARV drugs is still concentrated at the Ministry of Health and is currently fully subsidized by the government. The distribution is still centralized and sent directly to the services that need it according to service requests. This concentration is carried out to facilitate monitoring of drug use and availability in services, given the characteristics of the disease and the consumption of specific ARV drugs, where the drugs must be consumed on time and for life.

The budget for the procurement of ARVs has been allocated in the APBN and a grant from the AIDS Component Global Fund for AIDS, TB, and Malaria (GF-ATM). The budget with the State Budget (APBN) is increasing every year. In 2011, the comparison with GF-ATM funds was 70:30. It is hoped that next year it will get even bigger and in the end, the need for ARV can be met entirely with APBN funds.

The procurement process with APBN funds is carried out by the Directorate General of Pharmaceutical and Medical Devices through Electronic Procurement Services (LPSE), while the budget with GF-ATM funds is through the Voluntary Pooled Procurement (VPP) procedure, where the Ministry of Health submits the needs to the GF-ATM central procurement. Then they will buy directly from the drug factory. In this way, quality drugs are obtained at the cheapest price (breaking the trade chain). In the specifications for the procurement of ARV drugs, it is always stated that the

expiration period of the drug is at least 18 months since the drug is received by the goods receipt committee.

APBN funds are used to purchase first-line ARVs and a small portion of second-line ARVs, while GF-ATM funds are used mainly to buy second-line ARVs and some of the first-line ARVs that cannot be produced domestically, such as ARV for children and the combination of stavudine + lamivudine.

To cut delivery times, currently, there is a decentralization of ARV distribution in several provinces that are considered capable, in terms of human resources and infrastructure. The province that has implemented it is East Java. Provinces that have just started are Bali, Papua, and West Java.

In brief, the flow of drug delivery in the decentralized province is as follows: Every month the hospital reports on drug use and drug requests using the monthly report form. The amount of medicine demanded is the need for 1 month and 2 months of reserve stock by considering the final stock. So that the hospital has to be willing to supply medicine for 3 months. Every month the hospital reports on drug use and drug requests using the monthly report form. The amount of medicine demanded is the need for 1 month and 2 months of reserve stock by considering the final stock. So that the hospital must have a supply of drugs for 3 months. Reports are addressed to the Provincial Health Office. At the Provincial Health Officer, the request will be responded to by verifying drug calculations and making a Pre Order to the Provincial Kimia Farma to send the requested drug to the service. This process will take a maximum of 5 days. Kimia Farma Provinsi every 3 months submit a request for drug stock based on the calculation of the request from the service to Kimia Farma Pusat. The Provincial Health Office sends a copy of the report from health services to the Sub-Directorate for AIDS & Sexually Transmitted Diseases, Directorate General of Disease Control and Environmental Health.

After receiving new drugs, both domestically produced and imported through the above mechanism, the Ministry of Health directly distributes ARVs to all referral hospitals. Availability of drugs depends on reports of use and requests for drugs from the designated referral hospital. With the supervision mechanism and the pattern of procurement and distribution of ARVs, it is hoped that there will be no longer the administration and use of ARV drugs that have expired.

What should also be emphasized to the public is that ARV drugs only reduce the amount of HIV circulating in the blood and do not cure the disease. ARV drugs must be taken every day at the right dose and time and continue to be taken for life. Non-adherence to taking ARVs can lead to resistance.

HIV / AIDS prevention policies, especially concerning the provision of antiretroviral drugs, are largely determined by the government's perspective on HIV / AIDS. However, the government is considered not to be siding with PLWHA regarding HIV / AIDS prevention efforts because the availability of antiretrovirals, which is often limited, causes a decrease in the welfare of PLWHA.

Regarding the procurement of antiretroviral drugs that are equitable for PLWHA, it can refer to the perspective of the welfare state law. As stated by Satjipto Rahardjo, the most serious problem when talking about problems of the state and justice is about the 'culture' of community happiness (community welfare). In the form of a question, it will read, "state for what?" and answer with "the state to make the community happy (welfare of society)". From this opinion, it can be concluded that the aim of the modern state is none other than to bring about happiness, prosperity, and human glory[8].

The birth of the concept of a modern state as a mission to realize the welfare and happiness of society is marked by the development of a concept of a 'law state', namely a social service state (welfare state). Such a rule of law has a duty as a public servant, namely to organize and strive for justice / social welfare which Lemaire calls bestuur zorg. The concept of a welfare law state is the result of the development of the polizeistaat law state concept and the national law state concept[9]. This understanding of the rule of law is contained in the 4th paragraph of the Preamble to the 1945 Constitution of the Republic of Indonesia (UUD NRI 1945), that one of the objectives of the Indonesian State is to advance public welfare[10].

Based on the concept of the welfare state law, some of the obligations that must be done by the state are to fulfill, provide, serve, and protect its citizens. All of these are then reduced and introduced in the concept of Human Rights, in the form of rights to be free from and free to[11]. One of the manifestations of human rights referred to is the right to health, as regulated in Article 28 H paragraph (1) of the 1945 Constitution of the

Republic of Indonesia, which states that everyone has the right to live in physical and mental prosperity, to live, and to have a good and healthy living environment and the right get health services. The guarantee of the right to health is also contained in Article 12 paragraph (1) of the International Convention on Economic, Social and Cultural Rights established by the United Nations General Assembly 2200 A (XXI) dated December 16, 1966, namely that states participating in the convention recognize the right of everyone to enjoy the highest attainable standard of physical and mental health[12].

Efforts that can be made by the government to realize the right to health for its citizens are by carrying out various activities to maintain and improve the highest public health status, which is then carried out based on the principles of non-discrimination, participatory and sustainable development in the framework of the formation of Indonesian human resources, as well as increasing the resilience and competitiveness of the nation for national development. Further regulation of the right to health is contained in Article 13 of Law Number 36 Year 2009 concerning Health, one of which is that everyone has the same rights to gain access to resources in the health sector. The government's responsibility regarding the rights of citizens is stated in Article 15, that the government is responsible for the availability of the environment, structure, health facilities, both physical and social, for the community to achieve the highest health status.

Medicine is an important component in health care. Limitations of drugs will certainly cause health facilities to be less than optimal in carrying out their duties, namely serving the community[13]. ARV is the life of PLWHA, the scarcity of ARV is endangering the health of PLWHA, undermining efforts to stop the pandemic and discrediting efforts to optimize the process of procuring essential drugs, especially ARV. PLWHA are also citizens whose rights must be fulfilled and protected by the state. When issues of health and medicine are made commodities and government policies tend to be discriminatory, the rights and needs of PLWHA will be threatened, therefore the government is obliged to form a policy for administering ARV drugs that is just for PLWHA.

David R. Holtgrave in his book "Handbook of Economic Evaluation of HIV Prevention Programs"[14], said that discrimination against PLHIV, especially in any government policymaking, must be immediately overcome, because discrimination will affect the quality of life of PLHIV in society, therefore every policy-making especially concerning PLWHA, in this case, the policy for administering ARV drugs needs to apply the principles of good governance. In a good governance perspective, policymakers are required to produce policies that lead to the goals of political philosophy, so that they can be ethically justified, for example, policies carried out without discrimination. A policy will lose its ethical value if it is carelessly disregarding the interests of the lives of many people. In the Cochran and Malone category, a policy is interpreted as a good policy, if the policy is related to government decisions and actions designed to save people's problems, in this case, PLWHA[15].

Following the principles of good governance, policies can be formed based on the philosophy of feminism. The influence of feminism in the study of legal science is known as the feminist legal theory which focuses on legal reasoning to reach a certain conclusion, feminists will consider things that are not universal, not general, but rather reflect women as a part that does have certain needs and uniqueness. starting point of reasoning[16]. Feminism develops specific legal arguments that pose challenges to discriminatory and unfair laws[17].

Feminist legal theory considers that good law is law that sided with women and of course anyone including PLWHA as a minority group is often socially weakened by various government policies, laws based on feminist studies must be based on the objective of fighting oppression, domination and discrimination. . Departing from the principles of good governance and feminist legal theory, the policy of administering ARV drugs for PLHIV must be formed based on the principle of minority-centered social policies, that in public policy PLWHA is not only positioned in the target group but rather social actors who can determine and fight for their rights. , especially the right to good health through public policy. PLWHA in the process of making public policies must be given several rights, for example, the right to existence and the right to freedom of opinion and to be critical in making a public policy. The right to freedom for PLWHA in policy-making must be carried out by government officials, this is following the provisions in Article 7 paragraph (1) letter f of Law 30 of 2014 concerning Government Administration, that government officials are obliged to provide opportunities for citizens to be heard before their opinion. Make

decisions and/or actions following the provisions of laws and regulations[18].

The policy for administering ARV drugs that are centered on PLHIV must respect the rights, needs, and voices of PLWHA. The policy of delivering ARV drugs that are centered on PLWHA is expected to produce a fair policy output which then has implications for the high welfare of PLWHA in Indonesia, this is following the formation of a policy based on the study of responsive legal theory initiated by Nonet and Selznick[19]. Responsive legal theory which requires that laws that have implications for PLWHA should always be sensitive to the needs of PLWHA, especially regarding the availability of ARV drugs. Based on the study of responsive legal theory, the policy of administering ARV drugs must be oriented towards justice which is more than just procedural justice, but emphasizes substantial justice. Such a policy model is following the objectives of the state set out in the Preamble to the 1945 Constitution of the Republic of Indonesia, namely to promote public welfare.

Conclusion

The fair administration of antiretroviral drugs for people with HIV / AIDS in Indonesia, that Law 36 of 2009 concerning health stipulates that every person has the right to obtain information about his / her health data, including actions and treatments that have been or will be received from health workers. Based on Article 36, the government guarantees the availability, equity, and affordability of medical supplies, especially essential medicines, including antiretroviral drugs for people with HIV / AIDS. HIV / AIDS prevention policies, especially those related to the provision of antiretroviral drugs, are largely determined by the government's perspective on HIV / AIDS. However, the government is considered not to be siding with PLWHA regarding HIV / AIDS prevention efforts because the availability of antiretrovirals, which is often limited, causes a decrease in the welfare of PLWHA. Departing from the principles of good governance and feminist legal theory, the policy of administering ARV drugs for PLHIV must be formed based on the principle of minority-centered social policies, that in public policy PLWHA is not only positioned in the target group but rather social actors who can determine and fight for their rights. , especially the right to good health through public policy. PLWHA in the process of making public policies must be given several rights, for example, the right to existence and the right to freedom of opinion and to be critical in making a public policy. The policy for administering ARV drugs that are centered on PLHIV must respect the rights, needs, and voices of PLWHA. The policy of delivering ARV drugs that are centered on PLWHA is expected to produce a fair policy output which then has implications for the high welfare of PLWHA in Indonesia, this is following the formation of a policy based on the study of responsive legal theory initiated by Nonet and Selznick. Responsive legal theory which requires that laws that have implications for PLHIV are always sensitive to the needs of PLWHA, especially regarding the availability of ARV drugs.

Ethical Clearance: Yes.

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Forensic Examination of Torture and other Forms of ill-Treatment

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Abstract

Forensic documentation is crucial for documenting torture, and is generally treated as a central component of any effort to prosecute torture. As a result, it is central to ending the impunity for torture which is key to its perpetuation. Forensic evidences have been the centerpiece of the judgment by the court of cassation. This shows the important of the quality, independence and impartiality of the forensic examination for any strategy for the prevention of torture in Jordan.

Keywords: *Forensic Examination, Torture, ill-Treatment, Justice.*

Introduction

All allegations of torture or other cruel, inhuman or degrading treatment or punishment should be promptly and impartially examined by the competent national authority, that those who encourage, order, tolerate or perpetrate acts of torture must be held responsible and severely punished, including the officials in charge of the place of detention where the prohibited act is found to have taken place, and that national legal systems should ensure that the victims of such acts obtain redress and are awarded fair and adequate compensation and receive appropriate social and medical rehabilitation[1].

Jordan is a party to the main treaties including the UN Convention against torture and other Cruel, Inhuman or Degrading Treatment or punishment (Convention against Torture (CAT)), but Jordan did not sign in the Optional Protocol to the Convention against Torture (OPCAT), International Covenant on Civil and Political Rights (ICCPR) and Arabian Charter for Human Rights.

Jordan ratified the CAT on 13/11/1991, entered into force on 15/6/2006[2].

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However, concerns related to torture in detention in Jordan after the decision of the court of cassation No 4050 / 2019 on Sep 2020 against 3 of the accused, members of the Criminal Investigation Department, to imprison them for a period of 7 years. The court's decision came against the background of the case of the death of a citizen under torture at the hands of members of the Criminal Investigation Department.

Relevant International Legal Standards

a- Convention against Torture

Each State Party shall ensure that any individual who alleges he has been subjected to torture in any territory under its jurisdiction has the right to complain to, and to have his case promptly and impartially examined by, its competent authorities. Steps shall be taken to ensure that the complainant and witnesses are protected against all ill-treatment or intimidation as a consequence of his complaint or any evidence given[3].

b- Declaration on the Protection of All Persons from Being Subjected to Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment:

Any person who alleges that he has been subjected to torture or other cruel, Inhuman or degrading treatment or punishment by or at the instigation of a public official shall have the right to complain to, and to have his case impartially examined by, the competent authorities of the State concerned[4].

c- Arab Charter for Human Rights

Article 8:

1. No one shall be subjected to physical or psychological torture or to cruel, degrading, humiliating or inhuman treatment.

2. Each State party shall protect every individual subject to its jurisdiction from such practices and shall take effective measures to prevent them. The commission of, or participation in, such acts shall be regarded as crimes that are punishable by law and not subject to any statute of limitations. Each State party shall guarantee in its legal system redress for any victim of torture and the right to rehabilitation and compensation[5].

The Policy against Torture in the Jordanian legislation

1- The Jordanian legislation has criminalized the practice of torture through the provision of article 208 of the Penal Code. Perpetrators of torture have been subject to various penalties based on the seriousness of the offence committed. Such penalty may ascend to temporary hard labor punishment (extended period of imprisonment) if the torture has lead to serious illness or injury. Thus, the offence of torture would be criminally classified as a felony (art. 208/3 of the Jordanian Penal Code). Torture has also been criminalized under article 49 of the Military Penal Code.

2- The Jordanian legislation currently in force, namely article 208 of the Penal Code, imposes punishments on perpetrating the crime of torture, inciting its exercise, or approval or acquiescence thereof by any official or any person acting in an official capacity. The penalties imposed on the perpetrator of this crime have been set forth under articles 208/1 and 208/3 of the Penal Code including imprisonment for six months to three years against exercising any kind of torture to obtain confession of a crime or information in connection thereof. This penalty would be increased to temporary felony with hard labor if torture has lead to illness or serious injury. Furthermore, the court may not stop the enforcement of the sentenced punishment in the crimes listed in the said article 208, and it may not consider extenuating circumstances. As to the number of such cases and the statistics of the Public Security Directorate (PSD) on complaints received by the Grievances and Human Rights Office[6].

3- Jordanian legislation (article 100 of the amended Criminal Procedure Law No. 16 of 2001) has determined the period of detention of an accused person at police stations to be 24 hours, after which the accused shall be referred to the concerned public prosecutor as the legal authority in charge of the investigation.

4- Article 63 of the Criminal Procedure Law provides for the right of the defendant to have a defense attorney. Article 66/2 prohibits the public prosecutor from preventing the lawyer from contacting the defendant. In case the defendant claims that he has been tortured or has been subjected to physical or mental coercion by the law enforcement officer, torture being legally criminalized, the public prosecutor must register the incident in the investigation record and refer the defendant to the forensic doctor, if deemed necessary.

5- Also, no person with signs of any type of injury may be admitted to correctional and rehabilitation centers except after being examined by the forensic doctor to obtain a legal medical report and conduct the necessary investigations. The centre's doctor must examine the detainee and provide a report on his medical condition when he is admitted to the centre and before he is released there from, and when he is transferred from one centre to another pursuant to article 24 of the Rehabilitation Centers Law.

6- Article 159 of the Criminal Procedure Law stipulates that any evidence or statement obtained by physical or mental coercion and in the absence of the public prosecutor is considered void, and of no legal effect. It will not be accepted unless the prosecution provides evidence of the circumstances under which it was obtained and the court is convinced that the indicted, suspect or defendant has provided such evidence or statement voluntarily. The defendant may also dispute, before the public prosecutor and the court, the statement obtained from him by the law enforcement officer on the grounds that it was obtained under pressure or through physical or mental coercion.

Complaints of Torture and other Forms of ill-Treatment in Jordan

In 2018, the total number of cases registered against employees of the Public Security Directorate related to complaints of torture and other forms of ill-treatment reached 332 cases, of which 10 cases were referred to the Police Court, and the cases in which the conviction was made reached 51 cases, while it was decided to prevent

the trial of the defendant in 240 cases by the police public prosecutor, while 31 cases are still pending.

Compared with the year 2017, the total number of cases reached 45 cases, of which 4 were referred for trial before the unit commander, and 12 cases were decided to prevent the trial of the defendant by the police public prosecutor, while 2 cases were referred to the police court, and 27 cases are still pending, compared to 269 cases in 2016, 239 cases in 2015 and 140 cases in 2014.

As for the cases of ill-treatment committed against inmates in correction and rehabilitation centers in 2018, it amounted to 29 cases, in which 6 people were convicted, while the prosecution of 9 people was prevented by the police public prosecutor, and 14 cases are still pending, compared to 17 cases in 2017, and 12 cases in 2016, 8 cases in 2015.

As for the complaints that the National Center for Human Rights received from citizens related to allegations that they or their families were subjected to torture and ill-treatment at the hands of law enforcement officials and various security departments, whether in the preliminary investigation centers or in the reform and rehabilitation centers during periodic visits by its delegates to these places or through Complaints received by the center, as of 2018, 68 complaints were caught, including allegations of torture and ill-treatment at the hands of law enforcement officials and the various security departments, compared to 85 complaints in 2017, 63 complaints in 2016, 92 complaints in 2015 and 87 complaints in 2014.

As for the allegations of beatings, torture and other ill-treatment and complaints related to the rights of inmates in the correction and rehabilitation centers, the center received 39 complaints in 2018, compared to 17 complaints in 2017, 12 complaints in 2016, 8 complaints in 2015, and 11 complaints in 2014[7].

From the above statistics the forensic documentation is crucial to all factors of the fight against torture and ill-treatment, including prevention; denunciation, investigation, prosecution, punishment of perpetrators; and rehabilitation of victims and their families.

The failure to investigate allegations of torture by independent and impartial bodies and the lack of adequate guarantees in these investigations, in fact, means the existence of a climate of impunity resulting from the weakness of disciplinary or criminal prosecution against persons accused of committing torture. Prosecutions are currently being carried out under Article 37 of The Public Security Act of 1965, through disciplinary measures only. Although the Public Security Directorate formed 60 committees in 2012 to investigate allegations of torture, none of these committees ha send its investigations by referring any person to the Police Court under Article 208 of the Penal Code[8].

The Effective Investigation and Documentation of Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment

1- Medical experts involved in the investigation of torture or ill-treatment shall behave at all times in conformity with the highest ethical standards and, in particular, shall obtain informed consent before any examination is undertaken. The examination must conform to established standards of medical practice. In particular, examinations shall be conducted in private under the control of the medical expert and outside the presence of security agents and other government officials[9].

While medical personnel may be capable of identifying injuries or marks on the body during an examination, there may be need to consult health care personnel who specialize in the identification of the likely type(s) of torture or ill treatment inflicted on victims. Such specialists can deduce the specific type of torture or ill treatment by the injuries or marks inflicted on a given person that other doctors or other health care specialists may not be able to identify[10].

The victim may have injuries or marks visible on the body on admission that do not necessarily arise as a result of torture or other cruel, inhuman or degrading treatment or punishment, and the nature of these injuries or marks should be recorded, as well as any medical treatment, including types of medication, that is needed to maintain and protect a prisoner's health.

2- The medical expert shall promptly prepare an accurate written report, which shall include at least the following[11].

a- Circumstances of the interview: name of the subject and name and affiliation of those present at the examination; exact time and date; location, nature and address of the institution (including, where appropriate, the room) where the examination is being conducted (e.g., detention centre, clinic or house); circumstances

of the subject at the time of the examination (e.g., nature of any restraints on arrival or during the examination, presence of security forces during the examination, demeanor of those accompanying the prisoner or threatening statements to the examiner); and any other relevant factors;

b- History: detailed record of the subject's story as given during the interview, including alleged methods of torture or ill-treatment, times when torture or ill-treatment is alleged to have occurred and all complaints of physical and psychological symptoms; Physical and psychological examination: record of all physical and psychological findings on clinical examination, including appropriate diagnostic tests and, where possible, color photographs of all injuries;

c- Opinion: interpretation as to the probable relationship of the physical and psychological findings to possible torture or ill-treatment. A recommendation for any necessary medical and psychological treatment and/or further examination shall be given;

d- Authorship: the report shall clearly identify those carrying out the examination and shall be signed.

3- The report shall be confidential and communicated to the subject or his or her nominated representative. The views of the subject and his or her representative about the examination process shall be solicited and recorded in the report. It shall also be provided in writing, where appropriate, to the authority responsible for investigating the allegation of torture or ill-treatment. It is the responsibility of the State to ensure that it is delivered securely to these persons. The report shall not be made available to any other person, except with the consent of the subject or on the authorization of a court empowered to enforce such a transfer[12].

4- The health-care services should include somatic and mental health care 40, including specialized services for persons with mental illness of either acute or longer term character.

5- A broad range of gender-specific health-care services should be available to women prisoners, in line with the United Nations Rules for the Treatment of Women Prisoners and Non-custodial Measures for Women Offenders (the Bangkok Rules)[13].

Recommendations

1- The state has to provide a sufficient number of medical staff trained on the management of inter-prisoner violence, as well as in the identification and documentation of all forms of torture or ill-treatment, including sexual violence, is also essential to prevent violence. State should also monitor and document incidents of violence with a view to revealing the root causes and designing appropriate prevention strategies.

2- The states in numerous occasions to ensure that there are sufficient mental health professionals; Health-care should be available and accessible, without discrimination and without cost, to all victims. The health care service should be able to provide medical treatment and nursing care, as well as appropriate diets, physiotherapy, rehabilitation or any other necessary special facilities, in order to fulfill the victims' basic needs

3- Access to an independent doctor is of particular importance in the context of complaints and allegations on torture or ill-treatment, where there may be a need of/ request for assessment and documentation of injuries or other health related consequences stemming from torture or ill-treatment, including forms of sexual violence and abuse. Available and qualified medical personnel trained in and applying the Manual on effective investigation and documentation of torture and other cruel, inhuman or degrading treatment or punishment ("the Istanbul Protocol") is required in places where persons are kept deprived of their liberty.32

4- Medical confidentiality should be observed in prisons and places of detention in the same way as in the community at large. Keeping victims' medical files should be the doctor's responsibility.

5- The state has to initiate prompt and impartial investigations in such circumstances, particularly in prison settings, irrespective of whether a complaint has been received. Such an investigation should include as a standard measure an independent physical and psychological forensic examination as provided for in the Istanbul Protocol.57

6- Amending Article 208 of the Jordanian Penal Code to stipulate that torture is a criminal offense, and granting regular courts specific jurisdiction over torture cases.

7- Providing explicitly for compensation for victims of torture in accordance with the provisions of the Convention against Torture and Other Cruel,

Inhuman or Degrading Treatment or Punishment.

8- Establishing specialized centers for the rehabilitation of victims of torture in a way that guarantees the comprehensiveness of the concept of torture, as contained in the United Nations Convention Against Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment of 1984.

9- Transferring the supervision of reform and rehabilitation centers from the Ministry of Interior to the Ministry of Justice.

10- The state to sign in to the optional protocol to the Convention Against Torture (OPCAT, which allows the establishment of a system based on regular visits by independent international and national bodies to places of deprivation of liberty.

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Cultural Misconceptions and Public Stigma about Mental Illness

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Abstract

Background: This study aims to identify the demographic and cultural correlates of mental illness stigma towards patients and their family members from the perspectives of university students.

Methods: A descriptive, cross-sectional design was employed. A total of 398 students from four universities participated in the study. They completed the demographic data questionnaire, the scale of cultural misconceptions about mental illness and the Discrimination–Devaluation Scale.

Results: Results of independent t-test and one-way ANOVA indicated no significant difference in the levels of mental health stigma towards patients and their families based on students' gender, faculty and place of residency. Previous history of family mental illness was associated with stigma towards patients and their families. Old age was associated with few mental stigmas towards patients' families. Various cultural misconceptions about the causes and the treatment of mental illness were significantly associated with mental illness stigma.

Conclusion: Addressing cultural misconceptions is crucial in reducing mental illness stigma.

Keywords: *Cultural Misconceptions, Mental Illness, Stigma, Patients, family, University Students*

Introduction

Public stigma against mental illness negatively affects the lives of patients and thus leads them to avoid building relationships, working or seeking care [1,2]**.** Numerous patients with mental illness have low income and cannot get a job due to employers' prejudice [3]. In addition, those who experience social discrimination might internalise stigma and thus experience chronic low self-esteem [4]. Stigma does not only affect patients with mental illness, but it also harms family members of these patients [5]. Stigma against family members of patients with mental illness is a consequence of the social bias rather than the failure committed by these family members[6]. The parents of people with mental illness could be blamed for their child's condition due to possible parental negligence that could lead to mental illness[7]. Moreover, their brothers and sisters may be labelled as being contaminated by their sibling who has a mental disorder[8]. In numerous situations, the negative effects of stigma may be more incapacitating than the mental illness itself [1]. Considering stigma's negative effects, mental health research aims to identify the factors that could worsen stigma and take preventive actions[3].

Awareness of the causes of mental illness and its treatments could play a vital role in shaping public stigma against mental illness[8]. Various cultural misconceptions could be identified when investigating stigma. Numerous cultural misconceptions about mental illness exist, and they play a major role in shaping public stigma about mental illness [9]. Numerous people hold patients with mental illness accountable for their own disease and blame them for experiencing the symptoms[10]. In addition, other people may perceive patients with mental illness to be harmful or dangerous[10,11]. Cultural misconceptions regarding the causes and treatment of mental illness could also play a role in determining the degree of public stigma against mental illness[9]. Hence, poor knowledge about the underlying biological origins of mental illness may influence stigma[9]. In contrast to these perceptions,

most patients with mental illness can benefit from professional psychological help[12]. However, mental illness stigma could prevent patients from seeking professional psychological help to avoid being labeled as 'mentally ill'[13]. Therefore, mental illness stigma and its impacts on patients and their families is a concern among health care providers worldwide.

Mental illness stigma among university students in Jordan is significantly associated with attitudes towards seeking professional psychological help[14]. The negative effects of stigma indicate a need for paying additional attention to its antecedents from the society. Therefore, the current study aims to identify the demographic and cultural correlates of mental -illness stigma on patients and their family members from the perspectives of university students. The collected data may determine the cultural misconceptions about mental illness and reveal how people in the Jordanian society perceive patients or those who have history of mental illness. This research proposes recommendations on how to change public attitudes toward mental illnesses in the society.

Methodology

Design, Sample and Setting

A descriptive, cross-sectional design was employed in this study. A convenience sample of 398 college students from different scientific and human sciences schools participated in the study. These students were recruited from four Jordanian universities, whereas two of them were from government schools.

Data Collection Procedure

Zarqa University Review Board approved this study, and students from different scientific and human sciences schools were invited to participate. Participants were conveniently recruited by visiting them in the university campus during their free time. The research team explained the purposes and procedures of the study prior to administering the study questionnaire. Participants were assured that their participation is voluntary and that they were allowed to withdraw from the study any time. All of them signed the consent form. The questionnaire required 15–20 minutes to complete.

Instrument

Discrimination–Devaluation Scale DDS

The DDS is a 12-item measure which rates individual views about most people's acceptance of mental illness. Scores were ranked using a four-point Likert scale ranging from 1 strongly agree to 4 strongly disagree. Seven questions assessed patient-focused stigma, and five questions assessed caregiver-focused family stigma. High scores indicate great perceptions of mental illness stigma. The DDS required 15–20 minutes to complete. The Arabic version of the questionnaire was translated and culturally adapted by Dalky[15]. The Cronbach's alpha coefficient of the Arabic version[15] was 0.87.

Cultural misconceptions about mental illness

Cultural misconceptions about mental illness in Jordan were assessed using an eight-item scale developed by Rayan and Fawaz[9]. Participants were asked to identify their beliefs regarding various statements that reflect common cultural misconceptions about mental illness in the Arab world. In addition, they could answer the questions with 'yes' or 'no' and indicate their agreement with statements that describe the causes of mental illness and its treatment. The scale exhibited a well-established content validity, with a Cronbach's α reliability coefficient of 0.81.

Data Analysis

The Statistical Package for Social Sciences version 21 (SPSS Inc., Chicago, IL) was employed to analyse the data with a significance level α of 0.05. Descriptive statistics means, standard deviations and frequencies for the sample were reported. One-way analysis of variance ANOVA was used to detect whether participants' attitudes towards mental illness differ based on their gender, place of residence and previous contact with mental illness.

Results

Sample Characteristics

A total of 398 students completed the study-102 25.6% males and 296 74.4% females. The mean age of the participants was 20.09 SD = 2.40. About 61% of the participants were from faculties of science e.g. biology, engineering, computer sciences. About half of the respondents were in the first year of their university study, and 80% of the respondents live in a city. Only 14% of the respondents have a family history of mental illness Table 1.

Table 1: Sample Charactrastics

		Frequency	Percent
Gender	Male	102	25.6
	Female	296	74.4
Faculty	Scientific	242	60.8
	Humanity	156	39.2
Year of study	First	206	51.8
	Second	48	12.1
	Third	72	18.1
	Fourth	34	8.5
	Fifth	36	9.0
	Sixth	2	.5
Place of residence	City	282	70.9
	Village	98	24.6
	Al-Badia	18	4.5
Family history	Yes	14	3.5
	No	384	96.5

Cultural misconceptions about mental illness

Various cultural beliefs and misconceptions about mental illness are reported in Table 2. The table shows that 55.8%, 64.3%, 57.8% and 16.1% of the participants believe that mental illness is caused by evil eye Hasad, Seher, Jinn and a punishment from 'Allah', respectively. Most participants believe that mental illness is treatable. However, the majority of them believe that it can be treated by Sheikh 59.8%, Pray 81.4% and Rukia 69.3%.

Table 2:Cultural misconceptions about mental illness

Item		Frequency	Percent
Believe that mental illness is caused by evil eye (Hasad)	Yes	222	55.8
	No	176	44.2
Believe that mental illness is caused by "Seher"	Yes	256	64.3
	No	142	35.7
Believe that mental illness is caused by "Jinn"	Yes	230	57.8
	No	168	42.2
Believe that mental illness is a punishment from "Allah"	Yes	64	16.1
	No	326	81.9
Believe that mental illness is treatable	Yes	368	92.5
	No	28	7.0
Believe that "Shehk" can treat mental illness	Yes	238	59.8
	No	160	40.2
Believe that Pray might treat mental illness	Yes	324	81.4
	No	72	18.1
Believe that "Rukia" might treat mental illness	Yes	276	69.3
	No	122	30.7

Differences in Stigma According to the Sample Characteristics

The results of independent t-test and one-way ANOVA indicated no significant difference in levels of mental illness stigma towards patients and their families based on students' gender, faculty and place of residency. However, students with a history of mental illness in their families demonstrated high mental illness stigma towards families of patients and low mental illness stigma towards patients Table 3. Furthermore, age is significantly correlated with mental illness stigma towards families of patient's $r = -0.126$, $P < .05$ and the overall stigma towards mental illness $r = -0.114$, $P < .05$.

Table 3: Differences in stigma based on having a family history of mental illness

variable	category	Type of stigma	N	Mean	SD	P-value
Is there a mental illness in your family	Yes	Total Stigma	14	28.7143	3.91110	946
	No		384	28.8125	5.33732	
	Yes	Individual Stigma	14	14.2857	2.26779	042
	No		384	16.2552	3.58303	
	Yes	Family Stigma	14	14.4286	2.92770	013
	No		384	12.5573	2.75678	

Differences in Stigma according to Cultural Misconceptions about Mental Illness

An independent t-test was used to examine the differences in levels of mental illness stigma according to cultural misconceptions about mental illness. Students who think that 'Jinn' might cause mental illness exhibit high overall stigma and mental illness stigma towards patients and their families $P < .05$. Additionally, students who perceive that mental illness is a punishment from Allah demonstrated high mental illness stigma toward patients. Students who think that 'Sheihk' might treat mental illness using the Holy Quran or another holy book have high overall mental health stigma and high stigma towards patients. Finally, students who think that 'Rukia' and prayer might treat mental illness have high overall stigmas towards mental illness and patients' families.

Discussion

Using the perspectives of university students living in Jordan, the current study identified the demographic and cultural correlates of stigma against mentally ill patients and their family members. Studies were conducted in several Middle Eastern countries, such as Lebanon, Oman and Qatar, to assess the perception of the university students about mental illnesses. However, to the best of the researchers' knowledge, few studies addressed university students' cultural misconceptions and their role in stigma against mentally-ill patients and their family members. This study is the first in the Jordan context to identify the demographic and cultural correlates of stigma against mental illness in the said country from the perspectives of university students. The outcomes of this study have identified several cultural misconceptions that were associated with mental illness stigma among university students. Addressing stigma towards mental illnesses is an important issue in Arab world. Although the participants in the current study received a high education, they still hold misconceptions about the causes and treatment of mental illness and consequently stigmatise individuals with mental illness and their families.

The findings of the current study revealed that students with a history of mental illness in their families demonstrated high stigma towards families of patients with mental illness and low stigma towards patients with mental illness. About a quarter to half of family members hide their relationship with a family member who suffers from mental illness to avoid shame Jon et al 2008. This result might be attributed to misconception of the causes of mental illnesses.

The participants in the current study believed that 'Jinn' might be a cause of mental illness. This finding is consistent with the results of several previous studies that were conducted on participants from different countries in the Arab world. Rayan and Fawaz[9] studied Lebanese university students and found that about 71% of the students who participated in the study believed that 'Jinn' might be the cause of mental illness. The researchers of the current study suggest that culture and religion might affect Arab people's perception of mental illnesses. Jinn were mentioned in the Holy Quran, the religious book of Islam. Large proportions of Muslim people have traditionally seen Jinn, evil eye Hasad and God's punishments as the causes of mental illness and neurological diseases [9,16,17].

Misconceptions about the causes of mental illness might consequently lead to misconceptions about treatment. Students in the current study believed that reading Holy Quran or other holy books, praying and using 'Rukia' might treat mental illness. This finding is consistent with the results of [18], which revealed that a member of an Arab family who has symptoms of mental illness usually consults to family practitioners 33%, family members 21.6% and to the Sheikh 19%. Only 11% consult mental health physicians. Interestingly, certain Arab families might wait years before seeking mental health treatment. They usually decide to do so when the patients exhibit severe symptoms that can be observed by others. However, the cost of mental health care, the cultural view of mental illness, the ineffective media and the lack of trust in mental health professionals are important barriers to seeking mental health treatment[9].

Future research may develop specific intervention programmes and examine their effectiveness to reduce mental illness stigma. Such intervention programmes should address cultural misconceptions about mental illness among university students in the Arab world. Media may also play an important role in reducing negative attitudes towards patients with mental illness and their families. Furthermore, health care providers should help educate patients and family members to fight misconceptions about mental illness in the Arab world.

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The Effect of *Artemisia Dracunculus* L. on Mitotic Index in Bone Marrow and Spleen Cells of Mice: *In Vivo* Study

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Abstract

Artemisia dracunculus L. (tarragon) is a rich source of herbal remedies with antioxidant and anti-inflammatory properties. In the present study, the proliferation of bone marrow and spleen cells of mice was evaluated after extraction of *Artemisia dracunculus* with 80% of ethanol. Two doses of the extract (500mg/kg body weight and 1000mg/kg body weight) were given to the mice for seven successive days. On day eight, mice were sacrificed and cells from bone marrow and spleen were collected; mitotic index was calculated and results were compared with that of methotrexate at a dose 20mg/kg body weight (positive control) and distilled water (negative control). The results showed that the dose 500mg/kg body weight of *Artemisia dracunculus* extract caused significant increase in mitotic index in both bone marrow and spleen cells of mice when compared with negative control. While, the dose 1000mg/kg body weight of *Artemisia dracunculus* extract caused significant decrease in mitotic index in both bone marrow and spleen cells of mice when compared with negative control.

Keyword: *Artemisia dracunculus, bone marrow cells, methotrexate, mitotic index spleen cells*

Introduction

Artemisia dracunculus (*A. dracunculus*) L., commonly as tarragon, is a perennial herb in the Asteraceae (daisy) family that has a long history of use in culinary traditions. *A. dracunculus* possesses a wide range of health benefits and has therefore been widely used as herbal medicine[1]. The botany and chemical constituents are well described in the literature, the latter mainly focusing on its essential oil composition that determines its distinct flavor. Additionally, a wide range of secondary metabolites (flavonoids, phenylpropanoids, coumarins, etc.) are reported, deter-mining *A. dracunculus* biological activities and its potential useas a source for plant-derived pharmaceutical chemical entities and complex extracts[2].

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In traditional medicine, *A. dracunculus* is commonly used to improve a malfunctioning digestive system by increasing appetite, to flush toxins from the body, and as a digestive stimulant, especially in cultures with a high consumption of red meat[3]. Arabic cultures have used *A. dracunculus* to treat insomnia and to dull the taste of medicines. Additionally, *A. dracunculus* has also been used as an anesthetic for aching teeth, sores, and cuts. It has been used widely in central Asia and Russia for the treatment of skin wounds, irritations, allergic rashes, and dermatitis[4]. In the traditional medicine of Azerbaijan, tarragon was used as an antiepileptic, laxative, antispasmodic, and carminative remedy[5].

A. dracunculus have been reported to have an important groups of biologically active secondary metabolites like essential oil, coumarins, flavonoids, and phenolic acids[6]. *A. dracunculus* usually contains >1.0% coumarins including, herniarin, coumarin, esculetin, esculin, capillarin, 8-hydroxycapillarin, artemidin, 8-hydroxyartemidin, artemidinol, and others[7]. Coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic,

hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities[8].

Different extracts from the aerial parts of *A. dracunculus* have revealed IC50 values ranging from 80 to 150 µg/mL against L5178Y lymphoma cells, and that 100 mg/kg of acetonitrile extract administered for 15 successive days has resulted in significant decrease in L5178Y lymphoma tumor size in mice[9].

Mitotic index is a measure for the proliferation status of a cell population, and defined as the ratio between the number of cells in mitosis and the total number of cells. The mitotic index can be worked out from a slide, even with light microscopy[10]. There is a direct relationship between cancer and value of mitotic index; cancer cells have high mitotic index and they grow uncontrollably and divide fast. In a normal lung tissue, the percent of dividing cells is 5% while in a cancerous lung the percent of dividing cells is 25%[11]. Low mitotic index indicates slower proliferation kinetics. Cytotoxic or genotoxic agents may interfere with mitosis rate by different mechanisms; including, interfering with tubulin polymerization or tubulin associated proteins[12].

The study aims to study the effect of the ethanolic extract of *A. dracunculus* on the mitotic index of cells obtained from the lymphoid organs bone marrow and spleen.

Materials and Methods

Plant Collection

The plant was brought from the Iraqi market. A voucher sample was kept at the department of pharmacognosy & medicinal plants, College of pharmacy/ University of Baghdad. The plant's aerial parts were air-dried at room temperature and crushed by mortar and pistol.

Preparation of an ethanol extract of *A. dracunculus*

One hundred fifty grams of dried aerial part were heated to 80°C with 1 liter of 80% ethanol for 2 hours. The extraction was continued for an additional 10 hours at 20°C. The extract was then filtered through filter paper and evaporated with a rotary evaporator; the yield weight was 23.2 gram[13].

Animals and Treatment Protocols

Twenty-four albino Swiss mice, weighing 23-27 g, were used in this study in accordance with the guidelines of the Biochemical and Research Ethical Committee at College of Pharmacy, University of Baghdad (Canadian Council on Animal Care guidelines). Animals were purchased from the animal house of College of Pharmacy, University of Baghdad. They were housed for 2 days under standard conditions (well ventilated, temperature 22±2°C, relative humidity 50–60% and 12 h day and night cycle). Food consisted of normal animal chow and water was provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed from eight to ten a.m. All the experimental work with the animals was carried out after obtaining approval from the Institutional Animal Ethical Committee. The animals were allocated into 4 groups (6 mice in each) and treated as follow: the first group was treated with the vehicle (distilled water) and served as negative control; the second group was treated with methotrexate (20mg/kg) dissolved in water and administered intraperitoneally (i.p.) as a single dose; the third and the fourth groups were treated with 500 and 1000mg/kg of *A. dracunculus* extract dissolved in distilled water; respectively. Negative control group and test groups' treatments were administered as i.p. daily doses for seven consecutive days.

Preparation of Bone Marrow Cells

After seven days of treatment, all animals were injected i.p. with 1mg/kg colchicine (Sigma, USA); two hours later, they were scarified by cervical dislocation. Bone marrow samples was aspirated from the femur bone and processed using aseptic technique for evaluation of mitotic index as previously reported elsewhere[14].

Assessment of Mitotic Index: the number of cells in division expressed as a percentage of the total number of cells was calculated, according to the following formula[15] :

Mitotic index = (Number of cells in mitosis /Total number of cells) × 100

Statistical Analysis

Data are expressed as mean±SD; unless otherwise indicated, statistical analyses were performed using unpaired *t*-test. If the overall F value was found statistically significant ($P<0.05$), further comparisons

among groups were made according to post *hoc* Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

Percent of the response is calculated as follow:

Response %=A-B/B×100

Where; A= values for tests groups or positive control B=values for negative control

Results

As shown in table 1 and 2, the dose 500mg/kg of *A. dracunculus* was associated with a significant increase in mitotic index of bone marrow and spleen cells (39.5% and 47.4%; respectively) (P<0.05); and the dose 1000mg/kg was associated with a significant decrease in this parameter of bone marrow and spleen cells (-51.5%, -46.8%; respectively) (P<0.05), both compared with the distilled water treated group. Methotrexate treated group showed (-74.3% and -84.5%) (P<0.05) decrease in mitotic index in bone marrow and spleen cells; respectively, compared with the distilled water treated group.

Table 1: Mitotic index and response percent in bone marrow of mice treated with distilled water, methotrexate and different doses of *A. dracunculus* extract in distilled water

Mitotic Index of Bone Marrow	Mean ±SD	Response %
Distilled water (negative control)	8.62±0.42	
Methotrexate (20mg/kg) (positive control)	2.21*a±0.14	-74.3%
A. dracunculus 500mg/kg	12.02*Ab±1.16	39.5%
A. dracunculus 1000mg/kg	4.18*Bb±0.73	-51.5%

Data for mitotic index are expressed as mean±SD; *significantly different compared to negative control (P<0.05); values with non-identical superscripts (a,b) among treatment groups are significantly different (P<0.05), values with non-identical superscripts (A,B) between test groups are significantly different (P<0.05).

The mitotic index levels in methotrexate treated group, in both bone marrow and spleen cells, were significantly lower than that of *A. dracunculus* treated groups; moreover, mitotic index in animals treated with the dose 1000mg/kg of *A. dracunculus* was significantly lower than those treated with the dose 500mg/kg.

Table 2: Mitotic index and response percent in spleen of mice treated with distilled water, methotrexate and different doses of *A. dracunculus* extract in distilled water

Mitotic Index of Spleen	Mean ±SD	Response %
Distilled water (negative control)	7.11±0.24	
Methotrexate (positive control) (20mg/kg)	1.1*a±0.25	-84.5%
A. dracunculus 500mg/kg	10.48*Ab±1.28	47.4%
A. dracunculus 1000mg/kg	3.76*Bb±0.45	-46.8%

Data for mitotic index are expressed as mean±SD; *significantly different compared to negative control ($P<0.05$); values with non-identical superscripts (a,b) among treatment groups are significantly different ($P<0.05$), values with non-identical superscripts (A,B) between test groups are significantly different ($P<0.05$).

Discussion

Cancer is ranked second, just after the cardiovascular diseases, as most common cause of noncommunicable diseases death, with global socioeconomic burden[16,17]. Cancer chemoprevention has received a great deal of attention and variety of plants and herbs extracts have been shown to provide some protection against cancer[18-21]. Anti-proliferative effect of certain species of genus *Artemisia* have been described previously[22.23].

A. dracunculus have been reported to be rich with coumarins[7], which have been presented in several researches as promising anticancer agents[8, 24-27]. The anticancer activity of coumarins and their derivatives is generally mediated by caspase dependent induction of apoptosis[27]. The cytotoxic and antitumor effects of *Artemisia dracunculus* extracts has been reported in murine model of lymphoma[9]; the present study investigated the effect of ethanolic extract of the plant on the mitotic index as a marker of cellular proliferation in normal murine bone marrow and spleen cells.

In the present study, the ethanolic extract of *A. dracunculus* at dose (500mg/kg body weight) caused significant increase, and the dose (1000mg/kg body weight) caused significant decrease in mitotic index in both bone marrow and spleen cells when compared with distilled water treated animals. That refers to dose-dependent effect of *A. dracunculus* extract on cell division and proliferation in both bone marrow and spleen cells. The proliferative effect of low dose of *A. dracunculus* extract needs further study for confirmation and elucidation of the potential mechanism(s). The anti-proliferative effects of *A. dracunculus* extract might be attributed to coumarins that present in this plant.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional ethical committee

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Anti-Cancer and Acute Toxicity Activity of Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl) Imino Propanal

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Abstract

Background: The new indole Schiff base compound benzo[e]indol-2-ylidene)-3-(4-methoxyphenyl) iminopropanal](2P)was investigated for antioxidant, toxicity and anti-tumor ability for A547 cell, PC3 cell and WRL 68 cell.

Results: 2P has antioxidant ability for IC50 (75 µg/mL) compared with control ascorbic acid (27 µg/mL). Antitumor ability to 2P for A547 cell and PC3 cell were decreased significantly after treated with 2P in dose dependent manner at 24,48 and 72 hours, while no toxicity effect on the WRL 68 cell. The antitumor effect was through accelerated apoptosis, the alteration in nuclear morphology. Increasing in the cells membrane permeability, decreasing in the nucleus intensity with the increasing of concentration of 2P compound was observed.

MMP is disturbed in the apoptosis by forming of permeability and the result was showed that 2P activated release cytochrome c significant higher than control.2P break down the cell cycle progress in G1 phase ($P < 0.05$) compared with control. Conclusion: this result was conducted the new 2P compound has antioxidant and antitumor ability that may be used 2P a new anticancer drug.

***Keywords**: Acute Toxicity, Anti-tumor, Apoptosis, Synthetic*

Introduction

Cancer is the world's second most prevalent cause of death, killing more than 8 million people per year; the prevalence of cancer is predicted to rise by more than 50 per cent over the coming decades[1]. It is resulting from uncontrolled cell proliferation with an absence of cell death making abnormal cell mass or tumor, new vascularization with time, this primary tumor develops and spread to another body sites, causing metastasis then death. Cancer caused by damage or mutations in the genetic material of the cells due to environmental or inherited factors[2]. Between 2005 and 2015, cancer cases increased by 33%, for men, the most common cancer globally was prostate cancer (1.6 million cases). Prostate cancer is the serious problem among worldwide men society, in the UK, with a prevalence of 105 per 100 000 population, also common in northern Europe and the USA (mainly in the African American population) It is uncommon in India but the incidence is increasing, although the overall number of deaths from prostate cancer has declined in the past 10 years, due to the increasing use of screening methods focused on prostate specific antigen (PSA)[3]. In the second hand lung cancer (LCA) had become epidemic and come up as a leading cause of cancer related death in USA and Europe, killing three times much more than prostate cancer in men also twice as many women as breast cancer[4]. According to the recent statistics which illustrate the new cases in Iraq in 2018 for both sexes and all ages that 8% as lung cancer incidence and 14% of total deaths, according to the recent World Health Organization WHO announced report [5].

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Chemotherapy is still the best way to treat many type of cancers, considering the site of it , how it big, if it is spread to other body parts and if it effects on normal body function or health (side effect)[6]. Antioxidant can enhance the immune defenses and lower the risk of cancer and degenerative diseases by minimize cell oxygen toxicity or injury to prevent, intercept and repair damage resulting from (ROS). It is work at three levels: (a) prevention , by maintains formation of (ROS) to the minimum level, e.g., desferrioxamine; (b) interception, scavenges (ROS) by using catalytic and non catalytic molecules, e.g., ascorbic acid and tocopherol; and (c) repair, damaged target molecules, e.g., glutathione [7].

Schiff bases that prepared from the condensation reaction of aromatic primary amines with aromatic aldehydes or ketones are most stable then that prepared from the condensation reaction of aliphatic primary amines with aliphatic aldehydes oraliphatic ketones. They containing a carbon nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group but not hydrogen[8]. Indole derivatives are an important class of organic heterocyclic which have played vital and significant role in curing so many ailments throughout the history of pharmaceuticals and medicines. Also they are one of the most attractive frameworks with a wide range of biological and pharmacological activities[9]. This study aim to synthesize a new Indole Schiff base to find anti -tumor for PC-3 cell.

Methods

Synthesizing a new Indole Schiff base

(2g, 0.01mol) of 1,1,2-Tri methyl-1H-benzo [e] indole was dissolved in10 ml of DMF and cooled in to ice bath. The solution of (4.6 ml, 0.03 mol) of phosphoryl chloride in 10 ml of dimethylformamide was cooled in an ice bath also and then was added dropwise to first solution with stirring for 1 h at below 5°C. After that, the reaction mixture was reflux for 3 hours. The mixture of reaction was poured on ice water, the pH was mend to 8.0 by added aqueous solution of (25%NaOH), the solid product was precipitated and the product was filtered, washed with water and then dried to afford solid yellow product. Yield (91%), m. p.199-200°C.

Synthesis of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((4-methoxyphenyl) imino)propanal

A solution of (0.4 g, 1.5 mmol) of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)malonaldehyde was dissolved in ethanol 20 ml and (0.19 g, 1.5 mmol) of 4-methoxy aniline was dissolved in ethanol 10ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in a water bath at 78 ºC for 1h. A solvent was reduced to one quarter; yellow precipitate was formed direct, filtered off, washed with ethanol and dried in oven. The completion of the reaction was checked by using TLC (3:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. Rf=0.7 cm. Yield (88%),IR data in (cm-1):3301 υ(N-H) 3120 υ (CH aromatic) 2923υ (CH aliphatic), 1665υ (CH=O), 1640 υ(C=C), 1607 υ(CH=N),1376 υ(CH3), 1254υ(C-N), 1076υ(C-O), and 829 υ(C-H bending).

1H NMR (400MHz, CDCl3) δ (ppm): δ = 14.37ppm (s, 1H, NH), 9.74ppm (s, 1H, CHO), 8.32 ppm (s, 1H, CH=N), 8.05-6.92ppm (d, 8H, Ar-H) and(t, 8H, Ar-H) 3.8ppm (s, 3H, OCH3), 1.87 (s, 6H, 2xCH3). APT13C NMR (100 MHz, CDCl3) δ (ppm): δ = 186.91 (CHO), 185.09(NH-C=C), 153.00 (CH=N), 157.75, 148.01, 133.80, 132.36, 129.75, 129.03, 128.17, 126.44, 124.14, 122.78, 119.70 and 118.41(Ar-H). 115.11 (C=C-CHO), 56 (OCH3), 55.61 (CH3-C-CH3) and 22.69 (2x CH3).

Antioxidant Activity

To investigate the antioxidant activity for new Indole Schiff base synthesis compound by applied FRAP and DPPH method as described by Walaa,et al [10].

Toxicity Effect of New Synthetic Compound

This in vitro technique was conducted to examine the potential cytotoxic effect on tumor cell lines (PC3 and A547) and standard cell line WRL 68 at various concentrations of 2P compounds.The cell sheet washed with PBS after growth medium was aspirated.Trypsin solution have been added to the cell by (2-3mL). To cover the monolayer full with gentle rocking, the flask have been flipped over. The flask was needed 1 to 2 minutes of incubation at 37 ° C before the cells were disconnected from the flask.Fresh complete medium RPMI (15-20 mL) was applied and cells were distributed by pipetting from the wedding surface into growth medium.At the required concentration, cells were redistributed to culture flasks, or plates whatever was desired and incubated in a 5 % CO2 incubator at 37 ° C. MTT assay was done as describe by Walaa,et al [10].

Apoptosis Activity

To investigate apoptosis activity for 2P used Cytotoxicity 3 Kit. Solutions of 2P were diluted at different concentration (5, 10, 15μg/ml) was prepared and 25 μl was added to the PC3 cells. The cells were incubated at 37 ° C for 24 hours. A 50 μl of live cell staining solution was added to each well.The cells were incubated at 37°C for 30 minutes.The medium and the staining solution and 100 μl/well of fixation solution and was plate incubated for 20 minutes at room temperature. The fixation solution was gently aspirated and 100 μl/ well of 1x wash buffer was added.The wash buffer was removed and 100 μl / well of 1X permeabilization buffer was added and incubated for 10 minutes at light free room temperature.The permeabilization buffer was aspirated and the plate cleaned three times with a 1x wash buffer of 100 μl / well.Wash buffer was Aspirated, and at room temperature 100 μl of 1X blocking buffer was added and incubated for 15 minutes.The Buffer of Blocking have been aspirated and (50 μl/well) of the primary antibody Solution was added, with 60 minutes of Incubation and protection from light at room temperature.The primary antibody solution was aspirated and plate washed three times with 100 μl/well 1x wash buffer.

Wash Buffer was aspirated and secondary Antibody / Staining Solution added 50 μl / well. with 60 minutes of room temperature and protection from light Incubation secondary antibody / staining solution was aspirated and plate washed three times with 1x wash buffer at 100 μl / well One hundred μl/well of 1x wash buffer was added. The plate was sealed and evaluated on the array scan HCS reader.Sealed plates were Stored in dark at 4°C. Within 24 hours after assay completion the plates has been evaluated. Sealed plates were stored in dark at 4°C. Within 24 hours after assay completion the plates has been evaluated.

PC3- cell cycle analysis

Using the commercial CycleTestTMPlus DNA Reagent Package (BD Bioscience system), for PC3- cell cycle analysis.Cells were seeded in twelve-well plates at 5 x 10, 5 cells per well. The plates were incubated at 37°C, 5% CO2 for 24 hrs.

A- After incubation, the medium was removed and the cells were treated with the compound at 100 and 200 μg/ml for 48 hrs for the cell cycle analysis. The next steps were followed in order to obtain a fine cell suspension:

1. The growth medium was aspirated and the cell sheet washed with PBS.

2. Two to three ml trypsin/EDTA solution was added to the cell. The flask was turned over to cover the monolayer completely with a gentle rocking. The flask allowed incubating at 37°C for 1 to 2 minutes, until the cells were detached from the flask.

3. Fresh complete RPMI medium (15-20 ml) was added and cells were dispersed from the wedding surface into growth medium by pipetting.

4. Cell suspension was placed into a labeled 17 x 100 mm tube.

5. Cells were centrifuged for 5 minutes at 300 g at room temperature.

6. The supernatant was aspirated and 1 ml of Buffer Solution was added. Cells were re-suspended by gently vortexing at low speed.

7. Cells were centrifuged for 5 minutes at 300 g at room temperature.

8. Steps f and g were repeated. After centrifugation, the supernatant was aspirated and the pellet was re-suspended in 1 mL of Buffer Solution.

9. The cells were counted using a hemacytometer and cells concentration was adjusted to 1.0 x 10 6 cells/ ml with Buffer Solution for immediate staining and flowcytometric analysis.

B- Staining Protocol:

1. Cell suspension was centrifuged at 400 g for 5 min at room temperature (20°C–25°C). All the supernatant was carefully decanted and 250 μl of Solution A (trypsin buffer) was added to each tube. The tube was gently mixed by tapping the tube by hand.

2. The mixture was incubated for 10 minutes at room temperature

3. Aliquot of 200 μl of Solution B was added to each tube with gentle mixing by tapping the tube by hand.

4. The mixture was incubated at room temperature for 10 minutes.

5. Aliquot of 200 μl of cold (2°C–8°C) Solution C (PI stain solution) was added to each tube with gentle mixing by tapping the tube by hand.

6. Mixture was incubated for 10 minutes in the dark on ice or in the refrigerator (2°C–8°C).

7. Finally the mixture was filtered using 50 µm nylon mesh into a new sterile tube for flow cytometer analysis.

C- Data Acquisition and Analysis:

With forward scatter (FSC) and side scatter (SSC) detection, BD FACS may provide linear fluorescence amplification capability for flowcytometers. In the blue-to-green range, the flowcytometer was fitted with an argon-ion laser emitting PI excitation at 488 nm. At an acquisition rate of at least 60 events per second, all samples were run and histograms were analyzed with suitable DNA analysis tools.

4. Solution C (8 ml), this solution have been used to binds to the DNA at a final concentration at least125µg/ml. It was kept away from light and stored at 2°C–8°C.

Statistical Analyses

A one-way ANOVA (Duncan) variance analysis was performed to evaluate whether or not group variance was significant, and statistical significance was defined as $p < 0.05$. Data were represented using Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, CA) as mean± standard deviation and statistical significance.

Results

Synthesis of compound

The a new compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-(4-methoxyphenyl) imino) propanal has been synthesized according to condensation reaction of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde with 4-methoxy aniline in equivalent ratio 1:1

Infra-Red Study (FT-IR)

The results of the IR Spectrum for the new synthesized compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-(4-methoxyphenyl) imino) propanaldisplayed absorption bands in range between 400 - 4000 cm^{-1}.

IR Spectrum shows disappearance one absorption band of (NH_2) group which belonged to p-methoxy aniline and appearance a new absorption band of imino groups (CH=N) which is approved to formation new compound. The FT-IR results figure 2 showed absorption band at 3301 cm^{-1} which assigned to (N-H) of indole ring [11], absorption bands at 3120 and 2923 cm^{-1} were attributed to stretching vibration of aromatic (C-H) and aliphatic (C-H) respectively [12], strong absorption band at 1665 cm^{-1} was belonged to the carbonyl group (C=O) also strong absorption band at 1640cm^{-1} referred to (C=C) group as well as a new strong absorption band of azomethine group (CH=N) which appeared at 1607 cm^{-1} was approved the formation of a new compound, other absorption bands were appeared on spectrum such as, at 1376cm^{-1} which belonged to bending vibration of CH_3, at 1254cm^{-1} which attributed to (C-N) at 1076 attributed to (C-O) group and finally absorption band at 829 cm^{-1} was attributed to out -of-plane (C-H) group.

4.1.2 Nuclear Magnetic Resonance Study (NMR)

Nuclear Magnetic Resonance Study (NMR)

The ^1H and APT^{13}C NMR spectra of the a new synthesized Schiff base were recorded in deuterated chloroform $CDCl_3$ with chemical shifts expressed in ppm using tetramethylsilane TMS as an internal standard. The ^1H NMR and APT ^{13}C NMR spectrums were confirmed the suggested chemical structure for a new synthesized compound through disappearance signals and appearance of newsignals. Such as disappearance of one proton atom of the carbonyl group of aldehyde and a signal of NH_2 group of 4-methoxy aniline on spectrum as well as appearance new signal of one proton atom of imine group (Schiff base).This is approval to the formation of the new compound.

^1H-NMR spectrum of the new synthesized compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e] indol-2-ylidene)-3-(4-methoxyphenyl)imino) propanalshowedfigure 8 shows single singlet with chemical shift δ = 14.37 ppm which belonged to NH for indole ring . Also single singlet at δ = 9.74 ppm which attributed to one proton of carbonyl group .Important singale signal at δ = 8.32 ppm which assigned to new functional group of Schiff base CH=N) [13], single and doublet signals were observed in region between 6.92-8.05ppm which attributed to ten protons of aromatic rings .single singlet at δ = 3.8 ppm which attributed to three protons of methoxy group . Finally the spectrum shows strong single signal at 1.87ppm assigned to six protons of two methyl groups .

APT ^{13}C NMR was supported ^1H NMR results through appearance a number of signals of carbon atoms on APT ^{13}C NMR spectrum which were corresponding

to a number of signals of carbon atoms of the chemical structure of the a new proposed compound. Since a number of carbon atoms bearing protons of quaternary carbons were appeared at the positive side (above base line) such as some of carbon atoms of aromatic rings and indole ring, and a number of carbon atoms bearing protons of CH and CH_3 appeared at the negative side (under base line), like carbon atoms of carbonyl group, methoxy group, methyl groups and some of carbon atoms of aromatic rings.

Anti- oxidant activity

Activity of antioxidant of 2P compound was assessed via DPPH and FRAP test and compared with ascorbic acid in concentration gradient from (25, 50.100,200) µg/mL as shown in table 1. The result was revealed that the 2P compound has higher antioxidant activity.

Table 1 : Antioxidant activity value

	FRAP µmol Fe^{+2}/mg	DPPH inhibition %	IC 50 µg/mL
Ascorbic acid 25 µg/mL	45 ±1.414*	48±2.828*	27
Ascorbic acid 50µg/mL	52±1.414*	62±2.828*	
Ascorbic acid100µg/mL	63±2.828*	75.5±2.121*	
Ascorbic acid200µg/mL	73±2.828	83.5±2.121	
2P 25 µg/mL	26.5±2.121	20±2.828	75
2P 50µg/mL	38.5±2.121	37.5±3.536	
2P 100µg/mL	49±4.243	51±4.243	
2P 200µg/mL	69±1.414	77±2.828	

Data was presented as mean ±SD , *Significant at P< 0.001

Anti cancer ability

Concentration (12.5,25,50,100,200 and 400) ug/ml the viability for A547 cell and PC3 cell compared with viability of WRL 68 cell at 24h , 48h and 72 h were presented in the (Table 2) that showed effect of 2P in the viability of A547 cell and PC3 cell significantly (P< 0.05) compared with the viability of WRL 68 cell.

Table 2: Showed the Viability of the A547 cell,PC3 cell and WRL cell after treatment with Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl) IminoPropanal in different doses and different time.

Cells		Viability of cell in Concentration 2P/ ug/ml					
		12.5	25	50	100	200	400
A547 cell	24 h	95.2± 1.3	86.2±3.9	79.2±3.1	63.8±1.9	54.8±1.2	46.4±4.1
	48 h	83.2± 1.7	72.8±0.9	76.2±5.2	61.7±0.7	52.7±3.3	47.3±3.4
	72 h	65.1± 3.4	62.1±0.9	53.9±4.6	50.7±2.9	42.8±1.7	37.9±1.6
PC3 cell	24 h	96.6±1.4	96.2±0.7	90.0±2.4	63.8±2.7	37.8±1.7	25.1±1.8
	48 h	85.1± 1.2	73.2±0.7	63.3±3.0	56.1±1.2	47.4±4.1	44.9±2.4
	72 h	65.6± 4.5	60.1±1.3	50.8±1.3	44.8±1.3	40.7±1.7	33.7±2.4
WRL 68 cell	24 h	95.9± 1.0	95.2±0.8	95.3±1.2	93.6±2.1	84.8±1.2	69.3±2.6
	48 h	95.9± 1.0	95.2±0.8	95.3±1.2	93.6±2.1	88.3±1.5	79.3±0.7
	72 h	85.6± 2.0	85.4±1.1	76.9±5.8	78.4±2.3	69.6±5.2	65.7±3.7

Data was presented as mean ±SD , Significant at P< 0.05

2P induced apoptosis in PC3 cells

To prove of happened the apoptosis, the alteration in nuclear morphology of PC3 cells was examined by using Hoechest staining after treated the PC3 cells with different doses (5,10,15) µg/mL (Figure 1A).

The density of the nucleus has been found to be closely linked to apoptosis and chromatin modifications. in (Figure 1B). An rising in the permeability of the cell membrane was observed in (Figure 1C). The result was showed decreasing in the nucleus intensity and increasing in cell permeability with the increasing of concentration of 2P compound treated the cells.

2IP induced MMP disruption and release of cytochrome c.

MMP is distrusted in the apoptosis by forming of permeability, this ability of 2P on the MMP of PC3 cell was studied by using of mitochondria specific Dye, as seen in Figure 1D, the mitochondria membrane permeability in the PC3 cell processed with 2P showed significant ($P < 0.05$) diminution by decrease in the intensity of fluorescent compared with control (Figure 1A).

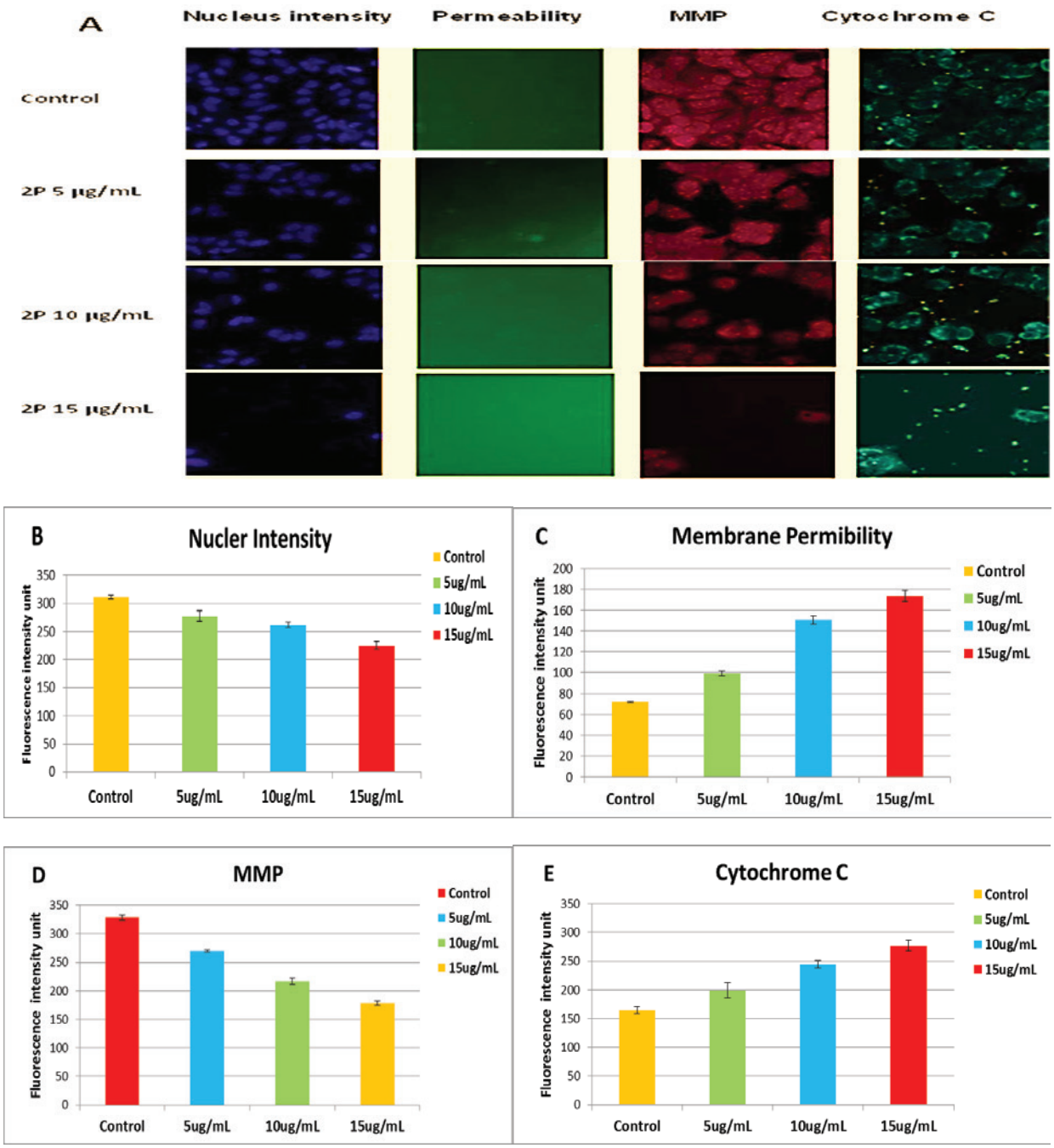

Figure 1:Effect of Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl) IminoPropanal (2P) on MMP, permeability and cytochrome c release. (A) Represented images of PC-3 cells treated with doses 5 µg/mL, 10 µg/mL, 20 µg/mL of 2P, and with Hoechst for nuclear, cell permeability dye, MMP and cytochrome c. The image from each row were obtained from the same field of each sample (magnification 20X0. (B□E) Average fluorescence intensities of Hoechst ,cell permeability dye, MMP, and cytochrome c in PC-3 cells treated with 2P. Data were presented as mean ±SD of florescence intensity readings measured from different photos taken. * Significant at P< 0.05

PC3 Cell cycle analysis

For detecting the effect of 2P on the DNA materials of PC3 cells applied the cell cycle phase divided into (G1) (G2) and (M) after therapy (Figure 2). The result was showed that 2P break down the cell cycle progress in G1 phase (P <0.05),(57.00 ± 0.283, 59.35 ± 0.354)% in 100 and 200 ug/mL respectively compared with control is (53.20 ± 0.283)% . The result display there is a significant G1 step arrest in PC3 in a concentration-dependent manner whereas, the cells in both G2 and M phase was reduced with an increased in the treatment.

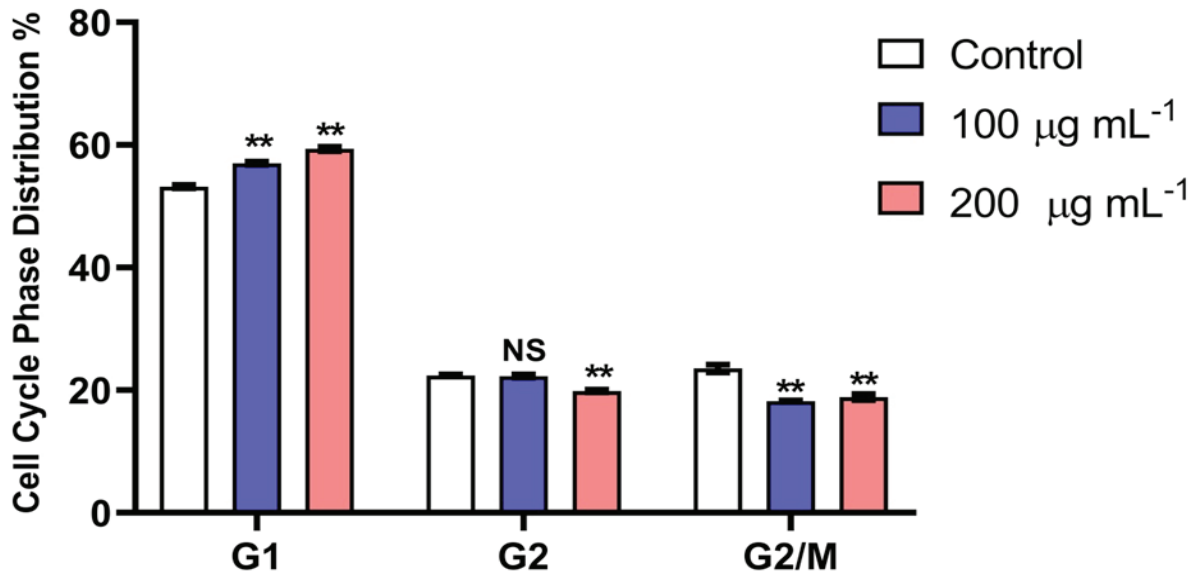

Figure 2: 2P induced G1 phase cell cycle arrest in PC-3 cell. Indication of G1 phase arrest in the cell cycle progression of PC-3 cell by 2P. **Indicate a significant difference (P < 0.05). Abbreviation: Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl)IminoPropanal (2P).

Discussion

Indole ring is an aromatic heterocyclic compound, and it is a white powder at room temperature. The indole chemical formula is C8H7N. It has an aromatic bicyclic structure consisting of a five-membered pyrrole ring fused together with a benzene ring to form two isomeric benzopyrrole. Indole derivatives are an important class of organic heterocyclic which have played vital and significant role in curing so many ailments throughout the history of pharmaceuticals and medicines. Also they are one of the most attractive frameworks with a wide range of biological and pharmacological activities [9]. Recent research has shown that the indole ring is the reactive center which handles oxidants because of its high resonance stability and very low energy activation barrier to free radical reactions[14].In this study the result of antioxidants activity was revealed that 2P compound has high level of antioxidant capacity upon the FRAP and DPPH assay as previous study reported phenols are involved in radical scavenging [15].

Many nutrient and synthetic drugs were followed to avoid or prevent oxidative damage which may lead to cell disruption to cancer cell depending on several mechanisms and means such as oxidative stress and apoptosis. This properties has been examined in this study and the result exhibited the ability of 2P compound significantly has anticancer activity for PC-3 cell.

Previous study appeared potent inhibitory effect of heterocyclic compounds which considered important class of molecules widely used for candidate as antitumor drug synthesis. The effect on proliferation in various types of aggressive prostate cancer cell lines (PC-3) dose dependent, the result significantly reducing to 80% of vehicle-treated controls [16].

A synergistic effect of new drug with effect of Titanocene−Gold Complexes Containing N-Heterocyclic Carbene Ligands Inhibit Growth of human Prostate cancer cell (PC-3) resulting heterometallic species which are highly apoptotic, exhibit strong antimigratory effects on the (PC-3) cell line [17]. A study gives a first prove in vivo that α-tomatine has antitumor affection of human androgen-independent prostate cancer(PC-3) cell line, potentially advantages was to prevention, thus acting as a therapy will need for more investigation [18]. A Natural dietary compound koenimbin reported as inhibitor of human prostate cancer, targeting PC-3-derived prostate cancer stem cells with koenimbin remarkably by induced nuclear condensation, forming of apoptotic bodies, and G0/G1 phase arrest of PC-3 cells [19].

The inspection of 2P compound anti-tumor activity effect on the PC-3 cell was achieved through Hoechst stain to tracing out nuclear morphology changing which following the treatment of the cell by using different dosage of 2P. The result was showed decreasing in the nucleus intensity and increasing in cell permeability with increasing of 2P compound concentration of treated cells. MMP changes or alteration after treating the PC-3 cells with 2P compound was studied by using mitochondria specific dye, the MMP in the PC3 cell treated with 2P showed significant ($P<0.05$) reduction by decrease in the intensity of fluorescent compared with control. The effect of cytochrome C was detected in the treated PC-3 cells with 2P compound, the result showed that 2P activated release cytochrome C significantly ($P<0.05$) higher than control. The data above reflect the ability of 2P compound to increase cell apoptosis by different ways using multiple strategies and scenarios, one of it is the alteration in membrane permeability.In this study the anti-proliferation activity of 2P compound was investigated on PC-3 cell which give a significant effecting on the cancer cells, in the other hand has less effecting on the normal cells WRL 68 cell where no effect on nuclear intensity, membrane permeability, MMP and cytochrome C. While there is a significant effect on the nuclear intensity, membrane permeability, MMP and cytochrome c in the PC-3 cell in different dose manner. Those effect because 2P improve the apoptosis processing by the antioxidant ability of 2P. Reactive oxygen species (ROS) Play a big part in the physiological process, involved in some important apoptotic signaling pathways, such as p38 and p53 pathways [20]. Programmed cell death is essential for animal development, homeostasis of the tissues and pathogenesis, apoptosis process has vital role in program cell death to maintain cell tissue homeostasis. The 2p compound has been found in this study inducing cell programmed death to PC-3 cell through apoptotic event including change in nuclear intensity, membranes permeability, release cytochrome C and DNA fragmentation in PC-3 cells. Activation of caspase-3, the primary executioner caspase in the majority of systems, irreversibly commits a cell to apoptosis. Therefore, caspase-3 activation serves as a reliable marker of apoptotic cells [21].

Appropriately to the cell cycle analysis 2P had induced PC3 cell cycle changing with increase in cell arrest in the G1phase. The result display that there is a significant G1 phase arrest in concentration dependent manner in PC3 cells. Whereas, the cells in both G2 and M phase was reduced with an increased in the treatment. The apoptosis process is highly related with cell cycle arrest. Controlling for cell cycle is essential for the maintenance of cellular division [22]. Many carcinogenicmaerial as natural and synthetic compounds were demonstrated in previous studies to enhance chemoresistance and tumorigenesis through cell cycle dysregulation and apoptosis inhibition in cancer cell [23],[24]. Like Koenimbin was induced PC3 cell cycle arrest with high percentage in the G0/G1 phase that revealed to inhibition of cellular proliferation through G0/G1 phase arrest [19].Generation of ROS interact with DNA caused alteration of the cell cycle phase [25]. DNA damage, and cell cycle perturbations induced by two representative gold(III) complexes in human leukemic cells with different cisplatin sensitivity [26].This made 2P effect on the disturb DNA lead to PC3 cell cycle arrest. The effect on the S phase arrest coupled with Bcl-2 down regulation cause anti- proliferation effect to PC3 cell [27]. Melatonin was shown that indole increases neuroendocrine markers and this cause increases cell sensitivity to apoptosis induced by cytokines [28].

proliferation properties for 2P compound have been related to effect on the transcription factors, protein kinases, Inflammatory pathways and growth signals pathways were activated by cell cycle proteins, cell adhesion molecules, and anti-proliferation proteins, but their function as antioxidants or pro-antioxidants was presumed [29].

Conclusion: 2P indole Schiff synthetic compound has anti-oxidant property and anti-proliferation effect could be promoted the role of bioactive compounds as

comparatively low toxicity, low cost, fast accessibility and potential role as adjuvant that potentiate the impact of chemotherapeutic drugs as chemo-preventive agents.

Ethical Clearance: Taken from College of Medicine, University of Diyala committee

Source of Funding: The source of funding self.

Conflict of Interest: Nil

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Detection of *Proteus mirabilis* in Healthy, sick and Diarrheal Dogs, Cats and Humans

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Abstract

The current work was conducted for isolation, identification and epidemiological frequency and distribution of *Proteus mirabilis* from humans and Dogs, cats, scanning Vitek in addition (PCR) assay was confirm detect isolates and Api 20E technique is also used in order diagnosis of *P. mirabilis* then determination of antibiotics susceptibility pattern of recovered isolates. The human isolates showed that (89.47%) were resistant Ampicillin, (42.10%) Ofloxacin, (57.14%) Cefoxitin, (57.14%) Gentamycin, (100%) Vancomycin, (57.14%) Chloramphenicol, (89.47%) Trimethoprim, (100%) Amoxiclav, (57.89%) Streptomycin, (100%) Tetracycline, (89.47%) Penicillin, (100%) Erythromycin. The dogs isolates showed resistance (64.28%) was resistant Ampicillin, (35.71%) Ofloxacin, (46.1%) Cefoxitin, (53.8%) Gentamycin, (64.28%) Vancomycin, (47.3%) Chloramphenicol, (64.28%) Trimethoprim, (64.28%) Amoxiclav, (42.85%) Streptomycin, (64.28%) Tetracycline, (64.28%) Penicillin, (64.28%) Erythromycin, otherwise the cat's isolates showed resistance (35.71%) were Ampicillin -resistant, for Ofloxacin (35.71%), for Cefoxitin (35.71%), for Gentamycin (35.71%), for Vancomycin (35.71%), for Chloramphenicol (35.71%), for Trimethoprim (35.71%), for Amoxiclav (35.71%), for Streptomycin (35.71%), for Tetracycline (35.71%), for Penicillin (35.71%), for Erythromycin (35.71%). The *Proteus mirabilis* was isolated from 33 out of 195 sample (16.92%) distributed as 19 isolated from human and 14 from Dogs and Cats. The human isolates showed that (89.47%) were resistant Ampicillin, (42.10%) Ofloxacin, (57.14%) Cefoxitin, (57.14%) Gentamycin, (100%) Vancomycin, (57.14%) Chloramphenicol, (89.47%) Trimethoprim, (100%) Amoxiclav, (57.89%) Streptomycin, (100%) Tetracycline, (89.47%) Penicillin, (100%) Erythromycin.

Keywords**:** *Proteus mirabilis*, *dogs and cats, VITEK®, Api20, Antibiotics.*

Introduction

Proteus spp. considered as G- bacillus of family of Enterobacteriaceae. Members of genus Proteus in the ecosystem are widely spread in animals and humans GI tract [(1)]. Proteus rod pathogens are opportunistic bacterial pathogens that cause infections in urinary tract (UT), infections of wound, kids or neonates meningitis, and Rheumatoid arthritis under favorable conditions [(2)(3)(4)] . E. coli have been was a widespread reason of infections being un-complicated [(5)]. Habitat of Proteus spp. is of normal flora part of gastrointestinal (GI) and urogenital tracts, and skin in dog, cat and human. It may be cross-linked with other Enterobacteriaceae, with transfer of plasmids and also, their transmission occurs as endogenous or exogenous infections[(6)] and genital infections tract, such as (epididymitis/ orchitis) and external otitis in cats and dogs, sometimes as part of mixed infections[(7)]. Other host effects, like the part of normal GI flora and part of normal skin flora in some dogs and cats. Maybe can the control via chemotherapies and this susceptibility of antibiotics, including beta-lactams, potentiated sulfonamides and cephalosporins[(8)(9)]. Also, it is a prevalent bacteremia cause following catheter-allied UT infections. At occasional circumstances, endocarditis, cellulite, empyema, osteomyelitis, and mastoiditis were seemingly caused[(10)]. It is suggested that intestines of human considered as are Proteus bacteria reservoir, particularly such belonging to the prevailing P. mirabilis species, and are several percent members of the human population of natural fecal microflora(11).

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Material and Methods

1. Collection of Samples:

105 urine Samples, stool, nasal and oral swab from diarrhea and stable people obtained, and a total of 90 samples were taken from cat and dog from urine feces, and nasal & oral swabs and usage containers.

2. Antimicrobial susceptibility test

Based on (NCCLS) recommendations, antimicrobial test of susceptibility was pereformed on media of MHA utilizing diffusion process of Kirby Bauer disc.

Results

1. Isolation of *Proteus spp*.

Table 1 Sample types, *P. mirabilis* number and % isolated from samples of human, cats and dogs

Sample types	Samples #	Isolates #	%
Human	105	19	18.09 %
Dogs and cats	90	14	15.55 %
Total	195	33	16.92%
Chi-Square (χ2)	---	---	1.608 NS
NS: Non-Significant. +			

2. Antimicrobial susceptibility

Table 2 Susceptibility as antimicrobial versus isolates of *P. spp*. in human

	Type of antimicrobial	*S	I	M.S	R
1.	Ampicillin(25)	0(0.0%)	15-16	2 (10.52%)	17(89.47%)
2.	Penicillin G(10)	2(10.52%)	11-12	0(0.0%)	17(89.47%)
3.	Trimethoprim-Sulfamethoxazole(25)	2(10.52%)	13-14	0(0.0%)	17(89.47%)
4.	Streptomycin(25)	7(36.84%)	12-14	1(5.26%)	11(57.89%)
5.	Chloramphenicol(30)	5(26.31%)	13-17	5(26.31%)	9(47.3%)
6.	Clavulanic /Amoxicillin Acid(30)	0(0.0%)	15-16	0(0.0%)	19(100%)
7.	Erythromycin(10)	0(0.0%)	14-22	0(0.0%)	19 (100%)
8.	Tetracycline(10)	0(0.0%)	15-18	0(0.0%)	19 (100%)
9.	Gentamicin(10)	8(42.10%)	13-14	3(15.78%)	8(42.10%)
10.	Ofloxacin(5)	9(47.36%)	13-17	2(10.52%)	8(42.1 0%)
11.	Cefoxitin(25)	0(0.0%)	15-16	0(0.0%)	19(100%)
12.	Vancomycin(30)	0(0.0%)	15-16	0(0.0%)	19(100%)
Chi-Square(χ2)		10.285**	---	8.452**	10.661**
** (P≤0.01)-Highly Significant.					

*S= sensitive, I= intermediate, M S= moderate sensitive and R= resistant.

Table 3; Susceptibility as antimicrobial versus isolates of *P. spp*. in dogs

	Type of antimicrobial	Dog			
		*S	I	M S	R
	Ampicillin(25)	0(0.0%)	15-16	0(0.0%)	9(64.28%)
2.	Penicillin G(10)	0(0.0%)	11-12	0(0.0%)	9(64.28%)
3.	Trimethoprim-Sulfamethoxazole(25)	0(0.0%)	13-14	0(0.0%)	9(64.28%)
4.	Streptomycin(25)	3(21.42%)	12-14	0(0.0%)	6(42.85%)
5.	Chloramphenicol(30)	1(7.14%)	13-17	0(0.0%)	8(57.14%)
6.	Clavulanic /Amoxicillin Acid(30)	0(0.0%)	15-16	0(0.0%)	9(64.28%)
7.	Erythromycin(10)	0(0.0%)	14-22	0(0.0%)	9(64.28%)
8.	Tetracycline(10)	0(0.0%)	15-18	0(0.0%)	9(64.28%)
9.	Gentamicin(10)	1(7.14%)	13-14	0(0.0%)	8(57.14%)
10.	Ofloxacin(5)	4(28.57%)	13-17	0(0.0%)	5(35.71%)
11.	Cefoxitin(25)	1(7.14%)	15-16	0(0.0%)	8(57.14%)
12.	Vancomycin(30)	0(0.0%)	15-16	0(0.0%)	9(64.28%)
Chi-Square(χ2)		8.239**	--	0.0 0NS	10.472**
** (P≤0.01)-Highly Significant., NS: Non-Significant.					

*S= sensitive, I= intermediate, M S= moderate sensitive and R resistant.

Table 3. Susceptibility as antimicrobial versus isolates of *P. spp*. in cats

#	Type of antimicrobial	Cat			
		*S	I	M S	R
	Ampicillin(25)	0(0.0%)	15-16	0(0.0%)	5(35.71%)
	Penicillin G(10)	0(0.0%)	11-12	0(0.0%)	5(35.71%)
	Trimethoprim-Sulfamethoxazole(25)	0(0.0%)	13-14	0(0.0%)	5(35.71%)
	Streptomycin(25)	0(0.0%)	12-14	0(0.0%)	5(35.71%)
	Chloramphenicol(30)	0(0.0%)	13-17	0(0.0%)	5(35.71%)
	Clavulanic /Amoxicillin Acid(30)	0(0.0%)	15-16	0(0.0%)	5(35.71%)
	Erythromycin(10)	0(0.0%)	14-22	0(0.0%)	5(35.71%)
	Tetracycline(10)	0(0.0%)	15-18	0(0.0%)	5(35.71%)
	Gentamicin(10)	0(0.0%)	13-14	0(0.0%)	5(35.71%)
	Ofloxacin(5)	0(0.0%)	13-17	0(0.0%)	5(35.71%)
	Cefoxitin(25)	0(0.0%)	15-16	0(0.0%)	5(35.71%)
	Vancomycin(30)	0(0.0%)	15-16	0(0.0%)	5(35.71%)
Chi-Square(χ2)		0.0 0NS	---	0.0 0NS	0.0 0NS
** (P≤0.01)-Highly Significant., NS: Non-Significant.					

*S= sensitive, I= intermediate, M S= moderate sensitive and R= resistant.

Discussion

This study revealed the prevalence of *P. mirabilis* (18.09%,) in human, this not agreed with [12], who stated that *P. mirabilis* infections prevalence is (90%). Although, it was higher than the other recorded with [13] [14]. In addition, our study recorded lower percentage than [16][17]. In clinical infections, *P. mirabilis* are further widespread compared to other *P.spp*., due to that *P. mirabilis* is normal flora part of human as well as mammalians which causes water contamination and this not agreed with [18], who reported (7.8%).According to gender distribution this study showed that the human females (11.42%) are more susceptible to infection with *P. mirabilis* than males (6.66%) and this agreed with [14]. The current work showed infections by Proteus were identified in all groups of age from <1 to 70 years where 20-35 years group of age recording as the highest infected group while age groups from 50 to 70 years recording as low group infected. On other hand the age group from <1 to 12 years which recorded moderate (18.09%) was the predominant species isolated from various clinical samples in different studies as reported [19][17], who reported (52.54%).Females were observed as more susceptible in Proteus acquiring infections comparing to males. In our study recorded 36.84% of *P. mirabilis* were isolated from whole human urine positive samples where these samples are higher than [20] [21], who recorded (19.64%) [19], who recorded (1.12%), which was highly level when compared with findings of [22], who reported a prevalence rate of (19.3%) in human urine samples while according to and[23] who reported an overall prevalence of 17.6%. In contrast, [24] reported a lower prevalence of *P. mirabilis* (4.8%) in urine samples of humans. On other side[25] mentioned *Proteus mirabilis* was (66%) this percentage not agreed with our study *P. mirabilis* has a greater tendency for UT colonizing because of pathogenicity difference [26]. *P. mirabilis* is associated commonly with both UT infections of community-assimilated and catheter-related, pyelonephritis and cystitis. They are associated less frequently in respiratory infections, endophthalmitis, and infections with CNS like [27][28].This study shows that is females infection are (63.15%) which is higher than[29][19] but the percentage of male's infection are (36.84%) lower than [29] as well as[19]. In the present study, the *Proteus mirabilis* show different percentage of resistance to antimicrobial drugs in human and animal (cat and dog). Such isolates are of non-lactose pale along fishy odor fermented, motile (swarming phenomenon) and lead to βhemolysis colonies on agar of blood, based on [30]. Furthermore, the latter reason is probably because the VITEK 2 system has a greater spectrum of microorganisms in its database with respect to the Micro-Scan system, and thus there could be differences in the software used in the equipment of both systems, as has been reported in other systems. Studies(31)(32) Although the results of identification of *P. mirabilis* using Vitek system showed all isolates were *P. mirabilis* and the percentage of identification was ranged from (95-99%), and this percentage was in agreement with[33] as it was stated that reported that *P. spp*. identification via system of Vitek 2 was in 97%. Furthermore, such isolates are of non-lactose pale along fishy odor fermented, motile (swarming phenomenon) and lead to βhemolysis colonies on agar of blood, based on [30]. In human isolates show (100%) resistance to Amoxicillin / Clavulanic Acid, Erythromycin, Tetracycline, Cefoxitin and Vancomycin. On the other hand, streptomycin was showed (57.89%) resistant and not agreed with [34] who was reported (0.0%) may be due to etiological UT infections agents and their patterns of susceptibility/ resistance differ based on locations of geography [35] may be because of likely recurrent UT infections attacks among such group causing recurrent antimicrobial use, catching antimicrobial being wrong for bacteriuria as asymptomatic, or others infections treatment [36]. The reason for this may be due to elderly people especially females are recognized to be disposed to develop bacteriuria as asymptomatic and UT infections as recurrent, that associated along factors of risk among such gender and age category[37].In addition, these bacteria were able to generate β-lactamases, in particular extended spectrum of β-lactamases (ESBLs), along their capability to transfer elements of genetic transporting the such enzymes genes, and mutations variety take place along such enzymes forms, causing increased resistance to antibiotic, in particular β-lactam, besides other mechanisms i.e., alt-site alteration or target site alteration (38)(39). Proteus mirabilis were isolated from dog urine with ratio (14.28%) to sick case and this isolation percentage frequencies vary among different reports as (9 - 32%) for Proteus mirabilis according to[40] and due to In UTIs an underlying abnormality exists that predisposes the dog to develop a UTI or fail treatment. This can include abnormal function or anatomy of the urogenital tract, or concurrent diseases, all of which may predispose the dog to acquire a UTI or make resolution difficult the prostate [41]. As for the isolation of dogs and cats, showed resistant many antibiotics in this study and this resistance can be due to diseases variety including

UT infections and diarrhea [42]. Likewise, the specific physiological bacterium characteristics promoted its resistance to drug [43]. Otherwise, acquired antimicrobial resistance in cats was determined by investigated population social environment. It is clear that healthy cat's flora may behave as resistance genes reservoir. Or may be attributable to differences of the antibacterial sensitivity tests performed.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

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Cryopreservation of *Dama. Dama* (Fallow Deer) Semen using Tris Extender Treated with Soya Milk and Omega3

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Abstract

The experiment was conducted in the Agricultural research's station due to the College of Agriculture/ Al-Muthanna University in the south of Iraq, to study the effect of adding soybean milk and omega3 to the tris extender on the quality of *Dama.Dama* (*D.Dama*) Gazelle semen under cryopreservation- thawing conditions. The semen was collected from four adults males of *D.Dama* Gazelle using electric ejaculator system .The experiment was contains four treatments :the control treatment using the traditional tris extender only , T1.adding 5% soybeans to the tris extender , T2.adding 2% omega3 to the tris extender, T3. Adding 5% soybeans + 2% omega3 to the tris extender. It was observed highly significant effect($p \leq 0.01$) of adding 5% soybean(T1) and adding 5% soybean+ 2% omega3(T3) to the tris extender on the sperm total motility% and progressive motility % associated with highest viability percentage(explained by the percentage of live sperms) and significant superiority in the results of the HOST test was noticed in this two treatments (T1 and T3).This results refers to better cryopreservation success of *D. dama* Gazelle spermatozoa in particular it was associated with a very good results of acrosome integrity in all treatments, this is the first study in Iraq deals with cryopreservation of *D.Dama* Gazelle semen, that helps to application of artificial insemination and (for the future) to conservation of the genetic resource for this endangered species.

Keywords: *Dama. Dama, fallow deer, Tris extender, soya milk, omega3*

Introduction

The geographic distribution of *D.Dama* (fallow deer) is expanded from North and South America, Islands, Europe, South Africa, Australia, New Zealand, southern European regions, Asia Minor, along the Mediterranean Sea, it's have an ability to live in a variety of climates ranging from cool-humid to warm-dry areas [1, 2]. The fallow deer includes two subspecies distinguished: *D.Dama* (European fallow deer and *D.Dama* Mesopotamia (Persian fallow deer) [3] . In the breeding season, the males of fallow deer are fight often and violently. Since they are easy to breed, they are present in almost all of the larger zoos. In addition, fallow deer are raised on large, fenced, unfertile meadows as domestic animals [2, 4], this reasons leads to searching to an ideas to reduce the number of males in the herd without affecting the reproductive efficiency of this endangered animals. Semen preservation and artificial insemination could become powerful tools for the genetic management of gazelle breeding programs since these assisted reproductive techniques would allow the storage of semen from genetically valuable animals extend generation times, circumvent husbandry or health problems that may prevent certain animals from breeding, and may facilitate transfer of semen between subpopulations that are geographically or biologically isolated [5, 6 7]. We have undertaken this study to explore the ability of cryopreservation of spermatozoa from D.Dama Gazelle semen. We sought to improve freezing protocols includes adding the soya milk and omega3 to the Tris extender.

According to our knowledge, this is the first study in Iraq deals with cryopreservation of D.Dama Gazelle semen, and the first study in the world to use the soya milk and Omega3 for cryopreservation of D. Dama Gazelle semen.

Materials and Methods

This study was conducted in the Agricultural research's station dependent to the college of Agriculture, Al-Muthanna University, to investigate the

effect of adding the soya milk and omega3 to the tris extender on some characteristics of fallow deer semen under cryopreservation conditions. A four adults male of fallow deer were uses as a semen donors subjected to veterinary health care and feeding protocol included wheat bran 500 gm/day and alfalfa .The semen was collected from all males using electric ejaculator * and initially assessed directly after collection then divided and diluted according to the treatment groups which: control (diluted with tris extender only), T1(diluted with tris extender +5% soya milk), T2(diluted with tris extender +2% omega3), T3 (diluted with tris extender +5% soya milk+ 2%omega3), the semen on all treatments was loading into 250 µl straw** and subjected to cryopreservation protocol to two months. Where the final concentration of sperm was estimated after dilution to be 200 million sperm / cm3. Three straw from any treatment ware thawed and assessed after two months, the results were recorded and subjected to the statistical analysis using SPSS program. The studied traits are as follows:

1**. Acrosome integrity:** Tested by reading the shape of the acrosome under a microscope with a magnification force of 100 using the oil Immersion.

2**. Sperm viability:** The semen vitality test was performed by mixing semen (whether cool or frozen), with Eosin-nigrosin stain, in a ratio of 1: 3, a smear was then carried out on clean, dry slides at body temperature, leave the slides to dry and then read under the microscope with a magnification strength of 40.

3**. General motility:** The general motility of all groups was evaluated, with a Computer Assisted Sperm Analysis; CASA device, it is estimated according to the random movement of the sperm.

4**. Progressive motility:** The previous device was used to estimate the natural movement (forward movement) and distinguish it from the other abnormal movements.

Preparation of soybean milk: Soybean milk has been prepared by weighing 10 g of soybeans with a sensitive scale, they were soaked overnight at 100 ml of distilled water, then the water was removed, the soybeans were washed thoroughly washed again with distilled water, add 100 ml of distilled water and heated between 80-100 m 0 for 20 minutes, immediately after heating, it is mixed with a high speed mixer for 5 minutes until the formation of the Slurry, after forming the Slurry it was heated again for 10 minutes with continuous stirring and maintaining the temperature between 80-100°C, to discontinue the activity of lipoxygenase, the Slurry was cooled and filtered with a clean gauze cloth, then the centrifuges were carried out at a speed of 4000 rpm for 20 minutes, for complete disposal of solids and set their PH level to [7, 8]. Preparation of semen Extender, semen Extender was prepared according to Baiee et al. [9].

Results

Effect of cryopreservation on the physical traits of *D.Dama* semen

The results of physical traits assessment of *D. Dama* semen after cryopreservation – thawing ware presented in the table (1). It was observed highly significant effect($p \leq 0.01$) of adding 5% soybean (T1) and 5% soybean+ 2% omega3(T3) on the sperm total motility% and progressive motility % . The highest viability percentage was noticed in T1 and T3 were 71.04±1.20 and 70.54±1.64 respectively as compared with control group, the effect treatment in the acrosome integrity showed a non-significant differences between the control group, T1 and T3 were significantly differs ($p \leq 0.05$) from T2 were 98.29±0.22, 98.54±0.14, 97.70±0.26 and 98.00±0.12 to the control, T1, T2 and T3 respectively. .In the HOST test there was non-significant differences between T1and T3 were significantly differs ($p \leq 0.01$) from the control and T2 were 59.33±1.06, 71.08±1.97, 62.75±0.88 and 68.91±1.72 to the control group, T1, T2 and T3 respectively.

Table 1. Effect of Treatment on some semen traits (Mean± Standard error).

Treatment	Traits				
	Motility%	Progressive motility%	Viability %	Acrosome integrity%	HOST test
Control	56.33±1.67c	45.83±2.20b	64.20±1.30b	98.29±0.22ab	59.33±1.06b
T1	64.17±2.02 ab	49.58±1.29ab	71.04±1.20a	98.54±0.14a	71.08±1.97a
T2	59.67±1.56bc	39.58±2.49c	66.91±1.53ab	97.70±0.26b	62.75±0.88b
T3	65.67±1.20a	54.17±1.92a	70.54±1.64a	98.00±0.12ab	68.91±1.72a
Sig.	0.01	0.01	0.01	0.05	0.01

control treatment: tris extender , T1: first treatment: 5% soybeans, T2: second treatment: 2% omega3, T3: third treatment: 5% soybeans + 2% omega3. * Averages with different letters within one row indicate significant differences (P≤0.05) between the treatments

Effect of cryopreservation on the total abnormalities % and dead sperms % of *D.Dama* semen.

The results of total abnormalities % in the *D.Dama* semen after cryopreservation – thawing ware presented in the figure (1). It was observed non- significant effect of adding different concentrations of soybean and omega3 on the sperm total abnormalities %, while it was observed highly significantly (P≤0.01) differences between the control and T2 that significantly differs from T1 and T2 in the dead sperms %, the means were13.33±1.20, 17.00±3.46, 20.67±6.11and 16.00±5.77 to the control, T1, T2 and T3 respectively figure (2).

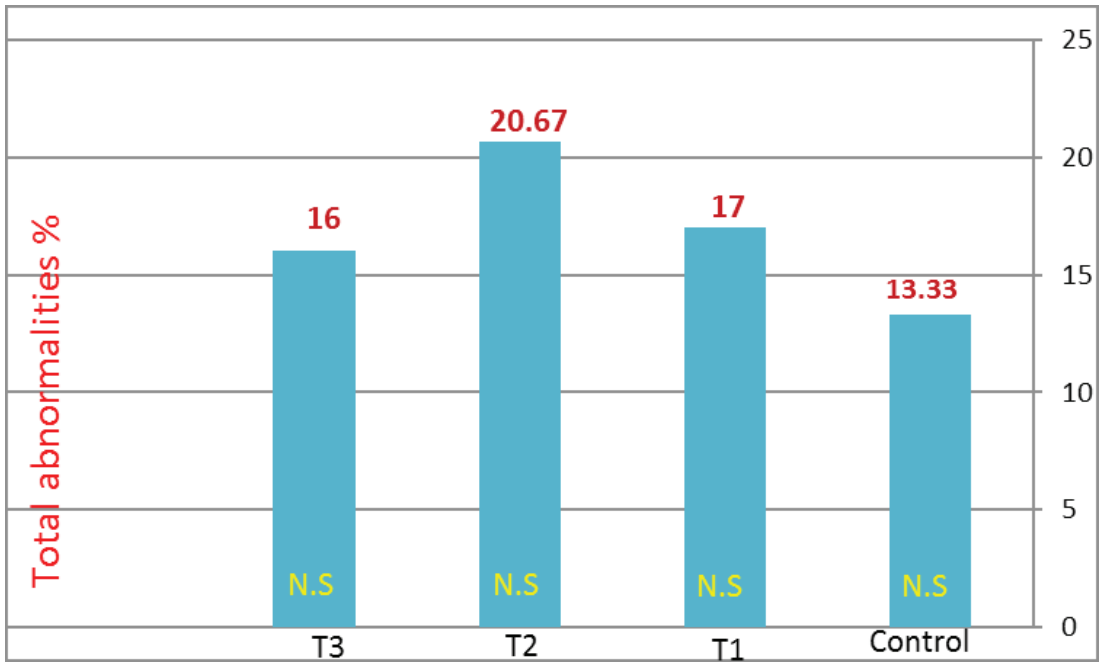

Figure 1 : Effect of treatment on the total abnormalities of *D.Dama* semen after cryopreservation - thawing

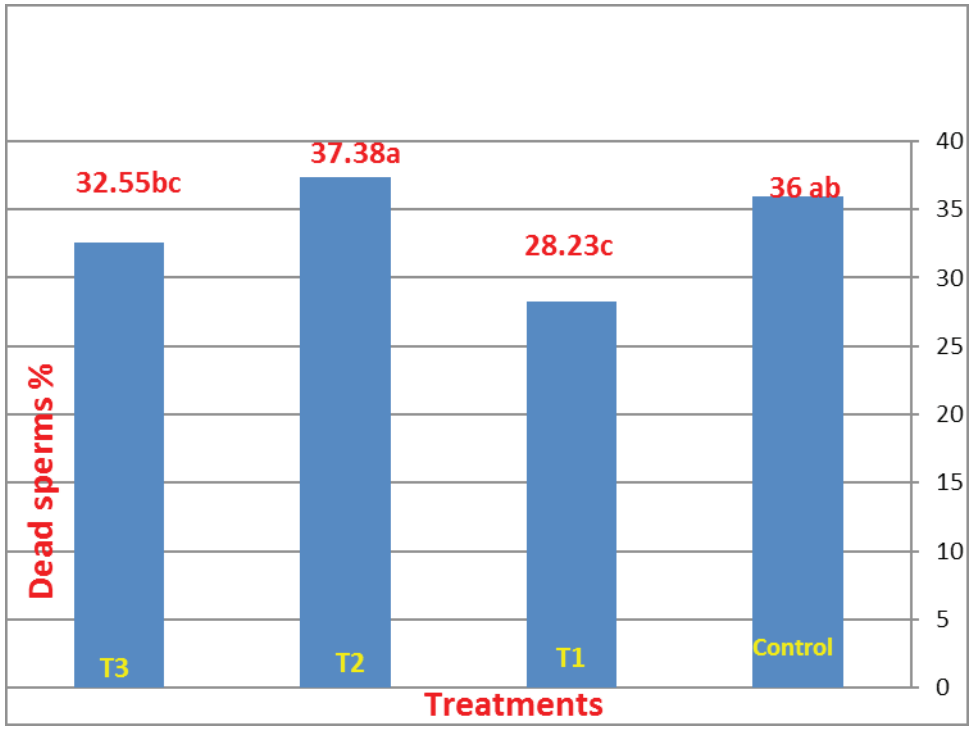

Figure 2 : Effect of treatment on the dead sperms % of *D.Dama* semen after cryopreservation – thawing.

Discussion

The experiment was conducted to find the effect of adding soybean milk and omega3 on the physical traits of cryopreserved semen of *D. Dama* Gazelle. It was observed highly significant effect ($p \leq 0.01$) of 5% soybean (T1) and adding 5% soybean+ 2% omega3 (T3) on the sperm total motility% and progressive motility % associated with highest viability percentage (explained by the percentage of live sperms) and significant superiority in the results of the HOST test was noticed in this two treatments (T1 and T3).This results refers to better cryopreservation success of *D. dama* Gazelle spermatozoa in particular it was associated with a very good results of acrosome integrity in all treatments, that helps for the future to conservation of the genetic resource for this endangered species. (10, 11) .There is a critical factors that affect cryopreservation success of spermatozoa from different species such as the equilibrium period [12] cooling rates before cryopreservation [13] the suitable buffer system, energy resources concentrations of egg yolk (14, 15) , type of cryoprotectant, [16].

The oxidative stress regards as one of very important factors that affect the semen quality after cryopreservation - thawing so that there is an necessity to addition of anti-oxidants and plant extracts to avoid the negative effects of peroxidation that affect the integrity of sperm membrane and the DNA damage[17, 18, 19] .Our results ware indicate that the spermatozoa of *D. dama* was better resist the freezing process whereas using the tris extender with Omega3 that decrease the production of the Reactive Oxygen Species (ROS) and increase the fluidity of sperm membrane [20] aside from the role of soya lecithin's for supporting the membrane structure, permeability,and elasticity influence the extent to which sperm will survive against the cryopreservation damage and the negative effect of peroxidation that occurs during thawing process [21].

Conclusion

Our results indicate that successful cryopreservation of D.Dama gazelle spermatozoa can be obtained using a tris extender containing 5% soya milk or 5% soya milk+ 2% Omega3 but that further studies is required for the improvement the sperm freezing protocols for this endangered species to ensuring the purpose of establishing the genetic resource bank.

Conflict of Interest: None

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Impact Evaluation of Gender and Age on Percentage Distribution of Candidiasis and their Control by Medicinal Mushroom in Laboratory

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Abstract

Biological control represents an important approach for controlling many pathogenic fungi. *Pleurotus ostreatus* is the most promising and effective bioagents against many pathogenic fungi. In this paper seventy clinical specimens were collected from hospitalized patients, who were aged between (1 and 71) years old for both genders. The results of this study showed that out of 70 specimens that collected from urine, Skin, Vaginal and Oral specimens, (61) specimens (87.14%) were positive for fungal infections and (9) specimens (12.86%) were negative. Out of (61) positive specimens, (50) specimens (82%) were positive for *Candida* spp, (7) specimens (11%) were *Trichophyton* spp and (4) specimens (7%) were *Microsporum* spp. Out of (50) positive specimens for candidal infections, *Candida albicans* was the more prevalence fungus (66%) followed by the fungus *Candida glabrata* (14%). The maximum infection incidence has been recorded in the age group of (21- 40) years and the women have been considerably more than the male in all tested age groups. *Pleurotus ostreatus* culture filtrate affected the growth of *Candida* spp and the effect had been increased with the increasing of the filtrate concentrations. The more sensitive species was *C. tropicalis* and C. *krusei* than the other species.

Key words: *Candidiasis, Biocontrol, Medicinal Mushroom, Pleurotus ostreatus.*

Introduction

Fungi were ubiquitous and might grow on the skin, in intestinal tracts and mucous membranes[1]. *Candida* species are normal microbiota of the respiratory tract, gastrointestinal tract, mouth and vagina, and cause opportunistic infections when altered host conditions enable the fungus to proliferate. Although genitourinary candidiasis can occur in both immunocompetent and immunocompromised persons, it is a cause of morbidity and mortality in immunocompromised patients in particular[2]. Gender and age, could be advanced as possible factors in community and hospital acquired infections[3]. Also, the life style of an individual or a group of people within a given community can primarily be a risk factor[4] (Aaron *et al*., 2017). The incidence of candidiasis varies in relation to gender, host susceptibility and hospital settings. The incidence is higher in women and among hospitalized patients[5]. Antimicrobial compounds played a necessary role in the treatment of these diseases. Antifungal resistance has been suggested with long-term antifungal use and with recurrent infections, such as those with persistent mucocutaneous candidiasis or recurrent oropharyngeal candidiasis in patients with uncontrolled human immunodeficiency virus infection[6].

Antimicrobial agent from natural biological supply such as fungal sources is needed. *Pleurotus ostreatus* is a mushroom that used now not for meals consumption since historical instances due to their dietary values and taste but for their medicinal properties. The bioactive substances contained in both the mycelium and fruiting bodies of *P. ostreatus* have been the major sources of exhibit antiarthritis, anti-inflammatory, antidiabetic, immunostimulatory, antioxidative properties, antifungal and antibacterial[7,8]. Emoghene and Onwudinjo[9] proved the antifungal activity of ethanol and aqueous extract of *P. ostreatus* against *C. albicans*. Similar studies were conducted about this field and revealed that the extract of *P. ostreatus* affect the growth of *Candida* species[10,11]. This study is intended for the evaluation of the distribution of *Candida species* isolated from patients in hospital along gender and age divides and

for the evaluation the anticandidal effect of *Pleurotus ostreatus* extract.

Materials and Methods

Collection of samples

Seventy clinical specimens were collected from hospitalized patients who were aged between (1 and 71) years old for both genders, suspected have infection with candidiasis and suffering from symptoms of stomatitis, dermatitis, endometriosis and Pain when urinating. The specimens taken from different sources include (Skin, Urine, Oral and Vaginal thrux). The pathogenic fungal isolate which grown on SDA medium has been identified morphologically according to the microscopic examination, cultural characteristics, growth rate, texture, colony size, germ tube test and Vitek 2 system.

Preparation of *Pleurotus ostreatus* extracts

Twenty grams (20g) of *Pleurotus ostreatus* dried fruiting bodies powder were soaked in 180ml of absolute ethanol for 24hrs and stirred every 6hrs and then filtered through Whatman No.1 filter paper, then concentrated using the rotary evaporator at 40°C until a paste was formed, solutions produced by dissolution of (0.5g) of the extracts in (10ml) of the normal saline to prepare stock solution (S) [12].

Biochemical test (VITEK 2 system)

Vitik-2 system was used in this study in order to diagnose the *Candida* spp to species level according to Sariguzel *et al*., [13].

Effect of *Pleurotus* extracts on Candidal growth

Effect of *Pleurotus* extracts by disc diffusion Method.

Sterilized agar plates have been utilized based on the disc diffusion assays[14], filter paper discs (5.0 mm in diameter) have been impregnated with a variety of the concentration values [50 (S), 25 (S/2) and 12.5 mg/ml (S/4)] of *Pleurotus* extracts. Impregnated disc have been placed at periphery of *Candida* culture plates, the activity has been assessed through the measuring of the inhibition zones of the fungal growth.

Effect of ***Pleurotus*** extracts by poisoned food technique.

The effect was performed according to Kumar *et al*.,[15] different volumes of *Pleurotus* extracts (stock solution) were mixed with the molten SDA media for obtaining the ultimate concentration values of 50%, 25% and 10%, the medium has been put in Petri-plate and inoculated with candidal inoculums, the Petri-plate were incubated at 30ºC. Colony forming unit were calculated by using haemocytometer.

Results and Discussion

Isolation and identification of *Candida* species

The *Candida* species have been characterized by the morphology of the colony, cultures which have dry, white-creamy color, opaque, pinpoint and fluffy colony morphology on the Sabouraud dextrose agar have been suspected as species of the *Candid.* The identification of the species-specific has been made by germ tube test and Vitek 2 system.

Germ tube testing

The results showed that 68% (n=34/50) species have been germ tube positive and have been identified as *C. albican*s. Approximately 32% (n=16/50) species produced no germ tubes and have been identified to be non albican *Candida* species. Two *Candida tropicalis* strains have given false positive results for the *Candida albicans*. The false negative results have been noticed for 1 *C. albicans* species. The sensitivity of this approach for the *Candida albicans* has been 97%.

Biochemical test (VITEK 2 system)

The results of this test confirmed the identification of *Candida* species by morphological features and showed that all *Candida* spp. strain has been successfully identified by Vitek 2 YST card. The diagnosis of *Candida* species gave the diagnostic results of (98%) unequivocal identification, (2%) low discrimination correct results and there was no false identification. *Candida albicans* has been the most common amongst all species of *Candida* which was 33(66%) isolates , *C. glabrata, C. Tropicalis* and *C. krusei* were 7(14%), 8(16%) and 2(4%) strains, respectively.

The result agree with the result of Sanguinetti *et al*.[16] who found that out of 750 isolates, (98.2 %) were identified correctly to the species level by using VITEK 2 system, including those isolates identified with low discrimination but resolved by additional tests, two isolates (0.3 %) were identified with low discrimination and not resolved by additional tests, eight isolates (1.0

%) were misidentified, and four isolates (0.5 %) could not be identified by the VITEK 2 system.

Distribution of fungal species according to the type of specimen and gender

The results of this study showed that out of 70 specimens were collected from different sources include (skin, urine, oral and vaginal thrux), 61(87%) specimens were positive for fungal infections and 9(13%) specimens were negative. Out of (61) positive specimens, 50(82%) specimens were identified as *Candida* spp, 7(11%) specimens were *Trichophyton* spp and 4(7%) specimens were *Microsporum* spp (Table 1).

Table (1): Distribution of fungal species according to type of specimen

Fungal spp.		Clinical specimens (n = 61)				Total	Total percentage
		Oral	Skin	Vaginal	Urine		
Candida spp.	C. albicans	4 (100%)	1 (8%)	12 (75%)	16 (55%)	33	50 (82%)
	C. tropicalis			1 (6%)	7 (24%)	8	
	C. glabrata			3 (19%)	4 (14%)	7	
	C. krusei				2 (7%)	2	
Trichophyton spp.			7 (58%)			7	7 (11%)
Microsporum spp.			4 (34%)			4	4 (7%)
Total		4 (100%)	12 (100%)	16 (100%)	29 (100%)	61	61 (100%)

The result was agree with the results of Al-Oebady[17] that found the most prevalent yeast isolates from vaginal swabs, urine samples and oral thrush samples are *C. albicans* 58/92 (63%), *C. glabrata* 13/92 (14.2%), *Trichosporon* sp. 5/92 (5.43%) and *Geotrichum* sp. 1/92 (1.08).

Sheevani *et al*.[18] referred that the infections caused by *Candida* species was the most significant opportunistic mycosis worldwide, in addition to being amongst the leading nosocomial infections causes.

The result also showed that out of (50) positive *Candida* specimens, 17(34%) specimens and 33(66%) isolated from male and female respectively (Table 2).

Table (2): *Candida* species gender wise distribution (n = 50)

Gender	Candida spp.				Total
	C. albicans	C. glabrata	C. tropicalis	C. krusei	
Male	11 (22%)	2 (4%)	3 (6%)	1 (2%)	17 (34%)
Female	22 (44%)	5 (10%)	5 (10%)	1 (2%)	33 (66%)
Total	33 (66%)	7 (14%)	8 (16%)	2 (4%)	50 (100%)
* P - Value = 0.0050 significant					

The result was compatible with the results of Heidrich *et al*.[19] who found that *Candida* genus has been of a higher prevalence in the females (5.84% males and 15.90% of the females), moreover, it had an impact over the females who have been older than the males (54 vs. 47 years, respectively).

Distribution of *Candida* infection in various age groups

The results also revealed that the *Candida* species distribution in the males and females has been different with the difference of age, it has been observed that the *C. albicans* has been the prevalent one amongst *Candida* species and in male and female and in all age groups. The result had been revealed that the maximum infection incidence has been recorded in the age group of (21 - 40) years, followed by the age group of (41 - 60) years and the minimum infection incidence has been recorded in the age group of (61 - 71) years (Table 3).

Table (3): Gender wise *Candida* species distribution in different ages

Age (Years)/Gender		Candida spp. (n = 50)				Total
		C. albicans	C. glabrata	C. tropicalis	C. krusei	
1 - 20	M	3	1	0	0	4 (8%)
	F	5	1	0	0	6 (12%)
21 - 40	M	4	1	1	1	7 (14%)
	F	9	2	2	1	14 (28%)
41 - 60	M	3	0	1	0	4 (8%)
	F	5	2	2	0	9 (18%)
61 - 71	M	1	0	1	0	2 (4%)
	F	3	0	1	0	4 (8%)
Subtotal	M	11	2	3	1	17 (34%)
	F	22	5	5	1	33 (66%)
Total	M+F	33 (66%)	7 (14%)	8 (16%)	2 (4%)	50 (100%)
* P - Value = 0.0427 significant						

The result was agree with the results of Loster *et al*.[20] who found that the maximum occurring of candidiasis has been recorded in age group of less than (50) years which was (61.4%) followed by the age group (50 – 60) years which was (43.7%).

The effect of *Pleurotus* culture filtrate

The effect of culture filtrate by poison food technique

The results showed different sensitivities of *Candida* species to the *Pleurotus* filtrate and the more sensitive species are *C. tropicalis* and C. *krusei* than the other species. Lowest cell account was recorded in case of *C. tropicalis* at the concentration of (50%) of the *Pleurotus* filtrate stuck solution on SDA which was (1.51 * 10^8 cell/ml) and the highest one was recorded at the concentration (10%) which was (1.95 * 10^8 cell/ml) for species *C. glabrata* which was more resistant to *Pleurotus* filtrate than the other species (Table 4).

Table (4): Effects of *P. ostreatus* culture filtrate on the growth of *Candida* spp. by poison food technique

Treatments	Concentration %	Cell account (cell/ml)			
		C. albicans	C. glabrata	C. tropicalis	C. krusei
Pleurotus ostreatus	50	1.56 * 108	1.58 * 108	1.51 * 108	1.52 * 108
	25	1.75 * 108	1.79 * 108	1.72 * 108	1.72 * 108
	10	1.93 * 108	1.95 * 108	1.92 * 108	1.93 * 108
Control	-	2.06 * 108	2.10 * 108	2.02 * 108	2.04 * 108

The result was agree with the study achieved by Phan *et al.*[21] to evaluate the anti-Candidial activities of the ethyl acetate, methanol and the aqueous extracts of the *Pleurotus giganteus* against the *Candida* species and revealed that the aqueous extract had minimum activity while the extract of the ethyl acetate entirely inhibited all *Candida* spp.

The effect of culture filtrate by disk diffusion method

The results showed that the highest inhibition zone that has been recorded for the *C. tropicalis* at the concentration (50 mg/ml) has been 8 mm, and the smallest inhibition zone was recorded at a concentration of (25 mg/ml) which has been 6 mm, there is no any inhibition at the concentration (12.5 mg/ml) (Table 5).

Table (5): Activity of *P. ostreatus* extracts against *Candida* species by disk diffusion method

Treatments	Concentration	Zone inhibition (mm)			
		C. albicans	C. glabrata	C. tropicalis	C. krusei
Pleurotus ostreatus	50 mg/ml	7	7	8	7
	25 mg/ml	6	6	7	6
	12.5 mg/ml	0	0	0	0
Control	-	0	0	0	0

The medicinal types of mushroom are rather less researched for the anti-fungal characteristics they have, none-the-less, in the past 5 years, there was an increased level of the interest in their use as antimicrobial agents, the oyster mushroom (*Pleurotus ostreatus*) as well as other medicinal mushroom types have been exhibiting anti-fungal activities against the *C. albicans*[22].

Conclusions

Candida albicans was the more prevalence fungus. The maximum infection incidence has been recorded in the age group of (21- 40) years and the women have been considerably more than the male in all tested age groups. *Pleurotus ostreatus* culture filtrate affected the growth of *Candida* species.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional ethical committee

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Sinus Tachycardia as a Predictor of Severe Carbonmonoxide Poisoning

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Abstract

Introduction: Carbonmonoxide (CO) poisoning is one of the most frequent intoxications in the world. The heart is one vital organ affected in CO intoxication. In this study, we aimed to investigate characterstics and electrocardiographic abnormalities of patients with CO poisoning.

Material and Methods: In a two-year period, we analyzed patient records admitted to our Emergency Department (ED), retrospectively. Demographical characteristics (age, gender, monthly distribution, sources of poisoning, etc), vital signs, laboratory findings, Glasgow Coma Scale (GCS) scores and ECG findings were investigated. Additionally, treatment method, source of exposure and length of stay (LOS) in the ED were investigated. Then, patients were divided into 2 groups as to normobaric oxygen therapy (NBO) and hyperbaric oxygen therapy (HBO) groups according to the treatment methods. Two groups were compared in terms of electrocardiographic abnormalities.

Results: Atotal of 75 patients were involved. The most common source of CO poisoning was stoves (n=71, 94.6%). The most common complaint was found to be nausea and vomiting (n=24, 32%). Mean GCS score of the patients was 14. Mean carboxyhemoglobin levels were found to be 15%, mean saturation was 95.9%. Five (6.6%) of the patients have undergone HBO in addition to NBO. The most common ECG finding was found to be normal sinus rythm in 52 patients (69.3%), followed by sinus tachycardia in 10 patients (13.3%), bradycardia in 4 patients (5.3%), ST depression in 2 patients (2.6%). Four of 5 patients who underwent HBO had tachycardia on ECG.

Conclusion: Use of stoves for heating purposes increases the risk for poisoning particularly in winter months. Although physicians must be aware of its ischemic effects of CO poisoning on cardiovascular system, sinus tachycardia may be a predictor of CO poisoning severity.

Keywords: *Carbonmonoxide poisoning, electrocardiogram, hypoxia, Emergency Department*

Introduction

Carbonmonoxide (CO) is an odorless, colorless, clear gas that attaches to the hemoglobin (Hb) with an affinity approximately 200 times higher than that of oxygen, and consequently blocks the capacity of hemoglobin (Hb) to carry oxygen[1]. Besides Hb, CO causes tissue damage by asphyxia and cellular hypoxia via binding to, myoglobin, and cytochrome p450, and by ischemia and reperfusion injury[2]. Since the central nervous system and cardiovascular system are known to be the areas most sensitive to this poisoning, CO can induce a tendency toward coronary vasospasm and thrombus formation by slowing the bloodstream, increasing the permeability of the vessel walls, and increasing platelet

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aggregability and polycythemia[3,4]. In addition, oxygen radical formation and subsequent lipid peroxidation, as well as induction of cellular apoptosis, are also potential explanations for the cardiotoxicity of CO[5,6,7]. These mechanisms of hypoxia may result in ECG alterations indicating myocardial ischemia[8,9]. In this study, we aimed to contribute to the literature by investigating demographical and cardiotoxic characteristics of CO poisoning. We also aimed to investigate the ECG findings which may predict the severity of CO poisoning in Emergency department (ED).

Materials and Methods

We retrospectively analyzed patient records admitted to Hitit University Medical School ED in a two-year period (January 1st,2014-December 31st, 2015). A verbal permission to use database system of the facility was obtained from facility administration. A total of 75 patients with diagnosis of CO poisoning were included into the study. Diagnosis of CO poisoning was mainly based on anamnesis, symptoms and clinical and laboratory findings of the patients. Demographical characteristics (age, gender, monthly distribution, sources of poisoning, etc), vital signs (blood pressure, oxygen saturation measured by probe and heart rate), laboratory (carboxyhemoglobin (COHb) levels), Glasgow Coma Scale (GCS) scores and electrocardiograhy (ECG) findings were investigated. Additionally, treatment method (normobaric oxygen (NBO) or hyperbaric oxygen (HBO) therapy), source of exposure and length of stay (LOS) in the ED were investigated. Then, ECG findings of NBO and HBO groups were compared.

Statistical Analysis

All statistical calculations were made using the SPSS for Windows version 17.0 (SPSS, Inc, Chicago, IL, USA) software program. Descriptive data were presented as mean ± standard deviation. The Shapiro–Wilk test was used to analyse normal distribution assumption of the quantitative outcomes. To compare two groups (NBO and HBO), Mann–Whitney U-test, chi-square tests and Spearman Rho correlation coefficient were used. $p < 0.05$ was accepted as statistically significant.

Results

Of the 75 patients involved, 42 were female (56%). Mean age of the patients was 46 (min: 18, max: 91) years. When month of admission was investigated, it was determined that 13 patients were admitted in January, 13 in February, 11 in March, 11 in April, 1 in July, 10 in November and 12 in December. When sources of CO exposure were investigated, it was determined that the most common source was stoves (n=71, 94.6%), followed by fires (n=2, 2.6%) and gas leaks from heaters (n=2, 2.6%). When complaints on admission to the ED were investigated, the most common complaint was found to be nausea and vomiting (n=24, 32%), followed by altered mental status (n=23, 30.6%), headache (n=17, 22.6%), fatigue (n=7, 9.3%), dizziness (n=2, 2.6%), dyspnea (n=1, 1.3%) and coughing (n=1, 1.3%). Mean systolic blood pressure (BP) was 119 mm/Hg and mean dyastolic BP was 74 mm/Hg. Mean carboxyhemoglobin levels were found to be 15%, mean saturation was 95.9%. Mean GCS score of the patients was 14. Of the patients, 11 had a GCS less than 14 (14.6%). It was determined that while 75 have undergone NBO therapy (100%), 5 (6.6%) of the patients have undergone HBO therapy in addition to NBO therapy. Mean LOS in the ED was found to be 4.6 hours.

When outcomes of the patients were investigated, it was found that 6 patients (8%) was transferred to an advanced center, 6 patients were hospitalized in a ward (8%) and 6 patients were hospitalized in intensive care unit (8%). The remaining 57 patients (76%) were discharged from the ED following NBO therapy with total recovery. Table 1 summarizes the basic characteristics of the patients with CO poisoning.

The most common ECG finding was found to be normal sinus rythm in 52 patients (69.3%), followed by sinus tachycardia in 10 patients (13.3%), bradycardia in 4 patients (5.3%), ST depression in 2 patients (2.6%). ST elevation was recorded in 1 patient. Four of 10 patients with sinus tachycardia were involved in severe group who underwent HBO therapy. Number of patients with sinus tachycardia in HBO Group was significantly higher than those in NBO Group. Electrocardiographic findings of the groups are listed in Table 2.

Table 1. Basic Characteristics of the Patients Admitted to the Emergency Department due to Carbonmonoxide Poisoning

Characteristics	Value n, (%)
Gender	
Male	33, (44)
Female	42, (56)
Months	
January	13, (17.3)
February	13, (17.3)
March	11, (14.6)
April	11, (14.6)
November	10, (13.3)
December	12, (16)
Sources	
Stoves	71, (94.6)
Fire	2, (2.6)
Gas leak	2, (2.6)
Complaints	
Nausea and vomiting	24, (32)
Altered mental status	23, (30.6)
Headache	17, (22.6)
Fatigue	7, (9.3)
Dizziness	2, (2.6)
Dyspnea	1, (1.3)
Coughing	1, (1.3)
Treatment method	
Normobaric oxygen therapy	75 (100)
Hyperbaric oxygen therapy	5 (6.6)
Mean Glasgow Coma Scale	14
Mean carboxyhemoglobin level	15% ± 7
Mean length of stay in the Emergency Department	4.6 hours

Table 2. Comparison of Electrocardiographic Findings of NBO and HBO Groups in Patients with Carbonmonoxide Poisoning

Finding	NBO Group n, (%)	HBO Group n, (%)
Normal sinus rhytm	51, (72.8)	1 (20)
Sinus tachycardia*	6, (8.5)	4 (80)
Bradycardia	4 (5.7)	0
ST depression	2, (2.8)	0
Right bundle branch block	2, (2.8)	0
Atrial fibrillation	1, (1.4)	0
ST elevation	1, (1.4)	0
Left bundle branch block	1, (1.4)	0
Prolonged QT	1, (1.4)	0
T wave abnormality	1, (1.4)	0

Discussion

According to the results of our study, CO poisoning mainly affect male subjects. In a study in the United States (the US), death rates were also found to be the higher in males males (2.28 deaths per million). Additionally, January was the month in which the CO poisoning mostly occur[10]. Accordingly in our study, most of the cases were seen in January. Due to cold weather in winter months people tend to be in search of alternative heating methods wasting CO gas. It is known that elderly population tend to expose to CO poisoning frequently and this fact may be associated with other factors such as comorbid conditions and misdiagnoses which leads to repeated exposure. This also explains the reason for their higher death rate[10]. In our study, the mean age of the patients was 46. Death due to CO poisoning in our study period was not recorded.

It was reported in the US that the most common sources of CO poisonings in homes are the use of gasoline-powered engines, such as electric generators, and malfunctioning heating and cooking appliances[10]. In a study with 140 patients, 81.9% were poisoned by stoves, 10.5% by hot water boilers and 5.8% by fires[11]. Open fireplaces, oil-burning stoves, gas or wood burners for heating the home are the most commonly reported sources[12]. In Turkey, the most common source of CO poisoning is known to be stoves[3]. In concordance, stoves were the main sources of the poisoning with a rate of 94.6%. Sources of the poisoning may vary in respect to socio-economical and cultural differences between regions.

A previous study revealed that 106 patients (46%) presented with a GCS less than 14[13]. In our study, ratio of the patients with a GCS less than 14 was lower. The ratio may be higher in advanced facilities accepting severe cases.

The most useful diagnostic test obtainable in a suspected CO poisoning is the COHb level, high levels of which confirm exposure to CO. The problem with using COHb levels as the basis for providing treatment is that there is a wide variation in clinical manifestations with identical COHb levels. Furthermore, particular COHb levels are not predictive of symptoms or final outcome[14]. CO exposure is generally confirmed by a COHb level >3% in nonsmokers or >10% in smokers[15]. In a study, COHb levels of 163 patients were measured and elevated COHb levels were observed in 114. Mean COHb value was 16.6. It was emphasized that elevated carboxyhemoglobin could confirm diagnosis of CO intoxication but normal levels do not exclude it[11].

In a study, the most common finding in patients with CO poisoning was loss of consciousness[3]. The clinical presentation of acute CO poisoning may vary, but generally, the severity of observed symptoms roughly correlates with the observed level of COHb. The patient is usually asymptomatic with levels less than 10%. As COHb increases above 20%, headache, dizziness, confusion, and nausea may develop. Coma and seizures resulting from cerebral edema are common with levels greater than 40%, and death is likely above 60% [48]. The mean COHb level was 15.04% and the most common symptom on admission was nausea and vomiting in our study. Hence, above-mentioned rough correlation between COHb level and symptoms was also observed in our study.

In a study, 123 of the 171 patients in the study were discharged in a healthy condition after receiving normobaric oxygen therapy, while 48 patients received HBOT[11]. In another study, 24 of 93 patients have undergone HBOT[9]. In a report a higher rate of HBOT was reported (51%). HBOT is recommended for the patients with COHb ≥ 25%, with a history of syncope, with an ECG abnormality, and pregnancy[16]. In our study, majority of the patients were discharged from our ED with full-recovery. However, particularly for severe cases, HBOT must be considered and delays must be avoided.

Although majority of the patients in our study revealed a normal ECG, the most common electrocardiographic abnormality was sinus tachycardia. Carbonmonoxide can induce a tendency toward coronary vasospasm and thrombus formation by slowing the bloodstream, increasing the permeability of the vessel walls, and increasing platelet aggregability and polycythemia; these factors are also responsible for myocardial damage in patients with CO poisoning. In addition, oxygen radical formation and subsequent lipid peroxidation, as well as induction of cellular apoptosis, are also potential explanations for the cardiotoxicity of CO[4,6,7]. It is well-documented in the literature that the overall incidences of arrhythmia, coronary artery disease, and heart failure is higher in the patients with CO poisoning than in normal subjects. The main mechanism of CO toxicity is ischemic hypoxia secondary to hypoxemia. The heart is the main target organ of acute CO poisoning due to its high susceptibility to hypoxemia[17,18]. Carbonmonoxide

poisoning creates an imbalance between myocardial demand and supply and thus, damage occurs[19]. It also affects hematocrit, blood viscosity, and platelet function[20]. In a study, in 41% of the patients, sinus tachycardia was observed in patients with CO poisoning. Also, ischemic changes were present in 30% of patients (4% with ST-segment elevation), whereas 41% had nonspecific ST changes and 27% had no ischemic changes[13]. Accordingly in our study, sinus tachycardia was determined in 4 of 5 patients underwent HBO therapy. Our study showed that sinus tachycardia is the most common ECG abnormality in patients with CO poisoning, but also in severe cases.

Limitations of the Study

Due to lack of proper medical history, we could not determine exact period of exposure to CO of the patients. In many patients, serial ECGs were not performed so that we could not make a comparison of ECGs. Serial troponin and CK-MB measurements also could not be obtained in all patients due to early discharge, hospitalization and transfer to other centers.

Conclusion

Our results revealed that sinus tachycardia may be an indicator of severe CO poisoning. The earliest symptoms associated with CO poisoning are often nonspecific and readily confused with other illnesses, typically a viral syndrome. The cardiovascular and central nervous systems are the organ systems most sensitive to CO poisoning due to their higher susceptibility to hypoxia. Continued exposure to CO may lead to symptoms attributable to oxygen deficiency in the heart. Myocardial infarction and dysrhythmias are described in victims of CO poisoning.

In our study, the most common abnormality in ECG was found to be sinus tachycardia and frequency of sinus tachycardia was higher in severe patients. In patients with CO poisoning, ischemic changes on ECG should also be evaluated carefully.

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Ethical Approval: Due to retrospective nature of the study, a permission from hospital administration to use hospital database system was obtained.

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Evaluation of Diabetic-Medications Adherence among Diabetic Type 2 Patients

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Abstract

Background : Diabetes mellitus (DM) is a worldwide health illness with deleterious consequences both medically and economically. When treated inappropriately, it can cause severe acute and chronic sequels. The purpose of this study was to assess the adherence to diabetic medications type 2 diabetic patients.

Method : The study was cross-sectional performed to assess the adherence of diabetic type 2 patients to diabetic medications. The study population was randomly enrolled from rural and urban regions. The participants of the present study were known cases of diabetic patients attending community pharmacies of the regions in which the study was conducted. After an explanation of the goal of the study, the patients were invited to be enrolled in the study. After obtaining of the written consent, the Arabic version of Morisky medication adherence scale was administered to patients to be reported by them but the exception for illiterate diabetic patients who interviewed by community pharmacists who were trained on the questionnaire.

Results: The total number of participants was 200 patients with a mean age of (50.65±8.91) and approximately half of participants (49.8%) have an age of greater than 50 years. More than 100 participants have primary and secondary education and about (43%) has higher education. The female participants were less than the third of the total number (29%) of the sample and the remaining was male. the majority of patients (75.5%) have poor adherence to medications while (21.5%) of participants have medium adherence to diabetic management and only (3%) have high adherence to medications.

Conclusion: In the present study, The adherence to diabetes medication was poor in 75.5% of participants and the most common reason for non-adherence as reported by patients was forgetfulness.

Keywords: *DM, medication adherence, Moriskey-8 scale*

Introduction

Diabetes mellitus (DM) is a worldwide health illness with deleterious consequences both medically and economically [1]. By the year 2035, the prevalence of diabetes increased to affect about 592 million individuals or one person from every ten individuals [2]. In Iraq, diabetes mellitus affects about 1,411,500 which is about 7.5% of Iraqi adults in 2017 [3]. It has been found that diabetes mellitus will be increased substantially in 2030 in Arab countries compared to remaining world countries. The adherence to diabetic treatment can achieve a major part of success in decrease the serious complications of the disease and lower the burden of cost in the treatment of such events. The adherence to medications is referred to as the level to which the drugs taken by patients as the health care professionals prescribed [4]. The non-adherence to medication can lead to great difficulties in improving the outcomes of health. It is considered a continuous problem because the patients take more drugs for the management of their chronic illnesses. This is obvious especially in elderly patients due to decrement in the physical and

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mental function of such population [5,6]. Moreover, non-adherence to medications also can affect the community due to elevated cost expenditures on health care due to frequent hospitalizations and it has an effect on patients who may lose their life chances due to the disease. The adherence to medications is influenced by many factors which comprise factors related to drugs itself (as medication regimen difficulty), patients (as health knowledge), health care administer (as the deficiency of communication between health-care administer and their patients), and disease (as the effect of positive clinical manifestation). Moreover, unavailability of medications can also affect the adherence to medications [7]. The most important issue in the achievement of good glycemic control is good adherence to diabetic treatment. It is estimated that approximately 30-50% of unsuccessful treatment was due to poor adherence to prescribed medications and this eventually lead to the failure of organs. So it is prudent to measure the adherence to medication by using valid Arabic tools to assess the poor adherers to diabetic medications [8]. Successful diabetic management depends on many patients' related factors like efficient education on diabetes, improvement of knowledge, skills, and attitude which finally leads to good control of diabetes. So the burden on systems of health care can be decreased by encouraging and guide the diabetic persons in the correct direction. Moreover, drug adherence has been affected by native culture and religious associations that impact a patient's medication compliance [9].

The direct methods to assess drug adherence include the measurement of drug or its metabolite product in the urine or blood while the indirect assessment of adherence includes the measure of pills taken, prescription refills, and self-reports. The self-report method for measurement of drug adherence can be considered a simple and cheap method. If the valid tool is used for assessment of adherence to the drug-like Morisky scale, the scores of self-reporting will be precise and of greater than 70% sensitive and specific [10]. Morisky scale which is denoted by (MMAS-8) because it has eight questions is considered one of the commonest and simplest tools utilized for measurement of adherence to medications. The MMAS-8 is characterized by measurement of its validity and reliability in different languages and assessed in various populations and illnesses [4]. The Arabic version of the MMAS-8 was stepwise managed with translation and back-translation with correspondence with global rules and was validated in a study of medication adherence in diabetic patients [8].

Method

1. Participants

The study was cross-sectional performed to assess the adherence of patients to diabetic medications. The study population was randomly enrolled from rural and urban regions. The participants of the study were known cases of diabetic type 2 patients attending community pharmacies of the regions in which the study was conducted. After an explanation of the goal of the study and obtaining the written consent, the Arabic version of the Morisky medication adherence scale was administered to patients to be reported by them but the exception for illiterate diabetic patients who interviewed by community pharmacists who were trained on the questionnaire. The inclusion criteria were if the diabetes was diagnosed after the age of 30 years, patients have taken the treatment regularly for a minimum of six months before acceptance in the study, and the patients should have at least one visit for the diabetic physician in the last month. The exclusion criteria were the pregnant, breastfeeding women, rejection to participate, the age of diabetic patients less than 30 years, and the patients who cannot understand the questionnaire due to mental illness. The prevalence of diabetes mellitus in Iraq was 7.5% [3], the size of sample can be calculated by following equation (n=[(z) 2 (p) (pm1)]/(d) 2 where n: size of sample , z: the coefficient of reliability [z=1.96 for 95% confidence interval], p: expected proportion of diabetic population =7.5%=0.075, d: desired interval width =4.0%, type I error α=0.05 and type II error β=0.2, and the interval confidence 0.95). Two hundred diabetic patients were enrolled. Twenty patients were involved in the pilot study to obtain the internal reliability of the questionnaire. The information regarding demographic data and disease-specific data like duration of disease and treatment taken has been reported by organized questions. The research was permitted by the scientific committee of research in a Faculty of Pharmacy at the University of Kufa.

2. Instrument used for collection of data

The medication adherence in this study was measured by the Morisky medication adherence scale (MMAS8). The permission for utilization and translation of the questionnaire was gained from the designer. The method of translation was standardized according to the international forward and backward method. The translated Arabic version of MMAS8 was utilized in the previous publications [8,10,11]. This questionnaire consists

of eight questions the initial seven questions with Yes\ NO responses but the last question has five Likert scale points [12]. The complete score of this questionnaire was (8) and the lower score was (zero) where the higher scores indicate better adherence to medication. According to the scores of this tool, patients were classified into three categories low adherence if the score is less than (6), medium adherence if score range (≥ 6 - < 8), and high adherence if the score was (8) [8]. The scoring of items depends on the administration of score (1) for each (Yes) response and zero for (No) response with an exception for the fifth question which was scored reversibly. The internal consistency of the questionnaire was greater than the acceptance level (0.74) [11].

Statistical Analysis

The data analyzed statistically by the utilization of statistical package of social sciences version 23 (spss 23). The mean and standard deviation were used to represent continuous variables with normal distribution while the median used for the representation of variables distributed in an asymmetric manner. The percentages used for the representation of categorical variables percentages. The Pearson chi-square has been utilized for the determination of the correlation between the categories of patient's adherence and the independent variables. One-way analysis of variance also utilized, the statistically significant level was of less than 0.05. Cronbach's alpha has been utilized to determine the internal consistency of the questionnaire.

Results

The internal consistency of the questionnaire was greater than the acceptance level of (0.74) as determined by Cronbach's alpha [11]. Table (1) show the total number of participants were 200 patients with a mean age of (50.65± 8.91) and approximately half of participants (49.8%) have an age of greater than 50 years. More than 100 participants have primary and secondary education and about (43%) have higher education. The female participants were less than the third of the total number (29%) of sample and the remaining was male. Table (2) shows most of the participants (89.5%) treated orally for diabetes and (3.5 %) of participants taken insulin as the sole treatment for diabetes and the remaining part mixing between oral and insulin therapy. Most of the patients (79.5%) have a duration of diabetes of ten years or less and the other part (20.5%) has a duration of greater than ten years. In descending order regarding the percent of patients, the most of patients treated with two agents (43.8%), one agent (31.8%), three agents (17.4%) and the remaining part (6.5%) taken four and five agents. Table (3) shows that the larger percent of patients (53.0%, 60.0%, 87.5%, 69.5%, 96.0% and 94.0%) selected the good choice of first ,second ,third ,fourth ,fifth and sixth questions respectively which were (no) response. While (47.0%, 40.0%, 12.5, 30.5, 4.0%, 6.0% and 86.5 %) of participants selected the bad response regarding adherence to medications which was the (yes) response of first, second , third, fourth, fifth, sixth and seventh questions respectively of Morisky medication adherence scale. Table (4) shows that the majority of patients (75.5%) have poor adherence to medications while (21.5%) of participants have medium adherence to diabetic management and only (3%) have high adherence to medications. Table (5) shows that there was a significant correlation ($p < 0.05$) between adherence classes of patients and both number of agents taken for treatment of diabetes and the type of treatment taken for the management of it. The association between the adherence to medications was non-significant ($p > 0.05$) with the gender, age, duration of diabetes, and educational levels of patients.

Table (1) Demographic Data Of Participants

Demographic variable	Percent (frequency)
Age (50.65±8.91)	
30-40 years	(18.9%) (38)
41-50 years	(30.8%) (62)
> 50 years	(49.8%) (100)
Education	
Uneducated	(6 %) (12)
Primary- Secondary school	(51%) (102)
Collage and higher	(43%) (86)
Sex	
Women	(29%) (58)
Men	(71%) (142)

Table (2): clinical features of respondents

Clinical variable	Percent (Number)
Treatment taken	
Oral medications	89.5% (179)
Insulin only	3.5 % (7)
Oral medication+ insulin	7% (14)
Hypertension coexistence	
Presence	28.5 % (57)
Absence	71.5% (143)
Diabetes duration	
≤ 10 years	79.5% (159)
> 10 years	20.5% (41)
Number of drugs taken	
single agent	31.8% (64)
2 agent	43.8% (88)
3 agent	17.4% (35)
4 agent	6.0% (12)
5 agents	0.5% (1)
Drug combination	
Fixed combination	54.5% (109)
No combination	45.5% (91)
Frequency of drug use	
Once daily	59.5% (119)
Twice daily or more	40.5% (81)

Table (3) responses of patients about questions of adherence in Moriskey-8 scale

Description of questions	Yes (%)	No (%)
(1)Asking about forgetfulness of taking medication	94(47.0)	106(53.0)
(2)Asking about not taking drugs in the past 2 weeks for reasons other than forgetfulness	80(40.0)	120(60.0)
(3)Asking about cutting or stopping drugs without referring to doctor due to felling worse if have the drug	25(12.5)	175(87.5)
(4)Asking about forgetfulness to bring medication when travel or leave home	61(30.5)	139(69.5)
(5) Asking about taking medication yesterday	192(96.0)	8 (4.0)
(6) Asking about stopping medication some times when feel the diabetes was controlled	12 (6.0)	188(94.0)
(7) Asking about feeling of hassling when sticking to the plan of treatment	173(86.5)	27 (13.5)
(8) Asking about the times of difficulties to remember to take all medicines · Never/Rarely (17) (8.5%) · Once in a while (120) (60.0%) · Sometimes (57) (28.5%) · Usually (3) (1.5%) · All the time (2) (1.0%)		

Table (4): adherence levels of patients

Adherence class	Frequency	Percent
High-adherence	6	3.0
Medium-adherence	43	21.5
Low-adherence	151	75.5

Table (5): the correlation between patients-adherence classes and the demographic and clinical variables of diabetic patients.

The variable		High adherence	Medium adherence	Low adherence	p-value
Gender		5 (2.5%)	34 (17.0%)	103 (51.5%)	0.432
Age	30-40 years	0 (0.0%)	6 (3.0%)	32 (16.0%)	0.53
	41-50 years	2 (1.0%)	16 (8.0%)	44 (22.0%)	
	>50 years	4 (2.0%)	22 (11.0%)	74 (37.0%)	
Education level	Uneducated	0 (0.0%)	3 (1.5%)	8 (4.0%)	0.419
	Primary-secondary	2 (1.0%)	27 (13.5%)	75 (37.5%)	
	College-higher education	4 (2.0%)	14 (7.0%)	67 (33.5%)	
Duration of diabetes	≤ 10 years	3 (1.5%)	34 (17.0%)	123 (61.5%)	0.138
	> 10 years	3 (1.5%)	10 (5.0%)	27 (13.5%)	

Cont... **Table (4): adherence levels of patients**

Number of agent taken	Single agent	5 (2.5%)	7 (3.5%)	53 (26.5%)	0.038
	2 agents	0 (0.0%)	23 (11.5%)	63 (31.5 %)	
	3 agents	1 (0.5%)	7 (3.5%)	28 (14.0%)	
	4agents	1 (0.5%)	5 (2.5%)	5 (2.5%)	
	5 agents	0 (0.0%)	1 (0.5%)	1 (0.5%)	
Treatment type	Insulin	2 (1.0%)	3 (1.5%)	2 (1.0%)	< 0.001
	Oral drugs	4 (2.0%)	36 (18.0%)	139 (69.5%)	
	Insulin+ oral drugs	0 (0.0%)	5 (2.5%)	9 (4.5%)	
Disease	Diabetes only	4 (2.0%)	32 (16.0%)	107 (53.5%)	0.950
	Diabetes and hypertension	2 (1.0%)	12 (6.0%)	43 (21.5%)	

Discussion

The poor adherence to diabetic therapies is a common health challenge with serious consequences like the occurrence of complications and increased hospitalizations so the poor compliance called an "invisible epidemic". The present study found that about 75.5% of patients have low adherence and this in accordance to the studies in which the low adherence reaches to 56% [13] and even to 73.6% [14,15]. Adherence to diabetic regimens was very poor due to low attitude and low literacy toward the disease in the general population [16]. The poor adherence level explains the lack of attention that is paid by diabetic patients toward their health [17]. It was found that many diabetic patients have forgetfulness to bring medication when travel or leave home and some cut or stop drugs without referring to doctor due to felling worse if they have the drug or stopping medication some times when feeling the diabetes was controlled. The differences in the proportions of adherence may be due to the utilization of various tools for the measurement of adherence. Forgetfulness was the most important cause of poor compliance in the present study and this also founded by many studies [14]. The forgetfulness has many manifestations like forgetting of taking drugs or forgetting the refilling of prescriptions. Some patients may not have adequate education about the importance of adherence to therapies. In spite of several studies indicated the association between certain factors like duration of diabetes, gender, and level of education [17,18,19], the present study has not found a significant association between these factors and drug adherence and this is in agreement with results that revealed by Rekha Thapar et.al study [14]. It was found a significant association between number of agents taken and adherence to drugs and this supported by a study found the association between use of single-agent for the treatment and high adherence level [20] and also reported that the number of oral agents for the treatment of diabetes can affect the adherence to medications [19]. It has been reported that about half of diabetic patients take more than seven drugs for management of diabetes and the comorbidities and this makes the regimen complex and the adherence to such regimen is of great challenge for the patients [17]. The most important cause of non-adherence was forgetfulness (47.0%) and this also founded by study report the forgetfulness as the main reason for non-adherence for oral hypoglycemic agents [19]. Other important cause was feeling of hassling when sticking to the plan of treatment (86.5%) and this corresponded with study found that the major reasons of non-compliance were unwillingness to use the prescribed drugs (31.53%) [21]. The reporting of this problem requires recurrent visits and sessions of counseling including members of the family of patients. Encourage the visiting of healthcare providers to the home of patients associated with a great increase in the adherence

to medications of diabetes, enhance glycemic control and so improve the outcomes of health. The utilization of cell phone for remembering of patients by sending messages led to enhanced adherence to medications in HIV patients [22]. So it is suggested that the application of pharmaceutical activities for the education of patients about the disease and its treatment can increase the adherence to therapeutic agents [15]. The improvement of drug adherence can be accomplished by the assurance of continuous education to patients and providing the patients with oral and written data and encourage the regular visiting of health care providers by the patients [15]. It was found in this study that the type of treatment has a significant correlation with drug adherence and this also supported in the study that reports the effect of treatment on adherence [23].

Conclusion

The adherence to diabetes medication in the participants was poor in 75.5% of participants. The most common reason for non-adherence as reported by patients was forgetfulness. The adherence to diabetic medication was correlated significantly with the number of agents used for treatment and type of treatment whether oral drugs or insulin or combinations. So identification of poor adherent diabetic patients is of great importance for ensuring the good management by encouraging regular counseling and education of such patients.

Conflict of Interest: The authors declare no conflict of interest.

Funding: Nil.

Ethical Clearance: This study was in accordance with the principles set out in the Declaration of Helsinki and approved by the ethical review committee of the University of Kufa, Iraq. The subjects were informed about the procedure of the study and consent letter was taken from them.

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Breast Cancer Screening: Comparison of Screening Modalities in Taiwan

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Abstract

Background: Screening for breast cancer is an important method for reducing the death rate in the Western world over the past 20-30 years. On this basis, since 2004 , the National Health Bureau of Taiwan has also begun providing free screening service. The purpose of the current research is to compare the efficacy of the three-mammography modalities for breast cancer identification.

Methods: Between July 2004 and December 2010, a total of 23,513 eligible women underwent screening mammography. 6804 of these received screen-film mammography (SFM), 1610 received computed-radiography (CR) mammography, and 15,099 received digital mammography (DM). The detection rate of carcinoma-in-situ, early detection rate of cancer, and the overall cancer detection rate of each modality was calculated. To determine the statistical significance of performance differences between these modalities Chi-square test was used.

Results: SFM callback rate was 12.23%, cancer detection rate was 5.29%, Ductal carcinoma in situ (DCIS) detection rate was 1.76%. Computed radiography's callback rate was 12.67%, with a cancer detection rate of 6.21% and DCIS detection rate of 1.24%. DM has the highest callback rate of 16.2%, but also the highest rate of cancer detection and DCIS detection rate of 9.21% and 3.58% respectively. DM was found to have better cancer detection rate and DCIS detection rate as compared to SFM and CR, it is only statistically significant when compared to SFM. Although DM has better cancer and DCIS detection rates, it is also associated with higher callback rate.

Conclusions: In terms of image quality and greater contrast, optical mammography has many advantages when opposed to screen-film and computed radiography mammography. In the Asian population, where women have higher breast densities, this is particularly vital. The incidence of Taiwanese breast cancer is steadily growing.

Key words: *Computed Radiography; Digital Mammography, Screen Film, Screening method*

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Introduction

Organized *mammographic screening at national level has been* adopted as the criterion for early *detection* of *breast cancer* in *western world for decades*. *Women older than 40 years* are *encouraged* to undergo *mammography screening every year. Successful screening programs proved to* increase the survival rate

of *breast cancer* patients because of its early detection. Large-scale randomized clinical trials (RCT) and Meta-analyses indicate a drop of 20-35% in mortality rate among women aged 50-69 who were asked to undergo mammography screening [1-6].

According to the Taiwan Cancer Registry Report of 2008 from the bureau of health promotion, department of health, breast cancer is the most frequent type of cancer among Taiwanese women. Among all types of reported cancer in women, breast cancer incidence was the second most frequent cancer in 2005 (42.3 per 100,000)[6], while in 2013 it became the cancer with the highest incidence (96.5 per 100,000)[7]. With the advances in awareness, screening and treatment, the mortality rate declined significantly between 1997 and 2013[8].

In 2008, a total of 56.1 cases (per 100,000 population; only invasive cancers) were diagnosed with breast cancer out of 8,136 cases. There was an increase of 5.6 percent in incidence as compared to 1995. The data from1995 also depicts that the number of breast cancer cases increased from 2838 to 8136; showing a standardized incidence rate of 28.4% to 56.1%. In 13 years, the standardized incidence rate increased by 97.5%. The lifetime risk for Breast cancer was (0-74 years) with a cumulative detection of 5.35%. It has also been found that breast cancer had an average lifetime probability of 1/19, with a cumulative lifetime risk of 1.06%. The average lifetime risk of death from cancer was 1/94 [9].

Although there are major resemblances, yet the mains difference is that the prime age for breast cancer is between 40 and 50 years in Asia, whereas the peak age in the European region is between 60 and 70 years. It is also an alarming fact that the breast cancer incidence is rising in Asia and it is linked with augmented mortality. On the contrary, in the West, although the incidence of breast cancer is increasing, but the mortality rate is definitely on decline. The collective lifetime risk of development of breast cancer is 6.31, 5.35, 4.38 and 3.73, while the average lifetime cancer risk is 1/16, 1/19, 1/23 and 1/27. Compared to detection in the United States and Britain, the cumulative lifetime risk 8.26, 9.49 while the average lifetime cancer risk is 1/12, 1/11. The cancer incidence has increased recently to alarming levels [9].

Compared to the United States, the domestic implementation of screening environment is somewhat different. In USA Screening for breast cancer targeting the 50-69 age group has been in place for decades since its implementation. The percentage of screening within the two years is about 70-80% in United States, while Taiwan the percentage is only 7%. Peak age of occurrence of breast cancer in Taiwan is ten years younger as compared to Europe and the United States among 45–55 years old women. Compared to the western women, the breast tissues are denser in Asian women (80%) and also smaller as compared to the Western counterparts (20%) [10].

A Study by the Bureau of health promotion report comparing staging of cancer between Taiwan and United States, the zero stage of the breast cancer rate was only 9.4%, as compared to the United States where it is 18.9 %. The ratio of the zero stage of the breast cancer is 25.9% in Taiwan which is 36.9 % percent in the United States representing a significant difference[11]. Early detection of the breast cancer to improve cancer outcome and survival is pivotal to the breast cancer control.

Data from Bureau of Health Promotion in Taiwan (years 1998– to 2002) shows that 5-year survival for all stages of breast cancer is 78.37%. The survival rates for 5- and 10-year are 98% and 95% for stage 0; 96% and 89% for stage I; 90% and 82% for stage II; 65% and 53% for stage III; and 22% and 10% for stage IV [12]. The study found a very high significance of early breast cancer screening in Taiwanese women. Bureau of health target of 1998 was 200,000 mammography screening for breast cancer. Since 1991, the bureau has stepped up efforts to promote breast cancer screening and the target is expected to increase to 400,00 people. For such a large screening program, some of the issues need to be addressed. The hospitals have different breast screening machines, because of a difference in the funding. All medical institutions use the three-screening equipment; Screen-film mammography, computer aided radiography and Digital mammography. However, there is no consensus or strategy for breast cancer screening in Asian women up till now. Breast cancer screening policymaking needs to be revised according to sensitivity, specificity and compliance. The aim of the current study is to compare the effectiveness of the three-mammography modalities to identify breast cancer.

Materials and Methods

According to the National Health Council in Taiwan local hospitals can undertake mammography screening for women, aged 45 to 69-year-old every two years. This study is a retrospective study for the mammography screening during the period between July 1st, 2004 to December 31st, 2010. Screening mammography for eligible women was interpreted by 52 radiologists representing 16 radiology groups in 26 facilities in Taiwan. We excluded 3,252 women from the research because they did not meet our requirements for eligibility. Major exclusion criteria include women who had gone through breast implants, believed they might be pregnant, had experienced mammography for any purpose within the previous 2 years, or had a history of breast cancer. A total of 23,513 women were eligible for inclusion in these analyses, all women in the study followed the regulations of the hospital in regard to screening. Mammographic outcome was based on the BI-RADS assessment and recommendation reported by the interpreting radiologist. The BI-RADS assessment category hierarchy was as follows: highly suggestive of malignancy, category 5; suspicious-looking abnormality, category 4; incomplete assessment, category 0; probably benign finding, category 3; benign finding, category 2; negative, category 1.

Mammograms that were assessed as highly suggestive of malignancy, suspicious, or incomplete assessment were considered positive. Mammograms assessed as negative, benign, or probably benign with no recommendation for biopsy or surgical consultation were considered negative.

Results and Discussion

In Taiwan, centers use various modalities for mammogram screening purposes, but a comparative analysis of the effectiveness of different modalities is lacking in Taiwan. In this research , various screening modalities used in Taiwan for early detection of breast cancer were evaluated. As shown in Figure 1, a total of 23,513 women were screened, 64 percent of them were screened by digital mammography, the most widely used method, and the second most commonly used method was screen film mammography. Computed radiography was only used to screen around 7% of the cases (figure 2). Just about 7 percent of the cases were screened using computed radiography (Figure 2). When a screening increases the suspicion of breast cancer and leads to further tests, false positive mammograms are normal. Typically, women are asked to call back in this case to do further tests to exclude the diagnosis of cancer.

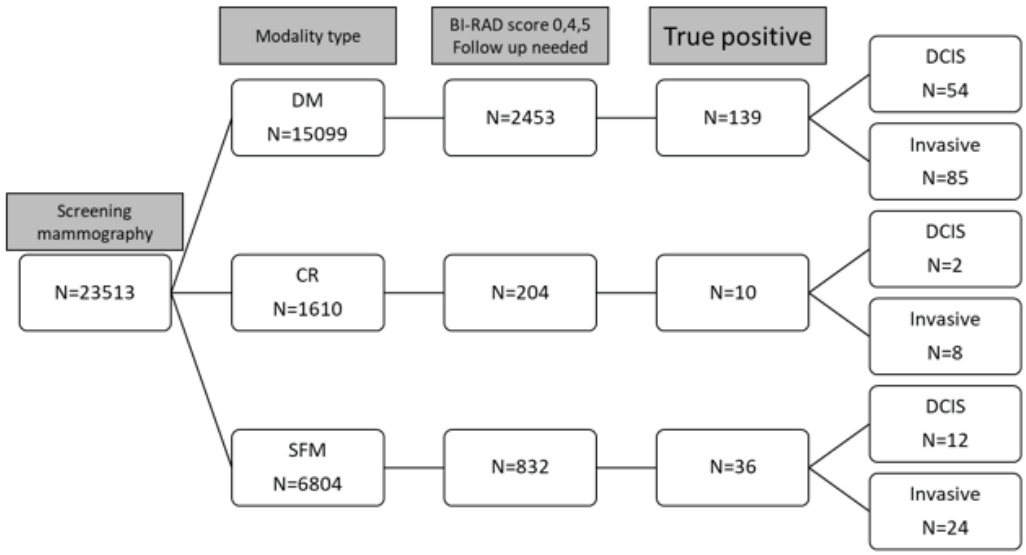

Figure 1: Schematic representation of the design of the breast cancer screening program and the number of women in each group. DM: Digital Mammography, CR: Computer-Aided Radiography, and SFM: Screen Film Mammograph

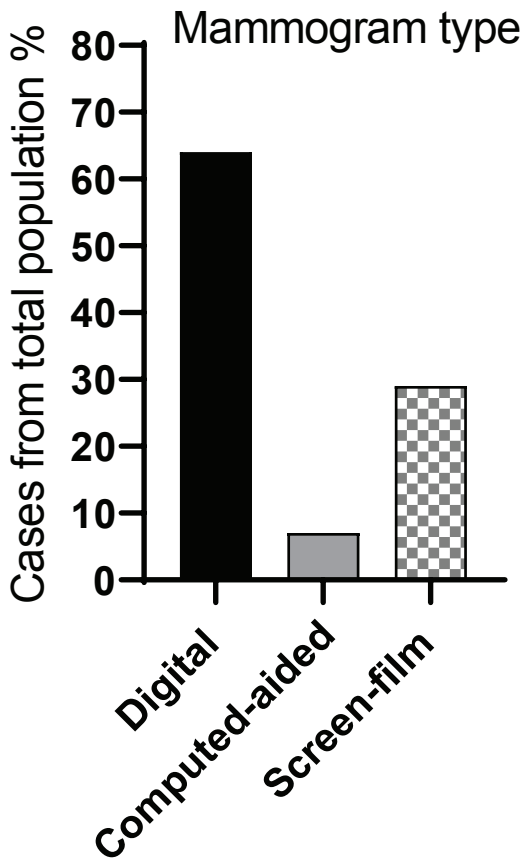

Figure 2: The percentage of different mammography methods usage in this study. The differences between all groups are statistically significant, p value <0.0001.

Although the majority of callback cases are negative for breast cancer, it is sadly related to increased stress and anxiety. Among the total population of this study, 3,489 women had initial positive mammogram, this represents 14.8% of the total population of the study who needed further follow up. As shown in figure 3, digital mammography group had a follow up rate of 16.2% where 2,453 women had positive mammograms out of the 15,099. Computed radiography was done for 1,610 women with 204 women (12.7%) needed follow up. Film screen mammography was done for 6,804 women, among these, 832 women (12.2%) had positive film mammogram and needed follow up. Callback rates in digital mammogram group was significantly higher than CAD and SFM groups.

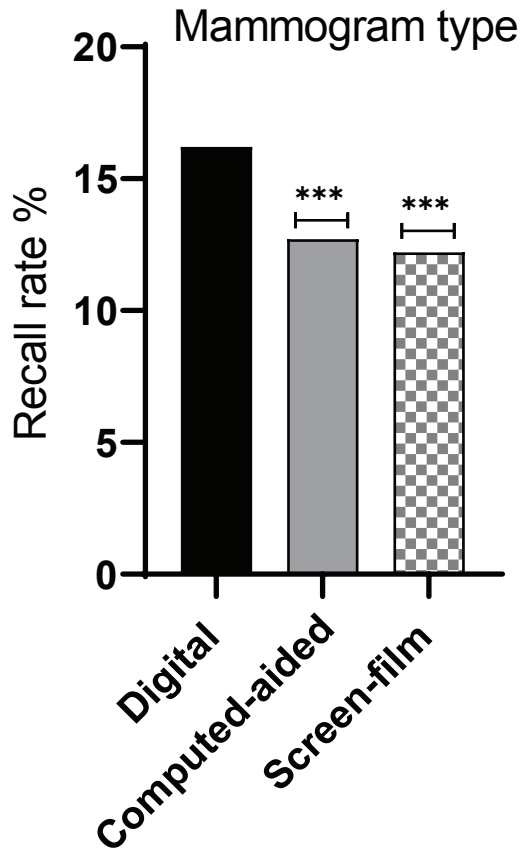

Figure 3: The percentage of recall rate in different methods. P value of the difference between DR and CAD is 0.0003, and <0.0001 for the difference between DR and SFM. The difference between CAD and SFM is statistically insignificant, p value is 0.6269.

We then aimed to calculate the Positive Predictive Value (PPV), among all 3,489 women recalled in this study, only 68 women were later diagnosed with cancer, this represents around 5.3% PPV in the population recalled from all three groups. As shown in figure 4, DM group has the highest PPV, representing around 5.7 %, followed by 4.9% for CAD group and 4.3% for SFM. Although PPV values differ slightly between groups, but this difference was statistically insignificant.

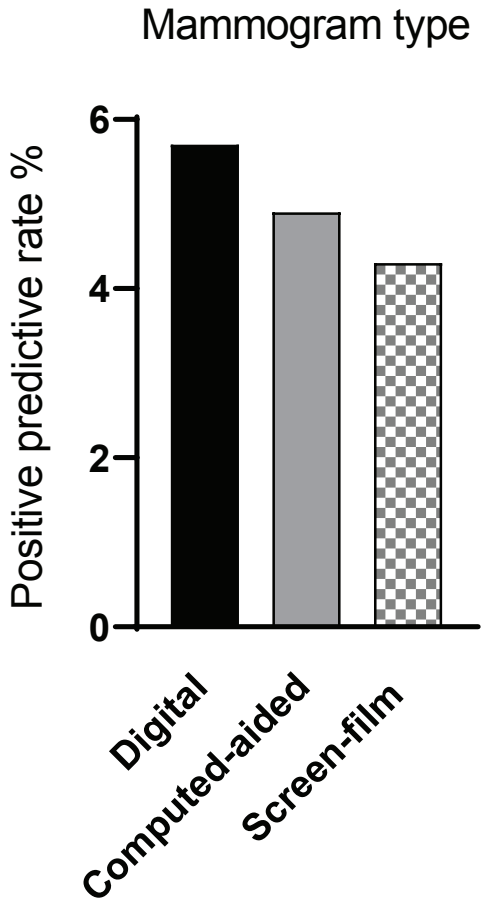

Figure 4: The positive predictive rates of different mammography methods. There was no significant difference between different groups, p value = 0.6483 between DR and CAD, and 0.1371 between DR and SFM and 0.7209 between CAD and SFM.

We then tried to measure from the original population the prevalence of positive breast cancer. The DR system has the highest detection rate of 0.92% of breast cancer, as seen in Figure 5, which is statistically better than that estimated for both the CAD (0.62%) and SFM (0.43%) methods. Positive patients with breast cancer were further classified into Ductal carcinoma in situ (DCIS) and invasive cancer. The earliest type of breast cancer is ductal carcinoma in situ (DCIS). In certain individuals, it could become an infectious cancer and spread to other tissues if DCIS is not treated.

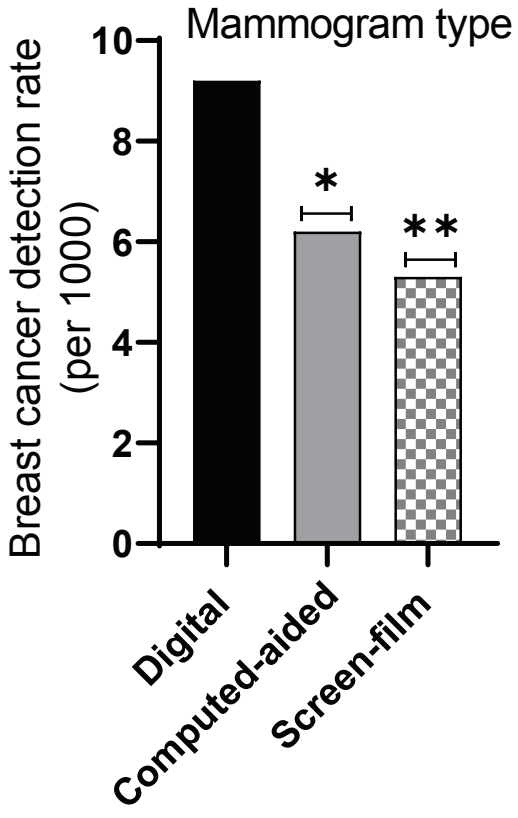

Figure 5: Breast cancer detection rate of different mammography methods. The difference between different groups, p value = 0.0234 between DR and CAD, and 0.0026 between DR and SFM and 0.4598 between CAD and SFM.

As shown in figure 6, among all breast cancers diagnosed by DR and SFM, comparable detection of more than 35-40% DCIS was reported, while only 20% of all cases diagnosed by CAD were DCIS. In all mammography methods in this study the majority of breast cancer cases were identified in the invasive disease which usually has bad prognosis.

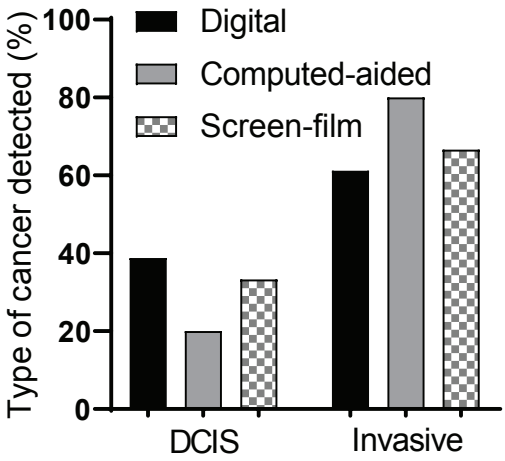

Figure 6: Comparison between stage of breast cancer detected by mammography methods. There was not significant difference between different groups, p value = 0.2373 between DR and CAD, and 0.5524 between DR and SFM and 0.4237 between CAD and SFM.

The findings of this study are descriptive of the 45-69-year-old woman undergoing mammography screening in Taiwan using the three distinct mammography instruments. In women with dense breasts, optical mammography was found to be more effective than screen film mammography and computer assisted radiography, showing a cancer detection rate of 0.92%, 0.53%, and 0.62%, respectively. The results show comparison of the three-mammography modalities. Digital mammography was found to be significantly better in detecting breast cancer in young women, and women with dense breasts, than conventional film mammography at. No significant difference in diagnostic accuracy between digital and film mammography in the population as a whole or in other predefined subgroups was observed. Nonetheless, digital mammography is beneficial as compared to film mammography by virtue of its other advantages like, convenient access to images and computer-assisted diagnosis; better ways of transmission, retrieval, and storage of images; and the use of a lesser average dose of radiation without any compromise in accuracy of diagnosis. Chi-square test was used to compare mammograms between computer aided radiography and the screen-film mammography, DCIS detection rate differences, and display of digital mammography verses screen-film mammography showed significant difference.

These results can more readily be compared with other results published in other literature. It confirms that breast density in Asian women is higher as compared to Caucasian women [13]; besides, mammography cannot be considered a perfect tool to screen women in Asia with dense breast parenchyma pattern (DBPP) [14]. Mammography has been shown to have lower sensitivity for invasive ductal carcinoma of breasts in patients with DBPP [15]. Breast density is defined as the amount of white area on the breast that appears to be black on a mammogram. The white and black balance echoes the composition of breast and comparative quantity of connective tissue, glandular tissue, and the fats. Breast density may be an important factor to affect breast cancer incidence [16]. According to a study conducted in 1998, densities accounting more than 60-75% have been found to escalate breast cancer risk 4-6 times more as compared to women with lesser or no breast densities [17]. Recent studies also reveal that breast density is a significant factor of development of breast cancer risk [18-21]. A recent study conducted on 106 women showed that 79.2% of Taiwanese women had high breast density as compared to 22% only of the western women [10]. Moreover, malignant breast tumors are more likely to appear in the areas with the greatest mammographic density than in the fattier areas of the breast [22]. Some studies show that digital mammography is significantly superior to conventional film mammography in detection of breast cancer in younger women, premenopausal and peri-menopausal women, as well as women with denser breasts [23 -24] According to the American College of Radiology (ACR) guidelines, the suggested recall rate should be lower than 10% for breast cancer [25]. Large-scale studies done in foreign countries recommend a recall ratio of less than 5% [26]. The digital mammography equipment used in the study were Hologic Selenia Digital Mammography unit with the R2 CAD (computer-aided detection mammography unit). Computer-aided detection systems for breast cancer can increase the detection rate of early breast cancer but does not help much in improving the revisit rate [27 - 28]. Based on our results, the need for return to clinic ratio for digital mammography is 16.2%, as compared to 12.2% for Screen-film mammography and 12.7% for computer aided mammography. Higher recall rate for digital mammography is probably because the it

aids radiologists in detection of micro-calcifications that may be neglected by film mammograms used traditionally. In general recall rate for all groups is greater than recommended rate by American College of Radiology which is less than 10% [29].

The high recall ratio does not generally improve the detection rates for breast cancer [30]. Western women's reports suggest a recall rate of less than 4%; follow-up to the clinic would improve the rate of breast cancer diagnosis and increase the rate of false-positive [31]. In Taiwanese women with dense breasts, while such a rule will not yet be fully applicable, there is still a need for more research in the future. Our study shows that optical mammography is a better way to detect dense breast cancer as well as carcinoma in situ in Taiwanese women than screen film mammography and computer assisted radiography.

Conclusion

The overall diagnostic accuracy for digital, screen film, and computed mammography was parallel, but in terms of DCIS detection rate, breast cancer detection rate, and recall rate ratios, digital mammography accuracy was significantly higher. From the results of this study, it can be concluded that digital mammography is a suitable diagnostic modality for breast cancer detection in the Taiwanese population.

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The Influence of Knowledge, Lifestyle and Culture on the Incidence of Tuberculosis A Literature Review

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Abstract

Background: TB is a public health problem whose incidence is still high in Timor Leste. TB is an infectious disease which is one of the leading causes of death in the world caused by an infectious agent Mycobacterium tuberculosis. The purpose of this study was to review scientific articles focusing on the Influence of Knowledge, Lifestyle and Culture on the Incidence of Tuberculosis. **Method**: The method used was a literature review. Articles used as literature review materials came from SagePub, BMC Public Health, Elsevier, Google Scholar, Pubmed, Proquest and others. The articles were selected based on the research focus, which was the Influence of Knowledge, Lifestyle and Culture on the Incidence of Tuberculosis. **Results and Conclusions**: Knowledge is very important in TB control efforts. Good knowledge needs to be followed by a good perception. Such knowledge is not only for the TB patients but also for the families and health workers. Lifestyles that are very risky to increase health vulnerability in TB patients are smoking, drinking alcohol, using illegal drugs, inadequate consumption, living in a slum environment, lack of air ventilation, lack of exercise, lack of sleep, urban life, lack of glycemic control, sugar consumption, and living with TB patients. Cultural influences include inter-racial disparities, culture of seeking local treatment or not going to health facilities, delay in initial screening, TB sputum examination, lack of PPE, poor local TB surveillance, traditional medicine, stigma and culture carried by

Keywords: *Tuberculosis, Knowledge, Lifestyle, Culture*

Introduction

TB is an infectious disease which is one of the leading causes of death in the world caused by an infectious agent Mycobacterium tuberculosis. TB can affect anyone, but generally occurs in adults. TB is curable and preventable. As much as 85% of TB sufferers recover after receiving TB treatment(1).

The incidence rate of tuberculosis in Indonesia in 2018 was 316 per 100,000 population and the death rate for tuberculosis sufferers was 40 per 100,000 population. In 2019, the number of tuberculosis cases found was 543,874 cases. The highest number of cases were reported from provinces with large populations, including West Java, East Java and Central Java. The Case Detection Rate of tuberculosis cases in 2019 was 64.5%. However, it is still far from the CDR figure recommended by WHO which is ≥ 90%. The national success rate for tuberculosis treatment has been achieved, including in 2019 which amounted to 86.6% (2). Timor-Leste has the second highest TB incidence rate in the Southeast Asia Region. According to data released by WHO in 2018, the total incidence of TB in Timor-Leste was 498 per 100,000 population. In comparison, the incidence rate in Indonesia is 316 per 100,000, in India 199 per 100,000, and in China 61 per 100,000 population. This article conducted a literature review on the risk factors for TB in the form of knowledge, lifestyle and culture to increase TB prevalence in Timor-Leste(1).

Tuberculosis problem in Timor-Leste is quite complex, from MDR TB, relapse, treatment compliance, stigma, community lifestyle, smoking culture, income, poverty, public trust, surveillance that needs to be improved, late arrival to health facilities and other factors. Dewi et al (3) stated that the patient's lack of knowledge, the delay in going to health facilities, the patient's ignorance of TB symptoms, as well as the

delay in health workers to take sputum also contributed to the TB problem in Yogyakarta. Family support and knowledge are also very important in TB control in Indonesia[4]. This article conducted a literature review on the risk factors of TB in the form of knowledge, lifestyle and culture to increase TB prevalence in Timor-Leste.

Methods

This article is a Literature Review article that focuses on Tuberculosis and the factors of Knowledge, Lifestyle and Culture. Articles used as literature review materials were from SagePub, BMC Public Health, Elsevier, Google Scholar, Pubmed, Proquest and others. The articles were selected based on the research focus, which was the Effect of Knowledge, Lifestyle and Culture on the Incidence of Tuberculosis.

Result

Risk Factors

1. Knowledge

Knowledge about the symptoms, treatment and prevention of TB is one very important factor in the effort to TB control [5]. Increased knowledge about TB is very important in the treatment and control of TB. TB Patient Knowledge can be changed and improved. The results showed that, when TB was diagnosed initially, TB patients had a moderate of 52.4% knowledge. The knowledge is significantly associated with the condition of the ability to read, education and income. Gradually, after doing the treatment program, the patient's knowledge about TB increases. This is certainly very important to succeed TB control and treatment. Knowledge is also very important to encourage the adherence to take TB drugs[6].

Recent research on the importance of Knowledge to the success of TB programs was conducted in Lesotho. Lesotho is one of the countries with the largest TB cases in the world and country with the worst incidence of TB-HIV co-infection in the world. This study focused on the knowledge and attitude associated with risk factor of TB in the general population. Some questions about TB were asked to respondents. The research questions consisted of 15 questions consisting of questions related to TB, TB symptoms, transmission and TB treatment. In general, TB knowledge in the community of Lesotho was good. However, efforts to increase knowledge need to be made on people who cannot read, rural people, unmarried people and farmers. This is because there is a very significant difference in knowledge between these groups[7].

Related research on knowledge was done at the Health Center in Iraq. This research was conducted in a special health facility for children, women and the elderly. The results of the study were quite biased because the selection of respondents was based on the position of the new patient in the Polyclinic. The results showed that there were differences in the patient's knowledge about TB for each room in the Policlinic. The result was certainly quite biased because the selection of respondents was not done well[8].

The measurement of knowledge regarding infection prevention and infection in household contacts was carried out by Researchers in India. This study was conducted considering that household contacts are the people most at risk for contracting TB from TB patients[9]. Knowledge measured was knowledge about TB, mode of transmission and prevention of transmission. The research result from the verifiers indicated that their level of knowledge was low and the economic conditions which was poor is one of the indirect risk factors of TB transmission in households. It is important for India to be able to develop family level communication tools and media so that transmission at the family level can be minimized properly.

Research related to TB knowledge among students was conducted in Indonesia. The research stated that the factors of gender, educational background before school and college courses will affect knowledge associated TB. The knowledge of female student is better than men's. Meanwhile, students who chose health as the main interest have a better knowledge than those who chose other interests[10].

In the general public in Indonesia, knowledge is very important in TB Control efforts. In urban communities, knowledge about TB is very good[11]. The level of knowledge is influenced by the educational level. Perceptions related to TB disease are also influenced by family income with a positive correlation. Perception will be important for successful TB treatment[12]. Good knowledge also needs to be followed by a good

perception regarding TB treatment. Organization of health workers is also important[13]. Studies in Indonesia still found delays in case contact tracing, delays in sputum collection and suboptimal TB surveillance.

Research on community was done in Somalia to measure individual's knowledge and attitude against TB. The interesting thing about this research is that knowledge about TB care is not followed by knowledge of the causes and transmission of TB. Such thing makes the number of TB incidence remains high. Health education needs to be done in order to increase knowledge about TB comprehensively so that TB control efforts can be carried out properly[14].

2. Lifestyle

Lifestyle such as smoking is one of the risk factors that can aggravate TB. A study in Mexico found that 60 adults smoked[15]. The government's efforts to increase the tobacco tax have proven to be able to provide changes such as a reduction in the number of smokers and a decrease in TB prevalence. This is because these efforts can improve the national TB prevention program. However, other risk factors that need to be considered are alcohol consumption, nutrition, housing conditions and food security.

Smoking behavior is one of the major obstacles in efforts to prognosis and outcome of TB. Study in Malaysia[16] stated that 83% of TB patients have smoking behavior. Smoking behavior has a very strong influence on the success and treatment of TB. Smoking is statistically a major barrier to TB treatment[17]. Furthermore, smoking cessation is one of the most appropriate ways to increase the success of TB treatment and to prevent TB drug resistance.

A study in Spain stated that smoking is one of the keys to the success of the TB program. TB patients in Spain are mostly smokers[18]. One of the government's efforts to control TB is to control the smoking behavior. This is done to support TB control and monitoring the programs in the country.

Likewise in South Africa, smoking is one of the major obstacles in the effort to control TB and HIV[19]. In southern Africa, the TB-HIV program is an important program. The interesting thing is that most of them know that smoking can worsen their health condition so they voluntarily quit smoking. However, this certainly needs to be evaluated in order to support the success of the TB Program.

TB problems and smoking behavior are major problems in *many low- and middle-income countries*[20]. Several efforts need to be made to stop smoking behavior, especially in TB patients. Treatment assistance and ongoing evaluation are still needed to support the success of the national TB control program. In *low- and middle-income* countries, smoking plays a major role in increasing the risk of TB, treatment failure and is very susceptible to cause relapse.

The Colombo study was conducted on the lifestyle of TB patients. In this study, it was informed that when TB patients were convicted of having TB, they restricted various activities that were usually carried out at home. In terms of employment, 27.7% of them were required to retired voluntarily, some of them change jobs, even temporarily laid off. On an income basis, 51% stated that they lost some of their income. When they became TB patients, they stop having recreation in public places and stop hanging out with friends. The most interesting thing is that 72% said that they wanted to stop smoking totally and 64.7% stop drinking alcohol. This is a form of positive life change due to the health promotion efforts undertaken by the local health authorities[21].

A cohort study was conducted in Indonesia regarding the No Smoking Area program and the Stop smoking program for TB patients that has been implemented[22]. Local regulation about KTR (No Smoking Area in Public Facility) has been implemented in all Indonesian health facilities. All forms of activities and promotions related to smoking are prohibited in health facilities and certain places in Indonesia. The existence of this KTR also helps efforts to reduce smoking behavior in public places and health facilities. This is certainly very important considering smoking can significantly affect the incidence of TB, the treatment and the outcome of TB. Smoking behavior can also cause delay in diagnosis, failure of TB treatment and even cause death due to TB. Efforts to quit smoking in TB sufferers are one of the best ways to prevent bad things as a result of TB[23]. Research in Indonesia stated that 77.6% of TB sufferers are active smokers. One of the efforts made

by the Indonesian government is an effort to promote health for 5-10 minutes to reduce or even stop smoking every time a TB patient comes to a health facility. This effort is running maximally, in which 86.1% of TB patients stated that they had stopped smoking completely for 6 months. This is certainly very good for the efforts to deal with TB in Indonesia.

The lifestyle of TB patients who adhere to treatment will change in a positive direction. This is evidenced by research conducted in Sri Lanka. During treatment, all TB patients tended to reduce risky behaviors such as smoking, hanging out with friends or family, consuming alcohol, and using illegal drugs[24]. Socio-economic conditions will greatly affect the life style of TB patients. However, when they are in the TB treatment program, they try to change their lifestyle to be better. This is certainly very good to support the success of the treatment program that is being implemented.

Nutritional consumption patterns will also have an indirect effect on TB disease[25,26]. Study in Canada[27] on 200 patients at Quebec in Canada generated information that nutritional deficiencies can lower a person's body immunity. This makes a person more susceptible to contract diseases, including TB. Vitamin and mineral intake are not proven to reduce the immunity with OR equals to 2.1. In addition, carbohydrates and vitamins are also important nutrients to increase endurance. The new fact states that living in a small flat with poor air circulation, poor access to clean water, alcohol consumption and smoking are risk factors that can aggravate TB[28].

TB with DM is one of the things that needs to be considered in the TB control program. Studies showed that someone with DM and insulin consumption, is more susceptible to be infected by TB. This study was conducted in Australia[29]. DM can increase the risk of developing TB to become more severe, failure of TB treatment, relapse, and can even lead to death[30].

Other lifestyle that also affects TB is the lack of exercise which causes obesity (OR = 4.40, 95% CI 1.27-15.25 and 2.38 (1.61-9.22), sleep less than 8 hours at night (OR = 1.75, 95% CI 1.01-3.01), and physical activity of less than 3 days/week [OR = 1.41, 95% CI 1.21-3.47] [31]. Besides urban life, monotonous lifestyle, glycemic control and high sugar consumption in TB patients with DM is also a risk factor that can worsen TB patients[32]. Maintaining a safe distance from people with TB symptoms, avoiding rook smoke and not smoking are behaviors that can prevent to be infected by TB[33].

3. Cultural Influences

A study was conducted in Michigan, US to look at disparities in existing races with TB incidence. Until now, disparities related to TB still occur in Michigan, especially related to race, age and origin. This makes transmission of the TB still common. This study was conducted on TB Trends for 9 years by analyzing 1,245 TB cases in that state. Blacks have a potential TB incidence 24 times greater than Whites. The existence of very far disparities certainly needs to be followed by a comprehensive approach to tackle the existing TB[34]. Different cultures and life styles between Black and White are the basis for future TB control programs. A follow-up study stated that a social environment also needs to be considered in solving TB which also involves Race[35,36].

The culture drivers and TB in Vanuatu are very unique. TB sufferers still consume alcohol, cigarettes and still do *Kastom* (local culture). Stigma in the community is still high. Most of them still believe in Traditional Medicine compared to Modern TB Medicine which has complied with WHO guidelines[37]. They even spend up to two weeks before going to a health facility. Health education is very important so that TB treatment can be carried out properly and in accordance with WHO guidelines, and efforts to prevent transmission can be prevented as early as possible.

Sweden is a developed country in Europe. However, TB-related problems can arise from immigrants who come from various countries with a high burden of TB cases. This is certainly a little difficult, considering that immigrants are certainly still thick with the culture they come from[38]. Culture is also related to the level of knowledge they have. A migrant who has a length of education less than 12 years is more prone to prevent the transmission of diseases including TB[39]. The same thing happened in London. Migrants will be more susceptible to be exposed or suffered from TB related to genetics, Vitamin D deficiency, comorbid, socioeconomic and migration-related[40]. The Difference related TB cases Culture and Gender also happened in Italy[41]. The number of TB cases in native Italy and other countries

is always higher than the migrants[42,43].

Valid, complete and reliable TB data is a problem in various countries in Africa[30]. Data originating from TB surveillance will certainly have various impacts on the decision making. Evaluation system of surveillance conducted obtained information that the data from various levels of health institutions are not the same. TB surveillance improvement is very necessary to produce valid and reliable information. Standard surveillance guidelines, good systems, and surveillance officers who can work well are needed in addition to related agencies to periodically monitor the operation of the surveillance[44]. Active case finding, case tracking and good cooperation between the government and NGOs will play an important role in the success of TB surveillance program. Therefore, the collaboration must be done well[45,46]. The research was also supported by the results of research in China stating that 25% TB of data in hospitals is not reported well. Various problems encountered are, surveillance officers who are less reliable, workload that exceeds the ability, poor supervision and records at the local and national levels that are not well integrated[47].

Different language cultures between patients and health workers can be a barrier to the success of TB suppression program. Besides traditional medicine, stigma, and the desire of people who prefer traditional medicine to treatment in health centers also become obstacles. This is also exacerbated by the insufficient availability of self-protecting devices for health workers. Inadequate training for health workers and TB patients add to the complexity of TB resolution in South Africa[48]. In South Africa, it was also mentioned that 20 types of herbs were developed which were used for treating TB. Allium *sativum L. (Liliaceae) and Strychnos decussata (Pappe) Gilg.* Strychnaceae is the most frequently used and claimed to have proven successful use[49]. This requires further action and research by WHO to prevent MDR TB.

Conclusion

The results of the literature review state that knowledge is very important in TB control efforts. Good knowledge needs to be followed by a good perception. This knowledge is not only in TB patients but also in families and health workers. Lifestyles that are very risky to increase health vulnerability in TB patients are smoking, drinking alcohol, use of illegal drugs, inadequate consumption, living in a slum environment, lack of air ventilation, lack of exercise, lack of sleep, urban life, lack of glycemic control, consumption of sugar, and living together with TB patients. Cultural influences include disparity on race, culture of seeking local treatment or not going to health facilities, delay in initial screening, TB sputum examination, lack of PPE, poor local TB surveillance, traditional medicine, stigma and culture carried by migrants.

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A Rare Case of Cervical Epidural Abscess Post Posterior Cervical Laminotomy with Isolation of Neisseria Meningitides Bacteria

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Abstract

Spinal epidural abscess is a rare condition, although it requires an urgent surgery to decrease the pressure on the epidural sac in addition to the other structures of the spinal cannel cord and nerve roots.

A 29 years old female entered the clinic with large neck swelling. She also had a history of headache with neck pain, both arm paresthesia with mild fever. Her MRI showed an epidural collection at level of cervical 5 to cervical 7 compressing the dural sac narrowing the spinal cannel and extending to the paraspinal intramuscular area in the neck.

She had undergone a surgery with a 2cm mid line incision above the swelling mass in order to suck out the pus. In addition, the intermittent Valsalva maneuver was performed to enhance the epidural pus gat out. The culture and sensitivity test to pus indicated a Neisseria meningitides bacterial growth. Penicillin G and cefotaxime as antibiotics were prescribed. Improvement occurred within 2 weeks.

Although rare, the isolated Neisseria meningitides spinal epidural abscess requires an urgent treatment through evacuating and decompressing. Spondylodiscitis or collapse may occur, therefore, a surgery for spine correction may be needed, fellow up is recommended in all forms of epidural abscesses.

Key words: *Neisseria meningitides bacteria, cervical spine, spinal epidural abscess*

Introduction of the Case

A 29-year-old female entered the clinic suffering from severe neck pain, head ache, paresthesia, myelopathy of both arms and a history of posterior cervical laminotomy for cervical 5 to cervical 6 before 11 months. Her MRI revealed an intramuscular cyst containing pus and 2 pieces of foreign body in the large neck swelling of 6 X 3 cm diameter. Its size was 5.5cm long X 1.5mm width of epidural abscess at level of cervical 5 to cervical 7 vertebral body on the right side.

On examination, she seemed confused, uncomfortable and pale. Her pulse rate was 100 beats per minute, respiratory rate 16 cycle, blood pressure 100/70, temperature 37.9^{O}C.

Methods

Musculoskeletal examination

Musculoskeletal examination showed a large posterior cervical tender mass, with painful neck movement and there was no stiffness and head ache.

Neurological examination

The examination showed that the patient had a paresthesia over the RT, LT shoulder and upper limb predominantly RT side.

Motor examination

Motor examination was conducted on the patient. This examination revealed the followings:

RT side

1. Shoulder grade 2
2. Wrist flexion and extension both grade 2
3. Elbow flexion grade 3, extension grade 2

LT side

1. All examination of grade 4
2. Reflexes
3. Diminished elbow and wrist reflex

Result

Laboratory findings

The patient had also underdone laboratory examinations showing the followings:

1. White blood cell count (WBC) of 4,631 cells/ul
2. Neutrophils sedimentation 56%
3. Lymphocyte 26%
4. Monocyte 15%
5. Hemoglobin level is 11.7 g/dL
6. Platelet is 476, cell/ul
7. Glucose concentration of 129 mg/dL
8. C-reactive protein (CRP) 2.77mg/dL (normal range <0.5 mg/dL)

MRI FINDING

The MRI findings showed a large intramuscular cystic pus of 6 x 3 cm diameter containing two suspicious bodies. In addition, the epidural pus collection extended from cervical 5 to cervical 7 of 5 cm long X 1.5 width (figure 1).

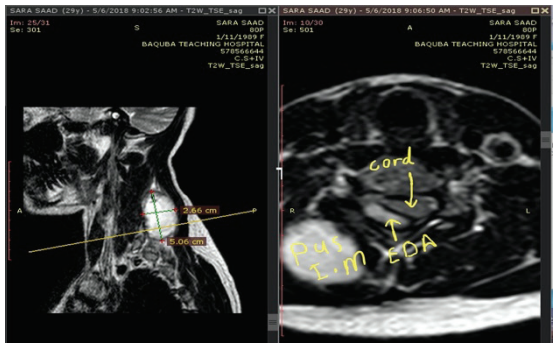

Figure 1, a large oval cyst intramuscular, and epidural abscess compressed spinal cord

Procedure

Under general anesthesia, prone position, amid line 1.5 cm long skin incision was made over the cyst at cervical 5 level. In addition, the paraspinal muscle was separated and the cyst wall was incised. All the pus was evacuated removing the 2 foreign particles (resulted from solidification of wax inserted in the previous operation) as figures 1,2 and 3 show. The anesthesiologist performed irrigation and suction with normal saline and an Appling intermitting Valsalva maneuver to increase the dural and vascular pressure. The pressure forces the epidural pus go out through previous laminotomy window (figure 2). This means that there was no need for another laminectomy. When clear fluid of irrigation was seen. A 350 ml ready vac drains were inserted (kept for 2 days) and the pus sent for lab examination. Intravenous antibiotics started during the operation including cefotaxime 1g by 2 pulse amikacin 1g by one a day. The result showed Neisseria meningitides bacteria, as a result, the antibiotics was changed to penicillin G and cefotaxime for 10 days post-operative. The patient remained 3 days at the hospital after the operation.

picture 1

picture 2

picture 3

Picture 1, color of pus, picture 2,3 a harden bone wax from previous operation (grasped by artery forcipes)

Figure 2, laminotomy site from previous operation

Sample Results

The colonies that grow on the Macconky and chocolate agar media are circular, convex, entire, opaque, shiny, smooth. They were positive for oxidase and catalase. The gram stain was used to investigate the shape of the bacteria and the stain was gram negative diplococcus bacteria that confirm the species is Neisseria meningitidis.

Out come and follow up

Anti-Neisseria antibiotics started by only changing amikacin to penicillin (ceftriaxone 1g injection plus penicillin 1.2 mega I.U intravenous injection for 10 days).

Postoperatively, gradual improvement occurred in both upper limbs. At one month follow up, both upper limbs showed a complete motor recovery (grad 5). MRI was conducted 6 months post-surgery showing an abscess complete resolution and the spinal cord had well decompressed (figure 3).

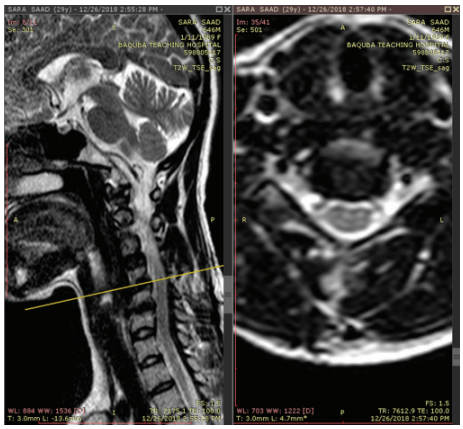

Figure 3, a complete resolution of abscess

Discussion

The reason of the disease of invasive meningococcal is Neisseria meningitidis which is a very quickly progressive sepsis syndrome. It could be non-classically presented as epiglottitis, septic arthritis, pneumonia and pericarditis.

The N. meningitidis clone is caused by a single introduction event. In Victoria, this event undergoes an extensive endemic transmission[1, 2].

In the case report paper, the patient was 78-year-old female patient presented to emergency department. She had a throat pain 5-day and hoarseness. She also had a dolorous and progressive submandibular swelling for the same period.

A neck CT scan with intravenous contrast has been performed for her. The scan displayed an acute epiglottitis typical thumbprint sign. In minutes, her health conditions deteriorated. The patient was near the respiratory exhaustion. The primary sample of blood cultures showed Neisseria meningitidis bacteremia. Then, the treatment of the patient was successful[3].

Another case was a 44-year-old woman. She suffered from rhinitis and sore throat. These two illnesses developed to dysphagia, neck swelling, dyspnea at night. Therefore, she was required to sleep sitting upright with dramatic external cervical swelling because of cellulitis. The blood cultures showed Neisseria meningitides positive[4]. In many countries such as the USA, Colombia, Sweden, Norway, Finland and Venezuela, the cases with N. meningitidis have risen over the previous 20 years [5].

For Neisseria meningitides, the natural habitat and reservoir are the human nasopharynx. Nasopharyngeal mucus components specifically bind the meningococci that cause cytotoxicity. This cytotoxicity breaks down the tight junctions of epithelial cells which slough the ciliated cells and ciliostasis. The most vital components that mediate between initial meningococci attachment and non- ciliated epithelial cells of the human nasopharynx are pili. The meningococci occupy the epithelial surface in order to arrive at the submucosa in different routes. Although meningococci are divergent, they have become successful. It is the mechanisms that infect the nasopharynx in humans [6]. The vital element to activate the classical pathway of component

is the fourth complement component (cervical 4). It is a key defense that protects from the activities of microorganisms. The total risen cervical 4 deficiency cases were in patients suffering from Neisseria meningitidis infection, Haemophilus influenzae or Streptococcus pneumoniae[7]. N. meningitidis could be defined as a strict human pathogen. With endothelial cells, this pathogen could interact very firmly. Type IV pili mediate meningococcus adhesion for the induction of a sub cortical cytoskeleton localized remodeling. This forms endothelial membrane protrusions anchoring bacterial colonies at the endothelial cell membrane face of endoluminal. This helps to better resist the flow of blood. In the recent studies, the ability of N. meningitidis to recruit the polarity complex Par3/Par6/aPKC has been reported. This re-routs the adhesion molecules of endothelial cell of interendothelial junctions which open a paracellular route for bacteria to pass the endothelial barrier [8].

Conclusion

Isolated Neisseria meningitidis spinal epidural abscess is an infrequent illness. However, it requires an urgent treatment through evacuation and decompression. Some signs of spondylitis or spondylodiscitis could exist later. Thus, a long follow-up is suggested for N.meningitidis patients with this type of abscess.

Ethical Clearance: Taken from College of Medicine, University of Diyala committee

Source of Funding: The source of funding self.

Conflict of Interest: Nil

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Hepatocellular and Immuno-toxicopathological Effects of Acrylonitrile in Albino Male Mice

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Abstract

This study to provide details on role of Acrylonitrile (AN) in blood profile and histopathological changes in liver in male albino mice, the aims of current study were detected influence and toxication of AN in the male mice, the present article was include (30 albino mice) divided into 3 groups, the age ranged from 8-9 week. The dose of Acrylonitrile (25mg / kg of BW) and the period of this study 60 days.

The hematological examination showed increased in WBCs (9.32±1.11 / 11.802±0.75), Lymphocyte (9.25±2.20 / 11.05±1.95), Neutrophile (5.55±0.88 / 7.03±1.50) and Monocyte (0.19±0.97 / 0.33±0.18) in 2nd and 3rd group respectively, while showed decreased in RBCs (6.40±1.11 / 5.82±0.70), PCV (38.65±1.20 / 36.40±2.13), MCV (44.70±1.49 /41.95±0.49), MCH (14.85±1.44 / 10.00±1.84) and Hb (13.24±1.82 / 12.63±1.91) in 2nd and 3rd group respectively, The histopathological changes in liver showed extensive necrosis, thickening and congestion of blood vessels, infiltration of inflammatory cells, mast cell aggregation in hepatocytes, hepatocyte atrophy and extensive areas of abscess in 2nd group, while in 3rd group showed perihyperplastic bile duct, large extensive granuloma, multiple giant cells, dystrophic calcified and hepatic necrosis.

Keyword: *Acrylonitrile, albino male mice, toxication.*

Introduction

Hazardous chemicals have dangerous toxic effects on the environment and human health, moreover producing these chemicals involve the use and handling of other hazardous chemicals are used in the manufacturing process. Harmful effects of chemical pollutants and their metabolites are due to their ability to generate reactive oxygen species (ROS) due to ingestion, inhalation or absorption chemicals and their products [(1)]

Acrylonitrile (AN) is an important industrial hazard chemical used extensively in the manufacture of synthetic fibers, resins, plastics, elastomers, and rubber for a variety of consumer goods, such as textiles, drinking cups, automotive parts, and appliances[(2)]. It is also used as a monomer for acrylic and modacrylic fibers, in plastics, surface coatings as a chemical intermediate, in organic synthesis, in home furnishings, in nitrile rubbers, and as a modifier for natural polymers and pesticides[(3)].

Human may be exposed to the AN around factories where it is made for used or near chemical waste sites which it has been improperly stored or disposed, two most likely exposure pathways are breathing acrylonitrile that has evaporated in to air or drinking water that has been contaminated due to AN is highly soluble and stable in water[(3)].

The AN induced pathological changes in lab. animals, specially in the gastrointestinal tract and liver, the overexposure to the AN characterized by mild jaundice, cyanosis (dark red, blue or purple skin colors in lips, gums or fingernails), irritation with inflammation of eyes and respiratory system including nose and threat[(4&5)].

Materials and Methods

The central objective of this study is to evaluate the toxicopathological effects of Acrylonitrile in albino male mice, this study was applied on albino male mice (30) , the age range was (8) weeks and the weight range was

(40-50) gms , the animals were housed in animal house in College of Veterinary Medicine, University of Diyala, by using plastic cages with temperature maintained at 25 ±2 C°, this plastic cages containing hard-wood chip as bedding and the bedding was changed continuously to ensure a clean environment, the experiment design consist of 3 groups:

1- 1st group (10 mice) were act as control group feeding on usual rat diet.

2- 2nd group (10 mice) were administered daily by stomach tube Acrylonitrile (25 mg/kg BW) for 30 days.

3- 3rd group (10 mice) were administered daily by stomach tube Acrylonitrile (25 mg/kg BW) for 60 days [6].

Sample collection: Heart blood samples were collected and separated into EDTA tubes for complete blood count (CBC). Tissue fresh liver were collected from the scarified rats directly and was left in 10% formalin solution to make a paraffin block for histopathological examination.

Hematological analysis: Blood samples were collected from the heart of each mice in EDTA tubes. the complete blood count (CBC) were collected blood samples by using Auto Hematology Analyzer (Huma count 30ts- Germany) including white blood cells (WBCs), lymphocytes, monocytes, neutrophils, red blood cells (RBCs), PCV, MCV, MCH and Hb.

Histopathological examination: After (30 and 60) days of experiment the animals were sacrificed by the anesthetized intramuscular with xylazine and ketamine 10%. Specimens (liver) were taken and the tissues were kept in 10% formalin solution for fixation and then processed routinely by using the histokinette. Tissue sections were embedded in paraffin and sectioned by rotary microtome and all sections were stained with hematoxylin and eosin stain [7].

Statistical analysis: The all grouped data were statistically read by SPSS program, Version 17 software (2010).Testing methods including one way ANOVA for comparisons among groups. P values of $p < 0.05$ were considered to record statistical significance. All data were expressed as means ± standard error (SE) [8].

Results

1- Hematological analysis:

Complete blood count revealed that the Red blood cells (10^6/µl) showed significant decreased count in 2nd group (6.40±1.11) and 3rd group (5.82±0.70) compared with the control group (8.24±0.52), also there were significant difference between the 2nd and 3rd group.

Packed cell volume (PCV) % showed significant decreased count in 2nd group (38.65±1.20) and 3rd group (36.40±2.13) compared with the control group (42.20±1.32), also there were significant difference between the 2nd and 3rd group.

Also the mean corpuscular volume (MCV) (fl) showed significant sharp decreased count in 2nd group (44.70±1.49) and 3rd group (41.95±0.49) compared with the control group (51.68±1.82), also there were significant difference between the 2nd and 3rd group.

Mean corpuscular haemoglobin (MCH) (pg) also showed significant decreased count in 2nd group (14.85±1.44) and 3rd group (10.00±1.84) compared with the control group (18.10±1.60), also there were significant difference between the 2nd and 3rd group.

The Hb (g/dl) showed significant decreased count in 2nd group (13.24±1.82) and 3rd group (12.63±1.91) compared with the control group (14.95±1.02), also there were significant difference between the 2nd and 3rd group.

Complete blood count revealed that increased in white blood cells count (103/µl) in 2nd group (9.32±1.11) and 3rd group (11.802±0.75) compared with the control group (6.22±1.60), also there were significant difference between the 2nd and 3rd group.

Also this complete blood count showed the significantly elevated lymphocytes (10^3/µl) difference count in 2nd group (9.25±2.20) and 3rd group (11.05±1.95) compared with the control group (4.85±1.10), also there were significant difference between the 2nd and 3rd group.

Neutrophile (10^3/µl) showed the significantly elevated in 2nd group (5.55±0.88) and 3rd group (7.03±1.50) compared with the control group (2.35±0.95), also there were significant difference

between the 2nd and 3rd group.

Finally the monocytes (103/µl) showed the significantly elevated in 2nd group (0.19±0.97) and 3rd group (0.33±0.18) compared with the control group (0.05±0.86), also there were significant difference between the 2nd and 3rd group.

Table (1): Effects of AN on complete blood counts in male mice groups.

Parameters	Groups		
	C (1st group)	2nd group	3rd group
RBCs (106/µl)	8.24±0.52 a	6.40±1.11 b	5.82±0.70 c
PCV (%)	42.20±1.32 a	38.65±1.20 b	36.40±2.13 c
M CV (FL)	51.68±1.82 a	44.70±1.49 b	41.95±0.49 c
MCH (pg)	18.10±1.60 a	14.85±1.44 b	10.00±1.84 c
Hb (g/dl)	14.95±1.02 a	13.24±1.82 b	12.63±1.91 c
WBC (103/µl)	6.22±1.60 c	9.32±1.11 b	11.802±0.75 a
Lymphocyte (103/µl)	4.85±1.10 c	9.25±2.20 b	11.05±1.95 a
Neutrophile (103/µl)	2.35±0.95 c	5.55±0.88 b	7.03±1.50 a
Monocyte (103/µl)	0.05±0.86 c	0.19±0.97 b	0.33±0.18 a

Values are expressed as means±SE with different letters are significantly different (P<0.05).

2- Pathological examination:

There are non-pathological changes (macroscopic and microscopic) lesions observed in control group.

In macroscopic changes in liver showed enlarged size, severe congestion with depressed irregular multifocal areas mostly necrosis in 2nd and 3rd group.

The microscopic changes in liver of 2nd group showed irregular macrovascular fatty change, infiltration of mononuclear cells, congested of blood vessels with aggregation of inflammatory cells and vacuolation of hepatocyte (fig. 1), also appear massive hepatic necrosis, bile pigment accumulative in hepatocyte, hepatocyte atrophy with thickening of blood vessels wall (fig. 2 and fig.3), Liver abscess consist of liquefactive necrosis surrounded with dead basophilic, neutrophils and zone of fibrous connective tissue with calcification (fig. 4).

The microscopic lesion in liver of 3rd group showed hyperplasia of bile duct, congested of blood vessels, infiltration of inflammatory cells with perihyperplastic bile duct (fig. 5). Massive hepatic necrosis with bile accumulates in the hepatocytes, epithelioid granuloma, numerous giant cells, fibrous connective tissue, hepatic necrosis with hepatocyte atrophy (fig. 6).

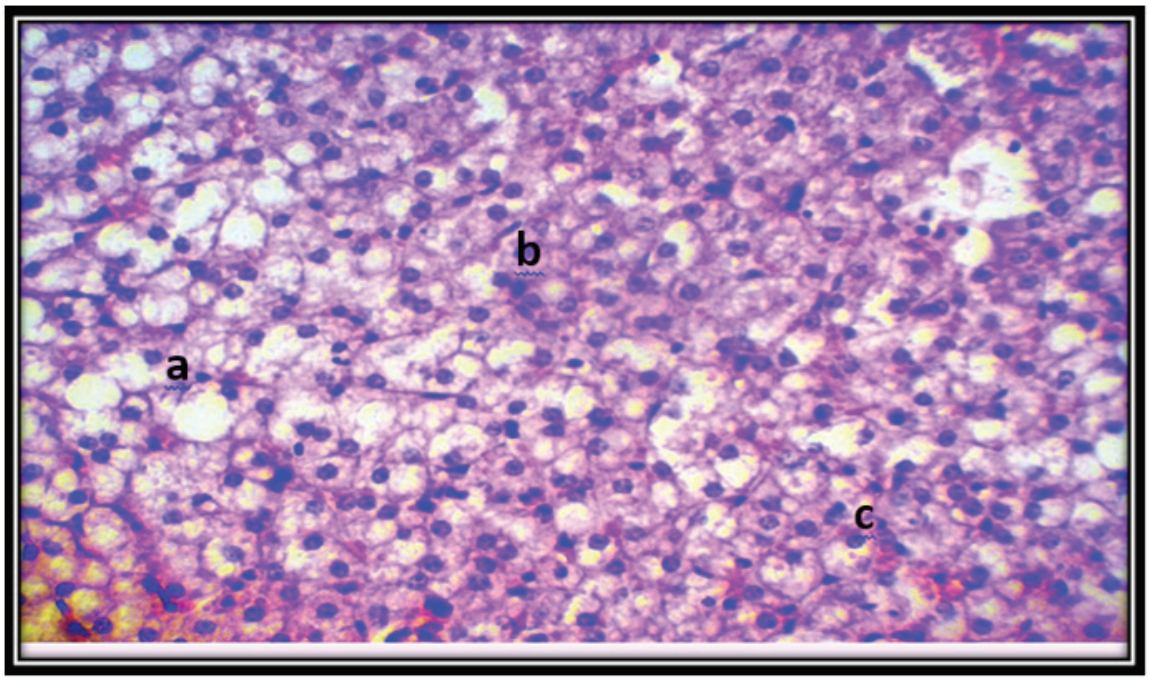

Figure 1: Histopathological section in liver in second group at day 30 showed (a) irregular macrovascular fatty change (b) infiltration of mononuclear cells (c) congested of blood vessels (X40 H&E stain).

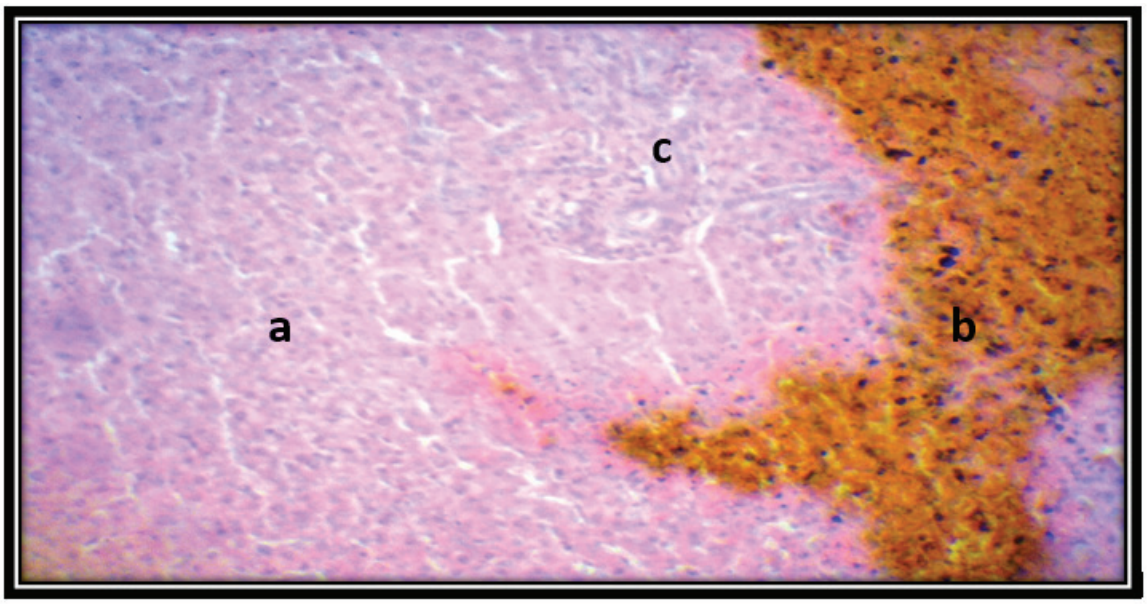

Figure 2: Histopathological section in liver in second group at day 30 showed (a) massive hepatic necrosis, (b) bile pigment accumulative in hepatocyte (c) hepatocyte atrophy (X20 H&E stain).

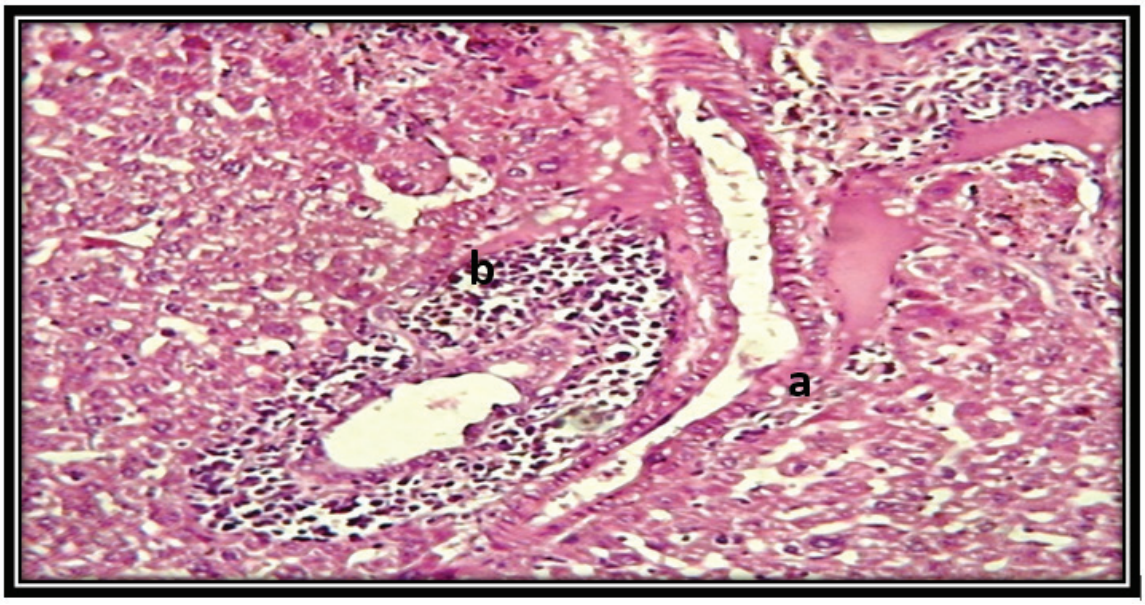

Figure 3: Histopathological section in liver in second group at day 30 showed (a) thickening of blood vessels wall (b) infiltration of inflammatory cells (X20 H&E stain).

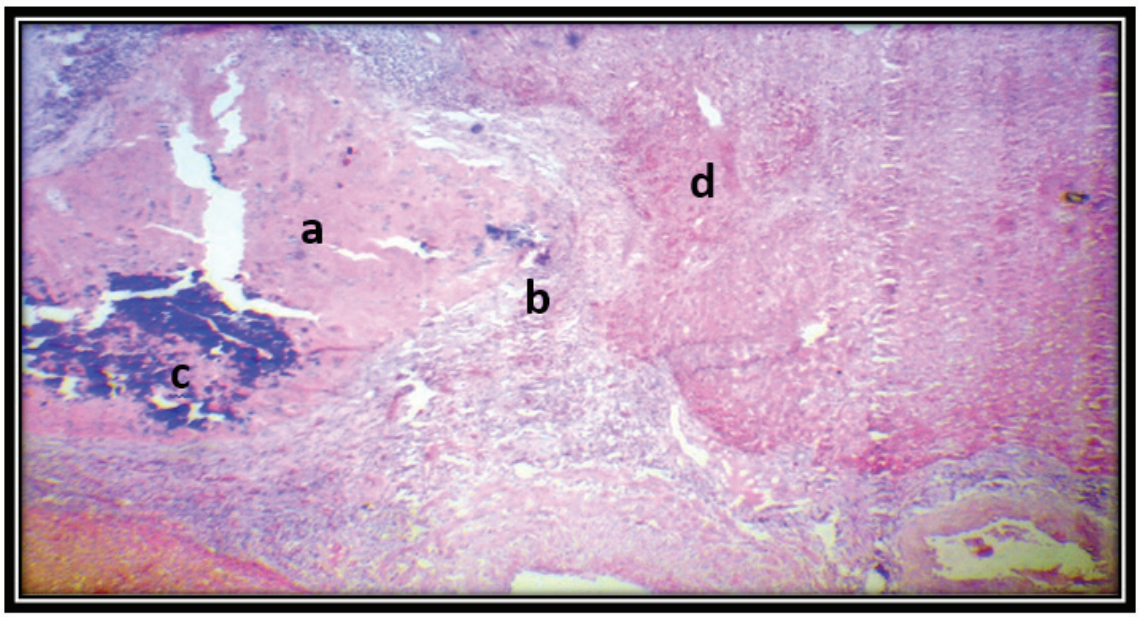

Figure 4: Histopathological section in liver in second group at day 30 showed (a) hepatocyte abscess consist of liquefactive necrosis,(b) few fibrous connective tissue zone, (c) calcification (d) extensive hepatic hemorrhage (X20 H&E stain).

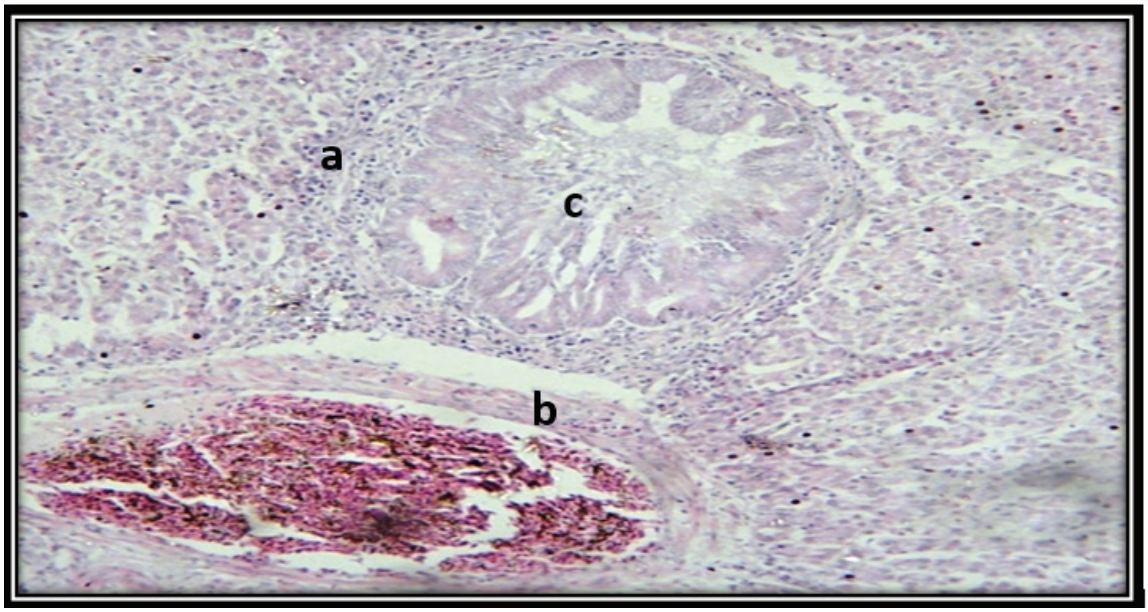

Figure 5: Histopathological section in liver in second group at day 60 showed (a) severe infiltration of inflammatory cells (b) fibrosis perivascular (c) perihyperplastic bile duct (H&E X20).

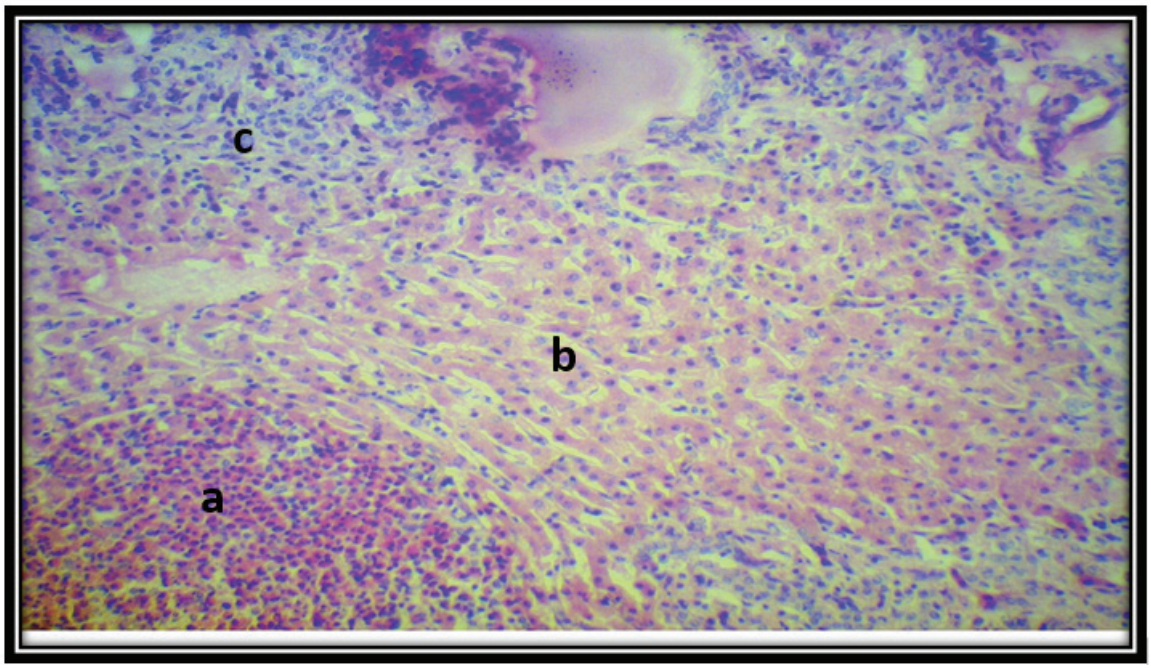

Figure 6: Histopathological section in liver in second group at day 60 showed (a) massive granuloma (b) hepatocyte necrosis (c) infiltration of inflammatory cells (X40 H&E stain).

Discussion

Exposure to different organic solvents and toxic substances has been reported to cause adverse effects on the hematological profiles in animal and humans and the adverse effects reported to be associated with exposure to different chemical substances are likely results from the interaction of the metabolites of these solvents with blood components[9].

The 2nd and 3rd group showed decrease of Hb concentration, this result is in similar with [10] who found decrease Hb concentration possibly due to suppression of erythropoiesis and heme synthesis and devastation of erythrocytes in hemopoietic tissue which lead to different disease such as anemia. while showed increase

in WBCs these results are in agree with[11] who observed activation of immune system of the body may result in increase in release of WBCs into the blood leading to an increase in number of WBCs.

[12] who showed a decrease in red blood cells (RBCs) count, PCV and Hb concentration after acute or chronic exposure to the AN and the mechanism of haemotoxicity were not clear but may be due to bonding of AN covalently with RBCs membranes and hemoglobin molecules. These results are in agree in current results of 2nd and 3rd group.

Also the decrease of RBCs, MCV and MCH and increase of WBCs in 2nd and 3rd group, these results are in agree with[13] who observed significant decrease in RBCs, HCT %, MCV,MCH, MCHC, platelets count and Hb concentration, and increase significantly of WBCs count in albino male rats exposed to AN.

The liver is the main organ responsible for the metabolism of drugs and toxic chemicals and so is the primary target organ for many organic solvents and the main pathogenic mechanisms responsible for damage caused by toxins are inflammation, dysfunction of cytochrome P450, mitochondrial dysfunction and oxidative stress. So the present study showed that the liver damage by prolonged exposure to toxins may be due to development or complications of liver cirrhosis which lead to liver cancer and the most common type of cancer caused by cirrhosis is hepatocellular carcinoma[14].

The histopathological lesions observed in liver in 2nd and 3rd group was due to that the AN has ability to induce progress liver cell injury, hyperplasia and cirrhosis. These results are in agree with[15] who reported that the liver was considered as a first organ responsible for metabolism of AN, also AN may have ability to cause lipid peroxidation via reactive oxygen species which lead to fatty changes in addition to hypoxia.

Also the damage in liver in 2nd and 3rd group including extensive necrosis, thickening and congestion of blood vessels, infiltration of inflammatory cells, mast cell aggregation in hepatocytes, hepatocyte atrophy, perihyperplastic bile duct, large extensive granuloma, multiple giant cells, dystrophic calcified and hepatic necrosis. These results are in agree with[13] who recorded hepatocytes swelling with nuclei enlargement, dilation of central vein and in portal areas congestion of all blood vessels, the hepatocytes with irregular arrangement, smaller than centrally located nuclei with multiple hyperplastic lobules, hyperplastic parenchymal nodules separated by fibrous band with distortion of normal liver architecture and moderated infiltration of mononuclear cells mostly lymphocytes and macrophages, blood vessels appear dilated and congested after exposure to AN in albino male rats.

The inflammatory cells infiltrating were also appeared mainly MNCs, lymphocytes and neutrophils in liver and other organs in 2nd and 3rd group, these changes may be due to the toxic effect of AN and phagocytic cells (macrophages, dendritic and B lymphocytes) that resulting in increased the susceptibility to toxicity and infection[16].

Conclusion:

Oral administration of AN (25 mg/kg BW) cause toxic effects characterized by significantly decreased the RBCs count, Hb concentration, platelets count and significantly increased of WBCs count, also histopathological changes in liver specially fatty change, congestion in blood vessels, infiltration of inflammatory cells, hepatocyte atrophy and granuloma.

Recommendations:

1-Adoption of control and preventives measures to minimize the effect of AN in the community.

2-Public education about the dangerous effects of pollution with plastic materials through lectures, TV, social media is recommended with the cooperation with public civil for organizations.

Ethical Clearance- Taken from Department of pathology and poultry disease committee

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Risk Factor Analysis for Placenta Retention in Maternal Delivery at the Tenriawaru Regional Hospital in Bone Regency

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Abstract

Placenta retention issues have complications that can endanger mothers after delivery because of their strong association with bleeding. This research aims to determine the risk factors for placental retention in maternal. This research was an observational analytic study with a case-control design with a sample size of 68 mothers. The research was conducted at the Tenriawaru Hospital in 2020. Data collection was carried out by interview using a questionnaire. Then it was analyzed using the chi-square test. The results of the research for the Age variable (p-value = 0.027) show that there is a relationship between age and the incidence of placental retention, with the OR value: 3.4 (CL: 1.26–9.41) and for the parity variable (p-value = 0.44) there was no relationship between parity and the incidence of placental retention, with the OR value: 0.47 (CL: 0.12–1.82). It can be concluded that of the two factors the incidence of placental retention including age and parity, only age is associated with the incidence of retained placenta.

Keywords: *Placenta Retention, Age, Parity*

Introduction

The issue of maternal and child health remains a problem that has not been resolved. World Health Organization data states that every day an estimated 800 women die as a result of preventable causes related to pregnancy and childbirth[1]

The Millennium Development Goals (MDGs) target are to improve maternal health and reduce up to ¾ of the risk of death to reach 102/100,000 live births by 2015[2]. One indicator of the impact is to reduce the maternal mortality rate (MMR) from 262 per 100,000 live births in 2005 to 74 per 100,000 live births in 2025[3]. The importance of efforts to reduce the incidence of retention of the placenta by providing counseling about the age at high risk of experiencing complications during pregnancy and advising pregnant women of high-risk age to make antenatal care visits at least four times to identify abnormalities early during pregnancy[4]

Maternal age is one of the factors that influence the mother's health status during pregnancy. Pregnant women with a relatively young age or on the contrary too old tend to be younger to experience health complications compared to mothers with a healthy reproductive period of 20–35 years[5]

Causes of maternal death in Indonesia include bleeding, infection, abortion, prolonged labor, and indirect causes of death such as cancer, heart disease, tuberculosis, or other illnesses suffered by the mother where bleeding became the second-highest cause after the indirect cause of death, namely 30.3%[6]. WHO mentions that one of the causes of bleeding after childbirth is placental adhesions or retained placent[7]

The causes of maternal death were bleeding 28%, eclampsia 25%, infection 11%, complications of abortion 5%[8]. The high MMR is also caused by three incidents of being late, namely being late in knowing, being late in referring and being late in making a decision and four incidents of being too young, too old, too many children, too close the distance between births is still high[9]. One of the causes of bleeding is placental retention with a frequency (16–17%) and other causes of uterine atony with a frequency (50–60%), birth canal laceration (4–5%), blood disorders with a frequency (0.5–0.8%)[10].

Other predisposing factors that influence the occurrence of placental adhesions are age, parity, the uterus is too large, short gestational distances,

and socioeconomic conditions. Other literature adds education, history of labor complications, and anemia status as factors that are associated with the incidence of placental retention. This research aims to determine the risk factors for placental retention in mothers who give birth at the Tenriawaru General Hospital.

Material and Methods

This research was conducted at the Tenriawaru Regional General Hospital, Bone Regency, South Sulawesi Province in July–September 2020. Tenriawaru Hospital is a hospital with a high incidence of placental retention due to receiving referrals from all Community Health Centers in the various bone regency.

This type of research is analytic observational with a case-control design. The population in this research were all mothers who gave birth at the Tenriawaru Hospital. The sample selection of all mothers giving birth at the Regional General Hospital Tenriawaru, then the sample was selected using a simple random sampling technique. The independent and dependent variables were analyzed by bivariate. The hypothesis test used is the chi-square test. The amount of the risk factor is calculated using the odds ratio (OR).

Findings

Table 1 Distribution according to the characteristics of respondents in the Tenriawaru Regional Hospital, Bone Regency

Characteristic	n	%
Retention of Placenta		
Yes	35	51.5
No	33	48.5
Education		
Graduated from elementary school	12	17.6
Graduated from junior high school	10	14.7
Graduated from high school	32	47.1
Graduated from college	14	20.6
Occupation		
Entrepreneur	2	2.9
Civil Servant	7	10.3
Housewife	54	79.4
Private	5	7.4
Age		
Risky (<18 – >35 years)	31	45.6
Not risky (18–35 years)	37	54.4
Parity		
Multiparous	11	16.2
Primiparous	57	83.8
Total	68	100.0

Table 1 shows the characteristics of the respondents studied. All respondents in this research were mothers with placental retention, which amounted to 35 or 51.5% of respondents, and mothers who gave birth without placental retention were 33 or 48.5% of respondents. Thus, the number of respondents in this research were 68 mothers giving birth.

Based on the characteristics of the level of education, most of the respondents had graduated from high school, namely, 47.1%, based on the characteristics of the job, namely 79.4% were housewives, it can be seen that the average age of respondents 18 years to 35 years (not at risk) is 54.4%, while based on parity characteristics most of the primiparous respondents are 83.8%.

Table 2, The Relationship Between Age And Placental Retention Of Respondents At The Tenriawaru Regional Hospital, Bone Regency 2020

Respondent Age	Placenta Retention						
	Yes		No		Total		Statistic Test
	n	%	n	%	N	%	
Risky	21	67.7	10	32.3	31	100	p = 0,027
Not risky	14	37.8	23	62.2	37	100	OR : 3.4
Total	35	51.5	33	48.5	68	100	CL : (1.26–9.41)

Table 2 shows that of the 31 respondents' at-risk age, 67.7% had placental retention and of the 37 respondents with no risk age, 37.8% had placental retention. Based on the test results obtained p-value < 0.027. This means that there is a relationship between age and the incidence of placental retention. Placental retention tends to be experienced by age at risk, namely <18 years– >35 years.

The results of the calculation of the odds ratio show that respondents with the risky age category have a 3.4 times greater chance of experiencing placental retention than respondents with no risk age category.

Table 3, The Relationship Between Parity And Placental Retention In The Tenriawaru Regional Hospital, Bone Regency 2020

Parity	Retention of Placenta						
	Yes		No		Total		Statistic Test
	n	%	n	%	N	%	
Multiparous	4	36.4	7	63.6	11	100	p = 0.44
Primiparous	31	54.4	26	45.6	57	100	OR : 0.47
Total	35	51.5	33	48.5	68	100	CL : (0.12–1.82)

Table 3 shows that of the 11 respondents with multiparous parity, 36.4% experienced placental retention and of the 57 respondents with primiparous parity, 54.4% of respondents had placental retention. Based on the test results obtained p-value < 0.44. This means that there is no relationship between parity and the incidence of placental retention.

Discussion

The results show that there is a relationship between age and retained placenta. Placental retention tends to be experienced by age at risk, namely <18 years–> 35 years. The results of the calculation of the odds ratio show that respondents with the risky age category have a 3.4 times greater chance of experiencing placental retention than respondents with no risk age category.

The results of this research are in line with Aminah which states that statistically there is a significant relationship between maternal age and the incidence of placental retention with an OR=3.8 value, which means that women aged 35 years have a nearly four times greater risk of experiencing retained placenta when compared to mothers aged 20–35 years[11]. This research is also in line with research conducted by Hardiana, the majority of respondents with no risk age are 61 people (76.2%)[12]. The majority of respondents did not experience placental retention, 64 individuals (80%). There is a relationship between maternal age and the incidence of placental retention (P-value = 0.001).

This can occur because at the age of under 20 years a woman's reproductive function has not developed completely. Meanwhile, in women over 35 years of age their reproductive function has decreased or deteriorated, so that in labor complications such as postpartum hemorrhage caused by retention of the placenta can occur. Therefore, consideration of age in pregnancy or childbirth is one of the things that must be considered.

The results of this research indicate that there is no relationship between parity and the incidence of placental retention with a p-value < 0.44. Parity is not a factor in the incidence of placental retention, it can also be related to the health status of pregnant women. However, theoretically, the incidence of placental retention is related to maternal parity in which the occurrence of deterioration and defects in the endometrium resulting in fibrosis in the placental implantation mark in previous labor, so that vascularity is reduced.

This research is in line with research conducted by Permatasari et al, obtained the results of the bivariate test between the parity variable and the incidence of placental retention obtained a P-value of 0.356 which means that statistically there is no relationship between parity and placental retention[13]. This research is also in line with the research of Mariana obtained by the results of the chi-square statistical test obtained a p-value of 0.102 (p>0.05) thus it can be concluded that there is no relationship between parity and the incidence of placental retention at the Raden Mattaher Regional General Hospital, Jambi Province. Besides, OR = 2.143, this indicates that respondents who have parity are at risk of experiencing a 2–3 times risk of experiencing placental retention when compared to parity who are not at risk[14].

The incidence of placental adhesions or retained placenta is one of the biggest contributors to the occurrence of postpartum hemorrhage in childbirth mothers. Therefore, it requires handling from various parties to overcome this. It is important for pregnant women to carry out regular antenatal care, to increase the nutritional intake needed during pregnancy.

Conclussion

This research shows that of the two factors, only age is associated with the incidence of placental retention in the incidence of placental retention including age and parity.

Conflict of Interest: *None*

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Ethical Clearance: Health Research Ethics Committee, Faculty of Public Health, Universitas Hasanuddin

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Nanocurcumin Potential Effect of SOD Enzyme and Caspase-3 Expression in Lead-Acetate Induced Rats Ovarian Granulosa Cells

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Abstract

Aim: This study investigated the potential effect of nanocurcumin on the increase of SOD enzyme expression and decrease of caspase-3 in lead acetate-induced rats ovarian granulosa cells.

Materials and Methods: Forty five female rats were divided into 5 groups, the negative control group (rats receiving corn oil, one hour later receiving distilled water), positive control group (rats receiving corn oil, one hour later receiving lead acetate of 40 mg/kg bw) and experimental groups 1, 2 and 3 (rats receiving nanocurcumin 50 mg, 100 mg and 200 mg/kg bw). One hour after administering the nanocurcumin, the rats received 40 mg/kg of lead acetate. All groups received oral treatment once a day for 26 days. On day 27 the rats were sacrificed and the expression of SOD and caspase-3 enzymes were measured using immunohistochemical methods.

Results: This study found that lead acetate decreased SOD enzyme expression and increased caspase-3. In contrast, nanocurcumin increased SOD enzyme and decreased caspase-3 expression in lead acetate-induced rats ovarian granulosa cells.

Conclusion: Nanocurcumin has potential as a strong natural antioxidant by affecting the increase of SOD and the decrease of caspase-3 cells in lead acetate-induced rats ovarian granulosa cells.

Keywords*: Antioxidants; lead acetate; SOD and Caspase-3; nanocurcumin*

Introduction

Lead (Pb) is one of the environmental pollutants that are dangerous, toxic, carcinogenic, biomagnificative, and bioaccumulative, as well as causing disorders and damages to reproductive system in animals and humans.[1] Continuous lead exposure will accumulate in the body, causing toxicity and potentially resulting in infertility,[2] reproductive system failure,[3] abortion, premature birth and fetal death.[4] Lead can also inhibit the work of enzymes, interfere with mineral absorption and easily bind to cysteine, lysine and histidine imidazole, and replace endogenous ions of the enzyme. The binding between Pb and protein will form a protein-metal binding, the metallotionin-Pb, and this binding will cause the enzyme to become inactive. Inhibition of this enzyme's action will affect physiological processes and can disrupt cell structure in the body's organs.[5]

Lead can affect SOD (superoxide dismutase) and caspase-3 enzymes. SOD is a metalloporfirin-protein that functions to convert *O2- to O2 and H2O2 and is an enzymatic metallo-enzyme antioxidant in the body. In its activity as an antioxidant, SOD depends on metal cofactors Cu, Zn, Mn and Fe. One of the highest activities of SOD is in the ovaries. Pb can bind to SOD

and replace endogenous ions from metallo-enzymes, forming Pb-metallotion which results in decreased SOD activity as an antioxidant.[6] Caspase-3 has an important role in cell apoptosis regulatory mechanisms.[7] The activity of caspase-3 is produced as an inactive enzyme (procaspase).[8] Pb exposure can cause an increase in caspase-3 through oxidative stress mechanisms. Lead causes an increase in ROS (Reactive Oxygen Species) followed by an increase in oxidative stress and Bax/ Bcl-2 ratio, as well as an increase in caspase-3 from the mitochondria.[9]

Curcumin functions as anti-bacterial, anti-inflammatory, chemopreventive, wound healer, anti-parasite and antioxidant.[10] Curcumin has antioxidant and ROS-reducing effects resulting from the action of its phenolic group (-OH).[11] Curcumin can reduce oxidative stress caused by lead toxicity[12] and can increase superoxide dismutase (SOD).[13] Curcumin is also able to inhibit the increase in caspase-3 by preventing the formation of hydroxyl radicals (OH*). The prevention of (OH*) formation by curcumin is by preventing Haber Weiss reaction and Fenton reaction.[14]

Materials and Methods

Chemicals

Lead acetate (PbAc) was purchased from Sigma-Aldrich.co. USA, Linear formula: Pb (CH3CO2) 2.3H2O), (Product No: CAS 6080-56-4, molecular weight (MW): 379.33 g/mol and Curcumin Product No: CAS 458-37-7 molecular weight (MW): 368.38 g/mol, product of China. (Curcuma longa (turmeric) powder, > 65%.

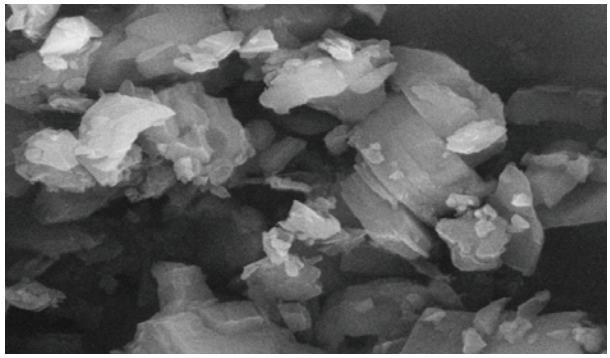
Making process of nanocurcumin

Nanocurcumin was made using curcumin powder from the rhizome of Curcuma longa (turmeric) using the miling method. Curcumin was milled by pounding by cubic zirconia balls, with a ratio of curcumin : cubic zirconia balls = 1: 10. The curcumin and cubic zirconia balls were put into a tube and milled on a High Energy Miling (HEM) machine. The setting time of milling was 5 minutes milling, 5 minutes rest until the effective time of milling, which was 20 minutes outside break time, was reached. Nanocurcumin was made at the Physics Laboratory, Airlangga University, Surabaya, Indonesia.

Analysis of nanocurcumin characteristics

After milling, the morphology of curcumin showed a more regular crystal shape with an average diameter of less than 200 nm. The morphology of curcumin prior to milling appeared as irregular plates with mean diameter of more than 1000 nm. The characteristic analysis of nanocurcumin size was carried out using Scanning Electron Microscopy (SEM) at Robotics Laboratory, ITS, Surabaya, Indonesia. The differences in the characteristics of curcumin before and after millling are shown in Figure 1.

(A)



(B)

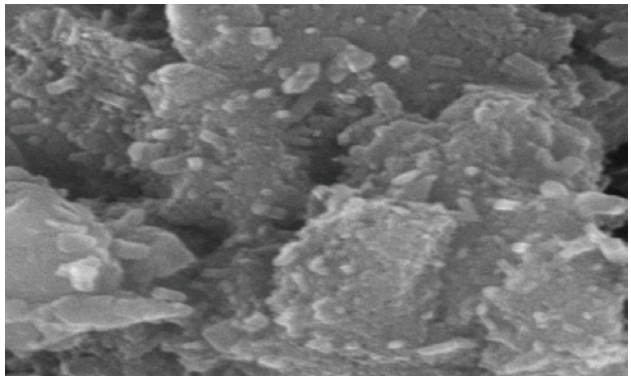


Figure 1. Curcumin before and after milling. (A) Curcumin before milling with a size > 1000 nm; (B) Curcumin after milling with a size of < 200 nm.

Making of nanocurcumin solution

Corn oil is the best solvent for nanocurcumin compared to butter, milk and water,[15] so this study used corn oil as a solvent. A solution was made by dissolving 2 grams of nanocurcumin with corn oil to 200 ml, so that 1 ml of the solution contained 10 mg of nanocurcumin.

Experimental animals

This study had passed the ethical test of the Ethics

Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, and obtained ethical eligibility No: 2.KE.170.08.2019 dated 29 August 2019. The experimental animals were female wistar rats weighing around 180-200 g, aged 2.5 - 3 months, obtained from the Bandung Institute of Technology (ITB), Bandung, Indonesia. The rats were placed in cages in an air-conditioned room with temperatures maintained at 26C ° - 2C ° and 12 hours in a light and dark cycle.

Experimental design

This study used 45 female rats which were divided into 5 groups, the negative control group (C-), where the rats received corn oil and one hour later received distilled water; positive control group (C +) where the rats received corn oil and one hour later received lead acetate of 40 mg/kg bw/day; experimental group 1 (E1), where the rats received nanocurcumin of 50 mg/kg bw/day; experimental group 2 (E2), where the rats received nanocurcumin of 100 mg/kg bw/day; and experimental group 3 (E3) where the rats received nanocurcumin 200 mg/kg body weight/day. One hour after giving nanocurcumin, experimental groups 1,2 and 3 were given with lead acetate of 40 mg/kg bw/day. The treatments were carried out every day at 09.00 AM for 26 days. On day 27 the rats were sacrificed, the ovaries were cleaned of connective tissue, washed with 0.9% physiological NaCl and implanted in paraffin blocks. Furthermore, the examination of SOD and caspase-3 enzyme expression in the granulosa cell was carried out using immunohistochemical methods.

Immunohistochemical examination

Subsequently, serial paraffin blocks, in which the ovary had been already implanted, were incised to a thickness of 4 - 6 □m. Representative incisions were selected for immunostaining procedure. The tissue incisions were stained with streptavidin method using immunoperoxidase. The next step was calculating the expression of the enzymes SOD and caspase-3 in 400x magnification. The presence of SOD and caspase-3 enzymes was indicated by the intensity of dark brown color. Observations were made quantitatively by counting the number of positive cells in each visual field, counting up to 10 visual fields. The number of positive cells for each visual field was summarized and divided by 10, and the final result was the mean number of positive cells per visual field.

Statistical Analysis

Data are presented as mean ± standard deviation. The comparative test used was Kruskall-Wallis Test to determine the differences between groups, followed by Mann-Whitney test to determine differences between the groups.

Results

Potential effect of nanocurcumin on the increase of SOD enzyme expression in lead acetate-induced rats ovarian granulosa cells

The results of Kruskal-Wallis test showed differences in the expression of the enzyme SOD (Kruskal-Wallis H = 23.625; df = 4; p = .000). Furthermore, to determine the differences between groups, analysis using Mann-Whitney Test was carried out, as in Table 1.

Tabel 1: Potential effect of nanocurcumin on SOD enzyme expression in lead acetate-induced rats ovarian granulosa cells (mean ± standard deviation)

Groups	n	SOD expression (%/micro)	Minimum	Maximum
Negative control	9	1.6 ± 1,2 a	0	3.5
Positive control	8	0.4 ± 0,3 b	0	1.2
Experimental 1	9	5.1 ± 3,5 c	0.6	12.4
Experimental 2	9	8.2 ± 10,5 c	0.3	28.8
Experimental 3	8	15.0 ± 12,6 c	1.0	35.5

[a, b,c] Different superscript within each column indicates significant difference between the means ($p < .05$).

Table 1 shows that the highest mean of SOD enzyme expression in rats ovarian granulosa cells is in experimental group 3 (x = 15.0; sd = 12.6%/micro), and the lowest is in positive control group (x = 0.4; sd = 0.3%/micro). Negative control group was different from positive control group (p = 0.017), experimental group 1 (p = 0.010), experimental group 2 (p = 0.038), and experimental group 3 (p = 0.005). Positive control group was different from experimental group 1 (p = 0.001), experimental group 2 (p = 0.001) and experimental group 3 (p = 0.001). Experimental group 1 was similar to experimental group 2 (p = 0.627) and experimental group 3 (p = 0.102). Experimental 2 group was similar to experimental group 3 (p = 0.178).

These results indicated that the administration of nanocurcumin increased the expression of SOD enzyme in lead acetate-induced rats ovarian granulosa cells starting from doses of 50, 100 and 200 mg/kg bw. This difference can be seen in Figure 2.

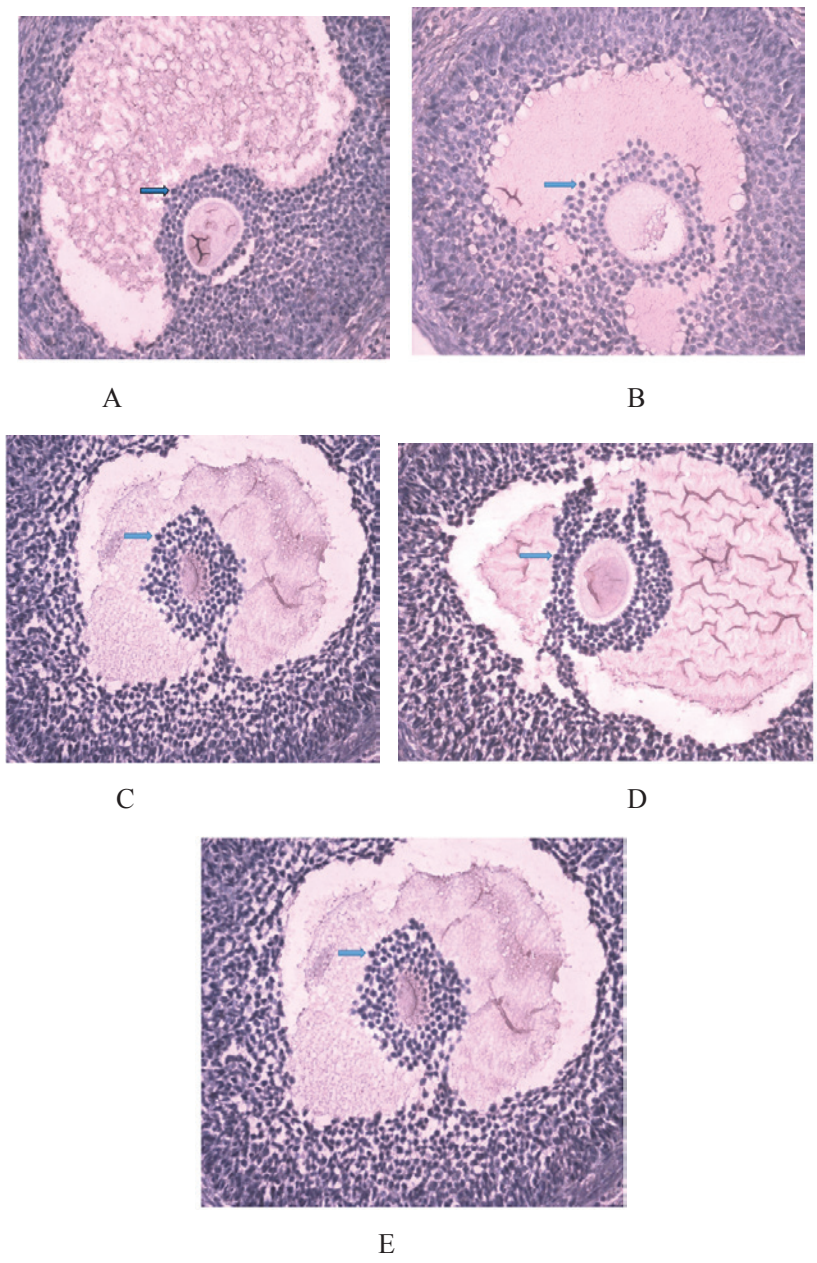


Figure 2. Expression of SOD in rats ovarian granulosa cells (A) K- group; (B) K+ group; (C) E1 group; (D) E2 group; (E) E3 group. Observation used light microscope with a magnification of 400x

Potential effect of nanocurcumin on decrease in caspase-3 expression in lead acetate-induced rats ovarian granulosa cells

The Kruskal-Wallis test showed differences between groups in the expression of caspase-3 (Kruskal-Wallis H = 27.321; df = 4; p = .000). Furthermore, to identify differences between groups, analysis using Mann-Whitney Test was carried out, as shown in Table 2.

Table 2. Potential effect of nanocurcumin on the expression of caspase-3 in lead acetate-induced rats ovarian granulosa cells (mean ± standard deviation)

Groups	n	Caspase-3 expression (%/micro)	Minimum	Maximum
Negative control	9	3.3 ± 5.9 a	0.1	17.3
Positive control	8	45.1 ± 42.2 b	2.5	103.6
Experimental 1	9	2.0 ± 1.1 a,d	0.6	3.6
Experimental 2	9	1.0 ± 1.5 a,c	0	4.8
Experimental 3	8	0.1 ± 0.1 c	0	0.3

[a, b, c, d] Different superscript within each column indicates significant difference between the means ($p < .05$).

Table 2 shows the highest mean is in the positive control group (45.1 ± 42.2%/micro) and the lowest in the experimental group 3 (0.1 ± 0.1%/micro). Negative control group was different from positive control group (p = .003), similar to experimental group 1 (p = .169) and experimental group 2 (p = .449), but different from experimental group 3 (p = .003)). Positive control group was different from experimental group 1 (p = .001), experimental group 2 (p = .001) and experimental group 3 (p = .001). Experimental group 1 was different from experimental group 2 (p = .042) and different from experimental group 3 (p = .000). Experimental group 2 was similar to experimental group 3 (p = .059). This proves that nanocurcumin starting at doses of 50, 100 and 200 mg/kg bw can reduce caspase-3 in lead acetate-induced rats ovarian granulosa cells. This difference can be seen in Figure 3.

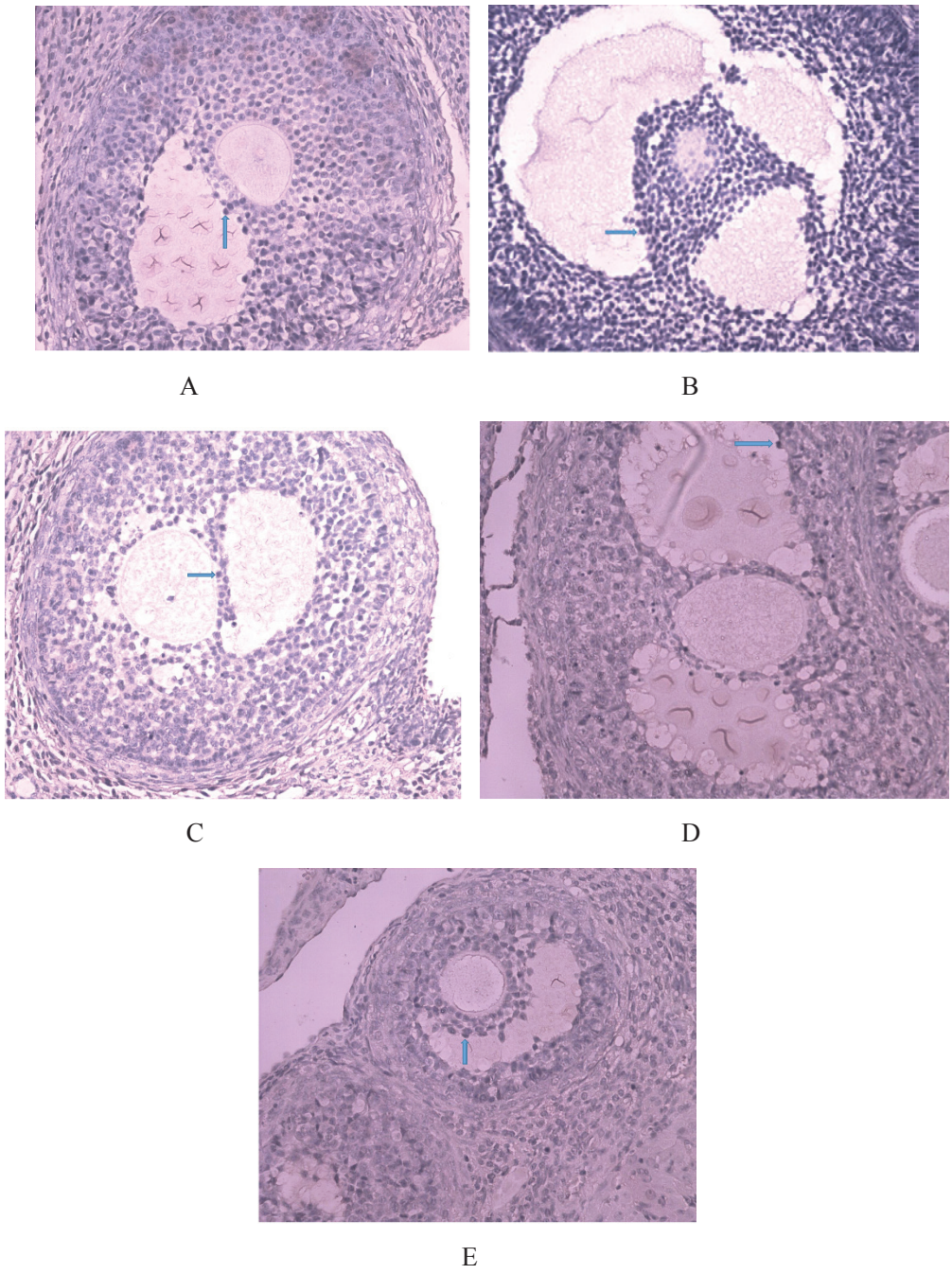


Figure 3. Expression of caspase-3 granulosa cells in rats ovaries with 400x magnification. (A) K- group; (B) K+ group; (C) E1 group; (D) E2 group; (E) E3 group. Observation used light microscope with a magnification of 400x. Arrow ( ) indicates an example of caspase-3 expression.

Discussion

This study proved that exposure to lead acetate as much as 40 mg/kg bw in rats decreased the expression of enzyme SOD in ovarian granulosa cells. We found that mean SOD enzyme expression in control group (K+) was lower than that in control group (K-). This finding was in line with the previous research that the administration of lead acetate in a dose of 24 mg/kg bw/day per sonde for one month resulted in lower blood erythrocyte SOD enzyme levels compared to control group that received water,[16] also stated that exposure to lead acetate of 100 mg/kg bw mixed with corn oil in rats decreased hepatic

SOD enzymes compared to that of control group.[17] Other researchers also reported that white rats injected with lead acetate of 20 mg/kg bw for 11 days had lower mean testicular SOD enzymes than control group.[18]

Lead acetate within cells is degraded, releasing Pb^{2+}.[19] Pb^{2+} can inhibit the action of SOD enzyme, which is a metalloenzyme, and requires metal cofactors for its antioxidant activity. SOD binds metal ions of zinc (Zn) and copper (Cu) that act as the cofactor.[20] Pb^{2+} works by binding to proteins and replacing endogenous ions (Cu/Zn) of SOD enzyme, so that this enzyme is not active as an antioxidant.[21]

In this study, the administration of nanocurcumin was shown to increase the expression of the enzyme SOD. The higher the nanocurcumin dose, the higher the expression of SOD enzyme in ovarian granulosa cells of rats exposed to lead acetate in a dose of 40 mg/kg bw. This can be observed from the mean SOD enzyme expression in E3 rats which was higher than that in E2 group and mean SOD in E2 group which was higher than that in E1 group. This finding was in line with the previous research who found that the administration of curcumin of 200 mg/kg bw/day orally and Pb of 50 mg/kg bw intraperitoneally for 7 days caused SOD expression in the liver of white rats to be higher than that in control group receiving Pb in a dose of 50 mg/kg bw intraperitoneally for 7 days.[22] Another study found that the provision of lead acetate of 100 mg/kg bw/day to white rats orally for 4 weeks, followed by curcumin of 400 mg/kg bw/day diluted with corn oil, caused a higher hepatic SOD expression than in control group.[23]

Curcumin has antioxidant and ROS-reducing effects due to its phenolic group (-OH).[24] The activity of free radical scavenging by curcumin is influenced by its phenolic hydroxyl groups. The energy of the curcumin phenolic OH group is lower (5.04 kcal mol) compared to the dissociation energy of the C-H bond in beta-dicetone curcumin group, so that the tendency of the antioxidant mechanism of curcumin is on the H atom in the phenolic group.[25] The antioxidant activity of nanocurcumin in increasing the expression of the SOD enzyme in rats granulosa cells exposed to lead acetate 40 mg/kg bw was by inhibiting the formation of superoxide radicals (O_2^-*)[26] through the inhibition of oxidative phosphorylation by suppressing the activity of cytochrome c oxidative enzymes.[27] Inhibition of oxidative phosphorylation by cytochrome P450 will reduce superoxide free radicals and increase the enzyme superoxidant dismutase (SOD).[26]

The results of this study also showed that oral administration of lead acetate (Pb) of 40 mg/kg bw/day increased the expression of caspase-3 enzyme in rats ovarian granulosa cells compared to that of negative control group (K-) which received distilled water. This finding was in line with a previous study,[28] who found an increase in testicular caspase-3 expression of white rats receiving lead acetate (Pb) of 30 mg/kg bw/day per sonde for 60 days compared to that of the control group. In vitro studies by He *et al.*,(2016) [29] showed that lead acetate in doses of 0.5; 1.0; 2.0 mol/l in normal rats renal epithelial cells for 12 h led to an increase in caspase-3 and caspase-9. Another study concerning intra-peritoneal injection of lead acetate as much as 20 mg/kg bw for 11 days in white rats resulted in increased caspase-3 testicular tissue compared to that of control group.[18].

Pb exposure can cause an increase in caspase-3 expression through oxidative stress mechanisms. The results showed that lead acetate caused an increase in ROS followed by an increase in oxidative stress. The presence of oxidative stress triggers an increase in the permeability of mitochondrial membrane which triggers the release of Smac/DIABLO and activation of pro-apoptotic protein (Bax/Bcl-2). Increasing Bax/Bcl-2 ratio increases the release of chytochrome c from mitochondria to cytosol.[9] In the cytosol, cytochrome-c binds to Apaf-1 (apoptotic protease activating factor-1) using energy from ATP and activates pro-caspase-9 in the form of an apoptosome. This apoptosome is what makes caspase-9 active. Active Caspase-9 will activate caspase-3.[30]

The results of this study also proved that nanocurcumin had the effect of reducing caspase 3. Nanocurcumin acts in the reduction of caspase- by inhibiting the formation of hydroxyl radicals (OH*) which are formed due to the encounter of superoxide ions (O2*-) with hydroperoxide (H2O2). This reaction requires the transition metal Fe^{++} (Cu^{+}) which is called the Haber Weiss reaction. Hydroxyl radicals (OH*) are also formed from H2O2 that reacts with the transition

metals Fe^{++} and Cu^{+}, which is called the Fenton reaction. To prevent Haber Weiss reaction and Fenton reaction, the presence of Fe++ and C+ ions must be prevented by binding Fe^{++} and Cu^{+}.[31] The inhibition of OH* formation results in increased mitochondrial phosphorylation, thus inhibiting the expression of Bcl-2 associated killer (Bak) and Bcl-2 associated death molecule (Bad) as pro-apoptotic proteins known as Bax subfamily.[32] Inhibition of Bak and Bad expression leads to an increase in anti-apoptotic proteins Bcl-2 and Bcl-xL. The increase in Bcl-2 and Bcl-xL inhibits the release of cytochrome c (cyt c) so that the binding of cytochrome c, apaf-1 and caspase-9 is minimal and the activation of procaspase-3 by caspase-9 does not occur, which will result in decreased expression of caspase-3.[33]

Conclusion

Nanocurcumin has the potential as an antioxidant to increase the expression of the first-line SOD enzyme and reduce caspase-3 in lead acetate-induced rat ovarian granulosa cells.

Conflict of Interest: There is no conflict of interest in this article.

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Possible Pain Caused in Judicial Modes of Execution: A Physiological and Pathological Analysis

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Abstract

Undoubtedly, the application of Capital Punishment has attracted a torrent of worldwide criticism. Primarily the cause behind this criticism is the excruciating pain caused by ancient outdated and uncivilised methods like crucifixion, hanging, beheading, etc., unlike the cotemporary scientific processes and international instruments. In opposition to the backdrop of enumerated counterattacks, most of the developed countries devised/formulated and implemented some contemporary procedures like shooting, lethal injection, gas chamber, etc. These contemporary procedures were alleged by the formulating nations to be swift and causing minimum pain. In this research paper, each of these modern modes have been critically analysed after reviewing the physiological and pathological aspect of pain associated with each of these methods. The author finds out that these so called modern method do inflict agonizing pain on the convicted offenders. In conclusion the author does suggest that medicalized process of execution may, to some extent, lead to painless killings.

Keywords-*Capital Punishment, medicalized, contemporary, execution.*

Introduction

"The rights of every man are diminished when the rights of one man are threatened."

John F. Kennedy

Modes of judicial execution of death sentence have to be determined in light of various objective factors like prevailing atmosphere of the international opinion, international norms and standards, contemporary penological theories and ever progressing standards of human dignity. Diverse and numerous aspects varying from society to society, influenced by the sacred tradition and culture of that society, its philosophy and history and its sense of moral and ethical values, are the key determining factors which effect the evolving standards of human dignity.[1] With the gradual passage of time the standards and norms of human decency and dignity do vary within the same society as well. In a progressive society, these standards keep on elevating to higher altitudes.

Pain and suffering are not merely one but an important aspect of cruelty; lack of pain and suffering do not make the punishments free from being termed as "cruel and unusual". As broadly understood, any act that diminishes human dignity is termed as cruelty. The thought that separates the existence of human beings from other inanimate objects and even other living organisms and makes them unique creatures in the entire universe, is nothing but simple expression of dignity. The holy book of Quran has defined human being as "ashrafulmakluqat", which means supreme of all beings on the planet Earth. All inanimate objects and living creatures are only property existing at the disposal of man, as the only creation of Almighty capable of holding rights and performing duties are human beings.[2]

In this research paper, researcher will be analyzing the functioning and pathology of different methods of Death Sentence. All the Information for this analysis has been derived from observations on the condemned convicts, based on postmortem reports, physiological studies on animals where a similar procedure was applied for execution, and emergency medicine literature. It is extremely difficult to mark the intensity and duration of pain a person goes through when executed, because all the signs of pain are dependent on the procedure or on the physical restraints,[3] but one can certainly identify those methods which are likely more painful.

Physiology and pathology in different methods of execution

Shooting

This method was carried out by either a single soldier or a policeman at short range who fired from behind the condemned person's occipital (back of the head) towards the forehead, or by a firing squad comprising of up to thirty soldiers who stood or knelt opposite to the blindfolded prisoners.[4] The soldiers aimed at the chest usually, since it was easier to target, than the head. Shooting from a short range literally destroyed the vital centers of the medulla, just like when a captive bolt is used for slaughtering cattle. When firing squad aimed at the head it produced the same type of lesions as that produced by a single soldier, while bullets fired at the chest ruptured the heart, great vessels, and lungs so that the condemned person dies of hemorrhage. A high velocity bullet produces a cavity which has a volume several hundred times higher than that of it.[5] Cavitation happens probably due to the heat dissipated by the bullet on body, an impact that boils the water and volatile fats in the tissue when bullet strikes. Convicts when hit by bullets feel as if they have been punched, pain comes later only if the person survives long enough to feel it. Shooting as a mode of death penalty and a potential substitute to hanging was discussed by the Royal Commission on Capital Punishment, however, it was discarded on grounds like; it did not possess the first and the most important requisite of an effective method that has the certainty of causing immediate death.[4] The authorized team furnishing evidence to the Commission often stressed on the point that any process of judicial execution recommended by them must not only be clean and swift but dignified as well.[6]

Beheading

Beheading as a mode of Capital Punishment was practiced in Congo, Qatar, Mauritania, the United Arab Emirates, Saudi Arabia, and Yemen.[7] In this method, the axe was wielded by a strong man who made repeated targeted sword cuts to the neck with immense force, or by guillotines a device consisting of a tall, upright frame in which a weighted and angled blade is raised to the top and suspended for beheading. Beheading does not always result from a single blow as the skin, muscles, and vertebrae of the neck are tough. The convict becomes unconscious within a few seconds, but not immediately after the spinal cord is severed. When biochemists had guillotined small rodents their eyes moved only for a few seconds. Anaesthetized sheep lose the flash-evoked responses of their electrocardiographs about 14 seconds after both the carotid arteries were severed, and it took 70 seconds after one carotid artery and one jugular vein were cut. It took merely 12 seconds for the dogs to be unconscious after the blood supply to their brains was obstructed. While the human brain stores enough oxygen for metabolism to carry on for 7 seconds after the supply is cut off. Nevertheless, the brain could also derive some of its energy from facial neck muscles and substrate in the scalp.[8] It may be concluded that a beheaded person dies from anoxia conceded by hemorrhage.

Hanging

Prior to the abolition of capital punishment in 1973, hanging as a judicial method of execution was lastly used in 1964 in Great Britain.[9] It would probably be used again if Parliament were in favor to reintroduce it as a mode of death penalty. In death penalty by hanging, the convict was weighed the day before the execution; also a try out was done using a sandbag of the same weight as that of the condemned. This was done to fix the length of the 'drop' that would ensure a rapid fracture-dislocation of the neck. Just before the execution, the convict's hands and legs were restrained, he was blindfolded and the noose was positioned around his neck.[10] The execution happened when the trapdoor was opened and the convict fell through. The weight of the convict caused a rapid fracture-dislocation of the neck, unless the condemned person had really sturdy neck muscles or the 'drop' was too short, or the noose had been wrongly positioned.[11] Whatever the case, the face became inflated and then cyanosed. The tongue protruded out and rapid violent body movements occurred which was usually due to the spinal reflexes. Usually the convict used to micturate or defecate or both. The heart continued to beat up till 20 minutes after the drop.

This procedure of capital punishment was most used by the British, but in most other countries hanging was a much less sophisticated practice, executions were often carried out in public, and the bodies were often left dangling to frighten others intending a similar crime.

It was discovered through the post-mortem that the

noose under the chin caused rotation and hyper flexion of the neck, and between the body and the pedicle of the axis, fracture of the junction was found, anterolateral on one side of the noose, and posterolateral on the other. The spinal cord got transected, with avulsed medulla, and there were extensive lacerations and bruising of the spinal cord. Such similar lesions were also seen in rats killed for biochemical experiments, who had cervical dislocation and their hearts continued to beat for 7 min approximately after dislocation.

It's usually implicit that instantaneous loss of sensation is caused by fracture-dislocation. Rupture of sensory pathways below the neck must take place without doubt. However, the sensory signals from the trigeminal nerve and skin above noose keep on reaching the brain till locked by hypoxia.[12]

In case there is no swift facture-dislocation, it's the asphyxiation which causes the death. In comparison to fracture-dislocation, asphyxia results in much slower death, because the vertebrae continues to supply blood to the brain as it protects the spinal and vertebral arteries and it's only the carotid arteries and jugular veins which are occluded by the noose.[13]

Gassing

Nevada was the first country to use Gassing in 1921. In this method of capital punishment, the condemned person is strapped into a chair in front of a sulphuric acid container, in an airtight chamber. The warders after strapping the convict withdraw from the chamber and it gets closed.[14] A lever is operated from outside the chamber which drops crystals of sodium cyanide into the pail. The condemned is instructed to take a whiff and breathe. Most convicts try to hold on their breath, and struggle as much as they can. It is still not known what sensation is felt when a person inhales cyanide, but in the post mortem reports of the signs and symptoms of accidental cyanide poisoning are vomiting, giddiness, ataxia, hyperventilation headache, dyspnea, and finally collapse to death.[15]Death is due to anoxia, because the cyanide is an inhibitor of the enzyme cytochrome oxidase, which is a key respiratory enzyme. Cyanide disrupts the electron transport chain by binding to the enzyme cytochrome oxidase. This prevents the transport of electrons from cytochrome oxidase to oxygen, thus resulting in anoxia.

Intravenous injection

United States was the first country to introduce execution by intravenous injection in 1977. In this method the convict is bound supine to a trolley and the vein in the angle of the elbow is annulated by a trained nurse or technician. There are times when the convict's veins are difficult to penetrate, for instance, if he or she does not cooperate, in case of phlebitis due to injection abuse of addictive drugs, or if there is scarring due to an attempt to slash the arm, due to all this, the procedure is fraught with complications. After the cannula is lodged successfully into the vein, three substances are injected one after the other: sodium thiopentone (a rapidly acting anesthetic), pancuronium bromide (a muscle relaxant to paralyzerespiration), and potassium chloride (stops the heart).[16] The condemned becomes unconscious within 10- 15 seconds after injection where death results due to anesthetic overdose and respiratory and cardiac arrest. In this method the doctor does not have any part in the execution, but he/she will certify after examination that the convict is dead.

The assessment of pain during execution

We will only consider the physical aspects of pain during execution. The ability to assess the intensity of pain, considering these signs in different modes of execution in death penalty, is indicated in the table:

Signs of severe pain or distress in persons executed

(Sh) shooting, (H) hanging, (B) beheading, (G) gassing and (IV) intravenous injection.

+ indicates that the sign is often seen,

- that it is not seen,

* that the sign can either not be made or not be seen, and

? that there is no information.

TABLE 1.[17]

Signs	Method of execution				
	Sh	H	B	G	IV
Shouting or screams?	?	*	+	+	-
Perspiration	?	?	?	?	?
Dilated pupils	*	?	?	?	+
Withdrawal from stimulus	*	*	*	*	*
Violent movements?	?	+	*	*	-
Contraction of facial muscles	*	+	?	+/-	-
Micturition	?	+	?	?	?
Defecation	?	+	?	?	?

TABLE 2.[18]: Factors occurring in execution which are likely to cause pain Intensity of likely pain is graded as little (+), moderate (+ +), severe (+ + + j, or not known (?), The likely duration of the sensations is not known.

Method of exxecution	Pain		
	Cause	Sensation	Intensity
Shooting	rupture of skin fracture of bone	sting or punch cracking	+or+ + ?
Beheading	stretch of skin prior to cut lacerations of skin	Burning sharp pain	+++ +++
Hanging	stretch of skin fracture - dislocation of vertebrae asphyxia	burning, stretching dislocation, fracture suffocation, distress	+++ +++ +++
Gassing	tracheal irritation asphyxia	burning suffocation	+++ +++
Intravenous Injection	missing or going through the vein	intramuscular injection	+

Following conclusions are drawn after examination of these tables:

(a) It is doubtful to state whether most of these signs happen or not even if some occur, they could not be seen;

(b) Some of them do not occur due to physical restraint;

(c) Various signs, like; micturition, contraction of the facial muscles and dilation of the pupils appear because of extreme pain, electrical stimulation, fear or even dying. Moreover, the intense stressful environment and circumstances surrounding the entire execution process may also either contribute to or suppress the pain.

The condemned when being stoned screams and shouts, but one cannot identify whether any of the other signs occur as well. Nor it is easy to assess the intensity and duration of pain when the head is decapitated. There are important

areas of ignorance that cannot be measured, and certainly one cannot conclude for certain the extent of pain with respect to any particular method of execution. However, one can conclude the behavior which is likely to occur in each method and through them (table 2.), and hence the evidence for intensity of pain is derived (table 3).

Different stages at which pain occurs are shown in table 2. Based on the data, it is undeniable that with the exception of intravenous injection, all the methods of execution produce a similar sort of lesions as conditions. Also, it is highly likely that with the certain exception of intravenous injection and possibly the exception of shooting, all the modes of execution are likely to produce severe intensity of pain. Examples to justify this are given in table 3. The states practicing the modes of judicial execution like, hanging, electrocution, and gassing believe that these executions are painless and that the pain lasts only for a short time where the sentenced person does not suffer for long, or that they believe that the pain that a convict goes through is justified as based on the heinous crime for which the condemned has been found guilty.

TABLE 3.[19] Evidence for Pain Being Likely During Execution

Method of execution	Evidence
Shooting	Evidence of victims of gunshot wounds (Beecher 1949; Owen-Smith 1981; Melzack et a1 1982)
Hanging	Dislocations are painful (Watson-Jones 1976), fractures are painful (Wu 1987)
Beheading	Skin receptors are active until sensory pathways are hypoxic (Donald Hatcher 1965; Safar et a1 19821
Gassing	Symptoms of cyanide poisoning include headache (Arena 1988; National Poisons Treatment Service 1991)
Intravenous Injection	No pain reported with successful cannulation

Conclusion

Subsequent to a thorough analysis as to the likelihood of suffering and pain that is caused to the condemned person in several modes of judicial execution of capital punishment like intravenous lethal injection, hanging, shooting, beheading, gassing, etc., the scholar reasonably reaches to the conclusion that all the judicial modes discussed above have the potential to cause severe pain and suffering in one form or the other, with only intravenous lethal injection as a possible exception. Apparently, the usual signs of intense pain seem absent, generally because these are being masked by the kind of procedures involved or because the physical restraint of the convict does not let him/her to demonstrate the same. Hence, lack of adequate evidence due to absence of signs of intense suffering and pain, leads to indecisiveness as to it's occurrence. Nevertheless, evidence offered by the science of physiology and derived from comparisons with emergency and accidental medicine, makes it clear that almost all the procedures involved in judicial executions have the potential to cause severe pain and suffering to the condemned. However, in spite of the evidence shown and discussed above, it's extensively emphasized that processes involved in judicial executions are painless and humane, even though no evidence proving this has been published till date.

1. **Ethical Clearance -** Not Required

2. Source of Funding- Self

3. Conflict of Interest- Nil

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Effectiveness of Instructional Program upon Pregnant Women's Knowledge Suffering from Pregnancy Induced Hypertension Attending to Primary Health Care Centers in Al- Najaf City

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Abstract

Pregnancy induced hypertension is a common obstetric disorder. It is among the leading causes of maternal and fetal mortality and morbidity. This research aims to assess pregnant women's knowledge regarding pregnancy induced hypertension, and to find out the relationship between pregnant women's knowledge and their demographic and clinical data . A quasi-experimental design is used to investigate the effectiveness of instructional program upon women's knowledge suffering from pregnancy induced hypertension , from the 8th of January to 3th September2020, included 80 pregnant (40 women as study group), and (40 women as a control group). The current study found that the knowledge of the majority of women about pregnancy induced hypertension is poor in complication domain specialty before the implementation of instructional program. However, 100% of them have had good knowledge after two weeks of implementation of the instructional program. The women have developed and improved in their knowledge about pregnancy induced hypertension. The study revealed that there were high significant in the study group knowledge regarding the pregnancy induced hypertension in the pre-test and post-test measurements. The study has concluded that the instructional program has a positive effect on the study sample blood pressure. The instructional program has a positive effect on the study sample knowledge regarding the PIH. Levels of education, monthly income, residential area, occupational status, and the type of family are affect the pregnant's knowledge regarding the PIH.

Keywords-*pregnancy induced hypertension, instructional program*

Introduction

Pregnancy is a normal physiological state which a women experiences at some point of her life. During pregnancy a women may develop complications which pose a risk to both maternal and fetal health [(1)]. A common medical condition that can cause perinatal morbidity and mortality is hypertension induced through pregnancy or childbirth [(2)].

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Hypertension caused by pregnancy is a multisystem disorder characterized by edema production, after 20 weeks of birth, hypertension can be associated with proteinuria, a disorder called preeclampsia, which can be fatal for both the mother and the developing baby [(3)].

The elevated or uncontrolled blood pressure (BP) leads to early and cesarean delivery also caused various complications to both mother and fetus [(4)]. Hypertension is the most public medical problem during pregnancy, with a frequency of 5-10% of all pregnancies worldwide[(5)].

Pregnancy induced hypertension is a common obstetric disorder. It is among the leading causes of

maternal and fetal mortality and morbidity. This research aims to assess pregnant women's knowledge regarding pregnancy induced hypertension, and to find out the relationship between pregnant women's knowledge and their demographic and clinical data

Methods

A quasi-experimental design is used to investigate the effectiveness of instructional program upon women's knowledge suffering from pregnancy induced hypertension , from the 8th of January to 3th September2020.

A non- probability (purposive sample)of 80 pregnant women's who are divided into two groups; 40 women as study group exposed to the instructional program, and the other 40 women are not exposed to the instructional program considered as a control group. This study conducted at Primary health care centers in Al- Najaf city.

The study instrument consist of five parts .

A socio-demographic data consist of age, educational level, economic level, occupation status, residency, type of family, cigarette smoker and smoking type, BMI, Blood pressure measurement(Appendix).

The second part of the questionnaire consist of Age at marriage, Age of pregnancy now in weeks ,number of pregnancies, type of pervious delivery, number of live children, number of died children, number of abortion, number of underweight children, your visit to the primary health care center(regularly, irregularly), causes of irregularity of the visit to the primary health care center.

This part deals with past medical history of family and includes (Pregnancy induced hypertension, Heart diseases, Diabetes mellitus, Renal diseases, Chronic hypertension), and deals with present medical history of women such as(Pregnancy induced hypertension, Heart diseases, Diabetes mellitus, Renal diseases) and past surgical history of women.

It is constructed to assess general knowledge of women about pregnancy induced hypertension to increasing the improvement women's health and to have healthy pregnancy and healthy babies, and this part consists of definition of PIH and normal blood pressure in adults, the signs and symptoms of PIH, the risk factors of PIH, the complication of PIH, and the prevention and controlling of PIH. This part is covered with the relevant points from the major content responses on program.

This part contains the source of pregnant women's knowledge such as(Physician, Nurse, Pharmacist, Family and friends, Social media and Experiences).

Statistical Analysis

Descriptive data analysis covers the following: Frequency and percentage table, mean and mean of scores. Pearson correlation coefficient was used to test the reliability of the study instrument. Chi-square was used to investigate the association between the study sample knowledge and their demographic data. Independent sample t-test was used to determine the difference between the study and control groups knowledge according to the mean difference. Paired t-test was used to determine the difference in the study and control groups knowledge according to the periods of measurements (pre-test and post-test).

Results

Table (1) shows the sociodemographic data of the case study and control Study, where 35% of the study group's age are between 20-24 years, while 37.5% of the control group's age are 35-39 years 57.5% of the study group's monthly income are sufficient to some extent, while 62.5% of the control group's monthly income were sufficient to some extent. 80% of the study group and 75% of control group are residency of urban. 60% of study group were house wife, while 77.5% of control group were house wife.

In the study group 57.5% of are multiple family, while 50% of control group are nuclear family and 50% were multiple family. 92% of study group are not active smoking, and 95% of control group are not active smoking. 77% of study group are passive smoking, while 85% of control group were passive smoking. 62.5% BMI of study group are obesity, and 37.5% were overweight, while 95% were obesity, and 5% were overweight.

The study results confirm that the physician is the main source of knowledge among pregnant women regarding the pregnancy induced hypertension (table 2) .

The study results assert that the assessment of the study and control groups knowledge regarding the pregnancy induced hypertension is good in the post-test at the studied sub-domains (table 3 and 4) .

The study results reveal that the assessment of the study group knowledge regarding the pregnancy induced hypertension in the pre-test is good in all the studied sub-domains. Excepting the "complications among pregnant" the assessment is poor.

While for the control group, the assessment is good, poor, good, fair, good, and good in the general information, signs and symptoms, risk factors, complications among pregnant women, complications among fetus, and prevention sub-domains respectively.

The study results show that there is a non-significant relationship between the overall study sample knowledge regarding the pregnancy induced hypertension in the post-test and their demographic data at p-value more than 0.05, except with their occupational status, the study results indicate that there is a significant relationship at p-value less than 0.05 (table 5) .

Table 1. : Study Sample Demographic Data

Demographic data	Rating and intervals	Statistics	Study	Control
Age / years	less than 20 years	Freq.	9	4
		%	22.5%	10.0%
	20-24	Freq.	14	3
		%	35.0%	7.5%
	25-29	Freq.	7	6
		%	17.5%	15.0%
	30-34	Freq.	5	7
		%	12.5%	17.5%
	35-39	Freq.	3	15
		%	7.5%	37.5%
	40 years and above	Freq.	2	5
		%	5.0%	12.5%
Monthly income	Insufficient	Freq.	10	5
		%	25.0%	12.5%
	Sufficient to some extent	Freq.	23	25
		%	57.5%	62.5%
	Sufficient	Freq.	7	10
		%	17.5%	25.0%
Residential environment	Urban	Freq.	32	30
		%	80.0%	75.0%
	Rural	Freq.	8	10
		%	20.0%	25.0%
Occupational status	House wife	Freq.	24	31
		%	60.0%	77.5%
	Employee	Freq.	11	5
		%	27.5%	12.5%
	Free job	Freq.	5	4
		%	12.5%	10.0%
Active smoking	Yes	Freq.	3	2
		%	7.5%	5.0%
	No	Freq.	37	38
		%	92.5%	95.0%
Passive smoking	Yes	Freq.	31	34
		%	77.5%	85.0%
	No	Freq.	9	6
		%	22.5%	15.0%
BMI	Overweight	Freq.	15	2
		%	37.5%	5.0%
	Obesity	Freq.	25	38

Table 2. : Sources of pregnant women's knowledge about pregnancy induced hypertension

Rating and intervals	Statistics	Groups	
		study	Control
Physician	Freq.	18	16
	%	45.0%	40.0%
Nurse	Freq.	9	7
	%	22.5%	17.5%
Pharmacist	Freq.	3	2
	%	7.5%	5.0%
Family and friends	Freq.	3	6
	%	7.5%	15.0%
Social media	Freq.	1	7
	%	2.5%	17.5%
Experiences	Freq.	6	2
	%	15.0%	5.0%

Table 3. : assessment of the study and control groups knowledge in the pre-test

Main studied domains	Levels	Statistics	study	control
General information	Poor	Freq.	16	12
		%	40.0%	30.0%
	Fair	Freq.	7	7
		%	17.5%	17.5%
	good	Freq.	17	21
		%	42.5%	52.5%
Signs and symptoms	Poor	Freq.	12	18
		%	30.0%	45.0%
	Fair	Freq.	14	11
		%	35.0%	27.5%
	good	Freq.	14	11
		%	35.0%	27.5%
Risk factors	Poor	Freq.	2	8
		%	5.0%	20.0%
	Fair	Freq.	7	8
		%	17.5%	20.0%
	good	Freq.	31	24
		%	77.5%	60.0%
Complications among pregnant women	Poor	Freq.	14	9
		%	35.0%	22.5%
	Fair	Freq.	14	17
		%	35.0%	42.5%
	good	Freq.	12	14
		%	30.0%	35.0%

Table 4. : Assessment of the study and control groups knowledge in the post-test

Main Studied Domains	Levels	Statistics	Groups	
			Study	Control
General Information	Poor	Freq.	0	10
		%	0.0%	25 %
	Fair	Freq.	0	10
		%	0.0%	25 %
	Good	Freq.	40	20
		%	100.0%	50 %
Signs And Symptoms	Poor	Freq.	0	12
		%	0.0%	30.8%
	Fair	Freq.	0	13
		%	0.0%	33.3%
	Good	Freq.	40	15
		%	100.0%	35.9%
Risk Factors	Poor	Freq.	0	6
		%	0.0%	15%
	Fair	Freq.	0	6
		%	0.0%	15%
	Good	Freq.	40	28
		%	100.0%	70 %
Complications Among Pregnant women	Poor	Freq.	0	10
		%	0.0%	25 %
	Fair	Freq.	0	12
		%	0.0%	30%
	Good	Freq.	40	18
		%	100.0%	45%

Table 5. : relationship between the study sample knowledge (post-test) and their demographic data

Demographic data	Chi-square value	d.f.	p-value
Age / years	3.533	5	.618 NS
Levels of education	2.393	4	.664 NS
Monthly income	.758	2	.685 NS
Residential environment	.256	1	.613 NS
Occupational status	7.179	2	.028 S
Type of family	.758	1	.384 NS

Discussion

Generally, the present study highlights the contributions of some demographic variables for women who check in at the Primary Health Care Centers in Al-Najaf city, most of them aged (20-24 years) (35%)of the study sample, most of the study sample's monthly income are sufficient to some extent (57.5%), most of the study sample have had the weight status as overweight (37%) and obese(62.5%). The majority of study sample occupation are house wife(60%). Most of them are intermediate school graduated. As for residency, the result shows that the most of study sample are living in urban residential areas are(80%). The most of sample study were not active smoking(92.5%) and (7.5%) of them are passive smoking. The type of family are multiple about(57.5%) of study sample. This results agree with the results of Jasim and Chyad [6] the researchers found that most of the study sample had age at (15-25years), had an obese and overweight status. Gudeta and Regassa [7] have supported the findings, they found that most of the sample age<20 to 24 years, about half of them were urban resident, most of the study sample were not smoking. Also Saxena et al., [8] they have found that most of the study sample had age at (24.98±3.65), and had obese weight status. Also Dhaliwal et al., [9] have found that the women age between (17-25years). This study disagrees with the result of Ramesh et al., (1) who found the family type was nuclear and has sufficient monthly income.

This study disagree with the study of Safvan [10] in India that showed no significant association between the knowledge score of pregnant women and occupation, monthly income, gravida and gestational age.

Conclusion

The instructional program has a positive effect on the study sample knowledge regarding the PIH. Levels of education, monthly income, residential area, occupational status, and the type of family are affect the pregnant's knowledge regarding the PIH.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

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The Influence of Roleplay Simulation on the Moral Development of Children Aged 8-9 Years Old

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Abstract

Background : A lack of moral development might increase negative moral behavior. Drama, as a part of role-play, can stimulate the moral development of children aged 8-9 years.

Objective : The purpose of this study is to explain the effect of role-play on the moral development of children aged 8-9 years

Method : This research applied pre-experimental one-group pre-post test design. The population in this study was children aged 8-9 years with a total of 47 children. A sample of 15 respondents was taken using purposive sampling. The independent variable in this study is role-play stimulation, while the dependent variable is the moral development of children aged 8-9 years. Data collection was done using observation sheets, then analyzed using Wilcoxon Signed Rank Test with significance $\alpha = 0.05$.

Result: Wilcoxon Signed Rank Test shows that stimulation through role-play can influence the moral development of children aged 8-9 years with $p = 0.001$.

Conclusion: The results of this study conclude that role-play stimulation can improve children's moral development.

Keywords*: role-play stimulation, children's, moral development*

Introduction

There are still many children who have low moral behavior[1]. Moral development in school-age children is characterized by the child's ability to understand the rules, norms and ethics that apply in society. In character building in school, the teachers' role in giving direction to the child about things that are praiseworthy and despicable is very pivotal. Instruction given to the children to interact and be tolerant with fellow friends can be given through the activity of playing in groups. Moral value is an abstract concept; in this case, the child has not been able to directly accept the abstract concept that is taught by the teacher or parents quickly. Due to this reason, parents or teachers must be clever in choosing and determining the methods used to instill moral values in children so that the moral message delivered can be understood and utilized by students for their future lives[2].

The methods that can be used vary greatly, one of which is role-play. Playing is an activity where children can do and practice skills, give expression to thoughts, be creative, prepare themselves to act and behave in an adult manner. Role-playing and make-believe is one form of games that aims to instill a broader view of a new behavior in children[3].

In school-aged children, the cognitive development of children reaches the stage of maturity. This period is also called the intellectual period due to the openness and desire of children to gain knowledge and experience. The cognitive development of children aged 7-11 years is a concrete operational stage. At this stage the child has the ability to understand the rules of the conversation so as to produce logical and mental operational patterns. This research observes the effect of role-play stimulation on moral development in children aged 8-9. The purpose of this study is to explain the effect of role-play on the

moral development of children aged 8-9 years[4].

Material and Method

This study is a Pre Experimental research done using One Group Pre-Post Design This study applied purposive sampling where samples were selected among the population in accordance with what is desired by researchers, thus the sample can represent the characteristics of the population that were known beforehand[5]. The independent variable of this research is role-play stimulation. While the dependent variable is the moral development in children aged 8-9 years[6].

Research Instrument

In this research, in supporting the role-play, researchers use drama scripts and properties such as clothes, chairs, tables, etc. The play was done based on an activity unit under the guidance of researchers and class teachers. In addition, in assessing the moral development of children, researchers use observation sheets obtained from elementary school curriculum with indicators of social behavior assessment including: honesty, discipline, responsibility, courtesy, care, confident, and teamwork. There are 14 items observed with 4 answer choices. The answers "always" score 4, "often" 3, "sometimes" score 2, and "never" score 1. Measurement results are categorized on an ordinal scale[7].

Data Collection Procedure

The initial data collection was carried out using questionnaire sheets for 47 children aged 8-9 years with low moral development. The researchers selected the samples according to the inclusion requirements and obtained a sample of 15 children. Prior to the observations, teachers were asked to give assignments every day for 1 week. In addition, teachers also made a schedule of daily exams held 2 times a week. This is done to assess the child's moral development in accordance with the indicators on the observation sheet. Researchers divided the 15 children into 3 groups consisting of 5 children each[8]. Two days before the play the researchers distributed the role according to the theme and the storyline. This was done so that each group rehearse first before playing in front of the class. Each intervention was carried out by all groups with the same theme in turn. The theme of intervention 1 was honesty, intervention 2 was disciplined attitude, intervention 3 was themed courtesy, and intervention 4 was controlled attitude towards the environment. After the intervention was given 4 times for 2 weeks, researchers observed the children with the help of the home class teachers for 1 week. Observations were made before the school started class until after school[9].

Data Analysis

From the data was collected, the differences in behavior were analyzed before and after the intervention, the role-play stimulation, was given, wilcoxon signed rank test was used to compare the pre-test and post-test ordinal data intervention Overall statistical test was done on the significance level of $\alpha = 0.05$ (Windows SPSS 21 program). If the statistical test shows the value of $\alpha \leq 0.05$ then there is a comparison between the previous data and after an intervention on moral development in children aged 8-9 years [10].

Result

This respondent characteristic data outlines the characteristics of the child which includes age, sex, status in the family, parents' last education, and parents' occupation. Table 1 shows that most of the respondents (12 respondents) were in the 9-year age group (80%) and there were 3 respondents in the 8-year age group (20%). Table 2 shows that most respondents were male (8 respondents, 53.33%) and 7 female children (46.67%). Table 3 shows that the characteristics of respondents based on the status of children in the family which shows that there were 3 only-child (20%), 1 firstborn (6.67%), 3 middle child (20%), and 8 youngest child (53.33%). Table 4 shows the characteristics of respondents based on parents' last education, most revealed that the last education of respondents' fathers were high school students (12 persons, 80%) and there were 3 persons in junior high schools (20%). Whereas for the mothers, 10 persons were in high school (66.67%) and 5 persons were in junior high school (33.33%). Table 5 shows that the respondent's parents' work, both the fathers and mothers. There were 13 fathers who work as private employees (86.67%) and 2 as entrepreneurs (13.33%). While 3 mothers work as private employees are 3 people (20%), 2 are self-employed (13.33%), and 10 persons are not working (66.67%). Table 6 reveals the result of the

Wilcoxon Sign Rank Test with p = 0.001 <α, indicating that H_0 rejected and H1 accepted, thus there was an effect of the role-play stimulation on the moral development of children aged 8-9 years. The age of 8-9 years are the pre-conventional stage on moral development according to Kohlberg.

Table 1. Distribution of respondents by age

Age	Total	%
8 years	3	53.33
9 year	12	46.67
Total	15 children	100

Table 2. Distribution of respondents by gender

Sex	Total	%
Male	8	53.33
Female	7	46.67
Total	15 children	100

Table 3. Distribution of respondents based on status in the family

Status	Total	%
Only child	3	20
Oldest child	1	6.67
Middle child	3	20
Youngest child	8	53.33
Total	15 children	100

Table 4. Distribution of respondents based on their parents' last education

Parents' last education	Father		Mother	
	Total	%	Total	%
Junior high school	3	20	5	33.33
High school	12	80	10	66.67
Total	15	100	15	100

Table 5. Distribution of respondents based on parents' work

Parents' occupation	Father		Mother	
	Total	%	Total	%
Private employees	13	86.67	3	20
Entrepreneur	2	13.33	2	13.33
Not working	-		10	66.67
Total	15	100	15	100

Table 6. The level of moral development of children aged 8-9 years

No	Moral development value	
	Pre	Post
1	37	49
2	23	39
3	23	34
4	25	44
5	25	35
6	27	39
7	20	25
8	26	43
9	22	26
10	47	52
11	39	45
12	35	45
13	39	43
14	33	42
15	39	46
Mean	30.6667	40.4667
SD	8.217	7.698

Wilcoxon Signed Rank Test p = 0.001

Discussion

After being intervened with the role-play stimulation, the respondents experienced a positive increase on the moral development. The respondents did not cheat during exams, obey rules in school, carry out the tasks assigned by the teacher, do not bother friends, do not litter, carry out picket duties, and were shown to perform other positive behaviors. However, a small proportion of children with poor moral development was also found. In addition, there were obstacles in the interventions, namely the crowded respondents and audience that caused the message conveyed in the play to not be fully received by children. This caused the results of the intervention to not be optimal.

Respondents who received the intervention experienced an increase in moral development. This means the role-play activity is not just a fun game but is also a method of learning for children in school. In this case, nurses play an important role as well as opportunities to further enhance the competence and creativity of nurses, especially towards child and community in providing nursing care.

Table 6 shows that a small proportion of children do not experience an increase in moral development. This was because their parents work, thus the quantity and quality of parental care for their children was reduced. In addition, the status of the youngest and only child also affects the improvement of their moral development, because the youngest child and only child tend to be selfish, stubborn, spoiled, and impatient. Analysis of the data in table 6 shows that there is an influence of play on the moral development of children aged 8-9 years. Significant results were obtained ($p = 0.001$). Increased results quantitatively indicate that the moral development of children aged 8-9 years increased after being given the intervention.

Moral development is a change in reasoning, feeling, and behavior about standards regarding right and wrong[1]. Moral development is influenced by two factors, internal and external. Internal factors are influenced by the level of intellectual development, while external factors can be in the form of the influence from parents, peers, and society. Moral development is influenced by the atmosphere of the home, school, environment, and society[11]. Moral development is influenced by age, race, social status, level of intelligence and social environment. Sex and gender also play a role in influencing moral development[12].

Prior to the intervention, from the 15 respondents it was found that all of the children who had insufficient moral development were only child and last child. The second factor that causes moral development is the child's position in the family. An only child is accustomed to being the center of attention. As a result, they are often considered selfish and stubborn. Usually, these traits are a mixture of the oldest and youngest child. Similar thing applies to the youngest child, they get a lot of attention from parents so that he becomes spoiled, rebellious, careless, and impatient.

The researchers assumed that the oldest and youngest children tend to have higher negative behaviors[13]. In the opinion of researchers, working parents, in general, have less time for their children. Moreover, coming with the trend of working mothers in the family, spending time together with children becomes a problem that can affect the parenting method applied by the parents[14].

Playing is an activity where children can do and practice skills, give expression to thoughts, be creative, prepare themselves to act and behave in an adult manner. As an activity that provides stimulation in the ability of skills, cognitive, and affective, playing is fitting for a guidance, considering that playing for children is a necessity. By playing, children will have a broader view of the world, causing them to be able to develop physical, emotional, and mental maturity, and shaping them into creative, intelligent, and innovative children[15].

Role-playing is chosen to be applied in the teaching and learning process since it is believed that it might become a special attraction for students. Students are usually very enthusiastic about lessons relating to daily life in the community[16]. Drama and role-play are a medium for character education, because drama is the art of role playing, which might assist children's character development. By role-playing, children can have the ability to imitate without being taught or by following directions in the roles being played[17].

Conclusion

Based on the results of research, it can be concluded

that the moral of children aged 8-9 years, following the role-play stimulation, had achieved a good development. This can be seen from how the children stopped cheating during exams, started implementing the rules in school, carrying out the tasks given by the teacher, not disturbing their friends, not littering, and started carrying out picket duties. With drama and role-play that are done repeatedly, children can remember the moral message permanently. Thus, the child's moral development could gradually increase in aspects of honesty, discipline, responsibility, courtesy, and caring.

Ethical Clearance: This research was carried out after receiving a letter of approval from the parents or guardians of students to emphasize the ethical issues, and giving informed consent sheet as a way of agreement between the researchers and the respondents before the study was conducted. The research was done based on anonymity (without name), where the confidentiality of the responden's identity is maintained. The researchers maintained the confidentiality of the respondent's identity by not publishing the respondent's name. Researchers only used name codes in the data collection sheet. The confidentiality of research results was also guaranteed, only certain data groups will be reported on the results of research.

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Conflict of Interest : There is no conflict of interest related with this paper.

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Dental and Oral Care Treatment Needs in Children with Down Syndrome in Surabaya

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Abstract

Objective: Aims of this study was to observe dental and oral care treatment needs of Down Syndrome children who are members of the Parents' Association of Children with Down Syndrome (POTADS) in Surabaya. **Material and Methods:** This study used secondary data from dental and oral examination records of total sample 34 Down Syndrome children aged 5-17 years who attended community service program "SPECIAL CHILDREN DENTAL VISIT -1" from August to October 2017. **Results:** Only few samples showed good oral hygiene though most of them brushed their teeth twice a day which likely related to physical and mental limitations. Most samples had cariogenic dietary habits with def-t/DMF-T score were below 10. Malocclusion founded can be worsen by oral bad habits which has existed in most samples. **Conclusion:** From this study it can be concluded that the most indicative treatment for Down Syndrome children are restorations and extractions.Good collaboration between parents and caregivers is needed to prevent more severe oral health problems which need more complex dental treatment. Children with Down Syndrome are entitled to the opportunity to develop and get special services, including in terms of oral health. With good oral health, the quality of life of Down Syndrome children can improve.

Keywords: *Down Syndrome, dental and oral care needs, quality of life*

Introduction

Indonesia Law No.4 of 2017 by State Minister for Women Empowerment and Child Protection Republic Of Indonesia concerning Special Protection For Children With Disabilities, define children with disabilities are whom with physical, intellectual, mental and / or sensory limitations for a long period of time who, when integrated with the environment, may experience obstacles and difficulties to participate fully and effectively with other children based on equal rights[1]. The Indonesia's Basic Health Research (RISKESDAS) in 2018 reported that 3.3% of children aged 5-17 years had disabilities[2]. For the group of children aged 24 - 59 months, the RISKESDAS in 2013 reported the prevalence of disabilities including special needs condition of Down Syndrome was 0.13% which increased from 2010 prevalence of 0.12% and reached 0.21% which stated on 2018 RISKESDAS data[3,4]. According to World Health Organization (WHO), the estimated incidence of Down Syndrome is between 1 in 1,000 to 1 in 1,100 births and currently reach 8 million around the world. It is estimated that about 3,000 to 5,000 children are born with Down Syndrome every year[5]. Data from the Down Syndrome Association of Indonesia, stated that almost 300,000 cases reported in the country[6].

Down Syndrome which earlier referred to as mongolism, was first described by Langdon Down, physician from England in 1866. Down Syndrome is a condition of abnormality of chromosome 21 or also known as trisomy 21. Down Syndrome is 95% caused by failure in cell division or is called non-disjunction due to risk factors like viruses / infections, radiation, aging of ovum, thyroid function disorders, the age of pregnant women over 35 years. The clinical appearance of a Down Syndrome sufferer can vary from unseen and minimal appearance to characteristic features. Some cardinal sign of physical characteristics included muscle hypotonia and hyperflexiblity, short stature with

a relatively smaller head with flat facial profile, oblique eyes with epicanthal folds, dysplastic ear, simian crease. Characteristics of orofacial structures include middle facial hypoplasia, with a relatively small size of nose bridge, facial bones and upper jaw; and a high and narrow palate. Hypoplasia of the middle face causes relative macroglossia where the tongue has normal size but smaller jaw size which causes difficulty in speaking and chewing. Malocclusion is a common condition. Children with Down Syndrome are prone to suffer from periodontitis, whereas for dental caries incidence is very low. With more than 50% of them can be caries free was especially remarkable since oral hygiene was more often neglected[4,7].

People with Down Syndrome also have mental retardation characteristics which included in mild or moderate based on IQ classification. They may have cardiovascular disorders, hematopoietic problems, visual and hearing impairments. Developmental delays, such as in speech and language, are common. These unique characteristics of children with Down Syndrome have impacts on the service needs they obtain. The condition cause limitations that result in independence and require prolonged support from environment[7,8]. Children with special needs are entitled to special services aimed to protect and fulfill the rights so that they can grow and develop and participate in society[1]. Early professional treatment by dentists, especially pediatric dentists, and daily oral care at home by parents and caregivers, enabling Down Syndrome children to have not only a healthy mouth, but on their quality of life as well[8].

In Surabaya, parents of children with Down Syndrome form POTADS, Parents' Association of Children with Down Syndrome. The members are from East Java Province which routine gather and manage cooperation with supporting organization/institution like IDGAI JATIM (Indonesia pediatric dentist association, East Java branch) and Pediatric Dentistry Department, Faculty of Dental Medicine Universitas Airlangga. From this study through community service activity "SPECIAL CHILDREN DENTAL VISIT-1", we observe the dental and oral health condition of Down Syndrome children who are members of POTADS in Surabaya. From the result we hope that hence forward we can map the general dental and oral care treatment needs in children with down syndrome.

Material and Method

Study Design and Sample

This observational study involved samples of 34 children with Down Syndrome aged 5-17 years, members of POTADS who attended to Specialist Clinic of Pediatric Dentistry, Dental Hospital Universitas Airlangga Surabaya between August and October 2017, whom parents or guardians were unable to give consent form were excluded from the study.

Data Collection

All samples had received caries preventive program including oral examination, oral hygiene instruction and motivation, prophylaxis and topical fluoride application which were applied by undergraduate pediatric dentistry specialist students (chief residents) under the supervision of instructor, specialist staff of Pediatric Dentistry Department, Faculty of Dental Medicine Universitas Airlangga.

The study was conducted by analyzing secondary data from examination results on medical records which include the results of anamnesis on parents and the results of objective examinations on samples. Anamnesis includes medical history, eating habits, and habits of brushing teeth, while the objective examination includes examination of soft and hard tissue, namely oral hygiene, caries, and malocclusion. Analysis is also carried out on treatment needs based on the results of the examination and supporting OPG.

Data Analysis

Microsoft Excel (version 2007) and Statistical Package for the Social Sciences (SPSS) statistical software (version 16) was used for data entry and descriptive analysis, respectively.

Ethical Aspects

This study was approved by the Committee of Ethics Faculty of Dental Medicine Universitas Airlangga. Before starting the study, all parents or guardians were informed about the aim, nature, and potential distresses and benefits of the study. Then, informed consent was obtained for each sample.

Result

From 34 samples of children aged 5 and 17 years who participated in the study, most of the children were girls (58.8%), and mean age was 7. Of all samples, 58.82% had a history of systemic diseases such as heart disease, allergies, measles, chicken pox, diarrhea and pneumonia and 50% of samples had been hospitalized. From oral hygiene examination by checking their debris and calculus using OHI-S index, appears that only 14.71% of the sample had good oral hygiene and most of the samples showed poor oral hygiene (41.18%). The results of the examination showed malocclusions distribution in samples as shown in graph 1. From graph 1, most samples (23.53%) had Class I Angle malocclusions, then anterior crossbite and crowding. About 10% of samples had Class III Angle malocclusions and anterior open bite condition. None of the samples showed Class II Angle malocclusion.

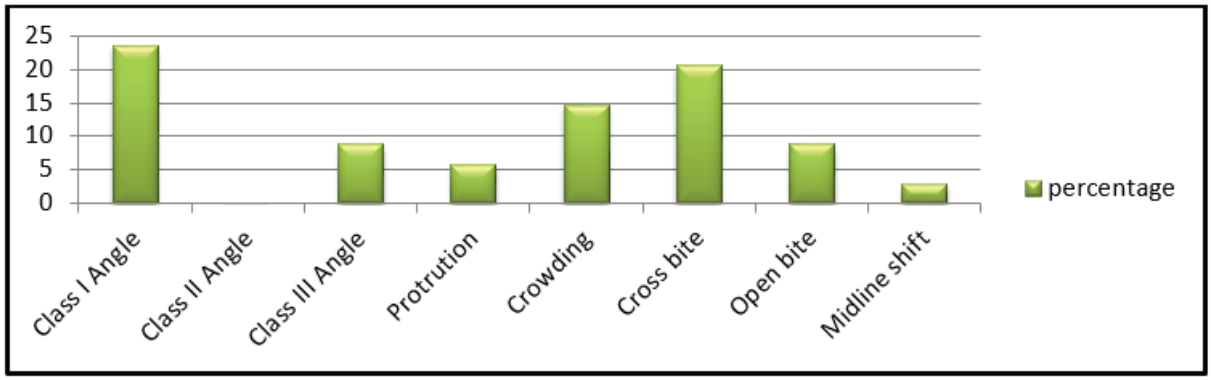

Figure 1. Distribution of samples based on malocclusion conditions.

Caries experience of samples was measured with def-t and DMF-T score ranged from 0 to 20, with 14.71% of the samples were caries-free and only 1 sample with the def-t / DMF-T score reach 20 (graph 2). Most of samples (58.82%) had def-t and DMF-T score below 10.

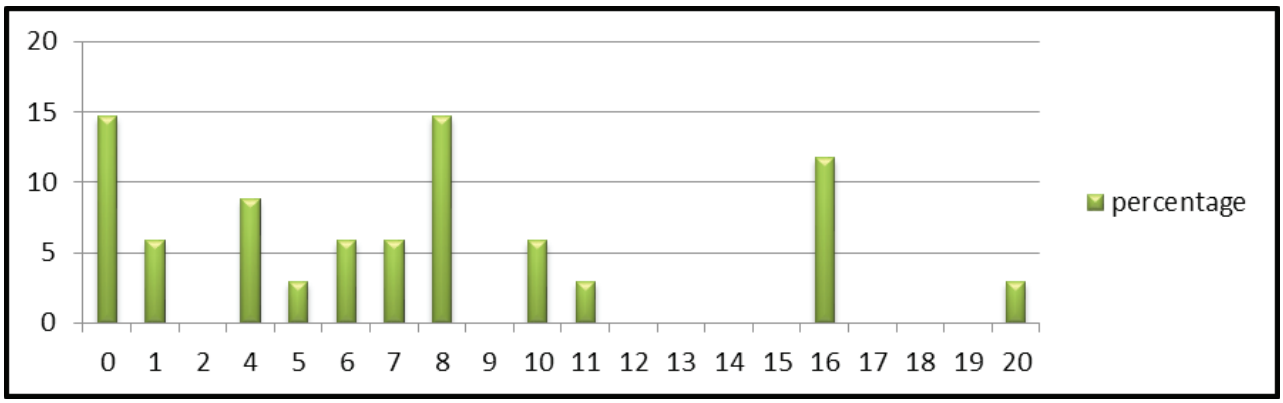

Figure 2. Distribution of samples based on def-t / DMF-t numbers.

Caries risk factor were assessed from eating habits and habits of brushing teeth in a day. From graph 3 showed that nearly 40% of the sample had habit of eating sweets, biscuits and cakes while they also have big appetite. All three are highly cariogenic foods, especially if they are not matched by efforts to maintain oral hygiene such as brushing teeth regularly and gargling after every meal.

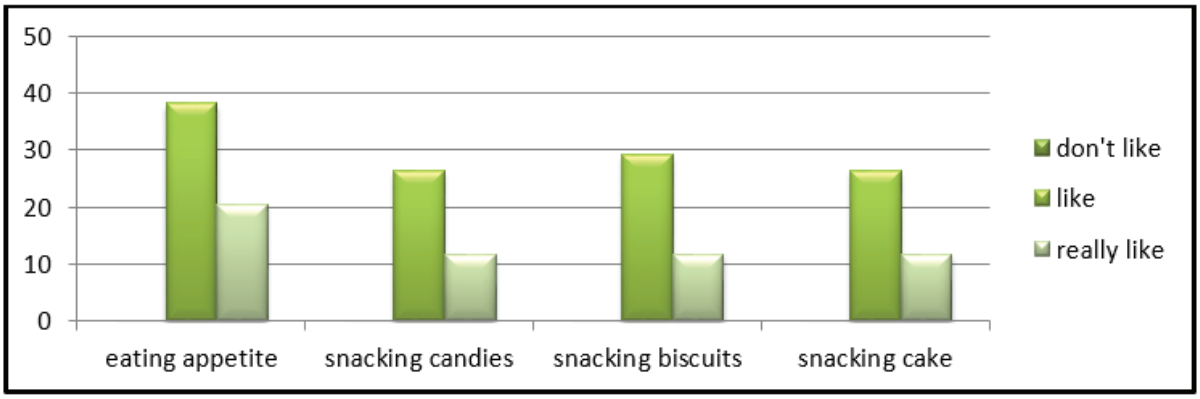

Figure 3. Distribution of samples based on caries risk factor: eating habits.

As shown on graph 4, most of the samples (55.88%) had the habit of brushing their teeth twice a day. But there are still a number of samples that only brush teeth once a day, even didn't have the habit of brushing their teeth every day. The graph also showed that few samples had brushing their teeth more in three or four times a day.

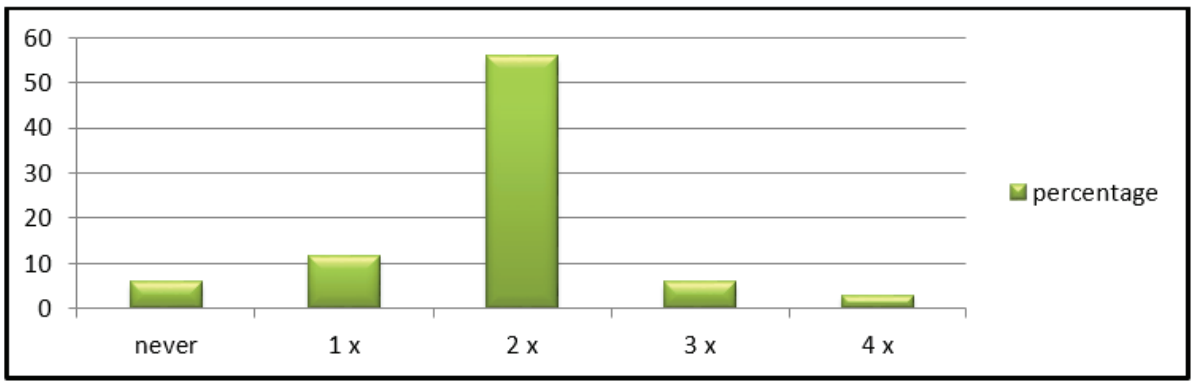

Figure 4. Distribution of samples based on caries risk factor: daily brushing habits.

From the graph 5, showed that the most indicated dental treatment for sample in this study was restoration in 61,76% and then extraction 55.88% and 38.23% from the total sample needed fissure sealants as caries prevention. Other dental treatments needed were scaling, space maintainer/partial denture and pulp treatment for more than 10% of samples, orthodontic treatment for 8,82% of samples, Preventive Resin Restoration and Stainless Steel Crown for about almost 6% of samples.

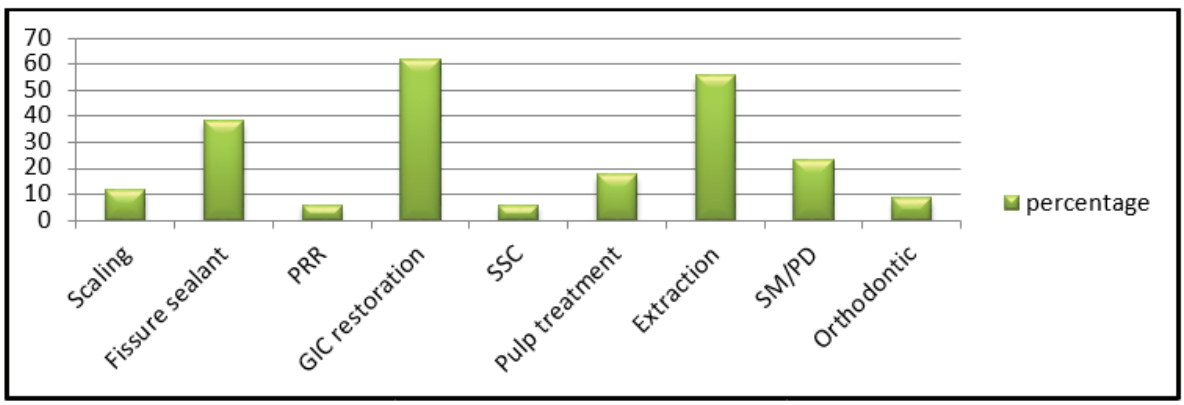

Figure 5. Distribution of samples based on the type of dental treatment indication.

Discussion

In this three months community service activity program "SPECIAL CHILDREN DENTAL VISIT -1" by Pediatric Dentistry Department, Faculty of Dental Medicine Universitas Airlangga in Dental Hospital Universitas Airlangga for children of POTADS Surabaya branch members, though most samples were girl, Down Syndrome prevalence in boys were reported higher (0.08%) than in girls (0.06%) which about 4% of Down Syndrome case were inherited from parents with the mother as carrier has bigger risk (10-15%) than the father (3%)[4]. From Ariani (2014) which reported each year addition of Down Syndrome new case confirmed from four cytogenetic laboratory in Indonesia, also emphasized more awareness about the importance of genetics and genomic medicine future implementation[6]. Cardiac disorders are common in 40-45% Down syndrome patient which shown in this study samples. People with Down Syndrome are at risk of developing valve dysfunction that leads to congestive heart failure, even if they have no known cardiac disease. Dentist must consult the patient's physician in the need for antibiotic prophylaxis[4,8]. From anamnesis result also showed that half of this study samples had been hospitalized. Ministry of Health Republic of Indonesia reported that from 2015 to 2017 there were about total 1000 Down Syndrome inpatient cases and also an increasing number of new Down Syndrome outpatient cases in more than 2000 hospitals in Indonesia[4].

Almost all samples of this study (88.23%) were cooperative on dental care. It can be seen from only a small sample were not able to take OPG after being examined oral and dental condition by the operator chief residents of Pediatric Dentistry Specialist. The cooperativeness of special need children as dental patient may vary depends on many factors but behavior management is not usually a problem in people with Down syndrome because they tend to be warm and well behave, only some can be stubborn or uncooperative[7,8]. From AAPD Reference Manual Of Pediatric Dentistry in Prescribing Dental Radiographs for Infants, Children, Adolescents, and Individuals with Special Health Care Needs, radiographic screening for the purpose of detecting disease before clinical examination should not be performed and should be taken only when there is an expectation that the diagnostic yield will affect patient care. It also mentioned that if radiographs of diagnostic quality are unobtainable, the dentist should confer with the parent to determine appropriate management techniques (e.g., preventive/restorative interventions, advanced behavior guidance modalities, deferral, referral), giving consideration to the relative risks and benefits of the various treatment options for the patient [9]. OPG is recommended for patient with Down Syndrome because it is more easily accepted than periapical and/or occlusal radiographic photographs which must be taken as well if necessary for the diagnosis[7,10].

In general, treating Down Syndrome patient just need a little extra time and attention to make it more acceptable. It is very important to set comfortable atmosphere for dental visit which can be done by scheduling appointment in the morning and shorter waiting time if possible[7,8]. In this community program, the appointment was conducted in early office hours and the clinic had also made an arrangement for other patients treatment schedule. The Pediatric Dentist Specialist Clinic of Dental Hospital Universitas Airlangga has special treatment room which particularly use for treating special need patient. In treating Down Syndrome patient, it is necessary to minimize disturbances in the form of both sound and light though music can be played during dental care so that Down Syndrome patient feel more relax. It is useful to note patient's preferences and other unique details that will facilitate treatment, such as music, comfort items, and flavor choices. People with Down Syndrome also have developmental delays which are generally in the form of speech difficulties, hearing loss and vision impairment which cause obstacles in communication. This condition is absolutely known prior to dental care so that the dentist and team can anticipate, such as maintaining eye contact, minimizing the noise of saliva ejector/suction machine and television, not using a mask when speaking in a soft tone so that the voice is clear and lip movements are readable. Through the pre-appointment, the operator contact parents/caregiver to gain information needed about special condition of each patient prior to dental visit for the dentist and team to be well prepared in establishing good communication. By establishing a good communication, will decrease the anxiety of the patient, gain trust and be able to motivate them to take a good care of their oral health[7,8,11]. As in this community program, at the end of the treatment a reward was given which can motivate and increase

patient cooperation.

Only 14.71% of the sample had good oral hygiene shown from the tooth debris and calculus (OHI-S index) and almost 12% of the samples need scaling prior to other dental treatment needed. People with Down Syndrome usually have poor oral hygiene, which characterized by inflammation of the gingival margins, acute and subacute necrotizing gingivitis, and advanced chronic periodontitis with abundant neither supra-nor subgingival calculus and material alba[7]. The prevalence of periodontal disease in patients with Down Syndrome ranges from 50-90% with increased severity compared to normal children of the same age and other mental disabilities[12]. But poor oral hygiene may not the only explanation of severe and generalized periodontal destruction found in Down Syndrome patients. This condition may be due to tissue anoxia of narrow arteries and peripheral capillaries and also associated with compromised imune system. There is a tendency to increase sensitivity to periodontal disease due to functional defects of cellular PMN and monocyte found in Down Syndrome children with lymphopenia[5,7,13]. As it may be an early sign of leukaemia, dentist should be alert to the presence of persistent lesions and spontaneous gingival bleeding. Down Syndrome patients have up to 20 times higher risk of developing leukaemia when compared to the general population[14]. It is important to treat acute necrotizing ulcerative gingivitis and other infections aggressively[8]. The severity of periodontal disease which tends to increase with age, if left unchecked can lead to the formation of pockets and bone loss in young children, causing teeth must be extracted[7].

Development of periodontal disease in people with Down Syndrome can also be caused by local disorders such as poor occlusal correlation, high frenum insertion, early mucogingival problems and advanced tongue position. Management of malocclusion must receive attention when treating periodontal condition[13]. High crossbite case as shown in this study have also been reported by previous studies, as well as cases of open bite. Some of the causes of malocclusion in people with Down Syndrome are due to the less developed maxilla, the prognathic of the mandible and the hypotension of the lips and cheeks. Macroglossia and tongue thrusting habits observed are important to correct by practicing the right tongue position when swallowing[7,14]. Another bad habit found in Down Syndrome patients is bruxism which associated to chronic anxiety, underdeveloped nervous system, malocclusion and TMJ dysfunction creates smoother occlusal surfaces enabling self cleansing but also lead to problems related to jaw reposition treatment need[14]. Malocclusion in Down Syndrome children may and can be successfully treated, depending on the severity of the malocclusion and influenced mental retardation conditions as well as the ability of cooperation between parents and caregivers related to the ability to maintain oral hygiene and control the use and care of orthodontic appliance[7]. In addition to periodontal treatment, Down Syndrome patients must also receive attention and management of obstructive sleep apnea and upper respiratory tract infections which can cause mouth breathing habits[7,13].

Down's syndrome children are resistance to dental caries and about half of them are caries free. As shown in this study, most of samples (58.82%) had def-t and DMF-T score below 10. Down Syndrome patient with caries development show far less number of cavities than would be expected in a normal child which can be due to dental anomalies[15]. Dental anomalies in primary and permanent teeth are very common, and has incidence five times greater in people with Down Syndrome when compared to general population. Anomalies in the number (hypodontia), size (microdontic teeth) and morphology (shallower teeth fissures) and the timing of their eruption. Delayed tooth eruption may occur as long as 2–3 years in an unusual order[7,14]. Another theory states that the results of the salivary electrolytes analysis showed that in Down Syndrome patient, sodium chloride and bicarbonate concentrations as well as pH are high although saliva flow was quite low and tend to be xerostomia. In people with Down Syndrome, caries develops can due to consumption of medicinal syrup containing sugar and cariogenic foods with a soft consistency from difficulty chewing due to hypotonus masticatory muscles which also causes a lack of self-cleansing ability[7].

Caries treatment need for this study samples were restoration using Glass Ionomer Cement (61,76%) then Preventive Resin Restoration and Stainless Steell Crown for about almost 6% of the sample, pulp treatment for more than 10% samples, and extraction about 55.88%. It is critical to maintain the primary dentition as long as

possible with the delayed emergence of the permanent teeth and the high number of missing teeth[15]. Poor periodontal tissue health conditions in people with Down Syndrome can cause permanent teeth to be removed in their teens which had been reported of more cases in loss of lower anterior permanent teeth before 8 years of age[7,14]. Routine psychological (Tell-Show-Do) intervention along with visual distraction using video eyewear and use of CDS-IS (computerized delivery system-intrasulcular) system was reported as effective behavior management technique for anesthetic delivery during invasive dental treatment[16]. But since 40-50% of people with Down Syndrome in general have a condition of the heart disease as risk factor for extraction, then the treatment must be under general anesthesia[7]. The use of general anesthesia is indicated for cases of more complex dental problems, which involve extensive dental treatment. Preventive, restorative and surgical treatment can be performed and concluded in a single appointment[10]. Furthermore, the indication of extraction followed by space maintainer appliance, partial denture or orthodontic treatment, though Down Syndrome is not a barrier to orthodontic care but certainly requires a much greater effort to be successfully carried out. Orthodontic treatment should be carefully considered in people with Down syndrome, some may benefit, while others may not. The possibilities and success of ortohodonrictreatment in Down Syndrome patients are limited by the ability and skills needed to ensure compliance in oral hygiene and in the use of removable or fixed appliances[7,8].

In this study 17,64% of samples did not brush teeth twice a day. Some people with Down syndrome can brush and floss independently, but many Down Syndrome children have physical and mental limitations that cause difficulties in holding and moving a toothbrush whose need help. Specific recommendations on brushing methods or modification of the shape of the toothbrush handle or the use of power toothbrush is sometimes needed to help them to hold their own toothbrushes and dentist must involve patients in hands-on demonstrations of brushing and flossing. However, the role of parents and caregivers is still very necessary to ensure that all parts of the teeth have been brushed, which can be helped by prior applicating the disclosing agent. Dentist must assume parents or caregivers do not know the right position in helping children with special needs brush their teeth so it is important for dentist demonstrate brushing teeth by sitting or standing positions. In addition to the importance of frequency of brushing teeth in a day, the right time to brush teeth, the use of fluoride toothpaste and the habit of gargling after eating are also important factor regarding maintaining oral health to prevent caries. Antimicrobial mouthwash containing chlorhexidine twice a day is recommended for people with Down Syndrome who able to gargle, or either using a spray bottle or in gel form applied on toothbrush which equally efficacious for person who has swallowing difficulties or one who cannot expectorate[7,8,17].

Down Syndrome children first dental visit should be at age 12-18 month to monitor tooth development and eruptions. Caries preventive must be programmed intensively to avoid later oral health problem which include oral hygiene instruction and motivation, dietary counseling and control, topical fluoride application and fissure sealant[17]. Method about how to maintain the dental health of children with special needs is very important to be taught to parents and caregivers early on and it is important to be repeated adjusted to the child's growth and development. Oral Health Instruction should ideally be carried out on the first visit to the dentist and oral hygiene control is carried out at each regular visit every 3 months or more often for some children[7,15]. Using leaflet or module print or non print as media that can be re-learned at home by parents or caregivers still an option. In general, visual-spatial processing and perception of people with Down Syndrome viewed as strength, therefore advice ideally given using media picture or diagram and models[17]. In this digital era, Dental Health Education using audiovisual media with or without interactive game can gain interest from children too.

Increased knowledge of parents and caregivers of children with Down Syndrome is expected to be able to build awareness and increase collaboration with dentists, especially pediatric dentistry specialist, enabling Down Syndrome children to have maintained oral health and improve their quality of life. Dentist, especially pediatric dentist specialists have important role in improving the quality of life for people with Down Syndrome. Besides having knowledge and exceptional skills, it requires willingness, patience, compassion, and motivation to

overcome the barriers faced in treating children with special need condition like in Down Syndrome[11]. Pediatric dentist should always consider general health when planning the dental treatment of patients with Down syndrome, in order to achieve a holistic and interdisciplinary approach with following health professionals included: pediatricians, family medicine physicians, internists, speech-language pathologist, physical therapist, occupational therapist and special education teachers whom each of them play important role[14,16].

Conclusion

From this study it can be concluded that the most indicative treatment for Down Syndrome children is restoration and extraction which can be caused by poor oral hygiene, possibly related to physical and mental limitations in brushing teeth habit, as well as eating habits of cariogenic foods and oral habits that can worsen existing malocclusions. Besides the physical and mental limitations that require dentists to put more effort into the management of children's behavior during treatment, caries preventive such as fissure sealants and topical fluoride applications as well as restorative are very important to avoid indications of extraction due to dental conditions that cannot be maintained.

Extra effort also absolutely necessary for parents and caregivers to work together with dentists, especially pediatric dentists in the treatment of Down Syndrome children to prevent more severe dental and oral health problems. Children with Down Syndrome are entitled to the opportunity to develop and obtain special services, including in terms of oral health. From this study, it is also proven that Down Syndrome children can be free of caries which can be a motivation for Down Syndrome children and their parents and caregivers.

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Toothbrushing And Dietary Behavior of Children Aged 3–6 Years with Early Childhood Caries in Impoverished Community of Semampir District, Surabaya

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Abstract

Objective: This research aimed to observe tooth brushing and dietary behavior of children aged 3–6 years with early childhood caries (ECC) in impoverished communities, Semampir District, Surabaya City. **Materials and Methods:** This was descriptive observational with cross-sectional design study in 50 samples of mother whom their children diagnosed with ECC. Research instrument using questionnaires based on caries risk assessment (CRA) which modified. **Results:** Most samples showed good tooth brushing behavior, which includes brushing frequency and duration, routines using toothpaste, had tooth brushing initiative and independently brushing teeth. The majority of samples had poor dietary behavior which includes snacking habit frequency, time management, fruit and milk consumption. **Conclusion:** Most children aged 3–6 years in impoverished communities, Semampir District of Surabaya already had good brushing teeth behavior, but they had poor diet which explain high prevalence of caries.

Keywords: *toothbrushing, diet, caries, impoverished community*

Introduction

Caries is a biofilm (plaque)-induced acid demineralization of enamel or dentin, mediated by saliva. The disease of early childhood caries (ECC) is the presence of 1 or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger[1]. In Indonesia, 90% of children aged 3–5 years have caries with a def-t value of more than six[2]. The American Academy of Pediatric Dentistry recommends that caries risk assessment (CRA) shall be carried out as part of a child's first dental examination to facilitate an individual prevention plan. CRA currently generally assesses risk factors for early childhood caries in biological, protective, and clinical settings. Risk factors for early childhood caries include vulnerable hosts, excessive carbohydrate diets, the presence of dental plaque, a large number of cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacillus*, and the most important factor is time. Habitual diet, fluoride exposure, sociodemographic status, maternal behavior, oral hygiene habits, and drug intake are also triggering factors for this condition[3, 4].

In children, oral hygiene and dietary behavior is predominantly by parents which factors influenced include socio-demographic factors[5]. Children who come from families with low socioeconomic status have a higher risk of caries. Socioeconomic status factors include income, employment, and education. Socioeconomic status is inversely proportional to general health status. The lower socioeconomic status denotes a higher rate of occurrence of illness[6]. Socioeconomic status affects caries through behavior, especially habits of maintaining oral hygiene, diet, as well as smoking[7]. Low-income family tends not to pay attention to dental and oral health. Bad dietary choices due to limited food selection and not regularly having dentist checks increase caries prevalence[8].

In 2017, the city of Surabaya had a poverty line figure of Rp474,365 per capita per month, with a number of impoverished people as much 154,710[9]. According to the East Java Central Statistics Agency in 2012, there were 3 impoverished subdistricts in Surabaya, including Semampir District[10]. This research aimed to observe brushing teeth and dietary behavior of children aged 3–6 years old with early childhood caries (ECC) in impoverished communities, Semampir District, Surabaya. From preliminary study obtained caries prevalence for children aged 3–6 years in Semampir poor communities was 8.4, which was classified as very high[11].

Material and Methods

Study design and sample

This descriptive observational research with cross-sectional design, involving unemployed mothers who independently raise their 3–6 years old children diagnosed with early childhood caries (ECC). The sampling technique to be used was simple random sampling technique.

Data Collection

The study was conducted in July - August 2018 at Balai RW 3 Sidotopo, Semampir district, Surabaya. This study used questionnaires as research instrument based on modified caries risk assessment (CRA) of The American Academy of Pediatric Dentistry [12]. A reliability test was performed to questionnaires resulted in relatively the same output upon several tests. The reliability was measured statistically using Cronbach's Alpha in 0.778 reliability coefficient. The result score was above 0.60; therefore, it means that the instrument was reliable [13]. Validity test was then performed to examine the accuracy of the actual data and collected data per item. The score of the item was then correlated with the total items. The results of the validity test of this study indicate overall validity.

Data Analysis

Microsoft Excel (version 2013) and Statistical Package for the Social Sciences (SPSS) statistical software (version 23) were used for data entry, and the data were performed with cross-tabulation and then were analyzed using Chi-Square to determine the relation of toothbrushing and dietary behavior with early childhood caries.

Ethical Aspects

This study was approved by the Ethics Committee of Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia. Before starting the study, all mothers were informed about the aim, nature, potential distresses and benefits of the study. Then, informed consent were obtained from all of them. Samples who were unable to give consent form were excluded from the study.

Results

Total samples were 50 mothers from impoverished community whom met the inclusion criteria were interviewed of their children toothbrushing and dietary behavior using questionaire of AAPD caries risk assessment which being modified. Toothbrushing behavior includes brushing frequency and duration, toothpaste usage, tooth brushing initiative and independency in brushing teeth. Table 1 illustrates cross-tabulation of samples' brushing teeth frequency in a day and toothbrushing duration with caries score. From the result for brushing teeth behavior, both samples categories who brushed their teeth once a day and twice or more per day showed lower percentage (20%) of caries score def-t 14-20. Samples who brushed their teeth once a day with caries score def-t of 7-13, and 0-6, both showed same percentage of 40%. While for samples who brushed their teeth twice or more a day, showed 35% and 40% of def-t score 7-13, and 0-6, respectively. The chi-Square test showed no significant relationship between the frequency of tooth brushing and the level of caries in samples (P = 0.933).

About toothbrushing duration results also shown in table1. Samples who brushed their teeth with duration less than 1 minute, 40% of them had a caries score def-tof 14-20, 20% had caries score def-t 7-13, ad 40% had caries score def-t 0-6. Samples who brushed their teeth for 1 minute, as much as 20% had caries score def-t 14-20, 40% had caries score 7-13, and 40% had caries score def-t 0-6. While samples who brushed their teeth for 2 minutes or more, as much as 23.3% had caries score def-t 14-20, 36.7% had caries score 7-13, and 40% had caries score def-t 0-6. Chi-Square test showed no significant

relationship between the duration of toothbrushing and the level of caries (P = 0.9).

Table 1. Cross-tabulation between toothbrushing frequency and duration with caries score.

	Toothbrushing Frequency			Toothbrushing Duration			
Caries score	**Fair (1x/day)**	**Good (≥2x/day)**	**Significance**	**Poor (<1 min)**	**Fair (1 min)**	**Good (>2 min)**	**Significance**
def-t: 14–20	20%	25%	Correlation Coefficient = 0.418 P-Value = 0.933	40%	20%	23.3%	Correlation Coefficient = 0.555 P-Value = 0.900
def-t: 7–13	40%	35%		20%	40%	36.7%	
def-t: 0–6	40%	40%		40%	40%	40%	

Table 2 illustrates cross-tabulation of toothpaste use for brushing teeth with caries score. Samples who brushed their teeth without toothpaste were 100% have a caries score def-t 14-20. Samples who did not routinely use toothpaste, 40% showed caries score def-t of 14-20, 40% had caries score def-t 7-13, and 20% with caries score def-t 0-6. Whereas samples who routinely brushed using toothpaste were 20.5% had caries score def-t 14-20, 36.4% with caries score def-t 7-13, and 43.2% showed caries score def-t 0-6. Chi-Square test results showed no significant relationship between routine use of toothpaste and caries rate in children (P = 0.335).

Table 2. Cross-tabulation between toothpaste use and caries score.

	Toothpaste use			
Caries score	**Poor (no toothpaste)**	**Fair (not routine)**	**Good (routine)**	**Significance**
def-t: 14–20	100%	40%	20.5%	Correlation Coefficient = 0.688 P-Value = 0.335
def-t: 7–13	0%	40%	36.4%	
def-t: 0–6	0%	20%	43.2%	

Samples' initiative and independency in brushing teeth is presented in Table 3. Samples who lack initiative (poor) and being reminded to brush their teeth by parents were 36.4% with caries score def-t 14-20, 45.5% with def-t 7-13, and 18.2% with def-t 0-6. Samples who sometimes had own initiative but sometimes also being reminded by parents were 12.5% had caries score def-t 14-20, 25% with def-t 7-13, and 62.5% had caries score def-t 0-6. Whereas samples who always had own initiative to brush their teeth were 22.6% showed caries score 14-20, 35.5% with a caries score def-t 7-13, and 41.9% had caries score def-t 0-6. Chi-Square test results showed no significant relationship between samples' initiative in brushing their teeth against caries (P = 0.398).

Table 3 also shows samples' independence in brushing teeth cross-tabulation with caries score. The table shows that 22.2%, 44.4%, and 33.3% samples with poor independence had caries score def-t 14-20, 7-13, and 0-6, respectively. From the table also shows that 21.4%, 35.7%, and 42.9% of samples with fair independence which sometimes assisted by their parents, had caries score def-t 14-20, 7-13, and 0-6, respectively. And for samples who brushed their teeth independently 25.9%, 33.3%, and 40.7% had a caries score def-t 14-20, 7-13, and 0-6, respectively. The Chi-Square test showed no significant relationship between samples' independence in brushing their teeth against caries (P = 0.978).

Table 3. Cross-tabulation between toothbrushing initiative and independence with caries score.

	Toothbrushing Initiative				**Toothbrushing Independence**			
Caries score	**Poor (lack initiative)**	**Fair**	**Good (self initiative)**	**Significance**	**Poor (being brushed)**	**Fair**	**Good (by him/ herself)**	**Significance**
def-t: 14–20	36.4%	12.5%	22.6%	Correlation Coefficient = 0.518 P-Value = 0.398	22.2%	21.4%	25.9%	Correlation Coefficient = 0.490 P-Value = 0.978
def-t: 7–13	45.5%	25%	35.5%		44.4%	35.7%	33.3%	
def-t: 0–6	18.2%	62.5%	41.9%		33.3%	42.9%	40.7%	

The results of caries risk assessment for dietary behavior includes snacking habit frequency, snacking time management, and fruit and milk consumption. Table 4 illustrates that 13%, 34.8%, and 52.2% of samples who had snacks more than 3 times a day had caries score 14-20, 7-13, and 0-6, respectively. For samples who had snacks 3 times a day were 33.3% and 66.7% had caries score def-t 7-13 and 0-6, respectively. And for samples who had snacks less than 3 times a day, had caries score def-t 14-20, 7-13, and 0-6, as much as 33.3% each. Chi-Square test showed no significant relationship between the frequency of snacking with the level of caries in samples (P = 0.212).

Table 4 also shows that 10.3%, 34.5%, and 55.2% of samples had poor snacking time management with caries score def-t 14-20, 7-13, and 0-6, respectively. For samples with fair snacking time management showed 20%, 20%, and 60% had caries score def-t 14-20, 7-13, and 0-6, respectively. And samples with good snacking time management were 31.25%, 37.5%, and 31.25% had caries score def-t 14-20, 7-13, and 0-6, respectively. The Chi-Square test showed no significant relationship between the timing of snacking and the level of caries in children (P = 0.372).

Table 4. Cross-tabulation between snacking frequency and time management with caries score.

	Snacking Frequency				Snacking Time Management			
Caries score	**Poor (>3x/ day)**	**Fair (3x/day)**	**Good <3x/day)**	**Significance**	**Poor (No time setting)**	**Fair (Rarely with a time setting)**	**Good (With time setting)**	**Significance**
def-t: 14–20	13%	0%	33.3%	Correlation Coefficient = 0,662 P-Value = 0,212	10.3%	20%	31.25%	Correlation Coefficient = 0,776 P-Value = 0,372
def-t: 7–13	34.8%	33.3%	33.3%		34.5%	20%	37.5%	
def-t: 0–6	52.2%	66.7%	33.3%		55.2%	60%	31.25%	

Table 5 presents cross-tabulation between fruit and milk consumption with caries score. It shows that 26.6%, 26.6%, and 46.7% of samples with poor fruit consumption had caries score def-t 14-20, 7-13, and 0-6, respectively. For samples with fair fruit consumption 18.75%, 25%, and 56.25% had caries score def-t 14-20, 7-13, and 0-6, respectively. And for samples with good fruit consumption, 10.5%, 47.4%, and 42.1% had a caries score def-t 14-20, 7-13, and 0-6, respectively. Chi-Square test showed no significant relationship significant between snack types and caries rates in children (P = 0.524).

Result for cross-tabulation between samples' milk consumption with caries score, shows that 16.7%, 35.7%, and 47.6% of samples with poor milk consumption had caries score def-t 14-20, 7-13, and 0-6, respectively. It also shows 100% of samples with fair milk consumption had caries score 0-6, respectively. And for samples with good milk consumption, 28.5%, 28.5%, and 21% had caries index of 14-20, 7-13, and 0-6, respectively. The Chi-Square test showed no significant relationship between the type of milk consumed and the level of caries in samples (P = 0.791).

Table 5. Cross-tabulation between fruit and milk consumption, with caries score.

	Fruit Consumption				Milk Consumption			
Caries score	**Poor (Not accustomed)**	**Fair (Rarely)**	**Good (Accustomed)**	**Significance**	**Poor (Formula Milk)**	**Fair (Pure Milk)**	**Good (Breasts Milk)**	**Significance**
def-t: 14–20	26.6%	18.75%	10.5%	Correlation Coefficient = 0,399 P-Value = 0,524	16.7%	0%	28.5%	Correlation Coefficient = 0,330 P-Value = 0,791
def-t: 7–13	26.6%	25%	47.4%		35.7%	0%	28.5%	
def-t: 0–6	46.7%	56.25%	42.1%		47.6%	100%	21%	

Discussion

Early childhood caries (ECC) term was recommended in a workshop by the Center for Disease Control and Prevention, focusing on many contributing factors not just tooth brushing habits as the main cause. Prevalence and severity of caries can't be determined just by one factor, all factors must be holistically considered to promote exact caries prevention strategy and treatments[14]. Socioeconomic status is inversely proportional to general and oral health and also associated with dental disease has been demonstrated from previous studies, with education and income as the most common and relevant indicators used in epidemiology for socioeconomic status measurement. Poorer self-rated oral health and higher untreated caries reported in lower income and education groups. Children born to low socioeconomic families have twice the risk of caries than children born to high-income families[15,16]. From preliminary study showed that children aged 3–6 years in impoverished communities of Semampir District had very high prevalence of early childhood caries (ECC) which was 8.4[11]. Caries Risk Assesment (CRA) from American Academy of Pediatric Dentistry had been modified to focus on determining children toothbrushing and dietary behavior which results can be use as data for local government to make more specific strategy for impoverished community able to achieve Indonesia caries free in 2030 as goal set by Ministry of Health Republic Indonesia.

Statistic results for tooth brushing frequency to caries rate on poor community in this study showed no significant relation (P = 0.933). This result was in accordance with Kumarihamy *et al*., that stated there were no statistically significance difference in def-t average (including non cavity lesion) between children who only brush once or less in a day and twice a day (P = 0.18)[17]. It is widely believed that effective removal of biofilm by tooth brushing could decrease development on new carious lesion, but the evidence was so weak especially if it related to tooth brushing frequency[18]. According to the general consensus among the professionals, everyone has to brush their teeth with effective technique at least twice a day and spend at least two minutes. A study conducted by Gallagher *et al*. showed that brushing teeth for two minutes could remove plaque 26% more than brushing for 45 seconds[19]. Stronger remineralisation response requires longer tooth brushing duration as it alleviates fluoride concentration in saliva, which lubricates enamel for at least two hours after brushing[20]]. In the contrary, Klukowska *et al*. showed that brushing for more than a minute did not improve plaque removal[21]. These result is consistent with this study that there was no significant relation between children tooth brushing duration and caries level (P = 0.9). Tooth brushing is connected with many factors other than brushing duration such as the property and design of the brush, brushing method, and the type of toothpaste used[22].

Folayan *et al*. suggested to brush our teeth twice a day and use the toothpaste regularly[22]. Tooth brushing without fluoridated toothpaste has failed to decrease caries prevalence[23]. But, Kumarihamy *et al*. said that brushing teeth with fluoridated toothpaste versus unfluoridated toothpaste had no significance difference (P = 0.22)[17]. This finding was corresponding with this study that there was no significant relation between daily use toothpaste with def-t score (P = 0.335). The use of different kinds of toothpaste failed to demonstrate the significant effect, whereas tooth brushing frequency is related to the prevalence of carious lesions[18]. From Zero *et al.* showed that brushing teeth with fluoride toothpaste but in short duration will limit fluoride contacts and dose in the oral cavity[20].

Based on this study, 62% of children brush their teeth on their own will and 54% brush their teeth by them self with parental guidance. Some researchers reported that children should be able to brush their teeth since the age of two, and it was the parents' responsibility to teach how to clean their teeth and hold the brush correctly since early age; and parents help should be continued until around age 7 or 8[24,25]. The lowest def-t was found in children who brush their teeth with parental guidance, but still brush independently and by their own initiative. But this study showed no significant relation between independency of tooth brushing and caries score (P = 0.978) and also between tooth brushing on their own will and caries score (P = 0.398). Child behavior management skills of parents and the duration of parent-led toothbrushing were associated with better children's oral health. Collete *et al.* suggest that parenting skills are an important target for future behavioral oral health interventions[26]. AAPD suggest parents to make toothbrushing as a fun time together, for

example by playing favorite song while brushing or tell jokes before and after brush[25].

Postnatal diet influences the caries status. Children with poor dietary habits had greater risk of caries compared to children with good dietary habits[27]. The sequence and frequency of consumption are closely related to the incidence of tooth decay. The prevalence of caries was higher in children who consumed snacks more than 4 times a day compared to children who consumed snacks less than 4 times a day[28]. The frequency of consuming cariogenic foods, especially between meals, has a strong relationship with the risk of tooth decay because it inhibits the normalize of oral pH and inhibits the "self-cleansing", which both increasing potential of enamel demineralization. This rinsing period depends on food consistency and solubility; and individual characteristics such as chewing, and salivary flow and quality[29]. But from this study Chi-Square test results found that there was no significant relationship between level of caries and snacking frequency (P = 0.212), and the timing (P = 0.372). This study results is in accordance with the research of Zahara *et al.* where there was no relationship between feeding frequency (P = 0.800) and the incidence of dental caries[30]. Cariogenic potential of a food is influenced by several factors like fermentable carbohydrate content, cariostatic properties or a food's ability to remain in the mouth, dietary habit, also sequence and eating frequency[29].

Children with low socioeconomic status experienced more childhood caries[31]. Low socio-economic value relates to mothers' education. Mothers with low education, equal to or lower than high school, usually do not aware of their children's diet. Children who come from low-income families tend to have poor-quality diets, less nutritious but high sugar diet which promote caries development[32]. Based on the available evidence, it is very likely that there is no relationship between the consumption of fresh fruit and dental caries. The WHO on "Diet, Nutrition and Prevention of Chronic Diseases" report also concluded that despite the acidic nature of some fresh fruits, still not enough evidence to link the consumption of whole fruit with a higher risk of tooth erosion. Fruit does not pose a significant risk to dental health, and replacing high sugar foods with fresh fruit will reduce the chance of tooth decay. By contrast, a serving of fruit juice contains more energy, free sugars and fruit acids than whole fruits and so its consumption may contribute to both undesirable weight gain and dental caries. All dental health professionals should have skills to provide healthier eating advice for their patients, including how to limit free sugars intake. Ideally, all patients, or their parents or caregivers, should receive dietary advice at the time of dental examination based on current food-based dietary guidelines[32, 33].

Based on the results of data analysis obtained from this study it can be concluded that there is no significant relationship between milk type and caries level in children (P = 0.791). This contrasts with research conducted by Firdaus and Iswati which stated children who consumed formula milk had a greater tendency to develop caries compared to children who consumed breast milk and there was a significant relationship between formula feeding and the level of caries in children[34,35]. Formula milk has high carbohydrates and sugar compared to breast milk and whole milk, therefore promoting caries, especially milk consumption in the bottle before bed. In contrast to formula milk, both breast milk and cow's milk have anti-cariogenic properties and high concentration of calcium and phosphate. In addition, cow's milk has phosphoprotein, which can be absorbed by enamel to prevent enamel demineralization. Pure milk also contains 3.5% fat, which form a protective membrane on the teeth to facilitate the process of self cleaning and reducing bacterial activity[36]. On the other hand, Feldens *et al.* showed significant relationship between milk diet and caries risk by his research which conducted in 345 children aged approximately 12 months or 1 year who were still breastfeeding[37]. One of the caries factors that can underlie this different opinion maybe is time which is the length of time the teeth are exposed to sugar. The acid produced by bacteria can last for 20 to 40 minutes in the oral cavity. If teeth are exposed to an acidic environment for too long, tooth enamel will demineralize due to its susceptibility to acid. Duration of formula milk consumption as a risk factor for dental caries found in kindergarten students (P = 0.013). Children who consumed formula milk in more than 15 minutes duration had greater possibility exposured to dental caries[35].

Children under 2 years get the majority of nutrients from breast milk, while children over 3 years have received nutrition indifference to most adults. However,

the mother and the environment greatly influence children's diet. Mothers with a high sugar diet are likely to introduce the same diet to their children, promoting high sugar consumption in children[38]. Children with a high-sugar diet tend to continue the habit in their childhood and adolescence. Maternal habits play an important role in dietary choices and caries status of children[39,40].

Conclusion

Children have very complex conditions and caries is a multifactorial disease. Socioeconomic and environmental factors may have effect in behavior shaping. It is mothers as close related person for their children who have significant role in inculcate healthy behavior. Young children tend to imitate their parents habit so it is important to educate mother. Both good brushing and dietary habit needed to reduce ECC rate. High prevalence of chidren caries in impoverished community need more government attention in setting prevention strategy.

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Comparing Fracture Resistance of Provisional Restorations of 3-Unit Fixed Partial Dentures: An in Vitro Study of CAD/CAM versus Indirect Technique

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Abstract

Objective: To compare the indirect and CAD/CAM techniques and using acrylic and composites resins on the fracture resistance of provisional 3-unit fixed partial dentures (FPDs).

Materials and Methods: In in vitro setting, thirty samples were divided into three groups included 10 FPDs manufactured indirectly and made of composite based bis-acryl (Protemp 4) resin, 10 FPDs indirectly milled with polymethyl methacrylate resin (PMMA) and 10 FPDs fabricated by CAD/CAM PMMA blocks. Fracture resistance was measured using universal testing machine (UTM). The master model was made of NiCr-alloy which designed using CAD/CAM technology.

Results: FPDs fabricated using CAD/CAM PMMA blocks showed the highest fracture resistance values followed by Protemp 4 and then PMMA using indirect method. Statistically significant difference was found between the three groups (P=0.001). Bonferroni test was applied for pair wise comparison, in which the statistically significant difference was observed between FPDs fabricated by CAD/CAM which made of PMMA blocks and Protemp 4 and between CAD/CAM PMMA blocks and indirect/PMMA FPDs and between indirect/Protemp 4 FPDs and indirect/PMMA FPDs. Hence this suggested that PMMA significantly has minimum value of fracture resistance when compared to both CAD/CAM and Protemp 4.

Conclusion: This study proved the superiority of CAD/CAM PMMA blocks for fabricating 3-unit FPDs over Protemp 4 and PMMA using indirect method, and the superiority of Protemp 4 over PMMA in terms of fracture resistance using indirect methods.

Keywords*: computer-aided design; dental restoration- temporary; denture, partial, fixed; fracture resistance; polymethyl methacrylate*

Introduction

The word provisional, interim or temporary means established the time being.[1] A provisional restoration plays a considerable role during tooth preparation procedure in which the tooth structure becomes smaller in size and the time frame for the fabrication of the final restoration might exceed several weeks.[2] A provisional restoration has a wide range of functions as the intermediate stage of the treatment including easthatics, diagnosis in dentistry, gum health, protection of pulp, providing occlusal stability, improving masticatory efficiency, and determining the final form of the definitive restoration.[3,4]

At present, available provisional restoration materials can be divided into two categories according to their chemistry composition namely acrylic (monomethacrylates) resins including ploymethylmethacrylatic (PMMA) resins and polyethyl/

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butyle methacrylate (PEMA) resins or composite-resin (bis-acryle or dimethylmethacrylates) which include bisphonel A-glycidyl dimethacrylate (Bis-GMA) and urethane dimethacrylate (UDMA) resins. The light activated method is used for the polymerization of these two categories.[5] The chemical composition of these material has been improved in recent years to achieve good fracture resistance and aesthetics performance in clinical situations.[6]

The fracture resistance is the most important property influencing the success of interim restoration and may cause discomfort, financial and economic loads for the patient.[4] Clinicians should be aware of the range of available commercial materials and suitable fabrication techniques to minimize the probability of the fracture.[7,8]

CAD/CAM technologies definitely solve some of the issues raised with provisional restorations when fabricated by conventional methods (direct or indirect) such as reducing chairside time of the extra appointment prior to the preparation and fabrication of an interim bridge, eliminating the time of sending to the laboratory, and using resin based blocks which prevent voids within interims and increase the mechanical strength.[9]

Little in vitro research has examined the fracture strength of interim restoration materials such as Protemp 4 and CAD/CAM technique. Therefore, the aim of this in vitro study was to compare groups of indirectly fabricated provisional 3-unit FPDs (fixed partial dentures) using the PMMA resins and Protemp 4 materials and CAD/CAM fabricated FPDs milled of PMMA blocks, in terms of fracture strength.

Materials and Methods

The fracture resistance of the studied materials and manufacturing techniques were tested using an in vitro setup on a metal master model with a 3-unit FPD. The two resins based materials were PMMA molding powder and liquid (methyl methacrylate resin) and Protemp4 (bis-acryl composite resin). Table 1 summarizes the characteristics of materials used including their composition. All materials were cemented in accordance with the manufacturer's manual.

Table 1. Characteristics of materials used in the study.

Serial no	Name of the material	Manufacturer	Composition
1	PMMA resin	Yamahachi, Japan	Methyl-methacrylate resin
2	Protemp 4 composite	PARKELL inc Edgewood, USA	Bis-acryl composite resin
3	PMMA block	Ketten Bach, Japan	Bis-acryl composite resin

Preparation of master model using CAD/CAM technique

The master model was designed using CAD/CAM technique. For this goal, the dental impression tray was used to reproduce the cast from a patient with dental implant on positions of 37 and 35. The data of abutment on the cast was transmitted using laboratory scanning abutment (Arum Co., South Korea) and collected by a scanner (D850 3D scanner, 3Shape, Denmark). Then two anatomic abutments similar to shaved teeth (angle 6˚, chamfer margin thickness of 1 mm, and height 8 mm) were designed using CAD/CAM software (3Shape Co., Dental System, Dental). Then, 3D printed abutment with resin material was produced using 3D printer (3D printing system, Asiga Max UV, USA) and finally casted with alloy (Scheftner Dental Alloys , Magucera N, Germany) (Fig 1 and 2).

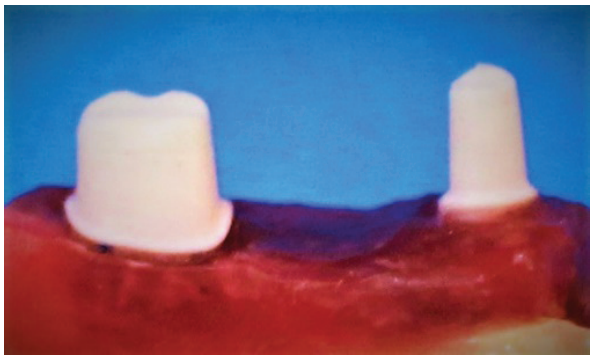

Figure 1. Final model in inlay wax;

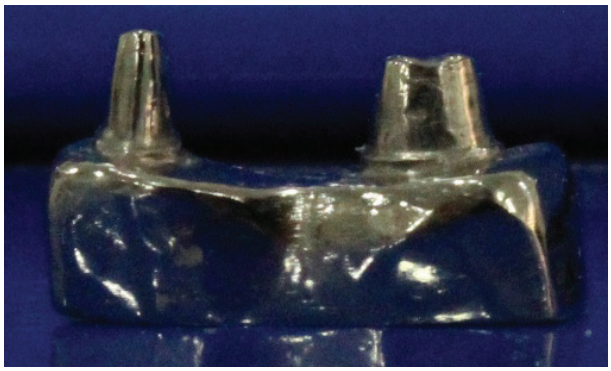

Figure 2. Final corpus model in NiCr-alloy

CAD/CAM fabrication of provisional 3-unit FPDs

The surfaces and margins of the master model were converted into data with the help of a scanner (3D scanner, 3Shape D850, Denmark), the data is then read and interpreted by the CAD/CAM (3Shape, Dental System, Denmark). The 3-unit FPDs was designed on tooth #36 as hygiene pontic with distance 2 mm from master model and retainers of buccal and lingual caps had 1.5 mm and 1 mm thickness, respectively. 10 samples were produced from PMMA blocks (Yamahachi, Japan) using milling machine (Arum model 5x 200, South Korea).

Indirect fabrication of provisional 3-unit FPDs

Ten samples of 3-unit FPDs from Protemp 4 materials (3M ESPE, North Ryde, USA) and Ten samples of PMMA martials (GC Tempron, Japan) were made based on the index of CAD/CAM 3-unit FPDs samples (Fig. 3).

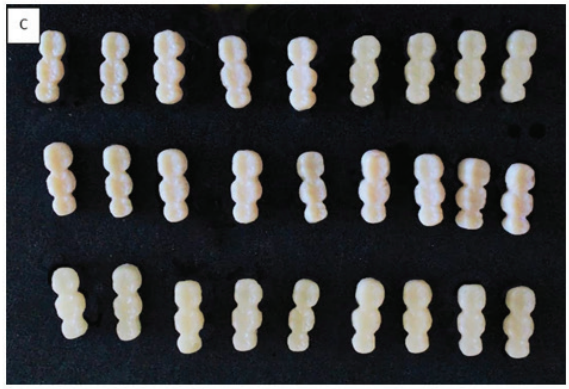

Figure 3. Trimmed temporary bridges

Fracture resistance Test

All FPDs were subjected to axial compression test at a cross-head speed of 1.2 mm/min until fracture occurred using a metal ball (6 mm in diameter) in a universal testing machine (Fig. 4). Fracture test was started at a crosshead speed of 1.2 mm/min until fracture occurred Maximum force at which the fracture occurred was recorded (Fig. 5).

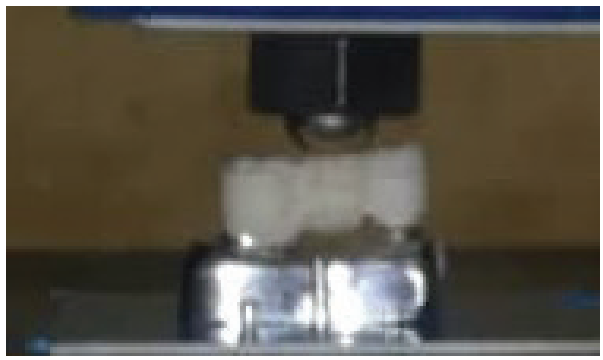

Figure 4. Setup with temporary bridge and sphere prior to fracture testing ;

Figure 5. Fracture of the bridge after load application.

Statistical Analysis

The one-way ANOVA analysis test was utilized to compare mean and SD of fracture resistance value between three groups. The P value was considered as <0.05.

Results

The maximum forces at fracture were recorded in Newton. These are listed in Table 2 and depicted in Fig. 6.

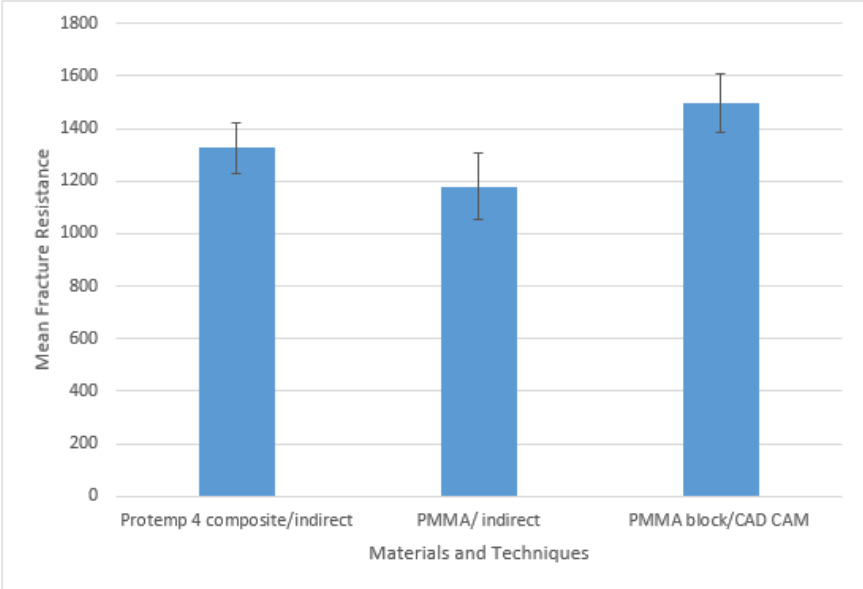

Figure 6. Comparison of fracture resistance between three groups.

Table 2. The maximum force at the moment of fracture (Newton)

Sample	Protemp 4/inderct	PMMA/indirect	PMMA block/CAD/CAM
Sample 1	1690	1208	1690
Sample 2	1375	1180	1375
Sample 3	1683	1002	1683
Sample 4	1460	1329	1460
Sample 5	1489	1199	1489
Sample 6	1500	900	1500
Sample 7	1470	1205	1470
Sample 8	1379	1225	1379
Sample 9	1345	1334	1345
Sample 10	1534	1209	1534
Mean ±SD	1326.6 ± 101.7	1179.1 ± 133.5	1494.3 ± 117.1

FPDs fabricated using CAD/CAM PMMA blocks showed the highest fracture resistance values followed by Protemp 4 and then PMMA using indirect method. Table 3 shows the comparison of the mean fracture resistance between the three groups using one way ANOVA analysis. Statistically significant difference was found between the three groups (P=0.001). Bonferroni test was then applied for pair wise comparison, in which the statistically significant difference was observed between FPDs fabricated by CAD/CAM which made of PMMA blocks and Protemp 4 and between CAD/CAM PMMA blocks and indirect/PMMA FPDs and between indirect/Protemp 4 FPDs and indirect/PMMA FPDs (Table 4). Hence this suggested that PMMA significantly has minimum value of fracture resistance when compared to both CAD/CAM and Protemp 4.

Table 3. Comparing the mean of fracture resistance between three groups.

Material/technique	Mean Fracture resistance (N)	Degree of freedom	P value
Protemp 4/Indirect	1326.6	29	0.001
PMMA/Indirect	1179.1		
CAD/CAM / PMMA block	1494.3		
One way ANOVA test			

Table 4. Comparison of mean between groups (Post HOC test)

Materials and techniques	Protemp4/indirect	PMMA/ indirect	CAD/CAM block (PMMA)
Protemp4/indirect	-	0.025	0.01
PMMA/ indirect	0.025	-	0.001
CAD/CAM block (PMAA)	0.01	0.001	-

Discussion and Conclusion

One of the most important steps in fixed prosthesis treatment is the fabrication of provisional restorations.[10] Various materials for provisional restorations are commercially available including PMMA, PR'MA, microfilled composite and light-cured polymerized composites.[11] Fabrication of temporary restorations are performed by two methods namely direct and indirect techniques. Recently, emerging of digital molding and CAD/CAM enhanced the accuracy of provisional restorations.[12]

In current study, the zero hypothesis indicating no difference in fracture resistance of three groups was rejected. Our findings showed that the fracture resistance of 3-unit FPDs interim restorations fabricated by CAD/CAM technology was significantly better than those fabricated by Protemp 4 indirectly (P value=0.025). In addition, the fracture resistance of 3-unit FPDs made by Protemp 4 was significantly better than PMMA. On the other words, CAD/CAM is superior to two other groups. Several studies showed similar findings with current study.[13-15] In addition, Balkenhol et al. in their study have found that fracture resistance of Protemp 4 is

higher than methacrylate (PMMA) in indirect method.[7] Moreover, Gujjari et al., in their study have reported that of bis acryl resin (Protemp 4) showed better mechanical properties than PMMA in indirect method.[16] A systematic review has showed that methyl methacrylate e.g. Protemp 4 has better fracture resistance than methacrylate due to its chemical structure and cross-link property.[5] The difference in fracture resistance of these materials is attributed to chemical composition variables such as monomer composite and polarity.[14,17] Bis-acryl contains organic matrix and filler non organic particles in addition to multifunctional monomers. Moreover, it contains cross-link polymer structure between monomer chains.[18] Whereas, conventional PMMA contains linear and mono functional molecules.[19] The researches have reported that usual PMMA resins absorbs more water in their polymer network.[20] However, polymer-based PMMA blocks have better homogeneities, less water absorption and solubility. In addition, PMMA blocks are kept under air prior to utilization which provides opportunity for the process of post polymerization and rest.[21] There are few studies have compared fracture resistance of 3-unit FPDs made of PMMA blocks with conventional materials (PMMA and Protemp 4). Alp et al.[22] and Karaokutan et al.[13] in their studies have compared fracture resistance crowns fabricated by PMMA blocks using CAD/CAM technology with crowns fabricated indirectly with PMMA and Protemp 4. Their findings are compatible with our results. Recently, a study conducted by Pop et al., in which fracture resistance of provisional restorations of 3-unit FPDs indirectly fabricated by two types of PMMA has been compared with those made of PMMA blocks by CAD/CAM technology.[23] They have demonstrated that fracture resistance in CAD/CAM technology was significantly higher than indirect method. In the study by Alt et al., it has been shown that fracture resistance of interim restorations of 3-unit FPDs fabricated directly by PMMA is lower than those fabricated by CAD/CAM PMMA blocks.[12] PMMA blocks own better mechanical properties, lack of air voids, reduced porosities in turn decrease water absorption and higher fracture resistance.[24] Previous studies have suggested to enhance the mechanical properties of bis-acryl and methacrylate.[25,26] A study by Haselton et al., found contrary results in which the PMMA and Protemp 4 did not have significant difference in terms of fracture resistance.[27] In contrast with our study, Sharma et al.[28] and Poonacha et al.[6] also found that PMMA is superior than Protemp 4 in terms of fracture resistance and elasticity.

The strengths of the current study were the evaluation of 3-unit FPDs fabricated by CAD/CAM PMMA blocks which rarely studied by previous researches. Moreover, the master model was designed by CAD/CAM technology which enhance the accuracy of the model. The relatively higher sample size and design and manufacturing of the samples according the clinical standards are other strength points of current study. The limitations of current research were no applying fatigue test and thermocycling. In addition, the effects of intra oral factors such as saliva were not assessed. At the same time, the dentist skill was ignored in indirectly manufacturing interim restorations.

In conclusion the results of the current study proved the superiority of CAD/CAM PMMA blocks for fabricating 3-unit FPDs over Protemp 4 and PMMA using indirect method, and the superiority of Protemp 4 over PMMA in terms of fracture resistance using indirect methods. The findings of previous research also support our results.

Acknowledgments: The study was approved at Ethical Committee of Ahvaz Jundishapur University of Medical Sciences. (Ethical Code: IR.AJUMS.REC.1397.270).

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GPx1-rs1050450 Gene Polymorphism Association with End Stage Renal Disease in Type2 Diabetic Patients of Babylon Province/Iraq

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Abstract

Background: Glutathione Peroxidase-1 is an antioxidant enzyme, play an important role in defense against oxidative stress. In Pro198Leu (C/T) polymorphism of Glutathione gene variation which result in lowering enzyme activity increasing microvascular complications of diabetic patients. **Aim:** This study was aimed to estimate the relationship between GPX1 gene variation and the risk of develop and progression of end stage renal disease in type I diabetic nephropathy patients. In this study, it has been examined 140 type I diabetes mellitus patients with nephropathy and 40 healthy control. **Results:** All samples have been genotyped by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. In diabetic nephropathy patients, there were a significant elevation in in the variation (TT) genotype and (T) allele frequencies compared with control group.

Conclusions: Using major Iraqi diabetic patients of Babylon province we studied the transforming effect of GPX1 gene polymorphism on the risk of development diabetic nephropathy, this study was found that the (TT) genotype of GPX1 SNPs increases the risk of development diabetic nephropathy, while (CC) genotype decreases the chance for developing disease. These results will gave the way for better perception of the genetic influencing on the development and progression of diabetic nephropathy.

Keywords: *Diabetes mellitus, Oxidative stress, ESRD, GPx1 gene polymorphism*

Introduction

Diabetes mellitus is a metabolic disorder with abnormal elevation of glucose levels as a result of insulin failure to metabolize elevated carbohydrates, proteins, and lipids leading to sever life threatening diabetic complications. The best known diabetic symptomes include polydipsia, increased urine volume, and excessive hunger, the persistent of disruption and abnormal functions of various body organs such as retinopathy, neuropathy, nephropathy, and atherosclerosis are strongly associated with chronic long lasting hyperglycemia[1,2].

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Oxidative stress is the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), these molecules play an important roles in the tissue damage and injury by several factors, then the body defense by several mechanisms such as tissuerepair, antioxidant and physical defenses, the association between oxidative stress and type2 diabetes is well revealed as that oxidative stress result from imbalance between oxygen free radicals and the endogenous antioxidant defense, diabetes is accompanied with the augmentation of free radicals formation andddiminished antioxidant ability[3,4]. The balance usually present in cells among free radical formation and their reduction through cellular antioxidants is damaged, these free radicals causese the destruction of cell components such as DNA, lipids, and proteins results in the development of diabetes mellitus and its complications[5,6].

Diabetic patients with renal disease have been a worldwide distributed with continuous increasing in numbers as the age and duration of disease increases, this forms dangerous burden on the presence of 'healthcare resources'. Nearly 30-40% of diabetic populations may develop diabetic nephropathy with high risk for end stage renal disease and several vascular complications. Despite both type1 and type2 diabetes lead to end stage renal disease, the vast majority of patients are those with type2 diabetes[7]. Glutathione peroxidase (GPx1), is the second line of enzymatic antioxidant defense system after superoxide dismutase enzymes, it has six isoforms and the best known isoforms is (GPx1), is the most distributed and the more abundant in body cells especially in vascular endothelial cells. GPx1, act by the conversion of peroxides such as hydrogen peroxide H_2O_2 and 'ROOH into 'water and 'alcohol[8] .Gene that are responsible coding for GPx1 is present on chromosome 3p21.3 with 2 exons (1.42 kb) region, numerous polymorphisms have been established in the GPx1 gene but, the best known one in located at 198 codon (C to T) leading to subsituation of proline (CCC) to leucine (CTC), this amino acid variation lead to modification of active site of enzyme changing its activity[9].

Material and Methods

1.Study subjects

The diagnosis of type2 diabetic patients was determined by the medical experts of Marjan teaching hospital in AL-Hilla City/Babylon province-Iraq. The patients with nephropathy history of 5-6 years with albumin excretion >300mg/day in two out of three urine samples. This study was comprised of end stage renal disease patients who were dependent on hemodialysis for 1-3 years. The questionnaire included diabetic nephropathy family history, age, smoking, hypertension, occupation, and the level of education. The physiological and biochemical parameters associated with diabetic patients include serum fasting blood glucose, HbA_{1c}, BMI, microalbuminurea, and albuminuria.

2.Extraction of DNA from blood samples

The blood samples were collected in EDTA tubes and stored at -20ºC. Genomic DNA was extracted from 140 patients blood samples and 80 control blood samples by using the extraction kit (Geneaid/Korea). An Proline/Leucine polymorphism in the (GPx1-rs1050450) gene was determined by PCR-RFLP technique. PCR amplification was performed with a total volume of 20 µl containing 50ng of genomic DNA, 1.25 U of Taq polymerase, 2mM dNTP, 2 mM $MgCL_2$ and 1X PCR buffer in the presence of 0.4 µmol/l of both primers, forward: 5'-TCCAGACCATTGACATCGAG-3' and Reverse: 5'-ACTGGGATCAACAGGACCAG-3'. The PCR amplification was performed in 38 cycles at 96ºC for 5 minutes (initial denaturation), 94ºC for 45 seconds (Denaturation), 59ºC for 30 seconds (annealing), and 72 for 10 minutes (final elongation). The amplification products then digested with the restriction enzymes ApaI (Promega/ USA), the digestion product then being electrophoresis in 3% agarose gel, it will show: 1 fragment of 314 bp for the Pro198 (C) wild type homozygous, 3 fragments of 314, 261, and 53 bp for the Pro198/Leu (CT) heterozygous, and 2 fragment of 261 and 53 bp for the 198Leu (T) mutated homozygous genotype.

3. Statistical analysis

Statistical analysis'were performed by using (SPSS) version (23.0). Chi square'test were used for the distribution of genotypes for deviations from'Hardy-Weinberg' equilibrium. Genotype 'distribution'of GPx1 gene in all 'subjects was analyzed by'Chi-square test.

The results

1. GPx1-rs1050450 genotyping

The results of GPx1-rs1050450 gene genotyping showed that the PCR product had one band about (314bp) for both diabetic patients and control group as shown in figure (1).

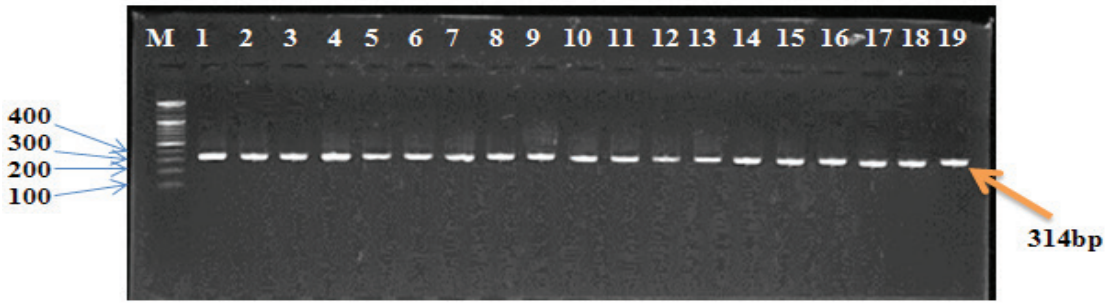

Figure (1): The electrophoresis pattern of PCR product for GPx1-rs1050450 gene. M: DNA marker (100bp), lane 1-10 from diabetic patients, lane 11-19 from control; 1% agarose, 75V, 20mA for 1h.

2. PCR-RFLP of GPx1-rs1050450 gene

The results of PCR-RFLP of GPx1-rs1050450 gene of diabetic patients and control group by using ApaI-restriction enzyme showed that the homozygous CC pattern has one band about (314 bp), the homozygous TT pattern has two bands about (53 bp and 261 bp) and the heterozygous Ff patterns has three bands (53, 261, and 314 bp) as shown in figure (2).

Figure (2): The electrophoresis pattern of PCR-RFLP for PCR product (314bp) with restriction enzyme ApaI, 3% agarose, 75V, 20mA for 2h. (7µl in each well). Lane M: DNA marker100bp, Lane: 3,4,7 ,8,10,11 showing homozygote type (CC) genotype, Lane:2,9 heterozygote mutant type (CT) genotype, Lane:1,showing homozygote mutant type (TT).

In this study, the results showed that the frequency of mutant homozygote pattern TT were significantly higher (P≤0.0001) in renal failure diabetic patients with (55%) than control group (15%), also the mutant allele frequency T was the most common in diabetic patients group (0.68 %) compared with only (0.25%) in control group. This means that the mutant homozygote pattern were significantly associated with renal failure in type 2 diabetic patients.

Table (1): GPx1-rs1050450 genotype and allele frequency among diabetic pateints with end stage renal disease and control groups.

Genotype Pattern	Genotype Frequency (%)				
	Diabetic Patients with ESRD No=140(%)	Control No= 80(%)	P-Value	Odds Ratio	95% CI
CC	28 (20%)	52 (65%)	Reference		
CT	35 (25%)	16 (20%)	0.39	1.3333	0.6835-2.6009
TT	77 (55%)	12 (15%)	0.0001**	6.9259	3.4455-13.9221
Allele Frequency%	C (0.32)	C (0.75)			
	T (0.68)	T (0.25)			

****signification at P value ≤0.001**

Discussion

Diabetic nephropathy is a common microvascular complications of diabetes. Candidate gene polymorphisms analysis in diabetic nephropathy could be important in determining patients have ability for the development and progression of this disease. Oxygen free radicals is associated in the evolution of diabetes and its resulting problems (Vincent *et al*., 2004). Many studies have documented that the glutathione C/T variation increases the occurrence of free radicals-related diseases (Cao *et al*., 2014).

Glutathione, a selenoenzyme which induces the liberation numerous of peroxides into O_2 and H_2O, it serve as a cellular barrier against damage by oxygen free radicals and it produced mainly by all tissues in the body. Previous studies were focused on the role of GPX1-rs1050450 which result in the substitution of Proline into Leucine (C/T), this mutation was studied by many studies to determine the risk of lung and bladder cancer[10]. In addition to the risk of cardiovascular disease. Some studies found that there is no association between GPX1 gene polymorphism and cardiovascular disease or chronic kidney failure in type II diabetic patients[11], but in this study it has been found a significant influence between GPX1 gene polymorphism and the risk for developing diabetic nephropathy in Iraqi population/ Babylon province. From results of this study it was determined that diabetes mellitus and TT genotype may act together to raise the risk for development diabetic nephropathy (Table 1). On the other hand, the patients were at lower risk for developing diabetic nephropathy if they have CC genotype of GPX1 gene polymorphism.

In some studies were performed on type I diabetic patients, these studies explained that the GPX1 gene polymorphism did not show any significant correlation with oxidative stress. Mohammedi *et al*. they studied three out from four GPX1 SNPs (rs-1987628, rs-8179164, rs-9819758, and rs-3448) and there analysis showed no any association with diabetic nephropathy or with oxidative stress in type I diabetic patients[12].

The process of the detected effect of glutathione gene polymorphism on predisposition of diabetic nephropathy is obscure. Various studies established the relation of Pro198Leu variation on glutathione activity[13]. The variation of antioxidant enzyme capacity perhaps result in elevation of oxidative destruction connected with the T allele. Earlier studies have been showed the relation between GPX1 activity and low density lipoprotein (LDL) oxidation, and it is very important in the distribution of microvascular injury of kidney disease[14,15].

The conclusion of our findings proposed that glutathione Pro198Leu genotypes are remarkably associated with the risk of nephropathy development in type II diabetic patients. Further studies is required for this type of mutations and it is association with other diabetic complications. The influence on detected genetic capability is little but it is important in the case of presence of other genetic factors. This study introduced novel clinical related information for genetic factors that plays a role in diabetic nephropathy susceptibility.

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Requirement Specification to Develop Digital Health Promotion Platform for Millenial and Post-Millenial Generations in Surabaya Indonesia

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Abstract

Background: Surabaya is facing various urban problems that are increasingly complex. Therefore, innovative solutions are needed such as digital health promotion for millenial and post-millenial as the largest segment of internet users. The aim of this study is to identify the requirement specification to develop digital health promotion platform for millenial and post millenial generations in Surabaya.

Methods: This research used qualitative methods with case study approach. This study used purposeful sampling on 24 informants, consists of several related parties who met the criteria. Data collection technique was carried out through in-depth interview and documents review.

Results: The requirement specifications were classified into 4 domains (functional, technical, interface, performance requirements). Functional requirements consist of procedures, menus, features, content, data, information, control mechanism, and parties involved. Technical requirements include programs, networks, size, and types of digital devices. Interface requirements consist of visual display related to user interface and operating system. Performance requirements include several aspects such as speed, accuracy, efficiency, effectiveness, security, etc.

Conclusion: Digital health promotion platform requirement specification must be tailored to the needs of millenial and post-millenial generations. If these requirements can be met, the target will be easier to accept and use digital health promotion platform.

Key words: *Requirement specification, health promotion, digital platform, millenial, post-millenial*

Introduction

Currently, more than half of the world's population live in urban areas, so cities have to bear the impact of urbanization which has implications for the emergence of various health problems. Cities also have a lot of potential as a place for 85% of science and technology innovation, so there is a great opportunity to develop solutions that are relevant to the challenges of the city today and future.[1] The rapid development of information and communication technology has also brought the world into a new era, from the conventional to the digital era. The digital era has also had a major influence in the field of health promotion. Digital platforms become a new means for health promotion and public communication, including health communication.[2]

Indonesia has many problems and issues related to development, including urbanization. In 2015, 59.35% of Indonesia's population lived in cities and it is predicted to be 82.37% by 2045.[3] On the other hand, Indonesia is one of the countries with the largest internet users in the world, reaching 143.26 million users in 2017. More than 120 million of them are mobile phone users who are

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active on social media. The number of users also shows an increasing trend. 72.41% users are in urban areas. 66.2% internet users in Indonesia are in the age range of 13-34 years who are included in the millennial and post-millennial generation groups.[4]

Surabaya as one of the largest metropolitan cities in Indonesia also faced various health problems, especially non-communicable diseases, smoking-related diseases, alcohol and drugs consumption, traffic accidents, universal health coverage, pollution, etc.[5] If these problems are addressed only by using conventional solutions, they will not be handled properly. Therefore, innovative solutions are needed to overcome them. One of the innovative solutions with great potential to be developed is digital health promotion for millennial and post-millennial generations as the largest segment of internet users in Surabaya.

Before developing a digital health promotion platform, it is necessary to identify the requirements so that its utilization can be more optimal and right on target according to the needs of millennial and post-millennial generations. This study aims to identify requirement specification to develop digital-based health promotion platform for millennial and post-millennial generations in the city of Surabaya. In this study, we will focus on determining the functional, technical, interface, and performance requirements that the targets really need.

Materials and Methods

This research used qualitative methods with case study approach. This study used purposeful sampling on 24 informants, consists of several related parties who met the criteria. Data collection technique was carried out through in-depth interview and documents review. The informants in this study were classified into key informants, main informants, and supporting informants.

Table 1 Informant classification and eligibility criteria.

Informant Status	Criteria
Key Informants	
Party from Surabaya City Health Office (Dinkes)	The current head of health promotion section or the party in charge of health promotion in Surabaya City Health Office.
Parties from health startup	Currently running a health startup within the scope of Surabaya or wider.
Main Informants	
Millenial generation	Born in 1981-1994, have an ID cards (KTP) as a resident of Surabaya, domiciled in Surabaya, as well as being involved in activities or organizations like youth clubs (karang taruna) and other community organizations or health cadres.
Post-millenial generation	Born in 1995 and thereafter, have an ID cards (KTP) as a resident of Surabaya, domiciled in Surabaya, as well as being involved in activities or organizations like youth clubs (karang taruna) and other community organizations or health cadres.
Supporting Informants	
Health promotion officer	Currently working as a person in charge or health promotor under the auspices of Surabaya City Health Office.

The key informants consist of several parties who have met the criteria in table 1, considered to understand and have the basic necessary information about the development of digital health promotion in Surabaya. The key informants in this study included party from Surabaya City Health Office, to be precise, the current Head of Health Promotion Section. The informants were determined according to the recommendation from The Head of Surabaya City Health Office. In addition, other key informants are 3 health startup players in the scope of Surabaya or wider, which are recommendations from Surabaya Communication, Informatics, and Public Relations Office.

The main informants in this study are certainly the millennial and post-millennial generations, according to the criteria specified in table 1. The informants included in these criteria were taken from the work areas of several public health centres (*Puskesmas*) in Surabaya after getting a recommendation from Surabaya City Health Office. Based on data obtained by researcher, there are 62 public health centres in Surabaya which are divided into 5 regions: Central Surabaya, West Surabaya, North Surabaya, East Surabaya, and South Surabaya. Informants who meet the criteria for millennial and post-millennial generations are taken from the working area of *Puskesmas* in these 5 regions. The selection of informants was based on recommendations of the person in charge or health promotor at the local public health centres by still referring to the eligibility criteria.

The number of informants considered sufficient if it has reached the level of redundancy. In-depth interviews with informants who are millennial generation achieved redundancy on 5th informant, but researchers still continued the interview until 8th informant. So that after adding 3 informants, the data obtained was completely saturated. Furthermore, interview with informants who are post-millennial generation were sufficient on 6th informant because they have achieved redundancy. The total number of main informants in this study were 14 people, taken from 14 working areas of *Puskesmas* in 5 regions of Surabaya. The 14 informants consisted of 8 informants from millennial generations group and 6 informants from post-millennial generations group.

In addition, interviews were also conducted with supporting informants, namely health promotion officers who were also taken from the working areas of several *Puskesmas* in Central Surabaya, West Surabaya, North Surabaya, East Surabaya and South Surabaya regions. The work area of *Puskesmas* that selected as the location for data collection of supporting informants were different from the previous *Puskesmas* work area which were designated as the location for data collection of main informants (millennial and post-millennial generations). While the determination of informants who work as person in charge or health promotor was based on the recommendation from head of the local *Puskesmas*. In-depth interviews with the supporting informants were sufficient on 6th informant because no new information was found anymore.

The total number of informants in this study were 24 people (4 key informants, 14 main informants, and 6 supporting informants). Informants identity is kept confidential in this study. Each informant who is directly interviewed by the researcher has an identity code that is known only to the researcher as well as the interviewer. This identity code is used in data processing and report writing. Informed consent was preceded by providing sufficient information regarding this research. If prospective informants agreed, they were asked to sign an informed consent form as a proof of willingness to be interviewed.

Results and Discussion

This study showed that digital health promotion platform requirement specification must be tailored to the needs of millenial and post-millenial generations. The requirement specifications were classified into 4 domains: functional, technical, interface, and performance requirements.

Functional requirements

These requirements consist of procedures that should be running, menus, features, content, data, information, control mechanism, and various parties involved.

1. Procedures

Regarding the required digital-based health promotion development procedures, there are several aspects that must exist, namely as follows.

a. Basic procedure for navigating digital platform.
b. Digital platform operation guide.
c. Liaison between users and the development team or customer care.
d. Setting and searching.
e. Procedures for assessing user's health status so that a platform can provide direction or service recommendations that most suitable with their health profile.
f. Payment procedures for paid health promotion services as well as payment procedures that can be integrated with BPJS via mobile JKN or other payment platforms.
g. Different procedures, but integrated between open access and closed access digital system.
h. Appropriate evaluation procedure of each digital-based health promotion services, including instruments and indicators to measure efficiency and effectiveness.

2. Menus and features

These requirements are quite diverse, including the following.

a. Virtual health communication, information, and education.
b. Social media functions with chatroom and timeline.
c. Nutrition and physical activity calculator.
d. Digital growth chart or digital *KMS (Kartu Menuju Sehat)*.
e. Digital health calendar.
f. Digital advocacy, such as online petition and suggestion box.
g. Geolocation or GPS navigation.
h. Digital financing for health promotion programs, especially for community empowerment activities. For example, latrine social gathering (*arisan jamban*) by adopting "Arisan Mapan" system initiated by go-pay.
i. Digital collaboration and partnership, particularly the development of crowdfunding into crowd-sourcing.
j. Other supporting menus and features, such as digital guiding for meditation and various healthy activities.

One of the most important things needed to accommodate the entire menus and features is the support of a qualified algorithm system, so that a platform can work automatically to select contents, features or services according to users preference and their health profile.[6] In addition, all features must be designed based on target characteristics, behavior of the digital device used and the issues raised. Furthermore, digital platform must also be supported by the propagation of issues via various social media channels.[7] This is important so that millennial and post-millennial generations are increasingly exposed to digital health promotion efforts.

3. Content

Millennial and post-millennial generations in Surabaya need content that covers the following topics.

a. Clean and healthy living behavior
b. Dengue fever and its prevention
c. Maternal and child health
d. Acute respiratory infection
e. Non-communicable disease and its prevention
f. Infectious disease and its prevention
g. Mental health
h. Adolescent health, especially adolescent reproductive health and nutrition
i. National health insurance

These various topics can be narrowed down based on data from Surabaya City Health Office regarding the top 10 health problems in this city.[8] The health problem is acute respiratory infection which includes as respiratory disease in the top rank of most common health problems (24.93%). Furthermore, dengue fever, which is included in the classification of infectious disease in the 4th rank of most common health problems in Surabaya (6.29%). In addition, non-communicable diseases and nutrition problems are in the 8th rank of most common health problems (2.575) in 2018. Meanwhile, many other health problems are also related to clean and healthy living behavior aspects, so these aspects should also be prioritized.

4. Data and information

One of the most important aspects needed to develop digital health promotion is basic data input by users

in the process of registration. In addition, integration and synchronization with available data banks is also required. For example, data related to population or other big data such as medical records and so on, according to the objectives and focus of each digital health promotion platform. Other data and information that also needed is the latest data regarding the description and magnitude of health problems in Surabaya, so that millennial and post-millennial generations increasingly understand the urgency of health promotion. Valid and reliable data and information are also needed to add value for the digital health promotion system that is developed to become a smarter system. This means that the digital platform being developed should be able to record and store data in a much tidier manner, so it can interpret the data into valuable information that can be used further. Digital technology is not only an enabler, but also a driver to improve public health.

5. Parties involved

The development of digital-based health promotion for millennial and post-millennial generations in Surabaya certainly requires the involvement of various parties. Internal parties involved include developers, UI/UX designers, platform managers, content creators with copywriting skills, graphic designers, data analysts, etc. While the external parties involved are stakeholders in the government (health office, population and civil registration office, communication and informatics office, etc), private sector, telecommunications providers, joint ventures, NGOs, communities, health workers, health professional organizations, religious leaders, community leaders include youth organizations, cadres, influencers, and also users. Collaboration and synergy among these various parties will determine the success of digital-based health promotion efforts.[9]

6. Control mechanism

Control mechanism needed in developing digital-based health promotion include the following.

a. In the internal system, it is necessary to have some parties that responsible for quality control in each division within their respective scope, such as in the content, media, program development, etc.

b. In a larger scope, it is necessary to have a team of experts known as BoA (Board of Advisors) who are responsible for conducting audits and quality control at a higher level.

c. Control mechanism through the flow built into the digital system. For example, in the consultation feature, experts or health workers who become consultants must be certified according to certain standards, go through a selection and curation process, then their account must be verified to be displayed on the homepage of a platform along with its profile. In addition, you can display ratings, reviews, and classifications of consultants based on their expertise.

d. From external elements, stakeholders and government authorities can be involved at a higher level, such as related to regulation and protection from macro threats like hackers and so on.

e. If social media is used as a tools, then each platform must have implemented a control mechanism in its digital system, such as Instagram and Twitter with a blue check mark indicating an account is verified.

f. Another form of control mechanism that actively carried out by the community as users. For example, to ensure the authenticity of an account, there are several parameters such as the number of followers, interactions, and comments on each post.

Technical requirements

Technical requirements include programs, networks, size, and types of digital devices.

1. Programs

Programs are closely related with menus or features that are functional requirements. This requirement is also related to 5 means of health promotion action based on the Ottawa Charter.[10]

a. Healthy public policy, can be realized through digital advocacy programs with kind of services such as digital suggestion boxes, online petitions, and digital-based recording and reporting.

b. Create partnership and supportive environment, can be realized through digital partnership programs such as implementing crowdsourcing practices.

c. Reorient health services, can be realized through digital community empowerment programs. For example, the maternal and child health programs in the form of online classes for pregnant women, mothers of babies or toddlers, pre-wedding classes

for couples who are getting married, digital bootcamp for capacity building, refreshing, and upgrading health cadres, etc.

d. Increase individual skills, can be realized in various programs such as virtual classes with serial thematic health education videos, promotive-preventive online consultations, digital public service advertisements, or other forms of health education, both audio, visual and audio-visual.

e. Strenghthen community action, can be realized through programs such as digital mentoring and features that are able to guide people in practicing a healthy lifestyle. For example, self-tracking feature, a healthy diet and meditation guide, etc.

2. Networks

Includes the availability of digital infrastructure which also determines the quality of internet signals, wifi networks and other digital systems. It is also necessary to increase the quantity and improve the quality of wifi hotspots or wifi corners at various points, especially public facilities in Surabaya. Moreover, millennial and post-millennial generations also need features that can be accessed both online and offline as well as the realization of quota-free services that can be achieved through collaboration with several internet providers.

As practiced today by several digital health platforms such as Halodoc and Alodokter in collaboration with Telkomsel. These digital applications make it easier for users to enjoy remote health services with medical and health professionals. Users also get free access up to 500 MB which can be used to access services at Halodoc and Alodokter such as chatbots, chat or consultation with doctors, purchasing medicines and medical devices, scheduling appointments with doctors, health education, and others.[11]

3. Types of digital devices

The type of hardware that most widely used by millennial and post-millennial generations in Surabaya to access digital health promotion services is smartphones. In addition, they also use laptops or PCs, although its use is not as often as smartphones. Based on data released by APJII (2017), 59.31% of users in urban areas such Surabaya use smartphones or tablets, 0.65% of users use laptops or PCs only, 38.31% use both smartphones and laptops, while the rest use other digital devices.[4] Therefore, the digital-based health promotion platform being developed must be appropriate and easily accessible at least on both types of digital devices (smartphones and laptops).

Meanwhile, the type of software that most widely used by millennial and post-millennial generations in Surabaya is digital applications. Other types of software or platforms that they also use a lot, namely specific applications in the form of social media. APJII survey results in 2018 showed that the most frequently visited social media are Facebook (50.7%); Instagram (17.8%); youtube (15.1%), followed by other social media such as twitter, linkedin, and so on.[12] Furthermore, they also mention the website or web-blog as a type of software or platform that still needed, although not as much as other platforms. Thus, digital health promotion platform for millennial and post-millennial generations in Surabaya should be able to be applied in all three types of software or platforms (digital application, social media, and website) so they have various alternative choices according to their various needs. One of the platforms can be the main channel, while the other platforms can be the support channel. Its existence can be a complete unit that complements each other.

Delivering messages via communication strategies with various digital platforms will expand the impact while strengthening the message conveyed. Today, various messages can very easily translated into digital media. There are few effectiveness studies to assess this, but various anecdotal reports show encouraging results. The platforms that successfully engage many users to participate and visit their channels have proven to be more dynamic, flexible, frequently changing contents and approaches to keep it interesting. Developing a community of active users is one way to keep contents updated and interactions alive.[13]

4. Size

Size of digital health promotion platform is also a consideration for some millennial and post-millennial generations to use a certain platform. Those who consider the size, have their own tolerance limits regarding the size of a platform that is still acceptable, which is an average of 50 MB and a maximum of 3 GB when first downloaded. Meanwhile, those who do not consider the size, prioritize the function and urgency considerations

as long as the device memory is sufficient.

Interface requirements

These requirements consist of a visual display related to the user interface and operating system.

1. User Interface (UI)

Millennial and post-millennial generations in Surabaya need user interface with the following characteristics.

a. Elegant visual design, capable of attracting attention, not stiff, causing a sense of pleasure and comfort.

b. User-friendly, uncomplicated, and doesn't cause confusion even when using it for the first time.

c. Visual design that fits with the type of device.

d. Features, menus, contents are well organized, neat and systematic.

e. Proper visualization of the algorithmic system on a platform, for example with certain layouts or highlights so that users can understand it more easily.

The UI of a digital platform can also influence the success of digital health interventions in addition to functional, technical and performance aspects. Millennial and post-millennial generations as users have various characteristics and different roles, so the UI must also be able to adjust. Likewise the needs, experiences, and perceptions of every users that very dynamic should also be considered. User responses and behavior need to be continuously tracked to support an adequate level of personalization, thus ensuring user adoption and engagement. This is needed to adjust the interaction experience between humans and digital devices related to visual aspects such as font size, themes, illustrations, and others.[14] These data are collected to analyze the user's ability to understand and use the system, for example in the aspect of content readability so that the User Interface (UI) component can support the User Experience (UX).

2. Operation System (OS)

Other aspects relate to the operating system used for at least the following 2 types of hardware.

a. Smartphone

Android is the most needed OS for millennial and post-millennial generations in Surabaya today. On the other hand, some of them also use iOS even though the number of users is not as many as android users. According to Statcounter (2020) data, the android smartphone leads market with 93.06% active users in Indonesia, followed by iOS smartphone which has 6.47% active users in last year. While the rest are recorded using other operating systems such as BlackBerry OS, Tizen, Series 40, and so on.[15] Therefore, the two operating systems (both android and iOS) should be accommodated by the digital health promotion platform that will be developed. However if the budget is limited, platform development in the android version should be prioritized because the segmentation can be wider than iOS.

b. Laptop atau PC

Millennial and post-millennial generations in Surabaya more require Microsoft Windows operating system (often called Windows). But there are also mention that the MacOS operating system (often called Mac) is also required, although the number of users is not as much as windows. As stated in Statcounter (2020) where Windows leads the market for desktop-based operating systems (laptop or PC) with 86.09% active users in Indonesia, followed by Mac or OS X with 9.54% active users in last year. While the rest are recorded using other operating systems such as Linux, Chrome OS, FreeBSD, and so on.[15] Therefore, a digital-based health promotion platform should be developed in 2 versions of the operating system for laptops and PCs, both Windows and Mac.

Performance requirements

The most required performance characteristics for millenial and post-millenial generations in the development of digital health promotion platform in Surabaya are related to efficiency and effectiveness. The platform that will be developed must be more efficient, effective, and capable to perform better than various existing platforms. Regarding the efficiency aspect, the informants hope that with the use of digital technology, more information and health promotion efforts can be done at one time. The coverage of issues is also more varied and more updated. In addition, digital technology

must be able to reach a much wider range of targets, even across geographic and administrative boundaries. Digital technology must also help accelerate health promotion efforts, so that it becomes more quickly achieve the desired objectives.

Various health promotion tasks and works are also expected to be done easier and cheaper because the use of digital technology. All the energy (including money, material, time, and other resources) that has been used up to do one thing, can be saved and then diverted to other things that are more important and substantive. The existence of digital health promotion platform must also be able to relieve the burden on users, because its application becomes more practical and can be accessed anywhere and anytime, without having to come and gather at a certain place which often spend more time. Moreover, digital health promotion platform not only relieve the burden on users, but also hoped that the workload of health workers will be reduced if many things can be carried out more efficiently, including health promotion services.

Meanwhile, related to the aspect of effectiveness, digital-based health promotion is expected to be more effective in reaching millennial and post-millennial generations whose level of interaction with technology is very high. At least, digital health promotion must be effective in increasing the knowledge, understanding and awareness of its users. Although in the context of behavioral changes, would still need a long process and many factors are involved. The available features must be sufficiently effective to solve problems and capable to answer users need. The content presented must also be more on practical rather than theoretical aspects.

The following are more specific details about the specification of performance requirements to develop digital health promotion platform for millennial and post-millennial generations in Surabaya.

1. Speed and accuracy

Millennial and post-millennial generations need a platform that can perform various functions more quickly and precisely. Opening up an opportunity that must be achieved in digital era with the need of speed and accuracy greatly determines the success of health promotion strategies. Digital technology also needs to support quick and precise decision-making processes.[16]

2. Security

Millennial and post-millennial generations also pay more attention to this aspect. This is reflected in the results of APJII survey (2017) which showed that 65.98% of internet users in Indonesia have the awareness that data can be retrieved. Therefore, 61.38% of users said it is important to maintain data confidentiality. In fact, 58.52% of users also feel it is important to install anti-virus as a form of protection to secure data and operation system.[4] The rapid advancement of digital technology requires sophisticated protection to ensure the security of users data and operating system in order to be protected from various threats such as hackers, viruses, and bugs that interfere system work.

Data security issues are also related to the norms and ethics that people believe as users. However, digital platform developers do not always take appropriate steps to protect users' personal data. In fact, personal data can include things that are very sensitive, such as data on reproductive function, sexual practices and partners that cannot be leaked carelessly.[17] Digital platform developers must pay close attention to this aspect, so that health promotion efforts do not violate ethical aspects and norms in society. Furthermore, the government also needs to establish clear and firm regulations to protect the data security

Good and adequate data security system can be built through privacy and policy system in the developed digital health promotion platform. It should also be separated between an open access platform for users with a closed access platform, which is specifically for administrators or managers, such as special account for health workers in health care facilities. One of the ways to do this is to ensure the confidentiality of users data, especially big data in the system that has been built. Moreover, human resources support to tighten the security protection of a platform is also very necessary. All parties involved must be able to hold back and if necessary also bound by regulation to ensure data security. For example, consultants is not allowed to divulge users data who consulted with them. If data security is guaranteed, then users will also feel safe and comfortable using digital health promotion platform.

3. Data center, server, and hosting capacity

Millennial and post-millennial generations need a health promotion platform with large capacity of data center, server, and hosting. The data center is a place to store servers and keep them working optimally. Server is a system that provides certain services in the computer network. While hosting is a place to store data in the digital system.[18] Large capacity is needed so that a platform can contain many features, menus, content, and various information without any significant obstacles even though it is accessed simultaneously by many users.

4. Frequency and reach

If the frequency of views is higher, the reach of a platform will be wider. It means that more users are accessing a platform.[19] This is an of the most important aspect to encourage millenial and post-millenial generations who can access the digital-based health promotion platform easily. Increasing frequency and coverage requires adequate digital management and systems, especially in terms of automation. It has been attempted since the beginning of digital technology development in health sector.[20]

5. Easiness and cosiness

This performance characteristics are also urgently needed for millennial and post-millennial generations to develop digital-based health promotion platform in Surabaya. The digital platform ease of use will have a significant impact on user convenience. Apart from developing a user-friendly system to make it easier for users, platform developers and managers also need to minimize aspects that can interfere user convenience, such as commercial advertisements that appear too often and other disturbing aspects because Indonesia is one of the countries with the highest adblocker installers in the world, reaching up to 65%. It means that more than half of Indonesians who access internet do not want to be bothered by advertisements.[21]

Conclusions

The development of digital-based health promotion for millennial and post-millennial generations in Surabaya should meet all requirements specification, both functional, technical, interface, and performance. Some mistakes that often occur in health promotion efforts are that the goals to be achieved is not chosen by the targets. The available resources are considered more than the target needs, so that the efforts made are not right on target. It has resulted in many health promotion efforts, both conventional and digital, hard to be long lasting and sustainable. In addition, the targets are not made to be aware in advance of the problems that will be overcome through health promotion efforts, so they feel no interest in getting involved. Therefore, all digital health promotion efforts must be developed based on target needs along with public awareness, then adjusted to the available resources. If the targets have been aware and these requirements can be met, they will be easier to accept and use the digital health promotion platform.

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Prevalence of *Eimeria* Species in Sheep and Goat in Tikrit City, Iraq

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Abstract

This study aimed to detect *Eimeria* sp. in sheep and goat, diagnose its species, and know its prevalence in Tikrit city and its surroundings during the wet and dry months. The results showed that the total rate of infection in sheep was 56.25%, while in goat was 69.05%, and it was also found that the highest rate of infection was in dry months 72.08% in sheep and 76%in goat, while the rate of infection in wet months was 32.5% in sheep, and 50% in goat. Eleven species of *Eimeria* were diagnosed in sheep and twelve species in goat, and the species *E. sundarbanens* was recorded for the first time in Iraq after made a survey among studies conducted in various governorates of Iraq, and depending on the phenotypic characteristics which were identical data in a specialized references . The mixed patterns of infection with different species of *Eimeria* showed the highest in sheep and goat 57.75%, 51.79% respectively.

Key word: *Epidemiological study, Eimeria, Sheep, Goat, Iraq .*

Introduction

Sheep and goat are infected with many species of parasites that infect the stomach and intestine [(1)] . The parasitic protozoans that infect sheep and goats have a negative impact on the productivity of infected animals in terms of affecting the weight, milk and health status of the animal in addition to the costs of treatments and their consumption of forage materials compared to healthy animals [(2)] . Despite the progress that has occurred in the methods of controlling and treating animals, sheep and goat are still exposed to many parasitic infections that may cause a serious health issues of animals [)3)], [(4)]. *Eimeria* is an important parasite that affects young sheep and goat, causing coccidiosis, which is one of the protozoan parasites that develop and spread in the small and large intestine of animals [(5)] . Because the disease has an economic impact, so many studies reported around the world. Toulah [(6)] indicated that the rate of infection with *Eimeria* parasite in Saudi Arabia was 41%, while In Iran, Tavassoli and Khoshvaghti [(7)] recorded (80.48%) in sheep. The researcher Mohamaden [(8)] was show the rate of infection with *Eimeria* parasite in Egypt was in sheep and goat 57.70%, 60% respectively.

In Iraq, Meyasar [(9)] in Mosul supervised the total rate of infection of *Eimeria* species was (63.6%) in sheep, while albayati [(10)] in Dohuk recorded the total rate of infection was 37.67%. karwan [(11)] has indicated the total infection rate in goat with *Eimeria* species was 96.9%.

This study was conducted to diagnose and find out the prevalence of *Eimeria* species in Tikrit city and in sheep and goat and its relation with some epidemiological factors.

Materials and Methods

Samples collection:

Fecal samples were collected from sheep (400 samples) and from goat (200 samples) directly using disposable gloves, for both sexes, and at the age between six months to four years. Information about the animals was recorded in questionnaire form, then the samples were transferred to the Postgraduate Laboratory / College of Science and then preserved in refrigerated at 4c until use.

Fecal examination:

Floating method was used to detect oocytes of *Eimeria* sp. [12] , and examined microscopically at (40X and 100X).

Statistical analysis

All data were analyzed according to chi-square model in order to determine the significant differences for the percentage of the comparative totals and calculate the rate of standard error (SE(and by using a program SPSS and at a significant level P > 0.05 and P > 0.05 according to (13).

Results

As show in table (1), the total rate of infection with *Eimeria* sp. was 56.25%, 69.5% in sheep and goat respectively.

Table (1) The total rate of infection with in *Eimeria* species in sheep and goat

Study groups	number of examined samples	number of infected samples	Percentage
Sheep	400	225	%56.25
Goat	200	139	69.5%

The results showed that the highest rate of infection in sheep was 72.08% in the dry months, while the rate of infection in the wet months was 32.5% ,table (2).

Table (2) The relationship between *Eimeria* infection and period of study in sheep

Sample collection period	number of examined samples	number of infected samples	Percentage
The wet months	160	52	%32.5 a
The dry months	240	173	% 72.08 b
Total	400	225	% 56.25

*** Vertically different letters indicate the presence of a significant difference and at the level of significance p<0.01.**

While in goat, the results showed that rate of infection in the dry months was 76% and in the wet months 50%. Tabel (3)

Table (3) The relationship between *Eimeria* infection and collection period in goat.

Sample collection period	number of examined samples	number of infected samples	Percentage
The wet months	50	25	50 % n-s
The dry months	150	114	76% n-s
Total	200	139	69.5 %

n-s There were no significant differences between the study months in goat.

Table (4) showed that there was a significant differences p<0.05 between male and female in sheep 65.42% and 45.69% respectively. The young animals under 2 years old were highly infected with *Eimeria* sp.

Table (4) The relationship between *Eimeria* infection and age and sex factors in sheep.

Sex Age	Male			Female		
	number of examined samples	number of infected samples	Percentage	number of examined samples	number of infected samples	Percentage
Less than 1 year	65	62	95.38 c	33	30	90.9c
1-2 years	72	71	98.61 c	45	42	93.33 c
2-3 years	35	7	20b	36	8	22.22 b
3-4 years	42	0	%0 a	72	5	6.94a
Total	214	140	65.42 B	186	85	45.69A

* The difference in small letters vertically indicate to a significant and level difference

P< 0. 05

* The different in capital letters horizontally indicate a significant and level difference

P< 0. 05

In goat, there was also a significant differences p<0.05 between male and female (68.04%,70.87%)respectively , and also found that the animals under 2 years old were completely (100%) infected with *Eimeria* sp.,

According to the patterns of infection it was found that the mixed patterns (3 species and more) showed high rate of infection in both sheep and goat(57.58% and 51.79%) respectively, table (5).

Table (5) Patterns of infection with *Eimeria* species in sheep and goat

Animals Patterns of infection	Sheep		Goat	
	No. of infected animals	%	No. of infected animals	%
Single infection	47	20.87a	26	18.70a
Bilateral infection	48	21.33b	41	29.49b
Mixed infection	130	57.78c	72	51.79c
Total	225	100%	139	99.98

* The difference in small letters vertically indicate to a significant and level difference P< 0. 05 .

This study diagnosed eleven species of *Eimeria* in sheep. The species *E. sundarbanens* was diagnosed for the first time in Iraq after make a survey of studies conducted on the diagnosed species of the *Eimeria* parasite in the various governorates of Iraq, and its phenotypic characteristics were similar to what is proven in the approved sources, figure (1). Twelve species of this parasite were also diagnosed in goat.

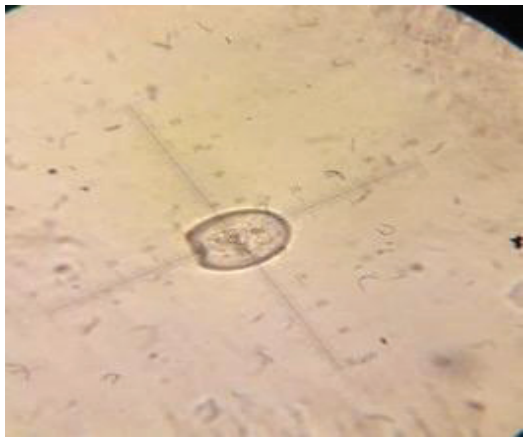

Figure (1) : Oocyst of *E. Sundarbanens* (100X)

Discussion

The total rate of infection with *Eimeria* sp. was 56.25%, 69.5% in sheep and goat respectively. These results agreed with Ali in Mosul [14], where they recorded an infection rate about 60.46%, and also consistent with what was recorded by Maisar, *et al*. in Mosul [9], where the total rate of *Eimeria* was 63.6% in sheep as well as agreed with Mohamaden in Egypt [8] 57.70% in sheep, while in Ethiopia Ayana recorded the rate of infection was 66.8% [15] .While in India, Verma [16] registered 71.45% in goat.

These results disagreed with Abdel-Wahab [17] in Iraq 70.83% in sheep. while in Sudan, Shmaon found that an infection rate in sheep was 86%. [18], and in Turkey the rate of infection was 100% in sheep [19] . In goat, Al-Amiri, , recorded the rate 91.86% [20] , and Ayez in Diwaniyah found that the rate of infection was 88.3% in the local goats [21] , while Karawan found that the total rate in goat reached 96.9% [22] . This variation due to the different in the numbers of animals examined, health statue, environmental and administrative conditions, in addition to the different time period for the examinations [22] .

Through the study, it was found that mixed patterns of infection with different species of *Eimeria* formed the highest rate of infection, reaching 51.79%. This consistent with studies conducted in Iraq, in Kirkuk [3-4] ,and also agrees with Maisar [9] , where the highest incidence of mixed type was recorded, reaching (62.5%) in Mosul city in a study conducted on sheep. Animals were infected with more than one type of infection, and this is an obvious matter because the pastures are often contaminated with more than one type and when animals graze on those pastures they pick up egg bags without discrimination [22] .

Eleven species of the *Eimeria* have been diagnosed in sheep as shown in the results of the study. *E. sundarbanens* was recorded for the first time in Iraq and this species is similar to the species isolated by Kheirandish in West Bengal in India [23] . The results of our study were similar to what was recorded by Abdul-Wahab in Baghdad [17] ; Arslan in Mosul [24] , and Khalaf *et al*, [25] in Baghdad.

Twelve species of the *Eimeria* have been diagnosed in goat. This result differs from what was recorded in the Governorate of Dohuk, as Al-Bayati recorded six species of the *Eimeria* in goat [10], and Karawan, recorded five species of the *Eimeria* in goat in Diwaniyah [22] .

Female sheep and goats are more receptive to infection than males, and this is in agreement with Khodakaram , the reason is that female animals are raised for a longer period than males in order to reproduce and benefit from milk [26] .

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Pathophysiology of Sickle Cell Anemia : Review Article

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Abstract

About 5–7% of the world population have an unhealthy hemoglobin (Hb) gene . The most common form of hemoglobinopathy globally is sickle cell disease. Sickle cell disorder (SCD) is the major predominant innate clutter of Hb amalgamation stamped through a change within the β globin gene, which result within the substitution of glutamate corrosive with valine at the 6th codon and union of Hb S a which is a hemoglobin, beneath hypoxic states, coming about within the abnormality of RBCs (Red Blood Corpuscles). The lysis of erythrocytes result in raise in extracellular hemoglobin, hence hoisting liking and official to open nitric oxide or antecedents of nitric oxide in this manner diminishing its levels and assist partaking to vasoconstriction . Sickle ruddy blood cells since of their hardness to stream by means of the micro-circulation, results in visit vaso-occlusive scenes, destitute micro-vascular blood stream, ischemic damage, and myocardial localized necrosis . The clinical highlights of sickle cell anemia are constant hemolytic weakness, an elevated vulnerability to diseases, repetitive difficult Vaso-Occlusive Emergency (VOC) and a brought down life hope .

Keywords : *sickle cell anemia, pathophysiology, hemolysis*

Introduction

Sickle cell anemia (SCA) is the major inexhaustible genetic clutter of hemoglobin union checked by a change within the βeta globin genome, which result within the substitution of glutamic corrosive with valine at the 6th codon and union of Hb S a hemoglobin which, beneath hypoxic states, gels coming about within the abnormality of the re blood corpuscles (red Blood corpuscles). The parchedness of the ruddy blood cells raises the level of the intracellular Hb with an raise within the inner consistency and thus a diminish within the RBC distortion [(1-2)] .

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Sickled RBCs and the fragile membranes of them are accessible to auto-oxidative stress because of endogenous activate oxygen species [(3)]. In vitro investigates on the presentation of sickle cells to oxidation distress with diverse oxidants (t-butylhydroperoxide, hydrazine, diamide, hydrogen peroxide) have displayed peroxidation of lipid and complete Hb de-naturation which is more articulated in sickled RBCs than typical one . In expansion, in sickled erythrocyte disease a lowered ability to neutralize the oxidation was clarified, and the impacts of cancer prevention agents, free radicals foragers, additionally press chelators on sickle red blood cells and their films have been assessed [(4)] .

Sickle cell disease (SCD) forms numerous basic and useful variations from the norm within the kidney, counting glomerular abnormalities. Albuminuria is the foremost plenteous appearance of glomerular harm, with a predominance between (26- 68%) in grown-up clients. The pathophysiology of albuminuria in SCD is likely multifactorial, with participations from hyperfiltration,

decreased nitric oxide (NO) bioavailability, glomerular hypertension, ischemic reperfusion harm, oxidative stretch, and endothelial clutter [5] .

Sickled RBCS since of their hardness to stream via the micro-circulation, results in visit vasoocclusive scenes, destitute micro-vascular blood stream, ischemic harm, and myocardial dead tissue [6] . In expansion, the brought down ruddy blood cells deformability causes an raised cell delicacy coming about in advanced haemolysis and a major part of haemolysis & oxidative push within the advancement of vessels brokenness have been displayed [7] . A lessening in ruddy blood cells deformability related with a hoist in oxidation stretch has been seen in numerous obsessive states such as diabetes and hypertension [8-9] .

Sickle cell disorder is brought about from a change within the Hb genes causing a valine for glutamate substitution at the sixth position of the βeta-globin chains of the hemoglobin atom coming about in a auxiliary variation of ordinary grown-up HbA, specifically sickle HbS, the term 'sickle' was clarified by some researcher who was in 1910 the primary to present the changes morphology that happen to the influenced RBCs, when sickle hemoglobin, which can be pressed by millions interior RBCs, makes polymerization on the off chance that exposed to low oxygenation [10] .

It is a result of hereditary of an alteration within the βeta-globin quality, a point transformation to one-base pair (i.e. GAG-GTG) comes about within the replacement of the amino acids glutamate (water liking) to Valine (water unliking) within the 6th location of the β-chain of hemoglobin called to as hemoglobin S (HbS) [11] .

Classification of sickle cell anaemia

The gebetics of homozygous HbS separated from alluded to as sickled cell disease (SCD) is a foremost transcendent shape of SCA, the extent varies with respect to the nation of beginning . Homozygosity for the HbS allele result within the plenteous sort of sickle cell illness; sickles cell disaese (SCD), other plenteous variations of the at slightest fourteen other genotypes, are the abnormal Hemoglobin SC clutter and Hemoglobin S/β- thalassemia [12] .

The another most inexhaustible shape of SCA is the coinheritance of Hemoglobin S and Hemoglobin C—called as Hemoglobin SC, usually more common in West Africa, uncommonly and the coast nations counting some countries, the co-inheritance with βeta thalassaemic anemia comes about in a sickle βeta thalassaemic anemia (hemoglobin S/βo or hemoglobin S/β+), depending on the hereditary injury on the thalassaemia gene, the introduction might become basic or similarly as hardas SCD (hemoglobin S/ hemoglobin S) [13] .

These with hemoglobin S/βo-thalassaemia have the most hardcourse of clutter like SS clients, whereas sibling with hemoglobin S/β+-thalassaemia relying on the βeta-globin transformation is related with different phenotypic from straightforward to difficult phenotypic SCD [14] .

Fetal hemoglobin (HbF) smother the polymerization of sickle hemoglobin (HbS), numerous of the results of sickle cell frailty (homozygosity for HBB, glu6val), like intense chest disorder , osteonecrosis, and difficult scene, are related with the level of HbF, HbF is conversely related with passing, examiners have diligently looked for pharmacological implies of hoisting HbF arrangement Hydroxyurea (HU), a ribonucleotide reductase silencer, could be a medicate that raises HbF level in clients with sickle cell weakness [15] .

Pathophysiology of sickle cell anaemia

Red blood corpuscles (RBC) that have hemoglobin S or hemoglobin S in together with other irregular βeta genes, when uncovered to deoxygenated status experience polymerization and gotten to be unbending. The unbending ruddy blood cells are obligated to hemolysis, as a result of hoisted thickness might influence blood stream and endothelial vessel divider keenness [16] .

The thick inflexible ruddy blood cells leads to vasoocclusion, ischaemia, dead tissue in addition hemolysis, the result of hemolysis could be a complicated group of occasions counting nitric oxide (NO) utilization hemolysis connected NO deregulation and endothelium brokenness that leads to results such as pneumonic hypertension , ulceration of leg, priapism and stroke, and [2] .

During deoxygenation ordinary hemoglobin re-organize itself into a distinctive con-synthesis, empowering official with carbon dioxide (CO2) compounds which returns to ordinary when launched out . In differentiate, hemoglobin S act by polymerising into inflexible non-soluble tactoids and strands, they are gelatin -like compounds h hemvingoglobin crystal, through intense sickle, intra-vascular hemolysis comes about in free hemoglobin within serum, whereas ruddy blood cells having Na+ and Ca2+ with comparing misfortune of potassium . The lift within the level of Ca2+ result in clutter within the calcium pump, Besides, hypoxia moreover smother the arrangement of NO, subsequently leading to the grip of sickled corpuscles to the vascular endothelial tissue [17] .

The hemolysis of RBCs result in hoist in extra-cellular hemoglobin, hence lifting fondness and authoritative to accessible NO or antecedents of NO in this manner lessening its levels and assist partaking to vasoconstriction . Not at all like typical red blood cells with half-life of around 120 days, sickle ruddy blood cells (red blood cells) may survive fair 10–20 days since of hoisted hemolysis [18] . A single quality change (GAG - GTG and CTC - CAC) comes about in a flawed hemoglobin that when uncovered to de-oxygenation polymerizes, may result within the blend of sickle corpuscles. Vasoocclusion can then happen .The clutter is additionally stamped by anomalous cement charateristics of sickle cells, fringe blood mono-nuclear cells and platelets follow to the sickled erythrocytes [19] .

SCD may be a qualitative haemoglobinopathy coming about from a auxiliary alter within the arrangement of the amino acids (AA) on the β chain of globin of the hemoglobin particle because of a point transformation. Sickling transformation results in a singlular base alter from the adenine to thymine upon the seventeenth nucleotide of the β globin chain quality . That perpetually interprets into replacement of (valine) for (glutamate) on the sixth AA of the beta globin chains. The anomalous natural chemistry of this mutated hemoglobin actuates polymerizing of hemoglobin S particles inside the red corpuscles, so that named sickling. Upon sickle hemoglobin, the glutamic acid protein atom, that is water liking ,polar ,and contrarily charged, is supplanted by a less polar, hydrophobic, impartial AA, (valine). Beneath de-oxy states, the unusual (valine) buildup results in intra-erythrocytic hydrophobic response of sickle hemoglobin tetramers, driving to their anticipation and polymer arrangement, therefore named (gelation) . Inevitably, all the cytosol hemoglobin atoms accelerate to 7 (1+6 internal and external respectively) twofold act with cross-linking that are named (tactoids) . On re-oxygenation, unsickling happens and the red corpuscles accept its ordinary form . Be that as it may, rehashed sickle and unsickle of the red corpuscles harms the red corpuscles cell film, because of herniation of sickled hemoglobin polymer via the (cytoskeleton), hence distorting the red corpuscles for all time sickled . These show up as an irreversible sickle cell (ISC) on fringe blood cellular state promptly [16-17] .

Red corpuscles energy sickling is profoundly heterogeneously . A few factors may be recorded to influence the pace and level of sickling of the red corpuscles. Intra-cellular lack of hydration of sickled red corpuscles increments average corpuscles hemoglobin level . Higher average corpuscles hemoglobin level favors sickling . As that, exceptionally tall hemoglobin S level of almost 80% to 90 % observed within the homozygous disorder is related with a more awful disorder whereas the nearness of α thalassemia (1-2 quality cancellations) improves infection. Other variables are the nearness of other connection non-sickle hemoglobin. [15] .

Higher extent of Hb F is related with gentle illness. When display, tall levels of Hb F are consistently scattered inside the red corpuscles and they impede (sickling) prepare. Hence, co-inheritance of sickled hemoglobin with innate tirelessness of the fetal hemoglobin is related with gentle disorder . Additionally, this benefit is emphatically used via medical utilize of fetal hemoglobin actuating medicate like hydroxyl-urea . Vessel beds which may have naturally drowsy venous outpouring like spleen , bone marrow, and/or aroused tissues are at high chance of infarcted occasions because of delayed micro-vascular travel time . At whatever point and wherever microvascular travel time gets to be more than delay time of sickling . The sickling and vessel closing gotten to be inescapable. Intra-cellular pH is the another critical variable . Acidosis with the hemoglobin atoms allow their O2 more promptly and/or sickling happens very promptly [20] .

Rehashed sickling of the red corpuscles actuates cell damages that have been appeared to enact film particle canals like the of pathway Gardos (Ca+2 gated K+ chan-nels) , K+C+2L co-transporter . There is convergence of Ca+2 particles and flux of K+ and H2O, consequently intra-cellular drying out. Tall intra-cellular Ca+2 levels incite action of proteolysis chemicals like phospholipase and protease resulting in the assimilation of layer phospholipid and protein , separately. Along these lines, there's irritation of film fats with exterior fats like phosphatidyl serine and/or ethanolamine that are ordinarily found within internal flyers of film fat bi-layer [21] .

The differing medical heterogenecity of SCD is associated to the second primary pathogenic forms: inveterate hemolysis and tall viscosity / vascular impediment. Infarcted occasions in SCD result from erythrostasis resulted from inflexible sickle corpuscles in different vessles beds particularly organ with drowsy blood stream like the bone marrow and spleen and. Blood capillary are approximately two-three micron in distance across. Sickled corpuscles due to misfortune of f legibility are incapable to travel the micro-vasculature, consequently vascular impediment. Aparte from these unthinking forms, sickled corpuscles are too appeared to show expanded adhesion to vessel endothelium, WBCs, and platelets . Sickled reticulocytes may be indeed very cement to the endothelial than sickled di-scocytes . Atomic interactions among the red corpuscles and the vessel endothelial incorporate (CD36) and thrombo-spondin, (VLA4) and (VCAM-1), separately [22] .

Long winded microvascular impediment in sickle cell malady indeed in unfaltering state comes about in ischemic-reperfusion damage that puts the arrange for the expanded provocative 'tone', in this way noteworthy heights in add up to leucocyte checks, platelet checks, positive plasma intense stage reactants. Indeed in unfaltering state , Sickles cells disease could be a inveterate fiery condition; the orderly aggravation initiated oxidative stretch encourage con-tributes to dynamic tissue harm . The WBCs in SCA expresses more levels of (L-selectin) additionally has invigorated adhesion . Expanded adhesion coupled along with (phosphatidyl serine) presentation on the red corpuscles surfaces lets SCA a pro-coagulant and a hyper-coagulable coditions [21-22] .

RBCs life expectancy in SCA midpoints approximately 20 days in differentiate to around 120 (days) in ordinary conditions . Hemolysis in sickled corpuscles disorder is extra-vascular and intra-vascular. Unusual form of the ISC makes an abnormal rheology, related to increased clearing by the reticulo-endothelial framework. Since of the expanded delicacy and/ or diminished (deformability), a few red corpuscles experience intra-vascular hemolysis. Tall blood hemoglobin concentration is related to decreased hapto-globin concentrations and tall concentrations of lactate de-hydrogenase (LDH), Alanine Aspartate Transferase and arginase-1 . Blood hemoglobin is an ardent forager of NO . Tall blood hemoglobin concentrations coming about from incessant hemolysis diminish nitric oxide bio-availability. Ordinarily, nitric oxide unwinds the endo-thelium and keeps up vasodilation as vessel tone [23] .

Decreased circulating concentrations of , nitric oxide engender vaso-spasm that is watched indeed in an expansive vessel in SCA . Including to this can be dys-function of endo-thelial NO synthetizing enzyme . Typically a fundamental premise of vasculo-pathic consequences like cerebro-vascular disorders, pneumonic elevation in blood pressure, and incessant leg ulcerations . The enzyme (Arginase 1) is ordinarily included in formation of urea (NH3) in the excretion of proteins . Quickened hemolysis of red corpuscles leads to higher levels of arginase 1. Exactly, more ornithine is created, assist exhausting plasma arginine lev-els. Abundance ornithine is channeled to interchange pathways which create abundance prolenes and polyamines. These byproducts advance endothelial sdecreasedth muscle proliferations, encourage narrowing the vascular chamber . Inveterate haemolysis comes about in abundance breakdown of hemoglobin particles and high levels of bilirubin, which is related with arrangement of bilirubin color stones within the irritate bladder (cholelithiasis) [24] .

A few specialists have endeavored to classify SCA clients to clinical sub-phenotypes according to the over-riding pathologic processes . In Clinical issues, there are few degrees of cover with the two bunches. A few clients encounter a great deal of viscosity-vasoocclusive consequences and act to possess more pattern hematocrit rate . The other encounter higher vasculo-pathic

consequences because of higher seriously hemolysis related with decreased standard hematocrit [25] .

Manifestations of sickle cell anaemia

The clinical manifestation of sickles corpuscles differ. Many people may have simple manifestations but other patients possess hard manifestations and should be set in hospital for therapy . Common manifestations in children are high temprature, enlargement of the upper limbs and lower limbs, chest pain, abdominal pain , pain in limb and joints, nasal bleeding and recurrent infections of upper respiration tract [26] .

Bone pain is the major abundant symptom in the child . Pain may be of high level, either chronic or acute, often from orthopedic problems in the lower limbs and back. Other manifestations are : irritability , weakness, difficult breathing, and high bilirubin. In adolescent or adult, manifestations of child progress into new manifestations like progressive anaemia, leg sores, , delayed puberty, hard joint pain, gum disorder and visual disorders. Pathological causes that result in sickled cell disorder correlated to consequences caused by both haemolysis and vasoocclusion [27] .

Consequences of sickle cell anaemia

SCA is marked by many consequences varying from general acute body pain to essential stroke, lower limbs ulcer disorders and the hazard of pre-mature mortality from multi-organ failure. Due to the influence of Hemoglobin F, medical properties may not start unless the first to the second portion of the initial year of child life that may be constantly changed to the adult hemoglobin [28] .

The clinical features of sickled corpuscles disorder are constant haemolytic anaemia, an elevated sensitivity to inflammations, frequent hard Vaso-Occlusive Crisis and lowered life expectancy [29] .

The genotypic form is the main common of medical hardness in clients with SCA by which clients with sickled cell anaemia (HbSS) and phenotypes compared to HbSβ0-thalassemic are done heavily hurt, then by Hb-SC and Hb-Sβ+-thalassemic patient . Hence , even-though inside the variant genotypic a great range of medical phenotype translation appear among clients, also fetus Hb levels and coinheritance of αlpha-thalassemic are crucial causes f disorder hardness [30] . .

Clients with SCA complain from a great variety of body consequences, involving leg ulcers, osteonecrosis, retinal disorders, renal disorders, , cardiac diseases, and Pulmonary Hypertension (PH) . Organ consequences may be believed to come from either micro vasoocclusion in addition to prolonged haemolysis [30-31] .

The common part was done among SCA clients with a higher vasoocclusive phenotypic (marked by somewhat increased Hb concentrations) may result in most common (VOC), (ACS) and non-vessels osteonecrosis compared with clients with a higher hemolysis phenotype (marked by decreased Hb concentrations , increased lactate dehydrogenase concentrations) that more commonly complain from consequences such as : renal disorders, (PH), stroke, and leg ulcer disorders [32] .

Complete caring program, involving neonates and baby scanning program, monitoring the cerebrovascular stenosis by trans-cranial (Doppler), the preventive intake of antibacterials (resulting in a great drop in infective-diseases correlated SCA morbidity) and a complete vaccinization programs, has contributed enormously to the elevated life expectation of babies with SCA [28] .

Ethical Clearance : Taken from University of Kufa ethical committee

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Molecular identification of epsA and ompA genes for *Acintobacter baumannii* Isolated from Burn Wounds

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Abstract

This study was designed to molecular detection of *epsA* and *ompA* genes for *Acinetobacter baumannii* Isolates that isolated from clinical. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from Ghazi Al Hariri Hospital and Baghdad hospital in Baghdad / Iraq. The results of the current study showed that seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %). From 70 samples inoculated on the Chromagar Acinetobacter, 10 isolate were grown on the medium, *A. baumannii* isolates on the Chromagar appeared as bright red colonies after 24h and incubation at 37 C . The results of *epsA* and *ompA* genes detection clarify that 8 isolate (80%) of *A. baumannii* isolates gave a positive result and carrying *epsA* and *ompA* genes while 2 (20%) of *Acintobacter baumannii* isolates were lack the gene and no cross reactivity could be observed with any of the AS-3 and AS-9 isolates. The identification of *A. baumannii* isolates were depended on culturing these isolates on CHROM agar Acinetobacter, MacConkey, blood, nutrient agar, biochemical tests and API NE 20 test VITEK 2 . Compact were carried out the confirms. Results showed the amplification of *epsA* and *ompA* genes for eight. *A. baumannii* isolates were AS-1, AS-2, AS-4, AS-5, AS-6, AS-7, AS-8 and AS-10. PCR product 531 bp of *ompA* gene and 451 bp of *epsA* gene.

Keywords: *Virulence genes, OmpA, EpsA, Acinetobacter baumannii*

Introduction

Acinetobacter baumannii is the most prevalent infection-causing organism in the hospital environment. *Acinetobacter baumannii* causes hospital acquired infections, such as ventilator-associated pneumonia, bacteremia, urinary tract infections, meningitis, and surgical wound infections [(1)] . *Acinetobacter baumannii* is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile bacteria; it has a high incidence among immuncommpromised *Acintobacter baumannii* is referred to as ‘’Iraqibacter’’ due to its seemingly sudden emergence in military treatment facilities during the Iraq War . *Acintobacter baumannii* produces high molecular weight capsular polysaccharide which surrounds the outer membrane [(2)]. Capsular polysaccharide forms a discrete layer on the bacterial surface providing protection from diverse environmental conditions, assisting in evasion of host immune defenses, and increasing resistance to a number of antimicrobial compounds [(3)]. *Acintobacter baumannii* is capable of entering and persisting inside host cells, it adheres to host cells, then translocate into nucleus. After killing host cells, it disseminates in bloodstream and tissues [(4)]. As a class of important virulence factors in bacteria, outer membrane proteins (OMPs) have attracted much more attention. Among those OMPs of *A. baumannii*, *OmpA* is the most deeply studied virulence factor which plays key roles in regulating the adhesion, aggressiveness, and biofilm formation of *A. baumannii* and immune response of host. Other virulence factors such as exopolysacharide also have an important role in the invasion of these bacteria . *Acinetobacter baumannii* produces a polysaccharide export outer membrane protein, called exopolysacharide or EPS, which is encoded by EpsA. EPS accumulates

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on the cell surface and provides protection to the cells against the harsh external environment [5] . The present work was aimed to molecular detection of some virulence genes (*OmpA* Gene and *EpsA* Gene) of *Acinetobacter* baumannii isolates

Materials and Methods

Sample collection

This study was conducted during the period from December 2019 to April 2020, During the study period, eighty clinical burn wounds swabs samples were taken. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from *Ghazi Al Hariri Hospital* (40 samples) and Baghdad hospital (40 samples) in Baghdad / Iraq.

Biochemical test

Biochemical test as Oxidase, Indole, Methyl red, Lactose fermentation, Citrate utilization, Catalase production and Urease test. were performed on the isolates to confirm their identification *Acintobacter baumannii*. All the tests were according to [6] .

API (Analytical Profile Index)

This test is used to identify *Acintobacter baumannii* bacterium, also used to determine and differentiate several types of Gram-negative bacteria .

Identification Using Vitek 2 System

The VITEK 2 is an automated microbiology system utilizing growth-based technology, according to the manufacturer's instructions (BioMerieux-France).

Molecular study

DNA Extraction

Genomic DNA was isolated from bacterial growth according to the protocol of ABIOpure . Primer sets used are shown in table (1) .

Table (1): Primer sets used in the present study

Primer name	Sequence	Annealing Temp. °C	Product Size (bp)	
epsA-F	5`-AGCAAGTGGTTATCCAATCG-3`	50	415	415
epsA-R	5`-ACCAGACTCACCCATTACAT-3`			
ompA-F	5`-CGCTTCTGCTGGTGCTGAAT-3`		531	531
ompA-R	5`-CGTGCAGTAGCGTTAGGGTA-3`			

Quantitation of DNA

Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5 min. incubation at room temperature, DNA concentration values were detected.

Polymerase chain reaction to check the quality of DNA

Polymerase chain reaction master mix was prepared (with final volume 20 µl per one reaction) containing 10µM forward and reverse primers, 2 X of master mix, and 5µl of nuclease-free water was added until the volume reached to 17 µl. Then, 3 µl of DNA was added

to mixture .

Results and Discussion

According to table (2) , Seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %).

Table (2): Total number of burn wounds samples used for the isolation of bacteria

Clinical wounds samples	Positive (growth)	Negative (no growth)
80	70	10
Percentage	87.5%	12.5 %

The increasing body of literature on this subject suggested that *Acinetobacter* infections occur mainly in four patient populations and settings . At least 25% of healthy individuals may carry *Acinetobacters* as part of their normal skin flora, but carriage of *Acinetobacter* spp. By healthy subjects at other body site is normally low, in contrast, high colonization rate of the throat, skin, respiratory tract or digestive tract of hospitalized patients with clinically significant strains have been reported during outbreaks of infection [7] .

The majority of infections caused by *A. baumannii* are contracted in hospitals, most often in critically ill patients hospitalized in intensive care or surgery *Acintobacter baumannii* can resist dehydration, detergents, UV radiation and common chemical sanitizers, making it extremely difficult to eradicate [8] .

The emergence of multidrug resistance *A. baumannii* and carbapenam-resistant *A. baumannii* have cause hospital outbreaks and threat in treatment due to its wide variety of resistance mechanisms and high survival rate on a biotic surfaces [9] .

A. baumannii traumatic wound infections have become a topic of recent interest with reports of increasing incidences of outbreaks among victims of combat injuries and natural disasters . Recent data from Iraq and Afghanistan identify highly resistant strains of *A. baumannii* to be some of the most common organisms causing severe and often lethal wound infections [10] .

A. baumannii infection occurs when the immunological barriers of the host are breached (e.g. mechanical ventilation) and is hence considered an opportunistic pathogen . *A. baumannii* causes various types infections complicating burn wounds [11] .

From 70 samples inoculated on the Chromagar *Acinetobacter*, 10 isolate grew on the medium . CHROMagar Acinetobacter is a recently developed medium for selective and rapid identification of *Acinetobacter* spp. [12] .

Acinetobacter baumannii is rod-shaped which grows well on MacConkey agar (without salt). Although officially classified as not lactose-fermenting ,they are often partially lactose-fermenting when grown on MacConkey agar . Growth and purity of cultures of *Acinetobacter baumannii* were determined by culture on MacConkey agar and Blood agar. On MacConkey agar it's formed pale coloured, Non lactose fermenting colonies and on Blood Agar it's formed non-hemolytic colonies. There was only one type of colonies attesting to its purity [13] .

Ten isolates of *A. baumannii* grow at 44°C were positive on blood agar medium after 24 hrs of incubation. A appropriate temperature for the growth of most *Acinetobacter* spp were 37ºC whereas *A. baumannii* can grow well at high a temperature of 44 °C [14] .

The suspected all isolates of *A. baumannii* and were then subjected to the related biochemical tests. All isolates of *A. baumannii* were found to be catalase positive and oxidase / indole negative. Tests on Lactose fermentation and motility test gave negative results. The positive results for the test appeared in methyl red and

Citrate utilization, finally we used the urease test which give negative results for *Acintobacter baumannii* . The isolates were identified as *A. baumannii* and have been confirmed by using Api 20 and VITEK 2 Compact, figure (1) .

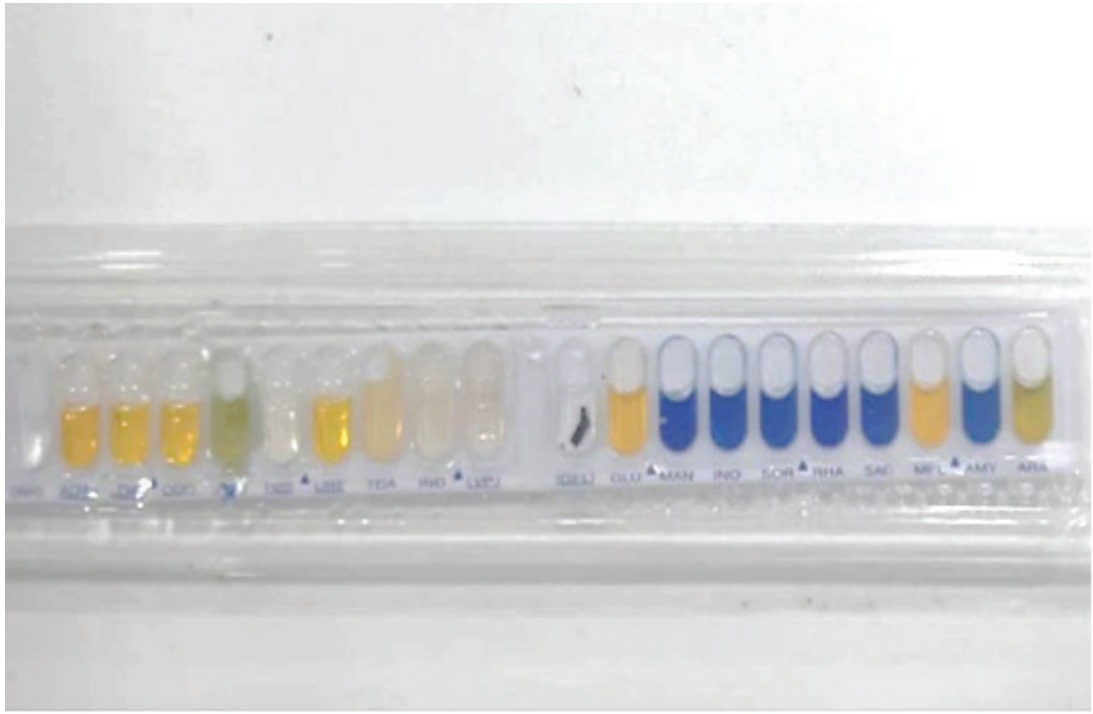

Figure. 1 : API test for identification of *Acintobacter bumannii*.

Acinetobacter was identified as non motile, oxidase negative and catalase positive. *Acinetobacter baumannii* is positive for citrate. Citrate in simmon citrate medium is important to detect weather the bacteria isolates able to grow on it as a unique carbon and energy source [15] .

Non-lactose fermenting and non-motile of *Acinetobacter baumannii* isolates [16] . Urease test was positive for *Acinitobacter baumannii* urease enzyme catalyzes the breakdown of urea, and the bacteria that can produce this enzyme is able to detoxify the waste products and to drive metabolic energy from its utilization which change the medium color from yellow to purple-pink, indicating urease positive test [6] .

Acinetobacter baumannii isolates were oxidase negative and catalase positive [17] . API NE 20 test VITEK 2 Compact were carried out confirms the biochemical tests regarding the species identification of the *A.baumannii* isolates. results showed that all 30 isolates were identified as *A.baumannii*, as shown in Figure (4). API 20 NE is a standardized system for the identification of non-fastidious, non-enteric Gramnegative rods, combining 8 conventional tests which include potassium nitrate (NO3), L-tryptophane (TRP), D-glucose (GLU), L-arginine (ADH), urea (URE), esculin ferric citrate (ESC), gelatin (GEL), and 4-nitrophenyl-β-D-galactopyranoside (PNPG), and also the 12 assimilation tests which include Dglucose (GLU), L-arabinose (ARA), D-mannose (MNE), D-mannitol (MAN), N-acetyl-glucosamine (NAG), D-maltose (MAL), potassium gluconate (GNT), capric acid (CAP), adipic acid (ADI), malic acid (MLT), trisodium citrate (CIT), and the phenylacetic acid PAC [18] .

Genomic DNA was extracted from the most 10 isolates of *Acintobacter baumannii* using genomic DNA kit. Figure (2). The result shows that the recorded range of DNA concentration was 50 ng / µl and the DNA purity was 1.8-2.0.

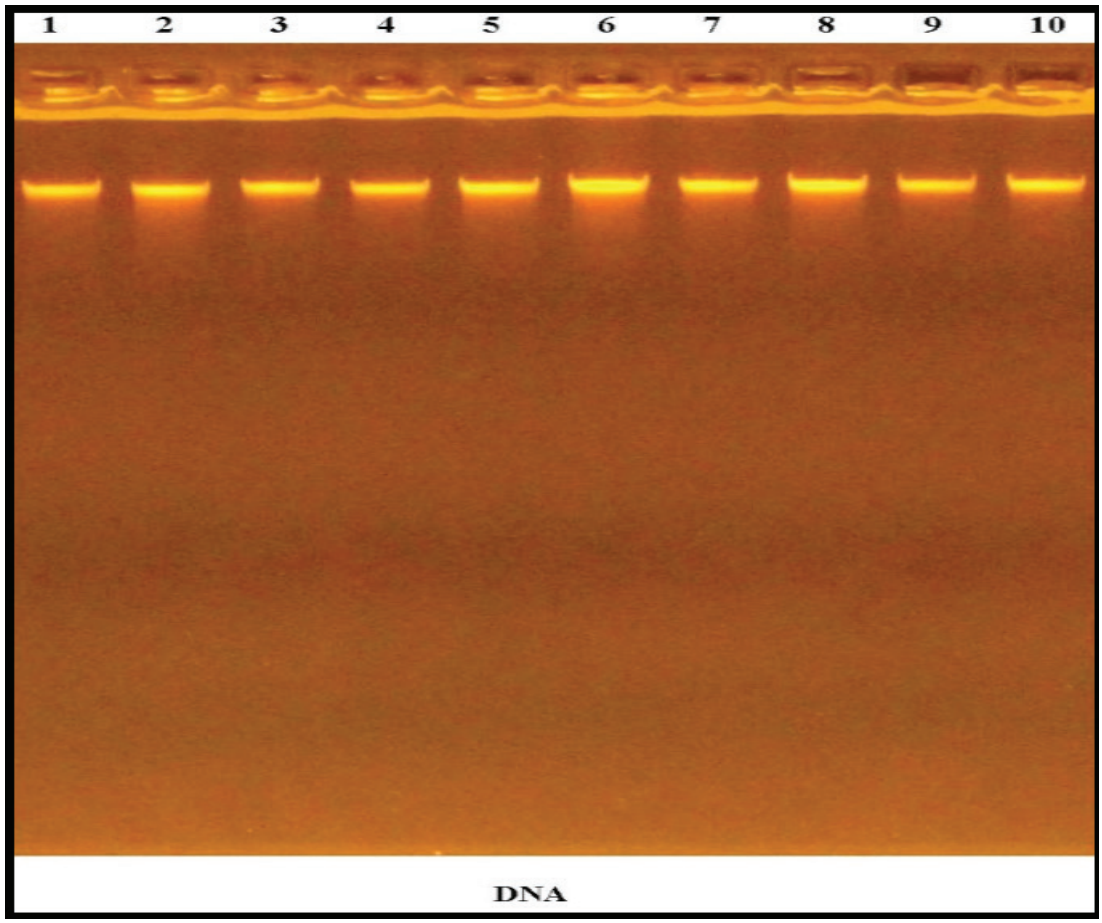

Figure. 2 : Genomic DNA bands extracted from *A. baumannii* isolates on (1%) agarose,75V, for 30 min stained with ethidium bromide. Lane 1-10: DNA of Lane.

The results of *epsA* and *ompA* genes detection clarify that 8 isolate (80%) of *A. baumannii* isolates gave a positive result and carrying *epsA* and *ompA* genes while 2 (20%) of *Acintobacter baumannii* isolates were lack the gene and no cross reactivity could be observed with any of the AS-3 and AS-9 isolates.

The results showed the amplification of *epsA* and *ompA* genes for eight *Acintobacter baumannii* isolates were AS-1, AS-2, AS-4, AS-5, AS-6, AS-7, AS-8 and AS-10. PCR product 531 bp of *ompA* gene figure (3) and 451 bp of *epsA* gene figure (4). The infection symptoms caused by *Acintobacter baumannii* are closely associated with its virulence factor. But the virulence genes distribution in clinical *A. baumannii* was rarely reported, except for *bap, omp* 33-36 and *traT* [19] .

This study examined the distribution of two virulence genes (*epsA* and *ompA* genes) in clinically collected *A. baumannii* isolates. The molecular investigation was performed to detect the amplification plots of the target genes and reference gene in order to find the threshold cycle value for each them as in figures (6) and (7).

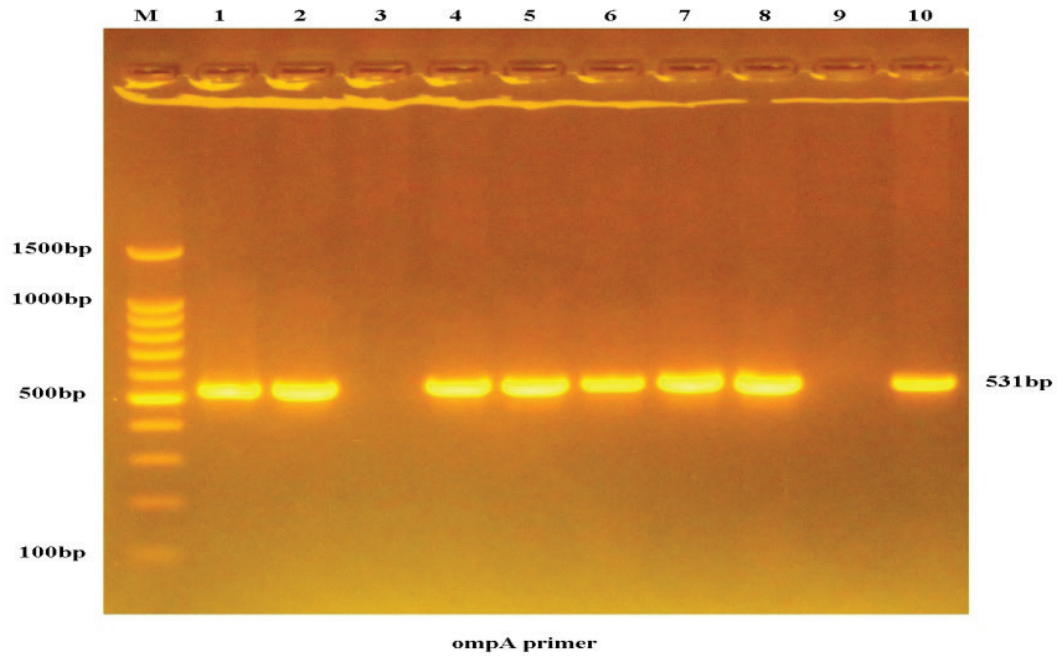

Figure. 3 : Results of the amplification of *ompA gene* of *Acinetobacter baumannii* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 531bp PCR products.

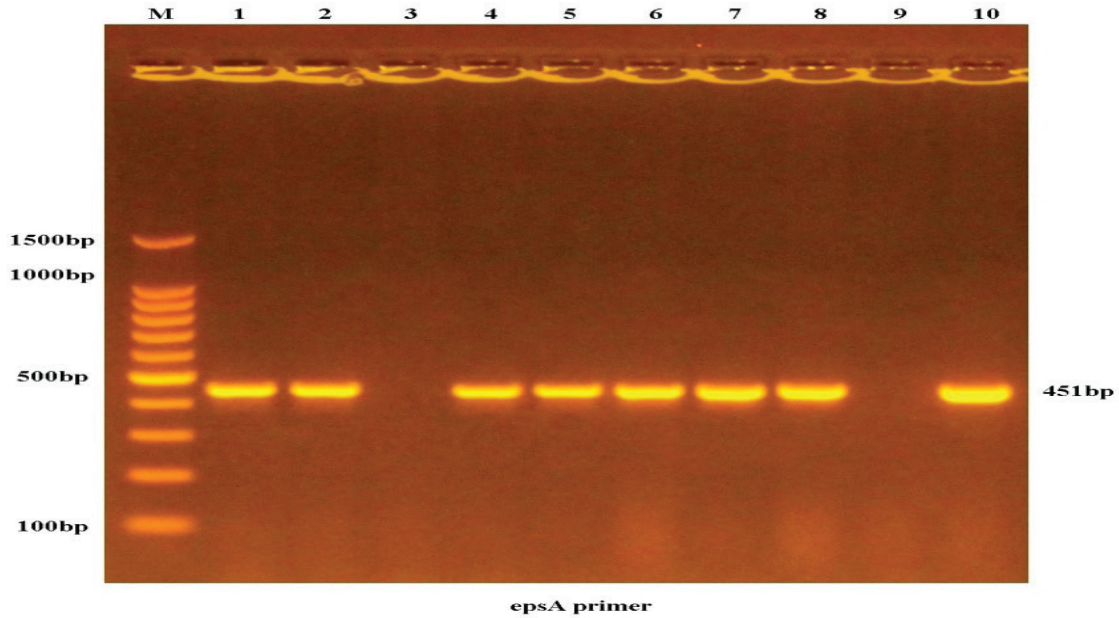

Figure. 4 : Results of the amplification of *epsA gene* of *Acinetobacter baumannii* samples were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-10 resemble 451bp PCR products.

Several virulence factors involved in biofilm formation of *A. baumannii* such as the outer membrane protein A (*OmpA*), biofilm associated protein (*Bap*)chaperon-usher pilus (*Csu*), extracellular exopolysaccharide (*EPS*). The *OmpA* of *A. baumannii* is essential for the attachment to human epithelial cells, development of biofilms and antimicrobial resistance [20] .

Previous results showed that all *A. baumannii* isolates carried at least one biofilm related gene. The most prevalent gene was *csuE* (100%), followed by *pgaB* (98%), *epsA* and *ptk* (95%), *bfmS* (92%) and *ompA* (81%). 98% of isolates carried more than 4 biofilm related genes, simultaneously [21] .

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Physical self-perception and Personality Traits among Women with Psychoactive Substance Abuse Compared to Normal Individuals

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Abstract

Introduction: Regarding to the role of physical self-perception and personality traits in abuse of psychoactive substance among women, this study aimed to compare the concept of physical self-perception and personality traits among psychoactive substance abusers and normal individuals.

Materials and Methods: In this causal-comparative study, 60 women (30 psychoactive substance abusers, 30 normal cases) referred to substance abuse treatment centers in Mashhad city (the second big-city of Iran) were selected in convenient sampling method. The Physical Self-Description Questionnaire (PSDQ) and NEO-Five Factor Test (Short Form) were performed. Data were analyzed using multivariate analysis and two independent means tests.

Results: Multivariate analysis revealed that women with psychoactive substance abuse have higher scores in neuroticism and lower scores in agreeableness, accountability and extraversion than normal women. The comparison of two independent means also showed that there is a significant difference between physical self-perception in normal and abusers, so that normal women have better physical self-perception.

Conclusion: The results confirm that physical self-perception and personality traits play an important role in involvement in substance abuse.

Keywords*: Physical self- perception, Personality traits, Substance abuse, Women*

Introduction

In the recent years, one of the most important issues of public health is the increasing rate of substance abuse among young women [1] . In addition, substance abuse is a multi-dimensional problem that successful treatment of it depends on the various factors So it is necessary that predisposing factors identified and considered to reduce tendency to substance abuse among different groups in terms of age and gender)[2] .

Research suggests that substance abuse- based on different psychological, physical, familial and social risk factors- usually starts from smoking cigarette in preadolescence which extends to using various substances in young adulthood[3] .

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The past studies suggested that physical perception and body image can influence the mental health and quality of life [4] . The researchers found that self physical dissatisfaction can lead to imaginary defects in body image and it finally resulted to unpleasant emotions and feelings[5] . It is indicated that continuous body dissatisfaction can cause depression, obsession, tendency to substance abuse and suicide[6-8] .

Also, studies suggested that women who are dependent to substance abuse or alcohol drinking show low body esteem [9].

In addition, women who worry about their weight and body image may tend to use stimulant substances. In this line, the results of a study showed that women who use ecstasy believe the aid of ecstasy in weight loss [10].

Different models propose an underlying psychological and personality vulnerability for substance abuse. Based on the previous studies, five personality factors can predict the high risk behaviors such as alcohol drinking, substance abuse and dangerous driving. It suggested that low scores of agreeableness and conscientiousness can predict impulsive behaviors [11,12]. Also, one study evaluated 3720 college students and showed that neuroticism and anti-sociality have important roles in prediction of substance abuse [13]. Also, some studies showed that the incidence of smoking among high school students significantly correlated with the incidence of smoking among the friends and family members[14] .

So, according to the importance of substance abuse especially psychoactive drugs and their related consequences such as impulsive and high risk behaviors among young women and the relation between physical perception and personality traits with tendency to abuse psychoactive substances and the lack of the same studies in Iran, the present study aimed to assess physical self-perception and personality traits among women with psychoactive substance abuse compared to normal individuals.

Materials and Methods

This causal-comparative study conducted in 2013 and its community included all women dependents to psychoactive drugs that they referred to treatment clinics of substance abuse in Mashhad city (The second big city of Iran). The sample size was calculated as 30 cases based on the formula. Inclusion criteria included women with psychoactive drugs dependency and they aged 18-45 years and they had educational level between middle grade to master degree. So, 30 cases were selected via convenient method and 30 normal cases without dependency to substances were matched based on the inclusion criteria as the control group.

Research instrument

A) Physical Self Description Questionnaire (PSDQ): This questionnaire was made by Marsh (1996) for measurement of body self-image in 2 total subscales and 9 specific subscales. The new and brief form of it has 47 items and with acceptable validity and reliability. The participant responses to each item in a Likert degree from 1 to 6 [15]. This questionnaire was standardized in Iran and total reliability was reported as 0.87 [16].

B) NEO five factor of personality inventory (short form): This scale includes 60 items which measure the five personality factors and it was made by Costa and McCrae (1992). The items are scored in Likert system as 5 degrees (completely disagreeableness to completely agreeableness). Each factor included 12 items and it is scored in a range 0 to 48. These factors included neuroticism, extroversion, openness to experiences, agreeableness and conscientiousness [17]. This inventory was standardized in Iranian studies and its Cronbach alpha reported as 0.64 [18].

The data analyzed by descriptive statistics, multifactorial variance analysis and independent t-test through SPSS software.

Results

In this study, 60 women as two groups were evaluated. The mean ages related to addict and control groups were 28.30 years (±6.46) and 29.30 years (±6.88) respectively.

The other demographic characteristics of cases such as marital status, level of education and number of children were presented in Table 1.

Table 1. The demographic characteristics of cases

	Variable	Case group Prevalence (percentage)	Control group Prevalence (percentage)
Marital status	Single	13 (43.3)	14 (46.7)
	Married	17 (56.7)	16 (53.3)
Level of education	Middle	12 (40.0)	9 (30.0)
	Diploma	12 (40.0)	13 (43.3)
	Associate degree	2 (6.7)	3 (10.0)
	Bachelor and higher	4 (13.3)	5 (16.7)
Number of children	0	16 (53.3)	16 (53.3)
	1	6 (20.0)	8 (26.7)
	2	8 (26.7)	5 (16.7)
	3 or more	0 (0.0)	1 (3.3)

As seen, there are not significant differences between two groups in demographic factors (P>0.05).

Table 2 shows the scores of two groups in NEO-inventory.

Based on the results, the highest scores are related to neuroticism and conscientiousness in cases and in controls respectively. In addition, there are significant differences between personality dimensions between two groups (exceptionally openness to experiences).

Table 2. The scores of two groups in NEO-inventory

Variable	Case group Mean (SD)	Control group Mean (SD)	P value	Effect size
Neuroticism	36.20 (2.60)	25.20 (6.18)	0.001	0.58
Extroversion	24.03 (4.51)	26.70 (5.29)	0.04	0.07
Openness to experiences	25.36 (2.84)	24.70 (3.22)	0.40	0.01
Agreeableness	19.60 (2.44)	29.56 (5.96)	0.001	0.55
Conscientiousness	14.16 (5.35)	32.66 (5.83)	0.001	0.73

Table 3 shows the scores of physical self description among two groups.

Table 3. The scores of physical self description

Variable	Case group Mean (SD)	Control group Mean (SD)	t	df	P value
Physical self description	122.10 (28.61)	186.96 (30.18)	-8.54	58	0.001

As seen, there is a significant difference between two groups in physical self description (P=0.001) and the women with abuse of psychoactive substances had lower scores than the controls.

Discussion

The present study reveals that there are significant differences between personality dimensions between two groups (exceptionally openness to experiences) and physical self description. So that women with abuse of psychoactive substances had lower scores in physical self description, extroversion, agreeableness and conscientiousness than the controls but they had higher scores in neuroticism and openness to experiences.

In this line, Fakhraei et al. studied 90 individuals (60 men, 30 women) with abuse of amphetamines (case group) and 90 normal cases without dependency to substances (control group). The personality traits of two groups were evaluated by NEO-inventory. The results showed the significant differences between dependants and normal cases in personality traits (exceptionally extraversion). Dependants had high scores in neuroticism, whilst they had lower scores in openness to experience, agreeableness and conscientiousness than control group. In addition, the demographic data showed that most of dependents were single and their academic level was lower than diploma. Also their mean age was 28.41 years. Unlike the mentioned study, the results of the present study show that the dimension of openness to experiences is not significantly different between two groups, also only female substance abusers were studied in our study [19].

In addition, the past same studies are concordant with the results of the present study [20,21].

Also, the higher scores in neuroticism dimension and lower scores in agreeableness and conscientiousness among addict persons compared to healthy controls was approved in different studies [22-25] that these results are concordant with the results of the present study.

In term of physical-perception, some of the past studies indicated that poor body image is more prevalent among addict women compared to health women and dissatisfaction about physical image can be concerned as a predisposing factor for addiction especially in women population [26,27] that is concordant with the results of present study.

Although some of studies concluded a different finding. Behzad et al. study evaluated the body image and marital satisfaction among 200 women (addict and healthy). They resulted that body image was not different significantly between two groups [28]. As same, Babaei et al. concluded physical self-description was not correlated significantly with alexithymia among substance abusers [29].

This study has some limitations. The researchers evaluated abuser women in initial period of complete remission that leads to the lack of generalization to all abusers. The lack of complete psychological record before substance abuse can be considered as another limitation of this research.

Conclusion

The results show that physical self-perception and personality traits are related to substance abuse in women.

It seems that regarding the importance of physical self-perception among addict women and the lack of

the studies in the field of relation between personality traits, body image and substance abuse especially psychoactive substances, further longitudinal studies should be conducted.

Ethical Clearance-Taken from Mashhad University of Medical Sciences Committee

Source of Funding- Self

Conflict of Interest- Nil

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The Potency of Kaffir Lime Leaf (*Citrus Hystrix*) Ethanol Extract Against Lead Acetate Toxicity on Spermatozoa Quality of Mice (*Mus musculus*)

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Abstract

Lead is a heavy metal which can affect male reproductive system. The accumulation of lead in the body becomes a precursor to the formation of ROS, which is able to damage spermatozoa. The toxicity of lead can be prevented by antioxidants. Kaffir lime leaves (*Citrus hystrix*) with their flavonoid content have the potential to be the source of antioxidants. The purpose of this study is to analyze the antioxidant effect of kaffir lime leaves on the quality of spermatozoa of the mice that have been exposed to lead acetate. **Methods:** The literature study was conducted using narrative review technique by collecting related articles using specified keywords. **Result:** It is found that kaffir lime leaves contain *hesperetin*, which is the dominant flavonoid in Citrus group. The antioxidant activity of kaffir lime leaf is able to neutralize lead-induced ROS, as well as to stimulate the formation of enzymatic antioxidants. **Conclusions:** Ethanol extract of kaffir lime leaves can improve the quality of spermatozoa of the mice that have been exposed to lead acetate.

Keywords: *Antioxidant, Citrus hystrix leaves, Lead acetate, Male mice, Sperm quality*

Introduction

Lead (*Pb*), as a pollutant substance, can be found in several places in Indonesia. One of the sources is the industrial fumes of used battery smelters, which do not have residual particulate controlling facilities. Citing from the website of Ministry of Environment and Forestry, based on the research conducted by P3KLL and PSTNT-BATAN on 2011, the *Pb* level in ambient air of used battery smelter industrial area, such as Parung Panjang, district of Bogor; Cinangka, district of Bogor and Manis Jaya, district of Tangerang are 17,503 µg/m^3, 5,186 µg/m^3, and 4,142 µg/m^3, respectively. These numbers have exceeded the standard on the Government regulations of Indonesia or *PP* number 41/1999 about Air Pollution Controlling, which stated that the standard is 2 µg/m^3 in an average of 24 hours. This will affect the people who live near the factory. Lead accumulation in the body can decrease the number of spermatozoa and cause male infertility (Panggabean *et al*., 2008). Lead is classified as an oxidant which becomes the precursor of ROS (Reactive Oxygen Species) formation. ROS can attack nitrogenous bases, such as purine and pyrimidine. It can also directly attack DNA, which is the collection of nitrogenous bases. It will activate *caspase* enzymes, which cause apoptosis of spermatozoa as well as degradation of Spermatozoa DNA (Hayati *et al*., 2005). Antioxidant is a substance which has the characteristic of *chelating*. This will enable antioxidants to overcome lead poisoning (Offor *et al*., 2017). Antioxidants can be found in nature which, in this case, is in a plant. Wulandari (2017) has found that kaffir lime leaf (*Citrus hystrix*) is one of the producers of antioxidants. It contains phenol, flavonoid, terpenoid and citronellal. Flavonoid is one of the natural antioxidants. It has *chelating* ability; which makes it able to bind metal and form a flavonoid-metal complex. This ability comes

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from the carbonyl and hydroxyl group which attach to its flavonol ring structure (Al-Megrin *et al*., 2019). Based on the background above, an analysis of the potency of kaffir lime leaf ethanol extract against lead toxicity on spermatozoa quality of mice is needed. The purpose of this study is to analyze the effect of antioxidant in kaffir lime leaf against the spermatozoa quality of the mice that have been exposed to lead acetate. The benefit of this study is that it can serve as a reference for readers regarding how to utilize kaffir lime leaf ethanol extract in order to maintain the spermatozoa quality of which has been exposed to lead acetate.

Material and Methods

This article was made by using literature study with narrative review technique. Narrative review is a comprehensive, critical, and objective analysis from recent knowledge on a certain topic. It is an important process of research in building the theoretical framework in order to identify patterns and trends in literature on many kinds of researches (Baker, 2016). The data used in this study originated from journals which have been published in both national and international journal platforms on the internet. The search engines used to find the journals are Pubmed, Science Direct, Clinical Key, NCBI and Google Scholar. The process of journal searching was focused on two different purposes; namely searching for reference and comparative journals. Reference journals are used to provide explanations related to the problem and strengthening the argument. Meanwhile, comparative journals are used to compare previous researches which are related to the topic of 'antioxidants in other herbs that are used to increase the quality of spermatozoa in mice.' Several keywords used in the search of comparative journals are citrus hystrix leaf, sperm quality, antioxidant, male mice, and lead acetate. Boolean technique with the word "AND" was also used, for example: Citrus hystrix leaf AND Sperm quality. The inclusion criteria for this study are: it has to be in English and available in full text. The absract and the full text of the comparative journals obtained were also analyzed. Journal analysis was conducted by classifying the author, year, sample population, study model and result. The result obtained from both reference and comparative journals were then written in narrative review.

Result and Discussion

Lead enters the body through inhalation. The size, shape, and phase of the particle will most likely affect its entrance to the body (Schlosser *et al*., 2010). Lead exposure can result in the decrease of spermatozoa quality, which can be seen in the amount of abnormal morphology of spermatozoa, motility, as well as the concentration of spermatozoa.

In the research by Li *et al*., 2017, it is stated that on ICR-CD1 mice that received lead in their water; the percentage of non motile and non progressive spermatozoa increased, while the amount of motile spermatozoa decreased. The percentage of abnormal spermatozoa increased after the administration of lead acetate.

Other studies have proven that a group of mice (*Mus musculus*) exposed to lead acetate (with a dosage of 100 mg/kg) has shown an increase in concentration of lipid peroxidation (LPO) and significant decrease in GSH level. Also, there are no appearance of primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa in seminiferous tubule. This means that no spermatogenesis processes occur. On histopathology examination of seminiferous tubules, degeneration of germinal cell, absence of spermatozoa, vacuolization of testicular tissue were found; while on histopathology examination of epididymis, no spermatozoa was found. This shows the reduction of the amount of spermatozoa. Spermatozoa morphology of a mice group exposed to lead acetate is significantly poor (Rao *et al.*, 2016).

ROS is also called free radical since it contains at least one unpaired valence electron and takes electron from other compounds - which in turn will cause oxidation (Durackova, 2014). ROS consists of superoxide (·O2-), hydrogen peroxide (H_2O_2), proxy radicals (·R OO), and hydroxyl (·OH-). Lead acetate causes an increase in ROS by inhibiting the work of antioxidant enzymes. This happens because lead has a high affinity towards the sulfhydryl group of enzyme, such as superoxide dismutase and glutathione peroxidase. Moreover, lead acetate also competes with essential ions in the body that have a role as cofactor for important reactions, such as the activity of superoxide dismutase and catalase enzyme (Sudjarwo *et al*., 2019). It is known that an increase in ROS can affect spermatozoa through several mechanisms, such

as lipid peroxidation, protein modification and damage to spermatozoa DNA. Lipid peroxidation can cause damage or disturbance to the structure of spermatozoa, transport function, ion and metabolite regulation, as well as signal transduction of receptor. This will decrease intracellular ATP, and later cause acrosomal damage as well as decrease membrane fluidity and permeability. This series of events will decrease spermatozoa motility (Sutanto *et al*., 2017). Plasma membrane of spermatozoa is susceptible to damage caused by oxidative stress, since plasma membrane mainly consists of Polyunsaturated Fatty Acids (PUFA) that contains double bonds such as DHA or Docosahexaenoic Acid (Drevet *et al*., 2019). The molecule of this membrane is reactive to oxygen radicals and will produce highly reactive lipid aldehyde, such as malondialdehyde. This will result in protein modification and DNA damage.

Depending on the type of ROS, protein can be converted through thiol oxidation, tyrosine nitration, sulfonation or glutathionylation. The most common protein modification is thiol oxidation. It targets the enzyme of glycolysis and krebs cycle, and later decrease ATP production efficiency; targets α-tubulin, which damages the microtubules polymerization; and targets protamine, which affects the remodeling of chromatin during spermiogenesis (O'Flahertty & Matsushita, 2017). The increase in ROS can also damage spermatozoa DNA integrity and cause apoptosis of germinal epithelial cells. Furthermore, it affects the amount and morphology of the spermatozoa. Oxidative stress causes decondensation of nuclear, which increases the susceptibility of spermatozoa DNA that is destroyed by free radical (Bashandy, 2007). On the other hand, it also causes spermatozoa DNA fragmentation by modifying bases such as 8-OH-guanine and 8-OH-20–deoxyguanosine (Drevet *et al*., 2019).

II. Antioxidant of kaffir lime leaf

(*Citrus hystri*x)

Antioxidant is a compound which is needed by the body to absorb or neutralize free radicals that damage normal cells, lipids and protein. This compound has a molecular structure which enables it to give its electron to free radical molecules and break the chain reaction of free radicals. Natural antioxidant compound can be found in several forms such as vitamin C, vitamin E, carotenoid, phenolic and polyphenolic compound which belong to the flavonoid group, derivative of cinnamic acid, coumarin, tocopherol, or other polyfunctional organic acids. The members of flavonoid group which have antioxidant activity are flavones, flavonols, flavanones, isoflavones, catechins, flavonols, and chalcone. Natural antioxidant compound has -OH group and double bond (>C=C<). These groups can give one of its hydrogen molecules so that ROS will become stable and non reactive free radical can be formed (Murray *et al*., 2009).

Kaffir lime leaf (*Citrus hystrix*) has the potential to be a source of natural antioxidant. According to Fidrianny *et al.,* 2016, kaffir lime leaves ethanol extract have antioxidant activity that can be seen by the number of total phenolic compound, which is 3,66 g GAE/100 g; total flavonoid compound, which is 4,46 g QE/100 g; and carotenoid compound, which is 0,36 g BE/100 g. On another study conducted by Butryee *et al*., 2009, it is found that the main flavonoid compounds of kaffir lime leaves are hesperetin, peonidin, myricetin, cyanidin, and isorhamnetin in the amount of 365±48, 206±47, 179±18, 123±7, and 113±22 mg/100 g dry weight, respectively. Based on the result, hesperetin is known as the dominant flavonoid compound of kaffir lime leaves. Hesperetin (3',5,7-trihydroxy-4'- methoxyflavanone, $C_{16}H_{14}O_6$) is a natural flavanone and an aglycone of hesperidin which dominantly found in citrus group (Samie *et al*., 2018) and has three hydroxy groups located in the position of 3'-, 5- and 7. It also has additional methoxy substituent in the position of 4'- (PubChem, 2020). Flavanone is a type of flavonoid which has a C ring and saturated double bond between the position of 2 and 3 (Panche *et al*., 2016). Hesperetin uses its antioxidant properties in two ways; which are direct radical scavenging and cellular defense augmentation. Direct radical scavenging plays a part in the protection of DNA, protein, and tissue against the damage caused by intrinsic factor (oncogene) or extrinsic factor (radiation, inflammation, toxin) (Parhiz *et al*., 2014). Meanwhile, cellular defense augmentation plays a part in the inhibition of cell damage by increasing the regulation of Nrf2 and ERK 1/2 gene expression (Elavarasan *et a*l., 2012; Martinez *et al*., 2009). Activation of Nrf2 and ERK 1/2 can increase the regulation of HO-1 expression, which later will decrease intracellular prooxidant and increase bilirubin as internal antioxidant. HO-1 expression increases the level of CO

by its anti apoptotic and anti inflammatory activity; as well as increases guanylyl cyclase level inside the cell (Chen *et al*., 2010). This shows that hesperetin is able to neutralize ROS. Other literature mentioned that the administration hesperetin in a diabetic rat group induced with STZ shows a decrease in MDA and carbonyl protein level as parameter of oxidative stres, with an increase in GSH, SOD, catalase and Gpx level - which are antioxidant enzymes – if compared to the diabetic group that did not receive administration of hesperetin. Other than that, Hesperetin administration in a diabetic rat group resulted in a significant increase of amount, motility and viability of spermatozoa compared to the diabetic rat group that did not receive administration of hesperetin. Histological analysis shows that administration of hesperetin in a diabetic rat group can increase the MSTD (Mean Seminiferous Tubule Diameter) and MTBS (Mean Testicular Biopsy Score) when compared to the diabetic rat group that did not receive administration - which result shows a decrease in amount, motility and viability of spermatozoa and damage of seminiferous tubules (Samie *et al.*, 2018). In table 1, analysis of the previous researches about the effect of antioxidant to spermatozoa quality can be seen.

Table 1. Analysis of comparative journals

No	Title	Author	Sample	Intervention Group	Result	Extract Content
1.	Effects of Red Dragon Fruit (Hylocereus polyrhizus) Skin Extract on Lead Acetate Toxicity in the Morphology of Balb/C Mice (Mus musculus) Spermatozoa	(Raharjo et al., 2017)	25 of male mice	NC group were given nothing. PC group were given 8mg/kg BW of lead acetate. T1 group, T2 group and T3 group each were given 250 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW of extract, respectively and 8mg/kg BW of lead acetate. The lead and extract given were intraperitoneally. The study conducted for 35 days.	a. Lead decreases the number of normal morphology of spermatozoa b. The extract increases the number of normal morphology of spermatozoa in the mice that have been exposed to lead, but the result of the number is always below the control group.	Phenolic, flavonoid and polyphenols
2.	Ferulago angulata extract ameliorates epididymal sperm toxicity in mice induced by lead and diazinon	(Naderi et al., 2017)	80 of male NMRI mice	C group were given nothing. T1 group were given 0,5% of lead acetate and T2 were given 400 mg/ kg BW of extract. T3 were given both extract and lead as much as T1 and T2 group . The lead and extract given were orally. The study conducted for 6 weeks.	a. Lead decreases the number of normal morphology, viability, motility, as well as the number of spermatozoa. b. The extract decreases the number of spermatozoa, but it increases the number of normal morphology of spermatozoa, as well as viability and motility of spermatozoa in the mice that have been exposed to lead. However, the result of the number is always below the control group.	9-octade cenoic acid, methyl ester, (E)-, carvacrol, thymol, 1, 2, 3-benzenetriol (pyrogallol)

Cont... **Table 1. Analysis of comparative journals**

3.	Protective effects of hydro-alcoholic extract of Quercus brantii against lead-induced oxidative stress in the reproductive system of male mice	(Soleimanzadeh et al., 2018)	42 of male NMRI mice	C group were given nothing, while NC group were given 0,2 ml of distilled water. T3 and T4 group were given 500mg/kgBW and 1000mg/kgBW of extract, respectively. While T5 and T6 group each were given 1000 ppm/kg BW of lead and additional 500 mg/kg BW and 1000 mg/kg BW of extract, respectively. The lead and extract given were orally. The study conducted for 35 days.	a. Lead decreases the number of normal morphology, viability, motility, as well as the number of spermatozoa b. The extract increases the number of spermatozoa, normal morphology of spermatozoa, as well as viability and motility of spermatozoa in the mice that have been exposed to lead. However, the result of the number is always below the control group.	Phenol, tannin, gallic acid, p-coumaric acid, ellagic acid, rutin , epicathecin and quercetin
4.	Carob (Ceratonia siliqua L.) fruit hydro-alcoholic extract alleviates reproductive toxicity of lead in male mice: Evidence on sperm parameters, sex hormones, oxidative stres biomarkers and expression of Nrf2 and iNOS	(Soleimanzadeh et al., 2020)	42 of male NMRI Mice	C group were given nothing, while NC group were given 0,2 ml of distilled water. T3 and T4 group were given 500mg/kgBW and 1000mg/kgBW of extract, respectively. While T5 and T6 group each were given 1000 ppm/kg BW of lead and additional 500 mg/kg BW and 1000 mg/kg BW of extract, respectively. The lead and extract given were orally. The study conducted for 35 days.	a. Lead decreases the number of normal morphology, viability, motility, as well as the number of spermatozoa b. The extract increases the number of spermatozoa, normal morphology of spermatozoa, as well as viability and motility of spermatozoa in the mice that have been exposed to lead. However, the result of the number is always below the control group.	Phenol, flavonoid, gallic acid, caffeic acid, chlorogenic acid, rutin, coumaric acid quercetin, cinnamic acid and apigenin
Note: C : Control, **NC** : Negative Control, **PC**: Positive Control, **T1** : Treatment Group 1, **T2** : Treatment Group 2, **T3** : Treatment Group 3 , **T4** :Treatment Group 4, **T5** : Treatment Group 5, **T6** :Treatment Group 6, **Ext** : Extract, **PbAc** : Lead Acetate, **Pb** : Lead.						

In table 1, it can be seen that all studies on the exposure of lead acetate in mice resulted in a decrease of the amount of spermatozoa. In each of those studies, the level of lead used for the experiment is different; however, each of the extract above has the ability to increase some or all of the quality parameters of spermatozoa - which are the amount, viability, normal morphology, and motility. However, the numbers are always below the ones in the normal spermatozoa/control group. Each of the extracts above contains phenolic and flavonoid as in the ethanol extract of kaffir lime leaf, although it belongs to a different type of flavonoid. From the studies

above, it can be concluded that flavonoid can improve the quality of spermatozoa.

III. Result Potential

Prediction of the result is that kaffir lime leaf ethanol extract can increase the quality of spermatozoa by using its flavonoid properties, which can serve as additional antioxidant to prevent the decrease of spermatozoa quality due to lead exposure.

Conclusion

Kaffir lime leaf ethanol extract can increase the quality of spermatozoa (amount, normal morphology, motility and viability) since it contains antioxidant compound, namely Hesperetin; which is classified as flavonoid.

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Pattern of Injuries Sustained in Cases of Four Wheelers without wearing Seat Belt Infatal Road Traffic Accidents in Mangalore

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Abstract

Introduction: Road traffic accidents (RTAs) have been the bane of the modern civilization accounting for considerable loss to the nation. General callous attitude for safety measures, poor law enforcement and uncontrolled motorization have caused significant increase in incidence of RTAs. World Health Organization (WHO) puts RTAs as the sixth leading cause of deaths in India, with a greater share of hospitalizations, deaths, disabilities and socioeconomic losses in young and middle aged population.

Aims and Objective: To study the pattern of injuries sustained in cases of four wheelers without wearing seat belt in fatal road traffic accidents in Mangalore.

Materials and Method: All cases of road traffic accidents involving fatal head injuries among four wheelers without seat belt which underwent autopsy at the Wenlock District Hospital and Justice K.S Hegde charitable Hospital, Mangalore from January 2009 to December 2013 were included in the study. A proforma was prepared, the details about the pattern of skull fractures and also about intracranial haemorrhages were obtained.

Results: Majority of the victims of four wheeler not using seat belt were in the age group of 30-39 Years and 40-49 Years (26.83% each). Majority of the victims were male (85.37%).Abrasion (46.34%) was the most common Injury to face and head followed by laceration (29.27%) was observed.Rib fractures was the most common associated injury (43.90%) while no associated injury was observed in 56.10%.Diffuse Extravasation of Blood from Scalp was observed in 53.66% while from occipital are was seen in 26.83% victims. Linear Type of fracture in vault was seen in 36.59% of victims. Occipital and frontal bone fracture was observed in 29.27% and 24.39% victims respectively. While studying the Base of skull fractures it was seen that ACF was seen in 24.39% victims while PCF in 19.51% and MCF in 14.63% victims. Most common intra cranial haemorrhage was Sub arachnoid haemorrhage (90.24%) followed by sub dural haemorrhage (82.93%). Brain Oedema was seen in 73.17% victims while Contusion to brain was seen in 26.83% victims. Frontal lobe of brain was injured in 46.34% victims while diffuse brain injury was seen in 29.27% victims.

Conclusion: We conclude that the four wheeler accident among the non seat belt users was common in middle aged males. Head injury and rib fracture was the most common type of injury observed. Liner fracture of skull with occipital and frontal bone involvement was common. Most common intra cranial haemorrhage was Sub arachnoid haemorrhage followed by sub dural haemorrhage. Brain Oedema and Contusion to brain were common brain injury. Frontal lobe brain injuries were common followed by diffuse brain injury.

Key words: *Four Wheelers without Wearing Seat Belt, Fatal Road Traffic Accidents*

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Introduction

Road traffic accidents (RTAs) have been the bane of the modern civilization accounting for considerable

loss to the nation. General callous attitude for safety measures, poor law enforcement and uncontrolled motorization have caused significant increase in incidence of RTAs. World Health Organization (WHO) puts RTAs as the sixth leading cause of deaths in India, with a greater share of hospitalizations, deaths, disabilities and socioeconomic losses in young and middle aged population.[1]

Trauma is a major cause of morbidity and mortality worldwide for death of majority of victims in road traffic accidents (RTA). According to World Health Organization more than 90% of deaths occur in low and middle income countries.[2] By 2020 Road Traffic Accident injuries will rise in the 6th place as a major cause of death worldwide.[3]

The United Nations General Assembly adopted a resolution on road safety on 2005 which invites Member States to implement the recommendations of the World Report on Road Traffic Injury Prevention; to participate in the first United Nations Global Road Safety Week; and to recognize the third Sunday in November of every year as the World Day of Remembrance for Road Traffic Victims[4]. This resolution follows the publication of The World Report on Road Traffic Injury Prevention by the World Health Organization in 2004.[5] This report highlights the fact that all over the world working age people are more likely to suffer hospitalization, permanent disability and death due to road traffic injuries than most other diseases. The situation in India is not very different.

About 82,000 persons were killed on Indian roads in 2002.[6] Official statistics regarding serious injuries are not reliable as they underestimate the actual number[7], but it is estimated that the number of people hospitalized may be 15-20 times the number killed.[8]

Aims and Objective

To study the pattern of injuries sustained in cases of four wheelers without wearing seat belt in fatal road traffic accidents in Mangalore.

Materials and Methods

The present record based retrospective study was conducted at the Wenlock District Hospital and Justice K.S Hegde charitable Hospital, Mangalore. All cases of road traffic accidents involving fatal head injuries which underwent autopsy at the Wenlock District Hospital and Justice K.S Hegde charitable Hospital, Mangalore from January 2009 to December 2013 were included in the study. Out of these cases patients involved in Four Wheeler accidents were further evaluated. Both of the hospitals being major tertiary care hospitals, situated in Mangalore. While all other fatal head injury other than road traffic accidents was excluded.

A proforma was prepared accordingly to collect the data based on the deceased's particulars, with complete external and internal examination in retrospective studies of those involved in fatal head injury cases due to road traffic accidents. Recorded details include name, age, sex, address, information furnished by the police in the inquest papers, postmortem reports, investigation reports if any and cause of death. All the data obtained was kept anonymous to protect the identity of the deceased and also for confidential medico legal information.

The details about the pattern of skull fractures and also about intracranial haemorrhages were obtained and entered in the proforma. The data from all the proformas were compiled in a master chart, analyzed by calculating sum, range, distribution and percentage. The data was also tabulated and appropriate inferences were drawn. These inferences were compared with other similar studies.

Results

Table no.1: demographic details of study subjects.

Parameter		No. of victims	Percentage
Age group	20-29 Years	3	7.32
	30-39 Years	11	26.83
	40-49 Years	11	26.83
	50-59 Years	8	19.51
	60-69 Years	4	9.76
	70 and above	4	9.76
Sex	Males	35	85.37
	Females	06	14.63
	Grand Total	41	100.00

It was observed that majority of the victims of four wheeler not using seat belt were young in the age group of 30-39 Years and 40-49 Years (26.83% each). Majority of the victims were male (85.37%).

Table no.2: Distribution according to injuries

Row Labels		No. of victims	Percentage
Injuries to face and head	Absent	4	9.76
	Abrasion	19	46.34
	Laceration	12	29.27
	Contusion	7	17.07
	Crush	0	0.00
	Surgical Scar	22	53.66
Associated Injuries	Rib fractures	18	43.90
	Long bone fractures	4	9.76
	Pelvis fractures	4	9.76
	Vertebral fractures	0	0.00
	Visceral Lacerations	4	9.76
	Only head injury	23	56.10
	Grand Total	41	100

Abrasion (46.34%) was the most common Injury to face and head followed by laceration (29.27%) was observed.

Rib fractures was the most common associated injury (43.90%) while no associated injury was observed in 56.10%.

Table no.3: Distribution of cases according to pattern of skull fractures

		No. of victims	Percentage
Scalp Extravasation of Blood	Absent	4	9.76
	Diffuse	22	53.66
	Frontal	7	17.07
	Parietal	0	0.00
	Temporal	3	7.32
	Occipital	11	26.83
Type of fracture in vault	Absent	15	36.59
	Linear	15	36.59
	Comminuted	11	26.83
Site of Fracture in vault	Absent	15	36.59
	Frontal	10	24.39
	Parietal	3	7.32
	Temporal	3	7.32
	Occipital	12	29.27
	All	4	9.76
Base of skull fractures	Absent	19	46.34
	ACF+MCF+PCF	4	9.76
	ACF	10	24.39
	MCF	6	14.63
	PCF	8	19.51

Diffuse Extravasation of Blood from Scalp was observed in 53.66% while from occipital are was seen in 26.83% victims. Linear Type of fracture in vault was seen in 36.59% of victims. Occipital and frontal bone fracture was observed in 29.27% and 24.39% victims respectively. While studying the Base of skull fractures it was seen that ACF was seen in 24.39% victims while PCF in 19.51% and MCF in 14.63% victims.

Table no.4: Distribution of cases according to details Brain Injury

		No. of victims	Percentage
Intracranial Haemorrhage	Absent	4	9.76
	EDH	0	0.00
	SDH	34	82.93
	SAH	37	90.24
	IVH	3	7.32
Type of Brain Injury	Absent	3	7.32
	Contusion	11	26.83
	Laceration	7	17.07
	Oedema	30	73.17
	Expelled out	4	9.76
Site of Brain Injured	Absent	3	7.32
	Frontal	19	46.34
	Temporal	7	17.07
	Parietal	0	0.00
	Occipital	4	9.76
	Base of Brain	0	0.00
	Diffuse	12	29.27

Most common intra cranial haemorrhage was Sub arachnoid haemorrhage (90.24%) followed by sub dural haemorrhage (82.93%).

Brain Oedema was seen in 73.17% victims while Contusion to brain was seen in 26.83% victims. Frontal lobe of brain was injured in 46.34% victims while diffuse brain injury was seen in 29.27% victims.

Discussion

The present study was conducted with the aim to study the pattern of injuries sustained in cases of four wheelers without wearing seat belt in fatal road traffic accidents in Mangalore.There is copious evidence that belt wearers are morecareful drivers than non-wearers.[9] When belted drivershave crashes, they are of lower severity than crashesof unbelted drivers.[10] Thus, the simple calculationincorrectly attributes to the reduction in injury due toseat belt, that are in fact caused by belted drivers beingin fewer, and less severe crashes. What other things aredifferent between persons who wear restraints and thosewho do not is 'Nearly everything'.[11]

It was observed that majority of the victims of four wheeler not using seat belt were male (85.37%) and in the age group of 30-39 Years and 40-49 Years (26.83% each). The similar results were drawn in the study conducted in Budapest, Hungary, where it was concluded that the Males outnumbered Females and the principal age group involved in fatal road traffic accidents was 20 to 29 years and 50 to 59 years with cases of 16.4% respectively[12]. In the YadukulS et al[13] study, majority of the victims were males constituting 94% of the cases and in age-wise distribution of cases, age group of 21-30 years (46.2%) followed by 31-40 years (21.4%) constitutes to about 2/3rd of the cases. Similar findings were noted in

Gururaj G et al[14] (84% males, 76% injuries were in 15-44 years age group), Arvind K et al[15] (88.22% males, 54.24% in the age group of 21-40 years), Singh B et al[16] (85% males, 58% in the age group of 11-40 years) and Mohammad KH et al (ref5-14) (76% males, 47% in the age group of 16-25 years).

There was a higher rate of face, head, and neck injuries in the present study. A possible explanation might be that motor vehicle occupants did not use seat belts, resulting in forward jerk during a collision and higher rate of injury. Abrasion (46.34%) was the most common Injury to face and head followed by laceration (29.27%) was observed. Singh, et al[17]also observed that abrasions and lacerations were the commonest types of injuries among the external injuries noted in this study. Similar results were also observed by Mehta SP. Et al[18] and Clark DW,et al[19].

Rib fractures was the most common associated injury (43.90%) while no associated injury was observed in 56.10%.These data were similar to the study done in Delhi, wherein most common injury was to the head followed by chest.[20]

Diffuse Extravasation of Blood from Scalp was observed in 53.66% while from occipital are was seen in 26.83% victims. Similar results were drawn from the study done in Bangalore, wherein scalp extravasation of blood was the most common external finding in 74 cases (45%).[21]Linear Type of fracture in vault was seen in 36.59% of victims. Occipital and frontal bone fracture was observed in 29.27% and 24.39% victims respectively. While studying the Base of skull fractures it was seen that ACF was seen in 24.39% victims while PCF in 19.51% and MCF in 14.63% victims. In contrary the study done in northeast Delhi, which had shown that posterior cranial fossa involvement in 40% of cases being common followed by anterior cranial fossa involvement in 20%.[22]

Most common intra cranial haemorrhage was Sub arachnoid haemorrhage (90.24%) followed by sub dural haemorrhage (82.93%). The present study showed the similar findings as seen by Dr V V Pillay.[23]Brain Oedema was seen in 73.17% victims while Contusion to brain was seen in 26.83% victims. Frontal lobe of brain was injured in 46.34% victims while diffuse brain injury was seen in 29.27% victims. While in a study done in Bangalore, showed diffuse involvement of brain in 39 cases (34%) followed by frontal lobe in 24 cases[21].

A retrospective study conducted by Arvind K et al[24] in New Delhi, northern India in year 2001-2005 was revealed that out of 7008 medico-legal autopsies conducted during the study period, 2472 (35.27%) were of vehicular accidents. Fatal traumatic brain injuries were seen in 1699 (68.73%) cases. Skull fractures were found in 1183 (69.63%) cases of head injury; common bone fractured was the temporal bone (47.25%). The most common variety of intra-cranial hemorrhage was sub-Dural hemorrhage (89.11%) cases. The craniotomy was done in 297 (17.48%) cases. Presence of skull fractures in cases of fatal head injuries is not a rule. In this study by Shobhana S. S et al[21] it was observed that linear fractures were the commonest fracture, accounting for 35.7% of cases. The findings were similar to the study done in Bangalore, where they observed that fissured fracture was the most common fracture in 41% of cases. Comminuted fracture was seen in 14.4% of cases in the present study which was in contrast to the study done in Bangalore wherein they observed comminuted fracture in 30% of cases.

RTAs (both crashes and injuries) are amenable toremedial action. Many developed countries havewitnessed drop in RTA and casualties by adoptingmulti-pronged approach to road safety that encompassestraffic management, appropriate infrastructure, roaddesigns, safer vehicles, law enforcement and provisionof accident care, etc. The challenge for us is to adapt andevaluate these approaches to suit our needs. The time forreforms is only now. Road safety laws should be properlyimplemented with emphasis on general awareness aboutsafety constraints and safe driving as primary preventivemeasures. This report may strengthen the database ofpolicy makers.

Conclusion

Thus we conclude that the four wheeler accident among the non seat belt users was common in middle aged males. Head injury and rib fracture was the most common type of injury observed. Liner fracture of skull with occipital and frontal bone involvement was common. Most common intra cranial haemorrhage was Sub arachnoid haemorrhage followed by sub dural haemorrhage. Brain Oedema and Contusion to brain

were common brain injury. Frontal lobe brain injuries were common followed by diffuse brain injury.

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Hypertension in Indonesia in 2018: An Ecological Analysis

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Abstract

Hypertension is a degenerative disease that is often found in conjunction with other degenerative diseases. The study was aimed at analyzing ecologically the factors related to the prevalence of hypertension in Indonesia. The study was conducted using secondary data from the 2018 Indonesia Basic Health Survey. All provinces were taken as samples. Apart from the prevalence of hypertension, 4 other variables analyzed as independent variables were the prevalence of obesity, the percentage of the population with less physical activity, the percentage of daily smokers, and the percentage of the population with salty food consumption habits ≥ 1 per day. Data were analyzed using a scatter plot. The results of the study found that the higher the prevalence of obesity in a province, the higher the prevalence of hypertension in that province. The higher the percentage of the population with less physical activity in a province, the higher the prevalence of hypertension in that province. The higher the percentage of daily smokers in a province, the higher the prevalence of hypertension in that province. The higher the percentage of the population with salty food consumption habits ≥ 1 per day, the higher the prevalence of hypertension in that province. It was concluded that the four independent variables analyzed ecologically were positively related to the prevalence of hypertension in Indonesia.

Keywords*: hypertension, ecological analysis, physical activity, healthy behavior.*

Introduction

Globally degenerative diseases such as cancer, diabetes, heart disease are the leading causes of death worldwide. Hypertension is one of the main dilemmas that lead to heart disease. Between 1990 and 2020, hypertension is estimated to increase by 120% in women, and 137% in men in developing countries in developed countries[1]. Hypertension causes death with the number reaching 10.4 million deaths per year in the world[2]. Hypertension or high blood pressure is defined as an increase in systolic blood of more than 140 mmHg and diastolic blood pressure of more than 90 mmHg at two measurements with an interval of five minutes in a well-rested state[3]. Currently, cases of hypertension in Indonesia are in a fairly large number with a prevalence of 34.11%[4]. This figure shows that one-third of Indonesia's population suffers from hypertension and has a risk of decreasing health status.

Hypertension is the biggest risk factor for cardiovascular disease. Hypertension is a condition in which the blood pressure in the arteries increases and causes the heart to work too hard. This disorder is popular as "the silent killer" because there are no specific symptoms in the early stages of its appearance. Hypertension rarely presents with specific symptoms, its identification is generally through screening or when a person seeks treatment for another disease[5]. Hypertension that does not get proper treatment or control can lead to cardiovascular system anomalies that endanger other vital organs in the body[6]. A prolonged increase in blood pressure can cause damage to the kidneys (kidney failure), heart (coronary heart disease), and brain (stroke)[3].

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If the right steps are not taken, the morbidity and mortality rate from cardiovascular disease will increase even more. Lack of attention to managing hypertension can have significant economic and social impact[7]. There are several micro and macro effects of the high prevalence of hypertension. If viewed from an economic point of view, the costs caused by hypertension include direct medical costs which are more expensive, and indirect costs include decreased productivity. These direct or indirect costs can be a significant economic burden[8]. Hypertension can cause a person to lose his/her workforce faster than people with normal blood pressure. Life expectancy in patients with hypertension is also shorter than in patients with normal blood pressure. Broadly speaking, the high prevalence of hypertension and the costs incurred in its treatment can have an impact on the Gross National Product and the inflation rate of a region or country[9].

Hypertension control can be done with the use of drugs and lifestyle modifications[3]. Modifications to a healthy lifestyle can prevent or delay hypertension and reduce cardiovascular risk. Changes in a healthy lifestyle are at the forefront of anti-hypertension therapy[2]. Based on this background, this study aims to analyze ecologically the factors related to the prevalence of hypertension in Indonesia.

Materials and Methods

Study Design

The study was conducted using an ecological analysis approach. The ecological analysis focuses on comparisons not individually but between groups. In the ecological analysis, the data analyzed is aggregate data at a certain group or level, in this study, it is the provincial level[10]. The variables in an ecological analysis can be aggregate measurements, environmental measurements, or global measurements. The purpose of the ecological analysis in epidemiology is to make biological inferences about effects on individual risk or to make ecological inferences about effects on groups[11].

Data Source

The study was conducted using secondary data from the 2018 Indonesia Basic Health Survey report. The 2018 Indonesia Basic Health Survey report is an official publication from the Ministry of Health of the Republic of Indonesia. The unit of analysis in this study is the province. All provinces in Indonesia were analyzed (34 provinces).

Data Analysis

The dependent variable in this study was the prevalence of hypertension. Hypertension was recorded based on the doctor's diagnosis history (internist and general practitioner)[4]. There were 4 independent variables analyzed in this study, namely prevalence of obesity, percentage of the population with less physical activity, percentage of daily smokers, and percentage of the population with salty food consumption habits ≥ 1 per day.

Data were analyzed bivariate using a scatter plot. The linear fit line was used as the basis for determining the tendency of the relationship between the prevalence of hypertension and the independent variable. The analysis in this study was carried out with SPSS 21 software.

Results and Discussion

Table 1 presents descriptive statistics of the prevalence of hypertension and other variables analyzed in this study. The information presented informs that the lowest prevalence is 4.4%, while the highest prevalence is 13.2%. The range of prevalence of hypertension among provinces in Indonesia is quite wide. Meanwhile, the prevalence range or the percentage of other variables also appears to be quite high. For example, in the variable percentage of the population with salty food consumption habits ≥ 1 per day, the range is between 7.0% -54.1%.

Table 1: Descriptive Statistics of Prevalence of Hypertension and Related Variables by Province in Indonesia, 2018

Descriptive Statistics	Prevalence of Hypertension	Prevalence of Obesity	Percentage of Population with Less Physical Activity	Percentage of Daily Smokers	Percentage of Population with Salty Food Consumption Habits ≥ 1 per day
N	34	34	34	34	34
Mean	8.181	21.703	34.879	23.494	20.891
Median	8.245	21.050	33.950	23.350	20.450
Mode	4.4a	18.7a	33.7	22.1a	13.2
Std. Deviation	1.8755	4.2801	5.7920	2.6014	10.0377
Range	8.8	19.9	22.6	9.3	47.1
Minimum	4.4	10.3	25.2	18.8	7.0
Maximum	13.2	30.2	47.8	28.1	54.1

Source: The 2018 Indonesia Basic Health Survey

Figure 1 shows a map of the prevalence of hypertension by the province in Indonesia. Based on this spatial information, it can be seen that the prevalence of hypertension tends to be lower in Eastern Indonesia. It can be seen that the provinces of Papua, North Maluku, Maluku, and East Nusa Tenggara, have a lower prevalence of hypertension.

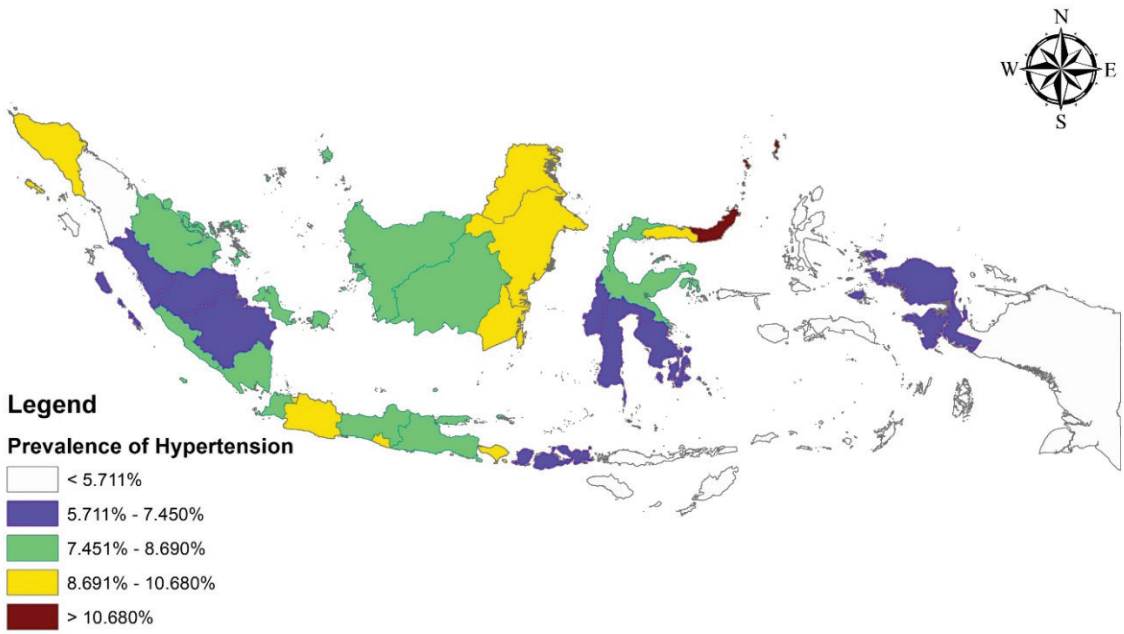

Figure 1. Map of the Prevalence of Hypertension by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

Figure 2 is a scatter plot of the prevalence of obesity and the prevalence of hypertension by the province in Indonesia. It can be seen that the relationship between the two variables shows a positive trend. This means that the higher the prevalence of obesity in a province, the higher the prevalence of hypertension in that province.

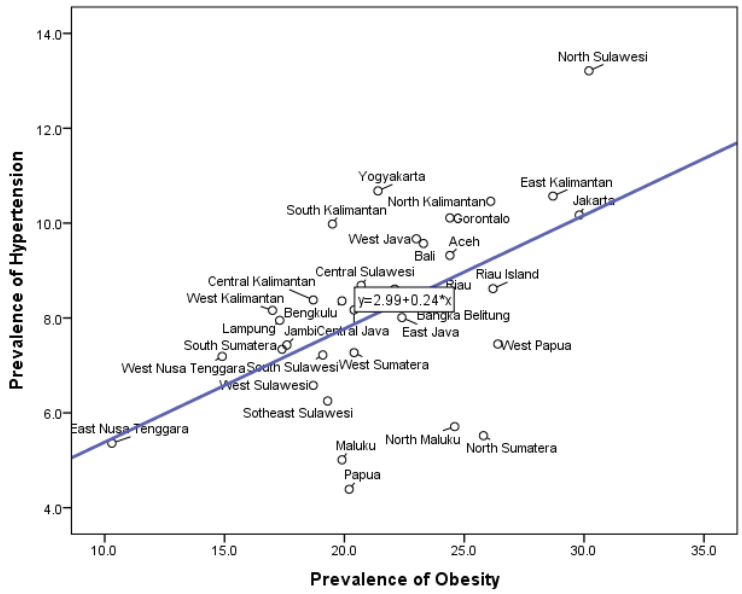

Figure 2. Scatter Plot of Prevalence of Obesity and Prevalence of Hypertension by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

The scatter plot of the percentage of the population with less physical activity and the prevalence of hypertension by the province in Indonesia can be seen in Figure 3. The results of the scatter plot indicate that the two variables tend to have a positive relationship. This means that the higher the percentage of the population with less physical activity in a province, the higher the prevalence of hypertension in that province.

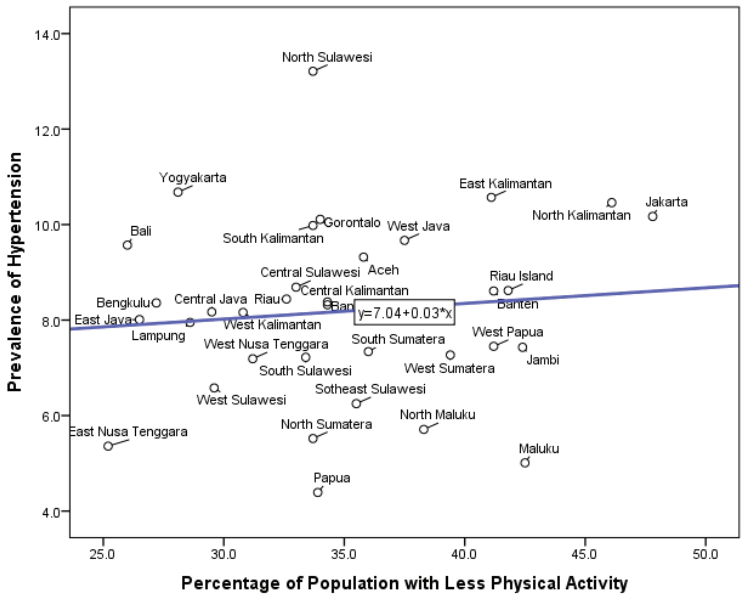

Figure 3. Scatter Plot of Percentage of Population with Less Physical Activity and Prevalence of Hypertension by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

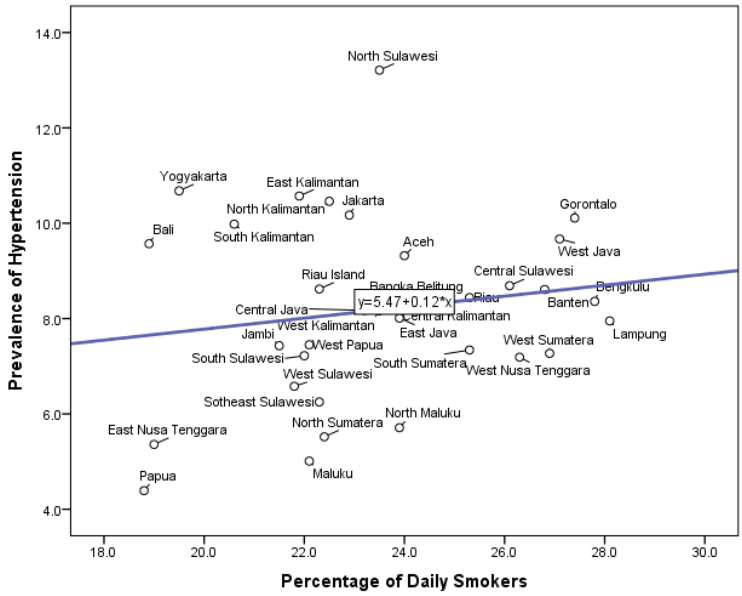

Figure 4. Scatter Plot of Percentage of Daily Smokers and Prevalence of Hypertension by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

Meanwhile, Figure 4 is the scatter plot of the percentage of daily smokers and the prevalence of hypertension by the province in Indonesia. Based on the scatter plot, it can be seen that the relationship between the two variables shows a positive trend. This means that the higher the percentage of daily smokers in a province, the higher the prevalence of hypertension in that province.

Moreover, the relationship between the percentage of the population with salty food consumption habits ≥ 1 per day and the prevalence of hypertension by the province in Indonesia can be seen in Figure 5. Based on the scatter plot, it can be seen that the relationship between the two variables shows a positive trend. This means that the higher the percentage of the population with salty food consumption habits ≥ 1 per day in a province, the higher the prevalence of hypertension in that province will be.

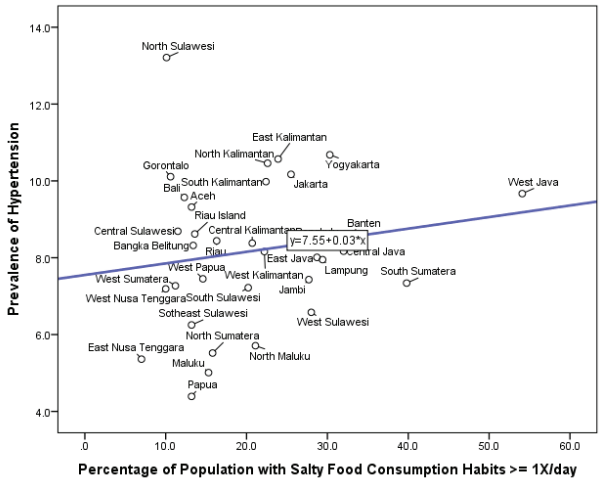

Figure 5. Scatter Plot of Percentage of Population with Salty Food Consumption Habits ≥ 1 per day and Prevalence of Hypertension by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

The information found in this study is consistent with the results of previous studies in Tanzania, Kenya, and South Africa. It is informed that in the study that obesity or overweight, low physical activity, and consumption of fatty foods increase the risk of hypertension[12–14]. Not only in adults, but previous studies have also informed that obesity in children and adolescents has also been found to increase the risk of hypertension[15]. On the

other hand, besides physical activity, risky lifestyles such as smoking and drinking alcohol are also reported to increase the risk of hypertension[16,17].

Although it has limitations because it is carried out by ecological analysis, the information on the results of this study is useful for the government in providing policy directions to reduce the prevalence of hypertension in Indonesia[18,19]. The government must continue to encourage people to have a healthy lifestyle and promote prevention efforts by doing enough physical activity, not smoking, and reducing salty food consumption to the recommended safe limit.

Lifestyle modification in society requires the participation of all parties. Prevention and control of hypertension in society are very complex and requires collaboration across stakeholders including government, academia, society, and others.

Conclusion

Based on the results of the study, it could be concluded that the four independent variables analyzed ecologically were positively related to the prevalence of hypertension in Indonesia. The four variables are the prevalence of obesity, the percentage of the population with less physical activity, the percentage of daily smokers, and the percentage of the population with salty food consumption habits ≥ 1 per day.

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Ethical Clearance: The study was conducted by utilizing secondary data from published reports. For this reason, ethical clearance is not required in the implementation of this study.

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The Effectiveness of Olive Oil to Combat Lice in Pregnant Women and Children

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Abstract

For the period from February 2019 until August 2019 it was checked 75 cases of pregnant women and children included girls and boys, and 28 pregnant women, 42 girls and 5 males were found to be infected . Head louse , as the study showed that the highest incidence of head louse was recorded among girls , it reached 56% , followed by pregnant women with a percentage of infection 37.33% , while males recorded less infection with head louse amounted to 6.66% , respectively , and olive oil was used to determine its effectiveness to combat head louse as a natural substance , and it used Statistical analysis was used Dunkin test .

Keyword : *Lice , Olive oil , Pregnant , Children*

Introduction

Pediculus humanus is a small insect the compact area of the dorsal and ventral region is lost to the wings . This insect hatch from eggs called nits . The infestation of them is called *Pediculus humanus capitis* . Which are attached to the individual hairs near the scalp . Nits can be found all over the hair , often located at the back of the scalp , behind the ears and the top of the head . Head louse not live more than 3 days away from warmth and moisture in the scalp . Head louse hatch in a period of 7 to 10days [1][2] . Female lice can live for 21-30 days , and lay about 6-8 eggs a day . This insect lives by biting and sucking blood from the scalp [3]. The most common symptom of lice infestation is itching. Other signs and symptoms may vary depending on the type of lice [4]. Severe itching from lice infestation can cause skin infections , especially when the itching is severe and persistent [5] . The olive tree is one of the perennial trees and is considered a wealth due to its economic and environmental benefits . Its fruit has many benefits , it is a complete food , and olive oil is extracted from it with health and nutritional benefits , as it does not contain cholesterol that is harmful to the heart [6] . In addition to auto oxidation and fat oxidation genes . The Olive oil is easy to digest , light and preserves . Beauty and eyeglasses are recommended for use in cooking food and add it to fresh vegetables as it is reduces blood sugar [7] .

Material and Method

For the period from February 2019 until August 2019 . Examining 75 cases of pregnant women and children in the city of Kirkuk . To find out the prevalence of *Pediculus humanus capitis* , some information was recorded about each case upon examination , such as gender , area of residence and climate factor , according to the most famous study . Fully examine the hair from the scruff and behind the ears with the naked eye , using the magnifying glass when needed . The insect has collected in a 10 mm bottle , contain 70% alcohol and forceps for the purpose of dispersing hair , thin wooden sticks were used to facilitate the process . It used olive oil to eliminate head louse , by painting the scalp well with olive oil, and covering all hair follicles with it to ensure oxygen cut off . Here's how to use olive oil : Shampoo and clean hair thoroughly . Ensure that the hair is cleaned of any other greasy substance , as it prevents olive oil from sticking to the hair and scalp , thus reducing the effectiveness of the olive oil recipe . If the hair is thick and long , separate it into tufts to make it easier to apply

it well , apply olive oil to the hair and scalp , and ensure that the oil covers all areas of the head . Tie the hair , wrap it and cover it with a cap , and make sure that the cap is narrow enough to prevent the lice from trying to escape from underneath , and leave the hair covered with oil for 4 to 6 hours . After this period expires , remove the cap from the hair and comb it with a special comb . Wash hair with shampoo twice , and clean it of the effects of olive oil . Dry hair well by dividing the hair into several sections to make it easier to dry it with a hair dryer and ensure that the scalp is dried . And repeat this method two or three times during the week . Statistical analysis was used Dunkin test .

Result and Discussion

Of the 75case included in the study , 28 were pregnant and 47children (42 girls & 5 boys) , (Table1) . The prevalence of infestation was significantly higher among girls (56%) than (37.33%) pregnant and boys (6.66%) .This increase in pregnant women is due to the ease of movement of lice between mothers and affected children and the difficulty in dealing with them as a result of the physiological condition experienced by the mother [8][9] , And that the difference in the rates of head louse infection between the sexes is not due based on physiological basis , it depends on the difference in hairdo and hair length where long hair is a safe haven for lice , through other studies conducted in Jordan [10] , boys have a different behaviour , as they have a short contact during daily play and sporting activities , while girls have a desire to direct and prolonged contact in small groups . Head louse are not a sign of people or impure homes . It can occur at any age and gender , whether male or female . Anyone who has close contact with an infected person or shares personal items can become infested [11] [12] . As for the (Table2) . Compare the treatments within each category , the results were as follows in pregnant women (37.5%) , for girls (55.55%) and for males (6.944%) . He knows that a woman's body is exposed to many changes during pregnancy , and those changes affect her scalp as her hair becomes weak and fast falling out , Lice treatments may adversely affect the safety of pregnancy and the fetus , as most of these medical treatments contain toxic chemicals . For the safety of the pregnant mother [13] . Olive oil has been used that is easy to digest and preserves beauty and skin glasses . And olive oil is used to nourish the scalp where it works to soften and ease the demobilization of hair as it works to kill the insect that the use of olive oil bridges the gaps through which the lice breathe . The cause of the lice 'death is due to the action of some plant compounds in killing the epithelial cells lining the insect's skeletal channel . It has also been used for children because their skin is thin and protect them from exposure to toxic chemicals [7][14] . The values followed by the same letter are not significantly different according to the Dunkin test (P≤ 0.05) .

Table No. 1. Ratios of head louse infection in pregnant women and children in Kirkuk

Sex	The injured	%
Pregnant	28 b	37.33
girls (6-9) years	42 a	56
boys (6-9) years	5 c	6.66
Total	75	

The values followed by the same letter are not significantly different according to the Dunkin test (P≤ 0.05).

Table No.2.Treating lice with olive oil in pregnant women and children in Kirkuk (Compare the treatments within each category)

Sex	The injured	Use olive oil	%
Pregnant	28 a	27 b	37.5
girls (6-9) years	42 a	40 b	55.55
boys (6-9) years	5 a	5 a	6.944
Total	75 a	72 b	

The values followed by the same letter are not significantly different according to the Dunkin test (P≤ 0.05).

Conclusion

After representing these results , we concluded that head lice that affect pregnant women and children can be cured by using olive oil as a natural substance .

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Profile of Determinant Factors on Drug Allergy Severity in Indonesian Children at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

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Abstract

Background: Drug allergy in children is a rare case that needs special attention. Identifying the factors that affect the severity of drug allergy is important for the improvement of drug allergy management in Indonesian children. **Objective**: to analyze the factors that influence drug allergy severity in Indonesian children. **Methods**: This study used a retrospective design and consecutive sampling in 2014-2018. The data collected included sex, types of allergy-causing drugs, previous allergy history, parental allergy history, and clinical manifestations. The data were analyzed by using a logistic regression test where the statistical test results were significant if $p < 0.05$. **Results**: The average age of the participants was 93.00 ± 50.54 months with a median value of 84 (12 - 204) months. Drug allergy severity in children is influenced by several things, such as the type of drug ($\beta = 5.76$; 95% CI 1.29 - 25.70; $p = 0.022$), sex ($\beta = 1.27$; 95% CI 0.30 - 5.36; $p = 0.743$), previous allergy history ($\beta = 2.44$; 95% CI 0.10 - 58.67; $p = 0.583$), and a drug allergy history on parents ($\beta = 0.65$; 95% CI 0.06 - 6.75; $p = 0.719$). **Conclusion**: There is a significant relationship between drug allergy severity and the type of drug whereas sex, previous allergy history, and a history of allergy on parents do not find a significant connection.

Keywords: *drug allergy, Indonesian children, severity*

Introduction

Drug Allergy is a serious health problem in children which is often causing concern for parents [(1)]. Drug hypersensitivity reactions cannot be predicted beforehand so it is rarely known the initial phase of the allergy reaction. This hypersensitivity reaction can also be life-threatening in high-grade drug allergies [(2, 3)]. Based on a survey conducted by the World Allergy Organization in 2011, the distribution of drug allergy in the world is as follows: Europe (18.2%), Asia Pacific (19.5%), North America (7.8%), Latin America (9.1%), and Africa Middles East (3.9%) [(4)]. The number of drug allergy in Indonesia is still not reported yet; the latest data only shows that from 2009 to 2013 in Bandung. Moreover, in Indonesia, which reported as many as 57 cases of drug allergy and children are still very rarely reported [(5, 6)].

The cases of children's drug allergy were reported as 6.5% of the total number of cases of drug allergy in all hospitals [(7)]. Management of drug allergy in children requires a caregiver role, in addition to medical management, it is also necessary to identify the factors that affect the severity of drug allergy symptoms in children [(8)]. Several factors need to be considered by

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medical staff including sex, type of drug that causes the allergy, previous history of allergy, history of allergy from parents, and clinical manifestations. Sex is a factor that affects drug allergies, which is in allergies, sex hormone receptors on lymphocytes, and leukocytes can modulate the type of immune reaction and regulate inflammation [9]. Currently, most of the types of drugs that cause allergies are the antibiotic class [5, 6]. Previous allergy history is a condition that is considered by medical staff in providing therapy to children with drug allergy [10]. Allergy history in parents based on previous research states that children who have parents with allergy have a higher risk of having the same allergy [11].

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia is the main referral hospital in the East Java Region. Indonesia shows that the number of pediatric patients with drug allergy has increased every year and the research that reports on the distribution of drug allergy cases in Indonesia are still very small. Based on the description above, analysis is needed to determine the factors that affect the severity of drug allergy in Indonesian children.

Materials and Methods

Participant

Participants in this study were the children with drug allergy that met the inclusion and exclusion criteria. Patient of inclusion criteria was patients that diagnosed with drug allergy according to guidelines [12, 13], aged >18 years, and patients undergoing hospitalization. Patients of exclusion criteria were patients having infectious diseases (measles, rubella, and staphylococcal scalded skin syndrome), immunodeficiency disease (HIV), and congenital disorders (Down syndrome). Caregiver/ parent participant gets an explanation regarding the research objectives and completes the informed consent form before using participant data.

Design

This study used a retrospective design that applied the consecutive sampling method. The data used in this study were patients from 2014 to 2018, which obtained 36 participants. This research was conducted in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia where this research was conducted with ethical approval prior to its implementation. The procedure of this study included collecting the medical records of participants who had been hospitalized. The data collected included sex, types of allergy-causing drugs, previous allergy history, parental allergy history, and clinical manifestations. The clinical manifestations of drug allergy are categorized into 2, namely: severe and non-severe [8]. After data collection and some incomplete data, the researcher contacted the caregiver/ parent participant to use the data and complete the missing data.

Statistical Analysis

The measurement of data results was analyzed by using the data entered into the IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). The data were analyzed by using a logistic regression test where the statistical test results were significant if $p < 0.05$.

Result

Participant Characteristics

The mean age of the participants was 93.00 ± 50.54 months with a median value of 84 (12 – 204) months. There will be a maximum number of participants aged 5-7 years, for 5 participants each. Most of the participants who experienced allergic reactions caused by non-antibiotic drugs were 52.78%, while participants who experienced drug allergy were male by 52.78%. Most of the participants did not have a history of atopy was 63.89% and only 8.33% of the participants' parents had a history of atopy. Then, most of the participants did not have a history of drug allergy was 88.89% and the majority of the participants' parents also did not have a history of drug allergy was 94.44%. Most of the participant's experienced clinical manifestations in the severe category were 55.56% (Table 1).

Profile of drugs cause drug allergy

Most of the participants who experienced drug allergy caused by antibiotic drugs were 47.22%, of which most were caused by amoxicillin (35.29%) and cotrimoxazole (29.41%). In the second position, NSAID types drug were the cause of the most drug allergy by 19.44%, of which 77.78% were mostly caused by paracetamol. Details of the types of drugs and classes

of drugs that cause drug allergy to the participant can be seen in Table 2.

Analysis of factors that influence the severity of drug allergy

Drug allergy severity in children is influenced by several things, namely the type of drug (β = 5.76; 95% CI 1.29 – 25.70; p = 0.022), sex (β = 1.27; 95% CI 0.30 – 5.36; p = 0.743), Previous allergy history (β = 2.44; 95% CI 0.10 - 58.67; p = 0.583), and a history of drug allergy in the parents (β = 0.65; 95% CI 0.06 – 6.75; p = 0.719; table 3). Based on the results of the analysis, the factors that affect the severity of drug allergy are the types of drugs that cause allergy itself.

Table 1. Participant Characteristics

Variables	n (%)
Hypersensitivity reactions	
Antibiotics	17 (47.22)
Non-antibiotics	19 (52.78)
Sex	
Male	19 (52.78)
Female	17 (47.22)
Atopy's children history	
Yes	13 (36.11)
None	23 (63.89)
Allergy history	
Yes	4 (11.11)
None	32 (88.89)
Drug Allergy in parent's history	
Yes	2 (5.56)
None	34 (94.44)
Atopy in parent's history	
Yes	3 (8.33)
None	33 (91.67)
Drug Allergy Clinical Manifestations	
Severe	20 (55.56)
Non Severe	16 (44.44)

Table 2. Allergy-causing drugs

Drug Classification	Types of drugs	n (%)
Antibiotics	Amoxicillin	6 (16.67)
	Thiamphenicol	3 (8.33)
	Cefriaxone	1 (2.78)
	Cotrimaxazole	5 (13.89)
	INH (Isoniazid)	2 (5.56)
NSAIDs	Ibuprofen	2 (5.56)
	Paracetamol	7 (19.44)

Cont... **Table 2. Allergy-causing drugs**

Anticonvulsants	Phenytoin Carbamazepine	5 (13.89) 2 (5.56)
Antidepressants	Amitriptyline	1 (2.78)
Chemoteraphy	Doxorubicin Leunase	1 (2.78) 1 (2.78)

Table 3. Correlation of determinant factors on drug allergy severity

Variables	**Drug of allergy severity**		
	β	**95% CI**	**p**
Types of drugs	5.76	1.29 – 25.70	0.022*
Sex	1.27	0.30 – 5.36	0.743
Allergy history in the parents	2.44	0.10 – 58.67	0.583
Previous Allergy History	0.65	0.06 – 6.75	0.719

Note: *significance $p < 0.05$

Discussion

There are 3 types of drug classification that most often cause drug allergies, including antibiotics, NSAIDs, and anti-congestion. Based on several previous studies, it was stated that antibiotics were the most common cause of drug allergy [14, 15]. Besides antibiotics, drug allergy caused by NSAIDs is relatively high. Based on previous research, it was reported that 20% of drug allergy cases were caused by NSAIDs [16]. Another study states that antibiotics and anti-consuls are drugs that often trigger hypersensitivity reactions [17].

In general, the mechanism that causes drug allergy is divided by IgE-mediated, which in this pathway will get the emergence of inflammatory mediators and non-mediated IgE. There are 3 reaction pathways, namely the first is cytotoxic/cytolytic originating from IgM or IgG reactions that react with complement. Second, there is the Drug Immune complex which can occur due to reactions with foreign proteins such as streptokinases, antitoxins, and antivenins. The third is a reaction pathway involving a specific T cell effector antigen in which the hypersensitivity response can be transferred to purified T cells or T cell clones. This reaction only appears after 24 - 72 hours which is often referred to as a slow type reaction [15, 18].

Based on the results of this study, it was concluded that the number of male participants was higher than female. This result is inconsistent with previous studies which stated that drug hypersensitivity reactions were more common in females. Previous research stated that based on the Health Insurance Review and Assessment (HIRA) database in Korea from 2009 to 2014, the number of females who experienced drug allergy was higher than male, which were 53,734 (57.55%), 52,139 respectively (57.90%), 52,028 (57.71%), 50,430 (57.27%), 49,992 (57.37%), and 49,464 (57.33%) [19]. Based on clinical observations, the relationship between hormonal status and allergic reactivity has been known for decades. In allergies, sex hormone receptors on lymphocytes and leukocytes can modulate the type of immune reaction and regulate inflammation. It is known

that estrogen exerts an effect on receptor-mediated mast cell release by affecting the threshold level in the allergy effector phase [9]. Hospitalized females were statistically and significantly more likely to experience drug allergy than male, although there were no significant differences in clinical manifestations and mortality between the sexes [4].

Several previous studies stated that atopy is a predisposing factor for drug allergy. Patients who use NSAID class drugs have a higher risk of drug allergy than patients without a history of atopy. In patients with a history of atopy, it is necessary to use alternative drugs that are used such as paracetamol and nimesulide [20, 21]. Recent research states that atopy patients are advised not to use penicillin-type antibiotics because they are proven to have a high risk for anaphylaxis [15, 18].

Previous studies stated that children with parents who have a history of allergy are more at risk of experiencing allergies than children without allergy history. Previous research states that children whose parents have a history of allergies to antibiotics have a 15x greater risk than children with parents without allergies [22]. A study of suspected drug allergic reactions in 97 children concluded that a family history of drug allergy was significantly associated with confirmed allergic drug reactions [11]. There is also a multicentre study conducted in Spain, reporting that patients with a family history of drug allergy are more likely to be allergic to some drugs [23].

Conclusion

Most children with drug allergy experience severe manifestations, most of them are male. Drug allergy severity in Indonesian children is influenced by several things, namely the type of drug, sex, previous history of allergy, and history of drug allergy in the parents. However, what is proven significantly in this study is only the types of drugs that cause drug allergy.

Ethical Approval: This research has gone through the ethical test stages based on the Declaration of Helsinki at Ethics Committee Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1739 / KEPK / XII / 2019).

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***Type of Manuscript**: Short Theoretical Article*

Early Surgical Management and Triple Antibiotic Regimen in the Management of Necrotizing Fasciitis of Scalp

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Abstract

Necrotizing fasciitis (NF) is a rare yet alarming condition faced by a maxillofacial surgeon. This article would emphasize the importance of early aggressive surgical management with triple antibiotic regimen of penicillinase-resistant penicillin, aminoglycoside and metronidazole in the successful management of NF. We would also like the emphasize the importance of 1% hydrogen Peroxide + Saline + Betadine followed by copius irrigation with Betadine+Saline + Metronidazole in NF along with a negative pressure drain to manage NF effectively.

***Key Words:** Necrotizing Fasciitis, Scalp, Antibiotics*

Necrotizing fasciitis (NF) is a rare infection of the deep dermis and subcutaneous tissue, that may lead to necrosis of subcutaneous tissues including the muscle layer, further progressing to septic shock and multi-organ failure[1]. In the 1990s, NF was popularly known to be infection of tissues infected with "flesh eating bacteria." Historically, group A beta-hemolytic *Streptococcus has been associated with NF*[2].However it is now known to be usually multi-microbial infection. Necrotizing fasciitis has been discussed as a syndrome predominantly due to polymicrobial infection involving pathogens such as *Streptococcus pyogenes* and species of *Bacteroides*, *Clostridium, Peptostreptococcus* and members of *Enterobacteriaceae*; most commonly affecting the abdomen, perineum, and extremities[3]. NF in the head and neck region is rare. Scalp is rarely affected by NF and when it does occur it is usually seen in neonates[4].

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Aggressive and fatal NF is usually a result of either late diagnosis, underestimation of the infection and septic complication[1]. In spite of advanced medical treatment, the mortality rate ranges from 24 to 34%[5]. This case report will highlight the importance of early aggressive debridement with triple antibiotic regimen in the management of NF.

Case Presentation

Patient was referred to our tertiary care center from a local hospital for management of deep infection of the scalp with associated fascial swelling and pyrexia, with an alleged history of slip and fall on the road 10 days back. This resulted in a lacerated wound of the scalp that was primarily sutured in the local hospital. Three days' post suturing, patient developed swelling at the wound site for which he was administered ceftriaxone and salbactum for 6 days, with no improvement. Thus patent was referred to us for further management. He presented to us with a gaping wound with pus discharge in the fronto-parietal area associated with swelling of the scalp which was tender on palpation. (Figure 1) It was pitting and doughy in consistency with surrounding cellulitis. Swelling spread up to the right periorbital

region with normal ophthalmic findings. CT scan showed diffuse soft tissue swelling with subcutaneous edema involving the right fronto-temporal region with a small area of fat density (-100HU) in the frontal region. Hypo-densities within the right preseptal region were observed likely to be edema. Both globe, intra-ocular lens and retrobulbar spaces appeared to be normal. Preoperative random blood glucose level was 504mg/dl with 10.4% glycosylated hemoglobin. Total leucocyte count was 22,200cells/mm^3 and all other biochemical and neurological findings were within normal limits.

Patient was planned for surgical drainage and exploration under empirical antibiotic cover of Inj. Cefotaxime 1gm i.v. TID and Inj. Amikacin 500mg i.v. BD. Glycemic control was achieved with injection Apidra (insulin glulisine) 10 units subcutaneous preoperatively.

A bicoronal approach was used to expose necrotic muscular and subcutaneous tissue (Figure 2). Necrosis of the subcutaneous tissues extended beyond the occiput posteriorly, till the supra-orbital rim anteriorly and the superior temporal lines on either sides. Thorough debridement was done with removal of all the necrotic tissues (Figure 3) and irrigated with normal saline + betadine + 1% H_2O_2 solution followed by copious irrigation with saline + betadine + metronidazole. Primary suturing was done after freshening of the margins. Wound was closed with negative pressure drain in place which was removed after 72hours. The debrided tissue along with pus was sent for culture and sensitivity. Gram staining revealed gram positive cocci in pairs $^{+++}$. Injection Apidra was continued for glycemic control postoperatively as per Q8H GRBS. Growth of Enterococcus faecalis was seen in conventional aerobic culture. Based on culture and sensitivity report, Inj. Amoxiclav 1.2gm i.v. TID, Inj. Gentamicin 80mg i.v. BD and Inj. Metronidazole 500mg i.v. TID was administered with regular wound care for 10 days. Patient recovered without any morbid status with total leucocyte counts within normal limits on postop day 10. Hence he was discharged with oral Amoxiclav 625mg TID for 3days. On regular follow up there were no signs of relapse with satisfactory wound healing at 1-month post op (Figure 4).

Figure 1 Photograph showing a tender swelling on the scalp

Figure 2 Photograph showing a bicoronal approach exposing the necrotic muscular and subcutaneous tissue

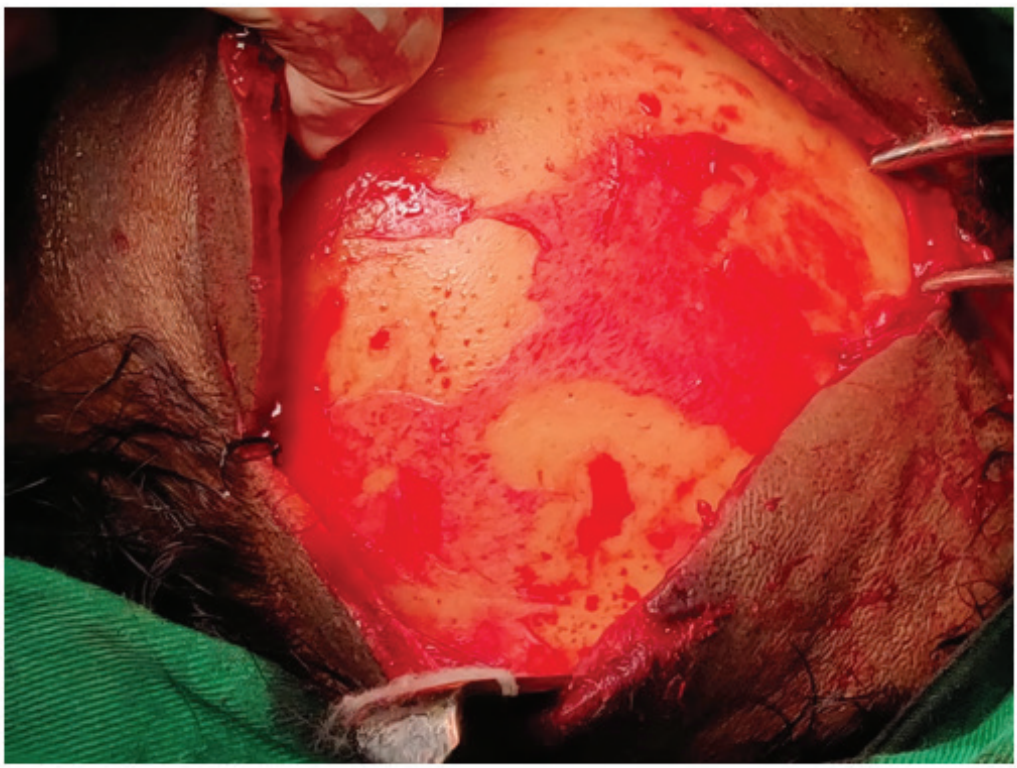

Figure 3 Photograph showing the affected area after debridement and removal of all the necrotic tissues

Figure 4 Photograph taken at the 1- month post-op review showing satisfactory wound healing

Discussion

NF is generally associated with immunocompromised individuals, commonly affecting limbs and perineum. Most NF in the head and neck region is due to odontogenic pathologies, and those involving the scalp are rare and is generally seen in neonates[1,4]. Keeping in mind the immunocompromised status and an unusual presentation of a minor laceration, we decided to debride the wound. A variety of solutions have been proposed for irrigation of wounds, including 1% hydrogen peroxide with normal saline, eusol and betadine[6]. We used 1% hydrogen peroxide + normal saline + betadine followed by copious irrigation with saline + betadine + metronidazole for irrigation and wound care. Since, NF is known to be a mixed aerobic and anaerobic polymicrobial infection with both gram positive and gram negative organisms been identified, triple antibiotic regimen of

penicillinase-resistant penicillin, an aminoglycoside and clindamycin/metronidazole has been well accepted for management of necrotizing fasciitis[1,6]. Also the use of negative pressure wound dressing emphasized by many authors, may have added to the success of our treatment outcome[7,8]. We believe that the early aggressive wound debridement with removal of all the necrotic tissue and use of triple antibiotic regimen were key factors in the successful treatment outcome of this case.

Conclusion

Necrotising fasciitis is a rapidly progressing infection, rarely encountered by a maxillofacial surgeon. These cases require an early diagnosis to manage them promptly and effectively. We recommend early aggressive surgical debridement with negative pressure closure, along with triple antibiotic regime of penicillinase resistance penicillin, an aminoglycoside and metronidazole, followed by good wound care for the management of necrotizing fasciitis.

Ethical Clearance- Taken from Institutional Ethics committee of MCODS, Mangalore

Conflict of Interest - Nil

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Relationship between Blood group and Chronic Myeloid Leukemia with the Role of the Oxidative Stress

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Abstract

Chronic myeloid leukemia characterized by the expansion of a clone of hematopoietic cells that carries the Philadelphia chromosome (Ph). The aim of study are to find if there is an association between blood groups and chronic myeloid leukemia, and to determine the role of the oxidative stress as risk factor. Ninety persons included in the study (45 as patient and 45 as control group). Patients were suffering from CML, the control are free from this disease. There are ranged from (21-60) years old where they are divided in to four group according to blood group (A, B, AB, and O). The sera were collected from the 90 persons and analyzed by using enzyme linked immunosorbent assay (ELISA) for MDA and GSH. The results of the study show that blood group O (44%) predominates in CML patients group followed by blood group A (22%), B (18 %) and AB (16%). The mean serum level MDA value shows highly significance at ($P<0.001$) change in the patients group in comparison with control. Also show that the level of MDA in male (900. 99 ± 603.89) is significantly higher than in female (582.28 ± 562.29). The mean serum level of GSH show that significant decrease at ($P<0.05$) in patients group (40. 12 ± 27.33) than in control groups (55.20 ± 28.79). As a conclusion from this study, the importance of MDA and GSH in CML patients can be used to detect the complications related to the among these patients.

Keywords *: ABO Blood Groups, Chronic myeloid leukemia, Oxidative stress*

Introduction

Chronic myeloid leukemia is a clonal my elopro life rative disorder of primitive hematopoietic progenitor cells. The BCR-ABL tyrosine kinase produced by the t(9;22)(q34;q11) translocation fuses the parts of the q arm of chromosome 9 to the q arm of chromosome 22, creating a hybrid BCR-ABL gene, also known as the Philadelphia chromosome and initiates the event of CML [(1)]. The mechanism whereby the (9:22) translocation occurs is unknown, although some studies suggest that oxidative stress may be involved in the genesis of CML. Moreover, BCR-ABL positive cells are a source of reactive oxygen species (ROS), which cause oxidative DNA damage and may contribute to the formation of additional abnormalities leading to disease progression to the more advanced stages (accelerated and blast crisis phases) [(2)]. Chronic myeloid leukemia occurs very rarely in children. In the Western world, the median age of onset is 50–60 years, which reflects the average age of the population. Although symptoms at presentation may include lethargy, weight loss, unusual bleeding, sweats, anemia, and splenomegaly, in more developed countries, 50% of patients are asymptomatic and are diagnosed as a consequence of blood tests performed for unrelated reasons. The incidence of CML is approximately 1-2 per 100,000 population per year. Males are affected more than females in CML, with a 2:1 ratio [(3)]. Oxidative stress is often described as a self-propagating phenomenon on the basis of observations that when oxidative stress–induced excessive ROS release triggers cellular damage,

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damaged macromolecules themselves may behave as and/or become ROS. Oxidative phosphorylation occurring in the mitochondria is a major source of ATP. As a by-product, this process produces free radicals or reactive oxygen species (ROS), reactive nitrogen species (RNS), and carbon- and sulfur-centered radicals [4]. The ABO blood group system consists of four basic groups, namely A, B, AB, and O, depending on the presence of the A and B antigens. These antigens are controlled by three allelic A, B, and O genes located on the long arm of chromosome 9 . The blood groups in the Rhesus system are classified as Rh− and Rh+, depending on the presence of the Rhesus D antigen located on the red blood cell surface [5]. The ABO blood group has been associated with a number of diseases or hemostatic complications. For example, meta-analyses have detected associations between increased risk of coronary heart disease, and venous thromboembolism and the non-type O blood types [6]. The first report describing a link between the A antigen and increased risk of stomach cancer was published in 1953 [7]. Other recent studies have reported the association between blood groups O and A individuals with increased incidence of duodenal ulcers and gastric carcinoma as well as, the association of B group type and pancreatic cancer, Hodgkin's lymphomas and cardiac cancer. Therefore, blood group antigens on the surface of cancer cells can be used as useful prognostic and diagnostic markers in different types of human cancers [7].

Materials and Methods

Patients

A case control study conducted at Middle AL-Furat Center for cancers in Al-Najaf Al-Ashraf city-Iraq, from the period of November (2018) to April (2019). This study include (45) patients (Male 30 and female 15), who were selectively collected and were suffering from chronic myeloid leukemia that sample taken. Their ages ranged from (20 to 60) year old. Patient are divided into four groups according to blood type (group A, group B, group AB, group O) and divided according age groups into two groups (20-40 and 41-60).

Control

Forty-five apparently healthy were selected as the control, the patient and controls (N: 90) were age and sex matched. All control group was had no family history of disease.

Sample collection

Five ml of blood samples were collected from CML patients and healthy control. A tourniquet was applied directly on the skin around the arm, the skin over the vein was sterilized with ethyl alcohol 70%, and then blood was collected by syringe. The sample divided into two part the first (2ml) transfer to EDTA tube for determination of ABO blood group and second part transfer to gel tube then left for 30 min at room temperature to clot, centrifuged at 3000 rpm for 10 min to get the serum for (MDA&GSH) estimation. The serum was separated and stored at -80 C until use for ELISA.

Immunological Assays

The study parameters estimated by ELISA kit ,the biochemical kits used in the study for MDA and GSH performed by Elabscience company (USA).

Statistical Analysis

The data was collected and transfer to statistical analysis using SPSS program (V. 23) in including the test of two independent -t- test to find the variances between study group. The results expressed as (Mean ± SD). P value at <0.05 was considered statistically significant and highly significant at $P<0.001$.

Results

The mean serum level MDA value shows highly significance ($P<0.001$) change in the studied groups in comparison with control. Table (1) show that the level of MDA in male (900. 99 ± 603.89) is higher than in female (582.28 ± 562.29). In the table the mean serum level of GSH show that significant decrease ($P <0.05$) in patients group (40. 12 ± 27.33) than in control groups (55.20 ± 28.79). In addition, the table show that the level of GSH in male is high (42.30 ± 29.04) in comparison with female (35.77 ± 24.94). Table (2) show that blood group B of patients has a significant increasing in the level of serum MDA (1387.07±520.89) in comparison with other blood groups in patients and blood group O(447.27±260.58) of controls also has a significant increase in the level of serum MDA in comparison with the other blood groups of controls. In addition, the table show the level of GSH

in different blood groups for patient and controls, which refers to that blood group A (53.23±12.76) has high level of serum GSH in comparison with other blood groups of patients. In addition, the table show that blood group AB (81.87±5.50) of controls has high level of serum GSH in comparison with other blood group of controls. The results show that blood group O predominates in the study groups (patients and controls), (44%) followed by blood group A (22%), B (18) %) and AB (16%) (Table2).

Table (1): Concentrations of MDA & GSH in Patients and Control

Parameters	Sex	Patient Mean± SD	Control Mean± SD	P-value
MDA ng/mL	Male (N=30)	900.99±603.89	396.89±215.12	<0.001
	Female (N=15)	582.28±562.29	367.76±328.40	<0.001
Total Mean		794.75±596.62	387.18±252.12	<0.001
GSH µg/mL	Male (N=30)	42.30±29.04	51.61±30.25	<0.05
	Female (N=15)	35.77±24.94	62.37±26.19	<0.05
Total Mean		40.12±27.33	55.20±28.79	<0.05

Table (2): Concentrations of MDA& GSH for Patients and Control Groups classified according to ABO Blood Groups

Parameter	ABO	Patients Mean ± SD	Control Mean ± SD	P-value
MDA ng/mL	A (N=10)	480.70±411.21	324.44 ±256.62	<0.001
	B (N=8)	1387.07±520.89	447.27±260.58	<0.001
	AB (N=7)	671.66±560.75	330.77±329.76	<0.001
	O (N=20)	757.94±559.78	414.25 ±231.10	<0.001
GSH µg/mL	A (N=10)	53.23±12.76	49.92±8.95	<0.05
	B (N=8)	30.75±22.28	52±40.6	<0.05
	AB (N=7)	17±4.30	81.87±5.50	<0.001
	O (N=20)	45.42 ±33.90	49.78 ±31.56	<0.05

Discussion

The results of the current study agree with study done in Kurdistan by Mohammed (2010) who found that blood group O has highest percentage (37.1%) ,followed by A (32.4%) , B (32.8%) and AB (6.5%) [8] . Another study done by Abdullahi (2009) in Iran found that blood group O also predominates (32.1%), then A (30.1%), B (23.3%) and AB (13.7%) [9] . ABO blood groups have also association with other diseases like hepatitis and gastric carcinoma [10]. A study conducted in republic of Bosnia revealed that O blood group have 40.9%, A have 37% and B have 16 % leukemia patients [11]. Another study was conducted in Iraq during 2008-10 and it was found that 41.8% of CML patients have blood group O while 28.8% have B [12]. In this study, the result of table (1) show that there is significant increase in the level of serum MDA is differ in each blood group in CML patients this agree with the study done by Rizwan Ahmed (2008) that found there is significant increase in the level of MDA n CML patients as compared to healthy volunteers [13] . In addition, the study agree with study done by Zelen (2010) that found the level of MDA is higher in compared with controls [14]. In patient blood group B has high level of MDA followed by blood group O has high level of MDA. Some previous studies shows increased MDA levels in different types of leukemia. Oxidative stress occurring in patients with leukemia may be due to the elevated number of mature and immature myeloid or lymphoid series cells. This can also due to increased formation or reduced clearance of free radicals by the cellular antioxidant system [15]. Table (1) show the result of the level of serum glutathione in patients and controls groups. The level of GSH in CML patients is lower than control group in different blood groups this agree with study done by Rizwan (2008) that found Antioxidant status was found to be significantly decreased ($p<0.05$) in Chronic myeloid leukemia patients and its phases as compared to healthy participants [13] . It could be concluded that oxidative stress may be associated with the pathophysiology of chronic myeloid leukemia. The actions of different antioxidants show different patterns during neoplastic transformation, and tumor, cancer or leukaemic cells, which exhibit abnormal activities of the antioxidant enzymes as well as the concentrations of non-enzymatic antioxidants, when compared with their appropriate normal cells. GR is a glutathione regenerating enzyme that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD+ [16]. Glutathione reductase is a secondary antioxidant enzyme that helps in the detoxification of reactive oxygen species by decreasing peroxide levels or by maintaining a steady state supply of metabolic intermediates like GSH (17).

Conclusions

As a conclusion from this study, the importance of MDA and GSH in CML patients can be used to detect the complications related to the among these patients.

Ethical Clearance: Taken from Al-Furat Al-Awsat Technical University ethical committee

Source of Funding: Self

Conflict of Interest: Nil

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Assessment of Psychological Wellbeing Among Elderly Residents at Nursing Homes in Middle Euphrates

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Abstract

As people get older, may show shifts in the main sources that contribute to their well-being, in response to the changing circumstances and abilities inherent in aging. The relocation to care homes considered as stressful event for the elderly people. A descriptive study is carried throughout the present study in order to achieve the early stated objectives. The selection of the sample through a non - probability purposive sample consist of 60 elderly persons that are selected from the total population of elderly residents at nursing homes. The data is collected by means of Ryff scales of psychological well-being (shorten version) . The data is collected by using structured interview technique with the elderly residents in nursing homes. The analysis indicates that the highest percentage of the elderly's group have moderate level of psychological wellbeing (mean of score=3.29) (81.67%). The level of psychological well-being is affected by their gender. The study recommends to focus on managing the problems existing among the elderly and monitoring their psychological state.

Keywords: *psychological wellbeing, elderly, nursing homes*

Introduction

As the population grows and changes in aging process, the most appropriate location for elderly people is entry into a nursing home[(1)]. Despite the fact the nursing home population is only a small segment of the elderly population, this number is also increasing. Approximately 1.5 million (4.5%) of the population resided in care homes in 2000 and it is believed this number will continue to increase by as much as 25% within the next decade[(2)]. The entry into the care homes has the mental implications such as rejection, anxiousness, psychological stress, loss of home, and the possibility of losing interaction with family, relatives and friends, therefore the elderly admission has the major influence on elderly people and concern about the elderly[(1)]. Psychological well-being is a topic of major interest at the level of national and international politics[(3)]. It is a positive concept in psychology referring to the personal opinions of one's self and the life[(4)]. "Psychological well-being" has been researched in more detail and is known to be more than just the absence of disease, which appears to be an obsolete concept of "well-being". It is widely studied because there is need to improve the status of mental disorders of people[(5)]. The analysis of well-being in old age is important, understood as a construct that includes a psychological aspect that influences the optimization and development of older people. Carol Ryff refers to "psychological well-being " as the significant efforts to maximize one's ability, related to the purpose of life, where the person's life acquires meaning for himself, through certain efforts to overcome and achieve valuable goals. Psychological wellbeing is conceptualized through an eudemonic vision of well-being and explained by a multidimensional model of personal development where six psychological dimensions of the person are specified: self-acceptance (positive evaluations of oneself and of the past life; knowing and accepting the different aspects of the person), autonomy (self-determination, independence and self-regulation, ability to think and act autonomously, resisting social pressure)[(6)].

The aim of the current study was to assess the level of psychological wellbeing among elderly residents at nursing homes.

Methods

A Descriptive study carried throughout the present study in order to achieve the early stated objectives. The selection of the sample through a purposive sample consist of 60 elderly persons . The study covers the elderly residents at nursing home in middle Euphrates in Iraq (Karbala, Najaf, Hilla , Diwaniya).

The study instrument comprises the following parts:

Part 1 : Demographic data form of the elderly which consists of (7) items, which including age, gender, marital status, level of education, number of children, length of stay in nursing home .

Part 2:- RYFF psychological wellbeing scale , 18 items version: A shortened version of Ryff Scale of Psychological Well-being has been adopted. This version integrate with longer version of Ryff scale and consists of 18 items divided into six dimensions[7] :- autonomy, personal growth, environmental mastery, positive relations with others, self-acceptance, purpose in life. The data has been collected through the utilization of the developed questionnaire and by using structured interview technique with the elderly people living at nursing homes. The current research only involved residents who could communicate with the interviewer physically and mentally. The data are organized and treated statistically by the Statistical Package for Social Sciences for Windows (SPSS version 24).

Results

Table 1: Statistical distribution of study sample by their demographic data

Items	Sub-groups	Study group (Total = 60)	
		Frequency	Percentage
Age / Years	65-74	40	66.7
	75-84	18	30.0
	≥85	2	3.3
Gender	Male	37	61.7
	Female	23	38.3
No. of Children	0	36	60.0
	1-2	15	25.0
	3-4	5	8.3
	≥ 5	4	6.7
Length of stay in nursing home/ Years	< 1	7	11.7
	1-3	18	30.0
	4-6	15	25.0
	7-9	6	10.0
	≥ 10	14	23.3
Levels of Education	Illiterate	11	18.3
	Able to Read and Write	21	35.0
	Primary School	10	16.7
	Intermediate School	6	10.0
	Secondary School	6	10.0
	Institute & College	6	10.0
Marital Status	Married	9	15.0
	Single	7	11.7
	Divorced	14	23.3
	Widowed	30	50.0

The table (1) reveals that the highest percentage of the elderly's subgroup are : The elderly with ages between (65-75) years old (66.7%), male patients (61.7%), widowed patients (50%), those who have no children (60%), those who are able to read and write (35%), those who stayed (1-3) years in the nursing home (30%), those who are satisfied with the services provided in the nursing home (70%) . Table (2) reveals that the highest percentage of the elderly's group have moderate level of psychological wellbeing (81.67%) .

The results reveals that there is a high significant in difference in psychological well-being level among the sample with regard to their marital status at ($P<0.01$) whereas no significant difference in the level of psychological well-being with regard to other demographic variables at $p \leq 0.05$, as in table (3) .

Table (2) : Distribution of the sample by the level of psychological well-being (Ryff scales)

		Moderate	High
Psychological well-being	Frequency	49	11
	Percentage	81.67	18.33

Table (3) ANOVA in the level of psychological well-being among the sample according to some of demographic data

Items	Sub-groups	Sum of Squares	df	Mean Square	F test	Sig.
Age / Years	Between Groups	0.47	2	0.23	1.49	0.23
	Within Groups	8.91	57	0.16		
No. of Children	Between Groups	0.64	4	0.16	1.001	0.41
	Within Groups	8.74	55	0.16		
Length of stay in nursing home/Years	Between Groups	0.85	4	0.21	1.36	0.25
	Within Groups	8.54	55	0.16		
Levels of Education	Between Groups	1.08	5	0.22	1.4	0.23
	Within Groups	8.3	54	0.15		
Marital Status	Between Groups	1.52	3	0.51	3.6	0.01
	Within Groups	7.86	56	0.14		

ANOVA : Analysis of Variance

Discussion

The study results indicates that the highest percentage of the elderly people with ages between (65-75) years old (66.7%), while the lowest percentage of their ages is more than 85 years old (3.3%).The high percentage of participants is males (61.7%). This result is reinforced by a study done by Heidari A, et al. (2019) [8]who indicated in their results that the majority of elderly 224 (74.6%) were males from the total number (300) of elderly people, who were being taken care of in public and private centers in Tahran, Iran. These findings may be related to the fact that the social culture of the Iraqi citizens does not allow women's status in such a place and may be indicator for a long life of males than females ,therefore; male were more than female and they were more among age group (65-75) years who live in the nursing home.

Regarding marital status, the majority of elderly residents at nursing home (50%) are widowed. Several previous studies are in agreement with the results of the present study[9-12] , they found that the high percentage of elderly were widows in the nursing homes. The reason for these results is that the majority of elderly who live in the nursing homes had no partner before entering the home due to the death of one of them[13].

The study results also appears that most of elderly don't have children (60%). These results coincide with the results of a study[13] in Iraq-Baghdad who revealed that the higher proportion of elderly people (43.7%) were residing with relatives before entering the home , this because they were either divorced, death of one partner, not married or they had no children. The study results also is similar to[10], they revealed that around half of institutionalized Egyptian elderly didn't have sons.

Regarding the education level, the study results demonstrated that the highest percentage of geriatric inmates are able to read and write (35%), while the percentage of elderly illiterate was observed to be (18.3%). This findings come along with[14], whose results indicate that the majority of the elderly at geriatric centers in Baghdad province (18.3%) can read and write.

Most of sample results indicate that 73.3% of the sample are received medical visits by a doctor, physician assistant or nurse practitioner, this because the home provides medical care to the inmates by visiting doctors to the home in order to follow up and check on patients, dispense the necessary treatment, supervise them, and follow up the health of the home's facilities, sleeping places and their contents. And the determination of the types of foods suitable for the elderly and the sickness, and follow-up on personal hygiene. An ambulance is also available to transport difficult cases to the hospital, as well as the presence of a permanent nursing staff in the home that takes care of the patients' health through daily examination and measurement of blood pressure and diabetes.

Concerning the visit frequency of relatives, the study result indicates that the majority of elderly received family, relative and/or friends visits (58.3%). This may be because that most the elderly residents at nursing homes in middle Euphrate and their relatives their religion is Islam, whose principles include the relationship of kinship and communication between its members and the increase in intimacy between them. This finding was consistent with a study[16], who n conducted a study to " Assessment of depression Older Residents at Nursing Homes in Al-Najaf City". He found that the majority of the sample (70%) visit

Regarding the chronic diseases, the highest percentage of elderly have chronic disease (88.3%). Such chronic diseases are occurred because physiological and biological changes in the body of older persons and as a result of age-related phenomenon. This result is reinforced by a study[17] who concluded in their results that most elderly people (73.3%) are suffering from chronic diseases.

With regard to self-report of health, the results demonstrated that most of elderly who categorize their general self-health as fair (41.7%). Previous studies showed that chronic diseases were associated significantly with perceived health status or subjective health, as well as different factors were correlated with self-reported health for different age groups. According to study[18] showed that the number of chronic diseases such as hypertension was the strongest predictor of self-rated health among elderly people aged 70 to 79 .

Concerning psychological wellbeing, the results indicates that the highest percentage of the elderly's group have moderate level of psychological wellbeing(**81.67**%).

This result may be due to the services provided in the nursing home that specified the defect in indoor and outdoor spaces. This refer to the association between environmental setting and psychological well-being, and the circumstances associated with the relocation to residential care and the changes inherent in aging that show a shift in the main sources that contribute to their well-being. This result is supported by a study[20] about "Assessment of the Client's Psychological Wellbeing at Outpatient Consultancy Clinics of Al -Sadder Teaching Hospital in Al Amara City". They indicated that the clients have moderate level of psychological well-being (MS=3.17) (most study sample at age 58 years and above).

The study results indicates that there is a significant increase (P<0.05) in psychological wellbeing scores in male subjects (MS=3.37) compared to female (MS=3.15). This result disagree with a study[14] about Psychological Well-being for Elderly People at Geriatric Centers in Baghdad City. They indicates that the high level of psychological wellbeing (21.6%) are female.

In addition, the results indicates that there is a significant increase (P<0.05) in psychological wellbeing scores in subjects that have frequently medical visits (MS=3.35) compared to those without (MS=3.11). The home provides medical care to the inmates by visiting doctors to the home in order to follow up and check on patients, dispense the necessary treatment, supervise them, and follow up the health of the home's facilities, sleeping places and their contents, these matters contribute to improve mental status and increase in psychological wellbeing [19] .

The results indicates that a high significant increase (P<0.01) in psychological wellbeing scores in subjects that can perform daily activities (MS=3.40) compared to those who can`t perform (MS=3.04). this result supported by a study[3]. Finally, the results show that there is a high significant differences in psychological well-being level among the sample with regard to their marital status at (P<0.01). This result is supported by a study[21] , who mentioned that marital status is an important determining factor in an individual's psychological well-being; those with poor marital quality experience more psychological distress that their non-married peers.

Conclusions

The study concluded that the level of psychological well-being for the elderly residents in nursing homes is moderate and affected by their gender, marital status, financial Import, medical visits to the nursing home, and difficulties in performing daily activities.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

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Assess Nurses Knowledge Regarding Extravasation Non-Cytotoxic Medication in Baghdad Teaching Hospital

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Abstract

Background: vesicant no cytotoxic drugs are used broadly in present medicine for therapy changes condition in hospitals most of them are registered to be peripheral cannulation. According to the literature, one vital complication caused by cannulation applied for intravenous fluid therapy is extravasation.

Objectives: to estimate knowledge about extravasation no cytotoxic drags.

Methodology: A non-experimental design was carried out to evaluate the registered nurse's knowledge regarding extravasation non- cytotoxic medication in Baghdad teaching hospital an indiscriminate sample consisting of 100 nurses was selected, and a questionnaire was distributed to measure their knowledge of the subject of the research. The statistical program version 23 Spss was used. The **Results**: were the majority of females with 60% and the rest males. Most of the study participants (55%) were ages between 20 and 29 years old. Study participants'. (43%) of the were secondary nursing school. The majority of participants (37%) experienced in hospital wards between 1-5years.

Conclusions: The current study concluded that most of the member's nurse's deficiency necessary knowledge about extravasations of no cytotoxic medications. this study recommends that nurses have to participate in more in-depth educational programs

Keywords: *nurses Knowledge, non-cytotoxic drug, extravasation, vesicant*

Introduction

No cytotoxic drugs are used extensively in recent medicine for the therapy of different conditions in hospitals most of them are giving by peripheral cannulation. According to the sources, one chief complication caused by cannulation therapy is extravasation. Nurses have vital duties in intravenous therapy. They administer intravenous fluid suggested by doctors, also they have other tasks such as creating, observing, discontinuing the liquid flow, detecting, and preventing complications caused by intravenous cannulation. (12) (24)

On The other hand, nurse's knowledge about type medication, solutions as well as its dose, concentration, physicochemical characteristics (e.g., ph., osmolality, and molecular weight) essential to diminish extravasation prevalence (7)(6)

However, Extravasation is the outflow of a vesicant drug into the enclosing tissue vesicants are drugs that have the potential to cause blistering and ulceration and which when delayed treated, may lead to additional problems such as permanent tissue destruction. (10) (13) (15) (14)

At hand various vesicant drugs and liquids that cause extravasation harms. Is cytotoxic and non-cytotoxic (Table 1) offers a list of no cytotoxic medications was reported by various published articles related to extravasation hurts. (2)(5)(6)(8) (6) (11).

Several different processes are behind the tissue damage in general, extravasations involve four numerous factors related to drugs harms: vasoconstriction, osmotic, pH related, and cytotoxic. (1)(2)

As the severity of the lesions ranges from a mild rash to tissue erosion, and it may require surgical intervention depending on the type and amount of the drug leaked. (3) (2) (24)

Clinical judgment making is mind-boggling. A mix of logical proof-based information related to instinct and relevant elements could empower nursing staff to use phenomenal clinical judgment (4) (9)

When hospital rises their staff knowledge concerning extravasation, they will respond positively to the extravasation cases via their experiences and handiness to preclude, identify, and heal extravasation. (8) (1)

vesicant medications and solutions reported causing extravasation injuries		
Antimicrobials	**Electrolytes and IV Solution**	**Other Medication**
Aciclovir Cefotaxime Gentamicin Levofloxacin Meropenem Metronidazole (Flagel) Piperacillin Thiopental Vancomycin	Dextrose 50% Mannitol 20% and 10% Sodium bicarbonate 8.4% Calcium chloride (10%) Calcium gluconate (10%)	Amiodarone Diazepam Digoxin Vasopressor Dobutamine Epinephrine Norepinephrine
This is not a comprehensive list. (2)(5)(8)		

Methodology and Materials

A non-experimental design was carried out to assess the registered nurse's knowledge regarding extravasation non- cytotoxic medication in Baghdad teaching hospital data were collected from 8 floors of surgery and medicine wards at the same hospital in Baghdad /Iraq. between June 2019 and February 2020. The study is optional for those who fulfill the specified conditions. and the questionnaire was distributed in the Arabic language.

A convenient sample consisting of 100 nurse's male and Female randomly chosen those who met the inclusion criteria were included Female and male, all educational level of nurses. Nurses who work the night and morning shift. Also, who have agreed to participate in the study.

The questionnaire in this study was developed by the researcher after conduct with an extensive review of the literature. To evaluate nurse's knowledge regarding no cytotoxic medication extravasation the assessment Toole consists of 2 parts. Part one about Socio-Demographic Characteristics of the Nurses including age, gender, level of education, and year's experiences in hospital and training courses. Part two Determined knowledge of the nurses consists of 28 items multiple-choice questions divided into 3 domains first domain viewing The vital phrases that are applied in extravasation. Second domain about prevention extravasation and the third domain conserving vesicant no cytotoxic drugs. The participants are requested to select one of the following answers (correct, incorrect, or I don't know) and recoding answer, each correct answer carried (2) mark while don't know carried (1) mark and incorrect was (0). The whole score series between 0 and 56 the upper scores show better levels of knowledge on the subject of extravasations of no cytotoxic medicines. First conduct pilot study was applied to ten nurses chosen randomly and excluded from sample study that result and need from 20–25 minutes

to be completed of the questionnaire. The reliability of the research instrument (questioners) had been evaluated through a Cronbach's alpha test result showed of 0.79. The normality of data was tested using the Kolmogorov-Smirnov test. Descriptive used Frequency, mean, Sander deviation, and percentage .and inferential statistics involving Chi-square test, independent T-test, ANOVA test, and Cronbach's alpha was. (0.79.). For each test, a p-value of less than 0.05 was considered statistically significant.

Results

Result of the present study shown most participants were females (60%) between 21 and 29 years and the greatest of them hold a secondary Nursing School (43%) over 37 % of the members had practices extending from 1 to 5 years, about 46%.

Table (1) Distributed Nurses Demographic Variables (n=100)

Variables	Categories	F	%
Age/Years	21-29	55	55.0
	30-39	24	24.0
	40-More	21	21.0
	Total	100	100.0
Gender	Male	40	40.0
	Female	60	60.0
	Total	100	100.0
Level Of Education	Secondary Nursing School	43	43.0
	Nursing Institute	37	37.0
	Nursing College	20	20.0
	Total	100	100.0
Years Of Experiences In Hospital Wards	< 1 Year	18	18.0
	1-5 Years	37	37.0
	6-10 Years	13	13.0
	11-15 Years	17	17.0
	16-20 Years	15	15.0
	Total	100	100.0

Table (2) The Mean of Score of all items Nurses Knowledge Regarding Extravasation Non-Cytotoxic Drugs.

domain	No	Items	True		False		Ms	SD	A.D
			No	(%)	No	(%)			
First domain	1	Define Extravasation	23	23.0	77	77.0	0.23	0.42	P
	2	Define Infiltration	27	27.0	73	73.0	0. 27	0.44	P
	3	Define Vesicant Medication	41	41.0	59	59.0	0.41	0.49	P
	4	Define Irritant Medication	33	33.0	67	67.0	0.33	0.47	P
	Total		31	31%	69	69%	0.32	0.45	P
Second domain	5	Forearm Best Site For Fix Cannula	79	79.0	21	21.0	0.79	0.40	G
	6	First Time For Fixed Cannula	58	58.0	42	42.0	0.58	0.49	M
	7	Some Patient Contributed Risk Factor Of Extravasation	61	61.0	39	39.0	0.61	0.49	M
	8	Malpractices Causes To Incident Of Extravasation	50	50.0	50	50.0	0.50	0.50	P
	9	Patient Education To Tall Nurses Staff	72	72.0	28	28.0	0.72	72.0	M
	Total		64	64%	36	36%	0.64	14.7	M
Third domain	10	Cefotaxime	30	30.0	70	70.0	0.30	0.46	P
	11	Gentamicin	23	23.0	77	77.0	0.23	0.42	P
	12	Metronidazole	19	19.0	81	81.0	0.19	0.39	P
	13	Vancomycin	25	25.0	75	75.0	0.25	0.43	P
	14	Amiodarone	16	16.0	87	87.0	0.16	0.36	P
	15	Digoxin	13	13.0	87	87.0	0.13	0.33	P
	16	Diazepam	20	20.0	80	80.0	0.20	0.40	P
	17	Levofloxacin	20	20.0	80	80.0	0.20	0.40	P
	18	Dopamine	21	21.0	79	79.0	0.21	0.40	P
	19	Vasopressin	25	25.0	75	75.0	0.25	0.43	P
	20	Epinephrine	22	22.0	78	78.0	0.22	0.41	P
	21	Propofol	15	15.0	85	85.0	0.15	0.35	P
	22	Dextrose Solution More Than 10%	22	22.0	78	78.0	0.22	0.41	P
	23	Magnesium Sulfate 20%	17	17.0	83	83.0	0.17	0.37	P
	24	Mannitol20%	28	28.0	72	72.0	0.28	0.45	P
	25	Sodium Bicarbonate 8.4%	24	24.0	76	76.0	0.24	0.42	P
	26	Calcium Gluconate 10%	25	25.0	75	75.0	0.25	0.43	P
	27	Calcium Chloride 10%	11	11.0	89	89.0	0.11	0.31	P
	28	Potassium Solution	20	20.0	80	80.0	.020	.040	P
Total			20.8	20.8%	79.3	79.3%	0.19	0.37	P

(No)=number (%)= percentage (M.S) =Mean of score, (S.D) = Standard Deviation, (Ass). = assessment. (p)= poor knowledge (0 – 0.50), (M)= moderate knowledge (0.51 – 0.75), (G)= good knowledge (0.76 – 1.00).

Table (3) Chi-square between demographic variables and the overall nurse's knowledge.

Variables		Score Level			X^2 – Value	P-Value
		High Score	Accepted	Poor Score		
Gender	Male	7	12	21	9.92	0.007
	Female	2	10	48		
	Total	9	22	69		
Level Education	Secondary Nursing	2	3	38	30.8	0.000
	Institution Nursing	2	8	27		
	College Nursing	5	11	4		
	Total	9	22	69		
Age	21-29	5	14	36	8.23	0.18
	30-39	2	8	14		
	40-More	2	0	19		
	Total	9	22	69		
Years of Experiences In Hospital	Less Than One Year	1	5	12	4.01	0.85
	1-5	4	10	23		
	6-10	1	3	9		
	11-15	1	3	13		
	16-20	2	1	12		
	Total	9	22	69		

Significant *: P ≤ 0.05

Table (4) Association between Overall nurse's knowledge and demographic variables

Level Of Education	**Overall Of Nurses' Knowledge**				
	No.	**Mean**	**S.D**	**F. Value**	**P-Value**
Secondary Nursing	43	6.76	4.08	18.006	0.000 / Hs
Institution Nursing	37	7.13	5.22		
College Nursing	20	14.25	5.73		
Gender	Overall Of Nurses' Knowledge				
	No.	Mean	S.D	F. Value	P-Value
Male	40	10.32	6.98	8.283	0.005 / Hs
Female	60	7.11	4.15		

Significant *: P ≤ 0.05

Discussion

Extravasation injury is a proven hazard of IV vesicant administration. Preventative actions can reduction the danger, so nurses should be prepared to treat extravasation injuries in a time-sensitive, evidence-based manner. (25)

Nursing knowledge is vital to all nursing field areas. It is to understand the relevance of nursing informatics to their routine. And necessary to best nursing care, patient approval, Decrease mortality, and morbidity. Nursing informatics gives the tools and skills required to enhance the data, information, knowledge, and perception passageway bui1ding decision (2) (1)

The present study explored nurse's knowledge of Extravasation Non- Cytotoxic drug that verifies a low percent of the participants (14%) have good knowledge about non-cytotoxic drugs that origin extravasation, prevention, and management. In general, nurses in the current study reported very low marks in the structured questionnaire

The Overall answer that Only (30%) of the study sample answered exactly and (70%) responded wrong "do not know". This finding agrees with the result obtained from other studies who reported respondents' knowledge regarding the non-cytotoxic medication extravasation is nineteen percent of students. (17)

logical knowledge essential for nursing care patients because nurses did not have advanced knowledge and not competent in their skills will be considered unsafe care and increases the chances of mistakes. (16)(20)(21)

questions knowledge concerning basic knowledge on extravasation medical terms only (31%) of the study sample answered correctly and (69%) responded incorrectly "do not know". knowledge questions. This finding is the same line with results found from other studies the researcher registered (46.5%) was the right answer. (17)

knowledge related to prevention of extravasation was answered correctly by (64%) majority of participation answer forearm best site for fix cannula this is a good starting with (79.0%)

The nurse is responsible for confirming that all actions have been taken to avoid extravasation include the choice of a suitable site for cannula placing is the first step,

And away from joint flexion such as the hand, wrist, the however antecubital fossa is always the best place for an IV cannula. [17]

Twelfth questions surveying nurses regarding knowledge on drugs that cause extravasation the results show that a low percentage of the participants (20.75%) have a correct and (79.5%) incorrect and don't know answer concerning no cytotoxic medications that cause extravasation the items Drugs was Cefotaxime (30%), while Vancomycin, Vasopressin (25%) and the minimum effect found produces of extravasation were metronidazole (Flagyl) (19.0%)), Digoxin (13.0%), and Propofol (15.0%). This result of the study are agreements with studies done by other researchers in Saudi Arabia whose reported that the same items by (16.8%) Flagel, Propofol (38.0%) while Vasopressin (36.7%) [18]

Outcome about the solution that because extravasation showed weak knowledge. was (18.3%) correct answer and (81.62%) incorrect don't know that exposed ineffective continuous education sector in hospital, this finding coincides with results obtained from another study [18] the outcome indicated lacking the role of continuing education units in hospital under the study, by the other hand activation of continuous nursing education courses and training programs have the assistances of maintenance nurses up-to-date and refining their knowledge, Nursing education permits fast access to information when and where it is needed to facilitate the delivery of safe, and effective, health care, support clinical judgments, and improve the education of nursing students strategic planning, patient satisfaction, and, finally, patient outcomes. [19][22][23]

Table 4 square between student's overall knowledge score and the demographic characteristics (gender, level education). The association was. There is a highly significant relationship between gender with P. Value less than 0.05, for men with a mean (10.32) this indicate men's interest and their attachment to work more than women because of the preoccupation of women with housework

This result disagrees with that of the other researcher was no significant between knowledge and gander [24]. As the level education, the result indicates that the academic study affects establishing a scientific basis and enriching the individual with information, the result was for university nurses through (14.25) this agree with study [19]

Through the research, Baghdad hospitals need to focus on continuing education for the nursing staff and to get acquainted with updates regarding medicines, their proportions, methods of prevention, or the use of vein detection devices, especially with people at risk such as the elderly and children, to avoid mistakes when administering intravenous treatment**.**

The results of this study could be used to design future educational programs for nurses according to their needs, to empower the registered nurses' knowledge regarding extravasations no cytotoxic drugs.

Conclusions

Most of the participants have poor basic information on the subject of study and the current study decided that knowledge and relying on a solid scientific base helps nurses perform their work properly and reduce health problems, and continuing to participate in educational seminars and workshops will help the nurse to understand how to avoid the risks of intravenous infusion and help to complete treatment.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Given by Technical Medical Institute - Baghdad /middle technical university. also, informed consent was obtained from all participants.

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The Indonesian Version of SF-36 Questionnaire: Validity and Reliability Testing in Indonesian Healthcare Workers Who Handle Infectious Diseases

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Abstract

Quality of life is a parameter to measure the position of life for high-risk people, including healthcare workers who handle infectious disease. Quality of life affects productivity and performance. A lot of instruments can be used to measure the quality of life, which one is the 36-item Short From Health Survey (SF-36). It is an international standard questionnaire which measuring health-related quality of life, so a translation process into Indonesian is necessary. The aim of this study is to evaluate the validity and reliability of SF-36 questionnare on Indonesian version. This study was an observational analytic with a cross-sectional design. This study divided into 2 stages, ie. translating questionnaire into Indonesian version, then testing it with respondents. We evaluated 45 healthcare workers working in the COVID-19 isolation room. Data were taken from September to October 2020. This study was analyzed by Pearson Correlation and Cronbach' alpha with α=5% and using SPSS v.23 software. Results showed that the overall cronbach' alpha coefficient was 0.883, while the five dimensions were >0.70 (physical function, vitality, mental health, bodily pain, and general health). But, the role physical, role emotional, and social function dimensions had cronbach' alpha coefficient 0.6-0.7. The Indonesian version of SF-36 was valid and reliable, so it could be used as a instruments for measuring health related quality of life among healthcare workers who handle infectious diseases, especially in COVID-19.

Keywords *: Health related quality of life, 36-Item Short From Health Survey (SF-36), Healthcare workers, Validity, Reliability*

Background

Quality of Life (QoL) is a multidimensional construct influenced by aspects of work and personal life, physical and phsychological health, social relationships, and environment in which a person lives. QoL has long been recognized as a measure in high-risk populations, including workers, because work is a social activity that can impact health[1,2]. Health-Related Quality of Life (HRQL) is a part of QoL which measure individual health status. It can capture individual's subjective evaluations of their current health status, and comparing it in last year[3].

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The health related quality of life in high-risk group has been the subject of extensive investigation, especially in patients with peacemaker, cancer, and others who are in emergency department. But study focussing on the health related quality of life in healthcare worker is rare and it need to be better appreciated, especially in healthcare workers who handle infectious diseases[3-5]. The quality of life aspects could be help to reduce negative effects on their lives.

The 36-item Short From Health Survey (SF-36) has been well-known as an international standard questionnaire which measuring health-related quality of life. It involves 8 domains from physical and mental aspects: physical function, vitality, mental health, bodily pain, role physical, role emotional, social function, and general health[6]. There is no appropriate Indonesian version of SF-36 for healthcare workers

who handle infectious diseases, maybe most questions to be constructed. The questionnaire to measure health-related quality of life have to not only be responsive, but also approriate, valid, reliable, and capable of being intrepreted[7,8]

Based on the background description, the aim of this study is to evaluate the validity and reliability of SF-36 questionnare on Indonesian version for measuring health related quality of life among healthcare workers who handle infectious diseases, especially in COVID-19.

Materials and Methods

This study was an observational analytic with a cross-sectional design. The population in this study was all healthcare workers who work in the hospital and according to the requirements of the inclusion and exclusion criteria, ie. Contact with the patients of COVID-19; and available to be reaserch subjects with informed consent. Sampling was multistage random sampling and obtained 45 healthcare workers.

Data were taken from September to October 2020. This study used the 36-item Short From Health Survey (SF-36) for measuring health related quality of life among healthcare workers who handle infectious diseases. It is an international standard questionnaire, so a translation process into Indonesian language is necessary. This study divided into 2 stages, ie. translating questionnaire into Indonesian version, then testing it with respondents. The translation version was then distributed to 45 healthcare workers via google form. It was to reduce the transmission of COVID-19.

The questionnare involves 8 domains from physical and mental aspects: physical functioning (PF), role physical (RP), vitality (VT), bodily pain (BP), perceptions of general health (GH), role emotional (RE), social functioning (SF), and mental health (MH). Each respondent filled out 36 questions were multiple choice with a score between 0-100. This study was analyzed by Pearson Correlation and Cronbach' alpha with α=5% and using SPSS v.23 software.

Results and Discussion

The respondent who participated in this study is healthcare workers who working in the COVID-19 isolation room in various region of Indonesia. Based on **Table 1**, 75,6% have living in urban area. This condition showed that in Indonesia, the hospital which capable of handling COVID-19 patients is a hospital that has complete facilities with ventilators. In Indonesia these facilities are mainly found in referral hospitals [9]

Based on the Decree of the Minister of Health of the Republic of Indonesia Number 391 of 2014 declared that all of governor in Indonesia has the right to appoint at least one hospital that will become a referral hospital in order to improve health services in the National Health Insurance or JKN Era. One of the criteria for a referral hospital is that it is able to handle patients across at least 4 districts or cities, and can be reached by land, sea and air transportation at least across 4 districts or cities. This ease of access is the reason why most of the referral hospitals are located in the central city area. All respondents who work at Referral Hospital for Specific Emerging Infectious Diseases as stipulated in the Decree of the Minister of Health of the Republic of Indonesia No. HK.01.07/MENKES/169/2020 [9]

Table 1 also shows that most of respondents are at 25-30 years old. The functioning of human organs is at its optimal peak during adulthood and tends to decline when you get older (>45 years). Decree of the Minister of Health of the Republic of Indonesia No. HK.01.07/MENKES/328/2020 explains that workers are divided into 3 field, there are low risk of exposure, moderate risk of exposure, and high risk of exposure. Health workers who have direct contact with COVID-19 patients are categorized as workers with a high risk of exposure. Therefore, one of the special criteria for health workers is that they do not have comorbidity factors. The comorbidity factor which we referred to is old age, the presence of comorbidities such as diabetes, hypertension, pulmonary disorders, kidney disorders, immunocompromised conditions or autoimmune diseases, and pregnancies[10,11].

Based on marital status and employment status (**Table 1**), 66,7% are single and 53,3% are work as contract employees at the hospital where they work. Based on hospital policy which is referred to the Circular of the Minister of Health of the Republic of Indonesia No. HK.02.01/MENKES/216/2020 concerning Protocol for the Prevention of COVID-19 Transmission in the Workplace. The circular also regulates self-isolation for

high-exposure workers or workers with symptoms of COVID-19. Health workers who are single more able to occupy the dormitory provided by the hospital as a self-isolated effort than those who are married[12]

Table 1. Respondents Characteristics

Variable	n	%
Living		
Rural	11	24,4
Urban	34	75,6
Age		
20 – 25 years	9	20,0
25 – 30 years	31	68,9
31 – 35 years	4	8,9
35 – 40 years	1	2,2
Marital status		
Single	30	66,7
Married	15	33,3
Employment Status		
Volunteer	5	11,1
Contract Employee	24	53,3
Permanent Employee	13	28,9
Civil Servants	3	6,7

Validity and Reliability Testing

SF 36 is one of the standard instrument to measure health-related quality of life. SF 36 consist of 36 items which is divided to measure 8 domains of health: physical functioning (PF), role physical (RP), vitality (VT), bodily pain (BP), perceptions of general health (GH), role emotional (RE), social functioning (SF), and mental health (MH). SF 36 is flexible and simple instruments so that it is easy to fill out both manually and digitally. This questionnaire is also widely used to measure general health status, including evaluating the effectiveness of an intervention[13-15]

The Validity analysis of SF 36 Indonesian version is carried out by comparing the Pearson Product Moment correlation value with a significance level of 5%, while the reliability analysis used Cronbach's alpha. The analysis of the validity and reliability of the SF 36 questionnaire was carried out per domain where each domain was represented by 2 to 10 questions. The question is said to be valid if the p value is <0.05 and it is said to be reliable if the Cronbach's alpha value is$>$ 0.6 [16]

Table 2. Validity and Reliability Testing for SF-36 Questions

Subscale and its items in Indonesian Version	r	p	Croncbach's Alpha
Physical Functioning (PF) Mengangkat benda berat atau olahraga berat	0,598	< 0,001	0,767
Memindahkan meja, menyapu, atau jalan santai	0,477	0,001	
Mengangkat atau membawa benda ringan	0,600	< 0,001	
Menaiki lebih dari 1 anak tangga	0,509	< 0,001	
Menaiki 1 anak tangga	0,477	0,001	
Menekuk leher/tangan/kaki, bersujud, atau membungkuk	0,488	0,001	
Berjalan lebih dari 1,5 km	0,806	0,001	
Berjalan melewati beberapa gang atau sejauh 1 km	0,809	< 0,001	
Berjalan sejauh 0,5 km	0,699	< 0,001	
Mandi atau memakai baju sendiri	0,271	0,152	
Role Physical (RP) Menghabiskan seluruh waktu untuk melakukan pekerjaan	0,681	< 0,001	0,610
Menyelesaikan pekerjaan tidak tepat waktu	0,590	< 0,001	
Terbatas pada pekerjaan tertentu	0,739	< 0,001	
Mengalami kesulitan dalam melakukan pekerjaan	0,737	< 0,001	
Role Emotional (RE) Menghabiskan seluruh waktu untuk melakukan pekerjaan	0,732	< 0,001	0,641
Menyelesaikan pekerjaan lebih lama dari biasanya	0,824	< 0,001	
Tidak teliti dalam menyelesaikan pekerjaan dari biasanya	0,732	< 0,001	
Vitality (VT) Merasa penuh semangat	0,790	< 0,001	0,775
Merasa memilikibanyak tenaga	0,829	< 0,001	
Merasa mudah bosan	0,686	< 0,001	
Merasa cepat lelah	0,792	< 0,001	
Mental Health (MH) Termasuk orang yang mudah gugup	0,425	0,004	0,738
Merasa sangat tertekan dan tidak bergembira	0,809	< 0,001	
Merasa tenang dan damai	0,792	< 0,001	
Merasa putuh asa dan sangat sedih	0,739	< 0,001	
Termasuk orang yang ceria	0,731	< 0,001	

Cont... **Table 2. Validity and Reliability Testing for SF-36 Questions**

Subscale and its items in Indonesian Version	r	p	Croncbach's Alpha
Social Function (SF) Kesehatan fisik atau emosi mengganggu interaksi sosial	0,835	< 0,001	0,662
Kesehatan fisik atau emosi mempengaruhi kegiatan sosial	0,657	< 0,001	
Bodiliy Pain (BP) Merasakan nyeri di tubuh	0,916	< 0,001	0,802
Rasa nyeri tersebut mengganggu pekerjaan sehari-hari	0,911	< 0,001	
General Health (GH) Kondisi kesehatan yang dirasakan sekarang	0,809	< 0,001	0,845
Kondisi kesehatan sekarang jika dibandingkan tahun lalu	0,607	< 0,001	
Saat ini mudah menderita sakit	0,796	< 0,001	
Saat ini sama sehatnya seperti orang lain	0,635	< 0,001	
Merasa kondisi kesehatan saat ini semakin memburuk	0,894	< 0,001	
Kesehatan saat ini sangat baik	0,750	< 0,001	

The question to measure physical functioning is to know that "Does your health now limit you in these activities?" We translating vigorous activities as activities which need a lot of energy or "membutuhkan banyak energi", moderate activities as activities that do not need a lot of energy or "aktivitas ringan" and add example such as sweeping, jogging and walking. Question about climbing several flights or stairs we translate as climbing some stairs or "menaiki beberapa anak tangga", and the next 3 question are walking more than 1,5 Km, walking a few alleys which are 1 Km/alley, and walk for 0,5 Km.

The PF domain consists of 10 questions that describe the ability of a physical function to do moderate to vigorous activities. **Table 2** shows that there is one item that has a significant value 0,152 (p>0,05) which is the last question about "bathing and dressing by yourself", this indicates that this question may get the misunderstanding by health workers. Other questions have a significant value <0,001 which indicates that the question or instruction can be understanding by health workers. In the conclusion for validity and reliability, the physical functioning domain consists of 9 valid questions and 1 non-valid question. After we deleted the non-valid question we conducted the reliability analysis and the results showed that Cronbach's alpha value is 0,767 (>0,6) which is indicated that the 9 questions to measure the PF domain of healthcare workers is valid and reliable[16-18]

The RP domain consists of 4 questions. The translating questions to measure role physical in Indonesian version can we described as "do you had difficulty to spend all of your time to do some work or other activities?", "do you had difficulty to finish your work on time?", "do you had a limitation to do your work or other activities?" and do you had difficulty to do your work or other activities that need extra energy like jacking, constructing work, or laundry?". All of the four questions have a significant level <0,001 its indicates that all of the questions are valid and can be the measurer of role physical domain of health workers. The high significants level of each question also indicates that the instruction or questions can be understanding by the respondents. **Table 2** shows that Cronbach's alpha value is 0,61 (>0,6), so all questions are reliable and can

measure the role physical domain of healthcare workers consistently[14,16]

The RE domain consists of 3 questions which are we described in Indonesian version as "do you had the emotional problem that can give you difficulty to spend all of your time to work or do other activities?", "do you have an emotional problem that gives you difficulty to finish your work on time?", "do you have an emotional problem that makes you did work or other activities less carefully than usual?". **Table 2** shows that all questions have a high significant value which is <0.001 this indicates that the instruction or questions is valid and can be understanding by the respondents. Cronbach's alpha value is 0,641 (> 0,6) it means that all questions are reliable and can measure the role emotional domain of healthcare workers consistently[16-18]

The VT domain can be measure by 4 questions which in the Indonesian version can we described as how the respondents feeling, is they always feel impassioned, have a lot of energy, feel bored, and feel tired quickly. All of this question has a significant value <0.001 which indicated that all of the questions are valid and can be used to measure the vitality domain of respondents. Besides that the Pearson Product Moment correlation value is high so all the questions is can be understanding by the respondents. **Table 2** shows that Cronbach's Alpha value is 0,775 (> 0,6) which indicated that all of the questions are reliable and can measure the vitality domain of healthcare workers consistently[14,16]

The MH domain can be measured by 5 questions, which can be used to predicting 5 main dimensions of mental health there are anxiety, depression, emotional control, and psychological well-being. In this Indonesian version the questions can be described as how the respondent feels in the past 4 weeks, including felt very nervous, felt depressed and nothing can cheer you up, felt calm and peaceful, and felt downhearted and sad. Besides that, for the last question, we translating the last question as "were you a happy or cheerful person?". The lowest score for the mental health domain is if the respondent answer that they felt nervousness and depression all the time, while the highest score is if the respondent answer that they felt peaceful, happy, and calm all the time, past 4 weeks[17]. Based on validity analysis results all of the questions have a significant value <0.001 which indicates that all of the questions are valid. Reliability results also give Cronbach's Alpha value 0,738 (>0,6) so the conclusion shows that the questions are reliable and consistent to measure the mental health domain of healthcare workers[16-18]

The SF domain wants to see the extent of the impact of physical health or emotional problems on someone's social activities on the past 4 weeks, these social activities are general social activities with family, friends, neighbors or groups and social activities like visiting friends, relatives, etc. The social functioning domain of the SF 36 questionnaire consists of 2 questions. **Table 2** shows that p-value<0.001, this indicates that 2 questions on the questionnaire are considered valid, while the Cronbach's Alpha value is 0.662 (>0.06) which can be concluded that the questionnaire is reliable or consistent[14]

The BP domain can measure the extent of pain that can interfere with a person's normal activities in the past 4 weeks. The questions to measure this domain consist of 2 questions. The lowest score is obtained if the respondent feels the pain that is very severe to the extent that can greatly interfere with daily work. **Table 2** shows that p-value<0.001 on both questions so that it can be concluded that the two questions to measure the bodily pain domain are valid. While Cronbach's Alpha value is 0.802 (<0.6) so it was considered reliable and consistent[16-18]

The last domain measured through the SF 36 questionnaire is the GH domain. The questions in this aspect consist of 6 questions, which are questions number 1, 2, 33 to 36. The questions in this aspect want to see how the respondent's perceptions of their health in general. The lowest score is obtained if the respondent believes or considers that his health condition is poor and tends to get worse. Based on the results of the analysis of the validity of the questionnaire from 45 respondents, it was found that the p-value of 6 questions <0.001, so it was concluded that the 6 questions to measure general health are valid. In the results of the reliability analysis with the same number of respondents, Cronbach's Alpha value is 0.845 (> 0.6) it indicates that this questionnaire is reliable or consistent (**Table 2**).

Conclusions

The Indonesian version of SF-36 was valid and reliable, so it could be used as a instruments for measuring health related quality of life among healthcare workers who handle infectious diseases, especially in COVID-19. In the the PF domain, we found that one question about "Bathing and dressing by yourself", is considered not valid. The authors will decide to modification of the questions instead to delete the question, so it can fully measure the PF domain.

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How do the Government and More than 1.3 Billion People Stand Against the COVID-19 Pandemic in the World's Largest Democratic Country? A Comprehensive Reflection from India, in a Public Health Perspective

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Abstract

Background- Coronavirus disease (COVID-19), which is caused by deadly novel corona virus has devastated the world causing a morbidity rate of more than 64 million and mortality rate of 1.5 million. India, being a Lower Middle-Income Country (LMIC) with a GNI per capita of nearly $2000, has proactively instituted various containment strategies. India has witnessed the effectiveness of primary prevention strategies in fighting various health problems in the years gone. The most worrying factor was about community spread of the pandemic, which would cause the spread of illness uncontrollable. Economic analysts had forecasted if appropriate strategy on containment of coronavirus is not initiated, the country would regress back to 20 years, in terms of economy and healthcare. This scoping review throws light into the efficacious preventive strategies adopted by India to fight COVID-19 pandemic and attempting to reduce mortality and morbidity rate, to flatten the illness curve.

Methods- We used a wide range of scientific database and news reports to conduct an extensive review on India's fight against COVID-19. Articles which has been published from January to November 2020 were reviewed and pooled the data to write this review.

Conclusion- Containment strategies are the key for prevention of any contagious illness. COVID-19 being highly contagious, and infecting millions of populations, is successfully being controlled in India to reduce the cases. The Case Fatality Ratio (CFR) of COVID-19 is very low in India, compared to other countries.

Keywords – *COVID-19, Public Health, Prevalence, Containment, India*

Introduction

On 31 December 2019, The World Health Organization (WHO) China Country Office was informed about cases of pneumonia with unknown origin detected in Wuhan City, Hubei Province of China [(1)]. International Committee on Taxonomy of Viruses (ICTV) announced the new virus as "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" on 11 February 2020, as genetic property of the new virus relates to coronavirus that caused the Severe Acute Respiratory Syndrome (SARS) outbreak nearly two decades ago[(2)]. Coronaviruses belong to large family of viruses and which might be a public health threat as it causes illness in animals and mankind [(3)]. Coronaviruses are causative organisms of common

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flu to critical illnesses like Middle East Respiratory Syndrome (MERS) and SARS among humans [4].

The youngest generation of corona viruses is known as novel coronavirus which causes novel coronavirus disease 2019 (COVID-19) in humans [5]. According to WHO estimation, around 80% of patients with COVID-19 recover without hospitalization, however, one out of six patients become seriously ill, demanding intensive treatment and illness management. Older people, and those with underlying medical conditions such as cardiovascular diseases, diabetes, chronic respiratory disease, and cancer are more prone to develop serious illness, and a few ends up battling for life (Table-1) [6].

Table 1: Conditions that worsen COVID -19 prognosis

Conditions and other risk factors that determine prognosis of COVID-19	
Asthma	Severe obesity
Chronic lung diseases	People above 65 years
Diabetes	People in long term care facilities
Serious heart conditions	Immunocompromised patients
Chronic kidney disease being treated with dialysis	Liver disease

Source (https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/groups-at-higher-risk.html)

Emergence of the Pestilence

COVID - 19, initially reported in the city of Wuhan, China by the end of December 2019, has affected the world, panicking the giants of health care delivery system in the world, with in a few weeks of its emergence. WHO has declared the illness as global pandemic, alarming the health care agencies to be very vigilant and pro-active to control the steep rise in the illness graph [7]. The uncontrolled morbidity and mortality rate, despite all available containment strategies trembled the nations and continents. The magnitude of the pandemic left the efforts and research activities of the medical fraternity and scientists in vein, stretching the graph bars into elongated lines of morbidity which is more than 64 million, mortality rate of 1.5 million and recovery rate of more than 42 million people across the globe. The world populations watched this in dismay as the numbers continued to rise [8].

Even though the CFR for COVID-19 has been much lesser than SARS of 2003, the disease transmission has been significantly greater, with a progressive mortality rate across the globe. WHO had estimated 3.4% mortality rate as of March 3rd 2020 [9], however the mortality rate is alarming the countries as it happened in the worst and the most severe pandemic in history which was the Spanish Flu of 1918, which lasted for two years with a morbidity of 500 million and a mortality of 50 million people globally [10].

The incubation period of the virus is 1- 12.5 days with an average of 7-10 days [11]. As the virulence and incubation period of the virus is varying among different people, and the clinical studies are progressing among active cases of 2019-nCoV, in particular whether transmission can and to the extent it may occur from "asymptomatic" or mildly symptomatic individuals or prior to symptoms onset [12].

Setting foot in India

Containment programs and massive strategies adopted by China has positively played a crucial role in the control of global spread of COVID-19. WHO reiterated that COVID-19 is a Public Health Emergency of International Concern (PHEIC). The spread of virus to countries with ill-prepared,

"weak" health systems with poor economy has bothered WHO to declare the pandemic as a global health emergency, inviting emergency measures, fortified with changes in policies and protocols [13].

In India, the very first case of COVID-19 was reported in a student returned from China, by the end of January in India from Thrissur district of Kerala, a southern state. The case was well managed and controlled in the isolation unit [14], incorporating the lessons learnt from the efficient management of Nipah outbreak in 2018 [15]. Adding the number of people returning from affected countries, the number of Covid-19 confirmed cases were threatening the health care, political, economic and trade sectors, with the first death of a COVID- 19 case, on 12 March, a Saudi Arabia returned 76 year old male from Karnataka state [16]. Even before the containment measures had been taken, the illness started spreading to most of the states and union territories in India, challenging the health care system [17].On May 10, 2020, the Ministry of Health and Family Welfare (MoHFW) have confirmed a total of 65,021 active cases, 19,665 recoveries and 2,153 deaths in the country, which had been reached to 4,44,746 active cases, 86,42,771 recoveries and 1,34,699 deaths as of November 25, 2020 [18]. India's testing rates are among the upper level in the world and this has crossed more than 13,48,41,307 as of November 25, 2020 and the nation tests more than one million samples a day as the country leads the world in the daily average number of reported new cases of COVID-19 [19]. The infection rate of COVID-19 in India was reported to be 1.7,which is significantly lower than in the worst affected countries as of end of April 2020, however the cases were outnumbered the mathematical predictions done by scientists, and reaching to more than nine million, yet leaving a ray of hope as the recovery rate peaking to 93.67 percent [20]. The COVID-19 mortality rate in India was lowest in the world which was marked as 3.2% by first week of May 2020 [21] which steadily increased and the CFR raised to 2.95% by end of June 2020, marking the highest among Asian countries.

Prevalence

The latest updates show that most cases were reported in the state of Maharashtra, with morbidity of 1.79 million (mortality 46,683), Karnataka being in second position, with 8,76,000 being affected (mortality 11,695) [21]. India reports CFR of 1.5%, compared to the US at 2.1%, Italy 3.5%, UK 3.6% and Mexico 9.7% as of November 25, 2020 [22]. The average CFR is estimated to be at 2.97% globally while it is 1.56% for India. India reports 73 deaths per million population while the global average is 130 deaths per million population. Experts opine that the relatively young Indian population could be one of the reasons of low mortality rate in India [23].

The Shaking Economy by Covid-19

The health expenditure of India is just 1.6% which is affected by other giant sectors of the country, keeping the level of GDP on a float, whereas The National Health Policy - 2017 stated that health spend by government should be 2.5% of GDP by 2025 [24].The developed countries like USA, France, Italy's economy is almost collapsed due to the rampage of COVID-19. The economic and financial predictions based on the current condition of India, compared with other COVID-19 hit countries put the experts in a dilemma. The economical standstill during the 40 days lockdown in the country certainly had an impact on its production, manufacturing, marketing, import and export sections. The health expenditure of government on coronavirus testing, quarantine and other measures of prevention and treatment of the pandemic has slapped the economic system of the country, in the production, export and sale of goods and services [25].

Apart from these general areas, where the impact of the new pandemic has made a difference, the other reasons why the pandemic has a great impact in spite of the rigorous containment strategies as a warfare situation is by analysing the following aspects [26].

a. Second Dense Population

The density of population in India is a serious challenge during the time of such catastrophes. The country's responsibility to each of its citizens will add the burden on the country not only in economical aspect, but in the other areas like, requirement of trained manpower and need for treatment facilities and extension of medical aid for the disease victims.

b. Unpreparedness to Meet a Calamity

A research study has estimated a shortage of 600,000 doctors in Indian health care system in 2013,

which is shocking [27]. Indian Public Health Standard (IPHS) has specified the guidelines and staffing pattern, including the infra-structure requirement for Public Health Units (PHU), Public Health Centres (PHC), Taluk hospitals and District hospitals. However, the findings and recommendations of many research studies on the existing lacunae in the health care delivery system in India is being overlooked. Doctor-patient ratio, nurse-patient ratio, availability of medical equipment and facilities, and such administrative issues need to be considered seriously to combat with COVID-19 like pandemics in the future.

c. Effect on the Growth and Development

Economic analysts forecast that global economic growth in 2020 will be limited by up to 0.3%, while the first-quarter growth of the US economy itself could be curbed by up to 0.4% [28].The Asian Development Bank has predicted that the global economic cost of the virus is as eye popping as $2 to $4 trillion [29].

Counting the estimated damages due to COVIDd-19, the figure goes up to an astonishing $6.5 trillion. Under complete lockdown less than a quarter of India's $2.8 trillion economy is functional. We are expected to lose over Rs 32,000 crore ($4.5 billion) every day during the lockdown [30]. The computed estimates for 40 days lock down in microeconomics to macroeconomics would be whooping. Major financial institutions have dropped growth estimates for India by 0.5 to 1.5 %. This is likely to put a downward pressure on markets and industries. As the economic analysts have forecasted major four areas where the worse hit of pandemic are supply chain (demand and supply uncertainty, disrupted custom clearance, workforce absenteeism) , consumer spending (most customers had low engagement in shared services), banking (disruptions to physical operations, impact on asset quality and liquidity, and demand pressure on digital channels) and oil economy (the growth in consumption of petroleum products falling to an abysmal 0.21 per cent to 213,686 thousand tonne) which has significantly created prostration to Indian economy. The estimated loss is almost 350 million USD, with two percent of reduction of Chinese export of intermediate inputs on Indian industries. However, the impact of this economic recession on a large proportion of the 40 million migrant labourers, those who provide the muscle power to India's construction, agriculture and other sectors would be reflected shortly [31].

Preventing Corona Virus Spread

The strategies of WHO include the identification and monitoring of sick people, testing of appropriate samples, treatment of patients, prevention of onward spread and controlling transmission in health care facilities, maintaining the right supplies, and communicating with the public about COVID-19, its causes and prevention [32].

The Government of India had initiated immense measures for containment of the spread of COVID-19, based on the general guidelines of WHO and policies of MoHFW in particular. Many models of the primary prevention and other information education and communication (IEC) strategies which had been implemented in India have proved to be very effective. Case detection, contact tracing, surveillance, data analysis, case management and patient transportation, risk communication, specimen collection and transport, disinfection and hazardous waste management and donning and doffing PPE were the major chunk of activities carried out by the country as per the advisory of WHO [33].

a. Disaster Management Act 2005 (DM Act 2005)

The lockdown in India, which has turned to be the *world's biggest lockdown*, has been imposed under the Disaster Management Act, 2005 (DM Act). DM Act was targeted to provide the guidelines and training for the effective management of disasters, '*to take measures for ensuring social distancing to prevent the spread of COVID 19*'. Section 51 to 60 of the DM Act explains the offences and penalties for violation of lockdown measures. The National Disaster Management Authority (NDMA) under the DM Act is the nodal central body for coordinating disaster management, with the Prime Minister as its Chairperson. The NDMA lays down policies, plans and guidelines for management of disaster [34]. The state of Kerala, in addition to the above, has legal authorities by enforcement of *'Kerala Epidemic Diseases Ordinance, 2020'* to deal with this biological disaster including punishments for disobeying order of a public servants and any malignant act of the public that is likely to spread infection of disease that can be

dangerous to life [35].

b. Epidemic Diseases Act 1897

The Epidemic Diseases Act (ED Act) was passed in 1897 in India, with the objective of better preventing the spread of dangerous epidemic diseases [36]. The Act was executed vigorously to control the plague epidemic that broke out in the 1890s. The Union Cabinet has approved amendment of the Epidemic Diseases Act, 1897 on 22nd April 2020 considering the turbulence in the healthcare system of India caused by COVID-19 pandemic. The decision on amendment of the Act was to protect healthcare service personnel and property including their living/working premises against violence during epidemics.

The amendment made any act of violence cognizable and non-bailable offences. Commission or abetment of such acts of violence will be punished with imprisonment for a term of three months to five years, and with fine of Rs.50,000/- to Rs.2,00,000/-. In case of causing grievous hurt, imprisonment shall be for a term six months to seven years and with fine of Rs.1,00,000/- to Rs.5,00,000/- under section 188 of the Indian Penal Code (45 of 1860). In addition, the offender shall also be liable to pay compensation to the victim and twice the fair market value for damage of property. Offences will be investigated by an officer of the rank of Inspector within a period of 30 days, and trial is supposed to be completed in a period of one year [37].

Preventive Strategies Adopted by India

India is the first ever country to develop specific national targets and indicators aimed at plummeting the number of global premature deaths from non-communicable diseases (NCDs) by 25% by 2025, in line with WHO's Global action plan for the prevention and control of NCDs 2013-2020 [38].

Despite the early setbacks, the response from Indian healthcare sector, both private and government, has been robust and proficient as of now, to meet the national emergency. Testing has been expedited up quickly and more than 1.6 million samples have been tested throughout the country [39].with a capacity of 70,000 tests per day, through 345 government labs and 131 private labs approved by the Indian Council of Medical Research (ICMR) as on May 10, 2020 [40].

Throughout India, a total of 586 hospitals with a capacity of over 100,000 isolation beds and 11,500 Intensive Care Unit (ICU) beds are reserved for COVID-19 patients in the early days of COVID-19 spread, with 80% of ICU beds in hospitals in Delhi alone[41].

Key Components of COVID-19 Prevention in India

Being the second most populous country in the world, India has the least mortality rate of COVID- 19. Various strategies and policies adopted by Government of India in the containment of coronavirus is a model for other nations and countries. All the stakeholders including decision makers, general practitioners on public health, administrators, political leaders and general public were given education and information on the illness and the measures to be taken on the preventive aspect. All the existing laws and policies pertaining to the containment of the epidemic were reviewed. All the healthcare workers who have direct contact with patients who are at high risk for transferring the disease were sensitized. Media are involved as a remote stakeholder for highlighting public health issues.

A greater importance was given to public health and environmental research to address the gaps in the emerging links between exposure to harmful environmental agents and the health outcomes. National, state and community level health issues were addressed, and remedial measures were taken by performing the root cause analysis. Environmental and public health system were fortified in all levels from the primary prevention to tertiary prevention strategies. Healthy lifestyles and use of eco-friendly products were encouraged. [42]

COVID-19 Prevention Strategies Enforced by India

a. Home quarantine and facility level quarantine

Homequarantine is initiated for the persons with travel history abroad in the last 14 days, but asymptomatic or with very mild symptoms, persons with contact history with affected person in the last 14 days but asymptomatic or with very mild symptoms, persons with no travel history or contact history, but having flu-

like symptoms [43].

Every suspected case detected in the containment/ buffer zones (till a diagnosis is made), are hospitalized and kept under isolation in a designated facility till such time they are tested negative. The positive cases of COVID-19 are hospitalized till such time of their samples are tested negative as per MoHFW's discharge policy. Ideally, patients can be isolated in individual isolation rooms or negative pressure rooms with 12 or more air-changes per hour. In resource constrained settings, all positive COVID-19 cases can be cohorted in a ward with good ventilation. Mixing up of these cases are prevented with utmost precaution. A minimum distance of one meter is maintained between adjacent beds. All COVID-19 patients need to wear a triple layer surgical mask throughout their stay in the hospital. Stringent infection control practices were followed in all healthcare facilities [44].

b. Travel restrictions

Imposing travel restrictions was one of the major strategies that was executed to prevent the transmission of COVID- 19 in India. Many states and interstate borders had been blocked to curb the migrants' and travellers' movement as a part of containment. International and domestic flights, trains, buses and all local travelling by the private vehicles were barred. The restrictions, however, have proved to be very much effective in reduction of unnecessary gathering and further spread of the illness [45]. Bureau of Immigration, Government of India has issued an advisory on travel and visa restrictions on their website for information [46]. By March 2020, spread of COVID-19 had become faster in the country, and hence the government had to put a halt for all commercial activities, ordering closures of schools and colleges and only allowed the functioning of certain enterprises and pharmacies with control measures, to curb the spread of illness [47].

c. Case Detection

From one laboratory in January, conducting COVID-19 test in India, by the end of November 2020, there are 2196 designated laboratories, both government (1186) and private (1010) with more testing and sample collection facilities to combat the pandemic. 47 By the first week of December 2020, total of 148 million cumulative total samples are tested in India, attaining the third position in the world for highest number of COVID-19 tests [39].

d. Contact Tracing

Contact tracing or patient movement map is a public health approach that has been adopted to contain infectious diseases including Tuberculosis, Polio, Ebola virus disease, and the first 2003 SARS epidemic**.** The goal of contact tracing is to identify, treat and isolate current and potential cases of an infectious disease in order to control and prevent further human-to-human transmission. Countries such as South Korea, China, Singapore, Germany and New Zealand have employed contact tracing with varying degrees of success to hinder the spread of COVID-19 and re-open their economies [48]. Patient movement map helps to identify the primary and secondary contacts of the patient and helps in keeping the asymptomatic persons who have the potential to become cases under quarantine and testing them subsequently for the presence of infection . Experts opined that, aggressive testing and contact tracing are the reason behind Kerala's success in fighting the pandemic [49, 50].

e. Telephone message

The medical and public health interventions are widely supported by mobile health (mHealth). Throughout the world there are almost 7 billion mobile phone users, 89% of them are from developing countries and the number is increasing yearly [51].

In India, the dial tone of mobile phones was replaced during anti-polio campaigns with a recorded message that reminded the public of National Immunization Day whenever a call was dialled. This strategy worked very well, making the campaign successful [52], and same is witnessed in the anti Covid-19 campaigns too. The telephone/ mobile based pre-recorded caller tune in different languages has made a measurable impact in the prevention of the illness by educating people on simple measures that would prevent the further transmission of this infection.

f. Blocking traffic and nationwide lockdown

On 24 March 2020, the Government of India imposed a nationwide lockdown for 21 days, limiting movement of the entire 1.3 billion population of India as a preventive

measure against the COVID-19 pandemic in India [53]. It was imposed after a 14-hour voluntary public curfew on 22 March, followed by enforcement of a series of regulations in the country's COVID-19 affected regions. The lockdown was placed when the number of confirmed positive coronavirus cases in India was approximately 500 [54]. The report analysis has highlighted that this nationwide lockdown had certainly made a crucial impact in the control of coronavirus transmission in the early period of its outbreak in India [55].

g. Arogya Sethu App

Government of India has proactively developed a smart phone-based application *'Arogya Sethu'* and insisted people including employees of private and government firms and general public to download the app and use it. This mobile application connects essential health services with people of India to fight COVID-19 pandemic. The mobile application is aimed at augmenting the initiatives of the Government of India, particularly the Department of Health, in proactively reaching out to and informing the users of the application regarding risks, best practices and relevant advisories pertaining to the containment of COVID-19.

Bluetooth based technology is used in the application which helps to contain the illness, if a smart phone user tests positive for COVID-19, everyone on their recent Bluetooth log would be alerted anonymously and advised to stay safe at home [56]. So far, the *'Arogya Sethu'* application has crossed more than 100 million downloads and getting popularized even among the common man.

h. Hand Sanitizer, Tunnel Sanitizer

Although Government of India motivates people to wash hands frequently with soap and water as it is one of the simple and easiest way to prevent the transmission of COVID-19, some of the researchers worry on the effectiveness of this strategy because of water scarcity in Indian villages. The sanitization protocol in India included hand washing with foot operated soap and water dispenser and fogging in a sanitizing tunnel for 20 seconds [57]. In disinfectant tunnel, which has been placed at many public locations in different states of India, Quaternary Ammonium Compounds (QAC) are used at concentration of 0.045 per cent, which is recommended by the health department. The principle behind India's new disinfectant tunnels is simple: wash your hands and walk through a tunnel in which overhead sprayers sprinkle disinfectant. This two-step process can substantially reduce the number of germs present on an individual [58].

i. Indian Railways

Indian railways, with 1.4 million employees and one of the largest employers of world has decided to go hand in hand with MoHFW, Government of India in fighting COVID-19. Many production units, and workshops of Indian railway has started manufacturing Personal Protective Equipment (PPE) Coveralls for medical and health-care personnel who get directly exposed to the COVID-19 infected patients. They had already developed prototype of a cost-effective version of ICU ventilator, that will be developed for using the converted ICU in the trains. The railways have transformed 5,000 train coaches across the nation into isolation or quarantine facilities with around 80,000 beds for COVID-19 patients. Indian Railways has also launched a mobile doctor booth named *'Charak'* to aid zero-contact check-ups of patients and maintain the safety of the medical professionals in the light of the COVID-19 outbreak. They have also converted several coaches into fumigation and disinfection tunnels in various spots across the country [59].

j. Police Force

Police are guarding containment zones, enforcing the lockdown and home quarantines, managing migrants trying to cross borders. They are also carrying out high-risk evacuations in coronavirus clusters with many of them not returning to their homes during this period of operation, as to avoid the transmission of infection to their family members. Many police personnel in India have been affected with COVID-19 while on duty and succumbed to this disease. [60]

Conclusion

India's response to COVID-19 pandemic with various measures is impressive, as mentioned by WHO. CFR of the illness is relatively low in India, compared to population size. Government of India has been pro-active in the preventive measures, including case detection,

quarantine and isolation with better treatment facilities for patients. The positive measures implemented by authorities in India indeed acted as a strong facilitator in protecting millions of people in the country. Researchers are yet to find the scientific reason for the slow pace of spread and high recovery of the deadly virus which has disrupted the life of millions in the developed nations. The announcement of Serum Institute of India, on the development of COVID-19 vaccine within a year is throwing light to hope of 30 million health workers who are directly battling the illness in the healthcare facilities to save lives of people.

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Hypoglycemic Effects of *Rosa damascena* Mill. Ethanolic Extract on Blood Glucose Levels and Diameter of Langerhans Pancreatic Islets

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Abstract

Background: Diabetes mellitus is a chronic disorder caused by elevated levels of high blood glucose (hyperglycemia). This chronic hyperglycemia causes ROS to accumulate and oxidative stress to increase. *Rosa damascena* is a plant that contains high levels of antioxidants and polyphenols, but this is not widely known to the public. **Purpose**: The aim of this study is to understand the hypoglycemic effects from *Rosa damascena* on blood glucose levels and diameter of Langerhans pancreatic islets. **Method:** Thirty Wistar albino rats (200-225gr) were divided into 6 groups. Group 1 was a normal control (KN), group 2 was hyperglycemic (KD), group 3 was metformin 250 mg/kg BW (KM), group 4 was treatment extract 250 mg/kg BW (P1), group 5 was treatment extract 500 mg/kg BW (P2) group 6 was treatment extract 1000 mg/kg BW (P3). All animals in the group were injected with STZ 50 mg/kg BW, except for group 1. **Result:** Data were analyzed statistically using SPSS- 22 software with the Kruskall-Wallis test ($p<0.05$). The result showed that ethanolic extract of *Rosa damascena* has decreased blood glucose levels in days-14 (382 ±21,97) with 250 mg/kg BW (P1) compare with another treatment group. In histological observed there are no significant different between group ($p>0.05$). This is showing that ethanolic extract of *Rosa damascena* has inability to repaired the diameter of Langerhans pancreatic islets. **Conclusion:** Ethanolic extract of *Rosa damascena* decreased blood glucose levels but did not repaired the diameter of Langerhans pancreatic islets.

Key words: *hyperglycemic, Rosa damascena, Langerhans islets.*

Introduction

Diabetes is a group of metabolic diseases characterized by increased blood glucose levels (hyperglycemia) due to impaired insulin secretion, insulin resistance or both[1]. Diabetes is classified into type 1 diabetes which results from failure of insulin secretion, type 2 diabetes occurs due to insulin resistance, gestational diabetes that occurs during pregnancy and other types of diabetes that occur due to autoimmunity[2]. According to data from the International Diabetes Federation, 382 million people worldwide suffer from diabetes, and this figure will increase to 592 million sufferers in less than 25 years[3]. Diabetes can lead to complications of micro vascular and macro vascular damage. Macro-vascular damage can be in the form of heart failure, stroke, peripheral vascular disorders, while microvascular damage can result in retinopathy, neuropathy and nephropathy in diabetic patients[4]. Hyperglycemia in diabetes can lead to increased production of ROS in various cell types. For example, there was an increase in AGEs in Type-2 DM in a diabetic mouse model. Increase in 8-hydroxydeoxyguanosine and modification

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of hydroxynonenal protein in pancreatic beta cells. This study shows that increased glucose levels and chronic hyperglycemia can further exacerbate oxidative stress during Type-2 DM[5].

Treatment of diabetes mellitus with herbal ingredients has been widely researched, especially in relation to its effectiveness, safety and low side effects. One of the herbs that can be used for diabetes treatment is rose flower (*Rosa damascena*). The rose (*Rosa damascena*) is a family of Rosaceae or commonly referred to as damask rose. Rose is a plant that is found almost all over the world, this plant is found in many areas including Europe, the Middle East, especially Iran and Turkey[6]. In Indonesia, this plant can be found in almost all regions, in East Java, this plant is found in many mountainous areas such as Batu Aji, Batu, Malang. Roses have many phenolic contents including flavonoids, glycosides, terpenes and anthocyanins isolated from various parts of *R. damascena* flowers[7]. The highest content of phenolic compounds is glycosides and quercetin which are obtained from methanol extract. Both of these compounds have high antioxidant abilities and are DNA protective agents. Based on the DPPH test conducted by Kumar[8] It is known that roses have the ability to free radical-binding by 43.6 ± 0.25% at every 100mg/ml. Study conducted by Gholamhoseinian[9] showed that *R. damascena* methanol extract with a dose between 100-1000 mg/kg BW can reduce blood glucose levels through inhibition of α-glucosidase activity by 98% compared to acarbose which only achieves 51%.

This study aims to determine the effect of giving different doses of rose flowers extract (*Rosa damascena*) on fasting blood glucose levels and the diameter of Langerhans islets in male albino Wistar rats (*Rattus norvegiccus*) after being induced with low doses of streptozotocin. The results of this study are expected to be useful for the development of plant effectiveness as an innovation in diabetes therapy.

Method

Animal

This study used experimental 30 animals Wistar white rat (*Rattus norvegiccus*) 3-4 months old male with a body weight of 200-225 grams. The maintenance and surgery of experimental animals was carried out in the Pharmacology Laboratory, Faculty of Medicine, Universitas Airlangga. Rats were acclimatized for 7 days at room temperature 25-30°C with a relative humidity of 55-65%. During the adaptation period, the experimental animals were fed a standard diet and drank clean water ad libitum. This research was conducted based on the letter of ethics no: 150/HRECC.FODM/III/2020.

Extraction Proses

Roses are obtained from rose plantations in the Batu Aji area, Batu, Malang, East Java. 800 grams of rose flowers that have been dried and soaked in 5 Liters of 96% ethanol solution for 3 x 24 hours, closed tightly and kept out of the sun. The liquid extract obtained was then removed from the moisture using the evaporation method with a rotary evaporator machine at a temperature of 50°C so that a thick extract was obtained. The extract obtained is then diluted and calculated according to doses of 250 mg/kg BW, 500 mg/kg BW, and 1000 mg/kg BW[10].

Animals Treatmenet

The experimental animals were divided into 6 groups, KN (control group), KD (hyperglycemic group), KM (metformin 250 mg / kg BW), P1 (RD 250 mg / kg BW extract), P2 (RD 500 mg / kg BW extract), P3 (extract RD 1000 mg / kg BW). The experimental animals were injected with a single dose of STZ 50 mg/kg BW. After 5 days of measurement of fasting blood glucose levels, the rats were considered diabetic if their glucose levels reached >200 mg/dL. Treatment according to each category was then given to the experimental animals for 14 days. Glucometers and glucostrip (*Easy Touch Blood Glucose*) were used to measure GDP levels[11], The animals were fasted for 8 hours, then slightly wounded at the tip of their tail, then dripped at the tip of the strip as the blood came out. Blood glucose fasting rate testing was performed on the 0^{th}, 7^{th} and 14^{th} days.

Preparation of Histological staining Pancreas

The experimental animals were euthanized using 0.67 ml chloroform / experimental animal for 60 seconds until the experimental animals did not move[12]. Furthermore, surgery and removal of the pancreas are performed. The pancreas wad washed with normal saline solution and then immediately put into a 10% *neutral*

buffer formalin solution. The preparation of HE staining slides was carried out at the Laboratory of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga. The pancreas organ that has been inserted into the 10% NBF is made paraffin blocks, then cut using a microtome knife to produce a thickness 4-5m. The results of the slices were transferred with a brush into warm water 38˚-40˚C to straighten any fine wrinkles. Perfectly stretched slices are taken with a slide. The pieces that have been taken are then dried and placed on a hot plate 38˚- 40˚C until dry. Subsequently, counterstaining was carried out with *Mayer haematoxylin* for 10 minutes and washed with tap water. The final step is to dry the preparations in the mounting using an entellan and cover with a glass cover[13].

Data Analysis

The data obtained were in the form of average fasting blood glucose levels and the average diameter of the pancreatic islets of Langerhans. In the process of determining the diameter of the Langerhans pancreatic islets, a qualitative observational study was carried out using an Olympus BX-50 light microscope combined with the *Olympus cellSens* Entry software with 4 fields of view. Data processing was performed with the help of SPSS version 22 software. Data were analyzed using the *Kruskall-Wallis* and *Mann-Whitney* tests with significance ($p < 0.05$).

Result

Testing of Blood Glucose Levels

Data on the results of blood glucose levels were tested for normality with Shapiro-Wilk with an abnormal distribution ($p > 0.05$) so that it was followed by the *Kruskall-wallis* test, it was obtained that the GDP data had differences between groups. The data then performed the *Mann-Whitney* test to determine significant differences between groups. GDP rate data is presented in Table 1.

Table 1. Fasting Blood Glucose levels before and after treatment

Treatment	N	Blood Glucose level 0 days treatment (mg/dL)	Blood Glucose levels 7 days after treatment (mg/dL)	Blood Glucose levels 14 days aftertreatment (mg/dL)
Control (KN)	5	103.8 ± 5,72a	106,20 ± 12,25a	98 ± 5,86a
Hyperglycemia (KD)	5	393.4 ± 101,37b	404,80 ± 91,75b	437,20 ± 75,96bc
Metformin 250 mg/kg BW (KM)	5	418 ± 102,39b	407 ± 149,39b	388 ± 166,12b
Extract RD 250 mg/kg BW (P1)	5	433.2 ± 43,67b	408,80 ± 21,97b	382 ± 21,97b
Extract RD 500 mg/kg BW (P2)	5	430 ± 20,71b	552,40 ± 46,94bc	402,40 ± 78,20bc
Extract RD 1000 mg/kg BW (P3)	5	417.6 ± 87,26b	491,20 ± 61,26bc	454 ± 75,80bc

Noted: *significant $\alpha = 0.05$

[a,b] Different superscript showed the difference between group significantly

From these data, it can be seen that the treatment group experienced a decrease in fasting blood glucose compared to the hyperglycemic group although it was not statistically significant. There can be seen that in the hyperglycemic group, which was only given STZ injection without extract, there was an increase in GDP levels on day 7 with a value of 404.8 ± 91,75 mg/dL and on day 14 to 437.2 ± 75,96 mg/dL. In the KM treatment group that was given metformin 250 mg/kg BW, there was a decrease in GDP on day 7 with a value of 407 ± 149,39 mg/dL and on day 14 to 388 ± 166,12 mg/dL. In the P1 treatment group given RD 250 mg/kg BW ethanol extract, there was a decrease in GDP on day 7 with a value of 408.8 ± 21,97 mg/dL and on day 14 it became 382 ± 21,97 mg/dL. In the P2 treatment group given RD ethanol extract 500 mg/kg BW, there was an increase in GDP on day 7 with a value of 522.5 ± 46,94 mg/dL and a decrease on day 14 to 402.4 ± 78,20 mg/dL. In the P3 treatment group given RD ethanol extract 1000 mg/kg BW, there was an increase in GDP on day 7 with a value of 491.2 ± 61,26 mg/dL and a decrease on day 14 to 454 ± 75,80 mg/dL.

Measurement of Pancreatic Langerhans Islet

The average results from the Langerhans islets diameter measurement results was checked using *Shapiro-Wilk* and reported that the data was normally distributed. The *Brown-Forsythe* test was then carried out and found a value ($p > 0.05$), which implies that there is no substantial difference between classes. Table 2 presents the data from the measurement results of the Langerhans islets diameter measurement.

Table 2. Diameter of Langerhans Pancreatic Islets

Treatment	N	Average Diameter of Langerhans Pancreatic Islets (mm)	0,592 p
KN (Normal)	5	124.01 ± 10.82	
KD (Diabetes)	5	107.96 ± 19.65	
KM (Metformin 250 mg/kg BW)	5	114.02 ± 7.95	
P1 (Extract RD 250 mg/kg BW)	5	111.27 ± 32.94	
P2 (Extract RD 500 mg/kg BW)	5	120.92 ± 15.00	
P3 (Extract RD 1000 mg/kg BW)	5	112.89 ± 16.21	

Noted: *significant $\alpha = 0.05$

From the data above, it can be seen that there are increase in the average diameter of the pancreatic islets of Langerhans although statistically there are no significant difference between groups. The histological of the pancreatic islets of Langerhans diameter showed that the hyperglycemic group (KD) had the least value (107.96 ± 19.65mm) compared to the all treatment group. The P2 group had the best value compared to other treatment groups (120.92 ± 15.00mm) almost the same as the control group.

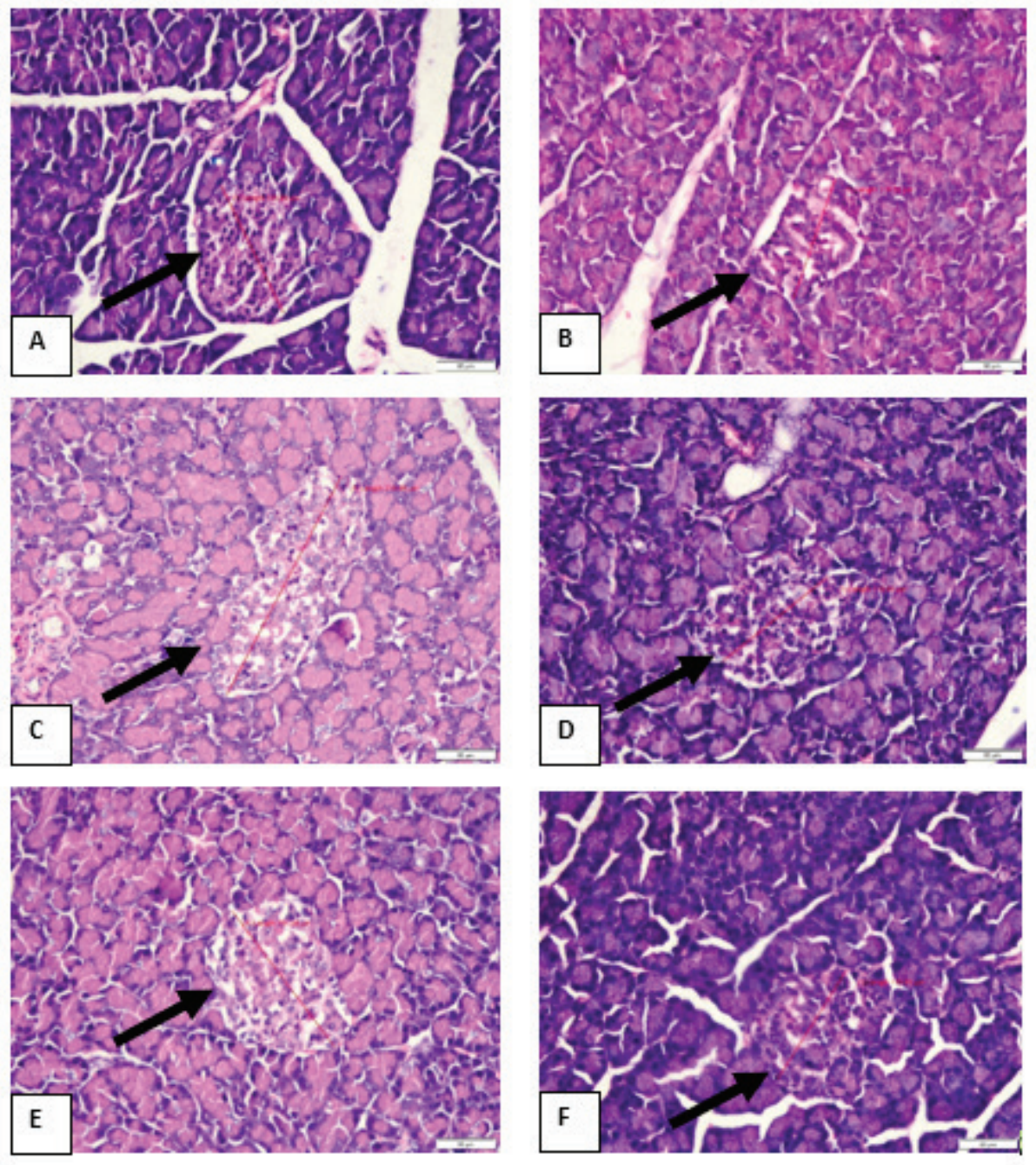

Figure 1. Histology Image of Langerhans Pancreatic Islets with 40x magnification.

A: Control group (KN), **B**: Hyperglycemic group (KD), **C**: Metformin group (KM) 250 mg/kg BW, **D**: (P1) Extract RD group 250 mg/kg BW, **E**: (P2) Extract RD group 500 mg/kg BW, **F:** (P3) Extract RD group 1000 mg/kg BW

Discussion

In normal rats, blood glucose levels can vary. According to research conducted by Gutierrez[14] normal fasting glucose rats levels ranged from 91.19 to 95.41 mg/dL. In this study, the fasting blood glucose levels in the control group showed 98-103 mg/dL. Meanwhile, the hyperglycemic group showed an increase in GDP after being injected with STZ 50 mg/kg BW with a range of 390-500 mg/dL. This shows that giving STZ at a dose of 50 mg/kg BW can increase fasting blood glucose levels in experimental animals. As already mentioned by Jinzi[15] the STZ mechanism enters pancreatic β cells

through the GLUT 2 receptor found on the pancreatic β cell membrane. Pancreatic β cells are specific target cells for STZ. As a glucose analog, via the GLUT2 transporter, STZ reaches β-cells and accumulates intracellularly. An alkylating agent, diazomethane (DAM), which induces DNA methylation and elicits diabetogenic action, forms STZ inside the cells. STZ also induces diabetes through various mechanisms, such as increased NADPH levels either through the development of glucose auto-oxidation or *diacylglycerol* (DAG) and increased free radical generation of O^2, activation of the protein kinase C pathway, glucose flux through the metabolic pathway of polyol, accumulation of *Advanced Glycation End products* (AGEs) and secretion of cytokine[16]. STZ selectively destroys β cells, allowing STZ to be considered a special compound in animals with an appropriate build and face validity for modeling diabetes.

From this research, it can be seen that the hyperglycemic group and the treatment group before and after being given the treatment showed different results. In the metformin group, the value of blood glucose levels on day 7 was 404.8 mg/dL and on day 14 it decreased to 388 mg/dL. This is in accordance with the research conducted by Shahlah[10] that metformin at a dose of 250 mg/kg BW can reduce fasting blood glucose levels. According to Ikeda[17] the mechanism action of metformin is related to the absorption process that occurs in the small intestine. The accumulation of metformin in the small intestine tissue after oral administration indicates that metformin increases the work of glucose absorption in the small intestine in rats in vivo, and this is believed to be a form of efforts to lower blood glucose levels. This maximum absorption of glucose cannot occur if metformin is given intravascularly or intraduodenal.

Another study suggested that metformin could decrease blood glucose levels through the (*Glucagon Like Peptide*) GLP-1 pathway. The gastrointestinal tract is the body's largest endocrine organ and accountable for the release of several hormones essential for homeostasis of blood glucose[18]. GLP-1 and *Glucose-dependent Insulinotropic Polypeptide* (GIP) which when compared to "isoglycaemic" intravenous glucose infusion, account for substantially greater insulin secretion in response to oral or enteral glucose, a phenomenon known as the "incretin effect." GLP-1 also inhibits gastric emptying, suppresses the secretion of glucagon and food intake and can minimize gluconeogenesis irrespective of insulin or glucagon shifts[19]. This study showed a decrease in fasting blood glucose levels in the KM group but the decrease was only slightly. This can be affected by the duration of metformin administration which is only given for 14 days. In an acute experimental study (850 mg/kg and 1500 mg/kg BW) it was found that metformin could increase postprandial GLP-1 in several groups. Another study states that giving metformin for 4 weeks and 18 months can consistently increase GLP-1 levels[20].

Based on report by Gholamhosseinian[21] *Rosa damacena* extract at doses of 250, 500, and 1000 mg/kg BW can reduce post prandial sugar levels in the 60th minute. In our study, there was a decrease in blood glucose levels in the P1 and P2 groups (250 and 500 mg/kg BW) compared to the hyperglycemic group, this is in accordance with the research conducted by Abbas [21] that methanol extract of *Rosa damascena* dose of 100 mg / kg BW can reduce fasting blood glucose levels in 2 weeks. From these studies it is known that *Rosa damascena* is able to reduce blood glucose levels and the HOMA-IR index on insulin resistance. *Rosa damascena* also increases sensitivity to insulin but has not been able to increase insulin secretion.

Study by Gholamhosseinian [9] *Rosa damascena* extract has been shown could reduce glucose levels by inhibiting the α-glucosidase enzyme, which slows down the absorption of carbohydrates from the small intestine and results in an antidiabetic effect by reducing the postprandial glucose level effect. In vitro inhibitory effect on lipid oxidation and antioxidant effect of *R. damascene*. Another study *R. damascena* has been shown to have a tocopherol-like effect and causes a strong antioxidant and lipid peroxidation inhibitor effect. This plant is proposed for the treatment and prevention of free radical diseases[22]. The study that did by Kumar [8] total phenolic contents of *Rosa damascena* methanolic extracts by DPPH-scavenging activity 43.6 ± 0.25 at 100mg/mL. The IC50 value for the methanolic extract of *R. damascena* was found relatively at 21.4 mg/mL. Thus, rose extracts displayed strong antioxidant activity due to the high content of polyphenols. These phenolic antioxidants play an important role as bioactive concepts in the conventional medicines used in rose flowers. In *Rosa damascena*, the flavonoid content is indicated to induce hypoglycemia and hypolidemia. A 16 percent

rise in insulin secretion from pancreatic β - cells is also induced by flavonoids. The action is activated by the *peroxisome proliferator receptor* (PPAR dan PPAR γ) arrangement[21]. Another study showed that the hypoglycemic effects of flavonoids can be partially due to increased hexokinase and glucokinase activity in the liver and their insulin-like effect, thus reducing the indications of diabetes mellitus[23].

In this study, the effect of decreasing fasting blood glucose occurred at doses of 250 mg/kg BW and 500 mg/kg BW, but at doses of 1000 mg/kg BW, the decrease in fasting blood glucose was not very visible. This is not in line with research conducted by Gholamhosseinian[9] at doses 1000 mg/kg BW, *R. damascena* was able to reduce post-prandial blood glucose levels. Several things that extract compounds are not well absorbed such as; because the doses used are non-toxic, the substances may not have been absorbed by oral gavage due to the presence of some materials in this plant, or they have been metabolized by the liver and produced inactive metabolite. Many things may affect the extract's ability to lower glucose, among others; Another potential reason is that, as shown in the present analysis, the small decrease in blood glucose achieved by obtaining the extract is neutralized due to the presence of absorbable carbohydrates usually found in extracts. It is also likely that there were substances that decreased blood glucose along with their additive in *R. damascena* extract to prevent blood glucose in rats[11].

The statistical analysis result showed that histological observations of the diameter Langerhans pancreatic islets there are no differences between groups. However, when seen from Figure 2, it shows that the hyperglycemic group has the smallest average value (107.96 ± 19.65mm) compared with the control and treatment groups, the P2 treatment group has the highest value (120.92 ± 15.00 mm) compared with other treatment groups. Figure 3 shows an overview of Langerhans islet cells in the hyperglycemic group that experienced the most severe damage due to STZ induction compared to the control and treatment groups. Pancreatic β cell damage can result in an inability to produce normal amounts of insulin; consequently, the size of Langerhans islets will be affected by a degree of damage. According to the number of their constituent cells, the Langerhans islets differ in size. These islets are responsible for the pancreatic β-cells' secretion of insulin. Under normal conditions, the presence of diabetic agents would not affect insulin secretion[24]. Histologically, the diameter of Langerhans islets in mice has a diameter approximately 2 times greater (116±80 μm) than human islets (50±29 μm)[25]. Pancreatic islets will experience a change in diameter under the condition of hyperglycemia.

In the KM, P1, P2, and P3 treatment groups, there was an increase in the diameter of Langerhans islets but it was not significantly different ($p>0.05$). This shows that the administration of metformin 250 mg/kg BW and *R. damascena* extract at a dose of 250 mg/kg BW, 500 mg/kg BW, 1000 mg/kg BW has not been able to increase the diameter of the pancreatic islets of Langerhans. This is due to the mechanism of action of metformin which increases the effectiveness of GLP-1 and *R. damascena* extract which inhibits α-glucosidase activity by inhibiting carbohydrate absorption in the intestines without stimulating the β cells of the pancreas so that there is no significant change in the histological cross section of Langerhans islets.

Conclusion

Based on the results of this study, it can be concluded that *Rosa damascena* ethanol extract can reduce blood glucose levels at doses of 250 mg/kg BW and 500 mg/kg BW. The ethanol extract of *Rosa damascena* has not been able to improve the diameter of the islets of Langerhans Pancreas in Wistar rats injected with STZ.

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Study on the Factors for Reporting Nosocomial Infections in Hospitalized Patients

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Abstract

Reporting nosocomial infections (NI) is a goal of alert and awareness, and an essential link in the current policy of controlling health risks.

From the health professionals and health authorities point of view, the reporting objectives are to set up an alert system allowing the quick detection of unusual INs, and to verify, if necessary, that corrective mesures are implemented

The present study did not fail to highlight a set of factors influencing the non-reporting of (NI) among the caregivers at El Idrissi Hospital in Kenitra, namely : a) personal factor, b) organizational factors and, c) institutional factors

The NI reporting plays an important role in the prevention of infectious risks, in the improvement of the quality of care. Thus, it would be intersting to examine the factors related to the non-reporting of NI

Keywords : *factors, reproting of NI, quality and safety of care, El Idrissi Hospital Kenitra.*

Introduction

Nosocomial infections represent a growing global public health issue, in view of the increasing population, the increasing frequency of immunodeficiences, the appearance of new microorganisms and the increase in bacterial resistance to antibiotics. Prevention and surveillance of NI, which require the involvement and investment of all stakeholders on a daily basis, are, today, the best weapon available to healthcare organizations to control them.

In France, the national prevalance survey of NI in 2016 involving 2337 health establishments and a population of 358353 patients, showed an overall rate of 4,97% [(1)].

In Morocco, the results of a national prevelance surver conducted in 1994 on an expanded sample of 24 hospitals revealed a prevelence of 5% in provincial hospitals, 10% in regional hospitals and reached 11% in national hospital structures [(2).] Therfore, the awarness of the importance and the seriousness of the NI is very recent. Thus, a delay has been registered in the surveillance, the reporting and the prevention of NI which remain an unknown problem and are not perceived as a priority within hospitals[(3)].

Object

The present study aims to explore and to describe the factors inducing the non-reporting of nosocomial infections by the nursing stuff at the care units of El Idrissi Hospital in Kenitra.

Methodology

In order to collect the necessary data from the selected population by the sampling, the authors recommanded to use a questionnaire adressed to caregivers to gather necessary data to get an overview meeting the objectives previously determined.

The survey through questionnaire is an observation tool that quantifies and compares data. This data is collected from a representative sample of the population targeted by the evaluation. 165 people.

II. Presentation and discussion of the study results the interest of reporting :

Table N°1 : the interest of reporting

The interest of reporting	%
An application of a ministerial note	0%
A risk management measure related to care	84%
A necessity to nursing practice	86%
A regulatory obligation	8%

84% of participants consider reporting the most important step for successful NI management, and is a risk management measure related to care. For 68%, it's a necessity for caregiving which confirms the importance of activities related to reporting. In fact, mastering this practice will lead to mastering NI surveillance, a cornertone of care quality. Therfore, the NI reporting is an indispensable link in the policy of nosocomial infection's control. As detected, the reporting is closely linked to the human nature, the latter can be influenced by an awarness campaign boosted by training sessions to prevent health risks.

In Fance, NI reporting is a legislative obligation and an alert and vigilance system put in place in August 2001[4], which specifies that every doctor, nurse, pharmacist, densits, midwife or member of the paramedical staff who notices one or more cases of infections must report them to the doctor in charge of the service where the cases have been detected and to the operational hygiene team (OHT). 64% of the surveyed hospital staff share this same finding.

Factors linked to caregivers :

In one hand, health professionals consider that the official definition of NI is difficult to operationalize, which makes it difficult to expore the responsible factors. According to 70%, reporting characteristics are difficult to identify and that the definition remains imprecise and vague, offering conductive conditions to indecision and produces, therfore, a non-report.

On the other hand, the non-reporting of NI by health personnel is a known fact all over the world according to a survey carried out on the perception of the NI reporting and which has disclosed several reasons gathered in the reluctance encountered within the establishment : fear of punishment, of prosecution and fear for branding [5].

The results of the present study confirm what has already been written (reluctance to report), 76% of careres noted that this reluctance is dur to fear of punishment and prosecution, and 66% ave a fear of branding.

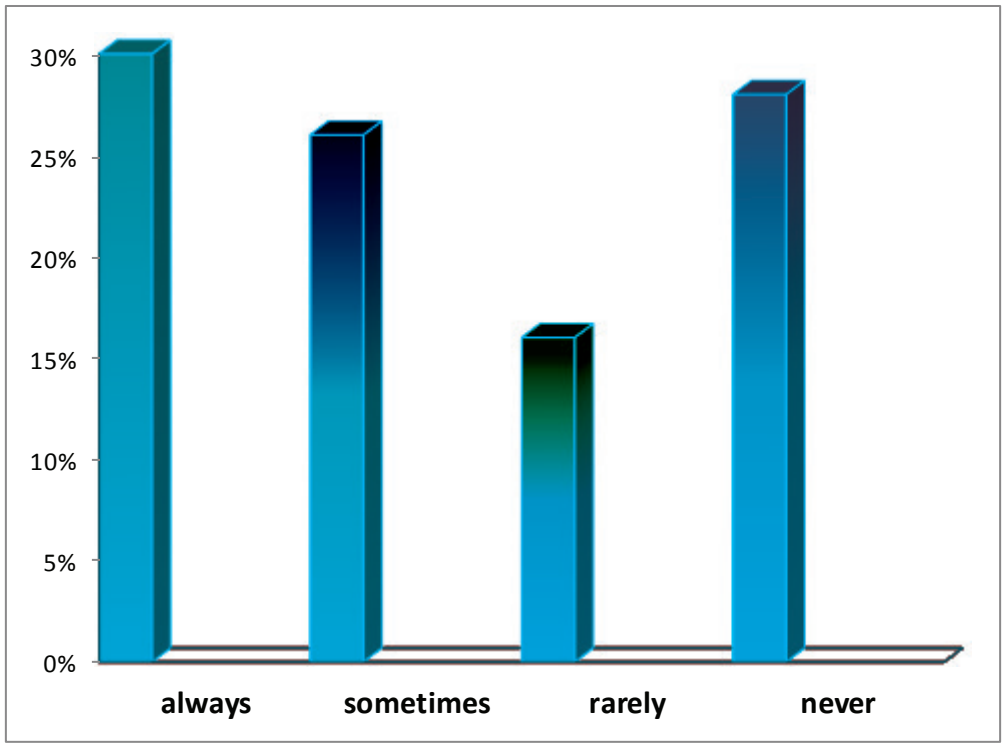

Figure 1: Perception of non-reporting and the absence of IN

However, according to the population under study, non-reporting is still linked to the real absence of NI for 30% and sometimes for 26%. The reporting of these infections is an essential link in the policy to combat INI.

Its implementation in a health institution will contribute to improving the awarness of health professionals to prevent health risks and coordinate between the different actors, identifying practices that do not comply with the recommandations and standards, detecting unusual infectuous events, not covered by usual surveillance network, and to complement NI surveillance.

Causes of non-reporting and these consequences

Table 2: Consequences in the event of non-reporting

Consequences	%
High economic cost	56 %
Outbreak of epidemics	44%
Development of multi-resistant bacteria	64%
Emergence of healthcare-associated infections	48%

The consequences according to the participants in the study can be economic 56%, and health Development

of multi-resistant bacteria 64%, emergence of infections 48% and outbreak of epidemics 44%.

As a result, any detected NI should be limited by mesures and precautions in order to stop its spread, therfore, any infetion unreported to the operational hygiene team (OHT) will escape these preventive mesures and precautions, therby causing adverse economical and social consequences for those who are cared for, caregivers and health establishments.

In Morocco, despite the creation of the CLIN (committe for the fight against nosocomial infestions) within hospitals (ministerial circular N°54/2008), the constitution of an EOH and the designation of a correspondant in hygiene, or a referent, at helath care units level, the dissemination of information in case of a NI remains marked by heterogeneity in the declarations since the regulatory texts obliging the declaration and the reporting of NI are not yet available[3].

On the other hand, the non-reporting of NI by health personnel is a known fact all over the world according to a survey carried out on the perception of the NI reporting and which has disclosed several reasons gathered in the reluctance encountered within the establishment : fear of punishment, of prosecution and fear for branding[5].

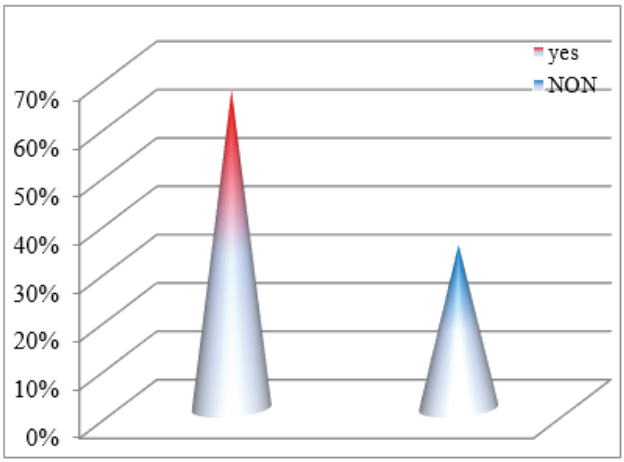

Figure 2: Fear in case of reporting for the service image

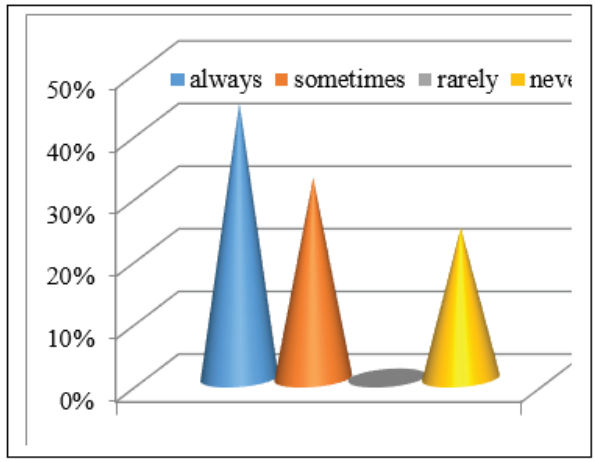

Figure 3: Fear of punishment and prosecution

The results of the present study confirm what has already been written (reluctance to report), 76% of careres noted that this reluctance is dur to fear of punishment and prosecution, and 66% ave a fear of branding.

The absence of intervention by the superiors has only brought effervescence to the existing situation, a developpement of a culture of taboos in the health sector which pushes us to question the way to resolve a problem not yet identified, as well as the non-reporting of 68% of participants when facing a NI.

Organizational factors :

This non-reporting remains a worrying situation by those in charge because it does not allow to specify the actual frequency of NI. Synthesis is made difficult by a set of brakes raised during NI reporting, namely: (a) uncertainty as to the accountability and (b) lack of information and training of professionals on the need to report NI and reporting procedures[6].

III. Staff training on reporting

Figure 4: continuing training on reporting nosocomial infections

84% did not receive continuous training in the reporting. Similarly, the survey conducted on the perception of nosocomial infection reporting by the South-West Coordinating Center for the Control of Nosocomial Infections (CCCNI) in 2006 revealed a range of reasons for not reporting, namely: (a)the lack of training of actors (mainly doctors) on nosocomial infections (NI), and (b) the lack of professional awareness of reporting. These studies confirm the results of our study, which highlighted the lack of basic and continuing training, as 88% did not have an initial training On one hand, it is necessary to identify, to recognize the object to report, from where the interest of the basic training. On the other hand, the development of the knowledge and the better management of the infectious risk will be dispensed by a complementary training in order to correctly interpret and identify the causes responsible for a detected NI.

Facteurs institutionnels

The hospital hygiene establishment policy is the main component of success of any system aiming the regression of NI and the security of care, that's why NI reporting must be included in the establishment priorities, especially since most international writings relate to hospital hygiene policy with the aim of improving quality and enhancing the safety of care, health care institutions need to move towards a clear policy on hospital hygiene and prevention that will focus on the development of action plans for contextual risk management and monitoring involving all stakeholders.

In the same context, many authors point out that uncertain protection and the aspects of legal protection of the reporting have been particularly studied and implicated in the non-reporting. Health professionals want to protect themselves from justice following complaints from victims (the patients) and the sanction of their immediate hierarchy[7]. The results of the present study have shown that the non protection of the reporting was evoked by 58 % of participants against 42%.

In the same perspective, the reasons for non-reporting are related to the fear of external control. This situation can be the subject of media coverage, sometimes even exploited by user associations and provoke an audit or external control of the general management, indirectly it can reproache the reporting professionals. Therefore, to avoid these judgments, they prefer not to report [8]. This finding, related to the fear of an external control, was evoked by 76% of the participants in the study.

Suggestions of the participants in the questionnaire :

Participants proposed a number of suggestions for improving the practice of reporting, namely:

Ø To sensitize the hygiene professionals and correspondents on their responsibility in the field of fight against the infectious risks.

Ø Provide continuing training as needed and during each incident.

Ø Display the reporting procedures and protocols at the different units.

Ø Adopt a permanent potential for support, based on the obligation to report.

Ø Guarantee protection of reporting professionals

Conclusion

NI reporting is a useful warning tool. However, progress still needs to be made to make the internal reporting circuits really operational. The development of a specific, accessible and simple computer tool is essential. In addition, the role of CLIN is seen as a positive help. Reporting a NI is part of a more comprehensive approach to transparency and risk management in health facilities which requires a major change in mentalities. . In France this device has proven itself (control of regional epidemics, national recommendations of good practices ...), at the national level, it is now necessary to extend the training around reporting and to convince the reluctant.

This system should be based on risk management, evaluation, traceability, transparency, collective responsibility and pooling of experiences.

Thus, we should understand non-reporting to situate it in a larger set, find levers to develop adherence to the quality-safety approach to care and overcome the issue of behavioral change.

Ethical Statement

To ensure the success of our study, a set of ethical

considerations were met, namely:

Ø Obtaining prior authorization from the establishment direction.

Ø Build trust with the participants of the study with regard to the confidentiality and the anonymity of the questionnaire and the self-determination interest.

In this context, respect to self-determination is achieved through the free and informed consent of all participants, and this, after explaining a) the content and the purpose of the study, b) its advantages, c) the possibility of withdrawing from the study at any time

Conflict of Interest: We wish to confirm that there are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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The Correlation of Congenital Anomalies of The Kidney and Urinary Tract with Renal Function in Children

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Abstract

Background: Congenital Anomalies Kidney and Urinary Tract (CAKUT) are the leading cause of kidney failure in children and cause of 30-40% end-stage renal disease with obstructive uropathy as the main cause. CAKUT obstructive type are: *vesico uretric junction obstruction* (VUJO), *pelvic uretric junction obstruction* (PUJO), *posterior uretral valve* (PUV) and non-obstructive type are reflux, neurogenic and hypoplasia. The aim of this study is to describe the effect of CAKUT on renal function in child.

Methods: Retrospective study from medical records of children with CAKUT at Dr. Soetomo General Hospital, Surabaya, Indonesia from January 2013 - March 2018. The characteristic of sex, age range, type of CAKUT and glomerulus filtration (GFR) value were recorded from medical record. Data processing using descriptive analysis with SPSS.

Result: A total of 94 Children on CAKUT abnormalities, both boys and girl's presentation equal 50%. Median age 3.0 (minimum-maximum 1.0-15.0) year. The most common CAKUT type in male were VUJO 17 (36.2%) and in female neurogenic type 19 (40.4%). Median initial glomerular filtration rate 2.7 ml/ min/1.73 m^2. Analysis of correlation between CAKUT and renal function p=0.072 (R^2=0,03). From 94 children of CAKUT, only 12 (12.8%) patients have undergo surgical intervention.

Conclusion: CAKUT with dominant obstructive type was more common in male and non-obstructive type in female pediatric patient. There was no significance correlation of GFR between obstructive and non-obstructive type.

Keywords: *Kidney, Urinary tract, Children, CAKUT, GFR*

Introduction

Congenital Anomaly of Kidney and Urinary Track (CAKUT) has a broad clinical spectrum and phenotype with clinical manifestations of obstruction and non-obstruction through different mechanisms [1]. CAKUT is a disease that is mostly found in children with a prevalence of 16.3 per million children with an incidence of CAKUT of 3-6 per 1000 live births. [2,3]. One third of these diseases will develop into end-stage renal disease (ESRD) [1]. It is important to determine the estimation of kidney function for each patient with CAKUT in order to minimize the occurrence of kidney damage and suppress the complications of kidney failure by periodic evaluation of renal function and adequate therapy [4]. Kidney function is assessed by calculating the estimated glomerular filtration rate, which depends on how many nephrons are still functioning normally. Currently, CAKUT therapy is largely conservative and maintains kidney function. This study aims to analyze the relationship between CAKUT and a decrease in the Glomerular Filtration Rate (GFR) in children.

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Methods

This study was an observational analytic study with a cross sectional study design to determine the effect of CAKUT and kidney function in children.

Subject Criteria

The subjects of the study were 94 pediatric patients experiencing symptoms of kidney and urinary tract disorders in the Pediatric Nephrology Clinic, Dr. Soetomo General Hospital, Surabaya in the period January 2013 - March 2018 using total sampling. All subjects who met the selection criteria (inclusion and exclusion) were included in the study. The inclusion criteria in this study included: age 1 month to 18 years, Fulfilling the diagnosis of CAKUT, there are results of renal function tests to determine the glomerular filtration rate. The exclusion criteria in this study were incomplete medical document data. Data from ultrasound results and kidney function tests were taken from the patient's medical records.

GFR calculation based on serum creatinine, age, body size, sex was calculated according to the formula [5]. CAKUT criteria were congenital abnormalities of the kidney and urinary tract, both structural and functional, which could occur in the kidneys, calix system, bladder, or urethra, as evidenced by urological ultrasound results[6].

Data Analysis

To test the correlation of CAKUT on both obstructive and non-obstructive kidney function, the simple liner regression test was used. Simple liner regression test is a test for correlation with categorical variables, if $p < 0.05$, a relationship between CAKUT and glomerular filtration rate was obtained.

Result

The results of this study indicate the characteristics of CAKUT patients as a whole, the sex and type of CAKUT have the same percentage, 50% each group. The most age is less than 5 years. The types of CAKUT in this study were divided into obstruction and non-obstruction types, the most subject type of CAKUT was the vesico uretro junction obstruction (VUJO) type followed by the pelvic uretro junction obstruction (PUJO) and the Posterior Uretral Valve (PUV) type, for the non-obstruction type respectively neurogenic followed by hypoplasia and reflux last (Table 1).

Table 1. Basic characteristics of subject

Variable				n (%)
Age (year), median (min-max)				5,0 (1,0-18,0)
Age at baseline (years), median (minimum-maximum)				3,0 (1,0-15,0)
Age (year), n (%)				
< 5 years old				41(43,6)
5-10 years old				36(38,3)
> 10 years old				17(18,1)
Sex, n (%)				
Male				47(50.0)
Female				47(50.0)
Type of CAKUT, n (%)				
Obstruction (n,%)	VUJO	PUV	PUJO	48(51,0)
				26(27,7)
				1 (1,1)
Non-obstruction (n, %)	Neurogenic			21(22,3)
	Hypoplasia			46(48.0)
	Reflux			28(29.8)
Therapy, n (%)				13(13,8)
Operative				5(5,3)
Conservative				12(12,8) 82(87,2)

The median GFR value in the obstruction CAKUT group was lower than that in the non-obstructed group. GFR values in CAKUT overall median were 102 (Minimum 2.7 - Maximum 269.0) ml / minute / 1.73 m2 (Table 2).

Table 2. Characteristics of GFR values in CAKUT

CAKUT	Median (Min-Max) (ml / menit / 1,73 m2)
Obstruction	98 (6.0 – 209.0)
Non-obstruction	103 (2.70 – 269.0)

In this study, based on the Kolmogorov-Smirnov test, it was obtained p <0.05, which indicates that the data distribution was normal. The simple linear regression test results obtained R2 = 0.035 and p = 0.072. p value was stated to be significant if p <0.05 so there was no relationship between glomerular filtration rate and obstruction and non-obstruction.

Discussion

The types of CAKUT in this study were divided into obstruction and non-obstruction types, where the obstruction type was divided into vesicoureteral junction obstruction (VUJO), pelvic-ureteric junction obstruction (PUJO), posterior urethral valve (PUV) and non-obstruction type divided into reflux, neurogenic and hypoplasia. The most obstruction group was VUJO type with 26 (27.7%), followed by PUJO type with 21 (22.3%) and PUV 1 type (1.1%). CAKUT was the most neurogenic type of non-obstruction group with 28 (29.8%), followed by hypoplasia 13 (13.8%) and reflux 5 (5.3%).

In contrast to the study from Soliman et al., 2015 showed PUV was the most common disorder found (36.4%), followed by VUR (19.6%), then PUJO (18.7%) [7]. Another study reported PUJO (62.7%) and VUR (16.6%). In a recent study from Radhakrishna et al., 2019, it was shown that although the majority of CAKUT has evidence of antenatal hydronephrosis, CAKUT sufferers begin to show symptoms of delayed presentation of various CAKUT, especially PUV and PUJO [8].

Characteristics of the GFR value in CAKUT each obstruction group median was 98 (Minimum 6.0 - Maximum 209.0 ml / minute / 1.73 m2) and the non-obstruction median group was 103 (Minimum 2.70 - Maximum 269.0 ml / minute / 1.73 m2). In this study, there was no significant difference in GFR values in each CAKUT group. In this study, it was found that there was no relationship between obstruction and non-obstruction of GFR (p = 0.072). CAKUT in children with stage 2 and 3 CKD has a high incidence of terminal renal failure over 5 years. Kidney anatomy abnormalities are more common than lower urinary tract disorders and often cause terminal renal failure [9]. The longer a patient suffers from CAKUT, the more likely it is to suffer from various diseases [10].

A study from Radakhrisna et al., 2019 in 81 children with CAKUT, there were 27% of children with hypertension, this group had a lower GFR value than the non-hypertensive group, but this result was not statistically significant, suggesting that the etiology of hypertension is multifactorial and not. depending on the level of renin plasm [8]. The number of functional nephrons formed at 32 to 34 weeks of pregnancy determines kidney function. Nephrogenesis stops after 34 weeks of gestation. The growth of the renal tubules and the expansion of the glomerular cross-sectional area increases the functional capacity of the kidney. The increase in glomerular base surface area after birth contributes to an increase in the glomerular filtration rate during infancy, childhood and adolescence. Reduced nephron count was associated with adult-onset hypertension. In severe dysplasia, it indicates renal insufficiency, whereas in renal dysgenesis disorders, it occurs at the level of tubule number, cross-sectional area, and renal cell maturation. Abnormal tubular differentiation such as kidney dysplasia limits the capacity of the kidneys to concentrate urine, absorb sodium and secrete potassium so that electrolyte imbalance will occur [6].

In this study, the research sample and kidney function, in this case the GFR data, were only taken once when the patient first came to the pediatric nephrology clinic, so that the average sample still had good kidney

function. This study did not assess the frequency of incidence of UTI, hypertension and proteinuria which can complicate children with ACE and have an effect on glomerular filtration rate. There is a strong relationship between CAKUT and chronic kidney disease, where the incidence of recurrent UTIs in the presence of functional morphological anomalies can lead to CKD. This was not done because of limited research time.

The benefit of this research was to know kidney function through glomerular filtration rate to determine the degree of severity or decrease in kidney function in CAKUT patients then kidney function evaluation and optimal therapy are given to minimize the occurrence of kidney damage and avoid complications of kidney failure

Conclusion

CAKUT with dominant obstructive type was more common in male and non-obstructive type in female pediatric patient. There was no significance correlation of GFR between obstructive and non-obstructive type.

Conflict of Interest : None declared.

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Ethical Clearance : Approved by the Health Research Ethics Committee of the Dr. Soetomo General Hospital, Surabaya No. 0225/KEPK/IV/2018

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Stress among Nurses Working in the Provincial Hospital of Kenitra, Morocco: a Job Demand-Control-Support Survey

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Abstract

The nursing stress literature points to an overwhelming culture of accepting and expecting stress at work, ironically linked to controlling the workplace to effectively and actively manage stress. According to the Karasek Job Demand Control Support (JDCS) model, stress-causing factors have been extensively studied in nursing-related workplaces, especially in critical and emergency situations. However, little is known about the impact of workplace stress on the cognitive functions of caregivers.

Method: A survey was carried out using the JDCS model among 96 nurses working in different departments of the provincial hospital in Kenitra (emergencies, surgical operating units, pediatrics…). Specific questions explored variables such as gender, length of service in the system, level of qualification and work experience.

Results: A high level of job strain was highlighted with a gender effect. Women Nurses are more stressed than men. According to the study, age over 40 was a key factor in high work stress, also for the work schedule; nurses adopting the 12/36 system were located in the high stress quadrant. Both of these factors can be predictors of poor mental health. Strategies to improve the personal and professional performance of nurses to cope with daily stressors in different hospital departments are needed.

Keywords: *Stress, Karasek, JDCS, Nurses, Hospital, Kenitra, Strain*

Introduction

Stress at work is a major concern [(1)]. Nurses working in emergency departments are particularly at risk [(2), (3), (4)]. They must focus on a wide range of acute care, on demand. Nurses also experience stress due to shift work [(5)], increased workload [(6)].

Nurses face complex interactions between stress [life-and-death emergencies [(7)], which are the defining characteristics of their work], sleep deprivation and fatigue due to repeated changes in shifts [(5)]. Work-related fatigue can lead to a variety of physical and psychological symptoms [(5)] and delays in decision making, including treatment or medication errors [(8),(9)]. In addition, prolonged stress puts nurses at a higher risk for multiple illnesses [(10), (5)], mainly systemic inflammation [(11),(12)], metabolic disorders[(13)], and coronary heart disease[(10),(14)].

All of this contributes to an increase in sick leave [(15)], to the premature departure of nurses towards early retirement. Among nurses, people working in emergency departments [(16)], pediatrics, surgical operating rooms or intensive care have higher levels of stress and burnout [(17), (18)]. However, the differences in job stress among nurses in these different types of acute care units have never been fully studied.

The Job Demand-Control (JDC) model [(19)] and the Job Demand-Control-Support (JDCS) model [(20)] is one of the most commonly used theoretical frameworks

relating characteristics of the workplace to health and well-being [21].

The JDC model recognizes the importance of daily environmental stressors for long-term stress experiences [22]. It is based on two main hypotheses: the workload hypothesis predicts that job demands such as heavy workload and time pressure increase worker stress, and the buffer hypothesis predicts that increased control can mitigate the negative effects of high demands. The use of the term "control" highlights two opposite ends of the continuum describing freedom of decision: the recognition of skills and decision-making authority [20]. The "active work" quadrant is defined by high demands and high control, and the "strain" quadrant with high stress is defined by high demands with low control [22].

The active work quadrant is associated with the facilitation of learning and skills development [19], [21]. Karasek also developed the JDCS, also known as the "Work Content Questionnaire" [20]. The JDCS model has added a buffer of social support that can mitigate the negative effects of high blood pressure on health and well-being [23].

Nursing has been part of the "active work" quadrant of this model on several occasions and around the world [22], [24].

However, less is known about Moroccan nurses and especially those working at the Kenitra provincial hospital. Therefore, the main objective of this study was to compare the differences in perceived needs, control and social support between nurses from different hospital departments of the provincial hospital center of Kenitra.

Methods

Participants

We conducted a cross-sectional study with 96 nurses working in different departments of the Provincial Hospital of Kenitra, Morocco. Being a nurse was the only inclusion criteria. The questionnaires were distributed to nurses through their hierarchies. Respect for anonymity and free consent have been clarified. The study was authorized by the management of the CHP in Kenitra, Morocco.

Outcomes

The questionnaires assessed the characteristics of nurses and demographic and employment variables: age, sex, experience as a nurse, seniority in the system, and experience in the current position. The JDCS assessed psychological demand, decision latitude and social support using 29 items from the French version of the content questionnaire [20], [25].

Statistical Analyses

Statistics were performed using SPSS software. Where possible, parameters were expressed as mean ± standard deviation (SD).

Also, a multi-component analysis was performed to detect the relationships between the variables in an exploratory factor analysis approach

Results

1-Distribution of nurses by sex and grade

The study was conducted with 96 respondents, of whom 41.4% (n = 41) were men and 55.6% (n = 55) were women. The sex ratio was found to be balanced (female / male) with p <0.189 (single sample binomial test). The ranking of these respondents indicates that 64.6% (n = 64) are state registered nurses (Nurses), including 30 male nurses and 34 female nurses, and 32.3% (n = 32) are nursing assistants. (N.A), including 11 men and 21 women. The chi2 independence test showed no significant relationship between these two factors (sex * category) (chi2 = 1.36; p <0.243).

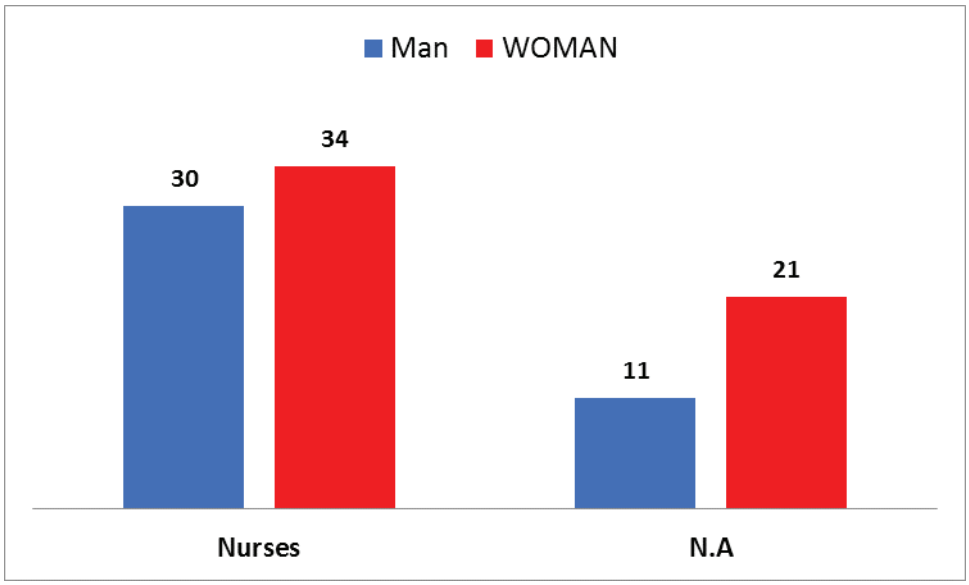

Figure 1: distribution of respondents according to sex and grade

2- Distribution of respondents according to sex, grade and age

The distribution of nurses surveyed according to age shows that 67.7% (n = 65) are over 40 years old, including 41 male nurses and 24 male nurses, followed by the class of nurses aged between 30 and 40 years old (8 women and 12 men) and which represents 20.83% and finally the category of respondents aged less than 30 years and which represents 11.45% (n = 11). The chi2 test did not show a significant difference between these two factors (age * sex) (chi2 = 3.37; p <0.186).

Regarding the distribution of nurses according to age and grade, the chi-square test showed a strong association between these two factors (chi-square = 18.94; p <0.000). In fact, among the category of nurses, 53.12% (n = 34) are over 40 years old, 31.25% (n = 20) are between 30 and 40 years old and 10 nurses are under 30 years old. In contrast, 96.87% of nursing assistants are over 40 years old and only one respondent is under 30 years old. However, the distribution of nurses by marital status shows that 75.8% declared married (n = 75) against 24.2% are single (with 5 divorced respondents).

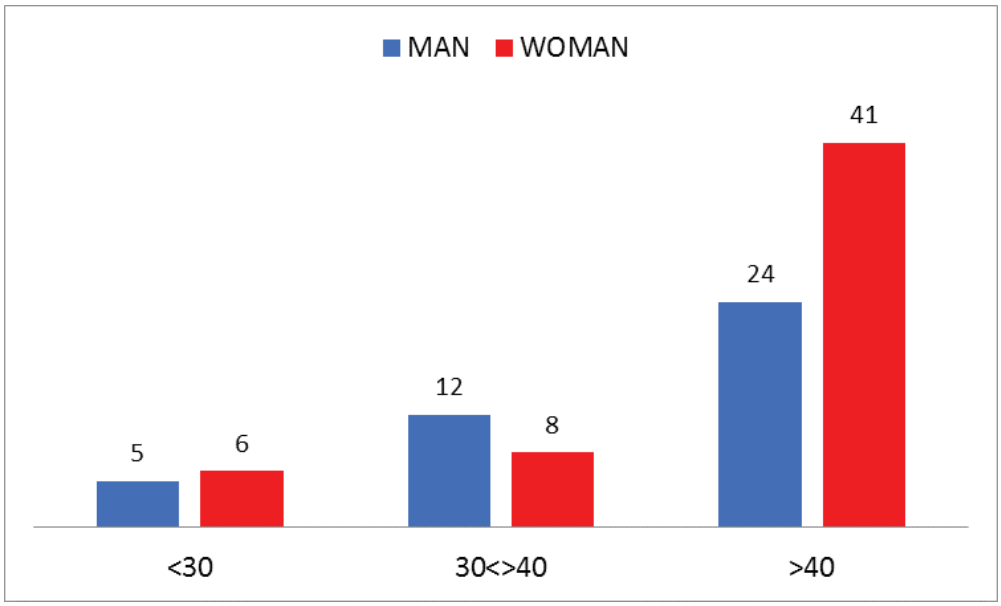

Figure 2: distribution of nurses by age and sex

3- Distribution by level of education, sex, grade and age

The table below presents the results of the chi-square test between the level of education and certain socio-demographic parameters. In fact, 33.33% (n = 32) have a university level against 28.12% (n = 27) have reached a bachelor's level or less, while the rest of nurses have other diplomas.

However, the chi-square test showed a strong association between education level and these three factors. In addition, and among the nurses who had a university degree (n = 32), 11 respondents were under 30 years old, 11 others were between 30 and 40 years old and 10 respondents were over 40 years old.

However, the category of nurses with a diploma less than the baccalaureate are all over 30 years old and for the rest of the respondents they are all over 40 years old.

The distribution of nurses by level of education and sex shows that among graduates of higher education (n = 32), 65.62% (n = 21) are female against 34.38% (n = 11) are male nurses.

For the category of nurses with a level of education less than the Baccalaureate, 17 respondents are female and 10 are male. On the other hand, among the auxiliaries (n = 32), only 3 people reached a higher level while among the nurses (n = 64), 29 respondents had higher degrees. There is a significant association between education level and grade (chi2 = 48.82; p <0.000).

Table 1: Chi-square test between the level of education and the factors Age, Gender and Grade.

		Educational Level (EL)				Total	
		Secondary	Bachelor	University	Other		
Age	<30	0	0	11	0	11	50,81 (p<0,000)***
	30<>40	0	9	11	0	20	
	>40	5	13	10	37	65	
Gender	Male	0	10	11	20	41	6,65 (p<0,037)*
	Female	5	12	21	17	55	
Grade	Nurse	5	21	29	9	64	48,82 (p<0,000)***
	N.Auxiliaire	0	1	3	28	32	
Total		5	22	32	37	96	

*: significant difference at 5%. ***: significant difference at 1 ‰

4-KARASEK results: relationship between decisional latitude, psychological demand and social support

The table below presents the results of the multiple correlations between the three dimensions. Indeed, only the association between decisional latitude and social support showed a negative significance (r = -0.465; p <0.000). Moreover, people who have shown decision-making autonomy do not have strong support and vice versa.

Table 2: multiple correlation between the three dimensions of Job Strain

Corrélations				
		DP	**LD**	**SS**
DP	Corrélation de Pearson	1	,184	-,039
	Sig. (two-tailed)		,073	,704
	N	96	96	96
LD	Corrélation de Pearson	,184	1	-,465**
	Sig. (two-tailed)	,073		,000
	N	96	96	96
SS	Corrélation de Pearson	-,039	-,465**	1
	Sig. (two-tailed)	,704	,000	
	N	96	96	96

**. The correlation is significant at the 0.01 level (two-tailed).

Discussion

From our study we deduced that:

- 11.46% of nurses are in a state of job strain. Indeed, they demonstrated low decisional latitude and a strong psychological demand. They are therefore people in a state of stress. This correlates strongly with other results which indicate that nurses working at the short-term care unit levels have a higher degree of stress than those at the medical service level [16], [17], [18]. Hot services, namely emergencies, operating theaters and pediatrics are stressful services which require a strong psychological demand and which influence the well-being of the caregiver.

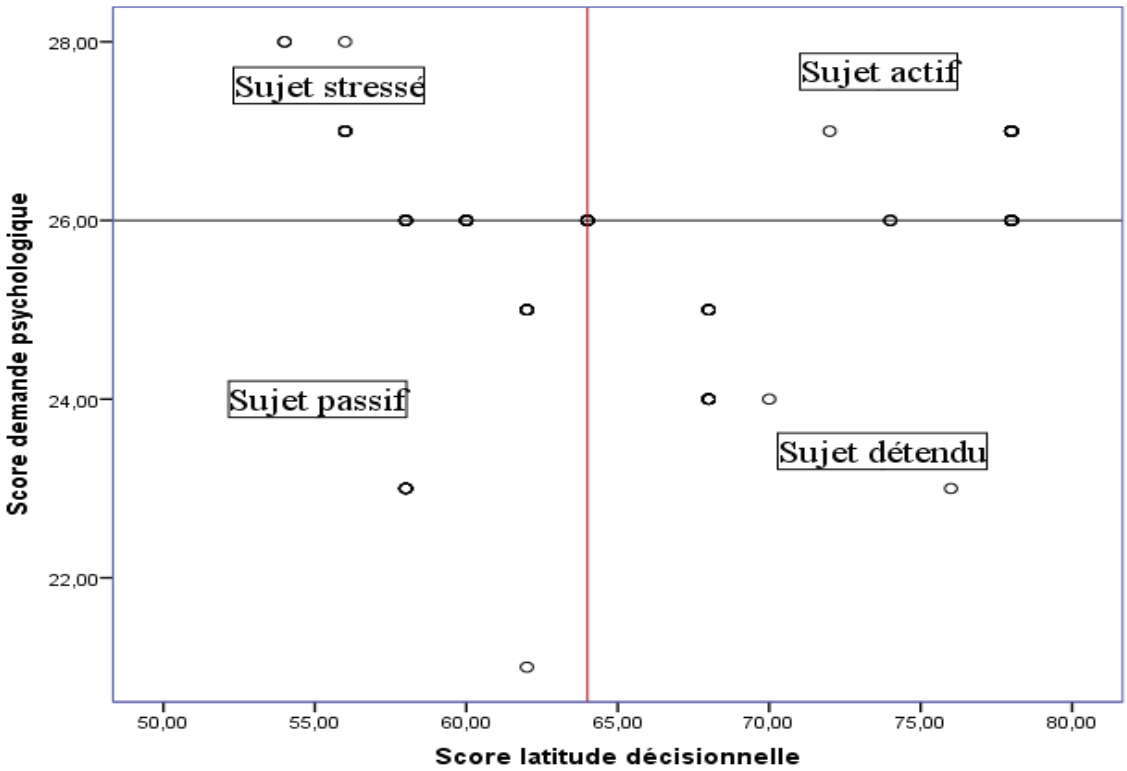

Figure 3: Average point cloud of the scores obtained by the nurses surveyed for the LD and PD dimensions

The table below shows the results of the description of the people who showed a state of stress (job strain situation). However, out of 11 people in job strain, 7 female nurses and 4 male nurses. 6 out of 11 people are over 40 years old and 7 nurses are married. Similarly, 7 nurses surveyed have spent 1 to 15 seniority in the post and 7 people follow the 12/36 system.

Table 3 : Distribution of nurses on job strain according to certain socio-demographic and professional parameters.

		workforce	Valid Percentage	Cumulative Percentage
Gender	M	4	36,4	36,4
	F	7	63,6	100,0
Age	<30	2	18,2	18,2
	30<>40	3	27,3	45,5
	>40	6	54,5	100,0
Marital status	SINGLE	4	36,4	36,4
	MARRIED	7	63,6	100,0
Educational Level	BACH	4	36,4	36,4
	UNIV	4	36,4	72,7
	OTHER	3	27,3	100,0

Cont... **Table 3 : Distribution of nurses on job strain according to certain socio-demographic and professional parameters.**

Seniority in the position	<1	1	9,1	9,1
	1<>5	2	18,2	27,3
	5<>15	5	45,5	72,7
	>15	3	27,3	100,0
Time system	Normal	3	27,27	27,27
	12/36	7	63,64	90,91
	Night Watch	1	9,09	100,00

Our results have shown that nurses over 40 are located in the high stress quadrant and even married nurses, something that has been cited by several studies that say that other potential explanatory variables of stress higher professional level were generally demographic factors such as age, marital status and education [(26), (27)].

In our study, there was a significant difference in the level of stress between the sexes reflecting that the demands and immediacy of stressful situations were likely to be perceived differently by gender.

Studies have already reported a higher level of stress among independent women of nationality and health unit [(26), (27)].

Conclusion

To conclude, a high level of tension at work was highlighted with a gender effect. Nurses are more stressed than men. According to the study, age over 40 was a key factor in high work stress, also for the work schedule; nurses adopting the 12/36 system were in the high stress quadrant. Both of these factors can be predictors of poor mental health.

Also, the results showed that nurses who exceeded a seniority in the post between 5 and 15 years began to feel stressed.

This imposes the adoption of strategies aimed at improving the personal and professional performance of nurses to cope with daily stressors in the various hospital departments in an urgent manner.

Acknowledgments: We thank all nurses of the provincial hospital in kenitra, Maroc, who participated in this study.

Compliance with ethical standard

Conflict of Interest : The authors declare that they have no competing interests.

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The efficacy of fractional erbium:YAG laser (2940 nm) compared to Microneedling of topical amniotic membrane stem cell conditioned medium (AMSC-CM) for photoaging

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Abstract

Background: Photoaging is a complex biologic process that affects various layers of the skin. Amniotic membrane stem cell conditioned medium (AMSC-CM) that contain growth factors and have capability to improve clinical picture of photoaged skin. Microneedling and laser devices are transdermal drug delivery methods which shows promising results to improve photoaging .

Objective: To investigate the efficacy of topical AMSC-CM after YAG erbium fractional laser compare to microneedling in photoaging.

Method :A clinical study, 60 adult women with photoaging. Randomized into two groups A 30 women received AMSC-CM after fractional erbium:YAG laser (2940nm) and group B 30 woman received AMSC-CM after microneedling. Both groups received the treatment 3 times with 2 weeks interval. The improvement of pore, wrinkle, and UV spot as was evaluate before, after and post treatment using computer-stimulated photograph skin analyzer.

Result: The evaluation of pore and UV spot showed greater improvement in the group of AMSC-CM with fractional erbium:YAG laser compare to AMSC-CM with microneedling group (pore p:0,00 and UV spot p:0,00). There was no significant different (p 0.43) of wrinkle improvement between both groups.

Conclusion: The combination of AMSC-CM and fractional erbium:YAG laser have better efficacy in the improvement in photoaged skins.

Keyword: *fractional laser, microneedling, photoaging,*

Introduction

Skin aging is the cumulative effect of intrinsic (genetics, cellular metabo- lism, hormone and metabolic processes) and extrinsic factors (chronic light exposure, pollution, ionizing radiation, chemicals, toxins). Among extrinsic factors, the effect of solar radiation on skin health is well characterized, resulting in coarse wrinkles, loss of elasticity, laxity, and rough- textured appearance.[1,2,3] Exposure to UV radiation is the primary factor of extrinsic skin aging; it accounts for about 80% of facial aging.Chronic photodamage of the skin manifests itself as extrinsic skin aging (photoageing). DNA photodamage and UV-generated reactive oxygen

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species (ROS) are the initial molecular events that lead to most of the typical histological and clinical manifestations of chronic photodamage of the skin.[2,3]

There has been great interest in understanding the regulation and coordination of the stem cells found within the skin in order to repair aged skin.[4] Amniotic membrane stem cells (AMSCs) are better prospects for cell therapy and regenerative medicine compared with the other adult mesenchymal stem cells because they are abundant and can be acquired easily and inexpensively. In fact, AMSCs can be obtained with little donor damage, have multipotency for all three germ layers and low immunogenicity and are ethically acceptable.[5] Several studies have identified the role of stem cells in improving and increasing dermal thickness and density of collagen fibers in aged mice as well as reducing skin wrinkles.[6]

The use of procedures stimulating the revitalization of skin, including fractional methods: fractional microneedling radiofrequency, fractional microneedling radiofrequency technology combined with LED light (light-emitting diode) and non-ablative, and ablative fractional laser therapy, fractional IR (infrared light) seems to be essential.[7] The clinical use of lasers in treating dermatological disorders such as skin spots, atrophic acne scars, chronological aging, and cutaneous aging has been growing in recent years.[8] This type of erbium laser can be found at wavelengths of 1540 or 1550 nm, which are classified as non-ablative erbium glass, and at 2940 nm, classified as ablative erbium YAG. Ablative fractional lasers destroy the superficial layers of the skin, to improve the appearance of the skin after epithelialization.[7]

Dermapens are also used in microneedling mesotherapy. Microneedling therapy is a progressively popular treatment for several dermatologic conditions. This cheap, relatively risk- free office maneuver reverses the clinical as well as histopatho- logical signs of aging and rejuvenates aged skin.Transdermal penetration of the drug was facilitated with the use of a needle length of 0.5 mm. A similar penetration of pharmaceuticals was con- firmed also with the use of dermaroller just before the substance application. Moreover, the increase in transepider- mal drug delivery is observed for at least 72 hr after the procedure. The microtraumatization creates micropores, which results in increased transdermal water loss, however, the result of the study has demonstrated that prompt recovery of skin barrier function is achieved within several hours from device usage.[7,9]

Moreover, combined minimally invasive modalities can improve collagen induction and facilitate penetration of topical agents for skin rejuvenation. We combine the use of fractional erbium: YAG laser (2940 nm) and product (AMSC-MP) in group A compared to microneedling with product (AMSC-MP) in group B. We will evaluate the effectiveness of these two combinations in terms of improving photoaging.

Method

Research Design and Subject.

This study is an analytical experimental study using controlled clinical trial methods, matching pair selection, and parallel designs that compare fractional erbium: YAG laser (2940 nm) and product (AMSC-MP) in group A compared to microneedling with product (AMSC-MP) in group B. The participants, who were patients with photoaged skin at the outpatient clinic of the Dermatology and Venereology Department, Dr. Soetomo General Academic Hospital Surabaya, Indonesia, were acquired by consecutive sampling. The inclusion criteria were patients with a Glogau score of II–III who had used tretinoin (0.25%) cream prior to therapy. The exclusion criteria were a history of keloid scars; active eczema; herpes simplex infection; a history of Botox injection, filler injection, chemical peels, or microdermabrasion; the use of cream or oral antioxidants; hemophilia/ physiological disorders of blood clotting using anti-platelets, diabetes mellitus, and HIV / AIDS and uncooperative behaviour. The final number of participants was 30.

Preparation of AMSC-CM and vitamin C mixture: AMSC-CM is a liquid culture product of AMSC that contains cytokines and growth factors that aid in wound healing and skin rejuvenation. In this study, the AMSC-CM was acquired from the Tissue Bank and Regenerative Medicine Department, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Procedure

We analyzed the level of pore, wrinkle, *polarized*

spots, UV spots, and skin tone by Janus. Thereafter, the subjects were instructed to apply 0.025% tretinoin and cream sunscreen (sun protection factor 30) for 2 weeks for priming. After 3 days of discontinuation of the tretinoin cream, the treatment procedures were initiated. For group A the AMSC-CM was applied after treatment with a fractional erbium:YAG laser. The therapy was performed three times with an interval of 4 weeks. All participants were provided sunscreen to apply every morning to afternoon and tretinoin cream (0.025%) to apply every night. Tretinoin cream (0.025%) was initiated 1 week after treatment. The progressive changes in wrinkles, spots, and pores were evaluated 4 weeks after the first treatment, before the second treatment (evaluation II), and 4 weeks after the third treatment (evaluation III). For group B A the AMSC-CM was applied after treatment *microneedling.* The *microneedling* used was in the form of dermapen, with a level 3 speed (45-50/times/sec), a depth of 0.5 mm, and 2 *passes*. Evaluation of results using *Facial Skin Scope* JANUS-II which assesses wrinkles, pores, *polarized* spots, UV spots, and *skin tones* was conducted after the action at week 0.4, and 8. The results of the data obtained are entered in the data collection sheet and accompanied by a photo analysis of the face with Janus-II to conduct data analysis.

Statistical analysis: The accumulated data were analysed using SPSS software version 21 (SPSS, Chicago, IL, USA), with the level of significance set at $P < .05$.

Results

This study was conducted on 60 women with photoaged skin who met the inclusion criteria. All the subjects were woman between 40-60 aged, mean age was 42±4,92 and the mean baseline Glogau score was 2,8±0,4. All patients work indoors; the most common outdoor activity was motorbike riding (100%), followed by morning exercise (50%; Table 1). Distribution of research subjects using matching pairs between groups based on photoaging criteria. There were no drop-outs in this study and all subjects completed the 8-week protocol.

Table 1. Patient characteristics

Variable	Fractional erbium:YAG laser + AMSC-CM n=30	Microneedling + AMSC-CM n=30
Age		
- 20-30	0	0
- 30-50	30	30
- 50-60	0	0
Glogau score		
- II	0	6
- III	30	24
Occupation		
- Indoor	30	30
- Outdoor	0	0

From the group A, the group given topical AMSC-CM after treatment with a fractional erbium:YAG laser obtained significant results in pores, wrinkles, UV spot and polarized spot. In the 1st and 3rd Janus observations there were significant improvements in pore, wrinkle and UV Spots (Table 2).

Table 2. Resume p value of pores, wrinkles, and UV spots between the 1st, 2nd, and 3rd janus in group A

fractional erbium:YAG laser+ AMSC-CM	Pores	Wrinkles	UV spot
Janus 1 vs 2	0,023	0,043	0,04
Janus 1 vs 3	0,023	0,043	0,00
Janus 2 vs 3	0,000	0,000	0,00

Table 3 shows of p values on the value of pores, wrinkles, and UV between the first, second and third janus from the group B microneedling combination of AMSC-CM.

Table 3. Resume p value of pores, wrinkles, and UV spots between the 1st, 2nd, and 3rd janus in group B

Microneedling+ AMSC-CM	Pores	Wrinkles	UV spot
Janus 1 vs 2	0,084	0,216	0,38
Janus 1 vs 3	0,032	0,429	0,258
Janus 2 vs 3	0,029	0,846	0,258

Table 4 shows the results of the comparison of mean value and p value between group A and groups B. The improvement was better in the group A. From the comparison of the two groups showed there were significant different of mean value in pore and UV spot and not significant different in wrinkle. Pore and and UV spots with p-value < 0,005.

Table 4. Comparison of mean value and p value between group A and group B

	P-value
Pore	0,00
Wrinkle	0,428
UV spot	0,000

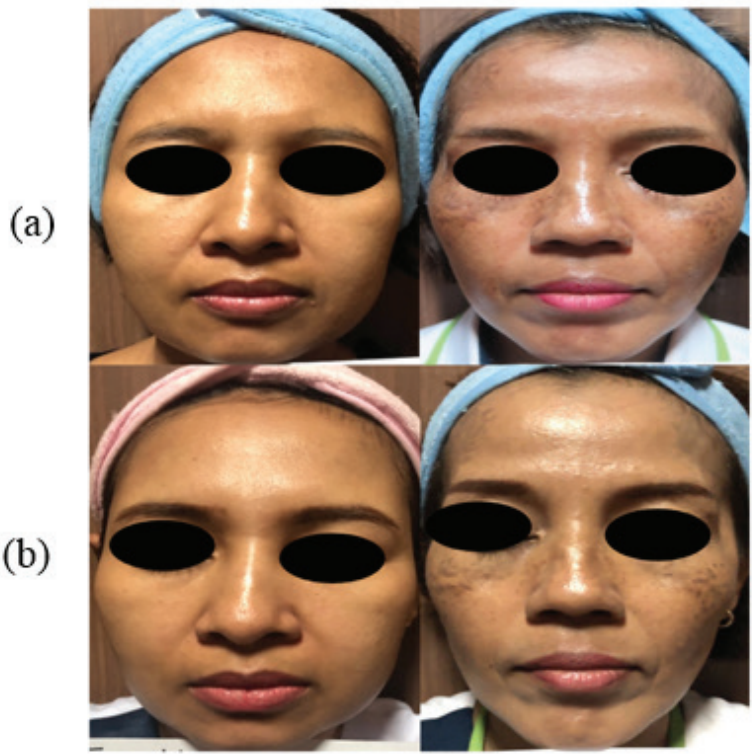

Figure 1. Photograph results (a) before fractional erbium:YAG laser + AMSC-CM (b) after fractional erbium:YAG laser + AMSC-CM last procedure.

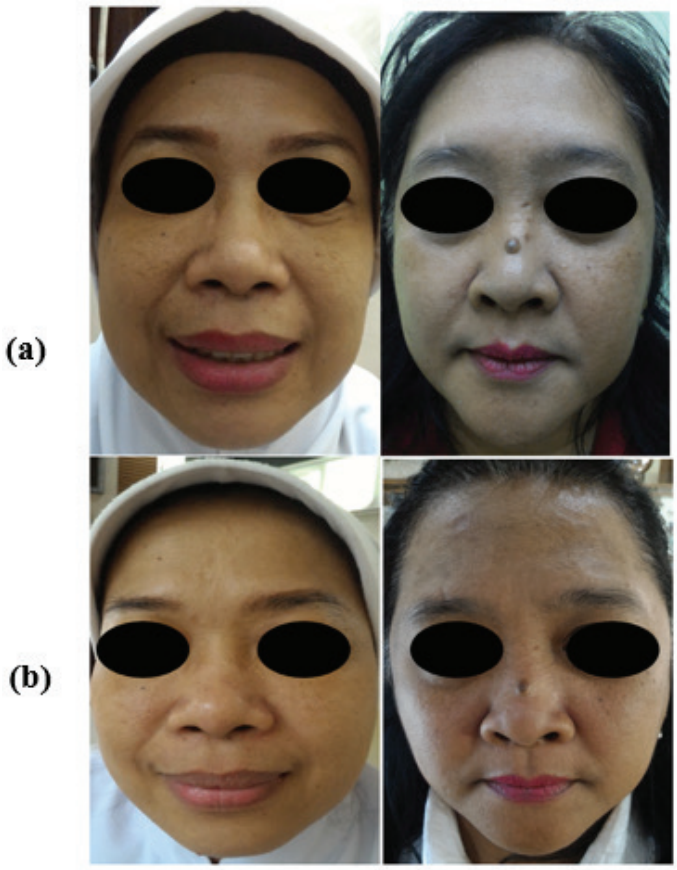

Figure 2. Photograph results (A) before mikroneedling + AMSC-CM **(B) after** microneedling+ AMSC-CM **last procedure.**

Figures 1 and 2 show the clinical improvement before and after procedure in group A and group B. Improvement are seen in patient's spot, wrinkle and skin tone.

Discussion

Skin rejuvenation has become the focus of cosmeceuticals, in which various medical treatments and products are used for anti-aging.[10] Aging process in the face area is a complex process that distorts the three-dimensional shape and textures.[6] The manifestations of photoaging investigated in this study were pore, wrinkle and UV spot. In the comparison of this study, it was found that the use of combination fractional erbium:YAG laser and AMSC-CM is better in terms of reducing photoaging compared to microneedling combination with AMSC-CM.

The skin extracellular matrix (ECM) that consists of glycosami- noglycans, collagen and elastin, is crucial for skin morphology and functions such as growth and elasticity [6]. The degradation of ECM that occurs along with skin aging is related to the increase of the ac- tivity of enzymes such as hyaluronidase, elastase and collagenase [7]. Photo-aging causes production of collagenase by Human Dermal Fi- broblasts (HDFs), which degenerates collagen production and is exposed on skin as wrinkles. Amniotic memban stem cell conditioned medium secrete many cytokines and growth factors such as Epidermal growth factor (EGF), basic Fibroblast growth factor (bFGF), Transforming growth factor-beta (TGF-b), which are important in cell growth and maintaining skin tissues.[10] Transforming growth factor-beta can induce a whitening protein, like TGF-β, hepatocyte growth factor, insulin-like growth factor (IGF) induce anti-wrinkle protein.[5] Recent studies revealed that HAMSCs have important roles in cell differentiation, promoting cell proliferation, enhancing cell viability and function, protecting cells from adverse effects and inhibiting apoptosis *in vivo* or *in vitro*. The present study aimed to determine whether HAMSCs involved in the protection of human dermal fibroblasts (HDFs) from UVA-induced senescence.[11] UVA radiation has the effect of reducing the telomere length of fibroblasts in the skin, inhibiting TGF-b1 secretion inducing G1 phase arrest, reducing superoxide dismutase (SOD), increasing malate dehydrogenase (MDH) levels and increasing MMP-1.4 expression. On histopathological examination, photoaging skin shows a reduced composition of the extracellular matrix.[12]

Fractional erbium:YAG laser is a technique for the treatment of skin lesions in which an array of microscopic thermal wounds (microscopic treatment zones) is induced into the skin to stimulate a therapeutic response deep in the dermis. In 2008, Lapidoth and colleagues reported results from a skin rejuvenation study of a fractionated 2940 nm Er:YAG laser. Pre-treatment biopsies, compared to those taken at 60 days post-treatment, clearly showed the epidermal and upper dermal ablation and healing process with collagen regrowth that is expected with Er:YAG fractionated resurfacing.[13]

Full effect of the series of microneedling procedures involving in the lowering of wrinkles visibility, and an improvement of skin elastic- ity, is achieved after about 3 months, due to the length of the neocollagenesis process.[7] El-Domyati et al. have demonstrated the effectiveness of microneedle mesotherapy in the fight against aging signs. Histopathological examinations of skin specimens taken from patients who underwent a series of six microneedling procedures (1 mm in depth) at 2-week intervals showed significant changes within both the epidermis and dermis. The prickle cell layer (stratum spinosum) of the epidermis thickened, which affected the development of rete ridges and the increase of skin layers integrity. The amount of collagen Type I, III, and VII increased significantly.Prakoeswa et all studied compared , microneedle plus AMSC-CM with microneedle plus NS, the result is microneedle plus AMSC-CM was more effective in improving the clinical signs of photoaging.[5]

The two modalities of fractional erbium therapy: YAG laser and microneedling have good effectiveness for photoaging, but because Fractional erbium: YAG laser can affect to the dermis, while the microneedling used in this study is 0.5 mm which does not penetrate to the dermis and the use of microneeling requires a longer evaluation time to see results in terms of reducing wrinkles. So the results fractional erbium: YAG laser is better than microneedling in overcoming photoaging.

Conclusion

The combination of AMSC-MP and fractional erbium:YAG laser (2940 nm) have better efficacy in the improvement of pore and spot in photoaged skins compared to AMSC-MP and microneedling.

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Conflict of Interest : There are no potential conflicts of interest relevant to this article

Ethical clearance: The study protocol has been approved by the Health Research Committee of Dr Soetomo General Hospital, Surabaya, east Java

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The Effect of Amniotic Membrane Stem Cell Metabolite Product (AMSP-MP) Combine with Vitamin E after Microneedling in Photoaging Progress

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Abstract

Introduction: The photoaging is a cumulative process. Amniotic Membrane Stem Cell Metabolite Products (AMSC-MP) that contain cytokines and growth factors that have a role in the skin's rejuvenation process. Vitamin E is an antioxidant that has a photoprotective effect. Topical combination of AMSC-MP and Vitamin E are expected to have an effect on clinical photoaging improvement.

Objective: To evaluate the effect of topical combination of AMSC-MP with vitamin E after microneedling compared with AMSC metabolite products after microneedling.

Methods: A total 60 photoaging women were included in this experimental analytic, controlled, matching research. Each participant's face was allocated to topical combination of AMSC-MP and vitamin E in intervention group and AMSC-MP only in control group. Microneedling modality was use to enhance epidermal penetration. Three treatment sessions were repeated at two weeks' interval.

Results: From the comparison of the two groups showed there were significant different of mean value in wrinkle, skin tone and UV spots with p-value < 0,005 in the intervention grup. While pore and polarized spot do not show significant differences between two groups.

Conclusion: The administration of a topical combination of MP-AMSC and vitamin E after microneedling provides clinical improvement in photoaging.

Keywords: *vitamin E, microneedling, photoaging.*

Introduction

Extrinsic aging (Photoaging) is caused by environmental factors such as sun exposure/ UV radiation. The effect of UV light exposure on the skin becomes a very significant factor affecting the extrinsic skin aging process. The degree of *photoaging* is directly related to the amount of UV light obtained in a certain period of time. *Photoaging* is clinically can cause soft and rough wrinkles, dryness, telangiectasis, loss of suppleness and changes in pigment.[1]

Amniotic membrane stem cells (AMSCs) are better prospects for cell therapy and regenerative medicine compared with the other adult mesenchymal stem cells because they are abundant and can be acquired easily and inexpensively. In the culture process, AMSC will secrete metabolite products. These metabolite products contain cytokines and growth factors and was reported to significantly improve the proliferation and migration of dermal fibroblasts and epidermal keratinocytes as well as increase collagen synthesis in fibroblasts.[2,3,4] Vitamin E has a photoprotective, anti-inflammatory and moisturizing effect. Hydrophilic molecules larger

than 500 Da have poor penetration through the stratum corneum.[3] Most growth factors are large hydrophilic molecules >20 kDa and are, therefore, unlikely to produce pharmacologic effects. To enhance the skin penetration of AMSCs, microneedle was used.

The aim of this is to examine the effects of Amniotic Membrane Stem Cell Metabolite Products (AMSC-MP) and vitamin E on photoaging.

Method

Research Design and Subject.

This study is an analytical experimental study using controlled clinical trial methods, matching pair selection, and parallel designs that compare topical therapies for AMSC metabolite combine with vitamin E after microneedling (treatment) and microneedling with AMSC metabolites (controls) in patients with photoaging.

The sample of the study were all photoaging patients who met the criteria for receiving samples, who came to the URJ Skin and Sex Hospital Dr. Soetomo Surabaya. Criteria for acceptance of samples are patients with Glogau II-III *photoaging* degrees or aged 40-60 years and patients who have used *priming* with tretinoin 0.025% cream for at least 2 weeks or new patients who are willing to be *primed* before treatment. Criteria for rejection of samples were patients with a history of keloids, active eczema on the face, hemophilia/ physiological disorders of blood clotting using anti-platelets, diabetes mellitus, and HIV / AIDS. All the subjects were randomly divided into two groups. First group was given AMSC-MP only as a control group and second group was given AMSC-MP with vitamin E as an intervention group. To enhance the skin penetration of AMSC-MP, microneedle was used.

Procedure

We analyzed the level of pore, wrinkle, *polarized* spots, UV spots, and skin tone by Janus. Thereafter, the subjects were instructed to apply 0.025% tretinoin cream for 2 weeks for priming. After 3 days of discontinuation of the tretinoin cream, the treatment procedures were initiated. In this study the *microneedling* used was in the form of dermapen, with a level 3 speed (45-50/ times/sec), a depth of 0.5 mm, and 2 *passes*. Evaluation of results using *Facial Skin Scope* JANUS-II which assesses wrinkles, pores, *polarized* spots, UV spots, and *skin tones* was conducted after the action at week 0.4, and 8. The results of the data obtained are entered in the data collection sheet and accompanied by a photo analysis of the face with Janus-II to conduct data analysis. This research has been examined by the Ethics Committee at RSUD Dr. Soetomo Surabaya.

Statistical Analysis

Analysis of variance (ANOVA) test was performed to evaluate the improvement in all parameters on Weeks 4 and 8. Data were analyzed using SPSS version 21 software (SPSS Inc., Chicago, IL). The level of significance was set at $p < .05$.

Result

This study involved 60 photoaging patients. All the subjects were woman between 40-60 aged. All research subjects were willing to take part in the study by signing information of consent, informed consent and medical approval sheets. Sixty patients consisted of 30 patients in the control group who were given AMSC treatment only and 30 patients in the treatment group treated with AMSC and vitamin E. Samples were taken in this study using consecutive sampling which took each photoaging patient at URJ Skin and Gender Cosmetic Division of RSUD Dr. Soetomo Surabaya. Distribution of research subjects using matching pairs between groups based on photoaging criteria. There were no drop-outs in this study and all subjects completed the 8-week protocol. From the control group, the group given topical AMSC metabolites after microneedling obtained significant results in pores, wrinkles, UV spot and polarized spot. In the 1st and 3rd Janus observations there were significant improvements in wrinkle, UV Spots, polarized spots. Significant results are also found in the pore seen in all of Janus's observations. (Table 1).

Table 1. Resume p value comparison of pores, wrinkles, skin tone, polarized spots, UV spots between the 1st, 2nd, and 3rd janus in control group.

	Pores	Wrinkles	Skin tone	Polarized spots	UV spots
AMSC-MP					
Janus 1 vs 2	0,001*	0,148	0,558	0,08	0,094
Janus 1 vs 3	0,001*	0,018*	0,055	0,002*	0,005*
Janus 2 vs 3	0,000*	0,206	0,126	0,081	0,261

Table 2 shows a comparison of p values on the value of pores, wrinkles, polarized spots, UV spots, skin tone between the first, second and third janus from the intervention group combination of AMSC-MP with vitamin E after microneedling. From the table it can be concluded that there are significant changes in pores, wrinkles, polarized spots, UV spots, and skin tones before and after the application of the topical mixture of PM-AMSC and vitamin E after microneedling with the most significant value on Janus 1 and 3.

Table 2. Resume p value comparison of pores, wrinkles, skin tone, polarized spots, UV spots between 1st, 2nd, 3rd janus in intervention group.

	Pores	Wrinkles	Skin tone	Polarized spots	UV spots
AMSC-MP+ Vit E					
Janus 1 vs 2	0,000*	0,227	0,000*	0,194	0,032*
Janus 1 vs 3	0,000*	0,000*	0,000*	0,002*	0,002*
Janus 2 vs 3	0,580	0,120	0,102	0,269	0,191

Table 3 shows the results of the comparison of mean value and p value between control and treatment groups. The improvement was better in the intervention group. From the comparison of the two groups showed there were significant different of mean value in wrinkle, skin tone and UV spots with p-value < 0,005. While pore and polarized spot do not show significant differences between two groups.

Table 3. Comparison of mean value and p value between control and intervention group

	Pore	Wrinkle	Skin Tone	Polarized Spots	UV Spots
AMSC	50,61	16,57	55,85	31,92	12,91
AMSC + Vit E	49,92	11,03	47,62	33,24	7,18
p-value	0,593	0,003*	0,000*	0,533	0,000*

Figure 1. Photograph results (a) before AMSC-MP microneedling procedures (b) after AMSC-MP microneedling last procedure.

Figure 2. Photograph results (a) before AMSC-MP and Vitamin E microneedling procedures (b) after AMSC-MP and Vitamin E microneedling last procedure.

Figures 1 and 2 show the clinical improvement before and after procedure in control group and intervention group. Improvement are seen in patient's spot, wrinkle and skin tone. The intervention group shown a better improvement in clinical and statistic analysis than the control group.

Discussion

The subjects of this study were women who experienced *photoaging*. Gender uniformity in this study aims to minimize the drop out of the study subject, because women pay more attention to the condition of their skin compared to men, so the level of compliance is expected to be higher than men. It can also be seen from *photoaging* patient visits in the cosmetics poly in IRJ Skin and Genital Health Dr. Soetomo is the women in major. This is supported by data on visits to cosmetics at RSUD Dr. Soetomo in 2018, there were 711 female patients and 24 male patients who came for skin rejuvenation treatment. This is also similar to the research conducted by Lee et al, which is all 25 subjects were woman.[5]

The amniotic epithelium and amniotic membrane stroma are sources of epidermal growth factor and keratinocyte growth factor, which promote wound healing. Their low immunogenicity and anti-inflammatory properties make them a suitable alternative material in the field of regenerative medicine. Furthermore, stem cells synthesize and secrete a variety of extracellular matrix proteins, cytokines, growth factors and other bioactive proteins that contribute to the healing process.[6,7]

In this study, the group given topical AMSC metabolite products after microneedling obtained significant results in pores, wrinkles, UV spot and polarized spot. Significant results are also found in the pore seen in all of Janus's observations. Concerning the research conducted by Seo and his colleagues with stem cell metabolite products and microneedling on photoaging showed improvements in skin roughness.[8] The histopathology results in the study also showed an increase in dermal collagen with minimal side effects.[8] Research conducted by Lee and colleagues comparing the use of stem cell metabolites after microneedling and with microneedling alone, showed significant results in wrinkle repair.[5] This is because the effect of AMSC-MP which contains various growth factors that can stimulate the proliferation and migration of dermal fibroblasts, epidermal keratinocytes, as well as increase collagen synthesis from fibroblasts, so there is an improvement in skin texture. Stem cell metabolite products have a brightening effect by inhibiting melanin formation, changing the shape of melanin and affecting the distribution of melanosome transfer.[6,7] Research conducted by Seo et al shows that stem cells have a whitening effect through inhibition of melanin synthesis and tyrosinase activity, and can reduce the expression of melanogenic enzymes.[8] Research conducted by Lee et al also showed a significant improvement in the melanin index of *photoaging* patients who applied *stem cell* metabolite products after *microneedling*.[5]

In the intervention group also showed significant results in wrinkle, UV spot, polarized spot, skin tone and pore. The most significant result showed in 1st and 3rd Janus. In accordance with the theory that topical vitamin E has a photoprotective, anti-inflammatory and moisturizing effect. The addition of vitamin E to this topical product is expected to have a synergistic effect on the clinical improvement of *photoaging*. Chung et al showed that the use of occlusive topical 5% vitamin E for 24 hours provided protection against UV induced metalloelastase *in vivo*.[9] Vitamin E protects cell membranes from lipid peroxidase by free radicals. The research shows that topical vitamin E that is applied topically has the potential to penetrate into the skin layer where oxidative stress occurs so as to protect from *photoaging* process, so the clinical signs of *photoaging* can be reduced.[9,10]

Comparative results of the control and treatment groups showed significant different mean value in wrinkle, skin tone, and UV spot. It means that addition of vitamin E in AMSC-MP give significant different that AMSC-MP alone.

Side effects that may arise from the actions of this study are persistent erythema (> 24 hours), urticaria, *post inflammatory hyperpigmentation*, hypopigmentation, or infection. In this study, the side effects after treatment were minor in 3 subjects, there were erythema in 2 subjects for 2 days and urticaria in 1 subject for 1 days in control group. Both side effects resolved after two days of treatment with topical 1% hydrocortisone cream and

antihistamine. This can occur because of *microneedling* itself is an act with minimal invasion, because the injury that occurs from the *microneedling* needle is only 0.5 cm deep which functions as a drug *delivery* to deeper layers of the skin. This is consistent with what was found in research conducted by Tamama et al, who found that MSC metabolite products secrete IL-8, chemokin ligand 1 (CXL1) which can accelerate the healing process.[11,12] In intervention group there were no side effect reported in all the subjects. It is maybe because vitamin E also functions as an anti-inflammatory agent and can affect scars *remodeling* by interacting with phospholipids on cell membranes, inducing molecular and maintaining membrane stability.[10,13]

Conclusion

In conclusion, compared with the AMSC-PM alone after microneedling, giving topical AMSC metabolite products with vitamin E after miconeedling, was effective in giving a positive impact on photoaging improvement.

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Ethical Clearance: The study protocol has been approved by the Health Research Committee of Dr Soetomo General Hospital, Surabaya, east Java

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The Impact Factors of Peripheral Diabetic Neuropathy Which is Along with Hyperglycemia, Hypercholesterolemia, and Hyperaggregation

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Abstract

Objective: The prevalence of T2DM case in 2018 has increased by 10.9%. The majority of T2DM patients often ignore some of the most important conditions to experience complications, which is complications of PDN. This study aims to analyze the dominant factors in PDF patients with hyperglycemia, hypercholesterolemia, and hyperaggregation**. Material and methods:** This research is a case control study conducted from April to October 2020. The sample is 85 patients who have been confirmed to have PDN by neurologists aged 40 - 65. Sampling was conducted at several hospitals in Surabaya with a purposive sampling method. Data analysis was conducted in three stages, namely univariate analysis, bivariate analysis and multivariate analysis. **Results**: 85 PDN patients who experienced hyperglycemia amounted to 81%, hypercholesterolemia 81%, and hyperaggregation 67%. The dominant factor affecting the three conditions (hyperglycemia, hypercholeterol, and hyperagregation) in this PDN patient was a history of hypertension with a value of p = {(0.012); (0.007); (0.001)} and also a long period of suffering from T2DM with p value = {(0.019); (0.025); (0.041)). Meanwhile, age, gender and regularity for treatment control were not the dominant influencing factors. **Conclusion:** Suffered for a long time from T2DM and a history of hypertension are the main determinants of peripheral diabetic neuropathy which is along by hyperglycemia, hypercholesterolemia, and hyperaggregation.

Key words: *Determinant Factors, Peripheral Diabetic Neuropathy, Hypeglycecimia, Hypercholesterolemia, Hyperaggregation*

Introductions

One of the causes of death in the world is a metabolic disease characterized by an increase in blood glucose levels due to insulin secretion disorders, this disease is known as Diabetes Mellitus [1]. Known by the data [2], there are 43% of deaths caused by hyperglycemia at the age under 70 years. This is also in line with what happened in Indonesia, based on [3] diabetes mellitus patients experienced increase of 6.9% in 2013 to 10.9% in 2018. The majority of diabetes mellitus patients are not aware of the symptoms they suffer until these patients experience complications. Diabetes and its complications are rapidly becoming the most significant causes of morbidity and mortality in the world [4].

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One of the most common complications of DM that can worsen quality of life is peripheral neuropathy. Diabetic neuropathy is diffuse nerve damage due to excessive hyperglycemia. Complications of diabetic neuropathy experienced by people with diabetes mellitus reach 50% [5].

In neuropathic diabetics, there is an endoneural vascular dysfunction that is recognized by platelet activity with an increase in platelet aggregation [6]. Platelet aggregation is the attachment between platelets that can occur through the secretion of Adenosine Diphosphate and ordering of Thromboxane A2.The increase in thromboxane production is a trigger for platelet aggregation [7]. Apart from being affected by hyperglycemia, and platelet aggregation, patients with 2TDM along with hypercholesterolemic states had a higher rate of complications than patients with hypercholesterolemia or DM. This is due to the fact that DM has an addictive effect on the development of arterial stiffness in patients with hypercholesterolemia, so that it can cause peripheral nerve disorders [8].Various conditions that occur in PDN can be exacerbated if there are external environmental factors that influence it. Environmental factors come from a bad lifestyle, such as lack of physical activity, hypertension, and irregular medical control [9].

It is hoped that the introduction of various risk factors in the various circumstances that can be experienced by PDN patients will serve as a reminder for patients not to aggravate their suffering. The purpose of this study was to analyze the dominant factors in PDN patients with hyperglycemia, hypercholesterolemia, and hyperaggregation.

Material and Methods

This research is a study with a case control study design. The sample of this research is PDN patients who control treatment at the Internal Medicine and Neurology Polyclinic in several hospitals in Surabaya. The sample calculation refers to the Lemeshow formula, in order to get 85 respondents. The inclusion criteria in this study were patients who had been confirmed by a neurologist with PDN aged 40 - 65 years, and were cooperative in being willing to participate in this study. The exclusion criteria in this study were patients with chronic kidney disease, chronic liver disease, HIV / AIDS, and cancer and were on heparin therapy and taking drugs such as aspirin, anti-retroviral, anti-platelet, chemotherapy, allupurinol, and estrogen.The sample in this study was taken by purposive sampling where all subjects met the inclusion criteria to be included in the study until the required sample size was met. Subjects were selected based on study inclusion and exclusion criteria until the minimum sample size was met. The study sample was taken when the patient left the examination room. Data were collected from April to October 2020 at the Internal Medicine Department in several hospitals in Surabaya. The dependent variable in this study was hyperglycemia, hypercholesterolemia, and hyperaggregation in PDN patients. The independent variables in this study were gender, age, regularity of treatment, length of suffering from T2DM, and history of hypertension. Data were collected through direct interviews with respondents and conducting laboratory examinations with several parameters, namely examination of BSN, cholesterol, and platelet aggregation activity. Laboratory examinations were carried out at the Kedungdoro Laboratory in Surabaya. This data that has been obtained, data analysis is carried out in three stages, namely univariate analysis, bivariate analysis and multivariate analysis. Univariate analysis to describe variables in terms of frequency. Bivariate analysis to see the relationship between variables in the study using the chi square test. Multivariate analysis to determine the dominant factor using logistic regression test.

Results

Characteristics of respondents based on age groups are divided into 2 age groups, namely age <53 years and> 53 years. Characteristics of respondents based on age mostly are> 53 years old as many as 56 people (66%). Characteristics of respondents based on gender were mostly female as many as 62 people (73%). The characteristics of respondents based on the type of work were mostly housewives as many as 27 people (32%). Characteristics of respondents based on education level are mostly high school graduates as many as 47 people (55%).Characteristics of respondents based on the domicile area of the patient's subjects mostly came from central Surabaya as many as 37 people (44%). Characteristics of respondents based on the results of most of the BSN examination, with results> 126 mg / dL or 69 respondents (81%) experienced hyperglycemia.

The characteristics of the respondents based on the results of the total cholesterol examination were mostly those with results> 200 mg / dL or 69 respondents (81%) had hypercholesterolemia. Characteristics of respondents based on the results of the examination of the Platelet Aggregation Test (TAT) were mostly hypergarge as many as 57 people (67%) (Table 1).

Table 1: Response Characteristics of Patients with Peripheral Diabetic Neuropathy complications (NDP)

Characteristics	Total	
	N	%
Age		
≤ 53 years	29	34
> 53 years	56	66
Gender		
Man	23	27
Woman	62	73
Type of Work		
Private Employees	13	15
Entrepreneur	25	29
Retired	11	13
Housewife	27	32
Does Not Work	9	11
Education		
Elementary	0	0
Junior High School	17	20
Senior High School	47	55
University	21	25
Domicile		
Central of Surabaya	37	44
East of Surabaya	19	22
North Surabaya	16	19
West of Surabaya	8	9
South of Surabaya	5	6
Blood Sugar Levels During Fasting		
< 126 mg/dL	16	19
> 126 mg/dL	69	81
Total Cholesterol Levels		
< 200 mg/dL	16	19
> 200 mg/dL	69	81
Platelet Aggregation Test		
Normoagregasi	28	33
Hiperagregasi	57	67
Total	85	100%

The Relationship of Gender Factors on Hypeglycemia, Hypercholesterolemia, and Hyperaggregation Conditions in PDN Patients

Table 2. shows that the majority of PDN patients were hyperglycemic women as many as 55 (80%). The results of the chi square analysis obtained p = 0.01 (p> 0.05), which means that there is a significant relationship between gender and hyperglycemia in patients with peripheral diabetic neuropathy with OR = 5.051, the patients are women and in a hyperglycemic state have a risk of suffering from diabetic neuropathy 5.051 times than male sufferers.Table 3 shows that most of the PDN patients were women with hypercholesterolemia as many as 51 (74%). The results of the chi square analysis showed that p = 0.757 (p> 0.05), which means that there is no significant relationship between the gender of men and women to the hypercholesterol state of PDN sufferers. Table 4 shows that the majority of PDN sufferers were women who were in a hyperaggregated state as many as 41 people (72%). The results of chi square analysis obtained p = 0.968 (p> 0.05), which means that there is no significant association between the gender of male and female genitalia on the hyperaggregation state of PDN patients.

The Relationship of Age Factors to Hyperglycemia, Hypercholesterolemia, and Hyperaggregation Conditions in PDN Patients

Table 2. shows that the majority of PDN patients were> 53 years of age who were in a hyperglycemic state as many as 49 (71%). The results of chi square analysis obtained p = 0.757 (p> 0.05), which means there is no significant association between age> 53 years and ≤ 53 years of age on the hyperglycemic state of PDN patients.Table 3 shows that most of the PDN patients were aged> 53 years with hypercholesterolemia as many as 46 people (67%). The results of the chi square analysis showed that p = 0.981 (p> 0.05), which means that there is no significant association between age> 53 years and ≤ 53 years of age on the hypercholesterolemic state of PDN patients. Table 4 shows that most of the Peripheral Diabetic Neuropathy patients were aged> 53 years who were in a hyperaggregated state as many as 37 people (65%). The results of chi square analysis showed p = 0.979 (p> 0.05) which means there is no significant association between age> 53 years and ≤ 53 years of age on the hyperaggregation state of PDN patients.

The Relationship of Regular Control of Treatment to Hyperglycemia, Hypercholesterolemia, and Hyperaggregation Conditions in PDN Patients

Table 2 shows that the majority of PDN patients who were hyperglycemic did not control for regular treatment as many as 52 (75%). The results of the chi square analysis showed p = 0.002 (p> 0.05), which means that there is a significant relationship between not having regular treatment and the hyperglycemic state of PDN patients with OR = 6.729, meaning that patients who are not regularly control treatment and in a hyperglycemic state have a risk of suffering from PDN 6,729 times than patients who regularly seek treatment.

Table 3 shows that the majority of PDN patients who were hypercholesterolemic did not receive regular treatment as many as 51 people (74%). The results of the chi square analysis showed that p = 0.008 (p> 0.05), which means that there is a significant correlation between not having control over regular treatment and hypercholesterolemia in PDN patients with OR = 4.722, which means that patients who do not control regularly seek treatment and are in hypercholesterolemic conditions at risk of suffering from neuropathydiabetic 4,722 times than patients who regularly seek treatment. Table 4 shows that the majority of PDN sufferers who are in a hyperaggregated state are not controlled for regular treatment as many as 45 people (79%). The results of the chi square analysis showed that p = 0.008 (p> 0.05), which means that there is a significant relationship between not having regular treatment and the hypercholesterolemia of PDN patients with OR = 4.722, which means that patients who do not have regular control over treatment and in a hyperaggregated state have a risk of suffering from PDN 4.722. times than patients who regularly control treatment.

The Relationship of the Old Factors of Suffering from T2DM to the State of Hyperglycemia, Hypercholesterolemia, and Hyperaggregation in PDN Patients.

Table 2 shows that the majority of PDN patients suffer from T2DM for> 5 years who are in a hyperglycemic state as many as 60 people (87%). The results of the chi square analysis showed p = 0.003 (p> 0.05), which means that there is a significant relationship between the length of suffering from T2DM and the hyperglycemic state of PDN patients with OR = 6.667 meaning that patients with T2DM for> 5 years and in a hyperglycemia

state have a risk. Suffer from diabetic neuropathy 6.667 times than patients who had T2DM for ≤ 5 years. Table 3 shows that the majority of PDN patients suffer from T2DM for> 5 years who are in a hypercholesterol state as many as 60 people (87%). The results of the chi square analysis obtained p = 0.003 (p> 0.05), which means that there is a significant relationship between the length of suffering from T2DM and the hypecholesterol state of PDN patients with OR = 6,667 meaning that the patient has had diabetes mellitus 2 for> 5 years and is in a hypecholesterol state have a risk of suffering from diabetic neuropathy 6.667 times than patients who have had T2DM for ≤ 5 years. Table 4 shows that the majority of PDN patients suffer from T2DM for> 5 years who are in a hyperaggregated state as many as 51 people (90%). The results of the chi square analysis showed that p = 0.002 (p> 0.05), which means that there is a significant relationship between the length of suffering from T2DM and the hyperaggregation state of PDN patients with OR = 5,500 meaning that patients with T2DM for a long time of> 5 years and in a hyperaggregated state have a risk. suffer from PDN 5.5 times than patients who have had T2DM for ≤ 5 years for a long time.

The Relationship of Hypertension History to Hyperglycemia, Hypercholesterol, and Hyperaggregation Conditions in PDN Patients

Table 2 shows that the majority of PDN patients have a history of hypertension who are in a hyperglycemic state as many as 49 people (71%). The results of chi square analysis obtained p = 0.007 (p> 0.05), which means that there is a significant relationship between the history of hypertension and the hyperglycemia state of patients with peripheral diabetic neuropathy with OR = 5,390, meaning that patients with a history of hypertension and a hyperglycemic state have a risk of suffering from PDN 5, 39 times than patients without a history of hypertension. Table 3 shows that most of the PDN patients have a history of hypertension who are in a hypecholesterol state as many as 50 people (70%). The results of the chi square analysis showed p = 0.001 (p> 0.05), which means that there is a significant relationship between the history of hypertension and the hyperglycemia state of PDN patients with OR = 7.895, meaning that patients with a history of hypertension and in a hypercholesterolemic state have a risk of suffering from diabetic neuropathy 7.895 times than patients who do not have a history of hypertension. Table 4 shows that the majority of PDN patients have a history of hypertension who are in a hyperglycemic state as many as 49 people (71%). The results of the chi square analysis showed that p = 0.000 (p> 0.05), which means that there is a significant relationship between the history of hypertension and the hyperglycemic state of PDN patients with OR = 7,917 meaning that patients with a history of hypertension and in a hyperaggregated state have a risk of suffering from PDN 7,917 times than the patient. who do not have a history of hypertension.

Table 2. Cross tabulation the effect of gender, age, regularity of treatment, duration of diabetes, and history of hypertension on hyperglycemia in PDN patients (p <0.05)

Variable	Hyperglycemia State				Total		P	Score of OR (95% CI)
	Yes		No					
	N	%	N	%	N	%		
Gender								
Male	14	20	9	56	23	27	0,010	5,051 (1,601 < OR < 15,934)
Female	55	80	7	44	62	73		
Age								
≤ 53 years	20	29	9	56	29	34	0,075	3,150 (1,032 < OR < 9,618)
> 53 years	49	71	7	44	56	66		
Regular Treatment Control								
Irregular	52	75	5	31	57	67	0,002	6,729 (2,046 < OR < 22,132)
Regular	17	25	11	69	29	33		

Cont... **Table 2. Cross tabulation the effect of gender, age, regularity of treatment, duration of diabetes, and history of hypertension on hyperglycemia in PDN patients (p <0.05)**

Long Suffering From T2DM								
≤ 5 years	9	13	8	50	17	20	0,003	6,667 (1,999 < OR < 22,238)
> 5 years	60	87	8	50	68	80		
History of Hypertension								
Yes	49	71	5	31	54	64	0,007	5,390 (1,659 < OR < 17,508)
No	20	29	11	69	31	36		

Table 3. Cross tabulation of the effect of gender, age, regularity of treatment, duration of diabetes, and history of hypertension on hypercholesterolemia in patients with peripheral PDN (p <0.05).

Variable	Hypercholesterol State				Total		P	Nilai OR (95% CI)
	Yes		No					
	N	%	N	%	N	%		
Gender								
Male	18	26	5	31	23	27	0,757	1,288 (0,393 < OR < 4,216)
Female	51	74	11	69	66	73		
Age								
≤ 53 years	23	33	6	37	29	34	0,981	1,200 (0,388 < OR < 3,712)
> 53 years	46	67	10	63	56	66		
Regular Treatment Control								
Irregular	51	74	6	37	57	67	0,008	4,722 (1,501 < OR 14,853)
Regular	18	26	10	63	28	33		
Long Suffering From T2DM								
≤ 5 years	9	13	8	50	17	20	0,003	6,6670 (1,999 < OR < 22,238)
> 5 years	60	87	8	50	68	80		
History of Hypertension								
Yes	50	72	4	25	54	64	0,001	7,895 (2,265 < OR < 27,522)
No	19	28	12	75	31	36		

Table 4. Cross tabulation the effect of gender, age, regularity of treatment, duration of diabetes, and history of hypertension on platelet aggregation activity in patients with complications of peripheral diabetic neuropathy complications (p <0.05).

Variable	Hyperaggregation State				Total		P	Nilai OR (95% CI)
	Yes		No					
	N	%	N	%	N	%		
Gender								
Male	16	28	7	25	23	27	0,968	0,854 0,304 < OR < 2,398
Female	41	72	21	75	62	73		
Age								
≤ 53 years	20	35	9	32	29	34	0,979	0,976 0,335 < OR <,293
> 53 years	37	65	19	68	56	66		
Regular Treatment Control								
Irregular	45	79	12	43	57	67	0,008	4,722 (1,501 < OR 14,853)
Regular	12	21	16	57	28	33		
Long Suffering From T2DM								
≤ 5 years	6	10	11	39	17	20	0,002	5,500 1,766 < OR 17,131
> 5 years	51	90	17	61	68	80		
History of Hypertension								
Yes	45	79	9	32	54	64	0,000	7,917 2,863 < OR < 21,891
No	12	21	19	68	31	36		

Dominant Variables that Affect the Incidence of Diabetic Neuropathy

The results of the logistic regression analysis showed that there was a significant influence between history of hypertension ((0.012 <0.05); (0.007 <0.05); (0.001 <0.05)} and a long period of suffering from T2DM {(0.019 <0.05); (0.025 <0.05); (0.041 <0.05)) against hyperglycemia, hypercholestetol, and hyperaggregation of patients with peripheral diabetic neuropathy. The dominant factor affecting the three conditions (hyperglycemia, hypercholeterol, and hyperagregation) in patients with complications of peripheral diabetic neuropathy is a history of hypertension, because the probability is lowest, 0.012; 0.007; 0.001 with OR = 6.880; 5,913; 6,437 means that patients with a history of hypertension are at risk of suffering from peripheral diabetic neuropathy which is in a hyperglycemic state

6,880 times, a hypercholesterol state 5,913 times, and a hyperaggregated state 6,437 times greater than patients without a history of hypertension (Table 5).

Table 5. Logistic Regression table of determinants in some of the main conditions often experienced by patients with complications of peripheral diabetic neropathy.

Main Parameters	Variable	P	OR	95% CI
Hyperglycemia State	Historyof Hypertension	0,012	6,880	1,515 – 31,234
	Long Suffering From T2DM	0,019	5,664	1,327 – 24,182
Hypercholesterol State	History of Hypertension	0,007	5,913	1,611 – 21,706
	Long Suffering From T2DM	0,025	4,420	1,207 – 16,185
Hyperaggregation State	History of Hypertension	0,001	6,437	2,249 – 18,430
	Long Suffering From T2DM	0,041	3,684	1,054 – 12,880

Discussion

The state of hyperglycemia in PDN patients was found in this study with a percentage of 81%. The hyperglycemic state in T2DM causes glucose levels to increase 4-fold in peripheral nerves [10], creating an oxidative environment and high carbon dioxide accumulation, which result in an inflammatory process that causes oxidative stress and neuronal cell injury [11]. This phenomenon is referred as glucose neurotoxicity with clinical manifestations in the form of peripheral diabetic neuropathy [10].

The subjects with PDN in this study who experienced hypercholesterolemia were also high with a percentage of 81%. This isaccordance with the study [12], where patients with T2DM and hypercholesterolemia had a higher rate of complications than patients with hypercholesterolemia or diabetes alone. This is because DM has an additive effect on the development of arterial stiffness in patients with hypercholesterolemia, so that it can cause peripheral nerve disorders [8]. The lipid profile potential to activity in platelet by apolipoprotein E and its interaction with platelet low density lipoprotein receptors [13]. In patients with DM, administration of dissolved high density lipoprotein has been shown to suppress aggregation by increasing the removal of cholesterol from the platelet membrane [14]. A further contribution to platelet hyperactivity, however,mediated by lipid and glucose interactions with low density lipoprotein formation, leading to impaired nitric oxide production and increased intraplatelet calcium concentration [15]. It is well known that hyperglycemia, hypercholesterolemia, and hyperaggregation can cause peripheral endothelial dysfunction which is commonly known as PDN [16].

Diabetes mellitus improves health with an increased risk of vascular disease. T2DM shows increased reactivity and activation of the platelet base,result in increased events such as thrombosis then platelet activation that initiates the coagulation cascade and thrombus ordering [17]. Platelets in patients with T2DM exhibit an irregular signaling pathway with platelet hyperactivation and by chronic and acute hyperglycemia via elevated levels of protein kinase C [18]. Increased hyperglycemia-induced interactions will make the surface membrane of platelets in patients with DM decrease membrane fluidity and increase platelet sensitivity to agonists [19]. Platelet surface agonist receptors (P2Y12) and targets of the antiplatelet agent thienopyridine have also been shown to increase in platelets in patients with DM due to changes in membrane fluidity dynamics, thus complicating DM patients with peripheral diabetic neuropathy [20]. In this study, 67% of PDN patients experienced hypergaregation. This is consistent with a study [20] where patients with DM PDN

complications have been shown to have a higher P2Y12 signaling rate compared to non-diabetic complications [16].

The majority of subjects with diabetic neuropathy complications were > 53 years old with a percentage of 66%. Accordance with the results of this study in line with research [21], namely the highest incidence of diabetic neuropathy at the age of 45-65 years. The number of neuropathy patients in the age range 45-65 years is due to tissue damage caused by free radicals, such as increased levels of lipid peroxide and changes in enzyme activity [1]. Diabetes mellitus is a degenerative disease, a disease that appears slowly as the patient's age increases over the years so that the patient experiences complications of diabetic neuropathy [22]. Age is not a major determinant of complications of peripheral diabetic neuropathy because age is only related to patients with hyperglycemia, but hypercholesterolemia and hyperaggregation are not related.

There are more NDP patients than men with a percentage of 73%. The results of this study are in line with research [21] which states that women more than men patients from diabetic neuropathy. The reason women dominate suffering from NDP is that women are housewives. Housewives who tend to have low activity so that they are at risk of developing diabetes mellitus [23]. Hormonal differences in men and women affect the onset of neuropathy. High estrogen levels in women can interfere with the absorption of iodine, which plays a role in the formation of myelin nerves, while testosterone levels in men protect the body from T2DM, but not in women [24]. Gender is not the main determinant factor for complications of peripheral diabetic neuropathy because gender is only related to patients with hyperglycemia, but for hypercholesterolemia and hyperaggregation, there is no association.

This study shows that there is a relationship between regular control over treatment and diabetic neuropathy. The results are in line with research [25] showing that there is a relationship between routine treatment controls and diabetic neuropathy. Especially blood sugar, is important to prevent complications, like diabetic neuropathy. Control can be seen from instantaneous blood glucose and long-term blood glucose.

This study shows that there is a link between the length of suffering from T2DM and diabetic neuropathy. The results showed that the complications of diabetic neuropathy experienced by patients occurred in a relatively longer period of time after being diagnosed with diabetes mellitus, so that there is a relationship or influence between the duration of suffering from type 2 diabetes mellitus, patients after > 5 years experiencing T2DM will gradually experience complications, one of which is peripheral neuropathy [26]. A patient with peripheral diabetic neuropathy who has suffered from T2DM for > 5 years will be at risk 3.5 - 5.5 times than those who suffer <5 years, especially triggered by a state of hyperglycemia, hypercholesterolemia, and hyperagregation. This is consistent with research [27], diabetic neuropathy generally occurs after 5 years of exposure to T2DM which is then triggered by low glycemic control and dyslipidemia, which will increase the occurrence of diabetic neuropathy. The longer experiencing T2DM, the higher the incidence of complications experienced. The duration of T2DM with high blood sugar levels will affect changes in blood vessel walls [28]. Chronic high blood sugar levels lead to decreased insulin secretion. The glucose will turn into sorbitol which causes nerve cell damage. The longer a person has diabetes, this process will last longer and aggravate the damage to nerve cells [29]. The duration of suffering from T2DM is one of the main determinants of complications of peripheral diabetic neuropathy with p value ((0.019 <0.05); (0.025 <0.05); (0.041 <0.05)), so that the duration of suffering from T2DM associate with patients has hyperglycemia, hypercholesterolemia, and hyperaggregation.

This study explains that there is a link between a history of hypertension and diabetic neuropathy. The results of this study are in line with research [30] which states that there is a relationship between hypertension and the incidence of complications of T2DM. Hypertension can cause thickening of the arteries so that the diameter of the blood vessels is narrowed. Narrowing of blood vessels will affect the transport of metabolism in the blood, so that glucose levels in the blood will be disturbed. When blood glucose levels are disturbed (hyperglycemia) and accompanied by elevated cholesterol levels (hypercholesterolemia) are risk factors for the development of systemic arterial hypertension [31]. The incidence of metabolic syndrome is 2/3 higher

in patients with hypertension so that hypertension is also a contributing factor for complications of diabetic neuropathy [32]. Hypertension history was one of the main determinants of complications of peripheral diabetic neuropathy with p value ((0.012 <0.05); (0.007 <0.05); (0.001 <0.05)), so that the history of hypertension is associated with hyperglycemia, hypercholesterolemia, and hyperaggregation.

Conclusion

The determinant factors in patients with peripheral diabetic neuropathy who are along by hyperglycemia, hypercholesterolemia, and hyperaggregation are long suffering from T2DM and had hypertension. The dominant factor that has the most influence is a history of hypertension, so PDN patients who are along by hyperglycemia, hypercholesterolemia, and hyperaggregation are expected to be able to control by knowing the factors that influence it, especially in maintaining a psychological state or food that can trigger an increase in blood pressure so that later it doesn't get worse.

Abbreviations: DM (Diabetes Mellitus); PDN (Peripheral Diabetic Neuropathy); T2DM (Type 2 Diabetes Mellitus); BSN (Blood Sugar Nechtar)

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Ergonomic Workstation Design for Fish Smoking Workers in the Coastal Area of Surabaya

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Abstract

Introduction: Workstation design should be made adjusting the workers' anthropometry to not put additional burden on the worker's body. Fish smoking workers in the coastal area of Surabaya work sitting on a chair as high as their heels which cause them working with unnatural postures. This study aimed to determine ergonomic workstation design for fish smoking workers in the coastal area of Surabaya.

Methods: Respondents were the total population consisting of 12 male and 18 female workers. The worker's body was measured in a sitting position using a measuring tape. Dimensions measured included the reach of the thumb as measured from the back, elbow height when seated, calf height, hip width, buttock-popliteal length, and sitting height. Each dimension was calculated on the 5th and 95th percentiles to determine the size of the ergonomic workstation.

Result: Workstations were made with an adjustable concept. The size of the ergonomic workstation design for male workers includes table width 71.93-77.57 cm, table height 51.81-55.86 cm, chair height 28.64-32.03 cm, chair width 38.66-41.84 cm, chair length 43.24-50.10 cm, and chairback height 89.21-95.46 cm. In addition, for female workers, the size of the ergonomic workstation design includes table width 60.55-68.00 cm, table height 39.21-43.57 cm, chair height 23.64-27.47 cm, chair width 29.13-34.54 cm, chair length 35.80-39.87 cm, and chairback height 74.28-79.72 cm.

Conclusion: It is necessary to make improvements to workstation of fish smoking workers in the coastal area of Surabaya, as an attempt to make it ergonomic.

***Key words**: design, ergonomic workstation, fish smoking workers*

Introduction

Ergonomics has the main principle of "fit the job to the man". It is adjusting the work system for workers, so that they feel safe and comfortable during work. The workstation design should be made to adjust the human user (human centered design), considering the user's anthropometry to match the body dimensions. Workstation design that is incompatible with the workers' anthropometry can increase the load on the worker's body, which has an impact on the comfort and efficiency of workers in doing their work[1].

Fish smoking is a method of processing and preserving fish which is popular in Indonesia by absorbing various chemical compounds from wood smoke into the fish so that the fish has a distinctive color and smoky flavor[2]. Fish smoking can be easily found in coastal areas, one of which is coastal area of Surabaya. Fish smoking workers in the coastal area of Surabaya carry out the smoking process, which amounts to approximately 1.5 quintals, starting at 05.00 a.m. every day. Fish smoker used is conventional and made of traditional chimneys. In addition, the process of

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smoking fish is still done at home.

Fish smoking workers in the coastal area of Surabaya sit on a small stool, called *dingklik,* while working. However, the stool is only as high as their heels and has no backrest or chairback, making it too short and less ergonomic. Moreover, all stools are the same size even though the anthropometry of fish smoking workers is different. Additionally, there is still no special table used for work. Therefore, it is important to make improvements to workstation of fish smoking workers in the coastal area of Surabaya.

This study aims to determine an ergonomic workstation design for fish smoking workers in the coastal area of Surabaya.

Materials and Methods

This research was conducted on fish smoking workers in the coastal area of Surabaya. The total population in this study was 30 people. Sampling was conducted using a total population sampling technique, so that all fish smoking workers were the respondents consisting of 12 male workers and 18 female workers. Data were collected in October 2020.

Anthropometric measurements of fish smoking workers were carried out when they were working in a sitting position using a measuring tape. Dimensions measured included: the reach of the thumb as measured from the back (A), elbow height when sitting (B), calf height (C), hip width (D), buttock-popliteal length (E), and sitting height (F).

Data were analyzed using descriptive statistics, covering the 5th (5%) and 95th (95%) percentiles of the data for each dimension for each gender. The calculation formula used is as follows.

$$5\ \% = \bar{x} - 1.645\ (\sigma) + \text{Allowance}$$

$$95\ \% = \bar{x} + 1.645\ (\sigma) + \text{A}$$

$$\text{Standard deviation } (\sigma) = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

The allowance factor used was 0.5, while n was the total number of respondents, namely 12 for males and 18 for females. The 5th percentile was used as the lowest measure and the 95th percentile was used as the highest size in ergonomic workstation design since the workstation was made with an adjustable concept.

Dimension A was used as a measure of width, while dimension B was used as a measure of the height of an ergonomic work table design. Furthermore, dimension C was used as a measure of height; dimension D was used as a measure of width; and the dimension E was used as a measure of the length of the ergonomic work chair design. Finally, the dimension F was used as a measure of the back height of the ergonomic work chair design.

Result and Discussion

The results of anthropometric measurements of fish smoking workers in the Coastal Area of Surabaya are shown in Table 1.

Table 1. Anthropometric Measurements of Fish Smoking Workers in the Coastal Area of Surabaya

No.	Dimensions					
	A (cm)	B (cm)	C (cm)	D (cm)	E (cm)	F (cm)
Males						
1	75	53	30	39	45	93
2	73	54	29	40	47	94
3	74	52	30	39	48	94
4	75	51	30	38	43	92
5	76	55	29	41	42	91
6	77	55	29	40	49	91
7	72	54	29	40	48	91
8	76	54	31	40	47	93

Cont... **Table 1. Anthropometric Measurements of Fish Smoking Workers in the Coastal Area of Surabaya**

9	72	53	31	39	47	93
10	74	53	32	39	46	93
11	75	52	29	41	47	88
12	72	54	29	41	45	89
Total	891	640	358	477	554	1102
Average	74.25	53.33	29.83	39.75	46.17	91.83
Females						
1	64	41	25	33	37	77
2	65	42	24	31	37	78
3	61	39	24	31	38	77
4	64	42	24	30	38	77
5	65	42	25	30	35	77
6	61	39	27	30	35	79
7	61	39	26	30	38	78
8	67	42	26	33	37	78
9	63	41	26	34	39	74
10	61	41	23	30	38	75
11	64	42	25	30	37	73
12	65	39	24	30	40	74
13	61	39	24	34	37	75
14	68	42	26	32	38	77
15	62	41	26	32	38	78
16	67	41	27	34	36	76
17	64	41	25	31	37	77
18	65	43	24	29	37	77
Total	1148	736	451	564	672	1377
Average	63.78	40.89	25.06	31.33	37.33	76.50

Based on the anthropometric data in Table 1, the size of the ergonomic workstation design for fish smoking workers in the Coastal Area of Surabaya was calculated for each dimension using the 5^{th} and 95^{th} percentiles for each gender. The results of these calculations are shown in Table 2.

Table 2. Size of Ergonomic Workstation Design for Fish Smoking Workers in the Coastal Area of Surabaya

No.		Dimensions	Note	Lowest Size (cm)	Highest Size (cm)
Males					
1	Table Size	A	Table width	71.93	77.57
2		B	Table height	51.81	55.86
3	Chair Size	C	Chair height	28.64	32.03
4		D	Chair width	38.66	41.84
5		E	Chair length	43.24	50.10
6		F	Chairback height	89.21	95.46
Females					
7	Table Size	A	Table width	60.55	68.00
8		B	Table height	39.21	43.57
9	Chair Size	C	Chair height	23.64	27.47
10		D	Chair width	29.13	34.54
11		E	Chair length	35.80	39.87
12		F	Chairback height	74.28	79.72

Table 2 shows the lowest and highest sizes of each dimension in the ergonomic workstation design for fish smoking workers in the Surabaya Coastal Area. A workstation that is not ergonomic can have several impacts on workers, including an increase in workload, musculoskeletal complaints, fatigue, and decreased productivity[3,4]. More importantly, there will be a higher rate of pain and muscle spasms and a lower rate of productivity[5]. Based on a systematic review of 5 studies, it was found that there is a positive impact of improving chair design in reducing musculoskeletal symptoms[6]. Thus, it is substantial to make ergonomic workstation design.

Gender is one of the factors that influence anthropometry because there are statistically significant differences between male and female body dimensions; male body dimensions are greater than that of women[7]. Accordingly, ergonomic workstation designs between the genders are differentiated.

Table Width

The design size of the table width for fish smoking workers in the coastal area of Surabaya was determined based on the reach of the thumb which was measured from the worker's back, so that workers could easily reach the table without bending over. Other studies have also determined the table width using the length of the hand held in front of the user [8], but some are not caused by limited space but still consider ergonomic factors and flexibility to put work tools[9]. Another study used only the 95th percentile to determine the table width[10]. However, the table width for fish smoking workers in the coastal area of Surabaya used the 5th and 95th percentiles

so that workers with the smallest to the largest body anthropometry still matched the size of the ergonomic workstation design.

Table Height

The table height in the ergonomic workstation design for fish smoking workers in the coastal area of Surabaya used the height of the workers' elbows in their sitting position. Several other studies have also used elbow height in a sitting position to determine the table surface height[11,12]. A table that is too high can cause pain in the shoulders and neck, while a table that is too low causes the back to bend, causing tense back and shoulder muscles[11]. The table height in the ergonomic table design for fish smoking workers in the coastal area of Surabaya used the 5th to 95th percentiles from the workers' elbow height data. The table height in this range can accommodate anthropometry of workers with the smallest to largest body sizes.

Chair Height

Fish smoking workers in the coastal area of Surabaya sit on very low stool during work, so that they always kept their legs bent and their backs bent. This kind of work posture also have an impact on the muscles and skeleton since there is excessive stress on the tendons, ligaments and joints[13]. Ergonomic small chair height in each study was set according to the type of work. A study on batik crafters in Indonesia recommends small stool at the knees of workers[14,15]. However, work chairs for fish smoking workers in the coastal area of Surabaya could not be made as high as knee; the sitting position of the workers needed to adjust the height of the fish smoker. Thus, the chair height for fish smoking workers in the coastal area of Surabaya was made to be as high as the workers' calves.

Chair Width

The chair width in the ergonomic workstation design for fish smoking workers in the coastal area of Surabaya was determined based on the workers' hip width. Several other studies have also recommended chair width based on the user's hip width[8,15]. Chair that is too narrow cause workers to be unable to move freely while working[15]. A study determined the chair width with armrests on the chair based on the 95th percentile of the worker's hip width data, in order to tolerate workers with large hips. However, chairs without armrests were designed to the 5th percentile to tolerate workers with small hips[14]. The chair width for fish smoking workers in the coastal area of Surabaya was set using the 5th to 95th percentile, allowing it to accommodate all workers, whether they had large or small hips.

Chair Length

A study conducted in a company utilized the 5th percentile of the buttock-popliteal length data to determine chair length considering workers who had short buttocks-popliteal[16]. Another study conducted on tailors in a village used the 95th percentile of the buttock-popliteal length data to determine ergonomic chair length [4]. Chair that is too long make it difficult for users to reach the back of the chair, causing pain in the back and shoulders[17]. Accordingly, it is important to pay attention to the chair length so that it is not greater than the buttock-popliteal length of the user. The chair length in the workstation design for fish smoking workers in the coastal area of Surabaya used the 5th to 95th percentiles from the buttock-popliteal data of all workers, so that workers with long and short buttock-popliteal sizes could match the chair length.

Chairback Height

A study conducted on *songket* weavers in Indonesia has shown a decrease in the incidence of low back pain after the weavers were provided by chairback while working[18]. Thus, it is important to add chairback for fish smoking workers in the coastal area of Surabaya. A chairback that is too short can cause unnatural posture which can lead to back pain[19]. Besides, a study conducted in Bangladesh used back height to determine the chairback height[12]. Likewise, a study in Turkey recommends the chairback for the user[20]. The chairback height in the ergonomic workstation design for fish smoking workers in the coastal area of Surabaya was determined based on the workers' back height, so that it could accommodate the back height of all fish smoking workers in the coastal area of Surabaya.

Conclusion

Ergonomic workstation design for fish smoking workers in the coastal area of Surabaya are made by

adjusting the workers' anthropometry, using the 5th and 95th percentiles of the anthropometric data, so that the workstations are manageable. The size of the ergonomic workstation design for male workers includes table width 71.93-77.57 cm, table height 51.81-55.86 cm, chair height 28.64-32.03 cm, chair width 38.66-41.84 cm, chair length 43.24-50.10 cm, and chairback height 89.21-95.46 cm. In addition, for female workers, the size of the ergonomic workstation design includes table width 60.55-68.00 cm, table height 39.21-43.57 cm, chair height 23.64-27.47 cm, chair width 29.13-34.54 cm, chair length 35.80-39.87 cm, and chairback height 74.28-79.72 cm. Referring to the results of the study, it is important to make improvements to workstation of fish smoking workers in the coastal area of Surabaya, to make it ergonomic.

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Severe Pneumonia: Etiology and Outcome in a Tertiary Hospital in Indonesia

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Abstract

Background: Severe pneumonia represents a subset of life-threatening pneumonia. The mortality rate of patients with severe pneumonia is considerably high. This study aims to determine the etiology and outcome of severe pneumonia.

Methods: An observational prospective study was conducted from September 2017 to September 2018 on pneumonia patients in a tertiary hospital. Clinical and diagnostic evaluations were carried out to assess the severity of the disease, etiology, comorbidities, and several other factors associated with outcomes.

Results: 140 pneumonia patients were evaluated and 41 patients met the severe pneumonia criteria. A pathogen was found in 20 community-acquired pneumonia (CAP) and 13 hospital-acquired pneumonia (HAP). The most frequently isolated pathogen from the sputum culture of patients with either severe CAP or HAP was *Acinetobacter baumannii*. The mortality rate of severe HAP patients was higher than that of severe CAP patients (84% vs. 65%), but the difference was nonsignificant. Most of the subjects had comorbidities (CAP 75%, HAP 61.6%). Procalcitonin (PCT) and C-reactive protein (CRP) levels in severe CAP were higher than those in severe HAP (PCT 7.7 vs 6.0, p=0.658; CRP 163.1 vs 93.6, p=0.580), but the differences were also nonsignificant.

Conclusion: The most frequently isolated pathogens from the sputum culture of patients with severe pneumonia were Acinetobacter baumanii, which should be considered at the time of diagnosis and empirical antibiotic therapy. Severe pneumonia was often accompanied by comorbidities, inflammation responses increase in both severe CAP and HAP with a high mortality rate.

Keywords*: severe pneumonia, etiology, mortality*

Introduction

Pneumonia is defined as an infection of the pulmonary parenchyma. Causative microorganisms of severe pneumonia are one of the risk factors associated with mortality[1]. Pneumonia was caused by different microbes in different parts of the world. In the Middle East, the most frequently isolated pathogens in intensive care unit-admitted hospital-acquired pneumonia (HAP) is Streptococcus pneumoniae and for community-acquired pneumonia (CAP) is Acinetobacter baumanii[2]. In South Asia, Staphylococcus aureus and Pseudomonas aeruginosa are the most common causes of severe CAP[3]. Streptococcus pneumonia is the most common cause of severe CAP in Singapore[1]. The mortality rate in severe pneumonia (community or hospital-acquired)

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is considerably high, (30.3%)[2].

Clinically distinguishing CAP and HAP is relevant because there are distinctions in the causative pathogens and thus different therapies. In HAP, the initial illness that leads to hospitalization interferes with host defenses and can contribute to susceptibility to infections and host response disorders. This theory explains the difference in the immune responses in HAP and CAP[4]. This study aims to determine the etiology and outcome of severe pneumonia

Methodology

Population and study design :

This study is a prospective observational study of hospitalized pneumonia patients in Dr. Soetomo Academic Hospital from September 1, 2017, until September 1, 2018, who met the inclusion criteria of severe pneumonia. Of the 140 patients with pneumonia (CAP and HAP), 50 were excluded because of incomplete laboratory data, 1 was discharged against medical advice, and 11 passed away before sputum culture collection. Of the remaining 78 patients, 41 patients met the criteria for severe pneumonia; however, positive sputum culture was found only in 33 patients. All patients gave written informed consent, and this study received ethical approval from the Health Research Ethics Committee of Dr. Soetomo Hospital Surabaya (602 / Panke.KKE / X / 2017).

Inclusion criteria :

Adult hospitalized patients who met the criteria of severe CAP according to IDSA / ATS 2007[5] or severe HAP according to 2016 HAP guidelines[6] and did not suffer from pulmonary tuberculosis, experience an acute infection of other organs, or suffer from lung cancer were included. The severe CAP definition according to IDSA / ATS 2007 consists of minor criteria, respiratory rate > 30 breaths/min, PaO_2 /FIO_2 ≤250 mmHg, multilobar infiltrates, confusion/disorientation, uremia (BUN ≥20 mg/dl), leukopenia (WBC count < 4000 cells/mm^3), thrombocytopenia (platelet count <100.000 cells/mm^3), hypothermia (core temperature <36°C), and hypotension that requires aggressive resuscitation, and major criteria, requires invasive mechanical ventilation and septic shock with the need for a vasopressor that requires aggressive fluid resuscitation[5]. Severe HAP is defined as HAP with risk factors for mortality, i.e., the need for ventilatory therapy due to pneumonia and septic shock[6].

Examination procedure:

Sputum samples for culture were collected spontaneously (with a prior mouth rinse) in patients who were conscious and able to expectorate phlegm. Sputum suctioning with a suction catheter was performed for patients who were unable to expectorate phlegm or with an endotracheal tube. The samples were subsequently sent to the laboratory for aerobic culture examination. Sputum samples were inoculated on blood agar and MacConkey agar. The etiology of pneumonia was determined based on the pathogenic bacteria that grew on the culture medium. Inflammatory biomarker examination (PCT and CRP) was performed by withdrawing 3-5 mL of venous blood. The C-reactive protein extended range (RCRP) method based on the PETIA technique was used to measure CRP levels with a Flex reagent cartridge (Dimension, Siemens Healthcare Diagnostics Inc.). A CRP value > 10 mg/L was categorized as higher than normal. Serum procalcitonin levels were measured by VIDAS® B.R.A.H.M.S PCT® (bioMerieux SA. Marcy-I'Etoile-France).

Statistical Analysis

Descriptive statistics were analyzed using the mean ± SD for quantitative variables and frequency and percentage for qualitative variables. The chi-square test was used to evaluate differences in qualitative variables, and the t-test was used for quantitative variables. The Kaplan-Meier method and log-rank test were used for survival analysis. The results of statistical calculations were considered significant when $p < 0.05$.

Results

Clinical characteristics of severe pneumonia

The characteristics of the 33 severe pneumonia patients are shown in Table 1. Of all study subjects, 60.0% had severe CAP, and 40% had severe HAP. The mean age was 56 years old in severe CAP and 46 years old in severe HAP, and there were more patients with comorbidities than those without comorbidities. Of the 33 pneumonia patients, 26 (78.8%) were hospitalized in

intensive care, and 7 (21.2%) were hospitalized in non-intensive care. All patients have received antibiotics before sputum culture sample collection.

Table 1. Clinical characteristics and comorbidity distribution

Characteristics N (%)	**Severe CAP 20 (60.6%)**	**Severe HAP 13 (39.4%)**	**p-value**
Age [mean ± SD]	56.65 ± 18.37	46.77 ± 17.32	0.133
Sex			
Male	10 (50.0%)	9 (69.2%)	0.464
Female	10 (50.0%)	4 (30.8%)	
Clinical features			
Fever (°C) [median (min-max)]	38 (36-38.9)	38.1 (36.2-38.7)	0.605
Cough	20 (100%)	13 (100%)	
Respiration Rate	25.90±3.21	26.92±2.56	
Systolic blood pressure	111.45±24.31	120.23±23.43	0.312
Diastolic blood pressure	68.50±13.41	70.38±15.40	0.712
Comorbidity			
Without comorbidity	5 (25.0%)	5 (38.4%)	0.329
With comorbidity:	15 (75.0%)	8 (61.6%)	
Diabetes mellitus	9 (45.0%)	2 (15.4%)	0.132
Cardiovascular insufficiency	7 (35.0%)	4 (30.8%)	1.000
Stroke	2 (10.0%)	3 (23.1%)	0.360
COPD	3 (15.0%)	0 (0.0%)	0.261
Chronic kidney disease	2 (10.0%)	1 (7.7%)	1.000
Malignancy	0 (0.0%)	2 (15.4%)	0.148

Note: Results are expressed as the mean ± SD, median (min-max) or %.

SD; standard deviation, CAP; community-acquired pneumonia, HAP; hospital-acquired pneumonia, COPD; Chronic obstructive pulmonary disease

Causative Pathogen

Pathogenic microbes isolated from patients with CAP were mostly gram-negative (85.0% (17/20)) and were dominated by *Acinetobacter baumannii* and *Klebsiella pneumoniae*, while Gram-positive microbes accounted for only 15.0% (3/20). In HAP patients, only Gram-negative bacteria were found (13/13=100%), the most common being *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Table 2). All patients received antibiotics before sputum culture sample collection.

Table 2. Results of sputum culture based on the type of severe pneumonia

Pathogenic microbes	*Severe CAP n=20*	Severe HAP n=13	All severe pneumonia	Passed away	p-value
Acinetobacter baumannii	12 (60.0%)	3 (23.1%)	15 (45.4%)	11 (37.9)	0.373
Klebsiella pneumoniae ESBL+	3 (15.0%)	2 (15.4%)	5 (38.2%)	3 (10.3%)	0.533
Pseudomonas aeruginosa	1 (5.0%)	3 (20.0%)	4 (13.3%)	3 (10.3%)	0.705
Enterobacter cloacae	0 (0.0%)	2 (15.4%)	2 (6.0%)	4 (13.7%)	0.260
Staphylococcus aureus	2 (10.0%)	0 (0.0%)	0 (0.0%)	2 (6.8%)	
Enterobacter aerogenes	0 (0.0%)	1 (7.7%)	1 (2.7%)	1 (3.4%)	
Escherichia coli ESBL +	0 (0.0%)	1 (7.7%)	1 (2.7%)	1 (3.4%)	
Acinetobacter iwoffii	0 (0.0%)	1 (7.7%)	1 (2.7%)	1 (3.4%)	
Proteus mirabilis	0 (0.0%)	0 (0.0%)	1 (2.7%)	1 (3.4%)	
Streptococcus pyogenes	1 (5.0%)	0 (0.0%)	1 (2.7%)	1 (3.4%)	
Aeromonas caviae	1 (5.0%)	0 (0.0%)	1 (2.7%)	1 (3.4%)	
Total	20	13	33	23	

ESBL; Extended spectrum beta-lactamases, CAP; community-acquired pneumonia, HAP; hospital-acquired pneumonia

Inflammatory response

Total leukocyte numbers in CAP patients was higher than that in HAP patients, but the difference was not significant. Procalcitonin and CRP levels were elevated in patients with CAP and those with HAP. The mean PCT and CRP levels in CAP patients were higher than those in HAP patients, but the difference was not significant (Table 3).

Table 3. Laboratory results of severe pneumonia patients

Laboratory parameters	*Severe CAP n=20*	Severe HAP n=13	p-value
Hemoglobin (g/dL)	11.44±2.41	10.56±2.26	0.307
Leukocytes (/µL)	19.240±9.59	14.850±5.46	0.145
Thrombocytes (/µL)	258.500 (180.000-513.000)	269.000 (151.000-940.000)	0.450
Blood urea nitrogen (mg/dL)	23 (4-89)	16 (6-55)	0.672
Serum creatinine (mg/dL)	1.21 (0.52-6.29)	0.77 (0.3-2.58)	0.074
Potassium (mmol/L)	3.9 (2.1-6.1)	3.8 (2.3-7.0)	0.365
Sodium (mmol/L)	140 (122-164)	141(124-164)	0.970
CRP (mg/dL)	163.1 (8.6-315)	93.62 (4.7-316.75)	0.580
PCT (ng/mL)	7.72 (0.13-116.9)	6.08 (0.22-200)	0.658

Note: Results are described as the mean ± SD or median (min-max).

CRP; C-reactive protein, PCT; procalcitonin, CAP; community-acquired pneumonia, HAP; hospital-acquired pneumonia

Table 4: Severity of illness and mortality

Variables	Severe CAP n=20	Severe HAP n=13	p-value
SOFA score	7.75	7.15	0.614
Requiring mechanical ventilation	16 (80.0%)	11 (84.6%)	1.000
Need for a vasopressor	6 (30.0%)	3 (23.1%)	1.000
CRP >100 mg/dL	13 (65.0%)	6 (46.2%)	0,478
PCT >5 ng/mL	12 (60.0%)	8 (61.5%)	1,000
ICU admission	16 (80.0%)	11 (84.6)	1.00
Blood gas analysis:			
PaO2	72.70 (31-197)	88 (36-135)	0.418
PaO2/FiO2	138.62±71.80	121.03±40.41	
PaO2/FiO2			
≥ 200 mmHg	4 (20.0%)	0 (0.0%)	0.136
< 200 mmHg	16 (80.0%)	13 (100%)	0.118

Mortality	13 (65.0%)	11 (84.0%)	0.26
ICU	10 (50.0%)	9 (69.2%)	0.261
Hospital ward	3 (15.0%)	2 (15.3%)	0.667

SOFA; The Sequential Organ Failure Assessment , CRP; C-reactive protein, PCT; procalcitonin, ICU; intensive care unit, CAP; community-acquired pneumonia, HAP; hospital-acquired pneumonia

The severity of illness and outcome

The degrees of severity of patients with severe CAP and those with severe HAP were equal, as seen in the sequential organ failure assessment (SOFA) score in Table 4. The increased SOFA score in severe CAP, pulmonary organ dysfunction (PaO_2 /FiO_2 ratio <200), and use of vasopressors were not significantly different between sCAP and sHAP. The severity of the disease affects the outcome. Overall mortality was 72.7% (n=24). Higher mortality was seen in severe HAP than in severe CAP, but the difference was not significant. Survival analysis showed that there were no differences in the survival rate between the two types of pneumonia (Figure 1).

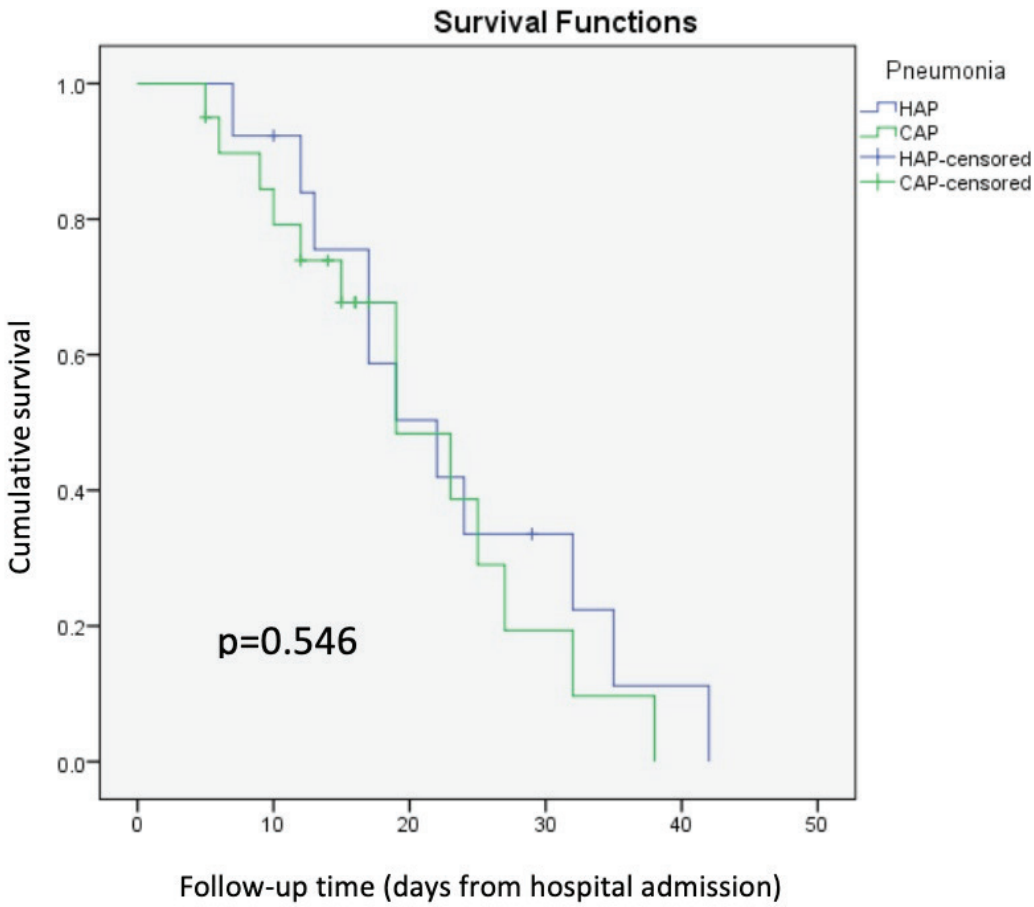

Figure 1. Kaplan-Meier survival curves in severe CAP and severe HAP.

Survival analysis of cumulative survival and follow-up time revealed that there were no differences in the survival rate between the two types of pneumonia

In this study, the most common microbes that caused severe CAP confirmed by sputum culture were Gram-negative bacteria (*Acinetobacter baumannii* and *Klebsiella pneumoniae*), followed by gram-positive bacteria (*Staphylococcus aureus*). Acinetobacter

baumanii has become the emerging cause of severe CAP in the Asia-Pacific region. In Australia, a reported 21% incidence of Acinetobacter baumanii from gram-negative bacterial pneumonia in the 1990s[7]. In the last few decades, severe CAP has been reported caused by A baumanii. The majority of cases were reported from Northern Australia and Asia, such as Thailand, Singapore, Hong Kong, Taiwan[8,9,10]. In this study, Acinetobacter baumanii sputum isolates were found in 60% of sCAP cases. Clinical characteristics of these cases were acute, severe with respiratory failure and septic shock, leading to a high mortality rate[8,11,12]. Several conditions, such as elderly patients, alcoholism, malignancy, diabetes, kidney disease, and liver cirrhosis are associated with Acinetobacter baumanii infection[8,9]. In this study, 75% of cases had an underlying disease but apart from alcoholism and cancer. Antibiotics administration might play a role as all patients have been given antibiotics from the referring hospital.

Patients` immune system is disrupted by the initial illness that leads to hospitalization and this immune disruption may contribute to susceptibility to infection[4]. Acinetobacter baumanii has become a potential pathogen of HAP in critically ill patients[13]. Acinetobacter baumanii sputum was the most common isolate found in HAP patients in this study (23.1%) as seen in HAP patients in Iranian ICU (73.9%) and 37% in Saudi Arabia[2,14]. In this study, 61.6% of HAP patients presented with comorbidities, similarly, Farid et al reported that 57.9% of patients with HAP had comorbidities[14]. This data is important in considering diagnosis and initial administration of antibiotics for severe HAP

Both severe CAP or HAP showed identical severity and outcome[15]. The mortality of severe pneumonia cases is considerably high. Several factors are associated with the mortality of patients with severe pneumonia. The causative pathogen, comorbidity, and host response are factors that influence intensive care outcomes. In the Middle East, the mortality rate of severe CAP patients admitted to the intensive care unit (ICU) was 46.6%[16]. A 2005 study in Japan reported that the mortality rate of severe CAP patients requiring intensive care reached 48.6%[17]. A noticeably high mortality rate (89%) was seen in cases caused by *Pseudomonas aeruginosa*[3]. Quah et al. reported that respiratory viruses that coexisted with bacteria in mixed viral-bacterial coinfection were associated with an increased risk of death[1]. In this severe pneumonia study, the results of sputum culture showed that Gram-negative bacteria, especially *Acinetobacter baumannii*, were the most frequently isolated microbes, with mortality reaching 65%. After suffering a critical condition, patients with few comorbidities tend to have a superior clinical outcome[18]. A study with a large number of CAP and HAP patients admitted to the ICU with a comparable number of comorbidities (69.7% CAP, 65.4% HAP), age, and severity index showed similar host responses[3]. Patients in this study who presented with prevalent comorbidities and a severe disease index (SOFA score > 7.0) had a high mortality rate.

Procalcitonin is a calcitonin peptide precursor released by the cell parenchyma in response to bacterial toxins. Procalcitonin not only is an inflammatory biomarker but also is used in the diagnosis of bacterial pneumonia[19]. Furthermore, the PCT level is also related to the severity of pneumonia[20]. Some studies reported that PCT levels were related to the severity of pneumonia, and patients who had an increase in PCT levels showed a tendency to pass away[21,22]. Procalcitonin is a useful predictor of pneumonia severity and mortality risk. A study reported that the level of PCT >1.1 ng/mL can be a guide to identify groups of ICU patients who are at high risk of suffering multiorgan failure and mortality[24]. Another study reported that high PCT levels (>10 ng/mL) increased mortality risk[23]. In this study, the mean levels of PCT were 7 ng/mL and 6 ng/mL in CAP and HAP, respectively, but the differences were not significant. A similar result was reported by Bloos et al.[20], showing that the initial PCT level in CAP was nonsignificantly different from that in HAP, albeit at a lower level (2.4 vs 2.2).

C-reactive protein is an alternative inflammatory marker to aid in the diagnosis of bacterial pneumonia and a predictor of pneumonia severity despite being less accurate than PCT[24]. It has been reported that higher accuracy was seen when CRP was used in combination with PCT than when it was used alone[25]. In this study, PCT (>5 ng/mL) and CRP (>100 mg/dL) elevation were found in 50% of severe CAP and severe HAP patients, although the difference was not significant. The clinical condition indicates that both severe CAP and severe HAP have comparable disease severity, as it was

evident that there was no difference in mortality.

In this study, the most common clinical features were cough, fever, and dyspnea in both severe CAP and HAP. In a study by Elshamly, the most frequent clinical features were fever, cough, dyspnea, and hypoxemia in patients with CAP[29], while Aya et al. reported that 72.2% of pneumonia patients complained of fever, 89.9% cough, 66.6% spasms and 35.11% pleuritic pain. The patients with HAP were dominated by elderly people and male sex, and they complained of less cough, sputum, and dyspnea than younger patients[27]. In this study, the PaO_2 of severe CAP patients was lower than that of severe HAP patients but not significantly different, and comorbidities were more common in severe CAP than in severe HAP (75% vs 61.6%). Diabetes mellitus (DM) was the most common comorbidity in severe CAP, while cardiovascular insufficiency was the most common comorbidity in severe HAP. A study of severe CAP stated that 51.85% of CAP patients had comorbidities, and the most common comorbidities were DM and hypertension[26].

A limitation of this study was that only routine aerobic cultures of sputum were performed, so other possible causes of severe pneumonia, such as viruses and atypical pathogens, were not detected.

Conclusion

The causative pathogens of severe pneumonia were dominated by gram-negative bacterias, mainly Acinetobacter baumanii. Underlying disease was an important risk factor for severe pneumonia. Severe CAP and HAP had identical clinical characteristics, disease severity, and high mortality rate.

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The Effects of Turmeric Extract (*Curcuma longa*) Loaded Hidrogels in Accelerating Wound Closure

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Abstract

Background: Disruption to healing process results on longer healing duration from the timeline. Povidone iodine and tulle are common management in wound care. The advance of industry in health sector and the availability of natural ingredients in Indonesia provide opportunities for developing alternatives for wound care. Turmeric (*Curcuma longa*) contains curcumin which is anti-inflammatory, antibacterial, and antioxidant; it is good for wound healing. Hydrogel nanocomplexes of carboxymethyl chitosan can maintain moisture in wound area and protect curcumin from hydrolysis. **Objective:** To analyze the potential of turmeric extract loaded hidrogels in accelerating wound closur. **Method:** experimental research with "post-test only control group design". The sample consisted of 30 mice which were assigned into 5 groups. **Result:** All experimental groups experienced a gradual decrease in wound length. The statistical test results for groups II and III showed significant advantages compared to group IV ($p<0.05$). Meanwhile, group I did not have significant differences in wound closure compared to group IV on day 1 and 3 ($p>0.05$), but it had significant results ($p<0.05$) on day 5. **Conclusion:** Turmeric extract loaded hidrogels can accelerate the reduction of wound length.

Keywords: *Carboxymethyl Chitosan, Curcumin, Hydrogels, Turmeric, Wound Healing*

Introduction

Wounds are damage to normal structures of the skin, either closed or open ones. Based on healing duration, wounds are classified into acute and chronic wounds[1]. Chronic wounds occur due to disrupted wound healing process and it takes longer to heal than the timeline[2]. There are more than 5.7 million chronic wound patients in the US and it is estimated that the annual medical costs reaches US $20 billion. The cost is expected to increase sharply to US $24.8 billion by 2024[3]. Therefore, proper wound healing is important in order to reduce costs and prevent chronic wounds[4].

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Wound healing process consists of several phases; coagulation and hemostasis phase, inflammatory phase, proliferation phase, and remodeling phase[5,6]. Povidone iodine is a common management in wound care. The advance of industry in health sector and the availability of natural ingredients in Indonesia provide opportunities for developing better alternatives for wound care.

Curcumin from turmeric extract (*Curcuma longa*) is a polyphenol that has anti-inflammatory, antibacterial, and antioxidant properties[7,8]. In addition, it increases granulation tissue formation, epithelial regeneration, and angiogenesis when topically administered to wounds[9]. However, curcumin has hydrophobic property which hinders its topical administration directly on wounds[3,10]. Carboxymethyl chitosan is a chitosan derivative that is non-toxic, biocompatible, and water-soluble[11,12]. In the process of wound healing, carboxymethyl chitosan can

stimulate cell proliferation, increase collagen formation, as well as accelerate regeneration and epithelialization of wound tissues[13].

The advance on health industry has provides alternatives for wound care modalities, one of which is the use of biopolymers[14]. Hydrogel is a cross-polymer network that is hydrophilic. Therefore, it is suitable for wound healing process because it can maintain moisture in wound area, ensure air exchange for tissue respiration, protect curcumin from hydrolysis, and provide comfortable sensation when used[15]. This research investigated the potential use turmeric extract loaded hidrogels and answered the problem of the efficiency turmeric extract loaded hidrogels to accelerate wound healing process by indicators of wound length.

Materials and Methods

Turmeric Extraction:

Turmeric (*Curcuma longa*) were washed, drained, and weighed. Turmeric were cut into thin strips and air-dried in the shade. To maximize the drying process, turmeric slices were roasted at 45ºC for about 48 hours or until completely dried. The dried turmeric slices were crushed in a blender and sieved into powder. Turmeric powder then extracted with 96% ethanol using maceration method. Extraction process was continued with rotary evaporator to removed the alcohol content and a thick extract will be produced. The extraction process was carried out by the UPT Laboratorium Herbal Materia Medica Batu, Indonesia.

Preparation of Hidrogels:

One gram of carbomer 940 was dispersed in 100mL of distilled water. One gram of carboxymethyl chitosan was dissolved in 100mL of distilled water. Carboxymethyl chitosan and carbomer 940 disperses were mixed at 400 rpm for 5 minutes using a magnetic stirrer. Then, triethanolamine was added to the carboxymethyl chitosan-carbomer 940 hydrogel until its pH reached 6 and was homogeneous. Turmeric extract was added with different volumes in each group; 1 mL for group I, 2 mL for group II, and 3 mL for group III. Each solutions was added with carboxymethyl chitosan-carbomer 940 hydrogel until it amounted 100mL.

Research Design:

This research has been confirmed the ethical conduct by the Health Research Ethics Committee of Medical Faculty of Airlangga University with the issuance of statement No.58/EC/KEPK/FKUA/2020. The location of this study was carried out in the laboratory of the Department of Pharmacology of the Faculty of Medicine, Airlangga University in February 2020. The design was experimental research through testing on experimental animals (in vivo study) with a "post-test only control group design" approach. The sample size was obtained from the Federer formula, $[(t-1)(n-1)] \geq 15$ where t is the number of treatments / number of groups and n is the number of repetitions / sample size in the groups[16]. The calculation gave result of sample size at 30. Mice (*Mus musculus*) were used and assigned into five groups. The following was the division of the research groups:

Group I : Mice were given 1% turmeric extract loaded hidrogels with a topical dose of 2 time 0.5 mL/ day during treatments.

Group II : Mice were given 2% turmeric extract loaded hidrogels with a topical dose of 2 time 0.5 mL/ day during treatments.

Group III : Mice were given 3% turmeric extract loaded hidrogels with a topical dose of 2 time 0.5 mL/ day during treatments.

Group IV : Negative control, mice were given aquades with a topical dose of 2 time 0.5 mL / day during treatments.

Group V : Positive control, Mice were treated with tulle + povidone iodine with a topical dose 2 time 0.5 mL / day during treatments

Animal Model:

Adult male mice (*Mus musculus*), aged 8-12 weeks and weighed 20-30 grams, were used in this research and then acclimatized for 7 days. The hair on the back of the mice was shaved. Then, disinfection was carried out using 70% alcohol, followed by anesthesia which was carried out by intraperitoneal injection of ketamine 2 ml/kg/BW + 0.8 ml xylazine added to 10 ml of water and injected with a dose of 0.1 ml/kg/BW. The incisions were made 10 mm long and 2-3 mm deep in the back

of the mice. The data were in the form of reduction of wound length which were recorded on day 1, day 3, and day 5.

Statistical Analysis

Statistical analysis was performed using SPSS 22. The data presented as mean ± standard deviation (SD). After that, a two-way ANOVA statistical test was carried out followed by the Post Hoc LSD test to determine the effect of treatments. Significance was shown at $p \leq 0.05$.

Result

All groups in this research had good wound conditions and showed no sign of infection. On day 1, the wounds in all groups did not show any significant difference as they were still in inflammatory phase. On day 3, the wounds showed differences between groups I, II, and III, with group IV which was a negative control. In group I, there were granulation tissues but still looked red. In groups II and III, there were clear granulation tissues. Meanwhile, in group IV, the wounds looked paler and more yellowish, indicating a lack of vascularization in wound area. On day 5, there were clear differences between wounds in groups I, II, and III, with group IV. The wounds in groups I, II, and III had re-epithelialized and almost completely closed, while in group IV the wound was wider. (**Figure 1**)

Wound length reductions were measured on day 1, day 3, and day 5 of treatments. The results can be seen in **Table** 1. Wound length reductions occurred gradually in all treatment groups. The results of mean wound length reduction on day 1 and day 3 showed that there were significant differences between groups II, III, and V compared to group IV as negative control ($p<0.05$). Meanwhile, the mean wound length reduction in group I was not significant ($p> 0.05$). On day 5, the results of mean wound length reduction in groups I, II, III and V were significant to group IV ($p<0.05$). The statistical test of wound length reduction which compared groups II and III did not show significant results ($p>0.05$).

Table 1. Mean ± standart deviation (SD) of wound length

Day	Group I 1% turmeric extract loaded hidrogels (mm)	Group II 2% turmeric extract loaded hidrogels (mm)	Group III 3% turmeric extract loaded hidrogels (mm)	Group IV Aquades (mm)	Group V Tulle + Povidone iodine (mm)
1	8.0 ± 0.6	6.8 ± 0.7*	6.5 ± 1.0*	8.3 ± 0.5	7.3 ± 0.8*
3	6.1 ± 1.1	4.8 ± 1.1*	4.3 ± 1.2*	7.3 ± 0.8	5.0 ± 0.8*
5	4.6 ± 1.0*	3.1 ± 0.7*	3.0 ± 0.8*	6.0 ± 0.8	3.5 ± 1.0*

Figure 1. Wound closure for mice after 1, 3, 5 days of *in-vivo* incision wounds. (I) 1% turmeric extract loaded hidrogels (II) 2% turmeric extract loaded hidrogels (III) 3% turmeric extract loaded hidrogels (IV) aquades (negative control). (V) Tulle + Povidone iodine (positive control).

Discussion

This research attempted to analyze the potential of administering turmeric extract loaded hidrogels to reduce wound length in mice. From the results, it was found that turmeric extract loaded hidrogels could accelerate the process of reducing wound length.

In determining the concentration of turmeric extract with carboxymethyl chitosan nanocomplex hydrogel, this research considered that of in vitro study regarding the production of chitosan-based curcumin nanoemulsion gel where a concentration of 1% and 2% showed good spreadability and good gel quality, whereas a concentration of 4% showed poor spreadability[17]. Other research have shown that high curcumin concentration

increased the formation of ROS at the inflammatory stage that inhibit wound healing process[18]. These were used as the basis for determining the ratio of the active ingredient concentration 1; 2; 3 with the hope of producing quality hydrogel with optimal spreadability and minimizing material toxicity.

In group V, which was a positive control, povidone iodine covered with tulle was used. Tulle is a synthetic textile fiber which is a polyamide resin derivative which is elastic, anti-decay in nature, and contains lanolin to keep the wound moist with a semioclusive barrier to accelerate wound healing. Povidone iodine has antibacterial properties, so it can protect wounds from bacteria which can potentially prolong wound healing duration[19].

In group III, which was given 3% turmeric extract loaded hidrogels, had the smallest wound length with a value of 3.0 ± 0.8 on day 5. Meanwhile, group IV as the negative control had the longest wound length with a value of 6.0 ± 0.8 on day 5, and was statistically significant compared to positive control group and the treatment groups I, II and III. Group III had a shorter wound length than group II on day 5 with a difference of 0.1 mm, and it was not statistically significant. These results indicate that administering turmeric extract loaded hidrogels can reduce wound length.

Interestingly, on days 1 and 3, there were no significant differences between group I who was given 1% turmeric extract loaded hidrogels and group IV which was a negative control. However, it showed significant results on day 5. The inflammatory phase is a natural response immediately after injury is modulated by pro-inflammatory cytokines. Based on previous research, it was reported that pro-inflammatory cytokines such as (IL) -1α, IL-1β, IL-6, IL-12, and TNF-α play a role in initiating inflammation, angiogenesis, leukocyte recruitment, and epithelialization in wound healing process. Therefore, the inflammatory phase is an important natural phase in wound healing process[20]. However, an increased and prolonged expression of TNF-α and other pro-inflammatory cytokines can interfere with wound healing process[21]. The use of curcumin from turmeric extract can increase cells sensitivity to participate in the inflammatory response mediated by high TNF-α levels. After that, the TNF-α level will immediately return to the normal level to prevent prolonged TNF-α expression. In addition, it has been reported that curcumin can accelerate re-epithetelization of wounds. This confirms that curcumin does not eliminate the inflammatory process itself, but prevents the prolonged pro-inflammatory cytokine activation which causes hypertrophic wounds and play a role in wound re-epithelialization. These results are consistent with research conducted by Niranjan et al. (2019) regarding the role of poly vinyl alcohol-chitosan-curcumin polymers in wound healing process in vitro and in vivo whose results showed that wound closure in the treatment group was better than the control group[22].

Wound closure is the final stage of complex wound healing. This stage is influenced by previous phases; the inflammation and proliferation phase. In the process of reducing wound length, the results showed that the use of turmeric extract loaded hidrogels could accelerate healing because of the combined effect on each phase of wound healing by accelerating the inflammatory phase, helping in the proliferation phase, and playing a role by increasing MMP and inhibits TGF in remodeling phase[7,23,24].

Conclusion

This research proved that turmeric extract loaded hidrogels can accelerate the process of reducing wound length. The use of turmeric extract loaded hidrogels in group I did not show significant differences compared to the negative control group on day 1 and 3, but on day 5 there were significant results. Meanwhile, groups II and III showed significant advantages compared to the negative control group. Therefore, turmeric extract loaded hidrogels is a good modality in wound care management. However, it is necessary to carry out further analysis regarding the optimum topical dosage and the long-term effects of its administration.

Disclosure of Interest: The authors report no conflict of interest.

Ethical Clearance: This study had been approved by Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Dermatoscopy in Vitiligo: Diagnosis of the Stages

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Abstract

The aim of the Study is to identify the main dermatoscopic patterns of vitiligo and to establish their relationship with the activity of the process.

Materials and Methods: The study involved 63 patients with an established diagnosis of vitiligo. There were 34 patients with a progressive course, 11 with a stable and 18 in the repigmentation stage. All patients underwent dermatoscopy using a Delta 20T dermatoscope. Statistical processing of research materials was carried out using the SPSS Statistics software package.

Results. In the study, it was found that the most significant changes are manifested in the perifollicular region. So, progressive vitiligo is characterized by perifollicular pigmentation (91.2%), an altered pigment network (97.1%), a blurred spot border (94.1%), as well as specific structures - star-shaped formations and "comet tail". A stable vitiligo process is characterized by perifollicular depigmentation (81.8%), a sharp spot border (72.7%). For the stage of repigmentation, marginal hyperpigmentation (100%), perifollicular depigmentation (72.2%), blurred spot border (77.8%), "pigmentation islands" (77.8%).

Conclusion. For the first time, diagnostic dermatoscopic patterns of vitiligo have been developed, and their value has been shown. Dermatoscopy is a promising auxiliary non-invasive tool for diagnosing vitiligo and determining the stage of the disease.

Key words: *dermatoscopy, diagnostics, stability, vitiligo*

Introduction

Vitiligo is an acquired autoimmune disease, whose typical features are sharply demarcated spots resulting from the progressive loss of epidermal melanocytes[1-2]. Vitiligo is a topical problem because this medical condition is common among many ethnic groups and regions, it has major implications for the patients' psycho-social status, and there are no reliable treatment methods for it. It is the typical external symptoms that allow for establishing the correct clinical diagnosis in most cases. Though understanding the process activity is critical for making a decision on the subsequent treatment approach, this indicator cannot always be properly determined. The methods of unbiased and affordable vitiligo diagnosis are not available now. Confocal microscopy is an expensive diagnosis method[3]; tissue biopsy is not always an acceptable procedure for patients, so the diagnosis is based on visual assessment and auxiliary devices that are widely available to skin specialists, such as Wood's lamp. Dermatoscopy of skin disorders is becoming a more common method in dermatology now. For instance, dermatoscopic patterns can be observed in many general dermatoses[4-6]. This method is widely used to diagnose melanocyte-based skin lesions, such as melanoma[7-8]. There are also some publications on dermatoscopic features of parasitic and viral dermatoses[9-12], psoriasis[13-14], lichen planus[15-17], Kaposi sarcoma[18], rosacea and seborrhea dermatitis[19-20]. The knowledge of particular dermatoscopic pattern features of these dermatoses may become a critical additional argument during differential diagnosis of these dermatoses in case of doubt. Foreign literary sources contain occasional publications on

vitiligo dermatoscopyonly[21], but no clear-cut emphases or dermatological patterns or terms in describing vitiligo. Therefore our study was designed to determine the key dermatoscopic patterns that can be useful in diagnosing vitiligo and in assessing the process activity.

Materials and Methods

The study was carried out in the V.A. Rakhmanov Department of Skin and Sexually Transmitted Diseases of N.V. Sklifosovsky Clinical Medicine Institute. The study enrolled 63 patients diagnosed with vitiligo who had been under observation in the Department from October 2018 to December 2019. Vitiligo was diagnosed based on conventional examinations and clinical signs well described in literary sources.

The patients with both stable and unstable (progressive) vitiligo course were enrolled. The disease course was regarded as stable if the patient did not report any new spots and the enlargement of the existing ones in the last 6 months. Stable course with repigmentation (that is normally used during treatment) is accompanied with the shrinking of available foci and pigmentation in the center of the focal spots. Vitiligo progression means the enlargement of the existing depigmentation foci and emergence of new ones during the last 6 months.

All patients underwent dermatoscopic assessment of a single vitiligo spot using the Delta 20T dermatoscope at 20-fold magnification in polarized mode, and photos were shot on Iphone 11.

The selection of dermatoscopic patterns included into the assessment process was based on the publications and own experience. Dermatoscopic structures included into the dermatoscopic assessment included: perifollicular changes, altered pigment network, borderline marginal hyperpigmentation, the spot boundary, the pigmentation islets inside the spot, availability of specific features, such as appearance of starlike formations, appearance of the comet's tail.

All patients were divided into 3 groups (stable course, stable course with repigmentation against the treatment background, unstable or progressive course), depending on the combination of medical history and clinical research.

Statistical processing of the study materials was carried out using the SPSS Statistics software. To compare the symptom emergency rate in patients, Pearson's chi square criterion is used in three stages with calculation of the precise significance by Monte Carlo method and using z-criterion with Bonferroni's adjustment to compare the proportions by columns (i.e. by stages).

Results and Discussion

In our study, 63 patients were randomized into 3 groups:

1. Progressive vitiligo (n=34),

2. Stable vitiligo (n=11),

3. Stable vitiligo with repigmentation (n=18).

Even though several dermatological structures were found in each category(Fig.1), some of them were observed in one of the treatment arms either exclusively or frequently. In this study, we did not adjust any particular dermatoscopic patterns for the patient's age, gender, disease duration, lesion site or concomitant diseases.

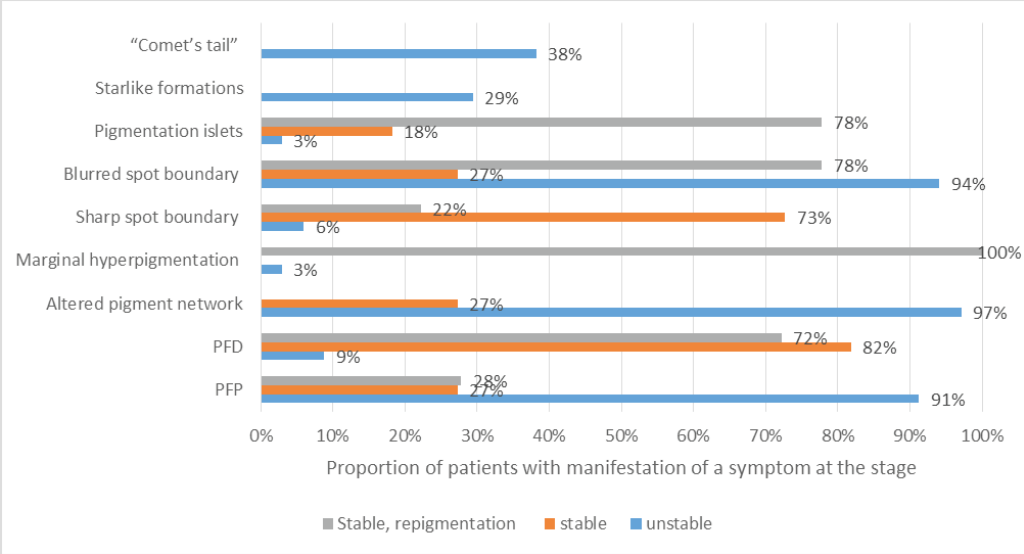

Figure 1. Frequency of the appearance of dermatoscopic patterns at different stages of vitiligo

The most significant changes in case of vitiligo are observed in the perifollicular region. The perifollicular changes included perifollicular pigmentation (PFP) and perifollicular depigmentation (PFD).

Perifollicular pigmentation (PFP) (Fig.2A) means the emergence of dotty pigment around the hair follicle on depigmented skin. Perifollicular pigmentation in patients at the unstable stage was observed at a significantly higher rate (91.2%, or 31 persons) as compared with patients at the stable and stable, repigmentation stages (27.3%, or 3 persons, and 27.8%, or 5 persons, respectively). Significant differences in PFP emergence (p=0.001) were found at stages by Pearson's chi square criterion and by the significance calculation by Monte Carl method.

Perifollicular depigmentation (PFD) (Fig.2B) is the lack of the pigment section near a hair follicle. Perifollicular pigmentationin patients at the unstable stage was observed at a significantly lower rate (8.8%, or 3 persons) as compared with patients at the stable and stable, repigmentation stages (81.8%, or 9 persons, and 72.2%, or 13 persons, respectively). Significant differences in PFD emergence (p=0.001) were found at stages by Pearson's chi square criterion and by the significance calculation by Monte Carl method.

Figure 2. Perifollicular changes. A- periffolicular pigmentation; B- periffolicular depigmentation

Altered pigment network (Fig.3) means partial disappearance or total absence of the network-like pigment pattern. The altered pigment network in patients at the unstable stage is observed at a significantly higher rate (97.1%, or 33 persons) as compared with patients at the stable and stable, repigmentation stages (27.3%, or 3 persons, and 0%, or 0 persons, respectively). Significant differences in the altered pigment network emergence (p=0.001) were found at stages by Pearson's chi square criterion and by the significance calculation by Monte Carl method.

Figure 3. Altered pigment network

Marginal hyperpigmentation (Fig.4A) is more intense, dark pigmentation around a depigmented spot. Its incidence in patients at the stable, repigmentation stagewas observed at a significantly higher rate (100%, or 18 persons) as compared with patients at the unstable and stable stages (2.9%, or 1 persons, and 0%, or 0 persons, respectively). Significant differences in the marginal hyperpigmentation emergence (p=0.001) were found at stages by Pearson's chi square criterion and by the significance calculation by Monte Carl method.

Pigmentation islets (Fig.4B) are homogeneous pigmentation of the healthy skin color as roundish lesions inside a depigmented spot. Their incidence inside the spot in patients at the stable, repigmentation stagewas observed at a significantly higher rate (77.8%, or 14 persons) as compared with patients at the unstable and stable stages (2.9%, or 1 persons, and 18.2%, or 2 persons, respectively).Significant differences in the emergence of pigmentation islets inside the spot (p=0.001) were found at stages by Pearson's chi square criterion and by the significance calculation by Monte Carl method.

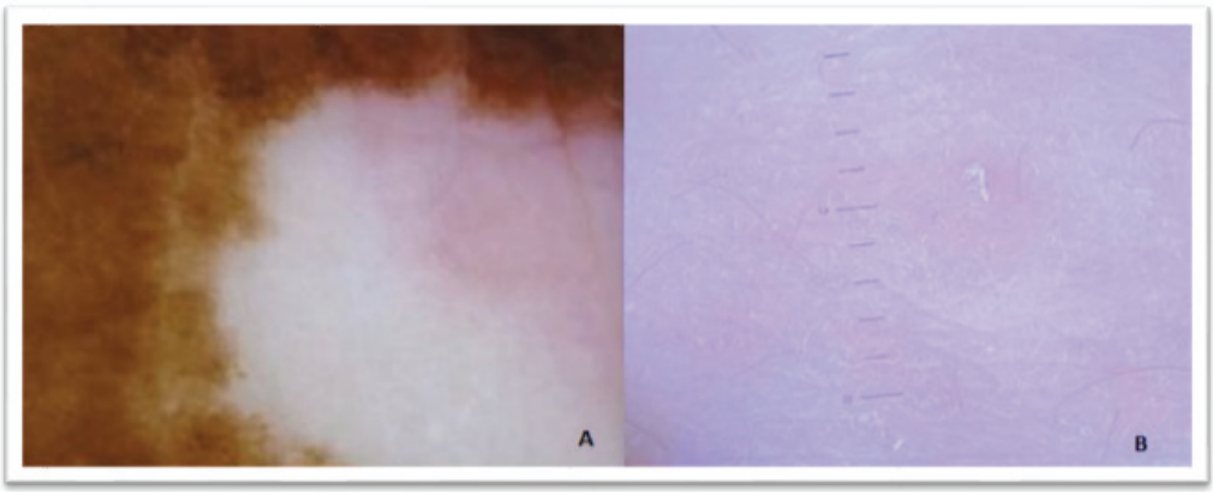

Figure 4. Dermatoscopic patterns: 4A- Marginal hyperpigmentation; 4B- Pigmentation islets.

Sharp spot boundary (Fig.5A) is a clear transition from the depigmented area to the normal skin color. The incidence of sharp spot boundary in patients at the stable stage was observed at a significantly higher rate (72.7%, or 8 persons) as compared with patients at the unstable and stable, repigmentation stages (5.9%, or 2 persons, and 22.2%, or 4 persons, respectively). Significant differences in sharp spot boundary emergence (p=0.001) were found at stages by Pearson's chi square criterion

and by the significance calculation by Monte Carl method.

Blurred spot boundary (Fig.5B) means a smooth transition from the healthy skin to the depigmented skin as the pigment intensity decrease. The incidence of blurred spot boundary in patients at the stable stagewas observed at a significantly lower rate (27.3%, or 3 persons) as compared with patients at the unstable and stable, repigmentation stages (94.1%, or 32 persons, and 77.8%, or 14 persons, respectively).Significant differences in blurred spot boundary emergence (p=0.001) were found at stages by Pearson's chi square criterion and by the significance calculation by Monte Carl method.

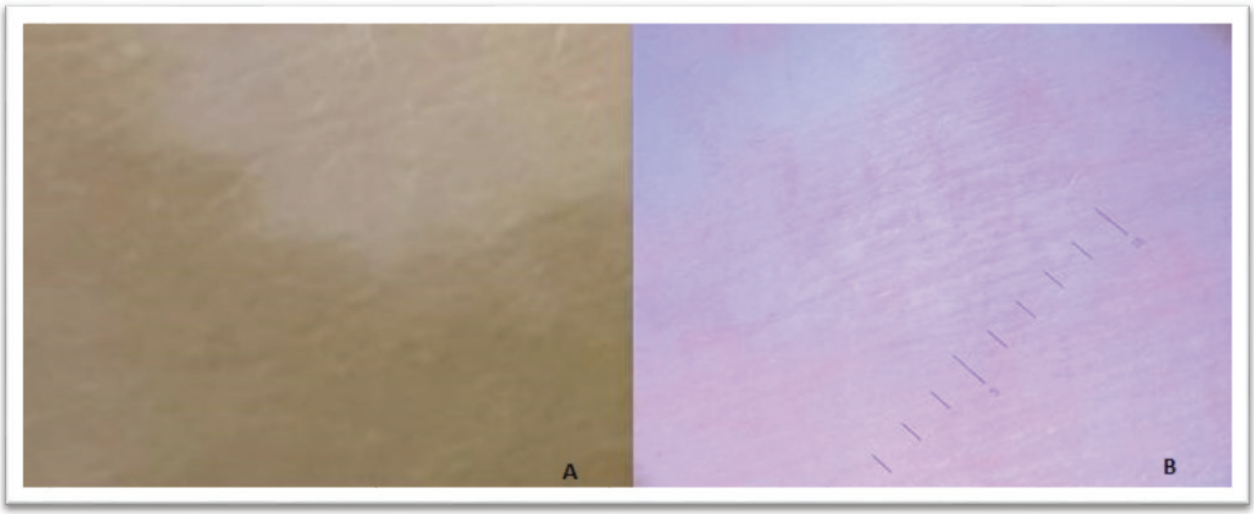

Figure 5. Spot border: 5A- Sharp spot boundary; 5B- Blurred spot boundary

The micro-Kebner phenomenon in vitiligo is the emergence of isomorphous depigmented strips along the injury lines, around the vitiligo spot, which demonstrates the appearance of the "comet's tail" andstarlike formations. Starlike formations (29.4% or 10 persons) and the comet's tail (38.2% or 13 persons) were only found at the unstable stage.

Thus, PFP, modified pigment network, blurred spot boundary and the specific structures, starlike formations and the comet's tail, are typical of the unstable disease course (vitiligo progression). Whereas PDF and sharp spot boundary are typical of the stable course of the disease. For the repigmentation state, the marginal hyperpigmentation, PFD, blurred boundary of the spot, "pigmentation islets".

Conclusion. Dermatoscopy is a promising auxiliary non-invasive tool for diagnosing vitiligo. It enables to assess the disease stages, which may influence the treatment approach selection in the future. The diagnostic dermatoscopic patterns were developed for the first time and demonstrated to be valuable. For instance, perifollicular pigmentation is typical of progressive vitiligo, and perifollicular depigmentation is rather a sign of stable vitiligo. Other dermatoscopic structures, such as modified pigment network, borderline hyperpigmentation and other auxiliary signs, will supplement the picture and enable a more precise diagnosis. Further research and development of the single, standardized dermatoscopic patterns as the diagnostic tests are warranted, which will relieve from the need for invasive histological examinations. Conflict of interests: the authors do not state any conflict of interests.

Ethical Clearance. The study protocol has been approved by The Ethics Committee of Sechenov University in Moscow, Russian Federation (approval number 01-19).

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Conflict of Interest – Nil.

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The Relationship between Nutritional Status and Physical Fitness in Pilot International Standard School (RSBI) at the Level of Junior High School in Padang

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Abstract

The problem of this research is the low of level of physical fitness of students of Pilot International Standard School (SMP level) in Padang. It happened because of lack of nutrional status and physical activity. The purpose of this research is to reveal the correlation between nutrional status and physical activity, individually and in group. This research is Quantitative Reaearch that used correlational technique continued by counting the contribution of independent variables to dependent variables. The accessible population of this research was all male students (448) of RSBI school in Padang, academic year 2011/2012. The sample of this research was 67 students or 15% of accessible population by using proportional technique of random sampling. The instrument of this research to measure physical fitness (Y) was Multistage Running Test (Tes MRT) ; Nutrional Status (X1) with the measure of weight and age converted to Classification Table of Society Nutritional Status, Directorate of Community Nutrition, Department of Health of Republic of Indonesia 1999 ; and Psysical Activity (X2) with questionaire. The results of the research are: (1).There is a significant relationship between nutrional status and physical fitness and contributed for 12.00% . (2). There is a significant relationship between physical activity and physical fitness and contributed for 26.00%. (3). There is a significant relationship between nutritional status and physical activity and contributed for 29.00%. Thus, it can be concluded that increasing the psysical fitness of RSBI students in Padang can be done by increasing Nutrional Status and Psysical Activity individually and in group.

Keywords: *The Nutriotion, Physical activity and Physical Freshness*

Introduction

As an attempt to improve the quality and competitiveness of education, an international standard education unit (school) is deemed necessary. This is mandated in Article 50 paragraph 2 of Law Number 20 of 2003 on National Education System, that: "The Government and/or Regional Governments organize at least one educational unit at all levels of education to be developed into an international standard education unit".

In accordance with the established mechanism, the realization of an International Standard School (ISS), , must start from the pilot stage called Pilot International Standard School. In terms of education units at the Junior High School (SMP) level in Padang, there are two schools which are in the level of RSBI called SMP RSBI including SMP Negeri 1 in West Padang Sub-District and SMP Negeri 8 in East Padang Sub-District.

The ideal condition or a point that must be realized as an SMP RSBI is the fulfillment of the Minimum Key Performance Indicator (IKKM) which contains eight elements of the National Education Standard, and the Additional Key Performance Indicator (IKKT) which contains various elements. One of the eight elements of the National Education Standards in the IKKM is the Graduation Competency Standards for Each Subject at the SMP level. One of these subjects is Physical

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Education, Sports and Health

Nutritional status is basically the state of a person's body health caused by consumption, absorption and use of food substances. Food substances consumed and absorbed by the body are very useful for the process of growth and development as well as a source of calories for a person to carry out activities. If a student's nutritional status is in the good category, he will certainly have a healthy body, strong, and have high resistance to disease attacks, as well as being able to carry out various daily activities well. On the other hand, students who experience more or less nutrition tend to experience impaired growth and development and are susceptible to disease. Thus, excess or lack of nutrition will surely have an impact on decreasing the level of their physical fitness.

More importantly, physical activity can simply be interpreted as various body movements produced by the muscles of the movement, which requires energy expenditure. These various body movements or physical activities are carried out spontaneously in various daily activities, both routine and free time. Students who have an active lifestyle tend to do more physical activity considering that they are more active and therefore have better physical fitness.

As it has been mentioned above, the two main factors (nutritional status and physical activity) are thought to be related to the problem of the tendency of low levels of physical fitness of SMP RSBI students, which is also exacerbated by the long study time at school. In other words, SMP RSBI students in Padang conduct learning activities from 7:00 a.m. to 3:20 p.m. or approximately 9 hours. Students who want to do homework (PR) can use the school internet which is open until 4:00 p.m. Student activity at school that is too long (7:00 a.m. to 3: 20/4:00 p.m.) can cause two of the following facts: (a) students have their lunch with the snacks they buy at school which affect their nutritional status; and (b) students have limited time to do various physical activities, such as exercising in their neighborhood. Both nutritional status and physical activity are certainly related to physical fitness.

According to experts, the components of physical fitness can be divided into two groups: (a) health-related physical fitness; (b) skill-related physical fitness. Gusril[1] explained that:

(a) health-related physical fitness consists of: cardiorespiratory endurance, muscle strength, muscle endurance, flexibility, body composition; (b) skill-related physical fitness consists of speed, power, balance, agility, coordination, reaction speed.

Physical fitness as referred to in this thesis is health related physical fitness. "health-related physical fitness is a person's ability to perform physical tasks that require strength, endurance and flexibility". Referring to the aforementioned quote,[2] here are some components of health-related physical fitness:

Cardiovascular endurance. The Center for Physical Fitness and Recreation affirmed:

Cardiovascular endurance is the ability of the heart, lung and blood vessel systems to function optimally at rest and work in taking oxygen and distributing it to active tissues so that it can be used in the body's metabolic processes. This is necessary to support muscle work.

Based on several components of physical fitness as stated above, Bafirman and the Center for Physical Fitness and Recreation had similar idea that[7]: "Cardiovascular endurance is the most important component of physical fitness". It indicates that cardiovascular endurance is the most important component of physical fitness since by having high cardiovascular endurance, a person will be able to carry out various activities without experiencing excessive fatigue. Meanwhile, the measure or parameter of cardiovascular endurance is a person's ability to consume oxygen optimally *(*maximal oxygen consumption) which is better known as VO_2max. It is in line with a, "The more oxygen the body uses, the more work it can do"[3].

Considering cardiovascular endurance as the most important component of physical fitness, the measurement of cardiovascular endurance is more appropriate to measure a person's physical fitness. It suggested several forms of tests to measure cardiovascular endurance:

The most ideal measurement of cardiovascular conditions is carried out in a laboratory using sophisticated equipment so that definite data is obtained for each measured component. However, looking

at various limitations, such as regional conditions, expensive equipment and experts, the availability of these tools is still limited and not every province has them. These tools include treadmill, ergocycle, spirometer. Therefore, without reducing the meaning of cardiovascular measurements, various field tests can be used, including the 4.8 Km Brisk Walk test and the Multistage Fitness Test.

Research Methods

This study aims to reveal the relationship between nutritional status and physical activity either individually or in group with physical fitness of SMP RSBI students in Padang.

Based on the research objectives, this research belongs to the type of quantitative research that uses correlational analysis techniques.

This research was conducted in SMP RSBI in Padang which included: SMP Negeri 1 Padang and SMP Negeri 8 Padang.

To find out the nutritional status, the results of measurements of body weight and data on age are used as described in the following guideline:

Table 1. Nutritional Status Instrument Guideline

No	Indicators	To be measured	Measuring instrument
1	Weight and Age	a. Weight (kg) a. B. Age (year-month)	Body scales date/ month/year of birth

Data on body weight based on the results of weighing and data on age obtained by calculating the date/month/year of birth of each sample (experimental students) were then converted to the WHO-NCHS Reference Standard/Weight Table (Kg) according to Boys Age 2-18 Years (Appendix 2). Here, the median by age of each sample (students trying) is obtained. The calculation was then done to get the median percent, by: weight divided by median x 100%.

The median percentage obtained was converted to Table Classification Table of Society Nutritional Status, Directorate of Community Nutrition, Department of Health of the Republic of Indonesia,.

To measure physical activity, a questionnaire was used based on a Likert scale. The instrument was structured based on several indicators described from the following sub-variables: (a) activeness in using the physical in Physical Education, Sports and Health (Penjasorkes) practices in schools; (b) activeness in using the physical in extracurricular activities at school; (c) activeness in using the physical while playing; and (d) activeness in using the physical, seen from the penchant for exercising in the neighborhood.

"One important way to determine cardiovascular fitness is to measure the amount of VO_2max. Therefore, VO_2max or aerobic capacity is not only a metabolic parameter but also a reliable measure of physical fitness".[8]

Considering VO_2max as a reliable measure for determining physical fitness, physical fitness measurements were carried out using the Multistage Fitness Test (MFT). This test aimed to measure the maximum oxygen uptake or VO_2max.

By using MFT, the maximum oxygen uptake (VO_2max) was determined based on the level and shuttle that the students could try.

The research data analysis technique consisted of: (1) analysis requirement testing; and (2) hypothesis testing using correlation and regression analysis..

Research Results and Discussion

The following session presents a description of the data, which is the variables to explain about data analysis to test the proposed hypothesis on Physical Fitness of SMP RSBI students in Padang. These variables are variable X_1 nutritional status and variable X_2 physical activity, and then variable Y physical fitness.

Table 2 Frequency distribution of nutritional status variable

No	Categories	F	%
1	Overnutrition	6	8.96
2	Good nutrition	40	59.70
3	Moderate nutrition	11	16.42
4	Undernutrition	5	7.46
5	Bad nutrition	5	7.46
Total		67	100.00

Based on the table above, it is known that 6 of the 67 sample students belonged to the > 120% interval class or in the category ***"over nutrition"*** with a percentage of 8.96%. Furthermore, it is also known that 40 students were included in the 80% - 120% interval class or in the category of ***"good nutrition"*** with a percentage of 59.70%; 11 students were included in the 70% - 79,9% interval or in the category of ***"moderate nutrition"*** with a percentage of 16.42%; 5 students were included in the 60% - 69,9% interval or in a category of ***"undernutrition"*** with a percentage of 7.46%; and 5 students were included in the < 60 % interval or in the category of ***"bad nutrition"*** with a percentage of 7.46%.

Table 3 Description of Physical Activity Data of SMP RSBI Students in Padang

No	Categories	F	%
1	Very Good	3	4.48
2	Good	23	34.33
3	Fair	19	28.36
4	Poor	18	26.87
5	Very Bad	4	5.97
Total		67	100.00

Based on the table above, 3 out of 67 students scored> 278 or were included in a ***"very good"*** category with a percentage of 4.48%. Moreover, 23 students scored 257 - 278 or were included in a ***"good"*** category with a percentage of 34.33%; 19 students scored 234 - 256 or were included in a ***"fair"*** category with a percentage of 28.36%; 18 students scored 210 - 233 or were included in a ***"poor"*** category with a percentage of 26.87%; and 4 students scored < 210 or were included in a ***"very bad"*** category with a percentage of 5.97%.

Table 4 Description of Physical Activity Data of SMP RSBI Students in Padang

No	Category	f	%
1	Very Good	5	7.46
2	Good	19	28.36
3	Fair	17	25.37
4	Poor	21	31.34
5	Very Bad	5	7.46
Total		67	100.00

Based on the table above, it is known that 3 out of 67 students scored > 278 or were included in a ***"very good"*** category with a percentage of 4.48%. Additionally, 23 students scored 257 - 278 or were included in a ***"good"*** category with a percentage of 34.33%; 19 students scored 234 - 256 or were included in a ***"fair"*** category with a percentage of 28.36%, 18 students scored 210 - 233 or were included in a ***"poor"*** category with a percentage of 26.87%, and 4 students scored < 210 pr were included in a ***"very bad"*** category with a percentage of 5.97%.

Requirement Analysis Testing

Research claimed that requirements that must be met for the use of the Compression technique are carried out with the t test, which functions as (a) data is sourced from randomly assigned data, (b) data comes from populations that are normally distributed, (c) population group has a homogeneous variance, (d) there is no significant correlation between independent variables, and (e) the relationship line between independent variable and dependent variable forms a linear line.

a.Data Normality Test

Data normality test analysis was performed using the Lilliefors test. The results of the analysis of the normality test for each variable are presented in the form of the table below, and the complete calculation can be seen in the appendix.

Table 5 Data normality test using Lilliefors test

No	Variables	Lo	Lt	Note
1	Nutritional status	0.0958	0.1082	Normal
2	Physical Activity	0.0958	0.1082	Normal
3	Physical Fitness	0.0843	0.1082	Normal

The above table shows that Lo values for nutritional status, physical activity and physical fitness variables of SMP RSBI students in Padang are smaller than Lt, or $L_{count} < L_{table}$. Thus, it can be concluded that data are normally distributed.

1. Homogeneity Test

Homogeneity test can be done by using the formula

1. Nutritional Status

$$F = \frac{535,82}{421,79} = 1,27$$

2. Physical activity

$$F = \frac{562,78}{343,50} = 1,64$$

3. Physical Fitness

$$F = \frac{37,28}{34,28} = 1,09$$

By using degrees of freedom (n1-1),(n2-1) and a significance level of 0.05 in the distribution table F, the limit of significance (F_{table}) is 1.78. Since F_{count} is smaller than F_{table}, it can be concluded that the two sample groups come from a homogeneous population.

2. Linearity Test

By using regression formula **Y = b0 + b1X1 + b2X2**, the following equation is obtained:

Y = 1.72 + 0.05X1 + 0.11X2

After getting the regression equation above, the calculation was continued by using a formula to test the meaning of multiple regression. The results are as follows:

JK (reg) = b1 ∑X1Y + b2 ∑X2Y
= (0.05) (2,940.6) + (0.11) (4,452.41)
= 147.03 + 489.77
= 636.80

JK (S) = ∑Y^2 - JK (reg)
= 2,316.71- 636.80
= 1,679.91

F_h = JK (reg) / k
JK (S) / (n-k-1)
= 636.80) / 2
1,679.91 / (67-2-1)
= 318.4
1,679.91 / 64
= 318.4
26.25
= **12.13**

Ft = $dk_{numerator}$ = 2

$Dk_{denominator}$ = n-k-1 = 67-2-1 = 64

Ft = 3.14

$F_h > F_t$ = 12.13 > 3.14

Conclusion= The multiple linear regression of X1 and X2 toward Y is significant (real).

Conclusion

Analysis of the correlation between nutritional status and physical activity on physical fitness of SMP RSBI students in Padang (X1X2 – Y). FCount 15 and F table 3.14.

Based on the results of the above calculations, it is known that the contribution of X1 and X2 to Y is 0.29 or 29.00%, while the rest is (100% - 29%) = 71.00%. It demonstrates that nutritional status and physical activity as independent variables can simultaneously contribute 29.00% to the dependent variable, namely physical fitness of SMP RSBI students. Additionally, the rest is explained by other variables outside the independent variables caused by other variables.

Source of Fund : Self

Ethical Approval : Universitas Negeri Padang Tahun 2019

Conflict of Interest : Nil

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Comparison the Serum Level of Paraoxonase Activity in Frequent Relapse and Infrequent Relapse Patients with Nephrotic Syndrome

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Abstract

Nephrotic syndrome (NS) is distinguished by heavy proteinuria (urine protein:creatinine ratio ≥2000 mg/g or ≥300 mg/dL, or 3+ protein on urine dipstick), hypoalbuminemia (≤2.5 g/dL), and edema. Leakage of massive amounts of serum proteins into the urine leads to a hypercoagulable state, a higher rate of infectious disease, and the dysregulation of fluid balance. Idiopathic nephrotic syndrome (INS)

is one of the most common renal problems in children encountered in day-to-day nephrology practices. The present study was aimed to evaluate the oxidant/ antioxidant status by measuring paraoxonase (PON) activities as well as total antioxidant capacity in steroid sensitive nephrotic syndrome (frequent and infrequent relapse) and compares it with healthy control children . Paraoxonase activity by spectrophotometer in three groups consisted of age and sex matched (30) patients with Frequent relapse nephrotic syndrome FRNS , (30) patients with infrequent relapse nephrotic syndrome (IFRNS) and (30) healthy control children. The serum level of paraoxonase activity in infrequent relapse group was 101.61±43.0 IU which far less than frequent relapse group (160.44±44.22 IU) or controls (228.35±32.35 IU) with highly significant differences. Of note, the difference between control group and frequent relapse group was highly significant.

Key words: *INS, Frequent relapse , infrequent relapse, PON*

Introduction

Nephrotic syndrome is characterized by the triad of proteinuria, hypoalbuminaemia, and edema[1]. NS may be classified according to aetiology (primary or secondary), age of onset (congenital, infantile, acquired or late onset NS), or histopathology (e.g. minimal change disease, mesangial hypercellularity, focal segmental glomerulosclerosis (FSGS), membranous nephropathy). However the most useful classification for management purposes is to define the disease according to its response to steroids (steroid sensitive or resistant with steroid sensitive disease being further classified into frequent relapses and steroid dependent NS) as patients who are steroid sensitive have an excellent prognosis with preservation of kidney function whilst those that are steroid resistant are more prone to complications with a high risk of having deterioration of kidney function and progression to end-stage kidney disease needing renal replacement therapy[2].

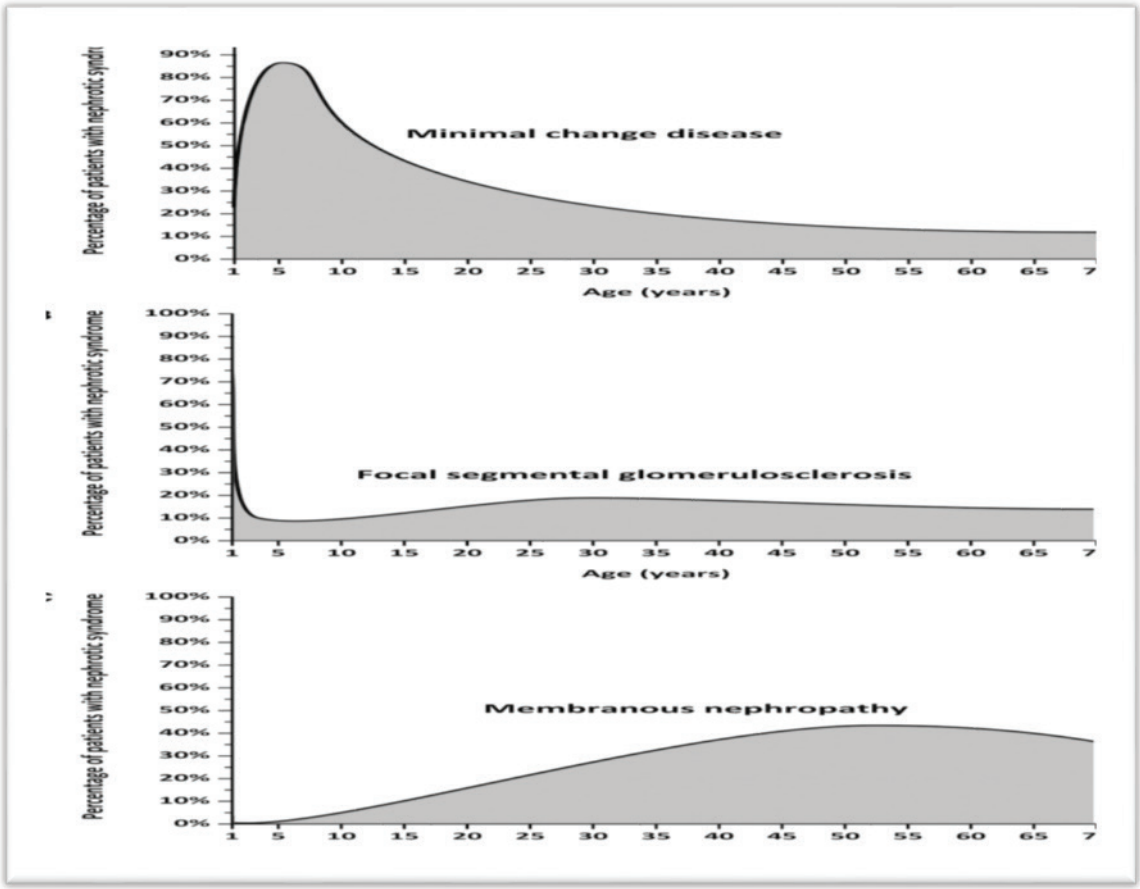

Figure 1 : Etiology of nephrotic syndrome according to age from 1 year onwards. Data are approximated from (Nachman,et,al.2008) and (Cameron,et,al. 1987).

Children with INS can be classified into well-defined categories based on the responses to the standard prednisolone therapy[3]. Prednisolone was used for children with nephrotic syndrome in 1956, four children (age 2-8 years) all of whom responded to prednisolone 60 mg daily[4]. Subsequently a dosage of 60 mg/m2/day is considered and accepted as standard treatment. There have been several studies that have looked at the effect of duration of prednisolone in respect of long term outcomes. The dose of prednisolone required to achieve remission has not been studied. Today most children with initial onset of NS of age greater than 1 year will be treated with corticosteroids without doing any initial biopsy[5]. Steroid sensitive nephrotic syndrome (SSNS) is considered a relatively benign condition, as progression to end stage renal failure is extremely rare and approximately 80% of child will enter long term remission[6,7]. About 80 % of children with steroid-sensitive nephrotic syndrome experience one or more relapses[8]. Those with frequently relapsing nephrotic syndrome are prone to suffer steroid-induced side effects such as obesity growth impairment, hypertension, diabetes mellitus, osteoporosis, and adrenal insufficiency. Many cases of steroid-resistant nephrotic syndrome, where steroids are ineffective, progress to renal failure[9]. Relapse is defined as recurrence of proteinuria with or without edema. It occurs in two thirds of cases of steroid sensitive nephortic syndrome . Opinions differ on the timing of treatment for relapses because upto 25% of the relapses can go for spontaneous remission[10]. Classically, a child presents with a history of progressive oedema, initially periorbital and noticeable in the morning. There can be an antecedent infection, typically of the upper respiratory tract. Urine output is described as frothy or foamy. Abdominal pain is relatively common and, if accompanied by fever, could signify spontaneous bacterial peritonitis. Headache with accompanying neurological signs or irritability should raise the suspicion for cerebral venous sinus thrombosis[1].

Since MCNS is by far the most common cause of nephrotic syndromes in childhood, initial efforts are devoted to the detection of features that are similar to MCNS. A course of corticosteroid treatment without a renal biopsy is indicated for children without atypical features, since responsiveness to steroids is a better indicator than kidney histology of longterm prognosis for renal function. Renal biopsy is done when there is poor or no response of the initial episode after 4 to 6 weeks of standard treatment (defined as steroid-resistant disease), and the child should be medically stable. The biopsy is essential to distinguish the nature and severity of the glomerular process, which may be primary or secondary[11]. Most children do not get a kidney biopsy at presentation. Historical studies have demonstrated that the most common pathological findings in childhood NS are either classified as minimal change and termed minimal change disease or FSGS. In minimal change, the glomeruli appear normal under light microscopy, with evidence of podocyte effacement by electron microscopy[12]. Characteristic histology in FSGS is segmental sclerosis of affected glomeruli, with the segment often adherent to Bowman's capsule by synechiae[1].

Treatment For induction of remission in nephrotic syndrome, corticosteroids (prednisone), cyclophosphoamide, and cyclosporine are used to decrease edema, diuretics are used. Inhibitors of angiotensin conversion enzyme (ACE) and blockers of angiotensin receptors may decrease proteinuria[12]. The sort of renal pathology that causes nephrotic syndrome should dictate treatment. Minimal-change illness has an outstanding reaction to corticosteroids, while only 20% of patients react well to corticosteroids in focal glomerulosclerosis. Renal biopsy is very helpful in distinguishing disease with minimal chang and its variations such as IgM nephropathy and C1q nephropathy. There are very few randomized studies to guid therapy[13].

Prednisolone is the drug of choice for the treatment of initial episode to induce remission. Other steroids such as dexamethasone, betamethasone and hydrocortisone are not recommended. Deflazocort, a steroid analogue, has been shown to have more stable remission and fewer side effects, but has not been widely used[14]. Children who have 3 or less relapses in a year usually respond promptly to prednisolone. It is administered at adose of 2 mg/kg/day, in single or two divided doses, until urine protein is trace or nil for three consecutive days. Subsequently it is continued at a dose of 1.5 mg/kg, single morning dose, on alternate days for 4 weeks and then discontinued Prolongation of therapy is not necessary in such patients[15]. Children with frequent relapses or steroid dependence should be managed in consultation with a pediatric nephrologist. Renal biopsy in these cases is not usually necessary. Prednisolone is administered a dose of 2 mg/kg/day until remission, subsequently it is continued at a dose of 1.5 mg/kg on alternate days for 4 weeks. Then prednisolone is tapered gradually to maintain the child in remission on alternate day dose of 0.5 mg/kg or lower. Alternate day prednisolone may be administered for 9-18 months. A close monitoring of height, weight and blood pressure and evaluation for features of steroid toxicity is essential[16]. Paraoxonase are a family of enzymes originally known for their ability to hydrolyze exogenous toxic organophosphate compounds such as the insecticide paraoxon. Studies have shown that the enzyme exists as Paraoxonase 1 (PON1), Paraoxonase 2 (PON2), and Paraoxonase 3 (PON3), encoded by 3 separate genes on the same chromosome 7 in humans. PON1 has been widely studied in human medicine with excellent reviews produced by different research groups[17]. Initially the interest on this enzyme arose from the toxicological point of view, by its protective role from poisoning by organophosphate derivates . But more recently research has been focused on other clinical aspects such as protective role in vascular diseases involving mainly three situation : (a) Oxidative stress ,since PON1 protect against oxidation ;(B) inflammation, being considered PON1 as negative acute phase protein[18] and (C) liver diseases, because PON1 is synthesized in this organ[19] .

Materials and Methods

Study subjects

This case control study was carried out on 90 children age ranged from 2 to 13 years, consist of 30 patients diagnosed as Frequent relapse INS , 30 with Infrequent relapse INS and 30 healthy control who matched for their age and gender with the cases. Samples collected between February 2020 and August 2020.

Exclusion Criteria: patients with

- Steroid resistant cases
- Secondary and congenital NS.
- Any nephrotic patient with heart disease.
- All patient with liver disease.

Sample collection:

5 ml of blood were taken from each patient, put in gel tube were centrifuged at 2000 rpm for 5-10 minutes. The basal levels of serum PON, Urea, Creatinine, Total cholestrol, Triglycerides, High density lipoprotein(HDL), Low density lipoprotein (LDL), total serum protein, Albumin, were measured.

Methods: The methods used to measure the markers was deferent ways according to the substance that measured the routine test measured by full automated machine but paraoxonase enzyme activity measured spectrophotomateric method via P-nitrophenyl acetate from agros organics as substrate.

Statistical analysis

The data that obtain could be analyzed using SPSS version Analysis of variance will be used to compare means between the different groups and person's correlation will be used to find out any correlation between serum concentrations of enzyme in Frequent relapse INS , Infrequent relapse INS and healthy control.

Result

Demographic Characteristics of the Study Population

Mean age of patients with frequent relapse was 6.17±3.14 years which was significantly lower than that of patients with infrequent relapse (8.3±3.3 years) or controls (8.47±2.93). In contrast, gender distribution was compatible between the different groups with no significant differences.

Table 1: Demographic characteristics of the study population

Variables	Frequent relapse NS (n=30)	Infrequent relapse NS (n==30)	Controls (n=30)	p- value
Age, years mean±SD Range	 6.17±3.14 1-13	 8.3±3.3 3-13	 8.47±2.93 2-13	0.013
Gender Males Females	 14(46.67%) 16(55.33%)	 16(55.33%) 14(46.67%)	 15(50%) 15(50%)	0.875

Paraclinical Characteristics of the Study Population

Blood urea level was much higher in frequent relapse group (7.17±2.51 mmol/L) than either infrequent relapse (4.5±1.36 mmol/L) or controls (3.5±0.5 mmol/L) with highly significant differences, while the reverse pattern was found in TSP in which the control group showed significantly higher level (64.0±4.15 mmol/L) than frequent (34.43±12.35 mmol/L) or infrequent relapse groups (38.67±11.19 mmol/L).

For lipid profile, mean serum level of triglycerides, cholesterol, LDL in frequent relapse group was (2.6±1.0 mmol/L, 8.43±4.0 mmol/L and 1.77±0.28 mmol/L, respectively) which was significantly higher than that in infrequent relapse group (2.17±0.54 mmol/L, 6.67±0.41 mmol/L and 1.35±0.25 mmol/L, respectively) or in controls (0.75±0.31 mmol/L, 2.16±0.44 mmol/L and 1.14±0.39 mmol/L, respectively). In contrast, control group showed significantly higher HDL (0.87±0.27 mmol/L) than frequent relapse (0.67±0.41 mmol/L)

or infrequent relapse (0.42±0.3 mmol/L). Interestingly, the disease duration was longer in infrequent relapse than frequent relapse group (43.6±17.68 months versus 29.87±16.33 months) with a significant difference (Table 2).

Table 2: Paraclinical characteristics of the study population

Variables	**Frequent relapse patients (n=30)**	**Infrequent relapse patients (n==30)**	**Controls (n=30)**	**p- value**
Urea, mmol/L	7.17±2.51a	4.5±1.36b	3.5±0.5b	<0.001
Creatinine	72.04±15.28a	45.99±9.07a	62.33±10.35a	0.192
Total serum Protein, mmol/L	34.43±12.35a	38.67±11.19a	64.0±4.15b	0.002
Albumin, mmol/L	34.27±11.58a	38.8±9.0b	36.9±2.52b	0.128
Triglycerides, mmol/L	2.6±1.0a	2.17±0.54b	0.75±0.31c	<0.001
Cholesterol, mmol/L	8.43±4.0a	6.67±0.41b	2.16±0.44c	<0.001
HDL, mmol/L	0.67±0.41a	0.42±0.3b	0.87±0.27c	<0.001
LDL, mmol/L	1.77±0.28a	1.35±0.25b	1.14±0.39c	<0.001
Disease duration, months	29.87±16.33	43.6±17.68	----------	0.003

Diagnostics value PON-1 Activity

The AUC for PON-1 activity in the context of discrimination between frequent and infrequent relapse NS was 0.836, 95%CI=0.738-0.934, p<0.001. The sensitivity and specificity of PON-1 activity= 135.75 IU were 0.70 for each (Figure 2).

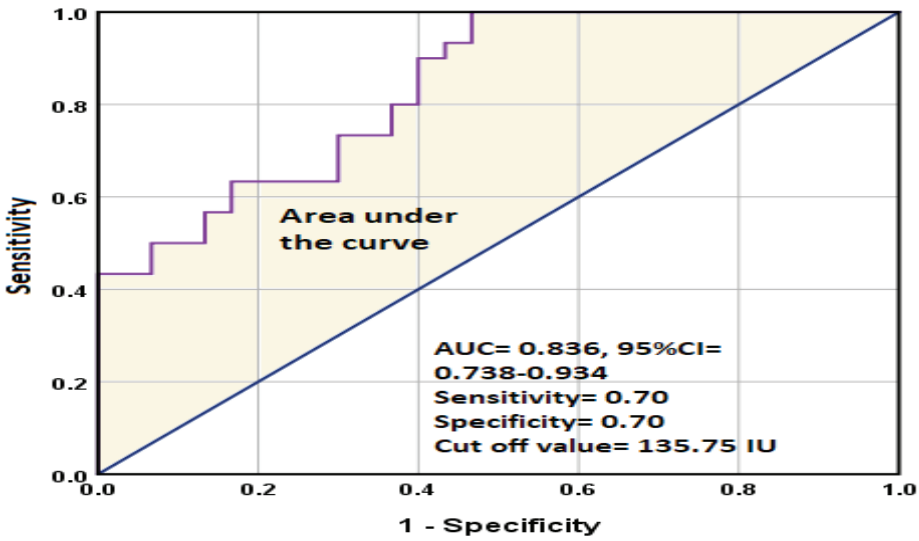

Figure 2: Receiver operating characteristic curve for PON activity in the context of discrimination between frequent and infrequent relapse NS (P<0.001).

In contrast, the mean serum activity of PON-1infrequent relapse group was 101.61±43.0 IU which far less than frequent relapse group (160.44±44.22 IU) or controls (228.35±32.35 IU) with highly significant differences. Of note, the difference between control group and frequent relapse group was highly significant (Figure 3).

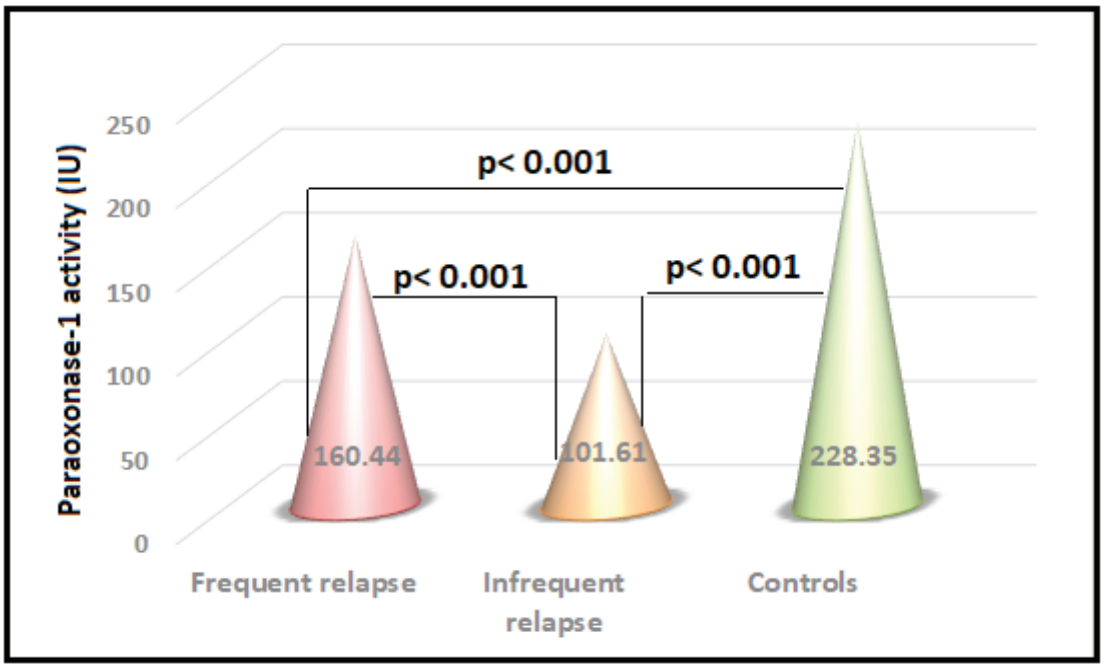

Figure 3: Mean PON-1 activity In NS patients and controls

Discussion

Accordance to the present result, age of patients with frequent relapse was significantly lower than that in patients with infrequent relapse or controls. This result be accordance with study includes The association between young age and relapses has previously been proposed by Takeda et al.[20] who reported that the sustained cumulative remission rate is significantly lower when patients present with NS at 7 years of age or younger.

This study includes these variations in the frequency of relapses have been related to many factors. Younger

age at the onset of the disease was reported to be correlated with the frequency of relapse, with more frequent relapses in children younger than 4 years. The authors found that children in the age group 1–5 years had significantly more relapses in comparison to other age groups[21].

The present study revealed that gender distribution was compatible between the different groups with no significant differences. These results deal with other studies[22]. The children with idiopathic NS the blood may be elevated (blood urea being disproportionately higher than severe creatinine), due at least in part to hypovolemia[10]. Both increase hepatic lipoprotein synthesis and reduce lipoprotein catabolism leads to increase level of serum cholesterol in children with nephrotic syndrome this agreed with Kronenberg et,al.[23] . In children with nephrotic syndrome reduce activity of lipoprotein lipase (LPL) and LPL associated with increase glomerular basement membrane permeability resulting hypertriglycerides, this result agreed with Wheeler et,al.[24]

In nephrotic syndrome, enhanced expression of the proportion of convertase subtilisin kexin type 9(PCSK9) results in enhanced LDL receptor degradation and decreased LDL intake by the liver, this result agreed with Nakatani et,al.[25]

The present study showed the mean serum activity of PON-1infrequent relapse group was 101.61±43.0 IU which far less than frequent relapse group (160.44±44.22 IU) or controls (228.35±32.35 IU) with highly significant differences. Of note, the difference between control group and frequent relapse group was highly significant.

Paraoxonase is an esterase that hydrolyzes organophosphate compounds. The enzyme is associated with HDL and could protect LDL against peroxidation, which suggests a possible involvement of paraoxonase in the antiatherogenic properties of HDL. Paraoxonase activity has been shown to be low in patients with myocardial infarction, diabetes mellitus, or familial hypercholesterobemia. Because cardiovascular disease is the main cause of death in renal diseases as nephrotic syndrome agreed with Dantoine e,al.[26]

Conclusion:

The significant decreasebin human paraoxonase enzyme activity level in patients with nephrotic syndrome suggest that PON-1 is considers as a one of parameters for Diagnosis of NS, PON-1 enzyme is easily to measure and adequate in its results.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional ethical committee.

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Effect of Cyanide on the Liver Histological and Biochemical Status

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Abstract

The biochemical and histological response of the rats following dosed on the potassium cyanide was examined in this study, about 36 rats were randomly divided into sex groups ; G0 which considers the control group, G1 oral dosed with potassium cyanide concentration 500ppm (500U/L), G2 dosed with concentration 1000ppm (1g/L), waiting for a long time 21 days before anatomy the animal. G3, G4, and G5 dosed with 2000ppm, 2500ppm, and 3000ppm, respectively to study the effect of high dose concentration on the liver histology, liver enzyme and lipid profile and determined LD_{50} (lethal dose), and LC_{50} (lethal concentration). After anatomy, the animal experiment to study the liver histological and determine the effect of potassium cyanide concentration on the liver enzymes such as HDL, triglyceride, and cholesterol, compared the result with the control group and previous studies . The study indicated the presence of a significant increase ($P \leq 0.05$) in ALP in the G1 , a non-significant increase ($P \geq 0.05$) in the G2 , the G3 and G4 groups suffered from a significant increase ($P \leq 0.05$) while the G5 group suffered from a non-significant decrease ($P \geq 0.05$) in comparison with G0. The present study also showed that there was a significant increase ($P \leq 0.05$) in GPT in the G1 , G2 , G3 , G4 in comparison with G0. The study also indicated the presence of a significant increase ($P \leq 0.05$) in GOT In the G1 , G2 and G3 compared to the G0.

Keywords : *cyanide , liver enzymes , histological and biochemical status, lipid*

Introduction

Cyanide is a chemical material that is popularly regarded as a highly toxic agent. Cyanide can be formed by some plants, bacteria, fungi, and algae [(1)]. CN occurs in several forms, including gaseous cyanide hydrogen (HCN), water-soluble potassium (K), and sodium (Na) salts, poorly water-soluble mercury (Hg), copper (Cu), gold (Au), and silver (Ag) CN salts [(2)]. Cyanide enters the air, water, and soil [(3)]. In the lungs, gastrointestinal tract, and skin, cyanide is absorbed [(4)]. Symptoms may occur within seconds of inhalation of HCN, within minutes of ingestion of cyanide salts, and may delay up to 12 hours following ingestion of cyanogenic glycosides, nitriles, or thiocyanates. Absorption time following ingestion depends on the pH of the gut and the solubility of the compound containing cyanide. Cyanide ready for use [(5)] .

Cyanide is one of nature's most potent toxins which can affect all species including cattle. Provided their feeding style, ruminants can be easily affected by this toxin [(6)]. Understanding of toxicity mechanisms is both practical and theoretical, providing a rational basis for evaluating descriptive toxicity data and estimating the likelihood that toxicity will occur, creating fewer dangerous drugs and synthetic chemicals, and creating more highly harmful pesticides for their target species [(7)]. Cyanide is a chemical asphyxiate that prevents the

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aerobic use of oxygen by binding to cellular cytochrome oxidase. The bulk of unbound cyanide (80 percent) is detoxified by thiocyanate metabolism, a substance that is much less harmful and is excreted in urine [8].

The liver is the largest gland in the body, and its concerned with the production and secretion of bile and many metabolic functions crucial to normal Homeostasis [9]. The liver is of great interest to the toxicologist, it is invariably examined and removed at postmortem examination and then sampled for histological sectioning [10]. The liver is one of the largest digestive organs and is located in a very strategic position. All absorbed nutrients and liquids from the intestines enter the liver through the hepatic portal vein [11]. It comprises two main lobes of almost equal size—right and left—separated by a falciform ligament and a round ligament (ligamentum teres) [12]. Many circulating plasma proteins are produced and secreted by the liver. The liver plays an important role in the uptake, storage, and distribution of both nutrients and vitamins from the bloodstream. The liver has a dual blood supply: a venous supply via the hepatic portal vein and an arterial supply via the hepatic artery [13]. Histologically the liver is composed of lobules, each lobule comprises a central vein (a tributary of the hepatic veins) with the portal tracts situated at the periphery [9]. About 80% of liver tissue in the adult is parenchyma consisting of hepatocytes arranged as a labyrinth of cellular plates. The remaining 20% is the stroma, a delicate supportive framework of connective tissue that forms the outer Glisson capsule [12] .

Elevated levels of one or more liver enzymes are commonly found in asymptomatic patients. Such findings may be important, because minimal elevation may be the only manifestation of the significant hepatobiliary disease [14] .

Liver function tests (LFTs)—these should include tests for liver secretory capacity (bilirubin, alkaline phosphatase, and gamma-glutamyl transferase (GGT)); synthetic capacity (albumin) and inflammation (aspartate aminotransferase (AST)) and alanine aminotransferase (ALT) [9].

Material and Method

Thirty-six White rats used in the experiment divided into sex groups. This animal set in a plastic cage and kept in a temperature room between 21-24 C^o.

This animal let for long 14 days to become acclimatized to naturalize, the animals were orally dosed with potassium cyanide at different doses (table 1) to determine the effect solution on the liver histological and the liver enzyme. Measurement included : ALP (Alkaline Phosphatase), GPT (glutamic-pyruvic transaminase), GOT (glutamate oxaloacetate transaminase) , Cholesterol, Triglyceride and
HDL (High density Lipoproteins) .

Table 1. : Showing the dose for groups)

ON	Group	Number	Concentration	Death
1	G1	6	Zero	Zero
2	G2	6	500ppm	Zero
3	G3	6	1000ppm	Zero
4	G4	6	2000ppm	3
5	G5	6	2500ppm	5
6	G6	6	3000ppm	6

Statistical Analysis

Data were subjected to analysis. The value was reported as Mean+ SE, while ANOVA and LSD were used to test for difference science (SPSS) version 16 (P≤ 0.05) was accepted as significant.

Results

The statistical analysis of the study indicated the presence of a significant increase (P≤ 0.05) in ALP in the G1 as well as a non-significant increase (P≥ 0.05) In the G2 group for a length period of 21 days' comparison with the G0 group. The G3 and G4 groups suffered from a significant increase (P≤ 0.05) while the G5 group suffered from a non-significant decrease (P≥ 0.05) in comparison with G0.

The Statistical analysis also showed that there was a significant increase (P ≤ 0.05) in GPT in the G1 and G2 for a length period of 21 days' in comparison with G0. The statistical analysis indicates a significant increase (P≤ 0.05) In the G3, G4 and G5 comparison with G0.

The statistical analysis indicated the presence of a significant increase (P ≤ 0.05) in GOT In the G1 and G2 for a length period of 21 days' compared to the G0. It was also found that there is a significant increase (P≤ 0.05) In the G3 and G5 groups and indicated a non-significant increase (P≥ 0.05) In the G4 group comparison with G0 (as shown in table 2) .

Table 2. : showing the effect of potassium cyanide on the groups compared with the control group (Mean + SE).

Group	ALP	GPT	GOT	Dose
G1	497.50±33.76	58.06±2.71	134.67±1.64	500 PPM
G2	131.42±18.33**	75.13±2.05	137.63±5.81	1000 PPM
G3	275.00±2.24	100.52±0.23	346.50±101.12	2000 PPM
G4	372.90±42.88	59.37±6.86	112.20±2043**	2500 PPM
G5	38.13±1.73*	50.45±3.68	141.46±22.84	3000 PPM
G0	125.49±18.03	38.83±1.35	35.17±1.35	ZERO
Lsd	68.34	9.73	79.96	

* non-significant decrease compared to G0 .

** non-significant increase compared to G0 .

The Statistical analysis showed there was a non-significant decrease (P≥ 0.05) in cholesterol In the G1 group, while the G2 suffered from a non-significant increase (P≥ 0.05) for a length period of 21 days' in comparison with the G0. The statistical analysis showed a significant increase (P≤ 0.05) in triglyceride in the G4 and G5 groups. the G3 suffered from anon significate increase (P≥ 0.05) in comparison with G0. The statistical results of the study showed a non-significant decrease (P≥ 0.05) In the G1 and G2, for a length period of 21 days compared to the G0. It also indicated a non-significant decrease (P≥ 0.05) In the G3, G4, and G5 groups, in comparison with G0.

The statistical analysis of the current study showed there was a non-significant increase (P≥ 0.05)in HDL in the G1 and a non-significant decrease (P ≥ 0.05). In the G2 for a length period of 21 days compared with G0 group. The results indicated a non-significant increase (P≥ 0.05) In the G3 and G5 groups, as well as a non-significant increase (P≥ 0.05) In the G4 group comparison with G0, (as shown in table 3) .

The results of the microscopic examination showed the hepatic tissue of the G1 congestion blood in the central vein, discrete apoptotic hepatocyte cells, and

reduction in the glycoprotein granules. Comparison with the control group. (figure 1-A).

The results of the microscopic examination also showed the hepatic tissue of the G2 dosed in solution KCN showing look – like normal structure but with the widening of hepatic sinusoid and reduction in the size of the hepatocyte cells, Comparison with the control group. (figure 1-B).

Table 3. : Showing the effect of potassium cyanide on the groups compared with the control group (Mean + SE).

Group	Cholesterol	Triglyceride	HDL	Dose
G1	45.00±1.34	70.50±7.38**	34.67±0.30*	500 PPM
G2	67.84±5.56	47.00±4.81*	53.56±5.24**	1000 PPM
G3	73.04±18.69	90.50±4.25**	43.17±1.42	2000 PPM
G4	101.58±14.12	196.60±31.67	95.99±18.60**	2500 PPM
G5	89.44±8.19	94.19±8.56**	61.57±5.64	3000 PPM
G0	142.67±13.51	67.00±3.58	41.03±2.44	ZERO
Lsd	26.30	38.95	18.01	

* Non-significant decrease compared to G0.

** Non-significant increase compared to G0 .

The results of the microscopic examination showed the hepatic tissue of the G3 treated by KCN showing congestion blood in the central vein Section congestion blood in the central vein, certain degeneration, discrete apoptotic hepatocyte cells with depletion of glycoprotein granules comparison with the control group. (figure 2-A).

The results of the microscopic examination also showed the hepatic tissue of the G4 congestion blood in the central vein, rare apoptotic hepatic cells and dilated congested sinusoid, comparison with the control group. (figure 2-B).

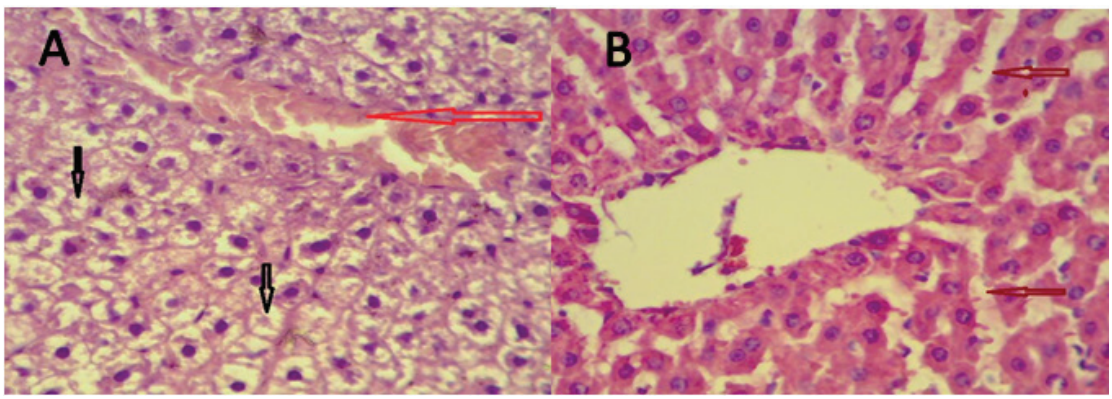

Figure (1) : A :rat liver treated with potassium cyanide solution at a concentration of 500 PPM, showing congestion (red arrow) and desecrate apoptosis hepatocytes and reduction (black arrow) in the glycoprotein granules (H & E)(X40). B : rat liver treated with potassium cyanide solution at a concentration of 1000 PPM, showing the widening (red arrow) of hepatic sinusoid and reduction in the size of the hepatocyte cells . (H&E)(X40).

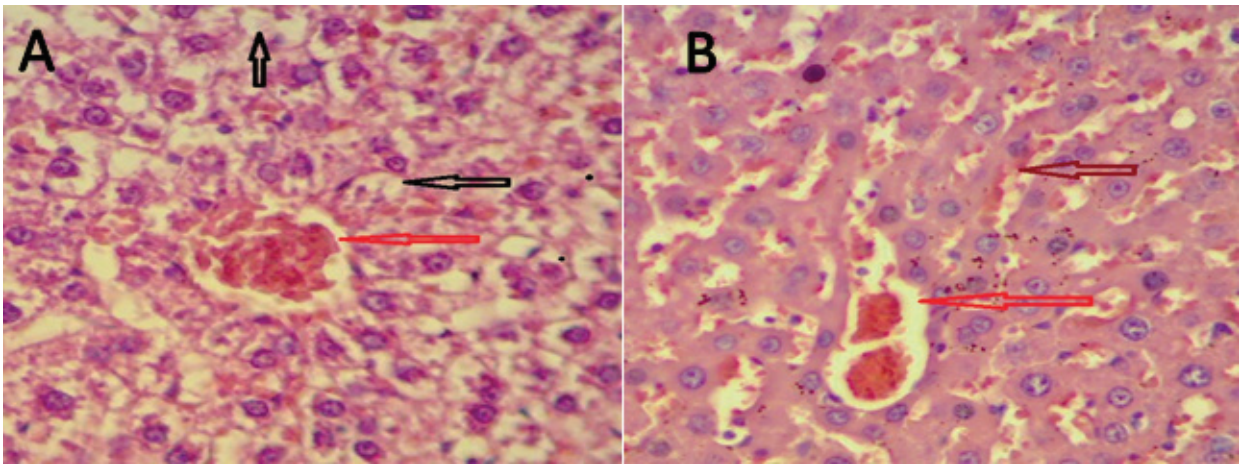

Figure (2) : A rat liver treated with potassium cyanide solution at a concentration of 2000 PPM, showing congestion (red arrow) and desecrate apoptosis hepatocytes and reduction (black arrow) in the glycoprotein granules. . (H & E)(X40). B : rat liver treated with potassium cyanide solution at a concentration of 3000PPM, showing congestion (red arrow) and widening of hepatic sinusoid (brown arrow). (H & E)(X40).

Discussion

Assessment of potassium cyanide toxicity concerning to rats, which is key to developing a toxicity assessment. Mortality of rats due to exposure to potassium cyanide (KCN) depended primarily on its susceptibility to the toxicant's concentration and duration, a strong idea of comparative le revealed the acute, chronic, sub-chronic toxicity test by earlier workers.

The liver histology shifts in coagulation, apoptotic hepatocyte cells, reduction of glycoprotein granules, depletion of glycoprotein granules, some degeneration and widening of hepatic sinusoid may be abbreviated for different dosages. This result agrees with more articles like [15] summarizes the impact of cyanide found in the article with the highest vacuum and hepatic degeneration

Showing in the Biostatistician for liver enzyme study in general found a significant increase for the liver enzyme parameters (GOT, GPT, ALP) except tow dose in alkaline phosphates. Most articles agree with the results, shown in article [16] a significant increase in the liver enzyme (ALT, AST, ALP) since an increase in these enzyme activity signifies hepatocellular injury and thus acts as biomarkers for liver damage. showing a non-significant change in the current study ($p > 0.05$) in all the dosage indicating that the cyanide treated does not affect cholesterol. When compared to the result study and data article [17], its discrepancy shows a significant increase in cholesterol in the worker's cassava compared to control in the article. Showing non-significant changes in the current study ($p > 0.05$) in all the dosages suggesting that triglyceride does not influence the cyanide handled. Relative to the outcome study and the data article [17], its disagreement shows a significant increase in triglyceride in the worker's cassava compared to control in the essay. showing the current non-significant change in the study except for G4 and G5 indicated a significant increase in high dosage, the difference between the current of the study shows no change in HDL for small dosages [17] . The impact of the article on the exposure of hydrogen cyanide to worker cassava is of interest since important data affect HCN on HDL. In the worker's cassava, show a substantial increase.

Ethical Clearance: Taken from University of Karbala ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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The Antioxidant Effect of Noni Fruits Extract on HDL and LDL Level of Wistar Rat Induced by High Fat Diet

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Abstract

Dyslipidemia is an important risk factor for myocardial infarction, cardiovascular diseases, and Non-alcoholic Fatty Liver (NAFLD) disorders. Inflammatory conditions, oxidative stress, and insulin resistance, as well as several risk factors for cardiovascular disease, are also risk factors for this disease. This study aims to determine the effect of noni (*Morinda citrifolia*) extract on the improvement of dyslipidemia condition, based on the Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) level. This research is a laboratory experimental study with a post-test only control group design. Male Wistar rat *(Rattus norvegicus)* were divided into four groups: negative control group with no induction and treatment (K-), positive control group given high-fat diet induction for 14 days (K+), noni extract treatment group given a high-fat diet and a noni extract at a dose of 100 mg/kg BB per day for 7 days (P1), and noni extract at a dose of 200 mg/kg BB per day for seven days (P2). Low Density Lipoprotein and High Density Lipoprotein serum levels were measured using the ELISA method. The results showed that there was a significant increase in LDL and a decrease in HDL in both treatment groups (P1 and P2) compared with K(+) group. Therefore, we conclude that noni extract *(Morinda citrifolia)* played a role as an antioxidant which can increase LDL and decrease HDL serum levels in rats induced by a high fat diet.

Keywords: *Noni extract (Morinda citrifolia), high fat diet, LDL, HDL, antioxidant*

Introduction

Dyslipidemia or atherogenic dyslipidemia is a term, which represents the occurrence of three things in humans, namely an increase in the concentration of Low Density Lipoprotein (LDL), a decrease in High Density Lipoprotein (HDL), and an increase in Triglycerides (TG). Dyslipidemia together with obesity, metabolic syndrome, insulin resistance, and type 2 diabetes mellitus are important risk factors for myocardial infarction and other cardiovascular diseases. Currently, several genes have been associated with this pattern of lipoprotein changes[1]. Apart from cardiovascular disease, dyslipidemia can also lead to non-alcoholic fatty liver (NAFLD) disease. Inflammatory conditions, oxidative stress and insulin resistance, as well as several risk factors for cardiovascular disease, are also risk factors for this disease[2].

According to data, from 2009 to 2012,> 100 million US adults ≥20 years had a total cholesterol level of ≥200 mg / dL; nearly 31 million had levels ≥240 mg / dL. The data also show the presence of insulin resistance - which increases plasma triglyceride (TG) levels, an increase in Low Density Lipoprotein Cholesterol (LDL-C) and a decrease in High Density Lipoprotein Cholesterol (HDL-C) levels - which are important risk factors for vascular disease disorders[3].

Riskesdas 2013 data shows, the prevalence in the population> 15 years of age, there is abnormal total cholesterol of 35.9 %, low HDL 22.9 %, LDL is not optimal with the combined category near optimal-

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borderline high 60.3 % and high-very high category 15.9 %, abnormal triglycerides with high borderline category 13.0 % and high-very high category 11.9 %, and abnormal serum creatinine 6.0 %.

The currently established therapy of choice for dyslipidemia is statins. For conditions that are resistant to statins, a combination of statins with ezetimibe, fibrates, and nicotinic acid is used as a second choice. The next option is anti-PCSK9 drugs (evolocumab and alirocumab). In the latest development, to overcome homozygous FH (Familial Hypercholesterolemia), lomitapide and mipomersen therapy are used, but the price is still very expensive[4].

Dyslipidemia requires long-term and repeated treatment, so the use of natural ingredients around us in the treatment of this disease is a very good breakthrough because drug side effects are expected to be minimal and costs incurred for treatment can be more efficient. Noni (*Morinda citrifolia*) or also called Noni, is a natural ingredient in the form of a plant with high antioxidant content, which is strong against free radicals, prevents oxidative damage to major biomolecules and provides significant protection against oxidative damage. This plant is rich in non-enzyme antioxidants such as ascorbic acid, α-tocopherol, carotenoids, flavonoids, phenols, tannins and carbohydrates. The contents of biological compounds in this plant include glycosides, polysaccharides, alkaloids, lignans, fattyacid esters, anthroquinones, scopoletin, morindin, vitamins and minerals, which can be extracted from the fruits, roots and leaves of Noni[5].

Morinda citrifolia, or noni and locally known as noni, is a member of the madder family, Rubiaceae. This plant is native to tropical Asia and Australia and is distributed throughout the Pacific region. This plant is an evergreen shrub or small tree that grows to about 4–8 m. The branches are elongated short circular shape, thick, shiny ovoid leaves, dark green and muscular. The flowers, about 75–90 in number, are ovate with a round head. The length of the handle is 10-30 mm and the calyx is, in the form of a cut edge. White corolla, five lobes, with a greenish white tube, 7-9 mm[6]. This fruit is used by people in all tropical regions of the world. The shiny green leaves are used by Caribs as a poultice for wounds, rheumatic joints, fevers and headaches. The leaves are applied directly to the affected area for pain relief. Mashed fruit can also be applied directly to the affected area, including deep wounds and broken bones. It is also reported to cure various diseases ranging from arthritis, rheumatism, wounds, ulcers, and even to get rid of head lice[6].

Antioxidants are substances that play an important role in inhibiting and scavenging free radicals, thus providing protection for humans against infections, degenerative diseases, and metabolic diseases. Much research has been focused on natural antioxidants derived from plants because they can produce safe therapies. Natural antioxidants can protect the human body from free radicals by slowing the progress of many chronic diseases as well as inhibiting dietary lipid oxidation. Many diseases related to oxidative stress are a result of the accumulation of free radicals in the body. Free radicals are involved in more than 80 diseases including Diabetes Mellitus, Arthritis, Cancer, Aging. Metabolic disease etc. In the treatment of this disease, antioxidant therapy is suspected to be the most important. In plants, there are free radical scavenger molecules that are rich in antioxidant activity. Free radicals are involved in more than 80 diseases including diabetes mellitus, arthritis, cancer, aging, and metabolic disease[3]. In the treatment of this disease, antioxidant therapy is suspected to be the most important. Therefore, this study was done to determine the effect of noni (*Morinda citrifolia*) extract on HDL and LDL serum levels in Wistar white rats (*Rattus norvegicus*) induced by a high-fat diet.

Materials and Methods

Experimental design

This study was a laboratory experimental research and used a post-test only control group design. There were four groups: two control groups and two treatment groups. Rats were randomly selected. This study was conducted in the Faculty of Medicine, Hang Tua University, Surabaya, Indonesia in 2019. The sample used in this study were 28 male white rats (*Rattus norvegicus*), aged 3-4 months with a bodyweight of ± 150-200 grams, obtained from the Biochemistry Laboratory of the Medical Faculty of Hang Tuah University, Surabaya. The white rats were acclimatized for two weeks. They were maintained under a controlled temperature of 25±2°C, humidity of 50±10%, and a

12 h light-dark cycle and had free access to food and water. Animals were randomly selected and divided into 4 groups consisting negative control group with no induction and treatment (K-), a positive control group was given high-fat diet induction for 14 days (K+), noni extract treatment group given a high-fat diet and a noni extract at a dose of 100 mg/kg BB per day for 7 days (P1), and noni extract treatment group was given a high-fat diet and noni extract at a dose of 200 mg/kg BB per day for 7 days (P2).

Preparation of noni fruits crude extract

The noni fruits (*Morinda citrifolia*) were peeled off the outer skin, then split into several parts. After being chopped, the fruits were dried out using an oven at 60^0C. The dried fruits were then mashed until it became powder. The powder was macerated with 70% ethanol for 24 hours and the filtrate was taken. Then, the filtrate was evaporated on the water heater[7]. Noni extract was obtained.

Preparation of Pulvis Gummi Arabicum solution

The 3% PGA solution was used in the negative control group during the treatment stage. Hot water was poured into the mortar. Around three grams of PGA were sprinkled and inflated for 15 minutes. It was stirred until homogeneous. Then, 100 mL of distilled water was added.

Blood serum analysis

On the 29th day, the rat's blood was collected. The rats were previously was fasten for ± 12 hours and were only given water to drink. Then the rats were anesthetized and blood was drawn from the rats' hearts (intracardiac) using a syringe. Each rat had approximately 3 ml of blood. Then the blood was collected in a serological container without anticoagulants and closed with a rubber cap. The sample proceeded for HDL and LDL levels analysis.

HDL and LDL level analysis

Blood samples were collected in tubes and then centrifuged at 3000× *g* for 20 min. The serum was obtained. The levels of HDL and LDL in the serum were performed using an ELISA kit (Bioassay Technology, Shanghai, China) according to the manufacturer's protocol. After adding 40µl blood serum to wells, around 10µl of anti-HDL and anti-LDL, and 50µl streptavidin-HRP was added. Plates were incubated for 60 minutes at 37°C and washed 5 times with wash buffer. Then, 50µl substrate solution A and B were added to each well and incubated for 10 minutes at 37°C in the dark. Lastly, a 50µl stop solution was added to each well. The optical density was measured using a microplate reader (λ=450 nm).

Statistical Analysis

Data from experiments were expressed as mean ± standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Duncan's post hoc test to compare the groups using SPSS version 21 (SPSS Inc., Chicago, IL). The significance level for analyses was set at $p < 0.05$.

Result and Discussion

Noni extract contains several active ingredients, namely phenolic compounds and flavonoids which function as antioxidants and reduce levels of lipid peroxidation and increase catalase activity. The main flavonoids that have been detected in *Morinda citrifolia* are catechins and epicatechins[8]. Inhibition of active ingredients of phenolic and flavonoid compounds will cause inhibition of Reactive Oxygent Species, which are produced from modified lipoprotein protein molecules due to oxidation, glycosylation and glycosidation processes[9].

In this study, 5% induction of a high-fat diet for 14 days was proven to increase the LDL blood serum levels of male Wistar white rats (*Rattus norvegicus*), and administration of noni extract (*Morinda citrifolia*) at a dose of 100 mg/kg BW for 7 days was proven to be effective in lowering LDL levels (Table 1). Rats given a high-fat diet will experience an increase in LDL levels, this is due to the intake of saturated fatty acids contained in goat fat, lard, egg yolks and cholic acid[10].

Table 1. Effect of noni fruits on HDL and LDL level in rats induced by a high-fat diet. *p< 0.05 compared to the positive control group. **p< 0,05 compared to both control groups.

Groups	LDL level (µg/mL)	HDL Level (µg/mL)
Negative control	6,86 ± 1,86	31 ± 2,16
Positive control	10,72 ± 1,38	25,86 ± 1,22
P1	5,86 ± 1,35*	30,29 ± 7,72*
P2	5,29 ± 0,95*	33,29 ± 7,50*

Other studies that are in line with this study are research conducted in vitro on animals and active smokers, and the protective effect of noni (*Morinda citrifolia*) / Noni Fruit juice on superoxide anion radicals and lipid peroxide. The effect of this noni fruit can contribute to lowering lipid levels. A double-blind placebo controlled study conducted in a small population showed a decrease in total cholesterol and triglyceride levels in Noni Fruit drinkers[11].

The study stated that the treatment group, namely the group given Noni fruit juice at a dose of 50 and 100 mg/kg BW showed a decrease in total cholesterol, triglycerides and VLDL-cholesterol at both doses when compared to control the disease. However, the reduction in total cholesterol and LDL-C in the administration of noni fruit juice at a dose of 50 mg/kg BW failed to show a statistically significant reduction compared to atorvastatin, a cholesterol-lowering drug that has been established. However, this study has sufficiently proven the hypolipidemic activity of Noni fruit juice in a high-fat diet induced hyperlipidemia in rats[11].

The study, which was conducted on 20 mice, used 40% sucrose induction for 10 weeks to make a metabolic syndrome model, then gave *Morinda citrophilia* extract at a dose of 200 mg/kg BW for 2 weeks in the treatment group compared to the standard diet. Observations were made on rat body weight, serum glucose levels, total cholesterol levels, HDL, TG, uric acid, total protein (PTOT), albumin and globulin. The results showed that the consumption of water extract from dried M. citrifolia leaves reduced abdominal fat and triacylglycerols which are associated with increased adiponectin levels and changes in adipose tissue cell dynamics[12].

Many diseases related to oxidative stress are a result of the accumulation of free radicals in the body[13]. The main mechanism of antioxidants maintained in mammals is through the neutralization of ROS with glutathione (GSH) and three main scavenging antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Besides, matrix metalloproteinases (MMPs) promote the degradation of the extracellular matrix (ECM) molecules that play a role in the development of liver damage or even fibrosis[14,15].

Morinda citrifolia can prevent free radical-induced oxidative-pathological events in the liver by inhibiting the inflammatory response and suppressing increased liver enzyme activity; thus preventing damage due to cell membranes. The addition of *Morinda citrifolia* extract shows hepatoprotection against alcohol-induced injury due to regulation of lipid homeostasis, antioxidant status, and alcohol metabolism[15].

Conclusion

In sum, the administration of noni fruit extracts have successfully increased LDL serum level and lowered HDL serum levels in dyslipidemia Wistar white rats induced by a high-fat diet.

Conflict of Interest : The authors declare that they have no conflict of interest.

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Original Article

Effect of Photoprotective Cream of Mangosteen Pericarp Extract (Garcinia Mongostana L) Against 8-OHdG After UVB Exposure On Albino Mice

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Abstract

Objective: This study aims to determine the effect of the application of mangosteen pericarp extract cream (*Garcinia Mangostana L*) on levels of 8-OHdG on the skin of mice after exposure to UVB.

Method: The study use a pure animal experimental research design with control group. The criteria for inclution were species Swiss albino mice, aged 6 – 9 weeks, weight 20-30 g, female gender and healthy.. The samples that met the criteria were divided into 5 groups : UVB, Base Cream plus UVB, 3 groups mangosteen pericarp extract cream with different concentration UVB radiation in mice using a 311 nm narrowband UVB (Dermalight 1000) with the power 450 mJ/cm three times a week for 4 week. Elisa examination was carried out before and after the experiment.

Result: This study used 25 mice albino selected randomly and were divided into 5 treatment group consisting of 5 group : UVB alone, UVB with base cream, UVB with mangosten pericarp extract cream with concentration of 1 %, 3 % and 5 %. The Comperative test levels of 8-OhdG between each group with Post Hoc test and found It was concluded that the decrease in OhdG was greatest in group 2 followed by groups 5, 4, 3, and 1 (control group), respectively.

Conclution: The ELISA levels of 8-OHdG was found that there are significant difference as between the various treatment groups (p>0,05). However, the increasing concentration of mangosten pericarp extract cream showed decreased level of 8-OHdG.

Keywords*: Mangosteen Pericarp Extract, 8 OHdG, Mice Skin, Ultraviolet B*

Introduction

Chronic reactions from sun exposure to ultraviolet rays can cause skin texture disorders, premature aging (photoaging) and skin cancer[1].Damage caused by UV rays can be seen both clinically, histologically, in anatomical pathology and functionally. Exposure to UV radiation from sunlight causes skin damage through several mechanisms, including the formation of burn cells, triggering an inflammatory immune response, the formation of thymine dimers. and collagenase production (Matrix Metaloproteinase).

The damage that occurs due to ultraviolet B is more in the damage to the cell DNA which is the

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chromophore. Much UVB rays are absorbed into the epidermis and penetrate the dermal papillae. Symptoms of damage caused by UVB absorption to the epidermis are erythema. The wavelength from ultraviolet that is most effective in causing erythema is 250-290 nm and the effect of erythema decreases with increasing wavelength[3].

Reaction with carbonyl groups and double carbon in area adjacent pyrimidine, lead the specific photoproducts formation such as cyclobutene pyrimidine dimers (CPDS), dan 6-4 pyrimidone photoproducts (6-4 PP) is a product of mutations in epidermal cells mutation in epidermal cell followed cancer formation[3]. At the high concentration, ROS can act as a mediator basis of structural cell damage, proteins and nucleic acid known as "oxidative stress". ROS mediated oxidative stress which resulted in damage to the DNA with the formation of 8-hydroxy-2-deoxyguanosine (8-OHdG), which is one major products of oxidative damage and mutagenic lot to detection. 8-OHdG is one of the major oxidative modified DNA base product, which was first reported by Kasai et al to be formed on interaction hydrocyl radical (OH-) and singlet oxygen photodynamic action with DNA[4.] To prevent the cellular damage associated with oxidative stress.

That is important to maintain the balance of oxidants with antioxidants, such as the mangosteen fruit (Garcinia mangostana L). especially the use of the fruit skin. Mangosteen pericarp oxidies clear resin that is rich in xanthone. Mangostin is a major Xanthone derivate and is present is mangosteen. In the mangosteen pericarp extract is found Xantghone content of 95 % beside it also contains isoflavonoid, tannins and flavonoid[5,6.] The mangosteen pericarp extrct similar to antioxidant have anti inflammatory- active[7].

Materials and Methods

The study use a pure animal experimental research design with control group. It was conducted in microbiology laboratoryof Hasanuddin University, Makassar, from July to August 2020, after obtaining the approval from ethics commite of Health Research

The samples were 25 mice that meet criteria. The criteria for inclution were species Swiss albino mice, aged 6 – 9 weeks, weight 20-30 g, female gender and healthy. Exclution criteria were the mice were sick and dying during the study. The samples that met the criteria were divided into 5 groups : UVB, Base Cream plus UVB, 3 groups mangosteen pericarp extract cream with different concentration. Mangosteen pericarp extract with a concentration 1 %, 3 %, and 5 % was made in the laboratory Pharmacognosy phytochemical , Politeknik Pharmacy. UVB radiation in mice using a 311 nm narrowband UVB (Dermalight 1000) with the power 450 mJ/cm three times a week for 4 week.

Elisa examination was carried out before and after the experiment. For Elisa measure, the first 100 ul of each standard or sample was added and then immediately added 100 ul Biotinylateddetection antibody. Incubatiom for 90 min at 37 ºC anda washed 2 times. After 100 uL enzyme conjugate wass added and incubated for 30 min at 37 ºC and wash 3 times. Add 100 uL substrate reagents, incubation 30 mi at a temperature 37ºC and wash five times. Add 100 uL colour reagent and stop abrution and immediately read at 450 nm, then the calculation result. The data were statically anlyzed using

SPSS.

Result

This study used 25 mice albino selected randomly and were divided into 5 treatment group consisting of 5 group : UVB alone, UVB with base cream, UVB with mangosten pericarp extract cream wit concentration of 1 %, 3 % and 5 %. From the result of the ELISA levels of 8-OHdG was found that there are significant difference as between the various treatment groups (p>0,05) as evidence by ANOVA (Table 1).However, the increasing concentration of mangosten pericarp extract cream showed decreased level of 8-OHdG. The level of 8-OHdG highest in UVb control group and lowest in mangosten pericarp extract cream 5 % groups.

Table 1 The comparison of the 8OHdG difference value in each treatment group (ANOVA)

Difference (ohdG)	Sum of squares	Df	Mean square	F	Sign
Between groups	13367.126	4	3341.781	39,946	0.000
Within group	1673.151	20	83.658		
Total	1540.277	24			

Comperative test the levels of 8-OhdG between each group with Post Hoc test and found It was concluded that the decrease in OhdG was greatest in group 2 followed by groups 5, 4, 3, and 1 (control group), respectively.

Table 2. Comparison of the levels of 8-OhdG among treatment groups

(I) Group	(J) Group	Mean Difference (I-J)	P
UVB	BC +UVB	53.98160*	.000
	Mangosteen1 % UVB	3.05840	.603
	Mangosteen 3 % UVB	36.78540*	.000
	Mangosteen 5 % UVB	50.78980*	.000
BC +UVB	UVB	-53.98160*	.000
	Mangosteen1 % UVB	-50.92320*	.000
	Mangosteen 3 % UVB	-17.19620*	.008
	Mangosteen 5 % UVB	-3.19180	.587
Mangosteen 1% UVB	UVB	-3.05840	.603
	BC + UVB	50.92320*	.000
	Mangosteen 3% UVB	33.72700*	.000
	Mangosteen 5 % UVB	47.73140*	.000
Mangosteen 3 % UVB	UVB	-36.78540*	.000
	BC + UVB	17.19620*	.008
	Mangoosten 1% UVB	-33.72700*	.000
	Mangosteen 5% UVB	14.00440*	.025
Mangosteen 5 % UVB	UVB	-50.78980*	.000
	BC + UVB	3.19180	.587
	Mangosteen 1% UVB	-47.73140*	.000
	Mangosteen 3% UVB	-14.00440*	.025

Discussion

8 OHdG is the best biomarker to determine DNA demage and measuring the levels of 8-OHdG is used an evaluation of the oxidative stress. This is due to the formation of 8-OHdG were trigged by UV on the skin indicates high levels in the epidermis 7 days after exposure. Reported 8-OHdG induced by UV in skin mice removal obtained slowly and remained at high levels in the epidermis 7 days after exposure[6]. One of the defenses against oxidative damage, human cells are equipped with various anti-oxidants which work through various mechanisms. In addition to primary anti-oxidants, secondary antioxidants can also be used which function to capture compounds and prevent chain reactions. Xanton is one of the secondary anti-oxidants, where we know mangosteen peel exudes yellow resin which is rich in xanton[8,9].extracting mangosteen peel found a content of 95% xanthone, besides that it also contained isoflavones, tannins and flavonoids[11].

The biochemical composition of mangosteen rind extract has been reported to contain flavonoids which play an important role in warding off free radicals, and xanthones which have various medical properties such as antibacterial, anti-fungal and anti-inflammatory. Of all the xanthones that have been identified, α mangosteen is the xanthones with the highest levels isolated from mangosteen pericarp.[9,10,12] The antioxidant molecules of the skin of mangosteens are very strong as catchers of free radicals (radical scavenging)[13].

Researches that have been conducted in Makassar using UVB and mice as experimental animals[4]. who reported the results of research on topical cacao extracts on albino mice that showed UVB exposure of 343 mJ / cm² 3 times a week caused epidermal hyperplasia14. The reports results of a study that showed UVB exposure of 450 mJ / cm² to albino mice affected the expression of 8-OHdG and PCNA[15].

In this study, from the result of the ELISA levels of 8-OHdG was found that there are significant difference as between the various treatment groups (p>0,05) as evidence. Then do the post hoc test and found no significant difference as between UVB base cream with UVB mangosteen pericarp extract 5 %. The base cream used is a cream without anti-oxidants which should not have a very protective effect on the skin. The addition of mangosteen peel extract to the base cream is expected to provide a stronger effect. It is necessary to further explore the content of the base cream which provides a protective effect. But of the three concentrations of mangosteen peel extract at a concentration of 5%, the reduction in 8-OHdG levels was greater with a mean (-) 54.13 ± 11,659 while at a concentration of 3% on a mean (-) 40.13 ± 11.060 and a concentration of 1% on the mean (-) 6.40 ± 2,834.

Theresearch about the effect of mangosteen pericarp extract cream with levels of 10% and 20% providing a protective effect on the skin after UVB exposure even though the base cream also provides a strong protective effect because it contains Titanium oxide[5,8.13]. research on the protective effect of cocoa extract on 8-OHdG levels after being exposed to 7,12 Dimfthylbenz (a) Antracene and 12-O-Tetradecanoylphorbol -13- Acetate gave results with a low concentration of 200 ppm of cocoa protection against DMBA / TPA[16]. A study conducted a solution of mangosteen pericarp extract with a concentration of 95% showed significant differences in MMP-1 and the amount of collagen in the dermis[18,14,16].

Conclution

The result of the ELISA levels of 8-OHdG was found that there are significant difference as between the various treatment groups (p>0,05). However, the increasing concentration of mangosten pericarp extract cream showed decreased level of 8-OHdG.

Source of Funding - Self-funding

Conflict Of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 551/ H4.8.4.5.31/PP36/2019), and all research subjects give written informed

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The Effect of Nitric Oxide Supplement Intake on the Value of Hematological Examination of Health Workers Exposed to Covid-19

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Abstract

Objective: Examination of the diagnostic covid-19 gold standard with real time reverse polymerase chain reaction (RT-PCR) is still limited, so that the initial hematological examination (leukocytes, lymphocytes, neutrophils, platelets, hemoglobin, and neutrophil lymphocyte ratio (NLR) plays an important role in monitoring the course of covid-19 disease Macrophages release nitric oxide (NO) to kill parasites NO inhibits migration and adhesion of leucocytes to the endothelium. **Metode:** The research design used pre-experimental with one group pre-post test, the total sample was 80 health workers who were exposed to Covid-19, the independent variable was the provision of NO and the dependent variable was the result of a hematological examination (leukocytes, lymposites, platelets, neutrophils, hemoglobin and NLR). The intervention was given NO 500 mg mixed with warm water 250 cc, given 3 times a day for 5 days, data analysis used the T-test with a significance value of a<0.05. **Results:** There is a difference in the results of the pre and post-test hematology giving NO to the results of the examination of leucocytes a=0.001, lymphocytes a=0.000, platelets a=0.000, neutrophils a=0.000, hemoglobin a=0.031, and NLR a=0.000. **Conclusion:** Giving NO to health workers exposed to Covid-19 can improve the hematology and immune systems to fight the corona virus.

Keywords: *Nitric oxide, hematology, Covid-19*

Introduction

The body's reaction when infected with the Corona virus is to form the body's resistance to eradicate the virus [(1).] The immune system is unable to fight the Corona virus, severe Covid-19 symptoms can appear and there is a risk of complications [(2)] .During infection, the patient exhibits an uncontrolled immune response, which is caused by hyperactivation of macrophages and monocytes. This response results in a decrease in the total lymphocyte count [(3)]. This process will be detected by the immune system. After that, the immune system or immune system will react by sending white blood cells and forming antibodies to fight and kill the virus [(4)].

Macrophages are immune cells that are used to eat particles that enter the body and release nitric oxide. In its activity as cells that defend the body from parasites, macrophages release nitric oxide to kill these parasites.

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All parasites, including COVID-19 can be killed by using free radicals in the body, namely nitric oxide or NO. NO is released by macrophages in the body, macrophages are present in several organs such as the lungs and skin [5]. Nitric Oxide (NO) is a potent vasodilator for blood vessels, platelet inhibitor, is anti-inflammatory and anti-atherogenic, NO inhibits migration and adhesion of leucocytes to the endothelium [6]. NO is a signaling molecule that is widespread and plays an important role in every cell and organ function in the body. One of the factors that influence changes in hematological values is the role of Nitric oxide in the blood [7].

Nitric oxide is a molecule that is produced naturally by the body. NO production is essential for overall health as it allows blood, nutrients and oxygen to travel to every part of the body. Green leafy vegetables and some root vegetables (such as beets) have high concentrations of nitrates [8].

Table 1 Foods Containing NO

No	Foof	Content NO/100 g
1	Dark chocolate	1332 g
2	Beets	279 g
3	Melon	68 g
3	Pomegranate	1,3 g
4	Strawbery	9,4 g

Nitric oxide is a compound produced by many body cells. It relaxes vascular smooth muscle by binding to the heme portion of the cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of 3 ‘, 5’-monophosphate cyclic-guanosine, which in turn causes vasodilation. When it enters the body NO dilates the pulmonary blood vessels and, due to the efficient cleaning by hemoglobin, has minimal effect on blood vessels throughout the body [9].

Laboratory tests play an important role in handling COVID-19, starting from screening, diagnosis, monitoring therapy, determining prognosis, to surveillance [10]. A simple examination at an affordable cost, relatively fast processing time, and later results can be used for research purposes related to Covid-19 [11]. Apart from being an early detection, laboratory tests can be a means of monitoring the course of the COVID-19 disease. Several components of blood tests that are widely used as a monitoring tool and predictor of Covid-19 are leukocyte levels, lymphocyte levels, neutrophil levels, platelet levels, Hb, and neutrophil lymphocyte ratio (NLR) [12-14].

Method

The research design used pre-experimental with one group pre-post test [15]. The number of samples of 80 health workers who were exposed to Covid-19, the independent variable was the provision of NO and the dependent variable was the result of hematology tests (leukocytes, lymphocytes, platelets, neutrophils, hemoglobin and NLR). The group exposed to COVID-19 received food powder rich in NO from pomegranates, beets and dark chocolate contained in the powder, each patient received 1 sachet of powder every day for 5 days. The mass of 1 powder sachet is 500 mg NO which has been approved by BPOM RI MD 867013167071 and BPOM RI MD 867013219071 in 2020.

The groups exposed were taken from health workers who were in the tracing monitoring of the Kebonsari Health Center working area who carried out self-quarantine at home. Venous blood sampling was 5 cc and intervention was given NO 500 mg mixed with warm water 250 cc, given 3 times a day for 5 days, data analysis used the T-test with a significance value of $a<0.05$. The results of the leukocyte, lymphocyte, platelet, Hb and neutrophil counts were compared with the normal number of each variable [16]. Neutrophil and lymphocyte ratio or NLR was also compared with normal NLR [17].

Result

A. Characteristics of Respondents Data

The results of the study were data on the characteristics of respondents including gender, age, level of education, history of disease, BMI, and fasting blood sugar. This data is listed in the following table:

Table 2 Characteristics of respondents

VARIABEL		THE GROUP	
		N	%
Gender			
1. Men		10	12.5
2. Woman		70	87.5
Age			
1.	21-30 Year	31	38.8
2.	31-40 Year	29	36.3
3.	41-50 Year	18	22.5
4.	51-60 Year	2	2.5
Level of education			
1. Diploma		45	56.3
2. Bachelor		28	35.0
3. Post Graduate		7	8.8
Previous Disease History			
1. No		77	96.3
2. Yes		3	3.8
Body mass index (BMI)			
1. Low		13	16.3
2. Normal		39	48.8
3. Obesity		28	35.0
Fasting Blood Sugar			
1. Low		1	1.3
2. Normal		70	87.5
3. High		9	11.3

Based on table 1 data shows that the most respondents are gender 70 women (87.5%), age 21-30 years 31 (38.8%), education level 45 diplomas (56.3%), history of disease 77 (96.3 %), BMI was 39 normal (48.8%) and normal fasting blood sugar 70 (87.5%).

B. Hematology examination data

The results contained data on the number of leukocytes, lymphocytes, neutrophic platelets, Hb and neutrophil lymphocyte ratio NLR). The significance test is used to determine whether there is an effect of the independent variable, namely the provision of NO and the dependent variable is the result of a hematology examination. The test results are carried out with the criteria that if the P-value <significant alpha 5% or 0.05, then there is a significant effect of the independent variable on the dependent variable.

The results of the significance and model testing can be seen through figures and tables 2. Hypothesis testing on the effect of NO intake on blood hematological values includes NLR (p = 0.000), leucocytes (p = 0.001), hemogoblin (p = 0.031), lymphocytes (p = 0,000), neutrophils (p = 0,000), and platelets (p = 0,000), showed a significant effect on changes in blood hematological values after consuming NO intake. Thus it can be interpreted that giving NO to health workers who are exposed to Covid-19 is able to improve the hematology and immune systems to fight the corona virus.

Discussion

The role of NO intake is very important in increasing body immunity. NO plays a role in immunological processes, some of which are produced by tissue macrophage cells, due to the activation of various cytokines and endotoxin of pathogenic bacteria [18]. which is able to damage target cells or bacterial cells through its role as a cytotoxic material [19]. A study with a total sample of 41 people who were divided into 2 groups, namely the ICU patient group and the non-ICU patient group. In the ICU patient group, it was found that 54% had leukocytosis (leukocytes> 10 x 109 / L). Meanwhile, in the non-ICU group, it was found that 48% had normal leukocyte levels (4-10 x 109 / L) (20).

NO in the immune system is a nonspecific molecule and can cause damage to both host cells and pathogens [21]. Nitric oxide functions on a variety of cells, in the nonspecific immune system that occurs before the presence of a specific and complex infection involving lymphocytes [22]. NO production is increased during inflammation, so that if the production is not controlled, it will cause cell damage [23]. Another study showed that of the 40 COVID-19 patients, 13 were severe cases and showed a significant and sustained decrease in lymphocyte counts. The mean lymphocyte value in severe cases was 0.6 x 109 / L and in mild cases it was 1.1 x 109 / L [24].

The role of NO in regulating neutrophil migration has been investigated. Human neutrophil migration to interleukin (IL) -8 and NO NG-nitro-l-arginine methyl ester (L-NAME) synthase inhibitor significantly (P <0.001) increased IL-8-induced migration by up to 45% [25]. Antibodies to L-selectin or PSGL-1 had no effect on IL-8-induced migration but prevented increased migration to IL-8 induced by L-NAME. L-NAME produced neutrophil-derived microparticles that were significantly (P <0.01) larger than untreated neutrophils [26].

The accumulation and activation of inflammatory cells is essential for defense but can also lead to pathology. Neutrophils, for example, are essential for clearance of a wide range of pathogens but also cause injury and death to host tissue if their activity is misdirected or exaggerated [27]. In a study with 60 patients in Wuhan, the mean absolute neutrophil value was 2.8 x 109 / L. The percentage of COVID-19 patients with absolute neutrophil levels below the normal range (1.8-6.3 x 109 / L) was 11 patients (21%) while the percentage of COVID-19 patients with absolute neutrophil levels above the normal range was as much as 7 patients (13%) [28]. Another study in the same area, with a sample of 40 people, stated that absolute neutrophil levels were significantly higher in severe cases of COVID-19 than in mild cases. The magnitude of the increase in absolute neutrophil levels may indicate the intensity of the inflammatory response in COVID-19 patients [29].

Nitric oxide (NO), originally described as an endothelial-derived relaxation factor, is an important messenger molecule involved in many physiological and pathological processes [30]. Endothelium-derived NO causes vasorelaxation and also inhibits platelet adhesion and aggregation, thereby maintaining blood fluidity and preventing thrombosis [31]. An important alternative function of NO is in modulation of platelet production from megakaryocytes. Immune thrombocytopenic purpura (ITP) may be associated with decreased NO bioavailability contributing to thrombocytopenia

and other clinical characteristics associated with this condition [32]. At the same time, several treatment modalities for ITP may exert its beneficial effect by increasing NO levels and thereby increasing platelet production [33]. Several studies with mixed results have reported slightly lower platelet levels in mild cases of COVID-19 than in severe cases. The platelet level in mild cases was around 52.06-293.46 x 109 / L while the platelet level in severe cases was 129.98-393.78 x 109 / L (7). It was also stated that in mild cases of COVID-19, platelet levels decreased by about 5-17% [19].

NO, a multi-functional molecule, modulates the immune response mediated by the NLRP3 inflammasome [34]. Interestingly, our findings suggest that NO can be an intrinsic negative regulator of various diseases caused by abnormal inflammatory activation of NLRP3 [35]. Our observation that NLRP3 inflammation in humans is inhibited by NO further suggests the potential application of NO therapy in the treatment of inflammatory diseases. [36]. In this study, the group divided between acute and chronic COVID-19 infection, found that the NLR value in chronic infection patients (69 patients) was lower than in acute infection patients (24 patients). The mean NLR value of acute infection patients was 20.7 and chronic infection patients was 4.8. The results of this study indicate that the NLR value is related to the clinical outcome of COVID-19 patients and that the increase in the NLR value can be an independent prognostic parameter for patients with COVID-19 infection [37].

The rate at which hemoglobin reacts with nitric oxide (NO) is limited by how quickly NO can diffuse into the heme pouch [39]. The reaction is as fast as a ligand / protein reaction and the result, when the hemoglobin is oxygenated, is the formation of nitrates which is known as the oxygenation reaction. [40] Since nitrate, at the concentration produced by the oxygenation reaction, is biologically inert, the only role hemoglobin has ever thought to play a role in NO signaling is to block it [41]. Research has shown the importance of Hb compartmentalization in red blood cells in limiting the breakdown of NO by Hb. [42]. In addition, oxidation of Hb to form metHb reduces the ability of Hb to absorb or destroy NO [43]. The formation of N2O3 or S-nitrosthiols from the Hb / nitrite reaction may be the key to nitrite-mediated export of NO activity from red blood cells [44]. In a study with a sample of 41 people, divided into 2 groups, the mean hemoglobin level in mild cases of COVID-19 was 12.2 g / L, while the mean hemoglobin level in severe cases was 13.3 g / L. Another study, which sampled 67 patients, stated that the mean hemoglobin level in the ICU patient group was 13.2 g / L and that in the non-ICU patient group was 14.2 g / L (16,45).

Conclusion

The hematological profile of health workers exposed to COVID-19 can be an important supporting data to see the state of a person's immunity. Covid-19 patients with severe symptoms may experience a compromised immune response, which can lead to the development of hyperinflammation of the virus. Therefore, patients with severe COVID-19 symptoms should be examined for laboratory parameters for hyperinflammatory markers to improve mortality rates.

In its activity as cells that defend the body from parasites, macrophages release nitric oxide to kill these parasites. All parasites, including COVID-19 can be killed by using free radicals in the body, namely nitric oxide or NO. The provision of nitric oxide intake to health workers exposed to Covid-19 has an effect on changing the results of blood hematology examinations.

Conflict of Interest : Nil

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Immunotherapy Duration and Risk of Psychosocial Emotion and Risk of Attention Deficit Hyperactivity Disorder Prevalence and Relation in Allergic Rhinitis Children

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Abstract

Background: Allergic rhinitis (AR) is the most common chronic disease in children. Allergic symptoms affect daily activities and increase risk of psychosocial emotion and attention deficit hyperactivity disorder (ADHD). Immunotherapy has been proven in improving AR symptoms

Objective: To identify prevalence of the risk of psychosocial emotion disorder and ADHD and its relation with immunotherapy duration in AR children.

Methods: A cross-sectional study was held in AR children aged 4-18 years at Allergy Immunology Outpatient Clinic, Dr. Soetomo Hospital, during March 2017. Immunotherapy duration categorized into 0-6 months, 6 months-1 year, 1-2 years, 2-3 years. Psychosocial emotion disorder risk assessed using Pediatric Symptoms Checklist 17 (PSC-17), scored into four different subscales: Internalizing, Externalizing, Attention, and Total Score. ADHD risk was assessed using Abbreviated Conner's Rating Scale (ACRS). Statistical analysis using One-Way ANOVA and Eta test, with a value of $p< 0.05$ considered as significant.

Results: Total of 37 children included. Based on immunotherapy duration 0-6 months, 6 months-1 year, 1-2 years, and 2-3 years, prevalence risk of ADHD are 20.6%, 15.4%, 12.5%, and 12.5%, and prevalence of psychosocial emotion disorder risk are only in immunotherapy duration 6 months-1 year 12.5%. There were no correlation between immunotherapy duration with risk of psychosocial emotion disorder ($p = 0.945$) and significantly correlated to ADHD ($p = 0.049$, $r = 0.326$).

Conclusion: Prevalence risk of ADHD decrease as the immunotherapy duration increase and immunotherapy duration weakly correlated with risk of ADHD.

Keywords: *allergic rhinitis,children, immunotherapy duration, PSC-17, ACRS.*

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Introduction

Allergic rhinitis is the most common chronic disease in children, achieved 40% of the general population. Comprehensive management include education, allergen avoidance, pharmacotherapy, and immunotherapy [1]. Immunotherapy have been proven as an effective therapy either for intermittent or persistent allergic rhinitis. It

takes 3 to5 years to complete immunotherapy, and needs minimum 6 months to achieve maintenance dose [2]. As a house dust mite induced allergic rhinitis management, it provides early effects and long-term effects in reducing symptoms and treatment needs, as well as reducing further hyper-responsiveness [3, 4].

Psychosocial emotion disorder believed as morbidity in children, account for 14-20% school and 13% pre-school aged children. The risk increase 2-3 fold higher for children with low socioeconomic status, single parent, or mentally disorder parent. It could be measured using *Pediatric Symptom Checklist* (PSC), which recommended as psychosocial screening for children in primary healthcare [5, 6]. Prevalence of psychosocial and behavioral disorder in allergic rhinitis children are 24,4%[7]. Attention deficit and hyperactivity disorder in children could be measured using Abbreviated Conner's Rating Scale. Prevalence of attention deficit and hyperactivity disorder in allergic rhinitis children are higher than general population, which are 1% vs 0,5% [8].

Allergic rhinitis symptoms could significantly affect quality of life, including physical function, social function, and mental health. The symptoms bring limitation to daily activities, moreover physical and emotional [1]. Administration of immunotherapy effective in reducing symptoms, therefore the risk of psychosocial emotion and risk of attention deficit and hyperactivity disorder will decrease [3].

The aim of our study is to analyze the prevalence and relation of risk of psychosocial emotion and risk of attention deficit and hyperactivity disorder with immunotherapy duration in allergic rhinitis children.

Methods and Materials

This study was a cross-sectional study. The study were conducted in Pediatric Allergy Immunology Oupatient Clinic during March 2017. It was carried out on 4-18 years old allergic rhinitis children, who administered subcutaneous immunotherapy, and parent signed agreement to join the research were included. The patients were excluded if they had history of psychosocial emotion disorder, or attention deficit and hyperactivity disorder (ADHD), or neurological disorder, or incomplete medical records. Comprehensive informed consent was obtained from a legal representative of the patient. Patients characteristic including gender, age, family history of atopic disease, immunotherapy duration, risk of psychosocial emotion disorder (internalizing, externalizing, attention, and total score), and risk of ADHD. Immunotherapy duration categorized into 0-6 months, 6 months-1 year, 1-2 years, 2-3 years. SPSS ver. 19.0 was used for statistical analysis. One-Way ANOVA was applied to analyze data comparison between groups. Eta test was used to assess the correlation of immunotherapy duration to risk of psychosocial emotion disorder and risk of ADHD. Statistical significance was considered with p-value of <0.05.

Risk of psychosocial emotion disorder

Risk of psychosocial emotion disorder was assessed using Pediatric Symptoms Checklist 17 (PSC-17), scored into four different subscales: Internalizing, Externalizing, Attention, and Total Score. Risk of psychosocial emotion disorder internalizing aspect if internalizing score ≥ 5, externalizing aspect if externalizing score ≥ 7, attention aspect if attention score ≥ 7. Risk of psychosocial emotion disorder were assessed if total score of PSC-17 ≥ 15.

Risk of Attention Deficit and Hyperactivity Disorder

Risk of ADHD was evaluated using Abbreviated Conner's Rating Scale (ACRS), which total score ≥ 13 assessed as having risk of ADHD.

Result

The study was participated by 37 patients. Age range of patients were 4 to 14 years old, median 7 and mean 7.14 ±2.3 years old. According to skin prick test result, all patients allergic to house dust mite. Most of them, 89% have combination of house dust mite, pet, and food allergy (Table 1).

Table 1. Patient characteristics

Characteristics	**n=37 (%)**
Gender	
· Male	20 (54.1)
· Female	17 (45.9)
Age, median (years)	7 (4-14)
Family history of atopic disease	
· Yes	30 (81.1)
· No	7 (18.9)
Allergen	
· House dust mite, pet, food	33 (89.2)
· House dust mite, pet	0 (0.0)
· House dust mite, food	3 (8.1)
· House dust mite	1 (2.7)
Immunotherapy duration	3 (8.1)
· 0 - 6 months	8 (21.6)
· 6 months - 1 year	10 (27.0)
· 1 - 2 years	9 (24.3)
· 2 - 3 years	
Allergic rhinitis based on therapy	
· Mild intermittent	7 (18.9)
· Moderate-severe intermittent	6 (16.2)
· Mild-moderate persistent	8 (21.6)
· Severe persistent	16 (43.2)
Risk of psychosocial emotion disorder	
· Internalizing	5 (13.5)
· Externalizing	1 (2.7)
· Attention	1 (2.7)
· Total	2 (5.4)
Risk of ADHD	9 (24.3)

Prevalence of the risk of psychosocial emotion disorder and risk of ADHD were categorized based on immunotherapy duration. Risk of psychosocial emotion and risk of ADHD decreased gradually by number, as the immunotherapy duration increased. However, only risk of ADHD which consistently decreased, as the immunotherapy duration increased (Table 2).

Table 2. Prevalence of the risk of psychosocial emotion disorder and risk of ADHD based on immunotherapy duration

Immunotherapy duration	n (disorder risk prevalence)				
	Psychosocial emotion				ADHD
	Internalizing aspect	Externalizing aspect	Attention aspect	Total score	
0 -6 months	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (67.7)
6 months - 1 year	1 (12.5)	1 (12.5)	0 (0.0)	1 (12.5)	3 (37.5)
1 - 2 years	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)
2 - 3 years	3 (67.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)

There was significant relation between immunotherapy duration and risk of ADHD (p = 0.049), weak correlation (r = 0.326) (Table 3).

Table 3: Relation of immunotherapy duration with risk of psychosocial emotion and risk of ADHD in allergic rhinitis children

		n	Immunotherapy duration (mean), days	p
Risk of psychosocial emotion disorder	Internalizing aspect			0.283
	· Yes	5	844.2	
	· No	32	647.7	
	Externalizing aspect			0.267
	· Yes	1	258.0	
	· No	36	685.8	
	Attention aspect			0.227
	· Yes	1	1127.0	
	· No	36	661.7	
	Total score			0.945
	· Yes	2	692.5	
	· No	35	673.2	
Risk of ADHD				0.049
· Yes		9	461.0	
· No		28	742.8	

Discussion

Pediatric Symptom Checklist-17 were created to evaluate children psychosocial function. This tool is a reliable indicator, which clinical judgement are needed on its utilization [6]. Consist of three aspects, internalizing, externalizing, and attention. Total score of PSC-17 beneficial for general psychosocial function assessment in children [9]. Prevalence of psychosocial emotion disorder internalizing aspect in allergic rhinitis children were 24% (OR 2, CI 95%, 1,6-4,4). Increase to 31% in persistent rhinitis, 32% in multiple atopic disease, and 36% in allergic rhinitis children with another atopic disease [10]. In Indonesia, prevalence of psychosocial emotion disorder internalizing and attention aspects in allergic rhinitis children were 85,7% and 80% [11]. This study showed, in internalizing aspect, the prevalence was 1 (12.5%) and 3 (67.7%) patients whose got immunotherapy more 6 months-1 year and 2-3 years.

There are two mechanisms involved in triggering internalizing disorder in allergic rhinitis children. Those are behavioral changes and biologic mechanism. Behavioral changes caused by long term stress due to symptoms and therapy of chronic disease. Even the morbidity were low, but the low quality of life has been massively reported [10].

Potential biological mechanisms for this association between allergic diseases and internalizing disorders proposed include the release of interleukin-1β in hypersensitivity reactions,which activates the hypothalamic-pituitary-adrenal axis stimulating the release of cortisol and which modifies serotonin release leading to mood disturbances. However, mouse models have proposed a direct relationship between antigen exposure and altered brain function leading to increased anxiety. T helper 2 (Th2) cytokines production in the prefrontal cortex and olfactory bulbs of rats with tree pollen and Ovalbumin-induced allergic rhinitis has been demonstrated. These findings support the hypothesis that mediators of allergic inflammation may directly influence the centers of the brain involved in emotions and socialization [10].

Risk of psychosocial emotion disorder in school and pre-school aged children were 13% and 10% [5]. In this study, based on PSC-17 total score which represent risk of psychosocial emotion disorder, the prevalence only found in 1 (12.5%) patient whose got immunotherapy for 6 months – 1 year. Atopic disease and psychosocial has two way relationship, which atopic disease affect psychosocial more than the other way. Therefore, mental health of atopic disease patient needs special concern, in order to discontinue the relationship [12].

Allergic rhinitis children before treatment, have higher impulsivity and inattention rate [13]. Prevalence of ADHD are higher than general population, 1% vs 0.5% ($p < 0.001$) [8]. In Indonesia, prevalence of ADHD risk in allergic rhinitis children are 83.3%.[11] In this study, risk of ADHD consistently decrease, as the immunotherapy duration increase. Prevalence of ADHD risk are in 2 (67.7%), 3 (37.5%), 2 (20.0%), and 1 (11.1%) patients whose got immunotherapy 0-6 months, 6 months-1 year, 1-2 years, 2-3 years. Allergen exposure in allergic rhinitis children will induce neuroimmune inflammation, which clinically manifest as ADHD [14].

In this study, days of immunotherapy duration mean in allergic rhinitis patients with and without risk of ADHD are 461 vs 743 days. Immunotherapy duration are significantly related to risk of ADHD ($p = 0.049$), and has weak correlation ($r = 0.326$).

In allergic rhinitis, allergen exposure will be presented by APC to naïve T lymphocyte. Allergen bound dendritic cell interact with naïve T cell and induced polarization aim to Th2 response. Activated T cell migrate to bone marrow and produce inflammatory cell such as, basophil, eosinophil, and mast cell. The activation process involve (1) Newly activated TH lymphocyte by allergen, will have Th2 phenotype which release IL-3, IL-4, IL-5, IL-9, IL-13, eotaxin (CCL11), and GM-CSF; (2) Released of IL-4 or IL-13 will induce B cell differentiation into plasma cell which synthesize IgE. Next exposure of immunocompetent cells to the same allergen leads to mediator release and further activation of Th2 cell. IgE covered mast cell will bond to allergen, and release histamine, main mediator of allergy. Histamine is the most consistent stimulant in hyper-responsiveness, and also have H1, H2, and H3 receptor. H1 receptor found in T cell, B cell, monocyte, and lymphocyte. Stimulation of this receptor will induce inflammation of the respiratory, gastrointestinal tract, and skin. Signal of H1 receptor play a role in inducing proliferation process of T cell and B cell. It releases

cytokine and antibody through a pathway mediated by antigen receptor. H1 receptor have an extensive role in inflammatory process. H2 receptor play a role in itchy and painful sensation of the skin, permeability increase, and peripheral vasodilatation. H3 receptor increase neurotransmitter release [4].

Immune system and brain communication was important, because brain have a role in immune response (fever, sickness behavior, general behavior, and immunomodulation). It is mediated by several pro-inflammatory cytokine [15].

Cytokines have the ability to neuromodulate the brain during infection and inflammation. Its presence is also found in healthy brain, regulating the mechanism of homeostasis and behavior such as sleep, memory, and metabolism. Cytokine receptors are found in microglia, astrocytes, neurons, endothelial cells, and oligodendrocytes, which are distributed in the central nervous system (CNS) with different amounts, in different areas of the brain, of each individual. Cytokines are also produced in the brain in response to peripheral cytokine production, which indicates that cytokine formation signals are transmitted from the periphery to the brain.[16] The transmission occurs through several mechanisms [15].

- Cytokines in blood diffuse through blood-brain barrier which is permeable and adjacent to circumventricular organs. In this case, cytokines can interact directly with microglia and astrocytes.

- Interaction of cytokines and endothelial cells in the brain. Endothelial cells have IL-1 receptors and transmit cytokine signals to the brain

- Direct nerve activation by peripheral cytokines through the vagal nerve and catecholaminergic circuit of sympathetic nervous system

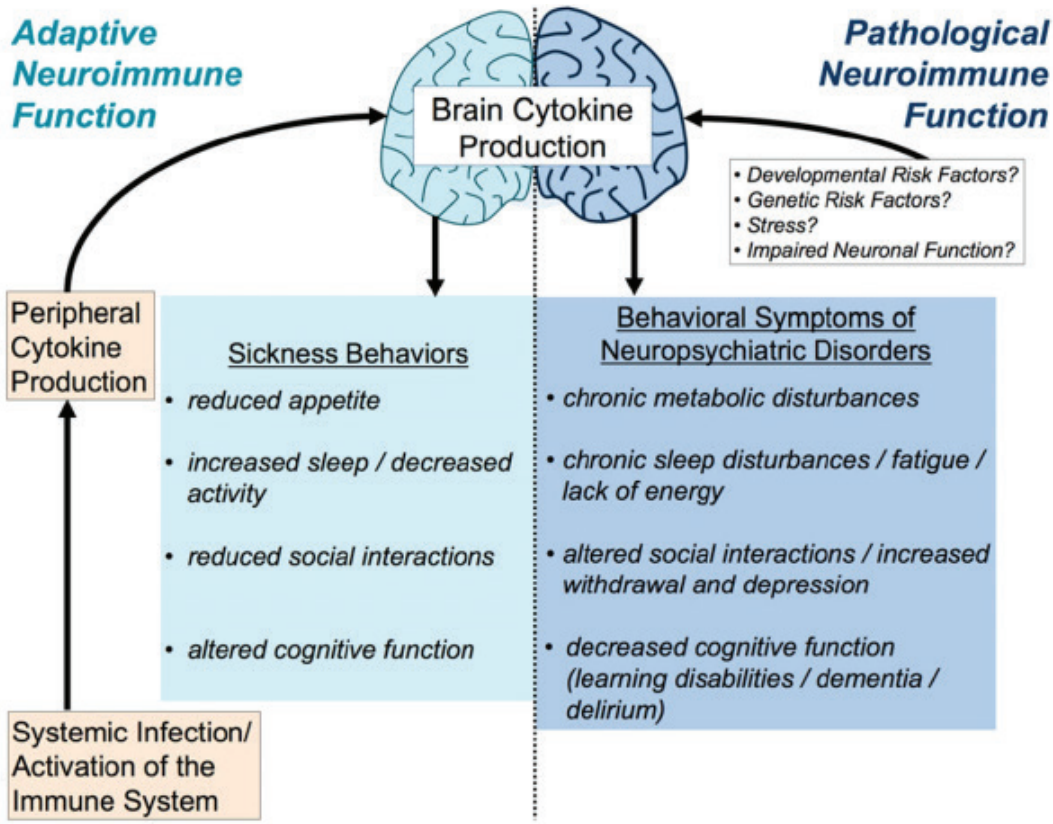

Figure 1 Adaptation function and neuroimmune pathology increase cytokine production and influence behavior.

Peripheral cytokine response induce brain cytokine production. Brain cytokine induce behavioral change including decrease social interaction and cognitive disorder.

Source: Bilbo SD, and Schwarz JM, 2012. The Immune System and Developmental Programming of Brain and Behavior. Front Neuroendocrinol, 33: 267-86.

Brain glia, consist of astrocytes (astroglia) and microglia, associated with behavioral disorders mediated by cytokines. Astrocytes are derivatives of nerve progenitors and have many functions, including neurotransmitter, maintaining the integrity of blood brain barrier, providing structural support, releasing neurotropic factors, and bind to synaptic transmission. Whereas microglia are derivatives of myeloid derivatives from bone marrow, which spread over the CNS, at least 15% of the CNS cell population. Initially myeloid cells migrate from bone marrow to brain parenchyma and terminally differentiate into microglia. Substitution of brain microglia is limited, in the absence of inflammatory stimulation microglia have a branched morphology, small sized cells with many arms and thin. In the resting phase, microglia actively monitor their microenvironment by emitting their projections. In the active phase, microglia appear unbranched, large and dense without arms. Activated microglia can have macrophages like function, such as scavenging, phagocytosis, antigen presentation, and produce inflammatory cytokines, so that they can affect neuron survival, proliferation, function, and synapses plasticity [15, 17].

Microglia and astrocytes work together in order to improve and regulate neuroinflammation response. Active glia will produce both inflammatory and anti-inflammatory cytokines. Inflammatory cytokines include IL-1β, IL-6, and TNFα which can induce and maintain behavioral disorders. The cytokine signal also triggers release of secondary inflammatory mediators such as prostaglandins and nitric oxide. Based on the glia response time, microglia are activated first and then astrocyte will increase the inflammatory signal. The neuroinflamation process usually occurs transiently, when the immune stimulus is finished, microglia will return in resting phase. However, the existence of a continuous neuroinflamation process will lead to maladaptation [15].

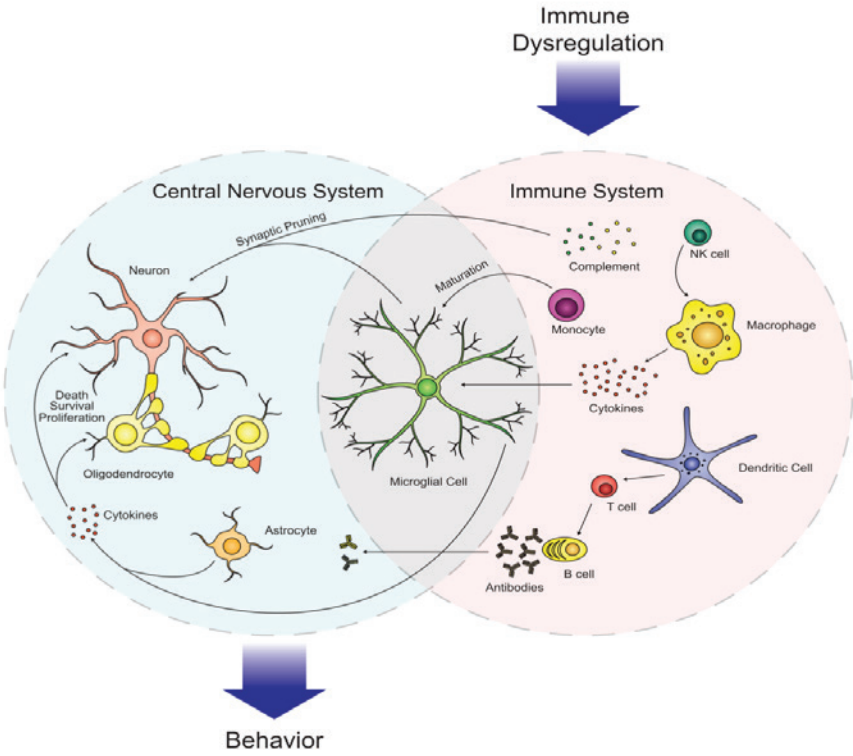

Figure 2. Immune dysregulation effect to behavior

Source: Onore C, Careaga M, and Ashwood P, 2012. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun, 26:383-92.

Immune dysfunction involves interaction of several types of cells, from natural and acquired immune systems. Several factors in immune system have an impact on CNS function. Increased cytokine production, such as TNFα and IL-1β, will inhibit neurogenesis and trigger cell death, whereas Il-6 can induce growth and proliferation of neurons and oligodendrocytes. Increased levels of complement protein can participate in synaptic scaling, opsonizing synapses and targeting for phagogocytosis by microglia. Activated microglia can also mediate synaptic pruning through MHC1 interactions. Therefore, it can be concluded that the presence of immune system dysfunction can cause behavioral disorders, such as cognitive function disorders, social environment withdrawal, and deviant behavior [17].

Relationship between allergic rhinitis and ADHD results from increased Th2 activity and secretion from the anti-inflammatory cytokines IL-4, IL-5, IL-9, IL-10, and IL-13. Th2 cytokines play an important role in induction process of the production of allergen-specific IgE (IL-4), and the entry of eosinophils into allergic inflammatory sites (IL-5). Inflammatory cytokines can activate neuroimmune mechanisms that involve brain circuits that affect behavior and emotions, and indirectly influence neuronal activity of brain structures through activation of hypothalamic-pituitary-adrenal pathway. In addition, inflammatory cytokines will also disrupt the metabolism of central neurotransmitters such as norepinephrine and dopamine which are known to play a role in the pathology of ADHD [18].

The mechanism of allergic rhinitis comorbidity with ADHD, is also thought to be due to stress exposure at an early age of child. Stress is related to the development of typical brain structure of ADHD, which affects cognitive function, and leads to onset of ADHD symptoms [18].

Sleep disorders are common finding in allergic rhinitis children. Minor sleep restriction cause specific circuits dysfunction in brain, especially prefrontal cortex, and negatively impact cognitive function and behavior. Impaired executive function is mostly found in children with ADHD. Therefore, sleep disorders are considered to have an important synergistic role in increasing risk of ADHD in allergic rhinitis children. In addition, allergic diseases and ADHD are thought to have similar genetic mechanisms. Signal transducers and activators of tranion 6 (STAT6) are involved in immune system regulation, cell proliferation, and apoptosis, which are thought to play a role in the pathogenesis of ADHD, and closely related to allergic diseases [18].

Immunotherapy has an allergen-blocking effect by IgG4 at the level of APC with the effect of preventing IgE production which facilitated by Th2 cells activation. In addition, immunotherapy immunologically worked by repressing eosinophilia, reducing immune reactivity, and shifting Th2 domination to Th1. Treg cells play a major role in immunotherapy. Treg cells are effective in regulating Th2 and Th1, where the clinical effect of immunotherapy is related to Th1 / Th2 balance. Increased production of allergen-specific IgG4 will block allergic inflammatory cascade (as a result of allergen and IgE bond in mast cells). Allergen-specific IgG4 has activity against IgE and is able to survive long term. So that specific immunotherapy also produces early effects and long-term persistent effects in reducing symptoms and treatment needs, as well as decreasing hyper-reactivity [4].

Improvement of ADHD symptoms in allergic rhinitis children is seen in the use of drugs that can regulate inflammation.[14] Administration of immunotherapy as a management of house dust mite allergy as a cause of allergic rhinitis is thought to be able to control allergic symptoms, so that the impact to risk of psychosocial emotion disorders and ADHD is low [3].

Main focus of allergy management is symptom reduction. Specific immunotherapy can reduce symptoms and treatment needs, and give long-term effects in preventing the development of further allergies. Specific immunotherapy is also useful as an anti-inflammatory, resolve causes, and prevent allergic airway diseases [4].

Limitations of this study, first the design used is cross sectional, so unable to detect increase or decrease trend of risk of psychosocial emotion disorders and risk of ADHD. Second, samples number inadequately represent allergic rhinitis children population. Third, immunotherapy duration is not long enough, so that the clinical effect might be not significant. Fourth, confounding factors not included in this study included

food allergies, exposure to environmental allergens, patient nutritional status, and allergic rhinitis severity.

Conculsion

The highest prevalence of the risk of psychosocial emotion disorder are in allergic rhinitis children whose got immunotherapy more than 3 years. Highest prevalence risk of ADHD were in allergic rhinitis children whose got immunotherapy more than 6 months. Immunotherapy duration weakly correlated with risk of ADHD.

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Ecological Analysis of Maternity Care in Indonesia in 2018

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Abstract

Policies that encourage maternity care to health care facilities are an important effort to reduce maternal mortality rates. This study aims to analyze the factors related to maternity care coverage in Indonesia. The ecological analysis was conducted using secondary data from the Ministry of Health of the Republic of Indonesia report in 2018. All provinces were taken as samples. In addition to coverage of childbirth in health care facilities as the dependent variable, there were 6 independent variables analyzed in this study, namely percentage of poor families, a ratio of primary health care/100,000 population, a ratio of hospital/100,000 population, a ratio of gynecologist/100,000 population, a ratio of midwife/100,000 population, and coverage of health insurance ownership. Data were analyzed using cross-tabulation. The results show the percentage of poor families tends to have a negative relationship with maternity care coverage. Besides, four other variables were found to tend to have a positive relationship with maternity care coverage, namely the ratio of primary health care/100,000 population, the ratio of hospital/100,000 population, the ratio of gynecologist/100,000 population, and coverage of health insurance ownership. Meanwhile, the ratio of the midwife/100,000 population was found to have no relationship with maternity care coverage in Indonesia. It was concluded that 5 variables show the tendency of the relationship with maternity care coverage in health care facilities in Indonesia.

Keywords: *ecological analysis, secondary data, maternity care, maternal health.*

Background

Health development is the implementation of health efforts to achieve the ability to live healthy independently by increasing the optimal degree of public health, improving the quality of human resources, and equal distribution of health service coverage. To equalize the reach of health services with a focus on basic health services, the government has built primary health care and its network throughout Indonesia. On average, each district has two primary health care[1].

Currently, births performed by health workers in health care facilities are recorded at 79.3%[2]. This situation still does not meet the target of the Minimum Service Standards that should be, namely 100% of mothers giving birth to health care facilities, following the Regulation of the Minister of Health of the Republic of Indonesia No. 741/Menkes/Per/VII/2008 concerning Minimum Service Standards in the Health Sector.

Provision of adequate health service facilities and improving the quality of health services is not sufficient to accelerate the reduction in maternal mortality. The condition of childbirth is an unpredictable process and bleeding is still one of the three main causes of death in Indonesia, which requires fast handling and adequate facilities so that if you experience an emergency maternal condition you can immediately get services compared to giving birth at home[3].

Previous studies that examined equity in health services informed that in countries that have not implemented a social insurance system, the cost of health services will greatly affect access to health services[4,5]. It was informed that the cost of health services is a barrier to accessing health services[4,6].

The quality and access to health services have a major influence on the successful implementation of health development. Where Indonesia is an area with quite complex geographical conditions and there is still an imbalance in the distribution of health facilities so that access to health services is still an important task to be resolved together so that all people experience fair and equitable benefits[7]. Based on the background description, this study aims to analyze the factors related to the coverage of maternity care in health care facilities in Indonesia.

Materials and Methods

The study was designed using an ecological analysis approach. Ecological studies focus on comparisons between groups, not individuals. The data analyzed is aggregate data at a certain group or level, which in this study is the provincial level. The variables in an ecological analysis can be aggregate measurements, environmental measurements, or global measurements[8,9].

The study was conducted using secondary data from the 2018 Indonesia Basic Health Survey report and the 2018 Data and Information of Indonesia Health Profile. Both reports were issued officially by the Ministry of Health of the Republic of Indonesia. The unit of analysis in this study is the province. All provinces in Indonesia were analyzed (34 provinces).

The dependent variable in this study was the coverage of maternity care in health care facilities. In addition to the coverage of deliveries in health care facilities as the dependent variable, 6 independent variables were analyzed in this study, namely percentage of poor families, the ratio of primary health care/100,000 population, the ratio of hospital/100,000 population, the ratio of gynecologist/100,000 population, ratio. of midwife/100,000 population, and coverage of health insurance ownership. Data were analyzed by univariate and bivariate. Bivariate analysis was performed using cross-tabulation. The entire analysis process utilizes SPSS 21 software.

Results and Discussion

Table 1 is a statistical description of the 7 variables analyzed. There is a very high gap in all variables. The highest coverage of deliveries in health care facilities was in Bali province (98.50%) and the lowest was in Maluku province (30.10%). Another variable that has a fairly high variation in values is the ratio of the gynecologist. The highest ratio of obstetricians was in Bali Province (39.40%) and the lowest was in West Sulawesi (3.90%). Besides, the variable percentage of health insurance coverage also shows a high variation in value.

Table 1. Descriptive Statistics of Maternity Care Coverage in Health Care Facilities and Analyzed Factors in Indonesia, 2018

Variable	N	Minimum	Maximum	Mean	Std. Deviation
Coverage of Maternity Care in Health Care Facilities	34	30.10%	98.50%	71.78%	17.08%
Percentage of Poor Families	34	3.55%	27.43%	10.74%	5.65%
Ratio of Primary Health Care/100,000 population	34	0.73	7.30	1.54	1.05
Ratio of Hospital/100,000 population	34	0.71	2.33	1.28	0.36

Cont... **Table 1. Descriptive Statistics of Maternity Care Coverage in Health Care Facilities and Analyzed Factors in Indonesia, 2018**

Ratio of Gynecologist/100,000 population	34	3.90	39.40	15.93	8.11
Ratio of Midwife/100,000 population	34	59.80	94.60	82.52	8.54
Coverage of Health Insurance Ownership	34	58.36%	165.45%	84.91%	21.97%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

This information shows that there is a fairly high disparity between provinces with high and low coverage of maternity care in health care facilities. Inequality shows that the trend is better in the West due to the uneven impact of health development[10]. This imbalance is further deepened by the geographic conditions of Eastern Indonesia which tend to be more extreme, which has further accelerated efforts to distribute development in the region[10,11]. Meanwhile, another study states that unfavorable geographical conditions will limit people's access to health care facilities[12].

Table 2 displays the results of the cross-tabulation of the percentage of poor families and maternity care coverage in health care facilities in Indonesia. It can be seen that there is a tendency for a negative relationship. The higher the percentage of poor families in a province, the lower the maternity care coverage in health care facilities in Indonesia. Previous research has found that poverty affects people's accessibility to health care facilities. Economic factors play an important role in determining the choice of place of delivery because the use of health facilities and facilities depends on the community's ability to pay[13,14].

Table 2. Cross-tabulation of Percentage of Poor Families and Maternity Care Coverage in Health Care Facilities in Indonesia, 2018

The Percentage of Poor Families	Coverage of Maternity Care in Health Care Facilities						Total	
	Low (30.10-52.90)		Middle (52.91-75.71)		High (75.72-98.50)			
	N	%	N	%	N	%	N	%
Low (< 7.25)	2	5.9%	5	14.7%	4	11.8%	11	32.4%
Middle (7.25-12.15)	3	8.8%	3	8.8%	6	17.6%	12	35.3%
High (> 12.15)	6	17.6%	4	11.8%	1	2.9%	11	32.4%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Table 3 shows the results of the cross-tabulation of primary health care/100,000 population and maternity care coverage in health care facilities in Indonesia. It can be seen that the higher the primary health care/100,000 population in a province, the higher the coverage in health care facilities.

Table 3. Cross-tabulation of Primary Health Care/100,000 Population and Maternity Care Coverage in Health Care Facilities in Indonesia, 2018

The Ratio of Primary Health Care/100,000 population	Coverage of Maternity Care in Health Care Facilities						Total	
	Low (30.10-52.90)		Middle (52.91-75.71)		High (75.72-98.50)			
	N	%	N	%	N	%	N	%
Low (< 1.31)	5	14.7%	5	14.7%	2	5.9%	12	35.3%
Middle (1.31-1.47)	5	14.7%	4	11.8%	2	5.9%	11	32.4%
High (> 1.47)	1	2.9%	3	8.8%	7	20.6%	11	32.4%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Moreover, Table 4 displays the results of the cross-tabulation of hospital/100,000 population ratio and maternity care coverage in health care facilities in Indonesia. It can be seen that there is a tendency between a high hospital/100,000 population ratio to have high maternity care coverage in health care facilities.

Table 4. Cross-tabulation of Hospital/100,000 Population Ratio and Maternity Care Coverage in Health Care Facilities in Indonesia, 2018

The Ratio of Hospital/100,000 population	Coverage of Maternity Care in Health Care Facilities						Total	
	Low (30.10-52.90)		Middle (52.91-75.71)		High (75.72-98.50)			
	N	%	N	%	N	%	N	%
Low (< 1.09)	5	14.7%	4	11.8%	3	8.8%	12	35.3%
Middle (1.09-1.39)	6	17.6%	3	8.8%	2	5.9%	11	32.4%
High (> 1.39)	0	0.0%	5	14.7%	6	17.6%	11	32.4%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

The higher the ratio of primary health care and hospital in a province, the higher the maternity care coverage in health care facilities. This is possible due to the impact of local health financing policies in several regencies/cities and provinces that eliminate service fees from basic health care facilities to hospitals[15].

Table 5. Cross-tabulation of The Gynecologist Ratio/100,000 population and Maternity Care Coverage in Healthcare Facilities in Indonesia, 2018

Ratio of Gynecologist/100,000 population	Coverage of Maternity Care in Health Care Facilities						Total	
	Low (30.10-52.90)		Middle (52.91-75.71)		High (75.72-98.50)			
	N	%	N	%	N	%	N	%
Low (< 12.71)	6	17.6%	4	11.8%	1	2.9%	11	32.4%
Middle (12.71-16.70)	4	11.8%	4	11.8%	4	11.8%	12	35.3%
High (> 16.70)	1	2.9%	4	11.8%	6	17.6%	11	32.4%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Table 5 shows the results of the cross-tabulation of gynecologist ratio/100,000 population and maternity care coverage in healthcare facilities in Indonesia. It can be seen that the higher the ratio of the gynecologist/100,000 population, the higher the maternity care coverage in healthcare facilities. This condition is possible because of the rare or uneven presence of gynecologists in each province[16].

Table 6 shows the results of the cross-tabulation of the ratio of midwives/100,000 population and maternity care coverage in health care facilities in Indonesia. It can be seen that there is no trend towards the relationship between the ratio of midwives/100,000 population and maternity care coverage in health care facilities. Although the ratio of midwives in a province shows a high number, this is not followed by high coverage of deliveries in health care facilities.

Table 6. Cross-tabulation of Ratio of Midwives/100,000 Population and Maternity Care Coverage in Health Care Facilities in Indonesia, 2018

Ratio of Midwife/100,000 population	Coverage of Maternity Care in Health Care Facilities						Total	
	Low (30.10-52.90)		Middle (52.91-75.71)		High (75.72-98.50)			
	N	%	N	%	N	%	N	%
Low (< 81.34)	1	2.9%	4	11.8%	6	17.6%	11	32.4%
Middle (81.34-86.23)	4	11.8%	4	11.8%	4	11.8%	12	35.3%
High (> 86.23)	6	17.6%	4	11.8%	1	2.9%	11	32.4%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Table 7 shows the results of the cross-tabulation of the percentage of health insurance ownership and maternity care coverage in health care facilities in Indonesia. It can be seen that there is a tendency that the higher the percentage of health insurance ownership in a province, the higher the maternity care coverage in health care facilities. This information is in line with previous research in Indonesia[17,18]. Although there are also studies that have found different information. Having health insurance does not guarantee a person to take advantage of health services[19,20].

Table 7. Cross-tabulation of Percentage of Health Insurance Ownership and Maternity Care Coverage in Health Care Facilities in Indonesia, 2018

Coverage of Health Insurance Ownership	Coverage of Maternity Care in Health Care Facilities						Total	
	Low (30.10-52.90)		Middle (52.91-75.71)		High (75.72-98.50)			
	N	%	N	%	N	%	N	%
Low (< 75.52)	4	11.8%	4	11.8%	3	8.8%	11	32.4%
Middle (75.52-86.57)	5	14.7%	3	8.8%	4	11.8%	12	35.3%
High (> 86.57)	2	5.9%	5	14.7%	4	11.8%	11	32.4%

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

The information generated from this research is ecological information. Although it can guide the direction of policies to be taken, further research is needed to study cases in each province. This is to dig deeper into the causes of inequality in maternity care coverage in health care facilities.

Conclusions

Based on the results of the study, it could be concluded 5 variables that show a tendency to have a relationship with maternity care coverage in health care facilities in Indonesia. The percentage of poor families was found to tend to have a negative relationship with maternity care coverage in health care facilities. Meanwhile, four variables tend to have a positive relationship with maternity care coverage in health care facilities, namely the ratio of primary health care/100,000 population, ratio of hospital/100,000 population, the ratio of gynecologist/100,000 population, and coverage of health insurance ownership.

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Ketogenic Diet Slows Down Weight Gain in Juvenile *Mus musculus* with Benzopyrene as Cancer Inducer

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Abstract

Objective: This study aims to analyze the ketogenic diet that slows down weight gain in juvenile *Mus musculus* induced with benzopyrene (BZP). **Method:** Thirty-six female mice (*Mus musculus*), 6-8 weeks old, weighted 15-25 grams were divided into six groups randomly. K1 (negative control, standard diet) induced with oleum olivarum, while K2 (positive control, standard diet), P1 (ketogenic diet 1), P2 (ketogenic diet 2), P3 (ketogenic diet 3) and P4 (ketogenic diet 4) induced with BZP subcutaneously in the right breast at a dose of 0.3mg/20gBB/day for 14 days. The ketogenic diet was given for the next 28 days. Data were analyzed using the ANOVA and post hoc LSD test. **Results:** After 42 days, the ketogenic diet mice experienced slower weight gain (p=0.000) because BZP induced neurotoxicity and the administration of the ketogenic diet in juvenile mice induced neurohormonal changes. The ketogenic diet increased ketone plasma level (p=0.000) and reduced blood glucose levels (p=0.002) due to nutritional ketosis state. **Conclusions:** The ketogenic diet with fat:protein ratio 60:30 slows down weight gain, increased ketone levels and decreased blood glucose levels in juvenile mice with BZP as cancer inducer.

Keywords: *ketogenic diet, weight gain, benzopyrene induced, ketone, blood glucose*

Introduction

The ketogenic diet is a high fat and low carbohydrates diet that has been used for various purposes such as treatment for epilepsy in children and adults, type 2 diabetes mellitus and obesity[1,2]. The ketogenic diet has the side effect of losing weight. However, children with epilepsy who are given the ketogenic diet have gained weight at a slower rate[3]. Benzopyrene (BZP) is a polycyclic aromatic hydrocarbon (PAH) compound that can be found in daily life. Humans can be exposed to BZP in air, water, and food through the skin, ingestion, and residual inhalation. As a food contaminant, BZP has been widely known to be a causative agent for some cancer. BZP metabolized in the liver by the CYP1A1 enzyme and produce carcinogenic metabolites that cause mutation in cells. Several studies in mice showed that BZP induction can cause tumors such as breast, skin, lung and liver cancer. In humans, exposure to BZP in the work environment is associated with the worker's cancer incidence, for example, coal workers, cigarette smokers, also paving and tile makers[4].

Since 1983, Rudolf Virchow has suggested the role of inflammation in carcinogenesis after discovering numerous inflammatory cells invaders surrounding tumor cells. Inflammation plays a role in two stages of carcinogenesis, namely initiation and progression. A clear association between chronic inflammation and cancer can be found in Hepatitis B virus infections

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and hepatocellular carcinoma[5]. The inflammatory and glycemic responses are interdependent. A sustained inflammatory response will increase glycolysis, followed by hypoglycemia as a follow-up process[6]. This is in line with Warburg postulate that cancer cells metabolized glucose to fulfil their energy needs[7].

Proinflammatory cytokines have an important role in insulin resistance, which is a key factor of metabolic changes in cancer. This changes will increase energy expenditure, systemic stress and normal cell function[8]. Unlike normal cells, cancer cells need more nutrients from their host to continue growing. These causes weight loss in cancer model animals[9]. Weight loss is one of the consensus criteria for cancer anorexia-cachexia syndrome (CACS). The ideal drug in the treatment of CACS should increase appetite, produce weight gain, improve quality of life, do not interfere with cancer treatment, and have an adequate tolerance profile[10]. Administration of the ketogenic diet is known to cause slower weight gain in juvenile wild type mice[11].

The ketogenic diet is expected to reduce blood glucose levels and the inflammatory process due to changes in the body's main energy source to ketones. Thus, it is expected there will be an improvement in the cancer conditions. This study aims to analyze the ketogenic diet that slows down weight gain in juvenile *Mus musculus* with BZP as cancer inducer.

Materials and Methods

Experimental Animal:

Thirty-six female mice (*Mus musculus*) aged 6-8 weeks old and weighted 20±5 grams were obtained from the Pusat Veteriner Farma Surabaya. Mice cages measuring 30x45x20 cm, made of plastic covered with gauze equipped with a place to eat and drink bottles, each cage filled with one group of six mice. Mice were placed at room temperature and the lighting was regulated by a light-dark cycle 12 hours respectively. The feeding was done at 09.00-10.00 a.m. *ad libitum*.

Chemicals:

The benzopyrene (BZP) was obtained from Sigma-Aldrich. BZP solution is prepared by dissolving BZP in oleum olivarum in the ratio of 100 mg of BZP to 100 mL of oleum olivarum[25].

Experimental design:

Thirty-six female mice were divided into six groups randomly. K1 induced with oleum olivarum, while K2, P1, P2, P3 and P4 induced with BZP subcutaneously in the right breast area as much as 0.3 mg/20gBB/day for 14 days. BZP was used to induce cancer. During 14 days (day 1-14) of induction, all groups were given a standard diet (60.3% carbohydrate, 17% protein, and 9.3% fat). Then, in the next 28 days (day 15-42) dieting namely K1(negative control, standard diet), K2(positive control, standard diet), P1(ketogenic diet with a ratio of 30% fat, 0% carbohydrate, 60% protein, and 10 % fiber), P2(45% fat, 0% carbohydrate, 45% protein, and 10% fiber), P3(60% fat, 0% carbohydrate, 30% protein, and 10% fiber) and P4(75% fat, 0% carbohydrate, 15% protein, and 10% fiber) were given.

Data collection:

Bodyweight was measured every two weeks using an HL-3650 heles digital scale (0-5 kg scale). Measurement of blood glucose levels using the Autocheck Multi-Monitoring System was done every two weeks and carried out 24 hours after the last ketogenic diet was given. Measurement of ketone levels using Abbott Freestyle Optium Neo Blood Glucose and Ketones was done every two weeks and carried out 24 hours after the last ketogenic diet was given.

Statistical Analysis

The statistical analysis was performed using SPSS software. The normality test used the Shapiro-Wilk test followed by the homogeneity Levene test, and ANOVA test continued with post hoc LSD test. All data displayed with mean±SD and used a significant level of $p<0.05$.

Results

The results of data analysis on the delta mean of final and initial bodyweight can be seen in Table 1.

Table 1: Delta mean of final and initial bodyweight

Group	Bodyweight (gram)			ANOVA Δ (p-value)
	Initial	Final	Δ	
K1	19.33±1.21	30.00±2.61	10.67±1.75a	0.038*
K2	19.83±3.97	26.33±3.72	6.50±2.43b	
P1	19.33±1.86	24.00±4.34	4.50±4.04b	
P2	18.33±1.63	23.00±4.20	4.67±4.68b	
P3	18.83±0.75	26.67±1.21	7.83±0.75a	
P4	19.17±3.55	27.00±3.95	7.83±4.83a	

Note: different superscript shows significant differences ($p<0.05$)

Based on table 1, the mean delta bodyweight of mice at K1 is higher than K2, P1, P2, P3 and P4 ($p=0.038$).

The results of the analysis of the average ketone levels post ketogenic diet can be seen in Table 2.

Table 2: Average ketone levels

Group	Ketone Levels (mmol/L)	ANOVA Δ (p-value)
K1	0.45±0.19a	0.000*
K2	0.42±0.12a	
P1	0.67±0.16a	
P2	0.75±0.24b	
P3	1.55±0.39b	
P4	1.20±0.30b	

Note: different superscript shows significant differences ($p<0.05$)

Based on table 2, the average ketone levels in P3 were higher than K1, K2, P1, P2, and P4 ($p=0.000$).

The results of the analysis on the mean delta of blood glucose levels post BZP induction and initial blood glucose levels can be seen in Table 3.

Table 3: Mean delta of glucose levels post BZP induction and initial blood glucose levels

Group	Blood Glucose Levels (mg/dL)			ANOVA Δ (p-value)
	Initial	Post BZP Induction	Δ	
K1	102.00+14.63	60.33 +3.72	-41.67+12.32a	0.000*
K2	98.33+14.47	49.67+6.06	-48.67+14.53a	
P1	71.50+28.22	59.50+7.56	-12.00+21.25bc	
P2	71.17+10.23	66.50+7.23	-4.67+9.91b	
P3	55.50+4.04	54.00+5.14	-1.50+2.26b	
P4	110.33+25.25	58.33+5.82	-52.00+23.53ac	

Note: different superscript shows significant differences ($p<0.05$)

Based on table 3, the mean blood glucose delta of mice post BZP induction and initial blood glucose at P3 was higher than K1, K2, P1, P2, and P4 ($p=0.000$).

The results of the analysis on the mean delta of final and initial blood glucose levels can be seen in Table 4.

Table 4: Mean delta of final and initial blood glucose levels

Group	Blood Glucose Levels (mg/dL)			ANOVA Δ (p-value)
	Two weeks post ketogenic diet feeding	Final	Δ (final-initial)	
K1	129.17+11.99	123.00+13.885	21.00+12.52a	0.002*
K2	123.83+16.06	127.33+7.528	29.00+9.27ab	
P1	116.67+12.40	87.00+12.665	15.50+38.28a	
P2	116.00+29.04	90.00+16.420	18.80+21.90a	
P3	109.17+9.04	104.17+19.438	48.67+16.08b	
P4	119.17+11.09	95.17+10.167	-15.17+27.80c	

Note: different superscript shows significant differences ($p<0.05$)

Based on table 4, the mean delta of mice blood glucose levels at P3 was higher than K1, K2, P1, P2, and P4 ($p=0.002$).

Discussion

The ketogenic diet has been known to cause slower weight gain in both mice and rats[11,12,13]. The present study showed the mean of delta bodyweight of mice at P3 was lower than those in the K1, K2, P1, P2, and P4 ($p<0.05$). This is in line with the study by Sussman et al. which stated that administration of a ketogenic diet with a ratio of 67.4% fat, 0.6% carbohydrate, and 15.3% protein wt/wt for 30 days in 6-weeks-old female mice significantly lead to a slower weight gain compared to the control[14]. The ketogenic diet change energy homeostasis, thus children who are given the ketogenic diet experienced slower growth than those on the regular diet. Administration of the ketogenic diet changes the calorie content and lead to several neurohormones changes, such as insulin and leptin which play a role in energy homeostasis regulation. The metabolic changes due to the ketogenic diet cause peripheral neurohormones levels to change[3,15]. The ketogenic diet administered for 4-6 weeks lead to visceral fat accumulation and increased both adiponectin expression and plasma leptin levels. This increased induce slower weight gain in mice because both hormones have an anorexigenic effect. In addition, high fats composition in the ketogenic diet provide a longer satiety effect and indirectly reduce calorie intake[13,16].

The K2 group experienced the lowest weight gain because it was induced by BZP without a ketogenic diet feeding. BZP induction causes neurotoxicity and inhibition of acetylcholinesterase and cholinesterase (ChE) which has a severe impact on the determinants of survival and performance functions, such as feeding and surviving toxic exposure[17]. The ketogenic diet exerts a neuroprotective effect that reduces injury to cells and modifies inflammation in the brain due to BZP exposure[18]. Increased expression of adiponectin also weakens the inflammatory response by decreasing the pro-inflammatory cytokines secretion[16].

The ketogenic diet contains high fat and limits consumption of carbohydrates will cause a nutritional ketosis[19]. The average ketone levels in the P3 were the highest ($p<0.05$), which is in line with the study by Greene et al. that a 3-months ketogenic diet with 69.1% fat, 22.9% protein, and 8.1% carbohydrates significantly increased fasting ketone levels compared to control[20]. Increased ketone levels in the P3 was happened due to the replacement of the main energy sources by fat. When the carbohydrate intake fewer than 20 g/day for 3-4 days, the oxidation of fat through the Krebs cycle is stopped and ketogenesis will occur to form ketone bodies as a substitute energy source for the brain. Ketone bodies can be used as an energy source when the concentration in the blood reaches 4 mmol/L (equivalent to glucose concentration). In contrast to ketoacidosis, nutritional ketosis does not cause changes in blood pH[19,21].

The present study showed the mean glucose levels in the standard diet groups (K1 and K2) were greater than the ketogenic diet groups. This is in line with the study by Ding et al. that mice in the control group have significantly higher glucose levels (7.0 mmol/L) than ketogenic diet group with fat-carbohydrate:protein ratio 3.1:1 (6.0 mmol/L)[12]. The ketogenic diet replaced the energy source for the brain into ketone bodies and causes blood glucose levels to remain low[19]. In contrast to the present study, Cooper et al. found that mice who got ketogenic diet, high-fat diet, and high-fat diet plus exercise for 12 weeks experienced moderate increases in blood glucose compared to control group because the ketogenic diet slows down weight gain and did not cause hyperinsulinemia[22].

The induction of BZP triggers an inflammatory response in mice and increases the use of glucose which results in a hypoglycemic state as a follow-up process[6]. The decreased glucose levels in the K2, P1, P2, P3, and P4 after BZP induction was caused by an inflammatory reaction. Inflammation triggers a glucostatic state where hyperinsulinemia occurs due to a rapid increase in glucose levels, followed by a more rapid decrease[23]. Hypoglycemic conditions trigger upregulation of glucose transporter 3 (GLUT3) on the plasma membrane of monocytes, which is an autoregulation mechanism to protect leukocytes from the detrimental effects of low glucose levels[24].

The present study showed that mice in the K2, which induced with BZP and given a standard diet, had the highest final glucose levels. This is caused by persistent inflammation due to BZP induction. Induction of BZP at a dose of 0.3 mg/20gBW/day for 14 days cause changes in the breast cells leading to malignant tumors[25]. The inflammatory condition can be exacerbated by blood

glucose increased. High blood glucose levels lead to low-grade inflammation, insulin resistance, and increased inflammatory markers[26]. Medroxyprogesterone (MPA) treatment in cancer patients decreased IL-1, IL-6, and TNF-α serum levels and increased appetite with bodyweight stabilization[27]. The ketogenic diet has similar anti-inflammatory effects by creating a relatively lower blood glucose level and decreasing TNF-α, IL-1, and IL-6 secretion[16].

Conclusion

The findings suggest that ketogenic diet with fat:protein ratio 60:30 can slows down weight gain in juvenile *Mus musculus* induced with BZP might be due to neurohormonal changes also its neuroprotective and anti-inflammatory effects. The ketogenic diet increased mice ketone levels and decreased glucose levels due to nutritional ketosis.

Conflict of Interest: The authors declare they have no competing interests.

Ethical Clearance: All research procedures were approved by The Airlangga University Faculty of Veterinary Medicine Research Ethics Commission No. 2.KE142.07.2019.

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Molecular Detection of *Toxoplasma gondii* in *Helix pomatia* in Wasit Province Iraq

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Abstract

DNA of a total of 96 samples of (32) enteric, (32) gastric and (32) pod of *Helix pomatia* has been obtained through the use of the Genomic DNA Extraction kit and tested by the use of the Real time PCR and specific set of the primers and probe for the detection of the *Toxoplasma gondii* B1 gene. Six enteric samples and only 2 gastric samples have been positive while the others have been negative, that result indicates the presence of the *T. gondii* in that snail species, and based upon those results infection can either be acquired from the contaminated environment or as a result of consuming the grass that has been contaminated with parasite oocysts. The negative result of pod samples may be due to the failure of filtration the infectious stages of parasites into the organs that close contacts with environment or inability of the parasites in surviving in those organs.

Key-words: *Toxoplasma Helix IRAQ real time PCR*

Introduction

The protozoan *Toxoplasma gondii* can be described as an obligatory microscopic parasite, it is avery common eukaryotic pathogens worldwide, cause Toxoplasmosis that infects most warm-blooded animals including birds, humans, domestic and other terrestrial animals [(1)]. Wild and domestic cats and other felids play as final hosts shedding oocysts in their feces; the infection can be acquired by the exhaustion of the undercooked or raw meat that contains tissue cysts, from soil, food or water that has been contaminated by the oocysts; or contact with infected carcasses during evisceration, processing or dressing, handling of the organs and other tissues of game animals, which risk of infection to humans [(2, 3, 4)]**.** The toxoplasmosis infection produces the generalized parasitemia involvements of liver, lungs, brain as well as other organs, and usually results in death [(1, 5, 6, 7)]. The ability of the oocyst to resist fluctuating environmental conditions such as dryness, freezing and sunlight, which is excreted with cat litter. It makes it a permanent and available source of infection [(8, 9)]. The *Toxoplasma gondii* parasite is characterized by its great ability to move in the water, so oocysts can remain able to infect in the water and soil for several months or several years [(10, 11)]. The 1st explosion of the *T. gondii* in the marine mammal has been designated by Dietrich and Van Pelt in the year of 1973 in harbor seal [(12, 13, 14)]. The infection with the *Toxoplasma* parasite took another turn that is not limited to marine and terrestrial mammals, as the infection acquired other types of invertebrates, including aquatic mollusks, as the parasite eggs were detected by microscopic examination of feces and snail tissues 10 days after exposure to the parasite oocyst [(15)]. Aquatic invertebrates can act as primary hosts through the accumulation of *T.gondii* eggs and their concentration in their tissues, so transporting food can be an important pathway for the transmission of *T. gondii* oocyst. Where the ability of abalone to swallow and accumulate *T. gondii* oocyst was proven in its tissues and concentrated, as *T.gondii* DNA has been detected in its tissues following the exposures to the parasite oocyst [(16)]. In Italy, Putignani *et al.,* (2011) [(17)] obtained in their investigations of *T. gondii* in oysters, using Real time and Nested PCR, a positive rate of 6.6% of the total number of samples examined.

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Esmerini *et al.,* (2010) [18] and Miller *et al.,* (2008) [19] have identified the naturally acquired *T. gondii* in the mussel through the use of the multi-locus PCR and DNA sequencing, and as well by nested PCR targeting B1gene locus in 3.30% of a 300 samples of the oysters. The bivalves'capacity in acquiring the pathogens from the sea water has been well known [18, 21]. The analyses have shown as well that *T. gondii* holds the responsibility of the high rates of the mortality in the otters in some California coastareas, and this percentage has been related to consuming infected marine snails [3, 22]. Sea otters which preyon the marine snails have an 11-fold increase in the *T. gondii* infection risk, while the predation on the bivalves has not been one of the considerable factors of risk [23]. In the case of the natural conditions, *T.gondii* has been found lately in the wild shellfish like the *Crassostrea rhizophorae* and *Mytella guyanensis* in Brazil [18] and *Mytilus californianus* in California [19]. As well as some of the cold-blooded organisms that have the capability of concentrating the *T. gondii* oocysts through the consumption of other marine mammals like the eastern oysters *Crassostrea virginica* and California mussels *Mytilus californianus*; *Toxoplasma gondii*is unable to infect those organisms, however, the oocysts can be concentrated in their digestive tract [24, 25]. The bioaccumulation of *T. gondii* oocyst in consumed oysters or mussels, which are often eaten raw, can transmit pathogens present in the water and directly indicate health risks to humans [26]. Bivalve mollusks, although not domestic animals, can act as carriers and hosts for *T. gonii* oocyst [27, 28]. These pathogens are stored in their tissues [20, 30]. The objective of the present work is detecting *Toxoplasma gondii* parasites in the *Helix pomatia* and identifying infected organs (such as the intestines, stomach, and pod).

Materials and Method

Sample collection

One hundred specimens of *Helix pomatia* snail were collected from a variety of agricultural fields in Wasit province - Iraq, between (February - March / 2020), and samples were taken from (stomach, intestine and pod) These samples were kept at a temperature of (-20°C), until they were used to extract DNA.

DNA Extraction

Genomic DNA has been obtained from the samples of the tissues through the use of the gSYNCTM DNA Extraction Kit (Geneaid. U.S.).

The probe and primers

Certain probe and primers targeted 94bp fragment amplification from B-1 gene of the *Toxoplasma gondii* in a variety of the samples. Those probe and primers have been designed by [31] and supplied by (Macrogen company, Korea), the sequences of primer TOXO-R (5'- AGCGTTCGTGGTCAACTATCGATTG - 3') and TOXO-F (5'- TCCCCTCTGCTGGCGAAAAGT - 3'); and the sequences of TaqMan probe which are specific for the B-1 gene in the *Toxoplasma gondii,* was (5-6 FAM-TCTGTGCAACTTTGGTGTATTCGCAG -TAMRA-3).

Real-Time PCR

This method has been conducted based on the method which has been presented in [31]. qPCR master mixture has been produced with the use of the (GoTaq®q-PCR Master mixture) which has been made following the instructions of the manufacturer, totally of 20µL of the solution of reaction (DNA template 5.0µL; B1 Forward primer (10pmol) 1.0µL; B1 Reverse primer (10pmol) 1.0µL; B1 probe (20pmol) 1.0µL; q-PCR master mixture10µL; PCR water 2µL) placed in the Real-time PCR Thermo-cycler (Bio-Rad . U.S.).

Results and Discussions

The snail enteric sample results in the real time PCR have been summarized in the figure1, there were 6 positive samples out of 32 enteric sample, the maximal concentration level of the DNA fragment of the B-1 gene of the *Toxoplasma gondii* amplifications of those samples has been (0.844) and the minimal has been (0.716), while there were 2 positive sample out of 32 gastric sample with maximum concentration of DNA fragment of B-1 gene (0.744), Shown in Figure2. The pods samples have been negative.

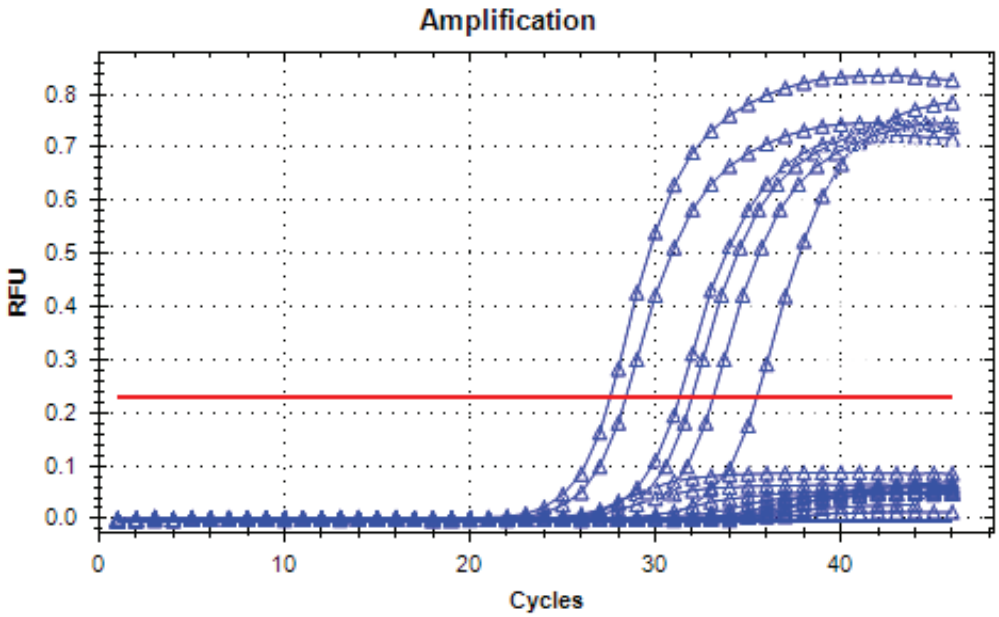

Fig.1: The plot of the Real-Time PCR amplification of the B-1 gene in the positive *Toxoplasma gondii* from the enteric snail samples

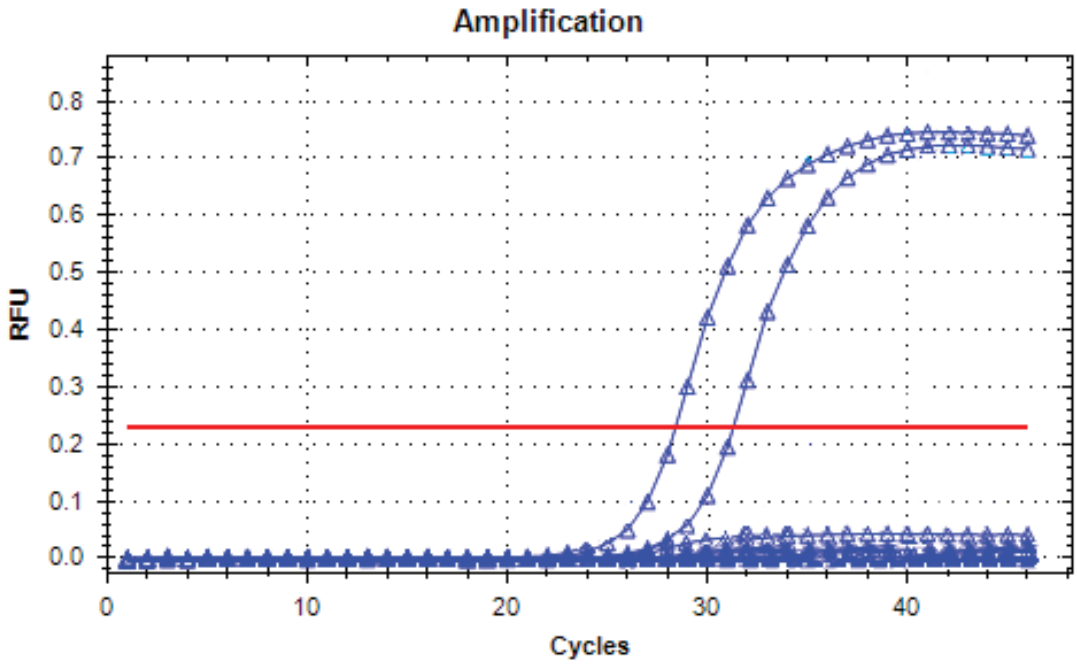

Fig.2: The plot of theReal-Time PCR amplification of the B-1 gene in the positive *Toxoplasma gondii* from the gastric snail samples

Earlier researches have shown that the parasites of the *Toxoplasma gondii* have been entirely terrestrial organisms, however, some of the authors' researches elucidated that *T. gondii* are infecting some of the marine animals like the sea otters as well as other types of the marine mammals [32, 33], which seldom consume the intermediate host containing parasites.

Cool *et al.*,(2000) [34] have shown that different types of invertebrates are vectors for the parasite, where the oocyst of the parasite are concentrated in some types of invertebrates such as bivalve oysters as a result of contamination of the aqueous medium, which leads

to transmission of infection to other organisms when consuming them as food [35]. However, in the present research, Iraqi rivers (Euphrates and Tigris) have a small number of the filter-feeding invertebrate species [36] Therefore, the likelihood to obtain positive samples was small.

Massie *et al.*, (2010) [25] have suggested that *Toxoplasma gondii* do not have the capability of infecting the filter-feeding invertebrates however, the oocysts can be concentrating within their organs like the digestive tracts as well as other organs which are in close contacts with the water that contains those organisms**.** The adverse result of pod samples may be due to the failure of filtration the infectious stages of parasite into the organs that close contact with the environment or the inability of the parasite to survive in these organs.

experimentally Arkush *et al.,* (2003))24) demonstrated that samples of mollusks of the type *Mytilus galloprovincialis* contained ssRNA of the *Toxoplasma gondii* parasite after using different concentrations of the *T.gondii* oocyst. and showing that the *T. gondii* oocyst stay infectious even after they pass through the marine invertebrate digestive system like in the oysters and mussels. On the other hand Coupe *et al.,* (2019) [37] used a variety of PCR-based methods to confirm the presence of the *Toxoplasma gondii* parasite›s DNA in the green mussel *Pern canaliculus* using 2 Nested-PCR assays that target the dhps gene and B1 gene and 2 Real time-PCR assays that target B1-gene and the repeating element 529-bp By comparing the results of the two assays, it was concluded that the rep529 qPCR test may be preferred for future mussel studies, but the DNA sequence is required for final confirmation of the detection of *T. gondii* DNA. However, there remain no evidences about the human infection by the *T. gondii* as a result of the consumption of the raw meat of the sea animals or their products.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Source of Funding: Self-funding.

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New Insight in the Using of Core Needle Biopsy As First Line in Preoperative Diagnosis of Thyroid Nodule

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Abstract

Background: The diagnosis of thyroid nodule by core needle biopsy is different from post resection samples because of difficulties in applying histological criteria for both samples due to limitation in core needle biopsy sample .

Objective: To assess the utility of core needle biopsy in diagnosis of thyroid nodule.

Material and Method: We use core needle biopsy as first line method instead of fine needle aspiration and to reach final diagnosis after resection sample . Core needle biopsy results were divided to six groups) unsatisfactory, benign, indeterminate, follicular neoplasm, suspicious for malignancy and malignant) according to The Bethesda system for reporting thyroid cytopathology [1,2,3] .

Result : A total of 45 thyroid nodules taken from 45 patients (30 female and 15 male) . Malignant changes were seen in 19 nodules after surgery and 26 after core needle biopsy. Benign nodules were diagnosed in 11 cases after surgery and in 12 cases after core needle biopsy . Final diagnoses were concluded for 34 nodules (76%). Twenty one nodules were diagnosed as malignant nodules and 13 nodules were diagnosed as benign nodules .

Conclusions: Core needle biopsy is reliable and valuable tool for the diagnosis of thyroid nodule and can been used as an alternative to fine needle aspirate especially in patients with inconclusive results .

Keywords*: core needle biopsy, pathological diagnosis, thyroid nodule*

Introduction

The diagnosis of thyroid nodule before surgery is done by fine needle aspirate (FNA) is useful diagnostic method [1,2]. Although cytological aspiration of the thyroid by large needle had been introduced since many years, FNA by using a needle has been an important diagnostic modality for thyroid nodules and usually it is safe and effective. An alternative to FNA, core needle biopsy (CNB) is now widely used as a preoperative diagnosis of thyroid nodule, with dependable results due to the new advances in core needle devices as thin needle and new advances in the procedure under ultrasound guidance.

On the other hand, the using of CNB for the diagnosis of thyroid nodules before surgery leads to new challenges for pathologists. The absence of widely accepted histologic classification for CNB has led to difficult connection between pathologists and clinicians and inefficient results. Therefore, we need to achieve efficient classification for thyroid nodule by CNB for both pathologist and clinicians. New advances suggest that CNB can be safe with low complication rate (0%-4.1%) . To reduce the danger that result from the complication of CNB, it should be done by well trained hands and should be undertaken under ultrasound guidance. So careful awareness about the anatomy of the neck is required to minimize such complication [18,19.20] . Nodules can be detected by clinical examination and by imaging technique. Thyroid nodule can be benign but even though may require surgery especially when they reach large size or if there is a suspicious for malignancy. The diagnosis may need ultrasound imaging or cytological findings to reach the final diagnosis. Markers analysis may be useful in detecting malignancy and may

be used as adjunct to cytology if available.

v In addition to that , findings of physical examination of the thyroid gland such as increasing in nodule size , hardness and fixation of lymph nodes are important step for the diagnosis of malignant nodules . If malignancy is detected preoperatively, total thyroidectomy is the treatment of choice .

Aim of the Study

In our study, we assess the utility CNB for the diagnosis of thyroid nodues .

Material and Methods

Samples of patients with thyroid nodules followed at Baghdad medical city between July and December 2019.

We use CNB as first line method instead of FNA and to reach final diagnosis after resection sample, review process can be achieved for some cases with inconsistent or final diagnosis. CNB can be done by using thin needle after application of Lidocaine as a local anesthesia . The needle was introduced into the thyroid gland after applying firm compression then the specimen is taken. All ultrasound examinations and ultrasound guided CNBs were performed by expert radiologists . CNB results were categorized into six categories according to "The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) [1,2,3] into unsatisfactory, benign, indeterminate, follicular neoplasm, suspicious for malignancy and malignant".

Statistical Analysis

"Analysis of data was carried out using the statistical package of SPSS-22. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range". "The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means. The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test (2-test). Statistical significance was considered whenever the P value was equal or less than 0.05"

Result

A total of 45 thyroid nodules taken from 45 patients (30 female and 15 male) were included in the analysis . Malignant changes were seen in 19 nodules after surgery and 26 after core needle biopsy. Benign nodules were diagnosed in 11 cases after surgery and in 12 cases after CNB . Final diagnoses were concluded for 34 nodules (76%). Twenty one nodules were diagnosed as malignant nodules and 13 nodules were diagnosed as benign nodules (Table1).

Table 1 shows the majority of cases were diagnosed as malignant in the initial and final diagnosis but some cases appear as suspicious in CNB then later on appear as malignant in the final diagnosis. These differences in diagnosis between CNB and resection sample may be related to size of the biopsy, in CNB is smaller and less informative than the resection sample from which we can take many pieces and from different places so the diagnosis appear more perfect and valuable.

Table 1: CNB Results and Final Diagnosis for suspicious Thyroid Nodules

		Benign (n=13)	Malignant(n=21)
Non diagnostic	0	0	0
Benign	12(26.7%)	11(84.6%)	0
indeterminate	5(11.1%)	2(15.4%)	1(4.7%)
FNA or SFN	0	0	0
Suspicious of malignancy	2 (4.4%)	0	1(4.7%)
Malignancy	26(57.8%)	0	19 (90.5%)

Table 2 shows the basic histopathological features to differentiate benign from malignant nodule and we depend on many factors like age of the patient. Thyroid nodule in older patients is more likely to be malignant . Another important factor is the sex of the patient were the thyroid nodule in female sex carry a higher risk for malignancy. Nodule with a size of more than 1 cm, is more likely to be malignant . In addition to these factors , the features of the nodule like irregularity of the nodule border or presence of with microcalcification are important for the diagnosis of malignant nodules .

Table 2. Basic and histo-pathological characteristics

	Malignant (n=21)	**Benign (n=13)**	**Inconclusive (n=11)**	**P value**
Age (year) 40-60 >60	 15 6	 11 2	 7 4	 0.071 0.218
Sex (male: female)	8 13	8 5	5 6	0.018 0.019
Nodule size (cm) <1cm >1cm	 7 14	 3 10	 3 8	0.221 0.019
Shape , n (%) ovoid Taller than wide irregular	 1 17 3	 10 1 1	 3 8 1	 0.017 0.018
Margin ,n (%) Smooth Speculated Ill defined	 1 18 2	 9 2 2	 5 6 1	0.218 0.081 0.285

Cont... **Table 2. Basic and histo-pathological characteristics**

Echogenicity,n(%)				
Hyperechoic	2	10	6	0.221
Hypoechoic	12	2	4	0.218
Markedly hypoechoic	7	1	1	0.317
Calcification ,n(%)				
Non	2	9	4	0.221
Microcalcification	18	1	5	0.172
macrocalcification	1	3	2	0.391

Figure 1: Core needle biopsy shows macrofollicular proliferation

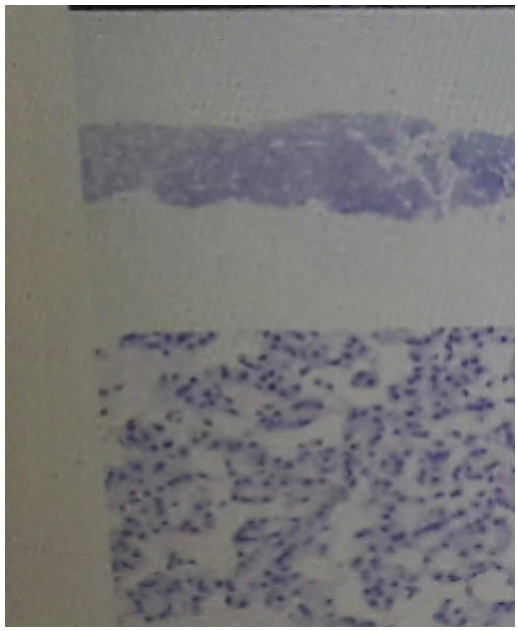

Figure 2: Core needle biopsy shows only microfollicular proliferation.

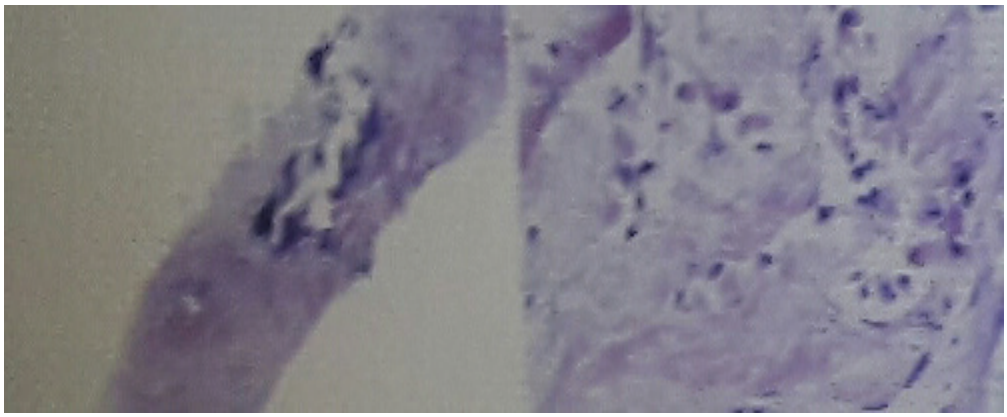

Figure 3: core needle biopsy shows calcification and suspicious atypical cells looks like malignant cells.

Discussion

Thyroid nodules are present in approximately 50 to 60% of healthy people. Usually, most of these nodules do not cause clinically significant symptoms, therefore, the main challenge in their management is to rule out malignant changes. Traditionally , FNA was the main diagnostic procedure for assessing thyroid nodules . Recently, CNB immerging as an alternative diagnostic procedure for thyroid nodules .

According to previous reports [17], CNB for thyroid nodule may help in improving the diagnostic results and can decrease the percentage of indeterminate or inconclusive results of traditional FNA and may be used a first-line diagnostic procedure in the assessment of thyroid nodules at high risk of malignant changes .

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) established a category-based reporting system for thyroid FNA samples. The 2017 revision states that thyroid FNA finding should be classified into six categories : "(I) nondiagnostic or unsatisfactory; (II) benign; (III) atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS); (IV) follicular neoplasm or suspicious for a follicular neoplasm; (V) suspicious for malignancy; and (VI) malignant".

The risk of malignancy in each category ranging from 0% to 3% for the "benign" category and may reach to 100% for the "malignant" category.

In one of previous studies [15] "CNB showed low rates of Bethesda category 1 and indeterminate results and a high diagnostic accuracy. CNB also reduce unnecessary thyroid surgery".

In our study , there was obvious difference between original diagnosis and final diagnosis after surgery . The diagnosis of some samples in CNB was inconclusive or benign while after surgery appear as different diagnosis, it could be benign or malignant, Therefore, we need urgent and perfect histological classification that is valuable and operable .

Thyroid nodules can be diagnosed around the world mainly by FNA , however the role of CNB is also seems to be dependable.

Among forty five CNB sample, only 5 samples (11.1%) were classified as indeterminate. Twenty six samples were diagnosed as malignant after CNB (57.8%) while after surgery 19 samples (90.5%) given this diagnosis. This difference in the results between CNB and resection samples may be related to sample size. Nevertheless the features of malignancy appears as atypia and other features of neoplasia are useful in diagnosis in CNB . Although, the features of malignancy present, it could be insufficient for the diagnosis of malignancy.

The main limitation of CNB is the sample size , so the diagnosis may be difficult or inconclusive .

All CNB and surgical samples were reviewed by a thyroid cytopathologist with more than 10 years of clinical experience in thyroid cytopathology. Although the classification of thyroid nodules by CNB finding is not well established , we try to apply the bethesda system

classification that used for histologic finding in FNA .

CNB results were classified as non diagnostic if the sample is insufficient for clear diagnosis, indeterminate or suspicious for malignancy when the features are inconclusive for complete diagnosis.

Our study results were comparable with many previous reports regarding the utility of CNB as an important additional promising tool for the diagnosis of thyroid nodule tumours .

Hahn SY et al [5], reported in there study that "ultrasonography-guided CNB is important for the diagnosis of thyroid nodules with inconclusive ultrasonography-guided FNA results. However, it is still not helpful for the differential diagnosis in 36% of nodules that are suspicious for follicular neoplasm seen on ultrasonography"

Na DG et al [10], reported in there study that "CNB show better results than FNA for reducing the frequency of inconclusive diagnostic results. CNB will improve the diagnostic accuracy for malignancy more than FNA in thyroid nodules that on the first FNA had nondiagnostic results"

Yeon JS et al [13], study stated that "CNB of the thyroid nodule demonstrates high rates of conclusive and accurate diagnoses in patients for whom previous FNA results were nondiagnostic, thereby reducing the need for unnecessary diagnostic surgery"

Similarly , Hakala T et al [14], found in there study that "CNBs had a high definitive sensitivity for malignancy (61%, CI 41% to 78%) whereas the definitive sensitivity for malignancy of FNA was significantly lower (22%, CI 10% to 42%). Moreover, CNB was not beneficial in the diagnosis of follicular thyroid lesions. When all suspected follicular tumours were excluded, the definitive sensitivity of CNB rose to 70% (CI 48% to 86%)".

Trimboli P et al [16], found in there study that "the overall rate of malignancy was of 80% (CNB, 77%; FNA, 83%). However, the diagnostic accuracy of CNB (97%) was significantly ($P<0.05$) higher than that of FNA (78%). In one benign lesion, CNB was inconclusive. Four (12%) of the 34 cancers of the FNA group were not initially diagnosed because of false negative , indeterminate or not adequate samples".

Furthermore , in Lee SH study [32] , they found that "the rate of a second non-diagnostic result was significantly higher on repeat FNA than on CNB (33.2 vs. 2.4 %; $p < 0.001$). There was no significant difference in the malignancy risk among patients initially non-diagnostic, twice non-diagnostic, and thrice or more non-diagnostic, nor did this differ from the rate following CNB. No further malignancy was found in cases with ≥2 non-diagnostic CNBs. The malignancy risk was 51 % in those who underwent thyroid surgery. The sensitivity for detecting malignancy was 65 and 70 % for repeat FNA and first CNB, respectively, with no false positives seen in either test. So they reported that about one-third of repeat FNA after an initial non-diagnostic aspirate are non-diagnostic on repeat examination, and the malignancy risk may not reduce following repeated non-diagnostic FNA. However, a single CNB may be enough to exclude malignancy risk for patients with a non-diagnostic aspirate"

Study limitations

Our study was performed in a single specialist center with a small sample size and different results may be seen in other centers or general hospitals with less experienced operators .

Conclusion

CNB is reliable and valuable tool for the diagnosis of thyroid nodule and has been used as an alternative or additional diagnostic procedure to FNA especially in patients with indeterminate or inconclusive results .

Ethical Clearance: core needle biopsy and resection samples were reassess by number of expert thyroid histo-pathologists.

Source of Funding: Self

Conflict of Interest: in this article there is no any problem in regard to interest.

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Species Composition and Seasonal Activity of Phlebotominae Sand Flies in Kuhdasht City, Lorestan Province, Iran

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Abstract

Background: leishmaniasis causes ninth largest burden among infectious diseases and has been known for several hundred years in Iran. Phlebotominae sand flies are proven vectors of leishmaniasis and some other infectious diseases such as sand fly fever, summer meningitis, vesicular stomatitis, Carri´on's disease and Chandipura virus encephalitis to human and animals.

Aim: To reveal the species diversity and distribution of leishmaniasis vectors, the present study was conducted with the aim of investigating the fauna and monthly activity of sand flies of Kuhdasht.

Methodology: This cross-sectional descriptive study was done on sand flies distribution and seasonal activity in Kuhdasht city. Using sticky traps and light traps sand flies were collected during activity season from the end of March to mid-December, 2016.

Results: During 8 months of study, totally 4455 sand flies (4157 with sticky trap and 298 with light trap) were collected. Sandflies include a collection of 7 species composed of *Phlebotomus* (*Phlebotomus*) *papatasi*, *P* (*Paraphlebotomus*) *sergenti*, *P* (*Paraphlebotomus*) *alexandri*, *P* (*Adlerius*) *halpensis*, *P (Paraphlebotomus) caucasicus*, *P (Phlebotomus) salehi* and Sergentomyia (*Sergentomia*) *antenata*. Activity of sand flies started in late May and continued until the end of November. *Phlebotomus papatasi*, *P. sergenti* and *P. alexandri* were three prevalent species in studied areas and two activity peaks were recorded in the end of June and in early-September.

Conclusions: Regarding the presence of proven vectors of leishmaniasis in studied areas and neighboring with important foci of cutaneous leishmaniasis, monitoring the status of vectors and epidemiology of the disease in the city and suburb of the Kuhdasht is very important in the prevention of forming a new focus of leishmaniasis.

Key words: *fauna, seasonal activity, sandflies, Kuhdasht city, distribution, leishmaniasis*

Introduction

leishmaniases are a group of arthropod-borne infections and one of the seven most important tropical diseases[1]that cause ninth largest burden among infectious diseases[2]. The causative agent is belonging to *Leishmania* genus in Trypanosomatidae family. More than 20 species of *Leishmania* can cause four types of Leishmaniasis namely; cutaneous, mucocutaneous, diffuse and visceral leishmaniasis[3]. Although in some foci human is the host-reservoir, in many cases the cycle of diseases is completed in the animal population. With few exceptions, the transmission is exclusively through

the bite of infected Phlebotominae sand flies[4]. There are 6 genera within the subfamily of phlebotominae of which three genera including *Phlebotomus*, *Sergentomyia* and *Lutzomyia* are more important because of their blood sucking behavior. Phlebotominae larvae breed in damp and dark places, fortified with organic materials and since they are weak fliers biting usually occur in nearby places[5].

The known number of sand flies species and subspecies estimated to be more than 900 [6] which about 98 species of them are proven or suspected vectors of leishmaniasis and only female sand flies attack vertebrate hosts to take blood meals which are required for egg development[7]. Phlebotominae also are proven vectors of some other infectious diseases to humans and animals such as sand fly fever, summer meningitis, vesicular stomatitis, Carri´on's disease and Chandipura virus encephalitis [8, 9].

Cutaneous leishmaniasis in Iran has been known for several hundred years, the age of Iranian physicians such as Avicenna[10]. The disease has been reported from 29 provinces and many cities of Iran, including Poldokhtar and Kuhdasht in Lorestan Province [11, 12].

Studies on phlebotomine sand flies of Iran has been started since 1930 by a limited number of Iranian and foreign entomologists[13]. Since then many investigations have been carried out on the different aspect of vectors of leishmaniasis by researchers in the country. So far more than 47 species of *Phlebotomus* and *Sergentomyia* genera have been described from Iran[13-15]. Although sand flies are distributed worldwide, due to the favorable climate they occur predominantly in warm, humid and tropical climates. Kasiri et al. [16] collected 11455 specimens in south-eastern Iran and identified 23 different species. In one of recent investigation in Paveh county of Kermanshah province (which has usually lower temperature than the southern part of Iran), Mawloudi et al. [17] collected 2110 phlebotominae sand flies comprising of 12 species of Phlebotominae. Sand flies have complex ecological behavior and the climatic conditions are very determining factors. Rafatbakhsh-Iran et al. [18] found that the highest total frequencies in Hamadan (west of Iran) occurs between 23 July to 30 August when the maximum and minimum registered temperatures were 31°C and 13°C, respectively, so they conclude that ambient temperature is one of the most important factors in the activity of sand flies in this area.

The high prevalence of sand flies in most parts of the country causes the transmission of the disease at a high cost of treatment, with long treatment time and, if left untreated, the effects of the disease in the individual will remain for the lifetime of the infected person [19]. Effective control of vector-borne diseases is possible if accurate and comprehensive information on the epidemiology of the disease and distribution of vectors are available. Meanwhile, knowledge of the diversity of species and the abundance of the vector are important in regulating control measures.

Materials and Method

Study area

This research is a cross-sectional descriptive study that was conducted in Kuhdasht, Lorestan province in 2016. The Province is located between Ilam, Isfahan and Khuzestan provinces (three important foci of cutaneous leishmaniasis in Iran). Kuhdasht is located at latitude 33°53´ N and longitude 47°6´ E. (Fig. 1). The city has an elevation of 1195 meters above sea level with an average of 450 mm of rainfall. According to the four main geographical directions of the city and nearby villages (North, South, East, West, and Center) 5 collection sites were selected and in each site, two stations were chosen. Using sticky traps (21.0 cm×29.5 cm paper soaked in castor oil) sand flies were collected once a month during the period of Phlebotominae activity from indoor and outdoor stations (30 traps for each station) from the end of March to mid-December. Also after a few weeks of the beginning of sampling and estimation of more populated station two light traps were added to sticky traps to compare the efficacy of these two sampling methods. The collection sites were as following:

A. Dawood Rashid and Kaleh gav Rah in the east and north of Kuhdasht

B. Dalab and Aoladghobad in the west of the city

C. Kora pa azadbakht and Goralivand in the south of the city

D. The end of Boo-Ali Street and the end of the Ghodar Pahn Street, inside the city.

E. Malmir and Tazeh abad in the south and west of the city

Collection and identification of sand flies

The sticky traps were installed in the afternoon and collected just before sunrise in the collection sites such as animal burrows, basements of old buildings, near mud walls, and other places likely to harbour sand flies. After transferring to the laboratory, specimens were removed from the sticky traps by a fine dissection needle and acetone was used to remove the oil. The samples were then transferred to tubes containing 70% ethanol and kept for further preparation in a cool place. Mounting of samples was done using puri's medium after cutting off the head and genitalia of individual sand flies on a slide. Later using the valid diagnostic key [20-22] and the standard samples in the laboratory sand flies were identified to the extent possible.

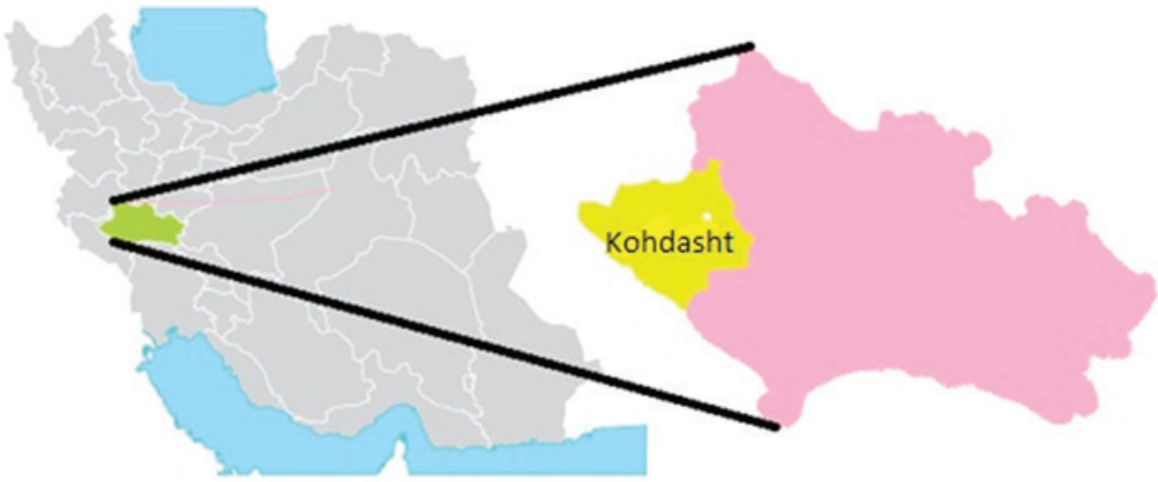

Figure 1: Map of Lorestan Province and Kuhdasht city

Results

Trapping at the interval of 15 days and using 4200 sticky traps and two light traps during active seasons of sand flies in Kuhdasht city resulted in the collection of 4455 sand flies (4157 of them with sticky traps and 298 with light traps). The activity of sand flies started in mid-May and continued until the end of November and then in December neither sticky traps nor light traps caught sand flies. During the study period, there were two peaks of activity. The first at the end of June and the second in early-September, which the first peak showed a higher population of sand flies (Fig.2). As Table 1 shows most samples (72%) were collected in outdoor places.

Also, Table 1. shows that Dawood Rashid and Kaleh gav Rah in the east and north of Kuhdasht had the highest population of phlebotominae with 1427 samples, whereas Malmir and Tazeh abad in the south and west of the city with 111 specimens had the lowest population. Using light traps in Dawood Rashid station resulted in 298 samples.

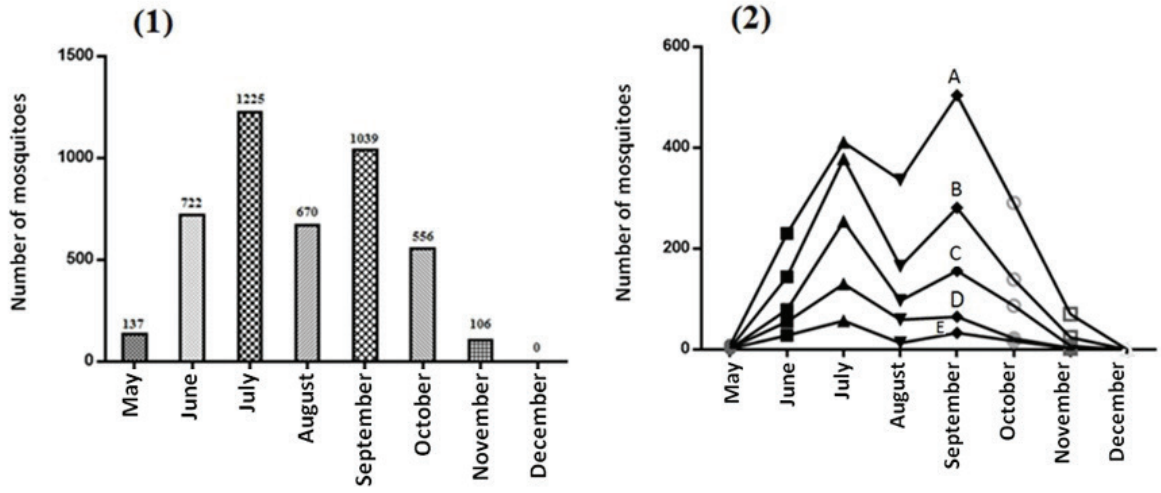

Figure 2: (1) Density of the sand flies species during activity season in Kuhdasht city, 2016. (2) Sand flies seasonal activity in Kuhdasht, 2016. A: Dawood Rashid-Kaleh gav Rah, B: Dalab and Aoladghobad, C: The Kora pa azadbakht-Goralivand, D: At the end of Boo-Ali Street, the end of Ghodar Pahn Street, E: Malmir-Tazeh abad

Table 1: Distribution of phlebotominae sand flies collected from Kuhdasht city, 2016

Studied areas	Outdoor places	Indoor Places	Total
Kaleh gav Rah- Dawood Rashid Area	1427	424	1851
Aoladghobad – Dalab Area	727	407	1134
Goralivand - Kora pa azadbakht Area	443	239	682
The end of Boo-Ali Street Area and the end of the Ghodar Pahn Street	210	128	338
Tazeh abad - Malmir Area	111	41	152
Light traps *	298	-	298
Total	3216	1239	4455

* Light traps were used only in Dawood Rashid station during July and Agust 2016

Table 2. indicates that in all station the number of samples collected from outdoor stations were higher from indoors collections. It also shows that more than 75% of samples belong to *Phlebotomus* (*Phlebotomus*) *papatasi*. It follows by *P.* (*Paraphlebotomus*) *sergenti* , *P.* (*Paraphlebotomus*) *alexandri* and *Sergentomyia* (*Sergentomyia*) *antennata* with 10.6%, 8.3% and 7.9% of samples, respectively. *Phlebotomus* (*Adlerious*) *halepensis* , *P.* (*Paraphlebotomus*) *caucasicus* and *P.(Phlebotomus) salehi* comprised less than 1 percent of samples altogether.

Table 2: Species and relative abundance of phlebotominae sand flies collected from Kuhdasht city, 2016

Species	Sex		Place		Total
	Male	Female	Outdoor places	Indoor Places	
Phlebotomus papatasi	2507	843	2269	1081	**3350**
Phlebotomus sergenti	347	27	408	66	**474**
Phlebotomus alexandri	221	49	228	42	**370**
Phlebotomus halepensis	3	0	3	0	**3**
Phlebotomus caucasicus	0	2	2	0	**2**
Phlebotomus salehi	0	1	1	0	**1**
Sergentomyia antennata	163	192	305	50	**355**
Total	3241	1214	3216	1239	**4455**

Discussion

The insect-borne pathogens have always been the cause of major health, social and economic problems among different communities and leishmaniases, which are considered to be zoonotic parasitic diseases, are no exception to this rule. One of the key factors in determining the distribution of arthropod-borne diseases is the geographical distribution of their vectors[23]. Phlebotominae sand flies are often referred to as the sole or most important vectors of Leishmaniases among humans and animal reservoirs. During more than 8 months study in Kohdasht city, 6 species of *Phlebotomus* genus and one species of *Sergentomyia* genus were collected and identified. As Table 2. shows *Phlebotomus papatasi* was the most abundant species collected during the present study, followed by *P. sergenti* and *P. alexandri*. The first two species are the proven vectors of zoonotic and anthroponotic cutaneous leishmaniasis, respectively and presence of the causative agent of leishmaniasis in their gut has been demonstrated in many occasions in Iran and other old world countries [13, 24, 25]. Also *P. alexandri* the other prevalent species has been recognized as the suspicious vector of visceral leishmaniasis in Iran [26] and as proven vectors of *leishmania donovani* in some other countries [27]. The activity of *P. papatasi* has been reported in almost all ZCL foci of Iran. The close association of this species and gerbils with humans has made a very suitable cycle for the transmission of the disease in foci of zoonotic cutaneous leishmaniasis [28] and studies in different parts of Iran have shown that natural infection to *Leishmania major* in this species was from 0.2 to 10.9% [13]. *Phlebotomu sergenti* has a wide distribution in the country. This fly has an endophilic behavior in rural and urban habitats [25] so can easily transmit the anthroponotic cutaneous leishmaniasis from infected man to another host in residential areas. Regarding the presence of important foci of cutaneous leishmaniasis such as Ilam, Isfahan and Khuzestan in neighboring provinces [29] and the presence of proven vector (The mean catch per trap was 0.99) the emergence of a new a focus in Kuhdasht seems likely. Table 2 shows the ratio of male to female 2.97, 12.85 and 4.5 for *P. papatasi*, *P. sergenti* and *P. alexandri*, respectively. This ratio is similar to previous studies in Iran [6, 30] which have used the sticky trap. Although *S. antennata* exceptionally has a male to female ratio less than 1(about 0.8). Comparing the light trap with sticky trap in this study suggested that at least in our studied area, sticky traps are preferable to the light

traps with UV lamps. One of the interesting findings of the present study is the effect of socioeconomic factors in the density of sand flies. As Table 1 shows Kaleh gav Rah- Dawood Rashid area and Aoladghobad Dalab area which is located in outskirts of the city and with mostly deprived dwellers and old buildings (some of them have been built with mud and sidewalks were without suitable coverage) had the highest number of sand flies. In contrast, Tazeh abad - Malmir area which is located in an area with new buildings, pathways made of asphalt or concrete and population with relatively higher income had the lowest density. This is also in agreement with results of Mirhoseni et al. in Yazd [6] who showed a significant difference in the number of sand flies captured in areas with old or new buildings. Presence of two activity peak has been reported in most studies related to sand flies in Iran such as Mawloudi et al. [17] and Kalantari et al. [31] although the timing of peak activity may change due to the difference in ambient temperature as has been mentioned in the study of Rafatbakhsh-Iran et al. [18]. Regarding the density of sand flies in first and second activity peak interesting point is that in contrast to results of studies in cities like Hamadan [30] or Paveh (Mawloudi et al) [17], in Kuhdasht the second activity peak shows a lower population of sand flies which, suggests higher temperature or some other factors may have had negative effects on population or activity of sand flies in this city on second activity peak.

Conclusion

Although at present Kohdasht is not considered as a focus of cutaneous or visceral leishmaniasis, regarding the presence of *P. papatasi* , *P. sergrnti* and *P. alexandri*, their importance in epidemiology of leishmaniasis and also presence of important foci of cutaneous leishmaniasis in neighboring provinces, to prevent emerging of a new focus in Kuhdasht, monitoring the status of vectors and epidemiology of disease in the city and suburb of the city is very important. Considering the reconstruction or improvement of old buildings and coverage of pavement in deprived neighborhoods also can be helpful in reducing the breeding places of phlebotominae larvae.

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Therapeutic Effect of Gum Arabic on some Biochemical Parameters in Nephrotoxic Albino rats

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Abstract

The aim of the current study is to evaluate some biochemical parameters in rats treated with gentamicin to induce nephrotoxicity . The experimental study was conducted using 42 healthy adult male rat weighing 250-300 grams . The results showed a significant increase (P≤0.05) in the levels of urea and creatinine in the blood serum, an increase in the activity of liver enzymes AST, ALT, ALP and a significant decrease in the concentration of albumin in adult male rats injected with gentamicin (80 mg / kg IP daily for 10 Days) to induce nephrotoxicity compared to control group. The results showed a significant increase (P≤0.05) in the concentration of urea and creatinine in the blood serum and a significant increase (P≤0.05) in the activity of liver enzymes AST, ALT, ALP, and albumin concentration in (untreated induced nephrotoxicity) compared with (control group). The results showed a significant decrease (P≤0.05) in the levels of urea and creatinine in the blood serum and a significant decrease (P≤0.05) in the activity of liver enzymes AST, ALT, ALP, kidney weight and a significant increase (P≤0.05) in albumin concentration, from the induced nephrotoxicity group treated with (15% w / v / day) Gum Arabic compared to the untreated nephrotoxic group, but the values In all treated groups were less than the control group. The present study concluded that the induced nephrotoxicity group treated with Gum Arabic showed improvement in some biochemical and hematological parameters associated mainly with nephrotoxicity .

Keywords: *Gum Arabic, Biochemical, Hematological, nephrotoxic, Albino rat*

Introduction

The kidneys are the major organ" that the human" body" needs"to realize and perform various "essential "functions" inclusive extracellular fluid control, homeostasis ,detoxification and toxic metabolite excretion(1) . It has important and essential role in excretion of many drugs and chemicals, So the failure of kidney to remove there materials may lead to retention of these compounds which may accumulate gradually to toxic levels (2). Gentamicin can cause the nephrotoxicity and ototoxicity and for this reason ,that can restrict its use in severely (3) . Nephrotoxicity is a specific disease that affects the kidneys occuring as a result of the accumulation of toxic metabolic discharge in the kidneys due to drugs and other toxic factors, in percentage"20%"of nephrotoxicity "is caused and occurs due to drugs ;"this" percentage increases in the "elderly "due"to the"increased"life"span (4) . The main mechanism of gentamicin in inducing nephrotoxicity is continous production of mitochondrial reactive oxygen species (ROS) which leads to a decrease in key endogenous antioxidant enzymes. Elevated levels of ROS are capable to cause injury to numerous cellular molecules, inclusive lipids, nucleic acids, and proteins, thus impairing function of the cell and leading to its death

ROS generation is capable of causing cell death by necrosis or apoptosis (6). Gum"Arabic"also known"as Gum Acacia, (GA) is known as an edible, water-soluble, dietary fibrous heteropolysaccharide,it is

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obtained from the dried gummy exudate of the branches and stems of Acacia seyal or Acacia senegal trees [7] . GA"possesses"biological" effects" including" nephro-protective activity.It has several characteristics such as being antibacterial, antiviral, anti-inflammatory and antioxidant[8] Gum Arabic is antioxidant because it contains four antioxidant elements; copper, iron,manganese and zinc. Also it has positive effect on the antioxidant enzyme expression [9] AG's protective effects are likely linked to its antioxidant and anti-inflammatory and cytoprotective properties [10] It is known that it is very rich in antioxidant, which were reported to have antioxidants and reduced effect of lipid peroxidation [11] The current study aims at evaluating the possibility of the therapeutic effect of Gum Arabic in concentration (15%w/v) on induced nephrotoxic by gentamicin in male albino rats .The study also aims at identifying the ideal concentration of treatment for the nephrotoxic by investigating Creatinine , Urea , ALT,AST, ALP, Albumin

Methodology

The experimental study was conducted during the period from February 2020 to March 2020, and the experiment was conducted using 42 healthy adult male rat (Rattus norvegicus) weighing 250-300 grams . The animals were integrated with wooden shelves, under natural light (12 hours) and (12 hours) in the dark. The animals were placed in cages at laboratory temperature (23-25 ° C). Food and water were introduced daily, and kept for a week before the acclimatization experiment began. Adult Male rat divided into two groups as following:

1. Control group: 18 rat will be kept without treatment and given 2mL /kg ,normal saline.

2. Nephrotoxicity group: twenty male rat were induced nephrotoxicity in dose 80 mg/kg/body weight IP for 10 days, After ten days of the experiment, 12 male rat from induced nephrotoxicity .

Measurements include Estimation of kidney function (serum Urea and Creatinine). Estimation of liver function : ALT (Serum Alanine aminotransferase) , AST : (Serum Aspartate aminotransferase) , Serum Alkaline phosphate activity ALP , Albumin

Results

Table 1. : Effect of Gentamicin on levels of serum Urea and Creatinine in induced nephrotoxicity in male rat

groups	Urea Mg/dl (Mean±SE)	Creatinine Mg/dl (Mean±SE)
Control group	B 36.5± 1.23	B 0.35± 0.009
Nephrotoxicity group	A 99.66± 1.20	A 2.05± 0.208
Sig. (2-tailed)	3.834	0.464

The different Capital litters refer to significant change (P≤0.05) between groups

The result in table (1) shows a significant increase (P≤0.05) in concentrations of Urea and Creatinine in serum of male rats treated with Gentamicin (80 mg/kg IP daily for 10 days) to induced nephrotoxicity in male rats compared with control group .

Table 2 : Effect of Gentamicin on Serum AST, ALT, ALP and ALB Levels in induced nephrotoxicity group of the male rat:

Groups	AST U/ml	ALT U/ml	ALP U/ml	ALB g/dl
Control group	B 139.66±7.59	B 50.83±3.84	B 155.5±10.95	A 3.95±0.187
Nephrotoxicity group	A 319.66±13.02	A 99.83±3.02	A 388.3±9.94	B 2.53±0.197
Sig. (2-tailed)	33.94	10.89	32.97	0.607

The different Capital litters refer to significant change (P≤0.05) between groups

Table (2) shows a significant elevated (P≤0.05) in liver enzymes AST, ALT and ALP activity in serum of induced nephrotoxicity group by gentamicin (80 mg/kg IP daily for 10 days) in male rats compared with control group .whereas, the results in same table, indicated that there was a significant decrease (P≤ 0.05)in ALB concentration in induced nephrotoxicity group compared with control group.

Table 3. : Therapeutic Effect of the Gum Arabic on levels of Serum Urea and Creatinine of the induced Nephrotoxicity in Male rats:

Groups (n=6)	Parameters (Mean±SE)	
	Urea Mg/dl	Creatinine Mg/dl
Control group	B 34.33±1.20	B 0.36±0.00
Nephrotoxicity group	A 130.33±18.94	A 2.60±0.15
Gum Arabic only (15%)	B 37.67±0.88	B 0.38±0.006
Gum Arabic (15%)+Nphrotoxicity	B 37.67± 0.66	B 0.44±0.00
Gum Arabic (7.5%)+Nphrotoxicity	B 40.33±2.84	B 0.49±0.04
LSD	27.09	0.225

The different Capital litters refer to significant change (P≤0.05) between groups.

In Table 3 , the statistical analysis for kidney functions, creatinine and urea (Mg/dl) showed significant increase (p ≤ 0.05) in the levels of serum Urea and Creatinine in induced nephrotoxicity group compared with control group and There were significant decrease (p≤ 0.05) in the concentration of serum Urea and creatinine of the nephrotoxicity groups treated with Gum Arabic compared with nephrotoxicity group .

Table 4. : Effect of treatment with Gum Arabic on serum AST ,ALT , ALP and ALB concentration in induced nephrotoxicity group of the male rats.

Groups (n= 6)	Parameters (Mean±SE)			
	AST U/ml	ALT U/ml	ALP U/ml	ALB g/dl
Control group	C 138.66±5.60	C 49.33±2.40	D 151.66±4.05	A 3.93±0.26
Nephrotoxicity group	A 320.66±11.46	A 114.66±8.68	A 397.00±7.76	B 2.45±0.10
Gum Arabic only (15%)	C 139.00±1.73	C 52.00±2.51	DC 158.33±2.72	A 3.86±0.20
Gum Arabic (15%)+ Nphrotoxicity	B 178.00±2.64	C 58.00±0.57	C 168.66±0.33	A 3.76±0.16
Gum Arabic (7.5%)+ Nphrotoxicity	B 193.00±3.46	B 84.66±3.48	B 186.66±6.83	A 3.63±0.08
LSD	19.161	14.092	16.136	0.555

The different Capital litters refer to significant change (P≤0.05) between groups.

In the table **4 :** The AST activity showed significant increase ($p \leq 0.05$) in nephrotoxicity group compared with control group . Result showed that there is highly significant decrease ($p \leq 0.05$) in the levels of AST activity of the nephrotoxicity groups treated with Gum Arabic in comparison with nephrotoxicity group but no significant difference ($p \geq 0.05$) when compare between the two groups .

Discussion

Gentamicin (GM) is one of the aminoglycoside antibiotics frequently used in the treatment of serious infections with aerobic gram-negative and gram-positive bacteria , The most evidenced series side-effects of gentamicin are nephrotoxicity and ototoxicity [(12)],Gentamicin can cause renal damage and dysfunction which marked by increase of serum urea and creatinine levels[(13)] . The current study showed increase in the level of the urea and creatinine become higher, This may be due to disturbed in transport functions of the epithelial cells of the collecting tubules and diffuse impairment in the functions of proximal convoluted tubules [(14)]. Increased creatinine and urea levels in the blood serum are due to the inability of nephrons to put them with urine because of the damage that occurred in the convuluated tubules, which caused a defect in The efficiency of the nephron in ridding the body of wastes of cellular metabolism, as the kidneys ability to excrete Urea and creatinine are valuable biomarkers in assessing kidney performance and Gentamicin contributes in ROS levels Increased, such as hydroxyl radical and superoxide [(15)] . It was found that Gum Arabic protects rats against gentamicin-induced nephrotoxicity, likely in part, by inhibiting the product of oxygen-free radicals which causing lipid peroxidation [(16)] Gum Arabic may act to protection tubular epithelial cells of the renal cortex from free radical oxygen-mediated damage and inhibited the formation of oxidised producted,this is supported by the fact that Gum Arabic has elevated

affinity to the free radical [17] it has an additional beneficial effect on kidney function, which is related to its anti-inflammatory and antioxidative effects [18] . properties of antioxidant in the Gum Arabic may be due to the its ingredeint flavonoids and another polyphenolics, and it has been reported that these plant components possess lipid-antioxidant and antioxidant activities [19] . Data in table 2 show a significant rise (P≤0.05) in" serum of "ALT",ALP",AST levels"and"siginificant decrease in Albumin concentration of induced nephrotoxicity group by gentamicin, (80 mg/ kg IP daily for 10 days),GNT was also seen to catalyze hepatotoxicity secondary to ototoxicity and nephrotoxicity. It causes hepatic injury via enhancing the productin of free radicals that invade and destroyed hepatocytes [20] . ALT and AST are two enzymes that are the most accurate indicators of necrosis or hepatocellular damage. Their levels in a number of hepatic diseases are show elevated .of the two enzymes, ALT is believed to be more diagnosis to hepatic injurys because it is primarily found in cytosole in the liver and elsewhere in low concentrations[21] , High concentrations for these enzymes are outcomes of destruction of hepatocytes and increased cellular permeability [22] Observed reduction in activity of all enzymes may indicate normal metabolism as well as improving liver function. In this line, liver enzyme activities are indicators of hepato-toxicity and liver function [23] . The present study, shows reductions in albumin concentration may be attributed to impaired hepatocyte synthesis ,as a result of oxidative stress and hepatitis, along with increased protein breakdown and kidney loss [24] , In this study ,its reported that animals which sever from nephrotoxicity which is caused by gentamicin when given Gum Arabic for purpose of treatment for 4 weeks , Table (4) notes significant decrease in serum AST,ALT,ALP, of male rat and this agree with [25] who reported that the gum Arabic has a protective effect on the liver through reducing the blood level of liver enzymes; aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) and through decreasing the oxidative stress.

Present study, revealed that the Gum Arabic significantly decreased the activity of liver enzymes and may play a protective role against liver dysfunctions in rats [26] , In mice and rats, GA has been act as protecting agent against hepatic and renal toxicities[27] ,The protective effects of Arabic gum are likely to be related to its anti-oxidant and anti-inflammatory and cytoprotective properties[28] .

Conclusions

The study concluded that administration of Gum Arabic to rats via drinking water for 4 weeks decreased the side effects of Gentamicin-induced nephrotoxicity by inhibiting free radical , and enhancing renal and hepatic functions .

Ethical Clearance : Taken from University of Kufa ethical committee

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Conflict of Interest : Nil

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Factors Related to Postnatal Care Utilization in Indonesia in 2018: An Ecological Study

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Abstract

Postnatal care is very crucial in the postnatal period because it is a critical period for babies. This study aims to analyze the factors related to postnatal care utilization (1st neonatal visits) in Indonesia. The ecological analysis was conducted using secondary data from the Ministry of Health of the Republic of Indonesia report in 2018. All provinces were taken as samples. Apart from postnatal care utilization, 5 other variables analyzed as independent variables were the ANC 1st visit, ANC 4th visits, poverty depth index, percentage of health centers with sufficient midwives, and the ratio of gynecologist per 100,000 population. The results show there was a tendency for a positive relationship between ANC 1st visit and 1st neonatal visit. The higher the ANC 1st visit coverage, the higher the 1st neonatal visit coverage ($p = 0.034$). Meanwhile, there was also a trend towards a positive relationship between the ANC 1st visit and the 1st neonatal visit. The higher the coverage of the ANC 4th visit, the higher the coverage of the 1st neonatal visit ($p = 0.040$). Moreover, poverty depth index, percentage of health centers with sufficient midwives, and the ratio of gynecologist per 100,000 population were found to have no significant correlation with 1st neonatal visits. It could be concluded that from the 5 independent variables analyzed, 2 variables were proven to be ecologically positively related to postnatal care utilization (1st neonatal visit), namely ANC 1st visit and ANC 4th visit.

Keywords: *ecological analysis, secondary data, postnatal care, antenatal care, mother and child health.*

Background

The neonatal period, or the first twenty-eight days of life, is the most vulnerable time for child survival. WHO reports that in 2018, 47% of all under-five deaths occurred in the neonatal period[1]. Meanwhile, Unicef in 2018 recorded the global average neonatal mortality rate of 18 deaths per 1,000 live births[2]. Early neonatal mortality is more closely related to factors related to pregnancy and maternal health, whereas late neonatal mortality is more closely related to factors in the newborn environment[3,4].

One of the ways to prevent neonatal death is to do neonatal death. According to the standards of midwifery care released by the Indonesia Ministry of Health, all babies born healthy must receive comprehensive care until the age of one month. This care can be obtained through three home visits, namely the 1st neonatal visit in the first 48 hours, the 2nd neonatal visit on the 3rd to the 7th day, and the 3rd neonatal visit after one week to the 28th day according to the needs of each family through a neonatal visit, it can be seen if there are complications in the baby so that they can be resolved immediately and if it is not possible to overcome them, they are immediately referred to a more complete facility so that they can get optimal care The neonatal visit is expected to reduce infant mortality[5].

Previous studies have informed that antenatal care (ANC) is a predictor of postnatal care. Mothers who do ANC well tend to also have postnatal care visits after giving birth[6,7]. Meanwhile, socioeconomic factors are

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also reported to affect postnatal visits to health workers. This is related to the ability to pay service fees[8]. Besides, the government must be able to guarantee the availability of midwives and gynecologists as the personnel who provide midwifery services.

Based on the background description, this study aims to analyze ecologically the factors associated with postnatal care utilization (1st neonatal visit) in Indonesia. The ecological analysis carried out in this study is important to provide clear guidance on policy targets for policy-makers to accelerate postnatal care coverage in Indonesia, so that it is expected to reduce infant mortality.

Materials and Methods

The study was conducted using an ecological analysis approach. Ecological studies focus on comparisons between groups, not individuals. The data analyzed is aggregate data at a certain group or level, which in this study is the provincial level. The variables in an ecological analysis can be aggregate measurements, environmental measurements, or global measurements[9,10]. The ecological analysis was conducted using secondary data from the reports "the 2018 Indonesia Basic Health Survey" and "the 2018 Indonesia Health Profile". Both reports are official reports from the Ministry of Health of the Republic of Indonesia. The unit of analysis in this study was the province. All provinces were included in the analysis (34 provinces).

The dependent variable in this study was "postnatal care utilization". Postnatal care utilization in this study was the first neonatal visit in a health care facility. Besides postnatal care utilization, there are 5 other variables analyzed as independent variables. The five variables are ANC 1st visit, ANC 4th visit, poverty depth index, percentage of health centers with sufficient midwives, and the ratio of gynecologist per 100,000 population. The poverty depth index was an indicator to measure the average gap based on the costs incurred by each poor population compared to the poverty line. A higher index value indicates the average expenditure of the poor moving away from the poverty line.

The analysis was carried out by bivariate using cross-tabulation and significance test with Spearman Rho. The entire analysis process utilizes SPSS 21 software.

Results and Discussion

Table 1 descriptive statistics of postnatal care utilization and related factors in Indonesia. Based on the coverage of the 1st neonatal visit, there is a very high gap between provinces. The highest coverage of the 1st neonatal visit in health care facilities was in the Province of East Nusa Tenggara (56.3%) and the lowest was in the Province of North Sumatra (13.6%).

Table 1. Descriptive Statistics of Postnatal Care Utilization and Related Factors in Indonesia in 2018

Descriptive Statistics	1st Neonatal Visit	ANC 1st Visit	ANC 4th Visits	Poverty Depth Index	Percentage of Health Centers with Sufficient Midwives	Ratio of Gynecologist
N	34	34	34	34	34	34
Mean	37.36	81.6559	67.2765	0.9106	3.5426	2.9348
Median	37	84.4500	67.2500	0.6800	2.5350	2.7642
Mode	25.10a	88.30a	65.10a	0.36a	0.00	0.14a
Std. Deviation	10.36582	8.24368	11.71699	0.77139	2.99496	1.67275

Cont... **Table 1. Descriptive Statistics of Postnatal Care Utilization and Related Factors in Indonesia in 2018**

Variance	107.450	67.958	137.288	0.595	8.970	2.798
Range	42.70	30.90	46.40	3.95	12.08	9.30
Minimum	13.60	63.50	43.80	0.11	0.00	0.14
Maximum	56.30	94.40	90.20	4.06	12.08	9.44

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Table 2 shows the cross-tabulation between the ANC 1st visit and 1st neonatal visit in Indonesia. It can be seen that based on the coverage of the 1st neonatal visit in the high category, the ANC 1st visit in the low category (18.2%) had a lower percentage than the 1st visit in the high category (45.5). This information shows a trend towards a positive relationship between ANC 1st visit and 1st neonatal visit. The higher the ANC 1st visit coverage, the higher the 1st neonatal visit coverage. The results of the correlation test also showed significant results between the two variables (p=0.034).

Table 2. Cross-tabulation between ANC 1st Visit and 1st Neonatal Visit in Indonesia, 2018

ANC 1st Visit	1st Neonatal Visit					
	Low (< 2.16)		Middle (2.16-3.21)		High (> 3.21)	
	N	%	N	%	N	%
Low (< 32.27)	6	54.5	3	25.0	2	18.2
Middle (32.27-44.00)	4	36.4	4	33.3	4	36.4
High (> 44.0)	1	9.1	5	41.7	5	45.5
Total	11	100.0	12	100.0	11	100.0

Source: The 2018 Indonesia Basic Health Survey

Table 3 provides the cross-tabulation between the ANC 4th visit and the 1st neonatal visit in Indonesia. It can be seen that based on the coverage of the 1st neonatal visit in the high category, the ANC 4th visit in the low category (9.0%) had a lower percentage than the ANC 4th visit in the high category (54.5). This information shows a trend towards a positive relationship between ANC 1st visit and 1st neonatal visit. The higher the coverage of the ANC 4th visit, the higher the coverage of the 1st neonatal visit. The results of the correlation test also showed significant results between the two variables (p=0.040).

Table 3. Cross-tabulation between ANC 4th Visit and 1st Neonatal Visit in Indonesia, 2018

ANC 4th Visit	1st Neonatal Visit					
	Low (< 2.16)		Middle (2.16-3.21)		High (> 3.21)	
	N	%	N	%	N	%
Low (< 61.37)	6	54.5	4	33.3	1	9.1
Middle (61.37-73.23)	5	45.5	3	25	4	36.4
High (> 73.23)	0	0.0	5	36.4	6	54.5
Total	11	100.0	12	100.0	11	100.0

Source: The 2018 Indonesia Basic Health Survey

This finding is in line with the findings in previous studies, which informed that mothers who did ANC visits performed more postnatal care than mothers who did not carry out ANC visits[11,12]. This is because mothers who complete ANC will get counseling from midwives to carry out postnatal care so that mothers who do antenatal care will be more motivated to carry out postnatal care than mothers who do not make ANC visits[7,13].

Table 4 shows the cross-tabulation between poverty depth index and 1st neonatal visit in Indonesia. It can be seen that based on the coverage of the 1st neonatal visit category high, the poverty depth index visit category low (45.5%) has a higher percentage than the poverty depth index category high (27.3%). This information shows the tendency of a negative relationship between the poverty depth index and the 1st neonatal visit. The higher the percentage of poverty depth index, the lower the coverage of 1st neonatal visit. However, the results of the correlation test also showed insignificant results between the two variables (p = 0.128). These findings differ from previous studies which inform that socioeconomic or wealth status is associated with postnatal care[8,14].

Table 4. Cross-tabulation between Poverty Depth Index and 1st Neonatal Visit in Indonesia, 2018

Poverty Depth Index	1st Neonatal Visit					
	Low (< 2.16)		Middle (2.16-3.21)		High (> 3.21)	
	N	%	N	%	N	%
Low (< 0.53)	3	27.3	3	25	5	45.5
Middle (0.53-0.98)	3	27.3	6	50	3	27.3
High (> 0.98)	5	45.5	3	25	3	27.3
Total	11	100.0	12	100.0	11	100.0

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Table 5 shows the cross-tabulation between the percentage of health centers with sufficient midwives and 1st neonatal visit in Indonesia. It can be seen that based on the coverage of the 1st neonatal visit in the high category, there is no trend pattern of the relationship between the two variables. Moreover, the results of the correlation test also showed insignificant results between the two variables (p=0.663).

Similar information is also shared in previous studies. The study informed that although the input ratio of midwives was still below standard, several provinces were able to show better performance in the MCH program than those with a ratio of midwives above the standard. The program performance analyzed in the study was coverage of ANC 1st visit, coverage of ANC 4th visit, neonatal visits, postpartum visits, and delivery assisted by health personnel[15].

The ratio of midwives per 100 thousand population according to the standard is an effort to ensure the availability of services for the community. However, these indicators still have to consider other factors. Indonesia has extreme topographic variability. This condition makes the situation in some underdeveloped areas in Indonesia not available for midwives[16]. Often women who give birth end up choosing a shaman, a traditional birth attendant, who is relatively more available and closer to the community[17–19].

Table 5. Cross-tabulation between Percentage of Health Centers with Sufficient Midwives and 1st Neonatal Visit and in Indonesia, 2018

Percentage of Health Centers with Sufficient Midwives	1st Neonatal Visit					
	Low (< 2.16)		Middle (2.16-3.21)		High (> 3.21)	
	N	%	N	%	N	%
Low (< 1.67)	3	27.3	5	41.7	3	27.3
Middle (1.67-3.98)	4	36.4	3	25.0	5	45.5
High (> 3.98)	4	36.4	4	45.5	3	27.3
Total	11	100.0	12	100.0	11	100.0

Source: The 2018 Indonesia Basic Health Survey

Table 6 shows the cross-tabulation between the ratio of gynecologist per 100,000 population and 1st neonatal visit in Indonesia. It can be seen that based on the coverage of the 1st neonatal visit in the high category, the ratio of gynecologists in the low category (30.0%) has a lower percentage of the poverty depth index category high (40.0%). This information shows a trend towards a positive relationship between the ratio of the gynecologist and the 1st neonatal visit. The higher the percentage of poverty depth index, the lower the coverage of 1st neonatal visit. However, the results of the correlation test also showed insignificant results between the two variables (p=0.431).

Table 6. Cross-tabulation between Ratio of Gynecologist and 1st Neonatal Visit in Indonesia, 2018

Ratio of Gynecologist	1st Neonatal Visit					
	Low (< 2.16)		Middle (2.16-3.21)		High (> 3.21)	
	N	%	N	%	N	%
Low (< 2.16)	4	36.4	3	27.3	3	30.0
Middle (2.16-3.21)	5	45.5	4	36.4	3	30.0
High (> 3.21)	2	18.2	4	36.4	4	40.0
Total	11	100.0	12	100.0	10	100.0

Source: The 2018 Indonesia Basic Health Survey

The availability of gynecologists in Indonesia is often only in hospitals. The availability is uneven, often accumulating in big cities. Provinces with many large cities tend to have better access to gynecologists[20]. This situation makes the gynecologist's ratio variable less sensitive.

Conclusions

Based on the research results, it could be concluded that of the 5 independent variables analyzed, there were 2 variables which are proven to be ecologically positively related to postnatal care utilization (1st neonatal visit). The two variables are the ANC 1st visit and ANC 4th visit.

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The Effectiveness of Cucumber (*Cucumis Sativus*) and Ambon Banana Fruit (*Musa Acuminata Cola*) in Reducing Blood Pressure in Hypertensive Patients in Palangga Gowa District, Indonesia

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Abstract

Hypertensive patients who are not adhering to treatment cannot control their blood pressure so it is important to find solutions that can stabilize blood pressure with food that is consumed and can be found anytime around them. **Objective**: This study was to identify the effectiveness of cucumber and Ambon banana fruit in reducing blood pressure in hypertensive sufferers. **Method**: This type of research is a Quasy Experiment with a non-randomized pretest and posttest control group design. Population of this study were hypertensive patient in Pallangga subdistrict and Sample participated were 24 people with hypertension selected by purposive sampling with the sample formula of unpaired numerical analytic research. **Results**: There were differences before and after treatment in the two groups, there was no difference in the effectiveness of the sample in the two same treatments. **Conclusion**: Cucumber and banana fruit are effective for lowering blood pressure.

Keywords*: Cucumber juice, Ambon banana fruit, hypertension.*

Introduction

Currently, modernization results in lifestyle changes in society, such as excessive eating habits, too much activity, smoking a lot, and lack of rest. These patterns and lifestyles cause increased heart and blood vessel disease, especially for people over the age of 40 years. One of them is the emergence of high blood pressure or what is often referred to as hypertension.[1]

Hypertension or better known as high blood pressure disease is a disorder that occurs in the circulatory system where blood pressure is above normal limits. Normal blood pressure is 120 mmHg for systolic and 80 mmHg for diastolic. A person is stated to have hypertension and is at risk for health problems if after several measurements, the blood pressure value remains high, >140 mmHg for systolic and >90 mmHg for diastolic.[2]

Hypertension is often called the "silent killer" because it does not provide characteristic symptoms. But it can increase the incidence of strokes, heart attacks, chronic kidney disease, and even blindness if not controlled and controlled properly.[3]

Nearly 1 billion people worldwide have high blood pressure. Hypertension is one of the leading causes of early death worldwide. In 2020 about 1.56 billion adults will live with hypertension. Hypertension kills nearly 8 million people every year in the world and nearly 1.5 million people annually in the East-South Asia region. About a third of adults in East-South Asia suffer from hypertension.[4]

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Based on data from the 2016 National Health Indicator Survey, the national prevalence of the population with high blood pressure is 30.9%. The prevalence of high blood pressure in women (32.9%) was higher than that of men (28.7%). The prevalence in urban areas was slightly higher (31.7%) than in rural areas (30.2%). The prevalence increases with age.[5]

Basic health research in 2018 showed that the prevalence of non-communicable diseases has increased compared to 2013, including hypertension. From the results of blood pressure measurements, hypertension rose from 25.8% to 34.1%. The increase in the prevalence of non-communicable diseases is related to lifestyle, including smoking, alcohol consumption and physical activity.[6]

Most cases of hypertension (90%) are primary hypertension, the cause is unknown. As a result of this, not all hypertensive patients need anti-hypertensive drugs. The more important treatment effort is to eliminate risk factors that are thought to be associated with the incidence of hypertension. In principle, there are two types of therapy that can be performed to treat hypertension, they are pharmacological therapy using drugs, and non-pharmacological therapy, by modifying daily life patterns and returning to natural products. Referring to the concept of back to nature, it's doing by using local ingredients that are widely available in the community, because these ingredients are rich in antioxidants and potassium in the form of fruit juices as an effort to reduce blood pressure in hypertensive patients as indicated by a blood pressure reduction graph. This research was conducted to see the effectiveness of the fruit juice in lowering blood pressure.[7]

One of these natural products is the cucumber (Cucumis sativus) which is widely available in the community. Cucumber has long been used as a traditional medicine that is useful for lowering high blood pressure. Cucumber is a type of vegetable that is commonly consumed by people to lower blood pressure. Previous studies have shown that there is a significant effect of cucumber juice on blood pressure reduction, the largest decrease occurred at 2 hours and after treatment on days 4 and 5 of cucumber juice. This fruit contains high levels of potassium and low sodium, making it suitable for consumption by people with hypertension.[8]

The substances in cucumber which can help lower blood pressure, including potassium (potassium), magnesium, and phosphorus are effective in treating hypertension. Apart from that, cucumber is also a diuretic because of its high water content which helps lower blood pressure.[9]

Bananas can lower blood pressure because they have Angiotensin Converting Enzyme Inhibitor (ACE-I) activity in the body. As the name implies, this substance inhibits the action of the angiotensin enzyme in the process of increasing blood pressure. Apart from ACE-I, bananas also contain high potassium and low sodium so it is good for people with hypertension. Consumption of two Ambon bananas every day, potassium-rich foods such as bananas can help lower blood pressure.[10]

Likewise, consumption of other fruits such as bananas can also lower blood pressure. One study shows that consumption of Ambon banana (Musa acuminata colla) is very effective in lowering blood pressure. The decrease in blood pressure is caused by Ambon banana contains high potassium and low sodium. Potassium helps maintain osmotic pressure in the intracellular space while sodium maintains osmotic pressure in the extracellular space so that high potassium levels can increase sodium excretion in the urine (natriuresis), thereby decreasing blood volume and blood pressure, but on the other hand a decrease in potassium in the intra-cell space causes the fluid in the intra-cell space tends to be attracted to the extra cell space and sodium retention due to the body's response to the osmolality of the two compartments at equilibrium but this can increase blood pressure.[10]

The prevalence of hypertension in South Sulawesi which was obtained through measurements at ≥18 years of age was 28.1 percent, the highest was in Enrekang (31.3%), followed by Bulukumba (30.8%), Sinjai (30.4%) and Gowa (29.2%). The prevalence of hypertension in South Sulawesi which was obtained through a questionnaire diagnosed by health workers was 10.3 percent, 10.5 percent diagnosed by health workers or currently taking medication, so that 0.2 percent were taking their own medication.[11] For Gowa Regency, hypertension is ranked 4th out of 10 diseases with a total of 975 with a prevalence of 5.02% in 2016.[12]

Seeing this fact, to provide a solution to stabilize blood pressure, several treatments will be given that do not cause boredom and are burdened by having to take medicine that sometimes they forget. The research objective was to identify the effectiveness of cucumber and Ambon banana in reducing blood pressure in hypertension sufferers.

Material and Method

This type of research is a Quasy Experiment with a non-randomized pretest and posttest control group design. Population of this study were hypertensive patient in Pallangga subdistrict and sample participated were 24 people with hypertension selected by purpossive sampling with the sample formula of unpaired numerical analytic research. This research performed by intervening all subjects based on their group with giving consuming Ambon banana and cucumber for each group. Both of interventions hypotesized could lower blood pressure in people with hypertension.

The research design used was non-randomized pretest and posttest control group. The study was divided into 2 treatment groups, namely the Ambon banana group and the cucumber group, each group consisting of 12 samples that were given treatment then carried out measurements. Each of which was given treatment in the form of giving 2 pieces of Ambon banana fruit per day in the first group while the second group was given 1 portion of cucumber juice as much as 50 grams per day.

Results

The subject characteristics of this study distributed in age group 45-54 years old were 7 subjects (29.1%), 55-64 years old were 10 subjects (41.6%), and >64 years old were 7 subjects (29.1%). In addition, this study also measured physical activity habits and smoking habits of subjects. In the physical activity habits, 17 subjects (70.83%) used to do gymnastic while 7 subjects (29.16%) used to do jogging everyday. In the smoking behavior, 4 of 24 subjects (16.66%), had been smoking while others not smoking.

Blood pressure of subjects after having 2 pieces of Ambon banana fruit in the first group and cucumber juice in the second group showed in Table 1.

Table 1. Blood pressure measurement result pre and post intervention for both groups

Blood pressure	n	Mean	Medium	SD	Min-Max	95%CI
Cucumber group						
Systolic						
Pre	12	157.50	150.00	20.05	140-190	144.75-170.24
Post1	12	143.33	135.00	17.75	120-170	132.05-154.61
Post2	12	137.71	140.00	7.53	130-150	132.71-142.28
Diastolic						
Pre	12	96.66	100.00	4.92	90-100	93.53-99.79
Post1	12	96.66	100.00	4.92	90-100	93.53-99.79
Post2	12	95.00	95.00	5.22	90-100	91.68-98.31
Blood pressure	n	Mean	Medium	SD	Min-Max	95%CI
Ambon banana fruit group						
Systolic						
Pre	12	155.83	150.00	13.11	140-180	147.50-164.16
Post1	12	155.83	130	13.78	120-170	125.40-142.92
Post2	12	142.50	140	10.55	120-160	135.79-149.20
Diastolyc						
Pre	12	94.16	90.00	5.14	90-100	90.89-97.43
Post1	12	87.50	90.00	4.52	80-90	84.62-90.37
Post2	12	93.33	90.00	4.92	90-100	90.20-96.46

Table 1 showed that the patient's mean systolic blood pressure decreased after intervening of cucumber juice from 157.50 ± 20.05 mmHg to 143.33 ± 17.75 after the first intervention and 137.71 ± 7.53 mmHg after the second intervention. Meanwhile, diastolic blood pressure remained persistent after the first intervention and decreased after the second intervention from 96.66 mmHg to 95.00 mmHg. The lowest value for systolic pressure reduction was obtained by 140 mmHg before the intervention decreased by 120 mmHg, while the lowest value for diastolic blood pressure was 90 mmHg and the highest was 100 mmHg. The estimation results of the 95% confidence interval showed that the patient's systolic blood pressure was 132.71-142.28 mmHg and diastolic blood pressure 91.68-98.31 mmHg after being given cucumber juice.

In the second group that was given intervention with Ambon banana, the patient's average diastolic blood pressure had not decreased after the first intervention with Ambon banana. After the second intervention, diastolic blood pressure decreased from 155.83 ± 13.78 mmHg to 142.50 ± 10.55. Meanwhile, diastolic blood pressure decreased after the first intervention from 94.16 ± 5.14 to 87.50 ± 4.52 and again increased after the second intervention to 93.33 ± 4.92 mmHg. The results of the 95% confidence interval estimation indicated that the patient's systolic blood pressure was 135.79-149.20 mmHg and diastolic blood pressure 90.20-96.46 mmHg after being given Ambon banana.

Inferential analysis was used to determine the effectiveness of cucumber and banana interventions in reducing blood pressure in hypertensive patients. The effectiveness of cucumber and Ambon banana in reducing blood pressure based on the results of this study can be seen in Table 2 below:

Tabel 2: The effectiveness of cucumber and Ambon banana in reducing blood pressure in hypertensive patients (n=12)

Blood pressure	n	Chi-Square	p
Cucumber group			
Systolic	12	1.474	0.479
Dyastolic	12	0.750	0.687
Ambon banana fruit group			
Systolic	12	7.610	0.022*
Dyastolic	12	12.286	0.002*

*Uji Friedman

Table 2 showed that the Friedman test results found p value = 0.479 indicating that there was no difference in systolic blood pressure before cucumber administration, after the first intervention and the second intervention. The same thing happened to diastolic blood pressure, there was no difference before giving cucumber, after the first intervention and the second intervention. This table also showed that in the group that intervened with Ambon banana fruit, the Friedman test results showed a value of p = 0.022, which means that there was a difference in systolic blood pressure before giving Ambon banana, after the first intervention and the second intervention. The same thing also happened to diastolic blood pressure, it was found p value = 0.002 which indicates that there was a difference before giving Ambon banana, after the first and the second intervention.

Discussion

The results of this study illustrate that the age group of respondents is mostly in the 55-64 years age group (41.6%) and for the two age groups 29.1% respectively. This study shows that older people tend to suffer from high blood pressure. The prevalence of hypertension increases with increasing age. This situation is caused by the increase in arterial pressure with age, the occurrence of aortic regurgitation, and the presence of a degenerative process, which is more frequent in old age. At the time of increasing age until they reach old age, there is also an increased risk of diseases including neurological / psychiatric disorders, heart and blood vessel disorders as well as reduced sensory function and metabolic disorders in the body.[13]

Isotonic sports, such as walking, jogging, swimming, can reduce hypertension. Exercise can reduce non-adrenaline hormones and other hormones that cause

blood vessels to shrink, which can lead to increased blood pressure. Conversely for people with hypertension avoid isometric exercises such as lifting weights, because they can increase blood pressure. In this study, respondents who had a habit of walking were 29.1%, while those who did exercise were 70.83%. This is because the *Prolanis* group (Chronic disease manajement program) in the Palangga Public Health Care Center has a program for this community, and the pedestrian group is a group formed since the respondent is still active in work until when he retires, this activity is still continuing.

Most of the respondents did not smoke (83.3%) and 16.6% of respondents smoked, this was found because the respondents were male smokers. From the results of previous research, it was known that patients who smoke tend to have high blood pressure, the nicotine in cigarettes can increase blood clots in blood vessels and can cause calcification of the walls of blood vessels.

The smoking habit for men is common place in this area so it is rare to find people who do not smoke, even though efforts to eliminate smoking have been made by the government. If there are men who do not smoke, it is likely that they have been banned by health workers because they are related to the illness they are suffering from. So that when we find subjects who smoke, it is because they feel that there is no health disorders so far with their smoking habits.

This study shows empirical evidence that there was a significant effect of giving cucumber juice and Ambon banana on reducing blood pressure, this is possible because cucumbers contain potassium, magnesium, and phosphorus, where these minerals can effectively lower blood pressure. Apart from that, cucumber is also a diuretic because of its high water content which helps lower blood pressure. Potassium is the main intracellular electrolyte, in fact 98% of the body's potassium resides in cells, the remaining 2% is outside cells. What matters is this 2% for neuromuscular function. Potassium affects the activity of both the skeletal muscle and the heart muscle. Bananas can lower blood pressure because they have Angiotensin Converting Enzyme Inhibitor (ACE-I) activity in the body. As the name implies, this substance inhibits the action of the angiotensin enzyme in the process of increasing blood pressure. Apart from ACE-I, bananas also contains high potassium and low sodium so it is good for people with hypertension.

The results of this study indicate that the consumption of both types of fruit can reduce blood pressure in patients with hypertension. Although statistically the decrease in blood pressure was not significant in the group given cucumbers, a significant decrease was found in the group that received Ambon banana.

Subjects who were treated were patients who did not adhere to pharmacological treatment, who sometimes did not take their medication, the need to take medication felt only when symptoms occurs. Referring to the results of this study, cucumber and Ambon banana juice can be recommended as food ingredients that must always be present in every family meal. Because of the mineral content present in both of these, increase their concentration in the intracellular fluid so that it tends to draw fluid from the extracellular and lowers blood pressure.

When seen in various previous studies that describe a decrease in blood pressure both systolic and diastolic, the effectiveness of cucumber juice on changes in blood pressure occurs with a fairly rapid onset, but the duration of cucumber juice is not that long. Likewise, giving Ambon banana fruit whose effectiveness is almost the same as cucumber juice in reducing blood pressure. No reference has been found that clearly describes the onset of action and duration of action. Therefore, this research can be continued by looking at how long the effect of cucumber and banana juice in maintaining blood pressure in hypertension sufferers.

Conclusion

From the results of the research that has been carried out, it can be concluded that there was a difference in the average blood pressure before and after being treated with cucumber juice and Ambon banana fruit in hypertensive patients. Significant results were found in the group that received the intervention with Ambon banana 2 fruit per day, while in cucumber juice there was a decrease in blood pressure which was not statistically significant. Cucumbers and Ambon bananas are recommended to be the fruit of choice in the diet of people with hypertension and are suggested to be part of the Public Health care center Program in implementing community movements with a family approach in their

environment, considering that these two fruits are local fruit products. It is recommended that the Public Health care center be able to launch a local fruit consumption program for health for all people in Palangga sub-district. The Prolanis group can be encouraged to replace carbohydrates as snacks with bananas or cucumbers.

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E-learning Challenges in Nursing Education during COVID-19 Pandemic-A Narrative Review.

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Abstract

Background: The COVID-19 pandemic has alarmed an urgent change in all spectra on the lives of people including education sector. **Objective**: This narrative review summarizes the challenges of online education in nursing and explores the possible opportunities to improve or maintain the standards of nursing education. **Methods:** A comprehensive literature search was done to identify the challenges of online education in nursing. Seven primary studies meeting the inclusion criteria, published from the onset of COVID-19 until 5th September 2020 were included in this narrative review. **Results:** The available literature shows that, students are missing the practical aspects of nursing care, the real essence of nursing education during e-learning, cancellation of international training, loss of job opportunities due to lack of confidence in their skill are bothersome for students and educators. Success of e-learning is also affected by internet connectivity issues, problem with electricity and lack of computer literacy. At the same time, it is found that online education delivery is substituting theory learning to a great extent, however practical training remains indispensable for nursing education. **Conclusion:** Continuous engagement and motivation from the part of both the students and nurse educators is very essential in meeting the educational needs during this crisis.

***Key words**: e-learning, nursing education, challenges, COVID-19 pandemic*

Introduction

According to World Health Organization (WHO) COVID-19 (Corona virus disease) dashboard, on 10th September 2020, the COVID-19 has affected 27,738,179 cases globally, including 899,916 deaths. [1]The global COVID-19 pandemic has inevitably changed our needs to be dependent on technology to stay connected to people, education, health care, and all spectra of daily life. Though a necessity, people are not literate enough with technology at the moment to meet this crisis.[2] The COVID-19 has a transformative impact on all dimensions of nursing including service, education, research and administration. Educational institutions including schools, colleges and universities across globe are struggling to meet the educational needs of their students during the lockdown period[3]. Social distancing and travel bans had replaced in campus education to a virtual mode. The rapid shift in teaching and learning to a virtual paradigm has not ceased the continuation of nursing education.[4]

Any learning that takes place across distance mode and not in the traditional classroom is called online learning. This online education system helps the students to utilize the learning opportunity without being physically there with the teacher and when this learning happens at learner's convenience it is known as asynchronous learning. [5] Education plays a vital role in preparing nursing students to work well and remain resilient during challenging situations including pandemic. [6]Certain students will readily accept e-learning due to its flexibility, whereas certain others will resist it due to their lack of computer literacy or

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infrastructure.[7] Students prefer face to face training over virtual learning,[8]and also some of them had a negative attitude towards e-learning.[9] High fidelity simulation training is very beneficial in training the student nurses non- technical skills like communication, empathy, appearance, active listening and teamwork.[4] Theory learning can be substituted to a great extend via online teaching, but students are missing the essence of nursing education that is competency training. [3,4] Hence special care should be taken by administrators and educators to ensure maximum learning among students.

Significance of the Review

In the light of COVID-19 crisis, most of the nursing educational institutions have started emergency virtual training in order to continue education to their students and not to lose their academic years. While shifting from the traditional teaching to completely virtual teaching and learning there are various challenges faced by the administrators, educators and the students. Though existed e -learning was supplementary earlier, whereas it has become the only resource available to students at the moment. It is an added challenge in the developing countries where complete online training was not a practice. Nurse Educators are required to upgrade themselves with use of technology, preparing e- modules, e-lessons and their evaluations, the students' needs to have computer literacy and infrastructure to access the e-learning and administrators needs to make sure that adequate online resources are mobilized to meet the educational needs of their students. Since COVID-19 pandemic is an uncommon catalyst in facilitating e-learning opportunity, it requires an understanding of the challenges of online education and exploring the opportunities to tackle those obstacles. This narrative review showed light to the challenges and opportunities of e-learning and would help the nursing institutional heads to enhance the use of e -learning as an essential educational tool rather than as an emergency one.

Methods

A comprehensive literature search was done in Google Scholar, PubMed/MEDLINE, EMBASE, science direct, Directory of Open Access Journals, Complementary Index, SCOPUS, and Cumulative Index to Nursing and Allied Health Literature. The selection criteria included the following: articles addressing COVID-19 and its impact on online education of student nurses, available in English for free download from the researcher's institutional health sciences libraries. The search terms used were e-learning and nursing education during COVID-19, online education and nursing, virtual training and nursing, and challenges of e-learning in nursing education.

A total of 19 articles were identified independently by the researchers, after 5 duplicates were removed, 14 articles were included for title and abstract screening. Seven articles were further excluded either due to inadequate information, full text not available in English or not addressing challenges of nursing education. Seven primary studies meeting the inclusion criteria, published from the onset of COVID-19 until 5th September 2020 were included in this narrative review.

Two Qualitative studies with inductive thematic analysis and 5 descriptive surveys were analyzed to understand the e-learning challenges in nursing education during COVID-19 crisis. The studies were from various geographic locations like Spain (2), Croatia (2), Philippines (1), Egypt (1), and Nepal (1). The quality appraisal of qualitative studies is assessed by using a tool developed by Hawker S, et.al,2002[18]. This tool contains nine questions and every question has scores ranging from 1(very poor) to 4 (good) which makes a total of 9 to 36. The scores from 30-36 is of high quality, 24-30 is of moderate quality and 9-24 is low quality. The qualitative studies included in this review are of moderate quality.

The quality appraisal of descriptive studies is assessed by using STROBE checklist. It has a total of 22 items; title and abstract (item 1), introduction (item 2and 3), methods (item 4-12), results (item 13-17), discussion (18-21) and item 22 on funding information. 18 items are common to all type of observational studies and 4 items have subcategories to specific categories of observational studies. If a specific item is mentioned, it is marked as 1; and if not mentioned it is marked as 0. Average reporting percentage of 70% and above under each category is considered sufficiently reported. The included studies here had an average reporting percentage of 70. The included articles were addressing various challenges either from students or from faculty point of view. Hence a narrative analysis of the available literature is done and presented here.

Results & Discussion

The search results have shown very limited evidence on challenges of e- learning to nursing education during COVID-19 Crisis. Hence the researchers tried to analyze the available evidence from the seven studies which met the inclusion criteria. More research studies exclusively in the field of nursing to be done in order to understand the challenges from the perspectives of faculty, students and administrators. The main challenges to e-learning in nursing education, identified through the literature search is summarized as table1.

Table1: Summary of the included studies

Sl NO	Study ID	Country	Research Method	Sample	Study duration	Significant challenges identified
1	Morcillo R, et.al (2020)4	Spain	Qualitative	32	25th March to 20th April 2020	lack of competency training
2	Rodríguez D J, et.al 20206	Spain	Mixed method	93	2nd April to 21st May 2020	Technical issues with virtual learning and lack of technical skill learning with simulation.
3	Gehan M A E et.al 202010	Egypt	Descriptive correlational	627	1st April 2020 until the end of May 2020.	lack of infrastructure and technology, and non-availability of instructor online.
4	Lovric R et.al 20209	Croatia	Qualitative	33	13th March 2020	lack of motivation, poor concentration and other significant learning difficulties.
5	Subedi S 202013	Nepal	Descriptive	1012+104	Not specified	Poor internet access, and electricity issues.
6	Cacayan EB et.al, 202015	Philippines	Descriptive	15	Not specified	inadequate training and preparation time for online teaching, and headache and eye strain due to long term online instruction.
7	Puljak L et.al 202016	Croatia	Cross sectional	2520	28th April to 11th May 2020	Insufficient classroom lessons, and in person communication with the teachers.

Table 1 shows the summary of the included studies highlighting the significant challenges of e-learning in nursing education. Most of the students were concerned about the practical aspects of nursing care in online education, and they are missing the competency training which is the essence of nursing education, and some of them felt a real waste of their time and loss of job opportunities due to lack of confidence in their skill. [4] Students reported technical issues with virtual learning and lack of technical skill learning with high fidelity simulation.[6] Students had expressed a negative attitude towards e-learning.[10]The most common obstacles faced by nursing students were lack of infrastructure and technology, followed by technical support and non-availability of instructor online.[10] Students experienced various problems like lack of motivation, poor concentration and significant learning difficulties during e- learning, and found teachers are effectively implementing the online education.9 Some other challenges faced by the student nurses includes not having full internet access, having electricity issues, not familiar with joining the session, recording the session and submission of online assignments,[13] and difficulty to comprehend certain activities due to lack of contact with their instructors. [15]Half of the students expressed that they miss the classroom lessons, and in person communication with the teachers, more than half of them believe that e-learning cannot compensate the missing practical hours of training and direct involvement in patient care. [16]

Among the nursing teachers 93.3% were glad to be updated with use of technology, and thought that online class allow them to save traveling time and spend time with family and reduce accidents.[13] The faculty challenges also includes inadequate instructor training for online teaching, limited time for the preparation of instructional modules, exams and other activities related to student training, and some faculty are experiencing headache and eye strain due to long term online instruction.[15]

When a student gets exposure to COVID-19, they risk themselves, their family, other students, teachers and patients. Hence it is very essential to carefully design students' educational activities during this unusual and difficult times. [11]

E-learning can be a useful method in continuing the preparation of graduate nurses, however there are challenges in the delivery of e-learning and a constant need of quality improvement. There are a few studies available addressing the challenges of e-learning exclusively in nursing education. The researchers have attempted to summarize the available findings as challenges and opportunities in implementing e-learning in nursing education. A total of 8 studies (2 qualitative and 6 quantitative studies) were included in this review.

One of the main challenges in implementing e-learning is technical difficulties such as lack of technical support, poor connectivity and outdated computer systems. Older students and faculty had more struggles with online education due to their lack of confidence and less empowerment in using the technology. [2] In this review we found that not having full internet access, having electricity issues, and not familiar with joining the session, recording the session and submission of online assignments were some of the challenges expressed by the undergraduate nursing students in Nepal.[13]

Challenges affecting the online education in the middle east includes poor internet connectivity, lack of public appreciation for online education and insufficient online repositories. [10] Moreover student complained that, over domination of assignments, unstable and costly internet connection makes e-learning highly challenging.[14] In a descriptive survey among nursing faculty revealed that they were getting disturbed due to the students joining and leaving the session during the lecture, eye problems, headache and also anxiety related to connectivity issues during the lecture. [13]

Though simulation is identified as a powerful alternative to real clinical training, the effectiveness of simulation depends on various factors. This review also supports the fact that factors like individual skill levels already achieved by the students, the quality of simulation experience provided, degree of student's involvement in the scenario, and the scenario drafted for the particular learning experience etc. can affect the success of the simulation experience. [12]Although the course may be completed the students may not achieve all the objectives of the course through e learning.[13]

Student engagement during the e learning is a constant challenge to the educators. This review also supports the fact that though teachers are taking effort to implement e-learning successfully, students experienced lack of motivation, poor concentration and significant learning difficulties during e-learning[9]. Another descriptive correlational survey also supports this finding, where more than 50% of the undergraduate nursing students had a negative attitude towards e-learning in general. [10]

e-learning challenge was also identified as an opportunity to transform traditional teaching and learning in nursing education. The flexibility of e-learning approach, that is anyone with an internet connection can access it at any time, from anywhere and the use of asynchronous mode of teaching during online sessions available at learner's convenience; makes it more convenient over traditional teaching and learning. [7]

As clinical areas were struggling accommodate the increasing number of students, transition to online learning with necessary time table changes were quick during the crisis. Some institutions have moved the theory block of next semester and postponed all the clinical courses to the next semester, and some other have put a hold on clinical placement, with no clue on when it could be resumed, whereas some continued with minor adjustments to the delivery of clinical learning [12]

In a descriptive survey done among nursing faculty, about 93.3% were glad to be updated with use of technology, and thought that online class allowed them to save traveling time, spend time with family and deliver the teaching material at the same time. [13]

Recommendations to overcome the challenges:

Arrange a formal training for faculty and students on various e-learning platforms to facilitate their effective use during virtual teaching and learning process.[17] Revise the course syllabus to facilitate effective implementation of online lessons. Utilize multiple techniques during e-learning sessions to keep the students motivated like online discussion, game-based learning, interactive learning etc.

Conclusion

Based on the literature reviewed, we conclude that most of the institutions had adopted online mode of training for their students. Factors such as lack of infrastructure and computer literacy either from the student or from the faculty can hinder the use of e-learning as an essential tool, especially in developing countries. Although e-learning in nursing education is substituting theory learning to a great extent, practical training remains indispensable for students. The present uncertain nature of COVID-19 pandemic is mandating the nurse educators today to propose durable distance training strategies which would maximize the critical thinking and clinical skills learning of students in the upcoming academic semesters. Continuous engagement and motivation from the part of both the students and nurse educators is very essential in meeting the educational needs during this crisis.

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Alveolar Bone Density after the Orthodontic Treatment in Patients of Rsgm Universitas Airlangga

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Abstract

Background: There is a relation between the orthodontic therapy and the periodontal tissue. In the moving teeth and planning treatment, the periodontal tissue is very influential in an orthodontic therapy. During the movement of the teeth, all components of the periodontal tissue such as the bones, periodontal ligaments, and gingiva will change and move together. Some studies indicate a decrease in alveolar bone fraction and alveolar bone density. The changes in density are seen by comparing the new bone mineralization with the old bones. **Purpose:** This research aims to analyze the differences in alveolar bone density after an orthodontic treatment in patients of RSGM Universitas Airlangga. **Methods:** This study used a patient's panoramic radiograph at the time before the orthodontic treatment and the time after it. Photographs of panoramic radiographs were measured using a digital densitometer. **Results:** The results of the study revealed a decrease in alveolar bone density after orthodontic treatment, both anterior and posterior. The average decrease in the anterior was 35.4%, while in the posterior was a decrease of 31.5%. Conclusion: There was a decrease in the alveolar bone density after orthodontic treatment in the anterior and posterior sections.

Keywords: *Bone Density, Orthodontic Treatment, Panoramic Radiography, Remodeling*

Introduction

The community awareness of the orthodontic treatment is increasingly high and becoming popular in the dentistry [1–4]. This is based on the desire to improve facial appearance, the position of the teeth, as well as the jaw relations[5]. Orthodontic treatment has an influence on the periodontal tissue, such as inflammation, changes in tooth root length, alveolar bone resorption, and changes in gingival structure. In some cases orthodontic therapy can improve gingival health such as removing black triangle in the interdental area [6].

Orthodontic treatment is based on the principle that if a pressure is applied to the teeth for a certain period of time, a tooth movement will occur because the periodontal ligaments and bones around the teeth change. When a pressure is applied to the crown of the tooth, the pressure will cause the other pressure and strain areas in the alveolar bone and periodontal ligament. In the area of pressure, there will be bone resorption in response to this pressure. Whereas in the strain area, the bone apposition will occur to maintain the integrity and mechanism of the teeth attachment to the bone. As a result, the socket of the tooth will move along with the movement of the tooth through the alveolar bone. In a study indicates a change in the alveolar bone density. That changes in the density are seen by comparing the new bone mineralization with the old bones [7,8].

Several methods are available for measuring the bone density. Magnetic resonance imaging, ultrasound, computed tomography, Dual energy X-ray absorptiometry (DXA), and densitometry radiography have been used for the medical evaluation [9]. Panoramic and periapical radiographs are one of the conventional densitometry methods. It can be measured with digital densitometers or can be digitized and processed using special software programs for the digital radiography. Using conventional film, the bone density measurement has several clinical benefits, such as low dose exposure,

cheap, easily available, and not invasive [10].

Research on the alveolar bone density after orthodontic therapy is rarely done and the orthodontic therapy requires multidisciplinary knowledge to obtain the maximum treatment results. This is because the moving teeth will affect the condition of periodontal tissues, especially the alveolar bone. Based on this fact, this research aims to analyze the differences in the alveolar bone density after the orthodontic treatment in patients of RSGM Universitas airlangga.

Methods

The type of research used was observational analytic with a retrospective approach. With studies comparing radiographs before orthodontic treatment and radiographs after it by a measurement using a digital densitometer. The population in this study was panoramic radiographs of patients who had completed fixed orthodontic treatment in January-May 2018 at the RSGM (Hospitals Teeth and Mouth) Universitas Airlangga. The selection of samples using the secondary data from the panoramic radiographs of patients before and after the treatment with criteria for treatment, and the 3 years duration of treatment and have a panoramic radiograph from the Parahita lab. Those 20 samples radiographic were obtained before and after the treatment that matched the criteria.

Panoramic radiographs of the patients who fit the criteria are collected. The radiograph was measured in the anterior and posterior regions of the mandible. First, the measurements are taken in the anterior region before and after the treatment. The second one is the measurements on the alveolar bone radiographs between the anterior teeth, from the left canine to the right canine. Afterward, measuring the density using the digital densitometry then calculating the average results to obtain the average anterior bone density value.

The measurements then are carried out in the posterior region before and after the treatment. The next one, the measurements on the alveolar bone radiographs between posterior teeth from canines to permanent second molars of patients both on the left and right. Measured density using digital densitometry, then performed an average calculation of the results to obtain the posterior average bone density value. The data that has been obtained from the measurement results are carried out by the normality test using the Kolmogorov-Sminov test which then tested by the T-Test to distinguish the difference in the density on the sides.

Results

The results of measurements on 20 panoramic radiographs before and after fixed orthodontic treatment at the RSGM Universitas Airlangga. The results of the study revealed a decrease in the alveolar bone density after orthodontic treatment, both anterior and posterior. The average reduction in the anterior was 35.4%, while the posterior decrease was 31.5%.

Table 1. Mean Value and Standard Deviation of Alveolar Bone Density Data

Bone Density	Standard Deviation
Anterior Before Treatment	1,0644 ± 0,2368
Anterior After Treatment	0,6871 ± 0,2891
Posterior Before Treatment	1,207 ± 0,2157
Posterior After Treatment	0,8267 ± 0,2728

The research was tested using Kolmogorov-Smirnov Test with the result of all groups have a value greater than 0.05. It can be concluded that the data are normally distributed. Afterward, the statistical test during before and after the treatment were conducted using paired T-Test at the significance level of sig less than 0.05. It can be concluded that there are significant differences in the bone density between before and after treatment. In addition, it then continued by the statistical test for anterior and posterior density using paired T-Test at the significance level of sig more than 0.05. The result can be concluded that there are no significant differences between the anterior and posterior density.

Discussion

Movement of the teeth in the area of pressure will cause another pressure on the periodontal ligament and on the reverse side strain. When given a mechanical force it will affect bones and periodontal. These mechanical forces also influence the tissue vascularization and blood flow. It then also affects molecular release, such as neurotranmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic acid metabolism [11].

The mechanical force applied will cause hygiene of the periodontal ligament and cause resorption of the alveolar bone. Resorption in the alveolar bone is caused by osteoclast activity. This is initiated by the inflammatory cells such as interleukin-1 beta (IL-1β), PGE2, interleukin-6 (IL-6), and the other ones. These cells will regulate osteoclastic activity from activation of RANK - RANKL. Osteoblast cells also regulate osteoclastic processes by synthesizing RANKL. In the strain area new bone growth occurs by osteoblasts. That osteoblasts differentiate the local precursor cells, namely, mesenchymal stem cells. These activities are called bone remodeling and affect the density of the alveolar bone [11].

The density of the alveolar bone can be measured and analyzed through various methods. In several studies used radiographic photographs to determine alveolar bone density by computerized methods. There also used radiography photos to determine the bone density from the mandible which correlated with BMD of skeletal bone. Several methods are available for measuring the bone density. For example, magnetic resonance imaging, ultrasound, computed tomography, Dual energy X-ray absorptiometry (DXA), and densitometry radiography. All of that have been used for the evaluation in the fields of medicine and dentistry [12,13].

In this study, the measurement of panoramic radiographs of patients before and after the treatment using digital densitometer was used. A research stated that there was a correlation from the measurement of the bone density using radiographic films by computerization and digital densitometer. Using conventional film, the bone density measurement has several clinical benefits such as low dose exposure, cheap, easily available, and not invasive measures [10].

However, the panoramic radiographs have several lacks, including the presence of images that overlap with the other structures. Afterward, there is also an experience enlargement and distortion when compared to the actual size. That enlargement of panoramic radiographs can be >30%, especially when the patient is not in the optimal position. There is an error from the operator or patients that may have effects as well as affect the quality of the panoramic radiograph. Panoramic radiography also has low contrast and detail so that it can affect the density measurements [14,15].

In the several studies using CBCT radiographs to determine changes in the bone density occurring during orthodontic treatment. The advantage of using CBCT compared to panoramic radiographs is obtaining a picture of the 3 dimensions and density of each bone structure. However, in some developing countries, an examination is rarely done using CBCT as a result of low socio-economic conditions. In addition, CBCT also provides high exposure doses compared to panoramic radiographs [7,8,16].

Decreasing bone density can affect the success of orthodontic treatment. The use of a retainer will provide maximum results in the orthodontic treatment. The use of a retainer aims to maintain stability and prevent relapse because the gingiva and periodontal tissue tend to return to their original position and require time to reorganize when the orthodontic device is removed. A study was found that during the active orthodontic treatment, there was a significant decrease in the bone. However, after being given the use of a post-retainer retainer, there was an increase in the bone density in these patients [14]. Giving bisphosphonates can also improve the stability

of post-orthodontic treatment. The bisphosphonates can inhibit osteoclastic activity which stimulates new bone formation. However, the prolonged administration of bisphosphonates can cause osteonecrosis of the jaw [17].

Orthodontic treatment in patients with periodontal abnormalities will affect the capacity of the alveolar bone to regenerate the tissue. A study stated that fixed orthodontic treatment would maintain bone height, but was unable to maintain the bone density for patients with periodontitis. The study showed that changes in the bone density may be more susceptible than changes in the bone height of periodontitis patients. Further research is needed regarding fixed orthodontic treatment in undetected patients in the regeneration capacity of the periodontal tissue or in patients with periodontal abnormalities [18].

Conclusion

Based on the results of the measurements of alveolar bone density before and after orthodontic treatment on panoramic radiographs using a digital densitometer, we found a decrease in the bone density of 35.4% in the anterior, meanwhile in the posterior is 31.5%.

Ethical Clearance: This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

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Prosthodontic Management of Flabby Ridge with a Modified Window Impression Technique – A Case Report

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Abstract

The performance of a complete denture is often a reflection of its support and retention of the underlying hard and soft tissues. If the superficial aspect of alveolar ridge is compressible more than 2mm, this will result in a mobile soft tissue (flabby tissues). This occurs when hyperplastic soft tissue replaces the alveolar bone. During conventional impression procedures, the flabby tissues are compressed and will later tend to recoil and dislodge the resulting overlying denture. This article aims to presents a case report for the prosthodontic rehabilitation of a patient with a flabby tissue with a modified window impression technique.

Keywords: *Flabby ridge, Impression technique, Metallic denture base, Modified window impression.*

Introduction

The ultimate goal of any prosthodontic treatment is restoration of the aesthetics, comfort and function of the missing dentoalveolar and its supporting structures. The performance of a complete denture is often a reflection of its support and retention of the underlying hard and soft tissues.[1] A thickness of 1.5-2mm of masticatory mucosa of adequate soft tissue is required to support the denture. A complete denture master impression should record the entire functional denture-bearing area to ensure maximum retention, stability and support for the denture.[2] However this is difficult to accomplish in the case of a flabby ridge. If the superficial aspect of alveolar ridge is compressible more than 2mm, this will result in a mobile soft tissue. This occurs when hyperplastic soft tissue replaces the alveolar bone.

This is a common particularly in the upper anterior region of long term denture wearers. According to literature reviews the prevalence of flabby ridges can vary, occurring in up to 24% of edentate maxillae and 5% in the edentate mandibles.[3,4]

A common finding in combination syndrome is the presence of flabby ridges in the anterior maxilla. The flabby ridge was thought to occur as a result of a maxillary complete denture opposing mandibular anterior natural teeth, without proper posterior occlusal support. Flabby tissues could also result from unplanned or uncontrolled dental extractions. A variety of techniques have been suggested to circumvent the difficulty of making a denture to rest on a flabby ridge. [5,6]

During conventional impression procedures, the flabby tissues are compressed and will later tend to recoil and dislodge the resulting overlying denture. Hence, the need for a special impression procedure to obtain stability, support, retention. This article aims to presents a case report for the prosthodontic rehabilitation of a patient with a flabby tissue using a modified window impression technique.

Technique report:

A 45 year old male reported to the Department of Prosthodontics at Thai Moogambigai Dental College and Hospital with a chief complaint of a loose and fractured maxillary complete denture. On intra-oral examination, it was noted that there was a flabby tissue present on the anterior maxillary region. In the mandible, the incisors were missing. The treatment plan for this patient was to provide an upper complete denture with a metallic denture base using a modified window

impression technique and a removable partial denture in the mandible.

The technique is as follows :

1) The primary impression was made with a perforated stock trays using an irreversible hydrocolloid impression material for the maxilla and mandible. (Figure 2)

A primary cast was poured and the wax spacer design was outlined using a haematoxylin pencil. (Figure 3)

2) Wax spacer design: An even layer of baseplate wax was adapted 4mm short of the sulcus around the entire denture bearing area except the PPS region. Tissue stops were provided in the canine and molar region bilaterally. An additional layer of baseplate was adapted in the flabby tissue region. On top of that, four additional evenly spaced wax extensions of 4x4mm were adapted in that region. (Figure 3)

3) A special tray was fabricated using a light cure sheet (poly tray DELTA). (Figure 3)

4) Secondary impression procedure: Border moulding was accomplished using heavy body impression material (virtual refill heavy body regular set tray material IVOCLAR VIVA DENT). Following border moulding, the entire wax was scooped out and relief holes were provided in the mid palatal region using a straight fissure bur. The wash impression was made using monophase impression material (Aquasil Monophase Impression Material Dentsply). The impression material extending on the anterior flabby tissue region was removed using a number 11 BP blade. The tray was then reinserted inside the patients mouth and the anterior flabby ridge was recorded by injecting light body impression material in the prefabricated slots on the special tray. (Figure 4)

5) A master cast was obtained after beading and boxing of the impression. (Figure 5)

6) A Metal denture base was fabricated on the master cast. (Figure 6)

7) Occlusal rims were made and the jaw relation was done along with a facebow transfer. (Figure 7)

8) The maxillary and mandibular casts were then mounted in a semi-adjustable articulator (Whipmix Hanau wide-vue arcon articulator). (Figure 7)

9) Teeth arrangement was done followed by a wax try. (Figure 8)

10) The maxillary and mandibular dentures were then processed and inserted. (Figure 9)

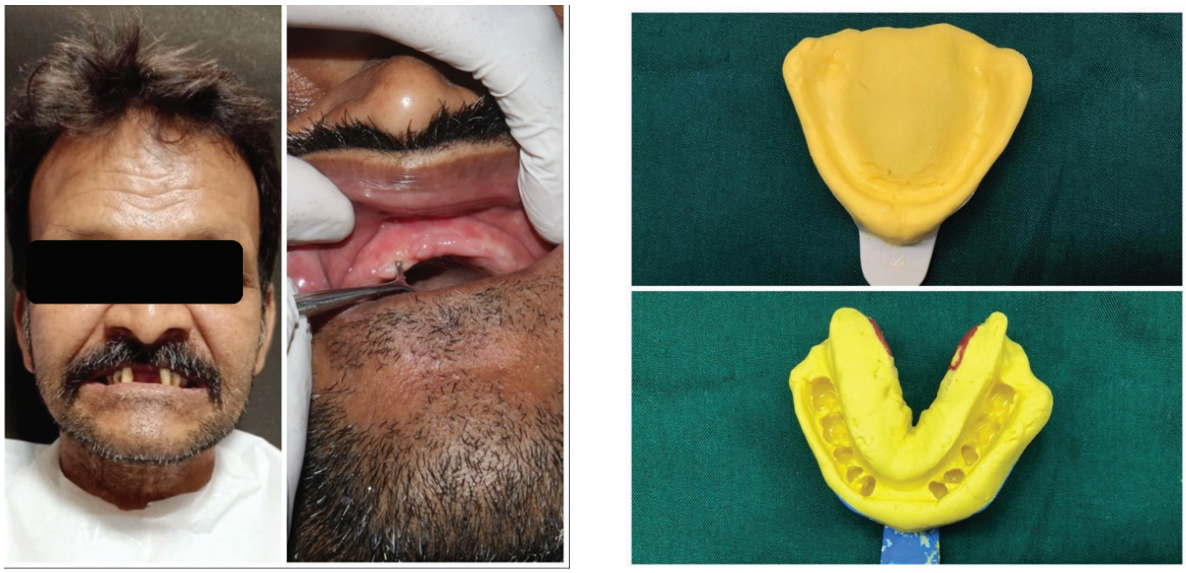

Figure 1: Pre-Operative phot,

Figure 2: Primary impression of intra-oral view of flabby tissue. maxilla and mandible.

Figure 3: Special tray design.

Figure 4: Secondary impression

Figure 5: Jaw relation and facebow transfer.

Figure 6: Teeth arrangement , Wax try in and. Denture insertion.

Discussion

Impression making plays a critical role in complete denture fabrication. The presence of a flabby tissue in the denture bearing area could present with problems. When utilizing a conventional impression technique to record such flabby tissues could often result in an unretentive and unstable denture. The Flabby ridges are compressed during conventional impression procedures. The elastic recoil of flabby fibrous tissue in its rest state results in the instability and loss of retention finally causing denture dislodgement. Hence, the need for an impression technique to record the flabby tissue in its anatomic form while recording the rest of the tissues in its functional form.

Several impression techniques and methods have been described for recording the flabby tissue during impression making. However, it is not proven to support that one particular impression technique will provide a stable and retentive denture on flabby ridges as compared to others.

Liddlelow et al[7] described a technique wherein two different impression materials are used in a custom tray ('plaster of Paris' over the flabby tissues, and zinc oxide and eugenol over the 'normal' tissues).

Osborne et al[8] described a technique where two separate impression trays and materials were used separately to record the 'flabby' and 'normal' tissues which is then related intraorally.

Watson et al[9] described the 'window' impression technique where a window or an opening is made over the flabby tissues on a custom tray. A mucocompressive impression is made using the custom tray and zinc oxide eugenol. Once set, it is removed, trimmed, and re-seated in the mouth. Through the window impression plaster is painted onto the flabby tissues. Once set, the entire impression is removed. However, special care needs to be taken when pouring a cast as the master impression is made with impression plaster.

Watt and McGregor[10] revisited by Lynch and Allen[11] described a technique where impression compound is applied to a modified custom tray. The impression compound is then manipulated to simultaneously compress the 'normal tissues', while avoiding displacement of the 'flabby tissues' using the same material and impression tray. A wash impression with zinc-oxide eugenol is made over this manipulated impression compound.

The advantage of our technique is that, there is no need to provide a window in the flabby tissue region. This design is incorporated during fabrication of the special tray itself thus, decreasing the clinical work time. The partial window design in this special tray supports the impression material. In addition, the slots provided in the anterior region and shows us exactly how much of impression material needs to be injected thus providing us a proper control and more patient comfort.

Prosthodontic management of flabby ridges are used in patients with medical conditions that do not allow for dental implant therapy or those who deny undergoing surgical intervention for correction of flabby tissue.

In this case, a metallic denture base was used for the maxillary complete denture. This is due to the fact that the patient presents with opposing natural teeth causing increased amount of amount of forces to be generated which could have been the causative factor for the patients previous denture to fracture. Hence, we planned to use a metallic denture base for this case.

Conclusion

This case report has described an innovative impression technique for the prosthodontic management of a denture bearing area containing flabby tissues. The materials used in this method are readily available and used in contemporary general dental practice. The time required for this specialized impression technique is not excessive. We conclude that this impression technique provides a stable and retentive denture for a flabby ridge.

Source of Funding- Self.

Conflict of Interest – Nil.

Ethical Clearance- Taken from Institutional Ethical committee M.G.R Educational and Research Institute.

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Molecular Detection of bla(SHV), bla(IMP) and bla(VIM) β-lactamases genes among *Klebsiella pneumonia* strains

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Abstract

Spreading of antibiotic resistance genes producing bacteria among patients usually caused potential infections for human with high morbidity and mortality especially *Klebsiella pneumoniae* and the fast development of Multi-drug resistant (MDR) strains has significantly increased and can be quite problematic. Thus, the current study was aimed to determine the prevalence of antibiotic resistance genes (β-lactamase) among *K. pneumoniae* isolates. Methods: Fifty K. pneumoniae isolates were collected from sputum samples for patients suffered respiratory infection. The detection of resistance genes (blaSHV, blaVIM, bla IMP) was performed by polymerase chain reaction (PCR). Results: The frequency of β-lactamase-positive K. pneumoniae was 28%, and the prevalence of the blaSHV, blaVIM and blaIMP genes was 100%, 0% and 0% respectively. Conclusions: These results β-lactamase genes believing multi-drug resistance genes. This help to decrease treatment problems as a result of resistant bacterial infections and prevention of transmission of drug resistance by rapid discovery infection-control programs.

Keyswords: *antibiotic resistance genes; blaSHV ; β-lactamase*

Introduction

Klebsiella pneumoniae is one of the most important pathogenic bacterium; it is a Gram negative rod in the Enterobacteriaceae family that cause various infections with high mortality and morbidity due to hospital-acquired infections and non-hospital-acquired infections [(1)]. That ranging from pneumonia, urinary tract infection (UTI), burns, surgical site, bloodstream infection and meningitis [(2,3)].

The most strains of *K. pneumoniae* can degrade a wide range antimicrobials classes such as penicillins, carbapenems, cephalosporins, cephamycins, fluoroquinolones and aminoglycosides by various enzymes called Extended-Spectrum Beta-lactamases Enzymes (ESBLs) [(4 ,5)]. These enzymes can be divided into four classes (A, B, C, and D) based on the Ambler classification[(6)].

On the other hand, these bacteria lead to difficult to treat diseases and called multidrug-resistant (MDR) bacteria [(7)]. This MDR bacteria and ESBL producing *K. pneumoniae* and other Gram-negative bacteria caused worldwide problems regarding the choice of effective antibiotic treatment hospital-acquired infections that high degree of prevalence in both hospitals and community [(8)].

Antimicrobial resistance is commonly related to the spread of transmissible plasmids [(9)]. The β-lactamase encoding plasmids, transportable genetic elements such as integrons can also contribute to the evolution and distribution of MDR genes (*blaSHV, bla*VIM, *blaTEM*, *blaCTX-M*, *bla*VEB, *bla*PER, *bla*GES, *bla*IMP, *bla*OXA, and *bla*KPC) in *K. pneumoniae* by vertical or horizontal transmission [(1011)].

The methods detected of ESBLs are not fully recognized by many clinical laboratories; therefore, there may be a lack of sufficient means in laboratories to decrease the spread of these mechanisms of resistance [(12)]. Thus, detection β-lactamase genes by molecular techniques in resistant bacteria could provide important data regarding high risk factors and infection epidemiology [(13, 14b, 15)].

Thus, the aim of the present study was to determine the prevalence of *bla*SHV*, bla*VIM and *bla*IMP, among

K. pneumoniae strains isolated from patients with suspected respiratory infections, Teaching Al-Hussain hospital in Nasiriya by polymerase chain reaction (PCR).

Methodology

Sample collection:

Fifty sputum samples were collected from patients suffering from a respiratory infection in Al-Hussain teaching hospital in Nasiriya city, Iraq during 2019. All samples transported to microbiology laboratory in medicine college for bacterial isolation and identification. All samples were cultured on MacConkey and blood agar plates and incubated for 24 hours at 37°C according to standard procedure [16]. Identification of isolates based on the morphology of colonies and by biochemical tests according to [17].

DNA extraction of bacteria genome:

The first step for extracted bacterial genomic DNA for each *K. pneumoniae* isolates by suspending a 1 µL loop-full of freshly cultured cells in 200 µL of sterile D.W. The suspension was incubated at 95°C for 5 min and complete other steps by DNA extraction kit according to manufacturer's instruction. The extract DNA was checked by nanodrop spectrophotometer and store in deep freeze until usage as template DNA for PCR [18].

Polymerase Chain Reaction (PCR) Amplification:

Antibiotic resistance genes (bla SHV, bla VIM and bla IMP) were determined using specific primers (Table 1). All primers used in this study and all PCR thermocycling conditions used for amplification of antibiotic resistance genes in the *k. pneumoniae* strains are listed in Table 1 , 2 and 3 respectively.

Table 1: Oligonucleotide primers sequences of target genes for PCR amplified

Target DNA	Primer [3' to 5']	Amplicon bp	Reference
Bla SHV	F:5'- GGTTATGCGTTATATTCGCC - 3'	867	(19)
	R: 5'- TTAGCGTTGCCAGTGCTC -3'		
Bla VIM	F:5'- CAGATTGCCGATGGTGTTTGG -3'	390	
	R:5'- AGGTGGGCCATTCAGCCAGA -3'		
Bla IMP	F:5'- GGAATAGAGTGGCTTAATTCTC -3'	188	(20)
	R: 5'- CGTGTGATGCYCCAAYTTCACT -3'		

The reaction was done by adding (5 µl) DNA template, (1.5 µl) forward and reverse primer (10 Pico/µl) to mixture tube of PCR then complete the volume by non-ionized distilled water to reach (50µl). Then mixed by vortex (Bioneer company, Korea). The reaction was done in PCR thermocycler apparatus (Mygene Bioneer company, Korea). The thermal program was optimized and performed in master cycler (Eppendorf) as follows:

Table 2: Program of bla SHv gene [19] (865bp)

Step	Temperature(°C)	Time	Cycle
Initial denaturation	95	3min	1
Denaturation	95	30 sec	30
Anneling	55.7	30 sec	
Extantion	72	90 sec	
Final extantion	72	5 min	1

Table 3: Program of bla VIM gene (523 bp)

Step	Temperature(°C)	Time	Cycle
Initial denaturation	95	3min	1
Denaturation	95	30 sec	30
Anneling	60.5	30 sec	
Extantion	72	60 sec	
Final extantion	72	5 min	1

Table 4: Program of bla IMP gene [20] (361 bp)

Step	Temperature(°C)	Time	Cycle
Initial denaturation	95	3min	1
Denaturation	95	30 sec	30
Anneling	57	30 sec	
Extantion	72	40 sec	
Final extantion	72	5 min	1

All PCR products were applied to the gel 1.5% (w/v) agarose gel and stained with 0.5 mg/mL safe stain and were tested by gel electrophoresis, the buffer which used in electrophoresis apparatus is Tris-borate-EDTA (TBE) (boric acid 5.5 g, Tris-base 10.8 g, EDTA (pH 8) 4 mL at (pH 8). A continuous voltage for 1 hour was used for product separation, then tested under UV transilluminator light (UVItec, Paisley, UK). The DNA ladder that used in this study consist of 100-1500-base pair that made in the USA in Roche Company.

Results

Fifty *k. pneumoniae* isolates were investigated in this study. Of the 90 patients diagnosed with respiratory infections.

Genomic DNA isolated from all isolates were exposed to PCR using specific primers. Only 28%(14/50) were positive isolates for one gene. The band size was 867 bp corresponds to amplification of bla SHV gene, Fig. (1). This study found bla SHV that was the most common virulence gene and was found in all isolates (100%). The bla VIM and bla IMP genes were not

detected (0%).

There were 14 strains tested positive for using phenotypic tests, but negative with PCR. The negative amplification in these isolates may be due to other ESBL genes, which could not be detected.

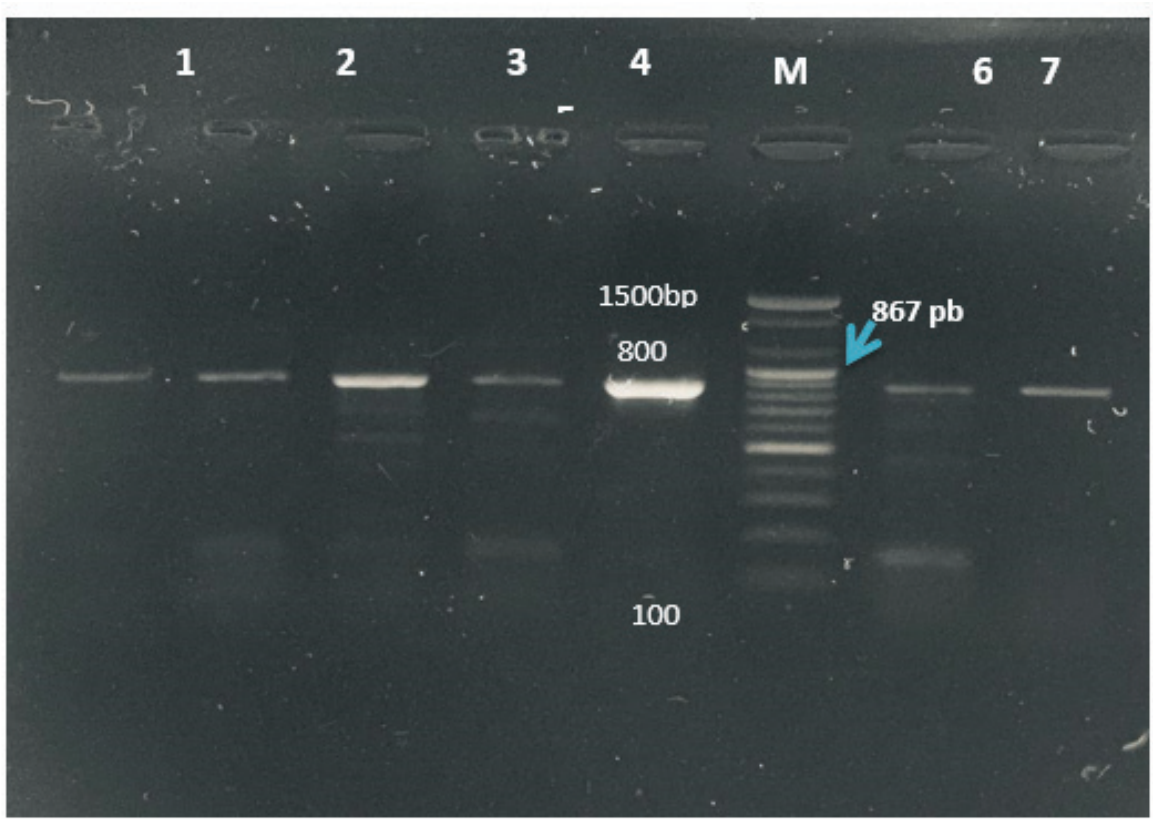

Fig. (1): Agarose gel electrophoresis of bla SHV gene amplicon, M: ladder, 1-7: positive results.

Discussion

The prevalence of resistance problem to antibiotics was increasing every year especially in hospitals and threatening the whole world. One of the most important resistance mechanisms of many bacterial species especially among gram-negative pathogens in the *Enterobacteriaceae* family, has been production of beta-lactamase, which hydrolyses and inactivates beta-lactam antibiotics.

The screening methods for detection of ESBLs is a challenge for the laboratories, requiring not only phenotypic methods, but also genotypic methods help us to confirm the genes responsible for ESBL production.

In recent study, the prevalence of ESBL-producing *K. Pneumoniae* in respiratory infections patients indicate that only 14 isolates were positive for bla SHV amplification, while bla VIM and bla IMP were not detected in any of the isolates. The negative amplification in the remaining isolates may be due to the presence of other ESBL genes, which needing more discover.

The present study noticed increasing in the prevalence of bla SHV gene in *K.pneumonia* isolates, this result is similar to many studies [21, 22, 23]. This gene may be related with increasing the pathogenesis of this bacteria [24] and may be of clinical significance.

SHV beta-lactamases are large families of enzymes production by blaSHV genes are responsible for the evolutionary line. Of the 172 enzyme types in the SHV family, 45 are extended spectrum beta-lactamases on transferable elements such as plasmids and transposons [25,26] . This localization of bla genes can facilitate horizontal spreading of antibiotic resistance among bacterial strains [27].

Conclusion

Beta-lactamase gene (bla SHV) was the highest prevalence in *K. pneumoniae* strains isolated from respiratory infections, this resistance gene may increase resistance of *K. pneumoniae* strains to beta-lactam antimicrobials. Thus, molecular identification are needed to detect the presence of different resistance-associated genes production in *K. pneumoniae* strains.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

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Improving Mental Health of Adolescents through Self-Presentation

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Abstract

Mental health is currently the problem faced by youth especially during the pandemic of COVID-19. Adolescents use of social media is also increasing due to this coronavirus pandemic. This situation have risk of increasing the risk of lower mental health of Adolescent as the impact of posting their self-presentation through a social media such as *Instagram*. *Instagram* is a social media platform that has a rapid user growth. Indonesia is one of the countries with highest number of users. *Instagram* users recorded as of May 2020 had reached 9.2 million users. *Instagram* as social media is a means for users to present themselves to wide audience. Self-presentation can be in the form posting photos or videos on their *Instagram* social media accounts. By posting photos, user hope to get feedback in the form of likes and comments. This research aims at the improving mental health of adolescent through self-presentation. This research used is secondary data obtained from journal database. The sample was 35 scientific articles obtained from the basis of screening journal that has been screened using pre determine inclusion criteria. The result found that self-presentation on social media *Instagram* was related to giving or managing impression, popular, self-disclosure, narcissistic behavior, self-image and self-esteem that in turn will result in lowering adolescent mental health if it is not manage well.

Keywords : *Mental Health, Instagram, Self-presentation.*

Introduction

Health is a maximum condition both physically, mentally, spiritually, and socially which enable everyone to live productively socially and economically. Mental health is a problem that always a concern of society. The many improvements in mental health such as an increase in mental illness patients, incidents of suicide, make mental health problems that hard to ignore.

According to WHO (World Health Organization), 20% of the population is experiencing mental health. The most common types of mental health are depression and anxiety. Meanwhile, 75% of mental emotional disorder are common before the age of 24 years or the teenage age range. According to RISKESDAS (2018) the number of people who experience mental emotional disorders is 19 million Indonesians aged 15 years and over, while more than 12 million other people were thoughts to have depression. This figure tends to increase from the previous years.

According to Santrock, several factors that can trigger mental emotional problems. They are the family environment, school environment, peer environment, community environment, and social media. Social media is media on the internet that allows internet users to present themselves and interact, cooperate, and share information with other users. One of the most used social media platforms, with 80% of people using the *Instagram* social media application.

The number of active *Instagram* users in the world has increased, from 300 million to 400 million people. The number of monthly active users of social media *Instagram* surpassed 1 billion as of June 2019, more significant growth compared to *Facebook* and Snapchat.

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As of June 2019, Indonesia is in the fourth rank of countries experiencing the highest growth of active *Instagram* users with 62 million increase from 2018. The growth of *Instagram* social media users in Indonesia reached 69.2 million as of May 2020. *Instagram* social media users are mostly women as much as 51%, while for men, 49%. 59% of *Instagram* social media users in Indonesia are 18-24 years old, while 30% of users are 25-34 years old, and the remaining 11% are over 35 years old. Based on description above, the age of 18-24 years were categorized as late adolescence.

The development of social media *Instagram* in society will shape the diversity of social media users. Users use *Instagram* social media as a medium to express themselves or also as a business medium. Currently, social media has its own power to influence individuals, one of which is by making *Instagram* social media a place to present themselves. When interacting with other people, especially in cyberspace, individual attention focused in how other people think according to that individual thinking. It is necessary to do self-presentation, by adjusting the impression that other people perceive about themselves. *Instagram*'s specialty in using photos of media for delivering message allows social media as a place to present oneself. The desire to make a perfect and suitable impression on others by doing your best to look perfect. The purpose of this research was to determine the relationship contained in self-presentation with self-esteem in social media *Instagram* using a literature review.

Method

The data used in this research was a secondary data from google scholar, *SprigerLink*, *Science Direct* and *DOAJ.org* articles that were screened were screened by the title, abstract using key words adolescent and self-presentation. Inclusion criteria were applied in selecting articles, namely the maximum journal publication period of 5 years, using Indonesian or English, adolescent and adult research subjects, full text journal type, open access and can be downloaded in Pdf format. The total number of 35 articles were found after screening using inclusion criteria.

Articles that match the inclusion criteria were then collected and put into database including the author's name, the publication date, the title of study, the research subject, and a summary of the research results. An analysis was carried out on the abstract and full text of the journal to be read and examined.

Result

1. Based on table 1 that Research on *Instagram*'s social media continues to increase over time. In 2020 research is still small because the year 2020 is still running so that it allows research Regarding social media, especially *Instagram*, it is still ongoing.

Table 1 Distribution Characteristic of Articles.

Category	N	%
Publication year		
2015	3	8.6
2016	8	22.8
2017	6	17.2
2018	9	25.7
2019	6	17.1
2020	3	8.6
Total	35	100
Research sites		
Indonesia	26	74.5
German	1	2.8
United States of America	5	14.3
South Korea	1	2.8
Russia	1	2.8
Spanish	1	2.8
Total	35	100

The country used for the study of the literature review article was Indonesia. This is because the growth of *Instagram* users in Indonesia is quite fast.This is because the growth of *Instagram* users in Indonesia is quite fast. This is evidenced by the fact that in 2019 Indonesia is the fourth country to occupy the country with the highest number of *Instagram* users. The review from 35 articles showed that self-presentation affects or relates to impression management [1,2,3,4,5,6,7,8], popularity [9,10,11,12] , self-disclosure [13,14,15,16,17,18,19,20] , narcissistic behavior [21], self-image [22,23] and self-esteem [24,25,26,27,28,29,30,31,32,33,34,35].

Discussion

The use of social media *Instagram* need to be done self-presentation to attract other users. Shaping social media self-presentation consist of a variety of things. *Instagram* is one of the social media that can be used as a mean to present yourself. *Instagram*'s social media focused on photos or videos so users can more easily organize the impression they want to present or want to show to a wide audience. In the cycle of life is a show of human audience, in its journey everyone is free to express as desired.

In Erving Goffman's Theory, social life is divided into two area of front stage is a place where one can display an impression that they want to be shown- unlike a backstage that only one person or few people can see what's not happening on the front stage. The front stage is the side that people want to show and know, the front stage here is *Instagram* social media. It support appearance such as costume, language style, place, setting, and expression that adolescent want to display.

Instagram users also want to show their existence in cyberspace by posting photos of themselves, with the aim of getting attention from other user or followers in the form of likes and comments. The more likes and followers on their *Instagram* account, user will assume that they are increasingly popular. User using *Instagram* usually aim to look increasing popular, follow trends, expand connection, fill free time, as a means to communicate, as a means to do business or make business, share experiences or moment, have many friends. Photo and videos posted were edited to make the resulting photos or videos more interesting. Location addition can also allow user to get feedback from other users. Sometimes the addition of popular hashtags can increase the feedback that users get in order to increase the like of the photos they post. This indirectly make user have their fun in playing *Instagram* social media.

Self-disclosure made on social media can be photos and captions. In addition, an individual who uses *Instagram* social media is more comfortable and satisfied with his need in presenting himself or also expressing himself by using *Instastory* contained in *Instagram* social media. Now *Instagram* has also started to become a lifestyle that must be done so that it can be makes its users addicted to *Instagram*;s social media use and it can also make individuals become someone who is too open so that the user's privacy is lees guarded. But nowadays there are also users who post photos and caption post about their personal issues. In addition to following lifestyle trends, user especially teenagers need recognition from their social environment even more in friendships. Teenagers would be more proud if he had a more modern lifestyle. They will more easily get recognition from the public when they have a trendy lifestyle. The behavior of coping photo/videos/stories o *Instagram* can also be a form of expression of the user's emotions.

Individual with high narcissism tend to post selfies and photos that he or she takes herself. Update their profile picture more often, and spend more time compare to their peers. Indirectly they also rate a person's profile picture directly physical. Adolescent post selfies they take to their *Instagram* social media. Besides that, one of the factors that influence narcissism is self-esteem. Individu with a narcissistic personality have a need to get appreciation and appreciation from others for the sake of forming their self-esteem. This is the reason someone uses *Instagram* social media to meet these needs. People who have narcissistic behavior are more likely to have low self-esteem. Narcissistic behavior prefers to show off themselves and think about how other people perceive themselves as more special than others.

Self-image is a perception of oneself pay as an identity card that is used to be introduced to the world about oneself. Individuals usually want to present a good self-image as well as an impression. Self-image is more often associated with physical appearance such as clothes hair, size and grooming. So that many individuals want

to wear clothes as possible, neat as possible, style their hair to display a good self-image. Likewise, *Instagram* social media users are competing to show their self-image, by making themselves unique and having their own characteristics, so that they can attract the attention of other users want to display usually wants to look wise, friendly, perfectionist, glamorous, etc. Self-image can also different from the individual's own personality. *Instagram* users deliberately create their self-image on social media to make them look or attractive by editing photos or videos to be posted.

Sharing selfies on *Instagram* social media is one form of activity to present themselves to others. A person's goal to present themselves on *Instagram* social media one of them is to increase their self-esteem. The social media of *Instagram* can influence user to judge themselves in front of others. This self-perception shows that the views of others have important value that can influence self assessment in assessing self-worth, self-acceptance of what they want to express through action. Such assessments can be referred to as self-esteem. Self-esteem was gained depending on other users' recognition and rating of photos or videos they post and present on *Instagram* social media.

Conclusion

Someone's self-presentation behavior on *Instagram* social media is shown to form their higher self esteem. *Instagram* users want them to be recognized in front of a wide audience so they are competing to show or show themselves as best and attractive as possible. so that suggestions that can be done for *Instagram* social media users are expected to have self-control in using *Instagram* social media. such as intensity of use and limiting hours on the use of *Instagram* social media besides that, give a moment not to use *Instagram* social media if someone is Experiencing saturation in its use. Because many *Instagram* social media users are teenagers, schools or universities can educate about the use of social media that is healthy and good for them Mental and physical.

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Comparison of Palatal Rugae Pattern among Indian and Malaysian Population

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Abstract

Background: Tools for establishing identity is invaluable part of forensic sciences form the beginning of the profession. Odontology is one of such branches which in early days started in its crude form with identification of different species and races within the species, currently is an individual subject with specialist societies, journals and courses. Palatal rugae, was identified to be a potential tool for establishing identity few centuries back itself but is not validated even today to be part of standard protocols. This is a pilot study comparing two racial groups i.e., Indian and Malaysian with established parameters for palate rugae aiming at testing the feasibility of the parameters to be used in identification of individuals and racial groups and also to provide preliminary data to make way to larger studies.

Aims and Objectives: The aim of this pilot study is to explore the feasibility of palate prints as tool for identification by, studying palate print in Indian and Malaysians and Comparison of plate Rugosity between Indians and Malaysians students for identification.

Methodology: Study population was male Indian and Malaysian Medical and Dental students, age between 18-25 years studying at Mangalore, India. Sample size 80 (40 in each group); were selected for studying of Palatal Rugae. The palate impressions were collected using maxillary alginate cast. The parameters assessed were: Total number of rugae, number of primary rugae (length- 5 to 10 mm), secondary rugae (3-5mm), fragmentary rugae (less than 3 mm). Comparison for two populations was done using non-parametric Mann-Whitney Test.

Results: There were no statistically significant differences among the Indians and Malaysians in total number and number secondary rugae in both sides more in Indians than Malaysians.

There was statistically significant difference in number of the primary rugae and the fragmentary rugae, which in both sides were more in Indians than Malaysians.

Conclusion: In conclusion, palatal rugae pattern are unique to an individual and their use in forensic identification has been advocated and applied. Our study demonstrated significant difference in the number of primary rugae on each side. It was more in Indians than Malaysians. This study demonstrated the uniqueness of rugae pattern in different individuals. Thus, it appears to be an effective & reliable source of identification.

***Key words**: Palate rugae, Odontology, Primary rugae, Secondary rugae, Fragmentary rugae.*

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Background

Establishing identity is crucial in Forensic Medicine. Forensic Odontology is a specialty or a branch of dental science which occupies the total spectrum

of methods applied to medico-legal identification. Forensic Odontology can be defined as it's a branch of dental science which deals with application of dental knowledge to civil or criminal laws that are enforced by police in a criminal judicial system.[1]

Dental, finger print, lip print, ear print, foot print and DNA are the most common techniques used in this context allowing fast and secure identification. Palatal rugae have been equated with fingerprint and are unique to an individual. It can be special interest in edentulous cases and also in certain conditions where there are no fingers to be studied, such as burned bodies or bodies that underwent severe decomposition. Rugae pattern may be specific to racial group facilitating population identification.

Palatal rugae otherwise called Plicae Palatinae Transverse and Rugae palatine, usually situated in the anterior third of the upper palate, Rugae are the asymmetrical and irregular elevation of the mucosa and each side of the side of the median raphe and behind the incisive papillae. In several branches of dentistry like Orthodontics, Prosthodontics, Anthropology, Genetics,Forensic Odontology and Comparative Anatomy, rugae pattern have been studied for various purpose.[2]

Palatal Rugae appear usually in the third month of intrauterine life and once rugae is formed, they experience changes in their size and but not in shape due to growth of palate. The Shape will be maintained throughout life. Because of the uniqueness and the overall stability of the rugae has been considered relevant for identification in forensic medicine.[3] The palate Rugae are protected from trauma and high temperature because of the internal position of rugae in oral cavity, surrounded by lips, tongue, cheeks and bone. During the growth also the palatine rugae are unique and stable to each individual. When the body is fully decomposed or burned or both upper and lower limbs are missing, in those cases the palatoscopy can be helpful for personal identification of body. In aeronautical accidents it is a valuable technique in order to ensure identification of pilots making use of antemortem data.[4] The palatalrugae is unique and permanent in each person's life. But some evens can contribute to changes in rugae pattern including persistent pressure of the Prosthodontics treatment and orthodontics treatment, extreme finger sucking in infancy, severe face trauma and cleft palate repair. The changes are more common in treated cases.[5] Hence the objective of this pilot study is to explore the feasibility of palate prints as tool for identification. Comparison of palate rugosity between Indian and Malaysian students for identification is done with palate print in Indian and Malaysians.

Materials and Methods

The present study was conducted at Yenepoya Medical College Hospital from November 2011 to September 2013. The ethical committee approved the study (Ethical clearance). Informed consent was taken from all the participants.

Inclusion Criteria for the study: Medical and Dental students in the age of 18-25 were included in the study.

Exclusion criteria for the study: 1. Subjects with the history of cleft palate and repaired cleft palate. 2. Subjects with the history of defect in maxillary palate. 3. Subjects below the age of 18 years 4.Those who had undergone surgery in anterior palate region 5.Individual undergoing orthodontic treatment, 6. those who had undergone extraction of upper permanent teeth and individual with palate lesions. 7. Subjects with trauma and facial accidents.

Participants were recruited by simple random sampling. The total sample size was 80.Permission was taken for inclusion of data for dissemination for research purposes.

The materials used for collection of palatal Prints were Alginate powder, perforated metal maxillary impression tray, Mixing bowl, Spatula, Dental Stone and Water. The participants were explained about the procedure and discomfort it may cause. After informed consent, Maxillary impressions were taken using Alginate and a dental cast was poured using type 2 dental stone to form an exact replica of mouth. The palatal rugae impressions were collected from the dental stone cast poured. The rugae seen as elevated impressions were marked on these casts using a black permanent marker pen (Fig 1 and 2).Prints that were collected was coded. Comparison of prints were made to see the similarity and palatal prints were classified.

The parameters assessed were: 1) Total number of rugae, 2) Number of primary rugae.

The rugae were classified based on their length as[6]:

1) Primary->5mm,

2) Secondary- 3 to 5mm

3) Fragmentary-<3mm

Results

A total of 80 maxillary dental casts obtained from 40 Indians and 40 Malaysians students' population were examined for the palatal rugae patterns by applying the classification proposed by Thomas & Kotze.[6]

Total Number of Rugae: Racial wise, there were no significant differences in the total number of rugae on the right side or left side of the palate among the Indians and Malaysians.

Number of primary rugae: The primary rugae in each side is more in Indians than Malaysians and statistically significant.

Number of secondary rugae: The Secondary rugae both does not show any statistically significant between Indians and Malaysians.

Number of fragmentary rugae: The fragmentary rugae in both sides more in Indians than Malaysians and statistically significant.

All the details from each dental cast were observed as mentioned and documented in the Performa. The data was tabulated and Comparison of each value between the two races were tested by Mann Whitney U Test. The tools used were Microsoft excel and SPSS software Version (18.0)

Fig:1Palatal rugae pattern

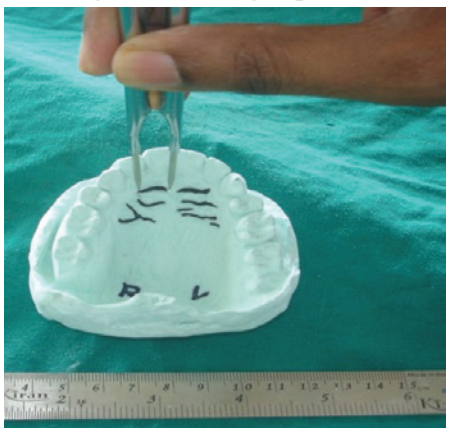

Figure 2: Length of the rugae measured using divider and measuring scale

Table 1: Comparison of each value between the two races: Mann Whitney u test

		N	25th Percentile	MEDIAN	75th	IQR	Mann-Whitney U	Z	P VALUE
LEFT SIDE LENGTH Primary	INDIAN	40	3	4	4	1	584	-2.221	0.026
	MALAYSIAN	40	3	3	4	1			
LEFT SIDE LENGTH Secondary	INDIAN	40	0	0	1	1	649.5	-1.753	0.08
	MALAYSIAN	40	0	0	0	0			
LEFT SIDE LENGTH Fragmentary	INDIAN	40	0	1	2	2	374.5	-5.142	<0.001
	MALAYSIAN	40	0	0	0	0			
RIGHT SIDE LENGTH Primary	INDIAN	40	4	4	5	1	474	-3.36	0.001
	MALAYSIAN	40	3	3	4	1			
RIGHT SIDE LENGTH Secondary	INDIAN	40	0	0	1	1	713	-1.154	0.249
	MALAYSIAN	40	0	0	0	0			
RIGHT SIDE LENGTH Fragmentary	INDIAN	40	0	0	2	2	491	-3.851	<0.001

Discussion

Establishing a person's identity can be very difficult process in Forensic Medicine. DNA analysis, lip prints and finger prints are the most scientifically reliable methods of identification. In Forensic Odontology field, still the palatal Rugoscopy in its infancy.[6]This study described the rugae pattern, shape and unification, quantitatively and qualitatively. Uniqueness to individuals has been clearly as providing the rugae are the reliable source of identification.[7,8,9,10,11]The present study was an attempt to determine the number, shape and unification, also to assess the predominant pattern of rugae in students in Yenepoya Medical College, Mangalore. An attempt was also made to determine the differences in rugae pattern among two races in the selected groups. Several studies done in the past have revealed and have statistically proved that the rugae patterns are highly individualistic and there are differences between races and gender.[12,13] In our study the number of primary, secondary or fragmentary rugae did not show any statistically significant differences among the Indian and Malaysians.

This observation was in contrast to that of Shwetha.et al,[12] who reported that Mysorean males and Tibetan females had more primary rugae than their respective counterparts.[13]Kashima in her study among Indian and Japanese children reported that Japanese children had more primary rugae than Indians.[14] Kapali in her study reported that Australian Aborigines had more primary rugae than the Caucasoids.[7]

This study clearly demonstrates that, palatal rugae are unique to each individual and rugae pattern with its different parameters can be used successfully as a tool of identification. In conclusion, palatal rugae pattern is considered to be unique to an individual and their use in forensic identification has been advocated and applied.[12]Although the study demonstrates the significant difference in unification of the palate rugae, the diverging patterns is more in Malaysians than Indians and diverging pattern more common in Malaysians than Indians. Although this study fails to demonstrate any significant racial difference in length and shape or rugae. This study also clearly demonstrated the uniqueness of rugae pattern in different individuals. Thus, it is an effective & reliable source of identification. However, this study was done in an institutional setting and had a limited sample size of 80 subjects. it will be beneficial to conduct studies with larger sample size and compare between various ethnic groups to validate the findings of this study and generalize the findings of this study. Another drawback of this method of identification is that it can be used only when an ante mortem record of palatal rugae is available.

Conclusion

In conclusion, palatal rugae pattern are unique to an individual and their use in forensic identification has been advocated and applied. Our study demonstrated significant difference in the number of primary rugae on each side. It was more in Indians than Malaysians. This study demonstrated the uniqueness of rugae pattern in different individuals. Thus, it appears to be an effective & reliable source of identification.

Source of Funding: This was entirely self-funded study

Conflict of Interest: All the authors involved in the study confirm that they have no conflict of interest in this study.

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Shape and Unification Pattern of Palatal Rugae as a Race Identification Tool- A Cross-Sectional Study

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Abstract

Background: One of the integral parts of forensic medicine is identification of individuals from biological samples and from the anatomical signatures. Identification of individuals, races, species, gender from the anatomical signatures of teeth and oral cavity is one of such branches which existed in practice in crude form even before origin of the Forensic odontology as a specialization. Individual variations in Palatal rugae pattern, and its biometric value as a potential tool for establishment of identity was reported centuries back. It's not standardised or included in the regular standard protocols even today in the age of artificial intelligence. In this pilot study we are comparing two racial groups i.e., Indian and Malaysian with established parameters for palate rugae with an objective of testing feasibility and validation for identification of individuals and racial groups and also to provide preliminary data for larger studies.

Aims and Objectives: The aim of this pilot study is to explore the feasibility of palate rougoscopy as a tool for race identification by studying the shape and unification Pattern of Palatal Rugae in Indian and Malaysian population.

Methodology: Study population was male Indian and Malaysian Medical and Dental students, aged 18-25 years studying at Mangalore, India. Sample size 80 (40 in each group); were selected for studying Palatal Rugae. The palate impressions were collected using maxillary alginate cast. The parameters assessed were: shape an unification based on classification of Thomas & Kotze. Comparison for two populations was done using non-parametric Mann-Whitney Test.

Results : Predominant Shape: The Curvy patterns were more common in Malaysians compared to Indian. The Wavy patterns were more common in Malaysians than Indians.The Circular patterns were more common in Indians than Malaysians.

Predominant unification: Diverging patterns were more in Malaysians than Indians and Converging patterns were more in Indians than Malaysians and statistically significant.

Conclusion : In the present study the Malaysians had predominantly curvy and wavy patterns and Indians had circular shapes. Straight patterns were seen equally in both the populations. Although unification of the palate rugae was seen, the diverging patterns is more in Malaysians and Converging patterns more in Indians.

Key words*: Palate rugae, Odontology, Shape of rugae, Rugae unification 2*

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Background

The oldest known parameter for identifying individuals and race is anatomical differences. Forensic sciences originated for the purpose of identification

of people or other matters from evidence collected. Differences in teeth and oral morphology was used even in animals as an identification tool, the same grew into a speciality known as forensic odontology. Palatal rugae have been equated with fingerprint and are unique to an individual. "Palatal Rugae'' consists of a series of irregular elevated ridges on the masticatory mucosa covering the anterior part of the hard palate. They originate from the incisive papillae and radiate transversely.[1] The rugae are well protected by cheeks, lips, tongue, teeth, premaxilla and palatine process of maxilla. This protects it from trauma, extreme finger sucking in infancy, and persistent pressure during orthodontic treatment and dentures. Such unique features, life-long stability and protection from damage makes palatal rugae one of the important candidates for individuals in forensic investigations.[2] Racial, ethnic, and geographical variations in the rugae patterns have been reported.[3,4,5,6] Hence the aim of this pilot study was to explore the feasibility of palate rougoscopy as a tool for race identification by studying the shape and unification Pattern of Palatal Rugae in Indian and Malaysian population.

Materials and Methods

The study was conducted at Yenepoya Medical College from November 2011 to September 2013. The ethical committee approved the study (Ethical clearance). Informed consent was taken from all the participants.

Inclusion Criteria for the study: Medical and Dental students in the age of 18-25 were included in the study.

Exclusion criteria for the study: 1.Subjects with the history of cleft palate and repaired cleft palate. 2.Subjects with the history of defect in maxillary palate. 3.Subjects below the age of 18 years 4.Those who had undergone surgery in anterior palate region 5. Individual undergoing orthodontic treatment, 6. those who had undergone extraction of upper permanent teeth and individuals with palate lesions. 7. Subjects with trauma and facial accidents.

Participants were recruited by simple random sampling. The total sample size was 80. Permission was taken for inclusion of data for dissemination for research purposes.

The materials used for collection of palatal Prints were Alginate powder, perforated metal maxillary impression tray, Mixing bowl, Spatula, Dental Stone and Water. The participants were explained about the procedure and discomfort it may cause. After informed consent, Maxillary impressions were taken using Alginate and a dental cast was poured using type 2 dental stone to form an exact replica of the mouth. The palatal rugae impressions were collected from the dental stone cast poured. The rugae seen as elevated impressions were marked on these casts using a black permanent marker pen (Fig 2 and 3). Prints that were collected were coded. Comparison of prints were made to see the similarity and palatal prints were classified.

The parameters assessed were according to classification of Thomas & Kotze[4] : 1) Predominant Shape - a) Straight, b) Curved, c) Wavy, d) Circular.

2) Unification of rugae -a)Diverged, b)Converged, c)Perpendicular, d)Forward, e) Straight.

The rugae were divided into 4 types based on their shape as:

1) Curved: They had a crescent shape and curved gently.

2) Wavy: If there was a slight curve at the origin or termination of curved rugae. 3) Straight: They run directly from their origin to termination.

4) Circular: Rugae that form a definite continuous ring were classified as circular. **Unification was said to have occurred when two rugae joined at their origin or termination:**

1) Diverging- If two rugae had the same origin from the midline but immediately branched.

2) Converging- Rugae with different origins from midline, but which joined on their lateral portions.

All the details from each dental cast were observed as mentioned and documented in the Perform. Comparison of each value between the two races were tested by Mann Whitney U Test.

Results

All the details from each dental cast were observed

as mentioned and documented in the Performa. The data was tabulated and comparison of each value between the two races were tested by Mann Whitney U Test. The tools used were Microsoft excel and SPSS software Version (18.0)

A total of 80 maxillary dental casts obtained from 40 Indians and 40 Malaysians students population in Yenepoya Medical College, Mangalore, were examined for the palatal rugae patterns by applying the classification proposed by Thomas & Kotze.[4]

Shape:

Curvy patterns were significantly more common in Malaysians compared to Indian. The Circular patterns were significantly more common in Indians than Malaysians Straight patterns were equal but not significant in Indians and Malaysians. The Wavy patterns were more common in Malaysians than Indians but not significant.(Refer Table1)

Predominant unification:

Converging patterns were significantly more in Indians than Malaysians. The Diverging patterns were more in Malaysians than Indians but were not significant (Refer Table 1).

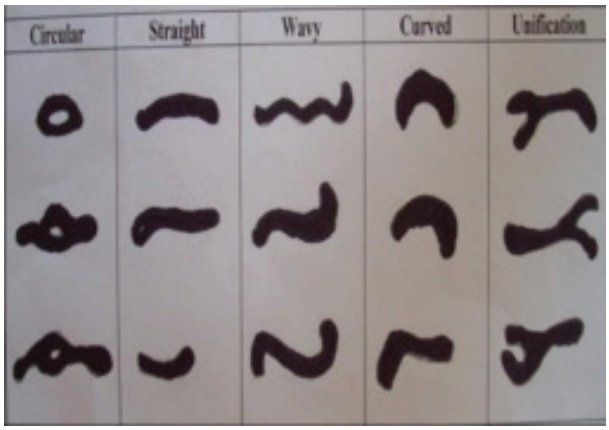

Figure 1: Diagrammatic representation of different types of rugae pattern

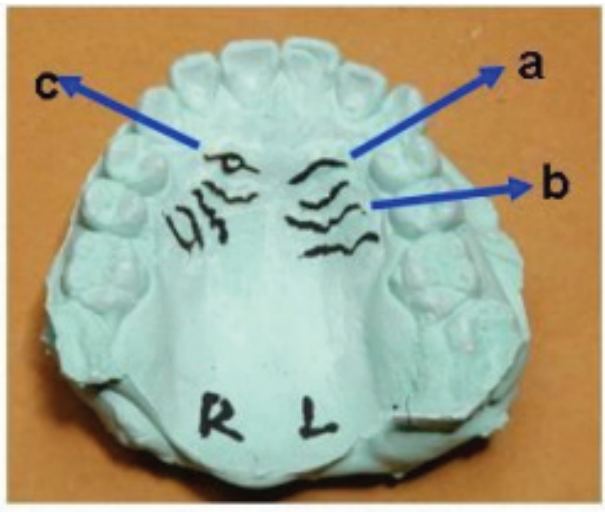

Figure 2 :Different types of rugae pattern delineated on maxillary cast (A-curved, B-wavy, N-C-wavy pattern)

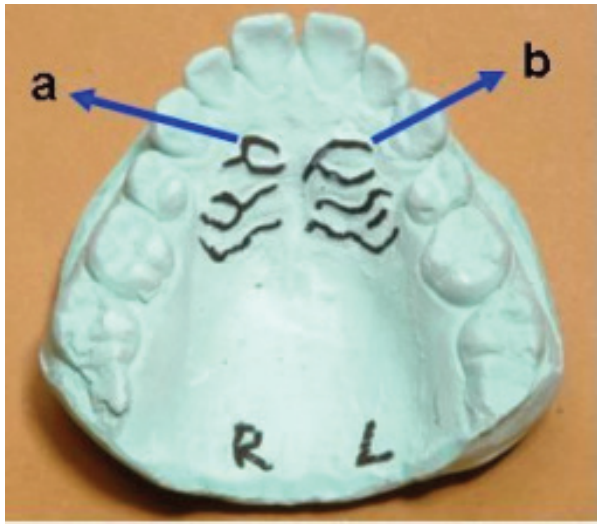

Figure3: Cast showing, a- Converging b - Diverging rugae

Table 1: COMPARISON OF EACH VALUE BETWEEN THE TWO RACES: MANN WHITNEY U TEST

Table 1. Comparison of Palate Rugosity based on shape and unification

		N	Minimum	25th	MED	Maximum	Percentile	IAN 75th	IQR	Mann-W	hitney U	Wilcoxo	n W Z P	VALU E
Straight	INDIAN	40	0	8	2	3.5	5	3	624	1444	-1.716	0.086	MALAYSIAN	40	0	8	2	3	4	2
Curvy	INDIAN	40	0	4	1	2	3	2	489.5	1309.5	-3.097	**0.002**	MALAYSIAN	40	0	5	0.25	1	1.75	1.5
Wavy	INDIAN	40	0	5	1	2	3	2	615	1435	-1.828	0.068	MALAYSIAN	40	0	7	2	2	4	2
Circular	INDIAN	40	0	7	1	2	3.75	2.75	281	1101	-5.309	**<0.001**	MALAYSIAN	40	0	3	0	0	1	1
diverging	INDIAN	40	0	2	0	0	0.75	0.75	711	1531	-1.078	0.281	MALAYSIAN	40	0	3	0	0	1	1
convergin g	INDIAN	40	0	2	0	0	1	1	538	1358	-3.547	**<0.001**	MALAYSIAN	40	0	1	0	0	0	0

Discussion

The aim of the present study was to explore the feasibility of palate rougoscopy as a tool for race identification by studying the shape and unification Pattern of Palatal Rugae in Indian and Malaysian population.

The predominant shape of the rugae in our study, the curvy and wavy pattern was more significant in Malaysians. The circular and curvy shapes were more in Indians. No significant difference in males and females. But Faisal in his study among males and females, reported an increased incidence of circular shaped rugae more in females than males in Saudi population.[7]

Our results were inconsistent with the study done by Sherif et al (2018) where they reported non-significant differences in all the shapes. Their study compared between Egyptian and Malaysian population wherein the age group ranged from 6-60 years. The mean age in our population was 18-25 and sample sizes were different. This could have resulted in the difference in results.

In our study diverging pattern was more in Malaysians and converging type was more common in Indians, This was in agreement with the observations made by Shwetha and in contrast to the findings made by Faisal who observed that the Saudi females had more converging rugae than males. [7]

This study clearly demonstrates that palatal rugae are unique to each individual and if there are earlier antemortem palate prints available, the palate rugae can be used successfully as a tool of identification.

Mahabalesh Shetty et al in 2011did study of rugae patterns among the students in Mangalore. Although the study fails to demonstrate any significant gender difference in number of primary rugae, it clearly demonstrates a distinct pattern of rugae associated with different gender. The study also clearly demonstrated the uniqueness of rugae patterns in different individuals. Thus it is an effective & reliable source of identification. [8]

Dekshan Kiran Shetty, Priyanka s et al in 2013 did a study of comparison of palate rugae pattern in Malayalees and Koduva of south India concluded Wavy pattern was highest in both the population groups. Circular pattern was totally absent in both the racial groups. Significant differences between the two populations were observed in wavy and unification patterns and a significant difference between Malayalee males and females was seen for straight rugae pattern.[9]

Patrones de Rugas Palatinas de et al in 2012 did study of Palatal rugae patterns of urhobos in abraka,south-southern Nigeria concluded his study clearly demonstrates distinct pattern of palatal rugae in different individual and in association with different sex, though it fails to establish any significant sex difference in number of rugae. The association between palatal rugae and ethnicity is also shown. The individualized pattern of palatal rugae and their low utilization costs make it a reliable guiding tool in forensic identification. (10)

Aravind Shivaraj et al 2013 did the study of significance of palatal rugae in orthodontics treatment, the rugae can be used to evaluate dental movements. They may be stable for their entire life. Rugae serve the reference landmark in various dental treatments and can help in identification also.(11)

Limitations of the Study:

This study was done in an institutional setting with limited sample and student population. The results cannot be generalized. A well-controlled large scale studies are required to validate the results of our study. This can be used only if ante mortem records of the individuals are available.

Conclusion

In the present study the Malaysians had predominantly curvy and wavy patterns and Indians had circular shapes. Straight patterns were seen equally in both the populations. Although unification of the palate rugae was seen, the diverging patterns is more in Malaysians and Converging patterns more in Indians. This study clearly demonstrates that palatal rugae are unique to each individual and if there are earlier antemortem palate prints available, the palate rugae can be used successfully as a tool Kotze of identification.

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Immune Dysregulation in Childhood Leprosy

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Abstract

Leprosy is a chronic infection which is caused by *Mycobacterium leprae*. Leprosy has long been considered a complex disease, in which host and environmental characteristics are as important as the pathogen to determine disease outcome. Leprosy is an excellent model for examining the immunoregulatory functions. Children are believed to be the most vulnerable group to leprosy infection due to their immature immunity. This review is aimed to summarize the findings of previous studies about immune dysregulation in childhood leprosy. This study highlighted the plausible maternal-fetal connection which are related in immune dysregulation in childhood leprosy.

Key words: *Immune dysregulation, Childhood leprosy*

Introduction

Leprosy is a chronic infection which is caused by *Mycobacterium leprae*. Leprosy has long been considered a complex disease, in which host and environmental characteristics are as important as the pathogen to determine disease outcome.[1] World Health Organization (WHO) has proposed the leprosy elimination program that aimed to reduce the global prevalence of leprosy to less than one case per 10,000 population by the year 2000 back in year 1991.[2,3] Despite the efforts, leprosy remains endemic in some country, with relatively high burden in children and an increase in the new cases detection rate.[4,5]

Even though leprosy occurs at all ages, children are considered more susceptible. However, clinical leprosy is more commonly observed in adults. Paucibacillary disease is more common in children and the incidence of reactions is lower in this age group. Epidemiologically, childhood leprosy is an index of transmission of the disease in a population and helps in detection of the source of infection. In 2019, 177,175 registered cases and 202,185 new cases of leprosy were observed globally.[6]

The proportion of new leprosy in children is one indicator of failure on termination of leprosy chain transmission and reflects the number of undiagnosed cases in the community. WHO has a target to reduce disease transmission and grade II disability, especially in pediatric cases.[7] A study has reported that the children appear to be more prone to leprosy than other family members.[8] Children are believed to be the most vulnerable group to leprosy infection due to their immature immunity.[6]

One peculiarity of leprosy is that the majority of the people (about 90%) do not become ill due to one's own natural defense against *Mycobaterium leprae*, which is related to genetic influences.[7] Clinical manifestations

of leprosy rely on the interaction between innate and acquired immune reactions involving interactions between bacterial proteins and host immune components. These interactions can either prevent invasion and infection or encourage the growth and development of leprosy. The immune system has developed mainly to suppress infection, but in leprosy, the immune response is responsible for the broad clinical spectrum of the disease and, similar to autoimmune disease, tends to cause more complications such as nerve damage.[9]

Leprosy is an excellent model for examining the immunoregulatory function of innate immune molecules and their association with the nervous system, which can impair homeostasis and lead to the production of inflammatory episodes over the course of the disease.[10] While innate immunity affects the clinical forms of the disease, it is the components of the adaptive immune system that tend to be closely associated with the characteristic continuum of leprosy.[11] This review is aimed to summarize the findings of previous studies about immune dysregulation in childhood leprosy.

Leprosy in Children: Current Findings

Currently, the proportion of leprosy in children has not improved significantly.[7] Epidemiological data from 150 countries show there were around 16,979 cases of child leprosy in 2017. Delay in early diagnosis and difficulty in assessing sensory loss in children may contribute to the high leprosy rate in children.[6]

A study by Palit et al. indicates that childhood leprosy differs from adult disease in many ways; there is a gender preference for male leprosy; pauci-bacillary cases, mostly borderline tuberculoid (BT) disease, are more common and episodes of reactions and associated deformities are less common in this age group.[12] Familial and non-familial close contacts play a significant part in the epidemiology of childhood leprosy. The type of disease in the contact and proximity to the child (household or neighborhood) are essential determinants of transmission.[12]

An important source of infection is close contact with cases of leprosy at home, especially in children who have a poor immune response. It confirms the significance of screening of family members in a case of leprosy.[13] A skin patch is the first symptom of leprosy in most childhood leprosy cases. In a study by Darlong and Govindharaj, 90% had patches as the first symptom, and 97% were seen either by the children themselves or by the parents.[14]

Long diagnosis delays and a high proportion of MB signify a lack of early case finding.[15] Diagnosis can be delayed by the failure of the child to comply with sensory testing.

Presenting the test as a feather game is a helpful tool, and another is demonstrating on the mother's skin as the child watches, ask the child to imitate his/her mother. The differential diagnosis can be supported by biopsy (an unpleasant procedure in a very young child) in a study, 35% of questionable cases, may be confirmed by histology.[16]

Skin biopsies are more likely to detect multibacillary cases compared to those with smears as it covers more tissues, including cutaneous nerve twigs, with a bacillary load 1000 times greater than that of inflammatory infiltrates. A single hypopigmented patch on the face of children is at high risk of misdiagnosis, as there are multiple causes of hypopigmented patches in children and biopigmented patients.[17]

In a study in India, deformity occurred in 0-24% of cases. Most of the studies from India measured deformity according to the WHO disability grading. At the presentation, many children had noticeable deformity. Some children developed deformity after being released from therapy.[18]

In older children, around puberty, where exposure to bacillus is expected to be longer, other symptoms may appear more similar to those defined for polar types of the disease. The only symptom of the disease is sometimes the thickening of the superficial nerves, especially the muscles of the auricular, superciliary and ulnar muscles.[19]

Even though previous studies have stated the male dominance in childhood leprosy, a study by Sashidaranpillai et al. found surprising female predominance in relapse cases. The higher susceptibility of teenage females to leprosy relapse can be due to hormonal changes and eventual alterations in the immune system with menarche.[20]

Immune Dysregulation in Leprosy

The immune system of the host is a crucial factor in leprosy. A healthy immune system must be nurtured from the beginning of life by improving the perinatal health status of both mother and child, and by improving environmental conditions at an early stage, especially in the first 1000 days of life.[21] Some immune components known as having an important function in leprosy are Th1, Th2, Treg, and Th17. The dysregulation of these four components makes the host susceptible to leprosy.

The groups at high risk of leprosy are individuals who have poor living standards, poor nutrition, and poor environmental conditions[22]. These are thought to cause dysregulation of the immunity[23] which is very prominent in leprosy endemic areas, and makes leprosy transmission easier.[11]

The immune equilibrium model suggests that the immune system depends on a balance between these various forms of immune response: a balance that determines homeostasis. As a result, the inhibition of the other forms of reaction is triggered by a microorganism or injury that causes one form of response. Conversely, the loss of stimulation of one form of reaction contributes to exacerbation of the other forms of response, with potentially pathological implications.[24]

Immune system that does not function properly results in immune system deficiency, hypersensitivity, autoimmunity, and uncontrolled inflammation. Ivanova and Orekhov[25] state that T lymphocyte plasticity is an important mechanism that makes the immune system able to respond to environmental changes by changing its function during exposure to parasites and infectious agents. Several specific subsets of T cells are regulated by complex cytokine signaling and transcription factors, and disruption in these signaling can cause a variety of disorders.

Immune dysregulation will occur when individuals cannot prevent infection, malnutrition, exposure to pollution, prevent exposure to smoke and cigarettes in children, provide an ideal physical and psychological environment for children, and the early usage of antibiotics in children.[26]

Polarized T cell response (Th1/Th2 bias) to *M. leprae* is known to be a crucial factor of leprosy pathogenesis and its numerous clinical forms. Generations of Th1 effector cells mainly developing interferon-gamma cytokines (IFN- γ) vs. Th2 interleukin-producing cells-4 (IL-4) are considered mainly accountable for the polarized state of immunity.[11]

The clinicopathological features of leprosy have distinct immunological implications. Immunologically, lepromatous leprosy is characterized by a Th2 T-cell immune response (interleukin-4 [IL-4] and IL-10), antibody complex formation, the absence of granulomas, and failure to restrain *M. leprae* growth. Tuberculoid leprosy features a Th1 T-cell cytokine response (gamma interferon [IFN-γ] and IL-2), vigorous T-cell responses to *M. leprae* antigen, and containment of the infection in well-formed granulomas.[27]

Lepromatous leprosy lesions are characterized by a lack of $CD4^+$ T cells, numerous $CD8^+$ T cells, and foamy macrophages, whereas tuberculoid leprosy lesions have a predominance of $CD4^+$ T cells and well-formed granulomas. In lepromatous leprosy, robust antibody formation occurs but is not protective, and cell-mediated immunity is conspicuously absent. In contrast, in tuberculoid leprosy, cell-mediated immunity is relatively preserved, and there is little evidence of *M. leprae*-specific humoral immunity. However, as noted above, the majority of patients are not found at the poles of the leprosy spectrum but in the intermediate categories of BL, BB, and BT disease, which are clinically 'unstable'. The immunology of these borderline states is poorly understood.[27]

Innate immunity responds to leprosy infection: macrophages and complementary natural killer (NK) cells; and adaptive immunity: lymphocytes and dendritic cells.[28,29] *M. leprae* entering through the skin will meet the dendritic cell (DC) as the first host to respond. DC in the epidermis is known as the Langerhans cells and in the dermis as the dermal DCs. Langerhans cells express CD1a and langerin in leprosy skin lesions. These cells efficiently display antigens to T-cells as part of the host response to leprosy infection.[10,30]

CD1a+ cells are associated with the results of leprosy reactions. CD1a is expressed in CD123+ cells located in the dermis in both lepromatous and reversal

patients. Quantitative study has indicated a significant predominance of dendritic cells in tuberculoid leprosy. In tuberculoid leprosy lesions, dendritic cells have been associated with matrix metalloproteinase (MMP)-12 and lead to the growth of granuloma.[10]

At first, *M. leprae* entering the host is recognized by toll-like receptors (TLRs), which then cause NF-Kb activation and increase pro-inflammatory cytokines (GM-CSF, IL-1B, TNF-α, IP-α, IP-10, IL-12) and chemokines, such as macrophages, which trigger the migration and activation of antigen-presenting cells. This antigen-presenting cells (ACP) then introduces *M. leprae* to lymphoid T-naive cells.[6,31]

Depending on its co-stimulators, inhibitors or other cytokines, naive T cells can develop into Th1, Th2, Treg, and Th17. Several previous studies have demonstrated differences in host response in PB and MB types[6] where PB type leprosy is more dominated by Th1-mediated immune responses. Th1-dominated immune responses are mediated by protective IFN-gamma and IL-2 with microbicidal properties. IFN-gamma induces macrophage activation resulting in induced synthase of nitric oxide (iNOS) and NO destroying *M. leprae*. Additionally, this immune response also produces IL-1b, IL-6, TGF-β, and IL-23; as later discovered, these cytokines are also involved in Th17 induction.[32]

A study stated that these cytokines could be used to predict protective factors against *M. leprae*. Th1 cells are also largely associated with leprosy reactions, in addition to being associated with PB types. Immune dysregulation causes low levels of Th1-produced cytokines that cause low clearance by activation of APCs, macrophages, and natural killer cells (NKCs). Unlike leprosy of the PB type, MB type leprosy has predominant immune response mediated by Th2. Th2 is mediated by IL-4, which was investigated to have the effect of suppressing macrophage microbicidal response, diminishing Th1 response, and promoting *M. leprae* survival.[23]

The role of regulatory T (Treg) cells in maintaining self-tolerance and balancing immune reactions in autoimmune diseases and chronic infections is well known. Regulatory mechanisms, however, can also lead to an extended survival of pathogens in chronic infections such as leprosy and tuberculosis (TB). Treg plays a role in suppressing the immune system by inhibiting the proliferation of T cells and reducing cytokine production. Treg are CD4+CD25+ cells that express CXCR4 and CCR5 on their surface, and have FOXP3+ transcription factors that play a role in inflammatory response regulation. Treg can identify autoantigens that are derived from damaged tissues and thus induce and maintain self-tolerance. Treg regulatory function is performed by inhibiting the activation of effector T cells such as Th1 and Th17; and by activating, proliferating and recruiting other Treg cells at the injury site through intermediaries of inflammatory and chemokine mediators.[23]

In leprosy, Treg has a role to play in keeping the balance of Th1 and Th2 responses. Immune dysregulation causes accumulation of Tregs found in MB leprosy which suppresses the immune response and causes the host to experience irresponsiveness to *M. leprae* infections.[33,34] Tregs, which are high in leprosy patients, are responsible for host immune suppression by producing IL-10 and TGF-β like cytokines. The first study describing the conversion of Tregs into Th1-like and Th17-like cells using in vitro cytokine therapy in leprosy patients was a study by Tarique et al.[35] Mononuclear peripheral blood cells were isolated from leprosy patients, and stimulated with MLCwA, rIL-12 and rIL-23 for 48 h. FoxP3+ expression in CD4+ CD25+ Tregs, intracellular cytokines IFN-γ around, TGF-β around, IL-10 and IL-17 in Tregs cells was evaluated after stimulation by flow cytometry (FACS). Treatment with rIL-12 increases pStat4 levels in Tregs and IFN-γ production. PStat3+ and IL-17A+ cells increase in the presence of the rIL-23. rIL-12 and r-IL-23 treatment down controlled Tregs development of FoxP3+, IL-10 and TGF-β and enhanced expression of co-stimulating molecules (CD80, CD86). rIL-12 converts Tregs through STAT4 signaling into IFN-γ producing cells while rIL-23 converts Tregs into IL-17 generating cells through STAT-3 signaling in patients with leprosy.[35] Th17 (CD4+ Th17) is one of the more recently identified effector T cells in contrast to Th1, Th2, and Treg. The presence of IL-23, IL-6, and TGF-β influences the differentiation of the naive T cells to Th17. Th17, like Th1, is pro-inflammatory, developing IL-17A, IL-17C, IL-17D, IL-17E, and IL-17F.[36]

Immune dysregulation in leprosy and associated cytokines

TNF is a multifunctional proinflammatory cytokine that is essential for the control of mycobacterial and other infectious diseases and is produced by monocytes and macrophages. Treatment with TNF inhibitors may be associated with the development of leprosy, and the withdrawal of TNF inhibitor treatment in leprosy patients may also enhance the formation of a type I reversal reaction. There is also evidence that TNF inhibitors may be effective for the treatment of recurrent erythema nodosum leprosum.[27]

Th17 has a major role in host immunity to extracellular pathogens and fungal infections, as well as involving autoimmunity, allergies, tumor development, and responses to organ transplants. Th17 is thought to have protective properties in leprosy and is found more frequently in PB type leprosy than MB[6]. Another study stated that Th17 is more common in patients with BT/TT leprosy than BL/LL, proven by the presence of high levels of IL-17 and IL-1. IL-17 also plays a part in producing inducible Nitric Oxide Synthase (iNOS), to help kill *M. leprae* mediated by reactive oxygen species.[23]

Evaluation of the cell-mediated immune response based on IFN-γ release assays has been used to classify *M. leprae*-specific antigens capable of discriminating against asymptomatic illness and/or early stages of infection in endemic areas. It's been seen that *M. leprae* peptides better discriminate contacts and patients against healthy controls. However, they induce low levels of IFN-γ compared to proteins, particularly when evaluated by whole blood assays. Peptides that provide specific reactions in patients with leprosy from an endemic setting could theoretically be developed into a rapid diagnostic test for early detection of infection and epidemiological studies of the prevalence of leprosy, of which little is known. One of the hindrances to T-cell-based diagnostic tests is cross-reaction of *M. leprae* antigens at the T-cell level with antigens found in other mycobacteria, such as MTB or *M. bovis* BCG.[37]

Immune Dysregulation in Childhood Leprosy

The dysregulation of the immune system early in life is crucial for the occurrence of dysregulation in older age, as explained in the concept of the immune regulation. Dysregulation will occur in the event of infection, stress, exposure to pollution, malnutrition and obesity, exposure to cigarette smoke, alcohol or drugs, unavailability of ideal physical and psychological environment for pregnant women and children and also to encourage vaginal delivery, as well as prevent infection, exposure to pollution, stress, malnutrition, and early antibiotics in children.[26]

Maternal-fetal connection in immune dysregulation

Previous findings indicate that the condition of increased Treg levels and decreased Th17 levels in healthy mothers will be continued in the fetus. There is evidence from previous studies showing that host-pathogen interactions are predominantly influenced by the genetic composition of the host. These findings are summarized in a brief review of genetic susceptibility in leprosy by Cambri and Mira[38]. An early study showed that the predisposition for infectious disease incidence was hereditary, in contrast to non-genetic factor-dependent cancers. The innate predisposition to infection appears to be crucial for leprosy, it is estimated that only 5-12% of individuals exposed to M. leprae are infected and symptomatic.[38]

Regulatory T cells (Tregs) and IL-17-producing Th17 cells are functionally and developmentally reciprocal to each other. Naive T cells develop into Tregs in the presence of transforming growth factor (TGF)-b, while the combination of TGF-b and IL-6/IL-21 introduce naive T cells into Th17. Tregs with tolerance or immunosuppression functions are the primary mediators maintaining peripheral tolerance and are essential for the prevention of autoimmune diseases and chronic inflammatory diseases. However, Tregs may also suppress the appropriate host immune responses against infections. IL-17-producing Th17 cells with immunity/inflammation functions are a recently discovered and characterized subset of effector T helper cells, which have a reciprocal relationship with Tregs in subsets of developmental programs.[39,40]

Sadhu and Mitra[11] stated that in leprosy patients there was an inversion of Treg and Th17 levels. Therefore, under conditions of increasing T-reg levels, Th17 levels will decrease. A study by da Motta-Passos

et al.[41] concluded that low Th17 expression in leprosy patients is a genetic feature of the patient. In addition, the study also showed that patients with leprosy had lower Th17 expression than in healthy controls.

According to Arsyad et al.[42] because the immune response takes some time to form, it can be assumed that household contacts of leprosy have more antigen load (*M. leprae*) in their bodies. This study conducted PCR examination of *M. leprae* on nasal swabs and anti-PGL-1 antibodies using ELISA in household contacts and non-contacts of leprosy. This study found that contact with leprosy patients in the same household can be the cause of the accumulation of antigens and high production of specific antibodies. In this study, healthy mothers in endemic areas had elevated Treg levels.

Previous evidence suggests that maternal cytokine levels influence the cytokine profile of children early in life. Longitudinal studies conducted on pairs of mothers and children in Indonesia that examined the relationship between maternal and child cytokine levels showed that the production of IL-10 in infants is influenced by maternal cytokine levels and environmental factors during pregnancy and breastfeeding that affect the mother.[43]

There has been a growing number of studies looking at immune responses in early life and their regulation by environmental conditions, which could predispose individuals to such diseases in later life.[43]

A study by Djuardi et al. suggest a strong association between maternal and infant cellular immune responses even after taking into account several environmental effects that could directly or indirectly influence the infant's response through the uterine microenvironment.[44]

A study in Indonesia found that most of the mother–child cytokine relationships became weaker over time (IL-10, IFN-γ and TNF-α). These findings indicate that the strong association between cytokine responses of a pregnant mother and her child is not directly due to genetic factors but could likely result from similar immune conditioning during gestational period extending into early childhood. Furthermore, fetal Th cell responses may be biased by the maternal immune system.[43,44]

There are anecdotal reports of leprosy in infants probably indicating intrauterine transmission of leprosy in humans. The proof of suspected intrauterine infection lay in the continued presence of IgA and IgM type of anti-*M. leprae* antibodies in the cord sera associated with an early and significant increase in these antibodies after birth. A decrease in serum IgG anti-*M. leprae* antibody was demonstrated in one of the babies after complete response to treatment.[6]

A previous study has highlighted that human breast milk cells contain a limited number of viable bacteria and bacterial DNA that might have been transported from the mother's intestine to the mammary gland through an endogenous cellular route. An animal study suggests that this process begins in late pregnancy. The results suggest a novel form of mother-infant connection. Elevated translocation of bacteria or their components in the mother should have an effect on her immune status and may explain the physiological activation of innate immunity that occurs during pregnancy.[45] This could potentially be a new mechanism of immune regulation in healthy individuals.

Immune dysregulation and child development

Activation of late fetal or neonatal T cells by foreign antigen results in a response biased to Th2 immunity, which is enhanced by neonatal dendritic cells and epigenetic features. Thus, tolerogenic reactivity, decreased allo-antigen recognition and weak responses to foreign antigens characterize rather early-life adaptive T-cell immunity.[46]

The immune repertoire is also formed by intercurrent infections and vaccines as the child grows. Pathogenic infections can be documented by symptomatic illnesses suffered

by the child or adult, but for many viruses, such as influenza, infection may be subclinical, but still sufficient to stimulate or boost immune responses.[46]

The transmission of protective antibody protection from a mother to her child is hugely important, especially in environments where 15%or more infants and children die of infection. Paradoxically, a mother who avoided a dangerous childhood infection, through herd immunity, may actually put her child at risk by being unable to

transfer specific protective antibodies.[46]

Previous studies show an age-associated increase of IFN-γ and TNF-α release, which is also consistent with the findings from flow-cytometry studies. The importance of the age-interval of investigated populations is further supported by a publication in Indonesian children showing that the greatest age-associated increase for IFN-γ and TNF-α was seen after the first year of life.[43,47]

It has been stated that the higher susceptibility of teenage females to leprosy relapse can be due to hormonal changes and eventual alterations in the immune system with menarche.[20] This can be explained by the correlation of cytokines and menstrual cycle. Factors that are extrinsic to the immune system also contribute to the heightened proinflammatory environment. For example, both estrogen and testosterone decrease IL-6 production in vitro and in vivo and age-associated decreases in estrogen or androgen production are associated with increases in basal proinflammatory cytokine levels.[48] A study also found that IL-4 is positively correlated with estrogen while TNF-alpha is positively correlated with progesterone. Females were found to have significantly higher concentrations of TNF-alpha across all phases of the menstrual cycle, compared to males across similar time points.[49] This shows a plausible mechanism of leprosy incidence and leprosy reactions in females, especially those who are born from mothers who had leprosy.

Conclusion

The proportion of new leprosy in children is one indicator of failure on termination of leprosy chain transmission and reflects the number of undiagnosed cases in the community. The immune system of the host is a crucial factor in leprosy. It is important to understand immune dysregulation in childhood leprosy. This study highlighted the plausible maternal-fetal connection which are related in immune dysregulation in childhood leprosy. The study provided promising approaches and targets for further researches in immune dysregulation, especially in childhood leprosy.

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Environmental Factors and Leprosy in Mother and Child: A Study in Endemic Areas in East Java, Indonesia

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Abstract

Background: Leprosy is a chronic infection caused by *Mycobacterium leprae*. In endemic locations, children become vulnerable as a result of being continuously exposed to foci of active transmission from a very young age, especially from their mothers. This study aims to find association between environmental risk factors and leprosy in mothers and children in endemic areas.

Methods: This study is a case-control study in endemic areas in Tuban Regency, East Java Province, Indonesia. Retrieval of data was done using structured questionnaire and direct measurement. Chi-square was used to assess the association between environmental factors and leprosy in mothers and children groups.

Results: 22 pairs of cases and 57 pairs of controls were examined. It is found that access to clean water is associated with leprosy in mothers in endemic areas (p value = 0.047, OR 3.080, CI 95% 2.232-4.251). Environmental factors are not associated with pediatric leprosy in endemic areas.

Conclusion: The results of our study show that environmental factors are not associated with pediatric leprosy in endemic areas. Access to clean water is associated with leprosy in mothers in endemic areas.

Key words: *Environmental factors, Leprosy, Mother and children, Endemic*

Introduction

Leprosy is a chronic infection caused by *Mycobacterium leprae*. In 1991, the World Health Organization (WHO) has proposed the leprosy elimination program that aimed to reduce the global prevalence of leprosy to less than one case per 10,000 population by the year 2000.[1, 2] However, leprosy remains endemic in some country, with relatively high burden in children and an increase in the new cases detection rate.[3, 4] In 2019, 177,175 registered cases and 202,185 new cases of leprosy were observed globally.[3]

Three countries with the highest leprosy cases i.e. India, Brazil, and Indonesia accounted for 80% global leprosy cases.[4] In Indonesia, registered leprosy cases reached 19,938 cases in 2019, in which 17,439 were new cases; leprosy on female add up to 10,741 cases (61.59%) and 2,009 (11.52%) cases were observed amongst children.[5] Despite rarely being lethal, leprosy

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cause numerous morbidity; ranging from skin and peripheral nerves manifestation to tissue damage, deformity, disability, and stigma.[6, 7]

Transmission of leprosy is accepted to be primarily person to person: the risk of developing leprosy is 5–10 times higher if one member of the family has developed the disease previously.[8] Previous studies showed that men were predominantly more affected by leprosy than women do. The significant difference could be attributed to underdiagnosis due to the sociocultural factors amongst women, such as illiteracy, limitation of mobility, and having low social status.[9] In addition, the position of women in the household increases the risk of leprosy transmission to their child.

Due to the long incubation period of this disease, leprosy is more common in adults. Nevertheless, in endemic locations, children and adolescents, theoretically considered the group most resistant to infection, become vulnerable as a result of being continuously exposed to foci of active transmission from a very young age.[10] Furthermore, it is known that new cases of leprosy in children depicts active transmission of leprosy in an area or a country.[11] Proportion of new cases of leprosy among children aged <15 years old also shows the high potential of transmission through household contacts. A study showed that cases of leprosy among children under 15 years old is a strong indicator of active leprosy source in a society.[12]

Previous studies have shown that pediatric leprosy most likely happens in an area with poor sanitation. A study in India has stated that low socioeconomic status, poor housing, and environmental factors are associated with leprosy. Bacteria can live in natural reservoirs such as land and water disposal unit, so this could contribute to the transmission of leprosy.[13] Another study has stated that social markers and environmental conditions are risk factors of *M. leprae* transmission in children and adolescents in Colombia.[14]

Finally, epidemiological studies of leprosy in children can point out the important aspects of the environment that influence the leprosy transmission in an endemic area. This is because children have lower mobility than adults.[15]

In previous studies, even though family contact increases the risk of leprosy, in a typical endemic area, the majority of new cases cannot be linked to intra-domiciliary contact with a leprosy patient.[8] This suggests the possibility that infection may result through prolonged or repeated unknown exposure to an environmental source containing viable bacilli. Evidence also suggests that the degree of vulnerability of the individual, the extent of exposure, and associated environmental factors could potentially influence the transmission. Complete understanding of ecological and environmental components may unfold the gaps in knowledge regarding the mode of transmission of leprosy.[16, 21]

Despite being one of the priority countries that are highlighted by WHO for leprosy elimination, there is a lack of studies regarding leprosy in mother and children in Indonesia, especially in endemic areas. Therefore, this study aims to analyse environmental risk factors for leprosy in mother and children in endemic areas.

Materials and Methods

Study area and population

The study was conducted from March until June 2020 in 10 sub-districts in Tuban Regency, East Java Province. Tuban Regency, a regency in northern area of East Java Province, is located 0-500 meters above sea level. This rather small regency (1.839,94 km², 3,8% of East Java) has about 1.2 million inhabitants, with paddy fields (31,6%) dominating its land. This regency is considered a leprosy pocket area, with 172 cases in 2018 of which 5.81% cases were cases among children. These 10 areas across 10 sub-districts (Bulu, Jenu, Jetak, Kerek, Palang, Soko, Sumurgung, Tambakboyo, Temandang, Tuban) are considered endemic areas of leprosy, where in the last 5 years there are always new cases every year. Study area is depicted in Figure 1.

Cases were selected from the local primary health center's registry data. The inclusion criteria for subject with leprosy was those with confirmed diagnosis of leprosy and aged between 5-18 years old for children; whilst the excluded were those with any leprosy reaction, poor general condition, and diagnosed with inflammatory or autoimmune disorder, allergy, or infection other than leprosy, and pregnancy. All of the

subjects were given informed consent. Thereafter, to confirm the diagnosis, the subjects underwent clinical examination done by a dermatologist and then acid-fast staining by trained health and laboratory professional from Dr Soetomo General Hospital and Tropical Disease Centre of Airlangga University. Controls were selected from mothers and children who visit the same primary health centers for other than skin problems and live in the same sub-districts.

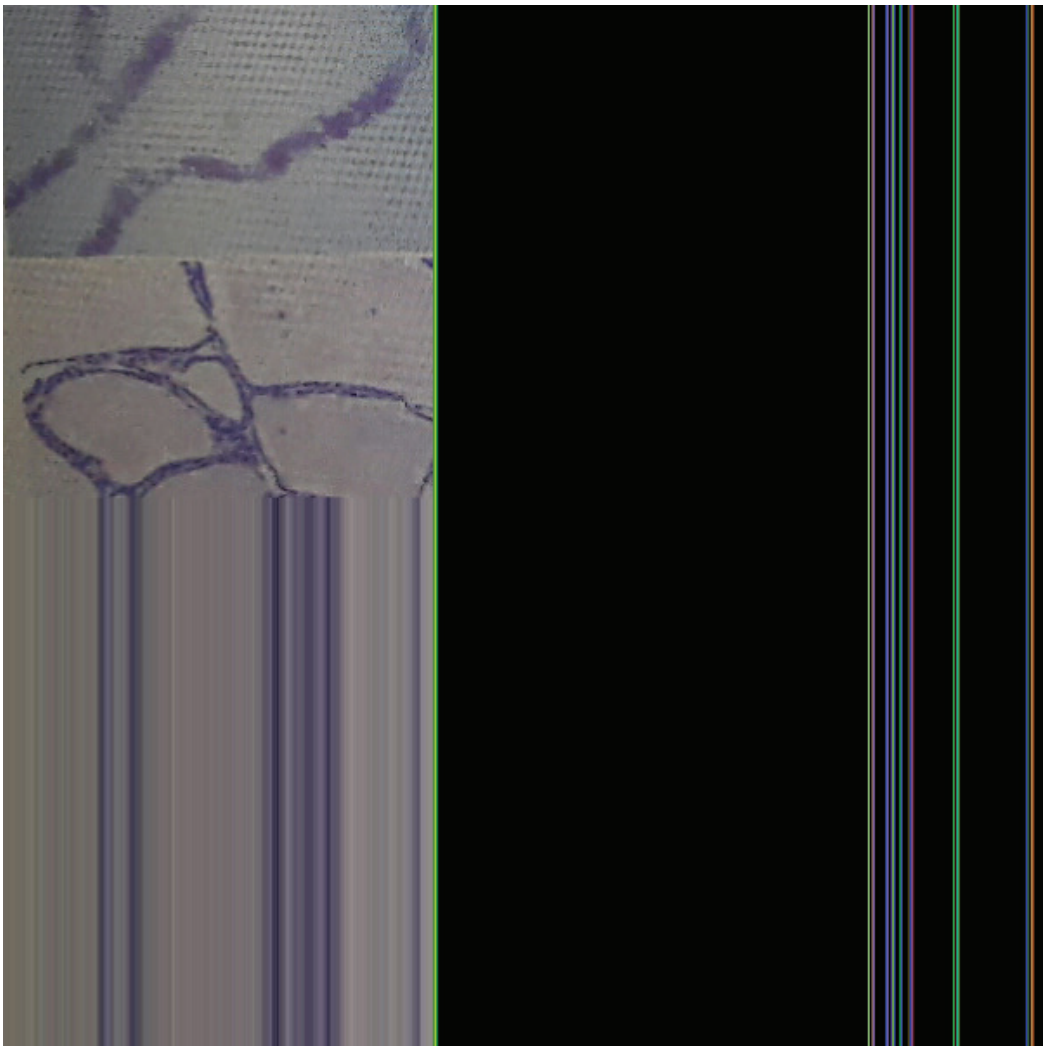

Figure 1. Study area. Ten sub-districts in which the study areas are located are colored in grey.

Data Collection

A structured questionnaire was used to collect demographic and environmental data from cases and controls (*see supplementary data*). Environmental data includes house ceiling, house windows, house walls, house flooring, lighting and house brightness, temperature, humidity, bedroom crowding, bedroom and house windows, ventilation, access to clean water, latrine availability, sewage disposal unit, trash disposal unit, and pre-tested house criteria score. Trained health professionals were responsible for interviewing cases and controls and measuring house components at the participants' houses.

Data Analysis

Data were analysed using SPSS® software (IBM Corp., Armonk, New York, USA). Variables were analysed using chi-square test to assess the association between environmental factors and leprosy in each mother and child populations.

Ethical considerations

The study protocol has been approved by the Health Research Committee of Dr Soetomo General Hospital, Surabaya (Ref. 1664/KEPK/XI/2019). Subjects were only included after written informed consent was obtained and they were reassured that non-participation would not affect their treatment.

Results and Discussion

Information was obtained from 22 pairs of cases and 57 pairs of controls in endemic areas. Within the children group, the proportion of male subjects is 48%. The mean age of all participants is 13.18 years (SD ± 4.649). The results of bivariate analyses are shown in Table 1. From bivariate analysis, it can be derived that none of the observed environmental factors are associated with pediatric leprosy in endemic areas.

Table 1. Bivariate analysis of environmental factors and pediatric leprosy in endemic areas

Variable	Diagnosis		Total	P Value	Crude OR (95%CI)
	Leprosy	Healthy			
	n (%)	n (%)	n (%)		
Having house ceiling					
No	20 (30.8%)	45 (69.2%)	65 (100%)	0.212	2.667
Yes	2 (14.3%)	12 (85.7%)	14 (100%)		(0.545 – 13.036)
House walls					
Bamboo webbing/not permanent	3 (37.5%)	5 (62.5%)	8 (100%)	0.521	1.642
Permanent walls	19 (26.8 %)	52 (73.2%)	71 (100%)		(0.357 – 7.544)
House flooring					
Soil	5 (50.0%)	5 (50.0%)	10 (100%)	0.094	3.059
Plestered/Ceramic	17 (24.6%)	52 (75.4%)	69 (100%)		(0.789 – 11.860)
Lighting					
Poor (<60 lux)	18 (31.0%)	40 (69.0%)	58 (100%)	0.294	1.912
Adequate (>= 60 lux)	4 (19.0%)	17 (81.0%)	21 (100%)		(0.563 – 6.498)
House brightness					
Dim/dark	18 (32.1%)	38 (67.9%)	56 (100%)	0.184	2.250
Brightly lit	4 (17.4%)	19 (82.6%)	23 (100%)		(0.667 – 7.586)
Temperature					
<18 oC; >30 oC	19 (26.8%)	52 (73.2%)	71 (100%)	0.521	0.609
18 oC – 30 oC	3 (37.5%)	5 (62.5%)	8 (100%)		(0.133 – 2.798)
Humidity					
>60%; <40%	21 (31.8%)	45 (68.2%)	66 (100%)	0.076	5.600
40% - 60%	1 (7.7%)	12 (92.3%)	13 (100%)		(0.683 – 45.947)
Bedroom crowding					
Crowded	14 (25.9%)	40 (74.1%)	54 (100%)	0.575	0.744
Not crowded	8 (32.0%)	17 (68.0%)	25 (100%)		(0.264 – 2.099)
Availability of bedroom windows					
No	14 (28.6%)	35 (71.4%)	49 (100%)	0.855	1.100
Yes	8 (26.7%)	22 (73.3%)	30 (100%)		(0.397 – 3.048)

Cont... **Table 1. Bivariate analysis of environmental factors and pediatric leprosy in endemic areas**

Availability of house windows					
No	13 (34.2%)	25 (65.8%)	38 (100%)	0.225	1.849
Yes	9 (22.0%)	32 (78.0%)	41 (100%)		(0.682 – 5.016)
Ventilation					
No	12 (27.3%)	32 (72.7%)	44 (100%)	0.898	0.938
Yes	10 (28.6%)	25 (71.4%)	35 (100%)		(0.349 – 2.520)
Access to clean water					
No	0 (100%)	2 (1000%)	2 (100%)	0.374	1.400
Yes	22 (28.6%)	55 (71.4%)	77 (100%)		(1.216 – 1.612)
Latrine availabity					
No	1 (14.3%)	6 (85.7%)	7 (100%)	0.402	0.405
Yes	21 (29.2%)	51 (70.8%)	72 (100%)		(0.046 – 3.570)
Sewage disposal unit					
No	5 (38.5%)	8 (61.5%)	13 (100%)	0.350	1.801
Yes	17 (25.8%)	49 (74.2%)	66 (100%)		(0.518 – 6.263)
Trash disposal unit					
No	17 (28.3%)	43 (71.7%)	60 (100%)	0.864	1.107
Yes	5 (26.3%)	14 (73.7%)	19 (100%)		(0.345 – 3.550)
House criteria					
Unhealthy (375 - 933)	13 (26.0%)	37 (74.0%)	50 (100%)	0.630	0.781
Healthy (<375)	9 (31.0%)	20 (69.0%)	29 (100%)		(0.285 – 2.142)

In the group of mothers, the mean age of all participants is 41.3 years (SD ± 7.943). The results of bivariate analyses are shown in Table 2. From bivariate analysis, it can be concluded that only access to clean water is significantly associated with leprosy in mothers in endemic areas (p value = 0.047, OR 3.080, CI 95% 2.232 – 4.251). Other factors are not are associated with leprosy in mothers in endemic areas.

Table 2. Bivariate analysis of environmental factors and leprosy in mothers in endemic areas

Variable	Diagnosis		Total	P Value	Crude OR (95%CI)
	Leprosy	Healthy			
	n (%)	n (%)	n (%)		
Having house ceiling					
No	21 (32.3%)	44 (67.7%)	65 (100%)	0.450	0.636
Yes	6 (42.9%)	8 (57.1%)	14 (100%)		(0.196 – 2.069)
House walls					
Bamboo webbing/not permanent	2 (25.0%)	6 (75.0%)	8 (100%)	0.564	0.613
Permanent walls	25 (35.2 %)	46 (64.8%)	71 (100%)		(0.116 – 3.267)
House flooring					
Soil	5 (50.0%)	5 (50.0%)	10 (100%)	0.259	2.136
Plestered/Ceramic	22 (31.9%)	47 (68.1%)	69 (100%)		(0.560 – 8.151)
Lighting					
Poor (<60 lux)	18 (31.0%)	40 (69.0%)	58 (100%)	0.328	0.600
Adequate (>= 60 lux)	9 (42.9%)	12 (57.1%)	21 (100%)		(0.215 – 1.677)

Cont... **Table 2. Bivariate analysis of environmental factors and leprosy in mothers in endemic areas**

House brightness Dim/dark Brightly lit	17 (30.4%) 10 (17.4%)	39 (69.6%) 13 (56.5%)	56 (100%) 23 (100%)	0.264	0.567 (0.208 – 1.544)
Temperature <18 oC; >30 oC 18 oC – 30 oC	25 (35.2%) 2 (25.0%)	46 (64.8%) 6 (75.0%)	71 (100%) 8 (100%)	0.564	1.630 (0.306 – 8.685)
Humidity >60%; <40% 40% - 60%	20 (30.3%) 7 (53.8%)	46 (69.7%) 6 (46.2%)	66 (100%) 13 (100%)	0.102	0.373 (0.111 – 1.250)
Bedroom crowding Crowded Not crowded	18 (33.3%) 9 (36.0%)	36 (66.7%) 16 (64.0%)	54 (100%) 25 (100%)	0.816	0.889 (0.329 – 2.401)
Availability of bedroom windows No Yes	19 (38.8%) 8 (26.7%)	30 (61.2%) 22 (73.3%)	49 (100%) 30 (100%)	0.271	1.742 (0.646 – 4.699)
Availability of house windows No Yes	15 (39.5%) 12 (29.3%)	23 (60.5%) 29 (70.7%)	38 (100%) 41 (100%)	0.339	1.576 (0.618 – 4.108)
Ventilation No Yes	19 (43.2%) 8 (22.9%)	25 (56.8%) 27 (77.1%)	44 (100%) 35 (100%)	0.058	2.565 (0.954 – 6987)
Access to clean water No Yes	2 (100%) 25 (32.5%)	0 (0%) 52 (67.5%)	2 (100%) 77 (100%)	0.047	3.080 (2.232 – 4.251)
Latrine availabity No Yes	3 (42.9%) 24 (33.3%)	4 (57.1%) 48 (66.7%)	7 (100%) 72 (100%)	0.612	1.500 (0.310 – 7.247)
Sewage disposal unit No Yes	5 (38.5%) 22 (33.3%)	8 (61.5%) 44 (66.7%)	13 (100%) 66 (100%)	0.722	1.250 (0.366 – 4.272)

Cont... **Table 2. Bivariate analysis of environmental factors and leprosy in mothers in endemic areas**

Trash disposal unit No Yes	23 (38.3%) 4 (21.1%)	37 (61.7%) 15 (78.9%)	60 (100%) 19 (100%)	0.166	2.331 (0.689 – 7.892)
House criteria Unhealthy (375 - 933) Healthy (<375)	18 (36.0%) 9 (31.0%)	32 (64.0%) 20 (69.0%)	50 (100%) 29 (100%)	0.654	1.250 (0.471 – 3.317)

In this study we analysed the association of environmental factors with leprosy in mothers and children in endemic areas in East Java, Indonesia. From our understanding, this is the first study that analyses the association of environmental factors with leprosy in mothers and children specifically in endemic areas. From bivariate analysis, it can be derived that none of the observed environmental factors are associated with an increased risk of pediatric leprosy. In the mothers population, however, access to clean water is significantly associated with leprosy in mothers in endemic areas.

This result in particular is similar to our previous study with female leprosy in a different regency with endemic and non-endemic areas.[17] The previous study concluded that there is a relationship between access to clean water and female leprosy. Matsuoka et al.[18] found that *M. leprae* DNA were detected by PCR from 21 out of 44 water sources used daily by villagers in Indonesia. The study also concluded that leprosy transmission through water contaminated by bacilli is likely to happen. Furthermore, Arraes et al.[19] stated that the finding of viable *M. leprae* in natural water sources which are associated with human contact suggests that the environment plays an important role in maintaining endemic leprosy in a region. Emerson et al.[20] also supported this statement, stating that water that is shared or reused from a source patient may become environmental reservoirs for infection, possibly by aerosolization of *M. leprae*. However, these findings need to be validated by further researches to assess viable bacteria in water samples.

The results are also in line with previous studies that stated that building or floor materials were not significantly associated with leprosy.[8, 22, 23] Furthermore, previous studies also found that sanitation (sewage system or the presence of a sanitary facility in the house) did not have association with lower incidence of leprosy.[8, 22, 24]

In this study, variable reflecting risk factors for person-to-person transmission, such as crowding, did not show a significant association with leprosy; this is probably because the study did not take detailed history of household contact with leprosy patient into account. It is accepted that household contacts with patients with leprosy are the most prone to catching the disease. A study of 12 children with leprosy in Colombia found that 9 of them had a household contact with a patient with leprosy. They reported that in a family where there are cases of undiagnosed leprosy, children are the ones most likely to get sick. Among the household contacts, the risk of developing the disease was up to nine times more, while for neighbourhood contacts the risk was four times more.[25, 26]

The present study has limitations in terms of the type of study used and the use of self-reported data, which may influence some findings. However, to ensure comparable groups and minimize possible biases, such as selection bias, participants were included from the same area.

Conclusion

In conclusion, the results of our study show that environmental factors are not associated with pediatric leprosy in endemic areas. Furthermore, access to clean water is significantly associated with leprosy in mothers in endemic areas. Further studies need to be conducted to analyse other environmental factors that are not analysed in this study.

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Ethical Clearance: The study protocol has been approved by the Health Research Committee of Dr Soetomo General Hospital, Surabaya (Ref. 1664/KEPK/XI/2019).

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Conflict of Interest Statement: Nil.

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Category: Original Research Article

Estimation of Stature from Hand Measurements. A Population based Study

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Abstract

A prospective study was carried out in the department of Forensic Medicine and Toxicology, Kanachur Institute of Medical sciences, Mangaluru in 2016. The aim of the study was to evaluate the stature from hand length and hand breadth in Dakshina Kannada population. Materials and method: The study group comprised of 100 individuals of ages ranging from 22-45 years of both males and females (50 males and 50 females) residing in Dakshina Kannada. Individuals were selected randomly who attended Kanachur hospital in the age group of 22-45 years of both males and females. Written informed consent was taken from each individual. Ethical clearance was obtained from Yenepoya University to carry out the study. Materials used were stadiometer, digital caliper. Results: The linear regression analysis and multiple regression equation were obtained for both males and females. Conclusion: Linear regression equation is useful in estimating stature than multiple regressions. To denote a population it is always better to do on a larger sample size to get accuracy of the results.

Keywords*: Stature, Estimation, Population, Hand length, Hand breadth, Anthropometry.*

Introduction

Forensic anthropology is the application of science of physical or biological anthropology to legal process. It deals with using anthropological data like age, sex, stature etc. to identify a person. Anthropometry (Bertillon System) was coined by Alphoso Bertillon, a French Criminologist in 1882. It is based on the fact that after 21 years measurements of various parts of the body do not alter and it is different for each individual. Identification is to determine individuality of a person. Identification means determination of the individuality of a person based on certain physical characteristics in living or dead persons, decomposed bodies, mutilated bodies and skeleton[1].

Article 6 of the Universal Declaration of Human Rights states that everyone has the right to recognition everywhere as a person before the law [2]. Establishing the identity of a person is a difficult task to the investigating officer especially in case of mass disasters, murders etc. The anthropological data like age, sex, stature, and race are important parameters in fixing the identity. In situations like only individual hand is brought for medico legal examination it is a challenge for a forensic expert to identify a person and several parameters like age sex stature which are important in identification could be looked for [3]. By knowing the hand measurements stature can be made out and help investigating officer to narrow down the investigating procedures. Though fingerprints remain the more reliable and economical method for identification, there should be antemortem data to match the fingerprints. If there are many parameters to match apart from only DNA or fingerprinting then the error in fixing the identity is less. There are many studies to correlate different body parts with that of stature and only limited study is done in this region. Most of the studies done are concentrating on students and not on population. Hence the present study is undertaken to estimate the stature from hand measurements in Dakshina Kannada.

Methodology

The study group comprised of 100 individuals of ages ranging from 22-45 years of both males and females (50 males and 50 females) residing in Dakshina Kannada. Individuals were selected randomly who attended Kanachur hospital in the age group of 22-45 years of both males and females. Written informed consent was taken from each individual. Ethical clearance was obtained from Yenepoya University to carry out the study.

The details about the individual were noted regarding age, sex, past history of surgeries etc. in the Performa.

Inclusion criteria: All healthy individuals of age 22-45 years both males and females residing in Dakshina Kannada.

Exclusion criteria: Below 22 years and above 45 years, pregnant women, deformity of the upper limbs, any hand deformity, fracture upper or lower limb, past history of any surgeries on the back and limbs.

Materials used are

Stadiometer, digital caliper

Methodology: The following measurements were taken.

Stature: The height of the individual was measured between the vertex and floor, with the person standing erect, in anatomical position and the head in the Frankfort plane, using a stadiometer. The measurement was taken in centimetres.

Hand length: The hand was placed on a flat surface with palm facing upwards and the fingers extended and close to each other. Care was taken to see that there was no abduction or adduction at the wrist joint, i.e., the forearm was directly in line with the middle finger. The Hand length was measured using sliding digital callipers as the straight distance from the metacarpophalangeal wrist crease to the middle finger **[Fig 1**].

Hand breadth: The breadth of the hand was measured as straight distance from the most laterally placed point on the head of 2nd metacarpal bone to the most medially placed point located on the head of 5th metacarpal bone **[Fig 1]**.

These measurements were taken in millimetres.

All the measurements were taken in the morning to avoid any diurnal variation and they were taken by one observer in order to avoid inter-observer bias. Only right hand measurements were taken.

The data obtained was entered in the Performa prepared for each individual.

The data was analysed using SPSS software to see the correlation between stature and hand dimensions.

Observation and Results

The total sample size was 100(n=100) 50 males and 50 females. In males the mean height was 162.42cm and standard deviation was 9.42, right hand length was 178.38mm SD was 9.81 and right hand breadth was 78.052mm SD was 3.026, **[Table 2].** In females the mean height was 154.81cm and standard deviation was 5.35, right hand length was 165mm SD was 9.53 and right hand breadth was 72.27mm SD was 3.50**, [Table 1].**

The linear regression analysis was derived for estimating stature using hand length in females by the formula,

Height= 106.432+ 0.293(LENGTH Expressed in centimetres)

For males

Height =129.265+0.752(LENGTH Expressed in centimetres)

Linear regression formula using hand breadth in females

Height=144.932+0.137 (Breadth Expressed in centimetres)

In males was

Height=116.650+0.599 (Breadth Expressed in centimetres)

Multiple regression equation obtained for females

Height= 103.203+0.291(Length Expressed in centimetres)+0.049(Breadth Expressed in centimetres)

For males was

Height= 114.543+0.742(Length Expressed in centimetres)+0.213(Breadth Expressed in centimetres)

Table 1: Showing Average Stature of Females using regression formula derived from Hand Measurements.

Descriptive Statistics					
FEMALES	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
HEIGHT	50	144.00	166.00	154.81	5.35
LENGTH	50	150.00	192.64	165.10	9.53
BREADTH	50	65.00	80.77	72.27	3.50

Table 2: Showing Average Stature of Males using regression formula derived from Hand Measurements.

Descriptive Statistics					
MALES	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
HEIGHT	50	140.00	183.50	163.42	9.42
LENGTH	50	164.89	212.00	178.38	9.81
BREADTH	50	71.00	83.00	78.052	3.026

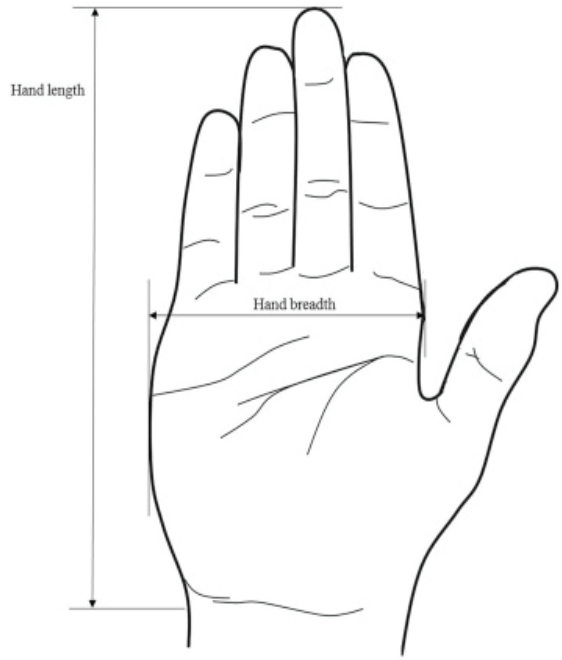

Figure 1: Showing standard landmarks used for measuring Hand Length and Breadth.

Discussion

Stature is one of the four main important parameters in physical identification that is age sex [4].Many studies have been carried out in different regions of the country to evaluate stature in different population. The regression formulas derived are different for different regions and even for both sexes it is not same. Hence attempt is made to evaluate stature from hand dimensions and to derive a formula for Dakshina Kannada population.

In the present study the multiple regression formula derived for females was Height= 103.203+0.291(Le ngth)+0.049(Breadth) and males it was Height= 14.543+0.742(Length)+0.213(Breadth) which when cross checked with known height and hand measurements found to be accurate with a +/_2cm error which are consistent with studies done by others [5-12].

The linear regression formula derived using hand length for females was Height= 106.432+ 0.293(LENGTH) and males was Height =29.265+0.752(LENGTH). Similarly linear regression formula derived using hand breadth for females was Height=144.932+0.137 (BREADTH) and in males was Height=116.650+0.599 (BREADTH) which was significant with minimum error. Rastogi et al [5] found out that multiple regression formula was more helpful in estimating stature than linear regression formula whereas in present study linear regression was more significant than multiple regression formula. It is consistent with studies done by Supare et al [8, 10].

In the present study only right hand measurements were taken and study done by Rastogi et al concluded that there were no statistically significant results when both hands were compared [5].

In the present study multiple as well as linear regression formulas have been derived and can be used for estimating stature in this region with reasonable accuracy.

Conclusion

Stature estimation in both males and females provided significant results. The multiple regression formula and linear regression formula derived can be used for stature estimation with reasonable accuracy. Linear regression equation is useful in estimating stature than multiple regressions. It has provided useful information in this region especially in cases of mass disasters, murders where identification is a difficult task for the investigating officer. To denote a population it is always better to do on a larger sample size to get accuracy of the results.

Ethical Committee Clearance: Obtained

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Potential Effect of Djambal Catfish *(Pangasius djambal) Gelatin as Biomaterial Product* on Healing Socket after Tooth Extraction in Rats

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Abstract

Purpose: Adequate wound healing after a tooth extraction is significant because the oral cavity has a masticatory function and plays a role in nutrient intake. Various scientific studies have been conducted to find the ideal biomaterial to support wound healing. This study aims to determine the wound healing activity using Djambal Catfish (*Pangasius djambal*) gelatin after tooth extraction.

Method: Gelatin was obtained from Djambal Catfish (*Pangasius djambal*). The sample was selected by random sampling, divided into 6 groups, namely the control group on the 3rd day (n=6), the control group on the 5th day (n=6), the control group on the 7th day (n=6), the treatment group on the 3rd day (n=6), the treatment group on the 5th day (n=6), and the treatment on the 7th day (n=6). Wound healing was examined by evaluating wound healing marker such as macrophages, fibroblasts, epithelialization, angiogenesis, and collagen. Data were processed using statistical analysis.

Results: The treatments had a positive result in wound healing as compared with the control groups. Histological assessment showed a remarkable sign of an increased number of macrophage, fibroblast, epithelialization, and collagen deposition.

Conclusion: Gelatin derived from Djambal Catfish (*Pangasius djambal*) can accelerate the wound healing process thus could be a component therapeutic potential product that may be beneficial in wound healing in the future.

Keyword*: gelatin, Djambal catfish, Pangasius djambal, wound healing, tooth extraction*

Introduction

The wound healing process after a tooth extraction is influenced by various factors, both internal and external. The healing process can be different for each individual. Generally, the wound healing process after tooth extraction includes the following stages: coagulation, inflammation, cell proliferation and migration, angiogenesis, matrix synthesis, remodelling, and wound contraction[1]. Various studies have reported the use of various materials to accelerate the tissue healing process, such as the use of hemosponge, bone graft, platelet-rich plasma, and platelet-rich fibrin[2,3,4].

Wound healing is the process of replacing and repairing damaged tissue function. The speed of wound healing depends on the location, severity, and extent of the injury. There are 4 main phases overlap between one and the other phase, namely the haemostasis phase, inflammatory phase, proliferation phase, and maturation phase. To ensure that the wound healing process runs well, several parameters can be used, including fibroblast proliferation, angiogenesis, collagen synthesis, epithelialization, and macrophages. Various

factors influence the acceleration of wound healing, such as the presence of local factors from intraoral and systemic factors[5,6,7].

This research aimed to obtain biomaterial products that can help accelerate the wound healing process after tooth extraction. This research applied gelatin gel material from Djambal Catfish (*Pangasius djambal*) in which the amino acid content consists of arginine, glutamine and glycine which can accelerate wound healing. There are no commercial products on the market that use Djambal Catfish as the basic ingredient, and there has been no research focused on wound healing using *Pangasius djambal*. Thus, this researcher was challenged and interested in conducting this research to determine the potential effect of applying gelatin gel made of Djambal Catfish on the tooth socket.

Materials and Methods

This research is a laboratory experimental research with Randomized Post Test Only Control Group Design in the laboratory in vivo to determine the effect of Djambal Catfish gelatin on the wound healing process in the socket after tooth extraction in Wistar strain rats with Ethical Clearance of Brawijaya University Number 399A/ EC/ KEPK-FKG/ 12/ 2017. The independent variable in this research was Djambal Catfish gelatin (*Pangasius djambal*) with a concentration of 100% while the dependent variables included the distributions of macrophages, fibroblasts, epithelialization, collagen, and angiogenesis.

The experimental animals were selected based on the sample criteria, then divided into 4 groups, each consisting of 7 rats kept in the experimental animal rearing place. The rats were cared for and adapted in the laboratory for 7 days. Before the extraction of the mandibular left incisor tooth of Wistar strain rats, an anaesthetic injection was carried out using 0.3 ml of ketamine peritoneally.

The production of gelatin comes from Djambal Catfish skin which was initially taken and separated from the meat and fat attached to it. Djambal Catfish skin was then stored at -20^{o} C. The stored Djambal Catfish skin was thawed at room temperature and cut to about 1 cm^2. After that, Djambal Catfish skin was rinsed with lemon water to remove any other material. A total of 100 gram of Djambal Catfish skin was rinsed and immersed in the citric acid solution for 12 hours to dilute the collagen fibers in order to be easily extracted. The sample was neutralized by washing it several times until the washing water was at a neutral pH (6-7). Djambal Catfish skin was extracted using a shaker water bath with distilled water at 60^{0}C for 6 hours. The gelatin solution was separated from the remaining skin using the Watchman filter cloth No. 1 and cooled at room temperature until the gelatin gel formed.

The administration of Djambal Catfish gelatin was performed by using a pipette and then the gel was slowly applied as much as 0.1 ml to the socket once after extraction and after the bleeding stop. Meanwhile, the control group was not treated. After being given Djambal Catfish gelatin (*Pangasius djambal*), the experimental animals were given a novalgin analgesic at a dose of 500 mg/ ml to reduce pain.

Tissue sampling was examined on the 3rd, 5th, and 7th day using ether. The rats were injected with a lethal dose of ketamine anaesthetic. The rats had the mandible decapitated where the tooth had been extracted. In the preparation, HPA staining was conducted by using a light microscope with a 400x magnification to ensure the location of the preparation to be counted. The sample on the preparation was divided into 5 fields of vision with the same size.

Results

Table 1. Results of Marker Calculation on Rats' Wound Healing in Control and Treatment Groups with *Pangasius djambal* gelatin

Group	Macrophage	Fibroblast	Epithelization	Angiogenesis	Collagen
C1	55.2 ± 7.92	2.9 ± 27.96	106.43 ± 13.63	1.76 ± 0.75	1.13 ± 0.35

Cont... **Table 1. Results of Marker Calculation on Rats' Wound Healing in Control and Treatment Groups with *Pangasius djambal* gelatin**

C2	42 ± 5.15	4.04 ± 32.04	96.66 ± 12.54	2.68 ± 0.72	1.76 ± 0.68
C3	33.2 ± 9.12	5.01 ± 36.52	125.28 ± 19.53	41.2 ± 0.30	-
T1	83.6 ± 4.83	6.44 ± 75.32	115.55 ± 24.33	2.92 ± 0.67	-
T2	65.6 ± 4.62	4.51 ± 88.4	131.66 ± 30.87	4.36 ± 1.29	1.66 ± 0.48
T3	33.2 ± 1.93	6.61 ± 91.02	187.29 ± 21.62	5.36 ± 0.99	2.93 ± 0.25

Note: Values were expressed as mean + SD. Values with the same letter in a row mean they are statistically significant different ($p<0.05$), and vice versa.

C1: control day 3 ; C2: control day 5 ; C3: control day 7 ; T1: treatment day 3 ; T2: treatment day 5; T3: treatment day 7

Examination Methods and Analysis of Macrophage

Based on Table 1, there was a difference in the number of macrophages between the control group not given and given Djambal Catfish gelatin. The one-way ANOVA test results showed a significance value of 0.000 ($p < 0.05$), indicating an increase in the number of macrophage cells in the wound after tooth extraction in rats between groups on the 3rd and 5th days. The results of the Post Hoc Tukey test (comparing the groups) showed that there was a significant difference in the mean number of macrophages between groups with a significant value of less than 0.05. However, several groups were being compared which had a greater value than 0.05, covering Group C1 and C2, C1 and C3, C2 and C3, and.T2 and T3.

Examination Methods and Analysis of Fibroblast

Based on Table 1, there is a difference in the number of fibroblasts between the control group not given and given Djambal Catfish gelatin. The one-way ANOVA test showed a significant value of 0.000 ($p < 0.05$), meaning that H_0 was rejected. This research indicated a difference in the number of fibroblasts in the wound after rat tooth extraction between the groups. The Post Hoc Tukey test results (comparing the groups) obtained that there was a significant difference in the mean number of fibroblasts between groups with a significant value of less than 0.05. However, several groups were being compared which had a greater value than 0.05, covering Group C1 and C2, C1 and C3, C2 and C3, and.T2 and T3.

Examination Methods and Analysis of Epithelization

Based on Table 1, there was a difference in the epithelial thickness in rats (*Rattus norvegicus*) post-tooth extraction socket between the treatment groups given and not given Djambal Catfish gelatin. The one-way ANOVA test showed a significant value of 0.000 ($p < 0.05$), meaning that H_0 was rejected. This research indicated that there was a difference in the average epithelium thickness of the wound after rat tooth extraction between the control groups and the treatment groups on the 3rd, 5th, and 7th days. The Pearson correlation test on the treatment group research data showed a correlation strength (r) of 0.761**, indicating a strong correlation between Djambal Catfish gelatin (*Pangasius djambal*) and epithelial thickness after rat tooth extraction. Furthermore, this correlation showed a positive direction, meaning that the increasing number of days (length of time) can thicken significantly the rat tooth epithelium after tooth extraction.

Examination Methods and Analysis of Angiogenesis

Based on Table 1, the administration of Djambal Catfish gelatin increased the number of blood vessels or angiogenesis in the treatment group of rats and accelerated the wound healing process in the treatment group. The One Way ANOVA test showed a significance value of less than 0.05 ($p < 0.05$). That is, the use of Djambal Catfish (*Pangasius djambal*) gelatin had a significant effect on angiogenesis in the wound healing process after tooth extraction of Wistar rats (*Rattus novergicus*). In other words, there was a significant difference in the number of blood vessels (angiogenesis) between groups on the 3rd, 5th, and 7th days. The Pearson correlation test on the research data showed a correlation strength (R) of 0.860 in the control groups and 0.736 in the treatment groups. Furthermore, this correlation had a positive direction, indicating a strong correlation between variables, in which the increasing number of days (length of time) can also increase the angiogenesis.

Examination Methods and Analysis of Collagen

The comparison between groups through the Kruskal-Wallis test was obtained a significant value (p) of 0.000, meaning that the null hypothesis was rejected. Hence, there was a significant difference in collagen formation in the healing process after tooth extraction in rats (Rattus norvegicus). The Spearman correlation test results showed that the correlation strength (r) on the 3rd day was 0.707*, indicating that Djambal Catfish (*Pangasius djambal*) gelatin was strongly correlated with collagen formation after tooth extraction in rats (Rattus norvegicus). This correlation on the 3rd day had a positive direction, meaning that the increasing number of days (length of time) can also significantly increase collagen formation after tooth extraction in rats (Rattus norvegicus). The correlation strength (r) on the 7th day reached 0.949**, indicating a very strong correlation between Djambal Catfish (*Pangasius djambal*) gelatin and collagen formation after tooth extraction in rats. This correlation on the 7th day had a positive direction, meaning that the increasing number of days (length of time) can also significantly increase collagen formation after tooth extraction in rats (Rattus norvegicus).

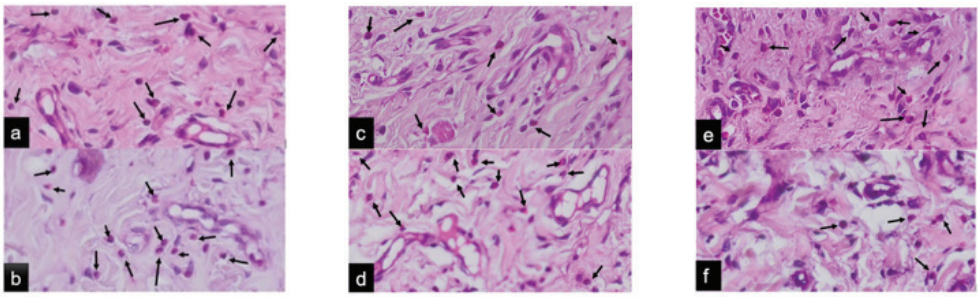

Figure 1. Histological view of macrophages using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

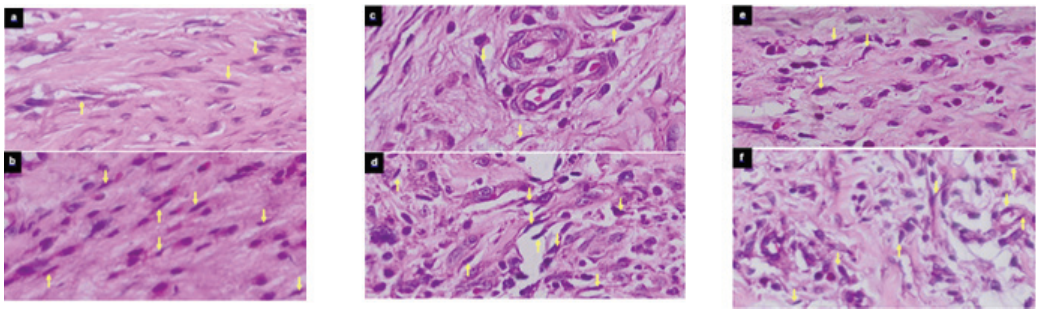

Figure 2. Histological view of fibroblasts using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

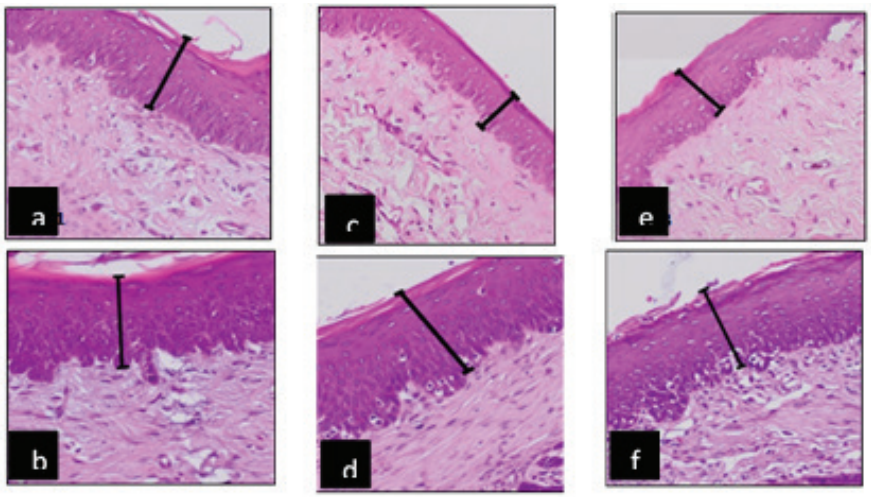

Figure 3. Histological view of epithelial thickness using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

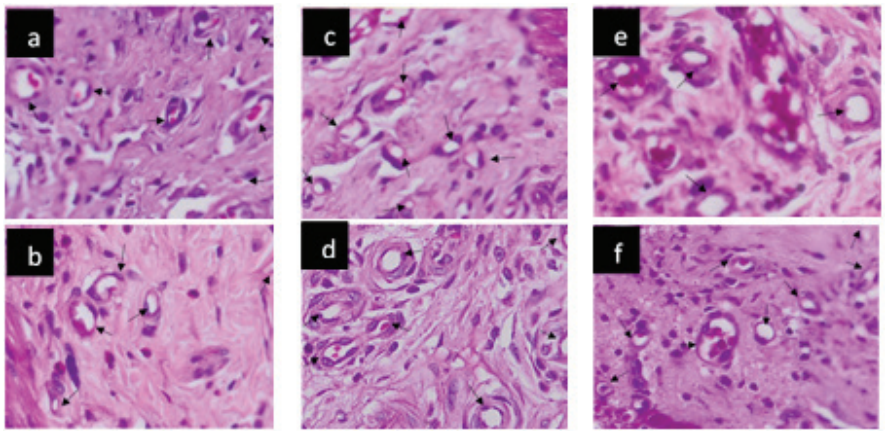

Figure 4. Histological view of the number of new blood vessels using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

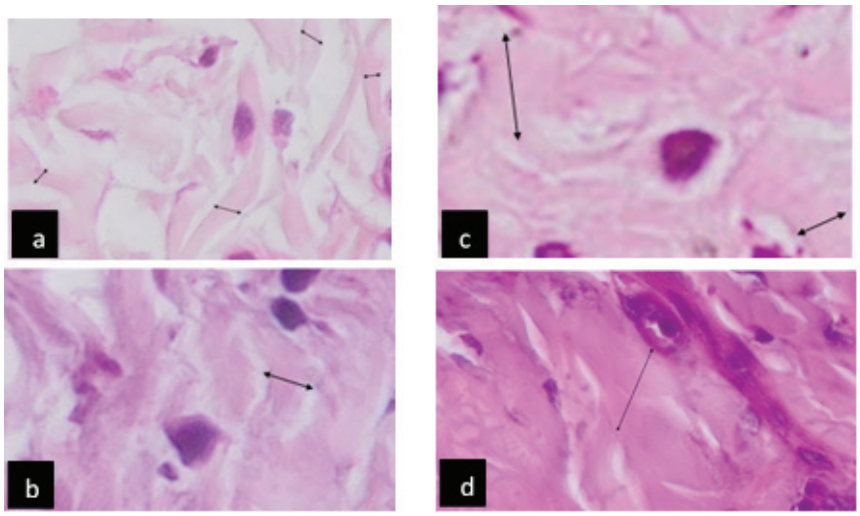

Figure 5. Histological view of collagen formation using H & E staining in one field of vision with a 400x magnification-light microscope at a) Day 3 Control Group, b) Day 3 Treatment Group, c) Day 5 Treatment Group, d) Day 5 Treatment Group, e) Day 7 Treatment Group, f) Day 7 Treatment Group.

Discussion

Based on the results of the study, the administration of Djambal Catfish (*Pangasius djambal*) gelatin in the post-tooth extraction wound resulted in an increase in the number of macrophages in the treatment groups compared to the control groups. When tissue damage occurs, macrophages will dominate the initial wound healing process for three to five days during the inflammatory phase[9,37]. Macrophages have a major role in the immune system, both as antimicrobial cells and immunoregulatory cells, which induce, suppress or modulate adaptive immune responses. In the immune response, this macrophage biology aspect is driven by the presence of macrophage arginine metabolic phenotype[10]. On the 7th day after the gelatin administration, the number of macrophages significantly decreased if compared to the control group. This is because the macrophages playing as phagocytic agents underwent apoptosis and replaced by fibroblasts. Besides that, the decreased number of macrophages can signify that the healing process has entered a proliferation phase, which is marked by the presence of fibroblasts and angiogenesis[8,12,13].

The increase in the number of fibroblasts after treatment using Djambal Catfish gelatin indicated an improvement in the wound healing process of the rat tooth socket. Fibroblasts began to emigrate to the wound area on the 3rd day after tooth extraction, so the lowest number of fibroblasts was on the 3rd day and increased to the 7th day[14,15]. The evaluation results showed a significant difference in the number of fibroblasts in the wound after rat tooth extraction, especially in the treatment groups (with the administration of Djambal Catfish (*Pangasius djambal*) gelatin), if compared with the control groups (without the administration of Djambal Catfish gelatin on the same day (C1 with T1, C2 with T2, and C3 with T3). This research suggests that the administration of Djambal Catfish (*Pangasius djambal*) gelatin can increase the number of fibroblasts in the wound after rat tooth extraction due to the glutamine and glycine amino acids which affect the wound healing process. The glutamine amino acid plays a role in increasing the fibroblast proliferation process and stimulates collagen formation[16]. Meanwhile, the glycine amino acid plays a role in regulating metabolism, anti-oxidative reactions, and neurological functions. The various beneficial effects of glycine make it an essential amino acid, including for mammals. Fibroblasts also influence the angiogenesis process. The absence of fibroblasts in combination with angiopoietin-1, angiogenin, hepatocyte growth factor, α transformation growth factor, and tumor necrosis factor will interfere the formation of blood vessel endothelial lumen[17,18,19,20,21]. The results of increasing fibroblasts make the wound healing process of the rat tooth socket would be faster with the use of Djambal Catfish gelatin.

The epithelization process begins at 12 hours after trauma with keratinocyte cell mitosis in the basal stratum. Keratinocytes will flatten and form bumps around it. These cells will lose the attachment of the hemidesmosomes to the surrounding basal cells and begin migrating at 24 hours after trauma. Within 48 hours, the proliferation of epithelial cells begins. Complete epithelial formation occurs on the fourth day to seventh days[22]. The presence of arginine plays a role to stimulate epithelial cell formation, strengthen the immune system and help synthesize amino acids. Arginine and glycine in protein can accelerate the maturation phase and the wound healing process[23,24].

In the proliferation of granulation phase starting from the 3rd to 7th day, the wound matrix will be occupied by proliferating endothelial cells. These cells will form new blood vessels in the process of angiogenesis, followed by the production of a temporary extracellular matrix and migrating from the wound edges to form layers covering the wound. The formation of blood vessels can occur from the 3rd to 7th day[26]. The findings of the increasing number of blood vessels (angiogenesis) are consistent with the study of Nofikasari (2016) showing that in the wound healing process on the 3rd day, the mean number of blood vessels seen on the histological preparation was higher than on the first day. The observation results showed that the highest mean number of blood vessels was on the 7th day because endothelial cells experienced peak proliferation[26]. The angiogenesis process also affects the formation of fibroblasts. The better vascularization of the wound area will increase fibroblast proliferation[27].

Glutamine is the main transporter of nitrogen from glutamine synthesis sites such as skeletal muscle, liver, and lungs to the endothelium. Endothelial glutamine together with asparagine and metabolism is significant

in the process of angiogenesis[25,28,29]. Arginine has the function of improving wound healing, triggering the release of insulin-like growth factor 1 (IGF-1), insulin, and prolactin[30]. Meanwhile, glycine together with VEGF increases the proliferation of endothelial cells and initiates angiogenesis both in vitro and in vivo[31]. The data analysis results from the angiogenesis examination based on the One Way Anova test on the control and treatment groups showed a significant difference in the number of blood vessels (angiogenesis). The higher number of blood vessels in the treatment group (P) indicated that the rats (*Rattus norvegicus*) experiencing tooth extraction and given Djambal Catfish (*Pangasius djambal*) gelatin affected the angiogenesis process.

Collagen is essential for the resilience and integrity of all tissues[32,33]. In the period of stress due to trauma, endogenous arginine synthesis is not sufficient to meet the increased demand for protein replacement. Thus, arginine supplementation becomes conditionally important in wound healing[34,36]. In this research, the collagen formation in the T3 group was higher than the C3 group. The mean collagen score of the T3 group was 2.93 while that of the C3 group was 1.76. This can occur because, at this stage, the inflammatory phase decreases and will change to the proliferation phase, in which growth factors will dominate. This process begins with the formation of fibroblast cells. Fibroblasts produce a basic substance, namely mucopolysaccharides, which unites collagen fibers. Collagen is useful in increasing strength in wound healing, accelerating the wound closure[35]. The higher collagen scoring in the treatment groups (T) than the control groups (C) was possibly due to the arginine contained in the Djambal Catfish gelatin.

Conclusion

Based on the research results and discussion on the use of Djambal Catfish (*Pangasius djambal*) gelatin after tooth extraction in rats (*Rattus norvegicus*), it can be concluded that Djambal Catfish (*Pangasius djambal*) has the ability to heal wounds, indicated from the activities of macrophages, fibroblasts, epithelialization, angiogenesis, and collagen formation. Although further research is still needed, it is learned that Djambal Catfish gelatin is likely to be an additional material used for wound healing therapy after tooth extraction.

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Declaration of Interest : The authors report no declarations of interest

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The D614G Mutation in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spike Glycoprotein: A Mini-Report

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a quickly spreading virus that poses a major burden on global human health. Thus, it is essential to develop COVID-19 vaccines. Vaccine development might not be easy, as a consequence of mutations. This study aimed to demonstrate the D614G mutation in SARS-CoV-2 spike glycoprotein from Indonesia. 102 isolates from the GISAID EpiCoV database and the Wuhan-Hu-1 isolate (reference sequence) from GenBank, NCBI were used in this study. BioEdit employed to examine the D614G mutation in the spike glycoprotein of SARS-CoV-2. In this report, there are 56 D614G mutations among 102 SARS-CoV-2 isolates. In summary, this report might suggest that the D614G mutation implicates the COVID-19 pandemic in Indonesia. Nevertheless, this report was a preliminary study and this study recommends expanding molecular epidemiology and surveillance programs to monitor COVID-19.

Keywords: *COVID-19, D614G Mutation, SARS-CoV-2.*

Introduction

The Chinese government first reported a novel pneumonia-causing disease in Wuhan (end of December 2019)[1,2,3]. The causative agent was identified and named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV). This new virus has rapidly spread across China and to many other countries across the world, including Indonesia. The World Health Organization has named the disease caused by SARS-CoV-2 as coronavirus disease 2019 (COVID-19)[4,5,6,7].

According to an online interactive dashboard hosted by the Center for Systems Science and Engineering at Johns Hopkins University (Baltimore, USA), which tracks reported cases of COVID-19 in real-time, about 70 million people have been infected by SARS-CoV-2 worldwide, with approximately 600,000 cases in Indonesia alone (December 2020)[8]. Currently, there are three coronaviruses that cause illness in humans: severe acute respiratory syndrome coronavirus (SARS-CoV), Middle-East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2[9].

The coronavirus family is composed of four different genera: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus*. SARS-CoV-2 belongs to Coronaviridae[10,11]. The SARS-CoV-2 genome is a single-stranded positive-sense RNA of roughly 30,000 nucleotides. This genome encodes four structural proteins: membrane (M), envelope (E), nucleocapsid (N), and spike (S)[12]. The spike glycoprotein has recently emerged as the primary target antigen in the formulation of a SARS-CoV-2 vaccine[13]. Previous study identified the candidate for a peptide-based vaccine against COVID-19 based on the spike glycoprotein[14].

The molecular epidemiological data of the SARS-CoV-2 outbreak remains unclear. Furthermore, a vital tool for discovering new emerging viruses is research

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on molecular epidemiology[15,16]. In addition, D614G is a common amino acid mutation in the SARS-CoV-2 spike glycoprotein globally[17]. Zhang *et al*. reported that this spike glycoprotein mutation transmits more efficiently[17,18]. Thus, this study aimed to demonstrate the D614G mutation in SARS-CoV-2 isolates from Indonesia.

Methods

SARS-CoV-2 Isolates

SARS-CoV-2 spike glycoprotein gene (3822 bp) sequences were obtained from GenBank (National Center for Biotechnology Information, USA) and the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database (Germany). This study used 102 SARS-CoV-2 isolates from Indonesia (end of December 2020).

Analysis of the D614G Mutation

In this report, the translation process of the genome performed by using BioEdit and then investigated the D614G missense mutation status of all isolates.

Results and Discussion

Coronaviruses infect both animals and humans and lead to many illnesses, including enteric, neurological, and respiratory diseases[3]. There are four distinct genera of coronaviruses. SARS-CoV, MERS-CoV, and SARS-CoV-2 are three highly pathogenic coronaviruses, capable of infecting humans, that emerged in 2002, 2012, and 2019, respectively[10]. Outbreaks of SARS-CoV-2 have led to a state of medical and economic emergency worldwide[15].

Many researchers worldwide have previously reported mutations in the SARS-CoV-2 genome[19,20]. In addition, Tang *et al*. have suggested that SARS-CoV-2 exhibits the characteristic high mutation rate of an RNA virus. Furthermore, the mutation rate of SARS-CoV-2 and other coronaviruses might be slightly lower than other RNA viruses because of its genome-encoded exonuclease[21]. The high mutation rate increases the potential of this zoonotic viral pathogen to adapt to efficient transmission from human to human and potentially allows it to become more virulent[15]. This report revealed that there are 56 D614G mutations among 102 SARS-CoV-2 isolates (Table 1).

Table 1. The Partial Results of D614G Mutation Analysis.

No	Isolate	D614G Mutation
1	Wuhan-Hu-1	No
2	JK-EIJK-0141	No
3	JK-EIJK-2444	No
4	JK-EIJK-0317	No
5	JK-EIJK-01	No
6	JK-EIJK-04	No
7	JK-EIJK-03	No
8	JK-EIJK-02	No
9	SA-EIJK-06	No
10	KI-EIJK-05	No
11	JK-EIJK-07	No
12	YO-UGM-202449	Yes
13	JB-TFRIC19-L4173	Yes
14	JI-ITD-853Sp	No
15	JI-ITD-1238Sp	No

This study is crucial for future investigations into the pathogenesis, prevention, and treatment of SARS-CoV-2. Development of this genomic data is vital work that will facilitate vaccine design, epidemiological investigations, viral detection, functional analysis, and evaluation of treatment options[22,15].

On the other hand, our molecular phylogenetic study reported the relationship of SARS-CoV-2 and other *Coronaviridae* based on the spike glycoprotein genes[14]. In line with this, SARS-CoV-2 is closest to *Rhinolophus affinis* coronavirus RaTG13, followed by pangolin coronavirus[13]. Therefore, Malayan pangolin is assumed to be the intermediate host before the virus began infecting humans.

Conclusion

In summary, there are 56 D614G mutations among 102 SARS-CoV-2 isolates. This report might suggest

that the D614G mutation implicates the COVID-19 pandemic in Indonesia. Nevertheless, this report was a preliminary study and this study recommends expanding molecular epidemiology and surveillance programs to monitor COVID-19.

Conflict of Interest : The author declare that they have no conflict of interest.

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Alpinia galanga or Lengkuas (Zingiberaceae): A Possible Treatment for COVID-19

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Abstract

Alpinia galanga (Zingiberaceae) or lengkuas is one type of herbal plant that is widely grown in Southeast Asia. Various countries cultivate this medicinal plant, including Indonesia. This medicinal plant has a variety of benefits and also be used as a treatment for many diseases. Although the lengkuas rhizome is the most widely used and studied part of the plant, the flower on the lengkuas can also provide additional advantages, such as antioxidant and antimicrobial properties. Therefore, this study aimed to demonstrate lengkuas for the future prospects against SARS-CoV-2. In summary, this study suggests that lengkuas might be useful as a treatment for COVID-19, although further studies could be conducted to confirm the results of computational studies.

Keywords: *Alpinia galanga, COVID-19, Medicinal Plant, Phytochemistry, SARS-CoV-2*

Introduction

Alpinia galanga (Zingiberaceae) or lengkuas is one type of medicinal plant that is widely grown in Asia. Many developing countries cultivate this plant, including Indonesia. This plant has a variety of benefits, ranging from being used as spices. lengkuas can also be used as a treatment for many diseases[1].

Lengkuas is widely used to treat breathing diseases, stomach diseases, and diarrhea. Lengkuas can also use as an antimicrobial replacement for antibiotics[2,3]. Lengkuas rhizome began to be used in several formulations to prevent cancer and is also used for the treatment of other diseases such as diabetes, neurological disorders, and so on[4,5,6,7,8].

In addition, there are various components in the lengkuas rhizome, such as flavonoids, phenolic acids, and terpenoids[9,10], while there are main active compounds found in the lengkuas rhizome, namely kaempferol, galangin, and etc[8,11,12,13,14,15]. Therefore, this study aimed to demonstrate lengkuas for the future prospects against SARS-CoV-2.

Plant Taxonomy

The lengkuas taxonomy as described: Kingdom (Plantae), Division (Magnoliophyta), Class (Liliopsida), Order (Zingiberales), Family (Alpinioideae), Genus (*Alpinia*), and Species (*Alpinia galanga*)[16].

Plant Description

Lengkuas is herb that can grow up to 3.5 cm, with underground rhizomes and minor adventitious roots. The flowers are compound and 3-4 long with a pleasant smell, with flower crowns green at the base and white buds. The rhizomes have a red-brown color on the surface, while the inside of the rhizome is brown-orange. Size and shape of the leaves are 3.8-11.5 cm, oblong-lanceolate, glabrous, distichous, and acute[17].

Geographical Distribution

Lengkuas grows in many Asian countries such as Indonesia (especially in Java)[18], Arabia, China, India, Malaysia, and Sri Lanka. It favors hot places exposed to extensive sun light, but it can also grow in forests[19].

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Traditional Use

Lengkuas rhizome is effectively used as a therapeutic treatment for various diseases, because it contains anti-inflammatory, antioxidant, anti-bacterial, anti-tumor, and etc[20]. It can be used to treat stomach pain, diabetes, and so on[21]. The tuber of the lengkuas is often used as a cough therapy, fever, and diabetes mellitus[22].

Phytochemistry

Lengkuas is one of the most common and widely studied galangal species because lengkuas has ethnobotany and medicinal benefits. Lengkuas has contains pharmacological attributes that are linked to phytochemical constituents in different galangal species. Phytochemicals in the lengkuas mostly are comprised of phenolic compounds and terpenes[23,24].

Biological and Pharmacological Actions

Lengkuas has recently become a popular medicinal plant for many studies. Therefore, lengkuas has been studied for its pharmacological effects. Various medicinal benefits of lengkuas are described below, including antiviral activity against SARS-CoV-2.

Antibacterial Activity

Lengkuas have shown significant results against *P. aeruginosa*, *S. aureus*, *E. coli*, and so on[25]. Essential oil compounds of lengkuas rhizome have significant activity against *P. aeruginosa*, *S. aureus*, *E. coli*, and etc[26,27].

Anti-inflammatory Activity

Lengkuas rhizome plays an important role in inhibiting the release of beta-hexominidase and also play a role in RBL-2H3 cells[28]. Additionally, lengkuas rhizome has anti-inflammatory potential in various animal models[29].

Antioxidant Activity

Lengkuas extract has antioxidant activity. Antioxidant activity was calculated using DPPH and ORAC[30,31].

Immunostimulatory Activity

In vivo study reported immunostimulatory activity of lengkuas[32].

Anti-Retrovirus Activity

The lengkuas rhizome extract contains the active compound which plays an important role againts HIV-1 (*Retroviridae*)[33].

Antiviral Activity against SARS-CoV-2

Medicinal plants in the Zingiberaceae family include *Alpinia*, *Kaempferia*, *Curcuma*, and so on, which contain compounds often used as herbal treatments[34]. The use of herbal treatments increased rapidly during the COVID-19 pandemic. In Indonesia, a study reported the potential inhibitory effect of *Alpinia*, *Caesalpinia*, *Citrus*, and *Curcuma* against SARS-CoV-2, so that the development may provide novel treatment and prevention strategies for COVID-19 treatment. One of the phytocomponents of lengkuas has been predicted as a possible potent antiviral agent against SARS-CoV-2[35] (Figure 1).

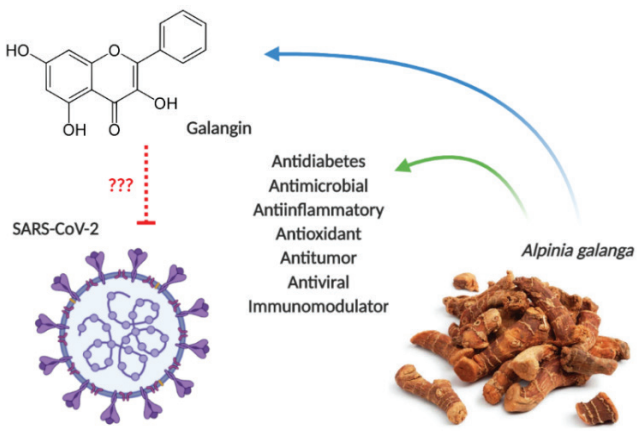

Figure 1. *Alpinia galanga* and its phytomedicine benefits.

Conclusion

In summary, lengkuas is commonly used as a medication for many illnesses. This study suggests that lengkuas might be useful as a treatment for COVID-19, although further studies could be conducted to confirm the results of computational studies.

Conflict of Interest : The author declares that there is no conflict of interest.

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Discovery of Therapeutic Targets and Potential Drugs to Fight Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Review

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Abstract

SARS-CoV-2, the causative agent of COVID-19, has led to more than two million deaths worldwide. There are currently no specific therapies approved to treat COVID-19 and drug repositioning, rather than new drug discovery, which may be the best short-term option for controlling the pandemic. In this review, the author discusses important viral targets such as the papain-like protease, RNA-dependent RNA polymerase, 3-chymotrypsin-like protease, and spike protein. The information about chemical compounds and targets provided in this review will aid further *in vitro* and *in vivo* studies to discover drugs to treat COVID-19.

Keywords: *COVID-19, drug discovery, SARS-CoV-2.*

Introduction

Infections with coronaviruses have resulted in major outbreaks of pneumonia during the twenty-first century. In 2002, SARS-CoV infections, with a lethality rate of 10%, were found on five continents[1,2]. In 2012, MERS-CoV, which has a lethality rate of 35%, emerged in the Middle East[3,4]. MERS-CoV and SARS-CoV are zoonotic viruses and their primary hosts are camels, and bats or civets, respectively[5,6]. Most recently, a novel coronavirus emerged in late 2019 and, on January 12, 2020, WHO recommended the interim name 2019-nCoV for this new virus. Later, in early February 2020, the International Committee on Taxonomy of Viruses categorized 2019-nCoV as SARS-CoV-2, and named the disease COVID-19[7]. General symptoms of individuals infected with coronaviruses include shortness of breath, cough, fever, and respiratory symptoms. In severe cases, kidney failure, SARS, and pneumonia may develop and lead to death[8]. No specific vaccines or drugs have yet been approved to treat coronavirus infections in humans[9].

Coronaviruses belong to the *Coronaviridae* family and have a positive-sense single-strand RNA genome (Figure 1). Coronaviruses are further classified into four genera, *Alpha-*, *Beta-*, *Gamma-*, and *Delta-coronavirus*. SARS-CoV-2 belongs to the genus *Betacoronavirus*. Coronaviruses contain four structural proteins, the spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N)[10] (Figure 2). Viruses bind to specific receptors on the surface of cells to gain entry. For example, MERS-CoV uses dipeptidyl peptidase-4 as its receptor[11], and SARS-CoV uses ACE2 as its receptor[12]. The spike protein aids attachment and membrane fusion and thus plays a key role in determining host range[7,8,13]. Potential therapeutic interventions against coronaviruses include disrupting viral RNA synthesis by targeting the genetic material of the virus, inhibiting viral replication by targeting enzymes, and blocking binding to cell surface receptors[14]. Blockade of cell signaling pathways needed for viral replication may also have a specific anti-viral effect.

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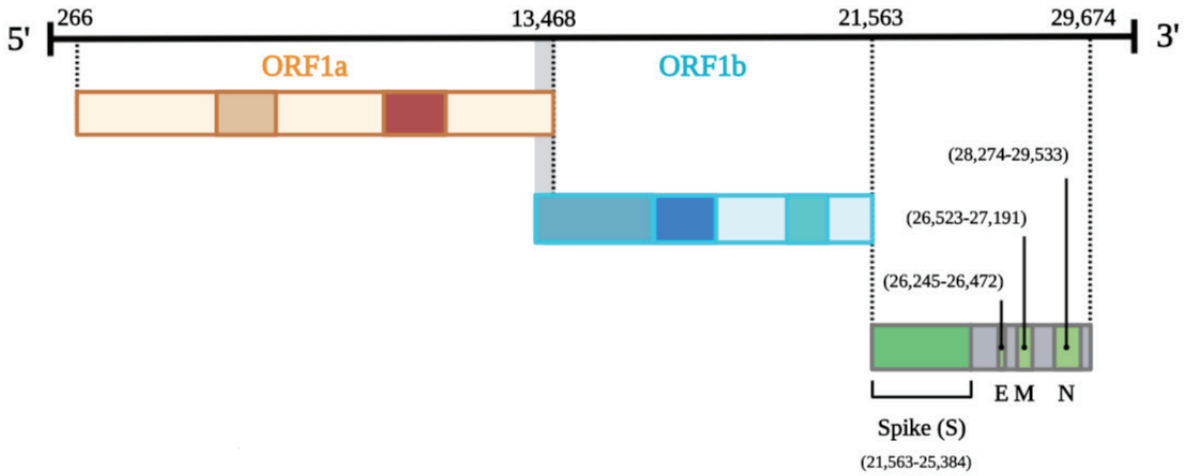

Figure 1: Genome of SARS-CoV-2. This figure created in BioRender.

In the fight against coronaviruses, there are three approaches for developing new drugs. The first strategy requires testing existing broad-spectrum anti-viral agents. The second strategy involves using molecular databases to identify molecules with the potential to act as therapeutic agents against coronaviruses. The third strategy uses the genomic data and pathological characteristics of different coronaviruses[14]. This review will provide information about chemical compounds and targets for further *in vivo* and *in vitro* studies to identify drugs to treat COVID-19.

Potential Drug Targets for SARS-COV-2

Drug targets for coronaviruses can be divided into different categories based on therapeutic pathways: (1) action on the host's specific enzymes or receptors to prevent the virus from entering the host's cells, (2) production of virulence factors to restore the host's innate immunity, (3) action on structural proteins of the virus, and (4) action on functional proteins or enzymes that are critical to the virus. The target proteins include Nsps, such as Nsp1, Nsp7-Nsp8 complex, Nsp9-Nsp10, and Nsp14-Nsp16, 3CLpro, Nsp3 (Nsp3b, Nsp3c, Nsp3e, and PLpro), ORF7a, E-channel, helicase, CRBD, TMPRSS2, ACE2, NRBD, RdRp, and spike protein[14] (Figure 2).

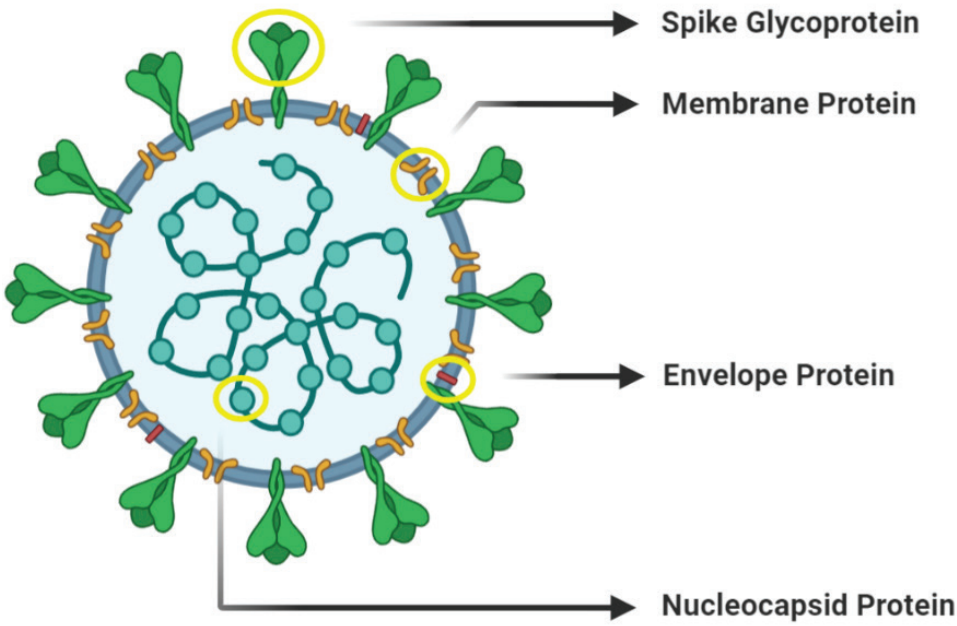

Figure 2. Structure of SARS-CoV-2. This figure created in BioRender.

Targets Preventing Viral RNA Replication and Synthesis

Nsps are important functional proteins of coronaviruses and are involved in RNA transcription, protein synthesis, translation, processing and modification, infection of host cells, and viral replication. The helicase, RdRp, PLpro, and 3CLpro are the most important targets for the development of inhibitors[14].

Papain-Like Proteinase

PLpro is important for the release of nonstructural proteins, such as Nsp3, Nsp2, and Nsp1, and for correcting the replication processes of SARS-CoV-2[15]. PLpro has also been confirmed to trigger host innate immunity[16]. Since PLpro is indispensable for SARS-CoV-2 replication and infection of the host, inhibitors of PLpro would likely be effective in combating SARS-CoV-2. Unfortunately, there are no approved inhibitors of PLpro[14].

Table 1. Potential PLpro Inhibitors.

No	Compound/Drug (Source)	Mechanism	Reference
1	Chloramphenicol	Antibacterial	14
2	Baicalin (*Scutellaria baicalensis*)	Antiinflammatory, Antiviral, Antibacterial, Antitumor	14
3	Ribavirin	Antiviral	14
4	Piceatannol (*Vitis vinifera*)	Antiviral	14
5	Sulfasalazine	Antibacterial	14
6	Rosmarinic acid (*Salvia verticillata*)	Antioxidant, Antiviral	14
7	Tigecycline	Antibacterial	14
8	Phaitanthrin D (*Isatis indigotica*)	Antiviral	14
9	Valganciclovir	Antiviral	14
10	Sugetriol-3,9-diacetate (*Cyperus rotundus*)	Anti-HSV-1, Anti-HBV	14

3-Chymotrypsin-Like Cysteine Protease:

3CLpro is first autoclaved from viral poly-proteins to generate mature enzyme, which then further cleaves downstream Nsps at 11 sites to deliver Nsp4-Nsp16[17]. 3CLpro is thus important in the SARS-CoV-2 life cycle and a study of the catalytic mechanism and structure of 3CLpro established this enzyme as an appealing target for anti-SARS-CoV-2 drug discovery[18]. Inhibitors of SARS-CoV 3CLpro are typically small molecules and peptides but, again, there are no approved inhibitors[14].

Table 2. Potential 3CLpro Inhibitors.

No	Compound/Drug (Source)	Mechanism	Reference
1	Oxytetracycline	Antibacterial	14
2	Andrographiside *(Andrographis paniculata)*	Antiinflammatory, Antiviral	14
3	Amprenavir	HIV-1 protease inhibitor	14
4	Theaflavin 3,3′-di-O-gallate *(Camellia sinensis)*	Antiviral, Antioxidant, Antitumor	14
5	Phenethicillin	Antibacterial	14
6	Phyllaemblinol *(Phyllanthus emblica)*	Antiviral	14
7	Cefpiramide	Antibacterial	14
8	Cosmosiin *(Scutellaria baicalensis)*	Anti-HIV, Antiinflammatory, Antioxidant	14
9	Demeclocycline	Antibacterial	14
10	Hesperidin *(Citrus aurantium)*	Antioxidant, Antiinflammatory	14

RNA-Dependent RNA Polymerase

Nsp12-RdRp, a conserved protein in coronaviruses, is a vital component of the replication or transcription complex[19]. Inhibitors of Nsp12-RdRp should not cause significant side effects or toxicity to host cells and this enzyme was an important drug target for MERS-CoV and SARS-CoV. Unfortunately, no specific inhibitors of Nsp12-RdRp were identified[20].

Table 3. Potential RdRp Inhibitors.

No.	Compound/Drug (Source)	Mechanism	Reference
1	Baicalin *(Scutellaria baicalensis)*	Antibacterial, Antiinflammatory, Antiviral, Antitumor	14
2	Idarubicin	Antitumor	14
3	Sugetriol-3,9-diacetate *(Cyperus rotundus)*	Anti-HBV	14
4	Novobiocin	Antibacterial	14

Cont... **Table 3. Potential RdRp Inhibitors.**

5	Phyllaemblicin B *(Phyllanthus emblica)*	Antiviral	14
6	Atovaquone	Antimalarial	14
7	Betulonal (*Cassine xylocarpa*)	Anti-HIV-1	14
8	Gniditrin (*Gnidia lamprantha*)	Antitumor	14
9	Itraconazole	Antifungal	14
10	Chlorhexidine	Antibacterial	14

Targets Inhibiting Virus Structural Proteins

The most important structural protein of coronaviruses is the spike protein, which binds to host cell receptors to mediate cell entry and determines host tropism[21]. The spike protein is cleaved into S1 and S2 subunits by a host cell protease. The main purpose of the S1 subunit is to bind to host cell surface receptors, and the S2 subunit then mediates virus-cell and cell-cell membrane fusion. Cleavage activation and structural integrity of spikes also play important roles in virulence and invasiveness[22]. Agents that prevent SARS-CoV-2 from entering cells by targeting the spike protein or specific receptors on the surface of host cells are thus likely to be effective antiviral therapies.

It has been widely reported that ACE2 is the unique receptor for the RBDs of both SARS-Co-V and SARS-CoV-2 spike proteins[23]. A recent study showed that the sequences of both spike RBDs are similar, and that there are critical interactions between several key amino acid residues of ACE2 and the RBDs[24]. ACE2 is thus a candidate host target for drugs to block SARS-CoV-2 from entering host cells[9].

Conclusion

In summary, this review provides information about chemical compounds and targets for further *in vivo* and *in vitro* studies to identify drugs to treat COVID-19.

Conflict of Interest : The author declare that they have no conflict of interest.

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In Silico Study of SARS-CoV-2 Vaccine Candidate: Spike Glycoprotein

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Abstract

This study aimed to perform the analysis of toxicity prediction, allergenicity prediction, and *in silico* cloning of peptides originated from SARS-CoV-2 spike glycoprotein in the previous study. Allergenicity prediction employed AllerPred to predict the non-allergen peptides and toxicity prediction performed using ToxinPred. Then, this study designed the *in silico* cloning of SARS-CoV-2 spike glycoprotein with pET-28a(+) using SnapGene software. Therefore, this study successfully constructed the SARS-CoV-2 vaccine candidate via *in silico* method. Therefore, these data could be used to design a peptide-based vaccine against SARS-CoV-2. However, the advanced study is recommended confirmation, such as *in vitro* and *in vivo* study.

Keywords: *Allergenicity Prediction, COVID-19, In Silico Cloning, Toxicity Prediction, Vaccine.*

Introduction

SARS-CoV-2 is the seventh coronavirus that has crossed the species barrier to infect human. The virus was first declared in China in 2019 and appeared sporadically all over China and many other nations worldwide[1]. In March 2020, the WHO declared that the infection was a pandemic. The sudden outbreak and quick deployment of COVID-19 have endangered the global health and economy[2,3]. At present, the virus has infected more than 60 million people globally with more than 1.5 million global deaths[4].

Taxonomically, coronaviruses belong to the *Coronaviridae* family in the order Nidovirales, with examples in four distinct genera: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus*[5,6]. The structural proteins are encoded by four genes, specifically the envelope (E), nucleocapsid (N), membrane (M), and spikeglycoprotein (S)[7,8]. Previous studies have shown that the spike glycoprotein plays a crucial role in binding to receptors on the host cell[9,10]. Therefore, this protein is a key target for a number of antiviral therapies and a promising antigen for generating vaccines formulated against many coronaviruses[11].

Scientists have shown that vaccines are being developed against SARS-CoV-2 by various research groups worldwide[12,13,14]. Despite these promising treatment options, COVID-19 remains a serious disease with no proven effective medication. Therefore, there is an urgent need to investigate the genome of SARS-CoV-2. This study performs the analysis of toxicity prediction, allergenicity prediction, and *in silico* cloning of peptides originated from SARS-CoV-2 spike glycoprotein in the previous study to design a peptide-based vaccine against SARS-CoV-2.

Materials and Methods

Allergenicity Prediction of the Predicted Peptides

The predicted peptides revealed from our previous study[9]. In this study, an extensive analysis of the allergenicity prediction of the predicted peptides was conducted using AllerTOP with default settings. Then, the predicted peptides submitted to this web server as demonstrated by Peele *et al*.[15].

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Toxicity Prediction of the Predicted Peptides

This study predicted protective non-toxic antigens performing ToxinPred. The standard thresholds as reported by Gupta *et al*.[16].

In Silico Cloning

Here, the pET28a(+) expression vector selected for cloning, and its nucleotides sequences were collected from the Addgene vector database[17]. Then, SnapGene software was used for pursuing the *in silico* cloning of peptide-based vaccine component against SARS-CoV-2[18].

Results and Discussion

In this study, the *in silico* analysis of allergenicity prediction and toxicity prediction of the predicted peptides presented in Table 1. The pET28a(+) vector was used to clone the vaccine construct DNA sequence (5485 bp) using SnapGene software (Figure 1). The *in silico* electrophoresis of the spike glycoprotein, plasmid, and the insert gene showed in Figure 2.

Table 1. The results of allergenicity and toxicity predictions of the predicted peptides.

Predicted Peptides	Allergenicity Prediction	Toxicity Prediction
KNHTSPDVDLG	Non-Allergen	Non-Toxin
VRQIAPGQTGKIAD	Non-Allergen	Non-Toxin
RTQLPPAYTNS	Probable Allergen	Non-Toxin
YGFQPTNGVGYQ	Probable Allergen	Non-Toxin
SGTNGTKRFDN	Probable Allergen	Non-Toxin
ILPDPSKPSKRS	Non-Allergen	Non-Toxin
LTPGDSSSGWTAG	Non-Allergen	Non-Toxin
RDIADTTDAVRDPQ	Non-Allergen	Non-Toxin

*Note: The results based on Ansori *et al*.[9]

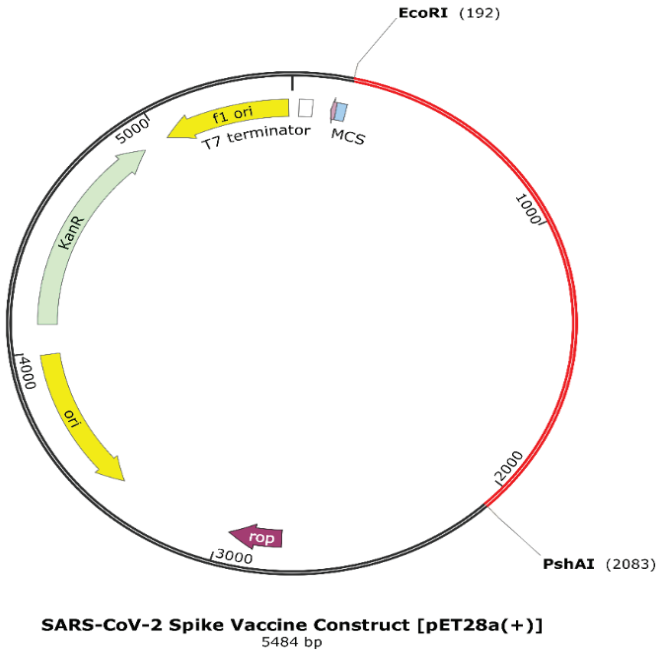

Figure 1. Schematic representation of *in silico* cloning of vaccine candidate within pET28a(+) expression vector.

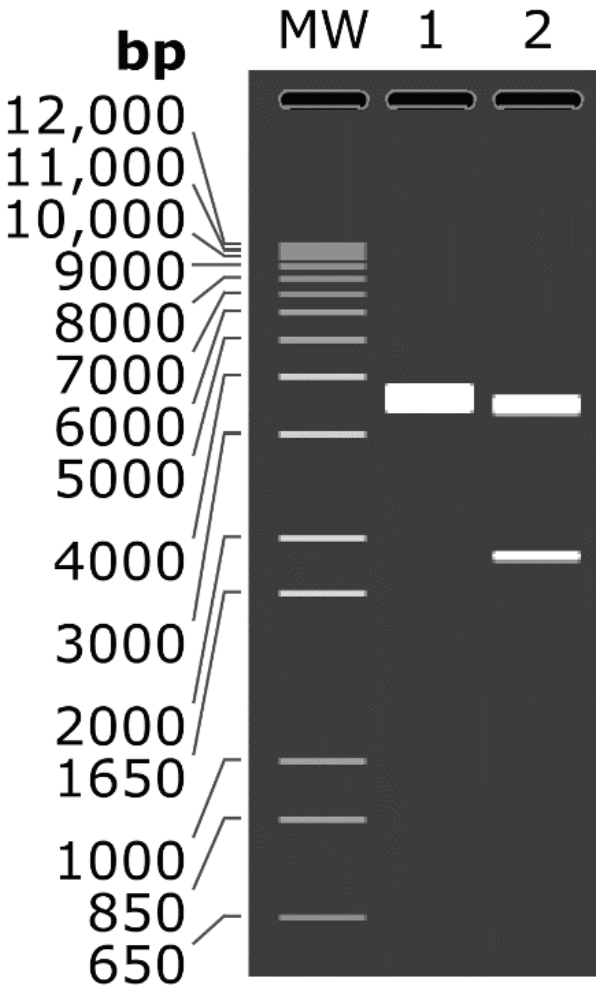

Figure 2. Schematic representation of *in silico* electrophoresis of the spike glycoprotein, plasmid, and the insert gene. 1: Spike glycoprotein of SARS-CoV-2; 2: pET28a(+) expression vector and the insert gene.

In addition, COVID-19 vaccine development has started in many research centers and pharmaceutical industries following the announcement of SARS-CoV-2 agent and its full genome recognized. Recently, the available assemble data stated that COVID-19 vaccine candidates were grouped into the following types: protein-based, epitope, inactivated or live-attenuated virus, virus-like particle, nucleic acid-based, and viral vectors[19,20]. Today, more than one year after the prevalence of novel coronavirus, vaccine and antiviral products are still in progress due to the pandemic paradigm development with several medication options and vaccines are in clinical trials globally[19,21,22]. Furthermore, scientists considered traversing the new concepts and latest cultivation in each type of vaccine to formularize a potent vaccine against COVID-19.

Previous research stated that *in silico* study is promoted as a useful method to generate vaccine against various diseases, such as dengue, zika, cancer, and HIV[23]. This method is employed by identifying MHC 1 and 2, B-cell and T-cell epitopes correlated with antigen presentation[24,25]. This type of vaccine consists of antibodies associated with the regions of foreign particles. These antibodies are straightforward and considered as the effective control. Based on the available data, the epitope-based virus might be a significant alternative vaccine formulation to fight SARS-CoV-2. Recently, there are various programs on vaccine developments[23]. Nevertheless, both *in vitro* and *in vivo* researches are further required for the advanced explanation of epitopes for the invention of SARS-CoV-2 vaccine.

Conclusion

In summary, the data of this study could be used to design an epitope-vaccine against SARS-CoV-2. However, the advanced study is recommended confirmation, such as *in vitro* and *in vivo* study.

Conflict of Interest : The author declare that they have no conflict of interest.

Source of Funding : This study supported by the Ministry of Education and Culture of the Republic of Indonesia.

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Ethical Approval : This study does not require ethical approval.

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Formulation and In Vitro Evaluation of Nimodipine as an Orodispersible Film

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Abstract

The objective of this study is to prepare nimodipine as orodispersible films by using solvent casting method in order to enhance its oral bioavailability and improve patient compliance. Nimodipine is a calcium channel blocker belongs to class II, it used to improve neurologic outcome following subarachnoid hemorrhage (SAH) by reducing the incidence and severity of ischemic deficits. ODFs were prepared using three types film forming polymers with different concentration HPMC E3, HPMC E5 and HPMC E15, using (Glycerin) as plasticizer and (Poloxamer 407) as surfactant. The effect of different concentrations and types of polymers, on mechanical properties such as (folding endurance and percent of elongation) and the in-vitro evaluation parameters such (disintegration time, surface pH, weight variation and dissolution profile) were evaluated. HPMC E5 films showed slightly higher cumulative % drug release than films of HPMC E3 and HPMC E15 at the same concentration of plasticizer and surfactant.

Keywords: *Orodisperible films (ODFs), Nimodipine, HPMC E5.*

Introduction

Solubility is an important factor in the drug development process. About 35–40% of the new chemical entities developed are less aqueous soluble results in low bioavailability that is why it is major concern for the scientist to develop and design formulation. The drugs with high permeability and low solubility fall in the category of BCS class II and class IV. Poorly absorbed drugs have slow drug absorption and lead to inadequate bioavailability and gastrointestinal mucosal toxicity [(1)].

Orodispersible films are polymeric matrices that meet numerous necessities for being utilized efficiently as a drug release platform [(2)]. These orodispersible films can be utilized for targeting sensitive site that may not be conceivable with tablets or liquid formulations, hence proving to be a promising candidate for delivery of the drug. Several other names of these thin films have been appeared, for example orodispersible films (ODFs), oral soluble films, oral strips, oral films, buccal films, wafers, ophthalmic films, and transmucosal films. A film that readily dissolves in the oral cavity is generally named as orodispersible film [(3)].

Orodispersible films are fast-dissolving films. It does not require water for the administration and gives quick absorption and instant bioavailability [(4)]. These orodispersible films have predominance over major drawbacks of rapid disintegrating tablets related to fear of choking, friability and can be utilized for dysphasic and schizophrenic patients. These orodispersible films are specialized in a way that the water is not required for administration because they quickly fragments within a few seconds, discharging the drug in mouth[(5)].

The various polymers used in ODFs are sodium CMC, HEC, polyacrylic acid, HPC, hypromellose (semisynthetic), etc., and the natural polymers are Chitosan, alginate, starch, maltodextrin, etc [(6)]. The various plasticizers used in ODFs are: Glycerol, propylene, glycol, polyethylene glycol, sorbitol, etc [(7)].

Nimodipine is a calcium channel blocker. It

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belongs to class II (low solubility – high permeability) according to (BSC) [8]. Nimodipine used as an adjunct to improve neurologic outcome following subarachnoid hemorrhage (SAH) from ruptured intracranial berry aneurysms by reducing the incidence and severity of ischemic deficits by crossing blood brain barrier due to its high lipophilicity and cause relaxation for cerebral artery due to calcium channel blocking [9].

The aim of this work is to prepare nimodipine as orodispersible films in order to enhance its oral bioavailability and improve patient compliance.

Materials and Methods

Nimodipine powder was supplied by Hyper Chem-China, hydroxypropyl methyl cellulose (E3, E5, E15) were supplied from Evonik Germany, polaxamer 407 and crosspovidone obtained from JP & SB Converting Services International S.L. Spain.

Nine formulas were prepared (F1-F9) as listed in (Table 1) by using solvent casting technique; every film of about 4 cm^2 area have 10 mg of nimodipine.

Each quantity of (HPMC) was dissolved in 20 ml of solvent (distilled water) constant stirring is needed for 2 h by using magnetic stirrer in order to form homogenous polymeric solution. Plasticizer was added to this solution and also stirring is required for additional 1 h.

Next step involve dissolving nimodipine, surfactant, saliva stimulating agent and sweetener in 20 ml of water and ethanol as co-solvent (equal quantity from each solvent) in another beaker to form clear solution, mixing the two medium using magnetic stirrer for 1 h, the solution was kept in sonicator for degassing.

Petridish was used to which the bubble free solution was casted on it and kept in hot air oven (50 ^oC) overnight. Dried film was then removed and cut into the desired shape and size (2 cm × 2 cm) for the intended application [10].

Table (1): Composition of Nimodipine Orodispersible Films Formulas

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nimodpine (mg)	10	10	10	10	10	10	10	10	10
HPMC (E3) (mg)	15	25	35						
HPMC (E5) (mg)				15	25	35			
HPMC(E15)(mg)							15	25	35
Polaxamer 407	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Glycerin (ml)	5	5	5	5	5	5	5	5	5
Crosspovidone (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Ascorbic acid	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Saccharin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

Evaluation of Nimodipine ODFs

Physical appearance of films such as homogeneity, consistency, color and clearness of films were estimated. These were tested by visual examination of films and evaluation of quality by feel or touch [11]. Weight variation test confirms the uniformity of the film formed. Ten randomly selected films from each batch (each of 2 cm × 2 cm) were cutted and weighed individually [12].

Thickness of ODFs was determined by using digital vernier caliper at five different sites (center and four corners). This is necessary to confirm the uniformity in the thickness of the film as it is directly correlated to the exactness of dose in each strip [13].

Folding endurance (FE) measures the elastic properties or the flexibility of films. The FE of ODFs was measured by repetitively collapsing a small stripe of ODFs at the same point until broken or folded up to 300 times. The number of times of folding at which the film remains intact indicates the value of FE [14].

Normally the value of percent of elongation (%E) of films is increases as the amount of plasticizer increases. %E is determined by calculating the differences between the initial and final length of stripe divided by the initial length [15].

Three films (2 cm × 2 cm) were cut randomly from each formulation batch; these films were placed in 50 ml of phosphate buffer pH 6.8 (0.5 % Tween 20) solutions individually and kept on magnetic stirrer for 1 h. Then the solution was filtered, diluted suitably and the amount of drug was calculated by measuring the absorbance of the drug at λ max 238 nm using standard calibration curve of drug in phosphate buffer pH 6.8 [16].

To measure the pH value of ODFs, one stripe was allowed to dissolve in 2 ml of distilled water and the pH of the obtained solution is determined using pH-meter. We expect different pH value due to using different film forming polymers in formulation of ODFs along with the drug [17]. In vitro disintegration time (DT) is measured by using petri dish method. DT is the time at which the ODFs start to breakdown or disintegrate (in second). The test is done by taking 3 films of each patch in petri dish and after adding 2 ml of distilled water for each film, the petri dish was shaken continuously, then measure the time at which the ODFs start to breakdown or disintegrate [18].

In vitro dissolution of nimodipine ODFs was determined through using USP type II dissolution apparatus (i.e. paddle). Nimodipine ODFs allow to dissolve in 500 ml of 6.8 phosphate buffer, the stirrer was modified to rotate at 50 rpm and the temperature of the medium was adjusted at 37 ± 0.5 ºC during the procedure. Then samples (5 ml) were taken by using a syringe at regular intervals (1, 2, 3, 4, 5, 10, 15 and 20 min); before analyzing the withdrawn samples using UV-spectrophotometry at λmax 238 nm they must be filtered by using filter paper (0.45µm). The withdrawn bulk at each time must be replace with fresh 6.8 phosphate buffer in order to maintain sink conditions. Then the dissolution profile of the drug is constructed by plotting the percent of accumulative drug release against the time [19].

Fourier transform infrared spectroscopy (FTIR)

The compatibility between drug and all excipients explained by (FTIR) spectroscopy. The study of compatibility by FTIR performed by making spectrums for nimodipine (pure powdered drug) and spectrum for selected formula.

Results and Discussion

Films that prepared from HPMC (E3, E5 and E15) were opaque white, thin and soft. The average weights for all the prepared formulations were uniform and fit to the referred values (Table 2). The average thickness values of nimodipine ODFs are shown in (Table 2). The results were found to vary between 0.12 to 0.18 mm. The formulated nimodipine ODFs showed an acceptable quantity of medicament ranged from 87.2 % - 109.6 % (Table 2). Nimodipine ODFs showed an acceptable surface pH value (5.7-6.3) (Table 2) when compared to that pH of oral mucosa indicating that it does not cause an irritation to oral mucosa.

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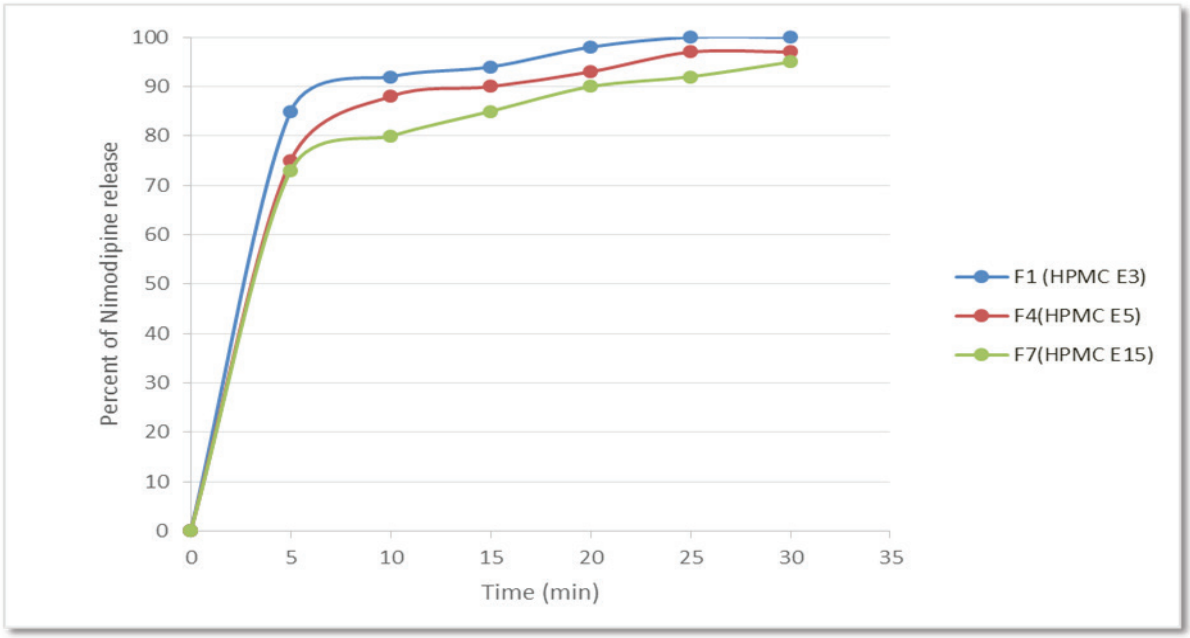
Table (2): Physical Evaluation Parameters of Nimodipine ODFs

Formula	Weight Variation	Thickness	Drug Content	Surface pH	In-Vitro DT
F1	39 ± 3	0.12 ± 0.02	93.4 ± 3.87	6.2 ± 0.2	33± 1.4
F2	46 ± 3	0.15 ± 0.02	95 ± 2.45	6.4 ± 0.1	35 ± 3.2
F3	61 ± 2.1	0.18 ± 0.02	95 ± 3.23	6.1 ± 0.1	38 ± 2.3
F4	41 ± 2	0.13 ± 0.01	97 ± 2.14	6.2 ± 0.22	40 ± 1.2
F5	53 ± 3	0.15 ± 0.02	95 ± 2.23	6.1 ± 0.14	45 ± 1.8
F6	57 ± 3.22	0.15 ± 0.019	96 ± 3.13	6 ± 0.12	50 ± 1.4
F7	48.3 ± 2	0.12 ± 0.02	99 ± 2.1	5.9 ± 0.1	55 ± 2.4
F8	51 ± 2.1	0.13 ± 0.01	98 ± 2.22	5.8 ± 0.12	59 ± 3.3
F9	58 ± 3	0.15 ± 0.01	97 ± 3.1	5.8 ± 0.2	60 ± 3.6

Effect of Different Types of Polymers

Formulas (F3, F6, F9) were utilized to study the influence of type of polymer on the mechanical, physical properties and on the dissolution profile of the nimodipine ODFs while the concentration of polymer remain constant. The mechanical properties of different film forming polymers are shown in (Table 3). The formula (F1) which contains HPMC E3 gave satisfactory % elongation (35 %) but it give low folding endurance values (84). While (F4) (HPMC E5) gave satisfactory % E (31.3) and good FE values (123), also HPMC E3 (F1) was difficult to handle.

shown in (Figure 1), 85% of the drug was released from F1 (HPMC E3) at the first 4 min compared to F4 (HPMC E5) which was released 85% at 7 min. The difference in dissolution may be due to the variances in viscosity grade of HPMC film forming polymer.



Figure(1):Effect of viscosity grade of HPMC polymer on the dissolution profile of nimodipine in phosphate buffer (pH 6.8) at 37°C

Effect of Concentrations of Selected Polymer (HPMC E5)

Formulas (F4, F5 and F6) were utilized to study the influence of different concentrations of HPMC E5 (15, 25 and 35 % w/w) respectively on the mechanical, physical properties and on the dissolution profile of the ODFs. The disintegration time of nimodipine ODFs (F4, F5 and F6) shows DT of the ODFs declined as the HPMC E5 concentration decreased from 35 % in formula (F6) to 25 % in formula (F5) (Table 4). This may be attributed to formation of thick layer of gel which make the diffusion of water inside the film difficult. Similar outcomes were established with lamotrigine fast dissolving films [20], losartan potassium fast dissolving films [21].

The results of mechanical properties exposed in (Table 4) revealed that the % E and folding endurance (FE) increased as the concentration of polymer increase.

This due to the fact that greater polymer concentration leads to heavily crowded chains of HPMC E5 that will need more force to distraction furthermore the increase in the concentration of polymer results in enhance the flexibility of ODFs resulting in high % E values. Similar outcomes were establish with cinnarizine fast dissolving films [22].

Table (3):Mechanical Properties of Nimodipine ODFs from different polymer types

Formula	Folding endurance	Percent of elongation
F1(HPMC E3)	84 ± 2	35 ± 5
F4(HPMC E5)	123 ± 4	31 ± 3
F7(HPMC E15)	136 ± 3	20 ± 2

Table (4): Mechanical Properties of Nimodipine ODFs Formulas (F4, F5 and F6)

Formulas	% E	FE	DT
F4	31± 3	123 ± 4	45
F5	35 ± 1.4	134 ± 3	38
F6	40 ± 0.9	148 ± 2	30

The dissolution profiles of nimodipine from formulas (F4, F5 and F6) are shown in (Figure 2). It was observed that the dissolution rate of nimodipine reduced significantly as the concentration of HPMC E5 was increased from 25 % (w/w) in formula (F5) to 35 % (w/w) in formula (F6). This may be due to the higher concentration of polymer, resulting in production of high consistency gel layer created by close interaction between the particles of HPMC E5 resulting in a diminished movement of medication particles in swollen lattices, which prompts a decline in dissolution rate [23].

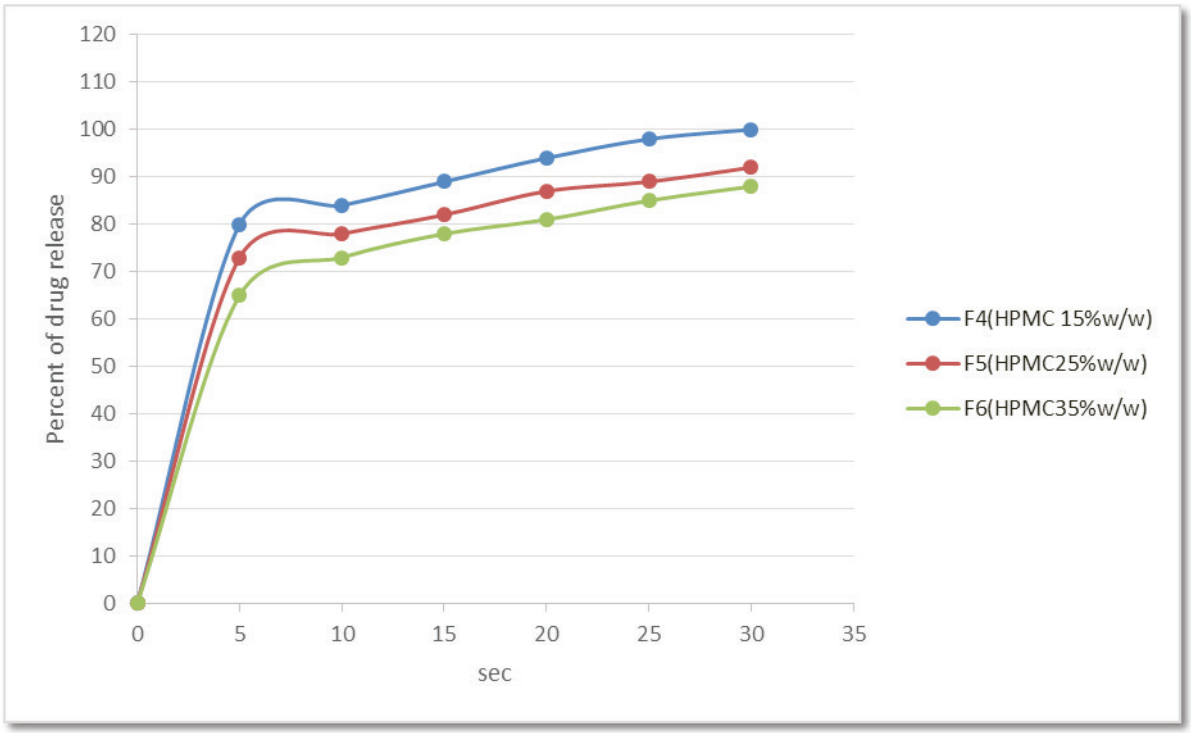


Figure (2): Various concentration of HPMC E5 effect on the dissolution profile of nimodipine in phosphate buffer (pH 6.8) at 37°C .

Conclusions

The best film forming agent among HPMC (E3, E5 and E15) was HPMC E5 because it gives fastest in-vitro disintegration time with an acceptable mechanical properties and dissolution behavior.

Ethical Clearance : Taken from University of Babylon ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Association of Genetic Polymorphisms with Risperidone Treatment Response in Schizophrenia: A Review

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Abstract

Background: Schizophrenia is a severe mental disorder characterized by positive, negative, and cognitive symptoms that affect the quality of life. Risperidone is widely known as second-generation antipsychotic that is effective to treat schizophrenia. Unlike the first-generation antipsychotic, risperidone has a lower impact on the extrapyramidal adverse effect. However, individually, the risperidone treatment response may be different caused by the genetic polymorphisms. This review aims to examine the association of genetic polymorphisms with risperidone treatment response in schizophrenia.

Method: The review was conducted using the Pubmed database and 49 articles included in this review among 80 articles obtained in an initial search. The result showed genetic polymorphisms which affects risperidone therapy include DRD2, genetic polymorphisms on serotonin, cytochrome P450, BDNF, COMT, and ABCB1. Those polymorphisms might increase or decrease pharmacokinetics and pharmacodynamic profile of risperidone. In addition, genetic polymorphisms also contributed to the risk of metabolic syndrome and hyperprolactinemia induced by risperidone treatment.

Conclusion: Based on those findings, several genetic polymorphisms had an association with therapeutic outcomes and side effect after risperidone treatment. Genetic polymorphisms screening may be useful for drug choices or dosage adjustment that safer and effective for patients.

Key words: *genetic polymorphism, risperidone, schizophrenia*

Introduction

Schizophrenia is a severe mental disorder characterized by positive, negative, and cognitive symptoms that affect the quality of life [(1)]. It is estimated that there are more than 21 million cases of schizophrenia worldwide. Individuals affected by this disease had two to three times higher risk to die early than the general population [(2)].

Schizophrenia is characterized by high activity of dopaminergic on mesolimbic and decrease activity on the mesocortical pathway. The main therapy for schizophrenia is using antipsychotic drugs that have a pharmacological effect as a D2 antagonist receptor [(3)].

Risperidone is one of the most widely used drugs on schizophrenia treatment and classified as second-generation of antipsychotics. This drug class has lower extrapyramidal adverse effect compared with first generation [(4)]. However, there are other side effects of risperidone, such as metabolic syndrome and hyperprolactinemia [(5,6)]. Both effectiveness and side effect of this antipsychotic affected by genetic variations among individuals.

Genetic polymorphisms can increase therapeutic response, decrease the therapeutic response, and also increase the side effect of risperidone [(7)]. The precise

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treatment for schizophrenia is needed because of the disease requires lifelong treatment and there are several adverse effects caused by risperidone [8,9]. Therefore, this review aims to examine the association of genetic polymorphisms with risperidone treatment response in schizophrenia.

Methods

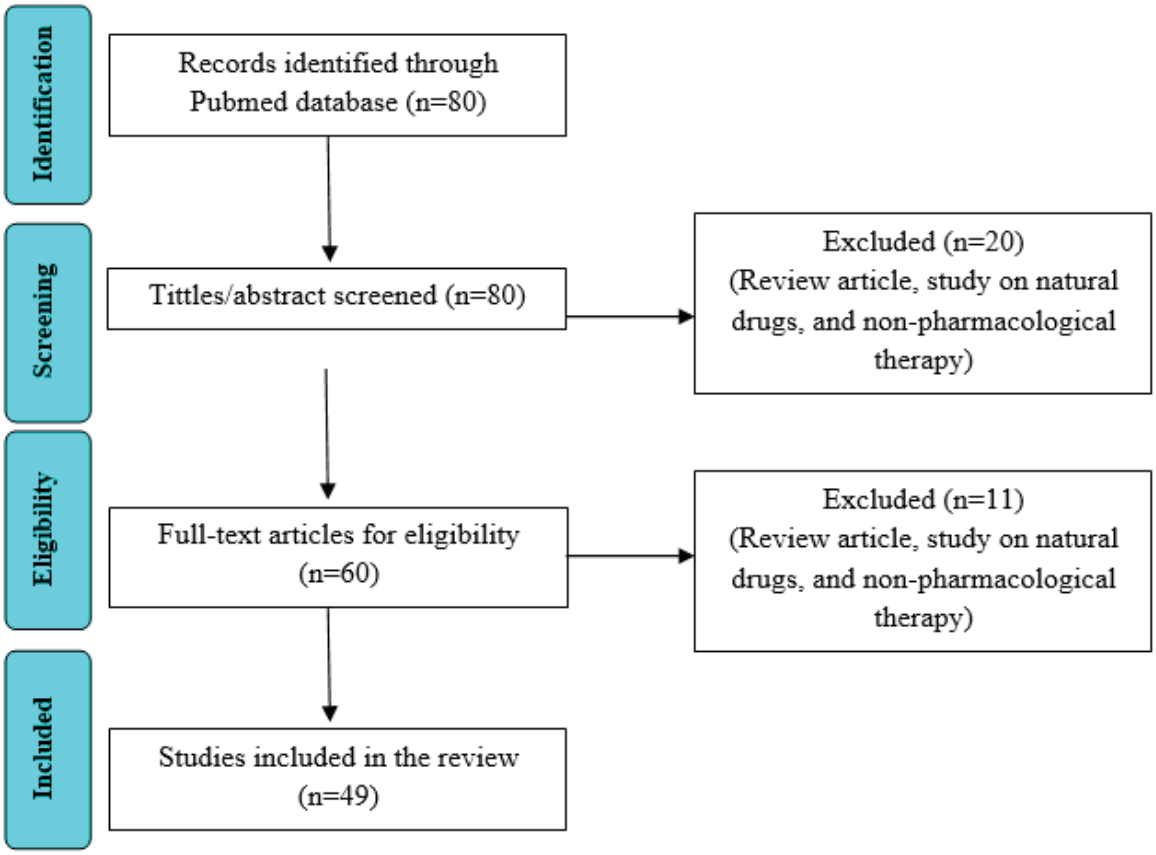

Figure 1. Literature review flowchart

Study literature on this review is using the Pubmed database with Medical Subject Heading (MeSH) terms. Detail keywords we used for study literature were "Genetic, Polymorphisms" [MeSH] AND "Risperidone" [MeSH] AND "Schizophrenia" [MeSH]. Studies included in this review are clinical trial study and published as a full paper. Review articles and study on natural drugs are excluded. Flowchart of literature review shown in Figure 1.

Findings and Discussion

Pathophysiology of Schizophrenia

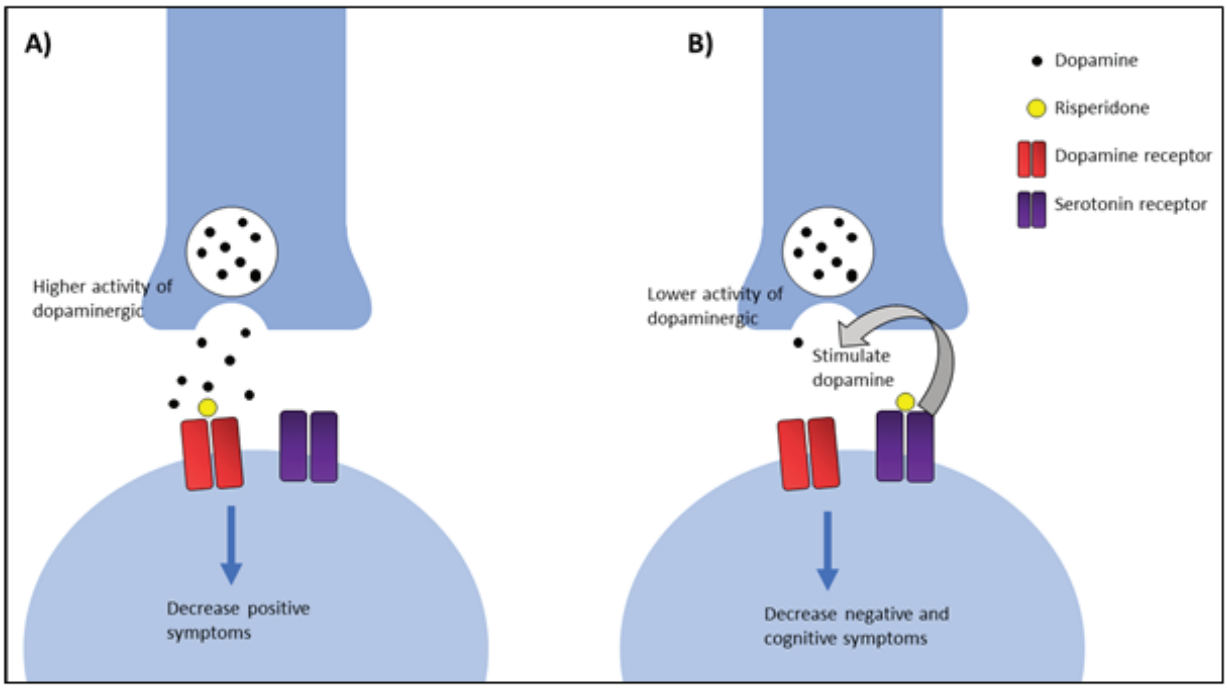

Figure 2. Role of dopamine and serotonin in schizophrenia. A) Mesolimbic. B) Mesocortical.

As mentioned above, the pathophysiology of schizophrenia is related to the high activity of dopaminergic on mesolimbic and decrease activity on the mesocortical pathway. The illustration of pathophysiology is shown in **Figure 2.** High activity of dopaminergic on mesolimbic leads to positive symptoms such as hallucination and delusion [10]. Risperidone is a second-generation antipsychotic that has the main mechanism of blocking dopamine receptors [11]. After that, excessive dopamine can not bind to its receptor so positive symptoms might be decreased. Besides, on the mesocortical pathway, there is a decreased activity of dopaminergic that impact on negative symptoms includes alogia, poor of attention, and loss of motivation. Antipsychotic can bind to serotonin receptor on mesocortical, stimulates dopaminergic releases, decrease negative and cognitive symptoms in schizophrenia patients [12]. Based on the literature review, there are several genetic polymorphisms affected therapeutic response and the adverse effect such as metabolic syndrome, body weight gain, increased lipid profile, and hyperprolactinemia. Review were conducted on 49 studies because there is no limitation on the year of publication.

Dopamine

Role of dopamine receptor on brain function is regulating mood, motoric, sensor, study, and motivation [13]. There are several genetic polymorphisms in DRD2 indicated had an association with therapeutic responses and side effects after risperidone treatment. Patients with the rs1801028 genotype had better improvement [14]. In rs1799978 polymorphism, patients with G genotype carriers showed faster and better response compared with A carriers [15]. On the contrary, a study in Japan with 120 subjects indicated that A genotype carrier has better response compared with G carriers [16]. The different therapeutic response was also caused by rs1800497 and rs1799732 gene. Polymorphism of these genes related to the responses of risperidone treatment but not related to hyperprolactinemia as a side effect of risperidone [17–20]. While rs1800497 and rs1799732 had no association with prolactin level, rs2514218 polymorphism leads to increase of prolactin level [6]. But, there is no significant association between genetic polymorphisms in DRD1 and DRD3 with treatment response [21,22].

Serotonin

The serotonin receptor is another target of risperidone beside of dopamine. Risperidone could form irreversible binding with the serotonin receptor and activated this receptor [23]. Mössner, et al. and Wang, et al. found that rs6295 polymorphism on 5-HT1A serotonin receptor associated with low response in patients with GG genotype [24,25]. In 5-HT3A, polymorphism associated with better improvement [26]. Other studies also examined the association of this gene with a side effect of risperidone such as metabolic syndrome and body weight gain. This is related to the role of serotonin in energy regulations and glucose homeostasis [27]. Some studies reported that rs6313 polymorphism in 5-HT2A, rs1414334 and rs498177 polymorphism in 5-HT2C, and rs6699866 polymorphisms in 5-HT6 associated with a higher risk of metabolic syndrome [5,28–31]. It was also believed that rs3813929 could induce metabolic abnormalities and weight gain after risperidone treatment [29,32].

Cytochrome P450

Cytochrome P450 (CYP) is an enzyme with a key function in drug metabolism in the liver. Main enzyme does metabolism risperidone is CYP2D [33]. Study by Almoguera, et al. showed that CYP2D6 poor metabolism has an impact in increase risperidone effect [34]. In addition, study by Weide, et al. showed that CYP2D6 poor metabolism might contribute toward higher plasma level of a drug [35]. Interestingly, other studies showed that there is no significant association between CYP2D6 poor metabolism and extensive metabolism on plasma risperidone level [36].

Besides CYP2D6, studies also observed on CYP2E1 and CYP3A4 gene. Both genes showed the same result that there was no association of gene polymorphisms with the therapeutic response or plasma level [37,38]. In another result, there was an increase in plasma concentration in CYP3A5 non-expressor compared with CYP3A5 expressor [36]. Besides of impact on drug metabolism, a study in China with 123 responders showed that 188C/T polymorphism in CYP2D6 has an association with body weight gain after therapy [39].

Others

Several genes well-known had an important role in weight gain. One of them is BDNF. Recently, some studies resulted in interestingly finding that rs6265 BDNF associated with weight gain and increase of triglyceride level [39,40]. However, a study reported that there is no association between rs6265 BDNF polymorphism with weight gain after therapy [41]. Other study observed that there is association of polymorphism in APOA5 and SLC6A2 with increase triglyceride level and weight gain [42,43].

In addition to BDNF, it is appealing to study about association of COMT polymorphisms with the treatment using risperidone. Polymorphism of rs9606186 is associated with better improvement in male patients [44]. Catechol-O-methyl transferase (COMT) enzyme has an important role in inactivating catecholamine post-synaptic. COMT is also believed to its function in dopamine regulation, which widely known related to the pathophysiology of schizophrenia. But some studies showed that there is no association between rs4680 polymorphism in the COMT gene with therapeutic outcome or prolactin level [16,20,45,46].

Different from some presented genes which observed in pharmacodynamic response, ABCB1 gene polymorphism observed in pharmacokinetic response. ABCB1 (ATP-binding cassette) is transporter which has an important role in DNA methylation. The high expression on ABCB1 related to higher elimination and lower in the absorption of the drug. Study on ABCB1 gene polymorphism on risperidone treatment outcome also observed on rs2032582. Suzuki et al. and Xing et al. studies resulted that there was no association between this gene with risperidone plasma level either therapeutic response [47,48]. But, there is an association between rs2032582 and rs1045642 with QTc interval, weight gain, and metabolic abnormalities [30,32,49–51]. However other study mentioned that there was an association between rs1045642 with increased of therapeutic response [34]. Besides ABCB1, a gene of ABCG2 and ADRB2 had been observed too. The result showed that C421 C4A in ABCG2 associated with adverse drug reaction risk and ADRB2 16Gly related to sex-related adverse effect [52,53].

RGS is a protein family consisting of 30 molecules which has a function for GTP-ase activated [54]. Decrease of expression of RGS may longer signal neurotransmission of glutaminergic so that dopaminergic and serotonergic can be changed [55]. On study observed in 120 subjects in China, rs10917670 polymorphism of RGS related to the low development of a social function and in rs2661319 associated with a low response [56]. In addition, patients with a short form of the MAOA polymorphism needed a higher dosage of risperidone for the treatment [57]. A study observed in SLC6A2 also found that different polymorphisms had different improvement after treatment [58]. On the contrary, polymorphisms in SLC6A3 had no significant association with treatment response [59].

Some studies reported other genes that impact on therapeutic response and metabolic syndrome. Gene related to therapeutic response included SULT4A1-1(+) that associated with low response compared with olanzapine, IL-1RN*2 which associated with an increase on negative symptoms and rs4795893, rs4586, and rs2857657 polymorphisms in CCL2 which associated with therapeutic response in risperidone [60–62]. And also in ESRα, GRM3, GRM7, HRH3, HRM3, and HRM4, showed a different response after risperidone treatment in individuals [61,63–67]. Meanwhile, in AKT found associated between polymorphism with a positive and negative symptom but no association with social function [20,68].

As presented, the common adverse effect of risperidone treatment is metabolic syndrome. Study in FTO gene showed rs9939609 polymorphism in TT carriers had lower weight gain than A carrier [46]. It was the same as NRXN rs11624704 and rs7154021 gene polymorphism which had an association with body weight gain too [69]. Different from FTO and NRXN, HCRTR1 indicated has an association with body weight gain although the association not significant [70]. Besides of body weight, another study also observed the risperidone adverse effect at the association with metabolic syndrome. Gene of INSIG2 and SREBF rs11654081 in T allele associated with a higher risk than others [71,72].

Challenges and opportunities

Pharmacogenomic in clinical application on psychiatry disease treatment shows the number of challenges. Studies about association of genetic polymorphisms and treatment sometimes shows different results [15,17,30,39,41], so it is hard to decide the precise genes that influence the treatment. Besides of genetic factor, there are several factors such as smoking, diet, substance abuse, and comorbidities that may also contribute to the variability of treatment response [73]. In addition, the study shows that pharmacogenomic application can be cost effective or cost saving [74]. Besides the challenges, there is a potential for clinical application of pharmacogenomics in the treatment of psychiatry disease include schizophrenia.

Conclusion

Several genetic polymorphisms had an association with therapeutic response and side effect of risperidone. Genetic polymorphisms which affects risperidone therapy include DRD2, genetic polymorphisms on serotonin, cytochrome P450, and BDNF which affects therapeutic response include changing in positive, negative, cognitive, and social function. Besides, the side effect that is affected with genetic polymorphisms includes metabolic syndrome, weight gain, increased lipid profile, and hyperprolactinemia.

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Evaluation of the Relationship between Ossification of Sternal Joints and Age with Multi-Slice CT Scan

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Abstract

Objective: Sternum radiograph is used in determination of age after 21 years old according to manubriosternal body (MB) and xiphisternal joint body (XB) ossification. In this study, we intend to estimate age using multi-slice CT images as radiographs have lower sensitivity and CT images demonstrate ossification better.

Materials and Methods: Multi-slice thorax CT images found in PACS archive are evaluated retrospectively. Cases between 21-65 years old are selected. Coronal reconstruction images were obtained. MB and XB joint ossification presence and degree are evaluated in respective age groups. MB joint ossification is graded as 0-3 and XB joint ossification graded as 0-1. Relation between variables is examined with Spearman correlation coefficient. Threshold for age variable is calculated using ROC analysis.

Results: Multi-slice CT images of 396 cases are examined (198 females, 198 males). In females and males, MB and XB fusion was seen to start between age group of 21-25 years but there were complete fusion in a few. In 61-65 age groups there were cases without complete fusion. There was statistically significant difference in males and females according to MB and XB joint ossification in between age groups ($p<0.001$).

Conclution: Evaluation of MB and XB joint ossification with CT scan in age estimation is not appropriate between wide age groups.

***Key words*:** *Age estimation, sternum, ossification, CT.*

Introduction

Age of the individual has great influence on his social relations and judgements. Degree of ossification in the bones is defined as bone age and evaluated with comparison between the individuals in same age. In people with normal maturation of bone, bone age is same with chronological age [1,2]. In radiological studies, maturation of bony structures, ossification of epiphyseal lines and degree of fusion between joints are evaluated. Determination of bone age can be made with wrist radiograph between 0-16 or 18 years, pelvic radiograph between 17-22 years and shoulder radiograph to inspect clavicle medial end ossification between 18-24 years of age [3-4]. Especially after 25 years old, age determination is not very accurate and can be estimated in wide range of values. Recent studies are mostly centered on fusion of sacral and sternal distal ends and changes in vertebral-sternal ends of ribs [5-8]. Yet it is known there is no substantial data on alterations in the bones to determine bone age of individuals between 25-40 years and after 50 years old.

CT is sectional imaging method and has great advantage in contrast resolution compared to radiograph. In this study, our aim is to evaluate application of thin section CT images in determination of age between 21-65 years of age using manubriosternal body (MB) and xiphisternal joint body (XB) fusion grading system and to obtain regression formula in males and females.

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Materials and Methods

Case selection

The study protocol is approved by the Clinical Research Ethics Committee of the Uludag University Faculty of Medicine. Chest CT scans performed on 396 individuals aged between 21 and 65 years are evaluated retrospectively. The date of birth subtracted from the date of the CT scan was used to calculate an individual's chronological age in decimals (e.g., age 21 was defined as between the ages of 21.0 and 21.9). Patients with nutritional or endocrine disorders, constitutional growth retardation or cerebral palsy, chronic illness, developmental abnormalities, musculoskeletal disorders, artifacts due to motion or contrast medium, missing or doubtful data on chronological age, clavicular fracture and anatomic shape variants were excluded from the study. Radiologic assessment and data analysis in Multi-detector computed tomography (MDCT) studies were obtained using a 128-row MDCT system (Somatom Defination AS, Siemens Medical Solutions, Erlangen, Germany). Technical settings were 120 kV, reference mAs 120 using Care Dose, Pitch 1.0, collimation 0.6, recon increment 1.0, and sharp kernel settings. All scans were obtained from the patients in the supine position. The CT images were evaluated on screen using a workstation with BARCO software (General Electric). In work stations, 0,6 mm coronal reconstruction images were obtained from original 0,6 mm-thick axial images (MPR technique). Evaluation of fusion between joints is made using coronal reconstructed images.

For determination of age, MB and XB joints were carefully examined for degree of fusion. We evaluated the MB joint ossification with four-grade classification system formed by using the five-stage classification system described by Schmeling et al. (9) and the substages of stages 2 and 3 by Kellinghaus et al. [9]; (Figure 1).

Grade 0 - no fusion present in the joint.

Grade 1 -fusion present but less than half of joining surface.

Grade 2 -fusion present half or more than half of joining surface.

Grade 3 -complete fusion present.

We described two-grade classification system for evaluation of the XB joint fusion (Figure 2).

Grade 0 - no fusion present in the joint.

Grade 1 -complete fusion present.

Image Analysis

Two radiologists (GG as R1 and SC as R2) evaluated all of CT images. R1 had 15 years of professional experience in CT, and R2 had 5 years of experience. R1 also had experience with the musculoskeletal system. All cases are classified according to MB and XB joint fusion on the consensus of both radiologists. Prior to and during the image assessments, examiners were blinded to the age of the patients.

Statistical Analysis

The data is statistically analyzed for the relationship between fusion of joints and age of cases and regression equations were obtained, separately for males and females. Statistical analysis of data is made using SPSS 23.0 statistical package software. Kruskal-Wallis test is used for comparison of more than two groups for data doesn't have normal distribution. Pearson Chi-Square test is used for categorical variables. Dunn test from multi-comparison tests is used for significant results. Significance level is set as α=0.05.

Results

396 cases in total were included in study. There were 198 males and 198 females. The subjects were divided into groups on the basis of their age and sex. Age groups were formed as 21-25, 26-30, 31-35, 36-40, 41- 45. 46-50, 51-55, 56-60, 61-65 years. There were nine groups with 44 cases and in each group males' and females' number were same (22 females, 22 males).

In females and males, it was observed that MB and XB joint fusion is initiated in age group of 21-25 years. In 21-25 age group a female and five males had complete fusion in XB joint and in more than 60 years' age group three females hadn't complete fusion. In males a case hadn't complete fusion in XB joint in groups of more than 50 years of age. In MB joint we detected that fusion starts at 21-25 age group (grade 1), there were a few cases with complete fusion in 26-30 age group (grade 3). In some cases in more than 60 years' age group there

wasn't complete fusion and in some fusion even haven't begun (grade 0); (Figure 3,4,5).

In MB joint, we detected 122 grade 0 (%61.6), 30 grade 1 (%15.2), 7 grade 2 (%3.5) and 39 grade 3 (%19.7) fusion in females. In males we detected 135 grade 0 (%68.2), 25 grade 1 (%12.6), 10 grade 2 (%5.1) and 28 grade 3 (%14.1) fusion (Table 1).

In XB joint, we detected 67 grade 0 (%61.6), 131 grade 1 (%15.2) fusion in females. In males we detected 64 grade 0 (%32.3), 134 grade 1 (%67.7) fusion (Table 2).

In males and females there was statistically significant difference in MB joint ossification between some age groups (p=0.004, p=0.001; respectively). In females, MB joint ossification grade of 61-65 age group is more than 21-25 age group (p=0,037). In males, there was significant difference between 21-25 age group and 31-35 (p=0,020), 56-60 (p=0,008), 61-65 (p=0,002) age groups. 21-25 age group males have less MB joint ossification grade than other age groups.

In males and females there was statistically significant difference in XB joint ossification between some age groups (p<0.001 in both). XB joint fusion is more likely in females with more than 46-50 age group. In males despite there is significant difference between some age groups, there is too much overlapping between fusion grade of the age groups. Therefore it can't be said there is more probability of fusion in between some age groups.

In our study there was significant difference in males and females when MB and XB joint ossification compared between age groups together (p<0.001). ROC analysis is made for MB joint fusion and in females 41 years is assigned as cut-off value with %69.74 sensitivity and %56.56 specificity, in males 30 years is assigned as cut-off value with %93.65 sensitivity and %29.63 specificity. For XB joint fusion in females 43 years is assigned as cut-off value with %66.6 sensitivity and %85.1 specificity using ROC analysis, in males 41 years is assigned as cut-off value with %71.64 sensitivity and %84.37 specificity (Table 3).

Table 1. MB joint ossification

Age groups	Female-MB ossification grade				Total	Male-MB ossification grade				Total
	Grade 0	Grade 1	Grade 2	Grade 3		Grade 0	Grade 1	Grade 2	Grade 3	
21-25	19	3	0	0	22	22	0	0	0	22
26-30	11	3	3	5	22	18	1	1	2	22
31-35	18	2	0	2	22	10	3	2	7	22
36-40	17	3	1	1	22	17	2	2	1	22
41-45	12	3	1	6	22	14	5	1	2	22
46-50	14	3	0	5	22	17	1	1	3	22
51-55	12	3	1	6	22	16	2	1	3	22
56-60	11	2	1	8	22	10	6	1	5	22
61-65	8	8	0	6	22	11	5	1	5	22
Total	122	30	7	39	198	135	25	10	28	198

Table 2. XB joint ossification

Age groups	Female			Male		
	XB ossification grade		Total	XB ossification grade		Total
	Grade 0	Grade 1		Grade 0	Grade 1	
21-25	21	1	22	17	5	22
26-30	13	9	22	14	8	22
31-35	9	13	22	12	10	22
36-40	6	16	22	9	13	22
41-45	10	12	22	6	16	22
46-50	2	20	22	5	17	22
51-55	1	21	22	0	22	22
56-60	2	20	22	1	21	22
61-65	3	19	22	0	22	22
Total	67	131	198	64	134	198

Table 3. ROC analysis results.

XB joint	Cut-off age	Sensitivity	Specificity	AUC value	P value
Whole groups	43	66,42(60,4-72,1)	83,26(79,2-91-6)	0,835	<0.001
Female	43	65,6(56,9-73,7)	85,1(74,3-92,6)	0,817	<0.001
Male	41	71,64(63,2-79,1)	84,37(73,1-92,2)	0,851	<0.001
MB joint	Cut-off age	Sensitivity	Specificity	AUC value	P value
Whole groups	41	67,63(59,2-75,3)	54,09(47,8-60,3)	0,641	<0.001
Female	41	69,74(58,1-79,8)	56,56(47,3-65,5)	0.636	<0.001
Male	30	93,65(84,5-98,2)	29,63(22,1-38,1)	0,650	<0.001

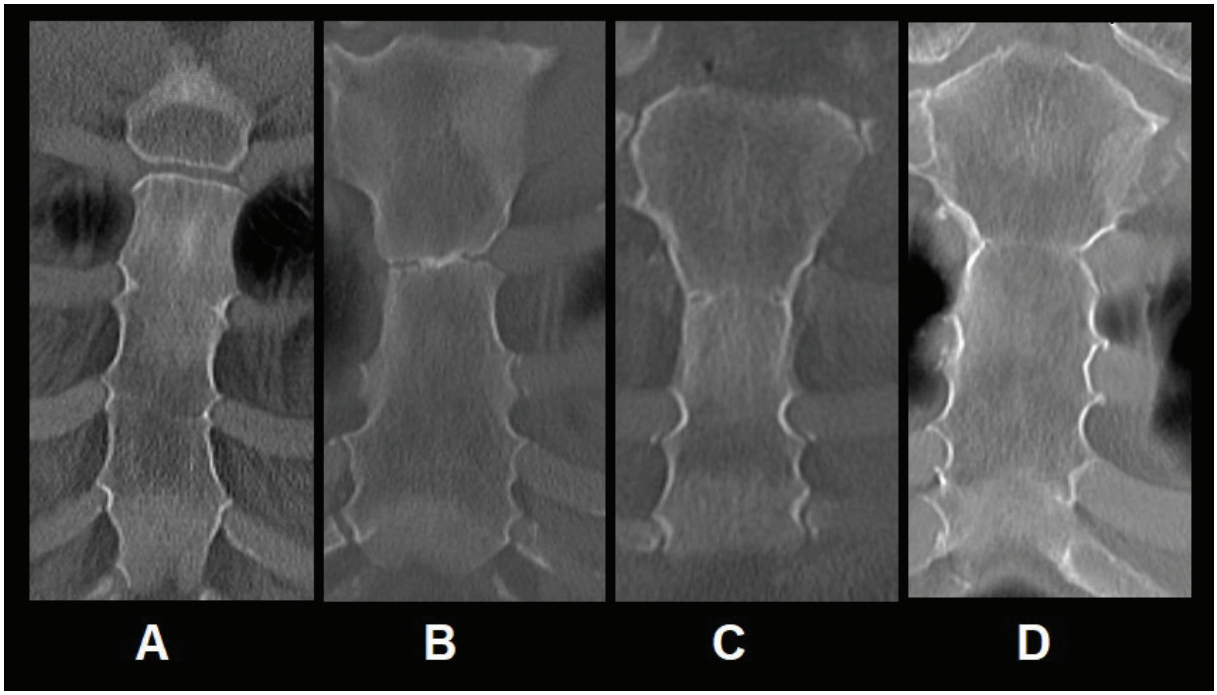

Figure 1 - MB joint fusion four-stage classification system. Grade 0 - no fusion present in the joint (A). Grade 1 - fusion present but less than half of joining surface (B). Grade 2 - fusion present half or more than half of joining surface (C). Grade 3 - complete fusion present (D).

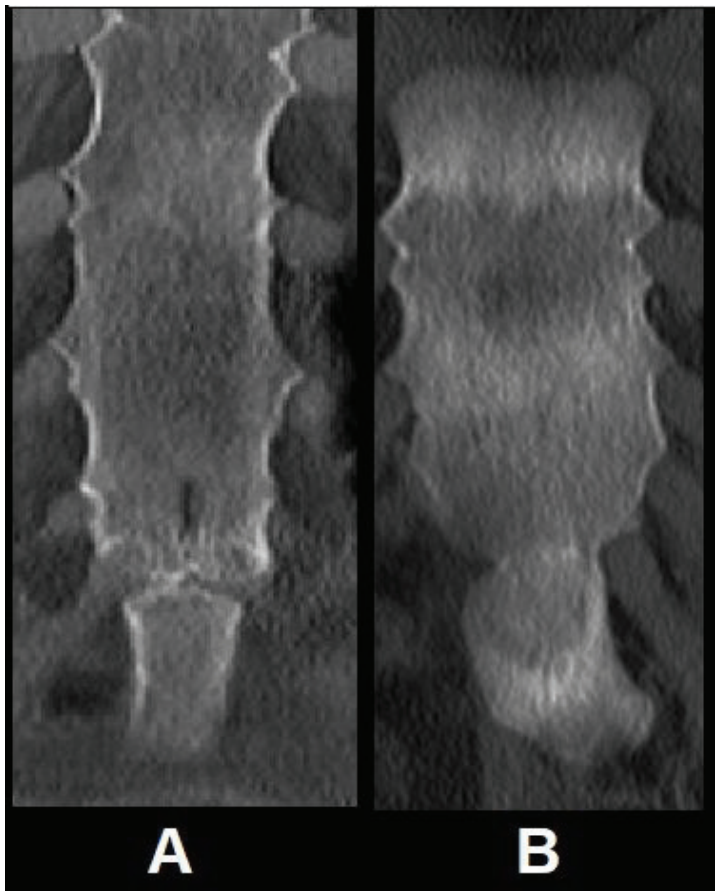

Figure 2 – XB joint fusion classification. Grade 0 - no fusion present in the joint (A). Grade 1 - complete fusion present (B).

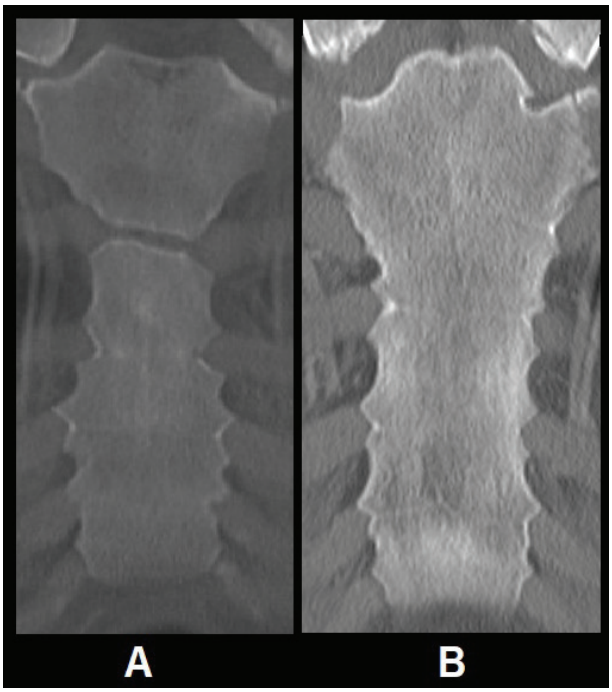

Figure 3 – At 21-25 age group there can be seen grade 0 (A) and grade 3 (B) fusion at MB joint.

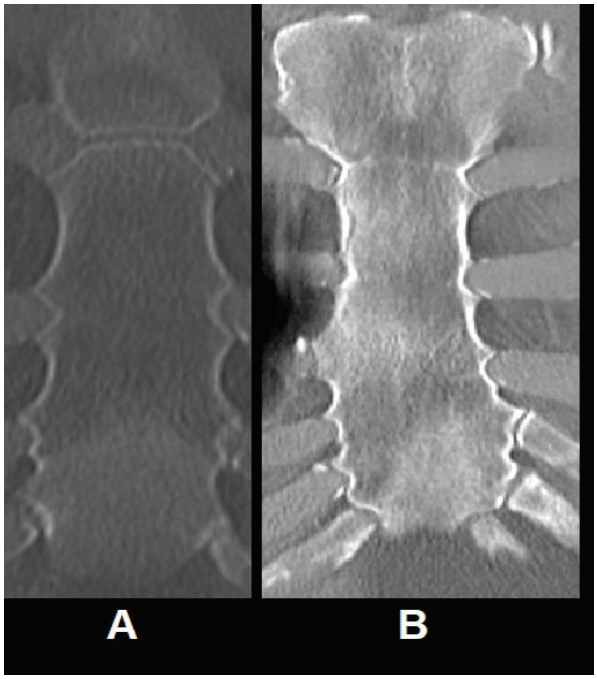

Figure 4 – At 61-65 age group there can be seen grade 0 (A) and grade 3 (B) fusion at MB joint.

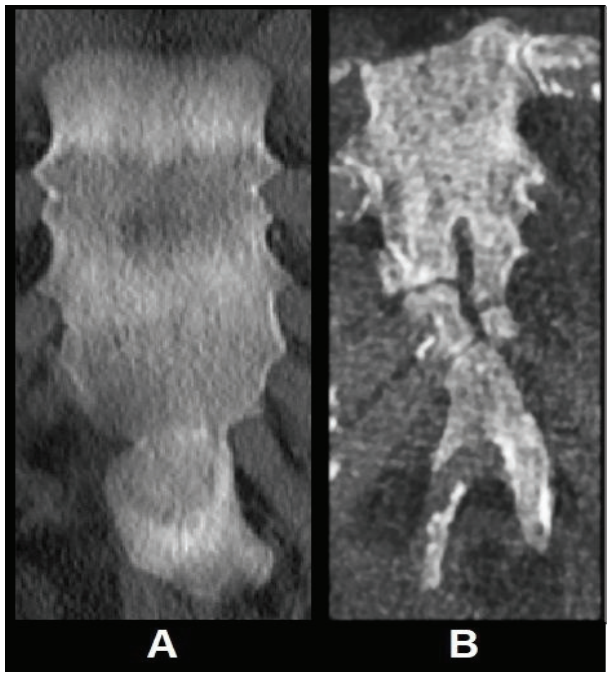

Figure 5 – At 21-25 age group there are patients with grade 1 fusion (A) and at 61-65 age group even patients with grade 0 fusion (B) could be detected.

Discussion

Radiological evaluation utilizing radiographs of certain bony structures are mostly used methods in determination of age. Lateral sacrum and coccyx radiograph is used between 23-40 years and lateral sternum radiograph around 40 years. Sternum has been studied by very few authors regarding its utility for estimation of age [6,7,10-18]. Authors have described fusion of xiphoid process with mesosternum at about 40 years of age and that of manubrium with mesosternum at "Very old age" i.e. 55-60 years [12]. Forensic medicine experts usually base their opinion on these observations. After 50 years old, fusion between manubrium and corpus is accepted as completed. For this reason, in our study we used sectional imaging method CT to evaluate accuracy of these methods. Our aim is to investigate utilizing MB and XB joint ossification grade as a method in determination of age.

In 1934 Trotter M [16], described the lack of a correlation of the manubriosternal junction fusion with age. Bacci N et al [17], the majority of sterna remain unfused throughout adult life, with complete fusion observed both in young and old individuals. In our study we found that there is no fusion and even ossification of MB joint in 21-25 years age group. In our study we

detected 4 cases in females and 2 cases in males with grade 3 ossification (fusion) in age groups less than 30 years. We found there wasn't ossification in 8 cases of females (%36.4) and 11 cases of males (%50) in more than 60 years' age groups. Despite there is significant difference of MB joint ossificiation in some age groups in males and females, utilizing MB joint in age estimation is not appropriate due to differences in ossification grades between each groups and excessive overlapping in wide age groups. It can be utilized in distinguishing young and elder population yet it is not our main aim and not convenient in clinical practice either.

According to Susan Standring et al [18], xiphisternal joint is usually transformed to synostosis by the 40 years and sometimes remains unchanged even in old age. In our study we detected cases with XB joint fusion in 21-25 years. There were 7 cases (%17.1) in females and 12 cases (%30) in males with grade 1 ossification (fusion) under 30 years old. 6 cases in females and a case in males greater than 50 years old had not ossification in XB joint (grade 0). Therefore, using XB joint in age estimation with CT scan is not appropriate in wide age groups.

There are several limitations in our study. Mainly determination of age using sternal joints is affected by geographic, ethnical, genetic, socioeconomic factors, nutritional habits besides diseases. Secondly due to investigation of wide age groups there is substantial overlapping in joint ossifications. That makes separation of age groups according to ossification more difficult. Thirdly there were fewer cases in each group after forming age groups. Our study can be repeated with more people and in closer age groups.

As a conclusion, sternal ossification can be evaluated well with thin section CT images. But it is arduous to estimate age in wide age groups using MB and XB joint ossification. As in earlier ages ossification can be detected, on the contrary in older ages there couldn't be any ossification. Despite there are statistically significant differences between some age groups, as there is no major differences CT is not convenient in age estimation. Only it can be said that XB joint fusion is seen more after 40 years. It should be reinforced with new studies in a greater number of population.

For Ethical Statement: Compliance with Ethical Standards

Funding: No

Conflict of Interest: No

Ethical approval: Yes

Informed consent: No (because retrospective study)

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Assessment of Nurses' Knowledge toward Sleeve Gastrectomy in Surgical Unit at Al-Najaf Al-Ashraf Governorate

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Abstract

A sleeve gastrectomy is a life changing surgery which used for helping patients who are severing from obesity (BMI 40+ or BMI 35+ with other health problems) for losing weight and enhance their health weight loss help to less the risk for development medical conditions related to obesity as high blood pressure, D.M, arthritis, respiration problems), the sleeve gastrectomy operation makes stomach small to change the amount of meals, and it helps to losing weight. This study aims to assess nurses' knowledge toward quantification sleeve gastrostomy in surgical unit and to identify the relationship between nurses' knowledge about quantification sleeve gastrostomy and their sociodemographic data in surgical unit. A quantitative descriptive design is used in the present study to perform objectives. A purposive sampling method; 40 nurses distributed on hospitals in surgical units, the data collection used through constructed questions divided to demographic data and nurses' knowledge, and data analyzed by descriptive analysis and an Inferential analysis. Nurses' knowledge about sleeve gastrostomy was ranging between poor to moderate, and as general the total score of nurses' knowledge was poor and no significant association between nurses' knowledge and their demographic characteristics.

Keywords: *sleeve gastrectomy, surgery, obesity, losing, weight,*

Introduction

Obesity is a rapidly growing health issue and is likely to represent a significant financial burden to the public healthcare system in most countries morbid obesity is considered the most serious stage of the disease, with a population prevalence of six to eight percent in the U.S management of morbid obesity through lifestyle, pharmacological, or surgical interventions has been described in the literature [(1)] . Obesity, identified as a 30 level or higher body mass index (BMI), was correlated with comorbidities such as D.M, disruptive sleep apnea, cardiac disease and dyslipidemia, and increased occurrence of certain cancers [(2)] .

Diet and exercise themed management require significant discipline and many morbidly obese individuals may find it difficult to instigate and maintain a consistent regimen pharmacologic therapy offers a possible adjunct when weight loss is not achieved through diet and exercise alone; however, its impact is modest and is limited by side effects, contraindications, and compliance rates [(3)] .

Surgical intervention traditionally is recommended only as medically necessary. It acts as an ancillary measure when patients who his body mass index (BMI) at ≥40 kg/m^2 or with body mass index ≥35 kg/m^2 with obesity assess with comorbidities fail diet, drug therapy and exercise, which bariatric surgery provides a durable and effective way of treating morbid obesity and related diseases, and the demanding of operation is growing nationwide[(4)] . Given that demanding of bariatric procedure increases as expected, strategies require to implement aims to optimizing perioperative care; to ensure efficiency and optimize outcomes the Metabolic and Bariatric procedure Accreditation and Quality Improvement Program, which joint program of the American College of Surgeons and the American Society for Metabolic and Bariatric operation, is the only nationally validated outcomes-based program to measure and compare the quality and safety for metabolic and bariatric surgical care in the U.S and Canada in 2016, the ACS and ASMBS published its updated MBSAQIP

Standards for best care of the metabolic and bariatric operation patient.

The Standards offer a nationally reliable and constant set of measures of safety and quality for a system in bariatric surgery care throughout the perioperative period under the Standards, both the ACS and ASMBS require established perioperative protocols that facilitate standardization of care related to the bariatric surgical procedure also, the ACS and ASMBS require the use of comprehensive protocols that outline the continuum of care of the bariatric surgery patient protocols are formal pathways that integrate evidence-based recommendations and algorithms for a specific patient population with a predictable clinical course. (5)

Protocols might be particularly helpful in bariatric surgery because they can define, optimize, and sequence different tasks performed by members of the multidisciplinary team the multidisciplinary team includes surgeons, anesthesiologists, nurses, psychologists, dietitians, and medical subspecialties as clinically indicated the implementation of protocols for use in the perioperative care had been related to improving outcomes in bariatric surgery patient and significant savings to the health-care system (6) .

Method and Material

The Study Design: A quantitative descriptive design is used in the present study to perform objectives. The (non-probability) purposive sampling method; the number of forms was with a generic form, 40 nurses' forms distributed on hospitals in surgical units

The Study Setting: The present study carries out in Al-Najaf Al-Ashraf City/ Al-Hakeem hospital, Al-Sadder Medical City, Iraqi specialist center, Al-Hayat national hospitals, Al-Najaf national hospital, and Al-Ameer . The Present part is comprised of (9) items, include: (gender, age, marital status, residency, occupational status, type of housing whether owned or rented monthly income, number of family members, level of educational, experience years in nursing filed, number of years' experience within the surgical unit and have you participated in obesity care courses after gastric sleeve surgery. Part two: nurses' knowledge includes general information about gastric sleeve comprises of (11) items, nursing care before gastric sleeve (8) items, nursing care after gastric sleeve (15) items, and complications (16) items as in appendix.

Statistical Analysis

The data was investigated with statistical processes and using the SPSS (Statistical Process for Social Sciences) version 23 application statistical analysis coordination .

Results

Table (1) Distribution of the Study Samples by Socio-Demographic Characteristics:

Items	Sub-groups	Study group Total = 40	
		Frequency	Percentage
Age / Years	21-27	11	27.5
	28-34	16	40.0
	35-42	5	12.5
	43-49	4	10.0
	50-56	4	10.0
Gender	Female	28	70
	Male	12	30

Cont... **Table (1) Distribution of the Study Samples by Socio-Demographic Characteristics:**

Marital Status	Single	18	45
	Married	20	50
	Widowed	1	2.5
	Divorced	1	2.5
Residency	Urban	38	95.0
	Rural	2	5.0
Levels of Education	Nursing School	12	30.0
	Nursing Institute	15	37.5
	Faculty of Nursing	13	32.5
	Postgraduate	0	0.0
Monthly Income (IQD)	Sufficient	9	22.5
	Sufficient to some extent	25	62.5
	Insufficient	6	15.0
Type of Hospital	Government	28	70.0
	Private	12	30.0
Year of Experience in Nursing	1-10	34	85.0
	11-20	4	10.0
	21-30	2	5.0
Year of Experience in Surgery Ward	1-9	35	87.5
	10-18	5	12.5
Training Courses	Yes	9	22.5
	No	31	77.5
No. of Courses	0	31	77.5
	1	5	12.5
	≤ 2	4	10

Table (1) show descriptive statistics (frequency and percentage) for nurses, it explains that the majority of the nurses subgroup are : nurses with ages between (24-34) years old (40 %), female nurses (70%), married nurses (50%), those who live urban residents (95%), nurses graduated from institute of nursing (37.5%), those with sufficient to some extent monthly income (62.5%), those with (1-10) years of experience in nursing (85%), those with (1-9) years of experience in surgery (87.5%), those with no training courses (77.5%) .

Table (2): Assessment and Mean of Scores for the Domain of Nurses' Knowledge about Sleeve Gastrectomy and Overall Assessment for Nurses' Knowledge about Sleeve Gastrectomy:

Domains	Mean of Score	RS	Assessment
General Knowledge	1.58	52.50	Poor
Nursing Care before Sleeve Gastrectomy	1.19	39.79	Poor
Nursing Care after Sleeve Gastrectomy	1.36	45.33	Poor
Complications	1.58	52.50	Poor
Total Score	1.43	47.53	Poor

F.=frequency. % = percentage Mean of score (poor=1–1.66 moderate=1.67 –2.32 high=2.33– 3) Relative sufficiency (poor =33.33-55.54 moderate =55.55- 77.76 high =77.77- 100)

Table (2) is about assessment and mean of scores for the domain of nurses' knowledge about sleeve gastroctomy, it shows that the assessment of nurses' knowledge is (poor) for the all four domains **.**

Table (3) is about overall assessment for nurses' knowledge about sleeve gastroctomy, it shows that the overall assessment for nurses' knowledge is (poor) with a mean of score (1.43) .

According to the tables (4) , there is no significant correlation between overall assessment of nurses knowledge about sleeve gastrectomy and their demographic data (P>0.05) .

Table (3): Frequency and percentage of nurses' subgroups according to their knowledge assessment about sleeve gastrectomy:

Nurses' subgroups	Poor	Moderate	Good
Frequency	37	3	0
Percentage	92.5	7.5	0

Table (4): Correlation between Overall Assessment of Nurses' Knowledge and Socio-Demographic characteristics

Demographic characteristics	Chi-Square	Contingency Coefficient	Significance p-value
Age	1.08	0.16	0.58
Gender	0.02	0.02	0.89
No. of Family Members	0.28	0.03	0.87
Residence	0.17	0.06	0.67
Marital Status	0.68	0.13	0.87
Educational Status	2.39	0.24	0.3

Cont... **Table (4): Correlation between Overall Assessment of Nurses' Knowledge and Socio-Demographic characteristics**

Types of Dwelling	0.94	0.15	0.33
Monthly Income	4.27	0.31	0.23
Type of Hospital	3.25	0.42	0.28
Years of experience in nursing	0.57	0.02	0.75
Years of experience in surgery	0.36	0.09	0.54
Training Courses	0.22	0.07	0.64
No. of Courses	0.94	0.15	0.62

Discussion

Table (1), shows and explains that the majority of the nurse's subgroup are: nurses with ages between (28-34) years old (40 %)this result nearly to result of study done by Hameed in (2012), he explained the most of the nurses (36.8%) are between the ages (22-27) years[7] , female nurses (70%), married nurses (50%) the present study demonstration married nurses 50% , the same results obtained by Griauzde et al., 2018 [8] . In United Kingdom and Twells et al.,2017 [9]. In Canada this tow study is similar to the present study found married nurses same result, those who live urban residents (95%), nurses graduated from institute of nursing (37.5%), those with a monthly income of (700-849) IQD (32.5%), those with (2-6) members of the family (55%), those that are owners of their houses (77.5%), those with (1-10) years of experience in nursing (85%), those with (1-9) years of experience in surgery (87.5%), those with no training courses (77.5%).

Table (2) is about Assessment and mean of scores for the domain of nurses' knowledge about sleeve gastrectomy; it shows that the Assessment of nurses' knowledge is (poor) for the all four domains and overall Assessment for nurses' knowledge about sleeve gastrectomy, it shows that the overall Assessment for nurses' knowledge is (poor) with a mean of the score (1.43). The present study shows questions about weight loss during 12 months by 65%, uncertain 25 (62.5%) assessment was moderate and mean of scores 1.75. This study resembles with results obtained in America by (Sharpton et al., 2019) and (Philouze et al., 2017). Because losing weight during this period is different because it follows each person's commitment [10,11].

The present study shows about, general knowledge, mean of scores 1.58, assessment was poor. This study is similar with results obtained in Russia by (Kirkil et al., 2018) [12] , but disagree with study in Franc by (Pizzorno, 2016) [13]. table (2)

In table (3) more than 92% of sample have poor knowledge about sleeve gastrectomy, the correlation between overall assessment of nurses' knowledge and demographic data. According to the Tables (4), there is no significant correlation between overall assessment of nurses' knowledge about sleeve gastrectomy and their demographic data (P>0.05). This study is agreeing with results obtained in India by. [14] but disagrees with study in America by.[15] Most of nurses don't have good knowledge about gastrectomy because this operation is considered new method in Iraq to decrease the weight and most of nurses don't read about this operation in there study program, that is mean all of nurses equal to have bad knowledge, for this reason association cannot be found between overall assessment of nurses knowledge about sleeve gastrectomy and their demographic data between overall assessment of nurses knowledge about sleeve gastrostomy and their demographic data, this result agree with Hameed (2006), he pointed no significant correlation between overall assessment of nurses' knowledge and their demographic data[16]

Conclusions

The age mean of nurses was (29.78) years and

ranging from (28-34) years them (40 %), most of them were female, half of the nurses were married, more than one-third of theme graduated from the nursing institute, most of the samples had employment in nursing from (1-10) years, and most of the samples study hadn't training sessions. Nurses' knowledge about sleeve gastrostomy was ranging between poor to moderate, and as general the total score of nurses' knowledge was poor in all dominos (general knowledge, pre sleeve gastrectomy, post sleeve gastrectomy, and complications related sleeve gastrectomy) and 3-There is no significant association between nurses' knowledge and their demographic characteristics. Special training and education programs should be designed and constructed for nurses in this surgical ward to reinforce their knowledge. 2-Publishing booklets for nurses related to pre and post sleeve gastrectomy. 3-Establishing a continuing education unit in surgical ward to improve the nurses' knowledge regarding sleeve gastrectomy.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

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An Physiological Enzymatic Study for Atherosclerosis Patients in the City of Fallujah

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Abstract

Atherosclerosis is a syndrome that affects arterial blood vessels. Arteriosclerosis thickens the walls of blood vessels as a result of accumulation of macrophages and white blood cells, and fat accumulation is more pronounced over time. Blood pressure, smoking, obesity, lifestyle, sugar and age are risk factors. Atherosclerosis. The symptoms and signs of atherosclerosis differ depending on the arteries affected by atherosclerosis.

This study was conducted in the Pharma lab in the city of Fallujah and samples were collected from the Fallujah General Hospital. 50 samples were collected, 30 of which were patients with atherosclerosis and the remaining 20 samples were for healthy people. The samples included both males and females between the ages of 30 to 78 years. A questionnaire was used to collect some information from patients and corrections that included (gender, age, blood pressure, smoking, Weight and blood sugar) and some tests were performed (Cholesterol , TG, HDL, LDL, VLDL, GOT, GPT, ALP, Albumin). The results showed a significant increase in concentrations of Chol, TG, LDL, VLDL, GOT, GPT, ALP) in patients with atherosclerosis and a significant decrease in concentrations of both HDL and ALB in patients with atherosclerosis at the probability level ($P \leq 0.05$). The results also showed that males are more susceptible to arteriosclerosis than Females, and also showed that the greater the age, the greater the risk factors for atherosclerosis. The more risk factors for atherosclerosis increase with increasing body mass. As for smoking, the results indicated that it has an effect on atherosclerosis, as people who smoke have increased risk factors for atherosclerosis, and disease risk factors increase with increased blood pressure and sugar.

Keywords : *Atherosclerosis, physiological study, Enzymatic study, Cholesterol, Triglyceride, LDL-C, HDL-C, VLDL, GPT, GOT, ALK, and Albumin.*

Introduction

Atherosclerosis is a disease that clinically affects all blood vessels, including the cerebral, coronary and peripheral arteries [1]. It is an inflammatory vascular disease caused by the deposits of fats in the walls of blood vessels, and it grows to a level that obstructs blood flow [3]. The accumulation of these deposits leads to narrowing of the artery and causing a clot, which leads to a stroke, dilated blood vessels in the abdomen, gangrene in one of the extremities, heart attack, myocardial infarction (angina pectoris) and ultimately, sudden death [2]. This build-up occurs over a period of years and shows no direct symptoms or signs until blood flow is severely affected, resulting in reduced organ nutrition [1]. Here the symptoms and signs appear, and this depends on the type of affected organ, and in most cases more than one member suffers from a lack of blood nutrition as a result of narrowing of the arteries that feed them due to atherosclerosis, and most cases these symptoms appear after the age of fifty or more, and angina is the first case [3].

Methods

1. Collections of Samples :

This study was conducted from (October 2019 to January 2020) in Pharma laboratory Fallujah city in Anbar governorate. During this period (50) samples (patients = 30, controls= 20) were collected from Males and females, the number of males was (28) and the number of females (22), and their ages ranged between

no Both sexes (30-78) years old.

2. Initial treatment of blood samples :

The blood was drawn intravenously in a volume of 10 ml and some blood was taken

Information for each, as blood samples were placed into test tubes Centrifuge at 3500 rpm for 15 minutes to obtain Blood serum, which was placed in new plastic tubes and recorded to and through all the information Lipid levels were checked in the central laboratory by measuring total cholesterol, triglycerides, and high-density lipoprotein concentration Density lipoprotein and low lipoprotein density.

3. Physiological study :

1.3. Determination of Cholesterol in Blood **:**

The enzymatic method was used to estimate the level of cholesterol in the blood serum by using the diagnostic kit (Cholesterol) provided by the Spanish company Biosystem [4], the light absorption was measured at (λ=500nm).

2.3. Determination of Triglyceride in Blood :

The enzymatic method was used to estimate the level of Triglyceride in the blood serum by using the diagnostic kit (Trigleseraide) provided by the Spanish company Biosystem [4], the light absorption was measured at (λ=500nm).

3.3. Determination of serum HDL-Cholesterol **:**

The enzymatic method was used to estimate the level of Triglyceride in the blood serum by using the diagnostic kit (HDL) provided by the Spanish company Linear [5]. the light absorption was measured at (λ=500nm).

3.4. Determination of Serum LDL-Cholesterol:

Low density lipoprotein in serum was determined using Fried Wald's equation The famous, it is clear from this equation that the value of VLDL = 5 / TG, then the Fried Wald equation states if : Ch = HDL + LDL + VLDL

LDL = Ch - (HDL + VLDL)

So LDL = Ch - (HDL + TG / 5)

4. Enzymatic study :

1.4. Determination of Glutamat-pyruvic (GPT) transaminase in Blood : The level of effectiveness of GPT was estimated using the diagnostic kit (GPT) provided by the Spanish company Linear [6]. The principle of this method is based on monitoring the reaction kinetically at 340nm at a rate of reduced absorption to the activity of ALT present in the sample.

$$\text{L-Alanine + 2-Oxoglutarate} \xrightarrow{\text{ALT (GPT)}} \text{L-Glutamate + Pyruvate}$$

$$\text{Pyruvate + NADH + H} \xrightarrow{\text{LDH}} \text{Lactate + NAD}$$

2.4. Determination of Glutmate-Oxaloacetate (GOT) transaminase in Blood :

The level of effectiveness of GOT was estimated the diagnostic kit (GOT) provided by the Spanish company Linear [6]. The principle of this method is based on monitoring the reaction kinetically at 340nm at a rate of reduced absorption to the activity of AST present in the sample.

$$\text{L-Aspartate + 2.Oxoglutarate} \xrightarrow{\text{AST (GOT)}} \text{L-Glutamate + Oxaloacetate}$$

$$\text{Oxaloacetate + NADH + H} \xrightarrow{\text{MDH}} \text{L- Malate + NAD}$$

3.4. Determination Alkaline Phosphate (ALP) in Blood :

The level of effectiveness of was estimated the diagnostic kit (ALP) provided by the Spanish company Bio system [7]. The concentration of (ALP) in the sample is calculated using the general formula :

$\Delta A/min \times Vt \times 106 / E \times I \times Vs = U/L$

The molar absorbance (E) of 4-nitrophenal at 405 nm is 18450, the light path (I) is 1cm, the total reaction volume (Vt) is 1.20, the sample volume (Vs) is 0.02, and U/L are 0.0166 μkat/L.

5. Determination Albumin in Blood :

The level of effectiveness of was estimated the diagnostic kit Albumin provided by the Spanish company Bio system [7]. the light absorption was measured at (λ=630nm).

6. Determination Glucose Test in Blood :

The level of effectiveness of was estimated the diagnostic kit (Glucose) provided by the Spanish company Linear [5]. the light absorption was measured at (λ=500nm).

Statistical Analysis

The Duncan test for statistics was used at the significance level ($P \leq 0.05$) for the studied variables in the blood serum of patients with atherosclerosis and compared them with controls.

Results & Discussion

1. Serum Test:

The mean concentrations (cholesterol, TG, -HDL-C, LDL-C, VLDL) and enzymes (GPT, GOT and ALK) and albumin were measured in patients with atherosclerosis compared to healthy controls, the study was divided into seven tables: the first for the level of lipid and enzyme concentrations for total patients (30) and total control (20). Second : classification of atherosclerosis patients and controls according to gender. Third : classification of atherosclerosis patients and controls according to age group. Fourth : the effect of body mass index (kg / cm) on the studied variants of atherosclerosis patients and controls. Fifth : The effect of smoking on the variables studied for atherosclerosis patients and controls. Sixth : the effect of pressure on the variables studied for atherosclerosis patients and controls. Seventh : the effect of blood glucose concentration on the variables studied for atherosclerosis patients and controls. Measuring a rate serum of concentration (Cholesterol, TG, HDL-C, LDL-C, VLDL, GPT, GOT, ALK and ALB) for patients with Atherosclerosis and controls, the results are shown as shown in table (1) where significant increase was observed in the mean at ($P \leq 0.05$) in the levels of concentration (Cholesterol, TG, LDL, VLDL, GPT, GOT and ALK) in patients with results (263.9±21.136, 321.2±20.095, 175.933±8.469, 33.6±4.952, 47.967±6.835, 41.767±6.452 and 161.3±8.074) respectively**.** This is accordance with a study (Chikkanna *et al.*) [8] and (Karajibani *et al.*) [9], where they notice a significant higher in levels (Cholesterol, TG) , the results also accordance with the study [10]. Where they observed a significant decreased for HDL-C in atherosclerosis patients and higher in concentration of (Cholesterol, TG) , Cholesterol increases above average normal when suffering from some disease, including atherosclerosis and heart disease [11]. This increase was caused for several reasons including nutrition, which causes an increase in the concentration of lipids in the plasma [12]. That increase cholesterol in the blood is one of the main causes of heart disease and atherosclerosis and plays an important role in the development of the injury in coronary heart disease, cholesterol deposits on the walls of blood vessels, causing them to narrow and blockage, and the increase in the concentration of triglyceride is due to an increase in fat-rich nutrition which leads to an increase in the production of chylomicrons in the intestine, which is then broken down it causes fatty acids to be released, so the liver cells will receive large amounts of fatty acids, causing increase in the release of triglycerides [13]. The high LDL-C level may be attributed to the degradation that occurs binding of (LDL-C) to receptor in the liver, which plays a large role in reducing the transformation of LDL-C to the liver tissue and then increase its concentration in the blood serum, and this leads to an increase LDL-C [14]. While the decrease in (HDL-C) concentration was significantly decreased at likelihood level ($P \leq 0.05$) for patients with atherosclerosis were (3.408± 33.067) compared to controls were (48.65 ± 8.592) the reason for decrease in (HDL-C) levels is to decrease in the activity of the enzyme lipoprotein LPL which leads to the degradation of (TG) to fatty acids and glycerol [15]. The results as shown in table (1) showed a significant increase in the level of probability ($P \leq 0.05$) in the level of (GPT, GOT) concentrations in patients with atherosclerosis, and the results were (6.452±41.767,6.835±47.967) compared with controls were (6.612±22.15, 5.906±22.4) respectively, and these results were accordance with a study (Alicia & Debabrata) [10]. Where noticed an increase in the levels of (GPT, GOT) that the increase in enzyme activity it can lead to abnormal structural and functional changes that the occur of hepatic cells, and these changes may increase the necrosis of hepatocytes (Necrosis). Thus, the enzymes are released into the bloodstream [16]. The results of the albumin concentration showed a significant decrease, and the result was (3.004±0.456) this result was accordance with the result of (Savini *et al*.), as this study that appears confirmed low serum albumin is associated with an increased risk developing cardiovascular disease in adults age.

Table (1) : A rate serum of concentration (Cholesterol, TG, HDL, LDL, VLDL, GPT, GOT, ALK and ALB) for patients with Atherosclerosis and controls.

Parameters Factors	Patients ± SD	Controls ± SD
Cholesterol	*263.9±21.136	177.174± .051
TG	*321.2±20.095	124.391±12.578
HDL-C	33.067±3.403	48.65±8.592
LDL-C	*175.933±8.469	99.1±11.248
VLDL	*33.6±4.952	26.615±4.214
GPT	*47.967±6.835	22.15±6.612
GOT	*41.767±6.452	22.4±5.906
ALK	*161.3±8.074	174.55±13.567
Albumin	3.004±0.456	4.268±0.302

* Significant differences according to the Duncan test ($P \leq 0.05$)

2. Effect of personal factors on Atherosclerosis:

1.2. Sex:

Table (2) shows the classification of the infected group according to sex, as the level of concentrations of each of (Cholesterol, TG, HDL-C, LDL-C, VLDL, GPT, GOT and Albumin) in the blood serum of patients with atherosclerosis (male-female) was measured compared to the healthy ones, as males notice more injury than females, and this is consistent with [17] it confirmed that males are more susceptible to cardiovascular disease. Testosterone increases the activity of the enzyme lipoprotein LPL, which increases the breakdown of (HDL-C) and thus high cholesterol and increases the amount of fat carried in the blood (VLDL, LDL-C and TG) which has a direct effect on heart disease [18]. In females, the female hormone estrogen has a role in the protection and prevention of heart diseases [19], as it helps in converting carbohydrates into fats in the process of storage under the skin and at the same time reduces the level of cholesterol in the blood and obesity and increases the level of (HDL-C), but in the case of Reducing or discontinuation of the hormone occurs an increase in the level of cholesterol and an increase in exposure to vascular and cardiac diseases, and this is what we observe after menopause, which leads to the occurrence of obesity, joint pain and osteonecrosis [20]. The results in table (2) showed a significant decrease at the probability level ($P \leq 0.05$) in the levels of (HDL and Albumin) concentrations in patients with atherosclerosis (male, female) compared with the controls, there are international studies that have demonstrated that the risk of atherosclerosis for both sexes is inversely related to levels of (HDL-C) in the blood, the higher the levels of (HDL-C) the oxidative damage is implicated in the development of atherosclerosis [21]. The results also showed that GPT enzyme increased in males when compared with women, and this study contradicts the study (Hussain) [22]. While the results showed that the concentration of ALK is high in healthy people compared with patients and this is due to the level of the ALK enzyme that varies in different people depending on blood types and is associated with an antigen present on the membranes of red blood cells [23].

Table (2): The effect of gender on the studied variants of atherosclerotic patients and control.

Parameters / Factors	Patients		Control	
	Male	Female	Male	Female
Cholesterol	*214.215±40.628	*243.05±47.509	165.57±9.65	167.66±16.13
TG	*222.917±97.041	*260.532±99.05	107.81±11.51	109.03±12.33
HDL-C	*38.5±8.511	*40.038±10.876	54.125±6.23	57.40±8.65
LDL-C	*140.083±41.182	*149.923±37.474	129.44±17.88	145.21±3.34
VLDL-C	*29.158±5.557	*32.32±5.652	27.122±3.23	22.15±6.5
GPT	37.792±15.322	37.5±13.831	38.287±16.81	33.22±12.4
GOT	33.333±11.846	34.654±11.17	32.45±12.5	31.24±10.13
ALK	*170.25±9.289	*163.231±13.99	166.45±7.33	160.33±10.9
ALB	3.5054±0.807	3.513±0.692	3.99±0.88	3.59±0.65

* Significant differences according to the Duncan test ($P \leq 0.05$)

2.2. Age:

Table (3) shows the classification of the group of people with atherosclerosis according to the age group, as it is noticed that people of late age groups (more than 50 years) are more susceptible to developing atherosclerosis to measure the level of concentrations of each of (TG, HDL-C, LDL-C, VLDL, GPT ALK and Albumin) and this is in accordance with (Costa *et al*.) [18] and those who indicated that progress Age is one of the factors that cause heart disease, as the incidence increases after the fifth decade and in both sexes [23]. Table (3) showed a significant decrease at the probability level ($P \leq 0.05$) in the concentrations of (HDL, albumin) for patients with atherosclerosis for all age groups compared to the controls, where the concentration of (HDL-C) decreased significantly in the age groups (40-50). Our results are not in accordance with (Attyha *et al.*) [24] which showed that it is positively correlated with GPT and negatively correlated with (GOT, Albumin), but it is in agreement with their study which showed that low serum albumin is associated with cardiovascular disease [22].

Table (3): The effect of age group on the variables studied for atherosclerosis patients and Controls

Parameters / Factors	Patients			Controls		
	40Less of ±SD	50-40 ±SD	50 More of ±SD	40Less of ±SD	50-40 ±SD	50 More of ±SD
Cholesterol	* 203.291±36.67	* 228.805±44.76	* 252.394±45.60	151.44±9.44	169.11±2.99	100.01±3.99
TG	* 193±92.515	*245.97±99.085	*281.401±90.53	99.04±2.88	106.11±23.87	113.34±8.99
HDL-C	* 40±6.725	* 39.75±11.525	* 38.125±10.02	56.98±5.88	54.10±4.88	53.65±6.00
LDL-C	*131.786±43.199	* 144.8±38.845	* 157.438±34.13	128.98±20.8	133.9±13.65	144.00±15.39
VLDL	* 26.914±4.875	* 30.425±4.833	* 34.688±5.326	24.98±2.67	26.33±5.6	30.11±5.87

Cont... **Table (3): The effect of age group on the variables studied for atherosclerosis patients and Controls**

GPT	* 33.214±14.61	*39.25±15.583	39.5±12.675	30.34±12.76	35.00±14.98	37.89±13.66
GOT	* 30.071±10.637	* 34.2±11.428	*37.25±11.642	28.65±9.55	30.23±10.8	36.22±11.80
ALK	* 157.688±11.965	*170.8±11.399	* 157.688±11.965	166.44±6.90	165.77±3.99	152.61±10.55
ALB	* 3.252±0.62	*3.606±0.775	* 3.252±0.62	2.88±0.23	2.98±0.54	3.00±0.45

* Significant differences according to the Duncan test ($P \leq 0.05$)

3. The effect of obesity with atherosclerosis:

Atherosclerosis is associated with obesity, as Table (4) shows the body mass index values (BMI_1, BMI_2, BMI_3) for a group of people with the disease compared to a group of Controls, as it is noticed that people who suffer from obesity are more likely to develop arteriosclerosis disease and this is confirmed [25]. The presence of high body mass index in Iraqi patients with atherosclerosis. The levels of lipid concentrations (Cholesterol, TG, LDL-C and VLDL) were significantly increased at the ($P \leq 0.05$) probability level with an increase in BMI in patients with atherosclerosis when compared with the Controls, The relationship of obesity, the rate of occurrence of heart attacks and arteriosclerosis is a direct relationship, because obesity is associated with the disturbance in the pattern of distribution of fat in the body and the deposition of excess fat under the skin, abdomen and muscles, as it represents an additional burden on the heart leading to a decrease in HDL-C and a high level of (Cholesterol, TG) As a result, it is a risk factor for developing heart disease [26]. Table (4) also shows that there are significant differences in the concentration of (GPT and ALK) for patients with atherosclerosis compared with the Controls, and this study came in line with (Hirakawa *et al.*) [27] when an increase in BMI was observed in people who suffer from obesity, which is a risk factor. In the development of oxidative stress associated with many diseases, including atherosclerosis in terms of an increase in oxidative products and a decrease in levels of antioxidants [27]. The study also showed a significant increase in albumin concentrations at the probability level ($P \leq 0.05$) compared with the controls. The activity of enzymes (GPT, ALK) in addition to albumin is an important diagnostic marker to infer hepatopathies [22].

Table (4): The effect of BMI (kg / cm2) on the studied variants of atherosclerosis patients

Parameters / Factors	Patients			Controls
	BMI1 (n=10)	BMI2 (n=10)	BMI3 (n=10)	BMI Controls (n=20)
Cholesterol	* 209.271±39.67	269.4±11.908	278.9±21.074	166.10±13.04
TG	* 205.252±96.287	* 329.4±19.126	* 329.3±11.441	107.90±10.20
HDL-C	* 41.629±10.606	34.4±4.45	33.6±3.565	55.266±7.77
LDL-C	132.143±39.884	174.2±9.094	* 176.4±10.554	130.34±11.04
VLDL	* 28.923±5.012	36±3.391	34.8±5.922	32.6±4.99
GPT	* 34.057±15.262	* 51.4±7.369	* 43.3±6.056	33.09±5.87
GOT	* 30.686±11.427	41.2±8.319	42.1±6.28	32.66±6.65
ALK	* 170.771±11.7	* 162.4±8.792	* 154.1±4.977	155.23±3.66
ALB	3.688±0.778	3.366±0.434	* 2.953±0.377	3.782±0.32

* Significant differences according to the Duncan test ($P \leq 0.05$), BMI_1= Normal weight, BMI_2= Above normal weight, BMI_3= Obese, $BMI_{Controls}$= Normal weight for Controls.

4. Effect of Clinical Factors on Atherosclerosis:

1.4. Blood Pressure:

Table (5) shows the effect of the blood pressure level on patients with atherosclerosis, as the results showed a significant increase in the concentrations of (Cholesterol, TG, LDL-C, VLDL, GPT, GOT and ALK) in both groups of patients, which are less than (10/15) and a group more than (10/15) at the probability level ($P \leq 0.05$) when Compare it with the controls group, This accordance with (Coresh *et al.*) [28], which showed that people with high blood pressure have a double risk of coronary heart disease at a rate of (2-3) times more than individuals with normal pressure. Our study also accordance with the study of people with high blood pressure and cholesterol relationship. In Nigeria, epidemiological studies indicated increased heart disease due to blood cholesterol [31]. High concentrations of cholesterol increase stress and are attributed to a combination of factors as diverse as genetics and lack of exercise [29]. The British Heart Foundation indicated that a decrease in pressure of (5) mmHg leads to a reduction in the risk of developing cardiovascular disease [29]. Studies have shown that angiotensin II, which is often elevated in hypertensive patients, has a role in narrowing the arteries, as it contributes to the establishment of stiffness by stimulating the growth of smooth muscles through its association with special receptors on the surface of smooth muscles, causing a stimulation of the phospholipase enzyme, which leads to the accumulation of calcium outside Cells, causing those muscles to contract further [30]. Increased angiotensin II stimulates increased activity of Lipoxygenase in smooth muscles, which in turn increases oxidation of (LDL-C) molecules it is thus a primary cause of inflammation by increasing H2O2 hydrogen peroxide and free radicals in the plasma [29]. The results showed a significant decrease in the concentrations of (HDL and Albumin) in both groups of patients at the probability level ($P \leq 0.05$) when compared to the controls, that (HDL-C) works to transport cholesterol from the peripheral tissues to the liver [28]. Our results showed that there was an increase in liver enzymes (GPT, GOT and ALK), It was found that negative hepatic congestion causes increased central vein pressure that may cause elevated liver enzymes [30].

The effect of stress on the studied variants of atherosclerosis patients and controls

Parameters Factors	Patients		Controls	
	10/15 نم لقا ±SD	10/15 نم رثكأ ±SD	10/15 نم لقا ±SD	10/15 نم رثكأ ±SD
Cholesterol	*205.59±13.984	* 275.059±51.458	188.05±12.09	198.67±11.09
TG	*197.085±20.839	* 330.588±104.98	190.56±14.19	199.89±10.89
HDL-C	* 42.242±3.097	* 33.588±10.13	38.12±8.09	30.88±12.09
LDL-C	* 129.485±6.36	* 175.706±40.1	119.78±7.90	170.78±6.87
VLDL	* 29.009±4.28	* 34.294±6.12	26.73±4.05	32.89±5.19
GPT	* 33.33±5.517	* 46±13.84	29.67±7.54	33.55±6.66
GOT	* 30.33±6.489	*41.176±11.293	27.03±4.54	38.32±10.42
ALK	* 171.091±5.732	* 157.882±14.031	160.56±8.37	150.88±6.11
ALB	*3.716±0.474	*3.109±0.686	2.089±0.567	3.001±0.231

* Significant differences according to the Duncan test ($P \leq 0.05$).

2.4. Concentration of Glucose in Blood:

The results showed as shown in Table (6), that there was a significant increase in the concentrations of (Cholesterol, TG, LDL-C, VLDL, GPT, GPT and ALK) in each of (less than 180 and 200-250 and more than 250) compared to healthy subjects, and a significant decrease in the concentrations of (HDL-C and Albumin). This is in accordance with Marks [31], who confirmed that individuals with diabetes have higher rates of cardiovascular disease compared to non-diabetics, and as it has been shown that people with type 2 diabetes have dangerously high levels of (LDL-C) and triglycerides that circulate in the bloodstream, and these high levels are associated with an increased risk of cardiovascular disease. The high level of triglycerides and cholesterol may be due to the body's use of fat as a source of energy instead of glucose sugar, as it consumes stored fats and then increases its level in the blood, and this increase leads to cholesterol deposition in the blood vessels to be a cause of atherosclerosis and heart disease [32]. Another study showed that there is a slight correlation between sugar and total cholesterol concentration in both atherosclerosis and type 2 diabetes, and this causes a long chain of fatty acids that inhibit the enzymes of the control points in the glucose analysis, which leads to an increase in the concentration of glucose and on the other hand, high glucose provides energy or adenosine. Triphosphate, which provides cholesterol metabolism [31]. The cause of high concentrations of (GPT, GOT and ALK) in patients with atherosclerosis in all groups, less than (180 and 200-250), and more than 250 result from the occurrence of abnormal structural and functional changes that may occur to hepatocytes and these changes may increase the laceration Or the hepatocyte necrosis (Necrosis) and thus the release of enzymes into the bloodstream [32]. The significant increase is also attributed to the increase in glycogen build-up in hepatocytes, which leads to Hepatic cirrhosis, which occurs as a result of the accumulation of fats inside hepatocytes [33]. The increase in the level of the enzyme (ALK) is also due to the release of the enzyme from the system, as diabetes may be associated with many bone diseases, especially osteoporosis, as a result of an imbalance in the secretion of the parathyroid gland (PTH), which plays a major role in maintaining the level of calcium in the blood [33].

Table (6): The effect of blood glucose concentration on the variables studied in atherosclerosis patients and controls.

Parameters / Factors	Patients			Controls		
	180 نم لقا ±SD	250-200 ±SD	250 نم رثكأ ±SD	180 نم لقا ±SD	250-200 ±SD	250 نم رثكأ ±SD
Cholesterol	*** 199.98±34.24**	*** 267.72±14.30**	*** 279.55±22.24**	**191.22±15.09**	**200.41±20.13**	**230.45±19.61**
TG	*** 182.99±85.24**	331.091±17.751	332.44±6.002	178.87±23.89	290.12±12.89	299.58±10.77
HDL-C	* 43.26±10.56	33.364±3.557	33.33±3.674	33.89±11.90	29.50±4.44	21.34±2.99
LDL-C	* 124.76±18.17	174.90±8.642	177±11.011	120.34±10.66	155.45±6.89	170.45±9.21
VLDL	*28.743±5.296	33.727±4.777	34.11±5.841	25.76±5.01	29.41±3.79	30.42±4.98
GPT	*30.867±13.788	* 52.182±7.040	* 42.44±5.747	28.91±10.76	33.81±6.87	37.90±6.90
GOT	* 28.4±10.11	43.455±8.513	41.22±5.975	21.86±6.33	35.81±5.00	34.84±7.22
ALK	171±12.407	164.72±9.001	* 154.22±5.26	161.80±5.64	157.51±5.87	144.90±4.82
ALB	* 3.79±0.781	3.12±0.47	3.025±0.317	2.75±0.22	3.801±0.23	3.98±0.239

* Significant differences according to the Duncan test ($P \leq 0.05$).

Conclusions

There was a significant increase in the concentrations of cholesterol and TG in patients with atherosclerosis compared to controls, and the subjects had a significant decrease in the concentration of (HDL-C) with a significant increase in the concentration of (LDL-C) in the blood serum of patients compared to controls. It was also observed that the level of the enzyme (ALK) varies in different people depending on the blood types and is associated with an antigen present on the membranes of red blood cells and the decrease in serum albumin within the normal range related to cardiovascular disease.

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Ethical Clearance: This study is ethically approved by the institutional ethical Committee.

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A Mini Review on Covid-19 Infection and Severe Outcome on Cancer Patient

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Abstract

Introduction: December 2019, the world witness a new strand of pathogen known as coronavirus-2 (SARS-CoV-2), later recognized as the novel coronavirus disease (COVID-19). By April 1st 2020, the confirmed cases were over 800,000 around the world with over 40,000 fatalities. Individuals with cancer are more vulnerable to COVID-19 impact because of compromised health-status.

Methods: A comprehensive search method was applied by searching the google scholar using the keywords to select eligible studies to be included in the review

Results: The findings showed that patients with cancer, especially thoracic malignancies that were infected with COVID-19 infection during the hospital stay developed severe cardiorespiratory symptoms that warranted use of a mechanical ventilator. The cancer patients with COVID-19 had increased rate of hospitalization and a poor outcome as well as a higher risk of mortality when compared to healthy individuals. It is recommended that cancer patients taking anti-tumour drugs should undergo early screening for COVID-19 because the anti-tumour drugs also suppress the immune system. Hence, their dosage should be regulated appropriately incase of COVID-19 co-infection.

Conclusion: In risk-mitigation pandemic scenario in COVID-19 period, efforts should be made not to compromise the prognosis of cancer in these patients by adhering to treatment guideline.

Key words: *cancer patients, COVID-19 novel coronavirus, exercises.*

Introduction

December 2019, the world was exposed to an unknown pathogen known as coronavirus-2 (SARS-CoV-2) which was later recognized as the novel coronavirus disease (COVID-19) [(1)]. By the 20th of March 2020, the number of infection has increased to 638,146 cases and 30,039 established deaths reported in more than 150 countries [(2)]. By the 1st of April 2020, the confirmed cases were more than 800,000 patients around the world with more than 40,000 fatalities [(3)]. Toward mid of 2020, COVID-19 has infected nearly 2.9 million individuals in over 200 countries with more than

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200,000 deaths [4].

COVID-19 is linked with manifestations ranging from an asymptomatic state to severe conditions of pneumonia, severe respiratory distress syndrome and fatality [1]. Individuals with cancer are more vulnerable to viral infections, especially if with concurrent history of chronic disease. Generally, poor health condition or immunocompromised status can mean poor treatment outcome alongside with anticancer treatments [5]. Dai, Liu [6] indicated that patients with cancer and infected by COVID-19 would experience a more unsatisfactory outcome than healthy populations. To the best of the researcher's knowledge, there are insufficient data on the systematic assessment of COVID-19 on patients with cancer within a representative population. Another study conducted by Liang, Guan [7] showed in their study that cancer patients with superimposed COVID-19 infection had complex outcomes and complications, some of the patients requiring admission into ICU for mechanical respiratory assistance, this finding was also similar in the study by Zhang, Zhu [8]. On the 10th of April, the city of New-York in the USA had reported a whopping number as high as 180,458 COVID-19 cases with acute respiratory distress syndrome, a severe complication that shorts down the respiratory system and recording about 9,385 deceased cancer patients, amounting to approximately 8.4% mortality figure [9].

During this sudden outbreak, the most vulnerable were the elderly especially those with the immunocompromised conditions such cancer, the rising death number was majorly among these age-groups, more so that, the health officers in Italy and the US were very concerned about the rising fatality in the nursing home because more deaths figure were reported during this short duration [10, 11]. Although the exact cause of deaths could not be unravelled, a research gap was created concerning which particular marker could be used to trace or monitor which stage in the pathogenesis of cancer and its treatment confers the risk of severe COVID-19 to prompt quick medical response [12]. Robilotti, Babady [13] showed in their study that having a profound knowledge or information about COVID-19 and cancer association is critical to be able to provide a standard and quality treatment to symptomatic cancer patient with confirmed COVID-19 infection. In their study, 40% (500 out of 2000 cases) were admitted for COVID-19, while 20 % developed acute respiratory disease with demand for mechanical ventilation while 12 % passed away within 30 days of confirmation of COVID-19. Generally, cancer patients with COVID-19 infection present with a significant rate of hospitalization and an acute outcome. Presently, the increasing incidence of COVID-19 in the United States is still at large, and there are still limited and comprehensive investigations or analysis to show that patients with cancer are at higher risk of severe condition [12, 14].

More so, there are few studies on the lasting adverse effects of COVID-19 in hospitalized patients with cancers especially haematological cancers, and because many these cancer patients would be treated with anticancer medications which suppress bone marrow function thus targeting the immune system and exposing them to a higher risk of public and hospital-contacted nosocomial infections and COVID-19 [15].

The Objective of the Review

There are many studies on COVID-19 pandemics in several countries. However, research on COVID-19 association and outcome on patients with cancer are still limited, and the address question is to understand whether cancer patients infected by COVID-19 have a higher risk and severe outcomes than the healthy population or whether these patients experience a worse prognosis and higher mortality.

Methodology

Selected studies were chosen by typing the keywords in google search wherein searched up till 2020 was obtained. Observational studies with retrospective studies, and comparative studies from various search-results included in the mini review. A total of 3000 studies and 89 full-text articles were assessed. Six studies met the inclusion criteria (COVID-19 impact and outcome on patient with cancer in 2020). The main outcomes of the review were overall survival, cancer-specific outcome and complication following COVID-19 infection on patients with cancer

Table 1. Summary of selected studies on effect of COVID-19 infection and severe outcome on cancer

Author/ Year	Title	Study Population	N	Methods	Results	Outcomes
(16)	Thoracic Cancers International COVID-19 Collaboration (TERAVOLT): Impact of type of cancer therapy and COVID therapy on survival.	Cancer patients with COVID-19	295	Data from Global consortium from 59 different hospitals in 9 countries Total of 295 patients with thoracic-malignancies infected with COVID-19 To determine the effect of COVID-19 on the cancer patients, provide information, direction and treatment outcome and identify the risk factors for the COVID-19 disease and fatality.	The result showed that COPD and HTN are the most recorded comorbidities The average age was 68, 31% female,69% male and total of 79% present/past smokers 73% NSCLC, 14% SCLC, 4% meso and thymic 49% of patients with stage IV disease 73% of patients require admission for cardiorespiratory features despite been on chemo or chemo-IO or RT and COVID-19 infection was treated with antibiotics 67%, antivirals 33%, and steroids 30%.	The study reported that patients with thoracic malignancies infected with COVID – 19 during the admitted time developed severe symptoms, more so are at higher risk of mortality
(4)	Case fatality rate of cancer patients with COVID-19 in a New York Hospital system	Cancer patients with COVID-19	218	Data on patients who tested positive for COVID-19 were selected from an electronic medical record in a single medical centre in New-York from the 18th of March to the 8th of April, 2020 and correlated with an existing cancer database from a period of one month	127 (58%) males and 91 (42%) females. HTN, DM and Chronic Lungs Disease are the most recorded comorbidities 164 (75%) patients have solid tumours, and 54 (25%) has hematologic Malignancies. COVID-19 related death on the cancer patient: Total of 61 (28%) death with a case mortality rate for 37% (20/54): hematologic malignancies 25% (41/164) for solid malignancies The number of fatalities was significantly higher among older age.	The Age-adjusted case mortality rate in cancer patients with COVID-19 contrasted with patients without cancer in the institution indicated a significant increase in the mortality rate among COVID-19 patients with cancer. Rates of ICU admission and ventilator use was slightly higher for the COVID-19 infected cancer patient
(15)	COVID-19 in persons with haematological cancers	Cancer patients with COVID-19	354	A cohort study conducted in China involving 128 patients hospitalized for haematological cancer (HC) and 226 healthcare-provider HCP 13 of 128 (10%) hospitalized-HC patients developed COVID-19 while, 16 of 226 of HCP had developed COVID-19, and 11 of whom were hospitalized Co-variates were contrasted with the 115 HC patients without COVID-19 with 11 hospitalized HCP with COVID-19	Most patients were adults over the age of 60 years (234, 56%) The median age of subjects with and without COVID-19 was 35 years (range, 23–53 years) and 49 years (range, 33–59 years; $P = 0.082$) Cardio- and cerebrovascular diseases were the most common comorbidities The result showed that there were no significant differences in baseline co-variates among patients who have HC positive or not-positive for COVID-19. The cases rate for COVID-19 was higher in hospitalized patients with HC: 10% (95% [CI], 6, 17%) compared with 7% (4, 12%; P = 0.322 in HCP. Case mortality rates were HC: 62% (32, 85%) and HCP: 0 (0, 32%; P = 0.002).	Admitted patients with HC had more severe presentations and at higher risk and mortality rate. The 13 patients with HC experienced more severe COVID-19 and more fatalities compared with HCP with COVID-19. However, the author was not able to detect the precise risk factors for COVID-19 in admitted patients with HC.
(13)	Determinants of COVID-19 disease severity in patients with cancer	Cancer patients with COVID-19	423	Record from the 10th of March to the 7th of April 2020 of 423 cases of symptomatic COVID-19 from 2,035 patients with confirmed cancer was diagnosed	The average age was 60, (234, 56%) Diabetes, hypertension, chronic kidney disease and cardiac disease were the most reported comorbidities	The cancer patients with COVID–19 had increased significant rate of hospitalization and a poor outcome

Cont... **Table 1. Summary of selected studies on effect of COVID-19 infection and severe outcome on cancer**

				at Memorial Sloan Kettering Cancer Centre	40% were admitted for COVID-19, of these: 20 % developed critical respiratory disease 9% of them needed mechanical ventilation 12 % passed away within 30 days.	
[6]	Patients with cancer appear more vulnerable to SARS –Cov-2: A multicentre study during the COVID – 19 outbreaks.	Cancer patients with COVID-19	641	A multicentre study on cancer patients with COVID-19 carried out in 14 hospitals over 2months in Hubei, China, among 105 cancer patients and 536 age-matched non-cancer patients tested positive with COVID-19. Four main outcomes analysed were death, admission into the ICU, development of severe/critical features, and requirement of mechanical ventilation.	Median ages of patients with cancer were 64.0, IRQ = 14.00) and patients without cancers were 63.5, IQR = 14.00) Comparing the outcomes in COVID-19 patients without cancer, the patients with cancer had 1.higher observed-death rates [OR, 2.34; 95% confidence interval (CI), (1.15–4.77); P = 0.03], 2.higher rates of ICU admission [OR, 2.84; 95% CI (1.59–5.08); P < 0.01] 3.higher rates of having at least one severe or critical symptom [OR, 2.79; 95% CI, (1.74–4.41); P < 0.01], and 4fold higher chances of mechanical ventilation requirement	The result indicated that cancer patients with COVID-19 had a higher risk than healthy individuals and at high risk of mortality and severe outcomes. More so, the result demonstrated that cancer patients appear more susceptible to COVID -19. Also, patients with COVID -19 who have non-metastatic cancer witnessed the same frequencies of severe health circumstances to those noticed in noncancerous patients.
					COVID-19 patients who did cancer surgery had a higher risk, severe outcomes and critical symptoms with higher mortality than those who have non-metastatic cancer who had only radiotherapy	
[8]	Clinical characteristics of COVID-19-infected cancer patients: a retrospective case study in three hospitals within Wuhan, China	Cancer patients with COVID – 19	28	A retrospective cohort study on cancer patients with COVID-19 Data were collected through medical record over two months: Jan.13 to the 23rd of February, 20202 Univariate and multivariate analyses were employed to determine the factors linked with severe outcomes, length of hospital stay, intensive care unit and requirement of mechanical ventilation, and death fatality.	From a total of 28, 17 (60.7%) patients were male. Median (inter-quartile range) age was 65.0 (56.0–70.0) years. Lung cancer was the most common type of cancer reported A total of 15 (53.6%) patients had severe events, and the mortality rate was 28.6%. 53.6% of the patients had severe events 21.4% were admitted to ICU 35.7% had life-threatening complications, and 28.6% of the patients died. The cancer patients had worsening conditions and poor outcomes from the COVID-19 infection with several features of pulmonary complication.	The study outcome shows the vulnerability of cancer patients in the current pandemic. Poor treatment outcome and the possibility of mortality

Discussion

COVID-19 has dealt a massive setback on most of the countries around the world. The consequences of COVID-19 is fatal, and it created severe outcomes among people with an underlying health condition, especially cancers patients. COVID-19 has infected nearly 2.9 million individuals in over 200 countries, with the devastating number of deaths over 200,000, and the US has the highest fatality [4]. With the on-going risk of COVID-19 transmission and concurrent adverse outcomes, the long term psychological effect on cancer patients health will be heightened, and treatment safety on patients with these chronic diseases will be compromised. The six studies in the table demonstrated that patients with cancer especially thoracic malignancies that were infected with COVID-19 infection during the hospital stay developed severe cardiorespiratory symptoms that warranted admission into ICU and requirement of a mechanical ventilator and eventually higher mortality risk.

The age-adjusted case mortality rate in the cancer patients with COVID-19 in contrast with patients without cancer showed a significant increase in the rates of admission into intensive care unit ICU with mechanical ventilator use and more mortality among COVID-19 patients with cancer. Patients with haematological cancer HC was shown to have a devastating outcome, admitted patients with HC had increased severe presentations, higher risk and highest mortality. From the review analysis, it was shown that thoracic and haematological malignancies were the two with the most recorded severe COVID-19 infection and more fatalities among the cancers. According to Mehta, Goel [4], the number of fatalities was significantly higher among the older age, and the cancer patients with COVID–19 had increased significant rate of hospitalization and a poor outcome. Also, patients with COVID-19 who have non-metastatic cancer showed the same frequencies of severe health circumstances to those noticed in noncancerous patients. The study outcome shows the vulnerability and poor outcome of cancer patients in the current pandemic, illustrating that patients with cancer demonstrated more susceptibility to COVID-19, poor treatment outcome and possibility of increase mortality rate. However, a contrary outcome was noted by only one study which indicated that there were no significant differences in baseline co-variates among patients with HC positive and no-HC patients infected with COVID-19.

Cancer is regarded as a chronic illness that gradually causes a significant decrease in the strength of the immune system, thereby reducing the individual capability to ward off infections [17]. A higher rate of severe outcomes and death were reported among the cancer patients with hematologic cancer (which included patients with lymphoma, myeloma, and leukaemia) thus recording the highest figure, followed by patients with lung cancer. [6]. These poor and worsen outcomes were associated with down-grading and weakening of the immune system because of the malicious or dysfunctional state of the lymphocytes, white blood cells or plasma cells. Generally, hematologic malignancy reduces the capability of the immune system to fence-off virus and other infections, which perhaps, is the main synergistic cause of rapid and deteriorating effect of COVID–19-associated high mortality rate and severe outcomes in these patients [6, 18]. Amongst solid tumours, patients with lung cancer have the utmost risk of contracting COVID–19 infections, because of the reduction in lungs function in lung cancer patients with superimposed acute COVID-19 infection, thus, contributing to the cardiorespiratory failure in this population [19].

Dai, Liu [6] indicated that the difference between the timeline in the hospitalization period and the severe events among cancer patient infected by COVID–19 and non-cancer patients infected by COVID–19 differs. Cancer patients with COVID–19 had a mean length of hospitalization of 27 days (SD 9.52) while non-cancer patients infected by COVID-19 had a mean length of hospitalization of 17.75 days (SD 8.64); which varies significantly (Wilcoxon test, $P < 0.01$). The severe medical events (composite endpoint) clearly defined as a circumstance demanding admission into an intensive care unit (ICU), the procedure of using mechanical ventilation, or mortality was very feasible and significant among the cancer patients infected with COVID-19 when compared to non-cancer patients with COVID-19 infection. In fact, a report by [20] concluded that the need for ICU intervention was seen to be 3 fold high among cancer patient infected with COVID-19 than when compare to the non-cancer patient with COVID-19 infection.

Recommendations

This review provides vital information on the vulnerability and outcome of COVID-19 on patients with cancer. Cancer patients infected by COVID –19 are linked with a significantly poor severe outcome and mortality rate, suggesting that the need for a dynamic and stringent approach is needed to decrease the spread of COVID-19 and to develop early accurate screening and pathogen identification in this susceptible population [4]. It is recommended that healthcare provider in-charge should increase surveillance and increase medical equipment availability. Special attention on cancers patients, especially hematologic cancer on admission for COVID-19 infection should be monitored adequately and precisely if on bone marrow suppressing drugs as well as those with progressive cancer. Persistent preparedness is another vital approach needed for adequate cancer treatment, especially during the on-going COVID-19 infection along with daily testing and screening of individuals for COVID-19. Cancer patients on anti-tumour drugs should have dynamic screening for COVID-19 because the chemotherapy drug are also associated with stiff adverse effects. Lastly, patient's drugs dosages should be routinely reviewed in patients with immunocompromised status like cancer and other chronic medical comorbid diseases.

The Benefit of Exercise for Cancer Patients.

The treatment of cancer patients is a critical health matter that creates physical, emotional, mental health and discomfort. Cancer leads to a massive effect on patients with a high burden of disease. Cormie, Trevaskis [21] stated that exercise is harmless and effective adjunct therapy in cancer patients' care. Exercise is significantly essential in all the health centre around the world because integrating exercise as part therapy represents a standard practice of keeping patient mentally and physically fit [22, 23].

Several studies have shown that introducing exercise into the management of cancer patient is significantly effective in improving patients' quality of life. If cancer patients perform moderate tolerable exercise frequently, they will have significantly immune boost, improve quality of life symptoms, possibly lengthening their survival, happiness, mental condition and physical status [24, 25]. Experts explained that cancer patients who perform exercise regularly would reduce their manifestations by stimulating changes in the immune system, metabolism, systemic inflammation, angiogenesis and redox status. Exercise outcome on cancer patient had a positive effect when patients performed it on a routine of six metabolic equivalent task 6-METs or more [26]. The American college of sports medicine (ACSM) admonishes the healthy person to perform twenty-sixty minutes of aerobic exercise at least 3-5 times/week and twenty repeated circle of resistance exercise with an intensity corresponding to 12-to-16 rate of exertion (20 is the maximum exertion rate) 2-to-3 times per week. Cancer patients can engage in 150 minutes of moderate-intensity aerobic exercise or aerobic combined to resistance exercise. The exercise-intensity is calculated using metabolic equivalent task (MET), 1-MET is equivalent to the expense-energy at rest. Moderate-intensity exercise is equivalent to 3-to-5.9 MET. High-intensity or vigorous exercise has a MET count of 6 and is referred to as an activity that produces perspiration and change in cardiac and breathing rates [26]. Dhawan, Andrews [27] indicated that high-quality exercise would significantly reduce the neuropathic pain level and improve functional quality of life QOL. High quality and regular aerobic exercise are highly advised for cancer patients or should be referred to an exercise physiotherapist: who has experience in cancer patients care [28, 29].

Conclusion

In a risk-mitigation pandemic scenario, efforts should be made not to compromise the prognosis of cancer in these patients by departing from guideline-recommended radiotherapy practice. If patients need for triage arise, important factors for triage such as potential for cure, relative benefit of radiation, life expectancy, and performance status should be employ in decision-making. The exposure and poor outcome of COVID-19 infection is increased in cancer patients as well as high mortality risk. Exercise is a harmless and effective adjunct therapy in cancer patients' care. Exercise will be significantly essential in all the centre around the world to integrate exercise as a portion of standard practice in cancer care because high-quality, regular and tolerable aerobic exercise would sufficiently boost the immune system and improve functional QOL.

Conflict of Interest Declaration

The research team declares that there are no conflict of interest in the course of this study

Ethics Approval For Main Research

The main study was carried out in conformity with the ethics requirement following which approval letter to conduct the main study was gotten in the Human Research Ethics Committee of the institution: Universiti Sains Malaysia (USM/JePEM/18120810).

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Assessment of Cervical Spine Mobility in a Subjects with Rheumatoid Arthritis Associated with Neck Pain

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Abstract

Background : Rheumatoid arthritis is an immunological mediated ,chronic inflammatory multisystem disease which involves inflammation of peripheral joints along with involvement of haematological , neurological and cardiovascular abnormality. Studies have shown that the involvement of cervical spine is common commonly affecting C1 and C2[1] due absence of intervertebral disc and synovial inflammation resulting in alteration in range of motion. Keeping in mind the inflammation and degeneration causes alteration in mobility . So that present study is conducted with the aim to find out proportion of alteration in mobility at the cervical spine range of motion in subjects with rheumatoid arthritis associated with neck pain.

Methodology: An observational study was conducted in 33 patients at karad with study duration of 6 months .The inclusion criteria was the patient with rheumatoid arthritis any age and gender willing to participate. The outcome measures were goniometer and inch tape method of assessing the range of motion .

Result: The results observational study suggest there is significant decrease in all movement of cervical joint with rotational components were hampered most around 26.36% loss of mobility for left rotation and 26.12 % loss of mobility for right rotation followed by right lateral flexion , flexion, extension , right rotation and left rotation .

Conclusion: the study concluded that rotational movements was more affected in a patients with rheumatoid arthritis associated with neck pain which is attributed to marked involvement of the C1 and C2 vertebrae followed by other movements at the neck.

Key words : *rheumatoid arthritis , cervical spine , range of motion ,goniometer and inch tape .*

Introduction

Rheumatoid arthritis is an immunological mediated ,chronic inflammatory multisystem disease. It involves inflammation of peripheral joints along with involvement of heamatological , neurological and cardiovascular abnormality.

Ra affect about 24.5 million people as of 2015 and the incidence rate is more dominated amongst females. The onset is evident in the age of 3rd and 4th decade of life.

Pathophysiology

It is an autoimmune disease predisposed by some environmental factors like smocking and bacterial

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infections. Genetic factors like present of human leukocyte antigen (HLA) specifically HLA-DRI and HLA –DR4 plays significant role in development of RA. Environmental factors triggers the antigenic activity in immunologically predisposed individuals and modifies our own antibodies like Ig-G , type 2 collagen fibres and vimentin. The process of Citrullation catalyst the autoimmune reaction as the Argenin converts into citrulline presenting type-2 collagen fibres and vimentin. And hence self antigens gets formed. This self antigens triggers the susceptible genes like HLA-DR4 AND HLA-DR1 and hence activation of antigen presenting cells occures which also activates the CD4+ T –cells which produces autoantibodies. The chemical mediators like cytokinesis i.e TNF-α , interferon- Gamma,IL-1 and IL-6 within the joint and leads to inflammatory response and hence activation of B- lymphocytes and macrophages along with endothelial cells. Activation of B-cells release Ig-M antibody against the Ig-G . This leads to proliferation of synovial cells and pannus formation and causes swollen synovial membrane due to granulation and scar tissue. The pannus damages the articular cartilage and erodes bone and damage other soft tissue structures.

Involvement of cervical spine in RA:

In 1952, Kornblum and colleagues3 were the first to recognize the relationship between RA and cervical spine instability[1]. The occiput –C1 and articulation of C1 –C2 are the only joint in the spine without intervertebral disc[1] and this are only consist of synovial joint make them more vulnerable in RA. Axis provide platform for skull to rest on it as it serve the purpose of ring. The transverse ligament of C1 articulates with the posterior aspect of send via synovial joint . This appearance allows the Atlas and axis permit increased motion at cervical joint . Odontoid process of axis articulates with the atlas-transverse ligament . And this allow the 50% of cervical spine rotation. The stability of the Atlanto-axial complex depend primarily on the integrity of transverse ligament . The alar ligament provides secondary stabilization. In RA , the transverse ligament is affected significantly and make it incompetent due to inflammation of the synovial articulation. There could be anterior subluxation of the Atlas if secondary protectors are intact . And if secondary stabilizes are involved then stability gets hampered. Synovial inflammation leads to erosion of the odontoid process . Axial skeleton involvement is a common feature of RA. It has a variable prevalence ranging from 25 to 80% (3), with the cervical spine being almost exclusively affected[2]. There are three types of cervical spine involvement in rheumatoid arthritis(RA). The commonest abnormality was erosions of the odontoid process (47%), followed by atlanto-axial dislocation and apophyseal joint involvement (24%) and only 5% of patients had abnormalities of spinous processes or vertebral bodies. The most common manifestation is AAS (49%), followed by superior migration of the odontoid (SMO, 38%) and subaxialsubluxation (SAS, 10-20%)[2] . The cervical mylopathy is also predominant in some cases[6]. The neck pain was present in 68% in a patiengts with rheumatoid arthritis as per the study conducted[4]. The condition of most patients with C1-C2 alignment abnormalities remained unchanged or became worse with time (i.e., the misalignment became fixed, subluxation increased, or AAI developed)[5].The study was done suggest that the degenerative changes in disc plates and osteophytes formation is also evident in upper cervical region is also prominent in some age group studied[7].

Aim

To study the alteration in mobility at the cervical spine in a patients with rheumatoid arthritis associated with neck pain**.**

Objectives

1.To find the alteration in mobility at the cervical spine in a patients with rheumatoid arthritis associated with neck pain with the help of goniometry.

2.To create awareness about the alteration in a mobility of cervical spine in a subjects .

3.To aware them about the future complications of

altered ROM and its effect on body

Materials :

1)data collection sheet

2) Consent form

Methodology

Type of study = observational

Study design = cross sectional

Place of study= karad, Maharashtra.

Sample size = 33

Sampling technique = convenient

Study duration = 6 months

INCLUSION CRITERIA : The individuals of Krishna institute of medical sciences , deemed to be university , Karad , Males / females suffering from rheumatoid arthritis, Patient with neck pain associated with neck pain with no specific age group criteria.

EXCLUSION CRITERIA : Individuals not willing to participate and individuals with symptoms other than rheumatoid arthritis.

DATA COLLECTION PROCEDURE : 33 subjects both male and female were selected for the study . Individuals were not willing to participate and individuals with symptoms other than rheumatoid arthritis were excluded. The written consent was taken from subjects those willingly to participate . Institutional ethical committee approve was obtained prior to beginning of the study. The conclusion was done based on goniometry and inch tape method of assessing the range of motion .

STATASTICAL ANALYSIS : Descriptive statastics such as mean , standard deviation and percentage was used to present the data . Association of altered mobility at cervical spine area was assessed by ENOVA P test . "P" value less than 0.030 were considered as significant . Data analysis was performed by using Microsoft excel and SPSS v16.0 .

Tables 1: By goniometer (in degrees)

movement	*avarage*	*normal - avarage*	*% of avarage*	*100 - %*
flexion	68.75	16.25	80.88%	19.12
extension	65.15	14.85	81.43%	18.43
left rotation	66.37	23.63	73.74%	26.26
right rotation	66.5	23.5	73.88%	26.12
L. lateral flexion	40.31	9.69	80.62%	19.38
R. lateral flexion	40.06	9.94	80.12%	19.88

Table 2: By Inch tape method : in centimeters

movement	*avarage*	*normal -avarage*	*% of avarage*	*100-%*
flexion	11.13	2.87	79.56	20.44
extension	6.43	1.6	80.39	19.61
left rotation	8.89	3.11	74.12	25.88%
right rotation	9.01	2.99	75.08	24.92%
L. lateral flexion	8.13	1.87	81.3	18.70%
R. lateral flexion	8.09	1.81	80.9	19.10%

Discussion

Cervical spine involvement in rheumatoid arthritis has been common feature of the disease. Due to long time standing of RA leads to cervical spine involvement. C1 and C2 joint is more commonly involved and there are previous studies that suggest atlantoaxial involvement of the cervical spine in RA is not so uncommon. Apart from atlanto-axial joint involvement , subaxial subluxation and other causes like cervical myelopathy are much common ultimately lead to joint erosion , altered mobility at the cervical joint . Loss of physiological lordosis , painful and limited AROM and passive cervical movements are prominent.

In this study , we've made an attempt to calculate and evaluate the cervical spine altered mobility by the means of goniometer and inch tape method.

Each and every movement at the cervical spine has been tracked out by both means of measurement methods . Values got recorded without any gender discrimination and majority of the patient were females as RA is a female dominant disease.

Age was ranging from 41 to 70 years as the rheumatoid arthritis with neck pain is prominent in this age only i.e the later half of life. There was no such criteria for gender and age but majority of the patient were female and above 40 years.

After comparing the data values with age wise distribution with particular movement were done i.e flexion movement was considered with age wise categorization. The age group strategy was straight forward as they belonged to 41-50 ,51-60 , 61-70. The mean and SD value was recorded then P value and F value were found out to find out age wise significance of RA and reduced ROM at the cervical joint. There was no as such relation between the increasing age leads to more hypomotility except for the rotational movement which had significant P value when measuring via inch tape method for both right rotation and left rotation p value showed significance with increasing age.

The observational study suggest there is significance decrease in all movement of cervical joint with rotational components were hampered most around 26.36% loss of mobility for left rotation and 26.12 % loss of mobility for right rotation by goniometric method due to more

involvement of C1 and C2 vertebrae in RA followed by right lateral flexion , flexion, extension , right rotation and left rotation.

Other movement were also affected ranging from 18 – 20 % .

Both method showed similar result in terms of percentile loss of ROM and there was minute difference in them suggest both method have similar effect while measuring the ROM with minimal difference and hence both method can be accepted to find out the percentage of loss of ROM.

So at the end of the discussion , we can suggest that rotational component get hampered in terms of ROM when cervical spine hypomobility than the other movements. Other movements also had significant loss but in lesser extent than the rotation. There is no significant relation between the age and reduced mobility except for rotational component. It could be because of severity of the RA differs patient to patient.

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Conflict of Interest : The authors declared that there are no conflicts of interest concerning the content of the present study.

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Maternal Risk Factors in Women With Breast Cancer in Al-Najaf Province

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Abstract

Recently, breast cancer has been considered as an increasing problem specially in Iraq . This study was designed to investigate maternal risk factors of breast cancer in Al-Najaf Province . A Convenience sample of (40) patients was taken in this study during the period from Dec. .2019 to Apr. 2020 . The result of the present study shows that the majority of the women were between the age (30-40) years show the breast cancer increase in this age group. The results of the current study also showed that the vast majority at the women were married. The current study also showed that about (27.5%) and (22%) at the studied women are suffering from hypertension and diabetes mellitus respectively. The present study reveal that about (32.5)of the women did not practice breast feeding ,(42.5) of them less than 2 years breast feeding. The present study revealed that about 60%of the women didn't exposed to passive smoking and 40% of the women exposed to passive smoking , other study explain the second hand smoke have been suggested to increase the risk of breast cancer. The present study indicated that approximately all investigated women (39of 40)have no medical history of ovarian polycystic disease . It was concluded that the most contributing risk factors in the occurrence Education, BMI , contraceptive use ,passive smoking and age. It is recommended to establish an education program to enhance the awareness of women about the risk factors that have been recorded in this study .

Keywords : *Breast Cancer, Maternal Risk Factor , Women*

Introduction

Breast cancer is the most common type of cancer in women and the second leading cause of cancer related deaths, next to lung cancer. Although men can also get breast cancer, cases of male breast cancer account for less than 0.05% of all breast cancer cases diagnosed. If eight women live to the age of 85, at least one of them will develop breast cancer in her lifetime. Two thirds of women diagnosed with breast cancer are over the age of 50, and the majority of the remaining women diagnosed with breast cancer are between the ages of 39 and 40 [(1)] .

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In Iraq the continuous rise in the incidence rate is associated with an obvious trend to affect premenopausal women [(2)] . Patients' data have recorded the following factors : age of onset, family history of breast cancer in 1st or 2nd degree relatives, histological type of cancer, tumor size in pathology reviews, axillary lymph node status, histological, nuclear grade, type of surgical procedure and adjuvant therapy administered. Disease was staged according to the American Joint Committee of Cancer (AJCC) system [(3)] .

Breast-feeding may modestly reduce the risk of developing breast cancer. Many studies, reported that women who breast-fed had a decreased risk of developing breast cancer (ranging from 10%-64%) compared to women who never breast-fed [(4)] . There is extensive evidence that breast cancer incidence is increased in current users of menopausal hormone therapy and that the risk returns to that of never users of hormonal therapy soon after use ceases and Virtually all published

reports that have compared the effects of different types of hormonal therapy found greater increases in breast cancer risk with use of estrogen – progestin than with use of estrogen-only formulations [5] . However, about 90% of the women in the estrogen only arm of this trial were randomly assigned to hormonal therapy more than 5 years after their menopause, and the Women's Health Initiative investigators recently reported that breast cancer risk was greater if hormonal therapy use began less than 5 years after menopause than after a longer gap, both for estrogen-only and for estrogen – progestin preparations [6]. The number of elderly patients with breast cancer is due to rapidly increase in the near future, as more than 20% of the population expected to be >65 years old by 2030 [7] . Some authors have compared the rate of breast cancer among women with age at menarche of at least 15 years compared with age at menarche less than 12 years. The rate ratio was 0.72 (95% CI 0.62-0.82), indicating increased risk with younger age at menarche. One study was identified that explored the relationship between timing of use of OCs in relation to defined age groups and risk of breast cancer [8]. A previous study has found a statistically significant association between oral contraceptives OC use before age 20 and risk of breast cancer (OR 2.10, 95% CI 1.32-3.33). However, this finding would need to be explored in other studies before forming any conclusions about timing of OC use by defined ages [9].

Materials & Methods

A cross-sectional descriptive approach was designed to meet the previously mentioned objectives of the current study. The period of the study is from Dec. .2019 to Apr. 2020. The study was achieved in Al-Najaf City Al- Forat Al -Awsad Center for tumors. A Convenience sample of (40) patients was taken in this study. Demographic data and maternal risk factors were assessed by interview .

Results

Table (1): The observed frequencies and percentage of risk factors related to the socio demographic characteristics for women with breast cancer

Characteristic	Groups	Frequency (Total=40)	Percentage (%)
Age	20-30 years	12	30
	31 -40 years	10	25
	41 -50 years	8	20
	51 -60 years	7	17.5
	61 -70 years	3	7.5
Education	illiterate	14	35
	read &write	4	10
	Primary school	18	45
	University	0	0
Marital status	Married	33	82
	Single	1	2.5
	Widowed	6	15
	Divorced	0	0
	Separated	0	0
Economic status	Sufficient	15	37
	Insufficient	10	25
	Sufficient to some extent	15	37

Table (1) shows the observed frequencies and percentage of the socio demographic characteristics for the studied women with breast cancer . The patients were grouped according to age into 20-30 years, 31 -40 years , 41 -50 years, 51 -60 years, 61 -70 years, The percentages of age group were (30 %), (25 %), (17.5 %) and (7.5 %) respectively .

Table (2) shows the observed frequencies and percentage of Types of breast cancer among women with breast cancer . The main type was Invasive ductal carcinoma which recorded (47.5%), followed by Ductal carcinoma in situ and Invasive lobular carcinoma with a percentage of (35%) and (17.5%) respectively .

Table (3) shows the observed frequencies and percentage of the socio demographic characteristics for the studied women with breast cancer . The table reveals that most of the patients were overweight (45%) and obese (35%) , it also shows that the main chronic disease are hypertension (27.5%) and diabetes mellitus (22%) . In relation to reproductive status, it was found that (15%) had a menarche before 12 years, (15%) had irregular menstrual cycle, (45%) had used contraceptive pills, (15%) have more than 6 children, (32.5%) did not experience breast feeding, (42.5%) had breast feeding for less than 2 years .

Table (2): Histological Types of breast cancer among involved women

Types of breast cancer	Frequency	Percentage %
Ductal carcinoma in situ	14	35
Invasive ductal carcinoma	19	47.5
Invasive lobular carcinoma	7	17.5

Table (3): The observed frequencies and percentage of risk factors related to the clinical characteristics for women with breast cancer .

Characteristic	Groups	Frequency (Total=44)	Percentage %
BMI	Under weight	0	0
	Normal	8	20
	Over weight	14	35
	Obese	18	45
Polycystic	Present	1	2.5
	Absent	39	97.5
Ovarian cancer	Present	1	2.5
	Absent	39	97.5
Chronic disease	Not found	21	52.5
	Hypertension	11	27.5
	Diabetes mellitus	7	17.5
	Other	1	2.5

Cont... **Table (3): The observed frequencies and percentage of risk factors related to the clinical characteristics for women with breast cancer .**

Menarche	Before 12years	6	15
	After 12years	34	85
Regularity of menstrual cycle	regular	34	85
	irregular	6	15
Duration of menstrual cycle	Less than 5days	5	12.5
	More than 5 days	14	35
	5 days	21	52.5
Menopause age	Less than55	27	67.5
	Less than 55 because chemotherapy	9	22.5
	More than 55	4	10
Family planning	Contraceptive pills		
	Yes	18	45
	No	22	55
	Having children		
	Yes	35	87.5
	No	5	12.5
	No. of children		
	0-3 child	16	40
	4-6 child	18	45
	More than 6	6	15
Breast feeding	Present	27	67.5
	Absent	13	32.5
	Duration		
	Less than2years	17	42.5
	2 years	12	30
	More than 2 years	1	2.5

Discussion

The result of the present study shows that the majority of the women were between the age (30 -40) years ,this result agrees with some researches that related a similar result ;which show the breast cancer increase in this age group [2,4] ; and other studies disagree with our result which show that the increase in the age was more than 65 years of age [7] .

The results of the current study also showed that the vast majority of the women were married , this may be attributed to the age classes of the scope that occur mostly more than 30 years , this result supported by other study which appear the married women were found to have increase risk of breast cancer [10] ; while disagree with other study which explain the marital status is not considered a risk for breast cancer , on the other hand ; a case control study done in Iran showed that never married women were at higher risk for breast cancer [11] .

According to table (2), about 80% of the women were either overweight (35%) or obese (45 %), this result supported by many previous data that consider high scores of BMI as one of the risk factor of breast cancer; because the metabolic change that accompany obesity include peripheral hyperinsulinemia , hyperglycemia and glucose intolerance , increase serum very low density lipoprotein ,increase serum leptin ,dyslipidemia , increase serum cortisol clearance ,increase serum c-peptide level ,down-regulation of insulin receptors, and an exaggerated insulin response to oral glucose load ,these changes ,especially when they occur in early adulthood , may be of fundamental important in the developmental of breast cancer [5]. The current study also showed that about (27.5%) and (22%) of the studied women are suffering from hypertension and diabetes mellitus respectively ,and it's agree with the presence of one or more of chronic non-communicable diseases appeared from this study to be significantly associated with the development of breast cancer in pre-menopausal women [12].

The present study reveal that about (32.5) of the women did not practice breast feeding ,(42.5) of them less than 2 years breast feeding while other study showed that there was no significant association breastfeeding practice and breast cancer [2]; and our result compatible with the result of another case control study made by Latif and his coworkers among women in Baghdad which shows the breast feeding may modestly reduce the risk of developing breast cancer ranging from 10%-64% [4]. A recent study shows women who breast fed (the failure to an association between breast feeding and breast cancer in the study may be related the prolonged breast feeding practice by most Iraqi women and prolonged breast feeding may be related to socioeconomic status, education and employment status of the women [13].

In response to the history of use of contraceptive pills, the current study shows that there is a slightly effect of contraceptive pills on the developing of breast cancer ; those result is in agreement with studies published by American Cancer Society , there was other studies disagree with our results which appear that the use of oral contraceptive pills found to be among the significant risk factors in the development of breast cancer among pre-menopausal women [5], furthermore ,we also found that nulliparous women and women who never breast fed their children were higher in patient population as comparing with controls [13], and some authors found significant association between the use of oral contraceptives and the development of breast cancer [4]. The present study revealed that about 60%of the women didn't exposed to passive smoking and 40% of the women exposed to passive smoking , other study explain the second hand smoke have been suggested to increase the risk of breast cancer in a number of studies that restrict the comparison group to women who report no exposure to second hand smoking [2],while other studies show there was no evidence of a statically significant difference in breast cancer risk between subjective who had ever smoked and those who had no [4].

The assessment of reproductive physiology showed that about (15%) less than 12 years but more than 12 years about (85%) . the age of menarche in women more than 12 years increase in women with breast cancer , these findings are in line with other studies, but also disagree with other findings which there was low risk for breast cancer with increasing age of menarche [4].

In current study menopause in women less than less than 55 years (67.5%), there is women's menopause less than 55 years because therapy about 55 years about (22.5%) and women more than 55 years is about (10%), But another finding disagree with this which late age

at menopause was also found to be strongly linked to the presence of breast cancer .this association is negative ,and another studies the distribution of patients according to menopausal status shows that the frequency of menopausal women in the patient group is statically higher than in the control group 64% versus 40% [13].

The present study indicated that approximately all investigated women (39 of 40) have no medical history of ovarian polycystic disease ,this may disagree with some research that which show Controversial data . However, the study of Atiomo and his coworkers detected a statistically significant positive family history of breast cancer among women with polycystic ovarian disease [14], and other study showed that obesity, hyperandrogenism, and infertility are features of polycystic, which were known to be associated with the development of breast cancer [15].

Conclusions

The most contributing risk factors in the occurrence of breast cancer are : age, BMI , contraceptive pills , absence of breast feeding and passive smoking Invasive ductal carcinoma type is the most prevalent type of breast cancer among the studied samples .

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Risk factors for prostate cancer in Kohgiluyeh and Boyer-Ahmad provinces, Iran

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Abstract

Background: Prostate cancer is the most common malignant cancer and the second most common cancer in men worldwide. It is the second leading cause of death after lung cancer in men. This study performed to determine the risk factors for prostate cancer in Kohgiluyeh and Boyer-Ahmad provinces.

Method: The present study was a retrospective and case-control study in which 80 prostate cancer patients and 140 controls matched in Kohgiluyeh and Boyer-Ahmad provinces. Data collected using a questionnaire and interviewed by the researcher. To analyze the data, Chi-square and logistic regression tests with odds ratio with 95% confidence conducted using SPSS v.22 software.

Results: The results showed that in the univariate conditional logistics analysis, The highest odds ratio for prostate cancer related to red meat consumption 2-4 times a week (95% CI: 2.60 – 26.40) was 20.74 and The smallest odds ratio was related to continuous physical activity 0.34 (95% CI: 0.19 – 0.61). In the multivariate conditional logistic regression model, after removing the confounding variables, it was observed that the history of pelvic imaging, red meat consumption 2-4 times a week, fast food consumption and processed meat consumption 2-4 times a week, Family history of prostate cancer, history of urogenital infections were identified as the most effective factors in prostate cancer.

Conclusion: Family history of prostate cancer, history of urinary tract infection, history of smoking, history of pelvic imaging, consumption of red meat, consumption of fast food and processed meats were the most important predictors of prostate cancer in this study. It can be considered by educational and health planners to prevent this cancer and take appropriate intervention measures.

Keywords: *Prostate cancer, risk factor, Kohgiluyeh and Boyer-Ahmad provinces*

Introduction

Prostate cancer is the most common malignant cancer and the second most common cancer in men worldwide[1]. It is the second leading cause of death after lung cancer in men. In general, 1 in 7 men will develop prostate cancer during their lifetime. The risk increases with age[2].The prevalence of prostate cancer in middle-aged and older men is higher among other cancers[3]. Studies have shown that diagnosing prostate cancer before the age of 50 is almost rare (less than 0.1% of patients). In most countries, 70% of all prostate cancers diagnosed in men over 65 years of age[4]. The various

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causes of prostate cancer are not yet well known and sometimes, conflicting results seen in various studies[5]. Differences in prostate cancer incidence and mortality in different parts of the world may be due to genetic, environmental and social factors that affect the patient[6]. However, studies of immigrants and temporal changes in prostate cancer suggest that modifiable factors such as diet may contribute to prostate cancer[7]. The incidence of prostate cancer worldwide is close to 50 per 100,000 population, ranging from 3.9 in India to 178.8 per 100,000 in black Americans[8]. Annually, more than 670,000 men diagnosed with prostate cancer in the world, of which about 225,000 are in Europe and 240,000 in the United States[9]. Despite the decrease in the incidence and death rate of prostate cancer in the United States and some other Western countries, the incidence and death rate of this cancer in the country is less developed and increasing[10]. The rate of prostate cancer in Asian countries is much lower than reported by the Western population, but this rate is also increasing in Asian countries[11]. Prostate cancer is on the rise in Iran, as in other developing countries[12]. This disease is the third most common visceral cancer reported in Iran, which causes about 7.75% of new cancer cases and is the seventh leading cause of cancer death in the country[13]. Knowledge about prostate cancer risk factors is limited and complex, focusing more on irreversible risk factors such as age, family history and ethnicity, and the role of modifiable risk factors such as obesity, smoking, poor diet, and inactivity less attention has been paid[14]. Hereditary history of prostate cancer is an important factor in the development of this cancer. Hereditary history of prostate cancer in close and primary family members, including siblings, increases the risk of developing it. Hereditary factors are involved in a small percentage (10%) of prostate cancers and are usually associated with early onset[15]. Due to the fact that the costs of this cancer are very high and most people unfortunately go for treatment when the disease is in an advanced stage and therefore because a study to investigate the risk factors for this disease has not been done in Kohgiluyeh and Boyer-Ahmad provinces. The aim of this study was to evaluate the risk factors for prostate cancer in Kohgiluyeh and Boyer-Ahmad provinces.

Materials and Methods

Type of study

The study was retrospective and case-control. The case group consisted of people with prostate cancer and the control group consisted of healthy people living in the area where the patient lived.

Statistical Society

The study population consisted of 80 patients with prostate cancer and 160 as controls. Participants were matched based on age and location variables. In order to conduct the study, after performing the administrative steps and the researcher's commitment to the confidentiality of patients' information, the relevant information extracted from the Cancer Registry of Kohgiluyeh and Boyer-Ahmad Center.

Sample size

240 people (80 as a case and 160 as a control) were required. The required sample size considering the significance level of 0.05, test power 90% and according to the risk factors related to cancer, the prevalence of prostate cancer history factor in the control group is equal to 82% and in the case group is 48%,

Sampling method

Sampling of patients done by census using information related to the years 2017-2019 of the Cancer Registry Office of Kohgiluyeh and Boyer-Ahmad Health Center. Sampling in the control group was selected by easy sampling method from the samples available at the residence of the case group.

Inclusion criteria

Inclusion criteria included all men living in Kohgiluyeh and Boyer-Ahmad provinces with prostate cancer with definite diagnosis of cancer, healthy people living in patients' neighborhoods and match in terms of age variables, willingness to participate in the study (inclusion with informed consent) and indigenous Kohgiluyeh and Boyer-Ahmad provinces.

Exclusion criteria

Exclusion criteria included non-natives and any type of cancer in the control group.

Methods and tools of data collection

The data collection tool was a researcher-made checklist that revised and developed under the supervision of an epidemiologist. The questionnaire consisted of six parts that after explaining the objectives of the study and obtaining informed consent, data collection done using a combination of face-to-face or telephone interviews.

Statistical Analysis

After collecting the data, the information entered into SPSS software. V.22 was analyzed using Chi-square test and multiple unconditional logistic regression with the corresponding odds ratio and 95% confidence level, and finally the risk factors for prostate cancer were determined.

Results

240 people participated in this study. 80 people were selected as case and 160 people as control. In both groups, the highest frequency related to the age group of 60-70 years (32.50% in the case group and 31.2% in the control group). The mean age and standard deviation of case and control groups were 72.40 ± 11.36 years and 72.50 ±11.22 years, respectively. The majority of the cases and witnesses lived in the city.

Table 1: Risk factors affecting prostate cancer using a univariate logistic regression model

Variable		Case		Control		OR	CI 95%	P-value
		Number	Percentage	Number	Percentage			
Family history of cancers other than prostate cancer	Yes	27	33.7%	19	11.9%	3.78	1.36-7.36	0.000
	No	53	66.3%	141	88.1%	1	--------	
Family history of prostate cancer	Yes	23	28.7%	11	6.9%	5.46	2.50-11.93	0.000
	No	57	71.3%	149	93.1%	1	----------	
Pelvic shooting history	Yes	35	43.7%	10	43.7%	11.66	5.36-25.39	0.000
	No	45	56.3%	150	56.3%	1	----------	
Continuous physical activity	Yes	44	55%	125	78.1%	.34	.19 – .61	0.000
	No	36	45%	35	21.9%	1	--------	

Cont... **Table 1: Risk factors affecting prostate cancer using a univariate logistic regression model**

Type of physical activity	walking	40	90.9%	95	76%	.36	.8-1.51	0.16
	Run slowly	4	9.1%	4	3.2%	3.2	1	
	Group exercise	0	0	21	16.8%	16.8	.5-3	
History of smoking	Yes	36	45%	35	21.9%	2.99	1.63-5.21	0.000
	No	44	65%	125	78.1%	1	---------	
History of urinary tract infection	Yes	27	33.7%	18	11.3%	4.01	2.04-7.89	0.000
	No	53	66.3%	142	88.7%	1		
Job status	Less than or equal to 48 hours per week	45	56.3%	117	73.1%	1	---------	0.003
	More than 48 hours per week	35	43.8%	43	26.9%	1.62	1.10-2.30	
Type of job activity	Light and medium	49	61.3%	127	79.4%	1	--------	0.003
	Heavy	31	38.8%	33	20.6%	2.87	1.24-2.83	

Univariate conditional logistic analysis performed to determine the most important risk factors for prostate cancer. The results of these analyzes showed that family history of cancer other than prostate cancer, family history of prostate cancer, history of pelvic imaging, continuous physical activity, history of smoking, history of urinary tract infection, occupational status, type of activity Occupation had a significant effect on prostate cancer ($p < 0.05$) (Table 1).

The odds ratio of those with a family history of prostate cancer to those who did not (95% CI: 2.50 – 11.93) was 5.46. Also, the odds ratio in those who had a history of urinary tract infection and a history of smoking (95% CI: 2.89 - 2.04) was 4.01 and 2.99(95% CI: 5.21 - 1.63) were obtained. In the univariate analysis, the highest odds ratio for prostate cancer was related to people with a history of pelvic imaging11.66 (95% CI: 5.36 - 25.39) and the lowest odds ratio were It was

related to continuous physical activity 0.34(95% CI: 0.19 - 0.61).

Table 2: Records of variables in case and control groups

Variable		Case		Control		OR	CI 95%	P-value
		Number	Percentage	Number	Percentage			
Consume vegetables per week	1 time and less	36	45%	38	23.8%	1	---------	0.001
	2-3 times	23	28.7%	76	47.5%	3.1	1.63-6.01	
	More than 4 times	21	26.3%	46	28.8%	2.07	1.04-4.13	
Fruit consumption per week	1 time and less	42	52.5%	48	30%	1	--------	0.002
	2-3 times	18	22.5%	66	41.3%	3.2	1.64-6.2	
	More than 4 times	20	25%	46	28.8%	2.01	1.03-3.92	
Frequent consumption of fast food per month	Once a month or less	32	40%	32	20%	1	----------	0.03
	1-3 times a month	31	38.8%	67	41.9%	2.16	1.12-4.13	
	1 time per week	12	15%	32	20%	2.66	1.16-6.08	
	2-4 times a week	5	6.3%	29	18.1%	5.8	1.99-16.8	
Red meat consumption per month	Once a month or less	28	35%	27	16.8	1	----------	0.01
	1-3 times a month	40	50%	55	34.4%	1.42	0.73-2.7	
	1 time per week	11	13.7%	58	36.3%	5.46	2.37-12.5	
	2-4 times a week	1	1.3%	20	12.5%	20.74	2.60-26.4	

According to Table 2, there was a significant difference between case and control in terms of fast food consumption and processed meats, red meat consumption, fruit and vegetable consumption history ($p < 0.05$). In general, red meat consumption is a strong factor in prostate cancer. The results showed that reducing the consumption of fruits and vegetables increases the chances of developing prostate cancer.

The results of the multivariate conditional logistic regression model showed that after removing the possible confounding variables. People who ate red meat once a week and 2-4 times a week were 5.86 and 44.29 times more likely to develop cancer. In addition, people who had pelvic radiation were 14.7 times more likely to get cancer, and people who ate fast food 2-4 times a week were 8.69 times more likely to develop prostate cancer. They had a genitourinary tract infection and were 4.09 times more likely to develop prostate cancer.

Table 3 :Fits of logistic regression model between case and control groups

Variable		B	Wald	df	Sig	Adjusted OR	CI
Red meat consumption per month	Once a month or less	---	----	----	---	1	-----
	1-3 times a month	0.5	1.20	1	0.27	1.65	0.67-4.03
	1 time per week	1.76	10.29	1	0.001	5.68	1.99-17.29
	2-4 times a week	3.79	8.85	1	0.001	29.44	3.64-537.7
Frequent consumption of fast food per month	Once a month or less	----	----	----	----	----	-----
	1-3 times a month	0.35	0.58	1	.44	1.42	0.57-3.5
	1 time per week	0.52	0.83	1	0.36	1.68	0.54-5.20
	2-4 times a week	2.16	8.83	1	0.003	8.69	2.08-36.23
Family history of prostate cancer		1.29	6.27	1	0.01	3.6	1.23-10.03
Pelvic shooting history		2.69	24.12	1	0.000	14.7	5.03-43.08
History of smoking		1.09	7.01	1	0.008	2.97	1.32-6.6
History of urinary tract infection		1.40	8.78	1	0.003	4.09	1.61-10.3

Discussion

This study performed to evaluate the risk factors for prostate cancer in a control case on all men diagnosed with prostate cancer in Kohgiluyeh and Boyer-Ahmad provinces. In the present study, control groups matched based on age and location variables to evaluate the effect of these factors on the development of controlled prostate cancer and other factors affecting the development of prostate cancer. Based on this study, no significant difference observed between case and control groups in terms of age and location variables. The mean and standard deviation of age in case and control groups were 72.42 ± 11.95 and 69.94 ± 14.51 years, respectively. In a real study, et al., The highest relative incidence of prostate cancer obtained for people over 70 years of age and the average age of patients with prostate cancer was 70.75 years, and no cases reported for people under 50 years of age[16]. Which was consistent with our study. In the study of Zeinalzadeh et al., Among the 10 most common cancers of men, prostate cancer with an average age of 70 years had the highest mean age of incidence[17]. In a study by Mohammad Naeem Bashir in Pakistan, most patients (95%) were in the age group of 55 years and older[18]. In a case-control study by Andersson et al. In Sweden, the risk of prostate cancer was higher in urban and densely populated areas than in rural areas[19]. In the present study, most patients lived in urban areas but the location of the risk factor do not identified as significant.

In the study of Pourmand et al., most of the patients lived in urban areas[20], which was consistent with our study in this regard. In univariate, analysis of the present study, increase in job activity and intensity of job activity were significant, but in multivariate regression analysis, they were not significant. According to Victoria Nadalin et al., Heavy work activity was a risk factor for prostate cancer[21]. In the study of Hosseini et al., With increasing work activity more than 48 hours per week and the intensity of heavy activity, the risk of prostate cancer increased[3], which was consistent with our study. In the present study, significant and positive relationships observed between smoking and prostate cancer, and the results of the regression model showed that smoking increases the chances of developing prostate cancer. In a study by Brijbhushan Tyagi et al.,[22]. Smoking history significantly increased the risk of prostate cancer. In the study of Pienta et al.,[23], A significant relationship was observed between smoking and prostate cancer. Coghlin et al [24]. In their study found that, the risk of men smokers increased 1.21 to 1.45 times. The results of the above studies were consistent with the present study[22,25]. Numerous studies have reported an increased risk of prostate cancer in men with a family history of prostate cancer. A study by Barber et al showed that a family history of prostate cancer was associated with a 68% increase in prostate cancer[26]. In the study by Gary D et al., Men who had a father or brother with the disease were twice as likely as men without relatives to be affected. Patients are 5 to 11 times more likely to develop prostate cancer[27]. In the present study, a significant relationship observed between family histories of prostate cancer in univariate analysis. The results of the regression model also showed that a family history of prostate cancer in the presence of other factors increases the chance of developing prostate cancer, which was consistent with the results of the above studies. Ali Dosti et al.'s study showed that accidental exposure of normal prostate tissue to ionizing radiation initially (within the first 3 to 5 weeks) causes the death of prostate tissue ascites cells[28]. Also an important result of the study of Zeitman et al. In the present study, the subjects in the case group had a history of radiotherapy due to pelvic malignancies other than prostate cancer than the control group and a significant relationship observed between pelvic radiotherapy and prostate cancer[29]. In the present study, in univariate analysis, a significant difference observed between the two groups in terms of consumption of processed meat (fast food) and red meat, and the results of the logistic regression model showed that with increasing consumption of processed meat (fast food) and red meat increases the chances of prostate cancer. In a research by Sinhar et al.,[30] and Rodriguz et al., [31] reported that high consumption of red and processed meat is associated with an increased risk of prostate cancer, In another study of Askari et al., it was observed the more types of red meat , visceral and processed meat is associated with an increased risk of prostate cancer. In the present study, in the univariate analysis, the history of chronic urogenital infection showed a significant relationship with the chance of prostate cancer and remained in the model with the presence of other factors. The logistic model showed that urogenital infection increases the chance of developing prostate cancer. . In a study by Dasilva et al., showed that chronic inflammation and infections are associated with an increased risk of prostate cancer, and there is considerable evidence to support a link between bacterial, viral and prostate infections[32]. Inflammation or infection of the prostate identified as a risk factor for prostate cancer in a study of a person matched by Rosebud in 2004 in the United States[33]. Also in the study of Hosseini et al. Conducted in Iran, inflammation or infection of the prostate recognized as a significant risk factor[34]. The above studies were consistent with the present study.

Conclusion

Family history of prostate cancer, history of urinary tract infection, history of smoking, history of abdominal photography, consumption of red meat, consumption of fast food and processed meats were the most important predictors of prostate cancer in this study. It can be considered by educational and health planners to prevent this cancer and take appropriate intervention measures.

Ethical Clearance: The article was approved by the ethics committee of Yazd University of Medical Sciences under the number IR.SSU.SPH.REC.1398.100

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Genetic Profiling of CYP19 Gene Haplotype in Holstein Bulls

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Abstract

The CYP19 gene is a protein-coding gene that stimulates the transmutation of androgens to estrogens, aromatase activity is related to haplotypes of the CYP19 gene, therefore this study aimed to detect haplotypes of the CYP19 gene in the Holstein bulls. This study, conducted from October 2018 to June 2019. Forty-two Bulls, aged between 4-6 years old were used, which belonged to local farmers in Basra city, south of Iraq. The DNA was extracted from semen, PCR amplification was carried out to acquire the DNA sequence. The sequences were analyzed by using some Bioinformatics tools. The results showed four haplotypes in the CYP19 gene as a result of silent and missense mutations in comparison with the same gene in GenBank, all the haplotypes are recorded in GenBank, DDBJ and EMBL. These findings call for further studies to reveal the relationship of CYP19 gene haplotypes and semen characteristics to uncover bulls' fertility.

Keywords: *CYP19 gene, haplotypes, Holstein bulls, phylogenetic tree, protein structure*

Introduction

In Iraq the livestock production is an important part of the national economy, however, this part of the economy suffers from many problems[(1)], these problems caused a huge decrease in the level of production that does not meet the food market needs of meat, milk, and derivatives, for many reasons, including climatic conditions, as well as the absence of scientific programs organized to elect animals with high production capacity[(2)], climatic changes significantly led to higher temperatures, which negatively affected the fertility of farm animals, especially the effect on the quality of semen in bulls[(3)], that non-fertile bulls can cost producers large sums of money without any economic return[(4)]. On the other hand, the molecular genetics techniques contributed to the diagnosis and treatment of infertility[(5)], via studying some of the molecular mechanisms that directly affect fertility[(6)] and improve it[(7)], one of these mechanisms is the study of the polymorphisms of the genes that directly affect the different semen characteristics[(8)]. CPY19 gene (cytochrome p450, family 19) is one of the genes that are related to the quality of semen because it directly effects on steroid hormones[(9)]. CPY19 gene located on chromosome 10q26 of the cattle genome[(10)], it works to encoding the aromatase P450 enzyme that catalyzes the conversion of androgens to estrogens[(11)], which has an important role on male productive by the effect on the function of Leydig cells[(12)]. Some studies indicated that the polymorphism of the CYP19 gene linked with semen parameters[(13)]. Because of the lack of studies that dealt with the CYP19 gene and there is no study mentioned the polymorphism of this gene in Iraq, this study aimed to detect the haplotypes of the CYP19 gene in Holstein bulls in Iraq.

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Methods and Materials

Animal and Sampling

This study was conducted from 10th October 2018 to the 15th of August 2019. Forty-two Bulls known fertility, aged between 4-6 years old were selected, which belonged to local farmers in Basra city, south of Iraq. Semen was collected using an artificial vagina.

DNA extraction

The extract of DNA was done by using Qiagen

Modified Protocol that described by Manuja[14].

Concentration and purity of DNA

The quality and quantity of extracted DNA were determined by using the Nano-drop according to the method mentioned by Muratori[15], taking into consideration that the value of the ratio 260/280 is 1.8 as a better index to the purity of DNA[16].

PCR Amplification

Amplification performed in 25 µL reactions as mentioned by Habib[17] table 1, with forward primer 5'-ACAACCAGGTAGGTGAGCCA-3' and reverse primer 5'-TCCCAGTAGGCTGCATCTGA-3' [18]. The PCR cycling protocol was done as mentioned by Vega[19] as follows: initial stage (95 °C, 1min), then 35 amplification cycles (with denaturation at 95 °C, 30s), annealing (61 °C, 30s), extension (72 °C,1min), and final extension come after the last cycle (72 °C,7min). the PCR product has been detected by using agarose gel 1.5%. ethidium bromide stained (Fig 1).

Table (1) PCR amplification reaction[17]

Components	Amount (µl)
2X PCR Master Mix	12.5
Water, nuclease free	9.5
Reverse primer, 10 µM	1
Forward primer, 10 µM	1
DNA template (75 ng)	1
TOTAL	25

Sequence Analysis

The sequence was detected in APICAL (First BASE) Laboratories/ Malaysia. The analysis of the BLAST has been carried out on the website http://www.ncbi.nlm.nih.gov, Multiple Sequence Alignment (MAS) was performed in http://www.ebi.ac.uk/Tools/msa/clustalo/ [20], to determine the differences in the resulting sequences

Phylogenetic Tree Analysis

All the resulting sequences were compared with Bos taurus partial CYP19 gene exon 1(Accession number Z69241) as a reference sequence, Mega – x (version 10.0.5) software[21] has been used to the analysis of the phylogenetic tree.

Three-dimensional Protein Structure

The 3D protein structure was done by using the Swiss model [22]

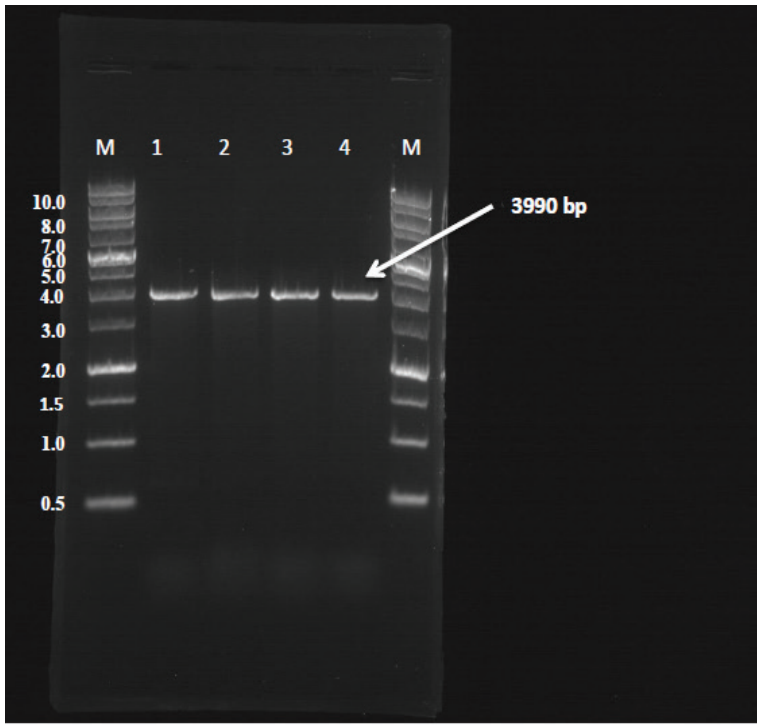

Fig. 1: Gel electrophoresis of PCR products to the CYP19 gene.

M: DNA ladder 10 kb

1-4 DNA templates.

Results and Discussion

The PCR product size was 3990 bp (Fig. 1). Compared to the reference gene from the GenBank (Accession number Z69241), the study found four different haplotypes according to the analysis of multiple sequence alignment (MSA). All the haplotypes were registered in GenBank, DDBJ and EMBL by the following accession numbers (LC510371, LC510372, LC510373, and LC510374) respectively.

All the mutations are summarized in table (2). These results are in line with Vega[19] about the multiplicity of haplotypes to the CYP19 gene, on the other hands, the results refer that the Holstein bulls in Iraq have new haplotype of the CYP19 gene because of no identity 100% with any sequence in GenBank.

Table (2) Type of mutations in the CYP19 gene in Holstein bulls in Iraq

	No.	Reference Gene	Haplotype	Position	Mutations	
					Type	Amino acid
Haplotype 1 (LC510371)	1	T	A	193	Missense	phenylalanine to isoleucine
	2	G	A	892	Missense	glycine to arginine
	3	T	G	1706	Missense	Phenylalanine to cysteine
	4	A	T	2058	Silent	
	5	G	A	3571	Missense	glycine to serine
	6	A	T	3667	Missense	Threonine to serine
	7	G	T	3927	Silent	

Cont.... **Table (2) Type of mutations in the CYP19 gene in Holstein bulls in Iraq**

Haplotype 2 (LC510372)	1	C	T	23	Missense	Proline to leucine
	2	T	A	193	Missense	phenylalanine to isoleucine
	3	T	A	208	Missense	Tryptophan to arginine
	4	C	G	254	Missense	Proline to arginine
	5	G	C	1296	Missense	Leucine to phenylalanine
	6	G	C	1918	Missense	glycine to arginine
	7	T	G	2043	Missense	phenylalanine to leucine
	8	A	T	2058	Silent	
	9	G	A	2096	Missense	Arginine to lysine
	10	G	A	2114	Missense	Arginine to lysine
	11	G	T	3927	Silent	
	12	A	G	3963	Silent	
Haplotype 3 (LC510373)	1	A	G	71	Missense	aspartic acid to glycine
	2	T	A	193	Missense	phenylalanine to isoleucine
	3	T	A	208	Missense	Tryptophan to methionine
	4	G	T	209	Missense	Tryptophan to methionine
	5	T	G	762	Missense	histidine to glutamine
	6	T	A	1099	Silent	
	7	T	A	2041	Missense	phenylalanine to isoleucine
	8	G	T	2271	Missense	lysine to asparagine
	9	G	A	2659	Missense	Glycine to serine
	10	C	G	3615	Silent	
	11	G	T	3927	Silent	
Haplotype 4 (LC510374)	1	A	T	57	Missense	leucine to phenylalanine
	2	G	T	1628	Missense	arginine to methionine
	3	C	A	1654	Missense	proline to threonine
	4	A	G	3340	Missense	Lysine to glutamic acid
	5	C	A	3360	Silent	
	6	G	T	3779	Missense	Serine to isoleucine
	7	A	G	3974	Missense	Tyrosine to cysteine

The multiplicity of the haplotype may be related to the traits that affected directly by the performance of the gene, this can greatly improve livestock production[23].

Silent mutations may be considered as molecular markers for the production traits[24], and it can affect the efficiency of mRNA splicing that impact the protein function[25].

On the other hand, missense mutation might be a candidate for production traits in cattle, there is a superior correlation between the gene polymorphisms and productive traits[26], also, the missense mutations could be lead to a change in the protein structure[27], this is consistent with the results of the study (Fig. 3), as the protein structure differed in all four haplotypes. These changes in structure can affect protein function[28]

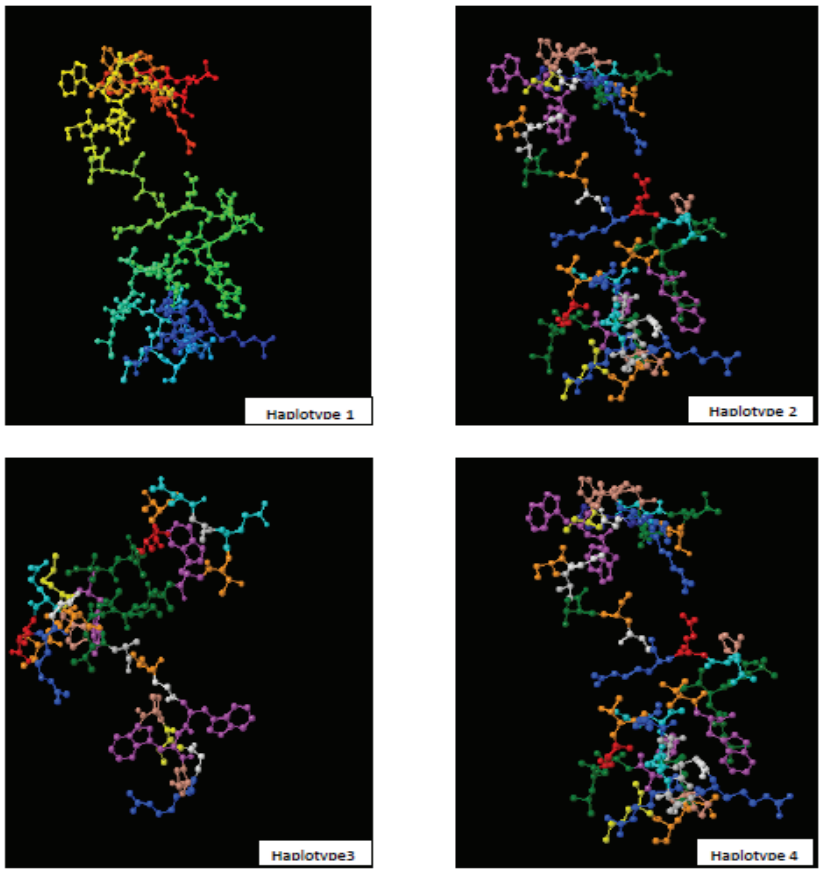

Fig. 2 Changes in Protein structure for haplotypes

Based on the result of the phylogenetic tree (Fig. 4) has found all the experiment animals belong to the same origin, they evolved from common predecessors[29] and closely evolutionary with the reference gene in GenBank (Z69241). These results may be useful to the positive selection[30]

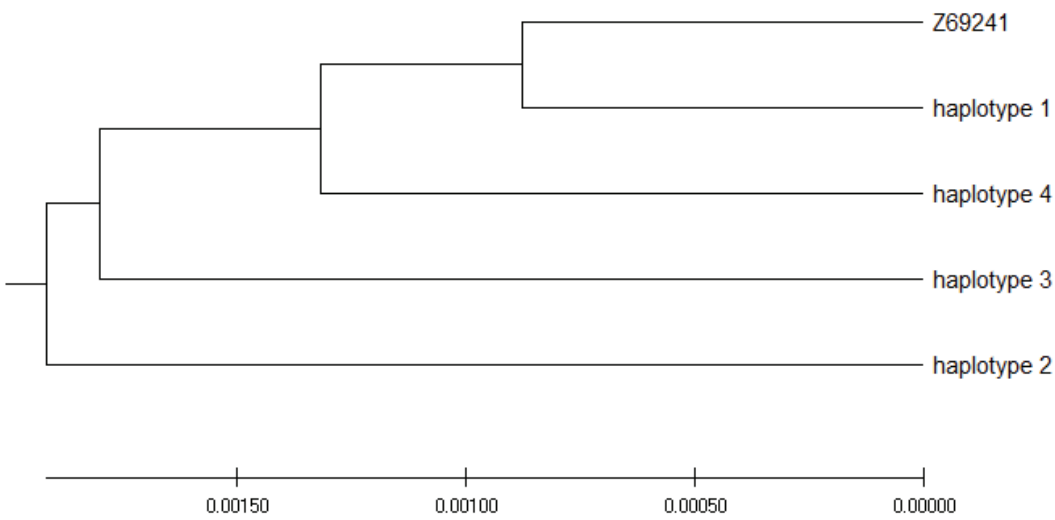

Fig. 3Phylogenetic tree of CYP19 gene in Holstein bulls

The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.00783139 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method(31), and are in the units of the number of base substitutions per site. This analysis involved 5 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 3990 positions in the final dataset. Evolutionary analyses were conducted in MEGA X(21).

Conclusion

The CYP19 gene demonstrated four different haplotypes in Holstein's bulls due to mutation occurrence these differences may indicate the CYP19 gene was affected by environmental conditions, therefore, more studies are needed using a greater number of animals and correlating haplotypes of CYP19 gene with bull fertility and semen characteristics.

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Determinant of Intention E-cigarettes Use Among a Sample of Senior High School Students in Surabaya

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Abstract

Background: The prevalence of smoking in Indonesia in 2018 among adolescents (10-18 years old) was 9.1%. Surabaya has 9,28% of boys who are active smokers and acknowledged smoking every day. Adolescents using conventional cigarettes are also tend to be interested to using e-cigarette.

Objective: The aim of this study was to analyze the factors that associate the intention of e-cigarettes use in adolescents in Surabaya.

Method: The type of study was observational analytic design with cross sectional. The sample size was determined by cluster random sampling, in 8 school which includes 291 students. Data collection was through filling out the online questionnaire and analyzed using logistic regression test to determine the association between variables.

Results: This results of this study show that the factors that have positively associated to intentions of e-cigarettes use by adolescent are instrumental attitudes (3,035), injunctive norm (2,561), descriptive norm (4,896) and self efficacy (3,526). Experiential attitude and perceived control did not associated the intention of adolescents e-cigarette use.

Conclusion: Providing education to adolescents, parents and educational stakeholders about the dangers of using e-cigarettes and restrictions on adolescents access to e-cigarettes should take considered the long-term impact of e-cigarette.

Keywords : e-cigarette, intention, adolescent

Introduction

In general, deaths by cigarette occur in several countries with low and medium income per capita. [18] WHO states Indonesia occupy on third in the world in the number of active smokers of 65 million (28%) after China with 390 million and India with 144 million from 718 million total of active smokers in the world.[18]

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One of the methods used by WHO to reduce the dangers of tobacco smoking is by using Nicotine Replacement Therapy (NRT). NRT is a method by providing nicotine needed by smokers without burning tobacco, one of which is an e-cigarette. This tool uses electricity from battery power to deliver nicotine in the form of vapor and WHO calls it the Electronic Nicotin Delivery System (ENDS).[20]

E-cigarette was initially considered as a safe product for health because the solution does not contain TAR and other toxic substances contained in tobacco cigarettes.[20] Adolescents who use conventional cigarette tend to be interested in using e-cigarettes.[14] America conducted to e-cigarettes research related and the results found that e-cigarettes contain toxic Tobacco Specific

Nitrosamines (TSNA) and Diethy Glycol (DEG) which is known as a carcinogen.[5]

The presences of toxic and carcinogenic substances causes the issuance of warnings about the dangers of toxic and carcinogens in e-cigarettes to restrict the distribution and sale of e-cigarettes in the United States and several other countries.[5] Another danger of e-cigarettes is that they can significantly increase plasma nicotine levels, plasma carbon monoxide levels and pulse rates within five minutes of use. Surely it would be detrimental to health if consumed long term.[17]

There was an increase in the use of e-cigarettes from 2011 to 2017 among adolescents in junior high school and senior high school in the United States.[13] The average proportion of population aged ≥10 years in Indonesia who use e-cigarettes was 2.8%. The most age groups that use e-cigarettes was the age group 10-14 years (10.6 %) and age 15-19 years (10.5%).[4]

The most adolescents motivation to use e-cigarettes because they think e-cigarettes are cool and fun.[10] Adolescents also belief that e-cigarettes can help them to stop using conventional cigarettes.[12] According to WHO, the awareness about the existence of e-cigarettes greater on the people who live in urban.[19] Surabaya becomes a research area because it becomes the second largest city in Indonesia after Jakarta with a population of 2,892,200 peoples.

The behavior of using e-cigarettes of adolescents which is associated by aspects of themselves and the environment can be studied using the Integrated Behavioral Model theory. Integrated Behavioral Model is a theory at the individual level to analyze the causes of individuals to take certain actions or not.[7]

Based on the problems above, this study aims to determine the association of experiential attitude, instrumental attitude, injunctive norms, descriptive norms, perceived control and self-efficacy of the intention of Surabaya adolescents to use e-cigarettes.

Methods

This study is observational analytical with cross sectional design. The population in this study was all students in senior high school from 22 public high schools in Surabaya with a total of 22,448 students. Sample size was determined by using cluster random sampling. This technique is used for sampling through two stages and involves more than one sample method. The first stage is to determine the school as a research sample so that eight schools are selected. The second stage is to determine the number of respondents using so that it produces 282 as the minimum respondent. Data collection is done through filling out online questionnaires and analyzed using logistic regression tests to determine the association between variables.

Results and Discussion

The questionnaire was filled by 291 students from eight senior high schools in Surabaya . Table 1 shows the characteristics of adolescents based on their e- cigarette behavior.

Table 1 Characteristics of Adolescents Based on E-cigarettes Use

Variable	E-cigarettes Use				Total	
	Current User		Never User			
	n	%	n	%	n	%
Age						
<16 years old	14	13,2	92	85,8	106	100,0
17-18 years old	47	25,7	136	74,3	183	100,0
19 years old	1	50,0	1	50,0	2	100,0

Cont... **Table 1 Characteristics of Adolescents Based on E-cigarettes Use**

Variable	E-cigarettes Use				Total	
	Current User		Never User			
	n	%	n	%	n	%
Gender						
Male	49	31,8	105	68,2	154	100,0
Female	13	9,5	124	90,5	137	100,0
Allowence						
<IDR500.000	25	20,7	96	79,3	121	100,0
IDR500.000 – IDR1.000.000	33	19,9	133	80,1	166	100,0
>Rp.1.000.000	4	100	0	0,0	4	100,0
Source information about e-cigarette						
Surrounding Environment (friends and family)	59	24,7	180	75,3	239	100,0
Internet	0	0,0	18	100	18	100,0
Social Media	3	8,8	31	91,2	34	100,0

Almost adolescents who actively use e-cigarette are 17-18 years old with a male gender and have an allowance of IDR500,000-IDR1,000,000 per month. The surrounding environment of both friends and family of adolescents becomes a source of information about e-cigarettes.

Table 2 Cross Tabulation of Behaviors, Perceived Norms and Personal Agency on E-cigarette Use

Variable	E-cigarette Use				Total	
	Current User		Never User			
	n	%	n	%	n	%
Experiential Attitude						
Positive	45	41,3	64	58,7	109	100,0
Negative	17	9,3	165	90,7	182	100,0
Instrumental Attitude						
Helpful	39	41,9	54	58,1	93	100,0
Harmful	23	11,6	175	88,4	198	100,0
Injunctive Norms						
Good	38	40,0	57	60,0	95	100,0
Less	24	12,2	172	87,8	196	100,0
Descriptive Norms						
Good	37	37,4	62	62,6	99	100,0
Less	25	13,0	167	87,0	192	100,0

Cont... **Table 2 Cross Tabulation of Behaviors, Perceived Norms and Personal Agency on E-cigarette Use**

Variable	E-cigarette Use				Total	
	Current User		Never User			
	n	%	n	%	n	%
Perceived control						
Easy	33	43,4	43	56,6	76	100,0
Difficult	29	13,5	186	86,5	215	100,0
Self Efficacy						
Strong	44	47,3	49	52,7	93	100,0
Less	18	9,1	180	90,9	198	100,0
Intention to electric smoking						
Strong	43	8,8	33	91,2	76	100,0
Less	19	56,6	196	43,4	215	100,0

Adolescents who have a positive experiential attitude think that e-cigarettes are useful tend to actively use e-cigarettes. Almost adolescents who have injunctive norms and descriptive norms that are good for e-cigarettes tend to actively use e-cigarettes. Almost adolescents who have good descriptive norms tend to actively use e-cigarettes. Almost adolescents who feel the ease of use of e-cigarettes tend to be actively using the e-cigarette. Almost adolescents who have strong self-efficacy for using e-cigarettes tend to be active in using e-cigarettes. Adolescents feel confident in their ability to keep using e-cigarettes despite some obstacles of using e-cigarettes.

Table 3 Association of experiential attitude, instrumental attitude, injunctive norms, descriptive norms, perceived control and self-efficacy of intentions

Variable	Intention to use e-cigarette				Total		p-value	Exp (B)
	Strong		Less					
	n	%	n	%	n	%		
Experiential attitude								
Positive	48	44,0	61	56,0	109	100,0	0,228	1,585
Negative	28	15,4	154	84,6	182	100,0		
Instrumental attitude								
Helpful	46	49,5	47	50,5	93	100,0	0,003	3,035
Harmful	30	15,2	168	84,8	198	100,0		
Injunctive norm								
Good	49	51,6	46	48,4	95	100,0	0,011	2,561
Less	27	13,8	169	86,2	196	100,0		

Cont... **Table 3 Association of experiential attitude, instrumental attitude, injunctive norms, descriptive norms, perceived control and self-efficacy of intentions**

Variable	Intention to use e-cigarette				Total		p-value	Exp (B)
	Strong		Less					
	n	%	n	%	n	%		
Descriptive norm								
Good	55	55,6	44	44,4	99	100,0	0,001	4,896
Less	21	10,9	171	89,1	192	100,0		
Perceived control								
Easy	40	52,6	36	47,4	76	100,0	0,180	1,699
Difficult	36	16,7	179	83,3	215	100,0		
Self efficacy								
Strong	49	52,7	44	47,3	93	100,0	0,001	3,526
Less	27	13,6	171	86,4	198	100,0		

A. Experiential attitude

Table 3 showed that there was no associated between experiential attitude to adolescent intentions to use e-cigarettes. The experiential attitude in this study was the adolescent positive or negative response to e-cigarettes use. Experiential attitude is a response that arises from the individual to the recommended behavioral idea. A person with a strong negative experiential attitude, it is unlikely that someone will display such behavior. Conversely, a person with a strong positive experiential attitude, then will be more likely to perform the suggested behavior. Fishbein's statement did not match the conditions on the ground in this study.[6]

The results are not in line with Thrasher & Sargents research which states that adolescents try to use e-cigarettes because of the tendency to look for something, sensations and new experiences from e-cigarettes.[16] Some adolescents consider that e-cigarettes are cool and a new trend. Its use of e-cigarettes in adolescents was associated with the positive perception of adolescents that e-cigarettes are more acceptable in society, the appeal of the taste of e-cigarettes, as well as the new experiences and sensations of e-cigarettes.[11]

B. Instrumental Attitude

Table 3 show that instrumental attitude have a positive association on adolescent intention to use e-cigarettes. Adolescents with a instrmental attitude had a strong intention in using e-cigarettes by 3,035 times greater. Instrumental attitude is a person's response based on cognitive beliefs determined by beliefs about the outcome of performing such behavior.[6] The response is based on the benefits and disadvantages resulting from performing a particular behavior.

Most adolescents who actively use e-cigarettes consider that e-cigarettes have benefits such as being able to help reduce the use of conventional cigarettes and not cause health problems. In line with the results of the research Bernat, Gasquet, Wilson, Porter, & Choi that there is a correlation between adolescents with the perceived benefits of e-cigarettes.[2] The benefits felt by adolescents of e-cigarettes include helping to quit conventional smoking, having more friends, looking cooler and making more confident. Bigwanto, Nurmansyah, Orlan, Farradika, & Purnama also mentioned adolescents who have the perception that cigarettes do not cause addiction and do not cause health

problems but will have a tendency to use e-cigarettes.[3] The instrumental attitude that actively use e-cigarettes can be caused by a lack of knowledge or information received by adolescents regarding the dangers of e-cigarettes.

C. Injunctive Norm

The injunctive norm had a significant effect on adolescent intentions in using e-cigarettes. Adolescents with good injunctive acidity on e-cigarette use were 2,561 times greater. Table 3 shows that there is positive association between injuctive norms on adolescent intentions to use e-cigarettes. The injuctive norm referred to this study is the belief of adolescents due to the opinions of people or groups of society that are considered important in e-cigarettes use.

The results of this study are in line with the research of Schoren, Karin, & Heinyang showed that the e-cigarettes use occurs because of the positive perception and support from the surrounding environment related to e-cigarettes use.[15] Positive opinions of the surrounding environment against e-cigarettes include family and friends are more agreeable using e-cigarettes than conventional cigarettes, and consider that e-cigarettes are better and safer.

In adolescence, it is common to spend more time with friends so it can be seen that the association of peers on attitudes to behavior greater than family.[9] This is also in line with field findings that many adolescent age groups choose to hang out with peers after school hours. Opinions or thoughts that arise also affect the pattern of behavior owned. They usually tends to choose something that considered their peers.

D. Descriptive Norm

Table 3 shows that descriptive norms have a positive association with adolescent intentions in e-cigarettes use. Descriptive norm have a significant assosiated on adolescent intentions in e-cigarette use. Adolescents with descriptive norms both have a strong intention in using cigarettes by 4,896 times greater than less intention.

The descriptive norm in this study is the belief of adolescents due to the actions of most people or communities around that is using e-cigarettes or not. Descriptive norms are a state of norms prevailing in society.[7]

The results are in line with research by Hua, Hua-Hie, Ron, Ann, & Sara which showed that adolescents who have close friends who use cigarettes will also be more likely to use e-cigarettes.[8] Research Bigwanto, Nurmansyah, Orlan, Farradika, & Purnama, also Barrington-Trimis, et al. found that adolescents who have families, especially parents who use e-cigarettes, cause adolescent tendencies and intentions to use it.[1,3]

The results showed that almost adolescents who actively use cigarettes such as close friends and parents also use e-cigarettes. In addition, e-cigarettes which is one of the growing trends today is also the reason adolescents use e-cigarettes. The habits of parents who use cigarettes give rise to a sense of inconsistance with the dangers and health problems caused by e-cigarettes. This can be seen by most adolescents consider there are examples or role models that are used as a reference in doing something, especially e-cigarettes.

E. Perceived Control

Table 3 shows that perceived control has no association on adolescent intentions to use e-cigarettes. Almost adolescents who have easy perceived control over their use of cigarettes tend to have a strong intention to use e-cigarettes. However, there was no significant difference with adolescents who had difficult behavioral controls over e-cigarette use.

Perceived control is a person's perception or feeling to control behavior determined by various environmental factors that can make it easier or harder to perform such behaviors.[7] The perceived control determined by the individual's beliefs regarding the availability of resources whether equipment, compatibility, competence and opportunities that support or inhibit such behavior. The stronger confidence in the availability of resources and opportunities, the stronger the control of the behavior that the individual feels towards the behavior.

Almost adolescents who find it easier to find seller e-cigarettes and the free use of e-cigarettes in public places feel the price of e-cigarettes is increasingly expensive and can reduce the intention to use e-cigarettes. The government set a maximum excise rate of 57% on cigarettes which led the intial price of e-cigarettes

quite expensive. Almost adolescents especially those who actively use e-cigarettes, have an allowance of IDR500.000-IDR1.000.000 per month. This is in line with the results of research Bigwanto, Nurmansyah, Orlan, Farradika, & Purnama that adolescents who have enough money to buy cigarettes will also tend to use e-cigarettes.[3]

F. Self Efficacy

Table 3 shows that self-efficacy has a positive association on adolescent intentions to use e-cigarettes. Self-efficacy have a significant associated with adolescent intentions to use e-cigarettes. Adolescents with self-efficacy had a strong intention to use cigarettes 3,526 times greater than those with less intentions. Adolescents who have high self-efficacy due to the large number of support from close friends to use e-cigarettes.

Adolescents with strong self efficacy encourage the strong intention of adolescents to use e-cigarettes. Almost adolescents who had strong self efficacy tended to have a strong intention to use e-cigarettes. Self-efficacy is a person's level of confidence in the ability to perform certain behaviors in various obstacles and obstacles.[7] Self-efficacy in this study is a adolescent's level of confidence in the ability to use e-cigarettes despite the obstacles that come with it. A person with high self efficacy does not necessarily have the ability to perform such behavior.[21]

The results of this study are in line with the research of Schoren, Karin, & Hein which showed the results that users of e-cigarettes with high self-efficacy will tend to continue to use e-cigarettes.[15] Current user will look at the situation and the surrounding environment that is able to support them to use e-cigarettes.

Almost adolescents have the confidence to keep using cigarettes because of the support of close friends who also use e-cigarettes. The findings in the field, there are some adolescents who claim the high price of supporting components e-cigarettes are still sure to use it and shares with their friends. Adolescents also claim that sharing or dues with their friends can be used as an opportunity to try more new variants, especially e-cigarette liquids.

Based on the findings in the field, some adolescents claimed to be allowed by their parents because it is considered healthier than conventional cigarettes. In addition, the increasing number of shops to online shops that sell e-cigarettes makes it easier for adolescents and sure to keep using e-cigarettes despite the obstacles faced.

Conclusions

The conclusion is adolescents intentions to use cigarettes are associated by attitude factors, perceived norms and self-efficacy. Educating adolescents, parents and education stakeholders about the dangers of using cigarettes are important. Restrictions on adolescent access to e-cigarettes should also be taken into account the long-term impact of e-cigarette use.

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Green Tea Suppresses Serum TNF-α and TGF-β1 Levels In Mice Model of Systemic Lupus Erythematosus

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Abstract

Objective: This study aims to investigate the effects of green tea administration on TNF-a, Hsp70, and TGF-b1 levels in the systemic lupus erythematosus (SLE). **Material and methods:** A total of 32 mice will be divided into four groups (each 8 mice), namely the control group, the SLE group, the SLE group who were given green tea extract at a dose of 500 mg/kg body weight, and the SLE group who were given green tea extract at a dose of 1000 mg/kg body weight. Analysis of TNF-a, Hsp70, and TGF-b1 levels was carried out using the enzyme-linked immunosorbent assay technique. **Results:** TNF-a and TGF-b1 levels were significantly increased in the SLE group compared to the control group ($p < 0.05$). This increase can be significantly reduced through the provision of green tea, even reaching levels comparable to the control group ($p > 0.05$). **Conclusions:** It was concluded that green tea containing EGCG can suppress TNF-a and TGF-b1 in the SLE model. Thus, green tea can be an alternative in immunology modulation in SLE.

Keywords*: green tea; SLE; inflammation; EGCG; stress protein*

Introduction

Systemic lupus erythematosus (SLE) is complex autoimmune and inflammatory disease marked by the presence of autoantibodies against nuclear and cytoplasmic antigens [1]. Worldwide, the average prevalence of SLE is 20-70 per 100.000 individuals annually. The annual incidence of SLE is 10 per 100.000 individuals [2]. The clinical manifestation of this disease varies between individuals. The pathomechanism of SLE is influenced by ethnic, genetic, environmental, and gender factors [3].

Tumor necrosis factor-α (TNF-β) is involved in pathomechanism of SLE, but this detail is still not fully understood [4]. TNF-α levels higher significantly in SLE and higher in active disease status than inactive [5]. TNF-α polymorphism gives sensitivity to individuals and correlates with the disease activity, organ damage, and specific clinical manifestations [6-8]. Increased TNF-α is associated with peripheral blood mononuclear cell apoptosis [9]. The administration of mesenchymal stem cell infusion can suppress TNF in SLE patients, thereby confirming that this cytokine is the target of SLE therapy [10].

Transforming growth factor-β is a multiregulatory cytokine for the immune system. TGF-β1 plays a role as regulatory T cell homeostasis in peripheral and T lymphocyte cells [11]. Previous studies have shown a decrease in TGF-β1 in the plasma of SLE patients, due to the production defect of TGF-β1 by lymphocytes [12]. This decrease in cytokines parallels the decline in peripheral regulatory T cells [13]. However, other studies have found no changes in TGF-β1 levels [14,15].

Heat shock protein (Hsp) is a high conserved protein expressed in physiological conditions at low levels. The levels will increase due to the response to stress stimulus [16]. Hsp70 plays a role in inhibiting apoptosis due to stress through various mechanisms. Hsp70 prevents the release of Bax, which is necessary for the

release of proapoptotic factors from mitochondria [17]. Some biofactors were involving in Hsp70 stimulation, including temperature change, inflammatory cytokines, oxidized LDL, and hypoxia. Previous studies have found an increase in CD70 expression in the serum of SLE patients [18].

Tea is a beverage that is consumed by all citizens of the world. This drink is made from a tea plant (*Camelia sinensis* L.). Various evidence states that consumption of green tea is beneficial for health. The biological function of green tea is played by catechin. Of all catechins, EGCG is the most abundant and most functional component [19]. To the best of ours, until now, there have been few studies that have applied EGCG from green tea to SLE. Therefore, this study aimed to know the pharmacological action of EGCG) for TNF-α, Hsp70, and TGF-β1 level modulation in the SLE

Materials and Methods

Animals

Female Balb/c mice, aged 26-28 weeks, weighing 25-30 grams, were randomly divided into four groups (n = 8 each): the control group, the SLE group, the SLE group who received green tea extract at a dose of 500 mg/kilogram body weight (SLE + GT1), and the SLE group who were treated with green tea extract at a dose of 1000 mg/kilogram body weight (SLE + GT2).

Animals were placed in a clean wire cage and maintained according standard laboratory conditions (a temperature of 25 ± 3°C and dark/light cycle 12/12 h). Standard diet and drinking water were provided ad *libitum*. Animals were acclimatized to laboratory conditions for one week prior to the experiment

Pristane-induced SLE

The systemic lupus erythematosus was made by an injection of 0.5 ml pristane (99% pure; Sigma-Aldrich Co, St Louis, MO, USA) intraperitoneally [20].

Plant material

The raw materials of green tea (GMB-4 tea clones) were obtained from the Tea and Quinine Research Center, Bogor, Wes of Java, Indonesia. EGCG as a standard with (95% pure; Sigma-Aldrich Co, St Louis, MO, USA).

Preparation of extracts for LC-MS/MS analysis

Green tea was cleaned and then cooled to dry (a moisture content of 5%). Dried herbs were cut and ground to make a powder. Three hundred grams of powder were macerated in 96% ethanol until the whole powder was submerged (1500 ml). Maceration was carried out for 6 hours with a shaker speed of 40 RPM. The powder bath was refluxed for 3 hours and filtered. The filtering pulp was refluxed again with 96% ethanol, repeated two times. Ethanol contained in the filtrate was removed by a vacuum evaporator at a temperature of 40^{O}C, so that a thick extract of 96% ethanol was obtained.

Standard and sample preparation

The EGCG standard stock solution is prepared by dissolving 5 mg of EGCG in 5 ml of ethanol. Concentrations of standard working solutions are 900 ng/ml; 1300 ng/ml; 1800 ng/ml and 3600 ng/ml.

The extract sample was weighed as much as 1.3 mg and then dissolved in 10 ml of methanol and sonificated for 15 minutes. Centrifugation was carried out at 4000 RPM for 10 minutes. Finally, the sample was taken 20 mL and added to 1 ml of methanol. Samples were filtered with a 0.2 µm filter membrane and were inserted for LC-MS/MS analysis.

LC-MS/MS analysis

LC-MS/MS analysis was performed using UHPLC (ACCELLA type 1250, Thermo Scientific). This instrument was equipped with a vacuum degasser, quartener pump, and thermostat Autosampler. Separation was performed with Hypersil Gold analytic columns (50 mm x 2.1 mm x 1.9 µm). The column temperature was set at 30^{O}C. The Autosampler compartment was set at 16^{O}C. Elution gradients use 0.1% formic acid in water (mobile phase A) and formic acid in methanol (mobile phase B). The elution gradient program is adjusted so that optimum separation occurs, namely 5% B (0-1 minutes), 50% B (5-6 minutes), and 90% B (7-8 minutes). The system is controlled at a constant solvent speed of 300 µl/min with an injection volume of 2 L.

The TSQ QUANTUM ACCESS MAX mass spectrometer (Thermo Finnigan) is equipped with an Electrospray Ionization source. The Selected Reaction Monitoring (SRM) was applied to quantification

method. The optimization was done in the evaporating temperature of 250^{O}C; capillary temperature of 300^{O}C; nitrogen gas at a pressure of 40 psi; and argon gas at a pressure of 10 psi. Quantitative EGCG analysis was conducted using LC-Quan (Thermo Scientific) software.

Measurement of serum TNF-α levels

Analysis of serum TNF-α levels was performed using an enzyme-linked immunosorbent assay technique. The analysis procedure is carried out according to detailed instructions in the assy.

Determination of serum Hsp70 levels

Analysis of serum Hsp70 levels was performed using an enzyme-linked immunosorbent assay technique. The analysis procedure is carried out according to detailed instructions on the kit.

Analysis of serum TGF-β levels

Analysis of serum TGF-β levels were performed using an enzyme-linked immunosorbent assay technique. The analysis procedure is carried out according to detailed instructions on the kit.

Statistical Analysis

Data were figured as mean ± standard of deviation. One-way analysis of variance (ANOVA) with SPSS 15.0 statistical package for Windows was performed for difference analysis. The probability values of $p < 0.05$ were considered statistically significant and later subjected to post hoc test.

Results and Discussion

The chromatogram of EGGC as standard (A) and green tea extract (B). The average concentration of EGCG at crude extracts was 8.08%. Figure 1 displays TNF-α expression in the control group and treatment group. TNF-α levels were significantly increased in the SLE group compared to the control group (p <0.05). This elevation can be significantly normalized through the provision of green tea, even reaching levels comparable to the control group ($p > 0.05$).

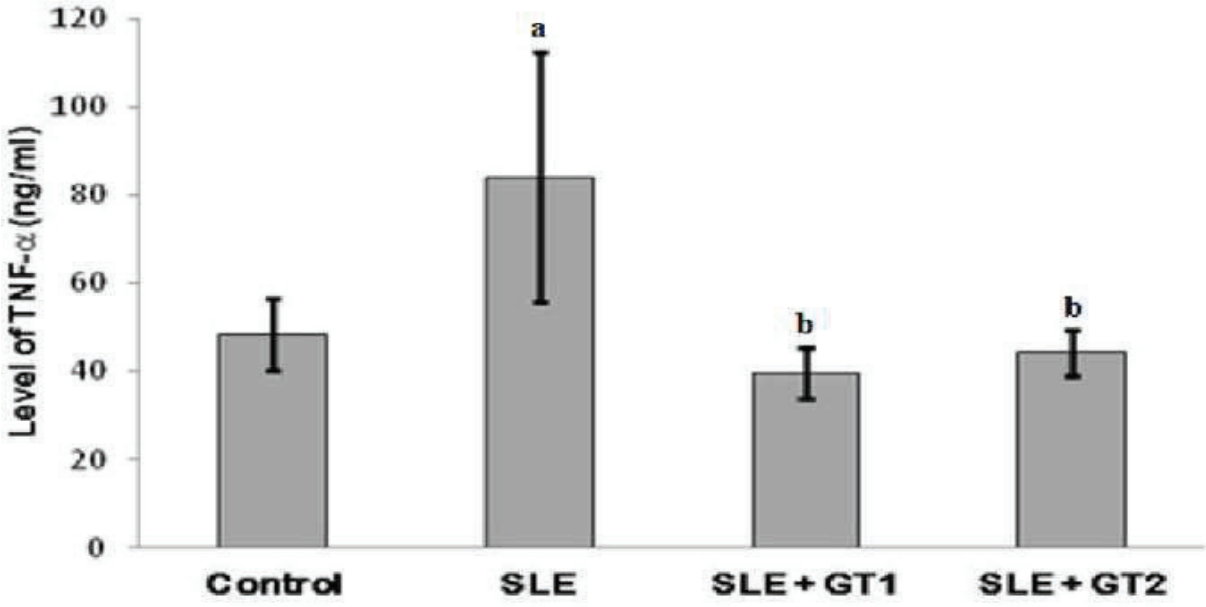
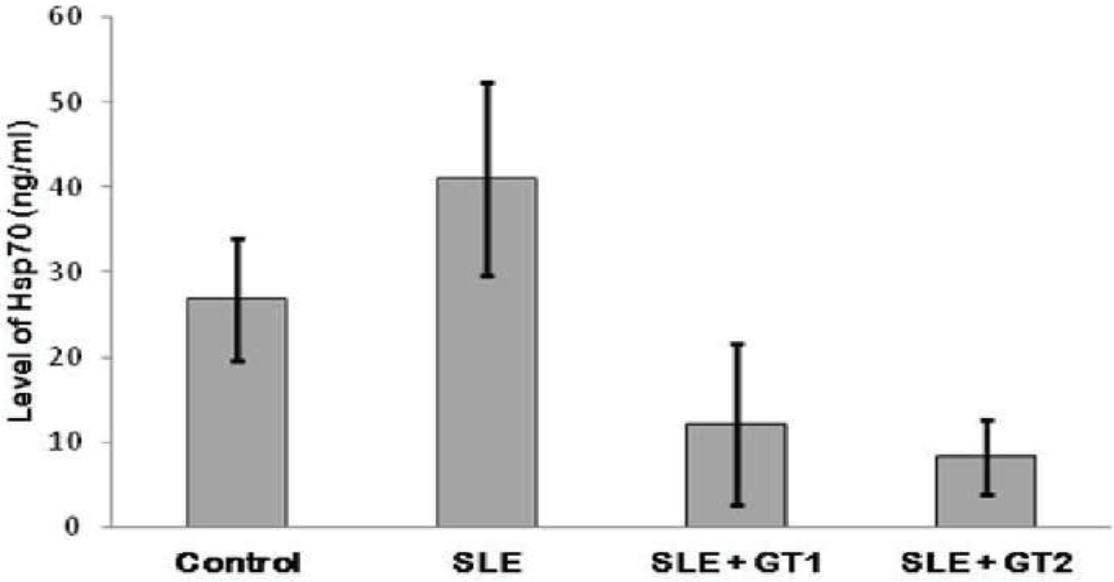


Figure 1. Levels of serum TNF- α in control and other groups. Note: [a]: there are significant differences compared with the control group ($p < 0.05$); [b]: there are significant differences compared with the SLE group ($p < 0.05$); SLE: systemic lupus erythematosus; SLE + GT1: systemic lupus erythematosus treated with green tea at dose of 500 mg/kilogram body weight; SLE + GT2: systemic lupus erythematosus treated with green tea at dose of 1000 mg/kilogram body weight.



Hsp 70 levels in various groups can be seen in Figure 2. There was an insignificant difference in HSP 70 levels in the control group and the treatment group ($p > 0.05$).

Figure 2. Levels of serum Hsp70 in different groups. Note: SLE: systemic lupus erythematosus; SLE + GT1: systemic lupus erythematosus treated with green tea at dose of 500 mg/kilogram body weight; SLE + GT2: systemic lupus erythematosus treated with green tea at dose of 1000 mg/kilogram body weight.

Figure 3 shows serum TGF-β1 levels in various groups. The TGF-β1 levels was significantly higher in SLE compared to control group ($p > 0.05$).The lowest dose of greean tea (SLE + GT1) significantly reduce this levels than that SLE group ($p > 0.05$), reaching comparable value to control group ($p > 0.05$).

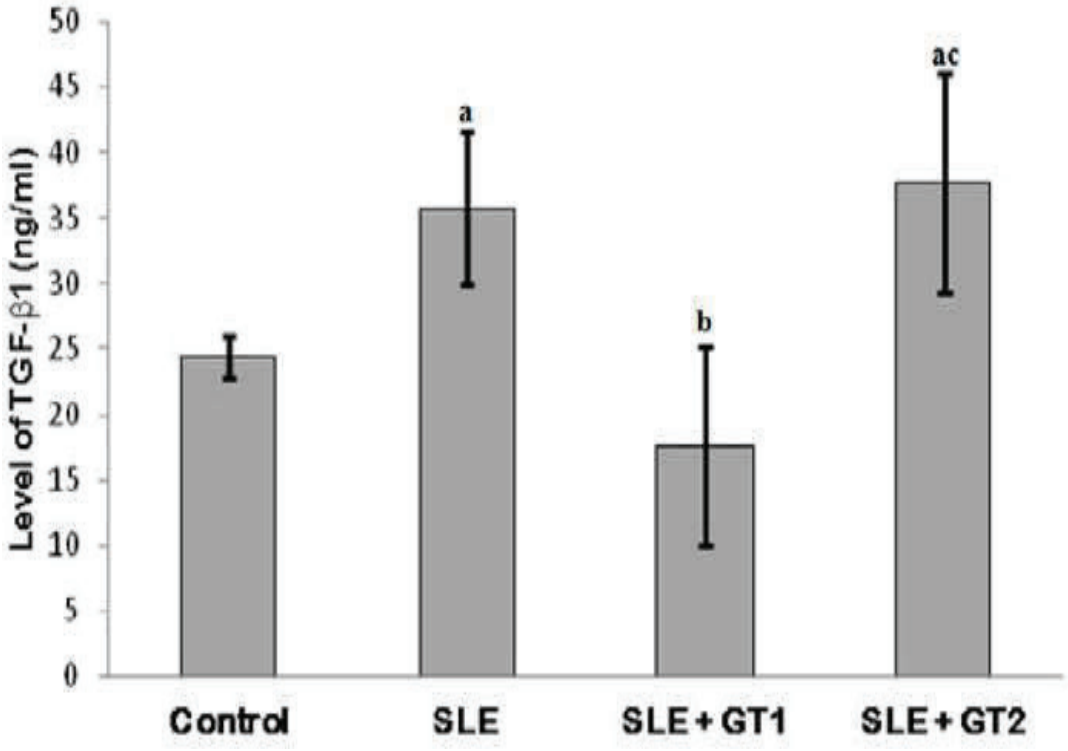


Figure 3. Levels of TGF-b1 in comtro and experimental groups. Note: [a]: there are significant differences compared with the control group ($p < 0.05$); [b]: there are significant differences compared with the SLE group ($p < 0.05$); [c]: there are significant differences compared with the SLE + GT1 group ($p < 0.05$); SLE:

systemic lupus erythematosus; SLE

+ GT1: systemic lupus erythematosus treated with green tea at dose of 500 mg/kilogram body weight; SLE + GT2: systemic lupus erythematosus treated with green tea at dose of 1000 mg/kilogram body weight.

Our study focus on the pharmacological effect of green tea in the modulation of TNF-α, Hsp70, and TGF-β1 in SLE model. We found that the TNF-α levels greater significance in the disease model compared to the control group. This increase indicates the involvement of inflammation in the pathomechanism of SLE. The rise in TNF-α in this SLE model indicated the involvement of kidney manifestations, autoantibodies, and inflammation [21]. Macrophages directly induce TNF-α production in response to immune complexes [22]. Besides, T cells from SLE will activate B cells, display inappropriate homing, and support the creation of proinflammatory cytokines [23.24]. Although this increase is consistent with clinical findings, that TNF-α levels increase in the serum of TNF-α patients [25], some studies state that higher levels of inactive SLE patients indicate that TNF-α can be a beneficial factor in SLE patients [26].

This increase in TNF-α levels in the SLE model of this study could be significantly reduced through the administration of green tea extract, even reaching levels comparable to the control group. This result shows that green tea can downregulate TNF-α production by macrophages in SLE. Our study is comfirmed with previous findings that EGCG can suppress inflammation [27]. We hypothesized that green tea and its active compound inhibits transcription factor for TNF-α production. Previous study shows that NF-kB inducing kinase is a therapeutic target for SLE [28].

For HSP70 levels, there were insignificantly differences between groups. This finding indicates that stress-induced by the administration of the pristane does not trigger Hsp70 production, so that extracellular levels are increased. Extracellular Hsp70 has two roles, namely to induce proinflammatory cytokines and increase antigenicity of antigens through modulation of antigen presentation. Also, extracellular Hsp is anti-inflammatory, triggering negative feedback in controlling inflammation [29,30].

In this study, SLE did not trigger changes in TGF-β1 levels. This finding is not consistent with previous clinical studies, that there is no difference in TGF-β1 levels of SLE patients compared to controls. The second dose of green tea extract can restore TGF-β1 levels to normal values. The pharmacological action of green tea is supported by previous study [31]. It was concluded that green tea containing EGCG can suppress TNF-a and TGF-b1 in the SLE model. Thus, green tea can be an alternative in immunology modulation in SLE.

Conclusion

It was concluded that green tea containing EGCG can suppress TNF-a and TGF-b1 in the SLE model. Thus, green tea can be an alternative in immunology modulation in SLE.

Ethical Clearance-obtained from the ethics commission of Medicine Faculty, Brawijaya University.

Conflict of Interest-no conflict of interests regarding the publication

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Knowledge and Attitude Towards HIV Patients among Dental Students

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Abstract

Introduction: The chances of dental professionals acquiring infection from HIV infected patient is very low yet significant. Hence most of the dental practitioners often hesitate to treat such cases reflecting their attitude and behavior on understanding about the pathogenesis and various aspects of the disease. **Aim:** The present study was aimed to evaluate the knowledge and attitude towards HIV patients among dental students. **Methodology:** A cross sectional study was conducted using self-administered questionnaire, through Google forms using various social media platforms among 206 dental students from second year to interns across dental colleges in Tamil Nadu, India during COVID-19 pandemic lockdown period. Statistics was done and results were tabulated. **Results:** In the present study it was observed that 77.7% students were aware of standard protocols to be taken for treating HIV patients and 71.8% were aware of various detection and management techniques. The overall knowledge and attitude **towards HIV patients among dental students** was 86% (highest) for interns group, 79% for final years, 75% for third years and 67% for second years (low score) respectively. **Conclusion:** The present study shows that there was adequate knowledge among the dental students regarding the oral manifestations and dental treatment of patients with HIV/AIDS however lack of attitude towards disease transmission was observed. Efforts have to be taken to improve the modes of educating the students about the HIV infection and its dental effects.

Keywords: *Acquired Immuno-Deficiency Syndrome, Awareness, Dental students, Human Immunodeficiency Virus, Saliva.*

Introduction

Human immunodeficiency virus (HIV), a retrovirus that invades the body's immune system results in decreased movement of cells towards any chemical stimulus, defective granuloma formation and impaired antigen production with generalized loss of CD4+ T helper cells [1]. Over a period of time, with progressive failure of the entire immune system, a life-threatening spectrum of disease known as Acquired immunodeficiency syndrome (AIDS) develops. Recent data suggests that AIDS as a globally emerging public health concern with approximately 38.8 million people are affected worldwide accounting for a massive 1.2 million death in 2015 [2] and 7.7million deaths in 2018 [3]. Numerous studies have shown that the average survival period following infection is only 11 years without appropriate management. In India alone HIV accounts for 2.5 million people with an estimated prevalence rate of 0.91% however the actual prevalence can be more than that because of the deliberate reporting in the subcontinent owing to social, cultural, religious and various other psychological factors [4, 5].

The probability of health care professionals and workers getting exposed to blood borne pathogens like Hepatitis B (HBV), Hepatitis C (HCV) and HIV is high. Among these health care professionals, dental professional's risk of occupational transmission of this virus has been estimated at 0.3% after a single exposure to HIV-infected blood. Dental professionals including practitioners, academicians and dental students should realize that they are ethically liable to treat their patients' with HIV/AIDS [6]. Conversely, HIV-positive patient's face challenges in disclosing their oral health status while seeking emergency or routine dental care. Engaging in such unacceptable behavior could be attributed to the lack of HIV-related knowledge on pathogenesis, modes

of transmission with subsequent fear of acquiring the infection while handling HIV infected cases [7].

In India, Bachelors program in Dentistry (B.D.S) is a five year course which includes compulsory one year training. Dental Students are introduced to the clinical practice only around 3rd year and are taught about various pathogenic blood borne infections including HIV/AIDS and its critical management. The exposure to handling HIV infected patients in a clinical scenario is very low owing to its low prevalence rate [8]. However, it is mandate that students should possess sufficient knowledge of HIV including its pathogenesis, related transmission, clinical and oral manifestations with positive attitudes in order to deliver their responsibility of treating patients with HIV/AIDS. It is indeed important to assess the knowledge and attitudes of dental students which helps in understanding their preparedness for treating HIV/AIDS patients and if any modifications have to be incorporated into the dental curriculum to inculcate positive attitudes in dental students. Thus, the present study was aimed to evaluate the knowledge and attitude towards HIV patients among dental students. In addition, we compared the differences in HIV/AIDS related knowledge and attitudes between the years of study.

Methodology

This study was designed to evaluate the knowledge and attitude towards HIV patients among dental students. A cross sectional study was conducted among dental students from second year to interns across dental colleges in Tamil Nadu, India during COVID-19 pandemic lockdown period. A total of 206 individual participated, in this study. The participants were informed about the study and assured that their participation was purely voluntary.

The self-administrated structured questionnaire consisting of 15 questions, in English, which is simple and easily understanding questions which helps the respondents. The questionnaire had a combination of selected response to the questions and closed ended questions (Yes/No/don't know). It took 5-10 minutes to complete the questionnaires for participants. The questions were converted into Google forms and shared to participants through social media platforms. The purpose and aim of the study were explained with requesting for participation, to the participants.

Statistical Analysis

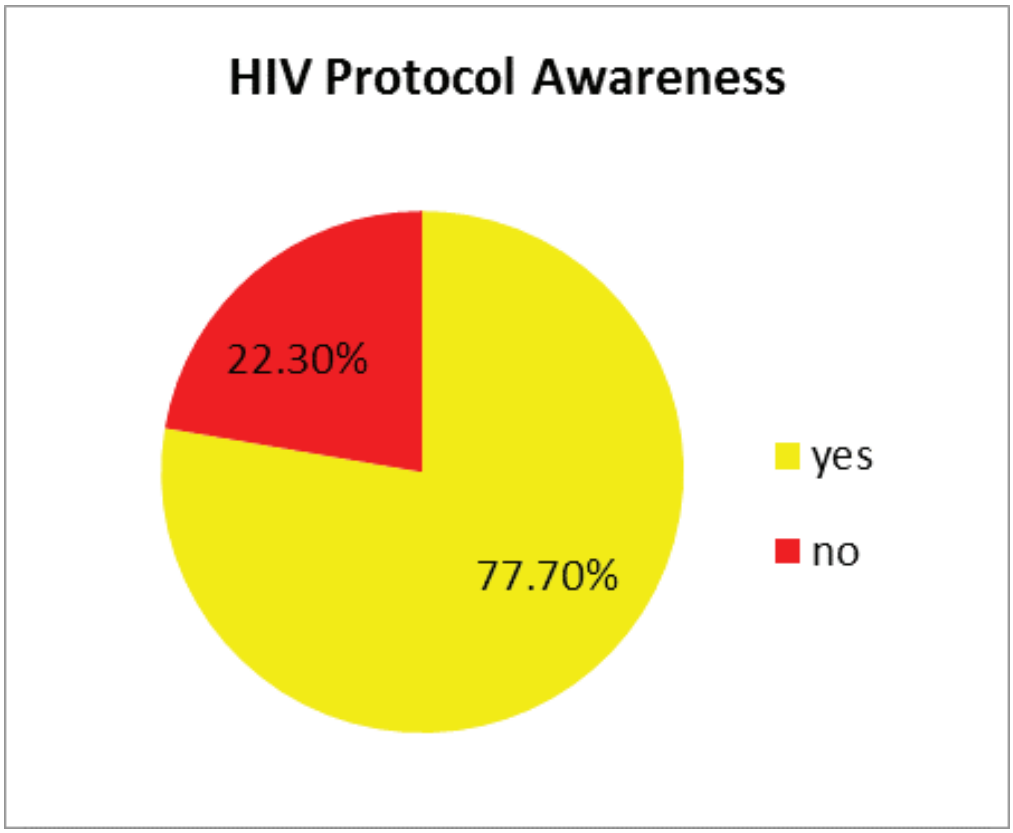
On statistical evaluation using SPSS version20.0 (Ilanos, NY, USA) it was observed all the 206 samples were valid for the study with Cronbach's alpha reliability score of 0.7836 (significant score).

Results

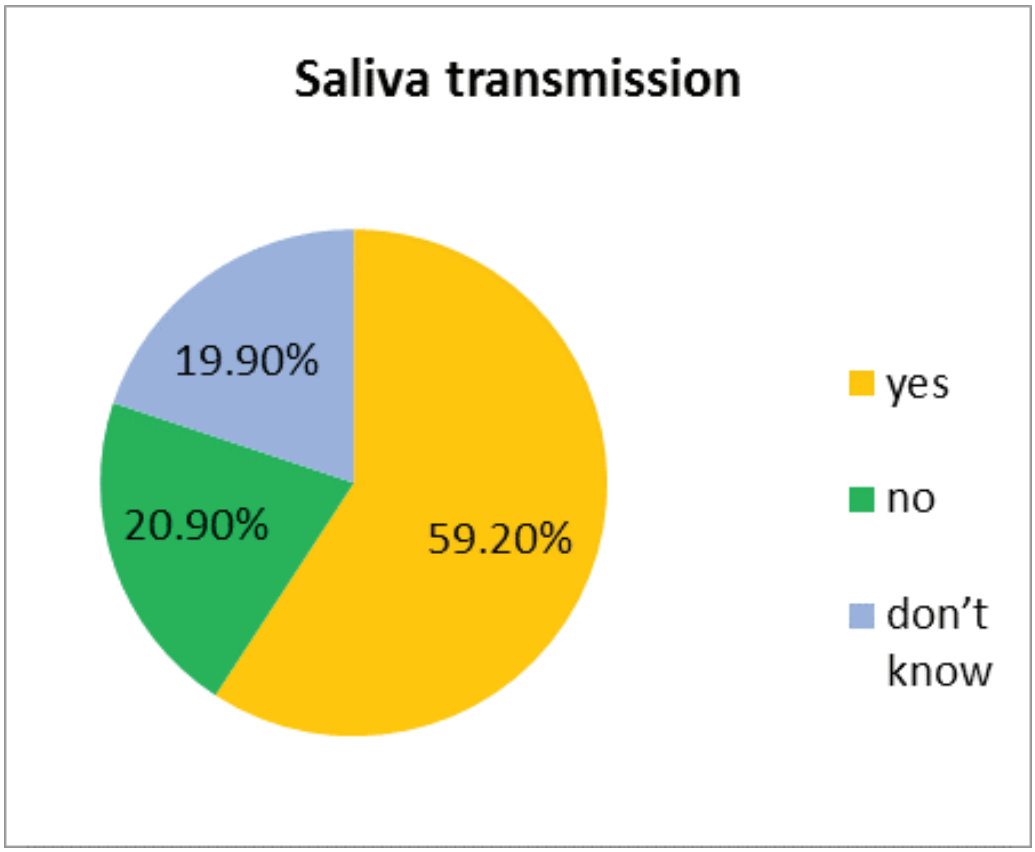
On analysis of the given data it was observed 31.55% of the study participants pursue second year followed by 30.58% final year students, 23.2% interns and 14.56% 3rd year students. On assessing the knowledge and attitude towards treating HIV patients, it was observed 77.7% students were aware of standard protocols to be taken for treating HIV patients (**Graph 1**). 54.9% respondents feel double gloving technique can prevent HIV transmission, and 59.2% consider saliva as a key vehicle in transmission of infection (**Graph 2**). About 56.8% of the participants are aware that needle prick injury is a route of transmission of infection apart from saliva and vertical transmission (43.2%). Only 46% participants were self-assured to consider aerosols generated from hand-piece can also cause HIV transmission (**Graph 3**). 63.1% believe infection control methods for hepatitis-B is adequate protection against transmission of HIV and only 34% opted for sterilization using autoclave for destruction of HIV.

On evaluating the knowledge about detection and management protocols for HIV patients 71.8% are aware of P24 screening test diagnosis of HIV infection (**Graph 4**). Most of the students were aware of oral manifestations which include xerostomia, Oral Hairy Leukoplakia, Necrotizing ulcerative periodontitis and candidiasis (**Graph 5**) and also agree that CD4+ count can be used as a marker to identify the progression of HIV and stage of immune suppression. Though 31.6% feel antiviral drug regime is effective against HIV/AIDS, 80.6% suggest educating the patient can prevent the spread of HIV. The overall knowledge and attitude **towards HIV patients among dental students** was 86% (highest) for interns group, 79% for final years, 75% for third years and 67% for second years (low score) respectively (**Graph 6**).

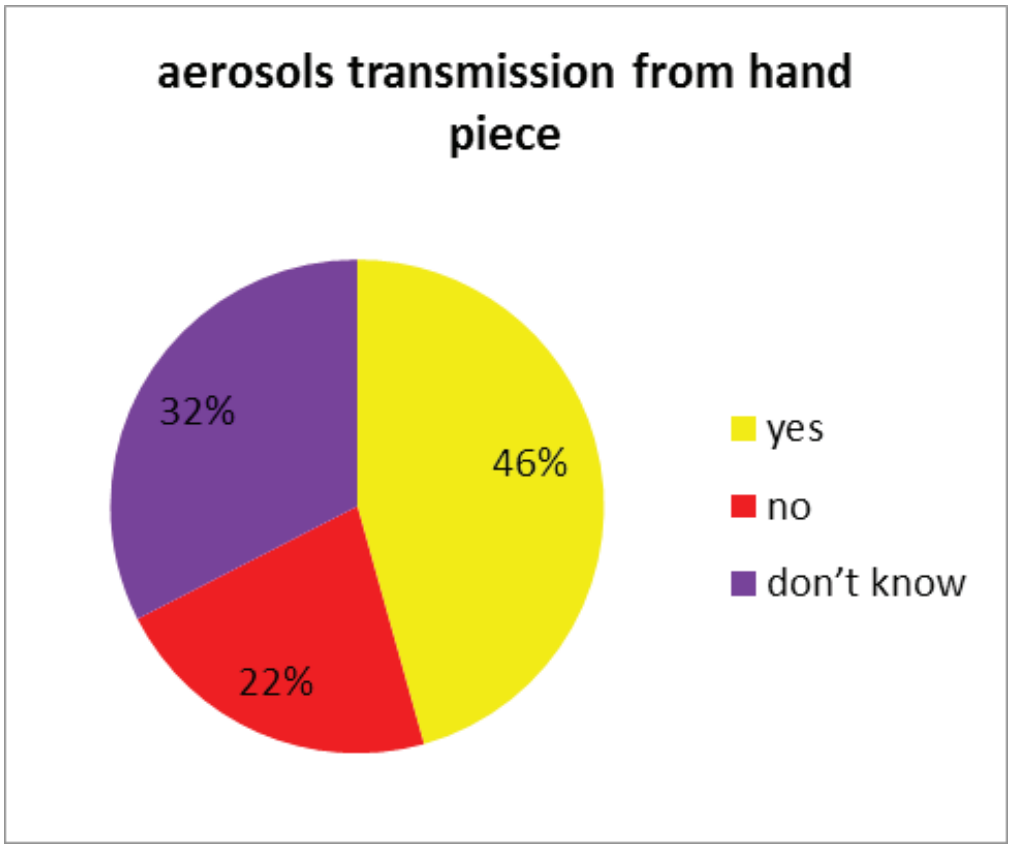
Graph 1: Awareness about the protocols to be taken for HIV patients



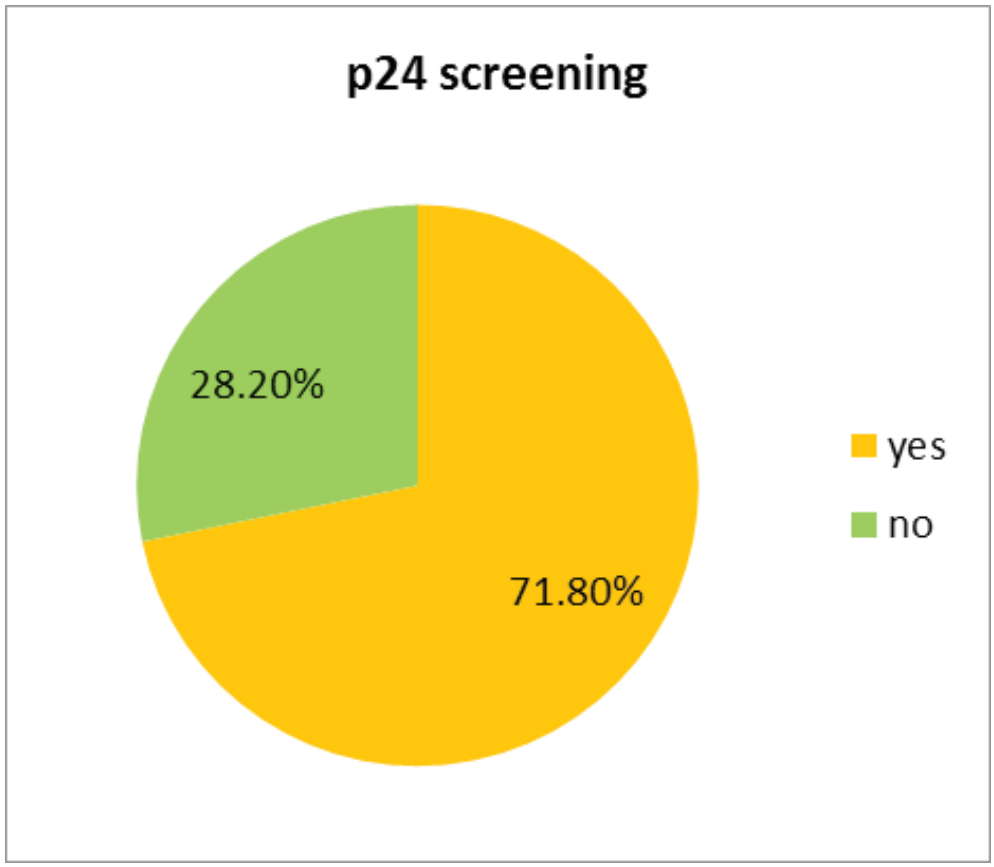
Graph 2: Saliva can be a vehicle of transmission for HIV infection



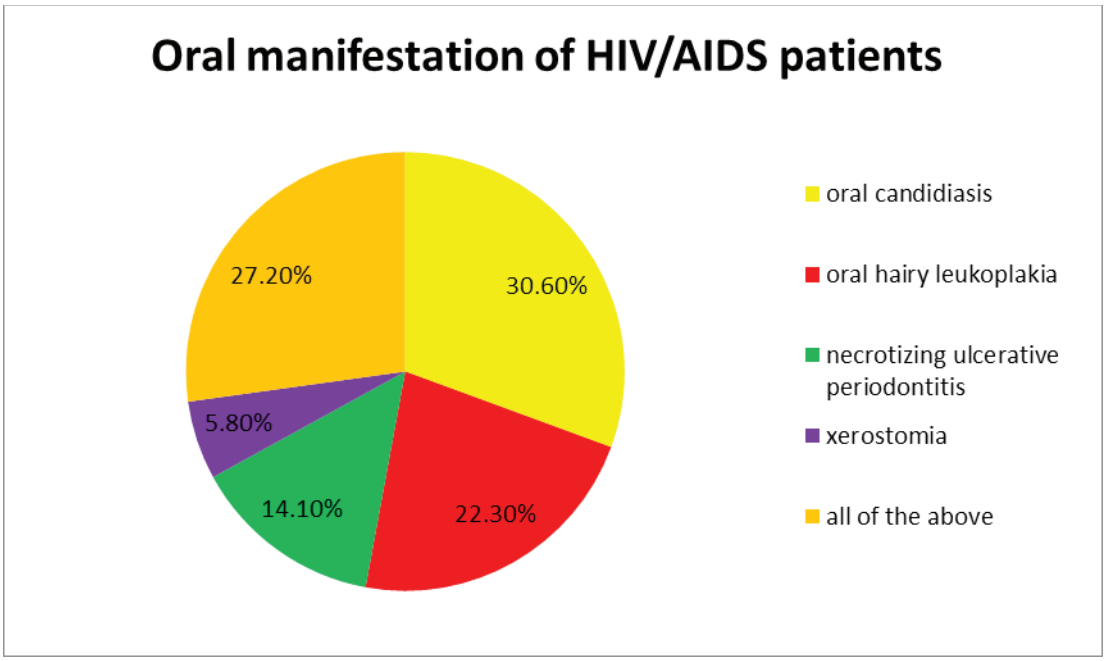
Graph 3: Aerosols from hand piece can cause HIV transmission



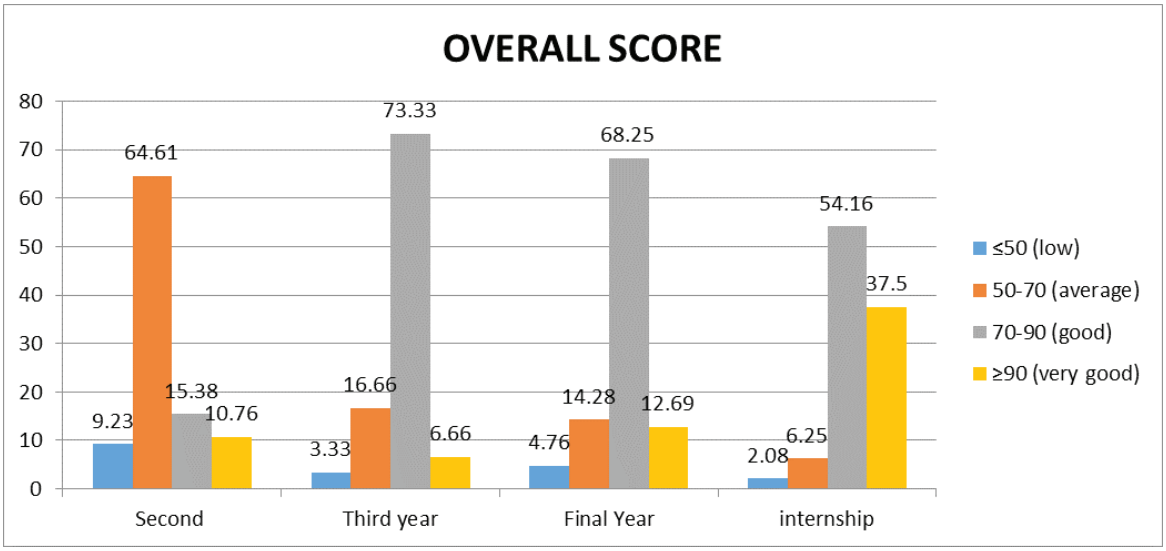
Graph 4: P24 screening test diagnosis of HIV infection



Graph 5: oral manifestation of HIV/AIDS patients



Graph 6: The overall Knowledge and Attitude scores among dental students and interns, n (%)



Discussion

In the present study it was observed that 77.7% students were aware of standard protocols to be taken for treating HIV patients. A similar study by Kumar S et al (93%) [6], Singh et al (78%) [9] also agreed that HIV/AIDS patients can infect a dental worker which suggests that most students were aware of disease transmission. However only 54.9% respondents feel double gloving technique can prevent HIV transmission which is slightly higher than study by Jain et al (37.2%) [10] and Champa et al [11]. These results suggest that lack of sufficient knowledge about standard protocols on double gloving has to be addressed in-order to provide protection from HIV transmission.

More than half of the students believed that saliva as a Key vehicle for AIDS transmission (59.5%) similar to Kumar S et al (57%) [6], Saheer et al (51.95%) [12], Rani et al (53.8%) [13], Oberoi et al [14], Sheikh et al [15] and Patil et al [16]. Various literature study also shows evident that exposure to saliva is a highly infectious source for transmission of HIV infection to the treating dentist.

About 56.8% students correctly responded to the question on needle-stick injury risk of contracting HIV

infection from an HIV-contaminated patient. A much higher results were shown by Oberoi (88%) [14], Kumar et al (73%) [6], Singh et al (98.5%) [9] and Saheer et al (92.65%) [12]. These findings clearly demonstrate that students were aware that sharps injuries as the more likely route of transmission as recommended by Centre for disease control (CDC) (1992).

About 43.2% of the participants were aware of various routes of transmission of HIV infection. Our study results are positively correlated to the studies done by Dhanya et al [7] among Indian students, Li et al among Chinese students [17], and negatively correlated with the study done by Rehan et al among Pakistani students [18]. Dentists should be taught that various transmission routes along with universal precautions since dentists and patients themselves will not always be aware of HIV-positive cases.

Around 63.1% believe infection control methods for hepatitis-B is adequate protection against transmission of HIV and only 34% opt for sterilization using autoclave for destruction of HIV which is similar to study done by Kumar S et al (28%) [6] and Jain et al [10]. Only 46% participants were self-assured to consider aerosols generated from hand-piece can also cause HIV transmission which is in accordance with the study by Rani et al (46.9%) [13] and Singh et al (19%)[9]. 80.6% suggest need for better understanding of various infection protocols can prevent the spread of HIV. This may be due to less or rare reported cases of aerosol HIV transmission. If the aerosol contains the infected blood of the HIV-positive patient, then there are likely chances of aspiration of oral fluids and blood from an infected patient into a dental unit waterline that could expose the microbes to the practitioner or the subsequent patient.

Most of the students were aware of oral manifestations which include xerostomia, Oral hairy leukoplakia, Necrotizing ulcerative periodontitis and candidiasis and also agree that CD4+ count can be used as a marker to identify the progression of HIV and stage of immune suppression which is similar to the study done by Kumar S et al (86%) [6], Oberoi et al [14], Sharma et al [19] but contrast to study by Singh et al (10.2%) [9] who were unaware that HIV/AIDS patients can be diagnosed with oral manifestations.

In our study 71.8% students were aware of various detection and management techniques which is high when compared to the study done by Kumar S et al [6], Singh et al [9] and Oberoi et al [14]. However the knowledge regarding the drugs to be taken under the antiretroviral therapy (ART) was found to be poor (31.6%) and our study is negatively correlated to the study done by Oberoi et al (72%) [14].

In the present study the overall knowledge and attitude **towards HIV patients among dental students** was 86% (highest) for interns group, 79% for final years, 75% for third years and 67% for second years (low score) respectively. Similar results were reported by Sadeghi and Hakimi (82.1%) [20], Aggarwal and Panat (78.8%) [21]. However studies by Kumar et al (74%) [6], Sharma et al (60%) [19], Saheer et al (55.87%) [12] and Singh et al (56.2%) [9] showed lesser percentage of knowledge/ attitude scores. This wide range of differences suggests knowledge and attitude depends on various factors like gender, ethnicity, age, and geographical locations apart from improved knowledge attained as one observes through the curriculum and also wide-range of aspects covered in the study curriculum.

Conclusion

The present study shows that there was adequate knowledge among the dental students regarding the oral manifestations and dental treatment of patients with HIV/AIDS, however lack of attitude towards disease transmission was observed. We recommend training should emphasize on problem-based techniques rather than conceptual understanding by incorporating experimental methods or affective module to improve self-esteem and also to include social activities in the curriculum to improve their empathic character and well-being.

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Ethical Clearance: None Declared

Source Of Funding: Self

Conflict Of Interest: Nil

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Awareness about Management of Pain and Anxiety during Dental Treatments among Dental Students

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Abstract

Introduction: Humans are more likely to feel contented with a familiar experience however discomfort with a novel one. The issues of pain, anxiety and fear are always a major part of dentistry. Dental anxiety will result in avoidance of dental care. The initial interaction between the dentist and the patient can reveal the presence of dental anxiety. **Aim:** This study was aimed assess the awareness about the management of pain and anxiety during dental treatment among dental students. **Methodology:** A cross sectional study was conducted using self-administrated questionnaire, through Google forms using various social media platforms such as whatsapp, facebook among 200 dental students from third year to final year in Tamil Nadu, India during COVID-19 pandemic lockdown period. Statistics was done and tabulated. **Results:** It was observed that 80% participants feel interrelation between dental anxiety and pain and 89% believe office environment and dental staff affects patient mind-set. 65.5% considers pharmacological route of pain management as effective strategy with 48.5% preferring conscious sedation and 36.5% accepts sedation can eliminate the use of local anesthesia. **Conclusion:** From the present study we would like to conclude that most of the dental students were aware about pain and anxiety management during dental treatments however further educational improvement regarding pain and anxiety in emergency conditions, especially concerning the assessment methods and continuity in the control of pain might result in better patient satisfaction.

Keywords: *Dental Anxiety, Dental Education, Local Anaesthesia, Pain Management, Sedation.*

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential or tissue damage, or described in terms of such damage. Dental pain is characterised as pain originating from the teeth and the supporting tissues [1].

Painful sensation is roughly classified as nociceptive pain, neuropathic pain and their combination. Pain is seen as an emotional reaction prior to its perception as a predominant physiological response and more recently as a combination of physiology and emotions [2, 3]. Both pain and anxiety are now perceived to have considerable overlap in their physiology and emotions [4].

Anxiety is an emotional state that precedes the actual encounter with the threatening stimuli. Toward dentists and dental treatments fear and anxiety are both significant characteristics that contribute to avoidance of dental care [5,6].

Treating anxious patients is stressful for the dentist because of reduced cooperation, requiring more time for treatment which finally results in an unpleasant experience. Cohen et al revealed that dental anxiety affects an individual in multiple ways. The physiological impacts of dental anxiety are signs and symptoms of panics and development of negative thoughts and fears [7].

Dental anxiety can arise due to multiple factors like previous negative or traumatic experience, vicarious learning from family members, lack of understanding, individual personality characteristics like neuroticism and the vulnerable position of lying back in the dental chair. Sensory triggers like sights of needle & air-turbine drills, sounds of drilling& screaming, smell of eugenol and cut dentine and also the sensation of high frequency vibration can provoke the dental anxiety [8].

This study was aimed assess the awareness about the management of pain and anxiety during dental treatment among dental students.

Methodology

This study was designed to measure the awareness about management of pain and anxiety during dental treatments among dental students. A cross sectional study was conducted among dental student of various dental colleges in Tamil Nadu, India during COVID-19 pandemic lockdown period. A total of 200 individual participated, in this study. The participants were informed about the study and assured that their participation was purely voluntary. The self-administrated structured questionnaire consists of 20 questions, in English.

The questionnaire had a combination of selected response to the questions and closed ended questions (Yes/No/option-based). It took 5-10 minutes to complete the questionnaires for participants. The questions were converted into Google forms and shared to participants social media platforms such as whatsapp, facebook. The purpose and aim of the study were explained with requesting for participation, to the participants. After collecting responses, the data was analysed by subjecting it to statistical analysis using SPSS Software version 18.0 with Cronbach's alpha reliability.

Results

In the present study 80% of participants believe significant interrelation between dental pain and anxiety. About 79% of participants have chosen both assessment and management technique as preferred mode of anxiety treatment , 45% of participants does not prefer the use of machined needles and 26% of participants prefer dental fear assessment strategies . On assessing the knowledge about emotional behaviour it was observed 42% of participants have chosen irrelevant speaking followed by hyperactivity 41% as responses from individual.

About 65.5% considers pharmacological route of pain management is effective in management of anxiety with 48.5% preferring conscious sedation and 36.5% accepts sedation can eliminate the use of local anesthesia. On evaluating the opinion about interrelation between virtual reality and music to treat dental pain and anxiety it was observed 57% of participants are positive in this approach. Most of the dental students consider previous traumatic experience as the predominant cause for dental pain associated anxiety.

Around 47% of participants have chosen relaxation breathing as effective treatment where as 68% consider cognitive behavioural therapy effective in treating dental anxiety/pain and 89% believes dental office environment and patient mindset influence these strategies.

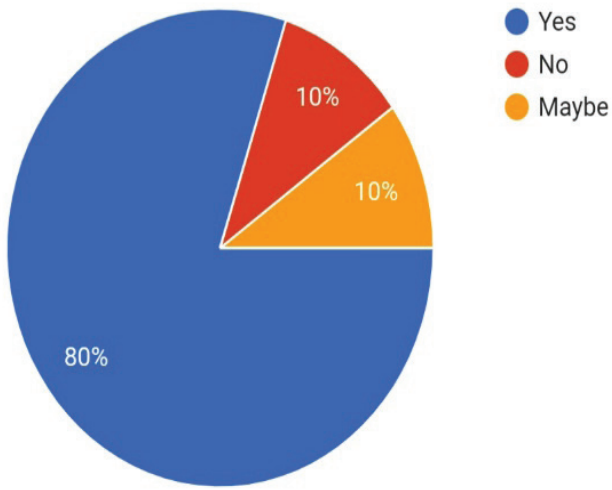

Figure1:-Interlink between anxiety and pain

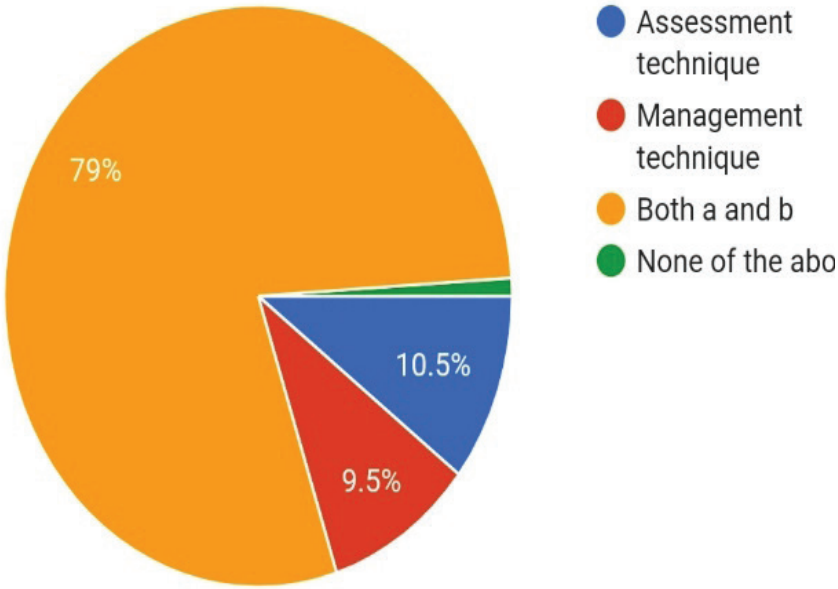

Figure 2:-Anxiety management technique

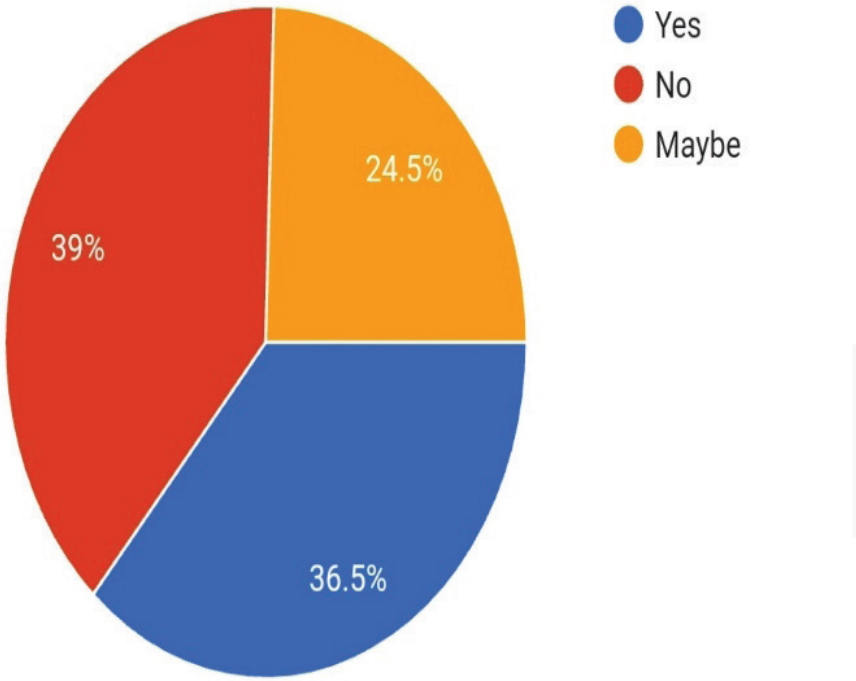

Figure 3:-Interlink between dental office environment and mindset

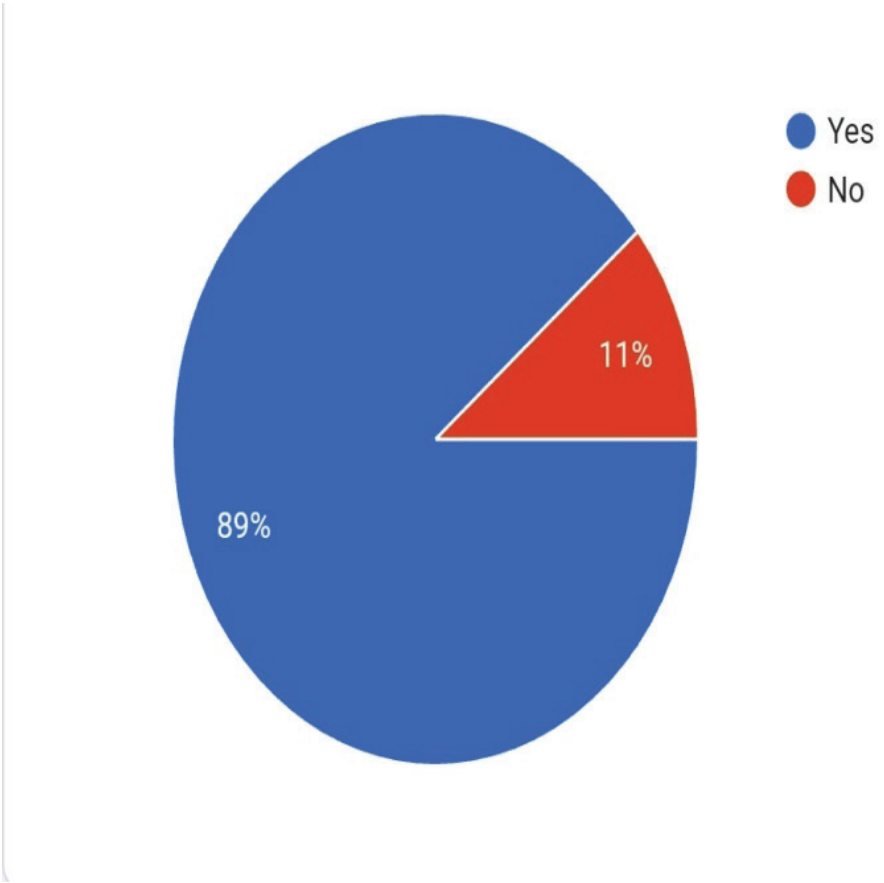

Figure:-4:-Sedation control eliminate pain and local anaesthesia

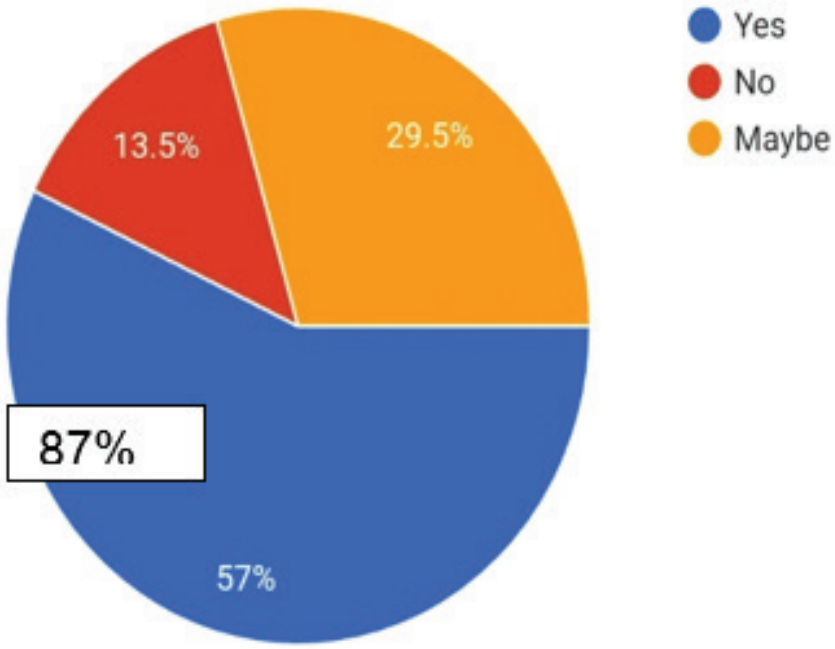

Figure:5:-Music and virtual reality used to treat pain and anxiety

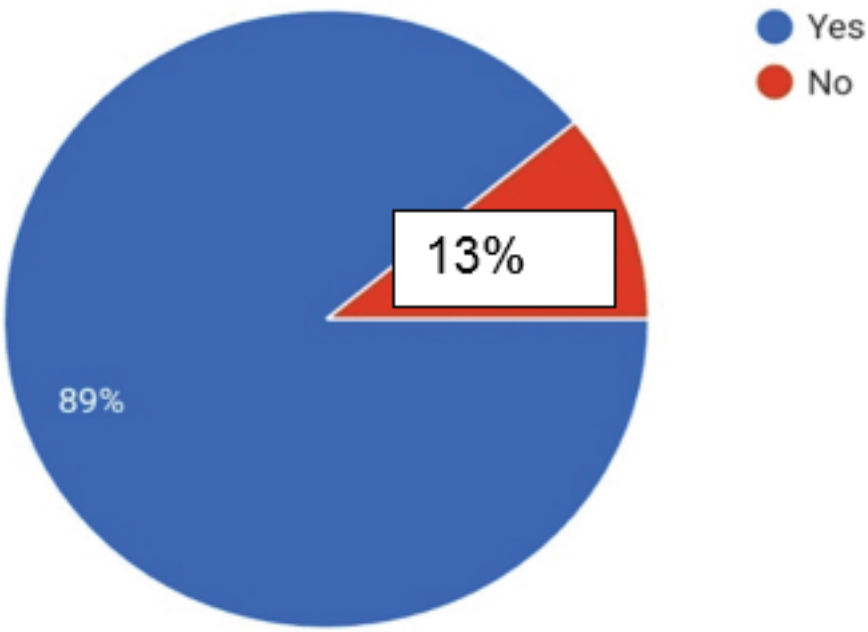

Figure 6:-overall result of the study

Discussion

Broadly dental anxiety can be managed by psychotherapeutic interventions such as behaviour or cognitively oriented therapy or pharmacological interventions like sedation and general anaesthesia or combinations of both depending on anxiety level and the clinical situations [9].In the present study it was observed that 80% of dental students are aware that there is an interrelation between dental anxiety and pain which is similar to global response average rate of 78.6% reported in other studies by Guivarchet al(2017) [10], Kaakko et al(1998)[11], Al-Omari WM et al(2009)[12]and Sghaireen MG et al(2013)[13] involving dental students.

In the present study 79% have chosen both assessment and management techniques are useful in managing anxiety, which is similar to studies reported by Spielberger CD et al(1985)[14] and Stouthard ME et al(1993) [15].89% believe office environment and dental staff affects patient mind-set. Appukuttan DP in his review observed introducing pleasant ambient odours to the dental environment can also help to reduce anxiety(2016)[16] and studies by McCaffrey R et al (2009)[17] and Muzzarelli et al Let al also showed similar results suggesting well-adapted dental environment plays an effective and crucial role in reducing anxiety and inducing relaxation(2006)[18].

In the present study 65.5% considers pharmacological route as best for pain management similar to the study done by Folayan MO et al(2002) [19] with 48.5% preferring sedation control as their first choice as mentioned in the previous study by Coult hard P(2013) [20] . 36.5% of dental students are aware that sedation can eliminate the use of local anesthesia. This could be attributed to the fact that moderate to severe chronic pain may counterattack localization in the body and migrate from one area to the another region, including the dentition. In such cases, overall pain control is necessary to reduce the dental pain hence opting for sedation is more effective.

Among the behavioural strategies 57% suggest use of virtual reality and music to treat dental pain and anxiety similar to the study by Nansi López-Valverde et al (2020) [21] where the author demonstrated Virtual

reality as an effective three-dimensional environment that provides patients with a sense of involvement, moving them to pleasing and co-operative surroundings.

Most of participants have chosen both visual and corah's anxiety scale as an anxiety tool indicator.63.5% of participants considers previous traumatic experience is the predominant cause for dental pain associated anxiety while 15.5%of participants considers listening to patients post traumatic experience as predominant cause of dental pain associated anxiety. This was similar to study by Narayan VK et al who believed behavioral measure is not always the ideal but often the only available technique for assessing dental fear especially in children (2019). [22]

Around 47% of participants have chosen relaxation breathing as effective treatment where as 68% consider cognitive behavioral therapy (CBT) effective in treating dental anxiety/pain. This is similar to the study performed by Shahnavaz S et al [23] (2016)which shows CBT is an effective treatment for children and adolescents with dental anxiety and should be made accessible in dental practice.

In our study, among intra-operative behavioural management strategies, reassuring words and providing control over treatment procedure were systematically implemented by 45% and 65% of dental students respectively . These percentages are lesser than those presented in a previous studies by Guivarch et al [10] (2017).In a study done by Corah N Let al(1988)[24]40% of the students declared giving reassurance to the patient during treatment is effective and only 8% declared offering the patient some control over the procedure as effective mode of behavioural management strategies.

Conclusion

From the present study we would like to conclude that most of the dental students were aware about pain and anxiety management during dental treatments however further educational improvement regarding pain and anxiety in emergency conditions, especially concerning the assessment methods and continuity in the control of pain might result in better patient satisfaction.

Acknowledgement: Nil

Ethical Clearance: None Declared

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Conflict of Interest: Nil

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Environmental Factors and Protective Effects of Epigallocatechin-3-Gallate to Systemic Lupus Eritematosus: A Review Study

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Abstract

SLE (Systemic Lupus Erythematosus) is one of autoimmune disease in which the immune system attacks its own tissues, causing widespread inflammation and tissue damage in the affected organs. Etiology and pathogenesis are not clearly mention until now by the scientist, but environmental factor is contribute to development of Lupus and Flare disease. The objective of this study to explain the relationship between environmental factors and pathogenesis development of SLE, and how the Epigallocatechin-3-Gallate (EGCG) can protect or prevent of organs damage and environmental effects.

Method: The research design of this study was used literature review. The data sources collected from Electronic database in Pubmed, Sage, Google Scholar and website of science including reports, journals and mostly published in the last 10 years.

Results: The pathomechanism of SLE was influenced by environmental factors and this was caused an increasing of oxidative stress, chronic inflammation, dysregulation of immune system and decreased the clean of immune complex and apoptosis of cells. The production of autoantibodies and immune complex are related with target tissue with is it was caused chronic inflammation and the end it caused irreversible damage in glomerulus of the kidneys, arteries, lungs and other tissues. EGCG plays a protecting role in environmental factors as a trigger because it works as an antioxidant, anti-inflammatory and immunomudulator. So EGCG can as a potential agent to protect SLE.

Conclusion: Environmental factors plays an important role as a triger of SLE and flare. Moreover, EGCG as a potential agent to protect the presence of oxidative stress, inflammation and immune dysregulation.

***Keywords**: Environmental Factors; Epigallocatechin-3-Gallate; Systemic lupus erythematosus*

Introduction

SLE (Systemic lupus erythematousus) is one of lupus type. SLE is an autoimmune disease in which the immune system attacks its own tissues, causing widespread inflammation and tissue damage in the affected organs. It can affect the joints, skin, brain, lungs, kidneys, and blood vessels. It is also one of leading killer among young women. In 2000 - 2015, SLE was ranked 10th occurred among people who aged 15-24 years, and it was ranged 5th occurred among women who has black skin and Hispanic and aged 15-24 years, and 6th of rank

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was occurred among people who aged 25-34 years [1].

At least more than 100 thousand new SLE cases suffered in the world. In 2012, the prevalence of SLE reached 1 in 1000 population in UK and 2-7.6 per 100,000 population occurred of SLE or 19-159 cases per 100,000 population in USA. In Asia Pacific an estimated 4.3-45.3 per 100,000 population or 0.9-3.1 cases per 100,000 population per year. The average of SLE in America was 7.9 and in Taiwan was 8.1. Now, this disease not one of rare disease occurred in every level of people. In some countries the prevalence of SLE was up to 80-120 per 100,000 occurred among adults people [2,3,4,5].

Mortality rates increased in 20th century with 60% of 10-year of life expectancy in 1950 and reached 90% in 1980s and 1990s. This was due to an increasing of the severity of disease activity although the development of diagnosis and treatment were progressed [4,6]. Now, the life expectancy of SLE was increased 80-90% among the patients and they can normal live [7] Previous research explained that people who diagnosed SLE will life up to 40 years. However, the deaths cases due to SLE still high in recent years. Mortality HRs estimated 2.15 in early (1999-2006) and 2.12 in late (2007-2014) cohorts [7]. In 2008-2016, In high-income countries mentioned that the survival rates was 0.95 among 5 years old, 0.89 among 10 years old, and 0.82 among 15 years old people of SLE. In low / middle income countries mentioned that the SLE were 0.92, 0.85, and 0.79, [8,9] At the past the death was caused by the own disease but now days it was caused the effect of the drug side effects (for the example, serious infections for patients who received potential immunosuppressive drugs) or cardiovascular problems [9].

The health problems impact of this disease was very large including bio-social psychology and economics, because most of the patients are young women and in childbearing age. SLE also affected the life of their partner such as financial problems, emotional, relationship and sexual condition. In addition, the disease and the treatment can inhibit the possibility of pregnant. Moreover, this disease also give an impact of quality of life such as marital relations, emotional problems (sad, depression, fear, anxiety, fear, anger, guilt), sleep disorders, pain, fatigue, rash, pregnancy, work and economy, daily activities, comorbid condition (hypertension, arterial diabetes, osteoporosis etc), as a result of treatment [10-13].

The causative factors of SLE are not yet clearly until now, there was relationship between environmental and genetic factors. Genetic factor was contributed 50% of the SLE pathogenesis and the various environmental factors [7]. The results of previous research described that the environmental factors was contributed to this disease. This review study will describe how the relationship between environmental factors and the disease activities. Meanwhile various kinds of treatments have been approved by the FDA as a drug for SLE treatment. Natural or herbal medicines also have potential effect to prevent environmental factors that aggravate the condition of SLE disease. How EGCG which found in green tea can protect SLE will also described in this review study.

Environmental Factors in SLE pathogenesis

Systemic pathophysiology of lupus erythematosus (SLE) begins with the presence of autoantibodies and immune complexes that bind together with tissues and give multisystem inflammation. One of the causes of SLE is environmental factors. Biologic mechanisms of environmental exposures, high oxidative stress, development of systemic inflammation and upregulation of inflammatory cytokine, and hormonal, and epigenetic modifications were influenced the development of SLE.

In immune systems of SLE patients both innate or adaptive were provided an inappropriate immune response to the body cell particles. The abnormal immune responses are: 1) activation of innate immune systems (dendritic cells, monocytes / macrophages) in DNA, RNA from self-antigen proteins; 2) activation of adaptive immune cells (T lymphocytes and 3) lymphocytes; 3) ineffective regulation of CD4 + and CD8 + T cells, B cells and suppressor cells; 4) decreased the cleansing of immune complexes and cells who apoptosis process [14, 15].

Furthermore, autoantibodies recognize self-antigens that exist on the surface of apoptotic cells and make immune complexes. Because the process of cell debris cleaning was disrupted so autoantigens, autoantibodies and immune complexes were available for a long time,

lead to inflammatory process and causing symptoms. Immune cell activation was also increased the secretion of type 1 and 2 interferons (IFN), tumor necrosis factors α (TNF-α), interleukin (IL) 17, stimulator of B cell maturation, and IL-10 which all support for inflammatory reactions. In this stage also has decreasing cytokines production such as natural killer cells which filed to produce IL-2 and transforming growth factor beta (TGF-β) which regulate CD4 + and CD8 + T cells, consequently the production of autoantibodies and immune complexes were uncontrolled and continues to autoantibody and complex, then binds to the target tissues, causes the activation of the complement system and released the cytokines, chemokines and vasoactive peptides, oxidants and proteolytic enzymes. This condition caused the activation of endothelial cells, tissue macrophages, mesangial cells, podocytes in tissue and causes B cells, T cells, dendritic cells and macrophages to reach the target tissue and also caused an inflammatory process. This chronic inflammation caused irreversible damage of tissue in glomerulus of kidneys, arteries, lungs and other tissues [4, 9,14, 16].

Lifestyle and Behavioral Factors

Lifestyle and behavior as triggers for SLE, it include eating patterns, activities, smoking, lack of sleep, stress and emotional factors. Obesity is one of the result of unhealthy behavior and lifestyle, and it also correlates with the incidence of SLE. The NHS studies said that more than 5,600,000 people / year were indicated that obesity was associated with an increasing of SLE incidence wit hazard ratio (HR) was 1.85. A meta-analysis study said that there was no relationship between lifestyle and SLE therefore further confirmation was needed [7]. A study showed that the risk factors for lupus occurred among people who had sleep patterns problems. Data obtained that less than 7 hours of sleep at night have a risk of lupus and the lack of sleep can occur due to stress, sunlight, smoking and others [17].

Triggers for fatigue in SLE are influenced by behavioral and lifestyle factors such as smoking, exercise, sedentary behavior, sleep and rest patterns. In group patients who have regularly exercise and low smoking habits were negatively correlated with fatigue. One of the reason was explained because smoking induces the pain [18]. Tobacco smoke has an effect on the immune system functioning. Macrophages were activated in alveoli, increasing myeloperoxidase activity and high production of free radical. In long-term smoking consumption can damage the secretion of proinflammatory cytokines and reduced the activity of natural killer cells [19]. There is a mechanical evidence which mentioned that there was an involving of smoking in the pathogenesis of SLE. Toxic components of cigarette (eg Ter, nicotine, carbon monoxide, polycyclic aromatic hydrocarbons and free radicals) were induced oxidative stress and corrupt the proteins and DNA endogen and lead to genetic mutations and gene activation. In a retrospective case-control study in SLE patients, mentioned that smokers were significantly more likely to have anti-double stranded DNA than who had never smoked (OR 4.0, 95% CI 1.6-10.4 [20].

Radiation, Nutritional and Diet Factors

Autoimmune diseases associated with daily of food consumption such as consuming foods exposed with chemicals like toxic oil syndrome and eosinophiliamyalgia syndrome [19]. Food intake was a potential risk factor for SLE pathogenesis because it was gave an effects on lipid and glucose metabolism, oxidative stress, and intestinal microbiomas [21]. The low level of SLE disease activity in patients was associated with lower intake of omega-3 of fatty acids and higher carbohydrates [22] and high alcohol consume [20, 23].

Nutritional risk index (NRI) associated with the increasing of organ damage and disease development of SLE. The excessive nutrition consumption was increasing the inflammation and it lead the risk for autoimmune diseases. Nutritional problems in SLE patients were associated with chronic weakness, depression and the presence of comorbidities so this changed in appetite and commonly marked by low serum albunin levels during inflammation and malnutrition [23, 24].

Various dietary factors play a role in the pathogenesis of SLE by using epigenetic changes (DNA methylation) and / or intestinal microbiota. Black tea Consumption and coffee showed that there was a relationship with SLE [25]. In addition, there was a mechanism of increasing salt intake and lupus autoimmunity by promoting the expansion and function of follicular helper T cells [7].

Meanwhile, there was relationship between serum trace and SLE development. Serum trace elements affected oxidative stress and involved the pathogenesis of SLE trace elements such as Zinc (Zn), Copper (Cu) and Selenium (Se). Zn has function to modulate the cellular interactions including DNA and protein synthesis, cell proliferation, electron transport and defense against free-radical damage. Zn has anti-inflammatory and antioxidant effects. Cu is a major component of antioxidant enzyme superoxide dismutase. Se is an essential constituent of anti-oxidative enzymes including selenoprotein P, glutathione peroxidases and thioredoxin reductases [26,27].

Various studies reported that low levels of vitamin D associated with increasing of B cell proliferation, polimorfisme reseptor of vitamin D (VDR) among SLE patients. Lack of sun exposure was a major cause of vitamin D deficiency among Caucasian race, because about 80% of vitamin D in the body comes from previtamin D which produced by the skin and induced by ultra violet light B. Many factors influenced the absorption in the skin, increasing of melanin pigmentation on the skin, topical sunscreen will absorbs ultraviolet B and it was significant with producing vitamin D in the skin. Moreover, geographical location, time/ hour, cloudy on atmosphere, haze were influenced the exposur of UV radiation and vitamin D synthesis. Various studies suggested approximately 5 - 30 minutes sunbathe when the sun come in the morning, and 3 times a week, especially some part of body such as hand, face, legs and back without used sunscreen. And for patient who experienced limited sun exposure were advised to supplement their vitamin D which contain on food and supplements. Vitamin D contains in some foods such as salmon, tuna, and mackerel and fish liver oil. Small amounts of vitamin D also found in beef, liver, cheese, egg yolks and mushrooms also contain ergocalciferol [28-30].

Ultraviolet (UV) rays is main environmental factors for SLE. UV rays effects DNA, apoptosis, autoantigen, cytokine, inflammatory cells process, and systemic flare induction. Kind of UV light are based on wavelength: UVA = 400-320 nm; UVB = 320-280 nm; UVC = 280-1100 nm, and some studies also said that there was relationship between shorter level of wavelength and high level of energy [31,32].

UV exposure also affects the production of ROS, DNA damage, and apoptosis. The decreasing of apoptotic cells clean in the skin contributed to induce inflammatory process in lupus and it increased automatic antigens exposure. Besides that, it also occurred dysfunctional and reduce phagocytosis, and in the end occurred the reduction in apoptotic cell clearance. $HMGB_1$ skewed polarization macrophage and diminished M_1-like phenotype in apoptotic cells. Another mechanism was regulated apoptotic clearance of complement opsonization [25].

Occupational factors was associated with the pathogenesis of SLE include exposure to Silica and Silicates, Solvents and Pesticides, Heavy Metals. Silica is stimulant immune. Silica increased proinflammatory cytokines, TNF and IL-1. Some studies said that there were relationship between silica and SLE with the risks was 3.0 - 10.0 [19, 33]. However, solvent or chemical liquid related with SLE pathogenesis. Chemical liquid are alcohols, glycols, aromatic hydrocarbons (e.g. benzene, toluene, xylene), and chlorine (carbon tetrachloride and trichloroethylene). Previous experimental study found that metals (cadmium and mercury) were associated with accelerating diseases of lupus [19]. Air pollution also associated with SLE pathogenesis. Kind of the pollution are Particulate Matter (PM_{10}), Sulfur Dioxide, Nitrogen Dioxide (NO_2), Ozone, and Carbon Monoxide [34].

Infection

Infection process also on of factors influence of SLE but the involvement was indirect relationships. The majority of previous studies said that there was a correlation between seroprevalence of EpsteineBarr virus (anti-virus capsid IgG antigen) with SLE (OR 2.08), while the other agents of infection were less evidences [7].

Protective Effects of Epigallocatechin-3-Gallate in Sistemic Lupus Eritematosus.

An estimated more than 50% of systemic lupus erythematosus (SLE) patients used complementary and alternative medicine (CAM) to reduce the symptoms and gain their health [35]. Chemosynthetic medicines such as non-steroidal anti-inflammatory drugs and corticosteroids are limited to find and in other this medicines also kind of medicines with extreme toxicity.

Heterogeneity of SLE disease is very difficult to managed by one component of the drug. Therefore, it is very important to use products of herbal medicines as additional treatment to SLE. Herbal medicines also has some functions such as to reduce of the symptoms, prophylactic drugs in cerebrovascular cases, liver, nephropatological, haematological, cardiopulmonary, mucocutaneous and musculoskeletal manifestations of SLE [36].

In this section will review how the natural products such as EGCG have potential to be used as complementary medicine. Epigallocatechin gallate (EGCG) is a type of vegetable compound and it called catechin. Catechins are categorized into a larger group of plant and known as polyphenols. EGCG and catechins are powerful antioxidants and can protect to cell damage which caused by free radicals. EGCG becomes a stronger lipid antioxidant than vitamins C and E [37,38].

Epigallocatechin-3-Gallate as an antioksidant

EGCG and catechins are work as potential antioxidants and they can protect cell damage caused by free radicals. EGCG is very hydrophilic and as an antioxidant in an aqueous compartment. The invitro antioxidant activity of EGCG reported in the plasma lipid compartment. Compared to vitamins, EGCG is a stronger antioxidant. The EGCG antioxidant is stronger than vitamins C and E to delay the LDL oxidation in cell free trials [37, 38]. EGCG neutralized the Reactive Oxygen Species (ROS) level such as the level of Superoxide Anions, Hydroxyl Radicals, Singlet Oxygen, Reactive Nitrogen Species, Nitric Oxide, Nitrogen Dioxide, and Peroxynitrite [34].

The chemical structure of EGCG is an antioxidant. The phenol ring in EGCG as an catcher of electron and free radical scavengers. EGCG is effectively inhibit the oxidation of tyrosine protein in blood platelets and improving mitochondrial function [39]. In autoimmune diseases such as Sjogren's syndrome (SS) and diabetes type-1 there are a decreasing of anti-oxidant and defense of enzyme levels in the affected tissue. PRDX6 is a general anti-oxidant enzyme which influenced by autoimmune because of high level of H_2O_2, ROS, and EGCG at physiological concentrations able to restore PRDX6 and anti-oxidant levels by using signal transduction of MAPK and it provide the protection to organs from oxidative damage. (40). In arthritis studies, EGCG improved the mice sample who has arthritis by using DC which this is a producing of indoleamine enzyme 2,3 dioxygenase (IDO), increasing the frequency of T regs and inducing the activation of the antioxidant pathway of NRF-2 [41].

Cells that express IDO can mediate strong local effects on nature immunology and adaptive toward the response of inflammatory disorders. The IDO activity was promoted local metabolic changes that affect cellular and systemic responses to inflammation and immunological signals. IDO helps to produce Treg cells and the NRF-2 activated in everywhere by the cell so this can fast protect against oxidative stress, inflammatory, and metabolic. Moreover, EGCG is known able to induce both IDO and NRF-2 [41.42].

Epigallocatechin-3- Gallate as an antiinflammation

The intervention of EGCG 120 mg/kg body weight per day to mice with lupus and 34 weeks old gain some effect such as (1) preventing of proteinuria, renal problems; (2) the increasing of E2 2 (Nrf2) in renal nuclear and glutathione peroxidase activity; (3) reduce renal oxidative stress, activation of NF-κB, and expression of mRNA/ NLRP3 protein and protein levels of mature caspase-1, IL-1β, and IL-18; and (4) increase Lien cell regulation (Treg). This data showed that EGCG has prophylaxis effect on nephritis lupus among mice and this really related with the effect of the increasing of antioksidan NRF2 signals level [43, 44].

The description of inflammatory was characterized by aggregation of immune cells in inflammation process, proinflammatory cytokines production and reactive oxygen / nitrogen (ROS/ RNS). ROS/ RNS associated with NF-*κ*B activation and activator of protein (AP-) 1. After the activation, NF-jB. and AP-1 will send to nucleus by cytoplasm and manage the inflammatory gene variation. EGCG also decreased the proinflammatory regulation by using P2X4 receptors than blocked the JAK1 / 2 tyrosine kinase signal in vascular endothelial cells [39].

Epigallocatechin-3-Gallate as an imunoregulator

EGCG as an immunoregulatory drug candidate, it can reducing the immune cells function or controlling the

inflammatory cytokines production, thereby maintaining immune homeostasis (45). The main mechanism of natural immunosuppressants action is depends on the antagonistic action of oxidative stressors, and in the end it leads to the formation of free radicals which targeted cytokines and autoantibodies. EGCG can also act as a modulator of cytokine production and B-T cell costimulation, therefore it eliminating the possibility of autoantibody formation and hyperactivation of the immune response in SLE cases [36].

The green tea effects / EGCG in innate immunity are 1) Neutrophils. The effect of EGCG on neutrophils are protects in limiting information and tissue damage caused by the inflammation, it has the effect to reducing migration and recruitment also decreasing proteulitic enzymes [46,47]. 2). Macrophages. EGCG affects the various aspects of macrophages including migration, cytokines and chemokines which they produced by suppressing method and including the suppressing of pro-inflammatory genes such as IL-6, IL-12p40, MCP-1, ICAM-1, and VCAM-1. Suppression of NF-κB, the main transcription factor regulates the expression of inflammatory molecular genes. EGCG inhibits inflammation process by induce lipopolysaccharides and turned off Notch signaling in primary macrophages of human [48, 49]. 3) Dendritic cells. EGCG inhibits and reduces the DC function. The main function of DC is catch and process Ag, as APC. So the effect is to reduce MHC class I and 2, CD80, CD86, CD11, IL-12, TNF-a, IL-6, IL-1b, IL-10 and NF-kB [50, 51]. 4) NK cells. EGCG has the effect of reducing the level and frequency of NK cells. 5) Mast cells and basophils. EGCG inhibits the degranulation by use histamine reducing and blocke the 67LR, inhibiting the expression of high-affinity IgE Fc3RI receptors and reduce the ability of mast cells to cytokines out (TNF-a, IL-6, and IL-8) by using NF-kB suppress activation [52].

The efek of green tea/EGCG in adaptive immunity are 1) as T cells (CD4+). T cells as an immunomodulator, it regulates T cell expansion, differentiation, and effector functions and manage the function of other cells like macrophage cells and B. The effect shows from the following parameters: proliferation and the decreasing of cell cycle, IL- 2, IL-10, IFN-g also decreas or increase, decreasing of IL-2R, IL-15R, IL-7 and pSTAT5. 2) EGCG inhibits the stages of T cell by activate Zap70, Cg1 of Phospholipase, extracellular signaling-regulated kinase, MAPK level and activates AP-1.3). CD4 + T cell differentiation. Specifically, EGCG can suppress Th1 responses but not affects Th2 responses. EGCG can inhibit the differentiation of T cell CD4 + naif to be Th1, Th9 and Th17 and small amounts prevents IL-6 induction of Treg suppression, EGCG suppresses T-bet, PU.1, and RORgt, specific transcription factors of Th1, Th9, differentiation and Th17. EGCG suppresses main transducer signals such as p-STAT1 for Th1 and p-STAT3 for Th17. The effect of EGCG on B cells may occur due to their effect on T cells [53].

Innate and adaptive immune systems express TLRs, NK cells express TLRs 1, 3 and 6, DC express TLRs 2,4,7,9, T cells of human peripheral blood express TLRs 1-5, 7 and 9. B cells express TLRs 3 , 4 and 9. TLR signals plays important roles in progenitors of myeloid and lymphoid. TLR activates the ligand to signaling cascade and produces chemokines and cytokines. After pathogenic recognition, TLR's signal transduction started through the Tol / Interleukin 1 receptor domain (TIR). Most TLR use MyD88 and TIRcontaining adapter to activate the signaling pathways of NF-κB to inflammatory cytokines process. While, TLRs are disregulated, there is high risk of serious inflammation and immunology diseases including autoimmune disease [54].

Pytochemicals was found in green tea because it have anti-inflammatory effects. This mechanism was explained that the targets affected are TLRs and downstream molecular signaling. It was also explained that the EGCG green tea flafonoid showed that modulator activity in specify pathways. EGCG flavonoids inhibit IKKβ downstream sinyaling from the MyD88 way and inhibit NFκB and it caused by TLR4 and TLR2 agonists. The TLR family and intracellular downstream have an important role in immunity of innate and adaptive. Activation of this signaling way shows in expression of various pro and anti-inflammatory molecules, which they are regulate homeostasis and metabolism. The changes or disability in this system will cause the infection, inflammatory diseases including autoimmune [54-58].

Conclusion

Environmental factors as triger and flare for SLE, EGCG also a potential agent to protect the presence of

oxidative stress, inflammation and immune dysregulation and also EGCG can be as an immunomodulation and inflammation respond by involving innate or adaptive immune functions. EGCG can be an antioxidant, anti-inflammatory and suppression of T cells. So, this can be as an alternative strategy to prevent and reduce autoimmune inflammation by T cells media. EGCG affects the human immune system and has therapeutic potential for autoimmune and inflammation process.

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A Prediction Model of Mortality in Patients Hospitalized with Diabetic Ketoacidosis in a Tertiary Referral Hospital in Surabaya, Indonesia

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Abstract

Background: The mortality rate of diabetic ketoacidosis (DKA) remains high in Indonesia. Different patterns were contributing to DKA mortality from previous studies. We aim to identify clinical and laboratory findings as mortality predictors of DKA.

Methods: This retrospective study was conducted in a tertiary referral hospital in Surabaya. Medical records of DKA patients from January 2016 to December 2018 were analyzed. Clinical and laboratory data were obtained from medical records for three years period. Clinical outcome was defined as either discharged home or death.

Results: Fifty-six among 116 DKA patients did not survive during hospitalized. Age of 60 or older, the depressed mental state with GCS below 13, potassium serum less than 3.5 mmol/L, and bicarbonate serum less than 15 mmol/L are found to be significant as independent factors of mortality in DKA patients.

Conclusion: The mortality rate of DKA patients in this study is still high. It independently associated with the age of 60 years or older, depressed mental state, hypokalemia, and low bicarbonate level. Early stratification of these predictors would help to treat patients accordingly.

Keywords: *prediction model, mortality, diabetic ketoacidosis*

Introduction

Diabetic ketoacidosis (DKA) is an acute complication of diabetes, still has a high mortality rate in Indonesia [1,2]. There is always a lack of annual incidence and mortality data in Indonesia, but two previous studies show that mortality was higher than another Asia and Africa countries [3–5]. The mortality rate of DKA in Cipto Mangunkusumo General Hospital compared to overall UK and US are 40% and <1% respectively [2,6]. This high mortality rate was associated with the characteristic of patient in tertiary hospital that tend to have low income, comorbidities, delayed in seeking medical treatment and severe infections [2].

DKA is characterized by a triad of hyperglycemia, ketonemia and metabolic acidosis [7]. It usually presents in short period of time associated to infections, drug or insulin noncompliance [8]. DKA could evolves rapidly and the diagnosis requires quick information obtained from Initial history taking, physical diagnosis and

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quick laboratory testing [6]. Treatment of DKA involves rehydration, correction of electrolyte derangements and metabolic acidosis, administration of insulin and treatment of precipitants [9]. Inadequate of either diagnosis or treatment of DKA could be a fatal prognosis [2,5,10]. Limited capacity of intensive care unit in Indonesia helps to stratify DKA patients to be treated at general or high-care wards.

The independent parameters associated to mortality of DKA were vary from each study [1–3,5,10,11]. Not only evaluated initial condition of DKA patients, some of these studies used patient's history or from evolving condition for following days [1,3,10]. Depressed mental state is most common variable that significantly independent to mortality of DKA [1–3,10]. Different study design, hospital regulation and demographic characteristic were taking account for different predictors of mortality. High mortality rate of DKA patients in Indonesia and in our hospital particularly, also limited availability of intensive- and high-care wards encouraged us to conducted a study using all available medical records.

The aim of this study is to analyze a prediction model for mortality of DKA using data obtained from initial patient's admission. It will help for better understandings in quick stratification and further management strategies despite a limited resources and facilities in our hospital.

Materials and Methods

This retrospective study was conducted in tertiary hospital of Dr. Soetomo General Hospital, Surabaya. All medical records under diagnosis of DKA from January 2016 to December 2018, aged ≥ 18 years were analyzed. All diagnosis of DKA in discharge resume were included, either in primary, diagnosis or complication diagnosis. Patients who discharged against medical advice were excluded.

Clinical outcome was defined as discharged home or death. Clinical and laboratory were obtained from emergency department at initial admission. All available data of demographic, clinical and laboratory were collected. Nine predictors were analyzed: age, Charlson's comorbidity index (CCI), depressed mental state, white blood cells (WBC), serum bicarbonate, osmolarity, albumin, potassium, and anion gap. The study was approved by the Local Ethics Committee.

All analyzed statistics were performed using Statistical Package for the Social Sciences. Subject's characteristics are presented as the mean with standard deviation or median with minimum and maximum range for continuous variables. The number of subjects for categorical data are presented as percentage. Chi-square test was used to analyze group differences for categorical variables. Multivariate analysis was done using all variable with p value < 0.25 from bivariate analysis. A p value < 0.05 from multivariate are considered as significant and formulated to be in prediction model.

Results

A hundred and twenty-three records were identified as DKA patients from discharged final diagnosis. Seven subjects were discharged against medical advice, left a hundred and sixteen subjects to be analyzed (**Table 1**). Deceased subjects had shorter length of stay (LOS) than discharged subjects (3 vs 8 days). Urinary tract infection (UTI) and pneumonia were most infections accompanied DKA subjects in this study. They took a proportion of 23.3% and 20.7% of infections respectively.

Table 1. Characteristics of subjects

Characteristics	Value (n=116)
Gender	
Male (n/%)	43/ 37.1
Female (n/%)	73/ 62.9
Outcome	
Discharged (n/%)	60/ 51.7
Deceased (n/%)	56/ 48.3

Cont... **Table 1. Characteristics of subjects**

Length of stay (days) Discharged (median/min-max) Deceased (median/min-max)	 8/ 3-19 3/ 1-11
Age (years) Discharged (median/min-max) Deceased (mean/SD)	 53/ 20-74 55.02/ 12.98
Referred from another hospital Referred (n/%) Non-referred (n/%)	 79/ 68.1 37/ 31.9
Ward Intensive/ high-care (n/%) Low care (n/%)	 45/ 38.8 71/ 61.2
Symptoms Depressed mental state (n/%) Dyspnea (n/%) Nausea (n/%) General weakness (n/%) Others (n/%)	 41/ 35.3 24/ 20.7 14/ 12.1 11/ 9.5 26/ 22.4
Glasgow Coma Scale Discharged (median/min-max) Deceased (median/min-max)	 15/ 3-15 12/ 3-15
Systolic blood pressure (mmHg) Discharged (median/min-max) Deceased (median/min-max)	 130/ 60-250 120/ 80-200
Diastolic blood pressure (mmHg) Discharged (median/min-max) Deceased (median/min-max)	 80/ 40-120 70/ 40-110
Infections Urinary tract infection (n/%) Pneumonia (n/%) Diabetic foot infection (n/%) Others (n/%)	85/ 73.3 27/ 23.3 24/ 20.7 11/ 9.5 23/ 19.8

Variables that had very high statistically significant were depressed mental status, potassium, and bicarbonate ($p < 0.001$). Osmolarity and WBC were found to be statistically very significant ($p < 0.01$). The rest of the variables in **Table 2** were not statistically significant as mortality predictors in DKA.

Table 2. Associations between categorical profiles and final outcome

Variables	Deceased		Discharged		p	OR	95% CI	
	n	%	n	%			Lower	Upper
Age*								
≥ 60 years	23	41.1	14	23.3	0.064	1.49	1.04	2.14
< 60 years	33	58.8	46	76.7				
Charlson's Comorbidity Index*								
≥ 3	12	21.4	13	21.7	1.000	0.99	0.63	1.57
< 3	44	78.6	47	78.3				
GCS*								
3-12	40	71.4	10	16.7	<0.001	2.53	1.76	3.65
13-15	16	28.6	50	83.3				
White blood cells**								
≥ 15000/mm3	15	26.8	2	3.3	0.002	1.89	1.30	2.74
< 15000/mm3	41	73.2	58	96.7				
Potassium**								
< 3.5 mmol/L	21	37.5	1	1.7	<0.001	2.56	1.94	3.38
≥ 3.5 mmol/L	35	62.5	59	98.3				
Albumin*								
< 3.5 g/dL	35	62.5	29	48.3	0.178	1.35	0.91	2.02
≥ 3.5 g/dL	21	37.5	31	51.7				
Bicarbonate*								
≤ 15 mmol/L	48	85.7	29	48.3	<0.001	3.04	1.60	5.78
> 15 mmol/L	8	14.3	31	51.7				
Osmolarity**								
≥ 320 mOsml/L	15	26.8	4	6.7	0.005	1.87	1.34	2.60
< 320 mOsml/L	41	73.2	56	93.3				
Anion gap**								
> 12 mmol/L	52	92.9	50	83.3	0.156	1.74	0.76	4.17
≤ 12 mmol/L	4	7.1	10	16.7				

*Chi Square test; **Fisher test

Eight out of nine variables that had $p < 0.25$ were analyzed in multivariate logistic regression. Statistically significant predictor from multivariate analysis were defined as $p < 0.05$. There were five variables in **Table 3** as

predictors of mortality in DKA patients. Depressed mental status had been highest statically significant predictors among others ($p < 0.001$).

Table 3. Multivariate analysis of variables independent to DKA mortality

Variables	p	b	OR	95% CI	
				Lower	Upper
Age ≥ 60 years	0.013	1.593	4.92	1.41	17.18
GCS < 13	0.001	2.144	8.54	2.47	29.49
Potassium < 3.5 mmol/L	0.001	4.844	126.9	7.82	2058
Bicarbonate ≤ 15 mmol/L	0.001	2.569	13.05	3.06	55.71
Constant (a)	<0.001	-4.320	0.013		

The prediction model of mortality was formulated from dividing the coefficient b by the standard error (SE) of all four were statistically significant variables ($p < 0.05$) in multivariate analysis. The prediction models were presented in **Table 4**. A Hosmer-Lemeshow test was shown good calibration in this prediction model ($p>0.05$). A good discrimination was shown from receiving operator curve (ROC) curve at **Figure 1** and presented area under the curve (AUC) value of 86.1% (95% CI 79.4–92.8).

Table 4. Formulated predictor scoring from significant variables

Variables	β	SE	β/SE	(β/SE)/2.114	Rounded value
Age ≥ 60 years	1.593	0.638	2.497	1	1
GCS < 13	2.144	0.633	3.387	1.35	1
Potassium < 3.5 mmol/L	4.844	1.422	3.406	1.36	1
Bicarbonate ≤ 15 mmol/L	2.569	0.74	3.472	1.39	1
Total score					4

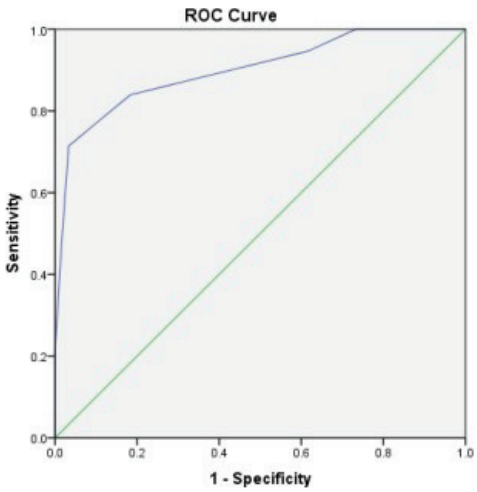

Figure 1. Area Under Curve (AUC) of prediction model after ROC

The probability of death for DKA patients based on 0–4 points for the total score in the prediction model as shown at **Table 5**. **Table 6** shows a prediction model of mortality in DKA patients. Sensitivity and specificity of this prediction model were 76.79% and 85% respectively.

Table 5. Mortality prediction based on patient score

Total score	y*	Probability (p)**
0	-3.175	4.01%
1	-1.047	25.98%
2	1.081	74.67%
3	3.209	96.12%
4	5.337	99.52%

$*y = -3.175 + 2.128(total\ score) y = -3.175 + 2.128(total\ score)$

$**p = 1/(1 + e^{-y}) p = 1/(1 + e^{-y})$

Table 6. Prediction model of mortality in DKA patients

No	Variables	Yes	No	Score
1.	Age ≥ 60 years	1	0	
2.	GCS ≤ 13	1	0	
3.	potassium < 3.5 mmol/L	1	0	
4.	Bicarbonate ≤ 15 mmol/L	1	0	
Total score:				

Discussion

The number of female subjects in this study were larger than male, that were consistent with other two previous study in Indonesia [1,2]. Out of 116 subjects, 56 subjects died (48.3%). This was higher mortality rate compared to other studies and was contrast compared to overall UK and US mortality rate that less than 1% [1–6,10,12,13]. This high mortality rate was associated with the characteristic of patient in tertiary hospital that tend to have low income, comorbidities, delayed in seeking medical treatment and severe infections [2]. three days (1-11 days) LOS of deceased subjects were similar with study from Efstathiou et al [10]. Most of DKA patients in this study were treated in low-care wards due to the limited intensive and high-care units. Most of DKA patients accompanied by comorbid conditions such myocardial infarction, congestive heart failure, acute renal failure, acute respiratory failure, altered mental status, coma, shock, hypothermia, sepsis, pancreatitis, gastrointestinal bleeding, uncontrolled hypertension end stage renal disease and hyperkalemia [14].

Depressed mental state was most clinical symptom found in the subjects (35.3%). Lower median of GCS in deceased subject compared to survivors that were related to the severity of acidosis and hyperosmolarity [15]. Other symptoms such dyspnea, nausea vomiting, and general weakness were consistent to be related to acidosis, electrolyte disturbance and dehydration [15]. Lower systolic and diastolic blood pressure in deceased group were consistent with study from Agarwal et al [5]. Lower blood pressure was related to severity of acidosis and dehydration [4].

Infection is most common precipitating factor of DKA in Indonesia [1]. In this study, infections were accompanied for 73.3% subjects. UTI and pneumonia had the largest proportion of infections that accounted for 23.3% and 20.7% respectively and consistent to other study [5]. The cause of death was mostly septic shock that could be associated to infections. Similar cause of death

was shown at study from Efstathiou et al. that systemic inflammatory response syndrome (SIRS) as cause of death [10].

Four variables that had highest statistically significant were known as predictor mortality in others study [1–3,11]. These were depressed mental state, leukocytosis, hypokalemia and low bicarbonate. Depressed mental state as predictor mortality in DKA and as most of clinical founding in the subjects, is related to severity of acidosis, hyperosmolarity and subject's comorbidity [4,16]. Leukocytosis in DKA may designate infection and need to be evaluated for source of infection [17,18]. Hypokalemia in DKA patient mainly caused by osmotic diuresis and may lead to decreased myocardial contractility and respiratory muscles [19,20]. Hypokalemia as one of significant predictor of DKA mortality in this study could be result as limited of intensive- and high-care wards that lead to suboptimal for evaluating electro-cardiac changes and potassium levels. Bicarbonate serum depletes during DKA, is a parameter of acidosis severity and could disrupt myocardial contractility [21]. Just like potassium, acid base correction with bicarbonate infusion requires close monitoring that were limited in our study center. Hyperosmolarity is related to osmotic diuresis and lead to hypovolemia and depletes electrolytes [6]. In this study, hyperosmolarity has statistically significant as predictor mortality of DKA subjects but not found to be significant in multivariate analysis.

Along with depressed mental state, hypokalemia and bicarbonate; Elderly was found to be significant as predictor mortality in multivariate analysis. This result was consistent with study from Mahesh et al [3]. Elderly as locally defined as age $\geq$ 60 years, tend to has severe comorbidity and lower insulin sensitivity were contributed to worse outcome and longer hospital stays [22–24]. The mortality prediction model was derived from these four parameters statistically significant from regression logistic. It is used as simple mortality prediction in term of risk at first admission. Each of four parameters is weighted as 1 in this scoring system as seen in table 6. Calibration and discrimination tests of this prediction model were found to be good with AUC is 86.1%. Sensitivity and specificity of this prediction model are 76.79% and 85%.

Our study provided simple risk prediction model that could help early assessment in admission. it consists of several clinical and laboratory parameter that can immediately obtained. Our study limitation that accessible medical records were only available in last three years' periods. Retrospective study design was failed to obtain some important data such history of previous ketoacidosis, type of diabetes, previous medical treatment and/or treatment compliance and complete history of subject's comorbidity.

Conclusion

The mortality rate of DKA patients in this study is 48.3%. High mortality rate in developed country still required specific attentions and further studies. Sixty years or older, depressed mental state, hypokalemia, and low bicarbonate level were associated with higher mortality rate.

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In-Vitro Differentiation Adipose-Derived Mesenchymal Stem Cells into Pancreatic Progenitor Cells

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Abstract

Background: Adult stem cells are currently reliable sources of mesenchymal stem cells for regenerative therapy, include diabetes mellitus. The aim of this study is to develop endocrine pancreatic progenitor cells characterized by Pdx1 and insulin expression from rat adipose-derived mesenchymal stem cells using two steps in-vitro differentiation.

Methods: In this experimental study, ADMSCs were isolated from rat adipose tissue and exposed to insulinogenic differentiation medium containing nicotinamide, activin A and glucagon-like peptide-1 (GLP-1). After induction, the existence of pancreatic progenitor cells (PPCs) was confirmed by immune-staining assay of Pdx1 and insulin.

Results: After three weeks of in-vitro differentiation, expression of Pdx1 and insulin proteins showed up as green in the immunofluorescence assay. Immunofluorescence intensity of Pdx1 was higher in PPCs than in ADMSCs control ($p<0.05$). Immunofluorescence intensity of insulin was also higher in PPCs than in ADMSCs control ($p<0.05$). Therefore, in-vitro differentiation was successful to develop PPCs from rat ADMSCs

Conclusion: This study has demonstrated the in-vitro differentiation of ADMSCs into PPCs that expressed Pdx1 and insulin

Keywords: *diabetes, adipose-derived mesenchymal stem cells, Pdx1, insulin*

Introduction

According to a report by the WHO, 442 million people suffer from diabetes worldwide[1] and more than 10 million people have diabetes in Indonesia [2]. Both type 1 and type 2 diabetes mellitus are characterized by destruction or dysfunction of pancreatic beta cells, regardless of the type of diabetes, the main focus of DM therapy is insulin therapy [3]. Currently, regenerative therapy is growing rapidly, including various studies that investigate the formation of insulin-producing cells (IPCs) from embryonic stem cells, umbilical cord, and various adult tissues [4].

Adult stem cells are currently reliable sources of mesenchymal stem cells for regenerative therapy. Among the various sources of adult stem cells, adipose tissue is an ideal population of stem cells. Adipose tissue contains an abundance of stem cells, which can be obtained by a procedure that is relatively easy, inexpensive and minimally invasive[5,6]. Several studies were conducted to differentiate MSCs into cells that were similar to pancreatic islets and which, when transplanted, proved to be functional and producing insulin in diabetic rats[6]. Pancreatic progenitor cells (PPCs) give rise to all cell

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types in the pancreas during development [7]. Pancreatic progenitors cells expressing the key transcription factor Pdx1 are recognized as the indispensable precursors of functional pancreatic β cells [7,8].

There are several main mechanisms of pancreatic regeneration such as replication of mature beta cells, differentiation of stem cells, cell fusion, and trans-differentiation of one type of stem cells to another type[9]. Adipose-derived mesenchymal stem cells (ADMSCs) are mesenchymal stem cells (MSCs) which obtained from adipose tissue and had unique characteristics making them suitable for differentiation into endocrine pancreatic progenitor[10,11]. Differentiation MSCs into endocrine progenitor pancreas can be carried out using many extrinsic factors such as nicotinamide, exendin-4, and GLP-1 receptor agonist[6].

The aim of this study is to develop endocrine pancreatic progenitor cells characterized by Pdx1 and insulin expression from rat adipose-derived mesenchymal stem cells using two steps in-vitro differentiation.

Materials and Methods

Isolation, culture and characterization of cells

Rats were obtained from Stem Cells Research and Development Center Universitas Airlangga, and this study was approved by the Ethics Committee of the same University. Subcutaneous adipose tissue from male Wistar rats was removed under sterile conditions, cut into small pieces and incubated to liberate the cells in 25 cm2 flasks containing Dulbecco's Modified Eagle's Medium (DMEM) and 1.0 mg/ml of collagenase. The cell suspension was centrifuged at 3500 rpm for 5 minutes at room temperature to obtain pellets containing cells from the stroma. Furthermore, erythrocyte lysis was carried out with ammonium chloride lysis buffer then washed with phosphate-buffered saline. Cells were maintained in DMEM (Invitrogen, Carlsbad, CA, USA) which had been supplemented with 20% Fetal Bovine Serum (FBS; Gibco, USA), 1% penicillin-streptomycin (Invitrogen) and 1% Amphotericin B (Gibco, USA). The cultures of ADMSCs were inspected every three days and passaged when the ADMSCs had reached approximately 80% confluence. The mesenchymal stem cells were isolated based on their ability to adhere to the bottom of the flask. The ADMSCs appeared spindle-shaped in the culture. They were harvested in passage 3 and characterized as mesenchymal stem cells using CD73 (1 mg/mL; BD Pharmingen), CD90 (1 mg/mL; BD Pharmingen, San Diego, CA, USA), CD105 (1mg/mL; BD Pharmingen), and CD34 (1mg/mL; BD Pharmingen).

In-vitro differentiation ADMSCs into PPCs

We performed the two-step differentiation protocol in this study for three weeks[12]. The cells that had been placed on multiwell plate M 24, each $5x10^3$ cells per well then cultured on insulinogenic differentiation medium containing 10 mmol nicotinamide (Sigma), 4 nmol activin A (Peprotech, Rocky Hill, NJ, USA), and 10 nmol glucagon-like peptide-1 (GLP-1, Sigma). During the first week of culture, cells were cultured in DMEM-high glucose (DMEM-HG) medium containing 10% FBS. Then two weeks later, culture in DMEM-low glucose (DMEM-LG) medium containing 10% FBS.

Characterization of Pdx1 and Insulin

The progenitor cells were placed on a multiwell plate M24 containing slides that were coated with poly-L-lysine with 6 slides of replication slides for each marker. The slides were fixed in 4% paraformaldehyde for 20 minutes, then added with Triton X-100 0.2% for 30 minutes and blocked with 5% normal goat serum (Sigma) for 20 minutes. Incubation of primary antibodies has been labelled with Pdx1 or insulin. It was continued with incubation of antibodies labelled FITC for 1 hour at room temperature, then washed with PBS-Tween 0.5% 2 times before reading it under a microscope. Images were captured using an Olympus CKX53 fluorescent microscope (Olympus, Japan). From each group, a minimum of ten slides were examined. Immunostaining intensity was estimated using quantitative score using digital software program ImageJ.

Statistical Analysis

Data distribution was analyzed using the Kolmogorov-Smirnov test. The comparisons of two group means of intensity were performed using Mann-Whitney tests, and $P<0.05$ was considered statistically significant.

Results

Cell surface markers of ADMSCs detected by

immune-staining revealed that ADMSCs expressed CD73, CD90 and CD105, whereas expression of CD34 was negative (Figure 1).

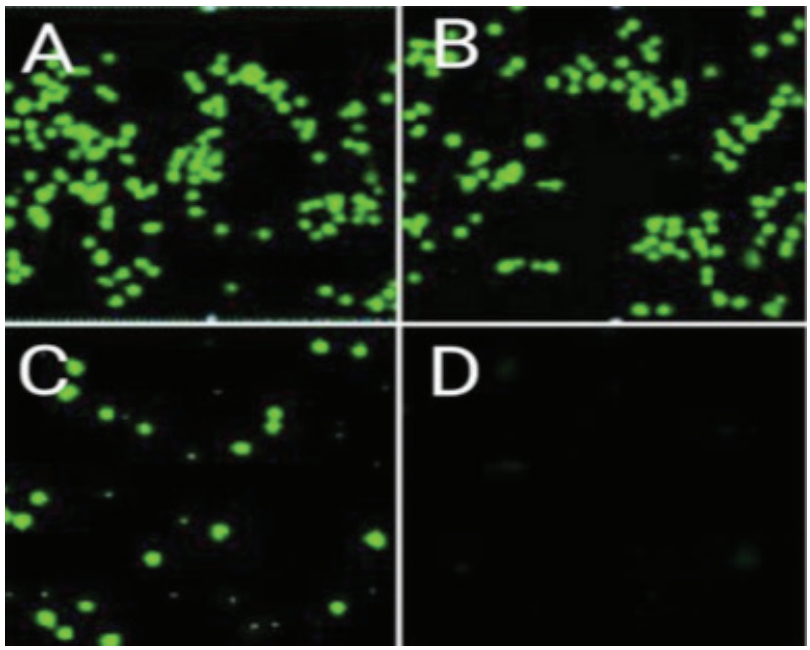

Figure 1. Immunofluorescence of cell surface markers. A. Expression of CD73, B. Expression of CD90, C. Expression of CD105, D. Expression of CD34 (magnifications: ×200).

After three weeks of in-vitro differentiation, expression of Pdx1 and insulin proteins showed up as green in the immunofluorescence assay (figure 2C and 2E). The ADMSCs cultured in non-differentiation inducing media (control group) showed very low expression of Pdx1 and insulin proteins (figure 2D and 2F).

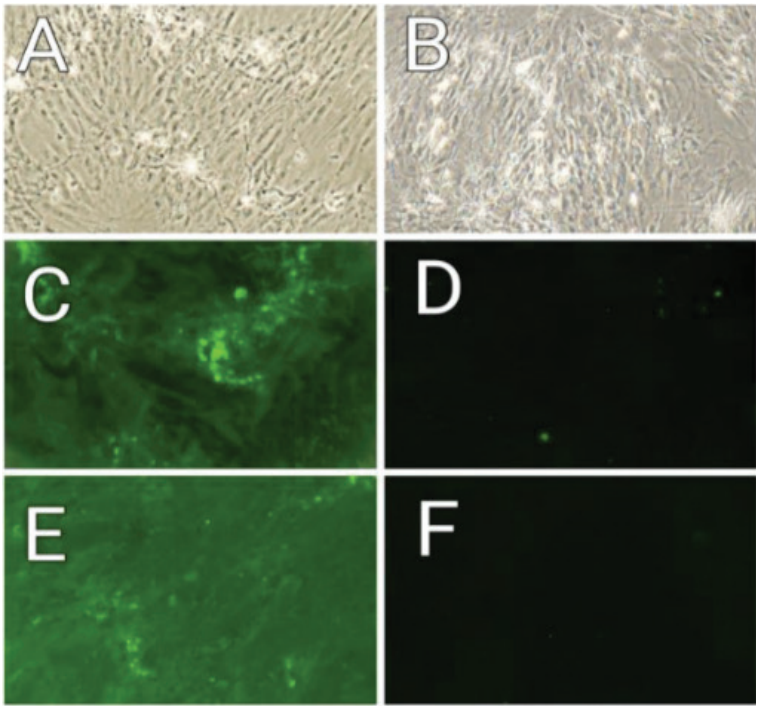

Figure 2. Progenitor cell identification and comparison with control. A. Progenitor cell proliferation. B. Control cell proliferation. C. Expression of Pdx1 on progenitor cells. D. Expression of Pdx1 on control cells. E. Expression of insulin on progenitor cells. F. Expression of insulin on control cells (magnifications: ×200).

Distribution of data was analysed using the Kolmogorov-Smirnov test and revealed that the distribution was not normal. Then the data were analysed using the Mann-Whitney test to compare immunofluorescence intensities of Pdx1 and insulin between PPCs and ADMSCs (control). Immunofluorescence intensity of Pdx1 was higher in PPCs than in ADMSCs control (p=0.0001). Immunofluorescence intensity of insulin was also higher in PPCs than in ADMSCs control (p=0.0001). Therefore, in-vitro differentiation was successful to develop PPCs from rat ADMSCs (table 1).

Table 1. Immunofluorescence intensities of Pdx1 and Insulin

Markers	PPCs		ADMSCs (Control)		Mann-Whitney Test
	Median	Range	Median	Range	
Expression of Pdx1 (/mm2)	12.455	8.479 - 15.547	5.486	4.263 - 6.187	P = 0.0001*
Expression of Insulin (/ mm2)	31.785	24.407 - 35.918	4.015	3.163 - 5.873	P = 0.0001*

*statistically significant (p<0.005)

Discussion

Stem cells are currently becoming the focus of beta cell replacement, due to flexibility and abundant sources. Stem cells derived from pluripotent embryonic stem cells (ESCs) have great differentiation potential, but ethical issues and malignant transformation are still the main problems[13]. Mesenchymal stem cells (MSCs) are found in various body tissues such as bone marrow, umbilical cord and adipose tissue and are immune-naive. MSCs derived from adipose tissue or called adipose-derived mesenchymal stem cells (ADMSCs) have the advantage of being easily obtained in abundance and have the potential to differentiate into various pathways, both mesoderm, ectoderm and endoderm[14]. Approximately 1-10% of the stromal fraction of adipose tissue are MSCs and they are capable to differentiate into multiple pathway[15]. Standard criteria for MSCs are positive phenotypes for CD105, CD73 and CD90, and negative phenotype for CD45, CD34, CD14 or CD11b, CD79 alpha or CD19[13,16]. The phenotypes characterization of MSCs in this study showed positive staining results for CD73, CD90 and CD105, as well as negative staining for CD34, according to the existing theory.

Pancreatic progenitor cells are multipotent cells derived from the endoderm that have the ability to differentiate into specific progenitor pathways that are responsible for pancreatic development[17,18]. In this study, ADMSCs were differentiated into pancreatic progenitor cells which later became insulin-producing cells. The induction of stem cells to become insulin-producing cells requires a multi-stage protocol. Most protocols require at least a two-stage protocol, while others can go up to 5-6 stages. It can last from several days to several months. Finally, the addition of combination extrinsic factors can stimulate the proliferation and differentiation of beta cells [19,20].

In this study, the differentiation of ADMSCs into progenitor cells was performed in two stages. The first stage used insulinogenic differentiation medium containing nicotinamide, activin A and glucagon-like peptide-1 (GLP-1) with DMEM-high glucose (DMEM-HG) medium containing 10% FBS for one week. Meanwhile, the second stage of the insulinogenic medium was cultured in DMEM-low glucose (DMEM-LG) medium containing 10% FBS for two weeks. Therefore, it needed a total of three weeks for the

differentiation of ADMSCs into insulin-producing cells[13,21]. The glucose medium had a function to increase beta-cell replication and hypertrophy. Nicotinamide is associated with the development of beta-cell outgrowth from the undifferentiated epithelial cell cluster. The growth factor activin A triggered beta cell regeneration by increasing the mass of beta cells. Meanwhile, GLP-1 accelerated the functional maturation of beta cells by secreting insulin [22,23].

Pancreatic and duodenal homeobox1 (Pdx1) is the first transcription factor produced by the pancreas. Loss of expression of this factor in humans and mice will lead to pancreatic agenesis due to the absence of endocrine, exocrine or pancreatic duct cells [24]. However, Pdx1 expression became limited to pancreatic beta cells only. If Pdx1 is removed from pancreatic beta cells, hyperglycaemia will occur due to reduced insulin-producing cells and an increase in glucagon-producing cells [25–27]. Pancreatic progenitor cells were characterized by expression of Pdx1, Myc and Ptf1A. Furthermore, cells expressing Ptf1A will differentiate into exocrine pancreatic cells [28]. In this study, the expression of Pdx1 and insulin were marker that showed ADMSCs differentiated into pancreatic progenitor cells. Expression of Pdx1 is a key factor that determines the fate and development of the pancreas, meanwhile, insulin was the target of progenitor cell treatment.

Conclusion

This study has demonstrated the in-vitro differentiation of ADMSCs into pancreatic progenitor cells that expressed Pdx1 and insulin. This method may help the formation of an unlimited source of cells for pancreas transplantation.

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Estimation of Lepitn, Melatonin , and Adropin Hormones in Gestational Diabetes Mellitus (GDM) in Tikrit City

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Abstract

The increased incidence of gestational diabetes (GDM), and its negative effect for the mother, and the fetus imposes the need to study the mechanisms which resulting in pregnancy complications development. This study was carried out to study the relationship between (GDM) and some hormones levels (liptin, melatonin, and adropin) in the three semester of pregnant women's, also estimation of (glucose, and lipid) profil). the study included (90) pregnant women divided to three groups (1st semester, second semester, and trimester) (30) women for each group. Also the study included (30) non infected women with (GDM). The results showed significant increase of leptin, and adropin($p \leq 0.05$) in first semester comparative with second and third semester and control group. There was significant decrease of melatonin in GDM comparative to control group. The level of lipid profile elevated in (GDM) comparative to non.

Key words: *liptin, melatonin, adropin, GDM, pregnant women's*

Introduction

Diabetes is defined as a chronic disease, which usually occurs because the body cannot use insulin in the correct form that produced from the pancreas or because the pancreas does not produce insulin in sufficient quantities[1] .One of the common disadvantages of diabetes are high blood sugar or what is known as "hyperglycemia". Over time, it causes serious damage to the body's systems, mostly blood vessels and nerves (WHO, 2016) [1.]

Gestational Diabetes Mellitus (GDM) is characterized when a pregnant has an onset, and first identification of glucose intolerance within (24 to 28) weeks of gestation. Mothers have a chance of GDM recurrence of up to 70 %, a five-to-10-year higher risk of type 2 diabetes, and can increase risk factor of cardiovascular disease[2.]

Leptin is hormone derived from adipose tissue , which is produced by some tissues, including many organs in humans body such as, the liver, intestine, and placenta[3,] the target organ of this hormone is hypothalamus receptors to minimize food ingestion, and raise the using of energy, previous studies suggest that "leptin also has a main effect on the regulation of whole body glucose homeostasis"[4] recent studies have demonstrated a strong association between obesity markers and plasma leptin levels, with a rises leptin concentration in pregnant women with improvements in mother fat stores, and glucose metabolism[5]

Melatonin is an omnipresent compound in almost living organism, including bacteria, animals, and to humans, in addition to being produced in peripheral tissues and work as an autocrine, and paracrine effect, is synthetized centrally by a "neuroendocrine organ, the pineal gland" [6.] Melatonin which produce by the pineal gland often released at the night, regardless of the species considered, and its development, and secretory event time are directly proportional to the night-time, Since its development is closely related to the light-dark cycle, the key Work integrative regular hormonal of melatonin is to organize behavioral approach, and physiological modifications to the day, and season of the geophysical environment[7] .

Adropin is a protein secretion from the liver, identified in 2008, [8] (Chen et al., 2017). It, encoded by "the *Energy Homeostasis Associated" (Enho)* gene, which is composed of (76 amino acids), That is present in many organisms such as humans, mouse, and rat [9]. Butler et al[10] mention that dietary fat ingestion in some persons which they have low concentrations of plasma adropin could increase circulating adropin levels shortly.

It also controls skeletal muscle energy metabolism and plays a significant role in the control of metabolic homeostasis. The liver has an important function in controlling glucose homeostasis in addition to muscle[11] .

Material and Methods

The samples the study:

this study included (90) pregnant women infected with gestational diabetes mellitus and (30) non- infected pregnant women as control group from Tikrit city / Salah El Din General Hospital and Outpatient clinics, infected women divided to three groups (first trimester (A), second trimester (B), third trimester (C), (30) women for each group. the period of this study was from 1/12/ 2019 to 1/3/2020.

Blood samples:

To estimation the biochemical variables in this study, (5ml) of blood was obtained from the pregnant of the all groups, then the blood samples were puts in centrifuge; to separate the plasma after (5000)rpm for 10 minutes. When the plasma was separating, puts in small plastic tub, and labeling, then transferred to refrigerator at (20-)C°. until the time of estimation.

Estimation of hormones:

(leptin, melatonin, and adropin hormones were estimated by ELIZA methods according to instruction of the kits by (bio-labo company).

Estimation of glucose, and lipid profile:

Lipid profile included(cholesterol, HDL, LDL, and triglyceride), and fasting serum glucose, were estimated according to colorimetric method by spectrophotometer at wave length 560 and 540, respectively [12] .

Statistical Analysis

The results were analyzed statistically by using the "SPSS program". The mean, and standard error were estimated, and the data were analyzed using the independent sample t-test

Results and Discussion

1. Level of lepitn, melatonin, and adropin

The results of Table (1) indicate a significant deference's in leptin, melatonin, and adropin concentration, since there was a significant increase ($p \leq 0.05$) in leptin level in groups (A, B, and C), which are the first, second, and third trimester of GDM, comparative with healthy pregnant women, while the results indicate that the hormone began to decrease in third trimester as shown in Table below. The mean level of melatonin hormone decrease significantly in three pregnant stages ($p \leq 0.05$) comparative with control group; especially in first trimester. the results of mean level of adropin showed significant increase in the all period of pregnant, comparative with healthy pregnant women at significant value ($p \leq 0.05$) .

Table (1) the mean and SD of the level of leptin, melatonin, and idropin in pregnant women

GDM groups	leptin	melatonin pg/ml	adropin ng/ml
first trimester (A)	18.9± 3.1a	32 ± 4.086 a	10.98 ± 2.09 a
second trimester (B)	16.2± 2.02 a	26±2.011 b	12.65± 1.04 a
third trimester (C)	13.6±5.06 b	24±2.021b	10.36± 2.054 a
Non-GDM	10.14± 2.33c	57±6.024 c	6.23± 0.11 b

Different liters indicate significant deference's at (p≤ 0.05).

In this study we indicate significant increase of leptin hormone in GDM Women, this results agree with previous study[3]. There was increase of serum leptin levels in (GDM) women comparative to non-infected women. The current findings are in accordance with research examining maternal plasma leptin. last studies estimated the linkage between the concentration of leptin, and insulin resistance[4]. Maghbooli et al (2007)[13] in his study institute that the concentration of leptin was "positively associated with insulin level and HOMA index" [14]. The positive and impotent relation between the levels of gestational leptin, and fasting insulin is indicated. Vitoratus et al (2001) have found such a relationship[15]. studies indicate that leptin also has role effect on the regulation of glucose homeostasis for whole body. the concentration of maternal leptin rises comparative with non-pregnant, and the peak arrived at 28 weeks of pregnancy [4]. Many factors may affect the level of liptin in which the determination of leptin concentrations were after the GDM diagnosis, observed variations in the concentrations of this hormone in plasma following the infection with GDM, or whether the discrepancies may be due to improvements in glucose metabolism linked to disease " leptin hormone play important role in metabolism process; that release the energy required for both fetus, and the mother; so the increasing of this hormone may be associated to increase the metabolism process during the pregnant period; also to regulate the blood glucose in GDM period.

Melatonin hormone was decrease significantly in this study (p≤ 0.05) of the GDM women comparative to non-GDM this result agree with previous study[16]. Melatonin has been detected in all body tissues, blood, and other immune system cells [4]. Hence, its production in eukaryotic cell mitochondria suggests the unusual defense of cellular melatonin organelles from oxidative harmful, and the preservation of their physiological functions. First, melatonin, and metabolites substances act as direct absorbers of free radicals produced at pregnancy period; to induce antioxidant enzymes, thus "providing stable defense against free radical damage at the level of cells and tissues in a single mother-fetuses placenta system"[16]. This explain the lowest of it level during the pregnancy because the GDM women consumption a lot of this hormone to keep the fetus health.

Proving the function of Melatonin in researchers' development of gestational diabetes data found less melatonin secretion; and the lack of the usual level in the second/ or third trimester of pregnancy problem; "the sleep disorder, the shortening of a night's sleep and quality features"[7].

In our study serum adropin in GDM women, diagnosed in the first , second, and third trimester, is significantly high for non-diabetics. The release mechanism remains uncertain, the rapid energy- and nutrition status regulations have been observed[17.] indicate the positive effect of adropine on glucose tolerance in their study of mice which suffering from obesity and insulin resistance. They concluded that "adropin improves glucose clearance, ameliorates insulin resistance" . this explain the increasing of adropin level in pregnant diabetic women.

One of many adaptive reaction to harmful glucose metabolism during pregnancy according to recent studies, may be hypothesized to be higher concentrations of adropin in women with GDM [18]. Adropin can be using as a diagnostic biomarker for insulin resistance as was reported. Topuz et al in there research of adropin in type 2 diabetes mellitus patients were considered an separate danger factors for endothelial dysfunction[19]. Endothelial dysfunction during pregnancy may lead to placental impairment in the 1st trimester, The high prevalence of late pregnancy complications such as preeclampsia, disorder of fetal growth, placental stopping, or fetal death during pregnancy. This explain the important role of this hormone during pregnancy period.

Level of glucose and lipid profile

The mean level of F.S. G (mg/dl) increase significantly (p≤ 0.05) in GDM patients which compared to control group for all pregnancy period as showed in table (2) below, this finding agree with previous study that indicate significant elevated in serum glucose during pregnancy[20]. The results indicate significant increase of total cholesterol, triglyceride, and LDL in the three gestational period (p≤ 0.05) as compared to non-GDM women, while there was significant decrease of HDL level (p≤ 0.05) in GDM compare to non.

Table (2) the mean and SD±, of serum glucose and lipid profile in GDM and non-GDM women.

Variables	GDM women			Non-GDM
	Group A	Group B	Group C	Mean
FSG mg/dL	188.7±6.03a	195.21±7.33a	178.38±5.04a	89.58±1.57b
T. cholesterol	179.1±8.23a	189.52±4.3a	185.47±6.05a	144.8±3.58 b
TG	169.58±8.48a	185.6±7.42a	178.05±11.58a	135.25±4.8b
HDL	35.25±3.58a	36.89±2.45a	39.58±1.35a	49.57±4.31b
LDL	110.03±7.35a	90.24±6.79b	120.35±8.25a	84.4±5.98b

Different liters indicate significant deference's ($p \leq 0.05$)

Fat metabolism is fundamental, for a healthy pregnancy growth. Plasma lipid profile are "the levels of TC, HDL-C, LDL-C, and TG changes apparently during normal pregnancy"[21]. "Due to estrogen stimulation and insulin resistance, plasma lipid concentrations increase during pregnancy[22]. There is an increase in maternal fat aggregation during the first two-thirds of gestation, associated with" both hyperphagia and increased lipogenesis" [23]. However, as a result of increased the activity lipolytic enzyme, and decreased the activity of lipoprotein lipase, maternal fat accumulation decreases in the last 3rd trimester of pregnancy, these changes are a reflection of maternal physiological adaptation to the fetus' energy demand, They are also essential for delivery, growth, and lactation preparations [24]. There has been extensive analysis of circulating lipid plasma patterns during normal pregnancy, and most studies have found that serum TG, HDL-C, LDL-C, and TC levels are "obviously elevated throughout the pregnancy" [25]

The TG / HDL-C ratio may be helpful in determining insulin resistance during pregnancy, and its level in early pregnancy may be a good predictor of GDM occurrence. These elevation of lipid profile in GDM may be due to; hormonal changes, administration of drugs during pregnancy, increase of blood volume and coronary heart disorders, or by the increase of body weight, metabolic disorders, the type of intake food, and insulin resistant.

Source of Finding: Self

Conflict of Interest: none

Ethical Clearance: taken

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Study of Laparoscopic Cholecystectomy in case of Complicated Cholecystitis

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Abstract

Cholelithiasis is one of the commonest disorders of GIT. Cholecystectomy was done traditionally by open or conventional cholecystectomy. But now Laparoscopic approach is believed to be the gold standard in treatment of calculous cholecystitis. Common problems encountered during Laparoscopic approach are Difficult dissection due to distorted Anatomy, Infection at GB Fossa, Frozen Callot's triangle [1], hypervascularity. In this study we included 30 patients having complicated calculous cholecystitis who went under laparoscopic cholecystectomy. They were assessed to determine the feasibility and safety of laparoscopic approach in complicated calculous cholecystitis. e.g. Gall bladder Mucocele, Gall bladder Empyema [2], Gangrenous changes in Gall bladder, Gall bladder Perforation, Common Bile Duct stones along with Cholangitis /Pancreatitis. Early post-operative ambulation (within 1 to 2 days) along with a lesser duration of post-operative hospitalisation, decrease chance of sepsis during post-operative period and decrease in morbidity along with lesser complication rate can be achieved with laparoscopic cholecystectomy in complicated calculus cholecystitis.

Keywords: *Cholecystectomy, Abscess, bile duct*

Introduction

Cholelithiasis is one of the commonest disorders of GIT. Cholecystectomy was done traditionally by open or conventional cholecystectomy. But now Laparoscopic approach is believed to be the gold standard in treatment of calculous cholecystitis. Advantage of Laparoscopic Cholecystectomy are having lesser morbidity, shorter hospital stay, less post-operative discomfort and cosmetic advantage. But it is difficult to decide about the preference of Laparoscopic approach in complicated cases. Such as ,Patients having Calculous Cholecystitis along with,

a) Gall bladder Mucocele

b) Gall bladder Empyema [2]

c) Gangrenous changes in Gall bladder

d) Gall bladder Perforation

e) Common Bile Duct stones along with Cholangitis /Pancreatitis

Difficult dissection due to distorted Anatomy, Infection at GB Fossa, Frozen Callot's triangle [1], hypervascularity are commonest problems which have to be face in such complicated cases. In such complicated cases laparoscopic approach may increase the chance of CBD injury and injury to surrounding structures.

Aims

To Determine if laparoscopic cholecystectomy is safe and feasible in case of complicated calculous cholelithiasis.

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Objectives

1. To identify cases of complicated calculous cholecystitis.

2. To analyse clinical features and radiological findings in complicated calculous cholecystitis.

3. To analyse outcomes of laparoscopic approach in treatment of complicated calculous cholecystitis.

Materials and Methods

30 patients having complicated calculous cholecystitis who went under laparoscopic cholecystectomy in Department of General Surgery, Dhiraj Hospital, SBKS Vidyapeeth, Vadodara from November 2018 to January 2020 were assessed to determine the feasibility and safety of laparoscopic approach in complicated calculous cholecystitis.

Patients should undergo laparoscopic cholecystectomy or ERCP followed by laparoscopic cholecystectomy.

Inclusion Criteria

1. patients having age more than 18 years

2. patients having Calculus cholecystitis with:

a) Gall bladder Mucocele

b) Gall bladder Empyema [2]

c) Gangrenous changes in Gall bladder

d) Gall bladder Perforation

e) Common Bile Duct stones along with Cholangitis /Pancreatitis

Exclusion Criteria

a) Asymptomatic Cholelithiasis

b) Incidental case of Cholelithiasis

c) Acute/Chronic cholecystitis due to Gall stones

Observation and Analysis

Figure 1,2,3,4,5,6,7A,7B,8

Discussion

} Patients with gall bladder gangrene or perforation presented with an acute abdomen while patients with Common Bile Duct stones presented with fever and jaundice.

} All 5 patients having Gall bladder perforation were having DM.

} Out of the 16 patients of calculous cholecystitis with cholangitis all had Common Bile Duct stones and were subjected to ERCP with stone removal and stenting followed by laparoscopic cholecystectomy.

} 2 patients of calculous cholecystitis with pancreatitis had raised serum amylase and lipase levels, out of which both had Common Bile Duct stone and were subjected to pre op ERCP with stone removal and stenting followed by laparoscopic cholecystectomy.

} Early identification of patients having calculous cholecystitis with complications mentioned above can be made by a proper clinical judgement and early radiological investigations like Ultrasonography and Contrast Enhanced Computerised Tomography of Abdomen.

} Out of 30 cases,

} 18 cases underwent antegrade cholecystectomy

} 12 cases had undergone retrograde cholecystectomy[3] due to Frozen Callot's triangle[1] or a large stone at Hartmann's pouch and if the stone size was equal or more than 2cm and mostly it was found to be impacted at the Hartman's pouch , in this scenario after opening the gall bladder wall , the stone along with sludge removed and put in retrieval bag.

} Out of 30 cases

} 16 cases had undergone Total Cholecystectomy

} 8 cases had undergone Subtotal Cholecystectomy, in which the gall bladder dissection was done in a retrograde manner and was excised just before the Callot'striangle [1] and intracorporeal suturing were done out of which 2 had undergone pre op ERCP.

} 6 cases had to be converted to open cholecystectomy

} Due to thickened gall bladder wall and frozen callot's triangle[1]in 2 case,

} 4 cases of Type 1 Mirizzi's Syndrome due to common bile duct injury out of which 2underwent hepaticojejunostomy and 2 case of Common Bile Duct rent was closed with T tube insertion and along with choledocoduodenostomy.

} Post operatively out of 55 patients

} 6 cases developed infection at port site (epigastric).[4]

} 2cases were having bile leak which was managed by conservative approach.

} 1 case developed post-operative gall bladder fossa collection which was also managed conservatively.

Conclusions

From this study of 30 patients having complicated calculus cholecystitis undergoing Laparoscopic Cholecystectomy the conclusions were made:-

} Not all the patients having Complicated Calculus Cholecystitis present with symptoms of pain at Right Hypo-gastric Region, fever and jaundice. So, a high index of suspicion is needed.

} Pre-op blood investigations combined with radiological investigations can help to detect patients with complicated calculus cholecystitis.

} Patients having Common Bile Duct stones, Laparoscopic Cholecystectomy was done post ERCP within 24 hours to minimise technical difficulties in dissection due to fibrosis and adhesions.[5]

} Patients having peritonitis due to gall bladder perforation, gallbladder empyema were operated immediately within 24 hours and early ambulation was done and had small hospital stay.

} Technical difficulties in dissection was encountered in complicated calculus cholecystitis due to :

} Adhesions between the gallbladder, omentum, duodenum and transverse colon.

} Frozen Calot's triangle.

} Gallbladder wall thickening.

} Pericholecystic collections along with contaminations.

} Increased bleeding/oozing.

} Easy friability of the gallbladder and surrounding structures.

Early post-operative ambulation (within 1 to 2 days) along with a lesser duration of post-operative hospitalisation, decrease chance of sepsis during post-operative period and decrease in morbidity along with lesser complication rate can be achieved with laparoscopic cholecystectomy in complicated calculus cholecystitis.

Ethical Clearance Taken from sumandeep vidyapeeth institutional ethics committee

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Immunohistochemical Expression of Myeloid Differentiation Adaptor Protein-88(MYD-88)and Toll like Receptor (TLR-4) in Human Leiomyomas

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Abstract

Leiomyomas are the most common benign tumor of the female reproductive tract. The aim of this study was to explore the role of TLR4/ MYD-88signaling pathway in the pathogenesis of leiomyomas. Benign nature and smooth muscle origination of leiomyomas was easily documented via histological examination, in this cross-sectional study of 20 conventional leiomyomas cases TLR4/ MYD-88 antibody status was positive in 90% for TLR-4 and in 95% for MYD-88 of leiomyomas. Leiomyomas were stained with TLR4/ MYD-88 marker more than those in normal myometrium cases (P<0.001). In conclusion leiomyomas growth may be associated with TLR4 and MyD88 expression

Key words*: Leiomyomas, TLR4, MYD-88, Myometrium,Immunohistochemistry*

Introduction

Uterine fibroids or leiomyomas of the uterus that arise from connective tissue and smooth muscle overgrowth of in uterus are benign tumors . Histologically, a monoclonal proliferation in smooth muscle cells occurs, it can develop wherever smooth muscle is present[1].

Women at reproductive age are mostly affected, incidence of fibroids is about 70% in women at 50 years age [2,3,4],most women develop uterine fibroid tumors before menopause[5,6] and are the leading indication for hysterectomy. When MRI examination revealed an abnormal increase in the size of a known uterine *leiomyoma* of the uterine wall. A treatment consisted of cyclophosphamide, adriamycin, and cisplatin with poor outcome [7], some women have painful, heavy periods while most women have no symptoms [8.] If fibroid become large, may push the bladder causing a need to urinate[8] . Female can have few or many uterine fibroid these make it difficult to pregnant, but this is uncommon[8]. Obesity and dietary components [8,] with hormone levels and genetic predisposition to leiomyoma growth often exists [9].

Estrogen and progesterone have a mitogenic effect on leiomyoma cells by influencing cytokines, hormones and a number of growth and apoptotic factors[10], estrogen promotes growth by increasing Insulin-like growth factor-1IGF-1, T*ransforming growth factor beta 1*,3 and decreasing p53. Progesterone promote the growth and survival of leiomyoma by up-regulating *Epidermal growth factor* EGF, B-cell lymphoma- 2 (Bcl-2) expression and down-regulating TNF-alpha[10] .

Five different fibroids classifications : 1-Intramural fibroids in the wall of the uterus the most common type.2- Subserosal fibroids in outer wall of the uterus can often grow to be larger and form a “stalk” on which the mass is attached (pedunculated fibroids). 3-Submucosal fibroids in muscle beneath the lining of the uterine wall (endometrium). 4-Intracavity fibroids in the cavity of the uterus. 5-Cervical fibroids form in the cervix of uterus,[11].

In humans the transmembrane protein Toll-like receptor 4 is a member of pattern recognition receptor (PRR) family encoded by the TLR4 gene. Its activation leads to an intracellular signaling pathway Nuclear factorNF-κB and inflammatory cytokine production which is responsible for activating the innate

immunity [12]. It is recognizing lipopolysaccharide (LPS) of Gram-negative bacteria as in Neisseria and Gram-positive bacteria also some proteins viral and a different endogenous proteins such heat shock protein [13]. TLR4 can detect on many tumor cells. It is capable of activating mitogen-activated protein kinase and pathways of NF-κB increased metastasis and carcinogenesis of tumor, inhibition apoptosis [14].On the other hand, TLR4 in inflammatory and immune cells of tumor microenvironment may lead to production of (Interleukin-6IL-6, tumor necrosis factorTNF, Interleukin 1 betaIL-1β, and IL-18) immunosuppressive cytokines, proinflammatory cytokines, (IL-10, TGF-β, etc.) and mediators angiogenic (Vascular endothelial growth factor VEGF, EGF, TGF-β), [14]. Myeloid differentiation primary response 88 (MYD88) is a protein that, in humans, is encoded by the MYD88gene[15]. The gene was originally discovered and cloned by Barbara Hoffman and Dan Liebermann in mice model[16], TLRs used an adapter proteinMYD88 to activate the transcription factor NF-κB., (TIR Domain Containing Adaptor ProteinTIRAP is necessary to recruit Myd88 to TLR 2,4, and MyD88 then signals by (interleukin-1 receptor-associated kinaseIRAK[17]. MyD88 mutant can act as a driver [18], promote tumor growth by its overexpression [19].

Materials and Methods

This retrospective study included 20 tissue biopsies of leiomyomas and 20 apparently normal myometrium biopsies collected from Al- Yarmouk Hospital in Baghdad. A specialist pathologist defined leiomyomas according to FIGO classification system [11].

Tissue preparation for Immunohistochemical diagnosis of TLR4 and MYD88 in section of paraffin-embedded.

Formalin10% were used to fix Biopsies, then biopsies dehydrated in ethanol and cleared with xylenes and finally embedding in paraffin [20].

· Immunohistochemical staining was performed using:-

a-Mouse monoclonal primary antibodies TLR-4(Santa cruz USA) Isotype: IgG

b- mouse monoclonal primary antibodies MYD88(Santa cruz,USA)Isotype: IgG,

c-PolyExcel HRP/DAB Detection System Secondary antibody, kit for Mouse and Rabbit Primary Antibodies (Pathnsitu, USA), which contain:-

1-Polyexcel HRP: Goat anti-rabbit Conjugate with HRP,50 ml (Pathnsitu, USA).

2-Polyexcel target binder :50 ml of rabbit anti-mouse (Pathnsitu, USA).

Procedure

· Four µm Sections of paraffin embedded leiomyomas specimens were cut and mounted on poly-lysine coated charge slides.

· Tissue slides deparaffinized with xylene, rehydrated in ethanol 95, 80 and 70% ,after that sections were subjected to antigen retrieval of citrate buffer (pH 9.0) by boiling in microwave oven for 10 min. Then endogenous peroxidase activity blocking with hydrogen peroxide. Following that for 1 h the sections were incubated with ready to use primary TLR-4 and MYD88 antibodies in a humidified chamber. The sections then incubated with polyExcel rabbit anti- mouse secondary antibody (Pathnsitu,USA) for 10 minutes then with PolyExcel HRP of Goat anti-rabbit Conjugate with HRP (Pathnsitu, USA) for 10 minutes then protein were detected with 3,3′-diaminobenzidine(DAB) substrate –chromogen that result in brown colored at the antigen site, finally section was stained with Mayer's hematoxylin for 1 min at room tempreture, rinsed with water, dehydrated in graded ethanol and xylene, covered with appropriate mounting media of DPX and cover slip.

Slides were examined by light microscope .The number of positive stained cells was evaluated as follows:

0, less than 5%;

1, 5% to less than 25%;

2, 25% to less than 50%;

3,50% to less than 75%;

4,more than 75%.

The intensity immunostaining was evaluated as: no staining 0; weak -light yellow1; moderate-yellow2; intense-brown yellow3. The total score was obtained

by multiplying the percentage and intensity score of stained cells. The final score less than 6 represented low expression and that of greater or equal to 6 represented high expression[21].

The prevalence rate was evaluated by the TLR4 and MYD88 index, known as the percentage and intensity of positive TLR4 and MYD88 cells. This indicator was determined by calculating TLR4 and MYD88 positive cells in at least 1,000 cells in different randomly selected areas at 400 magnifications.

Statistical Analysis

Chi-square was used as test to compare significant between percentage (0.05 and 0.01 probability) in this study.

The Results

A. The scoring of TLR-4 in leiomyomas and normal myometrium tissues

The percentage and staining intensity of positive stained cells in patients and controls were in (Table-1). The multiply of both percentage and staining intensity gave the final scores of TLR-4 in sample. The total score less than 6 represented low level expression and that greater or equal to 6 represented high level expression(20),(Table-2), it was found that TLR-4 was 90% highly expressed in leiomyomas tissue while 85% normal myometrium (biopsies) showed low/negative expression, with significant difference($P \leq 0.01$) between these groups (Figure 1). The cytoplasmic expression of TLR-4 was found in uterine smooth muscle wall of *leiomyoma* group.

Table-1: The percentage and intensity staining of positive TLR-4 cells of in leiomyomas, normal myometrium biopsies.

The Percentage score/TLR-4	TLR-4 intensity score						Total No.
	NO/scor0	Weak/score1	Moderate/score 2		Intense/score 3		
	Patients Num.(%)	Control Num.(%)	Patients Num.(%)	Control Num.(%)	Patients Num.%)	Control Num.(%).	
Score zero **<5%**	0	17	0	0	0	0	17
score1 5-≤25%	0	0	0	0	0	0	0
score2 25-≤50%	0	0	2	0	0	0	0
Score3 50-≤ 75%	0	0	0	3	12	0	15
score4 **≥ 75%**	0	0	0	0	6	0	6
Total No.	0	17	2	3	18	0	40

Table-2: Final TLR-4 score in leiomyomas , normal myometrium biopsies.

expression of TLR-4	Patients Num.(%)	Normal myometrium Num. (%)	Total Num. (%)	Chi-Square (χ2)
Highly expression (≥6)	18 (90.00)	3(15.00)	21	14.52 **
Low/negative expression (<6)	2 (10.00)	17(85.00)	19	14.52 **
Total No. (%)	20	20	40 (100%)	
Chi-Square (χ2)	14.10 **	12.94 **	---	---
** (P≤0.01).				

B-Scoring of MYD-88 in leiomyomas and normal myometrium tissues

The percentage and intensity of positive stained cells in leiomyomas and normal biopsies (Table -3), multiplied 0–12 then divided to: High expression (≥6) and Low/negative expression (<6).

It was found that MYD-88 was highly expressed in 95% of leiomyoma tissue and in 5% of normal myometrium biopsies, with significant difference between two groups was noted p<0.001 (Table-4 , Figure2). The cytoplasmic or membrane expression of M YD-88 was found in uterine muscle wall of *leiomyoma* group.

Table-3: percentage and intensity of MYD-88 positive cells in leiomyomas, normal myometrium biopsies.

Percentage score of MYD-88	Intensity score of MYD-88						Total No.
	No/ Score 0, weak/ score1		Moderate/Score 2		Intense/Score 3		
	Patients Num. (%)	Control Num.(%)	Patients Num.(%)	Control Num.(%)	Patients Num.(%)	Control Num.(%)	
Score 0 <5%	0	19	0	0	0	0	19
Score1 5-≤25%	0	0	0	0	0	0	0
Score2 25-≤50%	0	0	1	0	0	0	1
Score 3 50-≤ 75%	0	0	0	1	11	0	12
Score4 ≥ 75%	0	0	0	0	8	0	8
Total No.	0	19	1	1	19	0	40

Table -4: Final score of MYD-88 expression in leiomyomas patients and normal myometrium biopsies.

MYD-88 expression	Patients Num.(%)	Normal myometrium Num.(%)	Total Num.(%)	Chi-Square (χ2)
High expression (≥6)	19 (95.00)	1(5.00)	20	14.63 **
Low/negative expression (<6)	1 (5.00)	19 (95.00)	20	14.63 **
Total No. (%)	20	20	40(100)	---
Chi-Square (χ2)	14.63 **	14.63 **	---	---
** (P≤0.01).				

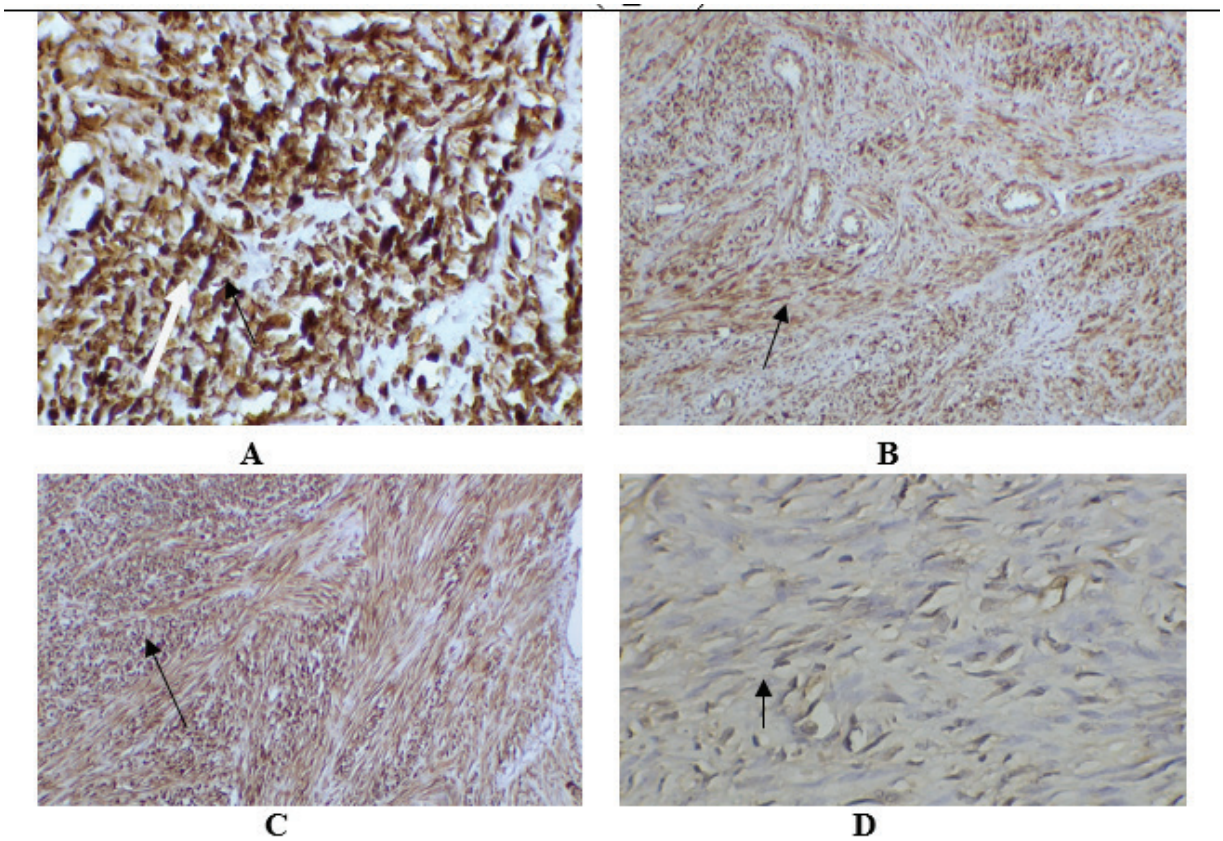

Figure (1): Immunoreactivity for TLR-4 in leiomyomas of uterine muscles, brown discoloration of cytoplasm,(A)high expression X 200;(B,C) high expression X 100;(D) negative expression X 200.

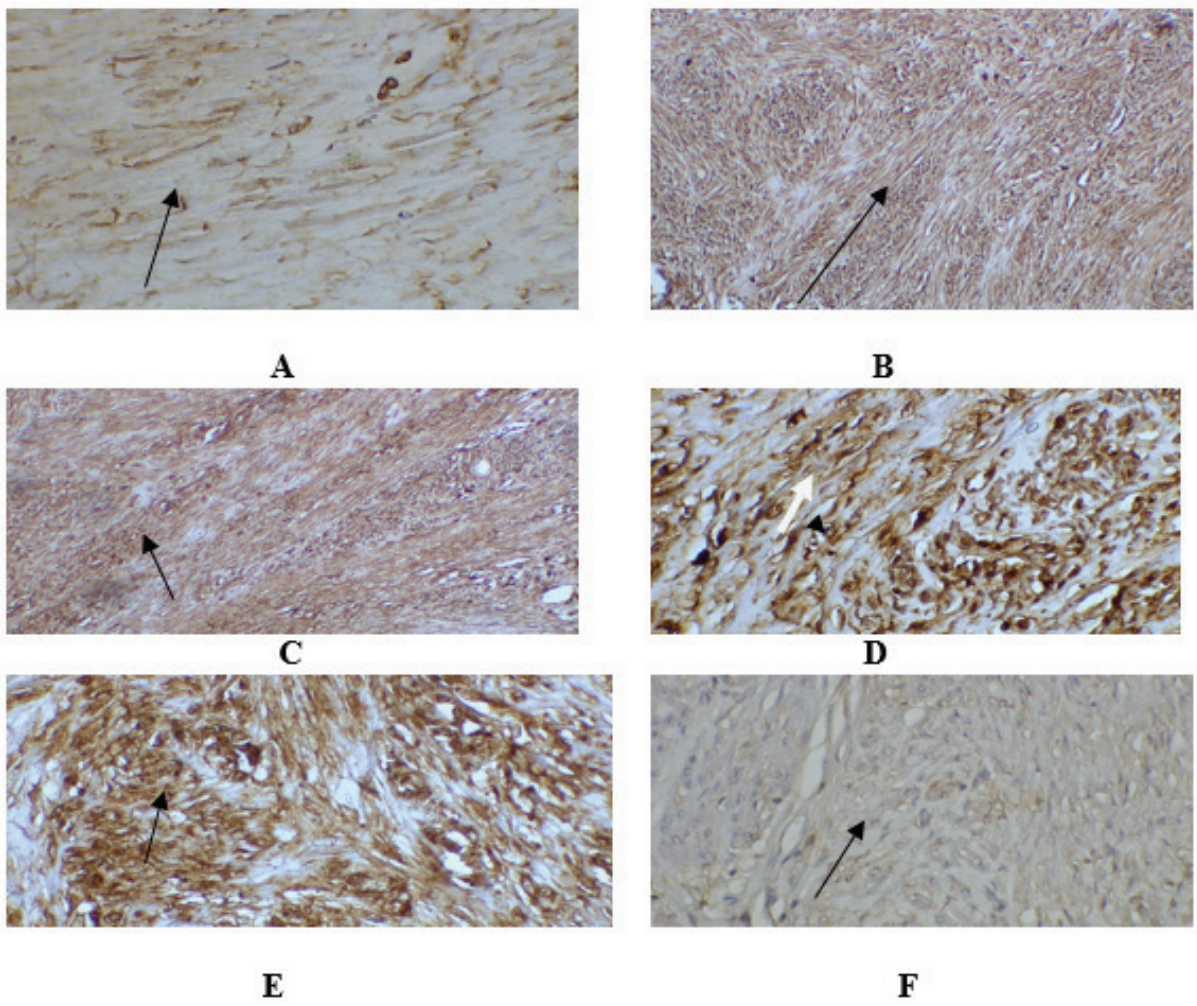

Figure (2):Immunoreactivity for MYD-884 in leiomyomas of uterine muscles, brown discoloration of cytoplasm or membrane, (A)low expression X 200; (B &C)high expression X 100; (D&E)high expression X 200; (F) negative expression X 200.

Discussion

Around the world most common neoplasms of the female genital tract arising from smooth muscle cells of uterus [22,23,] once leiomyomas are benign lesions of the uterus [24] Neoplasm of uterus constitutes 1% of uterine malignant smooth muscle tumours[25,26].

A distinguishable difference regarding expression of TLR-4; 90%cases of leiomyomas had highly cytoplasmic immunoreactivity, while15 % of normal myometrium was positive. This result is similar to what [27] reported and there was statistically significant difference was found.

The pathogen-associated molecular patterns (PAMPs) and damage-associated molecular pattern (DAMPs) recognize by the toll-like receptor4, which expresses on immune and tumor cells, activation of TLR4 in tumor can boost immunity antitumor in addition to raise immune surveillance and tumor progression[28]. TLR4 can signal through TIR-domain-containing adapter-inducing interferon-β ,*TRIF* to induce type I IFN through *Interferon regulatory factor 3* IRF3 and through MyD88 to induce pro-inflammatory cytokines *via* nuclear factor, NFκB[29], these signaling events are separated, signaling from TRIF-related adaptor molecule/TRIF (TRAM/TRIF) begin from endosomes while TIR Domain Containing Adaptor Protein TIRAP/MyD88 signaling start from the surface[30].

Out of 20 leiomyomas cases, (95%) was with high expression of cytoplasmic and/or membranous MYD-

88, while only (5%) of normal myometrium was with high expression, test Chi –square highly significant difference (P= 0.0001) in MYD-88 level of two group .The adaptor protein MyD88 regulate innate immunity against bacterial and viral diseases in the body. MyD88 recruit to activate the MyD88-dependent pathway after Interleukin 1 receptors (IL-1R) with Toll-like receptors recognize pathogens, but MyD88 mutation associated with development of lymphoma, inflammation progression and carcinogenesis. The signaling by MyD88 played dual duty in cancer by enhancing and promoting of tumor and inflammation, flora imbalance of intestine so to induce invasion and cell self-renew of tumor, and anti-tumor function to maintain homeostasis in host and immune responses against cancer cells and tumor cell cycle arrest [31].

During inflammation TLR4/MyD88 signaling mostly occurs [32], Repressing TLR4/MyD88 signaling lowering cell viability, enhancing apoptosis and up the levels of inflammatory factors following infection in macrophages [33]. Strong correlation between the inflammatory microenvironment of tumor and progression, prognosis of a number of cancer types as ovarian, uterus, rectal and prostate cancer [34].

Conclusion

Significant highly expression in the TLR4 and MYD88 in leiomyomas female cases than control group which indicate a strong relation between their high expression and tumor progression and inflammatory signaling induction in.

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Autopsy Death Profile Due to Railway Injury in Mangalore, A Coastal City of South Karnataka- A Retrospective Analysis

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Abstract

Background: Accident deaths is one of the most common types of deaths in India. In India railway related deaths are reported throughout the year. Fatal trains impacts cause extensive musculoskeletal injuries due to the nature of the object as well as the amount of force transmitted during impacts. The purpose of all accident investigation to take a positive step in correction of causation and to prevent such incidents in the future.

Objective: The aim of this study is to assess and learn demographic details, characteristics of the incident in fatal train crashes and recognize the injury profile.

Methods: This is a retrospective autopsy based study. We considered 59 cases of railway track related deaths in three years (2016-2018) conducted at Tertiary Care Hospital, mortuary, Mangalore and analyzed them statistically.

Results: The study showed most of the victims were men between 30-40 years of age. Most victims of incidents were pedestrians (72.9%,). The circumstance of incident in the majority was while walking on side of railway track (39%). The highest number of fatalities 42.4 % were observed between 6 Am to 12 pm. Analysis as to cause of death in accidental circumstances revealed that multiple injuries (35.9%) followed by blunt trauma of head (33.3%) and in suicidal circumstances, death due to blunt trauma to head (40%) and decapitation (40%) were the most prevalent.

Limitations: The study needs to be done for longer duration and more detailed parameters in the railway track accidents.

Conclusion: The study established that majority of railway related deaths are accidental. and there is a requirement of awareness and implementing strict traffic laws to strengthen their safety for such preventable deaths especially among the commuters of train and among the pedestrians using railway level crossings.

Keywords: *- Railway related death; Pattern of railway deaths; Accidental deaths.*

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Introduction

In India, deaths due to railway incidents are quite common and having the largest railway in the world which runs under a single management[1] Railway accidents have an implication in medical and legal implications of trauma due to railway incidents and traumatic disorders as a result of an accident[1]. The congestion and populated railway are mostly seen

in India and as being the cheapest mode of mode of transportation and thus, the possibilities of increase in such cases[2]. There are many cases reported where a person deliberately lie across the railway track or lie head on the track in order to meet self-destruction and hence the railway provides a convenient mode of suicides. The difficultly to differentiate death due crossing track, suicide or criminal violence, specifically in the absence of eyewitness[3].Railway related injury is severe, instantly and mutilated where the cause of death is clear.

In absence of history, determining the way of death is challenging. The most common dilemma in such cases is death is accidental or due to suicide[4]. Approximately one percentage of all fatalities submitted for medico legal autopsies are due to railway related death. The aim of all accident investigations is to establish the cause of incidents.

Accidental deaths were the major circumstance of death in railroad deaths. The most common injuries seen in suicide were a transection of neck, chest injuries and traumatic amputations of extremities[5]. The other factors for death would be a train and automobile collision, an impact between train or a passenger hanging out of compartment doors who are sandwiched by trees, posts or electric poles[6,7,8].To rule out criminal violence certain features deserves special observation, such as wheel marks on the body, dirt and grease contamination and manner of severance of tissues[8].

Despite many causalities are there, in the largest railway network in India, there are many places where unprotected railroad crossing is to be seen. Many cases of suicide over the railroad are reported as causalities are generally considered to be accidental. Laying oneself in front of running train is a common method of suicide. Less often, the person may jump in front of high-speed train. Generally, in cases of railway traumas the body is grossly mutilated with presence of grease and dirt materials on body surfaces[9]. To simulate suicide or accident, sometimes criminals may put the dead body of homicide victim over the railway track and mislead the autopsy surgeon as well as investigating agency. Autopsy surgeon need to be careful and able to distinguish postmortem injuries from ante mortem ones if a dead body recovered from a rail track where the circumstantial evidence has to be ascertained. Based on the crime scene investigation and autopsy findings the manner of death could be opined[8]. The present retrospective autopsy based study is carried out to prove patterns, socio-epidemiological features and other significant associated Co factors, manner of death and cause of railway related deaths.

Materials and Methods

An autopsy based retrospective study was undertaken using postmortem reports of three consecutive years. Post mortem reports of deaths due to railway incidents referred to the Wenlock District Government Hospital, Mangalore for medico legal autopsy between January 1, 2016, to December 31, 2018, were considered. Of the total autopsy cases 2120, A total 59 cases of railway track related deaths were studied.

The cases included were those who had fatal injuries following impact with the train while trying to catch to train, while crossing across the railway track, walking on the side of railway track, or committed suicide on the track. Information obtained included demographic details of the victim, nature of the incident, time and incident, place of collision, external and internal injury distribution and cause of death and manner of death as determined by autopsy were extracted and maintained confidential. The data were entered in Excel sheet and analyzed using SPSS. Ethical Clearance from Institutional Ethics Committee (IEC KMC MLR 04-19/167) was taken before the study

Results

Among the total 59 cases of railway accidents most of the victims were men between 30-39 years (32.2%, n=19) of age. The circumstance of the incident in the majority was while walking on the side of railway track (39 %, n=23). Among the fatalities, the highest number of fatalities 42.4 % (n=25) was observed between 6 AM to 12 PM and in a day. Accidents occurred in the morning hours 6AM to 6PM. Suicidal deaths occurred in the late evenings, night and early mornings 6PM to 6AM.

Majority of railway track related deaths were prevalent in the month of March (n=9, 15%) and in month of December (14%). **(Fig.1)**

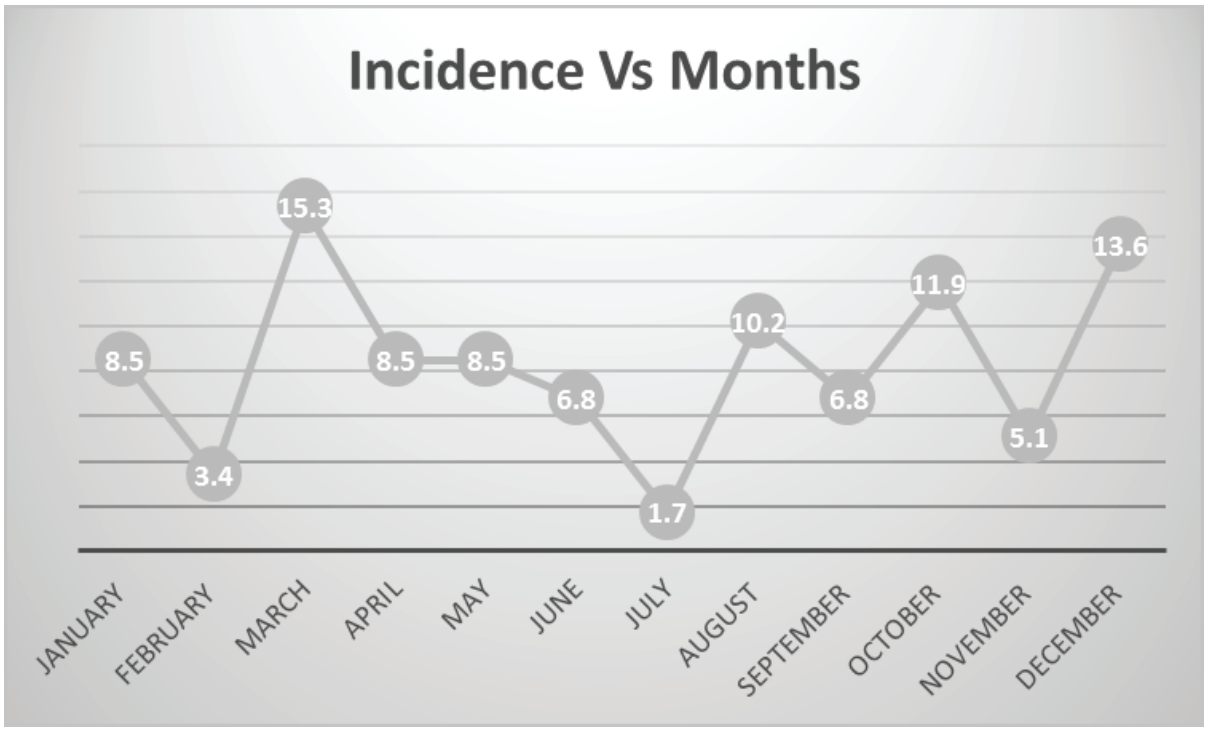

Fig.1. Month wise incidence of railway deaths

Among external injury head injury was the prominent injury observed in railway track deaths. In head injury abrasion and laceration were the most common injury. Skull fractures and facial injuries are common I the internal injuries in head and neck. Cervical vertebrae fracture (50%) and decapitation (50%) are the neck injuries observed in the railway deaths. **(Fig.2 ,3)**

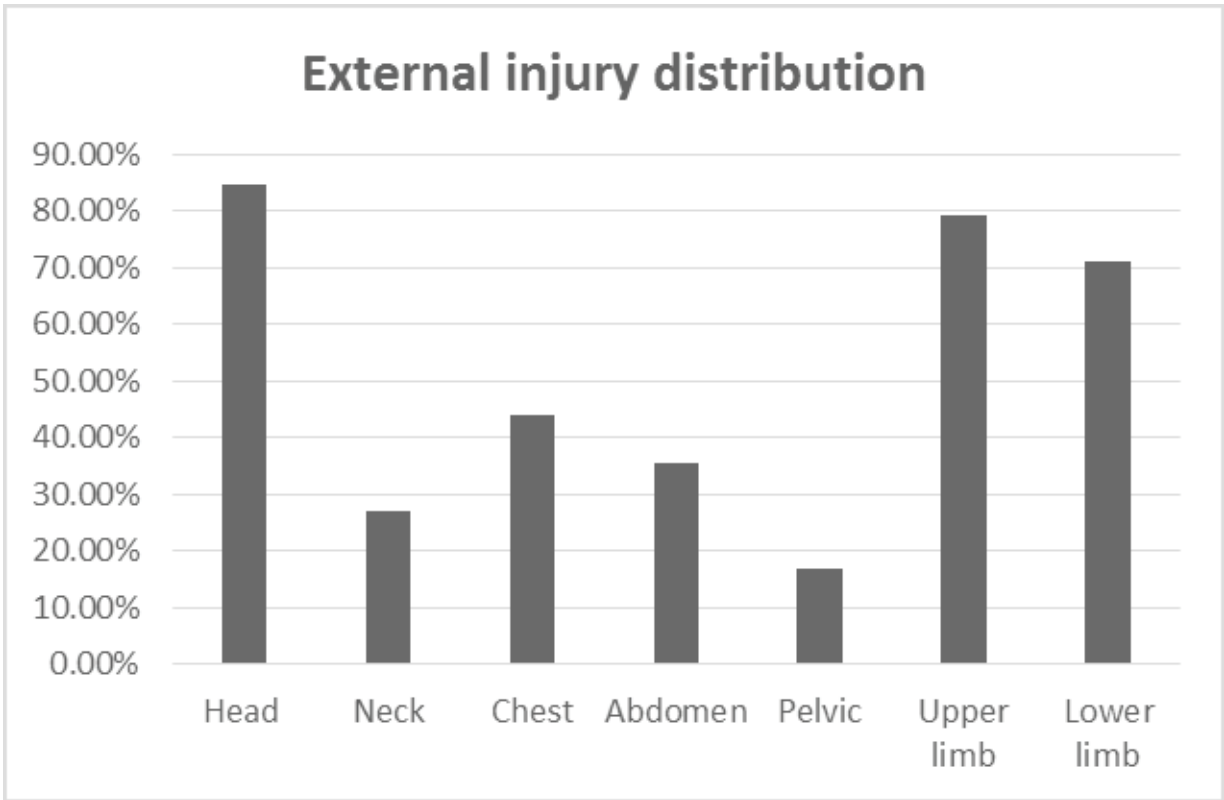

Fig.2. Distribution of External injury among the victims.

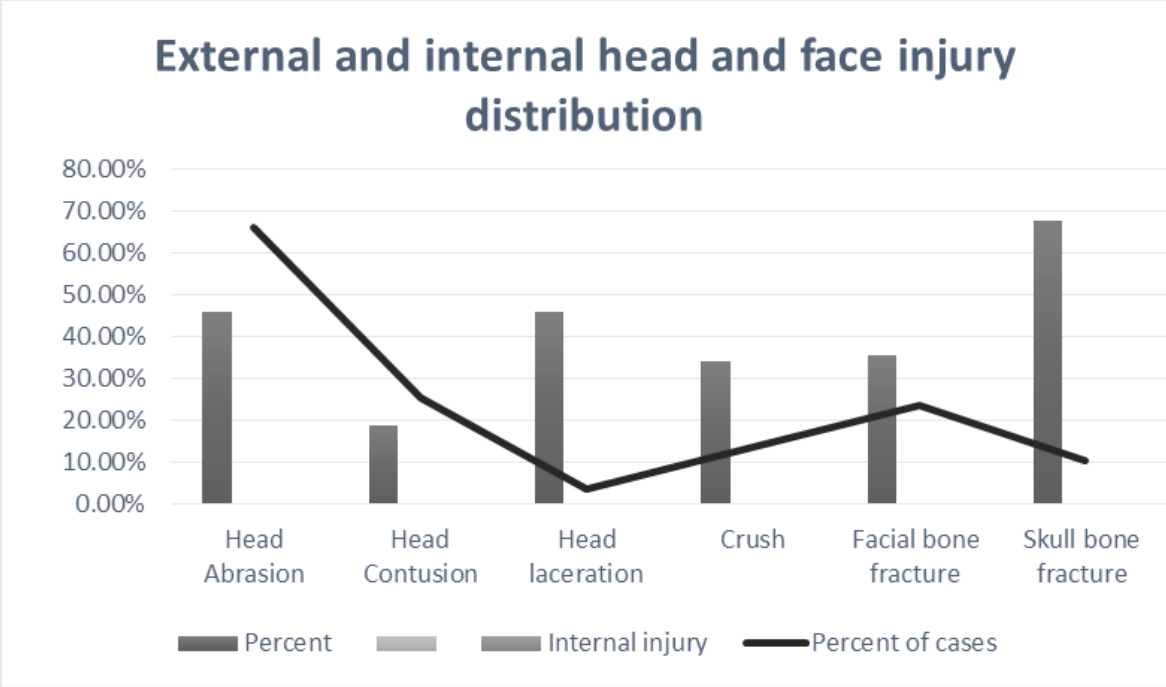

Fig.3. Distribution of Internal injury to head, face among the victims.

The study observed in all cases of suicidal deaths upper limb and head injuries were common and head, upper limbs, body were the prominent external injuries seen in accidental deaths**. (Fig.4)**

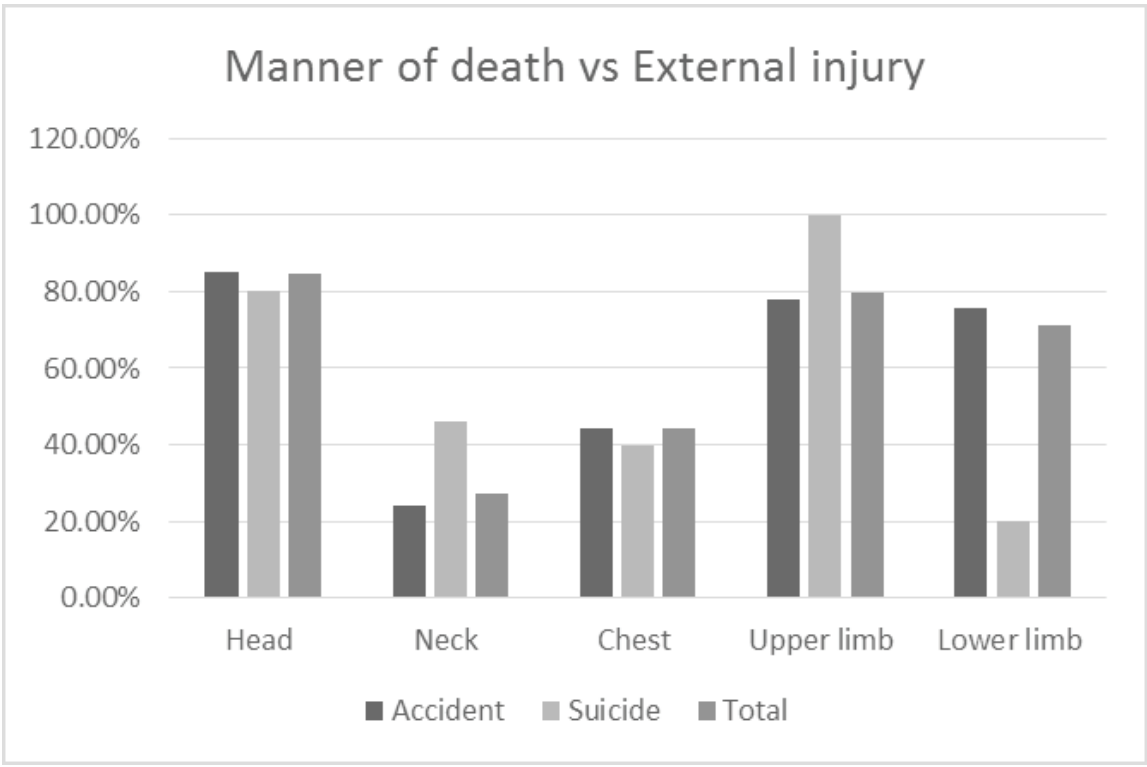

Fig.4. Distribution of injury versus manner of death among the victims.

Analysis as to cause of death revealed that multiple injuries (35.9%) was the most common cause of death in accidental death followed by blunt trauma of head (33.3%). In suicidal death blunt trauma to head (40%) and decapitation (40%) were the most prevalent cause of death. This was statistically significant **(Fig.5)**

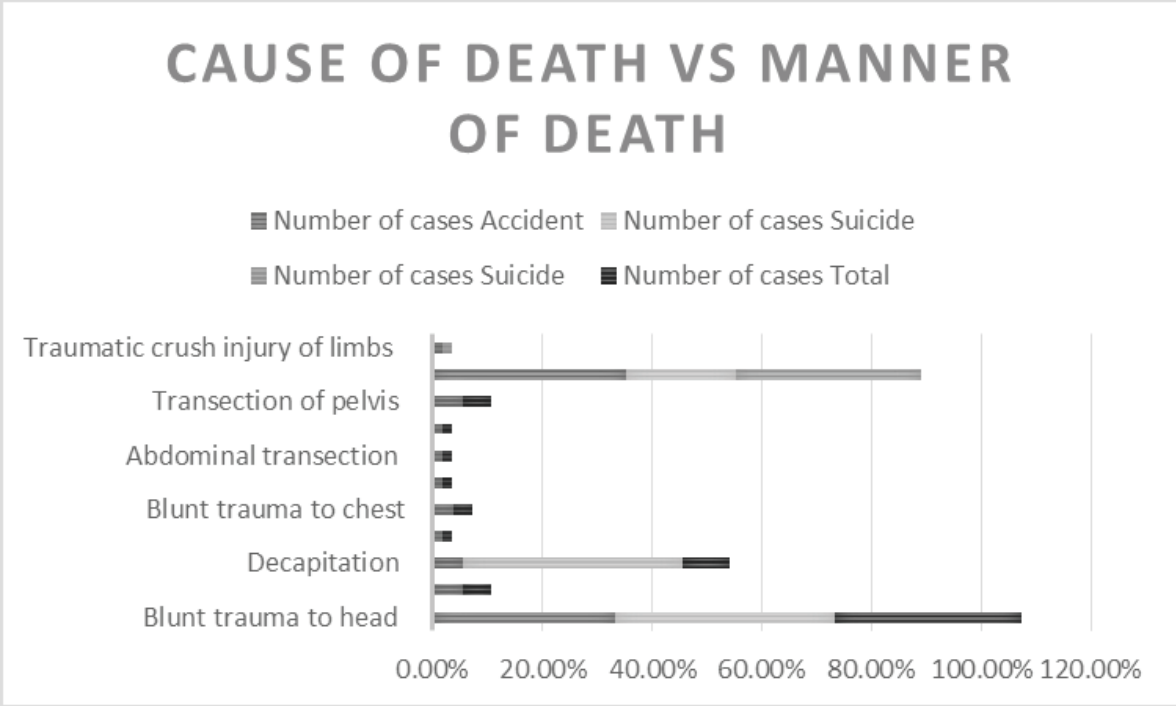

Fig.5. Distribution of Cause of death versus Manner of death among the victims.

Discussion

The present study understands vital information like the main circumstances, associated factors and other confounding factors from the railway related death profiles. The practical difficulties of history obtained from bystanders and other eyewitnesses, reliability about accurate history is an issue with railway accidents and suicides. Correlating circumstance by corroborating the injury profile with the given history is main task of forensic practitioners. Most of medico legal issues can be addressed by thorough investigation of external examinations, internal examinations, documentation and interpretation of injury patterns along with a crime scene visit is an important part of autopsy examination

The most vulnerable victims were in the age group between 30-39 years. The preponderance of this age group is mainly due to mobile behavior of this age group. This was consistent with other studies. (5,8) The majority of the victims were males again larger section of males commute and are mobile. In several studies male prevalence has shown. [(5,8)] 94 % of accidental deaths occurred in male, 80% of cases of suicide deaths occurred in male, 20% of suicidal cases in women and 6% of accidental deaths were in women. These findings are similar to an another study[10]. Differences between men and women indicate sex specific inheritance to suicidal behavior.

In most cases of railway incidents, victims were pedestrians. This is consistent with another study[8]. Most incidents took place while walking along the sides of the track (39%) than crossing railway tracks (30.5%) was contrary to a study[10].However the findings matched with another study observed by the author[11].Usage of railway tracks frequently as a path by pedestrians in absence of bridges, alternative roads and in the absence of road crossings. Negligence in personal safety could also be another reason[12] .passenger cases out of 71 cases died by falling from the train is similar to another study[8.]

The majority of accidents took place during the morning time (6-12.59p. m.), reasons could be due to work and for other activities most boards to train or rush to reach working area on time. Thus, the carelessness of the pedestrians might be the one of the leading Co factors.

43% of accidental deaths occurred in the morning time and suicidal deaths occurred in 40 % of midnight cases and in 40% of the morning cases but contrast to another study. [10] however correlates with a study[8].This

could be reasoned by the fact victims commit suicide after family goes to sleep and while they are hurrying to the workplace so that there is no help to rescue the offender.

Most of railway deaths (15%) occurred in the month of March followed by August month (14%). This may be reasoned due to seasonal variation among the incidents observed similar to the study[11] In 17% of cases ethanol was detected. This was contrasted with a study done by another author [8]. However, this was consistent with findings of other authors. This is reasoned by consuming some predisposing agents which can prepare to perform this act.

On the external examination head injury (87.2%) was the most common injury seen. Abrasions were mostly observed in upper limbs (50.2%) and lower limbs (45.8%). This was consistent to study done by another study[8]. Lacerated wounds were mostly distributed on the head region (45.8%) and lower limbs (45.8%). In 23.2% cases contusion was seen on the chest region. This was inconsistent to study[8]. Decapitation was seen in 40% of suicidal cases and in 7.4% of accidental deaths. This was contrast with the study[5]

The present study showed a large proportion of accidental deaths (91.5%) compared to suicidal deaths (8.5%). There were no homicidal deaths. A similar finding was observed by other studies [5,8] .However, these findings were contrary to the study carry out in New York, where suicide outnumbered to accidents[12]. Accidental deaths are often preventable with a great deal of good involvement of public and investigating authorities as seen in other studies.

The most common of cause of death in accidental deaths were multiple injuries (35.2%), consistent with previous study [5, 8] and the common cause of death in suicide were blunt trauma to head (40%) and decapitation

Conclusion and Recommendation

This study revealed that the majority of railway related deaths are accidental. Decapitation and blunt trauma to the head were common in suicidal deaths. Death due to railway incidents will be preventable by creating public awareness and implementing strict traffic laws. Public attitude for such preventable deaths could be improved through education, awareness drive, especially among the commuters of train and among the pedestrians using railway level crossings

The general attitude towards following the rules and regulations need to be improved and stricter enforcement can be made by law enforcement officials. There is a need to display these rules and penalties, so the law breakers are worried and aware of these rules at the railway stations and other areas notified and to generate a more eye-catching surroundings, enhanced public education messages are ingrained in the public.

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Access Rights to Health Services for Tolotang Believers as Minority Group, Sidrap District, Indonesia

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Abstract

The purpose of this paper was to analyze the forms of protection and fulfillment of human rights by local governments against the followers of the Tolotang faith in accessing their right to health service as minority group in Sidenreng Rappang District, South Sulawesi Province. The type of research used was normative legal research using a statutory approach. The results showed that The followers of the Tolotang faith have been recognized by the state, and weregiven space to worship based on their beliefs. Also Tolotang faith have recognize by the local goverment as part of the community in Sidenreng Rappang who have the same access in order to get health service from any medical stuff, doctor, clinic , hospital whenever they need it.The findings of this article can provide insights into the forms of protection and fulfillment of human rights by local governments against the followers of the Tolotang faith inin in accessing their right to health service as minority group in Sidenreng Rappang District, South Sulawesi Province.The followers of the Tolotang faith have been recognized by the state, and weregiven space to worship based on their beliefs. Also Tolotang faith have recognize by the local goverment as part of the community in Sidenreng Rappang who have the same access in order to get health service from any medical stuff, doctor, clinic , hospital whenever they need it.

Keywords: *Freedom of religion, ICCPR, Right to health, Tolotang faith, Policy*

Introduction

Human rights are the basic rights that humans have since they were born [1-5]. Human rights can be defined as rights that are inherent in our nature as human beings. If there is no such right, it is impossible for us to live as humans. This right is owned by humans solely because he is human, not because of gifts from society or gifts from the state. Therefore, human rights do not depend on the recognition of other human beings, other communities, or other countries. Human rights are obtained by humans from their Creator, namely God Almighty and are rights that cannot be ignored[6-9]. The right to freedom of religion and belief has been guaranteed by international law [10-16]and national law. Therefore, the state through the central and regional governments has an obligation and responsibility to respect, protect, and fulfill the rights of every individual as a citizen in embracing their respective religions and beliefs[17-21]. The state may not limit freedom of religion and belief in the forum internum which is non-derogable rights. The state can only limit freedom of religion and belief at the external forum level, and even then it must meet a number of requirements and the restrictions are made in the rule of law.

The Law of the Republic of Indonesia Number 7 of2012 on Social Conflict Management also pays attention to the possibility of issues related to religion. This law views religious issues as one of the potential conflicts through both inter-religious and/or inter-religious conflicts. However, this law emphasizes that handling conflicts, including those originating from religious issues, must reflect the principles of human rights. The followers of Tolotang faith in Sidenreng Rappang District live side by side with the Muslim

community in the local area because the belief religion has not yet got an equal place with other official religions in Indonesia, so the followers of the Tolotang faith were forced to use Hinduism as their religious identity so that they can still enjoy their rights as citizens. Even though they are ritually performing the worship performed by followers of Tolotang faith.It is very different from those of Hindus in general. This needs to be taken into account by the state, because basically the state cannot limit freedom of religion and belief in the forum internum which is *non-derogable rights*.

Based on 2015 data from the Statistics Indonesia for Hindus, the total of Tolotang people in Sidrap was 40,082. This makes Sidrap District as the area with the largest number of Hindus in South Sulawesi. Based on this, the authors were encouraged to study further the implementation of constitutional rights protection for followers of the Tolotang faith by the government based on the International Covenant on Civil and Political Rights.

Indonesia itself has ratified the International Covenant on Civil and Political Rights through the Law of the Republic of Indonesia Number 12 of 2005. Therefore, Indonesia as a state party was obliged to maximize respect and protection of the civil and political rights of its citizens including the right to freedom of religion while the research on the implementation of constitutional rights protection for followers of the Tolotang faith by the government based on the International Covenant on Civil and Political Rights is very important to be conducted. Therefore, referring to the ICCPR which was ratified in 2005, this paper needs to discuss further about the form of protection and fulfillment of human rights by local governments for the followers of Tolotang faith in Sidenreng Rappang District.

Indonesia has also ratified International Covenant on Economic, Social and Culture Rights trough the Law Number 11 year 2005, under the article 25 mentioned about the Right to health tha article 12 paragraph (1) ICESCR amely, that States parties to the Covenant recognize the right of everyone to the enjoyment of the highest attainable standard of physical and mental health. Also under article 25 on the covenant clearly stated that States Parties recognize the right of a child who has been assigned by the competent authorities for the purpose of care, protection or treatment of his physical or mental health or of periodic review of the care given to the child and all other circumstances relevant to his placement.

Of course this applies to all Indonesian citizens, including Local Beliefs, Tolotang, because as part of Indonesian citizens they also have the same rights including the right to access health[22-25].

Materials and Method

The type of this research was legal study [26-30]by examining policies and rules of national and international law, legal theories and legal materials related to the issues to be discussed, then supplemented with empirical data, namely seeing the implementation of these policies and regulations in society [31-33].The collected data and legal materials were analyzed qualitatively, then they were described to answer the problems in this study.

Results and Discussion

The Complexity of Adherents to Ancestral Religions in Indonesia

Since Indonesia's independence, several groups of citizens have categorized themselves as followers of the ancestral religion, or not adhering to one of the six (6) "official religions" (recognized) of the state. They are a group of citizens who demand state recognition and services for civil rights based on their diversity which throughout Indonesian history has been ignored and violated. Throughout history, they have been sued (or forced) for example self-identification, marry, give oaths and so on based on the official religion and belief of the state that they do not follow it[34-37]. This still happens a lot until now, while this Country is almost 75 years old, followers of ancestral religions can be categorized as a very vulnerable group as victims of persecution in society.

Religion, in religious politics, is defined exclusively based on the perspective of the dominant religion, which only accommodates (claimed by most) groups of citizens, but is used as a citizenship identity for all. Religion, as a policy, is defined to determine groups of citizens who are "religious" and "non-religious". Groups that are considered politically significant, and whose religion has been successfully adapted to this exclusive

definition, are considered citizens, and their religion is made by the official state religion. Hundreds of groups whose religion cannot, to be precise, are refused to be adjusted are considered not/are not yet religious, including mainly followers of ancestral religions. Their citizenship status is not legal to be recognized, protected or served by the state. In the name of religious politics, they are controlled and subdued[34].Indeed, the issue of religion is a problem in itself in Indonesia because of its sensitive nature, and even many conflicts have occurred with the trigger of being a matter of religion or belief.

Adherents to ancestral religions are a group of citizens whose "religion/belief" is interpreted, understood and treated differently from time to time. Their religion is claimed to be animist (primitive, pseudo-religion) so that it needs to be modernized. Ancestral religion is treated as a "culture" which on the one hand needs to be developed, but on the other hand its adherents are considered communists, and are required to convert to the official religion. At certain times, ancestral religions are allowed to develop, and are even considered equivalent to religion. Until now, the meaning of ancestral religion continues to develop in the context of religious politics[37,38].Motto "Bhinneka Tunggal Ika" [39-41]inherent in the eagle symbol, not just writing without meaning. The motto represents the soul and body of the Indonesian people in every area of life. "Different but still one thing" also reflects diversity religion and belief in Indonesia.

Indonesia officially recognizes six religions, namely Islam, Christianity, Catholicism, Hinduism, Buddhism, and Confucianism[34,42-45]. The majority of Indonesia's population adheres to Islam as much as 87.2% or equivalent to approximately 207 million people, while the rest are followers of five other religions.

Tolotang Believers

Religious rights and beliefs in Indonesia are recognized through the first principle of Pancasila, "God Almighty". Then it is described in the body of the 1945 Constitution of the Republic of Indonesia in article 29 paragraph 2 which reads, "The state guarantees the freedom of every citizen to embrace their respective religions and to worship for that religion and belief". If we examine more deeply, it will be found that in Indonesia there are not only religions, but also beliefs that are older than the six religions recognized in Indonesia today.

These beliefs are spread in almost all corners of Indonesia, one of which is the Tolotang belief. The Tolotang belief existed long before the six recognized religions existed today. Tolotang trust is spread across two sub-districts in Sidenreng Rappang District, which arein Watang Sidenreng District, Kanyuara and Amparita Villages.

Statistics Indonesia[46]in 2015 revealed that out of 11 sub-districts in Sidenreng Rappang District, the majority of the people adhere to Islam, which was as many as 310,461 people. Most Tolotang Believers are in Watang Sidenreng District, to be precise in Kanyuara Village.

Demographically, believersof the Tolotang faith can also be found in other sub-districts, such as Tellu Limpoe, to be precise in Amparita Lama Village. It is also spread partially in a limited number in almost all sub-districts in Sidenreng Rappang District. The daily lives of the followers of the Tolotang faith are quite peaceful, if connected with local people who do not adhere to this belief. Generally, they are reunited at a large event in the form of traditional rituals to commemorate their ancestors. Generally held every January, but the implementation still refers to the results of deliberations conducted by the uwa'or uwatta Tolotang.

Sidenreng Rappang District Government Policy

The Indonesian state only formally recognizes six religions, namely Islam, Christianity, Catholicism, Hinduism, Buddhism, and Confucianism. Thus, only these religions have representation in the Ministry of Religion at both the central and regional levels. Recognized religions have room to express teachings through religious practices such as worship and celebrations. These religions have a complete organizational structure that supports the continued implementation and dissemination of teachings. With an organizational structure like this, these religions will be spoiled with facilities to support their existence in the future.

This fact is a strong evidence of the state's fulfillment of the right to religion in Indonesia, which is regulated under the auspices of the ministry of religion. Nevertheless, along with the times, it is necessary to

absorb the aspirations and fulfill the rights of some people who are said to be adherents of local religions other than the official religions recognized by the state to date. Apart from the Ministry of Religion, the existence of several religious organizations such as the Indonesian Ulama Council (MUI), the Association of Indonesian Churches (PGI), the Indonesian Church Guardian Conference (KWGI), Parisada Hindu Dharma Indonesia (PHDI), WALUBI, and the Confucian Religious Council Indonesia (MATAKIN) also proves the partisanship of the state towards certain religions, especially the six religions. These organizations reflect the state's recognition of the religion represented by each organization. In fact, the existence of such a supporting forum is still limited to the community, especially for those who adhere to the belief.

Indonesia, which formally only recognizes or legalizes six religions, turns out to make an empty space for not getting the fulfillment of its legal recognition rights which have the value of legal certainty. The six religions are spread throughout the archipelago, competing with each other to maintain their existence in a heterogeneous society. This emerges the majority and minority religions. If it is conceived that religion has the meaning of belief in it, then many beliefs that are considered in accordance with the first principle of Pancasila begin to be isolated and have minimal fulfillment.

Six religions that exist and are legal in Indonesia, always get the right of protection and fulfillment by the state. However,this is minor for beliefs that predate the six religions. Even the nature of its protection and fulfillment seems too late, so it makes believers surrender to the situation. This also happened to Tolotang's belief in Sidenreng Rappang District, South Sulawesi.

The regional government exercises the widest possible autonomy, except for governmental affairs which are determined by law as the affairs of the Central Government. Local governments have the right to stipulate regional regulations and other regulations to implement autonomy and co-administration. The structure and procedures for the implementation of regional government are regulated in law. The implementation of regional autonomy is decided through a systematics of certain regulations involving various stakeholders, such as DPRD. Included in this is policy making by local governments, particularly by the Regional Government of Sidenreng Rappang District.

One of the representatives of Tolotang traditional leaders, namely Samang, SH., emphasized that because of this background, he was required to choose one of the six existing religions. Through a long process, Tolotang finally chose to join the Hindu religion, with the Decree of the Director General of Hindu Balinese/ Buddhist Community Guidance No. 6 of 1966 which stipulates that Tolotang is one of the sects of Hinduism. This decision was taken by Tolotang leaders on the grounds that Hinduism was more receptive to all Tolotang doctrinal entities without the slightest need to be degraded.

Tolotang's joining to Hinduism is referred to by Tolotang leaders as affiliation, that is, Tolotang's joining to Hinduism is only from the point of view of mentioning his religion in order to retain the right to protection and fulfillment. Meanwhile, the teaching entity has not changed and adapted to Hinduism. Argumentum per analogy. This indicates that Tolotang is like a minor country that joins a major state in order to be recognized and protected in the midst of the world community.

If viewed from the constitutional worldview, the district head's decision prohibiting the presence of the Tolotang belief contradicts Article 29 paragraph 2 of the 1945 NRI Constitution, namely "The state guarantees the independence of every citizen to embrace their respective religions and to worship according to their religion and belief". The absolute interpretation of this article is that the state does not only grants protection and fulfillment rights to religion, but includes beliefs as long as they are in accordance with Pancasila. Although it is understandable because at that time the Indonesian constitution had not been amended as is the result of the current amendments. However,empirically, followers of the Tolotang faith are still worried about the existence of the regent's decision, whether it has been abolished or it still exists today. Even though it is de facto, it seems that the district head's decision is no longer valid.

There are two classifications of rights in the ICCPR, namely Non-Derogable Rights and Derogable Rights. Non-Derogable Rights are rights that are absolute in nature which cannot be reduced by state parties under any circumstances. The rights that fall into this type

are:rights to life,*rights to be free from torture*, *right to be free from slavery*, Right to be free from detention for failing to fulfill debt covenants, the right to be free from retroactive punishment, rights as legal subjects, and the right to freedom of thought, belief and religion.

The second classification is Derogable Rights, which are rights that can be reduced or limited by the fulfillment of certain conditions. The scope of this type of right is:freedom rightof peaceful assembly, and freedom right of association; including forming and becoming a member of a trade union, and

The right to freedom of expression or expression; including the freedom to seek, receive and provide information and all kinds of ideas without regard to boundaries (either through orally or writing).

De facto (field facts), after the issuance of the Constitutional Court decision Number 97/PUU-XIV/2016 which finally provided the fulfillment of religious and belief rights to believers, the regional government of Sidenreng Rappang Districtrecognized and legitimized the existence of believers of faith, especially Tolotang. This is provedby the inclusion of representatives of Tolotang traditional leaders in the Religious Communication Forum (FKUB) in Sidenreng Rappang District. This information was obtained validly through the narrative of representatives of the Tolotang traditional leaders themselves.

In relation to the implementation of Article 18 of the International Covenant on Civil and Political Rights in Indonesia, it is interesting to note three important points related to the strengthening of the pro-contra discourse related to the making of public policies, both at the national and local levels written by Eni Puji Utami, et al[47]:

First, as a democracy with the majority of the population upholding religious identity, contestation to encourage the role of religion in the public sphere is inevitable. However, public expressions of religion in the form of violence and violations of the principle of citizenship should be avoided or prevented. Our research shows that most acts of violence and persecution against certain religious groups are repetitions of previous similar acts, some even taking place at the same place or with the same target group, and, as far as our research, have never occurred spontaneously. This shows that in fact such conflicts can be avoided and at the same time also indicates a weak diversity management capacity in terms of prevention strategies and responses to the risk of religious conflict.

Second, the level of ability of society and the state in managing diversity varies from place to place. In many places, communities with different identities are found but are able to live side by side (coexistent) for a long period of time. Unfortunately, however, there is not enough effort to explain, publicize and transfer the ability of one society to manage diversity to another so that the potential for violence in vulnerable areas can be overcome. On the other hand, not a few peaceful situations in one society are damaged by external factors including information with nuances of conflict originating from other places.

Third, the capacity of society and the state in managing diversity is often weakened by a lack of synergy between elements. Different perspectives, approaches and misunderstandings often create situations of mutual blame between the actors who should work together to create harmony. Worryingly, responses to diversity issues are often based on opinion, suspicion or misunderstanding rather than accurate research-based knowledge. As a result, it is not uncommon for the potential for violence not only to be prevented, but even the efforts to deal with religious conflicts actually perpetuate the problem.

The Right of Health Access of Tolotang Believer as a Marginal Group in Society

Based on the facts, Indonesia has its own legal instruments that guarantee and protect human rights. Therefore, the right to work, the right to health [48-50], the right to social security, along with all its services, the right to live in prosperity (the right to a decent life), the right to self-development are regulated and guaranteed in the 1945 Constitution, Law Number 39 of 1999 concerning Human Rights, Law Number 11 of 2005 concerning Ratification of the Covenant on Economic, Social and Cultural Rights. In Article 58 paragraph (2) letter h of the Population Administration Law, it has been stated that "religion/belief" is a part of individual data that must be recorded in a population database.

Whereas Article 58 paragraph (4) letter a of the Population Administration Law also explains [51]:

"Population data as referred to in paragraph (1), paragraph (2), and paragraph (3) used for all purposes is Population Data from the Ministry responsible for domestic government affairs, including for the use of: a. public service;....; Further explained in the explanation:

Point a: "What is meant by" utilization of public services ", among others, for the issuance of driving licenses, business licenses, taxpayer services, banking services, land certificate issuance services, insurance, public health insurance, and / or social security for workers. " From the explanation of the article above, it is increasingly clear that the existence of electronic identity cards (KTP) and family cards (KK) is the main entrance to be able to experience their use of access to public services such as the issuance of SIM, business permits, bank loans and also to fully access health rights which requires personal data / identity of each community. This is the basis for the position of believers in demanding access to health. Then referring to the International Covenant on Civil and Political Rights (ICCPR) which Indonesia ratified in 2005, Article 27 clearly states that; In countries that have minority groups based on ethnicity, religion or language, people belonging to that minority group must not be denied the right in society, together with other members of their group, to enjoy their own culture, or use their language.

As well as the International Covenant on Economic, Social and Cultural Rights, it reinforces again in Article 12, that the countries participating in this treaty recognize the right of everyone - in this case including minority groups who are members of society in a country- to enjoy the highest standard. that can be achieved for physical and spiritual health[52]. Therefore, regarding the right of the Tolotang community to get access to health services as a minority group in one of the regions in South Sulawesi, basically there is no special treatment for minority groups[53-56].

Conclusion

The Indonesian state only formally recognizes six religions, which are Islam, Christianity, Catholicism, Hinduism, Buddhism, and Confucianism. Thus, only these religions have representation in the Ministry of Religion at both the central and regional levels. Recognized religions have room to express teachings through religious practices such as worship and celebrations. These religions have a complete organizational structure that supports the continued implementation and dissemination of teachings. With an organizational structure like this, these religions will be spoiled with facilities to support their existence in the future. Even though Indonesia formally only recognizes or legalizes six religions, it turns out that it leaves an empty space where it does not get the fulfillment of its legal right to recognition which has the value of legal certainty.

Six religions that exist and are legal in Indonesia, always get the right of protection and fulfillment by the state. However, this is minor in the beliefs that predated the six religions. Even the nature of its protection and fulfillment seems too late, so it makes believers surrender to the situation. This also happened to Tolotang's belief in Sidenreng Rappang District, South Sulawesi. The Tolotang belief existed long before the six recognized religions existed today. Tolotang faith is spread across two sub-districts in Sidenreng Rappang District, namely in Watang Sidenreng Sub-district, Kanyuara and Amparita Villages. Therefore, the shape Government policy Sidenreng Rappang in the fulfillment and protection of the rights Tolotang faith is to provide privileges on Tolotang society; that at least two head of sub-villagein Watang Sidenreng Sub-district must belong to the Tolotang community and facilitate administrative licensing related to the implementation of various religious activities of the Tolotang faith.

Implication of the Study

The implications are in the form of the effect of Constitutional Court's decision on adherents of belief, especially Tolotong, as well as the implementation of Article 18 of the covenant on civil and political rights with the Constitutional Court decision. For the Tolotang community is that there is space for freedom and guarantees for the fulfillment of their rights to enjoy and implement their beliefs and duet o their faith, they also have the same right to access any other rights that provide and guarantee by the goverment, included the right to health services.

Limitations and Study Forward

The weakness of this research is that this research is normative since it only covers legal aspects so that more in-depth research is reuquired to ensure the application of the freedom rights of religion and adherents of belief at the individual level also regarding their access to get the right of health as a member of society even as marginal group. Social studies through quantitative research approach can reach these applications at all levels for adherents of the Tolotang faith. Comparison of similar belief groups in Indonesia needs to be made in relation to the fulfillment of the freedom right of believers.

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Cardiac Fibrosis Attenuation by Chlorogenic Acid and Epigallocatechin-Gallate Mediated by Suppression of Galectin-3 Gene Expression and Collagen Deposition in Rat Metabolic Syndrome Model

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Abstract

Objective: Metabolic syndrome (MetS) is a set cluster of risk factors for metabolic abnormalities that can develop cardiovascular disease (CVD), one of that is remodeling or cardiac fibrosis. Cardiac fibrosis identified from the high levels of the profibrotic molecule; one of them is galectin-3. **Aim:** This study intended to determine the effect of combination therapy of green coffee (CGA) and green tea (EGCG) on cardiac fibrosis by raise of the galectin-3 gene expression and collagen deposition in rat cardiac tissue in the rat metabolic syndrome model. **Methods:** Twenty-four male MetS rats (Sprague-Dawley) divided into two control groups and three groups therapy (n=5) administration of the CGA 200mg/kgbw (body weight in kilograms) and EGCG 300mg/kgbw orally. After eight weeks of treatment, rats euthanized, then mRNA expression of galectin-3 was measured. Furthermore, collagen deposition of cardiac tissue carried out in histology slides. **Results:** Research reveals that the expression of galectin-3 decreased in the metabolic syndrome model group, which given combination therapy compared with metabolic syndrome model mice that did not receive any therapy (P=0.000). Collagen deposition in cardiac tissue also found less than in the therapy group compared with the group not treated with both compounds (P=0.000). The correlation between the two parameters shows a positive association with low strength. **Conclusion:** This study shows that the combination therapy of CGA and EGCG is an engaging therapeutic candidate. It expected to reduce the progression of cardiac fibrosis in metabolic syndrome.

Keywords: *metabolic syndrome, chlorogenic acid, collagen deposition, epigalocathecin-gallate, galectin-3.*

Introduction

Metabolic syndrome (MetS) is a cluster of risk factors for metabolic abnormalities that can lead to many organs' dysfunction to death. Metabolic syndrome is defined based on several pathophysiologies: insulin resistance, central obesity, hypertension, hyperglycemia, and triglyceride dyslipidemia [(1)]. The number of metabolic syndrome events continues to increase from year to year. The prevalence of the metabolic syndrome is estimated to have reached more than 20% of the adult population worldwide [(2,3)]. Indonesian Ministry of Health (2013) reported the prevalence of metabolic syndrome in Indonesia reached 23%, with the proportion of adult obesity increasing to 21.8%, hypertension to 34.1%, and

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an increase in the prevalence of type 2 diabetes up to 8.5%

Obesity and insulin resistance, along with other risk factors in metabolic syndrome, also play a role in the development of cardiovascular disease. One of the cardiovascular diseases found in metabolic syndrome sufferers is a change in the histological structure of the heart or known as cardiac remodeling. Remodeling of the heart can be a direct effect of an increase in adipocyte cells or due to the risk of hypertension that is an agonist with obesity, resulting in excess pressure on the heart. Cardiac remodeling arises from an increase in extracellular matrix deposition, referred to as fibrosis[4]. Increased oxidative stress in response to fluctuations in glucose in blood plasma, can activate an inflammatory pathway that is significantly involved in the pathogenesis of reactive fibrosis that leads to the accumulation (deposition) of type 1 collagen in cardiomyocyte tissue [5]. An increase in collagen deposition, which is not compensated by its degradation, limits contractility and relaxation of cardiac cells and inhibits electrical conductivity and regional nutritional diffusion, leads to a cause cardiac dysfunction [6].

Cardiac fibrosis can be identified early from the high levels of profibrotic molecules; one of them is galectin-3. Galectin-3 expression consistently found in fibrosis models. Galectin-3 is a molecule consisting of an N-terminal domain, 100-150 amino acids, with a C-terminal domain consisting of about 135 amino acids, containing one carbohydrate recognition domain (CRD). Galectin-3 is formed in pentamer formation using ligands that go through the N-terminal domain. On the cell surface, galectin-3 plays a role in cell-cell interaction, and between cells with extracellular matrix (ECM), activation of growth factor receptors, signal transduction, and plays a role in the formation of a glycoprotein-galectin lattice [7].

Metabolic syndrome therapy has long focused on the individual risk factor, for example with metformin therapy in patients with insulin and hypertension resistance, and some anti-hypertensive agents, such as angiotensin II converting enzyme inhibitors (ACEi), or angiotensin II receptor antagonists [8,9]. However, in the last few decades, metabolic syndrome therapy through dietary interventions provides a reasonably good prospect of improving the condition of sufferers of the MetS. Functional food studies are quite interesting because medicines extracted from plants contain many essential compounds such as polyphenols, flavonoids, and terpenoids.

Green tea is one of the most frequently consumed drinks globally, occupying the highest production, about 20% of total global tea production. Epigallocatechin-3-gallate (EGCG) in green tea considered a significant contributor to various health benefits [10]. Previous studies revealed an increase in adiponectin and its receptor after administering green tea extract at a dose of 300-400 mg/kg bb for nine weeks[11]. Also, they increased PPARα and PPARγ expression in rat metabolic syndrome models[12]. Another popular beverage that is consumed by most people in the world is coffee. Green coffee beans are rich in chlorogenic acid, compared to other sources such as fruit and vegetables [13,14]. The administration of green coffee extract for seven weeks is known to reduce fasting blood glucose, lipid profile, blood pressure, increase adiponectin and HOMA-IR index in rat metabolic syndrome models[15].

However, the involvement of green coffee and green tea as a therapeutic agent for cardiac fibrosis in the metabolic syndrome remains unclear and needs further investigation. Therefore, this study will examine the effect of combination therapy of green coffee and green tea on cardiac fibrosis. It is characterized by increased expression of the gene galectin-3 and collagen deposition in rat cardiac organs in the metabolic syndrome model.

Methods

Research Design

The study was a true experimental design with a post-test only control group design with a simple random sampling technique. Sprague-Dawley rats aged 8-12 weeks, weighing 250-300gram acclimatized for seven days, which further divided into five groups, the negative control group was healthy mice (n=5) fed with commercial pellet feed. The positive control group (n = 5) were mice with metabolic syndrome through the high-fat diet (HFD) consist of powdered mouse pellets, 20% sucrose, 0.5% methionine, 2.5 salt, 2% MSG, 15% egg yolk, and 20% white fat) treatment for 14 days. Streptozotocin (STZ) injection 30 mg/kg body weight

(BW) given on the 15th day. Then, HFD continued until the end of the study [16]. Group mice must meet the criteria for the rat metabolic syndrome model, which is an increase in blood glucose levels >126 mg/dL, HDL levels <40 mg/dL, and triglyceride levels >150 mg/dL. Treatment group 1 was mouse metabolic syndrome model (n=5) who were given 200 mg/kg body weight green coffee extract (CGA) therapy for seven weeks via oral gavage. Treatment group 2 was metabolic syndrome model mice (n=5) which received green tea extract (EGCG) therapy with a dose of 200 mg/kgbw via oral gavage. Treatment group 3 (Combination) was a metabolic syndrome mouse model which (n=4) given therapy using a CGA combination of 200 mg/kgbw and EGCG 300 mg/kgbw by oral gavage.

Green Tea and Green Coffee Extraction Process

Coffea canephora robusta beans are roasted with an automatic coffee roasting machine at 180-200° C for 6-8 hours. Furthermore, the coffee beans were macerated with 95% ethanol to produce a crude extract. The crude extract is filtered using a filter cloth to separate the liquid phase from the solid phase. Besides, the liquid phase concentrated using a rotary evaporator at ±40°C. Green tea extracted from light green tea leaves. Green tea leaves 500 grams are dried using a drying cabinet (50ºC) for 8 hours to get green tea with a moisture 8-10%. Green tea blended and boiled at 80°C for 30 minutes. Then, the crude extract is filtered to separate the liquid phase from the solid phase—the concentrated liquid phase prepared using a rotary evaporator at ± 40 °C.

Dosage Determination

The dosage of administration of green coffee extract and green tea is determined based on preliminary studies. The optimal dose is 200 mg/kgbw for green coffee extract and 300 mg/kg BW for green tea extract.[12,15] Daily food intake and fluid intake are measured every day, and body weight is measured every week—fluid intake given *ad libitum*.

Blood Pressure Measurement and Biochemical Examination of Blood

Blood pressure was measured using the tail-cuff method with the sphygmomanometer at the beginning and the end of the experiment as systolic blood pressure (SBP). Serum concentrations of fasting glucose, triglycerides (TG), and HDL were measured enzymatically (Biolabo, France). The sample measured using a spectrophotometer.

Measurement of Galectin-3 Gene Expression

Total RNA of heart tissue isolated using Easy Blue (Intron Biotechnology) following the manufacture's protocol. Reverse transcription reactions carried out using the ReverTra Ace-α kit (Toyobo, Japan). Then the level of RNA expression was performed using the LightCycler 96 PCR system (Takara, Japan) using the GoTaq Green Master PCR Kit (Promega, Madison, USA) following the manufacture's protocol. The primary sequence is as follows: B-actin, forward: 5'- TGA GAG AAT CGT GCG TGA CAT-3′ and reverse: 5'-ACC GCT CAT TGC CGA TGA TGA TGA-3′; galectin-3 (LGALS3) forward:5′-GGCCACTGATTGTGCCTTAT-3′; and reverse: 5′-TCTTTCTTCCCTTCCCCAGT-3′. The PCR cycle is as follows: 5 minutes at 95°C pre-denaturation; 35 cycles of 30 seconds at denaturation of 95°C, 30 seconds of annealing at 57.1°C, followed by extensions for 30 seconds at 72 °C; and final extension for 10 minutes at 72 ° C. The mRNA level of the target gene normalized to the level of b-actin expression.

Histology Examination of Cardiac Tissue

The heart organ is put into a cassette, soaked in a level of ethanol solution that is 70% to 100% each for 60 minutes at room temperature. Cassette was removed, clearing using xylol for 15 minutes at room temperature three times. Infiltration with liquid paraffin for three times each transfer 60 minutes in an incubator temperature of 60 ºC, then removed to get a paraffin block. The paraffin block sliced 12 mm thick horizontally using a rotary microtome.

Analysis Data

Data obtained tabulated descriptively in mean (± standard deviation) for galectin-3 gene expression from each group. Data obtained from histological observations of cardiac collagen deposition by field of view, taken on average from five fields of view for each mouse model in each group treatment. The normality and homogeneity of the data tested using the Shapiro-Wilk test and the Levene test, followed by the Kruskal Walis test. The

confidence interval set to 95% (P = 0.05). Correlation of galectin-3 and the average area of collagen deposition from each group using Spearman's-rho correlation test.

Ethical Clearance: This experimental design has fulfilled and approved by the Ethics Committee of Faculty of Medicine, Brawijaya University, Malang, Indonesia, by registered number: 405/EC/KEPK/2020.

Result

Galectin-3 gene expression

The galectin-3 gene expression is relative to β-actin presented in Figure 1. It is indicating that there are differences in galectin-3 gene expression in each group. The negative control group (Normal) had the lowest gene expression, with an average of 0.2 ± 0.047. The positive control group (MetS) had the highest expression of the galectin-3 gene, among other models, which was 1.02 ± 0.228. The MetS + CGA group had an average of galectin-3 expression 0.94 ± 0.208, which was the highest value among the groups given the intervention. The MetS + EGCG treatment group, was known to affect the expression of galectin-3, which had the lowest average of all groups given therapy, 0.67 ± 0.124. The third treatment group (MetS+ CGA+ EGCG) produced an average expression of 0.70 ± 0.2901, which was the lowest mean after the second treatment group in the five groups used in this study. Based on Kruskall-Wallis analysis, showed the value of 19.251, obtained a statistical table value of 9.488 (df = 4; 5%). So that, there is an effect of giving a combination of CGA and EGCG to galectin-3 expression. Based on the independent T-test analysis compared with MetS group, all treatment groups produced significantly different levels of galectin-3 with a P-value <0.05 (Table 1).

Collagen deposition in heart tissue

Collagen deposition examination is a further histological procedure after collecting rat heart organs of each group (Figure 2). Data is converted into semi-quantitative data (%) in the percentage of collagen deposition compared to normal cardiomyocyte cells. The normal group had the lowest percentage of collagen deposition compared to other groups (1.8% ± 0.447). The highest collagen deposition was found in the MetS group, 8.52% ± 2.261. Meanwhile, the MetS + CGA group have a percentage of collagen deposition of 3.41% ±0.67, the MetS + EGCG treatment group with a percentage of 2.33% ± 0.095, and the combination therapy group with a percentage of 2.86 % ±0.58. Based on the Kruskal-Wallis analysis, obta11,175. This value, when compared to obtained a statistical table 9,488 (df=4; 5%), there is an effect of giving a combination of CGA and EGCG to collagen deposition. Compared with the negative control group, only MetS, MetS + EGCG, and combination treatment groups produced significantly different collagen deposition with a P-value <0.05 (Table 1).

Correlation between Galectin-3 gene expression and collagen deposition in cardiac tissue

The correlation test on the two dependent variables determines the closeness of the relationship between the two variables (Table 2). The results of correlation analysis between galectin-3 gene expression and collagen deposition for five treatment groups, obtained positive correlation (Sig.2-tailed; P-value> 0.05). The correlation coefficient is positive, indicating that the relationship between the two variables is unidirectional. It suggests that the higher the expression of the galectin-3 gene, the more collagen deposition in cardiomyocyte tissue.

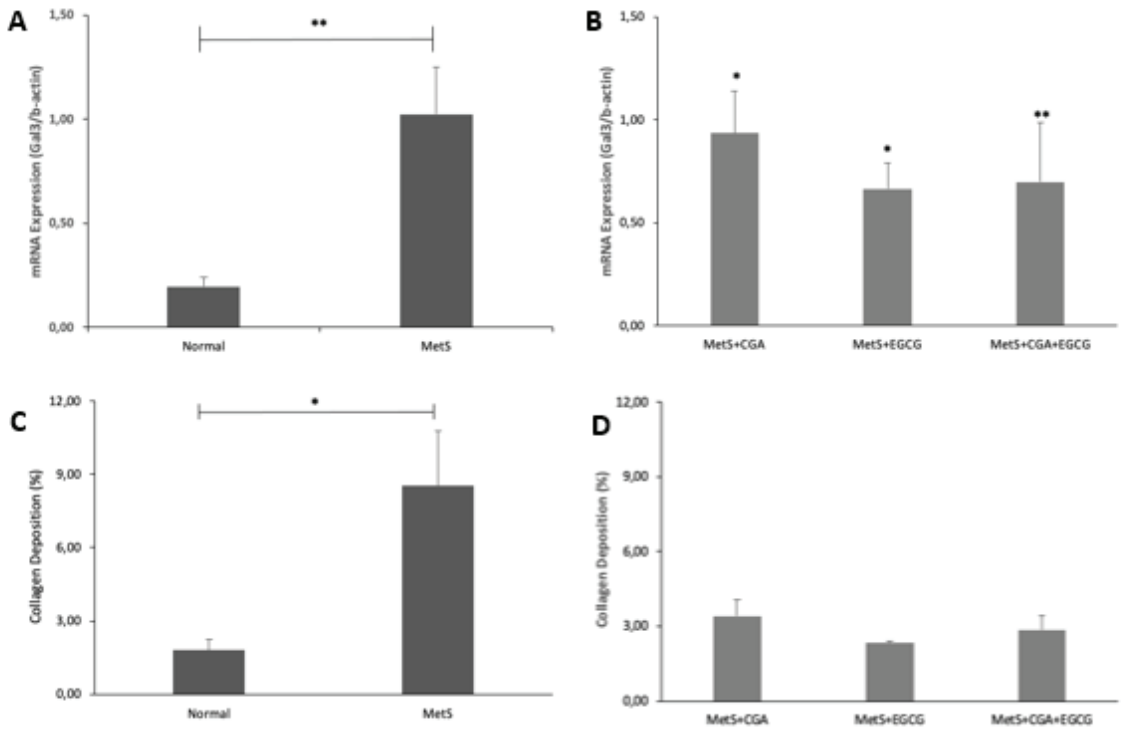

Figure 1. (A) Galectin-3 gene expression in (A) control groups and (B) treatment groups. Collagen Deposition percentage in cardiac tissue of in (C) control groups and (D) treatment groups.

Independent T-test		Sig. (2-tailed)	
		Galectin-3	Collagen Deposition
MetS	Normal	0.000**	0.045*
	MetS+CGA	0.003*	0,137
	MetS+EGCG	0.013*	0.088
	MetS+CGA+EGCG	0.000**	0,442

Table 1. p-value of Independent T-test analysis

*Significant for P-value <0.005; ** P-value=0.000

Discussion

Fibrosis is a tissue disorder that can be found in several vital human organs and is the most significant risk factor as a cause of organ dysfunction. Fibrosis is characterized by excessive accumulation of extracellular matrix (ECM) components that disrupt the function of a cardiac tissue, that composed by cardiomyocytes [17,18]. Besides, fibrosis can also be known based on protein levels or increased gene expression in the pathogenesis of cardiac fibrosis. One of the essential proteins that are pro-fibrotic mediator is galectin-3. Cardiomyocyte almost does not express galectin-3 under normal physiological conditions [19]. But in certain pathological conditions, cardiomyocytes can also act as alternative sources of galectin-3 which induces fibroblasts to synthesize collagen and other pro-fibrotic components

from the extracellular matrix [20,21].

In this study, researchers used a metabolic syndrome model that was given CGA therapy of 200 mg/kgbw and/or EGCG 300 mg/kgbw to investigate the potential of the two compounds in inhibiting galectin-3 overexpression. The treatment group with CGA therapy 200 mg/kgbb was known give effect on decreasing galectin-3 expression in cardiomyocyte tissue (P=0.000), compared with the group given EGCG therapy of 300 mg/kgbw alone. Meanwhile, the combination group of the two therapies had a good effect, not much different from the group given EGCG therapy of 300 mg/kgbw only. Although the lowest expression was obtained in the EGCG 300 mg/kgbw therapy group only, the combination of CGA 200 mg/kgbw and EGCG 300 mg/kgbw also suppressed galectin-3 expression (P=0.000). So, CGA and EGCG expected to reduce the progression of cardiac fibrosis. Raised galectin-3 expression in the metabolic syndrome model group, has the potential to make it possible that even in models that do not have cardiac fibrosis, the galectin-3 expression is a mediator that plays a role in the mechanism of diabetic cardiomyopathy. The correlation analysis between galectin-3 gene expression and collagen deposition for five treatment groups obtained a positive correlation coefficient, i.e. the higher the expression of the galectin-3 gene, it is possible to increase collagen deposition in cardiomyocyte tissue.

Other studies suggest that the use of EGCG as a supplement can significantly reduce systemic galectin-3 levels in wildtype mice and overexpress dual-specificity tyrosine phosphorylation-related kinase 1A (Dyrk1A) mice. It also followed by a decrease in the expression of collagen-1 and collagen-3, which are the main biomarkers for fibrosis heart[22]. Based on other studies, the administration of EGCG therapy to aged rats was able to provide a significant effect on increasing cardiomyocyte diameter and volume with a decrease in numerical density [23]. Oral EGCG therapy for one month after diabetes induction proved significantly inhibits over-expression of serum levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) as well as ICAM-1 and VCAM-1[24]. So overall, EGCG is showing anti-inflammatory potential to inhibit cardiac hypertrophy, fibrosis, and apoptosis that are triggered by ageing.

Research conducted by Tian et al. showed that CGA could repair the cellular injury caused by TNF-α[25]. Later, it has implications for increased cell viability, increased mitochondrial membrane potential, and inhibited cardiomyocyte apoptosis in mouse models of heart failure induced by transverse aortic constriction (TAC). Research on myocardial infarct (MI) mice reports that in the group given CGA therapy 30 mg/kg BW there was a significant increase in contractions compared to the MI vehicle group. Day-14 of the study showed an increase in macrophage infiltration in the MI vehicle group. This result shows the role of CGA in inhibiting fibrotic pathogenesis which is characterized by macrophage infiltration, without giving systemic side effects [26]. The fibrosis signalling pathway is a complicated mechanism. However, TGF-β1/α-smooth muscle actin (SMA)/ Collagen-1 pro-fibrotic pathway has widely used to explain the induction of cardiac hypertrophy and fibrosis in models or patients with heart failure [27]. TGF-β1 is known to bind to receptors in fibroblasts and myocytes, thereby activating the Smad 2/3/4 complex to facilitate nuclear translocation, and initiate the synthesis and secretion of pro-fibrotic cytokines, such as pro-collagen and α-SMA, which can induce fibroblasts to differentiate into myofibroblasts [28]. Galectin-3 is known to act by maintaining or increasing TGF-β receptor binding on the myofibroblast cell membrane, thereby promoting pro-fibrotic signalling through the Smad and PKB / Akt pathways and stimulating the production of the extracellular matrix (ECM) [29].

Based on this research, EGCG and CGA are synergistic in inhibiting cardiac fibrosis which is one of the complications due to diabetes, as seen from suppression of cardiomyocyte galectin-3 levels. This synergy can be caused by the two molecules being antioxidants that work to suppress inflammation. EGCG and CGA, which are a group of polyphenols, are known to be able to inhibit oxidative stress which is its central role as an antioxidant. Although the whole mechanism not widely understood, the anti-inflammatory effect given by the two components is quite good.

The difference between the two pathways in inhibiting fibrosis; EGCG that tends to be an anti-inflammatory agent, inhibiting the overexpression of cytokines and infiltration of inflammatory cells. Meanwhile, CGA is known to be quite useful in inhibiting

the pathogenesis of fibrosis through the TGFb pathway, so that it can reduce the expression of collagen 1 and 3, which expression has not measured in this study.

Conclusion

Therapy using a combination of chlorogenic acid (CGA) green coffee extract and epigallocatechin-gallate (EGCG) from green tea extract orally on animal models of metabolic syndrome is known to reduce the expression of galectin-3 gene percentage in collagen deposition in heart tissue. This study shows that the administration of combination therapy CGA and EGCG is an excellent therapeutic candidate, so that is supposed to reduce the progression of cardiac fibrosis in metabolic syndrome.

Declaration of Conflict: Author declares there is no conflict interest

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A Comparative Study to Evaluate the Effect of Blood Flow Restriction Therapy and Retro Walking on Pain, Strength of Muscles and WOMAC Score in Patients of Osteoarthritis of Knee

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Abstract

Background - Osteoarthritis of knee being of the major causes for disability in older population. Due to sedentary lifestyle this disease is becoming common in younger population as well. As prevalence of the disease is increasing it is a need of the hour to come up with a highly reliable exercise protocol which will be beneficial for the patients. This research aims to establish a beneficial and reliable exercise protocol which can be well tolerated by the patients suffering from osteoarthritis of knee.

Methods – 150 subjects will be selected. Randomly they will be assigned to two groups that is Group A and Group B using chit method. Group A will receive Blood Flow Restriction Training and Group B will receive retro walking training. Training will continue for 15 days. After 15 days patient will be reassessed for pain, strength and WOMAC score.

Result – Studies have proved that Blood Flow Restriction Therapy and retro walking both the intervention are beneficial in patients of osteoarthritis to reduce pain, improve strength and WOMAC score. This study will provide us an intervention which is beneficial in treating osteoarthritis and is well tolerated by the patients. We may get a result where blood flow restriction would have a better impact on few outcome measures while retro walking may fetch better impact on other outcome measures. We may also conclude that combination of both the interventions is a better option for the treatment of osteoarthritis patients.

Keywords *– Knee osteoarthritis, Blood Flow Restriction Therapy, Retro walking, WOMAC score*

Introduction

Osteoarthritis is a degenerative disease of joints. It causes wear and tear of joints as one ages. The structures which are affected are the bones, the cartilage and the synovium. Knee and hip being the weight bearing joints are susceptible to degenerative changes that interfere with physical activities and quality of life. Knee OA is the major source of pain and impairment in elderly population. Thirty three percent of individuals aged between 63 and 94 years suffer from knee OA which restricts their ability to stand, walk and climb stairs. In the population aged 60 years and above the occurrence rate of knee OA is 10% in males and 13% in females among.[1]

Osteoarthritis is a degenerative disease which is progressive in nature. This disease occurs mostly in weight bearing joints of lower limb. Pain is the earliest symptom which may be dull aching initially but may

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become worse and consistent in later stages of the disease. Swelling is also a common complaint associated with the disease. Pain and muscle spasm initially cause stiffness but later capsular contracture and incongruity of joint surface sets in. Decreased flexibility of muscles reduces the joint range of motion and thus contributes to increase in the stiffness of the joint. Physiotherapy is very beneficial treatment and provides symptomatic relief to patients of osteoarthritis of knee. Research also prove that some recent advances like blood flow restriction therapy and retro walking may be beneficial to the patients if added to their rehabilitation protocol.[1]

Blood flow restriction (BFR) exercise requires only mildly regulating arterial inflow and allowing relaxing (or "slowing") of venous outflow at the top of the arms or legs while training either very low-weight arm, leg or core muscles with good reproducibility to failure. Venous blood flow restraint is accomplished by applying an inflatable collar or brace to the extremity being exercised. The band must be tight enough to minimize normal venous transfer to the heart whereas remaining loose enough to facilitate arterial passage. BFR enables you dramatically improve muscle and strength by using as little as 20 to 30 percent of your maximum weight in one repetition (1 RM) as opposed to traditional strength training using 70 to 85 percent of your 1 RM. Alternatively one may contract the muscles with the BFR bands and move them very steadily on for those who can't or don't want to use weights.[2]

BFR makes use of lighter weight than Traditional strength training, and continuing to make it much widely available to variety of individuals including the elderly and disabled or injured patients. This intervention is indeed a vigorous training, as during the first set type I moderate jerk muscle fiber become exhausted, Therefore Type II moderate muscle jerk fibers are used as the exercise progresses, which is certainly known for many of the BFR 's physiological benefits.[2]

Retro-walking is protected shut dynamic chain practice as it decreases the compressive powers at the patellofemoral joint. During Retro-walking quadriceps flighty capacity is diminished, while the isometric and concentric quadriceps quality is protected. Retro-strolling preparing programs are known to expand quadriceps quality. Additionally, during retro walking cardiopulmonary interest is higher when contrasted with forward-walking. Henceforth, such advantages make backward walking a protected and viable segment of recovery schedule for patients of knee osteoarthritis. [3]

Methods/Design

Aim- To assess the impact of blood flow restriction and retro walking in knee osteoarthritis subjects.

Study Design

This study will be conducted in musculoskeletal sciences division, RNPC Sawangi, Wardha after endorsement from institutional ethic advisory group of DMIMS, deemed to be university. Before including all the participants will be told about the aim and procedure of the research. All the participants who meet inclusion criteria must give written consent. All the 150 participants diagnosed with osteoarthritis will be allocated to group A or Group B randomly by chit method and will be enrolled for the study for two weeks.

Participants

Inclusion Criteria

150 men and women of the age group 50 to 70 years will participate in the study. All the participants should be diagnosed patients of OA knee depending on the American College of Rheumatology criteria (unilateral or bilateral).[3] They must have symptomatic knee OA and must be graded from 1 -3 on Kellengren and Lawrence scale.[3,4]

Exclusion Criteria

Participants undergone any knee surgery to either knee inside recent months,[3] any sort of foundational ligament condition, some other solid, joint or neurological condition influencing capacity of lower appendage, experienced active recuperation treatment or taken intra-articular joint injections for knee joint for last previous three months will be excluded.[3] Also participants with fringe vascular illness, systolic pulse more prominent than 160 or under 100 mm Hg, diastolic circulatory strain more noteworthy than 100 mm Hg, profound vein apoplexy, previous myocardial localized necrosis, paralysis in the earlier year, or previous malignant growth which created confinements for work out will be excluded to avoid further complications.[4,5]

SAMPLE SIZE CONSIDERATION

Total 150 participants will be included in the study**.** Formula which was used to calculate the sample size was simple sample size calculation formula. Article of reference used was osteoarthritis in India: an epidemiologic aspect which was published in International journal of resent scientific research in October 2017. Participants will be randomly classified into two groups by chit method.

INTERVENTION DESIGN

Group A – Blood flow restriction therapy

All the participants will undergo 5 mins of warm up session followed by conventional

physiotherapy exercises. Then patient will undergo strength training using blood flow restriction therapy, followed by a 5 mins of cool down session.[5]

Warm up session - 1. Ankle toe movements

2. Heel slides

Conventional physiotherapy exercises - 3. Static quadriceps (10 sec holds with 10 repetitions)

4. Active hip movements (10 repetitions of each)

Strength training session - In the blood flow restriction therapy, resistance exercises will be done at twenty per cent of 1RM with external pressure on each leg's upper thigh. [4]

Exercises which will be performed[5] – 1. Dynamic quads

2. Hamstring curls

In each set counts of reps will be as follows [2]

In first sitting = 30 reps with rest of 20 seconds

In second sitting = 20-30 reps with rest of 20 seconds

In third sitting = 10-20 reps with rest of 20 seconds

Pressure of the cuff is set according to [pressure= 0.5 (systolic blood pressure) + 2(thigh circumference) + 5][6]

Cool down session – 1. Hamstring stretching

2. Calf stretching

3. Heel raises

Group B – Retro walking

All the participants will undergo 5 mins of warm up session followed by conventional physiotherapy exercises. Then patient will undergo 10 mins of retro walking session, followed by a 5 mins of cool down session. [3]

Warm up session – 1. Ankle toe movements

2. Heel slides

Conventional physiotherapy exercises - 3. Static quadriceps (10 sec holds with 10 repetitions)

4. Active hip movement (10 repetitions of each)

Strength training session -1. 10 mins of retro walking [3]

Cool down session - 1. Hamstring stretching

2. Calf stretch

3. Heel raises

Outcome Measures

1) Numerical pain rating scale – it is a 10-point scale which is used to measure pain where 0 signifies no pain and 10 signifies maximum pain. This scale has Excellent test-reliability with interclass association element of 0.95.[7]

2) Modified sphygmomanometer to measure strength of muscles – it will be used for calculating knee muscle strength. The Modified Sphygmomanometer Scale (MST) is an effective tool for intensity calculation, because it is inexpensive and provides objective values to measure the strength of knee muscles.[8]

3) WOMAC scale for functional activities – it is a 24-point scale which is used to rate day to day activities of person. Score out of 96 points will be calculated and depending on that the disability will be judged. One of the most commonly employed tests to test clinically

significant improvements in KOA patients is the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC). This contains three subscales that assess rigidity, pain and work. Substantial pain problems reflect a specific mechanical joint load and by recognizing behaviors with early signs, this can suggest when organized pain happens first and therefore whose operation is correlated with the initiation of pain.[9]

Statistical Analysis

Data will be coded and entered in MS excel worksheet and analyzed using appropriate statistical software.

Discussion

The study is aimed at assessing the impact of blood flow restriction and retro walking in subjects of osteoarthritis of knee. A recent study proved that retro walking plays a big role in increasing extension moment, improvising strength in functional range with improving physical function[10]. A study conducted in 2017 proved that as compared with normal quadriceps strengthening, low load with BFR produced pain reduction to a greater extend with improvement in daily living activities at 8 weeks in people with patella femoral Pain[11].Hence we can see that earlier studies have proved that both these interventions are beneficial in pain reduction and improvement of strength and WOMAC score. But amongst the two which one is better and well tolerated by the patients is not known. Hence aim of this research is to find out that which intervention amongst the two will be beneficial for the patients of osteoarthritis of knee.

To conclude we aim to find out effect of Blood Flow Restriction Therapy and Retro Walking on pain, strength and WOMAC score. Hence which intervention is more beneficial on these outcome measures is to be analyzed.

Result

This study will help us to find a better intervention amongst the two intervention. We may come to result where we may find that for some outcome measures blood flow therapy may provide better results and for others retro walking may be a better option.

Research Ethics Approval:

The trial will be performed in accordance with the Declaration of Helsinki.

Confientiality:

Specific patient's information will be kept separate from the central dataset, and will not be exchanged. All personal data will be stored securely before, during and after the court to preserve the confidentiality.

Funding:

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Environmental Conditions and Sincerity affects Cortisol and B-Endorphins Plasma Levels in Young Healthy Subjects Undergoing Dawood's Fast

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Abstract

Background: Prevention of Coronary Heart Disease by identifying risk factors facilitates the planning of prevention interventions. The prevalence of non-communicable disease (NCD) increases with one of the risk factors for NCD being an unbalanced diet. Dawood's fasting is one of the sunnah fastings that can be done at any time except at times that are forbidden to fast. This fast is a combination model of TRF and ADF (modified ADF = MADF).

Objective: To prove that MADF for six consecutive weeks can increase levels of the hormone β-endorphins and reduce levels of the hormone cortisol in peripheral blood circulation.

Materials and Methods: Quasi-experimental research with a comparative design of a non-equivalent control group with a population of PP students. Hidayatullah Surabaya with the purposive sampling method until the number of samples required was met as many as 34 people and divided into two groups, namely control and MADF treatment for six consecutive weeks. Sampling was carried out at the pre-test, at the end of the third week for the middle and at the post-test. All subjects in both groups received the same nutrition twice a day for 42 days. The subject gets spiritual motivation from competent resource persons to give spiritual spark twice.

Result: Dawood's fasting (MADF) for six consecutive weeks did not cause significant differences compared to control on cortisol and β-endorphins ($p > 0.05$). However, Dawood's fasting (MADF) for six consecutive weeks decreased the mean values of cortisol levels due to the effect of fasting, although there was also a significant difference in the control group. Meanwhile the β-endorphins mean values was increased at the middle of study, and then decreased at the end of the study, with a significant difference in the both group before and after treatment.

Conclusion: There was no difference in cortisol and β-endorphins levels in healthy young adult subjects undergoing Dawood's fast due to the influence of the same environmental conditions and a change in the sincerity of the subjects.

Keywords: *Modified alternate day fasting (MADF); Dawood's fasting; atherosclerosis; cortisol; β-endorphin*

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Introduction

The prevalence of death due to Non-Communicable Diseases (NCD) in Indonesia, amounting to 63% of all

deaths[1]. Pusdatin (2018) reports the results of Riskesdas in 2007 and 2013 NCD to tend to increase including heart disease[2]. Of 9.4 million deaths in the world each year due to cardiovascular disease, 45% are coronary heart disease (CHD)[2]. CHD is the seventh-highest NCD in Indonesia. The sample registration system survey results show that the death rate due to CHD is 12.9% of all deaths[3].

Prevention of CHD by identifying risk factors facilitates the planning of prevention interventions. This is following the results of Riskesdas 2017 which stated that the prevalence of NCD increases, NCD risk factors one of which is the diet which is out of balance. The control program on NCD is more focused on prevention efforts[2].

Atherosclerosis is the formation and growth of plaque in the arterial lumen together with loss of elasticity of blood vessels, an inflammatory response in the large arteries that begins with subclinical endothelial dysfunction can turn into life-threatening diseases that manifest as myocardial infarction or stroke. Atherosclerosis is not solely based on balanced lipid metabolism but is mainly driven by chronic inflammation in the walls of blood vessels involving many types of cells[4].

Intermittent fasting (IF) has been shown to increase life span and reduce the incidence of age-related diseases such as diabetes and effectively reduce the risk of heart disease in obesity and reduce risk factors for cardiovascular disease[5]. Ahmet et al (2010) in his study using rats with alternate day fasting (ADF) for six months monitored its echocardiography concluded that increased tolerance of heart muscle to ischemic damage[6]. Other studies on the two-week-old Drosophila fly model that was fasted 12 hours per day (time-restricted feeding = TRF) without reducing its daily calorie intake showed that there was no increase in weight the body from the age of 3 to 7 weeks, where free-eating flies experience the aging process of the heart which is characterized by, among others, an increase in the diastolic and systolic intervals, and arrhythmias[7].

Fasting affects the immune system through hormonal changes associated with the adrenal glands of the kidneys such as cortisol[8]. Several studies have reported that Ramadan fasting induces changes in the circadian rhythm of a number of body hormones including cortisol[9].

The fasting period is often associated with changes in diet, sleep and ritual habits[10], which can change the body's circadian rhythm and become a stressor that the body will respond to[11]. Fasting that is done with a strong intention will give a positive perception, so that it will form an adaptation response to fasting which was originally recognized as a stressor that can reduce the body's immunity to increase the responsiveness of the immune system. One of the causes of this is due to the release of endorphins from the anterior pituitary when a person intends to fast[12]. Ramadan fasting has a positive effect on inflammation by suppressing the expression of proinflammatory cytokines and reducing body fat and the level of circulating leukocytes[13].

Dawood's fasting is one of the sunnah fasts carried out by Muslims at any time except at times that are forbidden to fast. This fast is a combination model of TRF and ADF (modified ADF = MADF). The way to do this is not to eat from sunrise to sunset, and to do with one-day fasting and one-day not fasting[14]. Izzaturrahmi et al (2017) research on the effect of Dawood's fasting on body mass index (BMI) and abdominal circumference in 44 men and women over 50 years old in Yogyakarta, found significant differences in BMI and belly circumference of the Dawood's fasting and non-fasting respondents[15]. Research on students of the Al-Fithroh Islamic Boarding School in Yogyakarta found that perpetrators of Dawood's fasting tend to have a high level of emotional control so that they can withstand anger and lust[16]. This can prevent mental-emotional disorders which are one of the risk factors of CHD. Yatindra et al. (2019) proved that there was a significant decrease in white blood cells in a group of mice undergoing the Dawood's fasting model with a 10-hour fast by taking turns a day after being induced with acetaminophen[17].

Specific research aims to prove that MADF for six consecutive weeks can increase levels of the hormone β-endorphins and reduce levels of the hormone cortisol in peripheral blood circulation. The urgency of this research is to prove that MADF can be used as a model of prevention of cardiovascular disease, especially those related to the process of atherosclerosis associated with

stress and the hypothalamic-pituitary-adrenal (HPA) axis.

Material and Methods

Design

This study was a quasi-experimental study with a comparative pre-test post-test group design. Samples of Hidayatullah Islamic boarding school students in Surabaya 3, 5, and 7 semesters were 134 people with a purposive sampling method until the required number of samples were met.

The experimental protocol of the study was approved by the Medical Faculty of Universitas Airlangga ethics committee with No. 163/EC/KEPK/FKUA/2019, and the research reported here was carried out following the principles of the Declaration of Helsinki as revised in 2000. The research was carried out from August 2019 to June 2020.

Setting

This study was carried out in a young healthy male Indonesian Muslim setting at the Hidayatullah Islamic Boarding School in Surabaya, Indonesia during which fasting was conducted for approximately 14 hours. The participants received some information about Dawood's fasting before they fasted and given a weekly reminder to fast via Short Message Services (SMS), Whatsapp messages, or telephone.

Before the start of MADF (Dawood's fasting) and during the fast, respondents will receive spiritual motivation from a resource person who is competent to give spiritual splash. The aim is for the respondent to maintain a true and sincere intention in following this research until completion.

Participants

The subjects were young healthy male Muslim people divided into two groups, those who observed Dawood's fasting and a group who did not perform the fast. Samples were collected using the consecutive sampling method from January until August 2019. The inclusion criteria were 18-30 years old, Muslim, male, willing and able to do MADF (Dawood's fasting) for six consecutive weeks as evidenced by informed consent, healthy (no history of metabolic disease (HT / DM / CHD as evidenced by history, physical examination and screening Electrocardiogram (ECG) and Random Blood Glucose (RBG)). The number of samples per group required for this study with a correction factor of 30% was 17 samples. The total sample required for this study was 34 samples. Each respondent will fill in the research questionnaire instrument that has been prepared and sign an informed consent.

Intervention

The fasting group precluded any activities that could break the fast (such as eating, drinking, having sexual intercourse) from sunrise to sunset, preceded by the expression of intention to fast on alternate days[14]. This activity lasted for 42 days with 21 days of fasting starting from first following by third, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th, 21st, 23rd, 25th, 27th, 29th, 31st, 33rd, 35th, 37th, 39th, and 41st day. The research subjects in both groups were given the same food at dawn and breaking fast (fasting group), and at breakfast and dinner (non-fasting group) and were recommended to always eat the meal before dawn and break the fast immediately after the sunset. During the fasting process, activities of the subjects were observed by the researchers through personal contact with each of them via telephone.

Assessment

Fasting is done from sunrise to sunset (+ 14 hours) and alternating fasting a day and not a day[14]. A blood sample was obtained from each participant before, intermediate, and at the end of Dawood's fasting for 42 days at the Hidayatullah Islamic Boarding School in Surabaya, and was transferred to the Clinical Pathology Laboratory at Dr. Soetomo regional public hospital of Surabaya. Plasma's cortisol and β-endorphin were determined. Venous blood samples were drawn between 0700 and 0900 AM and centrifuged for 15 min at 4ºC. Immediately after centrifugation, the serum samples were frozen and stored at -80ºC. Plasma's cortisol levels were measured with chemiluminescence immunoassay (CLIA) examination using the ADVIA Centaur XPT Immunoassay System tool, Siemens Healthcare Diagnostics, with a ADVIA Centaur® Cortisol (COR) Assay kit by measuring serum range 0.50-75 mg / dL.

β-endorphin levels in serum were measured with an ELISA kit (Elabscience, USA, catalog number

E-EL-H0572). The range access was 15.63-1000 pg/mL. The inside and inter-assay coefficients of variance were less than 5.29% and 4.45%. the measurement of the β-endorphin levels in serum was done in June 2020, because of the trouble in the reagent, so the researcher has to reorder the reagent which takes a time about 6-8 weeks.

Statistical Analysis

The data have been analyzed the normality of distribution using the Shapiro-Wilk test because of the data number below 50. The next step was using the paired T-test (parametric data) or Wilcoxon-signed rank test (non-parametric data) to compare the pre and post-test data both in fasting and non-fasting group. If there were a significant difference, then the test will be continued to two independent samples T-test (parametric) or Mann-Whitney-U test (non-parametric).

Results and Discussion

Of 44 healthy men that volunteered to participate in this study, which were 24 men join the fasting group and 20 men join the non-fasting group. Respondents who entered the inclusion criteria as many as 40 people, 20 from the fasting group, and 20 from the non-fasting group. Before starting the MADF (Dawood's fasting) respondents will be drawn blood for a pre-test examination. At the time of fasting, all respondents both the control group and the treatment will get the same kind of nutrition twice a day for 42 days. At the end of the third-week blood sampling will be done mid, then at the end of the seventh-week respondents will do a post-test blood sampling.

At the end of the study, after 42 days of modified alternate-day fasting, there were only 34 subjects left to finish the study because in the fasting group, there were 2 subjects stopped fasting without any reason and 1 subject stopped fasting due to illness. On the other hand in the non-fasting group, there were 3 subjects could not follow the sampling schedule because of their activity.

The general description of the observed research subjects included age, weight, height, body mass index, and waist/hip circumference, to assess the nutritional status of the study subjects. Complete results can be seen in the table below.

Table 1 Characteristics of subjects based on age, weight, body mass index (BMI), and waist-hip ratio (WHR)

No	Variable	n respondent	
		Treatment n = 17	Control n = 17
1.	Age 18 – 20 years-old 21 – 23 years-old 24 – 26 years-old 27 – 30 years-old	 9 (53%) 7 (41%) 1 (6%) 0 (0%)	 10 (59%) 7 (41%) 0 (0%) 0 (0%)
2.	Body Mass Index (BMI) Underweight Normal Overweight Obesity 1 Obesity 2	 9 (53%) 7 (41%) 1 (6%) 0 (0%) 0 (0%)	 4 (24%) 12 (71%) 1 (6%) 0 (0%) 0 (0%)
3.	Waist – hip rasio (WHR) < 0,95 = low risk of CVD > 0,95 = high risk of CVD	 16 (94%) 1 (6%)	 17 (100%) 0 (0%)

Table 1 above shows the distribution of age, BMI, and WHR in research subjects. The age of the research subjects all met the inclusion criteria which ranged from 18-30 years with the greatest frequency in the treatment group being in the age range 18-20 years-old as many as 9 people (53%), then the age range 21-23 years-old as many as 7 people (41%) and the age range of 24-26 years-old is 1 person (6%). Most of the subjects in the non-fasting group were in the age range 21-23 years-old as many as 10 people (59%) and the age range 18-20 years-old as many as 7 people (41%). This shows that the age distribution in the treatment group is relatively younger than the non-fasting group.

The distribution of BMI calculated based on body weight and height of the subject with the formula BW (kg) / BH^2 (m^2) in the most treatment group with underweight BMI was 9 people (53%), followed by normal BMI of 7 people (41%) and BMI overweight as much as one person (6%). The distribution of the control group is different from the treatment group, namely the most normal BMI is 12 people (71%), BMI underweight is 4 people (24%) and BMI overweight is one person (6%). BMI can be used to assess nutritional status so that in general the subjects in the non-fasting group showed better nutritional status than the treatment group.

WHR calculated based on the ratio of waist circumference and hip circumference (in cm) showed that in the treatment group 94% or as many as 16 people had a WHR value < 0.95, and one person had a value > 0.95 (6%). All subjects in the non-fasting group had a WHR value of < 0.95 (100%). A WHR value < 0.95 indicates a low cardiovascular risk, while a value > 0.95 indicates a high cardiovascular risk.

Based on the general description of research subjects, it was found that all research subjects were in a state of nutritional status who were not obese.

The table below shows the results of the research for both cortisol and β-endorphins.

Table 2 Descriptive data of the cortisol (µg / dL) and β-endorphins (pg / mL)

Variable	Group	Pre-test	Intra test	Post-test
		Mean + SD	Mean + SD	Mean + SD
Cortisol	Not fasting	16,72 + 4,16	11,75 + 4,48	12,39 + 2,80
	Fasting	16,01 + 5,09	12,60 + 4,27	11,11 + 3,52
β-Endorphin	Not fasting	473,44 + 249,87	637,32 + 325,38	302,09 + 94,38
	Fasting	529,07 + 286,50	695,32 + 257,71	320,14 + 67,57

Table 1 above shows the descriptive data in the form of cortisol and β-endorphins mean values at the beginning (pre-test), middle (intra-test), and end (post-test) of the study for each group. These results can be illustrated in figure 1 below.

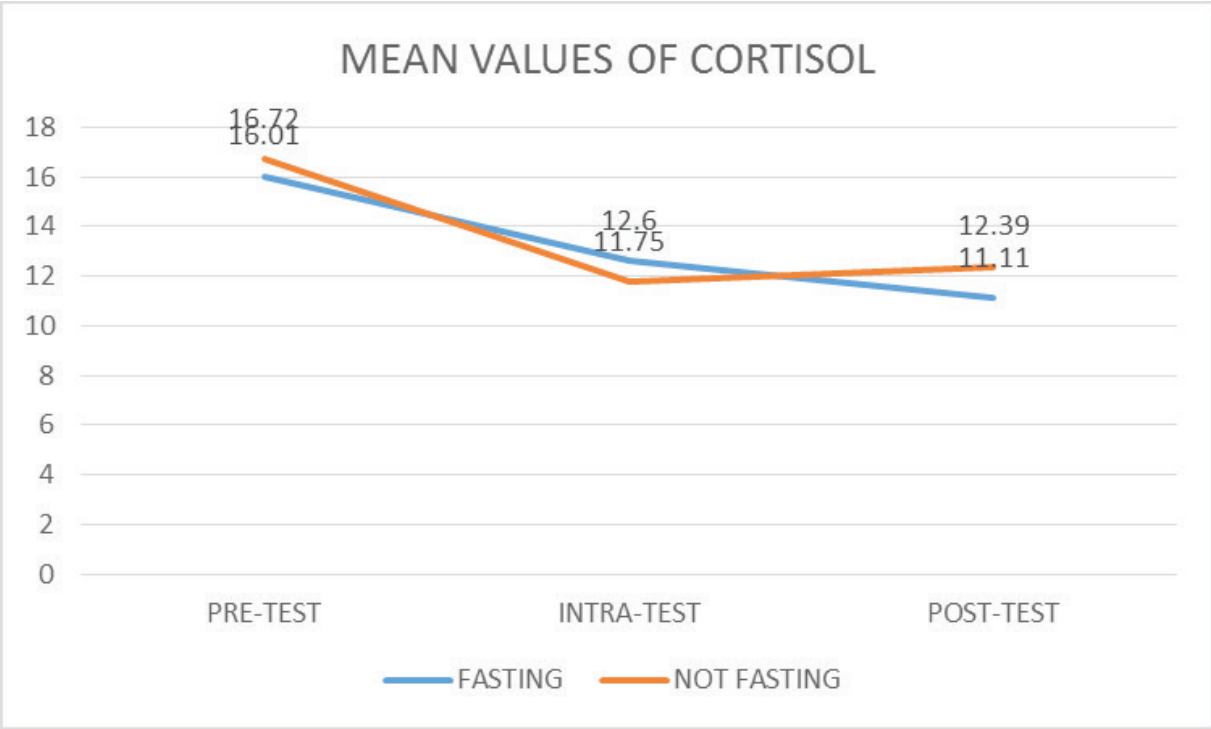

Figure 1 The graph of the cortisol mean values at the beginning, middle, and end of the study for each group.

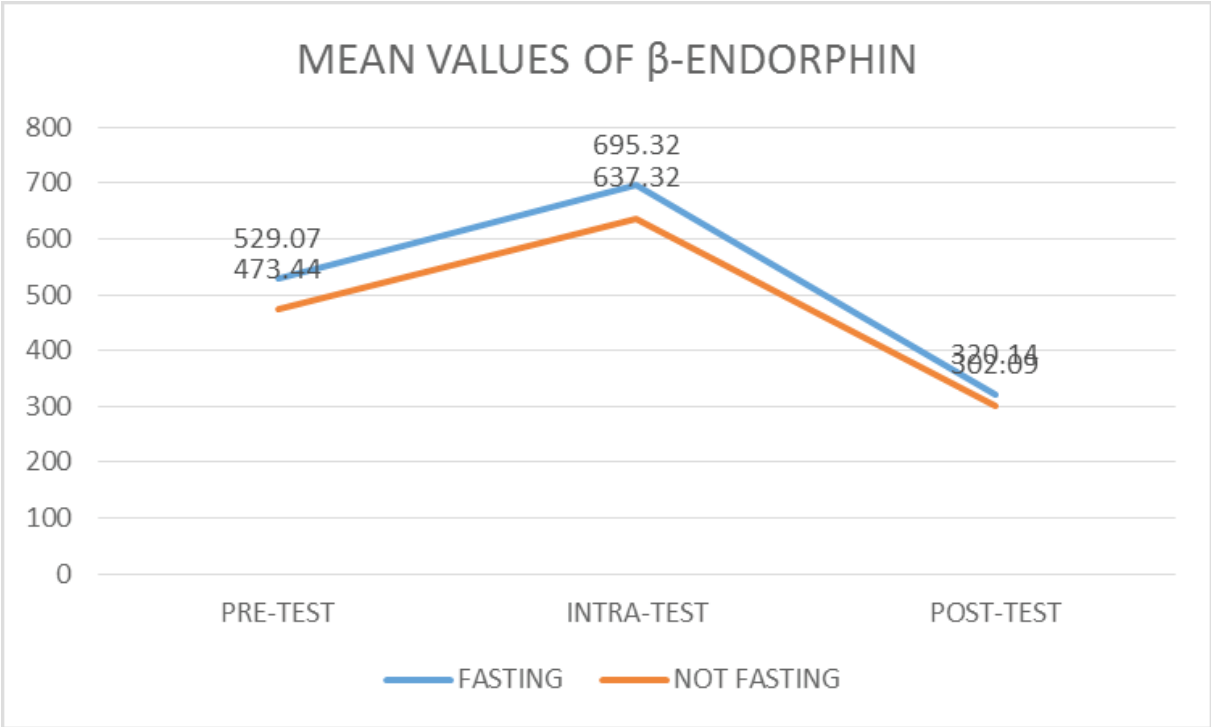

Figure 2 The graph of the β-endorphins mean values at the beginning, middle, and end of the study for each group.

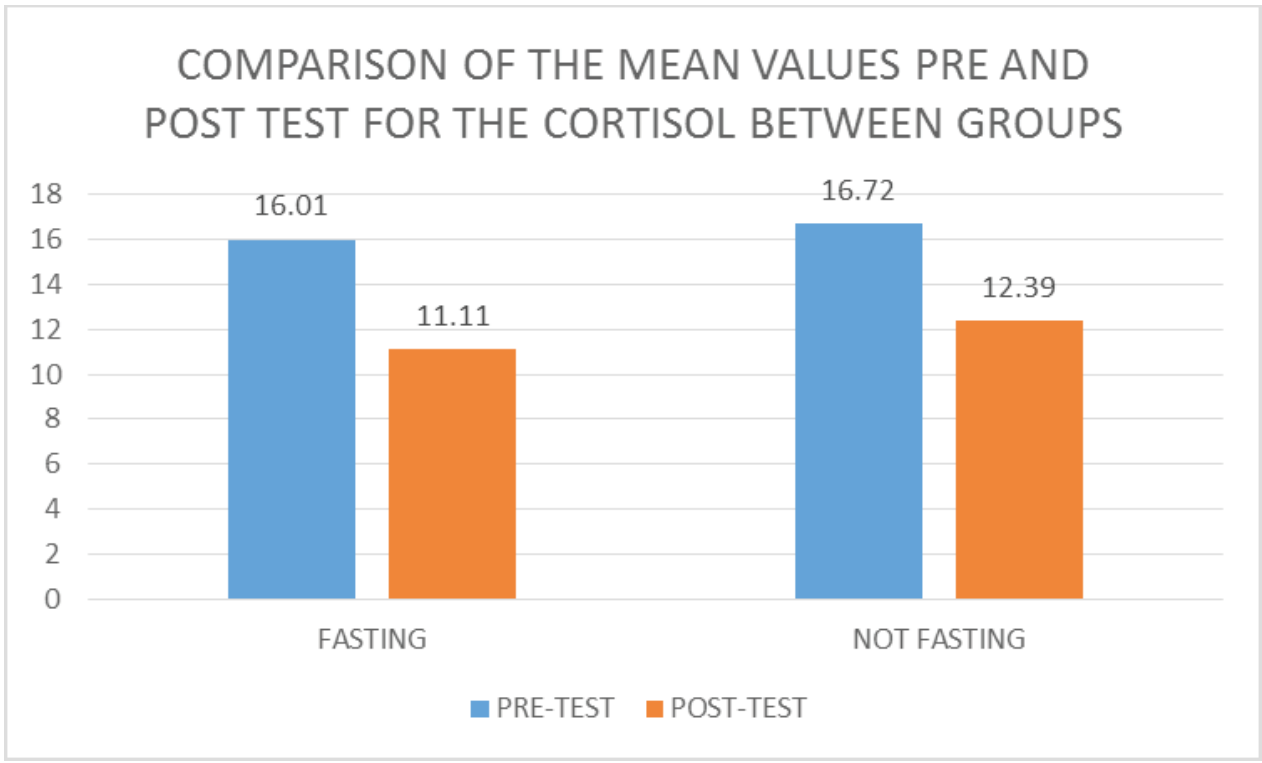

Figure 3 The comparison between mean values of the pre and post-test data for the cortisol

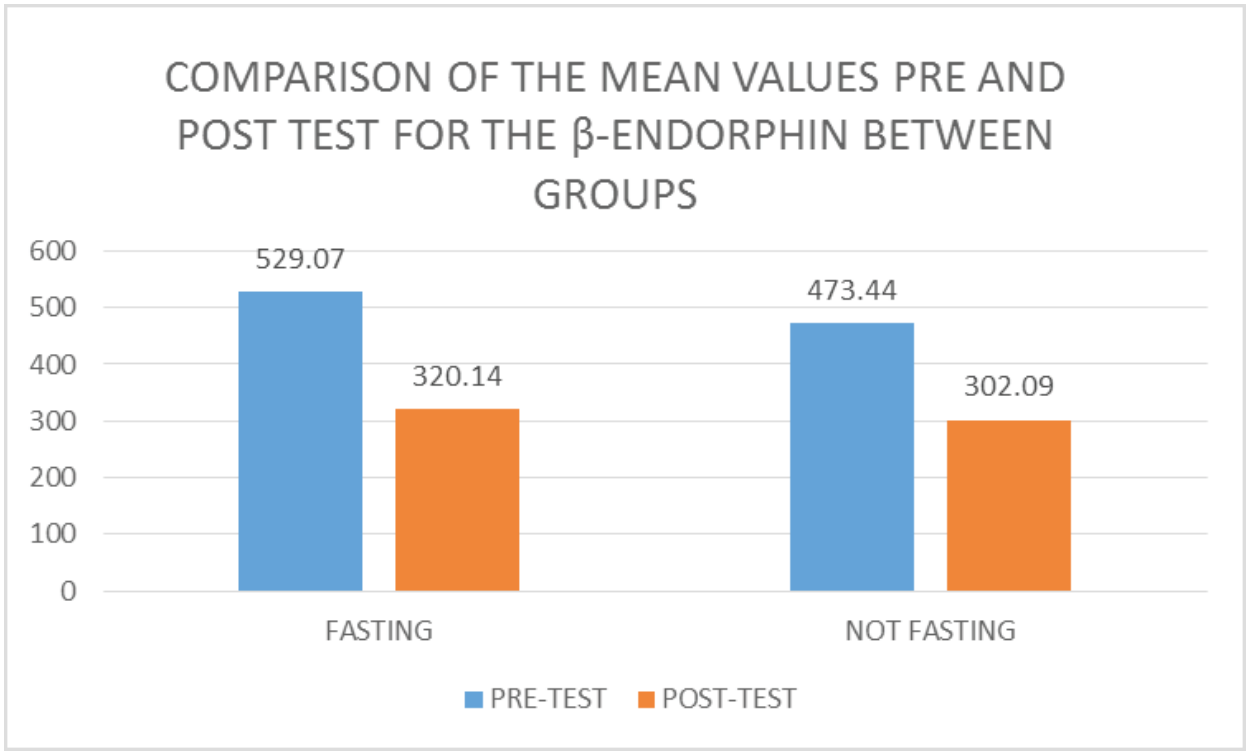

Figure 4 The comparison between mean values of the pre and post-test data for the β-endorphins

The mean values of the cortisol in figure 1 show that the mean values of the post-test cortisol level in the fasting group were lower than the non-fasting group, while figure 3 shows that in both groups the mean values were decreased at the end of the study.

The β-endorphins mean values in figure 2 show that the post-test β-endorphins mean values in the fasting group were higher than the non-fasting group, while in figure 4 it shows that in both groups the mean values were also decreased at the end of the study.

Table 3: The comparison between-time (pre and post-test) and between-group (fasting and non-fasting) for the mean values of the cortisol and β-endorphins

Variable	Group	p Between-time	p Between-group
Cortisol	Fasting	0,01[a]	0,71[c]
	Not fasting	0,01 [a]	
β-endorphin	Fasting	0,03[b]	0,67[d]
	Not fasting	0,01 [b]	

Note : signicantly different, if p < 0,05; a : paired-T test; b : Wilcoxon signed-rank test; c : two independent samples T-test; d : Mann-Whitney-U test.

Data from the normality test of the cortisol variable showed that all data were normally distributed (p>0,05) in all control and treatment groups. This caused the test to be continued for a paired-T test to compare pre and post-test cortisol data for both groups. The results of the paired T-test were showed significantly different with p = 0,01 in both groups. Therefore the test to be continued with the two independent samples T-test, because the normality test of the delta pre and post-test was normally distributed. The result of the two independent samples T-test showed p = 0,71, which means there was no difference between groups.

Meanwhile, the data from the normality test of the β-endorphins variable showed that not all data were normally distributed (p>0,05) in all control and treatment groups, the test to be continued for non-parametric Wilcoxon-signed rank test to compare pre and post-test β-endorphins data for both groups. The results of the non-parametric Wilcoxon-signed rank test showed that there was a significantly different both in fasting (p=0,03) and non-fasting group (p=0,01). Therefore the test to be continued with the Mann-Whitney-U test, because the normality test of the delta pre and post-test was not normally distributed. The result of the Mann-Whitney-U test showed p = 0,67, which means there was no difference between groups.

The aim of this study was that Dawood's fasting for six consecutive weeks resulted in lower cortisol levels and higher β-endorphins levels than non-fasting. The results of this study found that the post-test mean values of cortisol levels in the fasting group were lower than the pre-test mean values, with a significant difference in comparison test as shown in table 3 using the paired T-test. The non-fasting group also showed the same results that the post-test mean of cortisol levels was lower than the pre-test mean, with a significant difference of comparison test as shown in table 3 using the paired T-test. Because of the significant differences between both groups, the test was continued with the comparison test between groups using two independent samples T-test. The result of two independent samples T-test was p = 0,71 (p<0,05), which was not found the difference between groups.

For β-endorphins levels, it is shown in table 2 that the post-test mean values of β-endorphins levels in the fasting group were lower than the pre-test mean values, with a significant difference in comparison test as shown in table 3 using the non-parametric Wilcoxon signed-rank test on both groups. Because of the significant

differences between both groups, the test was continued with the comparison test between groups using the Mann-Whitney-U test, and the result was p = 0,67 (p<0,05), which was not found the difference between groups.

This means that there are no differences between people who did the Dawood's fast (MADF) and did not on cortisol and β-endorphins levels in this study. This is presumably because research subjects come from the same environment, a boarding school dormitory where their activities are almost the same for all subjects. Apart from that, motivation by the source is also thought to be the cause of the absence of significant differences between the fasting and non-fasting groups.

Cortisol is a steroid hormone from the glucocorticoid class which is produced by cells in the fasciculate zone of adrenal glands in response to the stimulation of the ACTH hormone secreted by the anterior pituitary gland. Cortisol is a hormone that responds to stress received by the body[18,19]. The high production of adrenal hormones and catecholamines will result in a narrowing of the heart blood vessels and an increased heart rate and ultimately will result in disrupted blood supply to the heart. Prolonged stress can increase the production of the cortisol hormone in large quantities by the body, which has been shown to cause decreased endurance and decreased cognitive function. Coronary heart disease sufferers usually tend to have higher stress levels[20]. Hypertension, mental-emotional disorders, and diabetes mellitus (DM) are the dominant risk factors for CHD in Indonesia[3]. Adult stress appears to have a more influential role in triggering cardiovascular events[21].

The Dawood's fast (MADF) ought to change a person's stress perception by increasing the stress threshold value, which results in people who undergo Dawood's fast not easily complaining of stress. Although from the comparison test between-groups there was no significant difference, it can be seen in figure 3 that the difference between pre and post-test mean values in the fasting group was greater than in the non-fasting group. This shows that although the statistical results did not differ significantly between groups, the decrease in cortisol levels in the fasting group was greater than the non-fasting group.

β-endorphins are neuropeptide hormones derived from proopiomelanocortin (POMC) neurons which are mostly produced in the anterior pituitary and the brain. β-endorphins have been identified in human plasma, and their release to the circulation in response to stress and pain is well known, but β-endorphins are rapidly degraded by blood proteases. β-endorphins can increase with exercise and increase more if accompanied by loud music but decreased if excessive exercise[12]. β-endorphins are mainly used in the body to reduce stress and maintain homeostasis. This is corresponding with the research conducted by Lahdimawan, et al. (2013)[12] which showed an increase in β-endorphins levels after Ramadhan fasting in healthy humans and PBMC.

Levels of β-endorphins are positively related to feelings of pleasure or happiness and sincerity. The greater the feeling of happiness, the higher the level of β-endorphins in the peripheral blood, the greater the sense of sincerity, the greater the feeling of happiness. So the greater the sense of sincerity, the higher the β-endorphins levels in plasma. Although the results of the statistical test in table 3 showed that there was no difference between the fasting and non-fasting groups, it can be seen in figure 2 that the pattern of changes in plasma β-endorphins levels was similar in both groups. This is presumably duet o the motivation given by the informants as well as similar conditions to subjects who live in the same environment (the dormitory) and have almost the same activities. The pattern of changes in the mean values of β-endorphins levels was seen to increase up to 3rd week in both groups, then decreased after 3rd week until the end of the study. The possibility that what happened is that there was a change in the sincerity that occurred after the 3rd week of the study that affected the plasma β-endorphins levels until the end of the study. The changes of sincerity can be related to increased activities or other things that can not be predicted. This is one of the limitations of the study, which was unable to measure the levels of happiness or sincerity of the subjects.

Conclusion

There was no difference in cortisol and β-endorphins levels in healthy young adult subjects undergoing Dawood's fast due to the influence of the same environmental conditions and a change in the sincerity

of the subjects. A longer fasting time may be needed to make a meaningful difference between groups, in addition to that is necessary to conduct a study on people who did Dawood's fasting for over 1 year compared to people who have never fasted at all.

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Pregnancy Disorders and Access to Female Workers' Health Services in Sidoarjo Industrial Area During Covid-19 Pandemic

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Abstract

Maternal health is part of public health that will show a positive trend if there is a decrease in maternal mortality and an increase in delivery assistance provided by health workers in health service facilities. to know the relationship between pregnancy disorders and access to health services for female workers in the industrial area of Sidoarjo. this research was cross-sectional The sample of this study was 159 midwives. ANC visits of female workers are still low and have not reached 90%. Many female workers have experienced various pregnancy disorders. These pregnancy disorders include; abortion, anemia, and Hyperemesis GravidarumThe biggest obstacle to antenatal care performed by female workers is time and cost. During the Covid-19 pandemic, antenatal care services have made adaptations by implementing the COVID-19 health protocol.

Keywords: *Pregnancy Disorders, Female Workers, Health Service, Industrial Area*

Introduction

Maternal health is part of public health that will show a positive trend if there is a decrease in maternal mortality and an increase in delivery assistance provided by health workers in health service facilities [(1)]. The reason is because according to the ICD-10, the definition of maternal mortality is the death of a woman that occurs during pregnancy or within 42 days regardless of the age and location of the pregnancy, which results from any cause related to or worsened by the pregnancy or its treatment, but does not

Basically, the health of mothers, especially those who are still in pregnancy, needs special attention because they are prone to problems caused by many factors and for working mothers the risk factors for experiencing these disorders increase due to the workload they get. Based on the research data, there were significant results between the workload of pregnant women and the incidence of maternal health problems, one of which is the incidence of preterm delivery [(3-4)].

The emergence of maternal health problems, especially for working mothers, could have been prevented if the mother receives special attention from the company owner, had good knowledge related to maintaining health during pregnancy to breastfeeding which the company owner could also facilitate, and had good access to the closest health services both with the work place and with the residence. The consequences of reproductive health for female workers are things that need to be studied and receive special attention, starting from the extent to which the legal system protects them, the work system they have to do during childbirth to breastfeeding, and their ease of access to health services, especially considering their time which is not flexible because their part of the time has to be spent at work.[(5)]

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Midwives are one of the health workers who can play a major role in preventing pregnancy problems experienced by pregnant female workers because midwives tend to be easier to reach both in terms of time and location, which are widely spread in several areas.

Sidoarjo is one of the districts in East Java, which has a large industrial area. Based on the data, most of the workers in industrial areas are actually female of productive age, which means that it is very possible for these workers to experience pregnancy while working. From a preliminary study that we conducted on several midwives in Sidoarjo area who often handle pregnant female workers, it was known that on the average, pregnant female workers experience various health problems including preterm, abortion, preeclampsia, anemia, and hyperemesis gravidarum. [6] It is assumed that the access of pregnant female workers to health services is one of the causes of the disorder that cannot be prevented from an early age, because pregnant female workers do not have flexible time to check their pregnancies at health care facilities.[7] Therefore, in this study, researchers wanted to know the relationship between pregnancy disorders and access to health services for female workers in the industrial area of Sidoarjo.

Material and Method

The type of this research was an analytical study with an observational research design. The data were collected using cross-sectional method, which was done at one time without repetition and any intervention. The population of this study was 159 midwives with the sampling process was performed using the total population. The variables used were health problems experienced by pregnant female workers and whether they regularly accessed health care facilities or not. The data were obtained from questionnaires distributed online via google form. The results of the data from the research were processed by describing the results of the cross distribution of the two variables.

Results

The respondents involved in this study were 159 people consisting of 16 non-midwives and 143 midwives. The midwives work in various health care centers in Sidoarjo District. All midwives involved in this study provided maternity services to formal, informal workers, government employees and others.

The majority of these midwives work for agencies, whether it is health centers, hospitals, or maternity homes. A total of 114 midwives did not open independent practices. Regarding the percentage of Antenatal Care (ANC), the number of complete visits performed by female workers vary widely at the range of 4% to 100%. The average ANC visit to female workers was 54.9%. There are many reasons why the ANC visit was incomplete, such as forgetting the control schedule and moving to the village and others, but the majority of the reasons the ANC visit was incomplete was because the female workers were busy working and only having holidays on Sundays so they did not have time to have their pregnancy checked.

Table 1. Problems of Pregnancy and Childbirth that Often Occur in Female Workers Patients According to Respondents in Sidoarjo in 2020

Problem	Total
Abortion	6 6
Anemia	51
Low Birth Weight (LBW)	23
Hyperemesis Gravidarum	52
Bleeding	45
Preterm Rupture Membrane	24
Preeclampsia	40
Preterm birth	27
SGA	3

There are many problems with pregnancy and childbirth that often occur in female workers patients, but the top three problems with pregnancy and childbirth that often occur in female workers are abortion, anemia, and Hyperemesis Gravidarum. In this question, respondents can provide information on more than one problem of pregnancy and childbirth so that researchers can obtained a lot of relevant data. The obstacle that may be experienced by female workers is being able to have regular pregnancy checks according to the scheduled timing of the check-up hours. The majority of them only get a day off on Sunday so that their check-up hours for midwives or doctors are limited. Many health services also take holidays on Sundays so they experience obstacles in carrying out checks on their pregnancies.

Table 2. Respondents' Constraints in Providing Pregnancy Examination Services in Sidoarjo in 2020

Characteristics	Yes	Not	Total
Constraints in providing antenatal care services	48	95	143

In connection with the Covid-19 pandemic that has entered Indonesia and is endemic throughout the world, the majority of midwives stated that there were no obstacles in providing antenatal care for female workers during Covid-19 pandemic (according to health protocols), but they were also worried about patients who checked themselves at the health service because they could have contracted Covid-19 while queuing and traveling to or from health services. On the advice of the midwife, pregnant women who work are pregnant women, if possible, do *work from home* (WFH) when the pandemic is underway, keep doing health protocol if forced to check into the health service, and if possible pregnant women should be diligent to undertake consultations with a midwife or doctor by *WhatsApp* or short message.

Discussion

1.Antenatal Care (ANC) visit

From the results of the study, it was found that almost all midwives involved in this study provided pregnancy services for female workers. Female workers prefer practice midwives to get ANC services due to several factors, among others;

a. location and cost factors; midwifery practice is available in all regions. Midwives check fee are cheaper than obstetricians.

b. checking time factor; midwives have more flexible examination hours than obstetricians at the hospital.

c. convenience factor; the comfort factor is the most dominant factor. The influence of Indonesian culture makes female workers feel confident and comfortable when the midwife carries out examinations and provides delivery services.

Nevertheless, ANC visit of female worker remains generally low. Antenatal care visits will greatly affect the health of female workers. ANC visits of less than 80% will have the potential to cause various health problems [(1).] If these health problems are not prevented from an early age, they can cause preterm birth and other labor problems.

Pregnancy disorders in female workers. Government Indonesia has recommended that pregnant women can do prenatal care at least 4 times during pregnancy, namely respectively 1 time in trimester 1 and II and twice in the third trimester [(9)]. ANC visit is certainly very important. It is expected that ANC can be carried out completely by all pregnant women, including sensitive women with the aim of monitoring the progress of the pregnancy process to ensure the health of the mother as well as the growth and development of the fetus in it, knowing pregnancy complications that may occur early on, improving and maintaining the health of the mother

and baby, prepare for childbirth, reduce morbidity and mortality of pregnant women, prepare mothers and their families to receive child births so that they can grow and develop properly, and prepare mothers to pass through childbirth well and support exclusive breastfeeding.[10]

Disorders of Pregnancy

Pregnancy disorders in female workers can be caused by various factors, both when going to work and go home from work, or at work [8]. These factors can be in the form of physical, chemical, biological, ergonomic, and psychological factors [5]. Various factors can have an impact on various systems that exist in the body so that the female worker is ultimately can lead to various health problems such as disturbed menstruation, bleeding, miscarriage, low birth weight, and problems of pregnancy and childbirth [11].

The three major problems of pregnancy and childbirth that often occur in women who work in Sidoarjo are abortion, anemia, and Hyperemesis Gravidarum. Many female workers face many health problems during pregnancy and complications during childbirth and poor health during the postpartum period. Preeclampsia causes pregnancy disorders, among others; premature babies and low birth weight[10]. Pregnancy disorders among female workers in Sidoarjo are caused by several factors, among others; physical activity, long working hours and distance from the workplace. Working hours are also an obstacle for workers to get health services. According to Akhtar, T., distance from work and work stress during pregnancy are strongly associated with pregnancy complications. [12] Female workers in Sidoarjo have a dual role, which is as mother for their children and as supporters of the household economy. This is what distinguishes women who do not work. The results of a study conducted by Khojasteh et al showed that the more female workers with potential complications, the higher the preterm labor in working mothers. Physical activity in pregnant female worker can predict a decrease in amniotic fluid and low birth weight [13]. Research conducted by Xu et al. also proved that there is a significant relationship between physical work and low birth weight [14]. Moderate to high physical activity at work is twice as likely to develop preeclampsia. Likewise, long working hours lead to a reduction in birth weight. Negative attitudes regarding pregnancy at work also contribute to job dissatisfaction with an increase in preterm labor. Duties and environmental exposures in the workplace increase the potential risk of fetal morbidity [15]. Apart from exposure to the work environment, working shifts also affects pregnancy. According to the research results of Peter E Bonde et al. Working at night is associated with the risk of miscarriage. Recurrent circadian rhythm disturbances in night workers are associated with an increased risk of spontaneous abortion [16]

The increasing number of female workers indicates the need for regulations/policies that protect female workers, especially during pregnancy. In addition, women with a history of pregnancy complications should receive additional attention [14]. Female workers do not work in a workplace with a high level of exposure [16]. This factor needs to be controlled so that companies can minimize the risk of pregnancy problems for female workers [10].

3. Service constraints

For female workers, examination time is one of the obstacles that often occurs. Research also stated that one of the problems that hinder ANC is the timing of the examination. This research also stated that in addition to time, the costs of both examination and transportation costs are the main problems in achieving ANC [3]. In addition to costs, knowledge and family income for pregnant women will also determine the ANC that will be carried out by a pregnant woman [4]. A pregnant woman with good knowledge and a family income above the UMR tend to be diligent in doing routine ANC.

4. Pregnancy screening services during Covid-19 pandemic

From the results of the study, it showed that 33.6% of midwives had difficulties in providing antenatal care and childbirth services to female workers during the pandemic. The transmission of Covid-19 is likely to occur if any of the female workers who come for pregnancy checks have contracted Covid-19. Protection against Covid-19 transmission must be provided to midwives and other female workers who come to have their pregnancy checked. However, services for examining pregnant women during the Covid-19 pandemic must still be carried out. In accordance with the MCH Service

Guidelines from the Ministry of Health, pregnancy examination services during a pandemic need to pay attention to strict health protocols to break the chain of transmission of Covid-19 . Currently, the government has instructed pregnant women to carry out swabs at the gestational age of 35-37 months. This is done in order to provide the best referral and services for pregnant women who are at risk of contracting Covid-19. It is also certainly a good move, especially for working mothers, so that she can either know about their health status of being exposed to covid-19 or not. If they are infected, then the clinic would offer referential Hospital that has adequate facilities and PPE level [17].

Conclusion

1. ANC visits of female workers are still low and have not reached 90%.

2. Many female workers have experienced various pregnancy disorders. These pregnancy disorders include; abortion, anemia, and Hyperemesis Gravidarum

3. The biggest obstacle to antenatal care performed by female workers is time and cost.

4. During the Covid-19 pandemic, antenatal care services have made adaptations by implementing the COVID-19 health protocol.

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Dosage Effectiveness of the Combination Calcium Hydroxide-Propolis as an Inhibitor for *Aggregatibacter actinomycetemcomitans* Biofilm

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Abstract

Objective: Calcium hydroxide is widely used as pulp-capping material, but it is resistant to *Aggregatibacter actinomycetemcomitans* bacteria. Because of this deficiency, many researchers are considering using alternative natural ingredients, such as propolis. Propolis has been widely used in dentistry due to its properties and because it has active ingredients that can act as antibacterial agents. It is hoped that when calcium hydroxide and propolis are combined, the combination can overcome the disadvantages. The aim of this study was to determine the dosage effectiveness of the combination calcium hydroxide-propolis as an inhibitor for *A. actinomycetemcomitans* biofilm**. Material and methods:** The sample was divided into five groups. The positive control group is a combination of calcium hydroxide-sterile aquadest, and the negative control group is *A. actinomycetemcomitans* bacteria. Group I is a combination of calcium hydroxide-propolis with a ratio of 1:1, group II has a ratio of 1:1.5, and group III has a ratio of 1:2. Each group consists of five replications. Combination of calcium hydroxide-propolis and *A. actinomycetemcomitans* bacterial suspension was incubated for 2x24 hours then washed three times using PBS. Furthermore, 2% of crystal violet was used for staining and covered with aluminum foil and then incubated for 15 minutes. Isopropanol 200µL was added, followed by a measurement of OD with an ELISA reader, and the OD values were calculated using an inhibition biofilm formula. **Results**: Group II has the largest mean inhibition with 76% compared to groups one and three. **Conclusion:** A combination of calcium hydroxide and propolis with a ratio of 1: 1.5 is more effective than 1:1 or 1:2 to inhibit *A. actinomycetemcomitans* biofilm.

Keywords: *Calcium hydroxide, propolis, herbal medicine, biofilm, Aggregatibacter actinomycetemcomitans*

Introduction

The most common cause of pulp damage is bacterial agents, which enters the pulp chamber through caries. According to Radman *et al.* [1] in their study using the Polymerase Chain Reaction (PCR) method, 80% of *Enterococcus faecalis* bacteria, 32% of *Aggregatibacter actinomycetemcomitans*, and 16% of *Porphyromonas gingivalis* were found. There was a significant decrease in the number of bacteria after the indirect treatment of pulp capping with calcium hydroxide, which decreased *E. faecalis* bacteria by 77.5% and *P. gingivalis* bacteria by 100%, whereas *A. actinomycetemcomitans* bacteria decreased from 32% to 16%. The decrease that occurred in *A. actinomycetemcomitans* bacteria was only 50%, whereby the percentage reduction was smaller when compared to the other two bacteria.

Biofilms in an oral cavity can be produced by some bacteria. One of them is *A. actinomycetemcomitans*, a gram-negative bacterium and facultative anaerob.[2] Biofilms are collections of microorganisms that attach to biotic or abiotic surfaces, and they are encased in a

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hydrated matrix consisting of exopolymeric substances, proteins, polysaccharides, and nucleic acids. Biofilm production in some pathogenic bacteria is regulated by a system of regulating the expression of bacterial genes called quorum sensing in response to the density of the microorganism populations through the production of extracellular signaling molecules or autoinducers. [3] Bacteria that grow in biofilms show higher resistance, both in antimicrobial agents and host defense mechanisms, compared to planktonic cells—cells that form biofilms 10–1000 times more resistant to antimicrobials. [3]

Pulp-capping treatment is required when there is inflammation in the pulp (reversible pulpitis) due to dental caries or iatrogenic factors. Pulp capping is the provision of a layer of protective/treatment material on the pulp. Calcium hydroxide is often used as pulp-capping material because it can stimulate the mineralization of scar tissue formation or reparative dentine, and the high pH of calcium hydroxide can kill microorganisms. [4.5] The inability of calcium hydroxide to eliminate some of the microorganisms found in the dentinal tubules is one of the deficiencies of calcium hydroxide, so many researchers considering using alternative materials derived from nature, such as propolis. [6.7]

Propolis is a natural bee product that has long been used in dentistry. It has anti-inflammatory, anti-microbial, and anti-fungal abilities, and it can heal scars. Existing studies have shown that propolis extract is effective in inhibiting anaerobic periodontal pathogens, namely *A. actinomycetemcomitans*. [8.9] According to Montero and Mori, [7] when calcium hydroxide is combined with propolis, calcium hydroxide can dissociate better into calcium ions and hydroxyl ions so that it can diffuse well into the dentinal tubules. In another study, it was shown that calcium hydroxide combined with propolis did not show any toxic reaction, and it could significantly reduce inflammation and was biocompatible material.[10]

Until now, there has been no research on the effectiveness of the calcium hydroxide-propolis combination in inhibiting the biofilm of *A. actinomycetemcomitans* bacteria; therefore, the aim of this study was to determine the dosage effectiveness of the combination calcium hydroxide-propolis as an inhibitor for *A. actinomycetemcomitans* biofilms.

Material and Methods

This research is an in vitro laboratory experimental study with a post-test only control group study design. The tools used include a Petri dish *(Pyrex, USA),* a brander, a spreader *(Pyrex, USA)*, an anaerobic jar *(Oxoid, UK),* an autoclave *(Hirayama, Japan),* an incubator *(Memmert, Germany),* an analytical balance (*Mettler Toledo, Indonesia*), a micropipette *(Socorex,* Switzerland *),* an osse, an *ELISA reader (Biorad model 550, California)*, a 96-well flat-bottomed plastic tissue culture plate, and a vortex (*Biomol, Germany).* The materials used were calcium hydroxide powder (analysis of Merck, Germany), propolis extract *Apis mellifera* Lawang, bacterial stock *A. actinomycetemcomitans*, trypticase soy broth (TSB) + glucose, aquadest sterilized, 96% ethanol, 1% glucose, phosphate buffered saline (PBS) pH 7.4, 2% crystal violet, aluminum foil, matches, and isopropanol. This research has been conducted ethically feasible test at the Faculty of Dentistry, Airlangga-Surabaya University with numbers 056/HRECC. FODM/V/2018.

Preparation combination of calcium hydroxide and propolis extract

The calcium hydroxide-propolis combination was made by mixing calcium hydroxide powder (analysis of Merck, Germany) and propolis extract (Apis Mellifera, Lawang) into a brownish thick liquid in a test tube at the following ratios: 1:1 (0.125 g calcium hydroxide powder with 0.125 ml propolis extract), 1:1.5 (0.125 g calcium hydroxide powder with 0.1875 ml propolis extract), and ratio 1:2 (0.125 g calcium hydroxide powder with 0.25 ml propolis extract). The combination of calcium hydroxide powder with Apis mellifera propolis extract mixed with sterile aquades to obtain a liquid form so that it can be tested on *A. actinomycetemcomitans* biofilm. To determine the concentration of the amount of propolis extract that is used, the MIC value of the extract against the *A. actinomycetemcomitans* bacteria was inspected. The concentration of the propolis extract was found by diluting 100% propolis extract with a dilution method of 100%, 50%, 25%, 12.5%, and 6.25%, and it was found that the MIC of the propolis extract against the *A. actinomycetemcomitans* bacteria was 12.5%.[11]

Bacteria Preparation

The stock of the *A. actinomycetemcomitans* bacteria was a subculture using the quadrant-streaking method on the *Tryptose Soy Agar* (TSA) medium in an incubator temperature of 37°C for 24 hours. After the pure colony subculture was obtained, one colony was taken into a 10 ml TS-Broth-glucose medium and incubated for 24 hours at 37°C. The subculture in the TS-Broth-glucose medium was measured to find the concentration of bacterial cells (optical density [OD] value) with a wavelength spectrophotometer of 625nm. The spectrophotometer OD results were diluted with 0.9% NaCl to OD 0.1, so we have obtained a 10^8 bacteria / ml suspension of *A. actinomycetemcomitans* bacteria, which is ready to be used for biofilm inhibition testing.

Biofilm formation preparation test

The method that has been selected to examine the formation of the biofilm is a biofilm microtiter plate. After the biofilm is formed, it was injected into the well microtiter plate culture to as much as 100µL / well, after which a combination of calcium hydroxide-propolis was added at 1:1, 1:1.5, and 1:2 ratios to each microplate well (treatment group) to as much as 100µL / well so that the final volume was 200µL / well. The well-positive control groups were given sterile calcium hydroxide and aquadest, and for the negative controls, only *Aggregatibacter actinomycetemcomitans* bacteria, without a combination of calcium hydroxide-propolis, was given. It was incubated again for 2x24 hours at 37°C. Then, the contents of each microtiter plate were aspirated and washed three times with 200µL PBS (pH 7.4) using a micropipette. This function removes planktonic bacteria. The microorganisms attached to the well were stained using 200µL / well crystal violet 2% and then covered with aluminum foil so that it was not exposed to direct light, after which it was incubated at room temperature for 15 minutes and cured overnight. Next, 200 µL / well isopropanol was added, which served to shed the color of the crystal violet on the wall of the well. The microplate was shaken for one minute and placed into an ELISA reader for measuring the OD of each sample with a wavelength of 570 nm. Each treatment group in this procedure was replicated five times.

Observing and measuring biofilm inhibition

The reading result is the absorbance value or OD, which describes the quantity of biofilm formation. To calculate the percentage of biofilm inhbition, it uses a formula with modification as follows. [12]

$$\text{Biofilm Inhibition (\%)} : \frac{\text{OD Control} - \text{OD Experimental Sample}}{\text{OD Control}} \times 100\%$$

Results

Based on the results of the measurements of the OD (Optical Density), the average results of the research are shown in Table 1.

Table 1. Average results of the research

Group	N	Mean (%)	*Std. Deviation*
Positive Control	5	39.0000	±13.03840
Group I	5	42.9540	±27.33372
Group II	5	76.2000	±13.62718
Group III	5	74.8000	±19.96747

Notes: Positive control = calcium hydroxide, group I = combination of calcium hydroxide-propolis 1:1, group II = combination of calcium hydroxide-propolis 1:1.5, group III = combination of calcium hydroxide-propolis 1:2.

Based on the research results of the mean values listed in table 1, it can be seen that the biofilm inhibition of *Aggregatibacter actinomycetemcomitans* bacteria which were treated with a combination of calcium hydroxide-propolis has a greater value than the positive control, so it could be stated that the combination of calcium hydroxide-propolis was effective in inhibiting bacterial biofilms *Aggregatibacter actinomycetemcomitans*, and the ratio of 1: 1.5 is more effective than 1: 1, 1: 2 in inhibiting the *Aggregatibacter actinomycetemcomitans* bacterial biofilm.

To see biofilm formation in the *Aggregatibacter actinomycetemcomitans* bacteria, SEM (Scanning Electron Microscope) was used with the Carl Zeiss (Evo MA 10) brand of England with magnifications used were

500, 1000, 4000, and 10,000x.

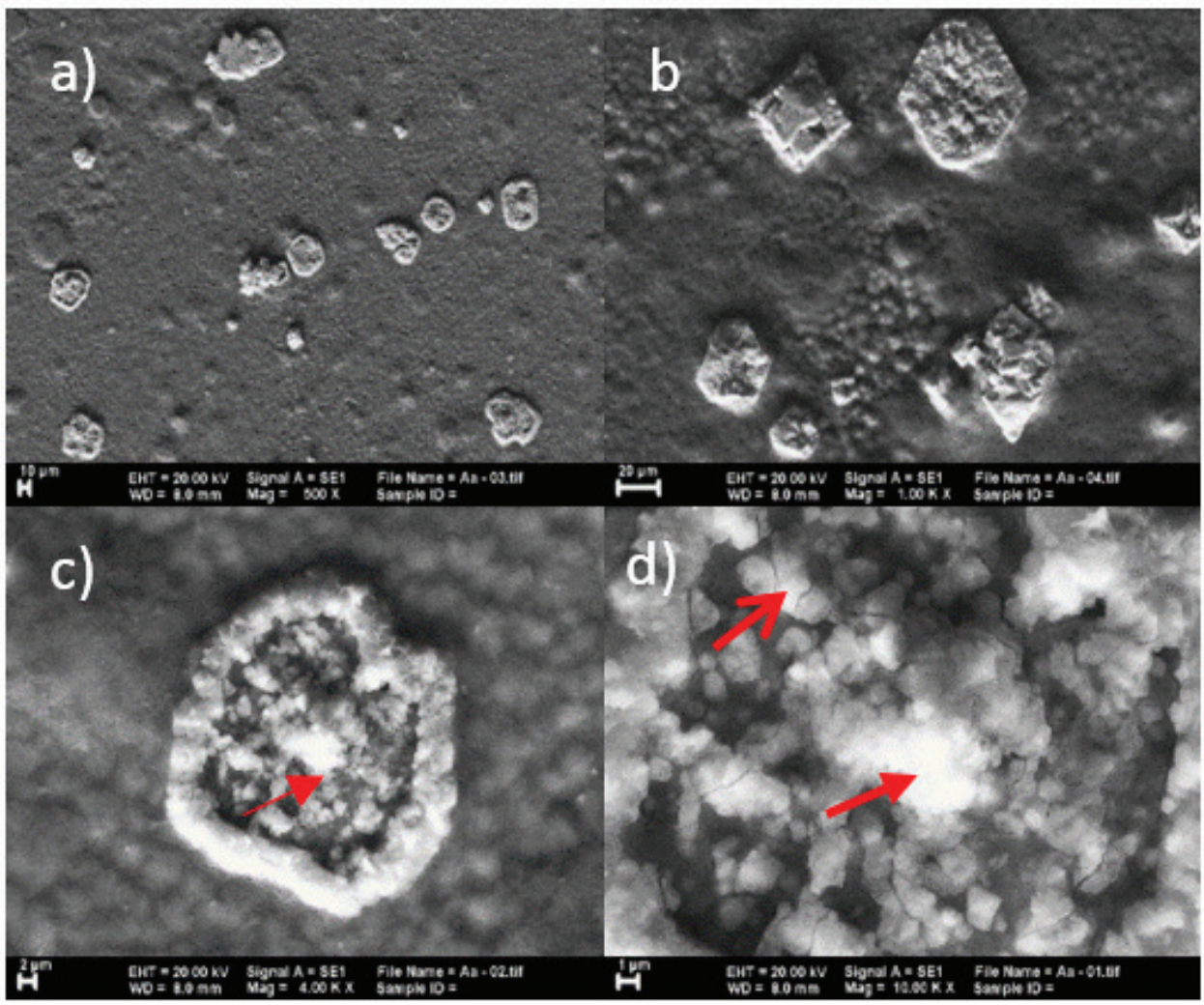

Figure 2. SEM Results *Aggregatibacter actinomycetemcomitans* **Bacteria Biofilms (Figure a and b image of colonies forming biofilms at 500 and 1000 magnifications; c and d. Figure biofilm matrix formed at 4000 and 10.00 magnification with arrows indicating bacteria coated with biofilm matrix)**

The normality test used is the One Sample Kolmogorov-Smirnov Test. The test results in all the research groups have a value of $p > 0.05$. This indicates that the data obtained is normally distributed. Homogeneity test with Levene's Test was conducted. The test assay shows a value of $p = 0.358$ ($p > 0.05$), which means that the data is homogeneous. Then, a one-way ANOVA test was conducted. The test results demonstrated a value of $p = 0.011$ ($p < 0.05$), which showed that there were significant differences in the treatment group. Then, the post-hoc LSD analysis was carried out. The test results in each treatment group can be seen below (Table 2). From the table above, it can be seen that there is no significant difference between the positive control group and group I, and there is no significant difference between groups II and III.

Tabel 2. LSD Post-Hoc Analysis Test Results

Group	Positive Control	Group I	Group II	Group III
Positive Control	-	0.736	0.008*	0.010*
Group I		-	0.017*	0.021*
Group II			-	0.911
Group III				-

Notes: * $p < 0.05$ = there was significant difference

Discussion

Pulp-capping treatment with calcium hydroxide is said to be successful if calcium hydroxide can dissociate into ionic forms Ca^{2+} and OH^-.[15] This is an ionization process in tissues that explains the antibacterial properties of calcium hydroxide. The dissociation of calcium hydroxide into Ca^{2+} and OH^- depends on the carrier that is used. The ion release being influenced by the carrier material is a major factor. The hydroxyl group creates the alkaline condition, which can activate an alkaline phosphatase enzyme that stimulates mineral tissue formation, which helps the repair process. When hydroxyl ions are able to diffuse in the dentine and remain in the pulp at adequate concentrations, the process becomes more effective. It can release the pH needed to destroy bacteria in the root canals and the dentinal tubules. One of the carrier materials that shows good diffusion in dentine is propolis.

Propolis is a combination of organic compounds containing weak acids. Its pH tends to be below 7, while calcium hydroxide is a strong base that has a pH above 8. If propolis and calcium hydroxide are combined, it is possible to observe salting reactions between caffeic acid and calcium hydroxide. If the amount of propolis extract mixed is small, it is possible to neutralize the process of propolis by $Ca(OH)_2$. When calcium hydroxide neutralized the acidic functional group of propolis with a low propolis amount, the inhibitory activity was dominated by calcium hydroxide. Because the active ingredient in propolis is not only acidic but also neutral, there will be synergy where the other active ingredients can suppress the formation of *A. actinomycetemcomitans* bacteria biofilm. Additionally, if the ratio of propolis increases, the inhibition of the biofilm produced will also increase. This happens because calcium hydroxide cannot neutralize all of the acid content in propolis.

The propolis extract is in the form of a brownish thick liquid. Meanwhile, calcium hydroxide is in the form of a powder (fine powder). When calcium hydroxide and propolis extracts are mixed, they create a suspension mixture. Suspensions are liquid preparations containing solid and insoluble particles that are dispersed in the liquid phase.[13] This mixture of suspensions, if allowed to stand, will separate (form deposits) so that the mixture of propolis and calcium hydroxide is not perfect. This imperfect mixture results in the activity of active compounds in propolis decreasing because of uninform impregnation, and the biofilm inhibitory results that are obtained will be different even though the concentration is the same (there are no significant differences).

The method used to observe the microbial biofilm attachments was the Microtitter Plate Biofilm Assay, with a combination of calcium hydroxide-propolis (1:1, 1:1.5, 1:2) put in a 100-µl microtiter plate well after the bacterial preparation on the plate to see the inhibition of the *A. actinomycetemcomitans* bacteria biofilm on the second day.[14] This biofilm inhibition occurs in the initial adhesion phase, which is a nonspecific biofilm formation and bacterial attachment to a reversible surface. If it is permanently attached, the bacteria will

begin to synthesize the insoluble exopolysaccharides (EPS), which attaches the bacteria to a three-dimensional matrix that functions to protect the bacteria from host and antimicrobial immune responses. Therefore, one effective method to inhibit the film formation is to prevent or reduce the initial adhesion of bacteria on the surface.[16]

The inhibition process of *A. actinomycetemcomitans* bacteria biofilm is not only related to the genes that synthesize polysaccharide intercellular adhesion (PIA) but also to the Quorum Sensing (QS) system, which also affects the inhibition of biofilm formation. The bacterium can recognize the presence of the other bacteria in its environment by detecting the autoinducer. Autoinducers, which are mainly in gram-negative bacteria, are AHLs, which have an important role in biofilm formation. [3,17]

The statistical test of this study was to determine the differences between the treatment groups using the LSD post-hoc analysis test. There is no significant difference between the positive control group (calcium hydroxide + sterilized aquadest) and group I (combination of calcium hydroxide-propolis 1:1). There is no significant difference in the value of $p = 0.736$ ($p > 0.05$), which means that the biofilm inhibition in the positive control group is the same as group I. This happened because the active content of the propolis in group I is low, or it can be said that the active ingredient has not been effective in inhibiting the A. actinomycetemcomitans bacterial biofilm, so the inhibitory power obtained is 42%, which is not that much different from 39% for the positive control group (calcium hydroxide + sterilized aquadest).

In addition, in group II and group III there were no significant differences with the value of $p = 0.911$ ($p > 0.05$), which means that the biofilm inhibition that occurs in group II is almost the same as group III. This happened because in group III, the mixture produced was more diluted than in groups I and II. According to Syamsuni (2006)[18], one of the factors that influence the manufacturing process of a suspension preparation is the thickness of a liquid (viscosity), which also influences the velocity of the liquid flow; the thicker the liquid, the lower the flow velocity or the smaller density. The flow velocity of the liquid will also affect the downward movement of the particles contained in it. Thus, by increasing the viscosity solution or the viscosity of the liquid, the movement of the particles they contain will be slowed down. In addition, the number of particles is also a factor regarding why there are no significant differences. If there is a large number of particles in a room, it will be difficult for the particles to move freely because there are frequent collisions between these particles. [18] The movement of ionization between the particles is too wide (free), so the effectiveness of the mixture is also low. Therefore, group III has a smaller inhibition average than group II.

Group II has a greater inhibition because at a ratio of 1:1.5, the suspension formed does not tend to be runny/thick, and the active can be sufficiently ionized so that the effectiveness is higher than group I and group III. In addition, group II is the saturation/ critical point of a concentration to be able to inhibit maximum biofilm formation. At certain concentrations, even though concentration is increased, surface tension will be constant by aggregating to form micelles and concentrations, which is called Critical Micelle Concentration (CMC). CMC is related to the minimum value of surface tension. If CMC is reached, the surface tension will decrease. After CMC is reached, the surface tension will be constant.[19,20] The constant surface tension causes no significant differences between group II and group III. This is in accordance with the results obtained where in group II the mean of biofilm inhibition is 76%, while in group III the mean inhibition is 75%. Therefore, it can be concluded that group II (calcium hydroxide-propolis 1:1.5) is the most effective ratio for inhibiting the biofilm of *Aggregatibacter actinomycetemcomitans* bacteria.

The effectiveness of calcium hydroxide in inhibiting the biofilm of *Aggregatibacter actinomycetemcomitans* bacteria is influenced by the active content in calcium salt compounds, which is the result of mixing calcium hydroxide with propolis extract. This compound is formed because of an acid-base reaction between caffeic propolis acid and calcium hydroxide. This calcium salt compound consists of hydroxyl groups and the active compounds that is derived from the extracts of propolis, such as flavonoids (apigenin), tannins, and terpenoids (tt-farnesol). These compounds are thought to be able to inhibit biofilms from the *Aggregatibacter actinomycetemcomitans* bacteria.

Inside the cytoplasmic membrane, the mechanism of the hydroxyl group as an antibacterial occurs because it is the location of enzymatic substance, both extracellular and intracellular. In addition, the pH gradient of the cytoplasmic membrane changes to alkaline (pH 12.5) because of the presence of high hydroxyl ions that affect membrane proteins (protein denaturation), and it also changes the integrity of the cytoplasmic membrane while ultimately damaging the bacterial DNA. This happens due to acidic bacterial DNA turning alkaline because the surrounding environment is alkaline.[21]

Flavonoids are polyphenolic compounds that have three mechanisms. The first relates to the inhibition of nucleic acid synthesis. The second involves the destruction of cell membranes through perforation mechanisms and the perforation mechanisms decrease membrane fluidity. The third inhibits the energy metabolism of bacteria. [22.23] The results of this study are supported by tests of propolis originating from China and Brazil, that the test has shown that propolis is effective A. *actinomycetemcomitans* bacteria. [24] Apigenin (4 ‘, 5, 7-trihydroxyflavone) is a compound of flavones derived from flavonoids that can inhibit the growth of biofilms from bacteria. Apigenin with tt-farnesol will cause a decrease in the number of polysaccharides in microorganism biofilms. Apigenin inhibits the activity of glucosyltransferase and affects the accumulation of S. mutans biofilms by inhibiting the formation of insoluble glucan and increasing the soluble glucan content of the polysaccharide matrix. [25.26]

A tannin is a plant-active compound of the phenol group that has the ability to inactivate the bacterial and enzyme adhesive. Tannins and flavonoids can play a role in inhibiting biofilm formation by inhibiting the expression of icaA and icaD genes. The icaA and icaD genes can regulate the formation of Polysaccharide Intercellular Adhesin (PIA) through the activation of the σ^B factor, which can activate the omega protomer. [27] In addition, tannins and flavonoids play a role in inhibiting biofilm formation by reducing the hydrophobic nature of bacteria, which plays an important role in the process of bacterial adhesion to the substrate. [28]

Additional research was carried out by looking at the morphological form of the *Aggregatibacter actinomycetemcomitans* bacteria using a scanning electron microscope with the British brand Carl Zeiss (Evo MA 10) to see the biofilms form of *Aggregatibacter actinomycetemcomitans* bacteria. The magnifications that have been used are 500, 1000, 4000, and 10,000x. The results of the *Aggregatibacter actinomycetemcomitans* biofilm images studied were not optimal when compared with the existing studies. This might happen because the *Aggregatibacter actinomycetemcomitans* bacteria used for the biofilms is pasase 4, which means that the characteristics were different from the original bacterial stock of *Aggregatibacter actinomycetemcomitans*.

Conclusion

There was no difference between the calcium hydroxide-propolis combination with a ratio of 1:1.5 and 1:2, but ratios 1:1.5 and 1:2 were more effective than 1:1 in inhibiting *Aggregatibacter actinomycetemcomitans* bacteria biofilms.

Ethical Clearance: No. 056/HRECC. FODM/V/2018

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Effect of Extract and Caffeic Acid Phenethyl Ester on Apoptosis of Odontoblast Cells

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Abstract

Background: Propolis is a resinous hive product collected by bees from tree buds and mixed with secreted bee wax to both avoid bacterial contamination in the hive and seal it. It is known to have a wide spectrum of pharmacological properties, including anti-inflammatory, antioxidant, antibacterial, antiviral, and anti-fungal abilities. Apoptosis tests were performed on odontoblast cells in rats (*Rattus novegicus*) to assess the viability of propolis extract and caffeic acid phenetyl esters (CAPE) as alternative candidates for pulp capping agents in conservative dentistry treatment. **Objective:** To examine the apoptosis activity of propolis extract and CAPE as capping materials on odontoblast cells. **Methods:** This study was designed as a post-test only control group laboratory experiment. The rats were randomly divided into three groups. Pulp exposures were performed on the occlusal surface of the right maxillary first molars. In the first group, the control group, glass ionomer cement (GIC) was directly applied to the pulp exposure. In the second group, the sample group, propolis extract was applied to the pulp exposure, and in the third group, CAPE was applied to the pulp exposure. All cavities were then filled with GIC as a permanent filling. Animals were sacrificed on the first and fourteenth days. The direct counting method of histological examination was based on the apoptotic odontoblasts, using the terminal deoxyribonucleotidyl transferase dUTP nick end labeling (TUNEL) assay technique. **Result:** There was a greater number of apoptotic odontoblasts in the control group, followed by the CAPE group and, lastly, the propolis extract group. **Conclusion:** The apoptosis activity of the propolis extract is lower than that of CAPE.

Keywords*: odontoblast cell, apoptosis, propolis, caffeic acid phenetyl esters, herbal medicine*

Introduction

Homeostasis has an important role to play in the balance of bodily functions. It is, however, influenced by different regulatory mechanisms, such as osmoregulation, thermoregulation, and chemical regulation, all controlled by various cell systems to maintain body stability.[1] One of these mechanisms, moreover, can also occur in dental pulp tissue. If a cell experiences a stimulus or mild stress, it will then react to maintain a balanced state. Some of the cell reactions that have emerged include cell adaptation, reversible injury, irreversible injury, or cell death.[2]

A type of cell death that is commonly found is necrosis. Necrosis involves cellular edema, denaturation, protein coagulation, cellular organelle degradation, cell destruction, loss of the entire plasma membrane, and cytoplasmic disorganization. In general, necrosis involves a number of cells simultaneously.[2]

Apoptosis is defined as internally programmed cell death and has a role in a variety of physiological and pathological conditions. It is also considered as a complex, tightly regulated and active cellular process

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in which individual cells self-destruct without injuring neighboring cells but causing non-necrotic inflammatory reactions. Apoptotic cell death plays an important role in maintaining normal physiological functions, as well as being responsible for the body's disease process. A dysfunctional apoptotic system is involved in the pathogenesis of various diseases, including oral pathology.[4]

Cell necrosis subsequently induces an inflammatory reaction. Necrosis is generally caused by bacterial invasion and heavy tissue damage. Inflammatory mediators involved are histamine, bradykinin, serotonin, interleukins, and arachidonic acid metabolites (PGE2). This inflammatory response causes vasodilation and increased vascular permeability. The tissue becomes swollen due to serum protein infiltration (electrolytes) and fluid from blood vessels, causing necrosis.[5]

The process of apoptosis is of broad biological significance, and is involved in the development, differentiation, proliferation/homoeostasis, regulation, and function of all systems, as well as in removing damaged or dangerous cells.[4] Apoptosis is a very well-programmed, active process, and is characterized by morphological and biochemical changes. In the early stages of apoptosis, chromatin is broken but the cell membrane is still intact (karyorrhexis). In the final stage of apoptosis, the membrane protrudes, the ultrastructural modification of cytoplasmic organelles occurs, and the integrity of the membrane is lost. Phagocytic cells, such as epithelial cells, macrophages, and fibroblasts will usually eat apoptotic cells before the apoptotic body is formed. Apoptotic cells that are not phagocytes, as in a laboratory-produced cell culture, will experience a degradation similar to necrosis, which is called secondary necrosis.[6] Necrosis and apoptosis are different types of cell death. The apoptotic process does not induce an inflammatory reaction, but it is induced by a previous inflammatory reaction.[7]

Propolis is often used in the dentistry and medical fields and is a non-toxic, natural, resinous substance that has antimicrobial, anticancer, anti-fungal, antiviral, and anti-inflammatory properties. The word "propolis" is derived from the Greek words "pro" (meaning outer wall) and "polis" (meaning city), reflecting the protective properties of the substance. Propolis is one of the natural substances created by bees to build and maintain their hives. It kills pathogens and protects the beehives from rain, and because of its adhesive properties, it can prevent external objects passing into the hive.[8]

Propolis is known to contain resins and bioactive materials that include bioflavonoids, artepillin, apigenin, and caffeic acid phenetyl esters (CAPE), which have a role in controlling inflammatory, antioxidant, antibacterial, and antiviral reactions; managing immunomodulators; and stimulating tissue healing. This is consistent with a study conducted by Bankova in 2009, which stated that CAPE can inhibit activation of Nuclear Factor κB (NFκB) so that Tumor Necrosis Factor-α (TNFα) secretion will also be inhibited.[9]

CAPE's anti-inflammatory activity mode inhibits the release of arachidonic acid from cell membranes, then inhibits COX-1 and COX-2 activity and suppresses the activation of genes responsible for COX-2 expression. In inflammation induced by carrageenan, CAPE suppresses the volume of exudate and the relocation of leukocytes.[10] Propolis displays anti-inflammatory activity that can increase the body's immune system. The benefit of propolis in oral health is as an anti-inflammatory agent since it contains CAPE. The CAPE in propolis has various characteristics, one of which is to act as an anti-inflammatory agent. This agent passes easily into cells to inhibit LOX and COX (both involved in metabolic pathways), inhibit the release of inflammatory cytokines, and simultaneously increase the production of anti-inflammatory cytokines, such as IL-10 and IL -4. CAPE can also reduce the infiltration of neutrophil and monocyte inflammatory cells.[11]

CAPE is the main active component of propolis, which is a soft and sticky lipophilic resin collected from beehives, and is collected by honeybees from various plants. Flavonoids and caffeic acid (CA) have antimicrobial, anticancer, anti-fungal, antiviral, and anti-inflammatory properties that have received special attention in medicine and dentistry.[8]

CAPE rapidly developed in dentistry. In vitro and in vivo studies have previously mentioned that propolis has a strong anti-inflammatory effect as a pulp-capping material,[8] and is quite effective in the formation of dentin.[12] Although the use of CAPE as a pulp-capping material is still controversial and requires further

research, it is known that it can reduce inflammation and bacterial infection and stimulate the formation of dentine in pulp degeneration.[12] In another study, it is even stated that the level of CAPE in certain doses can be cytotoxic to cell survival. This cytotoxic effect has been known to cause cell apoptosis.[13]

Moreover, the flavonoids and cinnamic acid derivatives contained in propolis, such as acacetin, quercetin, naringenin, CAPE, and CA, are able to inhibit silica, which induces reactive oxygen species (ROS), and then bind them to induce the release of arachidonic acid, CAPE, and CA. As a result, the release of arachidonic acid, the production of PGE2, and the release of histamine can be induced.[14]

Propolis, as an anti-inflammatory agent, is also able to block lipoxygenase and cyclooxygenase. Lipoxygenase is the main neutrophil enzyme producing leukotriene compounds, while cyclooxygenase produces prostaglandins, which will be mediators in inflammatory reactions. The existence of these obstacles then decreases the production of leukotrienes and affects the activity of neutrophil phagocytosis, so that the inflammatory process is suppressed. Following on from this, the inhibition of lipoxygenase and cyclooxygenase pathways by CAPE will reduce blood vessel vasodilation and blood flow, so that the migration of leukocytes (PMN) to the inflammatory area will also be decreased.[15]

Therefore, this study aims to reveal the effects of propolis extract and CAPE on the apoptosis of Wistar rat odontoblast cells. The apoptosis of odontoblast cells was analyzed after the administration of propolis extract and CAPE.

Materials and Method

The design of this research was experimental and was approved by the Faculty of Dental Medicine, Universitas Airlangga ethics committee (No. 14/KKEPK.FKG/II/2015). This laboratory research study used 42 male Wistar rats weighing 200–250 grams and aged 8–16 weeks as research samples. It was necessary for these rats to have fully grown molar teeth and healthy physiques. Once selected, the samples experienced adaptation for two weeks, and were then divided into six groups. Group (I) was given pulp roof perforation treatment with glass ionomer cement (GIC), and then was observed on day 1 (control I). Group (II) was given the same treatment as Group (I) but observed on day 14 (control II). Group (III) was given pulp roof perforation treatment with propolis extract, and then was observed on day 1. Group (IV) was given the same treatment as Group (III) but observed on day 14. Group (V) was given pulp roof perforation treatment with CAPE, and then was observed on day 1. Group (VI) was given the same treatment as Group (V) but observed on day 14. In groups (III, IV, V, VI) 0.5 mg of each ingredient was applied and then covered using GIC.

The samples were subsequently euthanized ready for tissue sampling followed by decapitation and maxilla separation. The molar teeth were cut, using microtomes, in a parallel series with a long tooth axis of seven microns. The preparations were then stained with hemotoxin and eosin. Finally, the data were analyzed using the Kolmogorov-Smirnov test to determine the distribution of data. As it was known that the data were normally distributed, a homogeneity test was performed using the Levene test. The independent t-test was then performed to find out whether there was an average difference between the two unrelated sample groups.

Result

A histological examination, counting apoptotic cells, was carried out using a 1000x magnification microscope. The following results were observed (Figure 1). The Mean and standard deviation of apoptotic cells on day 1 and 14 can be seen in Table 1.

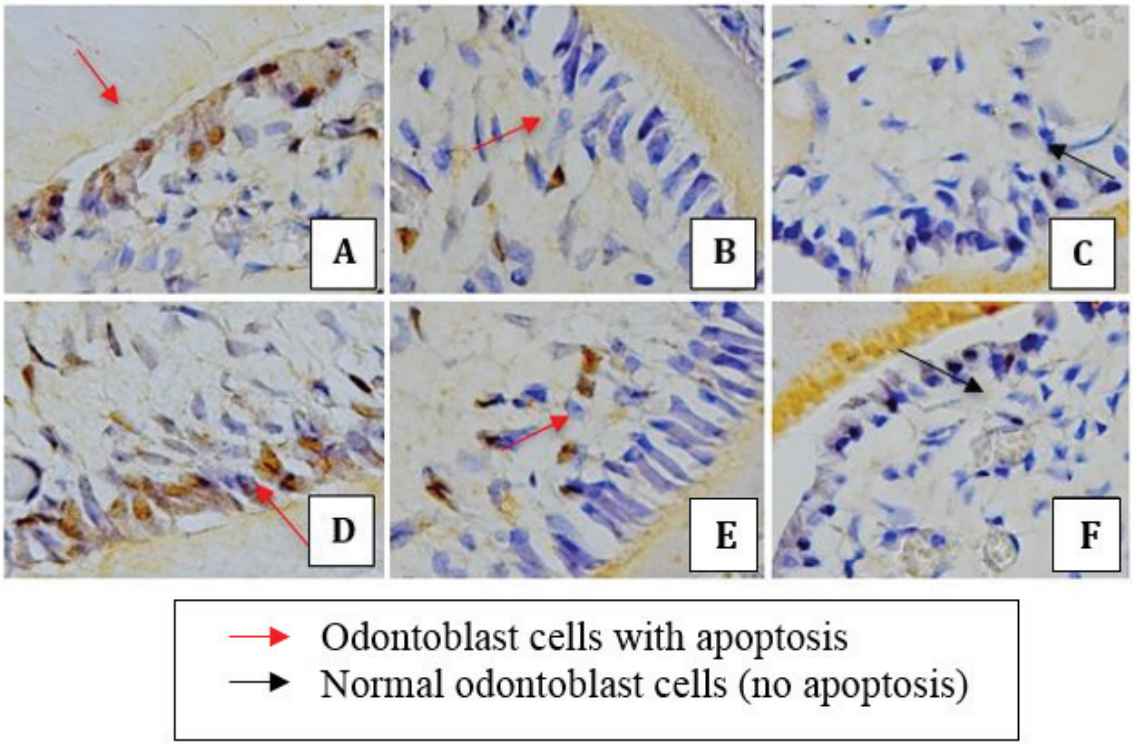

Figure 1. A. Odontoblast cells with GIC day 1, B. Odontoblast cells with propolis extract day 1, C. Odontoblast cells with CAPE day 1, D. Odontoblast cells with GIC day 14, E. Odontoblast cells with propolis extract day 14, F. Odontoblast cells with CAPE day 14.

Data analysis for apoptosis day 1

A normality test was performed for the three research groups to determine whether the data obtained was of a normal distribution. This normality test also determined the appropriate statistical test to be used in the research. A one-sample Kolmogorov–Smirnov test was used for the normality test and a result of $p > 0.05$ means that the data are normally distributed (Table 2).

Table 1. One Sample Kolmogorov - Smirnov Test

Substance	p
Control	0.73
Propolis Extract	0.93
CAPE	0.73

A Levene's homogeneity test was then performed. The Levene's test result of $p > 0.05$ means that the data are homogenous.

Following this, a one-way ANOVA test was performed to determine whether there were significant differences between groups. The test result of $p > 0.05$ means that there are significant differences between the three groups.

The analysis continued with a post hoc test for finding out the details of the differences in each group; the test used was Tukey's honestly significant difference (HSD) test (table 3).

Table 2. Turkey HSD test for apoptosis day 1

	Control (GIC)	Propolis Extract	CAPE
Control (GIC)	-	0.000*	0.000*
Propolis Extract		-	0.000*
CAPE			-

*: There were significant differences with $p < 0.05$

Data analysis for apoptosis day 14

A normality test was performed for the three research groups to determine whether the data obtained was of a normal distribution. This normality test also determined the appropriate statistical test to be used in the research. A one-sample Kolmogorov–Smirnov test was used for the normality test and a result of $p > 0.05$ means that the data are normally distributed (Table 4).

Table 3. One Sample Kolmogrov – Smirnov test

Substance	p
Control	0.71
Propolis Extract	0.74
CAPE	0.98

A Levene's homogeneity test was then performed. The Levene's test result of $p > 0.05$ means that the data are homogenous.

Following this, a one-way ANOVA test was performed to determine whether there were significant differences between groups. The test result of $p > 0.05$ means that there are significant differences between the three groups.

The analysis continued with a post hoc test for finding out the details of the differences in each group; the test used was Tukey's HSD test. The test result of $p < 0.05$ reveals that there are significant differences in the number of apoptotic cells between the groups (Table 5-8).

Table 4. Turkey HSD test for apoptosis day 14

	Control (GIC)	Propolis Extract	CAPE
Control (GIC)	-	0.000*	0.000*
Propolis Extract		-	0.000*
CAPE			-

*: There was significant differences with $p < 0.05$

Data analysis result for apoptosis in control group

Table 5. Significant difference test result between groups

Group	Significant difference test
Control day 1	p= 0.00*
Control day 14	
Propolis extract day 1	p= 0.006*
Propolis extract day 14	
CAPE day 1	p= 0.006*
CAPE day 14	

*p < 0.05: There was significant differences

Discussion

Oral cavities experience a variety of environmental conditions, and materials used in this environment must therefore be able to withstand those conditions. The safety of materials used in the oral cavity is very important, and other properties required for dental materials, such as not irritating the oral cavity and surrounding tissue and being non-allergic, non-carcinogenic, and nontoxic, must also be fulfilled.[16]

Toxicity testing is a part of dental material evaluation, and toxicity information is required for standard screening procedures. Hence, this study aims to determine the potential toxicity of CAPE and propolis extract against pulp cells, based on the number of cell deaths during the application of the pulp-capping material.

Moreover, apoptosis is a very well-regulated active process, which is characterized by morphological and biochemical changes. Apoptosis stimulated by physiological and pathological conditions plays an important role in maintaining normal homeostasis and the pathogenesis of several diseases.[6]

The initial stage of apoptosis is characterized by the expression of Phosphatidylserine (PS) passing through the inner layer into the outer layer of cell membranes. Apoptotic bodies formed at the end of apoptosis then trigger these dead cells to be recognized by macrophages without the release of cellular pro-inflammatory components.[6]

The selection of apoptotic identification method, nevertheless, depends on several factors, including experiment type, cell type, and experience. TUNEL testing is one of the apoptosis detection methods involving DNA fragmentation examination. Apoptosis examination with the TUNEL method can provide an overview of the apoptosis process at the single cell level, so that it is more specific and highly accurate.[17]

The results of apoptosis analysis on the first day using Tukey HSD showed that there were significant differences between the groups. The control group, with GIC, had the highest level of apoptosis, compared with the propolis extract and CAPE groups. Similarly, on the fourteenth day, the control group had the highest level of apoptosis compared with the propolis extract and CAPE groups. The high level of apoptosis in the control group is mostly due to the physical properties of soluble GIC. The level of fluoride contained in GIC can inhibit cell growth and proliferation and disrupt mitochondrial activity and protein synthesis, causing apoptosis in odontoblasts.[18]

Moreover, the results also revealed that the level of apoptosis on the first day in the CAPE group was higher than that in the propolis extract group. Similarly, the level of apoptosis on the fourteenth day in the CAPE group was higher than that in the propolis extract group.

Propolis is known to contain resins and bioactive

materials that include bioflavonoids, artepillin, apigenin, and CAPE, which have a role in controlling inflammatory, antioxidant, antibacterial, and antiviral reactions, managing immunomodulators, and stimulating tissue healing.[9] In this study, odontoblast cell death probably occurred due to the presence of phenol contained in CAPE. CAPE forms hydroxyl ions, which can change the transportation of organic material and nutrients that have toxic effects on cells. Free radicals have high reactivity since they tend to attract electrons, and can convert molecules into radicals, due to the loss or increase of one electron in another molecule.[19] Strong antioxidant activity correlates with high concentrations of CA and phenethyl caffeate.[14]

Cells have natural antioxidants, such as superoxide dismutase (SOD), catalase, reductase, glutathione peroxidase, and other antioxidants, that can protect against the influence of free radicals. However, when the number of free radicals is higher than the level of natural antioxidants the defense capability of cells can be disrupted, so that the normal reduction chain will be broken, and oxidative tissue damage will occur.[20]

The toxic effects of propolis extract can cause changes in the permeability of odontoblast cell membranes that will activate proapoptotic proteins in the cytosol (intracellular fluid). The activation of proapoptotic proteins will then induce the opening of mitochondrial permeability transition pores (MPTPs). The release of cytochrome c is related to the opening of MPTP, which will cause mitochondrial swelling, damage to the outer membrane, and trigger the release of inter-membrane proteins, including cytochrome c in some cells.[21]

Mitochondria contain proapoptotic factors, such as cytochrome c and apoptotic inducing factor. Both are dangerous substrates but can be stored safely in the mitochondria. When both are released into the cytoplasm, this protein can activate the caspase activation pathway. Cytochrome c acts as a water-soluble electron carrier in mitochondrial oxidative phosphorylation. Therefore, when an electron coil occurs via cytochrome c oxidase or complex IV, the change in ionic strength will trigger a wave matrix. Once the inner membrane of the mitochondria has achieved a wider surface permeability than the outer membrane, the wave matrix will cause the permeability pores inside the nonspecific membrane to open, so that cytochrome c passes through the cytoplasm. The cytochrome c that emerges from the cytoplasm then binds to Apaf1 to form a caspase recruitment domain (CARD). Several CARDs are bound into one to form an apoptosome complex, and then bind with pro-caspase-9 to become caspase-9 (caspase initiator). After that, the caspase-9 will activate procaspase-3 into caspase-3, an effector caspase stimulating apoptosis.[22]

Apoptosis of odontoblast cells after propolis extract application is of a lower level than following CAPE application. However, further research needs to be conducted to determine the physical properties of other propolis extracts to be used for pulp-capping treatment.

Ethical Clearance: No. 14/KKEPK.FKG/II/2015

Sources of Funding: Self-funded.

Conflicts of Interest: There are no conflicts of interest.

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Quality of Life of Post Stroke Patients and their Caretaker in Chennai

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Abstract

Stroke is one of the leading causes of death in India. Around 2 million Indians suffer from stroke every year in India, and the most important cause of severe adult disability. Stroke not only severely affects an individual's quality of life, but also their caretaker's. This burden can result in a deterioration of the caretaker's health, social life, and psychological well being such as, emotional health, morale, work life, finances, social mobility, interpersonal relationship and their own family. Here in this study, the quality of life of 50 stroke patients and their caretaker staying in a tertiary care hospital in Chennai, South India, is assessed using a pretested and validated questionnaire EuroQol EQ-5D-5L which contains questions in 5 domains in which each dimensions having five response levels and The EQ-VAS scale which records the respondent's overall current health on the day of examination on a vertical visual analogue scale from 0-100. The data were entered and coded in Microsoft Excel and analysed based on their frequency, proportion, and severity. About 80% of stroke patients return home after the acute hospitalization and at least one-half of them require permanent or temporary help from other people in the home setting. Many caregivers suffer from anxiety/ depression. This study will be useful in analysing the QOL of the patient's and their caretaker's and provide us a new perspective in not only treating the physical aspects of stroke but also its psychological impact.

Keywords: *stroke; quality of life; caretaker; self-care; anxiety and depression; usual activities*

Introduction

Stroke is one of the leading causes of death in India[1]. Around 2 million Indians suffer from stroke every year in India, and the most important cause of severe adult disability[2]. Stroke severely affects an individual's quality of life (QOL), compared to other illness, stoke is usually sudden and leaves a residual illness which affects both the individual and his family[3]. Patients are not only the individual affected in stroke as post stroke patients depend on others for most of their normal activities. This burden can result in a deterioration of the caretaker's health, social life, psychological well being such as, emotional health, morale, work life, finances, social mobility, interpersonal relationship and their own family[4]. Here in this study, the quality of life of patients and their caretaker staying in a tertiary care hospital in Chennai, South India, is assessed using a pretested and validated questionnaire EuroQol EQ-5D-5L which contains questions in 5 domains (MOBILITY, SELF-CARE, USUAL ACTIVITIES, PAIN /DISCOMFORT and ANXIETY / DEPRESSION), in which each dimensions having five response levels: no problems, slight problems, moderate problems, severe problems, unable to/extreme problems[5]. The EQ-VAS records the respondent's overall current health on the day of examination on a vertical visual analogue scale, where the endpoints are labelled 'The best health you can imagine' and 'The worst health you can imagine'. The EQ-VAS provides a quantitative measure of the individual's perception of their overall health on the day of examination.

Methodology

It's a cross-sectional Hospital based study on 50 post-stroke patients and their caretakers (50 patients and 50 caretakers of the patients) during March 2020-

May 2020. Study Tool: Basic demographic details along with a pretested and validated questionnaire EQ-5D-5L. Clearance from the Institutional Ethics Committee was obtained. All the eligible participants were identified and were invited to take part in the study. All the eligible participants were explained about the nature of the study and were given the choice of enrolling for the study. People who provide their written informed consent for the study were enrolled for the study. The enrolled individuals were given a questionnaire with include details about their basic demographic data along with brief details about their quality of life using a pretested and validated questionnaire EQ-5D-5L

Statistics and analysis of the data: The impact of stroke on quality of life of the patient and their caretaker using the questionnaire as the tool. The data were entered and coded in Microsoft Excel and analysed based on their frequency, proportion, and severity.

Results & Discussion

Test Data:

The study population comprised of 50 stroke patients and 50 caretaker and their basic demographic details can be found populated in Table1 and Table2.

Table 1: Demographic details of patients (N=50)

Variable	Category	Number (%)
Sex	Male	31 (62)
	Female	19 (38)
Age (in years)	41-50	3 (6)
	51-60	8 (16)
	61-70	23 (46)
	71-80	11 (22)
	+80	5 (10)

Table 1: Of the 50 patients, 31(62%) patients were male and rest 19(38%) were female. In that 3 people (6%) were between 41-50, 8 people (16%) between age 51-60, 23 patients (46%) between age 61-70, 11 patients (22%) between 70- 80 years and rest 5 patients (10%) above the age of 80 years with a mean age of 66.62 years.

Table 2: Demographic details of care-givers (N=50)

Variable	Category	Number (%)
Sex	Male	24 (48)
	Female	26 (52)
Age (in years)	31-40	9 (18)
	41-50	10 (20)
	51-60	11 (22)
	61-70	17 (34)
	71-80	3 (6)

Table 2: Out of 50 caretaker, 24(46%) were male and the rest 26(52%) were female. 9 individuals(18%) between the age of 31-40, 10people (20%) were between 41-50, 11 people (22%) between age 51-60, 17 people (34%) between age 61-70, 3 people (6%) between 70- 80 years, with a with the mean age of 54.88 years.

Table 3: showing the EQ-5D-5L frequencies and proportions reported by dimension and level of the stroke patients

Patient	Mobility	Self-care	Usual Activities	Pain / Discomfort	Anxiety/ Depression
LEVEL 1 (No problem)	6	6	1	9	4
LEVEL 2 (Slight problem)	8	7	12	10	5
LEVEL 3 (Moderate problem)	15	15	16	9	14
LEVEL 4 (Severe problem)	15	13	13	13	20
Level 5 (Extreme problem)	6	9	8	9	7

Table 4: Shows the EQ-5D-5L frequencies and proportions reported by dimension and level on the caretaker

Care-giver	Mobility	Self-care	Usual Activities	Pain/ Discomfort	Anxiety/ Depression
LEVEL 1 (No problem)	23	20	14	22	7
LEVEL 2 (Slight problem)	9	10	15	12	15
LEVEL 3 (Moderate problem)	11	9	14	10	17
LEVEL 4 (Severe problem)	6	10	5	6	8
Level 5 (Extreme problem)	1	1	2	0	3

Questionnaire and Result Analysis

Table3, Shows the QOL in each dimensions, here it clearly shows that only around 6(12%) of the people have no difficulty in mobility, around 6(12%) show normal self-care routine, only 1(2%) can do usual activities, 9(18%) have no pain or discomfort, and only 4(8%) show no anxiety or depression after the event. The usual activities are the one affected the most. Many show severe or extreme forms of anxiety or depression.

Table 4, Shows the QOL of caregivers in each dimension- around 23(46%) of the people have no difficulty in mobility, around 20(40%) show normal self-care routine, around 14(28%) can do usual activities, 22(44%) have no pain or discomfort, and only 7(14%) show no anxiety or depression, and more than 86% show some kind of anxiety/depression regarding their life or the patient's life.

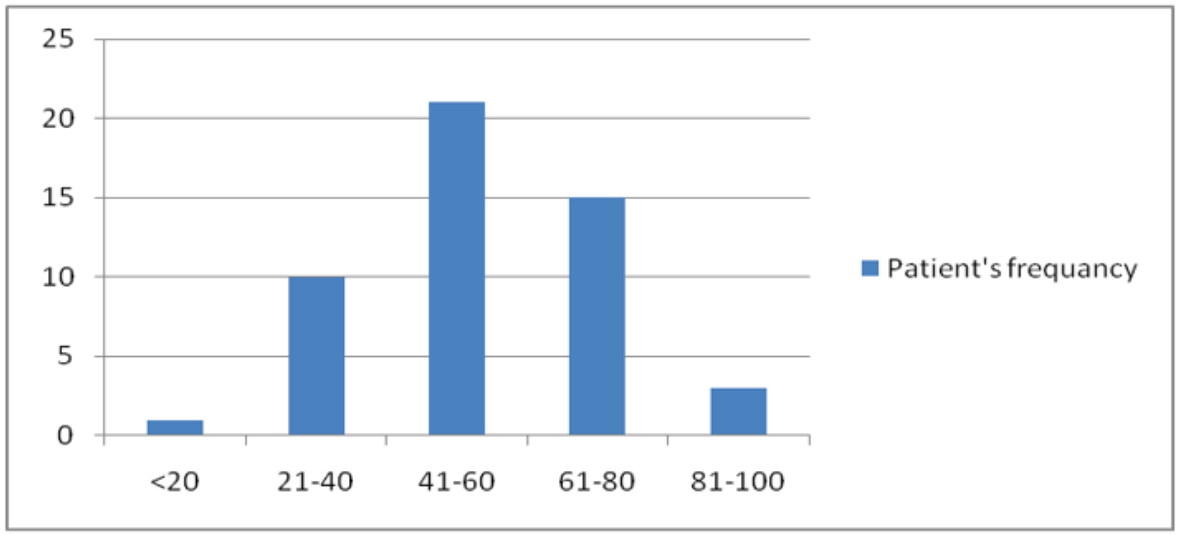

Figure 1: Shows the EQVAS of the patient

Figure 1, Shows that the overall health on the day of post stroke patients is evidently reduced as around 21 (42%) had only around 40-60 and the average was around 55.6% which is lower than the global average value of 86% in an individual leading a normal life.

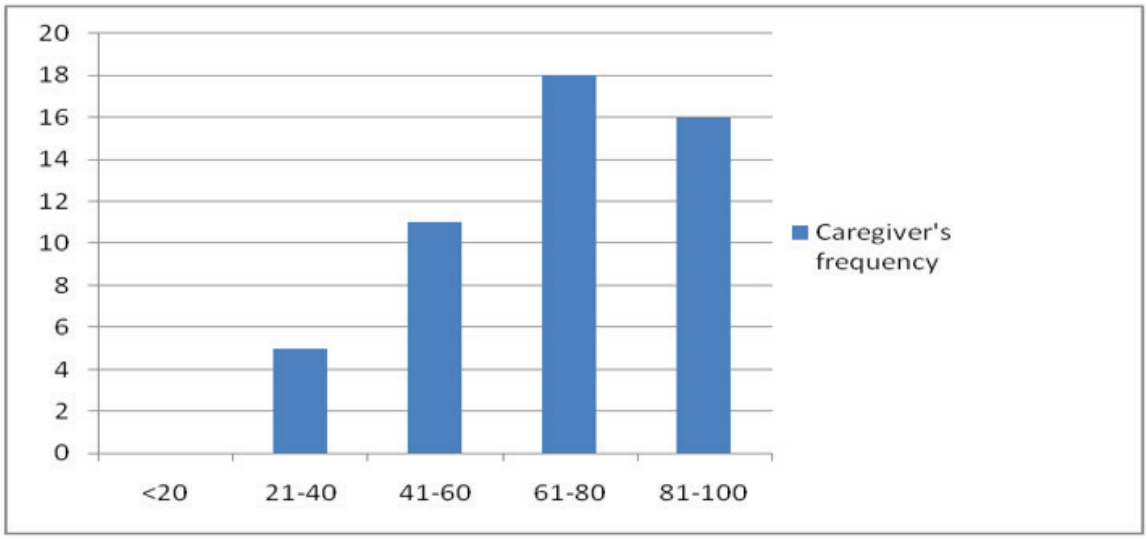

Figure 2: Shows the EQVAS of the caretaker

Figure 2, Shows that the overall health on the day of post stroke patient's caretaker is also reduced that average value is around 73% which is lower than the global average value of 86% in an individual leading a normal life.

Discussion

This study clearly implicates the fact that there is significant loss of quality of life on both patient and their caretaker. As it shows that the daily routine of the patient being affected the most, as they have some residual illness affecting their daily routine[6]. Due to the stroke affecting their nervous system, many show some significant disability in their mobility. Many often suffer from Anxiety/Depression after an episode of stroke, as they are eager to know their amount of disability and the impact of the disease on their family[7]. About 80% of stroke patients return home after the acute hospitalization and at least one-half of them require permanent or temporary help from other people in the home setting[8].

Caretakers are usually the closest family member to the patient. It's usually the spouse of the patient who takes care, along with their children taking care. As the disease significantly reduces the QOL of the Patient they depend on their caregiver[9]. This is one of the major reasons for reduction of QOL of caregivers. Many caregivers suffer from anxiety/ depression as they are not able to do their routine works freely or have enough time for self-care or excessive worrying about the outcome of the disease. Many also suffer from pain/ discomfort as it's the spouse taking care and due to their old age they need to put physical work for taking care of the patient. As the patients needs them for using the restroom or bathing or changing dress. Patient's and caregiver's routine is deranged causing excessive pain/ discomfort and also anxiety and depression[10]. Apart from physical support patients also require psychological support from their caretaker which causes excessive burden to the caretaker leading to reduction in their health status, social wellbeing and their productivity causing excessive anxiety and depression[11]. The burden of stoke can be significantly reduced by prompt intervention by family members and health care individuals. Health education plays a major role in reducing the burden of stroke, educating about lifestyle changes, diabetes, hypertension, obesity and warning signs of stroke can be of great help. Early rehabilitation proper counselling, physiotherapy, psychotherapy, behavioural therapy can also be useful[12].

There are few limitations to my study such as limited size, reduced correlation between the various types of stroke and their quality of life and not able to measure the positive emotions such as, happiness, gratification and satisfaction. QOL reflects well–being and according to WHO definition refers to 'an individual's perception of their position in life in the context of the culture and value system in which they live and in relation to goals, expectations, standards and concerns'. That's why the QOL needs to be broadened beyond the negative impacts on life but also accounting to the positive aspects.

Conclusion

This study clears concludes that the QOL of post stroke patients is reduced. Affecting their normal life, self-care and causing anxiety and depression. It not only affects the QOL of the patient's but also their caretaker's, affecting their normal routines and causing severe anxiety and depression.

Ethical Clearance: Saveetha Medical College and Hospital Institutional Ethics Committee (SMCH-IEC), Saveetha Nagar, Thandalam-602105

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Pain Relief and Post-Operative Outcome in Patients Receiving Tramadol via Thoracic Epidural versus Intravenous Method in Coronary Artery Bypass Graft Surgery

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Abstract

Background & Objectives: Acute pain is common after cardiac surgery and can keep patients from participating in activities that prevent postoperative complications especially respiratory complications. Accurate assessment and understanding of pain are vital for providing satisfactory pain control and optimizing recovery.

Our aim of the study was to compare pain relief and post-operative outcome in patients receiving Tramadol via Thoracic epidural versus intravenous method in coronary artery bypass graft surgery.

Methodology: Sixty patients aging 40-65 years posted for off pump coronary artery bypass graft surgery were selected. They were randomly assigned into two groups.Group IVA (n = 30) received Inj. Tramadol (1 mg/kgiv)and Group TEA (n = 30) received Tramadol 0.5 mg/kgepidurallyhalf an hour before shifting in cardiac recovery room. Hemodynamic parameters like Heart rate, systolic and diastolic blood pressure, pulmonary artery pressure were recorder for 72 hours postoperatively. We have compared extubation time in both the groups. Pain was assessed by visual analogue scale (VAS). Any patients with the VAS more than 4 were treated with rescue analgesic. Duration of analgesia and total no. of rescue analgesia were recorded. Duration of stay in cardiac recovery room and in hospital was also recorded.

Observation & Results: We have observed statistically significant difference in hemodynamic parameters between two groups with better stability in TEA group from shifting till next 72 hours. Extubation time was also earlier in TEA group. Mean duration of analgesia and VAS score was also better TEA group. All these led to shorter length of cardiac recovery stay and earlier discharge from the hospital with less complication.

Conclusion: Thoracic epidural analgesia is better than intravenous technique in terms of early extubation,maintaining hemodynamic stability and better postoperative analgesia with reduced length of cardiac recovery and hospital stay.

Key words: *Thoracic epidural analgesia, Tramadol,Coronary artery bypass graft surgery*

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Introduction

Pain is always subjective perception that cannot be objectively defined satisfactorily. The aim of an anesthesiologist is to render the patient pain free, during intra operative as well as post operative period.

Accurate assessment and understanding of pain are vital for providing satisfactory pain control and optimizing recovery.There are various methods for providing post-operative analgesia i.e. intravenous analgesia with various analgesic drugs like opioids, NSAIDs, etc; intrathecal or epidural administration of analgesics or local anaesthetic drug, patient controlled analgesia, nerve blocks & local infiltration etc.

Application of Thoracic Epidural Anaesthesia (TEA) to patients undergoing cardiac surgery during the modern surgical era was initially reported by Hoar et al in 1976[1]. Patients receiving TEA had significantly lower postoperative levels of pain mediators compared with control patients. TEA leads to more rapid recovery after CABG, with earlier extubation and shorter stays in intensive care and in the hospital[2].

Tramadol is an effective and well tolerated opioid analgesic used to reduce pain resulting from surgery, trauma, and also used for the management of chronic pain of malignant or nonmalignant origin. Tramadol appears to produce less constipation and dependence than equianalgesic doses of strong opioids.

Our aim of the study was to compareextubation time, pain relief and post-operative outcome in patients receiving Tramadol via Thoracic epidural versus intravenous method in coronary artery bypass graft surgery.

Aims and Objectives of Study

The aim of this study was to compare pain relief and post-operative outcome in patients receiving Tramadol via Thoracic epidural versus intravenous method in coronary artery bypass graft surgery.

Objectives of the study were to compare,

1. Cardiovascular Parameters & incidence of ischemia or arrhythmia

2. Post-operative analgesia & VAS score

3. Post-operative outcome such as extubation time, length of stay in cardiac recovery room and hospital

4. Any complications related with drugs or technique

Material And Methodology

This was prospective randomized study. Sixty patients aging 40-65 years posted for Cardiac Surgery at "Matsama" Heart Centre, Dhiraj General Hospital during the period of November 2012- June 2014 were selected. Clearance from institutional ethics committee was obtained. Patients were subjected to pre-anesthetic checkup and informed consent was obtained from all the patients.. They were randomly allocated into two groups; group TEA (Thoracic Epidural Analgesia) and Group IVA (Intravenous Analgesia) with 30 patients in each group by chit method.

Inclusion criteria:

Patient who were posted for off pump coronary artery bypass graft surgery (OPCABG)&willing to participate were included in our study.

Exclusion criteria:

1. Patient refusal.

2. Patients with coagulopathy.

3. Patients on potent anti-epileptics, analgesics, anti-platelets, or on anticoagulants.

4. Patients with spine deformity.

5. Patients with local skin infections at site of epidural injection.

6. Known allergy to the study drugs or local anesthetics.

Preoperative management

Tab. Alprazolam 0.25 mg was given on the night prior to surgery. Patients were asked to restrict fluids and solids by mouth at least six hours before operation. Anesthesia procedure explained and a written informed consent was obtained from them.

After taking patient inside Operation Theatre, multipara monitors were applied and base line respiratory rate, pulse rate, non-invasive blood pressure, SpO_2 and ECG were recorded. Intravenous line was secured with 18G IV line and the patients were started I.V. fluids. Premedication ofInj. Glycopyrrolate 0.2 mg, Inj. Ondansetron 4 mg and Inj. Ranitidine 50 mg ivwas

given.Radial cannulation was done and baseline ABG was taken. Then all other invasive (femoral, PA/CVP) cannulations were done. Arterial pressure monitoring and CVP/PA were started.

In TEA group, the epidural catheter was inserted with catheter tip was kept around T1-T2 level.The blockade require for thoracic surgery is T_1-T_8.

All the patients were preoxygenated with 100% O_2 for 3 minutes. They were induced with Inj. Fentanyl 5-10 µg/kg, Midazolam 0.05 to 0.1 mg/kg, Vecuronium 0.2 mg/kg i.vand intubated by direct laryngoscopy with appropriate sized Endotracheal tube. Tube was fixed after checking bilateral air entry and maintained with oxygen, Isoflurane, Muscle relaxants (top-ups) and fentanyl-midazolam infusion.

Group IVA (n = 30) received Inj. Tramadol 1 mg/kg iv half an hour before shifting in cardiac recovery room as a post-operative analgesia.

Group TEA (n = 30) received epidural dose of Tramadol 0.5 mg/kg, half an hour before shifting in cardiac recovery room for post-operative analgesia. Total volume required was calculated as per 1 ml for each spinal segment to be blocked.

Intravenous fluids and blood were replaced as per the requirement.

Patients were monitored intraoperative for pulse rate, blood pressure, SpO_2, ECG, PA pressure, Temperature and $ETCO_2$& other complications.

Post-operative management:

With stable hemodynamic, all patients were transferred to the cardiac recovery room with endotracheal tube in situ and ventilated with SIMV mode initially. Patients were extubated in cardiac recovery once the patient fulfilled following criteria.

Weaning criteria:

- No acute ischemia or new arrhythmia
- Hemodynamically stable
- Blood loss < 2ml/kg/hour for first 2 hours
- Urine output > 0.5 ml/kg/hour
- Demonstrating signs of awakening from anesthesia
- Core temperature 97.2° to 99.8° F

After fulfilling above mentioned criteria patient was weaned off to CPAP mode.

Extubation Criteria:

- Patient is awake and cooperative (follows verbal commands & moving all four limbs)
- Able to lift head for more than 5 sec.
- Spontaneous tidal volume > 6ml/kg
- Respiratory rate < 30/min
- ABG obtained in CPAP trial meets the following criteria

pH> 7.35, PCO2 < 45, PO2 > 80 with FIO2 ≤ 40

To minimize the complications related to epidural catheter such as infection or malposition, we have removed Epidural catheter after 72 hours. Thereafter, conventional rescue analgesics were given to control pain.

Postoperative monitoring:

All patients of both groups will be monitored for:

- Heart rate (HR).
- Systolic & Diastolic blood pressure (SBP & DBP)
- Arterial oxygen saturation (SpO_2).
- Extubation time
- VAS score
- Duration of Analgesia
- Total no. of rescue doses of analgesics in first 24 hours
- Total length of cardiac recovery room and hospital stay

Assessment of analgesia

Pain was assessed by visual analogue scale (VAS) immediate post-operatively and every 3 hourly for first 12 hour then 6 hourly till next 72 hours. Any patient with the VAS more than 4 was treated with rescue analgesic Inj. Paracetamol 15 mg/kg i.v.

For visual analogue score, the patient was taught on 10cm scale telling him that 0 (zero) represents "no pain" and 10 represent "worst pain". The patients then mark off on the scale the degree of pain he was suffering.

0	1	2	3	4	5	6	7	8	9	10
No	Mild Pain			Moderate Pain			Severe Pain			
	Good coughing on physiotherapy & no assistance in ambulation			Poor cough Require moderate assistance to ambulate			Unable to cough = Poor Require full assistance to ambulate			

Postoperative first analgesic requirement.

As in cardiac surgery, we can't allow the patients to suffer from pain. So as per the previous studies we have fixed the duration of top-up doses which was 8 hours in IVA and 10 hours in TEA.

Complications

Post-anesthetic side effects or complications, if any reported as:

Ø Nausea, Vomiting

Ø Itching

Ø Respiratory complications (such as atelectasis, pneumothorax, pleural effusion)

Ø Accidental dura puncture,Epidural hematoma

Ø Backache

Ø High Block,Catheter malposition

Ø Any newer onset of ischemia or arrhythmia

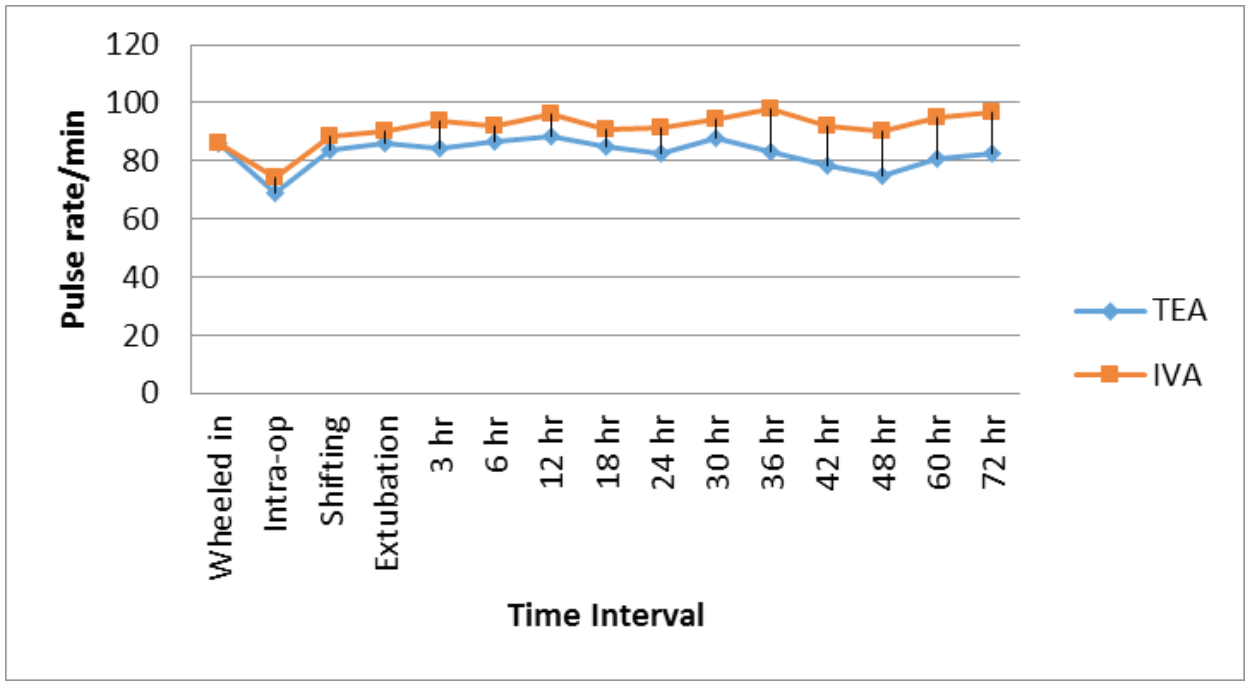
Ø Neurological injury

Observation & Results

Statistical methods: Data were collected, tabulated, coded then analyzed using GRAPHPAD PRISM computer software version 6.0. Numerical variables were presented as mean & standard deviation (SD) while categorical variables were presented as percent. Data were analyzed with unpaired student – t test. P value < 0.05 was considered significant and <0.001 was considered highly significant.

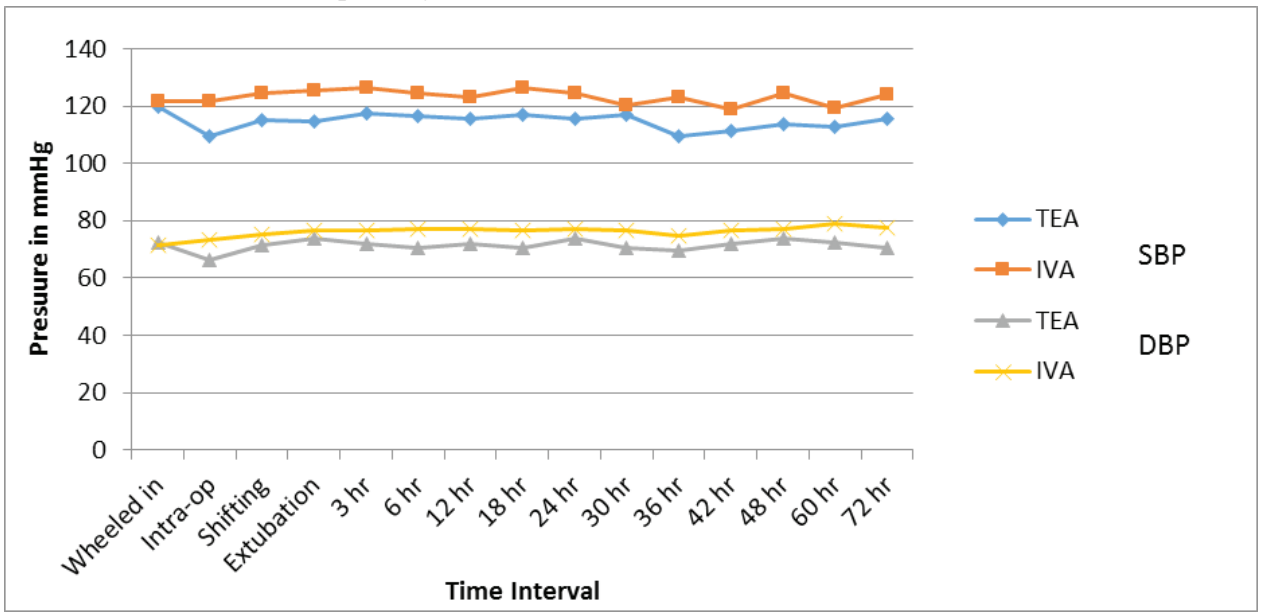
The distribution of patients with respect to age, height, weight and duration of surgery was comparable in both the groups (p value > 0.05).

Graph1: Pulse rate variation



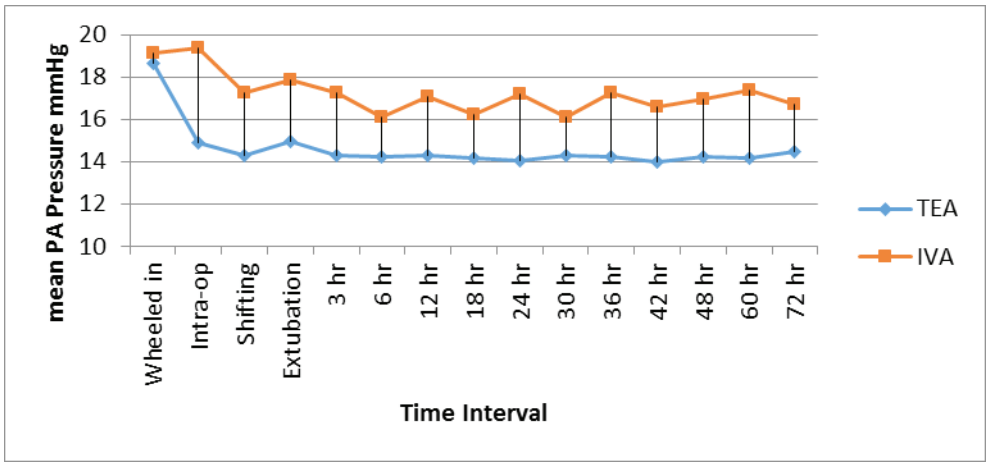
As shown in graph, there was statistically significant difference in pulse rate between the two groups (p value < 0.05). TEA group patients had lower pulse rate and had less pulse rate variation throughout study duration.

Graph 2: Systolic and Diastolic Blood Pressure Variation



As shown in graph, there was statistically significant difference in systolic blood pressure between two groups (p value < 0.05).TEA group patients had lower systolic blood pressure and had less systolic blood pressure variation throughout study duration.

As shown in graph, there was statistically significant difference in diastolic blood pressure between two groups (p value < 0.05).TEA group patients had lower diastolic blood pressure and had less diastolic blood pressure variation throughout study duration.



Graph 3: Mean PA Pressure (mPAP) variation

We had observed statistically significant difference in mean PA Pressure (mPAP) between two groups. (P< 0.05) TEA group patients had lower mean PA pressure and had less mean PA pressure variation throughout study duration.

Table1: Mean extubation time

Event	Group TEA		Group IVA		P value	Remark
	Mean	SD	Mean	SD		
Extubation Time (in minutes)	123.74	1.553	197.6	2.248	< 0.05	S

S= statistically Significant

As shown in table, there was significant difference in mean extubation time with early extubation in TEA group compared to IVA group.

Table 2: Analgesic Profile

Event	Group TEA		Group IVA		P value	Remark
	Mean	SD	Mean	SD		
VAS score at 1st Top up analgesia	2.133	0.124	2.833	0.136	< 0.05	S
No. of rescue Doses in 1st 24 hours	2.267	0.135	4.443	0.149	< 0.0001	HS
Overall VAS Score in 1st 24 hours	0.412	0.0112	1.862	0.0412	< 0.001	HS

S= Significant HS=highly Significant

We had observed statistically significant difference in VAS score at 1st top up analgesia between two groups (P<0.001). TEA group had less VAS score at 1st Top up analgesia as compared to IVA group. (P<0.001)

We had observed statistically significant difference in total no. of rescue analgesic doses and overall VAS score in 1st 24 hours between two group (P<0.001). TEA group patients required less rescue analgesic dose compared to IVA. Overall VAS score in 1st 24 hours was also less in \

Table 3: Total Length of Cardiac Recovery stay and Discharge from hospital

Event	Group TEA		Group IVA		P value	Remark
	Mean	SD	Mean	SD		
Total length of cardiac recovery stay in hours	83.43	0.62	100.4	1.32	< 0.001	HS
Total length of hospital stay in days	7.367	0.089	8.74	0.1282	<0.001	HS

There was statistically significant difference in cardiac recovery and hospital stay between both groups (p-<0.001) with less stay in TEA group.

In our study, we had observed newer onset of arrhythmias in 13% of patients in TEA group. It was VPCs, while 20% of patients in IVA group had developed newer types of arrhythmias which include 2(6.67%) patients of atrial fibrillation and 4(13.33%) patients of VPCs. Incidence of nausea and vomiting was slightly on higher side in IVA group which was controlled by antiemetic. In Chest X-Ray of postoperative day 1 and day 2, 10% of patients in IVA group had developed pulmonary complication such as 2(6.67%) had mild pleural effusion and 1(3.33%) had left lower lobe collapse. In group TEA, only 1(3.33%) patient had mild pleural effusion.

Discussion

The use of Coronary Artery Bypass Graft (CABG) surgery in the elderly population in the United States has doubled every 5 years since 1985 and in 2003 approximately 5,00,000 cardiac procedures were performed in the US at a cost of around $9 billion. Thusthe focus of cardiac anaesthesia started shifting in the early 1990's to lower dose opioids, earlier extubation and decreased ICU stay. This came to be labeled as 'Fast Track Cardiac Anaesthesia' (FTCA)[3]. There are no fixed, accepted definitions in this field but general consensus accepts the following:

- FTCA is an extubation within 8 hours of the end of surgery. Many major centers, however, aim for extubation 1 – 4 hour postoperatively.[4]

- There is also the notion of Ultra Fast Track Anaesthesia (UFTA) which refers to extubation of patients in theatre post cardiac surgery.

Application of TEA to patients undergoing cardiac surgery during the modern surgical era was initially reported by Hoar et al in 1976.[2]Subsequently, thoracic epidural anaesthesia and analgesia have been utilized in the intra operative and post operative anesthetic management of patients undergoing cardiac, thoracic and upper abdominal surgeries.[5]

Pain may be associated with many interventions, including sternotomy, thoracotomy, and leg-vein harvesting, pericardiotomy, and/or chest tube insertion, among others. Inadequate analgesia and/or an uninhibited stress response during the postoperative period may increase morbidity by causing adverse hemodynamic, metabolic, immunologic, and haemostatic alterations[6,7].

TEA lower the pain score, enable earlier extubation, and reduce the incidence of complications, including respiratory system infections, acute renal failure, and acute confusion secondary to intravenous opioid usage[8,9]and therefore earlier discharges[8-12]. Use of TEA also results in reduced infarct rates during and after surgery in patients with ischemic heart disease[13-14].

In our study patients in TEA group had lower pulse rate, SBP, DBP and also had less variation compared to IVA group. PA pressure was also less with less variation in TEA group compared to IVA group.Nagwa Ahmed EbrahimMegahedet al.[15] also observed Thoracic epidural analgesia with bupivacaine had better control on hemodynamic changes intraand postoperatively. Chakravarthy et al.[16] observed significant reduction in PA pressure after administration of thoracic epidural in patient planned for aortic valve replacement.

Extubation time is directly dependent on the anesthesia technique implicated and usage of opioids and inhalational anesthetics. Higher use of opioids and anesthetics leads to higher level of sedation. TEA had been associated with lesser sedation level secondary to reduced need of opioids.

In our study, mean extubation time was 123.74 ± 1.55 min in TEA while 197.6 ± 2.25 min in IVA group which was significant ($P < 0.05$). Length of ICU stay was 79.07 ± 1.17 hours in TEA while in IVA, it was 100.2 ± 1.32 hours which was strongly significant ($P<0.001$). Duration of discharge from hospital was 7.367 ± 0.089 days in TEA as compare to 8.74 ± 0.1274 days in IVA group. This effect was observed due to reduced need of opioids and inhalational anesthetics in TEA group leads to lower level of sedation. This causes early extubation and reduced length of ICU stay and early discharge from hospital.

Priestly et al.[17] studied use of thoracic epidural to have early extubation and reduce length of hospital stay. They have concluded that TEA leads to faster recovery and early extubation but it has no significant effect on length of hospital stay. Nevriye Salman et al[18] studied use of Thoracic epidural analgesia in CABG. They have observed similar results in terms of extubation time and early discharge from hospital.

Mean duration of analgesia which was calculated as time period from the administration of study drug which was half an hour before shifting till first rescue analgesia was given (VAS ≥4). It was 597.3 ± 3.89 minutes in group TEA compared to 435.2 ± 6.37 minutes in IVA group. VAS score at 1st top-up dose was 2.133 ± 0.124 in TEA in comparison with 2.833 ± 0.136 in IVA group.

Mean number of rescue doses in 24 hours was 2.267 ± 0.135 in TEA group compared to 4.443 ± 0.149 in IVA group. Overall VAS score in 24 hours was 0.412 ± 0.011 in TEA group as compared to 1.862 ± 0.043 in IVA group.In study done by Royse et al.[19], TEA was associated with lower pain scores compared to intravenous morphine, both at rest and during coughing, during the first two postoperative days.

Bloomberg et al.[20] observed that TEA increases the epicardial diameter of stenotic vessels along with dilatation of coronary vasculature. Thus, it increases coronary blood flow and reduces the incidence of post operative infarction in CAD patients. In our study, we have not observed any recent changes in ECG except VPCs in TEA group. Mehta Y. et al. also observed similar results in their study.[21]

Conclusion

Thoracic epidural analgesia is better than intravenous technique in terms of maintaining hemodynamic stability,goodpost operative analgesia and better postoperative outcome with reduced length of total hospital stay.

Source of Funding- Self

Conflict of Interest - None

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Detection for *Candida spp* in Periodontitis Chronic

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Abstract

The study aimed to isolate and diagnose some types of Candida yeasts *Candida Spp* Associated with periodontal infection extracted from deeply periodontal sinuses 3- 5mm The study focused on gender *Candida albicans*. Collection T. 120A clinical sample of patients with chronic periodontitis The patients Who return to the Center Martyr Nasser Amoososa Dental Specialist and outpatient clinics in the province of Najaf for the period from December 2019 To March 2020 The results showed that 44 Sample out of 120 It was a container of yeasts and a proportion 36.6 Three types of yeasts have been identified *Candida Spp* and *Candida albicans* It was the most frequent and prevalent over the remaining types of isolated yeasts, as they were 40 isolation And rate 91% And two isolates for*Candida dubliniensis* in the rate of 4.5% And one isolation for each *Candida ciferrii*And type *Cryptococcus laurenti* And the ratio of isolation2.25 % As they were diagnosed by a system VITEK2. Isolates were diagnosed *Candida albicans* On the differential mean Chromo genic agar And genetic diagnosis by polymerase chain reaction PCR The amplification size were shown(354) bp Base pair

Keywords: *candida spp, candida albicans, cryptococus, periodontitis Chronic, yeasts Candida spp ,pockets sinuses.*

Introduction

Many infections occur in the oral cavity due to the activity of microorganism present in the mouth. Oral flora Among these injuries, tooth decay Dental earies And cysts Absceses And Gingivitis[(1)] and periodontitis (Holt *et.al.* , 2000) Periodontitis and includes many of the most common diseases periodontitis chronic and inflammation of periodontitis aggressive and grunt, and included this study patients with periodontal chronic usually healthy gums are distinguished by color pale pink stick strongly teeth[(2)] not prone to bleeding when brushing either in the case of inflammation of Gingivitis The gums are swollen with redness and the appearance of an unpleasant odor from the mouth, and when untreated, the infection develops into chronic periodontitis, where penetration of the deep tissues around the tooth occurs and the bones are destroyed and with the progression of the disease exposed To give up, a chronic inflammatory disease[(3,4)] caused by multiple microbes which affects the gums, and it is one of the diseases that occur in a proportion[(5,6)] , It is a high and a major cause of tooth loss world wide[(7)] . (Nibali, *et.al* ;2013) this development of gingivitis is caused by the accumulation of bacterial plaque, causing inflammation[(8)] of the periodontal tissues and formation of the periodontal pocket, and damage to the alveolar bone. (ASK; Sham *et.al* , 2003)[(9)]

It occurs as a result of the breakdown of the collagen fibers surrounding the tooth, which leads[(10)] to sinusitis and tissue damage in the tooth (Watts, *et. al* ; 1998) . Chronic periodontitis spreads in adults and the elderly all over the world, the incidence of primary gingivitis increases with age, and the highest rate occurs between 50-60 Public[(11)]. There are several studies refers to chronic inflammation of periodontal link with many diverse forms of micro - organisms which include positive and negative bacteria of gram stain ,examples types of bacteria *Fusobacterium nucleatum* , *Tannerella forsythia , Prevotella*

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They are closely related to chronic periodontitis and the types of*Streptococcus gordonii , intermedia* It plays a major role in the formation of the biofilm of the dental layer and in accelerating the progressionof periodontitis and its types *Porphyromonas gingivalis* Bacteria gingivalis negative anaerobic associated with a significant correlation of Periodontitis[12] in adults (Duncan *et al* ., 2008) as well as the bacteria associated with fungal tumor associated with periodontitis in adolescents, including types[13] of *Aggregatibacter* (Fine *et al*;2008)

Showed the studies that Yeasts type Candida spp in gum pockets It has important role in inflammation periodontitis (Mac Neill *et.al.* , 1997) Where it was isolated *C.albicans* of a large number of patients with chronic periodontal pockets(Reynaud; 2001, Babitha et.al., 2018)As for the other[14-16] types of yeasts, there are low rates in periodontal infections, including*C.krusei, C.parapsilosis C.globrata, C.tropicalis, C.* guilliermondii *C.dubliniensis* It is one of the types closely related[17] to *C.albicans)* Hannula et al., 1997) .

Materials and Methods

Collection of speciement

120samples collected from the cases of patients Periodontitis infection Which was diagnosed clinically by specialized dentist.The Samples were taken from patients of different ages, ranging from)21-70(Year and For both sexes.

samples were collected at the Martyr Nasser Al-Mousawi Specialized Dental Center and Private outpatient clinics in Najaf Governorate for the period from December 2019 To March 2020, Where Swab was taken from infected patients using a sterile mouth swab With an agricultural medium vector and then the samples were transferred to microbiology laboratory where it conducted the following tests:

The tests used in the diagnosis

1-Morphology Examination

The study of the outer shape of the phenotypic colonies containing the shape and color of the Qtralamstamrh and height after Plant it on the medium SDA at °M37Temperature for 48hour (De Hoog; 2000)

2- Microscopic Examination

Study the microbiological characteristics of the yeasts by observing the shape of the yeasts, which are often round or oval . Remove part of the colonies cultivated and transfer them to a glass slide with a drop of solution Where was Normal Saline , It was stained with a gram stain and then examined[18] under a microscope strongly 40x. (Atlas *et al.,* 1995)

3-Growth test on Chrom agar medium

The test was performed by taking a portion of the developing yeast colony on a medium SDA aperiod for 24hour then planted it on the medium of Chrom agar and incubated for a period at (24-48)hour in37°M Temperature. where it is observed Species Colors. Those grown on this medium, use this test[19] to distinguish the types of Candida genus (Ellis *et.al.* , 2007)

4-Germ tube formation test

The test conducted according to the (Ellis; 2007) where taken part of the yeast colony was developing on the medium of SDA and transferring it to a clean, sterile, abendruff tube containing 0.5Ml of human serum, which was centrifuged at room temperature and the mixture was incubated 3 Do not add hour in degree 37 M ° for the Temperature , afterwards I took a drop of clamp and laid glass slide and examined microscopically to view the germination tube, this examination is characteristic of the species[20] *C. albicans*

As a diagnostic to distinguish it from other types of Candida, as mentioned[21] in (Keepeng *et al* ., 2015)

5- Test the ability of yeast to grow at 45 ° C

This test is used to distinguish yeasts growing on a medium SDA .,That appear converged with C.albicans Yeast color the test was stated in depending[22] on (Pinjon *et al* 1998) that developing yeasts in a SDA period (48-72) For a °M45 Degree The emergence of growth on the medium indication *C. albicans* yeast Existence

Results and Disccusion

Isolation and diagnosis

It was obtained 120 from a sample of patients with periodontal infection. The results of the injury appeared Periodontitis in isolation 44 a sample of percentage 36.6 % the total number. Three types of yeast return candida spp ,the candida albicans Species was most reluctant and indeed 40 samples in the rate of 91% , two isolate *C.dubliniensis* in the rate of %4.5 , and *Candida ciferrii* , *of Cryptococcus laurentii* Isolation is one of a kind the rate of %2.25 from the number of positive samples .The study focused on *C.albicans* because it has most Hesitation and it has important virulence factors associated with the disease . as follows in table(1) :

Table (1): Number of positive growth- isolate samples

Percentage %	**The number of samples**	Types of yeasts isolated
91	40	Candida albicans
4.5	2	Candida dubliniensis
2.25	1	Candida ciferrii
2.25	1	Cryptococcus laurentii
100	44	Total
6.8 Significant differences in favor Candida albicans		Arithmetic chi square
0.34		Chi- square tabularP <0.05

The results showed the dominance of the species *C. albicans*

As for the rest of the other types, these results are consistent with what was arrived (Babith e*t. al* ., 2018) For his research in India, he noticed the number of Candida albicans Increase chronic periodontitis And aggressive gingivitis .And that its appearance in this high percentage makes it a major cause of yeast infection, and this is in agreement with (Perera & Clayton; 1994) . About the prevalence of this type compared to the rest of the other species and thisWhat he mentioned. The dominance is due to its possession of highly pathogenic factors, such as the production of phospholipase enzymes , rotease and hemolysin. In addition to the formation of germination tube and chlamydial spore).Millon; 2001, Markus; 2002). Isolated patients with periodontitis*C. a lbi cans* a comparison was made for yeast For males and females, where the results of the statistical analysis showed that there are no significant differences between males and affected females. figure (1)

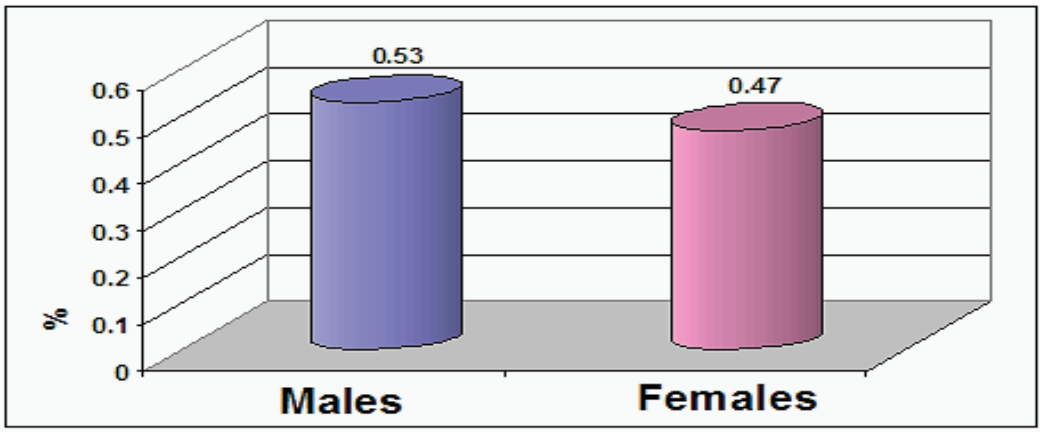


Figure (1) percentage of diagnosed with periodontitis relative to yeast *Candida albicans* **based on gender**

The statistical results showed that there are significant differences concentrated in the age group (41-50) Most frequent for people with periodontitis relative to yeast *Candida albicans* For both males and females in the rate of 32.5% and the least frequent in the age group (61-70) in the rate of % 7.5 ,figure (2)

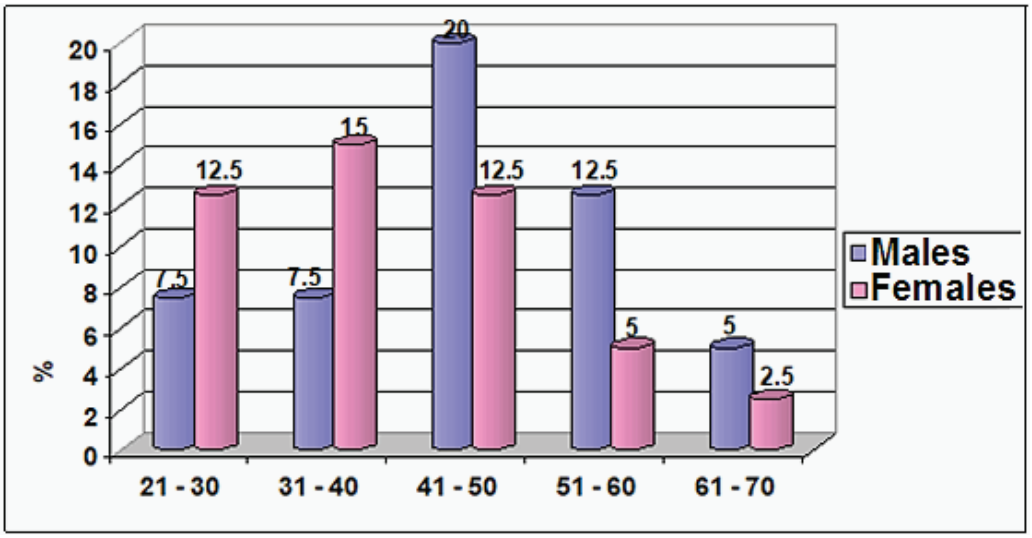
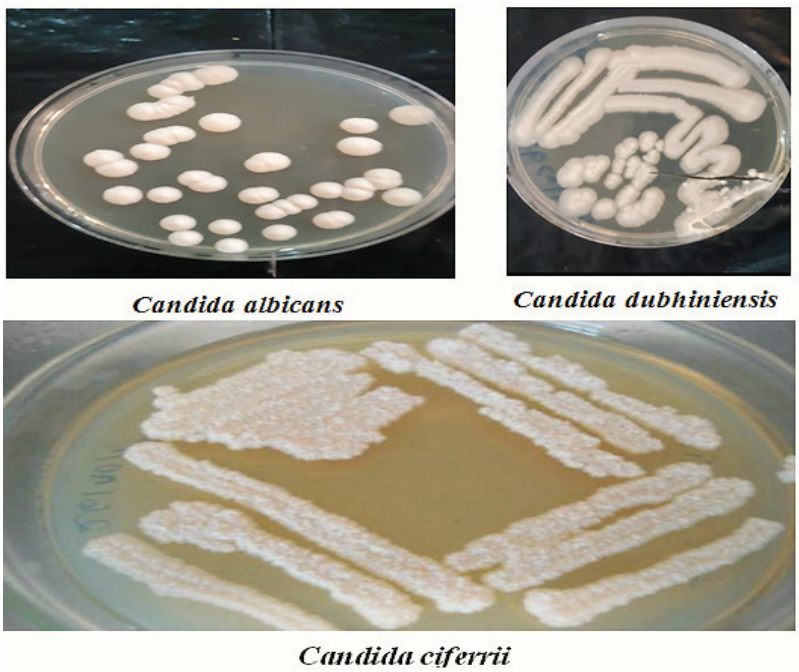


Figure (2) percentage of diagnosed with periodontitis relative to yeast

***Candida albicans* Based on age groups and gender**

Agricultural Characters

The Colonies *C.albicans, C.dubliniensis* When growing on a medium SDA at Temperature 37 M ° appeared white, creamy, smooth, shiny, circular in shape (Ellis *et.al* , 2007) .As for colonies *C.ciferrii* appeared alsoWhite , creamy color But Raised, rough and wrinkled This is consistent with what he mentioned. (Packia s . *et al* ., 2019) ز That colonies Candida spp It has phenotypic characteristics when grown on a medium SDA during duration (24-48) hour that is consistent with (Singh *et.al.* , 2013) as noted in Fig(3)



Figure(3) Isolated yeast growth on SDA medium at Temperature 37°M

Microscopic Characters

The isolated types of Candida yeasts gave positive results when interacting with the gram stain, as the cells appeared oval to spherical, oval, elongated or cylindrical, the fungus-shaped, and these results It was identical to (Boon *et al* ., 2013) . And that the appearance Candida cells are stained blue Because the layer on the cell wall retains this dye It was identical to (Sudbery *et. al* ,.2004) As noted in Figure (4)

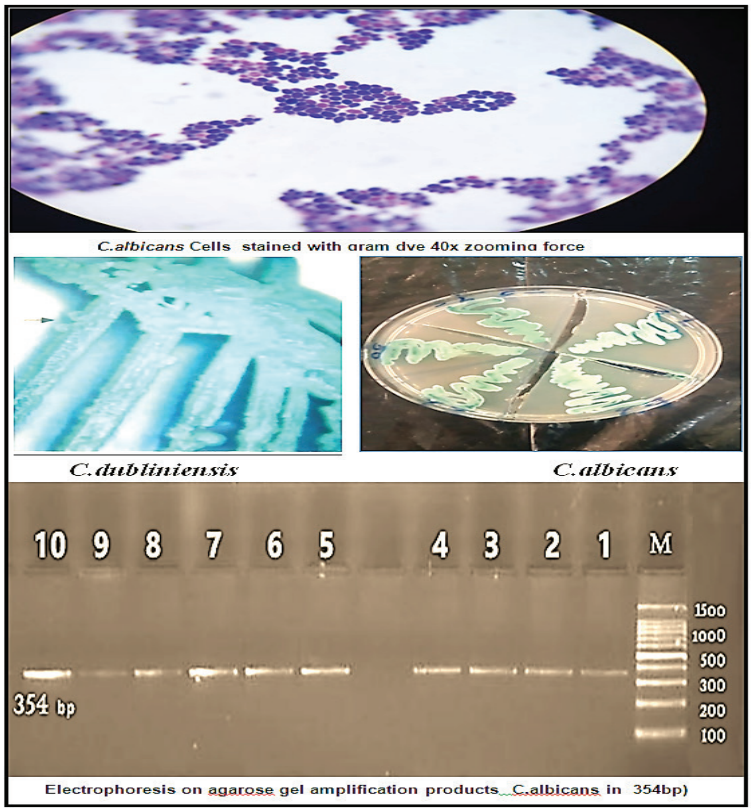
Growth on CHROM agar medium

The results showed that all isolates were able to grow well on the differential medium during Duration (24-48)hour. That colonies *Candida albicans* appeared light green color with convex shapes Smooth. either *C.dubliniensis* type his colonies appeared dark green with convex shapes rough ,This is consistent with what was arrived (Tintelnot *et* .al 2000) the Shown in **figure(4)**

Note: Some of the isolated yeasts that were not diagnosed on this differential medium were diagnosed by VITEK2 system

Molecular diagnosis of *Candida albicans*

Depending on the polymerase chain reaction to confirm *C.albicans* Molecular diagnosis of the genus was performed. Diagnosis of Candida isolates, as the current study showed a high susceptibility to the technique of polymerization reaction. Internal transcribed To inflate an area (ITS1, ITS2) Sequential by starting and infected patients were isolated For ten isolates of the yeast Candida albicans PeriodontitisIt was characterized by high accuracy and capacity compared with the traditional methods used, it has been shown. The results were amplification for the target region And the C.albicans Sequential polymerization of a type interaction amplification products (354bp(size the figure(4) .This method has been considered one of the quick and accurate methods of diagnosing Candida yeasts with less time is possible (Fujita et.al., 2001)



Fig(4): Electrophoresis , Growth *C.albicans* &*C.dubliniensis* on Chrom Agar medium at Temperature 37°M for (24-48)hour.

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Ethical Clearance: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

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Application of Two Sex Markers by Nested PCR for Gender Determination

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Abstract

Sex determination is one of the basic components in victim identification. There are many available methods, namely forensic anthropology method and conventional DNA typing method. In this study, nested PCR technique was employed in sex typing of burnt teeth through amelogenin (AMEL) and sex-determining region Y (SRY) markers. In this study, 17 teeth samples were burnt at temperatures that ranged from 100°C to 500°C for 2 min - 10 min. The whole tooth was used for DNA extraction by phenol-chloroform method. Accurate sex determination was achieved in 13 samples by both AMEL and SRY markers. The SRY marker achieved higher sensitivity as compared to AMEL marker. The sensitivity of both markers was improved consequent to nested PCR. Factors such as degraded DNA materials and the presence of tooth caries greatly affect sex typing results. Results showed that nested PCR proved to be a good method to amplify highly degraded DNA material as it greatly increased the DNA copy, and thus increased the possibility of sex typing.

Keywords*: amelogenin gene; burnt teeth; nested PCR; sex determination; SRY gene*

Introduction

In the victim profiling process, especially cases of mass disaster, sex determination is the basic tenet of forensic science with methods that include direct observation of genitals, forensic anthropology and conventional DNA typing. However, identification of victims is not always applicable in cases of explosion, burnt corpse or comingled remains when a complete body or skeletal remain is no longer intact. Therefore, an alternative method needs to be employed to determine the identity and sex of victims.

Conventional forensic DNA analysis utilises short tandem repeat (STR) to determine the sex based on the homologous amelogenin genes on X and Y chromosomes (AMELX and AMELY), which is incorporated in STR typing kit[1]. Previous study of a burnt corpse failed to produce result when STR typing was used to amplify due to degraded DNA materials[2].

Amelogenin gene is used as a sex marker for forensic samples since there is a length variation of amplified fragments between X and Y amelogenin[3]. This advantage of AMEL gene enables amplify by using one set of primers which target the X chromosome and Y chromosome. However, a study by Akane et al.[3] showed that this marker was unable to determine the sex of a drowned victim who was dead for more than a month. Based on a study by Zagga et al.[4], the success rate for sex identification by using amelogenin gene was only 33.3% for samples subjected to heating between 100°C to 300°C.

Besides, mistyping of male subjects as female was also reported due to AMELY deletion in the Y chromosome[5]. Primer site mutation of both AMELX and AMELY genes was theorised as one of the reasons for sex typing failure, causing the lack of an amplicon which caused confusion while interpreting DNA mixtures, especially in sexual assault cases[1]. A study conducted by Chang et al.[6] showed a significant number of AMELY

null individuals amongst Malay males (0.6%) and Indian males (3.2%), and none of Chinese male individual. A similar study also suggested that sex typing based solely on amelogenin is insufficient for use in routine forensic work[6].

As stated by previous studies, AMEL gene alone has high possibility of producing inaccurate results, thus it is important to utilise other sex markers with higher accuracy[5, 7]. In this study, sex-determining region Y (SRY) gene was chosen as it is the most closely linked to the male sexual phenotype and activation of SRY is required for testis formation[8]. This alternative sex marker is located at p11.31 on the Y chromosome[1], which is a part of the testis-determining factor (TDF) gene. The mutation of this gene has caused a sex reversal in XY females[8]. Currently, SRY gene is the only sex marker that has products which directly affects sex development in males. The strong association of SRY products and male phenotype suggests that SRY gene is the most accurate marker for male phenotype appearance when unreliable results were obtained from AMEL analysis[1]. According to Naik et al.[7], sex determination of human dental pulp through SRY gene showed 100% accuracy for all 20 tooth samples.

Forensic samples from the crime scene are always received in different conditions from normal samples, and thus application of nested polymerase chain reaction (PCR) is very helpful as compared to conventional PCR. Nested PCR is a modification of PCR which is designed to improve sensitivity and specificity of the amplified product through two primer sets and two successive PCR reactions[9]. The advantages of nested PCR are it reduces amplification error, consumes less time and involves a minimal cost.

In the present paper, two sex markers, namely amelogenin and SRY genes, were used to identify the sex of teeth samples treated at various temperatures and durations by employing the nested PCR amplification technique.

Material and Methods

Sampling

A total of 17 extracted teeth samples were obtained from School of Dental Sciences, Health Campus, Universiti Sains Malaysia and stored in dry condition. Prior to collection of teeth samples, ethic approval was obtained from the Human Research Ethics Committee USM (USM/JEPeM/16020081). A blind study was adapted, whereby the researcher had no information about the sex of tooth owner prior to analysis.

Then, all tooth samples were immersed in 10% bleach solution for 5 min, and followed by soaking them in 70% ethanol for 5 min. The teeth were later exposed to ultraviolet (UV) light for 15 min to eliminate exogenous DNA. All tooth samples were grouped based on designed experiment. Table 1 shows the specific temperature and exposure time applied for each tooth. The burning process was carried out in a burnout furnace. Teeth samples were ground into powder by using pestle and mortar prior usage for DNA extraction.

Genomic DNA Extraction

In this study, the phenol-chloroform organic method was employed for DNA extraction procedure, as suggested by Presecki et al.[10]. Approximately 0.15 g – 0.20 g of tooth powder was used as the starting material for DNA extraction. To increase the DNA extraction efficiency, 0.01 M of DTT (dithiothreitol) was added to the tooth powder mixture. The extracted DNA samples were then quantified by Thermo Scientific NanoDrop™ 2000 Spectrophotometer and stored at -20 °C for subsequent analysis.

PCR Amplification

In this study, the published primers were used for PCR amplification of amelogenin gene[1, 11] (Table 2). The PCR reaction mix was prepared in 20 µL which consisted of 1 µL of 10 pmol of each forward and reverse primer (Sigma, USA), 0.32 µL of 10 mM dNTPs (Bioline, USA Inc), 1X PCR buffer (Bioline, USA Inc), 2 µL of 25 mM $MgCl_2$ (Bioline, USA Inc), 1 U of Taq DNA polymerase (Bioline, USA Inc) and added with ddH_2O to make up to 20 µL of total reaction mixture. The external and internal amplifications have the same components in the PCR mix, with difference in volume of DNA template. The external PCR mix contained 2 µL of template while internal PCR mix contained 10 µL of DNA templates. The PCR applied for external and internal amplifications was as follows: 95°C for 7 min followed by 40 cycles of 95°C for 1 min; 56°C /58°C /60°C /66°C for 30 s; 72°C

for 45 s and the final elongation at 72°C for 7 min. For monitoring of contaminations, negative (without DNA) and positive (male and female DNA) controls were simultaneously amplified with samples.

Then, all amplified products were analysed by using 2% agarose gel and pre-stained with ethidium bromide solution (10 mg/mL). A total of 5 µL of PCR products was mixed with 2 µL Orange G dye and loaded for analysis. The electrophoresis was performed at 100 V for 1 h 30 min.

Results and Discussion

Genomic DNA Extraction

The analysis of extracted genomic DNA showed clear bands of high molecular weight DNA which were visualised in 6 out of 17 samples, with concentration that ranged from 27.3 ng/µL to 130.6 ng/µL after quantitating by using NanoDrop spectrophotometer. Other samples were undetected due to low concentration of DNA extracted and was supported by quantitation results which showed lower value of nucleic acid concentration. The purity value of $A_{260/280}$ reading ranged from 1.30 to 1.88, which indicated slightly lower than pure DNA

Table 1. Teeth sample information with respective exposure temperature and duration.

Sample ID	FDI Notation	Temperature (°C)	Duration (min)
A1	18	100	2
A2	13	200	2
A3	17	300	2
A4	47	400	2
A5	37	500	2
B1	12	100	4
B2	45	100	6
B3	23	100	8
B4	13	100	10
C1	17	200	4
C2	36	200	6
C3	26	200	8
C4	38	200	10
D1	25	300	4
D2	47	300	6
D3	37	300	8
D4	38	300	10

Table 2. Details of primer sequences.

Locus	Amplification	Primer sequences (5' – 3')	PCR products (bp)
AMEL	External	Forward (AMEF) ACC ACC TCA TCC TGG GCA C	281 (X) and 287 (Y)
		Reverse (AMER) TTA CGG CCA TAT TTA GGA	
	Internal	Forward (AMIF) ACC TCA TCC TGG GCA CCC TGG	212 (X) and 218 (Y)
		Reverse (AMIR) AGG CTT GAG GCC AAC CAT CAG	
SRY	External	Forward (SRYEF) CGG AGA AGC TCT TCC TTC CT	198
		Reverse (SRYER) CAG CTG CTT GCT GAT CTC TG	
	Internal	Forward (SRYIF) AGG CAA CGT CCA GGA TAG AG	85
		Reverse (SRYIR) TTT CGC ATT CTG GGA TTC TC	

Recommendation, although a few samples were within the suggested value. This may suggest that the phenol-chloroform method is still reliable and can produce sufficient DNA from the burnt and degraded tooth samples. As stated by previous studies, the DNA yielded from tooth can be affected by condition of tooth and chronological age of individual[12, 13]. Chronological age has positive effect on DNA yield because mature teeth are more mineralised and less porous, contributing to higher resistance to decomposition and more protection for endogenous DNA[13].

Comparison of Sex from DNA Typing and Known Sex

The results of PCR amplification were compared with the known sex of the tooth owner to verify sex of the teeth sample. Amplification products of single band indicated a female origin sample while double bands showed a male origin sample when amplified with AMEL primers. It was found that only seven samples were successfully amplified with AMEL external primer due to the degraded DNA condition. Second amplification by using AMEL internal primer showed that most samples were typed because smaller product size may increase the chances of amplification. Result showed that the accuracy of amelogenin typing was 76.47%. Application of SRY primers could distinguish male from female samples since the amplification only detect male samples. The accuracy of sex determination by using SRY primer was found to be higher than AMEL primer, with 88.24%accuracy. Therefore, it proved that the SRY gene is a suitable alternative marker for sex identification in situation when an individual cannot be resolved through AMEL gene.

Sensitivity of both AMEL and SRY genes were increased when nested PCR was applied (Table 3). A previously reported study showed that a negative result via classical PCR can be solved through nested PCR technique[11]. In addition, SRY marker showed higher sensitivity as compared to AMEL marker, which indicated a better sex marker. A study by Morikawa et al.[5] showed all samples were accurately typed by using

SRY gene marker, including AMELY-deleted male samples. Another research found that with the SRY gene in the pulp, 100 percent of the specificity was noticed, indicating that this gene may be a confirmatory test for male gender identity[14].

Discrepancy Upon Comparison

The findings showed that three male samples of AMELY gene had failed to be identified. However, typing was successful when SRY gene was used. This could be samples of those who were AMELY-deleted individuals, which required further verification by using an alternative sex marker for identification. Therefore, the situation of mistyped could happen if one sex marker was used rather than two sex markers. It was reported that the frequency of the AMELY-deleted amongst race of Malaysian population was 3.2%[6]. However, this study cannot be further investigated as the information on race of tooth owner was not collected. According to Shadrach et al.[15], lack amplification of AMELY product can be caused by mutation at the primer binding site in the amelogenin gene. A study showed that five out of six samples have primer site mutation, which was not amplified by amelogenin primer but amplified when Y-STR kit was used[16].

In this study, three out of ten male samples were identified as female, based on the amplification product of SRY external primer, but was corrected with internal amplification product. This shows that a false negative result can be obtained if internal positive control was not employed in amplifying SRY marker. Since presence of inhibitor or failure of amplification in the sample can also cause negative amplification, it cannot accurately define a sample with negative amplification which belong to a female sample. A study by Morikawa et al.[5] stated that the SRY primers were designed to amplify SRY marker on Y chromosome.

Table 3. Sensitivity results of each primer pair

Primer pair	Sensitivity (%)
AMEF-AMER	6.67
AME2F-AME2R	63.64
SRYEF-SRYER	70.00
SRYEIF-SRYIR	100.00

and two other homologous sequences in chromosome 7 and X chromosome which acted as internal positive controls by producing products of different lengths. This method found to eliminate the possibility of false negative while employing the SRY marker.

The absence of PCR product may suggest the possibility of PCR inhibitor in samples such as collagen and calcium in a great quantity, especially in tooth samples[17]. The incomplete removal of these PCR inhibitors during DNA extraction was found as one of the factors that contributed to false negative, which could interfere with the amplification process. Apart from that, the condition of highly degraded DNA as well as fragmented into small size can also cause false negative amplification. A study has shown that the highly fragmented DNA template was difficult to be amplified for product longer than 150 bp[18].

Conclusion

In conclusion, it was proven that genetic material can be typed by using sex markers AMEL and SRY from samples subjected to burning at 300 °C for 10 min. Apart from that, the nested PCR is found to be a useful technique in amplifying a minute and degraded genetic material of teeth samples. The findings also showed that the sex markers with a shorter length of PCR products could be considered when amplifying a compromised sample, especially from burnt samples.

Application of SRY gene to confirm the sex of individual can be proposed as alternative sex marker to avoid misinterpretation in situation when the amelogenin gene failed to discriminate an individual to provide a more conclusive result in sex determination.

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Structural Equation Modeling of Health Literacy, Self-Efficacy, Physical Activity, Dietary Pattern and Body Image in Obese Young Adults in Tomohon City

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Abstract

The prevalence of obesity is at a dangerous level. This situation is influenced by the extreme value of Tomohon city. This study aims to create Structural Equation Modeling of health literacy, self-efficacy, physical activity, dietary pattern and body image in obese young adults in Tomohon City and to analyze the influence between health literacy, self-efficacy, dietary pattern, physical activity and body image in obese young adults in Tomohon City. This research is a non-experimental research with a quantitative approach. Sample were 161 adults, aged 20-30 years, and had a total BMI ≥25. Data analysis was carried out by testing the structural equation modeling using Smart PLS. The results showed that structural equation modally of health literacy, self-efficacy, physical activity, dietary pattern and body image in this research can be used to show how strong the effect or influence of one variable was on another variable. Based on the equation modeling, several variables have a weak and strong influence so that the effect between variables also has different percentages.

Keywords: *structural equation modeling, obese, health literacy, self-efficacy, physical activity, dietary pattern, body image*

Background

In Indonesia, the prevalence of obesity is at a dangerous level. There was a two-fold increase from 10.5% in 2007 to 21.8% in 2018. This situation is influenced by the extreme value of Tomohon city; 33% in 2013 and around 40% in 2018. Campaigns to reduce the prevalence of obesity, such as Clean and Healthy Living Behavior (*Perilaku Hidup Bersih dan Sehat*) and Healthy Lifestyle Movement (*Gerakan Masyarakat Hidup Sehat*) carried out during the last decade have proven unable to prevent its increase[1,2]

If the aforementioned situation is not treated promptly, the number of sufferers of endocrine system disorders is likely to continue to rise to 77%, followed by an increase in the number of people with cardiovascular, gastrointestinal diseases, liver and kidney disorders as well as various types of cancer to mental problems. The combination of those issues will dominate global health financing and burden the state budget and spending by up to 20% annually (over US $ 2 trillion). It lowers the productivity of the population by up to $ 2000 US/capita, reduces life expectancy by 10 years, and increases the mortality ratio by 7.7%[3-6]

Furthermore, young adults are an important transition period in shaping long-term health behavior patterns that can affect a person's health status in the future. Failure to establish a healthy lifestyle that has become a habit during this period can lead to serious chronic health problems. Young adults who have hypertension and early T2DM are more likely to have long-term risks for poor blood pressure and glycemic control. They are also at greater risk for developing diabetes complications[7]

An initial step of public health efforts in controlling obesity in this age range is to carry out Structural

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Equation Modeling (SEM) to identify pathways of risk factors for the incidence of obesity in young adults such as individual characteristics including demographic and socioeconomic status in a region, dietary pattern, physical activity, self-efficacy. and body image. This study aims to create Structural Equation Modeling of health literacy, self-efficacy, physical activity, dietary pattern and body image in obese young adults in Tomohon City and to analyze the influence between those variables in obese young adults in Tomohon City.

Methods

This research is a non-experimental research with a quantitative approach. The research design used was descriptive correlational analytic with cross-sectional approach. It was conducted in Tomohon City, North Sulawesi Province. Sample were 161 adults aged 20-30 years and had a total BMI ≥25.

Variables in this study were obtained by:

1. Health literacy utilized food labels assessed by the Newest Vital Sign (NVS)

2. Self-efficacy was measured by the Weight Efficacy Lifestyle Questionnaire (WEL)

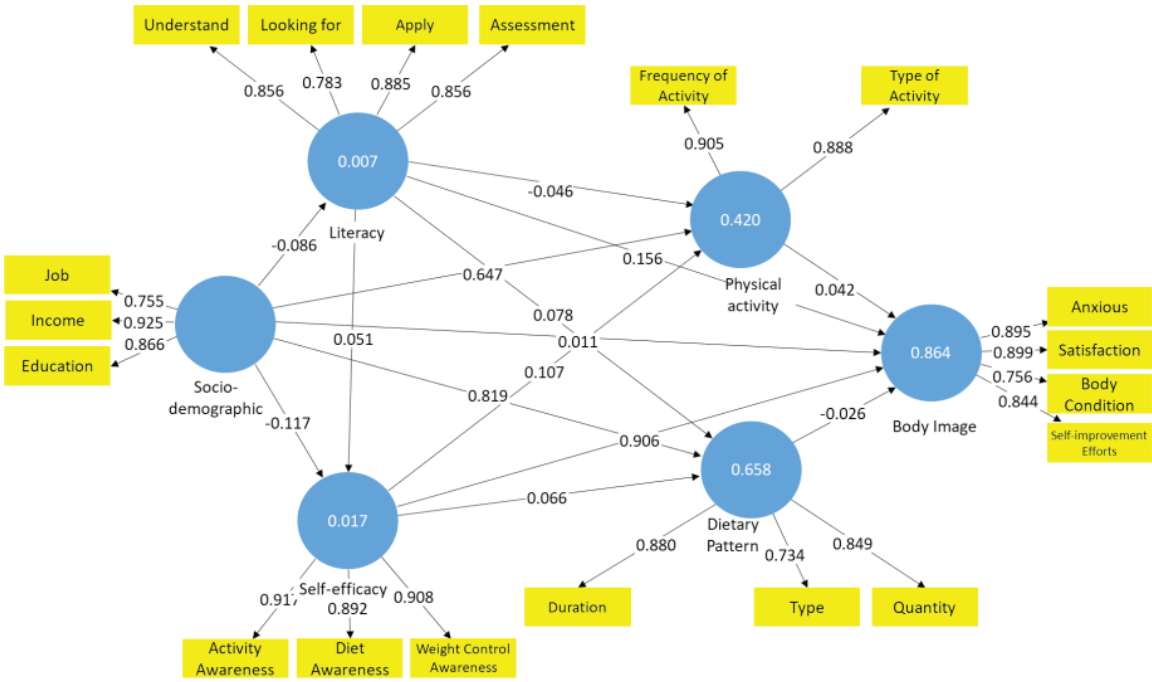
3. Body Image was measured by evaluating an individual's assessment of their appearance

4. Physical activity measured included intensity, frequency, amount of physical activity performed in 1 week and its duration.

5. Measured dietary pattern measured by Food Frequency Questionnaire (FFQ)

Data analysis was carried out by testing the structural equation modeling using Smart PLS. More importantly, Path Coefficient test was used to see the strength of the influence of each variable. Since this study utilized the Concurrent Transformative Strategy combination approach, data merging could be done by combining with the same weight (merging), connecting or mixing with unequal weights (embedding).

Results



Based on the PLS Algorithm, here is a model to be used in this research:

Figure 1 Structural Equation Model

After obtaining a fit model, the next step was to evaluate the inner model through the path coefficients test. It was used to show how strong the effect or influence of one variable was on another variable. Based on the test, variable with the greatest effect was socio-demographic on dietary pattern (0.819 or 81.9%).

Table 1 The Indirect Influence Between Variables

	Sample (O)	P-values
Physical activity à Body image	0.042	0.300
Self-efficacy à Physical activity	0.107	0.060
Self-efficacy à Body image	0.906	0.000
Self-efficacy à Dietary pattern	0.066	0.187
Literacy à Physical activity	-0.046	0.513
Literacy à Body image	0.156	0.000
Literacy à Self-efficacy	0.051	0.535
Literacy à Dietary pattern	0.078	0.124
Dietary pattern à Body image	-0.026	0.646
Socio-demographic à Physical activity	0.647	0.000
Socio-demographic à Body image	0.011	0.844
Socio-demographic à Self-efficacy	-0.117	0.142
Socio-demographic à Literacy	-0.086	0.291
Socio-demographic à Dietary pattern	0.819	0.000

Based on Table 1, it can be seen that:

1. Physical activity does not have significant relationship with body image (p = 0.300), while the effect of physical activity on body image is 4.2%.
2. Self-efficacy does not have significant relationship with physical activity (p = 0.060), while the effect of self-efficacy on physical activity is 10.7%.
3. Self-efficacy has significant relationship with body image (p = 0.000), while the effect of self-efficacy on body image is 90.6%.
4. Self-efficacy does not have significant relationship with dietary pattern (p = 0.187), while the effect of self-efficacy on dietary pattern is 6.6%.
5. Literacy does not have significant relationship with physical activity (p = 0.513), while the effect of literacy on physical activity is 4.6% with a negative direction.
6. Literacy has significant relationship with body image (p = 0.000), while the effect of literacy on

body image is 15.6%.

7. Literacy does not have a significant relationship with self-efficacy (p = 0.535), while the effect of literacy on self-efficacy is 5.1%.
8. Literacy does not have significant relationship with dietary pattern (p = 0.124), while the effect of literacy on dietary pattern is 7.8%.
9. Dietary pattern does not have significant relationship with body image (p = 0.646), while the effect of dietary pattern on body image is 2.6% with a negative direction.
10. Socio-demographic has significant relationship with physical activity (p = 0.000), while the effect of socio-demographic on physical activity is 64.7%.
11. Socio-demographic does not have a significant relationship with body image (p = 0.844), while the effect of socio-demographic on body image is 1.1%.
12. Socio-demographic does not have a significant relationship with self-efficacy (p = 0.142), while the effect of socio-demographic on self-efficacy is 11.7% with negative direction.
13. Socio-demographic does not have a significant relationship with literacy (p = 0.291), while the effect of socio-demographic on literacy is 8.6% with negative direction.
14. Socio-demographic has a significant relationship with dietary pattern (p = 0.000), while the effect of socio-demographic on diet is 81.9%.

Discussion

Physical activity does not have significant relationship with body image, while the effect of physical activity on body image is 4.2%. It is in line with a research conducted by Ferrari et al. on university students in Brazil and Rech et al. on students in Parana, Portugal[8,9]. Moreover, nutritional status is influenced by eating behavior, while eating behavior is influenced by opinions about the importance of health[10].

Self-efficacy does not have significant relationship with physical activity, while the effect of self-efficacy on physical activity is 10.7%. The impact of self-efficacy on a person's cognitive processes differs from one another. Most of behaviors change according to thinking to achieve goals. The higher a person sets goals or challenges, the more confident he/she will keep his/her commitments[11]. It is supported by a research by Anggraeni which found that self-efficacy has a significant relationship with physical activity[12].

Additionally, self-efficacy has significant relationship with body image, while the effect of self-efficacy on body image is 90.6%. Self-efficacy basically can determine how people feel, think, motivate themselves and behave, solve problems, or in the process of adjusting to stress. A person requires confidence in his/her own abilities since it will determine the action taken and the final result[13,14].

Self-efficacy does not have significant relationship with dietary pattern, while the effect of self-efficacy on dietary pattern is 6.6%. Strong belief in the ability to perform particular behavior increases the possibility that the behavior can be achieved[15]. This is related to the motivation to realize what is believed. The most important thing an individual must have in order to carry out healthy behavior is self-efficacy[16]. In contrast to the results of this study, self-efficacy does not have a significant effect on dietary pattern. Factors that generally influence the formation of dietary pattern include economic, socio-cultural, religious, educational and environmental factors[17]

Literacy does not have significant relationship with physical activity, while the effect of literacy on physical activity is 4.6% with a negative direction. The results of this study are in line with a research conducted by Rohmah et al. that also found that there is no significant relationship between literacy and physical activity[18]

Literacy has a significant relationship with body image, while the effect of literacy on body image is 15.6%. Literacy is related to what someone sees and hears, so that it can create perceptions about the subject of the information he/she sees. One of types of literacy sources that is also popular among women is mass media. Mass media and pictures in fashion magazines have an important effect on both teenage girls and adult women in perceiving their weight and body shape by making their own ideal beauty criteria[19,20]

Literacy does not have significant relationship with self-efficacy, while the effect of literacy on self-efficacy is 5.1%. Health literacy is important for every individual since it relates to the ability to obtain health information as an attempt to improve and maintain their

health. In general, health literacy is said to increase health knowledge and assist individuals/communities in making informed decisions about their health[21]. High level of health literacy skills enable a person to act independently in overcoming personal, structural, social and economic barriers[22-24]. Thus, people's skills in acquiring and applying health-related knowledge can have a significant effect on individual well-being[25].

Literacy does not have significant relationship with dietary pattern, while the effect of literacy on dietary pattern is 7.8%. Many factors can affect dietary pattern, such as economic, socio-cultural, religious, educational and environmental factors[17] . Thus, someone with a low level of literacy does not always indicate that he/she has a bad diet, and vice versa.

Dietary pattern does not have a significant relationship with body image, while the effect of dietary pattern on body image is 2.6% with a negative direction. Dietary pattern is a description of information about the consumption of various amounts of ingredients and types of food in every day and is a characteristic of certain groups of people[26]. Body image can be influenced by dietary pattern, but the results of this study indicate the opposite, which explains that there is no significant relationship between dietary pattern and body image.

Socio-demographic has a significant relationship with physical activity, while its effect on physical activity is 64.7%. Socio-demographic is needed since people and the environment interact with each other in which humans can act as subjects and objects. In this case, the number of humans will increase and environmental conditions tend to decrease[18]. In this case, socio-demographic factors play a role in the implementation of a person's physical activity pattern considering that it is attached to each individual and is carried on in daily activities.

Socio-demographic does not have a significant relationship with body image, while its effect on body image is 1.1%. Body image is the result of a reciprocal relationship between events in environment, cognitive, affective, physical processes, and individual behavior[27].

Socio-demographic does not have a significant relationship with self-efficacy, while its effect on self-efficacy is 11.7% with negative direction. The formation of perceptions in a person occurs along with the processes experienced during life, while undergoing certain experiences, seeing, hearing and feeling something. When someone has gone through these experiences, there is a tendency to form a perception that is believed, both towards that object and towards oneself.

Socio-demographic does not have a significant relationship with literacy, while its effect on literacy is 8.6% with a negative direction. Health information can be obtained from anywhere since literacy sources come in various forms, such as printed media, electronic media, and even direct delivery, so that access to health information is not only limited in terms of employment and economic factors.

Socio-demography has a significant relationship with dietary pattern, while its effect on dietary pattern is 81.9%. Many socio-demographic indicators can influence dietary pattern. Economic variable determines food purchasing power, therefore, the less income, the lower the quality and quantity of food consumed by the community. The abstinence from consuming types of food can be influenced by social cultural factors in local customs beliefs which become habits.

Conclusion

Based on the equation modeling, several variables have a weak and strong relationship so that the effect between variables also has different percentages. Thus, it can be concluded that efforts to reduce obesity in Tomohon City can be done by prioritizing variables with greater percentage of effect, including socio-demographic on dietary pattern.

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Reflections on the Community Empowerment in Giving Exclusive Breastfeeding

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Abstract

Background: Less than 40 percent of children under the age of six months were given exclusive breastfeeding (WHO, 2015). Therefore, this study will fundamentally analyze qualitatively the community empowerment model related to exclusive breastfeeding so that the target of achieving exclusive breastfeeding can be achieved progressively and effectively. **Method:** The type of research used was qualitative research The descriptive explorative approach was chosen in accordance with the research objectives of exploring the perspectives, experiences, and expectations of breastfeeding mothers and related parties. The study was conducted in five community health center in Surabaya. Data collection was carried out through indepth interview with breastfeeding mothers. Total respondents was 50 people. The process of data analysis used the nine-step data interpretation method according to Colluizi. **Result:** there were 7 themes and 24 categories. The theme were: miss perception, benefits of breastmilk, support, obstacles, myth, cureent prohram, and expectation. **Discussion:** Good cooperation between family, government, and institutions where mothers work is needed to achieve exclusive breastfeeding. in addition, massive exclusive breastmilk education through social media and advertising is an urgent need.

Key words*: Community, Empowerment, Exclusive Breastfeeding*

Introduction

Breastfeeding is a mother's behavior that brings many benefits for both mother and baby. Breast milk contains protective substances that can prevent babies from various infectious diseases. Nurmiati and Besral's research results (2008) show that the duration of breastfeeding greatly affects the survival of infants in Indonesia. Infants who are breastfed with a duration of 6 months or more have a survival rate of 33.3 times greater than those who are breastfed for less than 4 months and infants who are breastfed for 4-6 months have a survival rate of 2.6 times better than those who are breastfed for less than 4 month [1]. In addition, Roesli (2007) states that breastfeeding at least up to 6 months reduces the possibility of mothers suffering from breast cancer, uterine cancer, ovarian cancer [2]. However, the behavior of exclusive breastfeeding in Indonesia, including in East Java, has not met expectations.

One of the Sustainable Development Goals' targets by 2030 is to reduce neonatal mortality by at least 12 per 1,000 live births and deaths for children under 5 years of age at least 25 per 1,000 live births. This can be achieved by exclusive breastfeeding. Meanwhile, coverage of exclusive breastfeeding in Central Africa was 25%, Latin America and the Caribbean 32%, East Asia 30%, South Asia 47%, and developing countries 46%. Overall, less than 40 percent of children under the age of six months were given exclusive breastfeeding (WHO, 2015). The Ministry of Health Data and Information Center 2017 shows that exclusive breastfeeding in Indonesia is only 35%. In addition, the 2017 Indonesian Health Profile shows that babies who have been exclusively breastfed until the age of six months are 29.5%. Coverage of exclusive breastfeeding in East Java in 2017 was 41.17% (pusdatin, 2017).

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Meanwhile, the low coverage of breastfeeding is driven by the implementation of inadequate regulations even though the rules regarding exclusive breastfeeding have been clearly stated in Law Number 36 of 2009 concerning Health and Government Regulation Number 33 of 2012 concerning Exclusive Breastfeeding. Both of these rules stipulate the obligation to provide exclusive breastfeeding for six months supported by the procurement of lactation facilities in various places. In addition, there is a need for government efforts, cadres of the Maternal and Child Health (MCH), and breastmilk support groups in achieving the national exclusive breastmilk target. Efforts that can be pursued within the scope of community health are community empowerment. Therefore, this study will fundamentally analyze qualitatively the community empowerment model related to exclusive breastfeeding.

Method

The type of research used is qualitative research. The researcher makes a complex picture, examines the words of the detailed report from the viewpoint of the respondent, and conducts a study of the situation experienced (Creswell, 1998: 15). The descriptive explorative approach was chosen in accordance with the research objectives of exploring the perspectives, experiences, and expectations of breastfeeding mothers and related parties towards the community empowerment model.

The study was conducted in five community health center representing East, West, South, North and Central Surabaya. Data collection was carried out through indepth interview with breastfeeding mothers. Total respondents were 55 people.

The process of data analysis used the nine-step data interpretation method according to Colluizi [3] :1) Describe the phenomenon under study, 2) Collect a description of the phenomenon through the opinions of participants, 3) Read the entire description of phenomena that have been submitted by participants, 4) Reread the interview transcript and quote meaningful statements, 5) Describe the meaning contained in the statements significant statement, 6) Organizing a collection of meanings that are formulated into groups of themes, 7) writes a complete description, 8) meets the informant to validate the description of the analysis results, 9) Combines validation data into the description of the results of the analysis.

Results

The results of this study revealed several important things related to models and community empowerment in exclusive breastfeeding. In general, the practice of exclusive breastfeeding experiences many obstacles due to misunderstanding in the community, external factors of mothers that influence decision making to breastfeed, husband's support that is not optimal, exclusive breastfeeding education in the wider community, which is still not massive, and the obstacles encountered by working mothers when they have to leave their children at home.This research got 7 themes and 24 categories.

Some participants perceive that exclusive breastfeeding is milk directly from the mother without understanding the duration of administration. There was a category for reference for giving. In addition, there were four categories presented by participants regarding the benefits of breastmilk that they understand, namely: immunity, intelligence, economics, and emotional attachment. The support felt by the mother came from her husband, parents, and colleagues. Furthermore, the obstacles felt by mother were: role conflict, breast condition, difference perception, and work condition. The current programs were health education and smart mothers class. participants have expectations regarding to succesful breastfeeding, such as: TV commercials, lectures by religious leaders, support for personal devices, include an educational curriculum, and husband's attention in breastfeeding.

Discussion

Inaccurate knowledge regarding the limits of exclusive breastfeeding was found in some of the participants of this study. They understood that exclusive breastfeeding was breast milk given to infants without understanding its duration. So, as long as the mother gives breastmilk even though it is not for six months, it is considered exclusive breastfeeding. This is in line with Wulan's research (2019) that the mother's knowledge is related to her breastfeeding behavior. The mother's opinion about breastfeeding will correlate with the fulfillment of the baby's nutritional needs and nutritional status. Mothers with low knowledge about breast milk

cannot meet the nutritional needs of a balanced baby, one of which is the need for exclusive breastfeeding[4].

In the other hand, some mothers believe that breastfeeding can increase baby immunity, intelligence, and activity. Furthermore, mothers believe that through the process of breastfeeding, emotional closeness will be built between mother and baby. Another advantage that participants feel is economic issues. They revealed that breastfeeding babies was very helpful for the family's economy because the parents did not need to give formula milk. The results of this study related to children's intelligence were strengthened by research by Kathleen et al, who found that at the age of 5 years, children who were exclusively breastfed had the highest verbal intelligence score while children who were not exclusively breastfed had the lowest score [5]. This was confirmed by Deoni et al's study that exclusive breastfeeding for at least 3 months was associated with increased diffuse myelination in all parts of the brain at 2 years of age, including the brain regions associated with various cognitive and behavioral skills. In addition, there was an increase in overall cognitive abilities and levels of cognitive development, including verbal and non-verbal functioning in children who were breastfed compared to children who received only formula milk[6].

Some mothers also conveyed the economic benefits their families get by giving breastmilk. They feel more economical because they do not have to spend on milk for their children. This is in line with the results of research by Amistu Kuma 92015) which found that increasing the proportion of children who were breastfed would have saved an estimated $3.6 billion annually interms of both direct and indirect costs [7]. Globally, costly diversion occurs due to not breastfeeding. According to the research by Dylan Walters et al (2016) who exploring the cost of not breastfeeding in Southeast Asia, an additional contributor to economic losses is a decrease in cognitive abilities. Estimates arrive at a significant 0.5% loss of gross national income in Thailand [8].

Participants feel the impact of breastfeeding related to emotional closeness with children. Mothers feel that their children are calmer and more comfortable during the breastfeeding process. This is in accordance with the results of the study by Henrik Norlhot (2020) which shows that the effect of breastfeeding makes the bond between mother and child more qualified and social emotional development was better [9]. The results of Benjamin G. Gibset al (2018) 's research on infant attachment behavior points found that find that breastfed children are rated as having slightly higher scores on two measures ("warm and cuddly," "cooperative") and lower scores on one measure (" demanding / angry ") [10]. Babies need a sense of security and comfort to develop their social skills in the future so that good emotional closeness through the process of breastfeeding will help children fulfill their social competences.

The support that mothers feel during the breastfeeding process comes from their husbands, parents, and colleagues. This was inline with Research by Kris Yuet Wan Lok et al (2017) showed that the husband's preference for breastfeeding (aOR = 1.67; 95% CI 1.20, 2.31) were associated with significantly higher odds of intention to exclusively breastfeed [11]. It was also confirm by research by Shinta Krsitianti et al (2017) positive feedback and social support will likely encourage respondents to provide exclusive breastfeeding[12]. In addition, 73% of mothers planned to breastfeed If family and friends recommended breastfeeding [13].

Mother's occupation factor often made it difficult for some participants to continue providing exclusive breastfeeding. Almost all working mothers leave their children with their parents, either biological mothers or in-laws when they return to work. Meanwhile, the mother's parents suggested giving additional formula milk for reasons of nutritional adequacy. On the other hand, the facility of workplace to support breastfeeding was insufficient. Study from Ray Wagiu Basrowi et al (2018) revealed that the breastfeeding decreased significantly when mother return to work and the reason because the mother felt the production of their breastmilk decreased during the distance with their babies. In addition, there was a lack of facility in workplace to support breastfeeding[14]. This study also in line with Sylvia et al (2020) that found the working mother felt guilty and worry when they return to work because they think that the breastmilk would be not sufficient for their baby[15]. Meanwhile, maternal anxiety can have an impact on reducing breast milk production.

Another obstacle that mothers feel is differences of opinion with their parents or in-laws. Parents or parents-in-law believe that if a baby cries it means that he is thirsty and when the baby's mother tries to give breastmilk and the baby continues to cry, the grand mother will suggest giving additional formula milk because they feel that the breastmilk cannot fulfill the baby's needs. This was also found in the study of Christiana Nsiah-Asamoah et al (2020). The misconceptions revealed included beliefs that breastmilk is watery in nature and does not satisfy infants. In addition, there is also the provision of corn flour mixed with water or fine porridge for consumption by babies in the early days of life[16]. The similar perception felt by research's participant that show the perception that foods and other liquids were more nutritious than breast milk [17]. Breast condition also became an obstacles among the mothers. The primipara had difficulties to support their babies latched on easily during direct breastfeeding. The mothers showed that it was because of the shape and size of their nipples. So, it made the nipples sore and the breasts swollen because of the engorgement. This condition similar to the research by Jennifer S. C, et al (2019) that revealed the issues about obstacels in breastfeeding process related to breast condition were latch on process, pain, and insufficient breastmilk[18].

Some partisipants had expectation about lecturer from religious leaders about breastfeeding. They believe that it could be increase the positive perception of community and improve breastfeeding support. It was inline with the research by Rekawati et al (2017) showed information that the attitude and behaviour of public figure who support exclusive breastfeeding make easier transactional communication and affect the surrounding community because they are the role of society[19]. Indonesian society which is predominantly Muslim has a tendency to follow recommendations from religious leaders. Another hope of the participants is the massive advertisement for exclusive breastfeeding in the media. The results of research by Tuan.T Nyuyen , MD et al (2017) who studied the Television Campaign with Exclusive Breastfeeding showed excellent results related to exposure to breastfeeding campaigns through television media with exclusive breastfeeding [20]. In addition, the other research show that mother who saw breastfeeding ads on television and heard about breastfeeding on the radio had a positive perception about breastfeed their children for longterm health with the percentage 94% dan 100% respectively compare to the others[13].

Some participants complained that they felt pressured by the myths that were believed by their families regarding breastfeeding. They were annoyed by the illogical rules imposed by their parents or extended family. The prohibition to take a nap leaves mothers feeling exhausted from lack of rest after staying up all night to look after the baby. In addition, the prohibition of consuming certain types of food makes them less appetizing for food. This condition same as the research by Istiyaroh (2018) that showed the breastfeeding mothers who followed the myth related to the failure of breastfeeding[21].

Conclussion

Breastfeeding mothers need support from various parties in the community. The support expected by the mother comes from her husband and parents or in-laws. In addition, support from religious leaders is something that needs to be improved. Working conditions are a challenge in itself to continue providing exclusive breastfeeding for children. In addition, myths that are less favorable for breastfeeding mothers need special attention. Therefore, education related to breastfeeding needs to be given massively through electronic media and social media.

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Dandelion Effect on Broiler Representation

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Abstract

The aim of this study was to evaluate the dandelion as a source of probiotic. 300 day old chicks (Hubbard type) were chosen for this experiment. The results showed significant effect (p=0.05) for chicks fed with different levels of dandelion. 1.00% level shows the highest weight gain, not effects on eatable incentive in spite of there is found simple calculated of treatment significant effect (p=0.05). The study concluded that using dandelion fed as additive at 1.00% promote the performance of the whole chicks during the breaded chicks.

Key words: *Dandelion, Prebiotic broiler representation.*

Introduction

Dandelion is one of a nutritional herbs which is contain many important elements, particularly, K, Mn, Cu, Cl, Se, Ca, B, S and silicon according to [1] as well as vitamins. [2] found that Dandelion leaves is very rich in protein, fiber and linoleic acid and this lead to think that this plant could be use as food source.

The laboratory analysis of this plant yield that it contain terpenoids flavonoid glycosides and many phenolic compounds as well as sugar and mucilage [3].

[4] discovered that the leaves have more diuretic effect comparing to the root, with high influence comparable to that of furosemide , without causing potassium less because the leave content of high potassium.

Significant OH-radical removal activity was found by [5] from plant flower extracts, roots and stem.

Prebiotic like Inulin and oligofructose are not natural carbohydrates in banana, dandelion, chicory and others.

Normally all of these carbohydrates are completely fermented by intestinal microflora. [6] pointed out that one of the most important properties of inulin and oligofructose have influence on the growth of bacteria which founded in the intestine like bifidobacterial, lactobacilli and some of butyrate-bacteria. [7] observed similar phenomena in poultry gut flora.

Oligofructose and inulin have been shown their influence on body improvement, feed conversion and carcass weight and carcass percentage of female broiler [8]. Therefore, Dandelion could be used as a main source of inulin. Also (Taraxacum officinal) could be used as a source of prebiotic [13].

Materials and Methods

All experiments trail were carried at private poultry production field by Baghdad (Iraq). The sample (300 Hubbard chicks) was separated into 5 groups. Each group was then divided into two replicates. Dandelion was added as follows: 0.0%, 0.25%, 0.5%, 0.75% and 1.00%.

Ground pens (1.5 x 1.5 meter) were used to raise the chicks with mulch of wood shavings. The feeding program was divided for two levels, starter and finisher, for 21 and 42 ages respectively.

The same ingredients for each patch were mixed for each period. Based on National Research Council [9], all mixes are formulated to meet the nutritional requirements. The feed and water were provided *ad ibtum*.

Table one is reflects the constituents ration and its calculating feeding values.

Vaccines were given to all chicks against many common infectious diseases like Newcastle and bronchitis. Chicks weight gaining was measured during the feeding period, as well as feeding intake. 5 chicks were randomly chosen from each replicate to register the weight body gaining as live body weight (LBW).

Sharp knife was used to kill all chicks and picked off the feathers. All organs were weight separately as part of the carcass and also the whole bird and calculated as percentage. All data were subject to statistical analysis using the general linear model procedure of [10] because multiple test was used to expose the differences ($p<0.05$) through different group means.

Table 1: Feed mixes formulation in percentage used for the two rations.

feed materials	The initiator ration (1-21 day) %	The growth ration (22-35 day) %
Maize	548.20	58.70
Whole wheat	80	7.50
Vegetable protein (44% protein) Argentine	128.50	20.50
Proteins Center*	100	100
Vegetable oil	40	2.50
Limestone	10	0.50
Sodium Chloride	0.30	0.30
Total yield	1000%	1000%
	Chemical analysis (Theoretical)	
Energy (kCal / kg)	30790	3102.60
Cruder protein (%)	22.060	19.370
Lysiner (%)	1.210	1.030
Methionine + Cicin (%)	0.820	0.750
Raw fiber (%)	3.540	3.20
Calcium (%)	1.20	0.950
Phosphorus Ready (%)	0.440	0.420

*A Belgian protein center established according to [9] requirements.

Results and Discussion

Table (2) shows the results of the dandelion percentage used on the growth response (increasing of body weight), mixes conversion and conversion ratio of birds. Its clear from this experiment the significant effects ($p<0.05$) for chicks fed with different level of dandelion specifically at level of 1.00% which recorded the highest weight gained compared to zero dandelion. Significant increasing in the body weight may be associated with beneficial bacteria, Bifidobacterium and Lactobacillus and significantly less *E.coli* in the cecum and small intestine. Similar results was found by [11] who were reported that the main effect of prebiotic is the production of short-chain fatty acids and lactate which make a selective condition for increasing the beneficial bacteria like bifidobacteria and Lactobacillus resulting in an increasing of pathogen resistance as well as calcium and magnesium absorption.

Inulin supplemented by chicks lead to increase the villus height of jejunal and its paralleled increase in the digestion and absorption functions of the intestine due to the surface area absorption [12].

Table (3) shows that different levels of dandelion had no effects on the eatable incentive in spite of there is found simple calculated differ with result increase the percentage of treatment significant effect ($p<0.05$).

[13] found the same result using herbal plants and had no effect on dressing percentage. We conclude from this experiment that using dandelion feed as additive at 1.00% promote the performance of the whole chicks.

Table 2: Different levels of dandelion effects on representation ∓ SD on broiler

Treatments	parameters					
	3 weeks			6 weeks		
	BWG	FC	FCR	BWG	FC	FCR
control T_1	714.8 ∓ 8.7^{b}	$1243.7 \mp 7.4a$	1.74 ∓ 18.7^{bc}	2515.6 ∓ 19.4^{c}	4703.0 ∓ 14.7^{b}	1.87 ∓ 0.06^{c}
Dandelion T_2 0.25%	670.3 ∓ 9.2^{c}	$1159.6 \mp 8.5b$	1.73 ∓ 21.5^{b}	2615.9 ∓ 23.7^{b}	4734.8 ∓ 19.3^{a}	1.81 ∓ 0.06^{b}
Dandelion T_3 0.50%	678.7 ∓ 79^{c}	1175.9 ∓ 11.3	1.71 ∓ 16.3^{b}	2603.8 ∓ 27.4^{b}	4686.8 ∓ 18.7^{b}	1.80 ∓ 0.07^{b}
Dandelion T_4 0.75%	682.7 ∓ 10.2^{c}	$1146.9 \mp 8.5b$	1.68 ∓ 19.6^{a}	2590.7 ∓ 21.4^{b}	4636.1 ∓ 15.9^{c}	1.79 ∓ 0.007^{a}
Dandelion T_5 1.0%	739.8 ∓ 8.4^{a}	$1235.5 \mp 6.7a$	1.67 ∓ 18.5^{a}	2680.7 ∓ 23.1^{a}	4744.8 ∓ 16.7^{b}	1.77 ∓ 0.05^{a}

a, b, c – indicated the existence of significant difference between treatments in the same column

BWG- Body weight gain

FC- feed consumption

FCR- feed conversion ratio

Table 3: Different levels of dandelion effect on weight of eatable invective and dressing proportion ∓ SE On broiler performance.

treatments	traits			
	Eatable invective			
	Heart %	Liver	Gizzard %	Dressing proportion
Dandelion T_1	0.66 ∓ 0.3	2.85 ∓ 0.14^b	3.47 ∓ 0.13^c	73.12 ∓ 1.12^b
Dandelion T_2 0.25%	0.63 ∓ 0.5	2.93 ∓ 0.15^b	3.59 ∓ 0.17^c	73.01 ∓ 0.99^b
Dandelion T_3 0.50%	0.62 ∓ 0.4	2.91 ∓ 0.13^b	3.67 ∓ 0.21^b	74.78 ∓ 0.98^a
Dandelion T_4 0.75%	0.65 ∓ 0.3	3.02 ∓ 0.14^a	3.71 ∓ 0.12^a	73.98 ∓ 1.02^b
Dandelion T_5 1.00%	0.62 ∓ 0.4	2.84 ∓ 0.16^c	3.68 ∓ 0.11^a	74.65 ∓ 0.71^a

a, b, c, indicate the existence of significant difference between treatments in the same column.

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Comparative Study between Two Methods of Management of Thalassemia Patients Have Complicated Spinal Cord Compression

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Abstract

The aim of study to alert the neurosurgeons and ancologests about the significant of the use of radiotherapy in treatment of the thalassemia patients have intricate spinal cord compression. During the period of 15 years, 20 cases which were proved and diagnosed by spine M.R. I and hematological study, plain X. R as cases of intermediate and thalassemia *beta* which was associated with spinal cord compression divided these 20 cases into two groups. The 1st group was 10 cases which was treated by radiotherapy while 2nd group was treated by dorsal laminectomy only 10 cases were operated in Al-kademyia teaching hospital, Al-mosswy private hospital and Al-saady private hospital in Albassrha city and in Al hussian teaching hospital AL-Nasiriyah city. We found there is wide difference between these two types of management in relation to outcome in treatment of the spinal cord compression which is a complication of thalassemia. intermediate & beta. The use of the radiotherapy is indicated the need for minimal dose of radiotherapy which is the method of choice is recommended as usual method of treatment.

Keyword: *thalassemia intermediate, extra modularly, erythropoietin tissues, radiotherapy, paraplegia.*

Introduction

Thalassemia patients which are associated with the neurodificet are extremely rare [(1)]. spinal cord is usually compressed at dorsal region of the spinal cord because of erythropoietin over activity of dorsal spine where the lumen of the dorsal canal of vertebral column, it become narrowing [(2)]. This part of dorsal spinal cord is compressed progressively. so the neurodeficits will be appeared gradually, until decompression will be achieved by laminectomy [(3)]. The neurodeficits might be relived but from the reviews of the previous studies the neurodeficits will be appeared again after surgery when the use of the radio therapeutics techniques used the neurodeficits are disappeared completely with dramatic clinical improvement [(4)]. Extra modularly erythropoiesis (EE) is feature of severe anemia, with erythroblast tissue proliferating in an attempt to increase erythrocyte [(4)]. production in regularly transfused homozygote's severe thalassemia, (EE) is usually not observed but it is common in thalassemia intermediate in rare instances problem may arise, e.g when the (EE) tissue causes compression of spinal cord [(4)]. in these cases, prompt diagnosis and treatment are essential to prevent permanent neurological damage [(1)]. Traditional treatment of peripheral paralysis due to spinal cord compression by EE tissue consists of radiotherapy and regular blood transfusion [(1)]. Another complication which are related to spinal cord compression which results from(EMH), masses like Para paresis and paraplegia urinary – faecal incontinence which were treated by long term period with Hydroxy urea (H.U) which could improve the neurological deficits but the side-effects of the (H.U) and because its use required a long term period [(2)]. When (H.U) has been stopped after few months. The recurrence of the neurological deficits will be relapsed, AT this situation the use of the EBRT is obligatory [(4)].

Materials and Methods

Patient and Method

Beginning in September 1993 to the end of August 2018 ,The patients were divided into two groups ,each group consists of 10 cases ,the 1st group who were scheduled neurosurgical dorsal laminectomy necessitating dorsal laminectomy in order to do proper

decompression, while the 2nd group who were included 10 cases these cases were underwent only minimal dose of EBRT in order to suppress erythropoietin tissue over activity of the dorsal vertebral tissues were eligible for this study which was carried at the neurosurgical hospitals in the Baghdad ,AL-Busrha cities. We excluded the patients who were considered thalassemia minor, other neurodeficits complications apart from paraplegia and Para paresis cases were considered to proper candidate for this study including either Para paresis or recent paraplegic patient who were suffering from thalassemia intermedia. All the patients were male, the average age of both groups were 20 years, the range was between 13 – 30 years The type of treatment was done on the 1st group as dorsal laminectomy, this group of patients consists of 10 patients while 2nd group of patients who were underwent minimal dose of EBRT consists of 10 patients. The patients were operated in deferent theaters, with careful selection of patient was achieved on base of results of the HB-electrophoresis and dorsal spine MRI. When the results suggested that patients had actually Para paresis and paraplegia following well- known patients with thalassemia intermedia or beta, for 1st group, a bolus dose of 1gm of claforan (methotrixem) blues 1gm of Ampeclox which were given I.V 20 – 30 minutes before indication of anesthesia and repeated every one hour. The fellow up was done based on the clinical examination, dorsal spine MRI for both groups. The 2nd group which included 10 cases who were underwent minimal dose of EBRT one session. All those patient of 2nd group got well dramatic response. when the neurological deficits are improved. On fellow up this 2nd group there was no relapse or progressive neurological deterioration while in the 1st group who were treated by surgery as laminectomies, after short term they were suffering from progressive deterioration and relapse of the neurological deficits (Para paresis and paraplegia). We Referred them to the radiotherapy and they got-well dramatic neurological response.

Statistical Analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by chi-square using SPSS ver.19. Significant differences ($P<0.05$) among the parameters

Results and Discussions

The age of the patients two groups were studied the 1st group with an age above 20 years and the 2nd group below 20 years, it was established age-incidence table (1) and table (1) had showed no age deference. The age of the patient no function in outcome of the treatment in both groups. 100 % of cases in the both groups were males in both groups there was significant sex deference in both groups that mean neurodificit which following that thalassemia intermedia beta confined to the mainly Table (2). 75 % of cases were paraparesisas main neurodificat which is associated with those thalassemia intermedia beta patient Table (2). While 25 % of cases were paraplegia which was the least neurological deficit which is following the thalassemia intermedia beta patient. 50 % of cases of spinal while 25% of cases were located in the superior dorsal spine remaining 25 % of cases of the spinal cord compressor were located in the lower dorsal spine Table (2). Radio therapy model of treatment of neurodificet which is related to the thalassemia patient this model of choice of treatment Table (3). MRI study of the thalassemia patient compression is the diagnostic investigation to diagnose EE mass in 100 %, Table (4). Commonest site for EE mass is dorsal region of vertebral column in 100 % Table (5).

Table1: Clinical improving is related to the age (used laminate and EBRT)

	laminate			EBRT		
1st group	**Patient No.**	**%**	**Clinical improving %**	**Patient No.**	**%**	**Clinical improving %**
Above 20 years' old	10	50	100.00	15	25	100
Below 20 years old	10	50	100.00	10	75	100

X^2=0.00 df= 1 p-value= 1.00

Table2: Distribution of the patient is related to the neuroduficit and for the lesion is related to the site

Neuroduficit			site			Neuroduficit		
Cases	**Patient No.**	**%**	**Cases**	**Patient No.**	**%**	**Gender**	**Patient No.**	**%**
Para paresis	15	75	Superior dorsal spin	5	25	Male	20	100
Paraplegia	5	25	Midd dorsal spin	10	50	Female	0	0.00
			Inferior dorsal spine	5	25	Total	20	100
X2=25.00 df= 1 p-value= 0.00			X2=12.50 df= 2 p-value= 0.02			X2=0.00 df= 1 p-value= 1.00		

Table 3: Clinical improvement in relation to the type of model of the treatment

Treatment	Patient No.	Result
Surgical laminectomy	10 -50%	deteriorated
Radiotherapy(DXT)	10 -50%	curable
Surgery and radiotherapy after failure of the surgery	10&10 -100%	curable

X^2=25.00 df= 2 p-value= 0.00

Table 4: Radiological finding

Finding	Patient No.	%
MRI of D.S	20.00	100
Plain XR of D.S	0.00	0.00

X^2=0.00 df= 1 p-value= 1.00

Table 5: Site of the EE mass in relation to vertebral column

Vertebral region	Patient No.	%
Cervical region	0.00	0.00
Dorsal region	20	100
LSS	0.00	0.00

X^2=0.00 df= 2 p-value= 1.00

Discussion

Spinal cord complication which is resulting from thalassemia patient is mainly confined to included, thalassemia intermedia and Bet. This neurological complication is quite rare [1]. The most patient is young adolescent aged group according to our & previous studies [2]. The neurosurgeons saw that use of the radiotherapy in management of thalassemia patient with complication of the spinal cord is most preferable type of treatment in comparison with surgery "laminectomy" In last decades [3]. It seems the use of radiotherapy with or without hydro urea cytotoxic chemical medication lead to decline the adverse effect of this complication and improve the health of the thalassemia patient [4]. Some neurosurgeon said no guideline or common consensus are available in literature regarding the deferent treatment strategies and only single cases have been reported. Spinal cord compression (scc) is an unusual segaela of extra medullary MR imaging revealed long masses of EMH opposite T5-T4 and L5-L2 vertebrae with cord compuessa at T6 vertebrae, the patient who treated by with external beam R.T two weeks. All the patients had symptomatic relief of Para paresis by the 5 fraction and nearly regained, full recovery in lower limbs [4]. The location of EMH mass, usually seen mostly in the dorsal spine. Some cases which had of EMH mass, it had extension down to reach [3]. the lumbosacral region of the vertebral column. In our, series their associated neurodificit is part from Para paresis and paraplegia were very rare while other studies and articles, they mentioned presence of uncommon associated neurodicit e.g urine faccal in contenina, pareslhea numbers deriplleral neuro palhies. It seems in our study has similar style of (R.T) except that the use of hydrox urea (HU) past R.T courier became this cytotoxic medication is not widely available [4]. Most of cases of the (scc) had commonly EMH mass on MRI study. while some series that mentioned they saw multiple masses of EMH on MRI over the dorsal region [5]. The clinical features of Para paresis were present in 15 case 75% which is the most common complaint, the paraplegia were seen in 5 cases 25% all of them had single mass which is variable size and they had variable degree of dorsal canal stenosis. Plain X.R of the dorsal spine lateral A.P views, are negative previous studies showed widening interpetiolar distance [6-8]. The MRI of dorsal spine was the most reliable diagnostic method for EMH masses in all cases there were typical extradural lesion which dentition to the hyper density. Management of (scc)of (EMH) mass was surgery (laminectomies) which were achieved for 10 cases and RT with or without (HU)which were done for 10 cases all of them who were suffering from (scc) we saw that (R.T) preferable than surgery for many reason: -

1- More easier

2- Less cost

3- Less hematological risk

4- Relatively less contra –indications than laminectomies

5- No risky of anaotheoia or surgery

6- Give good results without recurrence or relapsing of signs and symptoms.

Near MR et al showed that blood hyper transfusion as treatment of thalassemia patient with (scc) improvement clinically and radiologically.

Although there are various options in the management treatment of such cases including decompression surgery and radiation treatment of hyper transfusion can be very effective even in severe compression of the spinal cord.

In our study had shown that the comments site is over the dorsal spines in 100% while other series had mentioned that EMH masses involved other regions of the vertebral column like lumbar and sacral regions. The location of spinal cord compression in our study had shown that the meddle dorsal spines are the commonest location 50%, while many studies are consistent with our –study. The previous studies and articles had mentioned that surgical method, laminectomy must be the method of choice in treatment of (scc) in thalassemia patient. Some neurosurgery suggested that use of hydrox urea. HU with surgery (laminectomy) without use of EBRT in minimal doses which was highly –effective treatment of (scc) which is followed the EMH masses which complicated Para paresis or paraplegia [9-10].

Conclusion

A- Thalassemia interned and Beta are commonly complicated sec in comparison with other types of thalassemia.

B- Neurological complication of sec are uncommon but benign one should suspect them particularly in our country where the disease is common in our locality.

C- Diagnosis should be done by MRT.

D- EBRT is curative if it is done properly to avoid hematological risky.

E- There are still quite many questions need to be answered in this field & will excite further researchers & studies medical treatment e.g. HU.

F- The medical fitness of patient must be assess carefully for radiotherapy.

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required

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Marked Dominance of methicillin Resistant *Staphylococcus aureus* among Iraqi Patients

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection in human beings and animals stands out as one of the leading pathogens causing nosocomial and community infections. Likewise, slightly increasing drug resistance in MRSA has narrowed the treatment choices. This work focuses on estimating the prevalence of MRSA in Baghdad, Iraq. A total of 130 specimens were collected from patients visiting various hospitals in Baghdad, Iraq. The present results revealed that 50 (92.6%) isolates were identified as *Staphylococcus aureus*. Noticeably, *mecA* gene was detected in 44 (88%) isolates. Hence, the light must be shed on this marked prevalence of methicillin resistant *S. aureus*.

Keywords: *MRSA, Staphylococcus aureus, mecA, Baghdad.*

Introduction

Staphylococcus aureus is very hardy pathogen for human and it is responsible for a wide variety of infections. Its pathogenesis is a complex process due to the release of different range of secreted and surface-associated virulence factors [(1)].

Strains of *S. aureus* harbouring mecA gene will resist all beta lactam antibiotics. Nevertheless, just after the presenting methicillin as a medicine to treat penicillin-resistant *S. aureus* strains in 1961, methicillin resistant *S. aureus* (MRSA) strains were reported, [(2)]. Thereupon, MRSA has emerged as a boundless challenge in human medicine, particularly as a nosocomial pathogen, nonetheless, this type of *S. aureus* has developed resistance against nearly all common antibiotics; rendering its treatment into a problematic concern. Moreover, since the 1990s, MRSA is considered as a worrisome problem in hospitalized patients or those who recently had intensive surgery; henceforth MRSA strains are known as community-acquired or community-associated MRSA [(3)].

During the past decades, MRSA has spread throughout the world and has become highly endemic in many geographical areas. Due to the changing pattern of antibiotic resistance in S. aureus and the prevalence of multidrug resistance (MDR) in MRSA, some investigators have suggested that the resistance patterns should be evaluated periodically and antibiotic therapy should be guided by susceptibility testing [(4)].

The prevalence of MRSA underlies a serious risk and potentiates a problematic concern that lead to the emergence of strains with enhanced virulence [(5)]. Upon that, the present work was undertaken to investigate the prevalence of MRSA amongst patients attending Baghdad hospitals.

Materials and Methods

Ethical statement

All participants agreed to provide the investigator with the specimens. Informed consent according to the Declaration of Helsinki was obtained from all participants.

Staphylococcus aureus isolation and identification

One hundred and thirty specimens included mid-stream urine, burn swabs, wound swabs, and blood were collected from hospitalized patients referring Al-Yarmouk teaching Hospital and Baghdad Medical City in Baghdad, Iraq.

All these specimens were cultured onto plates of Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hr. Colonies that appeared from primary cultures were re-inoculated by sub-culturing on BHI agar, then re-cultured onto MSA and incubated at 37°C for 24 hr to obtain purified bacterial isolates. Thereafter, discrete colonies were cultured onto blood agar for haemolysis behaviour detection. Oxidase, catalase, and coagulase were assayed as well.

Detection of methicillin resistance by cefoxitin disk method

All the isolates were subjected to cefoxitin disk (30 µg) diffusion assay. A 0.5 McFarland standard compatible suspension of the isolate was prepared and lawn culture was spread on Muller-Hinton agar plate. Plates were incubated at 37ºC for 18 hr. and zone diameters were measured. An inhibition zone diameter of ≤ 21 mm was reported as Methicillin-resistance [6].

Detection of ***16SrRNA*** and ***mecA***

Extraction of Bacterial DNA

Genomic DNA was extracted using Presto™ Mini gDNA Bacteria (Geneaid, Thailand). Upon the procedure itemized by the manufacturing company, DNA was extracted from overnight cultures of the carefully chosen staphylococcal isolates. Purified DNA concentration was measured using Biodrop (Biodrop, Canada).

Gene amplification protocol

To confirm the identification of *S. aureus* isolates, conventional PCR technique was carried out to amplify fragments of *16SrRNA* (108bp) genes. Two microliters of each primer Sa442-1 (5′-AATCTTTGTCGGTACACGATATTCTTCACG-3′) and Sa442-2 (5′-CGTAATGAGATTTCAGTAGATAATACA ACA-3′). *mecA* gene as a determinant of methicillin resistance was detected with primers MecA1 (5′-GTAGAAATGACTGAACGTCCGATAA-3′) and MecA2 (5′-CCAATTCCACATTGT TTCGGTCTAA-3′). Different concentrations of DNA (depending on DNA yield) extracted from each *S. aureus* isolate and deionized D.W. were added to PCR premix tubes in order to reach 20 µl as a final volume. The PCR conditions were as follows: 2 µl of template DNA in a 20 µl final reaction volume containing 0.2 µM for the primers with the thermocycling conditions (Bio-Rad T100, USA) set at 94°C for 3 min, followed by 35 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 15s for *16SrRNA* [7] and 94°C for 10 min, followed by 10 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 75 s and 25 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 75 s for *mecA* [8]. PCR products were visualized using 2% agarose gel stained with diamond nucleic acid dye (Promega, USA).

Results

Staphylococcus aureus isolation and identification

One hundred and thirty different clinical specimens collected from patients attending hospitals in Baghdad were streaked on MSA. Fifty-four isolates appeared as round yellow colonies and positive for beta haemolysis, catalase, oxidase, and coagulase; therefore, primarily identified as *S. aureus*. What's more, out of these 54 isolates, 50 (92.5%) developed methicillin resistance by cefoxitin disk diffusion method (CDD).

DNA extraction and preparation

After DNA extraction by Presto™ Mini gDNA Bacteria Kit, DNA concentration was between 22 and 81 ng/ml; whereas, purity was about 1.89- 1.92. A ratio of 1.8 -2.0 is generally accepted as "pure" for DNA [9]. Gel electrophoresis was done to confirm the integrity of extracted DNA.

Detection of ***16SrRNA*** and ***mecA*** genes by polymerase chain reaction

In this study, PCR technique was applied to confirm the presence of *16SrRNA* and *mecA* genes. The existence of genes was detected by presence of single band at a given molecular weight. (viz. 108 bp and 310 bp for *16SrRNA* and *mecA*, respectively) of the DNA marker.

The current results revealed that *16SrRNA* was located in 50 (92.6%) out of 54 biochemically *S. aureus* isolates. Correspondingly, four isolates were identified using traditional methods as *S. aureus*, they did not have this gene. Furthermore, of these 50 isolates, *mecA* gene was detected in 44 (88%) isolates. Of interest, 47 (94%) isolates were detected as MRSA by CCD, all of which carried *16SrRNA*.

Discussion

The increasing incidence of multi-drug resistant *S. aureus* strains, particularly, MRSA is a serious problematic issue in therapeutic strategies and simultaneously considered as a threat to both the clinical settings and community.

Karmakar et al. [10] mentioned that among 165 samples, 100 strains (60.60%) were isolated from a selective MSA media and then these isolates were identified as *S. aureus* by different biochemical tests. Gram staining, catalase, coagulase, and thermonuclease were important phenotypic identifying markers of *S. aureus*. They found that 100%, 92%, and 84% isolates were positive for catalase, coagulase, and heat-stable nuclease, respectively. The results of present study agreed with a study done by Rusenova and Rusenov [11] that total of 156 isolates suspicious for *S. aureus* were detected by a conventional biochemical method. The majority of *S. aureus* strains gave typical biochemical reactions with the exception of 30 (19.2%) and 25 (16%) that were VP negative and weak positive in fermenting mannitol respectively. Twelve strains were found to be non-haemolytic (7.7%). However, precise detection of *S. aureus* was done by combination of conventional and molecular methods. Ibraheem and Al-Mathkhury [12] reached similar findings as they highlighted the inaccuracy of traditional methods for the identification of *S. aureus*. Same authors recommend that all the traditional identification should be confirmed through molecular methods, in order to avoid false-positive results.

The *mecA* gene synthesizes penicillin binding protein (PBP2a) and it is the cause of methicillin resistance in MRSA. This protein able to reduced affinity for βlactam antibiotics**. This gene resides on the staphylococcal cassette chromosome (SCC). Staphylococcal cassette chromosome is a** large genetic mobile element which varies in size and genetic composition among the strains of MRSA (10). To treat staphylococcal infections, various classes of antibiotics including beta-lactams, glycopeptides, lipopeptide, oxazolidones, aminoglycosides, macrolides, and fluoroquinolones [13].

The present study agreed with a local study performed by Al-Dahbi and Al-Mathkhury [14] as they mentioned that the incidence of MRSA among *S. aureus* was 94.3%. However, it compatible with another local studies [12, 15, 16] demonstrated that most isolates of *S. aureus* developed methicillin resistance.

To investigate the distribution of methicillin resistance staphylococci among the patients, Muhammad and Al-Mathkhury (17) performed the antibiotic sensitivity test to 137 *Staphylococcus* isolates using CCD. The results revealed that 68% of *S. aureus* isolates developed methicillin resistance.

Interestingly, the present work revealed that MRSA detected by CDD outnumbered the PCR technique. The reason of such difference could be due to presence of other genes responsible for the methicillin resistance other than *mecA*.

Owing to unnecessary and unrestrained use of antibiotics, the **bacterial species developed multidrug resistance; hence narrowing the therapeutic choices for the treatment [18]. MRSA originated from nosocomial infections highpoints this species as a potential pathogen; which have the capacity to cope with different antibiotics [19].**

Conclusion

MRSA isolates in Iraq are increasing with time, an issue need to be highlighted.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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Successful Conservative Management of Premature Rupture Membranes of Twin Pregnancy with one Fetus Papyraceus

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Abstract

Fetus papyraceus is a rare obstetrics complication and is characterized by the death of one or more fetuses in the early gestation period, and the other fetus continues to develop. This report describes a twin pregnancy with one fetus papyraceus and both undergone premature membrane rupture. We reported a 25-year-old woman unbooking patient admitted to the Pusura hospital with a complaint of amniotic fluid leaking from the vagina after a traffic accident. The patient is a woman with a history of G2P1A0 at 22-week gestation with twin pregnancies (monochorionic diamniotic twin pregnancy). There was one live fetus with cephalic presentation and the other one was papyraceus. Conservative management with a corticosteroid regimen to complete the lung maturation was done. At 32-week gestation, the patient was admitted back with spontaneous rupture membranes and the live fetus was seen in lie presentation. She underwent a caesarian section and a premature life female baby was delivered with 1800g of weigh, APGAR score of 7 at 1 minute and 8 at 5 minutes. The fetus papyraceus was weighed 100g. Both the mother and the baby were discharged home in good condition. Fetus papyraceus is a rare condition that describes intrauterine fetal demise in early pregnancies. It can occur in both single or multiple gestation pregnancies. This case report reported case is a premature rupture of the membranes caused by a traffic accident. A life baby weighing 1800g was delivered and 100g of weigh of fetus papyraceus in a caesarian section. This case is a diamniotic – monochorionic placenta. Finally, the healthy baby was discharged 18 days after delivery.

Keywords: *twin pregnancy, fetus papyraceus, premature rupture membranes.*

Introduction

Increased use of Assisted Reproductive Techniques in obstetrics gynecology procedures in recent decades is followed by the rising incident of multiple pregnancies and fetus papyraceus[1]. Intrauterine fetal death of one twin is a relatively frequent complication of twin pregnancy and the single intrauterine fetal death rate in multiple pregnancies is significantly higher than that in singleton. This condition can be found in 2.7% of twin pregnancies and 4.3% of triple pregnancies[2].

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Usually, when the fetus dies in early pregnancy, the amniotic fluid and placental tissue are absorbed and the fetus is compressed in between the membranes with the co-living twin[3].

Premature rupture of membranes (PROM) is a frequent phenomenon that occurs in about 4-7% of pregnancies. Almost two thirds of PROM are spontaneous. The common risk factors are young mothers, multiple pregnancies, genital tract infection, and previous preterm delivery[4].

Here we report a case of fetus papyraceus in twin pregnancy with premature rupture of the membranes, which was diagnosed at the 22nd week of gestation. Conservative management was done, and the live baby was born at 32 weeks (1800g) by cesarean section in the

indication of premature rupture of the membranes and lie presentation of the fetus.

Materials and Methods

Case Report

A 25-year-old woman (G2P0A1) was admitted to the hospital with complaints of amniotic fluid leakage after a traffic accident approximately one hour before admission. Physical examination found a normal blood pressure (120/70 mmHg) and a good general health status, with no fever (37°C). Gynecology examination with a sterile speculum in the labor ward revealed dark brown amniotic fluid. While, abdominal ultrasound showed one fetus in cephalic presentation with cardiac activity and active fetal movements, and under the life, a baby has seen an irregular form or compressed of the fetus. The amniotic fluid of the life baby was in normal volume (AFI=10), but the other one appeared to have an extremely minimal amniotic fluid. Both amnions are separated by the amniotic membranes and there was only one placenta insertion in the corpus position. Thus, we came up with the diagnosis of monochorionic diamniotic twin pregnancy at 22 weeks gestation. According to the anamnesis, one month ago she had undergone an ultrasonography examination and both babies were in normal condition at 18 weeks of gestation. After the accident, the family of the patient was counselled about the condition and the risks that could affect the patient and the fetus if the pregnancy is to be continued. The patient agreed on doing conservative management with a corticosteroid regimen to complete the lung maturation was done. Sonography was planned to be done every three weeks and coagulation profile every fortnight. After the lung maturation management, the patient regularly came to the outpatient clinic for a check-up once every 3 weeks. During the first appointment, the life baby was seen to be in normal condition (25 weeks) and the other fetus (fetus papyraceus) was seen with extreme oligohydramnios.

At 32 weeks gestation, the patient was admitted to the hospital (Emergency Obstetrics and Gynecology Department) due to vaginal discharge of clear fluid (hydrorhea) with no uterine contractions and bleeding. The general condition was within normal limits. Abdominal ultrasonography showed the life fetus in lie position, normal fetal heart rate (FHR), normal placental insertion on the upper posterior side, AFI= 8, and the obstetric examination indicated 32 weeks + 2 days of gestation. A small fetus papyraceus was seen pressed flat against the uterine wall and the living fetus. Speculum examination revealed the release of clear amniotic fluid. Emergency caesarean section was performed: A premature life female baby was delivered with a weight of 1800g, APGAR score of 7 at 1 min and 8 at 5 mins, followed by a small papyraceus baby and the placenta. The fetus papyraceus weighed 100g and the placenta was 450g. The baby cried immediately after birth and was transferred to the Neonatal Intensive Care Unit for preterm management. On a careful examination of the placenta, two cords were noticed and identified as diamniotic – monochorionic placenta. The cord and placenta of the fetus papyraceus are seen pale and inserted in the placenta tissue (normal insertion). Finally, the healthy baby was discharged 18 days after delivery.

Discussion

Fetus papyraceus is a rare condition that can occur in monochorionic and dichorionic multiple pregnancies. The incident of fetus papyraceus is in one out of 12,500 cases. Fetus papyraceus is characterized by the death of one or more fetuses in the early gestational period, while the other fetuses continue to grow[5,6,7]. Other reports stated that the incident of fetus papyraceus is 1:12,000 live births, which ranged between 1:184 and 1:200 twin pregnancies. The term fetus papyraceus is used when the intrauterine fetal demise of a twin, early in pregnancy, occurs with retention of the fetus for a minimum of 10 weeks, resulting in mechanical compression of the small fetus, such that it resembles parchment paper[3,7,8]. The causative factors remain unknown in many cases. There are some reported risk factors including true cord knot, filamentous or membranous cord insertion, placental insufficiency, cord stricture, congenital anomalies, and nuchal cord[3]. While some cases were found to be caused by twin-twin transfusion syndrome. Fetus papyraceus was seen to occur more often with monozygotic twin pregnancies than dizygotic twins, but no association was found with maternal age, parity or gravidity. Fetus papyraceus can occur in both uniovular and binovular twins but is more common in uniovular twins[5]. In most cases, the fluid of the death tissue is gradually absorbed, the amniotic fluid disappears, and the fetus is compressed and incorporated into the membranes[5],

but in this case, the patient is admitted with premature rupture membranes. The degree of compression depends on the period between fetal death and delivery. Also, in the later weeks of pregnancy, due to larger fetus size, the probability of the fetus to become a fetus papyraceus is lower[3]. Based on the gestational period, there are three possible complications, which include a vanishing twin syndrome in the first trimester, fetal papyraceus in the second trimester, and macerated twin in the third trimester. The sign of single embryo death during the first 6-8 weeks may appear as a cyst on the fetal surface of the living fetus placenta. While, if the death occurs after 8 weeks, resorption of the amniotic fluid along with mummification of fetal parts may form FP[3]. Spontaneous reduction of one or more gestational sacs before the 12th week of gestation in 36% of twins, 53% of triplets, and 65% of quadruplets have been reported. Increased risk of fetal death has been associated with the monochorionic placenta, disproportionate sharing of the placenta, and monozygosity. Moreover, the intrauterine fetal death of one twin is found to be associated with serious mortality and morbidity of the surviving twin[5]. Fetal loss of the twin during the first trimester in not an uncommon event and does not appear to impair the development of the surviving twin. However, fetal death after mid-gestation (17 weeks of gestation) may increase the risk of IUGR, preterm labor, preeclampsia, and perinatal mortality[9]. The complications of single death fetuses in twin pregnancies can affect both mother and the surviving fetus. Complications affecting the surviving fetus include multicystic encephalomalacia, serious cerebral impairment, and twin embolization syndrome. It may also cause damage to high vascularized organs such as the brain and kidney. In the brain, it can cause central nervous system abnormalities, such as encephalomalacia, while in other organs it may cause extra cranial abnormalities such as small bowel atresia, gastroschisis, and aplasia cutis[5,6]. Meanwhile, the maternal complications include preterm labor, infection from the retained fetus, severe puerperal hemorrhage, and consumptive coagulopathy (rarely found in late fetal death)[3].

In our patient, the two factors that are found to increase the risk of mortality and morbidity of the fetus are prematurity and premature membrane rupture, with prematurity as the leading cause.

Preterm birth is defined as deliveries occurring from 20 to 37 weeks of gestational age[10]. Preterm birth is a condition that occurs in 6.0 – 15.0% of all deliveries and is the most frequent cause of fetal and neonatal death and morbidity. The incident of preterm birth ranges from 10 – 15%. Several clinical syndromes may lead to a pretem birth including preterm labor with premature rupture of the membranes (35%), preterm labor with the intact membrane (30%), incompetency cervix (15%), and the other such as hypertensive disorders (12%), fetal growth restriction (5%), and antepartum bleeding (3%)[11].

The common pathway of parturition has three components: cervical ripening, activations of the myometrium, and activation of the fetal membranes. It can be activated by multiple agents. The most clearly identified is a chorioamniotic infection. Excessive stretching of the uterus, such as that happening in multiple gestations or women with polyhydramnios, is also capable of initiating premature labor and/or premature rupture of the membranes[11].

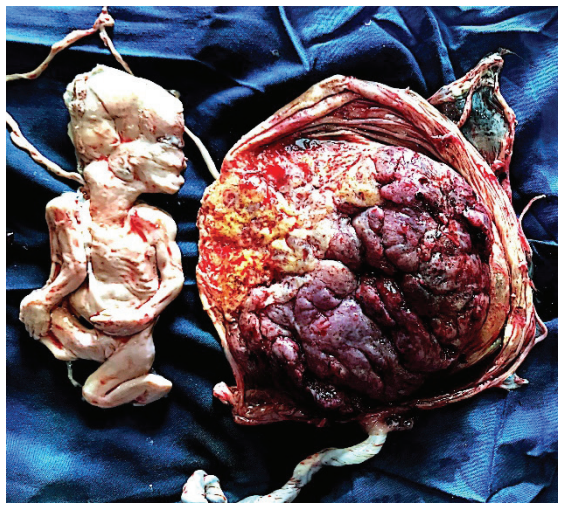

Figure 1: Fetus papyraceus (100g) with faded and its yellowish part of the placenta. The reddish part of the placenta and normal cord belongs to the surviving twin.

Figure 2: The surviving twin. (Female, 1800g)

Conclusion

Fetus papyraceus is a rare condition and the incident is one out of 12.500 twin pregnancies. Successful conservative management was done on the twin pregnancy of a live baby and a fetus papyraceus that had a PROM. A 25-year-old woman, G2P0A1, went to the hospital with a complaint to get out of amniotic fluid from the vagina and a history of traffic accident 3 weeks ago. A month before the accident happened, an ultrasound examination showed that both babies were in a good condition. The general condition of the patient was all within normal limits when the patient was administered to the hospital. Speculum examination: revealed brown dark amniotic fluid from the cervical canal. Ultrasound examination suggested a life fetus aged 22 weeks with AFI 10 and a fetus papyraceus with AFI 4 located just below the living fetus. Laboratory tests were within normal limits. The patient was treated according to PROM management. The subject delivered a live preterm female baby at 32 weeks by cesarean section for indications of PROM and lie presentation. The baby weighed 1800g, with an APGAR score of 5 at 1 and 7 at 5 mins. The fetus papyraceus weighed 100g, one placenta, biamnion, and monochorionic. The healthy baby was discharged 18 days after delivery.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical Approval: There is no ethical approval needed for this study.

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Comparison of Histological Changes in Mice Infected with the *Cryptosporidium Parvum* after Treatment with an Aquatic Leaf Extract of *Salvia Officinalis*, *Pimpinella Anisum* and, Spiramycin Drug

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Abstract

Background: *Cryptosporidium parvum* was cause Cryptosporidiasis .In this study the therapeutic effect of two aquatic extracts of plants in infected white mice with the *C. parvum were* examined and the results were compare with the therapeutic effect of Spiramycin through the study of the histological changes in mouse intestine. **Methods**: Thirty male mice were divided into six groups: group 1(non infected, negative control), group 2 (feeding with *C. parvum* oocysts, positive control), group 3 (infected and treatment with leaf extract of *Pimpinella anisum*), group 4 (infected and treated with *Salvia officinalis* extract ,the group 5 and 6 (infected and treatment with 5mg and 10mg Spiramycin Respectively) The existence of oocysts in the feces of animals was counted daily during the therapeutic period .After two weeks; the changes of cecum histological sections were examined. **Conclusions**: The oocysts counted in feces samples, showed significant differences between the treatments ($P < 0.05$). The cecum histological changes in the group 3 and 4 indicated that leaf extract of plants have moderate efficacy in treatment of C.*parvum* infection. The cecum appears close to normal at the concentration of 5mg Spiramycin there is no abnormal tissue in the concentration of 10 mg.

Keywords: *Cryptosporidium, plants extract, histological changes, Spiramycin.*

Introduction

One of the important parasites that belong to phylum Apicomplexa is Cryptosporidium parvum, which causes infection to the epithelium of the intestine. These obligate enteric protozoa found in human and most mammalian. [1,2]. Cryptosporidium parvum causes five to ten million deaths per year [3] .

Cryptosporidium parvum was known as the cause of the diseases Cryptosporidiasis transmitted by Food and water in humans and animals. The parasite is also a common pathogen related at self-limiting diarrhea in people with immune competence, but in low immune deficiency is life-threatening like AIDS patients [4,5]. Globally, Cryptosporidiasis is considered the second disease after rotavirus causing death among children lower than 2 years due to diarrhea, which may be severe or moderate. [6] *Cryptosporidium parvum* causes acute diarrhea, particularly in children lower than five years old and small animals [7].

In neonates Cryptosporidiasis cases, naturally and experimentally *C. parvum* not only influenced the distal small intestine, but affected proximal small intestine too and several portions of the large intestine. [8,9] .In infected animals with parasite, animal's intestine appear pathological changes like, villus atrophy, degeneration

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in villus, crypt hyperplasia, infiltrations in inflammatory cells and degeneration in microvillus. As a result of these changes many intestinal functions would be affected such as digestion (maldigestion), absorption (malabsorption) and osmotic imbalance [10, 11].

The use of chemical therapy for the disposal of parasites is useful, but in this way there are many problems like resistance to drugs, residue and side effects associated. Therefore, it is important to find a replacement medication. [12].Herbal medicine was adopted centuries ago to treat parasites in most parts of the world. Plants have been used by humans to eradicate several diseases. Medicinal herbs are used in current years, largely for their reliability High efficiency and low cost [13, 14] Spiramycin (Rovamycine) was examined and prove as an effective drug against cryptosporidiosis in patients infected with AIDS [15]. More than twenty years spiramycin was used in Europe and Canada in bacterial and protozoa disease, like *Toxoplasma gondii* [16].

Sage (*Salvia officinalis* L.) is a remarkable pharmaceutical herb to have aromatic characters used as a raw substance in different perfumery, medicinal uses, food, and in the cosmetics industry. *S. officinalis* is included in the family of Lamiaceae it is growing commercially for the production of essential oil [17]. Leaves used in extraction of essential oil are strongly aromatic, that have greater than 49 aromatic ingredients. Different pharmacological research proves that sage products hold a substance that inhibits oxidation, diabetic, inflammatory, microorganism causing disease and cancer. [18].

Pimpinella anisum called aniseed, belonged to Apiaceaeis family, a flowering plant. As a medicinal herb the uses of Anise are stimulating action of digestion and drug against parasites, bacteria and fungi. Aniseed is used in traditional European herbal medicine as treatment of diarrhea, colic and menstrual cramps [19]. Therefore, the purpose of this study was to know the impact of both *Salvia officinalis* and *Pimpinella anisum* on *Cryptosporidium parvum* infection and compare the result with treatment by spiramycin drug.

Materials and Methods

***Cryptosporidium parvum* oocysts:**

Oocysts of *Cryptosporidium parvum* were obtained from a feces specimen that arrived to the (Laboratory of parasite), at the Teaching Hospital for Obstetrics and Gynecology to four year child. The feces specimen was examined by using modified Ziehl-Neelsen (ZN) staining to ensure the presence of oocysts. A drop of the sample was spread on a glass slide, left until dehydration after boiling it with Sheather's sucrose solution fixed with ethyl alcohol, stained with the modified (ZN) and examined under compound light microscope on 40x objectives [20]. The infective specimen was maintained by 2.5% potassium dichromate as described by [21] .The number of *C. parvum* oocysts was detected by haemocytometer in the stock inoculums [22].

Experimental animals: Thirty of laboratory Seviss Albino Mice (strain Balb/C type Mus musculus), four weeks old, male, weighing about 23 -28 g each were used in the current study which was obtained from animal house at the College of Science / University of Anbar. The mice were raised in special cages and multiplied under appropriate conditions in terms of temperature and provision of food consisting of a cereal fodder Soybeans and protein. In this study we followed all ethical protocols of animal's treatment.

Determination of infected dose in experimental mice: 20 ml of oocysts were washed several times with distilled water and dosed 21 mice (for each dose three mice) with a suspensory of oocysts (10, 20, 30, 50, 100, 1000, and 10,000 oocysts / mouse). By using the Haemocytometer chamber, the highest dose and lowest dose were calculated to cause infection without fatality [22].

Collection of plants: The leave of *Pimpinella anisum* and *Salvia officinalis* were collected from different places from the College of Agriculture / Abu Ghraib, after being diagnosed by the national herbivore in the Department of Biology / College of Science / University of Baghdad, it was dried, ground and preserved at a temperature of 20 ° C.

Preparation of aquatic extracts: 100 grams of dry powder Leaves were infused in 1000 ml distilled water on magnetic mixer for 30 minutes then the solution was filtered and the filtrated liquid was placed in the centrifuge for 15 minutes, and then quit in electric oven at a temperature of 50 ° C, till gaining dry extract. The

stock solution was prepared by dissolving 1 g of each extract in 100 ml of distilled water. From this stock solution, a series of dilutions were prepared [23].

Design of Experiments: The mice, infection experiment with the parasite and treatment was designed as addressed in the table 1. All the animals were feeding orally by using the stomach tube, the animals in the second, third; fourth, fifth and sixth groups were infected by parasite by feeding the mice orally with 0.5 ml (10^3 oocysts). The mice feces were examined daily by direct stool examination to confirm the appearance of the infection, after 2 to 4 days the infection occurrence was confirmed. The aquatic extract was given to the affected mice experimentally to the third and fourth group with a concentration of 5 mg / ml while, the fifth and sixth group was treated respectively by Spiramucin in concentration 5 mg and 10 mg for each 20 g of body weight. Spiramycin obtained from (Samarra Pharmaceutical Factory) was dissolved in distilled water. Each mouse was given a dose every day during the experiment that continues for two weeks.

Table 1. The treatments of experimental animals.

No.of Group	Type of treatment	No.of animals	Dose of treatment
Group 1	Negative control Non infected	Five animals	0.5 ml PBS
Group 2	Positive control Infected	Five animals	0.5 ml (103 oocysts)
Group 3	Infected and treated with the aqueous extract, Pimpinella anisum,	Five animals	5 mg / ml
Group 4	Infected and treatment with the aqueous extract of Salvia officinalis	Five animals	5 mg / ml
Group 5	Infected and treatment with Spiramycin	Five animals	Spiramycin 5 mg / 20 g of body weight
Group 6	Infected and treatment with Spiramycin	Five animals	Spiramycin 10 mg / 20 g of body weight

Oocysts counting: Haemocytometer chamber was used to counts the oocysts in feces of experimental mice daily during 14 days, table 2 [22].

Preparation of the Tissue : After two weeks the mice were dissection , the cecum was harvested and fixed in formalin in concentration 4% for 24 hours before processing and then used paraffin wax for embedded stage ,The sections that in thickness 3-5 µm were stained with H&E according to[24].

Statistical analysis: The experiment is designed as a simple one-factor experiment. The results obtained from our current study were analyzed using SPSS Ver.22 statistical program, as a Randomize Complete Block Design (RCBD) was used. The significance of the averages was tested at a probability level ($P < 0.05$), and the meanings of the coefficients were compared using the Least Significant Difference (LSD) test using the Duncun test [25,26].

Rustles and Discussion

In this study, the therapeutic activity of sage leaf extract, anise, and spiramycin drug were examined in infected mice with *Cryptosporidium parvum*. The

comparison between the four treatments was adopted in terms of the effect of oocysts shedding number in the feces of infected mice, as well as the histological changes in the intestine of laboratory mice infected with the parasite.

To investigate the number of oocysts, the feces samples were examined in all groups of mice that infected with the parasite. The examination revealed that infected and untreated mice (G2 positive control) shedding more oocysts than infected groups that were treated with aqueous extract of anise and sage (G3,G4) [27]. Also the Spiramycin drug (G5, G6) show high effective in the reducing oocysts count during the experiment that continue (14 days) with significant differences ($P < 0.05$), this results identified with many studies [28].

Table 2. The number of oocysts during the period of experiment 14 days.

Type of treatment	Control negative	Control positive	Pimpinella anisum	Salvia officinalis	Spiramycin 5 mg	Spiramycin 10 mg
1st day	0 A	0 A	0 A	0 A	0 A	0 A
2nd day	0 A	0 A	0 A	0 A	0 A	0 A
3rd day	0 A	15 B	0 A	0 A	0 A	0 A
4th day	0 A	74 B	42 B	11 B	0 A	0 A
5th day	0 A	124 B	100 B	100 B	21 B	2 A
6th day	0 A	378 C	189 B	276 BC	22 AB	2 A
7th day	0 A	788 C	387 B	367 B	46 AB	0 A
8th day	0 A	2138 C	418 B	476 B	2A	0 A
9th day	0 A	6930 C	416 B	588 B	0 A	0 A
10th day	0 A	14678 B	321 C	790 C	0 A	0 A
11th day	0 A	24980 B	210 C	700 C	0 A	0 A
12th day	0 A	34529 B	103 C	389 C	0 A	0 A
13th day	0 A	55712 B	12 C	378 C	0 A	0 A
14th day	0 A	87645 B	0 A	301 C	0 A	0 A
Mean	0	16285.1	157	312.57	6.5	0 A

The different letters within the same row indicate significant differences between the treatments with a significance level of 0.05.

In this study, after two weeks the cecum was harvested and the histological section were prepared and the changes in the tissue were examined under compound light microscope. Histological sections of the positive control group fig2 showed severe histological changes such as vocalization in the secretory glands and the presence of large numbers of parasites around the secretory glands, compared to the negative control animals fig1 that revealed no changing in the histological section of cecum .

From the examination of the mice cecum tissue, that infected and treated with anise the study found that anise plant extract can assist to restore the tissue and secretory glands to normal state and minimize the number of oocysts fig 4 over time when compared with the positive

control group fig 2, as it is noticed vacuolization in the secretory glands and the presence of huge numbers of oocysts around the secretory glands, meaning that the anise extract is get rid of the parasite. Similar results reveal its antiprotozoal action, such as the anticoccidial activity against infection of poultry with Emeria tenella [29]. In another study, the methanolic extract of *Pimpinella anisum* proven effective against infection with Amoeba [30].

Also, the extract of the *Salvia officinalis* helps in the return of the secretory glands to a normal state fig 3 when compared to the positive control group fig 2. Similar results were reported regarding the effect of sage against worms, results showed significant reduction in the number of eggs excreted by *Hymenolepis nana*, *Aspiculuris tetraptera, Syphacia obvelata* [31] Similar results about antiprotozoal effects of sage extracts were recorded against *Cryptosporidium parvum* [32].

In the case of treatment with Spiramycin at a concentration of 5 mg, there is an increase in the thickness of the intestine wall as a result of slight fibrosis with slight bleeding, but in the concentration of 10 mg, there is emergence of the normal state after treatment and the return of the secretory glands to their normal state.

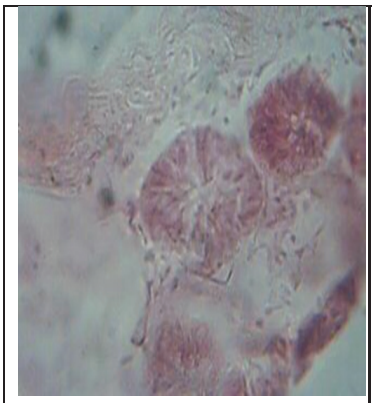

Figure 1.

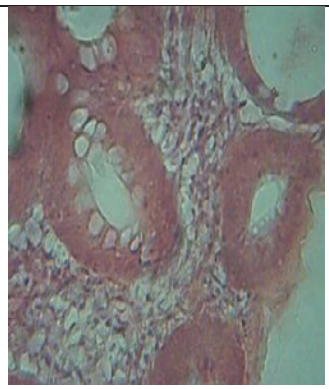

Figure 2.

Figure 1. Negative control .Histological section of the intestine mice, without infected parasite and without treatment, showed the cohesion of the secretory glands tissue H&E stain (40x).

Figure 2. Positive control. histological section of the intestine mice, the occurrence of vacuolization in the secretory glands and the presence of large numbers of parasites s around the secretory glands (H&E) stain 40x)

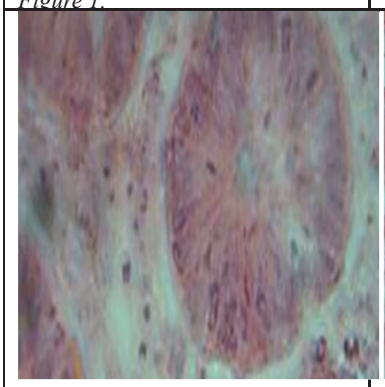

Figure 3.

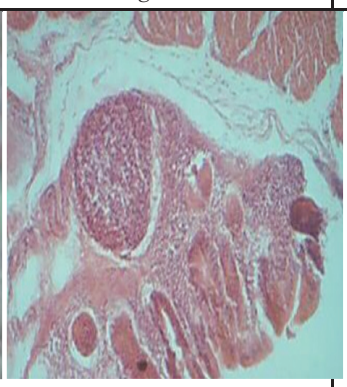

Figure 4.

Figure 3. Section in Intestine mice was treated with Salvia officinalis, notes the secretory glands and their return to normal, H&E) stain (40x).

Figure 4. Intestinal histological section of the treatment mice with Pimpinella anisum, noting the treatment attempt to restore the normal secretory tissue and glands, H&E stain (40x)).

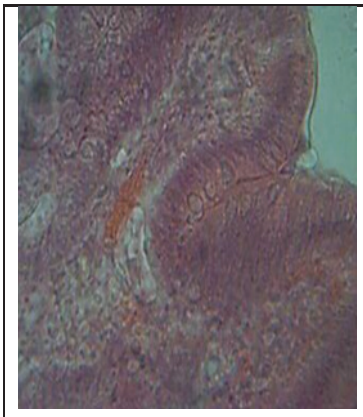

Figure 5.

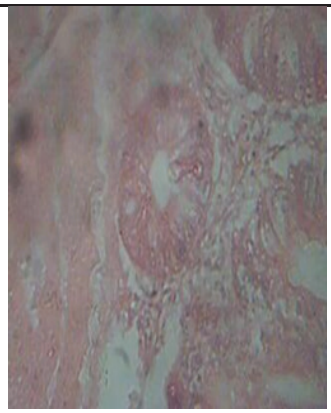

Figure 6.

Figure 5. Histological section of the intestine mice that treated with Spiramycin at a concentration of 5 mg notes the increase in thickness and thickening of the intestine walls as a result of minor fibrosis ( ) with slight bleeding ( ), H&E (40x).

Figure 6. Histological section of the intestine mice that treated with Spiramycin at a concentration of 10 mg. Normal condition appears after treatment, and the ejaculate glands return to normal

As for the drug spiramycin, it is highly effective, as it led to a high decrease in oocysts shedding rates as shown in table (1) this is consistent with the studies [33, 34] that the drug spiramycin is used to treat diarrhea it is caused by the Cryptosporidium parasite and can be used as a high-dose treatment for a long time, Sparimycin affects the protein synthesis process in the ribosome. Other study concluded that spiramycin a high influence on the *Cryptosporidium parvum* especially when mixed with vitamin E. [35].

Competing Interest statement:

The authors advertise no conflict of interest.

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Cardiac Arrest Induced by Anti-hypertensive and NSAIDS Drug Abuse Uses due to their Role Effect on Electrolytes and Aldosterone Levels in Hypertensive Patients with Renal Insufficiency

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Abstract

The present study aims to investigate the role of the drugs like angiotensin converting enzyme inhibitors (ACEI), non-steroidal anti-inflammatory drugs NSAIDs and their effects in the contributions of electrolyte fluctuation levels, as well as in an abuse uses. Forty four cardiac arrest subjects cases of hypertensive with renal failure (renal insufficiency) were taken, and forty participants as a healthy control without any heart and kidney problems with same of mean age (58±5). Done in Hilla city/Iraq were enrolled in this study. Results that collected from the present study were a significant in reduction of serum aldosterone levels, with very clear raises in plasma potassium and urinary sodium levels due to the action of hypertension drugs. Also results shown a significant decreases in the urinary potassium, otherwise plasma sodium levels within the normal elevated malady in patients, when compared with healthy control. There were significance results with moderately increases levels of the patient serums urea and creatinine, as when correlated with healthy control investigations. The current study found that BMI does not differ in patient groups (with cardiac arrest) when compared with the normal healthy control group and each were within the BMI range for normal weight of a person both in males and females. It was concluded that uses of antihypertensive drugs and NSAIDs lead to lowering aldosterone levels that relates hypertension treatments and inflammatory diseases.

Keywords: *ACEI, Aldosterone, NSAIDs, Potassium, Sodium*

Introduction

Nowadays, Blood pressure is the force that is exerted by the arterial blood to against vessels walls. Blood pressure forces depends on the heart work and the blood vessels resistance. The guideline of medicine was defined hypertension as a blood pressure higher than 130 over 80 millimeters of mercury (mmHg), according to guidelines issued by the American Heart Association (AHA) in November 2017 [(1)] . A high blood pressure can cause a damage of small blood vessels like in the kidneys and reducing their ability for properly work and may result in high stretch of blood vessels. Eventually, this stretching caused scars and weakens may including the blood vessels in the kidneys [(2)].

Historically a great relationship between hypertension and renal diseases. Renal failure may induced with hypertension as well as renal damage. Patients with uncomplicated obvious hypertension were separated into the distinct clinical and histological patterns like nephrosclerosis may compact with renal insufficiency symptoms [(3)]. The vascular lesions of hyaline arteriosclerosis develop slowly without overt proteinuria. Hypertension is the second leading cause of kidney failure in the United States after diabetes [(4)]. So the rate of kidney failure due to high blood pressure increased 7.7 percent [(5)]. The relative risk of serious

renal damage in patients with uncomplicated essential hypertension is less common, when its compared with other presented in cardiovascular complications [6]. An increased of a cardiovascular morbidity and mortality, that shown a risk of sudden cardiac death, has been presented in patients with rheumatoid arthritis (RA), that associated with heart rate variability and ventricular repolarization abnormality [7]. The interplay of these parameters and the inflammation for example RA made a known to exist that the inflammation induced and growing interest [8]. Whilst a higher incidence about world in a prevalence of the ischemic heart disease in many cases with rheumatoid arthritis, some authors have shown the increased of heart diseases that cannot be explained by traditional risk factors alone [9], so their interest in the role of inflammation as novel risk factor for atherosclerosis. Where potassium concentrations raised in the extracellular will be modify a for myocytes contractility, from -85mV to -65mV and -40mV in some cases [10]. Anti-hypertensive drugs likes Angiotensin-converting enzyme inhibitors, such as ramipril and enalapril inhibit the conversion of angiotensin I to angiotensin II and at the end lowering of aldosterone secretion, that's of the main role were blood pressure reduction by sodium reabsorption inhibition in the renal tubules, as well as the sodium was already losses due to renal failure [11]. When the nonsteroidal anti-inflammatory drugs (NSAIDs) administration are presence the clinical signs and symptoms were more obviously due to inhibition of renin secretion. So the hypoaldoserinisim was correlated with hyporeininmic that caused hyperkalemia. Whether remains inhibition of the aldosterone metabolism role by non-selective NSAIDs is a casual or causal factor in NSAID-induced cardiovascular toxicity [12].

Materials and Methods

Study Design

The study design was a case control study which was carried out between November 2017 to April 2018. Sample collection and examination was carried out at the Department of Biochemistry, College of Medicine, University of Babylon and Merjan Medical Teaching Hospital in Hilla City, Iraq. Sample size was calculated according to Daniel sample size formula equation with Iraqi disease prevalence. A total of 45 patients with hypertensive renal insufficiency were enrolled in this study.

Exclusion and Inclusion Criteria

The inclusion criteria included many cases of cardiac arrests and hypertensive patients with and renal failure (insufficiency) in presence of inflammatory diseases like rheumatoid and osteoarthritis. Only those with rheumatoid arthritis were selected. Patients who were under treatments where also taken. Participant who were smokers and patient who received antihypertensive treatment without NSAIDs or use angiotensin II receptor blocker like losartan and candesartan were excluded from the present study.

Measurements

Body mass index of patients and healthy controls were calculated using a mathematical equation, in which the weight in kilogram was divided by the square high measured by meters [13] . Determination of serum Aldosterone levels in patient and control groups were achieved using enzyme linked immunosorbent assay ELISA kit (Demeditec Diagnostics) Germany, and according to manufacturer instructions. Determination of Potassium and Sodium was examined using a spectrophotometric manual kit, blood collected without using tourniquet. Urine potassium and sodium was examined using a fully automated biochemistry mindray device. Urine sample were collected randomly and in a 1 litre volume. Determination serum urea was achieved using a spectrophotometeric manual prepared kits.

Results and Discussion

Table 1: The Number and Age of All Participants

Groups	Total	Male	Female	Mean ±SD Age (years)	
				Male	Female
Patients	45	25	20	58±5	55±5
Controls	40	20	20	55±2	50±2
P value				0.877	0.909

Table 2: Aldosterone ng/dl of Patients and Healthy Control Groups According to Sex, (normal 7.5ng/dl), P.value < 0.05.

Parameter	Gender	Patient Aldosterone Level	Healthy Control Aldosterone Level	P Value
		Mean ±SD	Mean ±SD	
Aldosterone ng/dl	Male	1.6±0.60	7.3±0.18	1.52 X10-9
	Female	1.2±0.53	7.1±0.38	9.12 X10-7

Table 3: Mean Difference of Plasma, urine and serum Parameters of Patients and Healthy Control Groups

	Parameter	Patient	Healthy Control	Normal Value
		Mean ±SD	Mean ±SD	
Plasma estimations Parameter	Na+2	1.6mmol/L	2.25mmo/L	2-2.6mmol/L
	K+	7.8mmol/L	4.3mmol/L	3.5-5.2mmol/L
Urinary estimations Parameter	Na+2 K+	18mEq/L 12.5mEq/L	50mEq/L 35mEq/L	40 - 220 m Eq/L 20 - 60 m Eq/L
Serum estimations Parameter	Urea Creatinine	77.5mg/dl 2.32mg/dl	43.75mg/dl 1.1mg/dl	45-50mg/dl 0.6-1.2mg/dl

Table 4: The Body Mass Index (kg /m2) of Participants.

Subjects	Total Number (Male)/ (Female)	Mean ±SD	
		Male	Female
Patients group	45 (25/20)	22.19±2.5	22.96±2.5
Controls group	40 (20/20)	21.55±1	20.92±1.5
P value		0.994	0.118

Cases with cardiac arrest were recruited, that are associated with a score level of symptoms and investigations. Disease severity in the current study found there was 45 patients with a significantly worsened hypertension and renal insufficiency, under treatment complexes such as NSAIDs and angiotensin converting enzyme inhibitors (ACEI). The other 40 healthy controls had no treatments or problems (table 1) .

The results of the present study revealed a significant decreases (P> 0.05) in serum aldosterone levels in patients with cardiac arrest when compared with healthy control (table 2) . A study by struthers [14] shown magnesium loss caused by aldosterone and by diuretics could contribute to coronary artery spasm like cardiac arrest and arrhythmias. The results were non-confined with a study by Chrysostomou [15] which depended on a Double-Blind Placebo-controlled or a triple mod therapy.

The Current study showed a significant decrease in the levels of plasma parameters sodium and potassium in patients groups when compared to healthy controls groups (table 3) . A similar result were revealed by Graudal and colleagues [16] and gu and colleagues [17] which showed the role mechanism of aldosterone in the sodium homeostasis and the reduction of aldosterone for reduce Na^+ in hypertension and in hypernatremia cases, as well as its correction level with the Serbian study by Tasic and colleagues [18].

The results of the present study included a significant decrease in the levels of urinary parameters potassium and in patients groups when compared to healthy controls groups, due to the reduction of aldosterone, similar results were revealed by [19] who showed the ability to distinguish hypovolemic by using the urine sodium alone was reasonably high (the accuracy with a cutoff value of 50 mEq/L was 82%). So the elevation of urine sodium levels up to 50 mEq/L depend on clinically meaningful response to isotonic saline infusion. And with experimental study [20] characterized high fat-fed mice which contribution of excretion of Na concentrations were mediated Na+ reabsorption in the presence of insulin resistance obesity and, so the high amount of fat-fed demonstrated impaired Na^+ excretion as well as elevated blood pressure. Our study agrees with the results of ueda and colleagues [21] where urinary potassium levels excretion reduced according to impairment in renal function, so the urinary potassium excretion mostly affected by urinary sodium excretion and estimated GFR in patients with chronic kidney disease. A study by weir and colleagues shows similar results to our study [22] that concluded the same results with our potassium studied. Although these results disagreed with a study by ghazi and team [23] were remembered that the antihypertensive treatments effect in spironolactone were positively related to urinary sodium excretion regardless of aldosterone status.

The present and other studies investigates the serum creatinine and urea levels, and found a clear elevation in these normal values due to the impairment renal function, and these results were in agreements with a study by Tesfaye& Jedlickova [24] have shown the nephrotoxic drugs contributions in the clinical state of spinal cord injury with chronic renal impairment , while the study by Córdova-Sánchez and team [25] showed elevation in creatinine results. The current study shows urea levels similar to those presented by Mackenzie & Chacko [26] who showed initiate in renal replacement therapy by treating acute kidney injury in the intensive care setting but without clinically significant uremia symptoms absence. The results of our study were in disagreement with a study by tujjar and colleagues [27] who showed patients who survived cardiac arrest were independent prognostic factors like age, epinephrine dose, cumulative fluid balance and presence of shock were independent predictors of the development of acute kidney injury in this population. Renal failure and neurological has no clear correlation.

The current study found that BMI does not differ in patient groups (with cardiac arrest) when compared with the normal healthy control group and each were within the BMI range for normal weight of a person both in males and females (table 4) . This was statistically insignificant between these groups and was coherent with a study by Gil and colleagues [28] who showed limited data between BMI and cardiac arrest.

Conclusion

Uses of antihypertensive drugs and NSAIDs lead to lowering aldosterone levels that relates hypertension treatments and inflammatory diseases. Hyperkalemia may contribute in the cardiac arrest pathogenesis . In the same time heart activity will be altered by abnormal depolarization like sudden cessation may occur.

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Study of Some Biomarkers and HLA-G for Early Detection of Multiple Sclerosis Disease

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Abstract

Multiple sclerosis is the most commonest a chronic inflammatory, demyelinating and neurodegenerative disorder in the central nervous system (CNS) of the brain and spinal cord. The disease commonly start onset in the age of (20-50)years, as for women, they are at greater risk of MS than men. The aim of this dissertation is to detected some biomarkers(IL-18, Caspase-1 and Opioid growth factor) in the serum and saliva in patients with MS and study the HLA-G genotype. Moreover, the current study indicated a comparison between the levels of biomarkers in two groups for MS patients (early diagnosis of MS without treatment and with treatment group) with the group of healthy controls. This study included the total number 150 case, it was classified to the group I early diagnosis without treatment (54 patients) , the group II using treatment (46 patients)and the group III (50) healthy controls. This study included its own questionnaire structural, where it includes the following: gender and age. Biomarkers were examined by ELISA methods and (HLA-G) it was done by PCR,PCR Sequencing methods. The age mean +SD of the untreated group of new early diagnosis of MS was recorded (35.54 ± 8.51) and the treated group MS (34.22 ± 8.24) while (36.5 ± 8.93) in normal population controls. This study showed the concentrations(IL-18, Caspase-1 and OGF) in the serum and saliva of MS patients for both groups comparison healthy controls. This study was conducted using a ROC chart for patients with MS in Iraq for biomarkers for both groups. In the current study investigated the effect of HLA-G 14bp polymorphism on MS. As well as, to genotype (+ 3142G>

Keywords: *Chronic inflammatory, biomarkers, HLA-G genotype, PCR Sequencing, ROC chart.*

Introduction

MS is not rare in Iraq but most common in chronic autoimmune inflammatory disorder , demyelination disorder and central nervous system (CNS) dysfunction in the brain and spinal cord. This disease is associated with physical disability, but due to health awareness of patients and early diagnosis in recent years, this disease has been identified, but we need more knowledge of the causes of the disease and methods of diagnosing the disease[1,2,3]. Women are mainly more likely to develop MS than males [4] .Lifestyle and environmental factors strong impact the risk of MS, such as impact of HLA-G in MS [5] . Therefore, the study aimed to evaluated in serum and saliva biomarkers such as (IL-18,Caspase-1 and OGF) as the diagnostics markers for MS [6] .

Materials and Methods

This study included the total number (100 case) and 50 healthy controls. diagnosed with MS of not less than (1) year period according to McDonald criteria by specialists in this field.

Specimen

The blood sample was collected with a capacity of 5 ml for each member and put a part of the blood sample (2 ml) in tubes EDTA for HLA-G test by PCR method and (3 ml) From each sample to be separated into serum for biomarkers test by ELISA method. As well as the saliva sample was collected with a capacity of 5 ml for biomarkers test by ELISA method. **Study protocols**

Quantitative measurement of OGF,IL-18 and Caspase-1 by ELISA Kit, according to (MyBioSource) manufactures protocol. Detecting of(14bp and 3142 bp) gene by PCR method and PCR Sequencing according to Macrogen Corporation Korea. **Statistical analysis**: It was measure statistical by the available statistical package from the SPSS-25 statistical program, then these data were analyzed with simple measures . As well as "ROC" curve technique was used.

Results

Demographic data

The distribution demographic data of studied population groups were showed , such as (age and gender) for (100) patients and (50) normal controls. Then the number of patients was divided into two groups. Age mean +SD for patients in the group with MS without treatment were registered (35.54 ± 8.51) and the other group MS with treatment (34.22 ± 8.24) while (36.5 ± 8.93) in normal population controls. The early diagnosis MS without treatment was showed (females 36, 66.7%; males 18, 33.3%) also showed the early diagnosis MS with treatment (females 30, 65.2%; males 16, 34.8%) consecutively versus (females 25, 50. 0%; males 25, 50.0%) in normal controls.

Parameters concentrations in general

Table (1**) shows parameters concentrations readings in general for both groups, as well as readings of natural controls.**

Table(1):parameters concentrations among MS.

Parameters	MS patients	Controls	P value
Serum IL-18	42.568±22.199	54.291±9.111	0.0001
Saliva IL-18	107.210±18.336	104.167±17.802	0.335
Serum Caspase-1	1406.819±1115.455	429.036±120.181	0.0001
Saliva Caspase-1	1933.957±1245.373	2385.581±929.264	0.025
Serum OGF	947.167±134.262	1046.642±63.605	0.0001
Saliva OGF	960.158±91.684	880.059±87.700	0.0001

This study showed the concentration IL-18 in the serum of MS patients for both groups(42.568 ± 22.199) and the normal controls (54.291 ± 9.111), while the concentration IL-18 in the saliva of the two groups suffering from MS without treatment and early diagnosis with treatment was the Mean +SD (107.210 ± 18.336, and the normal controls 104.167 ± 17.802, respectively).

With regard to the concentration (Serum Caspase-1) in the two groups (1406.819 ± 1115.455) and normal controls (429.036 ± 120.181). Where the level of saliva (Caspase-1) was showed significant differences in general and was at the level (p=0.025)for the early diagnosis group without treatment and treatment of early diagnosis for MS patients and normal control.

While taking into considered concentration OGF. The serum and saliva level scored a very big significant difference at (p=0.0001) for both groups in addition to normal control.

ROC for reading the biomarkers concentrations.

Table (2) indicates the measurement of the receiver operating characteristic or so-called ROC curve, which

works to measure the region of the trade-off between the sensitivity and specificity characteristic thru the sensitivity graphing against complementary values the specificity for examining that region, as well as measuring large levels to what is known as the test parameter which is less of 50% with a 95% confidence interval of region parameter concentration In each study group (MS early treatment group without treatment and the other group with treatment).

Table (2): ROC for MS patients.

Groups	Parameter	Area Under the Curve (AUC)	Std. Error	P value	95% Confidence Interval				
					Lower Bound	Upper Bound	CUT-OFF	Sensitivity	Specificity
Early MS treated	Serum IL-18	0.622	.065	0.040*	0.494	0.749	48.56	66.0	66.0
	Saliva IL-18	0.596	0.058	0.106	0.482	0.709	110.52	56.0	56.0
	Serum Caspase-1	0.766	0.056	0.0001*	0.657	0.875	729.00	62.0	100.0
	Saliva Caspase-1	0.545	0.063	0.450	0.421	0.669	2238.80	56.0	56.0
	Serum OGF	0.830	0.041	0.0001*	0.750	0.910	1009.43	72.0	71.0
	Saliva OGF	0.764	0.057	0.0001*	0.652	0.875	998.87	52.0	100.0
Early MS un treated	Serum IL-18	0.802	0.045	0.0001*	0.714	0.890	48.56	66.0	66.0
	Saliva IL-18	0.520	0.057	0.720	0.408	0.632	63.31	100.0	0.0
	Serum Caspase-1	0.789	0.053	0.0001*	0.684	0.894	522.60	74.0	74.0
	Saliva Caspase-1	0.655	0.055	0.006*	0.547	0.763	952.40	100.0	36.0
	Serum OGF	0.850	0.037	0.0001*	0.778	0.923	1009.26	72.0	71.0
	Saliva OGF	0.863	0.040	0.0001*	0.786	0.941	960.55	79.0	80.0

The current study showed that the area known as the region under the ROC curve is often quoted as follows: (0.622, 0.596, 0.766, 0.545, 0.830, 0.764) for Serum IL-18, Saliva IL-18,Serum Caspase-1, Saliva Caspase-1, Serum OGF and Saliva OGF, respectively) for the parameter concentrations used in the present study for the second group with early diagnosis treatment. This study was conducted using a ROC chart for patients with MS in Iraq.

In appending to that, the current study results showed that so called (AUC) area under the (ROC) curve is often quotable (0.802,0.520,0.789,0.655,0.850 and0.863) for Serum IL-18, Saliva IL-18,Serum Caspase-1, Saliva Caspase-1, Serum OGF and Saliva OGF of studied concentrations biomarkers respectively in light of patients with early diagnosis MS without treated .

Saliva(OGF) biomarker concerning so called (AUC) area under the (ROC) curve are accounted highly impact with high significant discernment at (P<0.01). It could be conclude, that precedent biomarker will be good pointer for diagnose MS and the disease was positively concerning early diagnosis MS without treatment.

Distribution of study results for the 14 bp gene.

This table (3) has shown the distribution of the variables that are related to the 14 bp gene studied in patients with MS and compared with healthy individuals.

Table (3): Distribution of variables HLA-G 14 bp gene.

		MS Not treated		**Control**		**P value**
		No	**%**	**No**	**%**	
14-bp rs66554220	Del/Del	-	-	16	32.0	0.0001*
	Ins/Del	36	66.7	24	48.0	
	Ins/Ins	18	33.3	10	20.0	
		MS Treated		**Control**		**P value**
		No	%	No	%	
14-bp rs66554220	Del/Del	10	21.7	16	32.0	0.001*
	Ins/Del	36	78.3	24	48.0	
	Ins/Ins	-	-	10	20.0	

The results showed that the early diagnosis MS untreated group and the control group a high statistical difference p = 0.0001. In additional the early diagnosis MS treatment group and control group at (p=0.001).

Distribution of study results for the 3142 bp gene.

Table (4) explains the distribution of the study results for the 3142 bp gene for patients with multiple sclerosis and the control group.

Table (4): Distribution of variables HLA-G 3142 bp.

		MS Not treated		**Control**		**P value**
		No	**%**	**No**	**%**	
HLA-G GG/rs180827037 G/A/T	GG	51	94.4	50	100.0	0.091
	GT	3	5.6	-	-	
CC/rs1063320 T/G/C	CC	-	-	25	50.0	0.0001*
	GG	28	51.9	13	26.0	
	GC	26	48.1	12	24.0	
AA/rs9380142 A/G	AA	45	83.3	28	56.0	0.002*
	AG	9	16.7	22	44.0	
CC/rs1610696 C/G	CC	21	38.9	12	24.0	0.040*
	GG	18	33.3	12	24.0	
	GC	15	27.8	26	52.0	

		MS Treated		**Control**		**P value**
		No	**%**	**No**	**%**	
HLA-G GG/rs180827037 G/A/T	GG	46	100	50	100.0	-
	GT	-	-	-	-	
CC/rs1063320 T/G/C	CC	-	-	25	50.0	0.0001*
	GG	13	28.3	13	26.0	
	GC	33	71.7	12	24.0	
AA/rs9380142 A/G	AA	29	63.0	28	56.0	0.483
	AG	17	37.0	22	44.0	
CC/rs1610696 C/G	CC	14	30.4	12	24.0	0.002*
	GG	-	-	12	24.0	
	GC	32	69.6	26	52.0	

Where the early diagnosis group appeared without treatment for patients with multiple sclerosis(CC / rs1610696 C / G)and control group significant statistically differences at (0.040 = p). As for were significant differences appeared (AA / rs9380142 A / G),CC / rs1063320 T / G / C and the control group at (p = 0.002), (p=0.0001), (0.091 = p),respectively.

Regarding to the treatment group for early diagnosis of MS, it was found that CC / rs1063320 T / G / C, CC / rs1610696 C / G,AA / rs9380142 A / G ,GG / rs180827037 G / A / T and the control group at (0.0001 = p), (0.002 = p), (p= 0.483),respectively.

Discussion

MS is a long-term illness that is also considered an autoimmune disease can affect the central nervous system(CNS) that leads to demyelination, sheath damage and advanced disability[7] . MS spread proportion increasing among women than men. This may be the result of sexual hormones that stimulate the inflammatory response that causes autoimmune diseases that lead to MS and play a key role in changing the course of this disease[8] . it was compatible with a studies conducted on MS, Which pose higher rate in the age group (30-39) years [9,10] .

The level IL-18 in saliva, where no statistical significant has been established between the studied groups of patients with multiple sclerosis and the normal controls, after I read a lot of studies and research on this topic, its not find any results that are close to the results reached .In this study, I found the results of the serum Caspase-1 level with high statistical significance between groups in general and healthy individuals P< 0.0001 comparison it to a level of Caspase-1 in saliva is of statistical significance between them and the groups included in the study and normal control (P<0.025). The role of the OGF is still in infancy , which made it the starting point for new studies in autoimmune diseases, especially in its role in multiple sclerosis. no confirmatory data for the low levels of OGF, which is one of the clear signs of autoimmune diseases, which led to an increasing number of studies and research on this [11] .

As far as we are concerned, we have not found results that support our current study according to the available researches. Through the study, we have found some explanations in this context. The present current study did not encounter any studying that valuated of (ROC) curve concerning studied of differently biomarkers according to groups included in the study for MS patients. In the current study investigated the effect of HLA-G 14bp polymorphism (rs66554220)on MS, found that it constitutes a significant statistical difference in the distribution of genotype results between MS patients and the normal control group.

A study conducted by Fredja et al, confirming the absence of any frequencies in the genotype , as showed The absence of any statistical bias between patients with MS and normal control. In addition, the HLA-G 14bp(ins / del) (rs66554220) genotype distribution was found to be the most prevalent among patients with multiple sclerosis and normal control [12] . Other studies conducted by (Kroner et al) reveal that there is no correlation between the HLA-G 14 bp of the insertion / deletion polymorphism(rs66554220) and patients with MS. Similar results have also been reported in MS patients in Iran and Poland[13,14,15] .

HLA-G is a non-classical gene. This gene is located in the 6p21.31 (6) chromosome region, which contains the polymorph (3142G>C (rs1063320 T / G / C), (rs9380142 A / G), (rs1610696 C / G) and (rs180827037 G / A / T), which is located in an untranslated region 3 ′ (3′UTR), where the level of HLA-G and its expression rate in promoter region are affected by polymorphism. [16] .

In other study , which was conducted on other autoimmune diseases such as RA that confirms that the +3142GG(rs1063320) genotype increases of risk of developing the disease and agree with the current study in early diagnosis group for MS only as it contains same the genotype (+3142G>C) in 3′-untranslated region (3′UTR) (rs1063320) of HLA-G polymorphism [17] .

Conclusions

It could be conclude, the role of the OGF is still in infancy , which made it the starting point for new studies in autoimmune diseases, especially in its role in multiple sclerosis. The current study indicates that the distribution of the results of the HLA-G14bp and (+3142 bp) among MS patients and controls group.

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The Covid-19 Pandemic and Developing the Legal Certainty on Bankrupcy for Health Institution in Indonesia

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Abstract

This research aims to analyze how to develop the legal certainty for health institution during the Covid-19 pandemic period. The method used in this research is the normative research method, which analyzes the related constitutional regulations and legal materials. In handling the impacts of the Covid-19 pandemic which leads to the weakening of the economy, the government has issued some economic stimuli which are not limited to direct cash aid to the impacted people, but also relaxation and restructurization programs for health institution and their business organs. The writer concludes that those things are not enough to cope with the bankruptcy which threatens the nation. Because of that, there needs to be restructurization agreements between the health institution. This is because until now, the Constitution on Bankruptcy and the Postponement of Debt Payment Obligations are the latest legislations which regulate the debtors and creditors' relation regarding their financial obligations during a financial crisis during the period of the Covid-19 pandemic to protect the health institution.

Keywords: *Health institution, Workers, Bankruptcy, Covid-19.*

Introduction

The 2019–2020 coronavirus pandemic or what is known as the Covid-19 pandemic is the phenomenon of the spread of a coronavirus disease, starting from 2019 to the whole world. This disease is caused by a new type of coronavirus, which is called the SARS-CoV-2.[1] The Covid-19 pandemic was initially detected in Wuhan City, Hubei Province, China, on December 2019. It is determined as a pandemic by the World Health Organization (WHO) on March 11th, 2020.[2] Until April 23rd, 2020, more than 2.000.000 Covid-19 cases have been reported from more than 210 countries and areas. It has caused the death of more than 195,755 people. Meanwhile, more than 781,109 were healed from this disease.[3]

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This pandemic has caused disturbances in the socio-economic sectors in the globe. There has been postponements or annulations of sporting and cultural events, and there were widespread concerns on the limited supply of goods, which causes panic-buying.[4] Misinformation and conspiration theories on the virus spread online. There have also been xenophobic and racism incidents rowards Chinese people, East Asian people, and Southeast Asian people.[5]

The International Labor Organization (ILO) recorded that as much as 81 percent of the 3.3 billion global labor, with the amout of around 2.67 billion workers have been impacted by the closing of the workplaces due to the Covid-19 pandemic. Meanwhile, 1.25 billion workers are threatened by work termination.[6] ILO estimates that the coronavirus crisis on the second quartal of 2020 has decreased 6.7 percent of the global-level work hours, which is equal to 195 million full-time workers.

Meanwhile, the sectors which are most impacted by the weakening economy due to the coronavirus include accommodation and food services, manufacturing,

retail, and also business and administrative activities. The coronavirus pandemic is the worst global crisis since the World War II.

Based on the newest study from the International Labor Organization, as much as 1.25 billion workers who are in the most impacted sectors are risked to face work termination, decreasing wage, and work hours.[6] In Indonesia, the Covid-19 disease has "forced the government" to issue some special policies by appealing the temporary termination of activities which create crowds, such as education activities at schools, work activities in companies, activities in public spaces, and also religious activities in places of worship.[7]

The Covid-19 pandemic has forced most health institution to increase their activities. Numerous health institution experience difficulties in surviving during this pandemic. The weakening of the economy due to the coronavirus has threatened health sector activities. It will cause work termination and even threaten the health institution to go bankrupt. Then, the worsening condition. In addition, many of the health institution not yet paid by government like the social security funds or even the Covid-19 subsidies fund from the government have the potential to the health institutionn to experience problems regarding liquidity and insolvency.

From January 2020 to March 2020, the number of Postponement of Debt Payment Obligations cases have increased compared to that in the same period last year. the trend of Postponement of Debt Payment Obligations cases kept on increasing because the health funds cant paid by the government because beaureaucracy circles.[8] This condition has the chance to trigger hospital or health institution to become bankrupt, which will lead to the increase of spending money but decrease income.[9]

The increase of health institution who go through the road of Postponement of Debt Payment Obligations. The slow process of the case resolution in health ministry and the long courtly process from the level of District to the ministry office pushes the health institution internal bleeding then seek credits or debt from bank or financials companies.

The increase of spending money for health services led some of them can't pay the debt. Surely the health institution seek mediation. Maybe, with the mediation do not prefer bankruptcy. This is because when bankruptcy is offered, the debtors and the lawyers may reject the proposal by offering Postponement of Debt Payment Obligations and simultaneously proposing the prospecting administrator's Postponement of Debt Payment Obligations. If the condition of the national economy causes condition to be weak, it will then cause many health institution debtors to fail in repaying the debt. The consequence is that it may cause many disputes, including the increasing cases in the Court of Trade.

The situation of the Covid-19 pandemic must be understood by health institution and workers, as parties which are both impacted. This is because no one wanted this situation to happen. The spread of the Covid-19 limits all activities in the society, including the operational activities. The operational limitation surely impacts the income of the institution. This causes the institution to have trouble in fulfilling its obligations in giving the normative rights to the workers in health institutions. The pandemic causes more spending in hazmat (protection suit, face shield, or other including respiratory device). For the workers like doctor, nurses, midwife, the Covid-19 has a wider impact, but it also regards the life of the workers and the family. [10] The Government's role is crucial in the condition of the Covid-19. The government must be able to issue some clear regulations which may protect the health institution and cut off the bureaucracy process.

To prevent more health institution from being bankrupt or health institution who take the option of being bankrupt or to temporarily terminate the operation of the health services, Shinta suggests that the government must give effective help in by relaxing the pressure of the institution's financial condition. This must be done from two directions: the first is from the aspect of the imposition of the costs, etc. The second is from the aspect of acquisition, especially in the forms or fresh capital injection and acceleration government health subsidies or health funds from government. To make sure that there are not many health institutions who experience bankruptcy, it must be guaranteed that the process would be shortcut. This is so that the financial pressure's relaxation effect of the health institution may be sensed significantly and may prevent bankruptcy.

Research Method

The research method used by the writer in this study is the normative research method. This method is used to find the suitable concrete laws and policy to be applied with the aim to solve certain legal problems.[11] In this research, the approaches used are the statute approach and the conceptual approach. The statute approach is carried out by analyzing all constitutions and regulations which are related to the legal issues being treated.[12]

Results and Discussion

The government has made efforts in making special policies for those impacted by Covid-19 through the Decree of the Financial Service Authority No. 11/ POJK.03/2020 regarding the National Economic Stimuli. With the issuing of this decree, the giving of stimuli had been applied since March 13th, 2020 to March 21st, 2020[13].

Problems arise from other companies which are not included in the Decree of the Financial Service Authority above. The number is still many, including those who have debt to non-financial service institutions like health institutions. They are impacted by the Covid-19, yet their condition is not protected because they are not part of the group which is protected by the Decree above. Another difficulty is that even though the institution is included as that which receives financial stimulus, the creditors (the banks and the funding institution) do not exactly give ease. The debt must still be repaid, meanwhile what is accommodated is perhaps only the period of repayment, for example from 1 year to 1,5 years.

Kartini Mulyani states that during the Postponement of Debt Payment Obligations, the debtors do not lose their power and rights over their wealth, yet they only lose their freedom in having authority over their wealth. In Postponement of Debt Payment Obligations, the Debtors and the Administrators must work together as one cannot act legitimately without the other.[14]

Postponement of Debt Payment Obligations or bankruptcy is the last resort in the case resolution (Ultimum Remeidium) when other efforts have failed. This means that both the creditors and the debtors must beforehand carry out some resolution efforts before going to court. In line with this, the first step of anticipation which must be carried out by the debtor is talk about his/her difficulties to the creditors as soon as possible. Considering that this difficulty comes due to the Covid-19 national disaster, which may be categorized as a force majeur, the creditors should give a credit relaxation in the form of debt restructurization. Yet, the debtors must be able to convince the creditors regarding the possibility of that restructurization.

In the view of the government, a policy which may carried out is by extending the Stipulations of Decree of the Financial Service Authority No. 11/POJK.03/2020. This is so that the financial stimulus is given to all sector impacted by the Covid-19. So, it should give financial stimulus to all sector impacted, so that it may save as many companies as possible.

The aim is surely not to think narrowly and take the side of the debtors, yet it is for the greater good, which is so that the nation's economy may grow again after the sluggishness due to the Covid-19 pandemic. There are at least three arguments which may be developed in facing the cases in court. First, is the force majeur, which is regulated in Article 1245 in the Code of Civil Law, which states that, "Cost of loss and interests must be replaced. If, due to a force majeur or an accidental phenomenon, the debtor is unable to give or to carry out their obligations, or due to the same causes, the debtor has done a prohibited action." Based on the stipulations of Article 1245 in the Code of Civil Law above, if the party which cannot carry out their obligations can prove that it is due to a force majeur, thus the other party cannot ask for the fulfillment of that achievement. Thus, in this case, the crucial point is that the debtors must be able to prove that their inability to repay is due to the force majeur of Covid-19.

Since March 2020, the government has issued some policies to prevent the spread of the coronavirus disease. One of them is through the Governmental Decree No. 21 of 2020 regarding the Large-Scale Social Restrictions to Speed the Handling of Covid-19 (Governmental Decree 21/2020). In this governmental decree, what is meant by the the Large-Scale Social Restriction is the limitation of a society's activity in an area which is allegedly infected by the Covid-19 so that the chance for the spread of that disease is minimized.

The Regional Government may also apply the Large-Scale Social Restrictions in a particular province

or regency/city by obtaining the approval of the minister who carries out the governmental role in the health sector beforehand.

The implementation of the Large-Scale Social Restrictions in various regions makes most of the industries apply the work-from-home system to decrease the mobility and others activity. Differentially with others sector, the health sector experiences increase activity. This may cause the decrease of income and profits, and may even lead to financial loss. The financial losses faced by the health institution will surely impact the creditors, which create a bad credit in repaying debt. If this keeps on going, it is not impossible that many health institutions will go bankrupt in a short time. The bankruptcy of a institution will the pandemic because the health sector collapse.

Apart from that, another regulation which regulates and protects the workers' health and safety during the Covid-19 pandemic is the Decree of the Minister of Labor 3/2020. It regulates some efforts to prevent the spread and in handling the Covid-19 at the work environment, by:

a. Carrying out guidings and monitoring towards the application of the constitutional regulations in the aspect of occupational health and safety.

b. Spreading information to all levels of the organization and the parties related who are in the Governor's area of guiding and monitoring.

c. Collecting data and reporting every case or every alleged case of the Covid-19 at workplaces to the related institutions.

d. Ordering all heads of the health sector to carry out efforts to anticipate the spread of the Covid-19 and take preventive actions such as applying the healthy and clean behavior in life by integrating the the occupational health and safety, empowering the Guiding Committee of the occupational health and safety, and optimizing the functions of occupational health services[15].

e. There may be many forms for intensive programs. One of the currently relevant ones is the production of masks, disinfectants, and other equipments needed to handle the Covid-19 pandemic. Later on, this health-intensive program for workers in health service but not yet fully transferred because a complicated bureaucracy process[16].

Conclusion

The impacts caused by this Covid 19 becomes the burden of the health institution. For example, the workers in wages in health institutions must still be paid, the increase spending for safety device for health service, Social security fund and Covid 19 Fund not yet paid by the government, etc. This causes severe legal consequences for health institution which may cause bankruptcy. The bankruptcy has big impacts to the nations. Because of that, the government needs to carry out direct treatment regarding the pandemic.

The government has had the vision of relaxation and restructurization which are only in the form of stimuli. Thus, in regulating the financial relations between the health institution, including those in the financial sector, the special civil law regulations are very significant as they give a more holistic solution and includes the resolution of all creditors owned by debtors. Because of that, according to the writer, there needs to be restructurization agreements between the parties of the health institution. The government must act and shortcut the bureaucracy process for the subsidies for health institution especially social security and Covid 19 funds including the incentives

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Impact of Active Release Technique and Core Strengthening on Pain, Muscle Stiffness, Muscle Hardness and Quality of Life on Non- Specific Low Back Pain: An Experimental Study

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Abstract

Background:- Bad posture while working puts excessive stress and strain on low back. These in turn contribute significantly to the development of the spasm–pain–spasm process, which ultimately results in the development of myofascial trigger points(MTrPs) in the muscle fibers. This is due to the reduced blood supply that leads to a decrease in the supply of oxygen and also minerals and vitamins to the muscle Active release technique(ART), a non-invasive soft tissue restoration procedure includes reducing the scar tissue that induces discomfort, stiffness, muscle weakness and unusual pain like mechanical dysfunction of the myofascial and soft tissue.

Methodology:- This experimental study will be conducted with population of 40 aging from 18 to 25 dividing them equally into two group with simple random sampling. The participants of one group will be given active release technique with hot fomentation while the other group will receive hot fomentation. Core strengthening exercises will be given to both the groups.

Conclusion:- This study intends to find the effectiveness of active release technique in reduction of pain, muscle stiffness and muscle hardness in patients with non-specific low back pain and also the resultant improvement in their quality of life

Key Words:- *Nonspecific lowback pain, ART (Active Release Technique), Myofascial Trigger Points, Quality of life*

Introduction

Lowback pain (LBP) a critical issue affecting quality of life is one of the main reason of work insecurity in developing countries[1]. It is commonly reported in late teens, early twenties or adult age[2]. Non-specific low back pain or "simple backache" is characterized as LBP not due to any identifiable disease such as pain in nerve root and infection such as spinal pathologies, tumour, rheumatoid arthritis, osteoporosis fracture or inflammation[3]. Bad posture while working puts excessive stress and strain on low back. This position of prolonged stress may lead to a continuous low intensity stimulation of the core muscles which in turn will contribute to the development of the spasm–pain–spasm process ultimately resulting in Myofascial Trigger Points (MTrPs) in muscle fibers due to the reduced blood supply as it affects the supply of oxygen, minerals and vitamins to the muscle[4].

Myofascial Trigger Points in skeletal muscles, identified with a hypersensitive palpable nodule or "knot" in the middle of the muscle belly are described as hyperirritable[5]. They are together with appreciable nodules in tight muscle fibre bands[3]. Myofascial Pain Syndrome also described as soft tissue pain syndrome with pain present in a single quadrant are often

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caused due to myofascial Trigger Points[4]. Myofascial trigger points cause tenderness in the muscle and can lead to characteristic referred pain, spasm and movement restriction[6]. These trigger points are often correlated with low-back pain, weakness of back muscles and subsequently restriction of motion. They can be a major causes of lack of mobility in individuals with nonspecific lowback pain thus impairing normal active movement control and causing sloppy trick action due to irregular patterns [7].

Despite low back pain being very common, the root cause of such aches is not well understood in many of the patients and are thus called as "nonspecific lowback pain". Stability of spine and muscular strength are prominent factors involved in the initiation and persistence of nonspecific LBP. Core musculature which is important for spinal stabilisation and also pelvic control play a cardinal role while performing functional movement. Alterations in the task of core muscles will lead to the spine becoming mechanically unbalanced with compressive forces. When the core muscle perform their task to the optimal level the muscle fibers are recruited adequately and the result is a maximal force production but with minimum shearing and compression forces when performing the functional movements[8]. The "core" is defined as a box of muscles with the abdominals on anterior and para spinals and gluteal on the posterior side. The diaphragm forms the roof and the pelvic floor along with the hip-girdle musculature is the framework[9].

Active Release Technique(ART) is a non-invasive soft tissue restoration procedure that includes reducing the scar tissue that would induce discomfort, stiffness, muscle weakness and unusual pain like mechanical dysfunction of the myofascial and soft tissue[10]. Locating and breaking down the scarred tissue and adhesions help in reducing the pain and discomfort as these adhesions and scar tissues are the primary cause of pain, stiffness, weakness as well as physical dysfunction commonly associated with soft tissue injuries. ART involves a combination of motions performed by the patient to release the adhesions between the tissue layers[11].

ART focuses on the soft tissue mobilisation by fibrosis removal, scar tissue eradication and breaking of adhesions formed in tissues through excessive and unpredictable tissue tension[12]. Deep digital pressure is applied over tender or trigger point asking the patient to move the muscle actively froma shortened to lengthened position thus breaking adhesions[4,13].

The role of musculoskeletal pain is significant in health related quality of life(HRQoL), primarily focuses on physical fitness and function. Hence, it is exceedingly instrumental in obtaining a pertinent metric assessing the quality of life of LBP patients from the patient's perspective. These utility ratings should be interpreted as quality adjusted life-years (QALYs) to enable cost-effectivenss analysis and cost-effectiveness of different LBP related interventions. The EQ-5D-5L is a standardized preference based metric for calculating HRQoL by the EuroQol committee and provides five-dimensional representation of health status. The EQ-5D-5L includes five significant indicators for each area (pain, self-care, mobility, daily tasks and distress, and depression and anxiety) and five level feedback. The questionnaire also include a visual analog scale (EQVAS) labeled with 0 (worst possible state of health) to 100 (highest practicable state of health) to determine general well-being. [14]

There is a paucity of research regarding the relevance and effectiveness of ART on lowback pain. It has proven to be helpful in pain reduction. This study aims to find out the impact of ART in nonspecific lowback pain and the expected positive results will certainly add to the treatment options available for treating nonspecific lowback pain.

Aim & Objectives

Aim:-

Through this research, we aim to evaluate impact of active release technique and core strengthening on pain, mobility and quality of life in non-specific low-back pain.

Objectives:-

i. Evaluating the impact of active release technique and core strengthening on non- specific low back pain.

ii. Analyzing the impact of active release

technique and core strengthening on muscle tenderness and hardness

iii. Examining the impact of active release technique and core strengthening on flexibility and range of motion.

iv. Studying the impact of active release technique and core strengthening on quality of life on subjects with non- specific low back pain.

v. Studying the long term effectiveness of active release technique.

Methodology

Study Design

This experimental study will be conducted in Musculoskeletal OPD of Ravi Nair Physiotherapy College, DMIMS(DU), Sawangi(Meghe), Wardha, the approval of which has been taken from Institutional Ethics Committee of DMIMS, DU, Wardha (ICE No:- 8977). Prior to participation, all subjects will be briefed on the purpose and protocol of the study and informed consent will be obtained from the subjects fulfilling inclusion ctiteria. This experimental study will be conducted with population of 40 aging between 18 to 25 divided equally into two group of 20 subjects each through simple random sampling. One group will be given active release technique in addition to hot fomentation and core strengthening exercises that will be given to both groups. Pre- test values will be noted followed by post test values which will taken after 6 sessions of ART. The patients will then be called for a follow-up every week for the next 4 weeks and all the parameters measured to know the long term effect of the treatment .

Experiment design

Experimental study in which the patients will be randomized into two group independent design. Group A or control group will undergo the classical rehab of hot fomentation with core strengthening exercises while Group B, the experimental group will receive active release technique in addition hot fomentation and core strengthening exercises.

Inclusion Criteria:- Subjects with nonspecific lowback pain aged 18 to 25 years.

Exclusion Criteria:- Subjects not willing to participate or having radiculopathies with or without neurological deficits, degeneration of spine, tumours or malignancies of back or thorax, pregnancy, any surgeries in back or thorax, anatomical deformities of spine and chest wall

Sample Size Consideration:- In this experimental study, two-group design that examines the effect of active release technique in nonspecific lowback pain. 40 individuals will be registered to the control group or experimental group and will be randomized using simple random sampling.

Interventions

40 participants will be selected from among the patients with nonspecific lowback pain coming to OPD and will be tested for algometer tenderness, durometer muscle hardness and biofeedback pressure muscle strength. They will then be randomly divided equally by sampling formula in experimental and control groups. Pre and post- test results will be reviewed[15–18].

Experimental group

These patients will receive ART in addition to the conventional treatment of hot fomentation and core strengthening exercises. The subjects will first be palpated for tender points in prone lying. They will then be given hot fomentation in supine. The subject will then be asked to sit and the therapist will palpate the tender point standing behind the patient and the subject will contract the muscle by doing its action. Pressure is added while the patient relaxes or does the muscle action and it will be done for 6 sessions of 8- 10 min. Core strengthening exercises will be given to prevent re-occurrence of pain[10–12,19–24].

Control group

This group subjects will also first be palpated for tender points in prone lying followed by hot fomentation. Core strengthening exercises will be given to prevent re-occurrence of pain.

Core Strengthening (to both groups)

This will include both static and dynamic exercises. The static exercises will be repeated 10 times with hold time of 10 sec in one set. The subjects should hold

the core muscle by drawing in manoeuvre. They will perform abdominal drawing-in manoeuvre[8]. Dynamic exercises will be repeated 10 times with hold time of 10 sec in one set[25].

The following exercises will be included in core strengthening:

Bridging:-Participants will maintain a supine position and with shoulder and elbow assistance they need to raise their hips so that a straight line is achieved. Hips are raised and lowered continuously at a slow level for 10 repetitions.

Quadrupedal stance:- Participants to remain in a quadruped posture with their knees and hands placed on surface and raise one arm and the contralateral leg in a horizontal position at a medium speed.

Cross curl-ups:- Participants will be supine with the hands crossed behind the head and elbows pointing towards the sides. With the legs in a flexed posture and feet on the surface, the individuals will turn left and right and rotate at a moderate speed[26,27].

Data Analysis

NPRS

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

ROM

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

Algometer

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

Durometer

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

Pressure biofeedback

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

Oswestry Low Back Pain Disability Questionnaire

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

EQ-5D-5L

	ART	Conventional	p	chi
Pre-test				
Post-test				
First Week				
Second Week				
Third Week				
Fourth Week/ 1 month				

Outcome Measures

o Algometer:- An equipment used to measure muscle tenderness.

o Durometer:- An equipment used to measure muscle hardness.

o Oswestry Low Back Pain Disability Questionnaire:- A questionnaire used to assess and evaluate the challenges encountered by people with low-back pain (with permission)[28].

o EQ-5D-5L:- It is a scale often used to evaluate health related quality of life and understand which component the patient is suffering due to his pain or disability (with permission).

o Pressure Biofeedback:- An equipment used to measure pressure given by the body while doing a particular exercise. It can be used to record the improvement by measuring the pressure applied.

o Numeric Pain Rating Scale:- Used for pain measurement. It has eleven point rating from 0- 10 where 0 is no pain 5 is normal or tolerable and 10 being worst bearable pain[29].

Follow- up

All patients will be monitored continuously for 4 weeks following the 6 therapy sessions. The time of the last rehabilitation training session will be recorded. When patients drop out of the study, the reasons for withdrawal will be recorded in detail. Comprehensive and supportive patient communication will be undertaken to avoid drop out. Data of individuals withdrawing from the trial would be included in the final report on the basis of the intention-to-treat analysis concept.

Data Management:- The data from the study will be stored in a safe, restricted storage facility where a bio-statistician and the researcher will have restricted access for later examination.

Statistical Analysis:- To perform the comparison between the two groups, collected data will be noted in tabular format. Data analysis will be done using SPSS.

Discussion

In this report we seek to establish the impact of the active release technique and core strengthening on pain, muscle stiffness, muscle hardness and quality of life in nonspecific low back pain. Active Release Technique works by simultaneously locating and breaking down of scarred tissue and adhesion which is one of the primary cause of pain, stiffness, weakness, as well as physical dysfunction associated with soft tissue injuries. It is a combination of motions performed by the patient to release the adhesions between the tissue layers. This should release the pain coming from myofascia and myofascial trigger point by breaking the adhesions in soft tissue thus re-lengthening the taught muscles and reducing spasm ultimately contributing to an enhancement in quality of life. Studies have been done on trapezius and hamstring but limited study in low back pain specifically in those with non- specific origin.

This study thus seeks to assess the efficacy of the Active Release Technique in nonspecific low back pain and also the impact on quality of life. This study will help to enhance the credibility of ART as a treatment alternative for patients with nonspecific low back pain.

Ethical Approval - Approval has been accorded by the IEC, DMIMSU

Consent - The principal investigator will obtain informed consent from all the participants of the study and the consent form will also include confidentiality statement with signature of the principal investigator.

Conflict of Interest – The authors claim no conflict of interest.

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Recruitment:- Ethics and dissemination

Study Period								
	Enrolment	**Allocation**	**Intervention**					**Result**
Duration			**Pre**	**1w**	**2w**	**3w**	**4w**	
Enrolment:-								
Pre-test Screen	X							
Obtained Consent	X							
(List other procedures)								
Allotment	X							
Interventions:-								
ART + HPK				X				
HPK				X				
Assessments:-								
Algometer			X	X	X	X	X	
Durometer			X	X	X	X	X	
Biofeedback			X	X	X	X	X	
ODI			X	X	X	X	X	
EQ-5D-5L			X	X	X	X	X	
NPRS			X	X	X	X	X	

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The Correlation of EMMPRIN and EGFR Overexpression toward Muscle Invasiveness in Urothelial Carcinoma of Bladder

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Abstract

Urothelial carcinomas represent 90% of all primary bladder cancers. Muscle invasion is a critical prognostic determinant in urothelial carcinoma. The overexpression of EMMPRIN and EGFR was found in urothelial carcinoma. The association between the two markers has not been reported in urothelial carcinoma, therefore we aimed to analyze the expression of EMMPRIN and EGFR and investigate their association with urothelial carcinoma invasiveness. Paraffin-embedded tissues were obtained from 54 urothelial carcinoma patients which then underwent immunohistochemistry staining for EMMPRIN and EGFR antibody. The comparison of EMMPRIN and EGFR expression was tested using the Mann Whitney U test. The correlation was analyzed using the Spearman test. Results showed a significant difference of EMMPRIN expression between non-muscle-invasive and muscle-invasive bladder cancer ($p = 0.000$), and EMMPRIN expression was significantly correlated with the muscle invasion ($r_s = 0.481$, $p = 0.000$). A significant difference of EGFR expression between the non-muscle-invasive and muscle-invasive bladder cancer was also found ($p = 0.020$), and EGFR expression was significantly correlated with the muscle invasion ($r_s = 0.319$, $p = 0.019$). The expression of EMMPRIN was positively correlated with EGFR in urothelial carcinoma ($r_s = 0.322$, $p = 0.018$). The expression of EMMPRIN and EGFR are two potential biomarkers for urothelial carcinoma invasiveness which may be helpful to differentiate between muscle-invasive and non-muscle-invasive bladder cancer.

Keywords: *Urothelial carcinoma, muscle invasiveness, EGFR, EMMPRIN.*

Introduction

Bladder cancer is the most common cancer in the urogenital system and urothelial carcinoma accounts for about 90% of all primary bladder cancer[1]. The most important factor for determining prognosis in urothelial carcinoma is the depth of invasion[2]. Depending on the presence of muscularis propria invasion, pT1 tumor is classified as non-muscle-invasive bladder cancer (NMIBC), while pT2 tumors and above are classified as muscle-invasive bladder cancer (MIBC)[3].

It is important to identify tumor invasion of the muscularis propria; the presence of which determines the therapeutic approaches. While accurate identification of muscularis propria invasion is crucial, it is considered as a challenge to evaluate muscle invasion solely on H&E. Because of this reason, the detection of abnormal expression of biological markers for urothelial carcinoma invasiveness, specifically muscle invasiveness, would be very helpful to determine the presence of muscle invasion in the tumor and assist doctors in providing a suitable treatment for patients[4].

Extracellular matrix metalloproteinase inducer (EMMPRIN), other names include CD147 and basigin, is a transmembrane glycoprotein of the immunoglobulin superfamily. It is highly expressed on the cell surface of many malignant tumors, including liver, breast, colon, prostate, and esophageal cancer[5]. Overexpression of

EMMPRIN is found in 71.2% of urothelial carcinoma[6]. EMMPRIN is best-known to stimulate the production of some matrix metalloproteinases (MMPs) in tumor cells and fibroblasts. MMPs are major proteases in degrading the extracellular matrix, leading to cancer cell invasion and metastasis[7].

Epidermal growth factor receptor (EGFR) is a tyrosine kinase transmembrane receptor that plays an important role in carcinogenesis[1]. It is overexpressed in many tumors, such as head and neck, lung, colon, breast, kidney, prostate, and bladder cancer[1]. EGFR is involved in the regulation of MMPs, several studies have shown that EGFR stimulation induces MMPs expression[8]. Therefore, this study was conducted to analyze the role of EMMPRIN and EGFR in urothelial carcinoma, also their relationship, specifically toward the tumor invasion.

Materials and Methods

Research Design and Sample

This study was analytic observational research with a cross-sectional approach. A total of 54 formalin-fixed, paraffin-embedded tissues were obtained from patients diagnosed as urothelial carcinoma and underwent Radical Cystectomy (RC) during 2010-2019 in the Anatomical Pathology Laboratory, Dr. Soetomo General Hospital. Samples were divided based on their muscle invasion, pT1 tumor was classified as non-muscle-invasive bladder cancer (NMIBC) and pT2-4 tumor was classified as muscle-invasive bladder cancer (MIBC).

Immunohistochemistry Staining

The tissues were cut into 4mm sections, deparaffinized three times with xylol for 5 minutes each, and rehydrated through graded alcohol. Antigen retrieval was accomplished by incubation for 10 minutes in sodium citrate buffer (pH 6.0). Afterward, the tissue sections were incubated with monoclonal antibodies for EMMPRIN (1.BB.218: sc-71038; dilution 1:250; Santa Cruz Biotechnology) and EGFR (0.N.268: sc-71034; dilution 1:100; Santa Cruz Biotechnology) overnight, followed by a secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol. Human placental tissue was used as a positive control for EGFR while human gastric tissue was used as a positive control for EMMPRIN.

Evaluation of Immunohistochemical Expression

All samples were evaluated by two pathologists in the blinded fashion. Any discordant was solved by interobserver agreement. Membranous and cytoplasmic staining of EMMPRIN and EGFR were evaluated on the basis of the percentage of stained tumor cells and the staining intensity of the tumor cells. The staining intensity for both EMMPRIN and EGFR was scored as 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). For EMMPRIN, the percentage of stained tumor cells was scored as 0 for no 0 - <10% positive cells; 1, 10% - 24%; 2, 25% - 49%; 3, 50% - 74%; and 4, ³75%. The sum of the percentage of stained tumor cells and the staining intensity score was then used as the final EMMPRIN score. Final scores of 0 to 1 were considered negatives, 2 to 3 were considered weak, 4 to 5 were considered moderate, and 6 to 7 were considered strong[9].

For EGFR evaluation, the percentage of stained cells score was slightly different from EMMPRIN, as follows: 0 for no positive cells; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, >75%. Then, a final score was obtained by multiplying the score of staining intensity and percentage of stained cells: a score of 0 was considered negative, 1–4 was weak, 5–8 was moderate, and 9 - 12 was strong[1].

Statistical Analysis

All statistical analysis was calculated using SPSS v.25.0. The comparison of EMMPRIN and EGFR expression was tested using the Mann Whitney U test. The correlation was analyzed using the Spearman test, with a significance level <0.05 (p <0.05).

Results and Discussion

The average age of patients was 60.6 ± 9.4 years with a male to female ratio of 50:4. In the study, muscle-invasive bladder cancer (MIBC) was found in 81.5% (44/54) cases, whereas non-muscle-invasive bladder cancer (NMIBC) was only found in 18.5% (10/54) cases. Around 87% of the cases (47/54) were high-grade, whereas 13% (7/54) were low-grade.

EMMPRIN Expression in Urothelial Carcinoma

EMMPRIN was found to be expressed at the plasma membrane and/or cytoplasm (Figure 1). We compared EMMPRIN expression between non-muscle-invasive and muscle-invasive bladder cancer, also between low grade and high-grade tumors. A significant difference of EMMPRIN expression was found between the non-muscle-invasive and muscle-invasive tumors (p = 0.000), while no significant difference of EMMPRIN expression was found between the low grade and high-grade tumor (p = 0.105). Strong EMMPRIN expression was mainly found in tumors with muscle invasion (52.3%), while none of the non-muscle-invasive tumors showed strong EMMPRIN expression. Spearman correlation test showed a highly significant positive correlation between EMMPRIN expression and muscle invasion of the tumor (r_s = 0.481, p = 0.000) (Table 1).

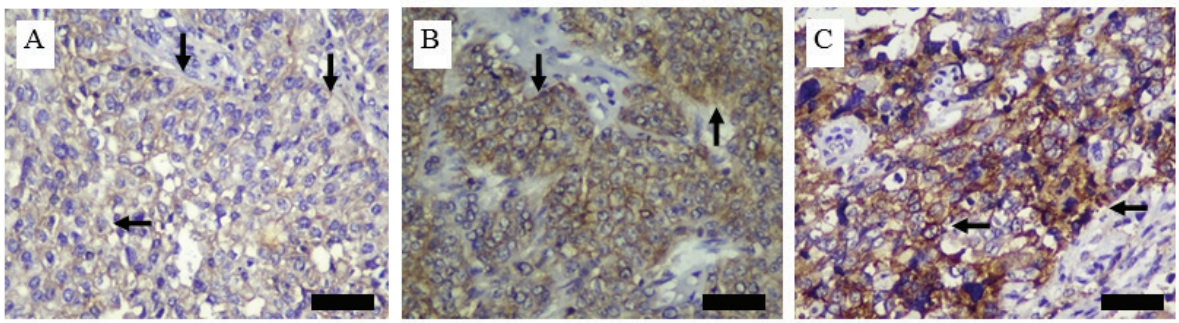

Figure 1. Immunohistochemical expression of EMMPRIN in urothelial carcinoma (brown stain in membrane and/or cytoplasm), 400x magnification. A: Weak intensity staining of EMMPRIN; B: Moderate intensity staining of EMMPRIN; C: Strong intensity staining of EMMPRIN. Black arrows: EMMPRIN expression; black bars: 50 µm.

Table 1. Association between EMMPRIN expression with muscle invasion and tumor grade.

	EMMPRIN Expression				p-value a	rs b	p-value b
	Negative n (%)	Weak n (%)	Moderate n (%)	High n (%)			
Muscle Invasion					0.000	0.481	0.000
Absent (pT1) / NMIBC	1 (10)	3 (30)	6 (60)	0 (0)			
Present (pT2-4) / MIBC	0 (0)	3 (6.8)	18 (40.9)	23 (52.3)			
Tumor Grade					0.105	-	-
Low Grade	1 (14.2)	2 (28.6)	2 (28.6)	2 (28.6)			
High Grade	0 (0)	4 (8.5)	22 (46.8)	21 (44.7)			

[a]Mann Whitney U test applied.

[b]Spearman correlation test applied.

p-value <0.05, considered as significant.

EGFR Expression in Urothelial Carcinoma

EGFR was found to be expressed at the plasma membrane and/or cytoplasm (Figure 2). We compared EGFR expression between non-muscle-invasive and muscle-invasive bladder cancer, also between low grade and high-grade tumors. We observed a significant difference of EGFR between the non-muscle-invasive and muscle-invasive tumors ($p = 0.020$), while no significant difference of EGFR expression was found between the low grade and high-grade tumor ($p = 0.079$). Moderate and strong EGFR expressions were only found in the muscle-invasive tumor, while the non-muscle-invasive tumor only showed negative and weak EGFR expression. Spearman correlation test showed a significant positive correlation between EGFR expression and muscle invasion of the tumor ($r_s = 0.319$, $p = 0.019$) (Table 2).

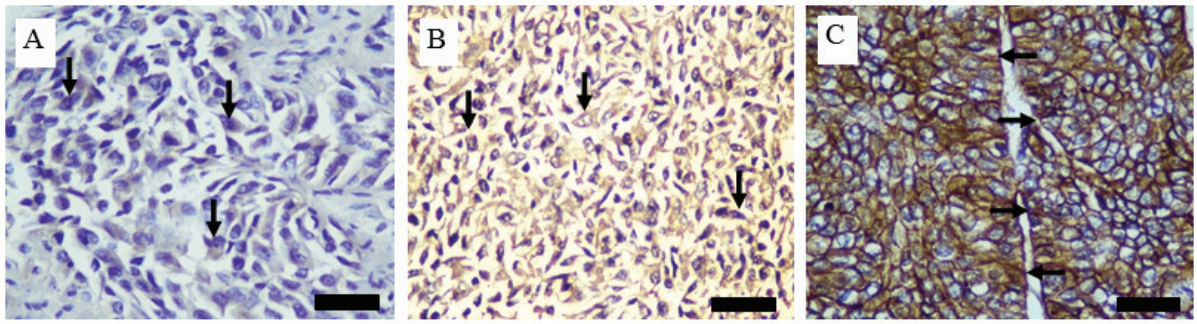

Figure 2. Immunohistochemical expression of EGFR in urothelial carcinoma with 400× magnification. A: Weak intensity staining of EGFR; B: Moderate intensity staining of EGFR; C: Strong intensity staining of EGFR. Black arrows: EGFR expression; black bars: 50 µm.

Table 2. Association between EGFR expression with muscle invasion and tumor grade.

	EGFR Expression				***p*-value [a]**	**r_s [b]**	***p*-value [b]**
	Negative n (%)	**Weak n (%)**	Moderate n (%)	High n (%)			
Muscle Invasion					0.020	0.319	0.019
Absent (pT1) / NMIBC	6 (60)	4 (40)	0 (0)	0 (0)			
Present (pT2-4) / MIBC	11 (25)	24 (54.5)	7 (16)	2 (4.5)			
Tumor Grade					0.079	-	-
Low Grade	4 (57.1)	3 (42.9)	0 (0)	0 (0)			
High Grade	13 (27.7)	25 (53.2)	7 (14.9)	2 (4.2)			

[a]Mann Whitney U test applied.

[b]Spearman correlation test applied.

p-value < 0.05, considered as significant.

Correlation between EMMPRIN and EGFR in Urothelial Carcinoma

A significant positive correlation was found between EMMPRIN and EGFR expression in urothelial carcinoma ($r_s = 0.322$, $p = 0.018$).

Discussion

The age of the patients observed in this study ranged from the age of 34 to 81 years (mean 60.6±9.4). In this study, the majority of the patients had muscle-invasive bladder cancer/MIBC (pT2-4) (81.5%), whereas non-muscle-invasive bladder cancer/NMIBC (pT1) was only found in 18.5% of the cases. Our study showed that EMMPRIN expression was significantly different between non-muscle-invasive bladder cancer/NMIBC (pT1) and muscle-invasive bladder cancer/MIBC (pT2-4), and EMMPRIN expression is significantly correlated with the muscle invasion, with a positive r-value of 0.481. Similar results were reported by other studies, showing a significant difference of EMMPRIN expression between superficial (£pT1) and muscle-invasive (³pT2) urothelial carcinoma[10]. Another study found that negative or low EMMPRIN expression was strongly correlated with non-muscle-invasive urothelial carcinoma (pT1 tumor) compared to muscle-invasive urothelial carcinoma (pT2-4 tumor)[6]. EMMPRIN stimulates the tumor cells and peritumoral fibroblasts to produce matrix metalloproteinases (MMPs), which then degrade the extracellular matrix of basement membranes, which normally play a role as mechanical barriers to tumor cell migration and invasion[11]. EMMPRIN induce MMPs production through the MKK-p38-MAPKAP signaling pathway, and the induction of MMPs by EMMPRIN occurs at the transcription level[12].

Regarding EGFR expression, a significant difference of EGFR expression was also found between non-muscle-invasive bladder cancer/NMIBC (pT1) and muscle-invasive bladder cancer/MIBC (pT2-4), and EGFR expression is significantly correlated with the muscle invasion in this study with a positive r-value of 0.319. This result is in accordance with a prior study that found a significant association between EGFR expression and muscle invasion in urothelial carcinoma, with strong EGFR expression, which was mainly found in the muscle-invasive tumor, while negative and weak EGFR expression was mainly found in the non-muscle-invasive tumor. A prior study found that EGFR overexpression was significantly associated with tumor grade which is in contrast with the result of this study that found no significant association between EGFR expression and tumor grade[4]. Several studies showed that basal tumors of urothelial carcinoma expressed high levels of EGFR, CD49 and Cyclin D1. A prior study showed no EGFR mutations were identified in all urothelial carcinoma cases observed, hence it was hypothesized that EGFR overexpression is the result of deregulation of the protein recycling and degradation pathway[13]. EGFR expression is higher in urothelial carcinoma than in normal urothelium[14]. EGFR signaling triggers Ras activation, this process can increase MAPK activity, which activates important regulators of the epithelial-mesenchymal transition[3].

We found that EMMPRIN expression was significantly correlated with EGFR expression with a positive r-value of 0.322. To our knowledge, this correlation has not been reported in urothelial carcinoma, although another study described a correlation between the two markers in colorectal adenocarcinoma[15]. A prior study suggested that EGFR might play a role in regulating EMMPRIN expression and is relevant for the tumor invasion in urothelial carcinoma[16]. Elevated EMMPRIN expression was able to stimulate hyaluronan production in tumor cells and hyaluronan is the main ligand for the cell surface receptor CD44. Hyaluronan and CD44 interaction induce the assembly of lipid raft-associated complexes containing EMMPRIN, CD44 and EGFR[17,18,19].

Conclusion

The expression of EMMPRIN and EGFR are potentially significant biomarkers of urothelial carcinoma invasiveness and that may be helpful to discriminate between muscle-invasive and non-muscle invasive bladder cancer, where urothelial carcinoma with EMMPRIN and EGFR overexpression is found more likely in urothelial carcinoma with muscle invasion. The EMMPRIN and EGFR overexpression further help to determine the patients who need more radical treatment.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Autopsy Study of Organ Weights in a Tertiary Care Centre in Kerala

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Abstract

Background : Organ weight and its morphometric parameters are one of the most important indicators of possible organ defects or diseases, therefore, many studies have evaluated the normal range of body and organ morphometric parameters, such as organ size, weight, and volume, in different ethnic groups all over the world. Weighing organs during Autopsy is not a mere physical exercise, but carries great medico legal significance.

Methods: A retrospective study was conducted by the Department of Forensic medicine at Government TD Medical College Alappuzha from autopsy data collected between January 2013 and December 2018. A total of 1206 cases (936 males and 270 females) were included in the study on the basis of inclusion criteria and the findings were recorded in the pre-designed proforma and analyzed.

Conclusions : The average age of patients was 46.81 (between the ages 18 and 98), males 46.68(between the ages 18 and 95) and females 47.23 (between the ages 18 and 98). In Males, the average weight of the brain was 1266.39g (between 700 and 1976g), right lung was 484.41g (between 110 and 1400g), left lung was 425.09g (between 83 and 1426g), Heart was 289.56g (between 112 and 732g), Liver in male was 1356.99g (between 116 and 2822g) , spleen in male was 107.79 (between 12 and 948g) , right kidney was 119.53g (between 16 and 270g) and left kidney in male was127.16 (between 46 and 886g).

In females the average weight of the brain was 1140.11g (between 725 and 1620g), right lung was 346.05g (between 98 and 992g), left lung was 303.43g (between 22 and 760g), was 241.13g between (68 and 479g); Liver was 1171.78g (between 550 and 2880g); spleen was 100.16g (between 18 and 509g); right kidney was 101.91g (between 36 and 624g) and left kidney was 104.23g (between 20 and 234g).

Keywords-- *Autopsy, Liver, Lung, Heart, Spleen, kidney, organ weight.*

Introduction

Organ weight and its morphometric parameters are one of the most important indicators of possible organ defects or diseases.[1]Therefore, many studies have evaluated the normal range of body and organ morphometric parameters such as organ size, weight and volume in different ethnic groups all over the world.[2] Weighing organs during Autopsy is not a mere physical exercise, but carries great medico legal significance.[3] Organ weights are important benchmarks in disciplines like surgery , pharmacology and radiation medicine.[4] Organomegaly is considered as a sign of disease and can only be established if the reference range of data for an internal organ is available. Identification of organomegaly helps identify risk factors for certain diseases like sudden cardiac death.[5]Organ weights also play a significant role in estimation of body weight and height and thereby establishment of identity of an individual.[6]Standard textbooks such as Gray's Anatomy and Ludwig give normal reference weight of organs.[7,8] But, most of these use Caucasoid population which might not correlate with Indian population. There is great variation in the organ weights amongst the various races in the world, just as there is in stature and appearance. Therefore, this study aimed to assess the reference range of values of various organs in the population of Kerala.

Aims and Objectives

- To obtain a reference value for various organ weights in the population of Kerala
- To compare the organ weights amongst sexes in the various age groups

Materials and Methods

Source of Data

This retrospective study is being conducted by the Department of Forensic medicine at Government TD Medical College Alappuzha. This study analyses data from January 2013 to December 2018. A total of 1206 cases (males and females were included in the study on the basis of inclusion criteria. The study was conducted after approval from IEC (50/2019 dated 20/06/2019).

Inclusion Criteria

All cases subjected for autopsy from Jan 2013 to Dec 2018 whose completed profile and data were known are included in this study.

Exclusion Criteria

Decomposed bodies

Gross organ pathology

People with congenital anomalies/ Manifest macroscopic disease or anomaly

History of prolonged medical illness

Procedure

The data was collected from the post mortem records and standard protocol was maintained for collection of parameters. The weight of the naked body was taken using an electronic weighing machine. The height of the decedent is measured in centimeters using measuring tape in the supine position from head to heel. Standard autopsy protocol and procedures proposed by Ludwig was employed for organ removal. Organ weight measurements were made using an electronic weighing scale having accuracy of +0.1gm, after the removal of foreign tissues.

Statistical Analysis

The data was entered into Microsoft Excel work sheet and was analyzed using Statistical Package for Social Service SPSS software version 16. Descriptive statistics such as frequency, percentage, minimum, maximum, mean ±SD and range were used.

Results and Discussion

1206 cases met with all criteria prescribed for the study during the period of January 2013 to December 2018. The data was obtained from the records stored in the department and the cross-sectional retrospective study conducted. (All results in table form Table 1 & 2)

The gender distribution of the study participants was that 936 were female (77.6%) and 270 (22.4%) were female. The age distribution was evenly distributed in the following manner: 18 -30 years- 263 (21.8%), 31-40 years - 212 (17.6%), 41-50 years – 224 (18.6%), 51-60 years – 242 (20.1%), 61-70 years – 159 (13.2%), 71-80 years – 79 (6.6%), more than 80 years. The mean age of the study population was 46.81+/- 17.07. In females the mean age was 47.23+/-19.60(18-98) while in males it was 46.68+/-16.27 (18-95).

The mean organ weight of Brain in males was 1266.39(1976-700) and in females was1140.11 (1620-725). The mean organ weight of Right lung in males was 484.41(1400-110) and in females was 346.05 (992-98). The mean organ weight of Left lung in males was 425.09(1426-83) and in females was 303.43(760-22). The mean organ weight of Heart in males was 289.56 (732-112) and in females was 241.13 (479-68). The mean organ weight of Liver in males was 1356.99(2822-116) and in females was 1171.18 (2880-550). The mean organ weight of Spleen in males was 107.79(948-12) and in females was 100.16 (509-18). The mean organ weight of Right kidney in males was 119.53 (270-16) and in females was 101.91 (624-36). The mean organ weight of Left kidney in males was 127.16 (886-46) and in females was 104.23 (234-20).

Table 1 : ORGAN WEIGHT IN VARIOUS AGE GROUPS IN MALES

Age group	Variables	Brain	Right lung	Left lung	Heart	Liver	Spleen	Right kidney	Left kidney
18-30 (n=192)	Mean	1306.73	443.27	396.02	262.05	1323.82	116.57	115.79	123.40
	SD	126.36	167.09	141.97	54.91	328.73	51.25	28.44	27.62
	Range	754	962	880	334	2334	310	254	200
31-40 (n=170)	Mean	1271.19	452.34	400.52	278.78	1501.23	118.81	121.83	128.08
	SD	133.48	174.15	151.73	63.93	353.22	65.19	29.36	29.90
	Range	820	1240	1194	382	1804	448	190	210
41-50 (n=184)	Mean	1276.96	505.92	448.36	290.83	1449.97	108.63	129.19	139.96
	SD	133.68	190.21	176.69	65.92	323.44	55.12	31.39	64.05
	Range	1276	1246	1290	584	2096	326	222	804
51-60 (n=199)	Mean	1260.08	502.74	446.45	306.70	1363.18	102.98	123.80	129.94
	SD	136.32	157.74	150.47	75.24	329.20	52.73	32.71	33.72
	Range	972	840	854	478	2060	392	161	210
61-70 (n=123)	Mean	1236.96	501.92	432.27	305.60	1203.04	91.03	109.49	117.02
	SD	120.08	154.68	129.90	66.39	267.47	44.66	27.37	31.94
	Range	634	992	840	360	1248	230	162	226
71-80 (n=54)	Mean	1195.61	553.89	441.33	314.43	1128.28	86.42	106.72	113.20
	SD	134.07	232.19	174.84	90.23	265.46	34.24	28.35	32.62
	Range	736	1020	867	508	1015	146	152	192
>80 (n=14)	Mean	1137.14	472.86	387.00	300.71	984.86	140.28	92.57	102.29
	SD	86.50	208.94	118.57	63.16	211.24	235.06	20.31	19.38
	Range	252	774	368	238	570	912	70	54

Table 2 : ORGAN WEIGHT IN VARIOUS AGE GROUPS IN FEMALES

Age group	variables	Brain	Right lung	Left lung	Heart	Liver	Spleen	Right kidney	Left kidney
18-30 (n=71)	Mean	1182.93	300.55	271.49	192.55	1130.65	99.66	98.76	104.24
	SD	99.90	101.62	106.82	42.21	283.89	41.27	32.57	29.13
	Range	594	574	622	240	1226	218	216	184
31-40 (n=42)	Mean	1152.86	407.86	352.02	237.29	1282.62	131.95	110.98	113.88
	SD	110.54	174.83	131.49	53.10	407.27	72.61	36.30	36.54
	Range	576	798	546	224	2240	444	214	180
41-50 (n=40)	Mean	1166.68	364.53	297.23	236.25	1232.10	102.05	103.83	112.93
	SD	130.22	128.80	92.06	55.23	321.52	74.51	26.67	34.54
	Range	716	566	410	256	1506	475	142	142
51-60 (n=43)	Mean	1116.02	358.30	332.47	263.70	1209.91	105.79	111.98	105.70
	SD	138.77	139.12	136.97	55.76	230.83	63.19	83.95	32.81
	Range	895	594	616	288	930	319	556	146
61-70 (n=36)	Mean	1128.44	333.75	291.14	280.81	1147.81	83.38	96.67	96.67
	SD	103.75	109.83	105.56	47.72	303.96	37.35	27.66	22.01
	Range	408	482	394	198	1164	194	106	82
71-80 (n=25)	Mean	1083.80	332.64	304.80	282.56	1126.84	82.56	89.88	95.76
	SD	100.85	147.56	105.06	51.99	265.75	33.80	24.34	21.82
	Range	360	624	422	236	1246	150	104	90
>80 (n=13)	Mean	1003.69	357.38	275.23	269.77	879.38	56.07	88.31	78.69
	SD	76.43	107.52	72.01	88.14	248.82	29.37	34.59	27.80
	Range	232	340	240	291	738	97	110	100

Table 3 : Comparative study of Mean Organ weight in Grams in Males

	BRAIN	RIGHT LUNG	LEFT LUNG	HEART	LIVER	SPLEEN	RIGHT KIDNEY	LEFT KIDNEY
Present Study	1266.39	484.41	425.09	289.56	1356.99	109.71	119.53	127.16
Kohli & Aggarwal Delhi9	1219.7	417.6	376.8	259.9	1210.9	131.3	108.3	104.2
Tanna1	1392-1025	560-435	546-392	352-260	1812-895	154-99	136-114	151-116
Modi's text book10	1342.86	514.55	469.65	283.5	1241.16	170.95	103.19	170.10
Sahni et al11							108.7±22.6	118.8±23.3
Singh et all4	1324.05	568.85	516.62	291.87	1426.62	155.17	128.15	137.74
Jain et al12	1255.3±170.8	980.6±144		250.5±58.4	1325.8±192	149±53.6	211.1±42.4	
Batra et13	1213.6	365.3	346.6	277.6	1205.3	191.6	113	114.3
Puttaswamy14	1325.06	569.9	516.65	291.9	1427.72	155.18	128.18	137.75
Molina DK5,15	1407	445	395	331	1561	139	129	137
Chandan et al18	1209.25±129.37	549.95±187.54	476.10±168	292.27±87.56	1246±357.59	142.47±107.37	133.86±50.73	126.26±49.47
Narongchai19	1311	321	296	291	1252	104	106	112
Mathuramon20	1330	831		302	1390	96	252	
Mubbunu21	1335±125.5	504.6±174.6	442±152.3	279.2±44.9	1285.3±270.1	169.9±107.2	110±22.8	117.9±27.4

Table 4 : Comparative study of Mean Organ weight in Grams in Females

	BRAIN	RIGHT LUNG	LEFT LUNG	HEART	LIVER	SPLEEN	RIGHT KIDNEY	LEFT KIDNEY
Present Study	1140.11	346.05	303.43	241.13	1171.78.	100.16	101.91	104.23
Kohli & Aggarwal Delhi	1156.2	369.7	340.6	217.7	1092.5	121.8	98.6	98.3
Tanna1	1198-845	515-348	506-324	325-226	1285-885	128-89	135-89	140-98
Modi's text book	1085.52	382.78	333.12	184.28	1084.39	145.27	87.32	83.63
Sahni et al							96.6±19.3	99.4±25.3
Singh et all	1195.73	421.2	392.87	235.69	1292.9	132.17	117.35	125.90
Jain et al	1220.4±119.5	966.2±166.5		238.7±37.3	1283.1±195.1	146.4±28.5	206.5±32.1	
Batra et al 13	1077.6	309.6	297	215.6	1055	113.6	86.3	89
Molina DK 16,17	1233	340	299	245	1288	115	108	116
Chandan et al	1125.16±107.16	438.04±125.8	382.56±104.8	272.01±76.79	1175.83±300.82	123.75±64.17	124.94±45.99	119.07±45.44
Narongchai19	1170	271	251	246	1106	77	97	98
Mathuramon20	1208	659		259	1211	81	222	
Mubbunu21	1228.3±76.4	405 ±116	365.7±119.8	268.4±64.2	1367.9±357.2	161.7±93.4	101.2±20.3	108.3±23

Brain

The weight of the brain is maximum in the age group 18-30 years and its mean is 1306.73 in males and 1182.93 in females in this group. In a study conducted by Batra [13] the weight of brain was maximum in the age group 31- 40 years and its mean was 1254 in males. Singh et al [4] also identified the 31-40 years age group to have maximum weight of brain in males which was 1336.85, while Murkey[22] identified the brain weight in this age group to be 1268.14 . In his study Batra [13] identified the female brain to weigh the most in the age group 11-20 years (1132), while Singh et al [4] (1210.21) and Murkey[22](1233.65) identified that it was highest in the age group 21-30 years. Irrespective of sexes, in the study conducted on Thai population[20] the maximum brain weight was obtained in the age group 11-20 years. Similar to this study it was noted that the weight of the brain decreases as age increases in both sexes.[13,4, 22]. It was also noted that irrespective of the age groups the brain weighed less in the female population.

Lungs

It should be remembered here that, in acute deaths the postmortem lung weight may primarily depend on an individual lung volume and persistent circulatory disturbance (congestion and edema) before death which is related to vital activity and survival time, this causes the lung weights to change easily after death[23]. In all the studies analyzed here it was noted that the right lung was heavier than the left lung in both sexes. The weight of the Right lung is maximum in the age group 71-80 years in males and is 553.89, while in females it is maximum in the age group 31-40 years (407.86). The weight of the Left lung is maximum in the age group 41-50 years in males and is 448.36 in males, while in females it is maximum in the age group 31-40 years and is 352.02. The data regarding the decrease of lung weights in females as age progresses correlates with the other studies [13, 4, 20, 22]. It was noted that the lungs were lighter in females than in males

Heart

The weight of the heart is maximum in the age group 71-80 years for both sexes and its mean is 314.43 in males and 282.56 in females in this age group. It was noted that the weight of the heart progressively increases up to the weight of 80years beyond which there is a slight decrease. Deepika et al noted that the weight of the heart increases with increasing age. She observed the maximum weight of the heart in the age group of 51-60 years with a range of 228 to 390 with the female hearts weighing lesser[24]. This relationship of weight of the heart was also observed by Singh D et al, Narongchai et al and Mathuramon P[4,19,20] Puttaswamy noted that after attaining the peak all organ weights tend to decrease, except the weight of the heart[14]. This was explained by many authors as due to deposition of epicardial fat and incidence of cardiomyopathy, and undiagnosed systemic hypertension amongst the elderly[14,20.22].

Liver

The weight of the Liver is maximum in the age group 31-40 years for both sexes and its mean is 1501.23 in males and1282.62 in females in this age group. The mean weight of the Liver showed a progressive decrease as age advanced, with the lowest mean weight being recorded above the age of 80years, their values being 984.86 and 879.38 respectively for males and females. In most studies it as observed that the peak weight of the Liver was in the age group of 51-60 years[4, 24]. The present study shows that the liver was heavier in males when compared to females, and this is consistent with Singh D et al, Narongchai et al, Mathuramon P and Deepika et al [4,19,20,24]. Mubbunu in a study conducted on Zambian people noted that the Liver weighed more in females when compared to males[21]. This was attributed to genetic factors.

Spleen

The spleen showed a progressive decrease in weight amongst males with advancing age commencing from the age of 40 onwards. It was noted that the mean weight of the spleen in the group aged above 80 years was 140.28. On perusal of data, it was noted that it was a case of an 82 year old male with portal hypertension and hematemesis with aspiration of blood into the lungs. The abnormal weight of the spleen in this case has increased the mean weight of the organ in this relatively small sized age group. Amongst the females, the maximum weight of the spleen was noted in the age group 31-40 years and the mean in this age group was 131.95, thereafter the organ showed decreasing weight with advancing age. As in all other organs the weight of the spleen was noted to

be more in males. This is consistent with other studies conducted all over the world[1,4,9,15, 19,20,21]

Kidneys

In our study, it as observed that the kidneys are slightly heavier on the left side in males, the maximum weight being on the age group 41-50years. In females, there was no significant difference in the weights of the kidney on either sides; the right kidneys weighing maximum in the 51-60year age group, and the left in 31-40 years. In both sexes the kidneys progressively decreased in weights after their peak weights were attained. P.N. Murkey noted that the weight of kidneys increased up to the age 50years in females while in males it increased up to the age of 70 years Batra also noted that the kidneys weighed maximum in the age group 61-70years, while Singh D observed the maximum weight of kidneys to be in the age group 31-40 years. Sahni observed statistically significant differences in weights of both kidneys amongst the sexes[11].

The normal values of organ volume, weight, and size change with time probably under the influence of genetic factors and environmental factors and it may act as a good diagnostic criterion for autopsy only if normality is accurately and regularly defined.

Conclusion

From this study, we can conclude that weights of all organs are more in males when compared to females. There is no significant differences in organ weights obtained in our studies when compared with other studies conducted in India. The brain and liver weights in both sexes were slightly lower when compared to studies conducted abroad. The weight of the spleen was less than the average weight recorded from the rest of India and abroad; but was consistent with studies conducted in Thailand. The weight of the heart positively correlates with increasing age.

Implications

With this study we have reference values for organs which can be put to practical use during autopsy studies. But for these values to be valid and reliable for forensic and non forensic evaluation of organ pathologies, these values need to be correlated with variables like height, weight, and body mass index.

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A 27 years old Woman with Drug Reaction with Eosinophilic and Systemic Syndrome (DRESS) induced by 2nd Line treatment of Multi Drug Resistance Tuberculosis: A Case Report

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Abstract

Introduction: Drug Reaction with Eosinophilic and Systemic Syndrome (DRESS) also known as Drug Induced Hypersensitivity Syndrome initially recognized as a severe drug – induce hypersensitivity occurring 2-8 weeks after drugs introduction.

Case presentation: We report a 27 years-old woman treated by pyrazinamide, ethambutol, capreomycin, levofloxacin, cycloserine, and bedaquiline as an individual treatment for relaps multiple drug resistance pulmonary tuberculosis, then after eighteen days of treatment she admitted to our hospital because of her complaint about progressive skin rash, periodic fever, multiple lymphadenopathy, increased of eosinophilia count, liver function test and renal function test. She have recovered after stopped the drugs and was given of corticosteroids.

Conclusion: Every patients with multi drug resistant (MDR) tuberculosis who receive 2nd line of MDR tuberculosis treatment have a possibility to develop DRESS. Giving of new individual treatment after DRESS resolved, can lead to patient's cure.

Keywords: *antituberculosis, DRESS, drug eruption, eosinophilia, levofloxacine, MDR*

Introduction

In 1959, Saltzstein and Ackerman described a cutaneous adverse reaction to anticonvulsant drugs that included fever, eosinophilia, lymphadenopathy and sometimes hepatosplenomegaly. It was called pseudolymphoma. It was subsequently defined by Bocquet et al. as drug rash with eosinophilia and systemic symptoms (DRESS)[(1)]. The pathogenesis of DRESS syndrome is not well understood and is hypothesized to consist of a complex interaction between two or more causes[(2)]. The clinical manifestations of DRESS include drug-induced general skin rash, fever, lymphadenopathy, abnormal hematological findings, liver damage, as well as damage to other internal organs such as kidney, lung, heart, and pancreas. The condition is potentially life-threatening[(3)]. The most common drugs associated with DRESS include anticonvulsants (mostly aromatase derivatives), antimicrobial agents, particularly penicillin and sulfonamide-based agents, and antipyretic/ antiinflammatory analgesics[(4)]. DRESS has a relatively long latency period (between 2 and 8 weeks), and symptoms may persist for up to 2 weeks after the discontinuation of the culprit drug. Mortality rate is approxi-mately 10%, the DRESS is a severe drug-induced reaction[(5, 6)]. Early diagnosis and immediate withdrawal of the suspected drug is a key of management of DRESS[(7)]. The patient had probably developed a hypersensitivity reaction to antituberculosis drugs. We summarize our experience with the diagnosis and treatment of this case of DRESS syndrome.

Case Presentation

On December, 31th 2018, a 28 years old woman presented to Emergency Department of Dr. Saiful Anwar General Hospital, Malang, Indonesia with fever and skin eruption in all over her body. She complaint this skin rash since 9 days ago, at first the little spot showed up on both hand of the patient, then spread into her chest, back, all of her extremities and her face on the last. She felt itchy and sometimes pain on her skin, and after 3 days the pustule showed with infection sign and spread more the day overcome (figure 1). And also the patient complaint about swelling on her neck and her face so she couldn't breathe normally. Fever, vomiting, nausea and general weakness because of her decreased of appetite and body weight also accompanied her chief complaint. There were no complaint about chest pain or her urinary and bowel production.

One month backed, she was stopped her 9 month - from 1 year treatment for her Tuberculosis with multiple drug resistance because of the Geneexpert result from other hospital. Then for almost a month she complaint severe cough and decrease of her appetite. So she was checked for her sputum culture and found that the bacterial result was positive, so she was started for given Tuberculosis treatment with individual therapy.

So, in the end of 2018, November, she was planned to given a new regimen, such as pyrazinamide, ethambutol, levofloxacin, bedaquiline, cycloserine, capriomycin, ofloxacine and B6. On December, 10th 2018, she was started for given the new regimen, but on day 18, she felt general weakness and the skin eruption showed up, so she was admitted to Pasuruan General Hospital. 6 days after initial treatment on General Hospital Pasuruan, Malang, she was transferred to Dr. Saiful Anwar General Hospital, Malang, Indonesia for intensive therapy because her complaint was spreading than before, and one of Tuberculosis drugs was take a responsibility of her skin rash complaint. The patient didn't have any history of allergic reactions before, she also didn't have any history of atopi on her family.

At admission, generalized papules and erythema were observed over his face, trunk, extremities, palms, and soles. The patient's weight was 70 kg, body temperature was 38.6 °C, blood pressure was 100/65 mm Hg, respiratory rate 20 times per minutes and heart rate was 105 beats per minute. Blood routine examination showed leukocytosis 23,400 /ul with a white blood cell count 25.2 % of eosinophils. No atypical lymphocytes were detected on peripheral blood smear. Blood biochemistry test results were notable for alanine aminotransfer-ase 45 U/L, aspartate aminotransaminase 44 U/L, and albumin 2.57 g/L. For renal function, there were increasing of ureum and creatinine serum which are 105 mg/dL and 5.4 mg/dL. And also there were decreasing of electrolyte serum for Sodium 120,3 mmol/L, Potassium 1.73 mmol/L, and Chloride 77.0 mmol/L. Urine routine examination was normal and stool occult blood test was negative. Serological tests for common viral infections and autoantibodies were negative. Blood culture was negative. Electrocardiogram were also normal. Radiological workup revealed infiltrate with fibrotic on both upper lung with multicavities around 0.3 – 0.5 cm in diameter on apex area, and 7 – 8 cm in diameter on right upper lung area, leaded to Pneumonia supection with Tuberculosis far advanced lesion with giant bullae (figure 2).

Histological examination of skin biopsy were all compatible with DRESS syndrome. Based on the clinical, laboratory, and pathological findings, he was diagnosed as a case of DRESS syndrome; the pulmonary inflammation was probably attributable to organ involvement of DRESS. After dermatological consultation, DRESS was possibly considered to be induced by levofloxacine, bedaquiline or ofloxacine was noted for our next discussion for new regimen therapy. And systemic corticosteroid 62.5 mg/day was initiated and continued with oral methylprednisolone (4 mg/day) until her skin lesions improved, temperature as well as liver enzymes normalized, and the percentage of blood eosinophils decreased.

The patient was discharged on day 40, after her skin eruption was replaced by hyperpigmentation completely. At 2-weeks follow-up, she was weaned off systemic steroids with fading skin pigmentation, there was no relapse of drug eruption and joint swelling. Then after a comprehensive discussion, successful therapy is expected to occur with the administration of this new regimen, isoniazid, pyrazinamide, ethambutol, capriomycin, levofloxacin, mixifloxacine, etionamide, cycloserine, and linezolide with strict daily monitoring. The patient signed an informed consent statement for

publication, and the study was a retrospective analysis of clinical case, so ethical approval to report this case was not required.

Figure

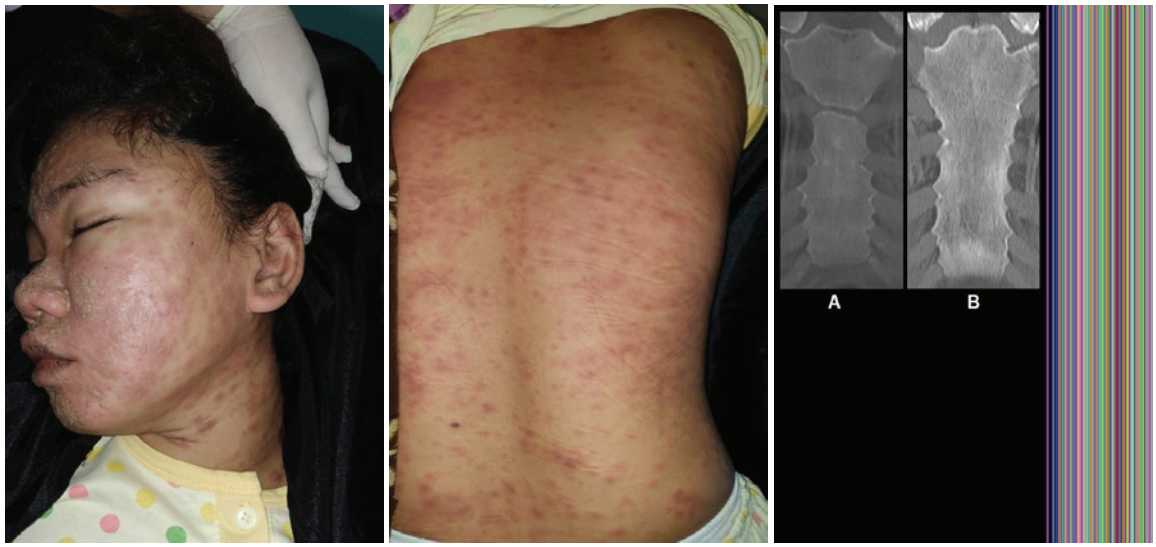

Figure 1. First admission of the patient showed makulo popular erythema in all over of her body

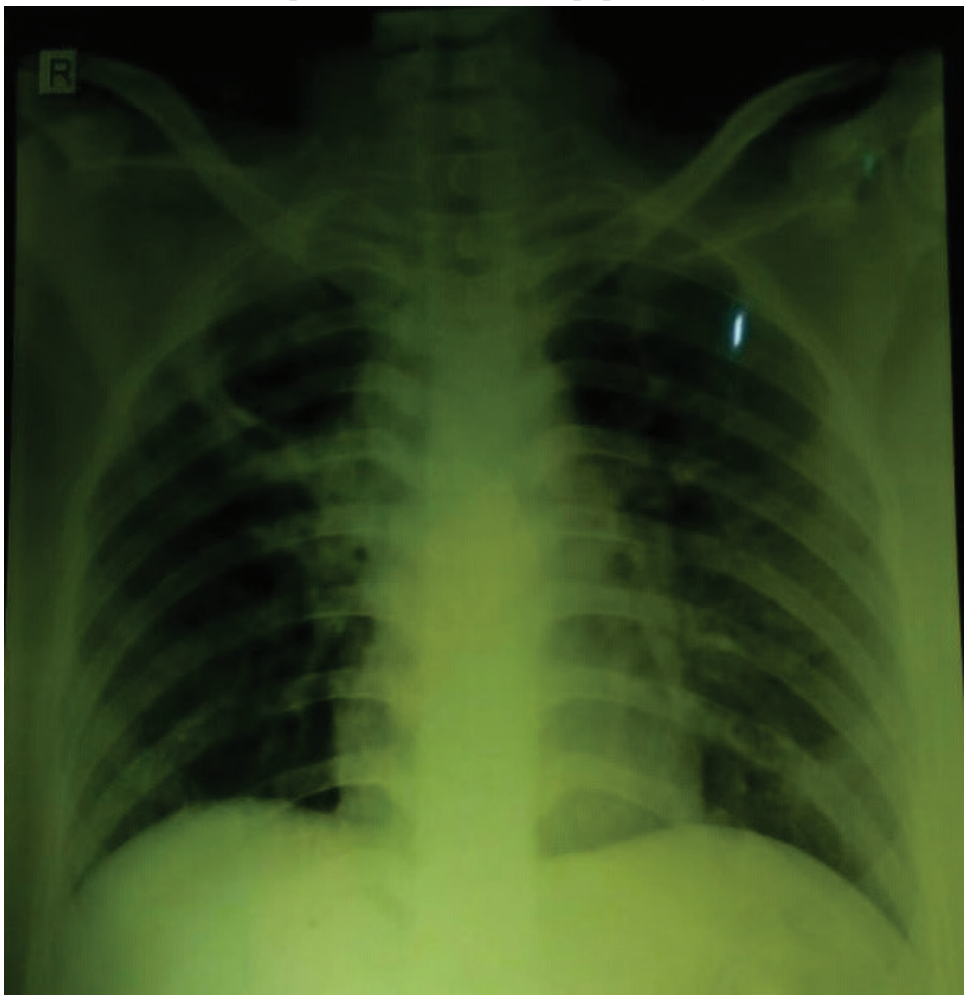

Figure 2. Chest X-Ray of the patient suggest Pneumonia supection with Tuberculosis far advanced lesion with giant bullae.

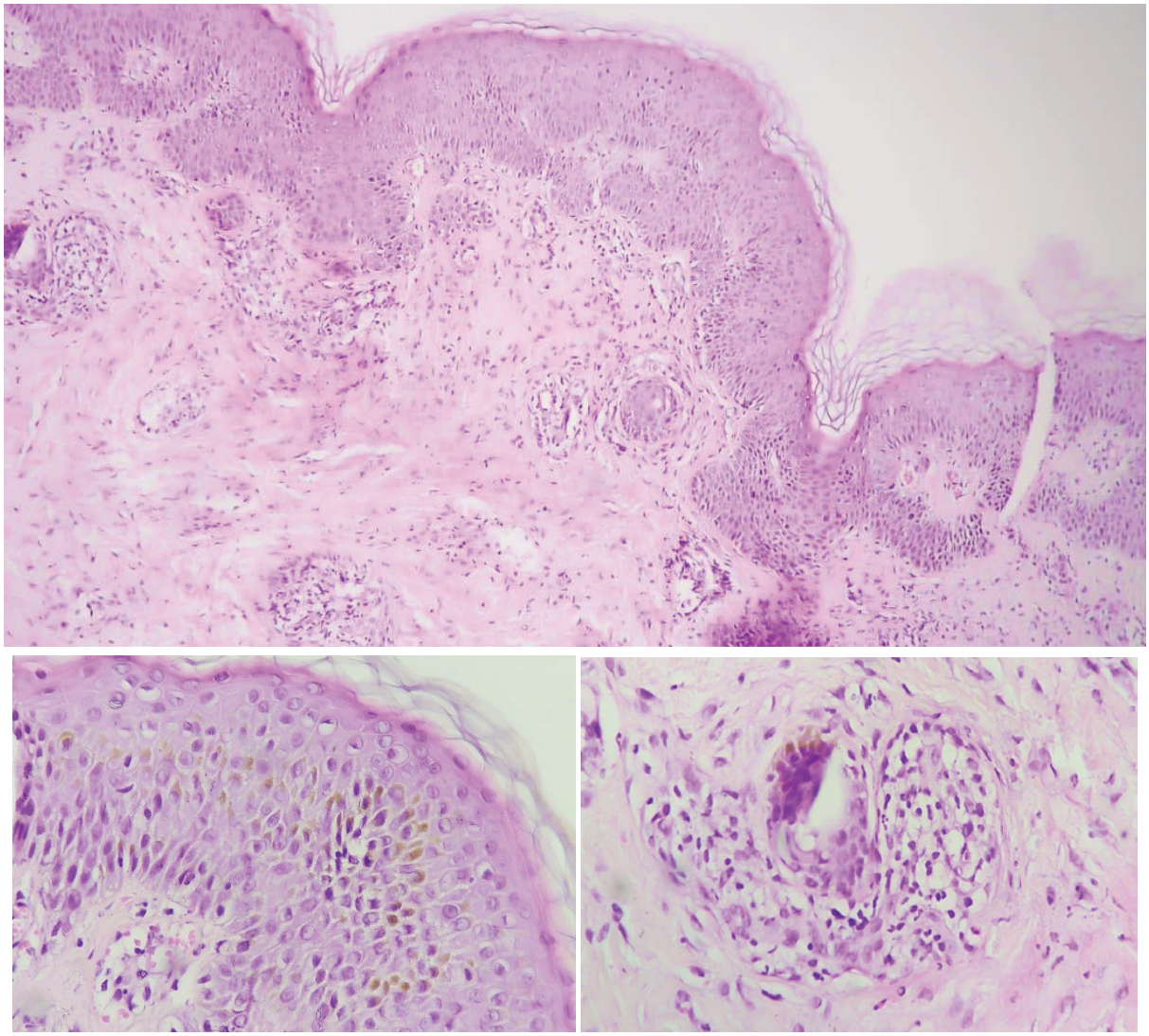

Figure 3. Histological examination of skin eruption shows intraepidermal spongiosis, perivascular lymphocytic infiltration, and presence of a few eosinophils in the dermis; hematoxylin and eosin (H&E), 200

Figure 4. In this picture, the patient showed good progession on her skin after corticosteroid used and withdrawal of MDR tuberculosis drugs after 40 days of treatment

Discussion

From the patient we got the information about her symptomps such as fever and diffuse erythema and papules over the entire body start from abdomen and lower extremities, then her face. Also she developed swelling edema on her face and neck. The symptomps showed eighteen days after the initiation of her MDR TB individual treatment. From physical examination, we found facial and neck edema, accompanied with enlargement of lymfonodi and maculo popular erythema covered all of the body. From laboratory findings, we found hig amount of eosinophilia, and increase of renal function test (Ur/Cr), and wild decreased of electrolyte. From REGISCAR, we found a probable diagnose to DRESS, suited to history present illness, physical examination and laboratory finding. We confirmed DRESS from skin biopsy (figure 3).

As theory said that to diagnose and differentiate DRESS as a separate entity from other severe cutaneous adverse reactions (SCAR), such as Stevens-Johnson syndrome or toxic epidermal necrolysis, a European registry for severe cutaneous adverse reaction was developed, called RegiSCAR.5 In this, diagnostic criteria for DRESS have been established. Patients must have three of the four following main criteria: an acute rash, fever above 38 °C, lymphadenopathy at two sites, involvement of at least one internal organ and abnormalities in lymphocyte and eosinophil counts. Additional criteria include hospitalisation and that the reaction is suspected to be drug-related. The RegiSCAR scoring system defines a case of suspected DRESS as 'no,' 'possible,' 'probable' or 'definite' case[8].

With Dermatologist, we suggested one of levofloxacine, bedaquiline, or ofloxacine, which induced DRESS on this patient. Use of corticosteorid to this patient was very helpful to decrease the progressivities of DRESS, but we should pay attention to her tuberculosis treatment (figure 4). Systemic corticosteroids or other immunosuppressive drugs are recommended for all cases of DRESS but need to be used with caution in patients with TB due to the risk of overwhelming infection, especially when adequate tuberculosis medications have been discontinued. Prednisone 1 mg / kg / day or equivalent is given until clinical improvement, and normalization of laboratory parameters are obtained and then tapered over the ensuing 3–6 months to avoid relapse[9]. Topical corticosteroids may be applied to skin lesions for symptomatic relief[10]. Successful therapy is expected to occur with the administration of this new regimen (isoniazid, pyrazinamide, ethambutol, capriomycin, levofloxacin, mixifloxacine, etionamide, cycloserine, and linezolide), with strict daily monitoring.

Conclusion

Our case report highlights the risk inherent in delayed diagnosis of DRESS and challenges in clinical management of this condition. The key to the treatment of DRESS is early diagnosis and immediate withdrawal of all suspected drugs. When an adverse drug reaction is observed, reuse of the same drug could cause more severe adverse drug reactions the 2nd time. When severe adverse drug reactions are suspected, a dermatologist should be consulted and the suspected drug should be discontinued immediately, and given of Corticosteroid is necessary and important. In this patient, successful therapy is expected to occur with the administration of this new regimen (isoniazid, pyrazinamide, ethambutol, capriomycin, levofloxacin, mixifloxacine, etionamide, cycloserine, and linezolide), with strict daily monitoring.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: None

Data Availability: The data set used and/or analyzed during the current study are available from corresponding author on reasonable request.

Ethics Statement: All procedures performed in studies / case report were in accordance with the ethical standards of the Ethics Committee in Dr. Saiful Anwar General Hospital, Malang, Indonesia. The authors explains the aimed, benefits, and rights of the participant during the process of collecting data to the patient's guardian, if the participant agrees we ask the participant to fill out an informed consent sheet.

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Time-dependent Expression of Caspase-3 and Degeneration of Lateral Rectus Muscle on Experimental Esotropia in Rabbits

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Abstract

Background: To identify correlation between caspase-3 expression and degeneration of lateral rectus muscle on experimental esotropia in rabbits by using time dependent analysis. **Methods:** Twenty four rabbits were divided into 4 groups, first month sham (S1); second month sham (S2); first month esotropia (E1); and second month esotropia (E2) model groups. Immunohistochemical and haematoxyllin eosin staining were performed. **Results:** In first month observation no significant difference in *caspase-3* expression between S1 (1.56 ± 1.03) and E1 (3.93 ± 1.66) groups (p >0.05) and in the degeneration score of lateral rectus muscle between S1 (1.33 ± 1.36) and E1 (3.83 ± 2.04) groups (p> 0.05). In second month observation no significant difference in *caspase-3* expression between S2 (1.80 ±1.08) and E2 (6.50 ± 3.26) groups (p >0.05); but there was significant difference in the degeneration score of lateral rectus muscle between S2 (1.16 ± 1.16) and E2 (4.50 ± 1.04) groups (p <0.05). There was a significant correlation between *caspase*-3 expression and the degeneration score of lateral rectus muscle (p=0.020; r=0.471). **Conclusion:** On the experimental esotropia with prolonged lateral muscle contraction in rabbits, the increasing trend of caspase-3 expression has a correlation with the increasing degeneration score of lateral rectus muscle.

Keywords: *caspase-3, degeneration of lateral rectus muscle, esotropia, rabbit*

Introduction

Esotropia is a type of strabismus that occurs when one or both eyes turn inward due to imbalance of the convergent and divergent tone of medial and lateral rectus muscles. The convergent tone of the medial rectus muscle causes the increased contraction of the lateral rectus muscle as its compensation that results in decreased visual function and binocularity as well as decreased quality of life. [1,2]

Prolonged strabismus often changes extraocular muscle structure as well as causes muscle degeneration which potentially makes difficulties in its surgical management and often requires repeated surgery. [3, 4]

Based on extrapolation from the mechanism of general muscle contraction it was suspected that the increased contraction of lateral rectus muscle in esotropia might increase cytosolic calcium level that had impact on cell survival and apoptosis through various pathways in intracellular organelles. [5,6]

Although the change of extraocular muscle structures is found in strabismic eye, the effect of prolonged esotropia on the degeneration of lateral rectus muscle through apoptosis pathway has not been studied. This study was conducted on rabbits as due to ethical and technical concern it was not possible to conduct this study in humans. The purpose of this study was to identify correlation between caspase-3 expression and degeneration of lateral rectus muscle on experimental esotropia in rabbit by using time dependent analysis.

Materials and Methods

Animal

Twenty-four New Zealand white rabbits weighing 3.4-4.0 kg were used in this experimental study. All animals were strictly cared in accordance with the Animal Ethics and Animal Welfare Regulations. This study was carried out during August 2019 – February 2020 at Faculty of Veterinary Medicine and approved by the Institutional Review Board of Faculty of

Veterinary Medicine, Universitas Airlangga no:2. KE.153.07.2019.

Surgery for esotropia model and sham

After calculating sample size, the rabbits were randomly divided into two groups, the experimental group (n=12) and control group (n=12). Surgery was performed on rabbits in the experimental group to create a model of esotropia by cutting and shortening the medial rectus muscle. The surgery was only performed on the right eye under general anesthesia. After surgery, esotropia model rabbits were randomly divided into two observation groups, the 1st month group (E1) (n=6) and the 2nd month postoperative observation group (E2) (n=6). In rabbits control group, sham procedure was performed by cutting without shortening the length of medial rectus muscle. Similar to the experimental group, sham rabbits were divided into two observation groups as described above. At the time of observation, the collection of 4 mm lateral rectus muscle specimen was carried out under general anesthesia in both experimental and control groups of rabbits.

Immunohistochemistry staining for caspase-3 expression and interpretation

Immunohistochemistry (IHC) examination for caspase-3 expression was performed by following the method that has been previously done using *anti-caspase3 antibody* ab44976 reagent Abcam USA. Immunoreactive scoring was assessed according to Remmele index scoring method, as follows [7] (Table 1)

Table 1 Immuno Reactive Score

A (percentage of positive cells)	B (intensity of staining)	IRS (multiplication of A and B)
0= No positive cells	0 = no color reaction	0-1 = negative
1=<10% Positive cells	1 = mild reaction	2-3 = mild
2=10-50% positive cells	2 = moderate reaction	4-8 = moderate
3=51-80% positive cells	3 = intence reaction	9-12 = strongly positive
4=>80& positive cells	Final IRS Score (A x B) : 0-12	

Hematoxyllin eosin staining and interpretation

Hematoxyllin eosin (HE) staining was performed by following the method that has been previously done. The degree of lateral rectus muscle damage on this examination was assessed according to the modified Yao method, with scoring as follows [1] (Table 2):

Table 2. Score of the degeneration of lateral rectus muscle

Score	Interpretation
0	Normal, if the thickness of the muscle cells (coir) is relatively uniform with the same direction of coir, muscle filaments is dense with little extracellular matrix
1	< 1/3 of the muscle tissue observed varies in thickness and the spacing between muscle filament is wider than normal
2	The same as score 1 with affected muscle tissue between 1/3 - 2/3 of the area
3	The same as score 2 with affected muscle tissue > 2/3 of the area
4	< 1/3 of the muscle tissue was atrophic, fibrotic and degeneratic
5	The same as score 4 with affected muscle tissue between 1/3 - 2/3 of the area
6	The same as score 5 with affected muscle tissue > 2/3 of the area

Statistical Analysis

Datas were presented with mean ± standard deviation. Normality test was performed using Shaphiro-Wilk test. Homogeneity test was performed using Lavene test. Furthermore, data difference was tested using ANOVA and the linear regression test was carried out. This study used a significance level of 0.05

Results and Discussion

Expression of caspase-3 as an executor of apoptosis

The expression of caspase-3 in myocyte was semiquantitatively calculated and shown in brown chromogen color in immunoreactive cells, as can be seen as follows (Fig.1)

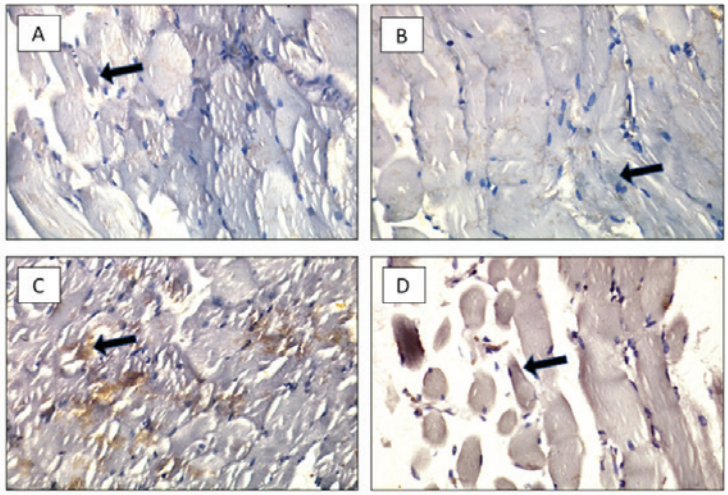

Figure 1. Caspase-3 expressions of sham group in 1st month observation/S1 (A), in 2nd month observation/ S2 (B), esotropia group in 1st month observation/E1 (C), and in 2nd month observation/E2 (D). *IHC Magnification 400x; microscope Nikon*

The expression of caspase-3 in S1, S2, E1, and E2 groups were 1.56 ± 1.03; 1,80 ±1,08; 3,93 ± 1,66; and 6,50 ± 3,26 respectively.

Degeneration of lateral rectus muscle

The degeneration of lateral rectus muscle was assessed semiquantitatively and can be seen as follows (Fig.2)

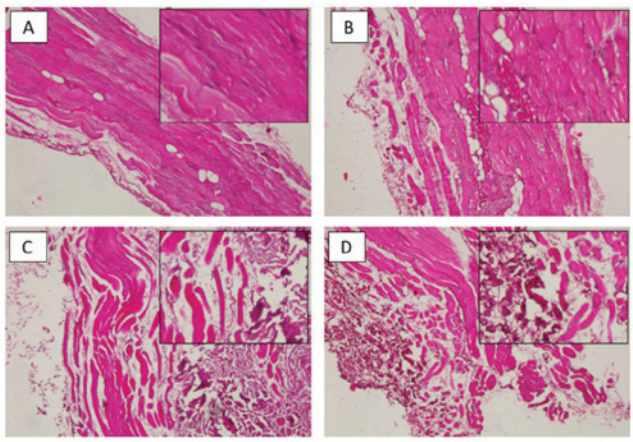

Figure 2. The degeneration of lateral rectus muscle of sham group in 1st month observation/S1 (A), in 2nd month observation/S2 (B), esotropia group in 1st month observation/E1 (C), and in 2nd month observation/ E2 (D). *HE Magnification 400x*

Degeneration score of lateral rectus muscle in S1, S2, E1, and E2 groups were as follows: 1.33 ± 1.36; 1.16 ± 1.16; 3.83 ± 2.04; and 4.50 ± 1.04, respectively.

In first month observation there was no significant difference in caspase-3 expression between S1 and E1 groups (p >0.05) and in the degeneration score of lateral rectus muscle between S1and E1 groups (p> 0.05) (Figure 3)

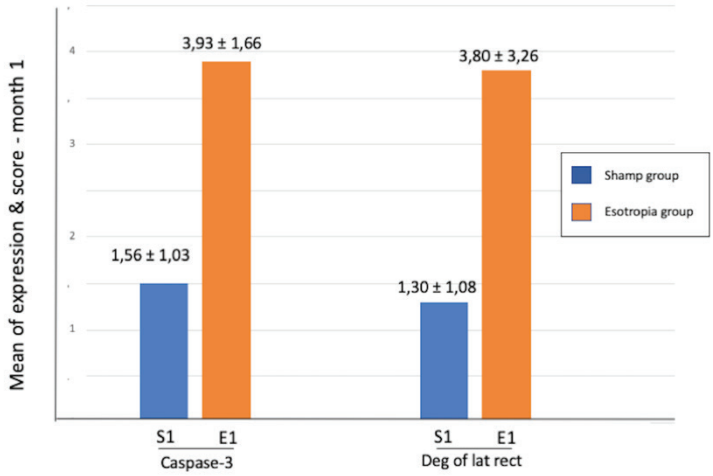

Figure 3. The bar diagram of 1st month observation on the expression of caspase-3 and degeneration score of lateral rectus muscle (Deg of lat rec=degeneration of lateral rectus muscle; S1=1st month observation on sham group; E1=1st month observation on esotropia model group

In 2nd month observation there was no significant difference in *caspase-3* expression between S2 and E2 groups (p >0.05); but there was significant difference in degeneration score of lateral rectus muscle between S2 and E2 groups (p <0.05) (Figure 4).

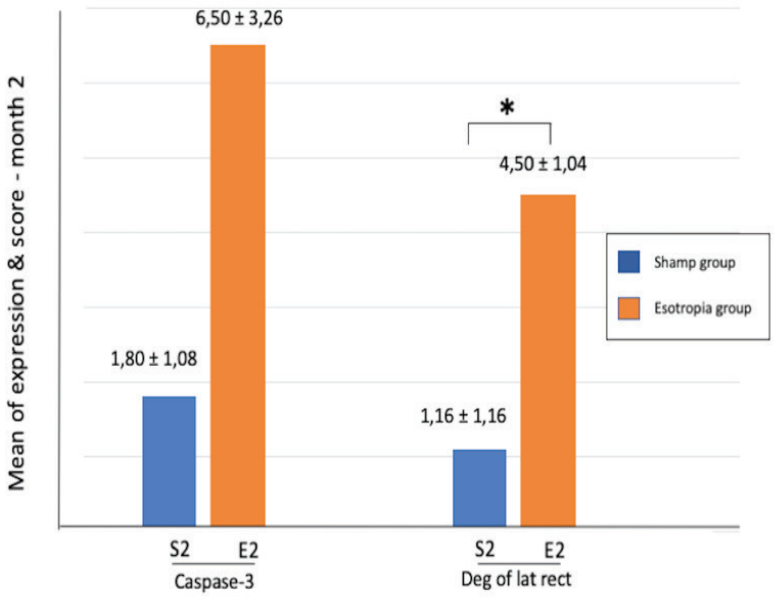

Figure 4. The bar diagram of 2nd month observation on the expression of caspase-3 and degeneration score of lateral rectus muscle (Deg of lat rec=degeneration of lateral rectus muscle; S2=2nd month observation on sham group; E2=2nd month observation on esotropia model group.

Misaligned condition with inward turning of the eye in esotropia may cause increased lateral rectus muscle contraction wherein the activity can mimic an exercise that can cause disruption of calcium balance. The changes in calcium homeostasis will affect metabolism processes in mitochondria and endoplasmic reticulum. [8, 9]

In this study, immunohistochemical examination of the lateral rectus muscles in the 1st month esotropia group showed an increase in caspase-3 expression compared with control group. This result indicated that the mitochondrial apoptotic pathway was activated. Mitochondria was important checkpoints of the apoptotic process, as they might release caspase cofactors. [10] Calcium (Ca^{2+}), which is released from endoplasmic reticulum storage to cytosol due to the need of lateral rectus muscle contraction, also enters mitochondria. Caspase-3 as a marker of programmed cell death is activated upon the initiation of apoptosis. Caspase-3 activation and subsequent apoptosis occurs through the release of cytochrome c from mitochondria. The pore on mitochondrial outer membrane, through which cytochrome c is released, is controlled by Bcl-2 family proteins. Altering Bax (pro apoptosis) and BCl2 (anti apoptosis) ratio results in cytochrome c release to cytosol. As a consequence, organelle swelling and fragmentation are detected as parallel with the release of cytochrome c. [8,11] In the control group caspase 3 expression also increased even though it was lower than that in esotropia group. The sham procedure, in which shortening of lateral rectus muscles were not performed, also triggered apoptosis but not in similar way with its mechanism in esotropia group. In esotropia group, the increased lateral rectus muscles contraction produced higher amount of apoptosis compared with that in the control group.

In the 1st month observation, it was found that the increasing degeneration score of lateral rectus muscles in esotropia group was higher than that in the control group, although it was not statistically significant. This result was in line with the expression of caspase-3 in the same month of observation. The lateral rectus muscle, as the skeletal muscles anatomy in general, is a unique network consisting of myofibrils with many nuclei and interstitial tissue. Beside of its occurrence in myonuclei, apoptosis also occurs in interstitial tissue with different apoptotic signals. As a consequence of increasing muscle contraction in shortening muscle in esotropia group, increasing exercise is followed by endoplasmic reticulum stress. It may cause perturbations and increasing of calcium release from endoplasmic reticulum which in turn produce apoptosis. [12-14] It is suspected that apoptosis occurs in lateral rectus muscle, which is indicated by increasing of caspase-3 expression followed by the degeneration of lateral rectus muscle. In the 2nd month observation, prolonged contraction of lateral rectus muscle may increase calcium release and further stimulate caspase dependent apoptotic pathways. The result in this study showed that increasing caspase-3 expression in esotropia group was higher than its expression in control group.

In the 2nd month of observation, prolonged contraction of the lateral rectus muscles caused significant increase of the score of lateral rectus muscle degeneration in esotropia group, which was significantly higher than its degeneration score in control group. The average score of 4 in modified semiquantitative Yao method scoring showed that atrophy, fibrosis and degeneration occurred in lateral rectus muscles. This result was in line with the finding in a research performed by Gong et al. (2017), who in different condition observed cat model exotropia and found that the longer the observation time (1st, 2nd, and 3rd months), the higher the irregular myofibril arrangement in the medial rectus muscle. [15] Similar to Gong's study, our research showed that in prolonged contraction of lateral rectus muscle (2nd month of observation) the higher the caspase-3 expression, the higher the degeneration score of lateral rectus muscle.

In this study it has also been proven that there was a significant correlation between *caspase*-3 expression and the degeneration score of lateral rectus muscle (p=0.020; r=0.471). However, it is still necessary to prove several pathways that correlate prolonged contraction of lateral rectus muscle with apoptosis through endoplasmic reticulum, reactive oxygen species and inflammation in esotropic eye. Extrapolation from a number of studies suggest that calcium homeostasis dysregulation in lateral rectus muscle activates the apoptotic pathway and further it will induce several pathological processes, including muscle atrophy and degeneration. [10,16]

As a conclusion of the study, on the experimental esotropia with prolonged lateral rectus contraction in rabbits the increasing trend of caspase-3 expression has a correlation with increasing degeneration score of lateral rectus muscle. Further studies are needed.

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Determination of Sex by Morphometry of Acetabulum and Acetabulopubic Index in South Indian Population

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Abstract

The capability of determination of gender from unknown human skeletal remains is indispensable and the techniques to find out this from different bones of human skeletal remains have been researched tremendously. Several investigators have asserted the necessity for population specific statistical data for methods that are dependent on specific measurements as there exist ample variations in body size among diverse ethnic populations. Most reliable bone exhibiting sexual dimorphism in human body is the pelvis and we have chosen it for derivation of population and sex specific data with respect to diameter of acetabulum and acetabulopubic index. 72 adult, dry innominate hip bones without any morphological deformities and defects (38 of male and 34 of female sex) belonging to South Indian population were carefully studied with respect to diameter of Right acetabulum and acetabulopubic index derived from the maximum distance between pubis symphysis and the anterior rim of acetabulum i.e. cotylopubic length or acetabulum-pubis symphysis length. All measurements were accurately taken with the aid of digital Vernier caliper and results were analyzed by means of SPSS software version 26 and the results including the demarking point, standard deviation, sectioning point etc. were found to be statistically significant and therefore acetabulum diameter and acetabulopubic index can be authentically applied for sex determination from dry adult hip bones belonging to the South Indian population. From the analysis of demarking point, AP index of >94.13 identified 73.84% of male sex and <87.15 identified 84.97% of females and therefore AP index can definitely be considered as the positive and decisive indicator for sex determination from adult hip bones.

***Key Words**: Acetabulum diameter, Acetabulopubic index, Sex determination, South Indian population*

Introduction

The exact determination of sex plays a crucial role in the establishment of identity of a deceased person from skeletal remains in challenging forensic and archaeological scenarios. For the purpose of establishing sex, the pelvis has been considered as the most reliable individual bone, providing the precise results. Krogman and Iscan[1] found 95% accuracy of sexing whereas Bruzek[2] established 59 to 96% accuracy pertaining to complete pelvis. Nevertheless, it has been universally accepted that skeletal traits differ among populations[3-7] and because of this regional variability, to enhance the veracity of sex determination for the purpose of identification, population specific standardized data have to be developed. Several studies involving various morphometry features and indices from pelvis have been done among different world populations with diverse degrees of precision in this context.[8-11]

Visual method of sex determination by studying morphological features of hip bone is very subjective and therefore less reliable. Discriminant analysis employing morphometry excludes observer bias.[2] As acetabulum being one of the highly reliable diagnostic pelvic indicators for gender determination and one among the bony parts that are relatively less susceptible

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to postmortem degeneration and damage, it was chosen for this morphometric study. The aim of our present study involves the analysis of morphometric sex differences pertaining to innominate hip bone using the diameter of acetabulum and acetabulopubic index in South Indian population as population specific studies in this regard were negligible and insufficient. We derived the demarking points for these parameters for gender determination of hip bone and estimated the overlapping values in studied parameters and indices.

Materials and Methods

The specimens used for this descriptive observational study included 72 adult dry Right hip bones of known gender (38 males and 34 females) native to South Indian population. Right hip bone was chosen because of the proven fact that the morphometric differences between right and left hip bones, pertaining to our study, are negligible. Specimens with defects and deformities secondary to morphological, pathological or traumatic etiologies have been cautiously rejected for analysis. The bones were chosen from the Departments of Forensic Medicine and Anatomy from Chettinad Hospital and Research Institute, Kelambakkam. Acetabulum diameter (AD) was assessed as the maximum distance from the acetabular ridge that is in close proximity with the body of ischium to the anterior iliac margin that intersects the acetabular ridge.[12] The pubis symphysis-acetabulum length or cotylopubic length (CP length) was estimated as the distance between pubis symphysis and anterior rim of acetabulum. After marking all the exact anatomical points on hip bone with skin marking pencil, measurements were precisely taken with the help of sliding digital Vernier caliper capable of measuring up to the least count of 0.01 mm. by the same investigator to eliminate the possible inter-observer errors and all the results thus obtained were analyzed with the aid of SPSS software version 26 to get various statistical parameters which were found to be statistically significant with respect to sex determination. From the above metric data, the acetabulopubic index (AP index) was derived applying the equation AD x 100 / CP length. The results were tabulated.

Results

Descriptive statistics of the morphometric variables were tabulated in Table 1 from which values of acetabular diameter (AD) and acetabulopubic index (AP index) were found to be comparatively higher in males than females while the cotylopubic length (CP length) was higher in females when compared to males and the results were seen to be statistically significant with p value of <0.001.

Table 2 shows the Demarking points (DP) pertaining to all variables taken for sex differentiation. The Demarking points specific for AD exactly classified 5.48% of male sex and 1.3% of females. The DP for CP length correctly differentiated 48.28% of males and for females it is 45.72%. The DP for AP index clearly classified 73.84% of male and 84.97% of female hip bones.

Table 3 exhibits the Sectioning points corresponding to various parameters applicable for sex differentiation. The accuracy of prediction of sex by employing the Sectioning points corresponding to AD and AP index in both sexes was 50% and 100% respectively while Sectioning point for CP length identified 46% and 55% of male and female hip bones respectively.

Table 1: Descriptive statistics of the variables

(SD: Standard Deviation; SEM: Standard Error of Mean)

Statistical parameter	CP length (mm.)		AD (mm.)		AP index	
	Male	Female	Male	Female	Male	Female
Mean	53.78	62.57	53.48	49.72	98.67	76.46
SD	3.35	3.28	3.64	3.26	3.84	5.89
SEM	0.68	0.59	0.75	0.51	0.73	1.08
t-value	-9.729		3.572		11.895	
p-value	<0.001		<0.001		<0.001	

Table 2: Demarking point (DP)

Variable	Sex	Mean±SD	Calculated Range	DP (mm.)	% beyond DP
CP length (mm.)	Male	53.78±3.35	43.73-63.83	<52.73	48.28
	Female	62.57±3.28	52.73-72.41	>63.83	45.72
AD (mm.)	Male	53.48±3.64	42.56-64.4	>59.5	5.48
	Female	49.72±3.26	39.94-59.5	<42.56	1.3
AP index	Male	98.67±3.84	87.15-110.19	>94.13	73.84
	Female	76.46±5.89	58.79-94.13	<87.15	84.97

Table 3: Sectioning point

Variable	Sex	Sectioning point (mm.)	Accuracy (%)
CP length (mm.)	Male	<58.17	46
	Female	>58.17	55
AD (mm.)	Male	>51.6	50
	Female	<51.6	50
AP index	Male	>87.56	100
	Female	<87.56	100

Discussion

Among all the bones of our human skeleton, the hip bone exhibits significant sexual dimorphism and therefore applied as the most reliable osteological tool for gender identification.[22] Several osteometric parameters and indices derived from the hip bone were successfully employed for its sex determination. As shown in Table 4, the present study indicates that the cotylopubic length was of smaller value while acetabulum diameter was showing higher value in males when compared to corresponding values in females and found to be consistent with the results of similar studies.[13-18] The present study found out that the acetabulopubic index showed highest sexual dimorphism followed by cotylopubic length while acetabulum diameter stood as the least dimorphic parameter.

The findings of the current study and analysis as tabulated in Table 5 indicates that the acetabulopubic index of >94.13 identified 73.84% of male hip bones and the value of <87.15 clearly identified 84.97% of females. The percentage of accuracy of sexing as found from our study was very high when compared to that of similar researches and therefore highly reliable with respect to applicability of demarking points for AP index. The research of Pal GP[20] showed that the AP index of >90.95 with identification percentage of 12.58% in case of males whereas index of <63.63 with zero percentage in case of female hip bones. Again, our study has better accuracy

of sexing when compared with that of Nagesh KR et al.[23] in which accuracy percentage for sex determination was 17% with AP index of >88.4 in case of males, whereas in case of females, AP index <66.9 made out 26% of female bones.

From our current study, the sectioning point found to be a highly dependable tool for sex determination as AP index of <87.56 identified female bones and value >87.56 distinguished males with accuracy of 100% and this can be compared with the results of the study done by Nagesh KR et al.[23] in which AP index of <77.5 determined 81% of female hip bones and value >77.5 picked out 83% of male bones.

Table 4: Comparison of present study involving Acetabulum diameter in male and female hip bones with data from other Researchers

(n: Number; AD: Acetabulum Diameter; SD: Standard Deviation; DP: Demarking Point; S: Significant; HS: Highly Significant)

Researcher	Year	Population	Sex	n	AD Mean (mm.)	SD (mm.)	DP (mm.)	p-value	Remarks
Chauhan et al.13	2002	North Indian	M (R)	36	47.1	2.9		<0.049	S
			F (R)	12	44.38	3.01			
Krebs et al.14	2012	Chinese	M	100	53.7	3.47		<0.001	HS
			F		56.0	3.33			
Zeng et al.15	2012	Chinese	M (R)	50	55.2	3.11		<0.001	HS
			F (R)	50	51.4	2.38			
Yugesh K16	2016	South Indian	M	60	48.66	0.33		<0.005	HS
			F		46.18	0.24			
Rajasekhar S et al17	2017	South Indian	M	121	48.13	3.1	55.55	<0.001	HS
			F	85	45.24	3.52	39.03		
Indurjeeth K et al18	2019	South African	M (R)	100	57.31	3.37		<0.001	HS
			F (R)		52.52	3.55			
Present study	2020	South Indian	M	38	53.48	3.64	59.5	<0.001	HS
			F	34	49.72	3.26	42.56		

Table 5: Comparative analysis of percentage of identified hip bones based on Acetabulopubic Index (AP index) in various populations

Researcher	Year	Population	Sex	n	AP index Mean (mm.)	SD (mm.)	DP (mm.)	% of identified bones	p value	Remarks
Mewalal19	1993	North Indian						8.3		
								2.5		
Pal GP20	2004	Madhya Pradesh	M	143	84	6.79	90.95	12.58	<0.001	HS
			F	62	74	5.65	63.63	0		
Nirmale VK21	2016	Maharashtra	M	124	90.51	7.85	109.02	0.8	<0.001	HS
			F	54	81.75	8.09	66.98	1.85		
Present study	2020	South Indian	M	38	98.67	3.84	94.13	73.84	<0.001	HS
			F	34	76.46	5.89	87.15	84.97		

Conclusion

The findings of the present study will surely aid in the determination of bone sex in cases of mutilated remains, extremely decomposed corpses, bomb blasts, charred bodies due to burns etc. where gender determination plays a crucial role in establishing the identification of the deceased. According to Krogman[1], the accuracy of sexing from pelvis is of higher percentage and therefore considered as a single best bone for gender identification among other bones like skull, long bones etc. with relatively lesser percentage. The results of our research indicate the existence of statistically significant gender difference in all morphometric parameters studied, with acetabulopubic index found to be having most reliable sexual dimorphism which was proven by the fact that 100% accuracy achieved with respect to identification of bone sex from AP index based on discriminant function analysis as well as sectioning point analysis. The results from this study further can be used for sexing archeological skeletal remains since only one side hip bone is sufficient, unlike other morphometric and morphological methods which require both sides innominate hip bones. The various parameters derived from this study can thus be reliably employed in conjunction with other conventionally applied parameters of hip bone to boost the accuracy of gender determination pertaining to South Indian population.

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Informed Consent: Not applicable

Ethical Clearance: Not required as the study was done on bone specimens

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Digital Dental Photography-A Modern Revolution

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Abstract

Digital photography has multilevel significance and represents the synonym of contemporary dentistry. Its application in dental practice is simple, fast, and extremely useful in documenting procedures of work, effectuating the education of patients, and pursuing clinical investigations, thus providing many dentists and patient. This article aims to review the various features and advancements in the field of dental photography and its application in prosthodontics.

Keywords: *Dental photography, DSLR, Macro lens, Light setup, Advancement in dental photography, Probe lens.*

Introduction

Digital photography has multilevel significance and represents the synonym of contemporary dentistry. Its application in dental practice is simple, fast, and extremely useful in documenting procedures of work, effectuating the education of patients, and pursuing clinical investigations, thus providing many dentists and patient. Digital photography is described as the images that are stored in a computerized file format referred to as a digital image file. Digital photography has become a vital part of dentistry [1].

History of Photography:

Photography provides the operator the ability to record patient data, events, and document scientific discoveries in a unique way. Alexander Wolcott played the key role in the history of photography. In 1848, Dr.R.Thompson and W. Elde Marked the first time use of before and after photographs of dental procedures. Digital photography arrived in mid-1990 with digital camera available at the marketplace. New software has come which allow things to be measured, changed, shared and integrated into new communication tools with just a click of a mouse. The images also can now be animated, used in reports, and published in websites. These applications are unparallel in film technology [2].

Classification:

Digital dental cameras can be divided into 3 categories.

***a) The compact point-and-shoot cameras, without* interchangeable lenses:**

This type of camera allows varying amounts of exposure control and mixed results in the unique setting of the oral cavity. Full-face photos can be acceptable, but intraoral and close-up views remain variable. The amount of noise in the images is also quite significant. A slightly higher variant is available called Prosumer cameras in which, the size and form of the Prosumer camera is smaller than a Digital SLR camera. Prosumer camera is light and equipped with a super zoom lens. It is cheaper than entry level Digital SLR camera.

b) The second type of camera used is the DSLR (Digital single lens reflex) camera:

DSLR cameras are designed for semi-professionals to professionals. DSLR cameras have the advantage of

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interchangeable lenses, including macro (or Micro in a Nikon System), telephoto and metered lenses. They also have ports for accessories such as a ring flash or a dual flash system. All controls can be set manually. An 85mm- to 105mm telephoto macro lens is used ideally- the reason is quite simple to maintain natural height-width ratio when reproduced in a photograph. A ring flash is placed around the lens to distribute the light evenly with intraoral exposures. Some cameras are also fitted with point flashes to reduce red-eye with full-face views. A drawback is that these cameras can be expensive and bulky.

c) Intraoral cameras:

An intraoral camera is a tiny device with a video camera that moves around inside the mouth and generates a surface video examination of the teeth. The images or videos can be stored, and later enlarged and showcased. Patients can see their teeth and any issues such as a fractured tooth, plaque, decay, and gingival disease, among others. This allows for better education of the patients and also allows dentists to catch problems in the early stages for analysis or to record patient progress.

BASIC ARMAMENTARIUM:

1.Digital camera

A. compact point –and-shoot camera

B. Digital single lens reflex

More the pixels, greater would be the detail of the image. In digital dental photography, minimum of 12 Mega pixels is required.

C. Intraoral cameras

2. Camera accessories.

A***. Lens:***

Fixed focal length of macro lens should be 24-105mm. The lens that mostly concerns us for documentation and record purposes are mainly the macro lenses. The lens system allows a sharper focus in the close-up pictures as they have larger diaphragm and we get a higher magnification than other immature lenses. These lenses have mechanisms that are defined by the term "diaphragm". It consists of sheets that let more or less light in it, similar to the function of iris in human eye. As smaller the diaphragm size, sharper would be the image. Hence with true macro lenses, the operator can take advantage of the depth of the field and obtain sharper and focused images at their original magnification[3].

B. Light and electronic flash systems

- Ring flash
- Point flash
- Twin flash

C. Memory card for storage of data.

D. Filter: It serves the dual purpose of lens protection and if required changing the lighting conditions.

E. Batteries: An extra battery pack with a quick charger ensures that we never run out of battery during shoot.

F. Camera bags: This is useful to protract the camera and be able to carry our lens, camera and other accessories in an organized fashion.

3. Clinical dentistry photographic accessories:

A. Cheek retractors.

Columbia wire lip retractor: This combines buccal mirror and cheek retractors like martin metal retractors.

Intraoral mirrors: It should have long handled with silver rhodium coated glass mirrors.

B. Black background: Contraster

The Shadow Box:

It is a portable photo studio for macro photography. Taking photos of restoration, models and small products has never been easier. Shadowbox can be configured for many different types of lighting and coloured backgrounds. As shown in the photos above, the Shadow Box starts with the "box". The base of the Shadow Box is reversible (white / black) and the back of the box is white with an extra panel that is also reversible (black / red) (Figure.1) [15].

Figure 1: Shadow Box

4. Other accessories equipment for intraoral photography:

- Plastic or glass spatula.
- Dental mirrors.
- Gauze strips.
- Air syringes or aspirators.

CAMERA ACCESORIES

A) Lens: A lens selected for dental purposes must be able to capture diagnostic and accurate views of teeth, gingiva and surrounding structures.

Specially fixed focal length macro lenses are able to capture an image of a subject while focusing at a very close range. Macro lenses with a fixed focal length designation of 85 to 105 mm provide the ideal combination of magnification ability and working distance convenience for dental purposes. The quality of the lens has a significant influence on the sharpness, clarity, and ultimate quality of the final image (Figure.2)

The magnification ratio is the ratio of the size of the image projected on the sensor compared to the actual size of the object. A magnification ratio of 1:10 means the image on the sensor is one-tenth life-size, while a 1:1 magnification ratio signifies a life-size image on the sensor. The 1:1 setting is ideal for close-up imaging of teeth while the 1:10 setting is useful for full-face views[4].

Figure 2- Macro lens

Probe lens

Figure 3: Probe lens Features

Probe lens is one of the new revolution in the field of digital camera lens, it has a feature of 2:1-macro and infinity focus, it also consist of tiny lens tip which is supported with ring light which gives full dept of coverage, since it has tubular barrel which is water proof it can be safely recommended for intra oral pictures (Figure: 3) [4].

B) Light and Electronic Flash Systems: Proper illumination is one of the most significant factors in achieving a quality image. Since natural ambient light is usually inadequate to illuminate all the dark areas in most intraoral photographic situations, a supplemental electronic flash source is needed. An electronic flash can provide light with neutral color temperature i.e. relatively high output light for a short duration[5].

In flash photography, the lighting effect is dependent on the form and arrangement of the flash sources. There are three types of electronic flash system configurations available for dental photography:

i. ***Ring Flash***: It is considered the universal flash system for general macro- photography. This system furnishes either a single ring flash tube or individual sector flash tubes that surround the lens. The specular or mirror-type reflection created by this type of flash tends to eliminate shadows in the image. The disadvantage in the reduction of shadows is that the image may appear to "flatten out" with reduced discernable contours (Figure: 4) [6].

Figure 4: Ring Flash

ii. Point flash: This provides a single strobe light source mounted in different positions around the lens to provide a unidirectional light from different angles. Photographic compositions for frontal, right lateral and left lateral views require the flash to be placed at the 12, 9, and 3 o'clock positions, respectively. Control of the light direction allows shadows to be cast by the three-dimensional topography of the objects in the scene. The appearance of shadows improves the visual definition of contour and texture to emphasize the apparent depth

within the image. The advantage of this flash system design is its ability to record surface texture detail and contour. However, it is suggested that multiple images with several flash positions be taken to establish adequate information. This type of flash system requires considerable experience and additional set-up time to maneuver the flash position before each exposure [7].

iii. Twin flash: Its configuration consists of two flash units that are mounted next to the lens. The light sources can be positioned to create custom mild shadowing to reveal texture with depth and life-like effects. Mastering the use of this lighting system will yield professional photographic results. While requiring more experience and thought for proper use, the twin flash design system may offer the best combination of soft, uniform illumination because it simultaneously reveals surface detail, color transitions, translucency variations, and crack lines (Figure:5).

Figure 5: Twin Flash

C) Memory card: Memory cards are available in different formats such as CF (Compact Flash), Micro SDHC (Secure Digital High Capacity), Micro SD (Secure Digital) and SDHC. They can reach up to 512 GB in size and store thousands of images. Blue/Standard for Point and Shoot cameras and Ultra for DSLRs should suffice the needs of the dental setup.

D) Filter: It serves the dual purpose of lens protection and if required changing the lightening conditions.

E) Batteries: It is required to get an extra battery pack with a quick charger, to ensure you never run out of battery during a shoot.

F) Camera Bags: This is useful to protect the camera and to be able to carry your camera, lenses and accessories in an organized fashion [8].

GUIDELINES FOR DENTAL PHOTOGRAPHY

To achieve a good digital photograph, standardization is very important, i.e. consistent lighting, exposure, patient positioning, perspective, depth of field and background. Photographs should be stored and presented appropriately for their use in publications. Three types of intraoral cameras are used like 35 mm film camera with Macro lens and ring flash, intraoral video camera (Orthoscan camera) or 5 and 6-megapixel extra oral digital camera. Several views should be taken for all the patients like frontal view that incorporates full facial profile and entire dentition. Other views

like lateral and oblique lateral, occlusal Mandibular-maxillary and a three quarters profile view for esthetic purposes [9]. Photography can be divided into three broad area namely, preparation of the patient, background and intraoral sites, preparation of camera. Comparison of light reflection on mesial and distal line angle with different flashes (Figure: 6).

Guidelines for Radiographic photographs :

Radiographs are only composed of white and black areas. Therefore, an imperfect photograph can be the result of four characteristics, non homogeneous illumination, variable color, flicker and limited brightness.

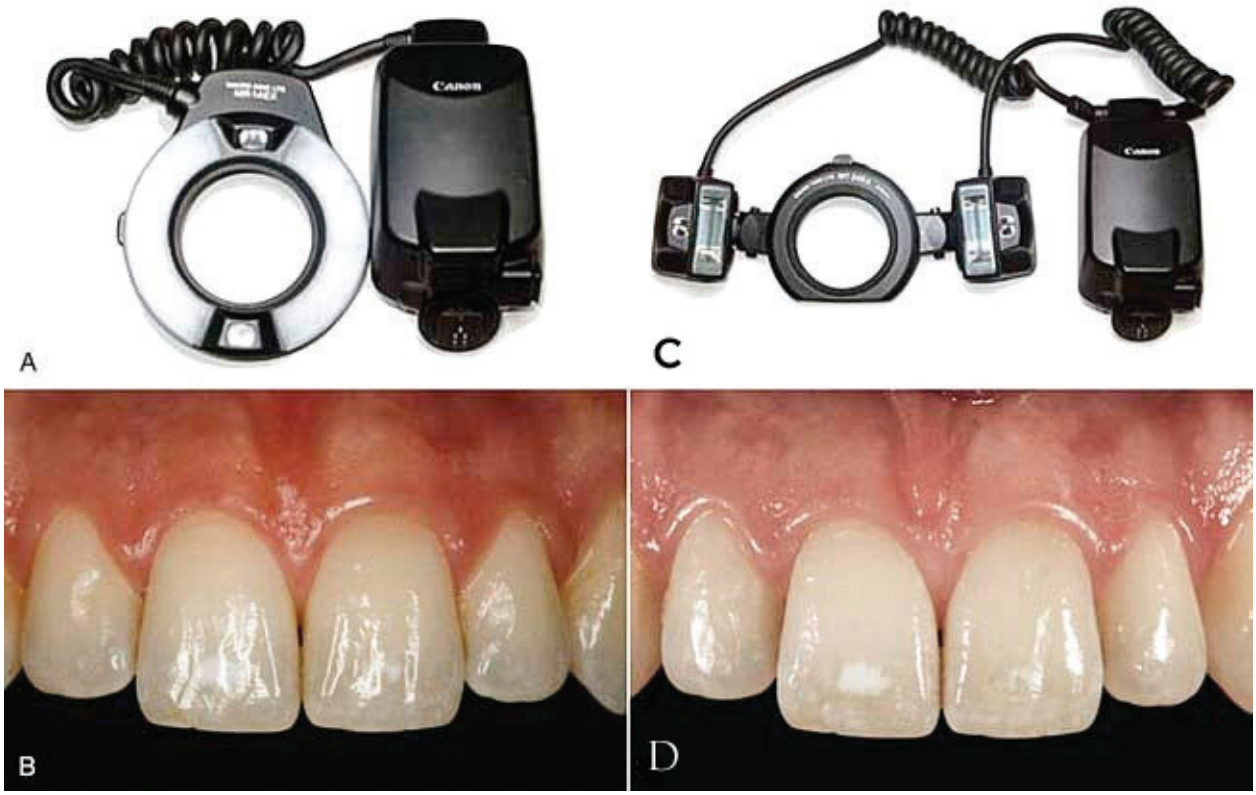

Figure 6: A – Ring Flash, B- image taken using ring flash, C- Dual Point Flash, D- Image taken using Dual point flash.

REQUSITIES OF DENTAL PHOTOGRAPHY IN DENTISTRY:

1. Diagnosis and treatment planning:

There are 34 views required for all clinical case examinations. Of the 34 views, 17 should take before treatment and 17 after treatment. Additional views are required for the technique documentation. The images should be captured in either manual or TTL mode. All intraoral images should be captured using high F-stops to maximize the depth of field. Intraoral and extra oral photographs provide a static, in-depth look at the patient's dentition.

2. Enhanced patient education and communication:

Utilizing a tablet display and presentation software, tailored presentation on dental procedures could be created with photographed cases. These detailed pictures showing anatomy, surgical steps, materials, and completed cases can help in educating the patients on diagnosis and proposed treatment and thereby improving their understanding and case acceptance.

3.Legal Documentation:

Digital photographs in their raw format can be used as a legal document proof. This can help a mistreated patient or defend a colleague who has provided appropriate treatment or can be helpful in malpractice lawsuits.

4. Insurance Verification:

Periodontal charting, radiographs, or a narrative is required by insurance companies before disbursement of benefits to the consumer. Therefore, a digital photograph can be used to support a narrative.

5. Specialist consultation:

A complete case history with high resolution photographs may be sufficient enough foe an over-the-phone consultation with a specialist. Similarly, photographs from referring dentist of mutual patients and their recent accomplishments could be transferred or received so that the operator may assess the condition without being physically present in the office [10].

6. Laboratory communication:

A shade guide is required to convey information on tooth or gingival character, shade, or color. This procedure is mostly accompanied by demerits like falling short in describing the complexity of depth and shadowing a tooth exhibit. Hence, here a color-corrected photograph can provide the much- needed information to create a final restoration with more accurate hue, value, and chroma.

7. Professional advertising and marketing:

Before and after photos are powerful aids to motivate the patients for accepting the treatment plan or for showcasing any particular skill.

8. Professional instruction:

Only texts and bullets are often inadequate in describing dental concepts or specific surgical procedures. A photo is worth more than a thousand words and sparks more interest and discussion than written matter.

9. Self-education and improvement:

As professionals, we continuously learn throughout our careers. Courses and other forms of continuing education are important educational vehicles. Digital photography in such a occasion is a boon.

10. Treatment philosophy and work ethic:

Talking efforts and time to clean surgical sites for photographs requires patience and attention to detail. This attitude propels us to execute our work at the high levels of accuracy [11].

APPLICATION IN THE FIELD OF PROSTHODONTICS

Photographic-assisted diagnosis*:*

Diagnosis and treatment planning of esthetic cases requires the use of photographs that are specifically designed to give the practitioner the information required to make that diagnosis and develop a sequential treatment plan.

Smile line, smile width and buccal corridor:

Analysis of smile width and buccal corridor can be done with frontal profile photograph of the patient. This can help in correct treatment planning and choice of material to be used for the restoration.

Midline, Incisal horizontal plane, and occlusal plane:

v A midline drawn on the photograph from the center of the forehead through the tip of the nose and chin or Cupid bow or philtrum can help the operator to determine any asymmetries if present. Similarly, interpupillary line drawn from the center of the pupils and facial midline form a cross, through which harmonious facial geometry can be assessed.

v Incisal plane can also be assessed in this view, revealing the length, Incisal curvature and horizontal symmetry of anterior arch.

v Occlusal plane can be evaluated by tracing the Incisal edges of the central incisors, cuspids, and first molar and compare its parallelism to the interpupillary lone and commissural line [12].

E-line, facial profile line, Frankfurt line, esthetic plane, camper's plane:

v E-line can be determined by the line that connects the tip of the nose to tip of the chin. This helps the operator to determine the lip support and in classifying anterior malocclusions such as overjet[13].

v Facial profile line can be evaluated by drawing a vertical line through the glabella and the tip of the chin. The angle formed by these designates a convex, concave, or straight facial profile.

v Frankfort plane can be assessed when the patient tilts the head slightly forward. Conversely, when the patients head is held erect with the eyes facing toward the horizon, the Frankfort plane forms an angle of about 8 degree with the arbitrary horizontal plane commonly referred to as the esthetic plane.

v The esthetic plane helps the operators to articulate the upper cast on a fully adjustable articulator in its correct superior vertical inclines.

v Camper's Line determined from the inferior border of the ala to the tragus helps making prosthetic considerations in denture, partial , or full mouth rehabilitation case.

v These statistics can be obtained by assessing a lateral profile photograph.

Smile width:

This reveals the number of teeth exposed from anterior to posterior. Analysis of smile width determines the correct planning of buccal preparation and the material of choice for the restoration. On frontal smile profile, the display of the teeth and gingiva is measured[14]. The Incisal most points of each tooth are depicted as line 1 and the l8p edge as line 2 (which is drawn parallel to the pupil line). The vertical distance between these lines is measured. This gives the lip position. Similarly, gingival margin (if visible) is marked as line 3. The difference between line 2 and line 3 gives the lip line height. If not visible, the length of the tooth is measured in intramural frontal view. These markings are important to determine the patients' gonial angle giving the operator an insight about the skeletal classification of occlusion and better understanding about the wear patterns of the remaining teeth[15].

Curve of Wilson / Curve of Spee:

Curve of Wilson, curve of spee and maximum intercuspation can be determined in anterior-posterior view. These observations have an important role in prosthetic rehabilitation from technical, esthetic and functional aspects.

The Golden proportion:

In esthetic smile makeovers or laminates, concepts of golden proportion can be easily applied and assessed onto a digital photograph.

Shade selection:

Photographs taken with an absolute dark or absolute white tab with 30-degree angled photos and a matching shade tab help a dentist to easily communicate with the laboratory technicians. By assessing these digital photographs, exact shade can be verified for the final result, eliminating shading errors and in turn excellent patient satisfaction.

Conclusion

In the field of prosthodontics, dental photography has its own impact. Through digital photography, the operator can communicate with the patient as well as among other dentists for referral or treatment documentation purpose. Technical aspects such as smile line, smile width, facial profile, emergence profile, occlusal plane, gingival anatomy, compensatory curves and shade matching can be better visualized through perfect intraoral and profile photographs. It also brings laboratory cases closer to the visualization of the actual patient. With more information at disposal, operators and technicians can deliver better skills to a greater precision and achieve the patient's desired restorative outcome with lifelike skills. Dental photography is very useful for the patient during and after treatment to satisfy form, function and esthetics. It improves the patient's oral health quality of life.

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Estimation of Time Since Death Using Vitreous Humour Potassium Values

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Abstract

The death erases all doubts from the dead's mind but inscribes new doubts in the living mind. The study of time since death gives the final turn in the crime to find the cause and time of death. The present study was conducted in Department of Forensic Medicine, Madras Medical College, Chennai during February 2006 to July 2006 to find out the time since death by analysing the potassium levels of vitreous humour after death. Total 75 cases were studied which is broadly classified into: Hospital cases and Non- Hospital cases irrespective of age, gender, religion, caste etc., who has died. All the data related to Non-Hospital cases were analysed to record the effect of nature and pattern of rise of vitreous humour in the deaths, whose detailed autopsy examination were also recorded subsequently for statistical analysis. From this study, it is concluded that the vitreous sodium and chloride was found to be totally unaffected and stable for 97 hours after death, irrespective of sex, age, environmental temperature, nature of death and cause of death.

Keywords: *Time since death, Vitreous Humour, Potassium levels, Vitreous sodium and chloride.*

Introduction

In India, medical literature on the Vitreous Potassium levels and its significance in the estimation of time since death has been focussed more as the facts of the study has its way in writing described by various authors. "Forensic Thanatology" - a medico legal study of death which offers invaluable and ingenious inroads into the quest of the investigator's and gives the final turn in the crime[1]. As there are numerous course of angles to perceive the crime and to comprehend when the crime has an intriguing aspect involved in the death, where the time since death becomes an important feature which leads to a new depth and dimensions to reveal the final part of the crime[2].

Vitreous humour fluid is protected from the post mortem degradation and contamination, hence it is suitable for the determination of "Time since death" [3]. This study aids various factors one of which is signs of death, which is classified into: Immediate, Early and late. The early signs occur between 12 and 24 hours, late signs occur after 24 hours, whereas immediate signs are clinically important to estimate the signs of death[4].

Considering the limitations in estimating the time since death, using routine methods through the estimation of elctrolytes concentration of various body fluids. Blood and cerebrospinal fluid has been studied extensively, therefore, the potassium levels of the vitreous humour is studied[5]. Time since death is an important parameter to the forensic expert and determines the accurate time since death at extremely difficult factors[13].

Thus the present study was conducted to find the correlation of potassium levels with the Postmortem interval, and also to know the changes in the level of Vitreous sodium and chloride. This study further illustrates, whether postmortem vitreous potassium levels have any relation with the age, sex, mode of death, Temperature in the Chennai region.

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Materials and Methods

The study was done on 75 cases brought for autopsy during the period February 2006 to July 2006 in Madras Medical College, Chennai. The selection was confined to cases, in which the time of death is known either from the history or from the Hospital records. Based on the general view, the intravenous fluids and other drugs administered during hospitalisation will alter the basal potassium level, which is scrutinised by the literature, hence the 75 cases were broadly classified into: Hospital cases and Non - Hospital cases.

2-3 ml of vitreous was aspirated from the eye using autoclaved syringes with no.19 needles by piercing the eyeball lateral to the sclero-corneal junction after cleaning the area with distilled water. After obtaining vitreous, the empty vitreous cavity was refilled with normal saline to maintain the shape of the eyeball. The estimation of electrolytes like potassium, sodium and chloride was done by FLAME PHOTOMETER by adding 0.99 ml of double distilled water to 0.1 ml of supernatant portion of the centrifuged sample and feeding it in the photometer[6]. The data obtained were tabulated and studied statistically analysed by Paired t-test, using statistical package for social sciences (SPSS) version 13.0.

Observations

Two samples of vitreous humour, one from thr right eyeball and the other from the left eyeball were taken at an interval of about one hour in each case, no significant difference was observed[7]. In the present study, the cases which are not Hospitalised were focused. Out of 75 cases selected at random, 20 cases were hospitalised and 55 cases were non hospitalised during the period and the minimum and maximum values of actual postmortem interval of 20 hospitalised cases noted were 10 hours and 52 hours respectively(Table 1). Taking the basal potassium concentration in the vitreous humour at the time of death showed 4.0 m Kg/L rise during the lapse of time between death and withdrawal of vitreous humour was calculated. Based on these, the rise of potassium and the concentration of sodium and chloride in vitreous humour was estimated at that time. It further provides the prediction for the nature and pattern of the rise of vitreous humour potassium after death. It conveys the PMI in ascending order with the corresponding potassium concentration of vitreous humour[8]. Plunging deep into the ultimate cause of death in these 20 cases, it reveals the nature and cause of death.

Table 1: POSTMORTEM INTERVAL & VITREOUS POTASSIUM VALUES IN HOSPITAL DEATHS

S.NO	POSTMORTEM TIME INTERVAL (HOURS)	POTASSIUM VALUE (mEq/L)
1.	< 10	NIL
2.	11-20	6.8 - 9.4
3.	21-30	6.6 - 14.0
4.	31-40	9.6 - 11.0
5.	> 40	10.26 - 14.0

The sodium and chloride ions in all these 20 cases were found to range between 110-150 mEq and 96-149 mEq with average values of 132.7 mEq and 115.14 mEq respectively (Table 2).

Table 2: SODIUM AND CHLORIDE VITREOUS VALUES IN HOSPITALISED DEATHS

S.NO	POSTMORTEM TIME INTERVAL (HOURS)	SODIUM LEVEL (mEq/L)	CHLORIDE LEVEL (mEq/L)
1.	< 10	NIL	NIL
2.	11-20	120 - 142	96 - 142
3.	21-30	110 - 150	120 - 133
4.	31-40	120 - 140	96 - 149
5.	> 40	130 - 140	97 -102

The study of 20 hospitalised cases revealed that the vitreous potassium values were found to be not steady for the given postmortem interval, hence the interval plot against the potassium values found to be insignificant. Hence, the further studies are carried with the 55 non - hospitalised cases.

The average rise and the least rise of vitreous potassium based on the age groups are tabulated (Table 3).

Table 3: REPORT OF AGE ON THE RISE OF POTASSIUM IN VITREOUS HUMOUR AFTER DEATH IN NON-HOSPITALISED CASES:

S.NO	AGE	NO.OF CASES STUDIED	AVERAGE RISE OF POTASSIUM / HOUR (mEq)
1.	21-30 years	39	0.21
2.	71-80 years	16	0.16

Postmortem time interval and the potassium values of the non hospitalised cases were recorded to be 6 hours and 97 hours as minimum and maximum time, 5.1 mEq/L to 20.0 mEq/L (Table 4).

Table 4: POSTMORTEM TIME INTERVAL AND VITREOUS POTASSIUM VALUES IN NON HOSPITALISED CASES

S.NO	POSTMORTEM TIME INTERVAL (HOURS)	POTASSIUM VALUE (mEq/L)
1.	< 10	5.1 - 6.4
2.	11-20	5.6 - 9.5
3.	21-30	8.2 - 10.0
4.	31-40	8.0 - 11.0
5.	41-50	9.0 - 12.4
6.	51-60	16.0
7.	61-70	14.0 - 19.6
8.	71-80	NIL
9.	> 80	17.0 - 20.0

The sodium and chloride ions in all these 55 cases were found to range between 100-150 mEq/L and 90-152 mEq/Lwith average values of 142 mEq/L and 109 mEq/L respectively (Table 5).

Table 5: SODIUM AND CHLORIDE VITREOUS VALUES IN NON - HOSPITALISED CASES:

S.NO	POSTMORTEM TIME INTERVAL (HOURS)	SODIUM LEVELS (mEq/L)	CHLORIDE LEVELS (mEq/L)
1.	< 10	120 - 140	NIL
2.	11-20	108 - 156	80 - 110
3.	21-30	120 - 170	102 - 129
4.	31-40	134 - 138	98
5.	41-50	124 - 145	120
6.	51-60	NIL	NIL
7.	61-70	120 - 140	101 - 152
8.	71-80	120	NIL
9.	> 80	100 - 132	112 - 115

Based on the classification of non hospitalised cases into natural cause and unnatural cause to study the effect of nature and cause correlation upon the rise of vitreous potassium after death (Table 6).

Table 6: EFFECT OF NATURE AND CAUSE OF DEATH ON THE RISE OF VITREOUS POTASSIUM AFTER DEATH IN NON HOSPITALISED CASES

S.NO	NATURE OF DEATH	CAUSE OF DEATH	NO.OF CASES STUDIED	RANGE OF RISE OF VITREOUS POTASSIUM / HOUR (mEq)	AVERAGE RISE OF VITREOUS POTASSIUM / HOUR (mEq)
1.	NATURAL	MYOCARDIAL INFARCTION	6	0.13 - 0.24	0.19
2.	UNNATURAL	HANGING	5	0.16-0.22	0.19
3.	UNNATURAL	DROWNING	3	0.11-0.22	0.17
4.	UNNATURAL	BURNS	3	0.14-0.18	0.16
5.	UNNATURAL	POISONING	7	0.14-0.26	0.20
6.	UNNATURAL	ASSAULT	3	0.17-0.24	0.21
7.	UNNATURAL	FIREARMS	3	0.16-0.27	0.22
8.	UNNATURAL	ROAD TRAFFIC ACCIDENTS	15	0.12-0.29	0.21
9.	UNNATURAL	RAILWAY ACCIDENTS	7	0.11-0.22	0.17
10.	UNNATURAL	OTHER INJURIES	3	0.12-0.20	0.16

Discussion

Out of 75 cases, 20 cases were found to be hospitalised during the period of study. The average rise of potassium per hour in these two cases from a very wide range with 0.20 sq ft for the former and just 0.02 cents for the latter. Considering the cause of death in both the cases to be of the same nature we could give proper explanation for such a wide range. Similarly the head injury causes in road traffic accidents show a range of 0.03 to 0.20 per hour. As this to second to be very wide range, an attempt at arriving at a reasonable answer was made but in vain.

In the burn cases which was studied one among these showed 0.01 as the rise of potassium per hour. But the analysis of vitreous humour taken from the same case one hour earlier gave a more favourable answer with 0.13 mg/hour. Considering the former value as due to some manual error the other four census with two samples each were studied. They gave rise to vitreous potassium/hour in a range of 0.11-0.60. This also seemed to be erratic and follows no predictable pattern.

In the case of railway accident the victim had survived for 2 hours after being knocked down by train. The rise of potassium per hour were 0.20 and 0.15 with an average of 0.19, though the hospital records show administration of intravenous fluid and other drugs, considering the survival time to be very small, this result could be taken as favourable one. Drowning case followed the erratic and unpredictable pattern of other groups. The rise of potassium / hour in the four poisoning cases were found to lie between 0.11 mEq and 0.26 mEq with a mean of 0.19 mEq.

Accidentally, all the 6 types of non - hospitalised cases of death due to natural cause were found to be due to heart failure as a result of coronary occlusion. The survival time had not exceeded 1 hour in any case or these 6. The highest value of rise of vitreous potassium per hour was 0.24 mEq and the lowest was 0.13 mEq with a mean of 0.19 mEq. The range and mean were found to be a reasonable answer for an ever expected easy and reliable method of estimating the time since death. None of the hanging cases survived for more than 10 minutes and hence discussion over the effect of survival time on the rise of vitreous potassium was abandoned.

Though the drowning cases gave a stronghold on the reliability of this method with a rise of vitreous potassium per hour ranging from 0.11 mEq to 0.22 mEq with a mean of 0.17 mEq. Through some cases herewith discussed had survived for a period of time between onset of the death, since they had not been attended, in such a way to alter the vitreous electrolyte values, the consideration of the survival time and its effect on the rise of vitreous potassium stands unnecessary.

The findings of the study showed a rapid increase in the level of vitreous potassium which increases. There is a correlation of potassium level of vitreous and the postmortem interval and found that the rise in the levels of potassium after death has a correlation with the postmortem intervals[9].

The postmortem interval for the 55 non hospitalised cases ranged from 6-91 hours. When this was compared with the corresponding vitreous potassium values, there is a progressive steady rise of vitreous potassium after death from a basal level of 4.0 mEq/Litre at -0 postmortem interval. Though in some cases the potassium values for the same postmortem interval differs slightly. This rise of vitreous potassium after death was found to be steady throughout and follows a predictable pattern till first 97 hours.

It is hypothesized that the concentration of vitreous biochemical constituents in both the eyes change at the same rate and the changes occurs is time dependent and it is used to estimate the postmortem interval[10].

Regarding the changes in the potassium concentration and its role in ages and in its vitreous humour after death. Out of 55 non hospitalised cases 46 were male and 9 were females, which were studies age wise and grouped as 21-30 years and 71-80 years. The age group of 21-30 years found to have maximum average rise of vitreous potassium per hour whereas the age group of 71-80 years, found to have the least of the average rise of vitreous potassium per hour. This is consistent with the results of other study[11].

According to Abhi and Garg[12], the observations that there is no effect of temperature on the levels of potassium concentrations in the vitreous after death is in concordance with the present study. Further, to study the effect of temperature on the rise of vitreous

potassium after death, out 55 cases 38 were stored in the cold chamber at a temperature below 0℃and the rest were in the room temperature, between death and the withdrawal of vitreous humour. This study reveals that the range of rise of vitreous potassium is 0.11 mEq - 0.29 mEq for the bodies stored in the cold chamber and 0.13 mEq-0.27mEq for the bodies at room temperature. The inference thereby arrived, that the temperature has no effect on the rise of vitreous potassium per hour for both the groups. Farmer et al[13] studied that in warm seasons with higher environmental temperature all the time since death which caused a marked enhancement of the observation of the potassium values in the vitreous humour.

We find that there is a average rise of vitreous potassium per hour for the non hospitalised cases which is classified into death by natural cause and death by unnatural causes.

Conclusion

This study confirmed that the method of using vitreous humour potassium rise after death in non hospitalised cases were estimated to find the time since death which followed a easy and reliable method. This method should be applied to non hospitalised death, because it has been proven that the intravenous fluids and other drugs administered during the hospitalisation definitely distort the progressive and steady rise of vitreous potassium after death resulting in an erratic pattern of total unpredictability. The nature of the cause of death has no effect on the rise of the vitreous potassium as described in the non hospitalised cases. It is proven that greater the survival time, greater will be the erratic pattern of the rise of vitreous humour potassium in hospital cases. It is fortunate enough to find that the nature of the cause of death has nothing to do with the steady rise of vitreous humour potassium after death in non hospitalised cases, so also the age, sex and the environmental temperature. This study concludes with the fact that the vitreous sodium and chloride are totally unaffected and stable for the first 97 hours after death.

Ethical Clearance: Obtained from institutional ethical committee, Madras Medical College.

Source of Funding: Self.

Conflict of Interest: Nil.

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Cross Sectional Evaluation of Females in Fatal Road Traffic Deaths

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Abstract

The unnatural deaths can be divided as per the manner as suicidal, accidental, homicidal or undetermined .the accidental deaths constitute a major portions of unnatural deaths. Female population in India were often not acquainted to travel by their own like by means of two wheelers and four wheelers in olden days but the proportion has a gradual rise in recent times. The total number of 107 cases constituted unnatural female deaths autopsied from June 2013 to August 2014 out of which 52 cases were confirmed to be road traffic deaths constituting 49% out of which 10 were pedestrians 33 travelled in two wheeler and 9 in four wheeler. On analysis of these 33 cases it was observred that most of them were pillion riders (73%), 95% of female victims did not use helmet when travelled on two wheeler and the injuries on the body were more on the left side (69%) than right. All the victims who travelled in the four wheeler did not use seat belt(9)

Keywords: *Unnatural deaths, Road traffic deaths, Helmet, Seat belt*

Introduction

According to the 2011 census, India recorded a sex ratio of 940 females per 1000 males, which is lowest among the 10 most populous countries of the world. The fascinating state of Tamil Nadu is the emerging hub of technology and modern lifestyle in India. Tamil Nadu is one of the most developed states of India with 44% of its population living in urban areas and seventh most populous state in India. The state witnessed a growth of 15.6% in its population between years 2001 to 2011[1]. Female population in India were often not acquainted to travel by their own like by means of two wheelers and four wheelers in olden days but the proportion has a gradual rise in recent times for education and employment and thus being exposed to road traffic deaths.

The pattern of road traffic deaths show in a study conducted in Sri Lanka shows an ever-increasing trend and an alarming number of fatalities. The circumstances of unnatural deaths of females in the above said study showed 40% were accidental deaths while remaining 60% were other causes of unnatural deaths[2]. The major factors leading to more number of two wheeler accident were due to increase in traffic and poor adherence to road safety rules & traffic laws. mobile phone usage while driving a vehicle remains to be a major contributory factor in road traffic accidents and thus use of such gadgets are strictly prohibited by. Many countries have imposed restrictions on the high speeding, legal age for driving license, graduate driver licensing, increase in fine & withdrawal of Driver's license and speed control interventions have led to significant reduction in traffic accidents[3]. According to National Crime Record Bureau report of 2015 – 2019 Tamilnadu stays as a leading

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contributor towards number of accidents and accident related deaths[4].The recent traffic laws focus mainly on strong implementation of wearing protective gears and imposing stringent fines on violators[5]. Studying the profile of unnatural deaths is of extreme importance to a country, especially to draw policy in preventive strategies.

Aims and Objectives:

- To study the incidence of various types of female road traffic deaths

- To analyze and indentify the preventable risk factors in such deaths.

Materials and Methods

All cases of unnatural female deaths brought to the mortuary of Sri Ramachandra Medical College and Research Institute, Chennai for postmortem examination during the period of May 2013 to August 2014, were studied in detail. This cross-sectional observational study was undertaken in which all road traffic deaths admitted in Sri Ramachandra Medical College and Hospital or admitted in any other hospital and referred to Sri Ramachandra Medical College and Hospital and received as brought dead to the mortuary of Sri Ramachandra Medical College and Hospital were included in the study and all other female deaths due to any cause other than road traffic deaths were excluded.

A standard proforma specially designed was used to collect information regarding their mode of travel and their usage of protective gear. The history and data were obtained after detailed interviews with investigating officers, deceased's kith and kin in addition to hospital records of the deceased. In cases of deaths associated with two wheeler riders and pillion riders their usage of helmets and in cases four wheeler deaths their usage of seat belts were collected. Detailed examination findings of all external and internal injuries were also recorded and along with their sides. Abrasions and superficial contusions (skin deep) were grouped as minor injuries and deep contusions, lacerations and fractures with or without internal injuries were considered as major injuries. Data were analyzed using computer software, Statistical Package for Social Sciences (SPSS) version 11.5 and were expressed in its frequency and percentage.

RESULTS:

The total no. of female victims in road traffic deaths (52) on segregation were found as 10 pedestrians (19%), 33 travelers in two wheeler (64%) and 9 (17%) travelers in four wheeler (Graph-1). Among the 33 victims of two wheelers most of the victims were pillion riders constituting 73% of victims (Table 1). Most of the victims (95%) who travelled in two wheeler did not use helmet as a protective gear (Table 2). The patterns of injuries in two wheeler victims were studied based on site of infliction of major injuries.it was observed that more injuries were present on the left side of the body of victims (69) than the right side (51). Among the injuries on the left side, lower limb injuries (38%) were maximum followed by head injuries (33%). But on the right side injuries the lower limb injuries (41%) were maximum followed by injuries to the torso (33%). Among 9 cases of four wheeler victims all the victims (9) did not wear their seat belts and 1 victim intentionally jumped out of the running car accounting to suicidal nature.

Table -1: Type of two wheeler occupants (n= 33)

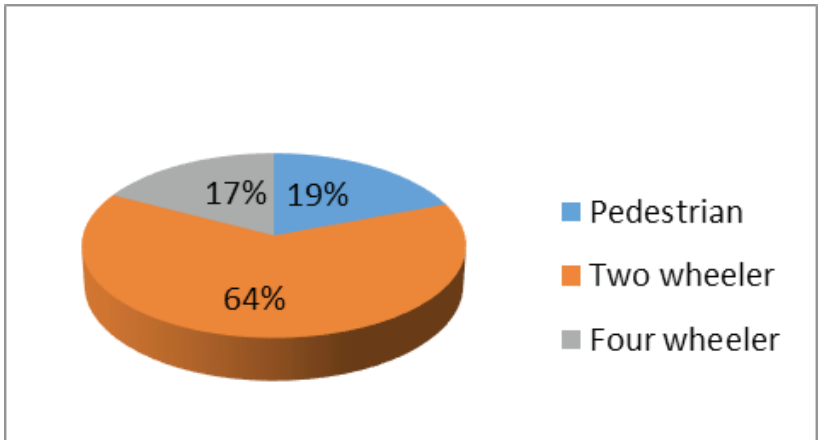
	Rider	Pillion rider
No. of cases	9	24
Percentage	27	73

Table -2: Use of helmet among female victims (n = 33)

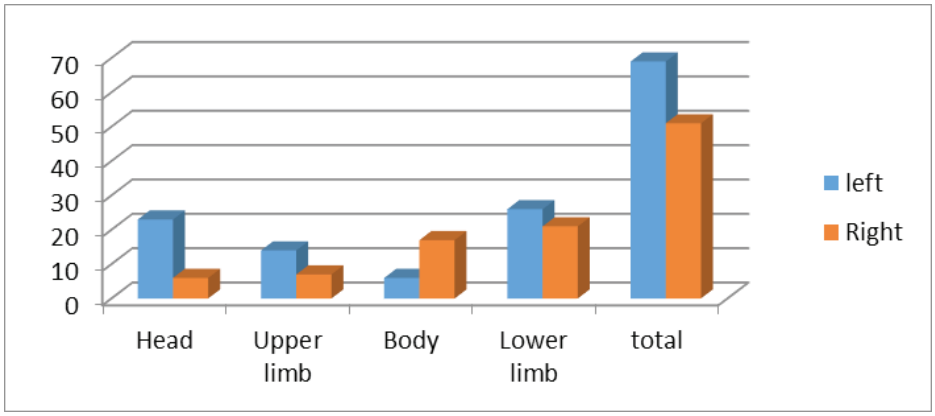
	With helmet	Without helmet
No. of cases	2	32
Percentage	6	94

Table -3: Distribution of injuries in female victims of two wheeler accidents

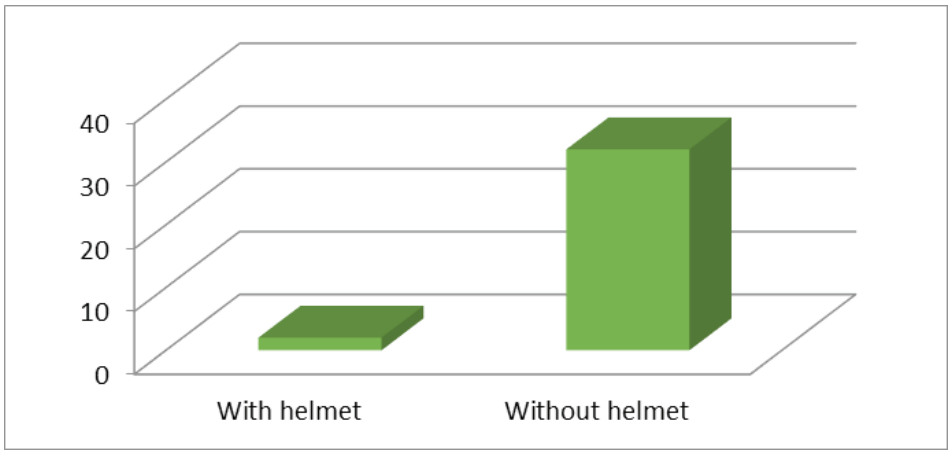
	Head	Upper limb	Body	Lower limb	total
left	23 (33%)	14 (20%)	6 (9%)	26 (38%)	69 (100%)
Right	6 (12%)	7 (14%)	17 (33%)	21 (41%)	51 (100%)



Graph-1: Types of female victims



Graph-2: Distribution of injuries in female victims of two wheeler accidents



Graph-3: Usage of helmet in victims of two wheeler accidents

Discussion

Among the three categories of the victims two wheeler was the predominant factor in road traffic deaths in our study. Similar finding was noted in Animesh Jain et al[6] where two wheeler road traffic accidents are commonest cause and it is seen that there is a rising trend. In a study done by Maria Segui-Gomez et al[7] it was observed that female two wheeler awareness is of a lesser scale as a result more victims noted on two wheeler commuters. Similar study result were found in national crime record bureau report were 38% of their study population belonged to two wheeler.

In two wheeler victims we found majority of them were pillion riders on the contrary in a study done by K. Prasannan et al[8] it was observed that more number of victims were riders this difference in result is mainly due to females riders are less when compared to female pillion riders. Not using helmet by two wheeler riders was the major cause of road traffic deaths similar findings were observed in K. Prasannan et al[8] were a considerable proportion (62%) of two wheeler travellers are not interested to wear helmets

In our study the injuries on the left side of the body were more than the right side. This could possibly be due to the position of females sitting as pillion i.e. facing the left side of two wheeler. So during the fall in road traffic accidents the left side of the body receives the secondary injuries (impact on the road / surface) more often. Due to fall the lower limb is more likely to get the first impact on the road which describes it to be the commonest site.

Among the victims travelled in four wheeler (9) none of them used seat belt while travelling. This explains the lack of sensitivity and awareness among the commuters of four wheelers. In India although seat belt is mandatory for driving it is not followed with spirit by the citizens. It is not even felt necessary to use the seat belt by the four wheeler occupants other than the driver.

In our study one four wheeler victim committed suicide on the road and reason was evaluated to be marital disharmony which was observed by Priyadarshee Pradhan et al[9] that Marital disharmony is the customary cause behind female suicides.

Summary and Conclusion

The females are more vulnerable to road traffic injuries and death in India due to increased employment among females, various reasons to commute and rapid growth of population and economy. More number of two wheelers on the road increases the number of female victims. Females as pillion riders are not safe during drive especially without protective gears. Non use of seat belts makes the vehicle occupants vulnerable during accidents.

In many states helmet is not mandatory for pillion riders and females which needs to be introspected. Separate lane driving and speed limit need to be implemented especially in urban roads and highways. Although the traffic laws have been amended recently it has not percolated to the citizens' mind that road safety is more of individual responsibility than the state.

Ethical Clearance: Obtained from Institutional Research Ethics Committee, Sri Ramachandra Medical College & Research Institute (Deemed to be University)

Source of Funding: Self funded

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Advances in Epidemic Control Laws & COVID-19 Pandemic in India: A Medico-Legal Analysis

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Abstract

This paper deals with the efficacy of the *Epidemic Diseases Act*, 1897 and *Epidemic Diseases Amendment Act*, 1937 in the context of Covid-19 pandemic. The colonial mould of epidemic control laws reviewed the World Health Organization (WHO) legal guidelines for disease preparedness and response. The public health strategies of epidemic-pandemic control need juristic innovation, and blanket application of British enactment fall short of the expectation of the right based approach health and equity. The enforcement of the *Epidemic Diseases Act*, 1897 during dengue, swine flu, influenza and COVID-19 criticized by the Indian medico-legal community in India. The emerging discourse can be tailor-made to suit the magnitude of the Covid-19 and pandemics needs a careful analysis. Though the WHO *Revision of the International Health Regulations*, 2005 offers new paradigm the Indian government resort to the *Epidemic Diseases Amendment Ordinance*, 2020 needs a critical appraisal in COVID-19 pandemic in India.

Keywords: *Epidemic Laws, Health Regulations, WHO Legal Guidelines, Epidemic Ordinance, COVID-19 Pandemic*

Introduction

The nationwide lockdown and enforcement of quarantine law under *Epidemic Diseases Act*, 1897 *Epidemic Diseases Amendment Act*, 1937 *Epidemic Diseases Amendment Ordinance*, 2020 needs a legal examination in the context of Covid-19 pandemic**.** Entry 6 of the State List of the Seventh Schedule to the Constitution of India, 1950, envisages health within the exclusive domain of the states. *T*he prevention of infectious or contagious diseases and pests affecting men, animals or plants placed under Entry 29 of the concurrent list. The central government sledgehammer declaration of national lockdowns logical sustainable and legally tenable for the implementation of coherent policies across the nation.[1] The catastrophic dimensions of Covid-19 pandemic necessitated either the national emergency under the constitutional power of the centre or promulgation of Ordinance on public health-giving a fillip to long-standing demands of health law reform.[2] The void seems to have filled by the Disaster Management Act, 2005's omnibus provisions of the 'catastrophe beyond the coping capacity of the community.[3] The *Indian Penal Code*,1860 and *Epidemic Diseases Act,* 1897 as colonial legislation analyzed in the precept of Britain's *Public Health Act,* 1875 and *Public Authorities Protection Act,* 1893. Independent India's health reform zeal reflected in the *National Health Bill*, 2009; *Public Health Bill,* 2017; and *Health Services Personnel and Clinical Establishments Bill,* 2019 did not mature in law in the emergency preparedness of COVID-19 pandemic.[4] India's legal preparedness reflected in *Epidemic Diseases Amendment Ordinance*, 2020 is dominantly 'policing' in nature. The scale of emergencies and health preparedness needs a critical mass of public health legislation having the right and equity approach.

Material and Method

The material and method of epidemic-pandemic driven study rooted in the World Health Organization (WHO) legal guidelines for disease preparedness and response.[5] The public health strategies of epidemic-pandemic control[6] in the Parmet & Sinha's methods offer the novelty of juristic pragmatism.[7] The legislative survey of the epidemic control laws also delineated in the right

based approach health and environment.[8] The review of *Epidemic Diseases Act*, 1897 in the context of Influenza response abounds in Indian writing by Kakkar,[8] Patro[10] and Rakesh.[11] The potential of these analyses can be tailor-made in the Covid-19 pandemics needs a thorough examination.[12] The *Revision of the International Health Regulations*, 2005 by WHO in H5N1 avian flu epidemic provides meaningful narratives in COVID-19 pandemic to this end the established statutory canons of statutory interpretation and case law methods applied to the understanding of the *Indian Penal Code*,1860 *Epidemic Diseases Act,* 1897 *Epidemic Diseases Amendment Act*, 1937 *Epidemic Diseases Amendment Ordinance*, 2020 in combating COVID -19 pandemic.[13]

Findings

The recourse to a vintage *Epidemic Diseases Act of 1897* empowering the government to adopt special measures and stringent quarantine enforcement during COVID-19 pandemic deserves critical scrutiny. Its antiquity combined with the *Indian Penal Code, 1860* trust the lawmaker placed in the executive to deal with epidemic and pandemic.[14] The repeal of the *Quarantine Act*, 1870 placed excessive reliance on section 271 of the *Indian Penal Code*, 1860 regarding the disobedience to quarantine rule.

Historical Legacy & Epidemic Laws

The *Epidemic Diseases Act,* 1897 adopt special measures and stringent quarantine enforcement in line with the *Indian Penal Code,* 1860 punitive import. The socio-legal dynamics of the law deserves historical and sociological inquiry. The bubonic plague which compelled Sir John Woodburn to bring a law on the prevention of the spread of epidemic diseases.[15] The Governor-General empowered to undertake special regulatory measures as may be deemed expedient. The foreign trade was likely to suffer on account of the contagion spreading through the suspected ship whose departure from India with sickness on board might give grounds for severe measures to be taken against Indian shipping abroad. [16] The *Epidemic Diseases Act, 1897* has little to offer about public health needs of the native masses. The law abdicated the legislative function in favour of the executive by conferring unbridled powers And legal immunity.[17] It is reasonably believed the imposition of the *Epidemic Diseases Act,* 1897 aimed at protection of trading interest of colonial master sought to protect the British from the contagion of bubonic plague.[18]

Anglo-Indian Epidemic Laws

The Anglo-Indian comparative law offers a fascinating account of deadly contagion in the historical annals of the Black Death and the Great Plague from 1665 to 1666. The sovereign English legislature enacted a comprehensive law as *Public Health Act,* 1875. The regulatory framework spread over to 343 sections having robust infrastructure for infectious diseases and hospitals management. Section 264 of the *Public Health Act,* 1875 empowers the citizenry for action only after the lapse of a months' notice on the local authorities.[19] The immunization of administrative action, and personal liability clause replaced by the *Public Authorities Protection Act,* 1893.[20] The British were neither strangers to epidemic nor the notion of the epidemic law. But they adopted double standard for epidemic control while dealing with Indian masses. The *Epidemic Diseases Act,* 1897 passed after the two elaborate enactments of *Public Health Act,* 1875 and *Public Authorities Protection Act,* 1893 but remained highly superficial in content and outlook. Thus the grounds for testing plenary legislation were far more limited in the pre-constitution era and applied in a wholesale manner in COVID-19 pandemic in India.[21]

Colonial Mould of Epidemic & COVID-19

A comparative study of the *Public Health Act,* 1875 and *Public Authorities Protection Act,* 1893 of Great Britain and *Epidemic Diseases Act,* 1897 impels to examine the epidemic Law in a holistic framework. The colonial mould of epidemic law allowed perpetuating in COVID-19 scenario in India. The Indian government health reform reflected in the *National Health Bill*, 2009; *Public Health (Prevention, Control and Management of Epidemics, Bio-Terrorism and Disasters) Bill,* 2017; *Prevention of Violence against Doctors, Medical Professional and Medical Institutions Bill,* 2018 and *Health Services Personnel and Clinical Establishments (Prohibition of Violence and Damage to Property) Bill,* 2019 did now see any progress in COVID-19 pandemic. After all the *Epidemic Diseases Act,* 1897 is a colonial relic of conflicting moralities applied while legislating for the English masses and native Indian.[22] The ambit

and scope such laws immunized all executive action and placed beyond judicial review. The *Indian High Courts Act*, 1861 generally upheld the excessive delegation in emergent situations of the epidemic. The Council of the Governor-General seems justified by the Privy Council ruling in *Empress* v. *Burah* 1878 tilting to the convenient mode of executive actions in an emergency.[23]

Discussion

The *Epidemic Diseases Act,* 1897 granted omnibus power to the Governor-General the power to epidemic regulations for the prevention and control of the dangerous epidemic disease. Later on *Epidemic Diseases Amendment Act,* 1937 assumed the federal character from robust centralized driven epidemic control measure relating to inspection of vessels at ports.[24]

Epidemic Diseases Act, 1897

The vintage law of *Epidemic Diseases Act,* 1897 dates back to 123 years despite two-page law and five sectional enactments. The laws of more distant antiquity such as the *Indian Penal Code, 1860, Indian Evidence Act,* 1872 and *Indian Contract Act*, 1872 in the currency in contemporary India cloaked the antiquity of *Epidemic Diseases Act,* 1897 during COVID-19 pandemic. The Central and state government have omnibus power for the prevention of infectious diseases. The disobedience of quarantine order dealt following Section 188 of the *Indian Penal Code*, 1860. The disobedience compounded with the tendency to cause danger to human life, health or safety converted with the offence of public order such as a riot or affray.[25] It may be mentioned that under Section 188 of *Indian Penal Code,* 1860, an intention to cause harm is not relevant as mere knowledge of the order gives sufficient cause for liability of committing the offence. In *J. Choudhary v. State of Orissa,* [26] the Orissa High Court in the wake of kala-azar epidemic punished a homoeopathic doctor from refusal to vaccination under Section 3 of the *Epidemic Diseases Act,* 1897. The Court held that the intention of the doctor was irrelevant and mere disobedience is thus actionable *per se* under the Act. The perusal of the law prevention of epidemic disease reveals that although it prevents the spread of contagious diseases, health care and access to medicine[27] to the people has never been a goalpost during the epidemic and pandemic.

Epidemic Diseases Amendment Ordinance, 2020

The novel outcome of the COVID-19 pandemic has been the promulgation of *Epidemic Diseases Amendment Ordinance*, 2020 which is ostensibly a security and safety law for the medical fraternity.[28] that is why the revamping of *Epidemic Diseases Act,* 1897 laced with punitive overloads and stringency of the executive action.[29] The salient features of the Ordinance revolves around acts of violence, healthcare service personnel

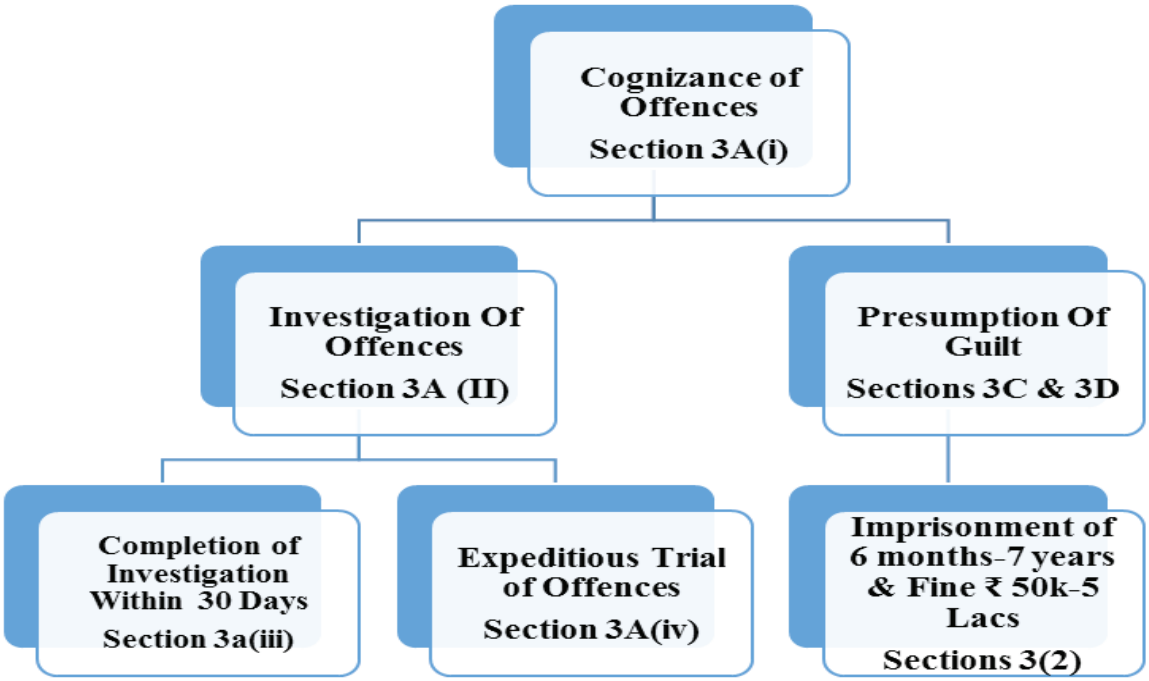
and protection medical property.[30]

Salient Features of Epidemic Diseases Amendment Ordinance, 2020

The triangular approach to epidemic control manifested in acts of violence, healthcare service personnel and protection of medical property. Section 2 B of the *Epidemic Diseases Act*, 1897.[31] The definition of '*acts of violence'* encompasses harassment, intimidation, harm and injury to doctors, nurses, paramedical workers, community health workers in the discharge of duties within the precincts of the hospital and mobile health delivery.[32] The acts of violence, healthcare service personnel and protection of medical property provided under Section 1A (a), (b) and (c) is attracted when a health care service and delivery has direct nexus concerning the epidemic control drive.[33] The ambit and scope of the medical property enlarged to cover clinical establishments, quarantine and isolation facility, mobile medical units, and damage to medical data and documents.[34]

Cognizance, Investigation and Trial Procedure

The cognizance, investigation and trial of offences are punitive as well as compensatory in under the ordinance nature. Sections 3(2) and 3(3) of the *Ordinance* provides for the punishment for commission as well as abetment of an offence.



The presumption of guilt for causing 'grievous hurt' under Section 3(3) entails that the commission of offence unless the contrary proved. The grievous hurt requires a culpable mental state for the commission of the crime by the accused. Section 3D (1) mandates that the Court shall presume the existence of such mental state with the caveat under Section 3D (2) that 'a fact is said to be proved only when the Court believes it to exist beyond reasonable doubt and not merely when a preponderance of probability establishes its existence.' The Ordinance couched in anomalies on the count of presumption of the existence of facts. Section 114 of the *Evidence Act*, 1872 envisages that the Court should draw a distinction of fact and culpable mental state on a preponderance of probability. The onus is upon a person prosecuted under Section 3(3) of the *Epidemic Diseases Amendment Ordinance*, 2020, cannot be derived from the mere preponderance of probability. On closer analysis, it appears that Section 3D (2) renders this presumption in Section 3D (1) perfunctory.[35]

Conclusion

The *Epidemic Diseases Act*, 1897 *Epidemic Diseases Amendment Act*, 1937 *Epidemic Diseases Amendment Ordinance*, 2020 is a continuum of the colonial and post-colonial mould of epidemic control and quarantine law. These enactments represent the state power to enforce sledgehammer enforcement with little regards to health right and equity. The objectives of the *Epidemic Diseases Act*, 1897 is to contain the contagion of the bubonic plague spreading and prevent a slump in foreign trade by the British in India. Therefore, the government assumed power of the medical inspection of suspected Indian shipping abroad. The recourse to a pre-Constitution vintage of the *Epidemic Diseases Act,* 1897 is antithetical to Entry 29 of the concurrent list of the *Constitution of India,* 1950. The President under Article 123 of the *Constitution of India*, 1950 promulgated the *Epidemic Diseases (Amendment) Ordinance*, 2020 on April 22, 2020, to deal with the emergent situations. They were, however slipping into the colonial mould of state action and executive power. The critiquing of the Ordinance discerns missed opportunities and misplaced priorities**.** The Ordinance miserably fails to envision a law in terms of Entry 81 of Union List dealing with the 'inter-state migration and inter-state quarantine.' The COVID-19 viewed as a catastrophe for the justification of the lockdown. Still, it adversely stifled health rights and public health delivery at national and the state *inter se* with silence on regulating inter-state migration of epidemic control.

Conflict of Interest-None

Source of Funding- Self

Ethical Clearance- Not Required

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Correlation of Age, Sex, and Symptoms to Number of Problematic Joints in Children with Idiopathic Juvenile Arthritis

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Abstract

Background: Juvenile Idiopathic Arthritis (JIA) is a rare case in Asia, including Indonesia. **Objective:** Analyze the correlation between age, sex, and symptoms on the number of joint problems in children with JIA symptoms. **Methods:** This study used a retrospective design from January 2016 to December 2019. The data collecting in this study was included age, sex, symptoms, and the number of joint problems. The analysis used was multiple linear regression with $p < 0.05$. **Results:** The correlation between sex ($\beta = 0.015$; 95% CI -0.002 – 0.002; $p = 0.916$), age ($\beta = 0.015$; 95% CI -0.197 – 0.291; $p = 0.916$) to the number of joint problems was not significant. While, the correlation between symptoms and the number of joints with problems was significant, which included swelling ($\beta = 1.012$; 95% CI 0.243 – 1.782; $p = 0.011$), fever ($\beta = 1.000$; 95% CI 0.082 – 1.918; $p = 0.034$), and pain. ($\beta = 0.931$; 95% CI 0.082 – 1.918; $p = 0.007$). **Conclusion:** There is a significant relationship between symptoms and the number of problem joints while age and sex are not significant.

Keywords: *Juvenile Idiopathic Arthritis, Age, Sex, Symptoms*

Introduction

Juvenile Idiopathic Arthritis (JIA) is a chronic arthritis disease found in children under 16 years with autoimmune causes [(1)]. The clinical symptoms that occur in children with JIA are varied and non-specific. Affected, due to the variety of clinical symptoms that arise, sometimes JIA disease in children can go undiagnosed. This is lead to short to long term disabilities in children [(1, 2)]. In Europe and North America, the incidence of JIA is reported as 16 - 150 out of 100,000. Meanwhile, in Asia (Japan) the incidence of JIA is reported very low as much as 0.83 per 100,000 [(1, 3)]. In various countries, there are only a few surveys that assess the epidemiology of JIA. Even in developed countries such as France, where rarely does anyone know about the course of a patient's disease accurately both from diagnosis and treatment [(4)]. In Indonesia there is a low of JIA reporting, the incidence of JIA in Indonesia that has been reported is 198 from 2001-2006 [(5)].

Pediatric rheumatology is still considered a youth subspecialty in many countries, and the standards of medical education and patient care have developed rapidly over time. (6). The academic recognition for this subspecialty and the opportunity to obtain rheumatology medical education in various pediatric centers is still insufficient, although some developed countries have shifted patient care towards highly specialized multidisciplinary pediatric rheumatology teams [(7, 8)].

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But not in various other countries, as a result, until now many pediatricians and general practitioners are still lacking knowledge about rheumatic diseases and clinical examinations of pediatric musculoskeletal [9].

The lack of attention to the JIA problem causes this disease to feel strange in society, especially health workers [2] such as in Indonesia. This has an impact on delays in diagnosing JIA, thereby reducing the success of JIA management. Even now, the etiology and pathogenesis of JIA are not known with certainty, so it still uses criteria in making the diagnosis [5]. The result is frequent misdiagnosis that is detrimental to the patient [10].

Moreover, based on the importance of diagnosing JIA, we as researchers wish to know the relationship of age, sex, and symptoms to the number of problem joints in children with JIA in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia as the largest specialist medical teaching hospital in Eastern Indonesia. Knowing the correlation between age, sex, and symptoms on the number of joint problems in children with JIA.

Methods

Participants

Participants in this study were children diagnosed with JIA. Participants must meet the inclusion and exclusion criteria. Participant of inclusion criteria was included patients aged >18 years, patients diagnosed with JIA based on the International League of Associations for Rheumatology (ILAR) [1, 10]. Participants' exclusion criteria included incomplete patient medical records.

Setting

The study used a retrospective design and the data used was from January 2016 to December 2019. The determination of participants number was 50 patients, obtained by using the total sampling method. This research was first carried out by ethical approval based on the Declaration of Helsinki in Ethics Committee (1528 / KEPK / IX / 2019). The data collection was included age, sex, symptoms, and the number of joint problems. This data collection by looking at hospital medical records in that period and then analyzed.

Statistical Analysis

The data obtained and analyzed by using IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). The data presentation technique was used table analysis of the form of frequency distribution and percentage. Meanwhile, the bivariate analysis used in this study was the multiple linear regression method with $p < 0.05$.

Results

Characteristic of Participant

The mean age of the participants was 105.12 ± 45.33 months with a median value of 108.00 (24.00 - 192) months. Most of the participants were 108 months old (12%), followed by 120 months and 132 months, which were 10% respectively. Most of the participants were male was 60%. Most of the participants who experienced pain symptoms were 88.0% and the number of participant joint disorders was at most 2 joints (46.0%; Table 1).

Correlation between sex, age, and symptoms on the number of joint problems in children with Juvenile Idiopathic Arthritis

The correlation between sex and the number of problem joints in children with Juvenile Idiopathic Arthritis was not significant ($\beta = 0.015$; 95% CI -0.002 – 0.002; $p = 0.916$). In addition, it was found that there was no significant correlation between age and the number of problem joints ($\beta = 0.015$; 95% CI -0.197 – 0.291; $p = 0.916$). A significant correlation was found between symptoms and number of joint problems, which symptoms included swelling ($\beta = 1.012$; 95% CI 0.243 – 1.782; p = 0.011), fever (β = 1.000; 95% CI 0.082 – 1.918; p = 0.034), and pain ($\beta = 0.931$; 95% CI 0.082 – 1.918; $p = 0.007$; table 2).

Table 1. Characteristic of participant

Variables	n (%)
Sex	
Male	30 (60.0)
Female	20 (40.0)
Patient Complaints	
Swollen	20 (40.0)
Fever	13 (26.0)
Pain	44 (88.0)
Number of Problematic Joints	
One joint	17 (34.0)
Two joint	23 (46.0)
Three joint	3 (6.0)
Four joint	0 (0.0)
Five joint	7 (14.0)

Table 2. Correlation between sex, age, and symptoms on the number of joint problems

	t	β	CI 95%	p
Sex	0.106	0.015	-0.002 – 0.002	0.916
Age	0.106	0.015	-0.197 – 0.291	0.916
Swollen	2.663	1.012	0.243 – 1.782	0.011*
Fever	2.205	1.000	0.082 – 1.918	0.034*
Pain	2.827	0.931	0.264 – 1.597	0.007*

Note: *significant $p < 0.05$

Discussion

In our study, it was found that JIA cases have more males than females. Based on previous research, it was stated that JIA cases were more common in females. 2004 In Spain was found 432 cases of JIA, of which 288 were female [11]. A study in France in 2010 also had the same cases of JIA in female than male [12]. In the case of JIA, it is often found in female than male [11-13].

Several literatures states that JIA cases often occur in children under 16 years, which is consistent with our study [1, 2]. This condition is supported by previous research where JIA cases occurred in the age range of 1.6-18 years and the mean age of the participants was 8.1 years [14]. Other studies also found that the age of children who experienced JIA was 7.4 years (median) with an interquartile range of 3.6 - 11.2 years [15].

Arthritis is clinically characterized by swelling, warmth, stiffness of joint effusion, joint pain and, limited range of motion, and limited movement due to pain [16]. Inflammation of the joints causes pain, loss of function, and morning stiffness. The distribution

of complaints varies between JIA subtypes. Systemic symptoms usually occur in systemic and polyarticular subtypes and can include fatigue, weight loss, anemia, anorexia, or fever. Growth abnormalities can complicate JIA and cause short stature or local growth disorders such as bone overgrowth, prematurely fused epiphyses, and leg length discrepancies. This may be of value in determining patient management [1, 17].

Characterizing the number of joints affected by each joint and analyzing the correlation of the chief complaint is essential for analyzing the basic mechanisms of AIJ and for efficiently predicting future joint damage. However, there is no detailed analysis to address the correlation between these joint symptoms. The most common symptom of AIJ is joint pain. Joints may swell and become tender, red, and feel warm to the touch. Joints can become stiff and lose mobility. This causes a loss of subtle dexterity, especially in the hands [16]. Children experience anxiety due to joint pain in the hip, knee, or ankle. As a result, the child becomes less physically active because of pain and limited movement. If it lasts for a long time, it can cause damage to the affected joint, causing disability. Joint pain from arthritis can interfere with sleep that causes tiredness. Inflammation can also cause fatigue. Physical examination shows a limp, hesitates to use an arm or leg, or, in younger children, has problems with fine motor skills [1, 18].

Conclusion

There is a significant correlation between symptoms and the number of problem joints in children with JIA. Meanwhile, age and sex did not have a significant relationship with the number of problem joints in children with JIA.

Ethical Approval: This research has gone through the ethical approve stages based on the Declaration of Helsinki at Ethics Committee Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

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Ending Human Trafficking in India- A Much- Awaited Goal

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Abstract

Discussion on the issue of human trafficking in 21st century is definitely quite shameful as the entire world is transformed into a small place due to globalization. Not only in India, but in almost all the countries of the world, this worst form of crime is still existing in some form or other. Through this paper the author has attempted to analyze the reasons for prevalence of this crime in India even after having concerted efforts by the successive governments to completely eradicate the evil through statutory enactments. Being the second largest populous country of the world and having been invaded by a number of foreign invaders, the country always suffered in its socio-economic growth. The demographic feature of the country is certainly another disadvantage that makes it quite difficult for the governmental schemes to reach the poor people living in the rear ends of the country. Another reason affecting the economy is the refugees coming into the country through its porous borders and making it their permanent shelter and expecting their shares from the limited resources available for the countrymen. It is, in fact, a matter of grave concern that even after having several enactments to deal with human trafficking, still the country is witnessing its prevalence in a larger scale. Prior to the enactment of the Indian Constitution, the Constitution makers too took it very seriously for which traffic in human beings or any forms of forced labour were inserted to be prohibited under Article 23. The judiciary has also taken stringent views to eradicate the crime of human trafficking through strict interpretation of the legislative provisions. But unfortunately it is not being eradicated till today, for which it becomes imperative for all to introspect the entire system responsible for prevalence of such crime.

***Key Words**: Constitution, Crime, Human Trafficking, India.*

Introduction

In whatever manner the term 'Human trafficking' be interpreted, but it refers to the worst form of crime as the victims of human trafficking always subjected to huge mistreatment and are dealt as material objects used for gaining benefit. In most of the cases of human trafficking, the victims are forced to be engaged in profitable sex business such as getting employment in organized brothels or in any other sex trade like doing pornographic etc. Even some instances are there where the victim is compelled to live with the offender as his house maid. Such instances mostly arise in cases where the victims are kidnapped or trapped by the offender with the commitment of giving fascinating employment or delightful marriage proposals. There are even cases where the victims are sold off by their own near and dear ones for lump sum amount. Human trafficking is also made for labour purposes, where the victims are engaged in bonded labour and many other forms of forced labour. The victims in such cases are tortured both physically and mentally so as to compel them to do all these inhumane acts due to the fear for their life.

Lots of factors are responsible for human trafficking in India, such as:

- Absence of adequate employment prospective.

- Vulnerable economic condition leading to acute poverty.

- Huge disparity in societal status between the haves and have not's.

- Practice of various Socio-cultural evils resulting caste and gender discrimination.

- Lack of proper education.

- Blind beliefs within traditional religious and cultural practices.

Human trafficking in India is mostly witnessed in the states having poor per capita income of people. Poor economic condition is one of the major reasons that easily makes people victims of human trafficking in states like Andhra Pradesh, Bihar, West Bengal, Odisha, Maharashtra etc. As per a study conducted by the government of India in collaboration with the UNICEF in 1998, it was revealed that about 83% of women indulged in sex work were coming from under developed areas having very low economic perspectives. In maximum cases the victims were trafficked with assurance of good jobs, advanced career opportunities and good marital life. In some cases the trafficked persons fall victims to the ambitions of their close friends, relatives and parents also. Poverty, unemployment, extremely poor economic condition and lack of legal awareness are the major reasons for the same.

The Global Report on Trafficking in Persons, 2009[1] reveals that of the child trafficking cases across the world an estimate of about 1.2 million children were trafficked for the purpose of sexual exploitation. As per the report of the UNICEF, 2009[2,] 'approximately12.6 million children are estimated to be engaged in hazardous occupations'. In India baring few international cases, maximum number of human trafficking occurs within the country itself. As per the report of the National Human Rights Commission, out of around 40,000 children abducted in India each year, nearly about 11,000 remain untraced.

From time immemorial India is considered as a centre for human trafficking for various reasons like forced labour, slavery, child marriage, commercial sex trade etc. The maximum trafficking cases in India takes place within the country itself. As per the report of the National Crime Records Bureau (NCRB) published by the Global Slavery Index in 2016[3] about 8132 human trafficking cases of were found across the country , of which more than fifty percent were found to be below 18 years. The recent report of National Crime Records Bureau, 2018[4] reveals that as many as 5264 numbers of cases of human trafficking were reported only in India in India, out of which about 64% include women and 48% to be minors. These reports show maximum victims of human trafficking in India include women and children belonging to vulnerable classes like scheduled castes, scheduled tribes and other backward and minority groups.

Legislative Provisions & Judicial Interpretation on Human Trafficking in India

It is certainly painful to note that after so many years of achieving Independence, there are discussions on human trafficking in India. But to root out this evil, the framers of Indian Constitution also thought of around seventy years back for which Article 23 was included as one of the Fundamental Rights categorically speaking for prohibition of traffic in human beings. Further, Article 39 of the Indian Constitution makes it obligatory for the State to act for protecting the children from any kind of exploitation.

Article 23 gives protection not only against the State action, but also against the action of private individuals. The Hon'ble Supreme Court in Peoples Union for Democratic Rights v. Union of India[5] while interpreting the scope of Article 23 has held that the it not merely prohibits 'begar' but also all other forms of forced labour, as any kind of forced labour is violative of human dignity thus being contrary to basic human values. Criticizing the stand of the government in Bandhua Mukti Morcha v. Union of India[6], the Apex Court held that when a public interest litigation is initiated alleging the continuance of bonded labour, it provides an opportunity for the government to examine the veracity of the allegation regarding existence of bonded labour in any form so as to take steps to eradicate the same completely, if it is found to be true.

With a view to take more effective steps to eradicate the evil practice of human trafficking through a specific legislation, in 1956 the Suppression of Immoral Traffic in Women and Girls Act, 1956[7] has come into force. The very objective of the Act was "to inhibit or abolish the commercial vice of traffic in women, men and children for the purpose of prostitution as an organized mean of living". The Madras High Court in Ratnamala, re[8] has defined the purpose of the Act for not being abolition of prostitution per se as a criminal offence but to inhibit the commercialized vice namely traffic in women for the purpose of prostitution. Giving a broader connotation to the objectives of the Act, the Hon'ble Supreme

Court in Vishal Jeet v. Union of India[9] held that "the Act not only deals with a social problem, but also a socio- economic problem. Thus the provisions of the Act are more preventive than punitive". The impugned case was filed in the form a public interest litigation to save the girls, Devadashis and the Joginis from forced prostitution and to seek for their rehabilitation. The Court not only ordered for evaluating the various actions taken by the government in dealing with the issues as per the provisions of the concerned Acts, but also issued several directions to set up separate Zonal Advisory Committees to look into the rehabilitation of the victims and the issues relating to the Devadashis and the Joginis.

Moving further, in Gaurav Jain v. Union of India[10] the Hon'ble Supreme Court by its order directed the government to constitute a committee to make an exhaustive study on the issues relating to the problems of forced prostitution and child prostitutes and to formulate appropriate plans for their release and rehabilitation. With a view to implement the directions of the Apex Court, the government in 1998 constituted a committee in the name of 'Committee on Prostitution, Child Prostitutes and Children of Prostitutes and Plan of Action to Combat Trafficking and Commercial Sexual Exploitation of Women and Children'. The Committee in its first report reflected the various issues relating to commercial sexual exploitation and also made comprehensive suggestions to deal with the problem with certain legal reforms[11].

The Human Trafficking Act, 2005 not only prohibits the trafficking of any individual but also prohibits the act of any person performing the role of an intermediary to commission of such crime. The Act makes the offender liable of imprisonment for a period of five years with direction to compensate the victim in certain cases[12]. Besides this Act, the Immoral Traffic Prevention Act, 1956[13] is also there that deals with the immorality of trafficking, but the lacuna lies with this Act is that it only covers cases relating to trafficking for prostitution purposes without covering the cases of children trafficking for any other purpose. As per the provisions of the Act, the victims of trafficking or any person having the knowledge of trafficking of human beings may file the complaint regarding the matter either with the police or the security services at places where the victim or the offender resides or at the place of occurrence of the crime. The complaint may also be filed by any of the family members of the victim on his/ her behalf. In case the victim is a child, the testimonial is to be recorded in the presence of his/her guardian. The Police officer is under an obligation to assist and protect the victim as well as the informant, failing which disciplinary action may be initiated against such officer. If circumstances compel, the Police officer can even arrest the suspect without a warrant.

After a trafficked victim is rescued, he/ she should be provided with immediate assistance of counseling and steps to be taken for his/her rehabilitation. It then becomes imperative upon the concerned officer to trace out his/her family, but before bringing them in contact with the victim, their opinions are to be collected. Steps should be taken to provide employment opportunities for the rescued victims as per their skills. In some cases financial assistance may be provided so as to ensure his/ her sustenance.

As per the report prepared by the National Human Rights Commission of India in 2005[14], India becomes a transit point and destination for children and human trafficking for both non-sexual and sexual purposes. As because of this factor India has become unable to uphold the very essence of the Palermo Protocols those provide for the prevention, suppression and punishment for trafficking in persons, especially women and children.

Effect of COVID-19 on Human Trafficking in India

As soon as the first case of COVID-19 detected in India, the citizens of the country become panic with a fear of unforeseen danger and the government at this point become proactive in taking preventive measures like locking down the entire country in different phases. But the most unfortunate issue that shattered the entire nation during this time is the pathetic condition of the migrant workers who wanted to return their homes to be with their family during this pandemic. The issue brought down the naked reality of the socio- economic conditions of lakhs of people who have left their motherland in search of livelihood. Even thousands amongst them were in reality trafficked to work as bonded labourers in various small scale industries and factories. At this juncture the government's policy to lock down the entire country for more than three months though was

the need of the hour to tackle the pandemic, but has definitely affected the country's economy adversely resulting loss of jobs for many, making their lives more precarious than ever. Such issues clearly point towards the more increase in the number of people below the poverty line after the COVID-19 pandemic resulting in the increase in the number of trafficking victims. As per the report published by the Centre for Science and Environment(CSE), India in its 'State of India's Environment in Figures 2020', the country is going to include about twelve million poor to its populace post this pandemic. Adding more authenticity to the data, the research brief published by the United Nations Office on Drugs and Crime (UNDOC) on 14th of May, 2020 apprehends the increase in the cross border trafficking and smuggling of human beings due to the measures taken by the governments in tackling the COVID-19 pandemic. The report mainly stressed on the fact that the global economic crisis resulting the loss of employment of huge number of people for a longer period in most of the countries may lead to increase in more number of human trafficking.

Conclusion

Even after having several stringent legislative provisions still every year around lakshs of people are estimated to be trafficked in India. The basic reason being the steps taken by the states to protect the trafficking victims are considerably inadequate and more particularly the various programs proposed by the government for the rehabilitation of the trafficked victims are not being implemented in its true letter and spirit. Due to lack of timely action by the Government authorities in identifying and rescuing the unfortunate individuals, they become victims of trafficking. Only having several laws to deal with the issue is not enough, it needs careful and honest implementation of the same. In reality proper legal, psychological or medical assistance are not provided by the state governments or the central government to the victims after repatriation. In most of the cases, rather the Non Governmental Organizations play better roles in providing protection services to the rescued victims. Though central anti-trafficking law enforcement 'nodal cell' was established by the government to look into the issue of trafficking, the government as such did nothing in spite of the fact that a number of issues were upheld by them. The human rights activists and NGOs are trying their best to compensate the negligence on part of the government. It is high time now for the governmental agencies to become proactive and to try their best to eradicate this evil from its root. Besides assisting the victims and implementing the provisions of the acts and laws more effectively, the concerned offices must involve themselves in making people aware of the veracity of such offences. India has always shown new ways for any revolution to the entire world. Now time has come to show its sincere effort to completely root out the evil of human trafficking from the country so as to declare it as the no trafficking country of the world.

Ethical Clearance- Not required.

Source of Funding- Self

Conflict of Interest - Nil

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Factors that Influence Self-Efficacy in Maintaining Personal Hygiene of Postpartum Mothers

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Abstract

Background: At present the health status of mothers and babies in Indonesia is still low, which is marked by high maternal mortality rate (MMR) and infant mortality rate (IMR). Mothers and families must be able to carry out their roles and functions related to postnatal maternal health care and the health of their babies. Mother's personal hygiene helps reduce the source of infection and increases the feeling of comfort in the mother and thus increase the self-efficacy of postpartum mothers to care for their babies. The purpose of this study is to determine the direct and indirect influence of the role of health personnel, family support, physical conditions, sources of information and social environment to self-efficacy in postpartum mothers in maintaining personal hygiene at Pasir Mulya Health Center of Bogor City in 2017.

Methods: This research study used cross-sectional design. The data collection with a total sample of 60 participants was taken from questionnaires. The analysis method used was the Structural Equation Model (SEM) using SmartPLS 2.0.

Conclusion: The self-efficacy variable of the postpartum mother in maintaining personal hygiene at Pasir Mulya Health Center of Bogor City was influenced by information source (2.61%), family support (6.39%), physical condition (31.62%), information source (39.40%) and social environment (24.72%). Total direct and indirect influence of self-efficacy of postpartum mother in maintaining personal hygiene was 56.44%. Thestudy showed that when the mother received more information, then the self-efficacy will be higher. If a postpartum mother has high self-efficacy, the personal hygiene will improve as well and lower the maternal and infant mortality rate.

Keywords: *Mother, personal hygiene, postpartum, self-efficacy.*

Introduction

Self-efficacy is an individual's belief in his ability to produce the expected action on events that affect their lives. Self-efficacy determines how individuals feel, think, and motivate themselves and act. Furthermore, Bandura explains that self-efficacy is related to self-belief that one is able to control difficult situations and believes that one is able to overcome adverse situations. High self-efficacy will lead individuals to overcome challenges and obstacles in achieving goals. So, it can be concluded that self-efficacy is a person's assessment of her own ability to do something and get the desired results.(1)

The postpartum or puerperal begins after the placenta is delivered and ends when the uterine uterus returns to its state before pregnancy. The puerperium lasts for about 6 weeks or 42 days, but overall it will recover within 3 months. Blood that comes out before giving birth is accompanied by signs of birth, so that includes postpartum blood as well. To help speed up the healing process during the puerperium, the postpartum mother requires an adequate diet of calories and protein, requires adequate rest and also especially hygiene of

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the mother. Mother's personal hygiene helps reduce the source of infection and increases the feeling of comfort in the mother. The health personnel should encourage mothers to maintain personal hygiene by taking regular baths at least 2 times a day, changing clothes, bedding, and the environment in which mothers live.[2]

Maternal mortality rate is one indicator of the success of health services in a country. According to the WHO report in 2014, maternal mortality rate (MMR) in the world reached 289,000 people, divided into several countries, including the United States reaching 9,300 people, North Africa 179,000 people and Southeast Asia 16,000 people. Whereas based on the 2012 Indonesian Demographic and Health Survey (IDHS), the Maternal Mortality Rate in Indonesia stands at 359 per 100,000 live births.[3]

According to the World Health Organization (WHO) in 2011, health is a healthy condition that is intact physically, mentally and socially and not only free from disease. Maternal and infant health problems are national issues that need to get top priority, because they determine the quality of human resources for future generations. The strength of a community lies in the health of the mother and the baby that is obtained from the family or the closest person to the postpartum mother. The maternal mortality rate (MMR) and infant mortality rate (IMR) in Indonesia are the highest compared to other ASEAN countries. Many factors can affect the self-efficacy of postpartummothers, one of which is physical appearance. Other physical conditions that also affect the physical and social health of the mother (social environment) are fatigue, perineal disorders, cesarean section, back disorders, hemorrhoids, headaches, constipation, urinal incontinence, sleep disorders, lack of sexual desire, and painful sexual relations.[4]

Maternal Mortality Rate (MMR) in Indonesia which is one indicator of the degree of women's health is still high. It is the highest in ASEAN countries. The latest Indonesia Demographic Health Survey in 2007 showed that Indonesia's MMR reached 228 per 100,000 live births (Maternal Mortality Rate). This figure is still high, although it has declined from 2002/2003 which reached 307 per 100,000 live births.[5]

Maternal mortality during pregnancy and birth has an impact on the increase in MMR in Indonesia. The main cause of maternal death according to Wiludjeng (2005) was dominated (91.7%) by obstetric death due to complications of pregnancy, childbirth and the puerperium which included bleeding, eclampsia and infection.

At present, the health status of mothers and babies in Indonesia is still low, marked by the still high maternal mortality rate (MMR) and infant mortality rate (IMR). Maternal mortality rate (MMR) is the number of maternal deaths due to birth and complications of pregnancy, childbirth and the puerperium (42 days after pregnancy ends) per 100,000 live births. While the Infant Mortality Rate (IMR) is the number of infant deaths calculated against 1000 or 10,000 live births.[7]

Optimal postpartum care at home will have a profound effect on the physical and mental health status of the mother.[8]

The purpose of maintaining the cleanliness of postpartum mothers is to meet the needs of mothers after give birth, including preventing postpartum infections. Maintaining personal hygiene is not only aimed at preventing infection, but also is a basic need that must always be met to maintain health, both physically and psychologically. Mother's self-efficacy in maintaining personal hygiene is influenced by various factors, including culture, social values in individuals and families, knowledge about self-care, and perceptions of self-care.[9]

The purpose of this study is to determine the direct and indirect influence of the role of health personnel, family support, physical conditions, sources of information and social environment to self-efficacy in postpartum mothers in maintaining personal hygiene at Pasir Mulya Health Center of Bogor City in 2017.

Method

This study was conducted using descriptive research method with cross sectional approach where the relationship of exogenous and endogenous variables was measured at the same time with the aim to determine the direct and indirect effects between the influence of the role of health personnel, family support, physical condition, sources of information and social environment on themselves efficacy in post-partum mothers in

maintaining personal hygiene in 2017.

The population in this study was postpartum mothers at Pasir Mulya Health Center of Bogor City. The total amount of sample in this study was 60 participants. The processing of this data uses Structural Equation Modeling (SEM) analysis, where the number of indicators is multiplied by 5 to 10. Since the number of indicators used in this study is 12, the sample is in the range of 60-120 people. Sampling is determined using the inclusion and exclusion criteria as follows:

1. Inclusion Criteria

a. Primipara postpartum mother

b. Willing to be a participant

c. Live around PasirMulya Health Center of Bogor City

2. Exclusion Criteria

a. Not willing to be a participant

b. Participants were sick when the study was conducted

c. Participants were not available when the study was conducted.

The method of sample collection in this study was random sampling. The measurement technique used a semantic differential scale to measure attitudes, perceptions and opinions of people or groups of people about social phenomena that have a 5-point scale. In regard to Semantic Differential Scale, the variables in this study were measured and will be explained into sub-variables which were then given indicators according to variables before it was distributed to participants. The response value was arranged at an extreme continuum which represented attitude, for example: good-bad, positive-negative, like-dislike.[11]

To obtain the required data, this study useddata collection through questionnaires. The researcher asked for approval from the selected sample through Informed Consent. The validity and reliability test aimed to check whether the instruments could measure the expected variable and indicator and whether the result will be accurate and reliable. In this study, the validity and reliability were tested using Smart Partial Square.If a loading factor of 0.5-0.6 is obtained, it is said to be valid (if the value of 0.5-0.6 can still be tolerated as long as the model is still in the stage development), but the recommended loading factor is above 0.7.[12]

Average Variance Extracted (AVE) value in this study is used to measure how much variance can be captured by the construct compared to the variance caused by measurement errors. The AVE value obtained must be greater than 0.5. A reflective indicator is declared valid if it has a loading factor above 0.5 for each construct that is intended based on its substantive content which can be seen from the significance of the weight (t = 1.96).[12]

The data was obtained in the form of composition and frequency of the sample, presentation of Structural Equation Modeling (SEM) analysis, presentation of this hypothesis in the form of research based on the output of data processing results.

Result

Out of 60 participants, the total participants aged 15-20 years were 22 people (36.7%) and aged 21-25 years were 38 people (63.3%). Based on educational level background, participants with low education (primary and junior high school) were 43 people (71.7%) and participants with high education (high school / college) were 17 people (28.3%). Meanwhile according to the work status, housewife group were found with 41 participants (68.3%) and entrepreneur group as many as 19 participants (31.7%).

The Smart Partial Least Square (PLS) output for loading factors produced the following results: all indicators used in the study were obtained by evaluating the results of cross loading (discriminant validity) on all indicators. Data processing analysis showed that the construct used in forming a research model, in the confirmatory factor analysis process has met the predetermined criteria. The probability valueshown a value bigger than 0.05.

Table 1. Evaluation of R-square of family support, physical condition, social environment, health personnel role, information resources factors toward self-efficacy in maintaining personal hygiene of postpartum mothers

Variables	R-square
Self-efficacy	0.763243
Family support	0.571830
Physical condition	0.494939
Social environment	0.172096
Health personnel role	0.000000
Information sources	0.615259

From Table 1, it can be seen that the variability of the role of health personnel contributing to self-efficacy is 76.32% and the remaining 23.68% is influenced by other variables not examined in this study. The role of health personnel and self-efficacy contributed to family support by 57.18% and remaining 42.82%, the role of health personnel, self-efficacy and family support contributed to physical conditions by 49.49% and the remaining 50.51%, the role of health personnel, self-efficacy, family support and physical conditions contribute to social environment by 17.20% and the remaining 82.8%, the role of health personnel, self-efficacy, family support, physical conditions and social environment contributed to information sources by 61.52% and the rest was 38.48%.

Table 2. Percentage of Influence Among Variables towards Self-Efficacy

Source	LV Correlation	Direct Path	Indirect Path	Total	Direct (%)	Indirect (%)	Total (%)
Health personnel role	0,124	0,211	0,9247	1,1355	2,62	0,46	3,07
Family support	0,164	0,390	0,3338	0,7237	6,40	0,42	6,82
Physical condition	0,236	0,134	0,2783	0,4126	3,16	0,40	3,56
Information sources	0,611	0,645		0,6449	39,40		39,40
Social environment	0,602	0,411	0,0749	0,4857	24,75	0,04	24,79
Total					76,32%	1,33%	77,65%

Based on the pattern of relationships between variables described in the conceptual framework, there were direct and indirect relationships. The results of the parameter coefficient test between the role of health personnel on self-efficacy showed direct influence of 2.62% while for the indirect effect of the role of health personnel on self-efficacy of puerperium in maintaining personal hygiene by 0.46%. There were 6.40% direct influence between family support and self-efficacy in maintaining personal hygiene of postpartum mothers and 0.42% indirect influence. While the direct influence between physical condition and self-efficacy in maintaining personal hygiene of postpartum mothers resulted in 3.16% and the indirect influence showed 0.40%.

Information sources directly influences self-efficacy in maintaining personal hygiene of postpartum mothers. The result of the parameter coefficient test between the source of information directly affects the self-efficacy of the postpartum mothers in maintaining personal hygiene

which showed the amount of 39.40%.

Discussion

Based on the test results, the direct influence of the role of health personnel is smaller than the indirect effect. The T-statistic value indicated there was a significant influence between the role of health personnel on the self-efficacy of postpartum mothers in maintaining personal hygiene at Pasir Mulya Health Center of Bogor City in 2017. If the role of health personnel isincreased then the self-efficacy of postpartum mothers in maintaining personal hygiene will be increased as well. Health personnel are expected to be able to help provide a way out in overcoming health and nursing problems and provide health education to postpartum women / individuals, groups, and communities both at home, in the community, as well as instill healthy behavior to achieve optimal health levels.

The role of health personnel and family support is an important part of a person, so that the post-partum motherswill be able to maintain personal hygiene during the postpartum period and appreciate themselves.The role of health personnel can be described as verbal or non-verbal information or advice, tangible assistance or actions obtained due to the presence of health personnel and have emotional benefits or behavioral effects for mothers especially during give birth, especially those relating to health reaction with other.[14]Postpartum mothers who get information from others and the mass media will tend to have better knowledge about self-care in maintaining personal hygiene during the puerperium. By getting good information about postpartum care will have an impact both on physical condition and self-efficacy.

Postpartum mothers who receive attention and family support can increase the mother's self-efficacy in maintaining personal hygiene during the postpartum period because they have received information and advice from the family.[15]

Family social support can be in the form of internal family social support, such as support from husband or wife and support from siblings or external family social support.[16] From the test results of the parameter coefficient between family support and maternal self-efficacy showed there was an indirect effect of family support through physical conditions and sources of information by 0.042%. Family support influences postpartum mother's self-efficacy through physical conditions. Much evidence is shown that postpartum mothers who are cared for and loved by their family and social environment during caring for themselves in the postpartum period will show fewer emotional and physical symptoms, more easily to adjust to their new role. This is believed because there are two main needs that are demonstrated by the achievement of the mother's role, namely receiving signs that she is loved and valued, also the need for acceptance of her partner. [17]Through its function, midwives as health personnel must be able to harmonize information about good care with the family and community so that the postpartum motherswill not feel afraid or hesitant to maintain personal hygiene during give birth. Midwife's ability to carry out tasks or decision-making that is compatible with their role in the organization that is relevant to all expertise, knowledge, and the presence of knowledge from the mother.

Conclusion

It can be concluded that information sources, role of health personnel, family support, physical condition and environment affected the self-efficacy of postpartum mothers in maintaining personal hygiene.

Ethical Clearance: An approval for the study was obtained from the protocol committee and institutional ethical committee of Wijaya Husada Academy of Midwifery.

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Conflict of Interest: Nil

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Do Lab Parameter Could be used as Indicators of Severity in Case of Acute Cholinestrase Inhibitor Insecticides?

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Abstract

Background: Cholinestrase inhibitor insecticides considered one of the most common causes of morbidity and mortality due to poisoning especially in developing countries like Egypt. This work aims to study the possible role of Creatine Phosphokinase (CPK), Amylase, Lactate Dehydrogenase (LDH), C-Reactive Protein (CRP) and blood sugar status in predicting the severity of acute cholinesterase inhibitor insecticides poisoning at the time of admission.

Subjects and Methods: This work was done at Poison Control Center of Ain Shams University Hospitals – Egypt, and was carried on 150 patients admitted with acute (CEI) insecticides poisoning. Clinical evaluation of the patients and poisoning severity was graded according to Peradynia organophosphorus poisoning (POP) scale, then venous blood sample was collected from patients and examined for CPK, Amylase, LDH, CRP and random blood sugar (RBS).

There is significant increase of CPK, LDH, CRP RBS and amylase enzyme specially with increase the severity in those patients admitted to ICU rather than inpatient, and in mechanically ventilated patients rather than non mechanically ventilated and in died patients rather than survivors.

Conclusion: CPK, Amylase, LDH, CRP and random blood sugar could be used to predict severity of cholinesterase inhibitor insecticides poisoning. They can also predict ICU admission, mechanical ventilation need and mortality. Amylase is a better predictor of respiratory failure and need of mechanical ventilation.

Keywords:*Amylase, , C-Reactive Protein , Lactate Dehydrogenase, Peradynia organophosphorus poisoning scale.*

Introduction

Cholinestrase enzyme inhibitor (CEI) insecticides, are most commonly used to get rid of agricultural, household and structural pests[1]**.** The easy reach to these insecticides with lack of knowledge about its serious hazards resulting in increased its accidental and suicidal poisoning[2]**.**

According to the World Health Organization (WHO), one million accidental and two million suicidal toxicity with insecticides take place worldwide yearly with approximately 200,000 die, most of them developing countries[3].

Organophosphates (OPs) irreversibly bind to cholinestrase enzyme by forming covalent bond between it and active site of enzyme leading to accumulation of acetylcholine at synaptic gap and myoneuronal junctions resulting into cholinergic crisis[4] . Carbamates also bind

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to cholinesterase enzyme ; however, the formed bond is weaker than that formed by OPs, hydrolyze more rapidly and enzyme returns functioning again within 2 days[5] .

Cholinergic symptoms are in the form of muscarinic, nicotinic and central. Muscarinic symptoms include excessive salivation, lacrimation, urination, diarrhea, gastrointes-tinal colics, vomiting, blurring of vision, miosis, slow pulse, and wheezing. Nicotinic symptoms include fasciculation, paresis or paralysis, increased blood pressure and rapid pulse. Central symptoms include anxiety, confusion, convulsions, psychosis and ataxia[6].

Peradeniya Organophosphorus Poisoning (POP) scale is a simple scoring system was used by by N Senanayake, HJ de Silva and L Keralliceede in 1993. The score is done at initial entrance to the Emergency Room on admission before doing any medical intervention[7].

Subjects and Methods:

This work presents a cross sectional prospective study carried out at Poison Control Center (PCC) of Ain Shams University hospitals, cairo, Egypt, during the period from March 2017 to January 2018 ,it was done on 150 patients admitted with acute cholinesterase inhibitor (CEI) insecticides poisoning. The study was approved by Institutional ethical committee and an informed written consent was obtained from each patient or from his/her guardian.

Inclusion criteria:

The selected patients were adults of both sexes with acute (CEI) insecticides poisoning presented to PCC within 12 hours of exposure to insecticide and without receiving any previous treatment. Diagnosis of cases was based on history of exposure to an insecticide, clinical manifestations according to POP score and low serum pseudocholinesterase activity.

Exclusion criteria:

Patients who consumed other poisons along with (CEI) insecticides, Patients with renal, hepatic, cardiac diseases or chronic lung diseases, Patients known to be diabetics or addicts, Patients with septic manifestations, pancreatitis, epilepsy, autoimmune diseases or known cases of malignancy, Patients who presented to PCC with delay time more than 12 Hours, Patients who received any previous treatment before reaching PCC and Patients less than 18 years old.

Clinical evaluation of patients was done on arrival to ER according to POP score (Table 1).

POP score ***(Senanayake et al., 1993)***

(0-3) mild poisoning, (4-7) moderate poisoning, (8-11) severe poisoning.

Sampling:

Venous blood sample (5 ml) was collected before admission and after stabilization of the patient under complete sterile conditions. The samples were centrifuged, the serum was stored at refrigerator in (-80 degrees) until they were examined.

Laboratory parameters:

Pseudocholinesterase (PCHE) level was measured to confirm the diagnosis using kinetic colorimetric method (reference range between 4900–11900 U/L), Serum Creatine Phosphokinase (CPK) was measured by kinetic method (reference range between 36–174 U/L for men and 26–140 U/L for women), Serum Amylase was measured by kinetic method (reference range Up to 100 U/L),

Serum Lactate Dehydrogenase (LDH) was measured by kinetic ultraviolet method (reference range between 240–280 U/L), C-Reactive Protein (CRP) was measured by turbidimetric immunoassay method (reference range < 6 mg/L) and blood glucose was measured by enzymatic colorimetric method (reference range 70–105 mg/dl).

Statistical Analysis

The collected data were statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc, USA). For quantitative data, the mean and standard deviation (SD) were calculated. One-way ANOVA was used in comparing between three groups while Independent t-test was used to compare between two groups. Qualitative data were presented as number and percentages, chi square ($\chi 2$) was used as a test of significance. ROC curve was used to determine the cut-off point in which highest sensitivity and specificity of several parameters as a predictor for severity.

For interpretation of results of tests of signifi-cance, significance was adopted at $P \leq 0.05$.

Results

Out of 150 patients included in this study, 41.3% were mild, 31.3% were moderate and 27.3% were severe according to POP scale.

The study showed that the mean age of patients was 29.9 ± 12.3 years and there was significant positive correlation between age of patients and severity of poisoning (Figure 1).

59.3 % of patients were females and 40.7 % were males, 56.6 % of patients were from urban areas and 43.3 % were from rural areas.

Organophosphorus compounds (OPCs) were responsible for poisoning in 54.7% of all patients and carbamates were responsible for poisoning in 45.3% of all patients and (OPCs) were associated with more sever poisoning than carbamates.

Suicidal exposure was responsible for 79.3% of all studied patients and accidental exposure was responsible for only 20.7 %.

60.7% of all patients were admitted in the inpatient department and 39.3% of the all patients were admitted in the ICU and there was significant positive correlation between severity and ICU admission (Figure 1).

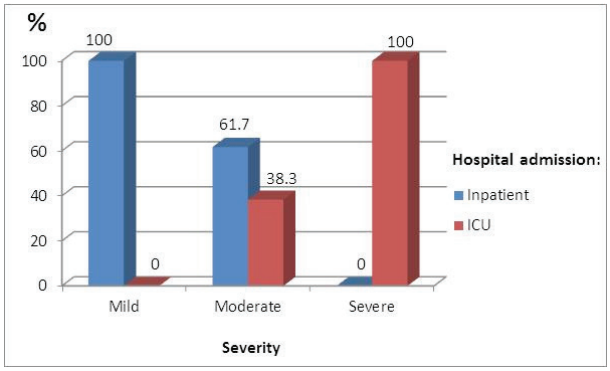

Figure (1): Place of admission and severity

As regard need for mechanical ventilation in ICU admitted patients, none of moderate patients admitted in ICU needed mechanical ventilation and 85.4% of sever patients admitted in ICU needed mechanical ventilation. There was significant positive correlation between severity and need for mechanical ventilation in ICU admitted patients (Figure 2).

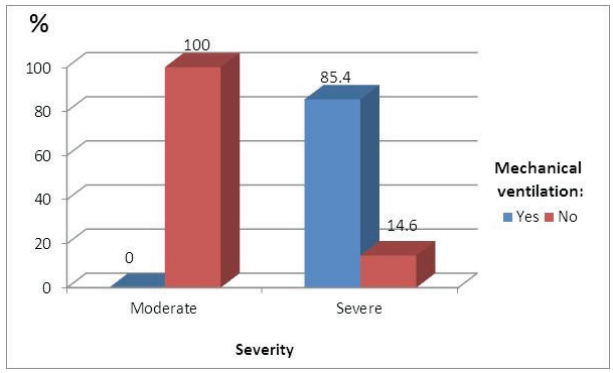

Figure (2): Mechanical ventilation need and severity

87.3% of all patients were discharged and 12.7% of all patients were died and there was significant positive correlation between severity and death outcome of the patients (Figure 3).

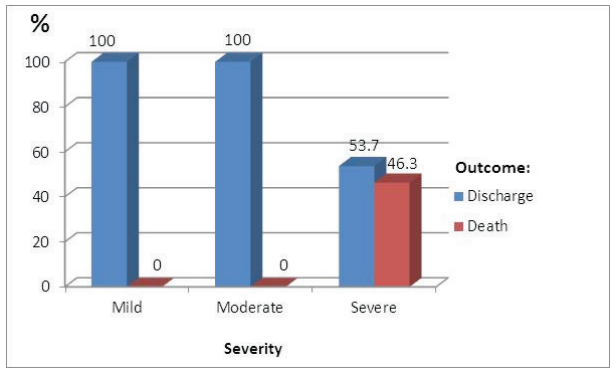

Figure (3): Outcome and severity

There was significant negative correlation positive pseudocholinestrase (PHCE) level and severity , also there was significant positive correlation between CPK, Amylase, LDH, CRP, RBS levels and severity according to POP score (Table 1).

Table (1): Differences in laboratory parameters levels according to severity

Parameters	Severity			P
	Mild (N=62)	Moderate (N=47)	Severe (N=41)	
	Mean ± SD			
PChE	1730.95 ± 765.33	1313.36 ± 419.76	939.46 ± 264.10	<0.0001#*
CPK	218.56 ± 15.29	329.19 ± 43.20	676.51 ± 77.42	<0.0001#*
Amylase	69.69 ± 13.71	127.72 ± 17.48	264.32 ± 41.95	<0.0001#*
LDH	521.60 ± 19.70	687.77 ± 54.16	884.66 ± 65.65	<0.0001#*
CRP	6.27 ± 2.37	17.34 ± 3.89	39.10 ± 11.40	<0.0001#*
RBS	104.40 ± 7.06	129.81 ± 8.84	176.90 ± 22.54	<0.0001#*

Independent t-test *Significant

There was significant negative correlation between pseudocholinestrase level and incidence of ICU admission while there was significant positive correlation between

CPK, Amylase, LDH, CRP, RBS levels and incidence of ICU admission (Table 2).

Table (2): Differences in laboratory parameters levels according to place of admission

Parameters	Place of Admission		P
	Inpatient (N=91)	ICU (N=59)	
	Mean ± SD		
PChE	1576.23 ± 716.03	1086.92 ± 364.65	**<0.0001#***
CPK	247.66 ± 49.56	580.05 ± 161.12	**<0.0001#***
Amylase	84.88 ± 25.76	227.75 ± 65.99	**<0.0001#***
LDH	570.64 ± 76.37	830.63 ± 106.26	**<0.0001#***
CRP	9.65 ± 5.67	32.69 ± 13.81	**<0.0001#***
RBS	111.24 ± 12.31	164.47 ± 26.91	**<0.0001#***

Independent t-test *Significant

There was significant negative correlation between pseudocholinestrase level and the need for mechanical ventilation while there was significant positive correlation between CPK, Amylase, LDH, CRP, RBS and the need for mechanical ventilation (Table 3).

Table (3): Differences in laboratory parameters levels according to mechanical ventilation need in ICU admitted patients

Parameters	Mechanical Ventilation		P
	Yes (N=35)	No (N=24)	
	Mean ± SD		
PChE	911.09 ± 273.97	1343.33 ± 329.70	<0.0001#*
CPK	698.40 ± 59.91	407.46 ± 88.09	<0.0001#*
Amylase	276.49 ± 32.02	156.67 ± 24.12	<0.0001#*
LDH	903.23 ± 51.19	724.75 ± 69.88	<0.0001#*
CRP	41.46 ± 10.45	19.92 ± 5.65	<0.0001#*
RBS	181.57 ± 20.62	139.54 ± 10.21	<0.0001#*

Independent t-test *Significant

There was significant negative correlation between pseudocholinestrase level and death outcome of patients while there was significant positive correlation between CPK, Amylase, LDH, CRP, RBS and death outcome of patients .

By ROC curve analysis to predict ICU admission and need for mechanical ventilation; CPK, Amylase, LDH, C-RP and RBS had higher sensitivity and specificity than pseudocholinestrase and Amylase had the highest sensitivity and specificity among these parameters in predicting ICU admission and MV need .

Discussion

The mean age of patients included in our study was 29.9 ± 12.3 years, this was in accordance with ***Kaumar and Sahna, (2017)*** [8]. ***and Banday et al., (2015)*** [9]. as they noticed that the highest incidence of poisoning was seen in patients in the middle age group which may be due to the increased stress conditions because of unemployment, poverty and depression that can happen during this age of life. Significant positive correlation was noticed between severity of poisoning and age, as noticed by ***Akdur et al., (2010)*** [10].

Female patients represented 59.3% of patients while male patients represented 40.7% with a female to male ratio of 1.46:1. This was also seen in the study of ***Hassan and Madboly, (2013)*** [11]. *and* ***Gündüz et al., (2015)*** [12]. where females were more affected than males. On the contrary, ***Sumathi et al., (2014)*** [13]. studies showed that males were more affected than females. This may be due to the emotional liability of females to stresses and easy availability of insecticides at home as household

products.

56.7% of patients were from urban areas compared to those from rural areas who were 43.3%. The same result was also reported by ***Dubey et al., (2016)*** [14] ***and Ismael et al., (2013)*** [15]**.** This may be explained by that the study was done in Cairo which is a wide urban community and most of rural patients included in the study came from only the near rural areas.

54.7% of patients were intoxicated by (OPCs) while 45.3% were intoxicated by carbamates, In their study, ***Sun et al., (2015)*** [16]**.** reported that 79% of patients were due to (OPCs) toxicity while 21% were due to carbamates poisoning.

79.3% of patients were suicidal while, the remaining 20.7% were accidental, these results agreed with ***Ismael et al., (2013)*** [15]**.** **,** ***Banday et al., (2015)*** [9]**.** .

60.7% of patients were admitted in the inpatient department and 39.3% were admitted in the ICU of which 59.3% needed mechanical ventilation (MV) and all of them were from the severe group of patients. The remaining 40.7% of ICU admitted patients did not need MV. Significant positive correlation was noticed between severity of poisoning and each of ICU admission and the need for MV, these results are in accordance with the results reported by ***Girish et al. (2016)*** [17]**.** *and* ***Kaumar and Sahna, (2017)*** [8]**.** **.**

Death represented 12.7% of patients while the remaining 87.3% were discharged. This in accordance with the results reported by ***Sun et al., (2015)*** [16]**.** where death was 12% of cases and cure was observed in 88% of cases, significant positive link was observed between severity of poisoning according to POP score and death with increased severity was associated with increased death rate and this in accordance with the results reported by ***Girish et al. (2016)*** [17]**.** and ***Vernekar and Shivaraj, (2017).*** [18]**.**

Significant negative relation was found between level of Pseudocholinestrase (PChE) and severity of poisoning, ICU admission, the need for Mechanical Ventilation and mortality. ***Muley et al., (2014)*** [19]**.** *and* ***Kaumar and Sahna, (2017)*** [8]**.** agreed with the present study regarding the associated relation between the severity and PChE level.

The present study showed that there was significant positive relation between CPK level and severity of poisoning, ICU admission, need for MV, and result, this was in agreement with those found by ***Hassan and Madboly, (2013)*** [11]**.** and ***Dubey, et al., (2016)*** [14]**.** whom found that CPK levels were significantly positively related with the severity according to POP scale, Mechanical Ventilation need and death. In contrast, ***Khan et al., (2016)*** [20]**.** found that there was insignificant link between CPK levels and patients' severity and mortality and ***Gündüz et al., (2015)*** [12]**.** found no significant difference in levels of CPK between patients who died and those who survived.

There was significant positive relation between Amylase level and severity, ICU admission, Mechanical Ventilation need, and result. In their studies ***Patil and Vasepalli (2014)*** [8-9]**.** *and* ***Dubey, et al., (2016)*** [14]**.** found that serum amylase levels related well with the severity of poisoning according to POP scale, and admission to ICU, the need for Mechanical Ventilation and end. It was found in Previous studies that acute pancreatitis may develop following organophosphorus intake, this may be due to excessive cholinergic stimulation and hypertension in ducts ***(Ikizceli et al., 2005)*** [22]**.**

There was significant positive relation between LDH levels and severity, ICU admission, Mechanical Ventilation need, and result. ***Coskun et al., (2015)*** [23]**.** ***and Gündüz et al., (2015)*** [12]**.** found that LDH levels linked well with the severity of poisoning and can be used as a predictor of severity and mortality. On the contrary to that , the study done by ***Sen et al., (2014)*** [24]**.** **,** showed no significant difference in serum LDH levels between the survivor and non-survivor patients.

There was significant positive relation between CRP level and severity, ICU admission, MV need, and result, this agreed with the results of ***Wu et al., (2016)*** [25]**.** where they also found significant link between CRP level and the patients' severity and prognosis. In contrast, the study done by ***Yeo et al., (2012)*** [26]**.** showed no significant link between CRP level on admission and death. CRP may increased in (CEI) insecticides poisoning due to induced tissue and body organ damage ***(Lee et al, 2013)*** [27]**.** **.**

There was also significant positive link between RBS level and severity, ICU admission, Mechanical Ventilation need, and result.

Panda et al., (2015) [28]**.** found that there was a rise in the random blood glucose with increase in severity and blood glucose status at time of admission may help in identifying the severity of poisoning. ***Rao and Raju, (2016)*** [29]**.** found that elevated blood glucose at the time of admission linked well with severity of cases and levels of RBS more than 200 mg/dl were fair parameters to guess mortality and the need for ventilatory support. The persistent increase of hormones (catecholamine and cortisone) associated with insecticides poisoning, lead to increased blood sugar level due to decrease the person's sensitivity to insulin ***(Clark, 2002).*** [30]**.**

The present study showed that Amylase had the highest sensitivity and specificity in prediction of respiratory failure and Mechanical Ventilation need, which was in accordance to results detected by ***Kozaci et al., (2012)*** [31]**.** and ***sumathi et al., (2014)*** [13]**.** which reported that the elevation of amylase levels was high in respiratory failure and could be used as a predictor for the need to mechanical ventilation.

Conclusion

Poisoning severity can be monitored by using POP score, it facilitate the grouping of severity state of patients and can predict the end of the case.

Severity of toxicity according to POP score was significantly positively linked with ICU admission, need for Mechanical Ventilation and death.

Significant positive link was noticed between CPK, Amylase, LDH, CRP and RBS and severity, ICU admission, the need for Mechanical Ventilation and death, so they can be used as predictive tools in cholinesterase inhibitor insecticides toxicity.

Amylase had the highest sensitivity and specificity between all parameters in the guessing of the ICU admission and need for Mechanical Ventilation so it can be used as predictor of respiratory failure and need for Mechanical Ventilation in acute cholinesterase inhibitor insecticides poisoning.

Conflict of Interest: The author declare that there is no prescence for conflict of interest

Source of Fund: None

Ethical consideration: This study was approved by the Fayoum Faculty of Medicine Research Ethical Committee. Study was conducted after explaining the study's aims. Verbal and written consents were obtained from all patients (or their relatives) included in the study and each person had the right to refuse to participate in the study.

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Potassium Sorbate Induces Oxidative Stress and Genotoxicity in Human Lymphocytes

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Abstract

Potassium sorbate, a potassium salt of sorbic acid, has been used as food preservative and antimicrobial agent. Some observations in rat and hamster cells suggest its toxicity. However, observations in human cells are limiting. Therefore, it has been intended to investigate impacts of potassium sorbate on human lymphocytes. The lymphocytes were cultured and treated with 0.5, 1.0, 1.5 and 2.0 mg/ml of potassium sorbate for 24 and 48 h. Evaluations of its impacts were done through the MTT assay, analysis of chromosomal aberrations, formation of micronucleus and activities of superoxide dismutase. The results revealed that potassium sorbate induced oxidative stress, genotoxicity, damages to chromosome, formation of micronucleus in human lymphocytes.

Keywords : *Potassium sorbate, Genotoxicity, Oxidative stress, Lymphocytes.*

Introduction

Food additive, substance or mixture of substances, is added to basic components of food in a scientifically controlled amount. It is used to color, preserve, blend, thicken or flavor foods. It also prolongs the shelf-life of products by protecting them from deterioration caused by micro-organisms. However, some of the food additives have been reported to cause sensitization, inflammation of tissues. They also act as potential risk factors of several chronic diseases and activator of inflammatory pathways. [1]

Potassium sorbate (E202) is the food preservative. It is the potassium salt of 2,4-hexadienoic acid (sorbic acid). It is used as antimicrobial and fungi static agent in cigarettes, cheeses, fishes, baked goods, syrups and jams. It has been proposed as a preservative for bacon, as an additive in conjunction with nitrite and ascorbate or erythorbate. It is also used as a preservative for dehydrated foods like jerky and dried fruit. It is commonly used in wine production because it stops the yeast from continuing to ferment in the bottles. [2] Many beauty products are prone to mold growth and use the preservative such as potassium sorbate to extend the life of skin and haircare products. Taking too much potassium sorbate preservative over a long period of time could lead to symptoms such as nausea, vomiting and diarrhea. The weak genotoxic effect was observed in Chinese hamster cells at 3-4 mg/ml concentration, with chromosome aberrations test and in human lymphocytes at 4 and 8 mM. [3, 4] Contrary to the other findings, many studies have reported that potassium sorbate is not genotoxic in different rat organs at 2000. mg/kg concentration with comet test and Hela cells. [5, 6]

Antioxidant enzymes are proteins involved in the catalytic transformation of reactive oxygen species and their by-products into stable nontoxic molecules. Their functions are the most important defense mechanisms against oxidative stress-induced cell damage. [7]

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Superoxide dismutase (SOD, EC 1.15.1.1) is one of the most important antioxidative enzymes in nearly all living cells exposed to oxygen. Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. [8]

The effects of food additive on antioxidative enzyme and oxidative stress were reported. The reports suggest significant elevation in MDA and reduction in GSH levels in mice administrated with sodium benzoate for 4 weeks and reduction in GSH and antioxidant enzymes in monosodium glutamate (MSG) treated Wister rats. [9, 10]

In vitro methods are useful for assessing the safety of chemicals including food additives. They also serve as indicators for specific toxic effects of discrete molecules and substances. Increased consumption of food preservatives causes some health problems such as asthma, headaches, migraines, urticaria and genotoxic effects. [11]

Potassium sorbate is the food preservative and there are controversial genotoxicity and/or toxicity data about this preservative. At present, there is no published data on the induction of oxidative stress by potassium sorbate in lymphocytes. Therefore, this report presents its evaluation on human lymphocytes.

For these reasons, the aim of this study was to further evaluate the potential genotoxic activity, oxidative stress induction, and safety of potassium sorbate in human lymphocytes.

Materials and Methods

Lymphocyte cultures

Human lymphocyte cell line (ATCC® PCS-800-013TM) was cultured in RPMI medium containing 10% fetal bovine serum (Invitrogen, USA.), antibiotics and phytohaemagglutinin M. The human lymphocyte cultures were incubated in a 5% CO_2 incubator at 37ºC. The cells were incubated with potassium sorbate (Sigma-aldrich, USA.) at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml for 24 and 48 h. There was no addition of potassium sorbate in the control experiments.

Cytotoxic study of potassium sorbate in lymphocytes by MTT assay

Genotoxic study of potassium sorbate in lymphocytes

Chromosomal aberrations

Lymphocytes ($1x10^6$ cells/ml) were incubated in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohaemagglutinin M at 37ºC for 72 h. The cells were treated with potassium sorbate with different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) for 24 and 48 h. Colchicines 0.06 µg/ml was added 1 h prior to the harvesting of the culture.

To collect the cells, the cultures were centrifuged (216 g for 15 min), treated with hypotonic 0.075 M KCl solution for 30 min at 37ºC and then fixed in methanol and acetic acid fixative in a 3:1 ratio for 20 min, at room temperature. They were then treated with the fixative twice. Finally, metaphase spreads were prepared by dropping the concentrated cell suspension onto slides.

For the chromosomal aberration assay, the slides were stained with 5% Giemsa (pH = 6.8) prepared in sorensen buffer solution, for 20-25 min, washed with distilled water, dried at room temperature and mounted. [13] The chromosomal aberrations were observed from 100 well-spread metaphases for each potassium sorbate concentrations. Mean of the numbers of metaphase chromosome (each metaphase chromosome contains 46 chromosomes) was calculated.

Micronucleus assay

Potassium sorbate with different concentrations were added in lymphocyte cultures for 24 and 48 h. At 44 h, after the start of the culture, cytochalasin B (3µg/ml) was added to arrest cytokinesis. Cells were harvested after 28 h, treated with a hypotonic solution (0.075 M KCl) and fixed with methanol and glacial acetic acid in a 3:1 ratio v/v, supplemented with formaldehyde, according to Palus et al. with some modifications. The slides were air-dried and stained with 5% Giemsa. Micronucleus was scored from 1000 binucleated cells per potassium sorbate concentration under microscope. [14]

Assay of SOD activity in lymphocytes treated with potassium sorbate

Lymphocytes ($1x10^6$ cells/ml) were treated with potassium sorbate at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml for 24 and 48 h. Then, the SOD activity

was measured by Superoxide Dismutase (SOD) Activity Kit (Biovision).

Statistics Analysis

For the percentage of the numbers of metaphase chromosome and micronucleus cells, the results were expressed as mean±SE (standard error). SOD activity was also expressed as mean±SE. The differences among the potassium sorbate-treated and the control groups were analyzed by ANOVA and $p< 0.05$ was regarded as statistically significant.

Results

Cytotoxic effect of potassium sorbate on lymphocytes

The results of MTT assay showed that the potassium sorbate affected viability of lymphocytes ($p<0.05$) at all concentrations (0.5 to 2.0 mg/ml) at 24 and 48 h. Lymphocyte viability was decreased when the concentration of potassium sorbate increased. After 0.5 mg effect was almost similar up to 2 mg but 24 h (Figure 1) was more effective than 48 h incubation time. The IC_{50} value at 24 h incubation time was 0.79 mg/ml and IC_{50} value at 48 h incubation time was 0.94 mg/ml.

Genotoxic effect of potassium sorbate on chromosome aberration and micronucleus induction

At 24 and 48 h incubation time, the potassium sorbate significantly reduced numbers of metaphase chromosome in a dose dependent manner ($p<0.05$) (Figure 2). The effect was concentration dependent in both incubation times. The sister chromatid separation and chromosome break were also observed (Figure 3).

The induction ($p<0.05$) of micronucleus was also observed in lymphocytes at all concentrations (0.5 to 2.0 mg/ml) of potassium sorbate both at 24 and 48 h incubation time (Figure 4 and Figure 5).

Effect of potassium sorbate on SOD activity in lymphocytes

The potassium sorbate affected activity of SOD in lymphocytes at 24 and 48 h incubation time. The concentration 1.0-2.0 mg/ml of potassium sorbate affected SOD activity. The effect was more after 24 h incubation time than the percentage activity inhibition of SOD after 48 h incubation time. At 48 h, the percentage of SOD activity inhibition was decreased when the concentration of potassium sorbate increased ($p<0.05$) (Figure 6).

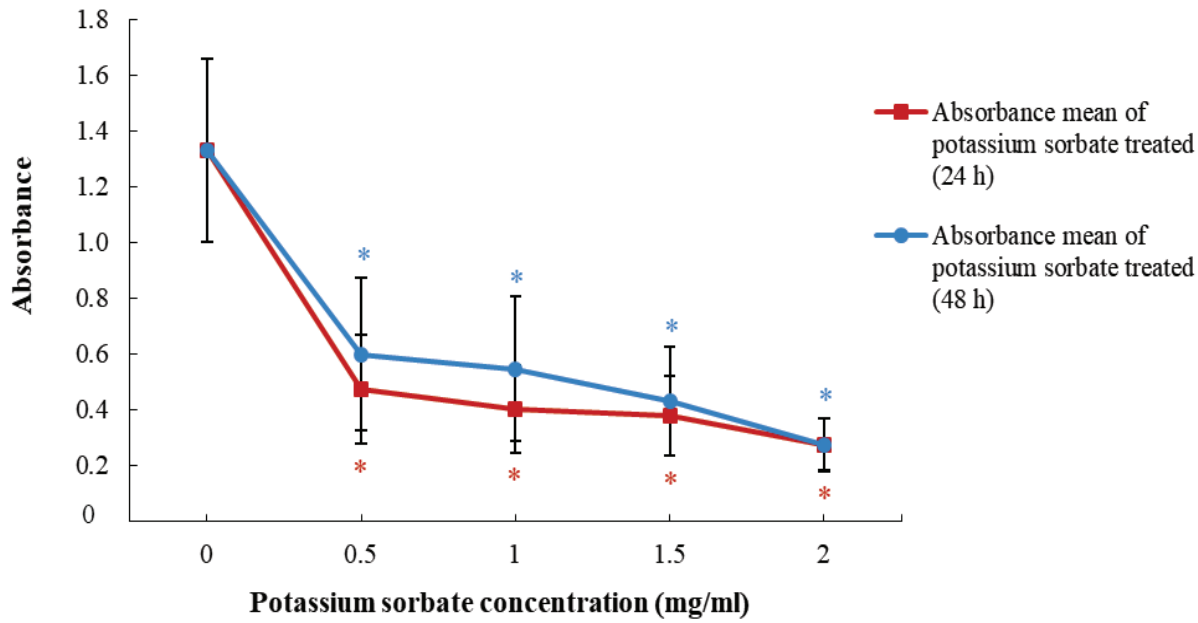

Figure 1 The MTT assay (measured absorbance at 570 nm) showed effects of potassium sorbate on the lymphocyte viability at 24 and 48 h incubation time (n = 3).

The value of absorbance depended upon viability of lymphocytes or the numbers of living lymphocytes. The lymphocyte viability was decreased when the potassium sorbate concentration increased.

* $p<0.05$, p value compared with control group.

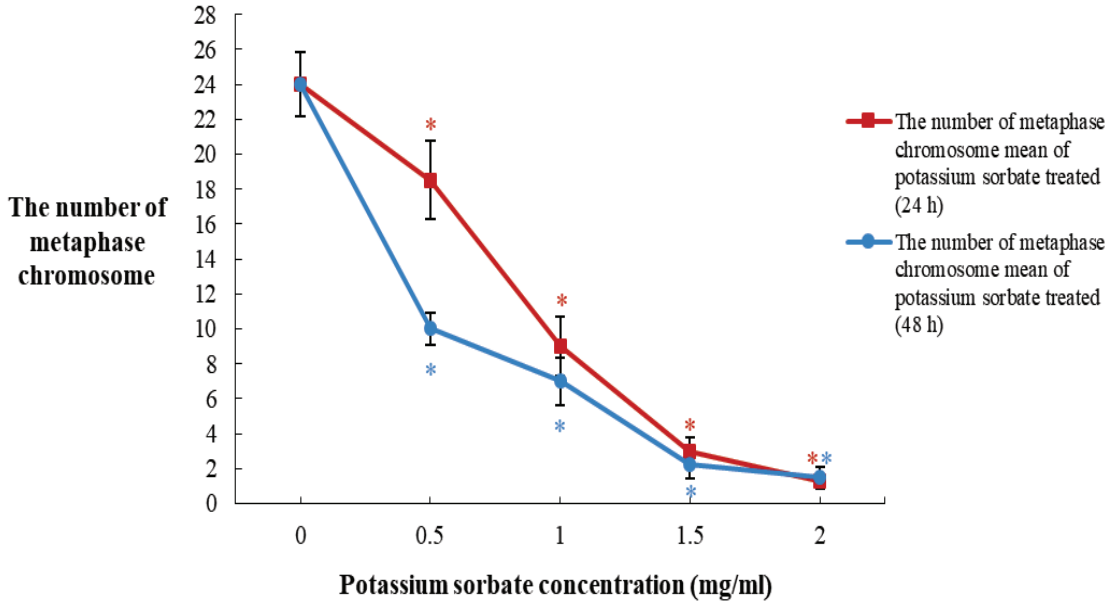

Figure 2 Effects of potassium sorbate on metaphase chromosomes at 24 h and 48 h incubation time (n = 3).

* $p<0.05$, p value compared with control group.

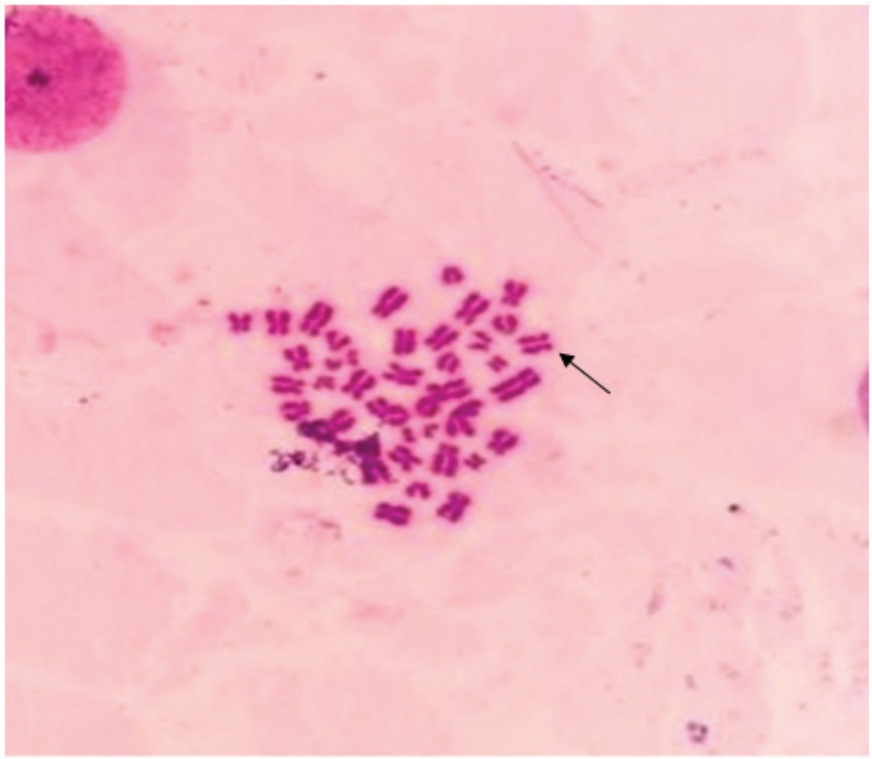

Figure 3 Sister chromatid separation (the arrow) caused by potassium sorbate.

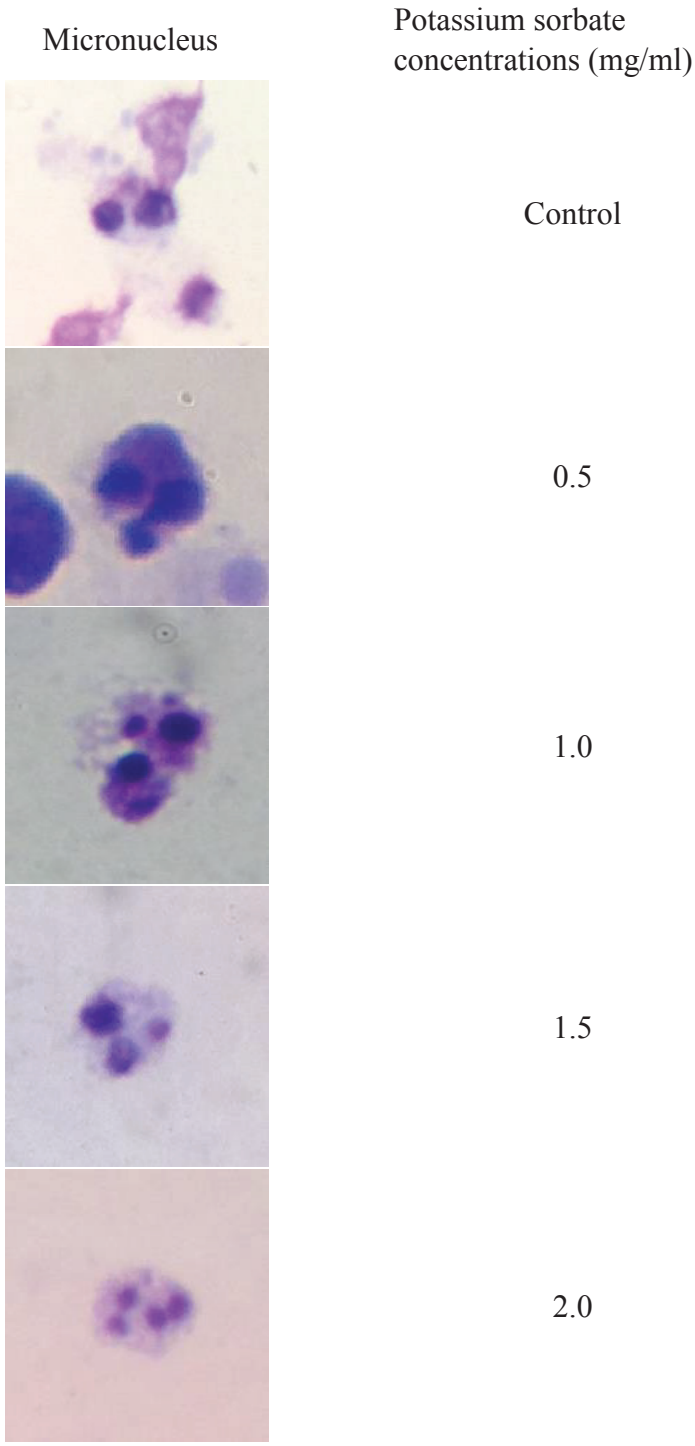

Figure 4 Micronucleus formation induced by the potassium sorbate concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml at 24 h incubation time.

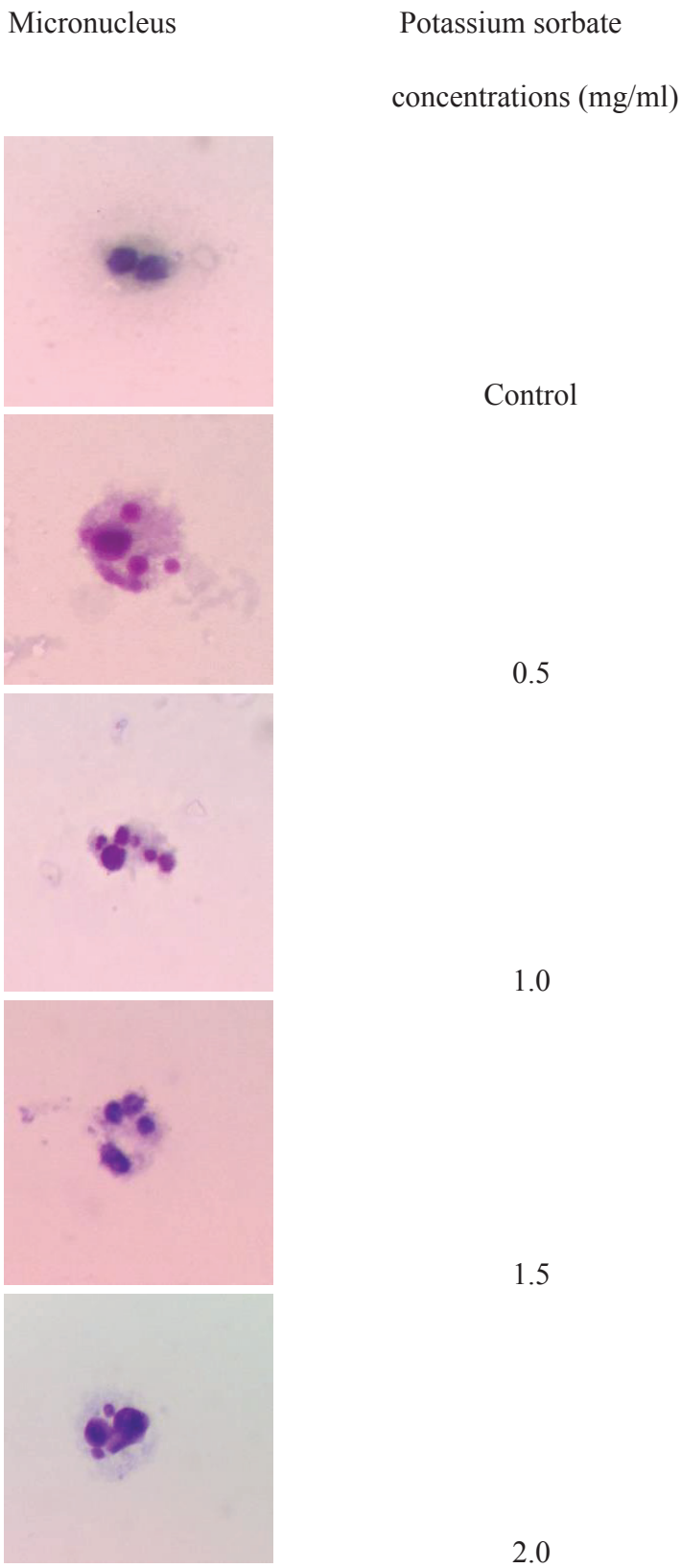

Figure 5 Micronucleus formation induced by the potassium sorbate concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml at 48 h incubation time.

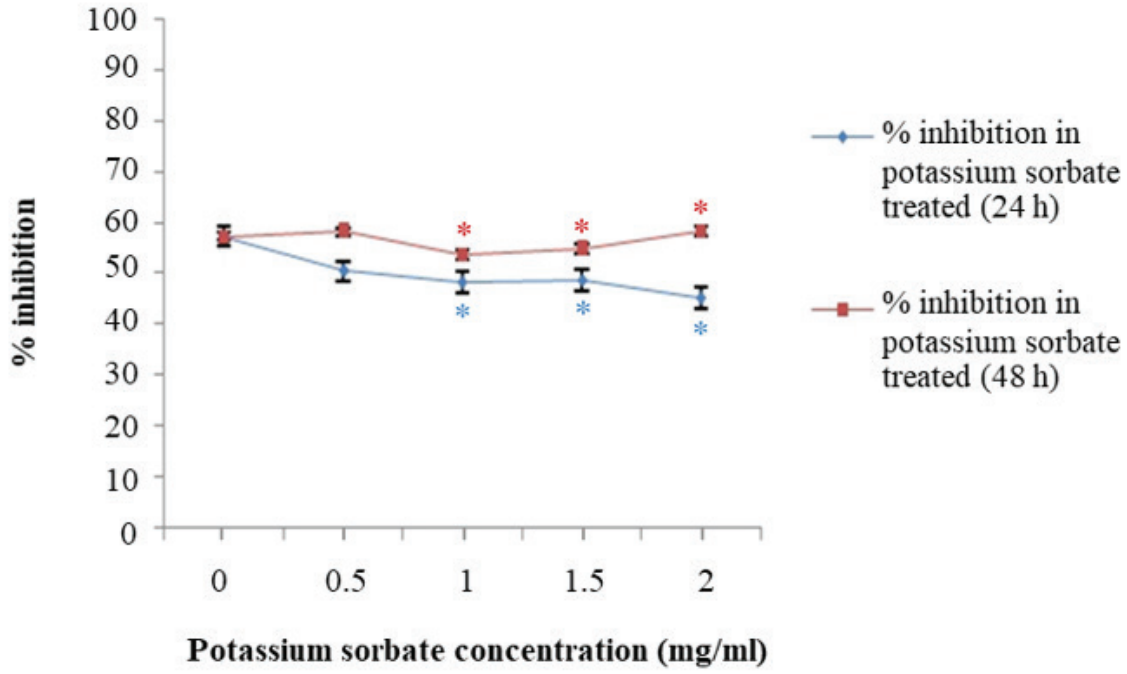

Figure 6 Effects of potassium sorbate on the percentage activity inhibition of SOD in lymphocytes at 24 and 48 h incubation time (n = 3).

* $p<0.05$, p value compared with control group.

Discussion

The damage to genome has been used as a biomarker of carcinogenesis. The status of micronucleus (microscopically visible round to oval cytoplasmic chromatin mass next to the nucleus) has been observed useful tool for measuring genotoxicity in vitro cultures. [15] This assay detects both clastogenicity (chromosome breakage) and aneugenicity (chromosome lagging due to dysfunction of mitotic apparatus). [16] An increased micronucleus also implies risk of cancer in human.

The report on potassium sorbate presented negative results in vivo. [17] Potassium sorbate did not show any adverse genetic effects in the hamster embryo fibroblast micronucleus assay or in the cell transformation test. [18] Sorbic acid and potassium sorbate were less genotoxic than the sodium sorbate. [19, 20, 21] On the other hand, potassium sorbate was found to cause sister chromatid exchanges in Chinese hamster cells. [22]

The mechanism of toxicity of sorbic acid and its salts are probably related to the alkylating activity. Sorbic acid and sorbate showed alkylating activity on the nucleophile 4-(p-nitrobenzyl) pyridine (NBP), which is used as a trap for alkylating agents having nucleophilic characteristics similar to DNA bases. [23] Alkylating agents can damage DNA by attaching the alkyl groups to the DNA bases or mispairing of the nucleotides or formation of cross-bridges, bonds between the atoms in the DNA. [24] Alkylating agents cause gross mitotic abnormalities and can effect gene mutations. This study indicated that potassium sorbate is a genotoxic agent. It clearly reveals the cytotoxic and genotoxic effects of potassium sorbate in cultured human lymphocytes. It significantly reduced the numbers of metaphase chromosome during the 24 h and 48 h incubation periods, in a concentration-dependent manner. Sister chromatid separation and chromosome break were recorded in these studied concentrations. It caused micronucleus formation in a concentration-dependent manner. The potassium sorbate concentrations (1.0-2.0 mg/ml) reduced SOD activity which indicates impact on enzymes of oxidative stress management. The results also indicate that the

impact was maximum within 24 h. As compared to SOD activities observed with same concentrations of potassium sorbate at 48 h, it is presumed that the cells tend to recover due to its defense. It is supported by increased activity of SOD after 48 h.

Several reports explain that potassium sorbate did not show any adverse effect on toxicity testing for DNA profile. [17] The reports are also contradictory. However, potassium sorbate has been used as food preservative. Our observations are in agreement with most of the reports that impact of potassium sorbate is less on SOD but genotoxic. Our observations in human lymphocytes suggest that potassium sorbate is toxic to cause chromosomal aberrations, formation of micronuclei and damage to oxidative stress management system.

Conclusion

Potassium sorbate which is commonly used in food as preservative is toxic to cause chromosomal aberrations, formation of micronuclei but shows mild damage to oxidative stress management system. Potassium sorbate may cause a wide range of long term health problems and side effects. The usage of food preservatives demands more awareness and surveillance.

Ethical Clearance : Not required

Conflict of Interest : Nil

Source of Funding : Self

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Effects of Trichloroethylene on *p53* and *Bcl-2* Expression, Chromosome Loss and Catalase Activity Inhibition in Human

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Abstract

Trichloroethylene (TCE) is a common organic solvent that has been widely used in industrial applications. TCE is highly lipophilic and readily absorbed into the circulation following oral, dermal or inhalation exposure. The purpose of this study is to examine the alterations of *p53* and *Bcl-2* expression, chromosome loss and catalase activity inhibition after treatment with TCE. Human lymphocytes were cultured with various doses of TCE for 24 h. Quantitative RT-PCR (qRT-PCR) technique, chromosome culture technique and catalase activity assay were performed in human lymphocyte cultures. The results of Quantitative RT-PCR (qRT-PCR) analysis showed up-regulated expression in the experimental groups comparison with the control groups of *p53* gene [0.016 mM/L (1.4-fold) and *Bcl-2* gene [0.008 mM/L (1.4-fold) and 0.016 mM/L (1.6-fold)]. Loss of chromosome 8 and 22 were observed. Catalase activity was reduced when TCE concentration increased ($p<0.05$). It is indicated that TCE effects on *p53* and *Bcl-2* gene expression, chromosome loss and catalase activity inhibition. These effects may lead to the onset of cancer in the future.

Keywords : *Trichloroethylene, p53 gene, Bcl-2 gene, Human chromosome, Catalase activity.*

Introduction

A number of environmental toxicants such as nickel, chromium, pesticides cause DNA alterations in genes of various tissues leading to both non-cancer and cancer effects. [1] Trichloroethylene (TCE) is a high-production volume chemical with numerous industrial applications. Human exposure to TCE is assumed to be mostly through inhalation, owing to its ability low solubility in water and high vapor pressures. TCE is common contaminant in ambient and urban air. TCE is the most commonly found in groundwater contaminant. TCE is a human carcinogen, with the kidney being the target tissue. TCE exposure was found for non-Hodgkin lymphoma, chronic lymphocytic leukemia, multiple myeloma, hairy cell leukemia and liver cancer. TCE has been classified as a known human carcinogen group I. [2]

The mechanisms of action for cancer and noncancer toxicity associated with TCE is dependent on multiple factors, including tissue and species. [3] TCE-induced toxicity to humans is due to the multiple metabolic pathways. Both apoptosis and enhanced cellular proliferation are associated with the prominent changes in expression of several proteins that regulate apoptosis and cellular growth, differentiation and stress response. The pattern of expression of those proteins can serve as molecular markers for TCE exposure and effects in human tissue. Epigenetic effects are recognized for their potential influence on carcinogenesis of environmental chemicals. TCE administered via drinking water can lead to DNA hypomethylation in liver tumor tissue in N-nitroso-N-methylurea-initiated hepatocellular carcinoma. [4]

The *p53* gene is located on the short arm of chromosome 17 (17p13.1). The gene spans 20 kb, with a non-coding exon 1 and a very long first intron of 10 kb. The coding sequence contains five regions showing a high

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degree of conservation in vertebrates, predominantly in exons 2, 5, 6, 7 and 8. An acidic N-terminus transcription-activation domain (TAD) is known as activation domain 1 (AD1), which activates transcription factors. The N-terminus contains two complementary transcriptional activation domains, with a major one at residues 1 - 42 and a minor one at residues 55 - 75, specifically involved in the regulation of several pro-apoptotic genes. *p53* plays a role in regulation or progression through the cell cycle, apoptosis and genomic stability. *P53* gene is damaged, tumor suppression is severely compromised. The person who inherits only one functional copy of the *p53* gene will most likely develop tumors in early adulthood, a disorder known as Li-Fraumeni syndrome. It is reported high prevalence of *p53* abnormalities in solid tumors, particularly ovarian, pancreatic, breast and small cell lung cancer. [5]

The *Bcl-2* gene, is the founding member of the *Bcl-2* family of regulator proteins that regulate cell death (apoptosis), by either inhibiting (anti-apoptotic) or inducing (pro-apoptotic) apoptosis. Bcl-2 is localized to the outer membrane of mitochondria, where it plays an important role in promoting cellular survival and inhibiting the actions of pro-apoptotic proteins. The pro-apoptotic proteins in the *Bcl-2* family, including Bax and Bak, normally act on the mitochondrial membrane to promote permeabilization and release of cytochrome C and reactive oxygen species (ROS), that are important signals in the apoptosis cascade. The Bcl-2 protein is a suppressor of programmed cell death that homodimerizes with itself and forms heterodimers with a homologous protein Bax, a promoter of cell death. The damaged *Bcl-2* gene has been identified as a cause of a number of cancers, including melanoma, breast, prostate and lung cancer. The overexpression of *Bcl-2* and the proto-oncogene *Myc* may produce aggressive B-cell malignancies including lymphoma. [6]

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four iron-containing heme groups that allow the enzyme to react with the hydrogen peroxide. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). The optimum pH for human catalase is approximately 7. Catalase protects cellular organelles and tissues from damage by peroxide, which is continuously produced by numerous metabolic reactions. In mammals, catalase is found predominantly in the liver. Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second. [7]

TCE is an environmental toxicant of public health concern owing to widespread exposure across the population and its high-production volume, persistence in the environment. This study was focused on the cytotoxic effect of TCE on human lymphocyte viability in vitro, human chromosome abnormalities, the impact of TCE genotoxicity on the expression of *p53* and *Bcl-2* genes and catalase activity inhibition. The potential of TCE can cause severe adverse health effects by *p53* and *Bcl-2* gene alterations and catalase activity effect. The knowledge about TCE toxicity helps in control and prevents pollution problems due to TCE toxicity.

Materials and Methods

Chemicals

Trichloroethylene (CAS No. 79-01-6) was purchased from Loba chemie (Mumbai, India). MTT (3-[4.5-dimethy-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) was procured from Bio Basic Canada (Ontario, Canada). Dimethylsulphoxide (DMSO)and Giemsa stain were purchased from Sigma-Aldrich (St. Louis, USA.). RPMI medium and chemical for cell culture were purchased from Thermo Fisher Scientific (Massachusetts, USA).

Specimen collection

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Thammasat University ethical committee (the institutional ethical committee which monitors research involving human subjects). The project identification code was EC 084/2018 and date of approval was July 17, 2018. Heparinized blood was collected from 20 healthy volunteers (age 20-25 years and no history of hematologic diseases and genetic disorders) after getting their informed consent. Approximately 10 mL of blood was collected from each healthy volunteer. The

peripheral blood mononuclear cell (PBMC) isolation was done following Debey's study. [8] Lymphocytes were cultured for a study on the effects of TCE on lymphocyte viability, human chromosome abnormalities, gene expression alteration and catalase activity inhibition.

Study on the effects of TCE on lymphocyte viability and human chromosome abnormalities

Human lymphocyte cultures from the same subject were separated to be both control (n = 20) and experimental (n = 20) groups. The studies about lymphocyte viability and human chromosome abnormalities were performed by added TCE conc. 0.002, 0.004, 0.006, 0.008, 0.016 and 0.032 mM/L to the experimental groups, respectively.

The $1x10^5$ cells/mL of lymphocytes were added in RPMI 1640 medium and incubated at 37 °C for 48 h. After 48 h, the concentrations of TCE 0.002, 0.004, 0.006, 0.008, 0.016 and 0.032 mM/L were added in lymphocyte cultures and incubated for 24 h. After 24 h incubation time, MTT assay was done to analyze the viability of lymphocytes. For MTT assay, the content was centrifuged at 600 g for 10 min and 0.5 mL of 300 µg/mL MTT in phosphate buffer saline solution (PBS) was added to each well and incubated for 4 h at 37°C. The medium was removed and formazan was dissolved in DMSO and the optical density was measured at 570 nm. using a Bio-assay reader.

For chromosome culture technique, lymphocytes were added to RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M). The cultures were incubated at 37 °C for 48 h and the cells were treated with TCE conc. 0.002, 0.004, 0.006, 0.008, 0.016 and 0.032 mM/L for 24 h. After 24 h incubation time, 0.06 µg/ml colchicine was added 1 h prior to the harvesting of the cultures. The cells were harvested and metaphase spreads were prepared by dropping the concentrated cell suspension onto slides. G-banding technique was done for chromosome abnormality observation. [9]

Study on the expression of *p53* and *Bcl-2* gene alterations

The expression of *p53* and *Bcl-2* alterations were studied by treated lymphocytes with TCE conc. 0.004, 0.008 and 0.016 mM/L for 24 h at 37 °C.

RNA extraction

RNA was extracted by UltRNA Column Purification kit (Applied Biological Materials Inc, British Columbia, Canada). RNA was isolated as described in the kit procedure. Then, quality and quantity of RNA concentration were checked with A260/A280 ratio.

Quantitative RT- PCR (qRT-PCR) analysis

The cDNA (RNA 20 ng) was synthesized by RT-PCR reaction using Precision nanoScript 2 Reverse Transcription kit. The RT master mixes were prepared to contain 4X Buffer 5 µl, 10 mM dNTP mix 1 µl, Enzyme 1 µl, 1X RT random primer 1 µl, RNAes/DNAse free water 3 µl and 9 µl of RNA. The total volume in the tube was 20 µl. The tube was loaded using a thermal cycler programmed at 25 °C for 5 min, at 42 °C for 20 min, at 75 °C for 10 min and at 12 °C for 10 min. The amplification of genes was carried out in a real-time PCR machine using SYBR green-based chemistry. The reaction mixture was prepared to contain 2X SYBR mix 10 µl, 5 µM forward primer 0.8 µl, 5 µM reverse primer 0.8 µl, sterile water 6.4 µl and cDNA template 2 µl. The total volume in the tube was 20 µl. The β-actin gene (*ATCB*) was used as an internal control. The primer sequences used for various genes were as follows : *p53* gene (NG 017013.2) (from nt.16453 - 17450, 998 bp) and *Bcl-2* gene (M13994.1) (from nt.1192 - 2010, 819 bp)) shown in Table 1. The thermal cycler was programmed at 95 °C for 2 min, at 95 °C for 10 sec and 60 °C for 45 sec. The cycle time was 58 cycles.

Study on the effect of TCE on catalase activity inhibition

The $1x10^5$ cells/mL of lymphocytes were added in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohemagglutinin M) and incubated at 37 °C for 48 h. After 48 h, the concentrations of TCE 0.002, 0.004, 0.006, 0.008 and 0.016 mM/L were added in lymphocyte cultures and incubated for 24 h. After 24 h incubation time, TCE treated lymphocytes were studied about catalase activity inhibition by Catalase Activity Colorimetric/Fluorometric assay kit (Biovision Incoperated, CA, USA.)

Statistics analysis

The toxicity of TCE on lymphocyte viability was tested by ANOVA. The effects of TCE on *p53* and *Bcl-2* expression and catalase activity were analysed by ANOVA and *p* values of less than 0.05 were considered statistically significant.

Results

Effects of TCE on lymphocyte viability and human chromosome abnormalities

Human lymphocyte cultures were examined by MTT assay to determine the TCE-induced cytotoxicity at concentration of 0.002, 0.004, 0.006, 0.008, 0.016 and 0.032 mM/L for 24 h. The value of mean absorbance was directly related to the number of the living cells. The result showed that the numbers of lymphocyte viability were decreased when the TCE concentrations increased comparing between control and the experimental groups ($p<0.05$) (Table 2). In addition, the 50% inhibitory concentration (IC_{50}) of TCE concentration was 0.0095 mM/L which effected growth or depletion of human lymphocytes.

For human chromosome abnormalities, chromosomes 8 and 22 were lost when lymphocytes were treated with TCE conc. 0.008 and 0.016 mM/L respectively.

Effect of TCE on *p53* and *Bcl-2* alterations

Gene expression analysis of *p53* and *Bcl-2* genes was done by qRT-PCR using beta-actin gene (*ATCB*) as housekeeping. The control group and the experimental groups with different TCE concentrations (0.004 mM/L, 0.008 mM/L and 0.016 mM/L) were studied about gene alteration.

Effect of TCE on *p53* expression

The expression level analysis revealed up-regulation of *p53* gene in the experimental groups at 0.016 mM/L (1.4-fold) in comparison to the control as shown in Figure 1. The result expression level analysis revealed up-regulation of *p53* gene showed a statistically significant difference between the control and the experimental groups at 0.016 mM/L ($p<0.05$). Comparing within the experimental groups, the experimental group at 0.016 mM/L was found to have a different statistically significant from the experimental group at 0.008 and 0.004 mM/L ($p<0.05$) as shown in Figure 1.

Effect of TCE on *Bcl-2* expression

The expression level analysis revealed up-regulation of *Bcl-2* gene in the experimental groups at 0.008 mM/L (1.4-fold) and 0.016 mM/L (1.6-fold) in comparison to the control as shown in Figure 1. The result expression level analysis revealed up-regulation of *Bcl-2* gene showed a statistically significant difference between the control and the experimental groups at 0.008 and 0.016 mM/L ($p<0.05$). Comparing within the experimental groups, the experimental group at 0.004 mM/L was statistically significant from the experimental group at 0.008 and 0.016 mM/L ($p<0.05$) as shown in Figure 1.

When we compared the effect of TCE concentrations between these two genes expression, it was shown that the expression levels of *p53* and *Bcl-2* genes, in the control group and the TCE concentration group at 0.004 mM/L, was statistically insignificant but they were statistically significant at 0.008 and 0.016 mM/L, as shown in Figure 2.

Effect of TCE on catalase activity inhibition

The result showed that TCE could inhibit catalase activity in the treated lymphocytes. The catalase activity was reduced when the TCE concentration increased as shown in Table 3. The catalase activity in the experimental groups were different from the catalase activity in the control group significantly ($p<0.05$).

Table 1. The primers used in qRT-PCR analysis for ATCB, p53 and Bcl-2	
Gene	**Primer Sequence (5′-3′)**
ATCB (housekeeping gene)	Forward: 5'- CCATCATGAAGTGTGACGTGG - 3'
	Reverse: 5'- ATCTTCATTGTGCTGGGTGCC - 3'
p53	Forward: 5'- TGGCCCCTGTCATCTTCTG - 3'
	Reverse: 5' - CCGTCATGTGCTGTGACTGC - 3'
Bcl-2	Forward: 5' - AAGCCGGCGACGACTTCT - 3'
	Reverse: 5'- GGTGCCGGTTCAGGTACTCA - 3'

Table 2. Percentage of lymphocyte viability in different concentrations of TCE

TCE concentrations (mM/L)	Percentage mean of lymphocyte viability±SD
0	98.8±0.2
0.002	90.4±0.4 *
0.004	87.3±0.1*
0.006	85.7±0.5*
0.008	77.7±0.4*
0.016	74.6±0.3*
0.032	51.1±0.6*

* $p<0.05$, p value compared with control group.

**The numbers of lymphocyte viability were decreased when the TCE concentrations increased comparing between control and the experimental groups.

Table 3. Effect of TCE on catalase activity

TCE concentrations (mM/L)	Absorbance mean at 570 nM in control±SD	Absorbance mean at 570 nM in test sample±SD
0	0	0
0.002	175.35±0.05	157.27±0.04*
0.004	173.94±0.03	150.71±0.05*
0.006	173.33±0.04	150.30±0.05*
0.008	171.72±0.06	149.70±0.04*
0.016	171.11±0.05	147.71±0.07*

* $p<0.05$, p value compared with control group.

** Absorbance mean related to the catalase activity. The catalase activity was reduced when the TCE concentration increased comparing between control and the experimental groups.

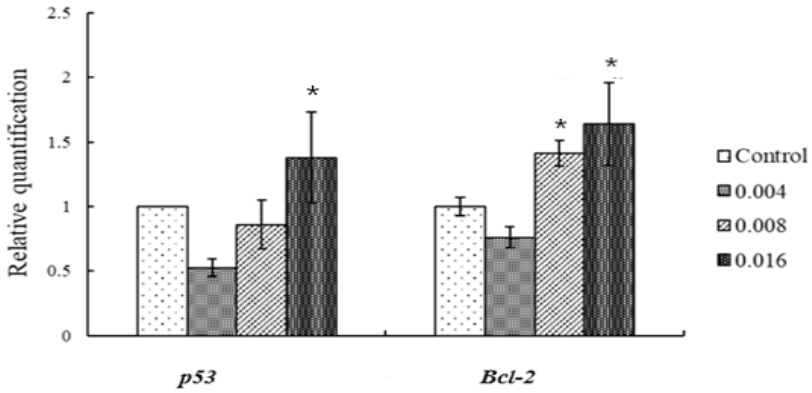

Figure 1 The comparison between gene expression and the different TCE concentrations

(0.004, 0.008 and 0.16 mM/L) in human lymphocytes. The expressions of *p53* and *Bcl2* were progressively higher due to increase in TCE concentration. The expression level analysis revealed up-regulation of *p53* gene showed a statistically significant difference between the control and the experimental groups at 0.016 mM/L ($p<0.05$). The expression level analysis revealed up- regulation of *Bcl-2* gene showed a statistically significant difference between the control and the experimental groups at 0.008 and 0.016 mM/L ($p<0.05$).

* $p<0.05$, p value compared with control group.

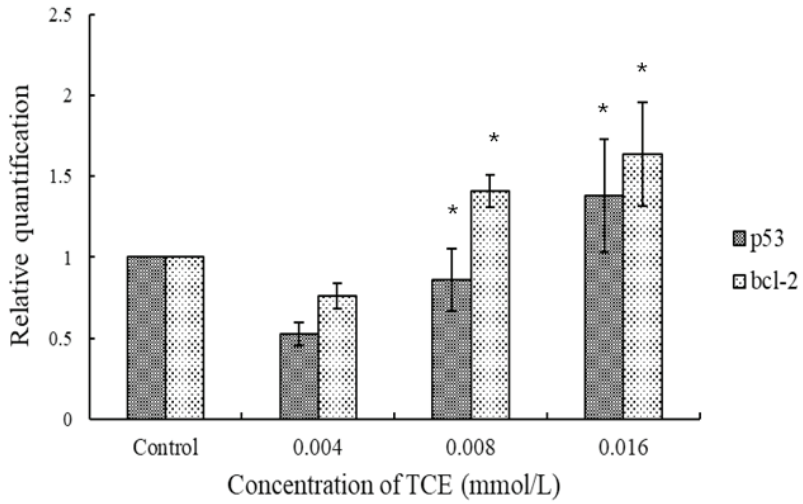

Figure 2 Effects of TCE concentrations on *p53* and *Bcl-2* gene expression by

qRT-PCR comparing between the same TCE concentrations and the two gene expression. The expression levels of *p53* and *Bcl-2* genes, in the control group and the TCE concentration group at 0.004 mM/L, was statistically insignificant ($p>0.05$) but they were statistically significant at 0.008 and 0.016 mM/L ($p<0.05$).

* $p<0.05$, p value comparing between the same TCE concentrations and the two gene expression.

Discussion

Trichloroethylene (TCE) is the organic compound that is primarily inhaled through the respiratory system. [10] It is a solvent used in numerous industries, as degreaser in the metal manufacturing industry, solvent for oils and resins, or the production of rubber and as a chemical intermediate in the production of refrigerants.[11] DNA analysis provides an opportunity for detection of unique genetic alterations representing a signature of TCE exposure. These alterations can arise from genotoxic and nongenotoxic pathways at multiple points throughout tumorigenesis. [12] TCE can be metabolized via cytochrome P450 oxidative and glutathione transferase conjugation pathways, producing dichloroacetate (DCA), trichloroacetate (TCA), and S-(1,2-dichloro-vinyl)-L-cysteine (DCVC). [13] These oxidative and conjugate-derived metabolites might act through genotoxic and nongenotoxic mechanisms to induce tumors. The *K-ras* and *H-ras* genes have been commonly examined in TCE-,DCA-, and TCA-induced liver tumors in mice. [14] With regard to epigenetic changes, global hypomethylation has been reported in DCA- and TCA-induced liver tumors in mice. The animal studies provided evidence that TCE may alter genomic status via genotoxic pathway, which may consequently lead to chromosomal abnormality. [15] Genome-wide alterations, including genetic and epigenetic mechanisms, are essential for tumor development. [16]

TCE can readily cross biologic membranes. TCE has low solubility in water. Pulmonary uptake of TCE is rapid, with steady-state levels being attained within a few hours after the start of exposure. [10] Occupational exposure to TCE and renal cell carcinoma were observed in individuals with an active *GSTT1* genotype and *CCBL1* genotypes. Renal cell tubular adenocarcinoma was reported in male and female rats after chronic exposure to TCE.[17] TCE and its metabolites have been reported to decrease the methylation of promoter regions of the *c-Myc* gene, leading to an increased mRNA expression in the liver of B6C3F1 mice. [15, 18, 19]

In this study, TCE effected lymphocyte viability and chromosome loss. The numbers of living lymphocytes were reduced when the TCE concentrations increased and chromosome loss was found. TCE toxicity effected catalase activity inhibition lead to defect on cellular organelle and tissue protection. Inhalation of TCE may damage the enzymes that are responsible for synthesizing the pulmonary surfactant and antioxidative enzyme protection. [20] This study supported the association between TCE toxicity and disturbances in antioxidative enzyme. We observed the effect of TCE exposure on apoptosis-related genes. Various DNA - damaging agents that trigger programmed cell death also induce *p53* expression. *p53* mediated cell death may involve activation or suppression of other gene like *Bcl-2*. [5, 21] This study reported about the up-regulation of *p53* gene in the experimental group at 0.016 mM/L and the up-regulation of *Bcl-2* gene in the experimental groups at 0.008 mM/L and 0.016 mM/L in comparison with the control group in TCE- human lymphocytes. It showed the association between TCE toxicity and disturbances in cell cycle regulation. TCE could induce alteration of apoptosis-related genes and antioxidative enzyme function. TCE may effect apoptosis and antioxidant system and lead to carcinogenesis in human.

Conclusion

TCE effected on lymphocyte viability, chromosome abnormalities and catalase activity inhibition. It caused modulation in expression of *p53* and *Bcl-2* genes in human.

Ethical Clearance: Taken from Thammasat University ethical committee, Thammasat University, Rangsit Campus, Patumthani, Thailand

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Methodological Study for RAS Development for Chronic Pediatric Population

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Abstract

Adolescence is considered as a difficult period filled with conflicts in search of self-autonomy, which culminates the rediscovery of an individual towards adult life introduction. In addition to being sick there are many other factors i.e. health care staff rotation, away from parents, experiencing pain during the procedures, peer detachment and loss of self-esteem during hospitalization. The objective of the study was to develop a scale for assessing resilience among children with Renal diseases. Methodological research approach was used for development of an effective Resilience Assessment Scale for children admitted with renal diseases. Study was done on 75 children aged 10-18 years admitted with renal disease in MMIMS&R Hospital, Mullana, Government Civil Hospital at Ambala and Kalpana Chawla Government Hospital at Karnal. The related review of literature ,experts guidance and investigator's personal experience, a list of factors/domains were identified and compared with standardized Resilience scales i.e., Wagnild and Young Resilience assessment scale, Child and Youth Resiliency measure. Modified Delphi technique was used for Content validation of Resilience assessment scale with 9 experts Content validity was ascertained by expert's opinion and was found to be 0.79.The range of rating score was 31-155 with the assumption that higher the scores, the greater the resilience. At a cut-off point of ≥102, the best balance between the sensitivity and specificity was achieved. Cronbach's alpha and inter item correlation were used to measure internal consistency of the tool, coefficient alpha was 0.81. Pearson correlation between resilience assessment scale and Wagnild Young resilience scale was 0.84. In order to estimate stability test re-test reliability was calculated and it came out to be 0.84.

***Keywords:** Resilience, Assessment scale, Children, Renal Disease*

Introduction

Background of the study

As the age progress with time development in a child takes place along with growth in physical, psychological, social, and spiritual domains as a whole.

Around the age of 10-18 years, the theorists explained the overall development of the individual right from the childhood in aspects of cognitive development, psychosexual development, psychosocial development, moral development and development of faith respectively. Chronic kidney diseases are emerging to be an important aspect which needs close supervision in therapeutic as well as behavioural concept globally[1]. Stress was significant associated with risk of emotional and behavioural problems along with chronic diseases in adolescents interferes with adherence to treatment regimens and alters the psychological functioning. By the decade of the 1990's, researchers became increasingly focused on a phenomenon known as resilience. Resilience is the ability of the individual to bounce back from difficult experiences.[2-3]

This psychological abilities and positive attitude among children goes on deteriorating when the child goes through the pathway of chronic treatment. Coping during this(chronic) period and identifying the coping has become a major concern which is termed as "Resilience". Resilience is the word derived from "Resile" [4]which

means to "bounce or spring back"(re-"back"+ salire-"to jump, leap;) . Resilience is a resistance to be sick, adopted, thrive and ability to leave the state of illness by recovering out of it. [5]

Need Of The Study

Measures that have been developed to assess "resilience" have focused the factors and resources that make them possible to achieve.[6]

Adolescents and youngsters who confront chronic illness, usually experience a wide variety of stressors that can pose the threat of producing psychological disorders. There is a need for chronically ill child to alleviate the symptoms , which needs physical and lifestyle modifications, besides its affects the routine activities of child too like sports and school. One of the reason can be regular ongoing treatment of the child. These factors confine the chances of regularity and, produce hurdles in social relationships and create positive concerns about the future.

It is necessary to regain the lost activity, positive perception and functional outlooks and above all educational strengths and behavioural ratings among the children suffering from the renal diseases. Very few studies and literatures have reported to the development of tool to measure resilience and other related resources specifically for the renal diseases in paediatric population. Children with chronic diseases undergo various health challenges such as inability to maintain nutritional balance, metabolic defects, physical and mental challenges. These challenges further pose a serious disability threat among children to follow their developmental deprivations and for their families to render those children the desired cooperation.

So in this instance resilience assessment scale was designed up for the children aged 10-18 years suffering from chronic diseases in order to rule out the extent of the resilience acquired in relation to social domain, personal attributes, physical domain, psychological domain and also to gather various coping strategies used by the child to regain back the maximum potential for health and well-being. Nurses besides therapeutic regimen should also understand the psychological need and mental abilities of the child hospitalized with chronic disease in order to render quality and effective care and thus promote rehabilitation and resilience.[7]

Methodology

Methodological research was conducted on 100 children aged beyond 10 years for upto 18 years with chronic diseases in Paediatric wards and Intensive care units of Mullana, Government Civil Hospital at Ambala and Kalpana Chawla Government Hospital at Karnal.

Assent form was created in Hindi language and the consent was obtained from parents, which confirm the willingness of participants to be a part of this study. The aim of research study was explained to the subjects and confidentiality of the study subjects was ensured.

1. Phases of the Study

Phase 1: Preliminary Preparation

1) Review of literature

Extensive ROL was studied and the factors/domains affecting resilience were selected for the preparation of preliminary preparation of first draft.

2) Item pool generation

An exhaustive list of the factors which plays an important role in attaining resilience among children with chronic diseases was prepared from literature review.

3) Preparation of Preliminary Draft

The blue print of resilience assessment scale for children with chronic disease was prepared. Suggestions from the children and their parents suffering with chronic diseases followed by qualitative analysis from the same helped to rule out their concerns in the diseases conditions and strategies to cope up with their diseased condition.

Phase 2: Validation of first draft and subsequent drafts.

Validation of resilience assessment scale was done by four rounds of modified Delphi technique. Drafts were given to seven experts from the field of paediatric nursing and paediatric medicine and their suggestions were incorporated for the preparation of subsequent drafts. Modifications were made as per their suggestions

and content validation and content validity index of the final draft of resilience assessment scale was calculated. The final draft of the resilience assessment scale contained 29 items related to Personal Attributes , Social Domain , Psychological Domain and Coping Strategies opted by the child during the journey of chronic illness. The scoring criteria for resilience is as under:

S. No.	Levels	Range of Scores
1.	High Resilience	107-145
2.	Moderate Resilience	68-106
3.	Low Resilience	29-67

Phase 3: Pilot study

The Resilience Assessment Scale was administered to 10 children with chronic diseases from MMIMS&R hospital, Mullana after finalization of the second draft. The items were converted to Hindi and given to the children as self-administered questionnaire. The result of the pilot study after first administration indicated that the language of items was clear but somewhere misinterpreted by the children. The investigator has to restate and re-comprehend the sentence for its easy understand ability for the child. The average time taken for responding to the components of the Resilience Assessment Scale from one patient was 30-40 minutes .So, in subsequent drafts, structured interview was opted that was comfortable for the patient as well as for the investigator.

Phase 4: Final try out of Resilience Assessment Scale

Resilience assessment scale was translated to Hindi for understanding of the child. Data was collected from patients with chronic diseases after translating the Resilience Assessment Scale from English to Hindi and further from Hindi to English for sentence Re-verification. The scale was administered to 100 children suffering with chronic diseases in the month of August to October 2015. The average time taken in completing the Resilience Assessment Scale from one patient was 40-45 minutes.

Scoring criteria was modified for easy understanding for the child and further 5 coloured pens were used for Likert scale interpretation such as:-

5=All of the time (>8 times/ 10 times)

4= Most of the time (6-8 times/10 times)

3=some of the time (4-6 times/10 times)

2= A little of the time (2-4 times/10 times)

1=None of the time (<2 times)

RELIABILITY

a) Internal consistency

Data was analysed by using SPSS (version 21.0) .To ensure the internal consistency, reliability of measurement scale; the Cronbach's alpha was used. The scale was consist of 29 items and the overall calculated Cronbach's alpha coefficient was 0.81. (Cronbach's alpha coefficient should be >0.70).[8]The resilience assessment scale for the patients suffering with chronic diseases was found to be highly reliable in terms of assessing the child's ability to spring back to previous state of well being.

Table 1 Reliability analysis of resilience assessment scale, by using Cronbach Alpha

S.NO	Items	Corrected Item–total correlation	Cronbach's alpha if item deleted
1. O	I like to play with my friends whenever I feel.	.432	.805
2.	I report any unusual sign or symptom to my parents.	.458	.803
3.	I take my favorite story books, games along with me during hospitalization.	.342	.808
4.	I like to watch T.V, play video games, mobile handling during my free time.	.414	.802
5.	I like to play outdoor games like badminton, boll dosing, going a new place to a market, fun park, water park etc. with siblings and neighbors.	.439	.813
6.	I discuss my personal problems with my teachers (disease related).	.326	.805
7.	I try to compensate my absenteeism by studying extra hours and with help of my class mates.	.455	.805
8.	I take help of my teachers in completing my homework and assignments.	.551	.796
9.	I like to take part in co-curricular activities (school games, drawing competitions, and art and craft workouts.)	.422	.802
10.	I like to do my daily care activities myself.	.344	.805
11.	I feel hard to snap back when something bad happens (sensitive to situations).	.225	.809
12.	I have a mentor with whom I share my worries and feelings.	.374	.814
13.	I know that I would get well as early as I take my all medication on time. .	.451	.800
14.	I go for regular follow up as and when advised.	.421	.817
15.	I feel confident with parent's presence during hospitalization.	.546	.796
16.	I sleep for 8-10 hours daily.	.568	.796
17.	I feel confident with parent's presence during hospitalization.	-.039	.801
18.	My past record in school gives confidence for newer achievements and challenges.	.478	.800
19.	I try to see the positive side of the happening situation.	.450	.802
20.	I believe that there stands a supernatural power that holds me safe during my illness.	.629	.798
21.	I do meditation daily.	.060	.813
22.	I like to be with myself as and when troubles face me.	.319	.806
23.	I learn from my mistakes.	.318	.806
24.	I come up with different ways to handle a tough situation.	.418	.802
25.	My parents feel pride for my achievements.	.069	.814

Cont... **Table 1 Reliability analysis of resilience assessment scale, by using Cronbach Alpha**

26.	I try to work hard more in a situation when my teacher expects best out of me for my school work.	.451	.801
27.	My family helps me whenever I need them.	.032	.814
28.	I try to work out problems by talking or writing about them.	.360	.808
29.	I think myself as a strong person to deal with difficulties.	.329	.806

b) Equivalence of resilience assessment scale:

Inter-Rater Reliability

Inter Rater reliability calculated with Cohen's Kappa and it was found to be 0.76 for the resilience assessment scale for the children suffering from chronic diseases.

c) Stability of resilience assessment scale:

Test Retest Reliability

During the final try out of the resilience assessment scale, the test retest group was formed from 10 patients suffering with chronic diseases. The tool was coded with order number 1 to 10.The patients were interviewed with Resilience Assessment Scale. After 5 days, same patients were interviewed with same code number. Karl Pearson test was applied. The total instrument test retest reliability was 0.80.(normal value of Test Retest reliability is .70-1).[8]

Validity

a) Content validity

The content validity index was calculated and the Performa was circulated to experts .The CVI was 0.71 (If the values CVI is > 0.78, it shows good content validity)

b) Concurrent validity:

Wagnild and Young Resilience scale (Variable 1) was used as a Gold Standard because the items in the scale are similar to the items in the Resilience Assessment Scale (Variable 2) and the scale was also used for the adolescents. Pearson correlation between the resilience assessment scale and Wagnild and Young resilience scale, was calculated for concurrent validity.

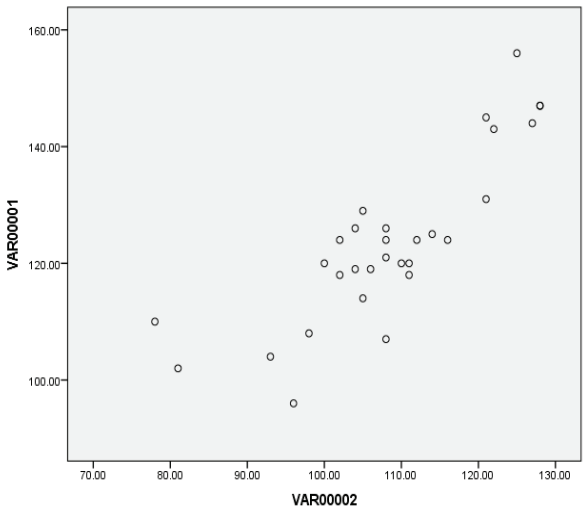

Figure 1: Positive correlation showing relationship between Wagnild and Young resilience scale and Resilience Assessment Scale for children suffering with chronic diseases. .

Table 2 Methodological analysis

Cut of Point Total Score	Sensitivity %	Specificity %	Ppv %	Npv %
>=59	100	0	70	-
>78	100	11.11	72.4	100
>81	100	22.22	75	100
>93	100	33.33	77.8	100
>96	100	44.44	80.8	100
>98	100	55.56	84	100
>100	95.24	55.56	83.3	83.3
*>102	100	100	86.4	75
>104	80.95	66.67	85	60
>105	76.19	77.78	88.9	58.3
>106	71.43	77.78	88.2	53.8
>108	57.14	88.89	92.3	47.1
>110	52.38	88.89	91.7	44.4
>111	47.62	100	100	45
>112	42.86	100	100	42.9
>114	38.1	100	100	40.9
>116	33.33	100	100	39.1
>121	23.81	100	100	36
>122	19.05	100	100	34.6
>125	14.29	100	100	33.3
>127	9.52	100	100	32.1
>128	0	100	-	30

c) Construct Validity :

Factor analysis

Exploratory factor analysis was used in identifying the key factors of resilience assessment scale. The value for Kaiser –Meyer Olkin (KMO) and Bartlett's test of sphericity was applied to assess the tool for its eligibility for carrying out factor analysis statistically. The value for Kaiser –Meyer olkin was <.60 and whereas p value of Bartlett's test of Sphericity was >0.05, which was not significant. It shows that the data was not appropriate for factor analysis.so, factor analysis findings were not considered.

Discussion

The Resilience Assessment scale constituted various domains/factors that affect the resilience among the children with chronic diseases during the course of the illness. The Resilience Assessment scale consisted 29 items including personal attributes , social domain , psychological domain and coping strategies opted by the child during the journey of chronic illness. Child and youth resiliency measure, CYRM was developed for the adolescents which included personal attributes, community domain, peer domain and contextual connectedness.

Wagnild and Young developed a 25 item Resilience Scale, RS for the adolescent children to check for the resilience achieved in overall domains including personal competence and acceptance of self and life.

Also, Resiliency tool was developed for chronic diseases. The questionnaire was divided into three domains interpersonal characteristics, characteristics of coping, and intrapersonal characteristics. Factor analysis has shown five factors; positive self-understanding, self-reliance, resourcefulness, perception of positive family relationships, and intimacy.

Investigator calculated internal consistency of resilience assessment scale and found it to be highly reliable that is 0.81.Similarly, Wagnild and Young developed a resilience scale and similarly calculated internal consistency and was also found to be reliable that is 0.91.

A study conducted on development of tool calculated the concurrent validity by correlating with other resilience measures and found it to have positive correlation. Also,

The correlation of Resilience assessment scale was found to be highly correlated with another measure that is Resilience scale.

Conclusion

The study was concluded with the help of findings that the resilience assessment scale consists of total 29 items including personal attributes , social domain , psychological domain and coping strategies opted by the child during the journey of chronic illness.

The resilience assessment scale developed by the investigator is valid and reliable and can be used effectively to predict the patients for resilience in chronic diseases.

Ethical Clearance: An ethical clearance was granted by the research and ethical committee of M.M College of Nursing, M.M University, Mullana, Ambala.

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Conflict of Interest : Researcher hadn't any conflict of interest

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Influence of Neck Pain on Pectoralis Minor Muscle Length among Young Adults

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Abstract

Background : Students are prone for posture and works related to neck pain due to their desk jobs. It leads to postural abnormality and muscle imbalance. Neck pain leads to alteration in the length of the pectoralis minor muscle length. Checking pectoralis minor muscle length helps in clinical discussion making in patients with neck pain. **Objective**: To find out the prevalance of pectoralis minor muscle tightness among students having neck pain. **Methodology**: A convenient sample of 100 participants were categorized into symptomatic and asymptomatic group. Neck pain was assessed by NORTHWICK PARK neck pain questionnaire. **Results**: Neck pain has positive correlation with pectoralis minor muscle tightness in both right(r=0.887) and left(r=0.828) side.The pectoralis minor muscle tightness is more in symptomatic group than in asymptomatic group (p=0.00). **Result& Conclusion**: The study confirms that neck pain has influence on pectoralis minor muscle length

Keywords*: Neck pain, pectoralis minor, tightness, prolonged sittting, young adults*

Introduction

Neck pain is becoming increasingly common throughout the world.Neck pain has a considerable input on all individuals and health care system.Neck pain accounts for 50% of all soft tissue problems seen in general practice and are a common reason for referral to PHYSIOTHERAPY

Prolonged sitting, improper posture of head using mobile phones/devices are major cause of neck pain in STUDENTS. Bad posture is certainly at top of list for most of the people.Pectoralis minor muscle attaches to the coracoid process of scapula and inserts on three, four,five ribs near the costosternal junction.The muscle orientation determines that it will produce scapular downward rotation,anterior tilt an internal rotation. Measuring the pectoralis minor muscle length is of clinical importance/ interest as the short pectoralis minor length is identified as a part of pain and the muscle imbalance. In the upper extremity adaptive shortening of pectoralis minor may be repetitive use of the upper extremity and also due to VARIETY OF COMPENSATION PATTERNS.This shortening leads to postural abnormalities and upper body muscle imbalance.

In neck pain the neck muscles work extra hard and continuously contract to maintain the head posture. The neck pain that leads to forward shoulder causes the muscle of shoulder blade to lengthen and the chest muscles to shorten.Clinical test have been recommended to test shortening of the muscle. Measuring the pectoralis minor muscle length helps in CLINICAL DISSCUSSION MAKING and aidsin Determining the risk factors when treating patients with neck pain. This muscle contributed to the host of compensation patterns. This muscle has a significant role and should never be forget when treating neck pain.

Physiotherapy has become an independent practice among the health care profession has a vital role in

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making life better. Physiotherapist has the right to asses and plan a proper treatment legally

The prevalance of neck pain among college student are common due to the nature of the tasks such as writing and reading habits,the usage of computer and other electronic gadgets.The head and neck posture which assumed for a long period of time desk works and usage of electronic gadgets contributes to mechanical neck pain

The anatomical and kinematic relationship between cervical spine and thoracicSpine contributes to mechanical alignment changes in thorax and shoulder among mechanical neck pain students.The mechanical changes cause slouched shoulder and pectoralis minor muscle tightness

The tightness of the pectoralis minor muscle contributes to range of symptoms in shoulder,upper extremity and thorax. The screening of the pectoralis minor muscle tightness among neck pain subjects will be used as a prophylactic measure to prevent upper extremity and thorax symptoms and abnormalities in future

To know about the prevelance of the pectoralis minor muscle tightness in students with mechanical neck pain ,the pectoralis minor muscle length was measured in this study.

Methodology

A convenient sample of 100 participants age 18-25 were involved.50 volunteers with neck pain and 50 individuals were selected .The procedure of the study is explained and a consent form is obtained from the participants. The symptomatic individuals were given northwick park pain questionnaire and their pain was measured with visual analogue scale and score were calculated based on their response. Pectoralis minor muscle length was measured using a rigid standard transparent right angle (height of 12 cm and base of 8 cm) for both symptomatic and asymptomatic group. People with history of cervical pathology,shoulder gridle pathology, chestwall deformity, rib fracture, sternum injury, clavicle fracture, scapula fracture were excluded from the study. To find out the prevalence of the pectoralis minor muscle tightness among the subjects those who are having neck pain studies show that the muscle imbalance in upper quarter of body is common among the neck pain subjects this study emphasize on the significance of screening the pectoralis minor muscle tightness among neck pain subjects, will b used as a prophylactic measure tp prevent upper extremity and upper thorax symptoms due to it in future among the neck pain subjects

Procedure:

Based on selection criteria 100 students were selected (50 subjects with neck pain- symptomatic and 50 subjects without neck pain asymptomatic) . the subjects were explained in details about the need of the study and those who are willing to participate are selected. The NORTHWICK PARK NECK PAIN QUESTIONNAIRE was given to the symptomatic group. The greater the score the greater the pain intensity. The pectoralis minoe muscle length was measured using a rigid standard transparent right angle (height of 12 cm and base of 8 cam) for both symptomatic ans asymptomatic group and were correlated with the neck pain score

Statistical Analysis:

The correlation between neck pain and pectoralis minor tightness was done by PEARSON CORRELATION and the comparison of pectoralis minor tightness between the symptomatic and asymptomatic group was done by INDEPENDENT SAMPLE t TEST

TABLE 1- CORRELATION BETWEEN PECTORALIS MINOR TIGHTNESS AND NECK PAIN

	RIGHT PECTORALIS MINOR TIGHTNESS	LEFT PECTORALIS MINOR TIGHTNESS
NECK PAIN	R= 0.887**	R= 0.828**

the values are the pearson correlation and R value. The results are statistically significant and shows that there is a positive correlation between the neck pain and the pectoralis minor length on right and left sides

TABLE 2. COMPARISON OF PECTORALIS TIGHTNESS BETWEEN BOTH THE GROUPS

		MEAN	SD	MEAN DIFFERENCE	T	DF	SIGNIFICANCE
Right pectoralis minor tightness	SYMPTOMATIC GROUP	6.34	0.78	2.93	22.58	98	0.000
	ASYMPTOMATIC GROUP	3.40	0.47				
Left pectoralis minor tightness	SYMPTOMATIC GROUP	5.60	1.02	2.51	15.87	66.88	0.000
	ASYMPTOMATIC GROUP	3.09	0.45				

Table 2 shows the values mean +/- standard deviation. The mean value of the right pectoralis minor tightness of neck pain group was 6.34 (SD 0.78) while that of the asymptomatic group was 3.40 (SD 0.478) and a stastically significant difference (p = 0.000**)

TABLE 3 COMPARISON BETWEEN THE RIGHT AND LEFT SIDE PECTORALIS MINOR TIGHTNESS

		MEAN	SD	MEAN DIFFERENCE	T	DF	SIFNIFICANCE
OVERALL PARTICIPANTS	Right pectoralis minor tightness	4.87	1.60	0.52	10.36	99	0.000
	Left pectoralis minor tightness	4.34	1.49				

Table 3 The mean value of the right pectoralis minor tightness of neck pain group was 6.34 (SD 0.78) while that of the asymptomatic group was 3.40 (SD 0.478) and a stastically significant difference (p = 0.00***)

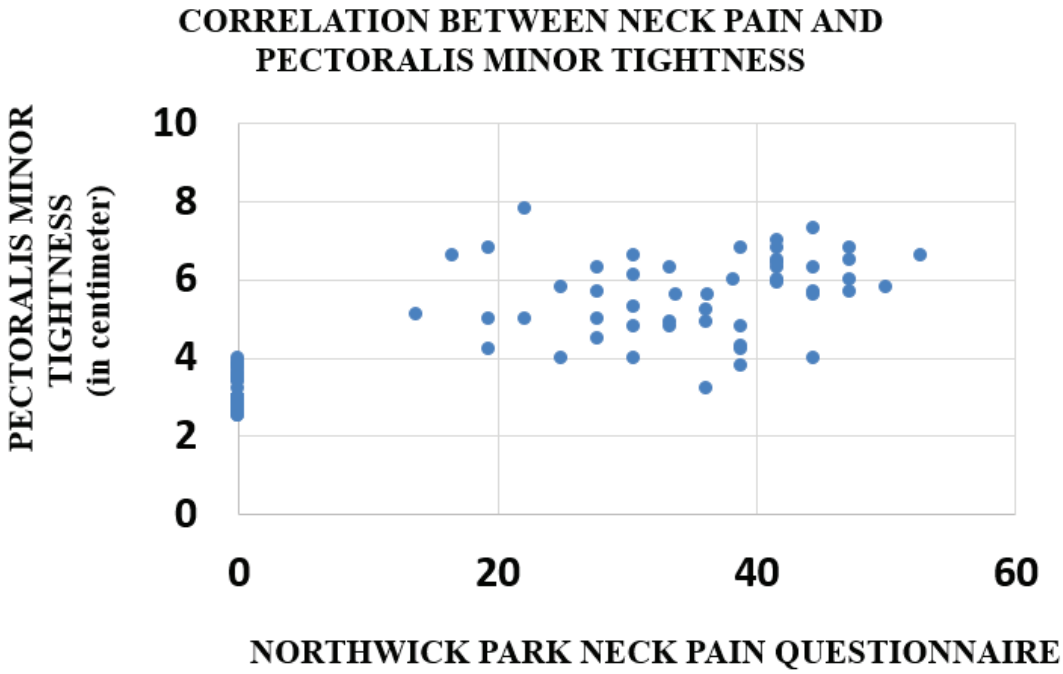

Fig 1 shows the correlation of the Northwick park neck pain score and pectorlis minor tightness. The blue dots in the scatter plot represent the neck pain score. The right and the left pectoralis minor tightness is more in symptomatic group than in asymptomatic group (p = 0.00)**

REPRESENTS THE COMPARISON OF RIGHT PECTORALIS MINOR TIGHTNESS IN SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS

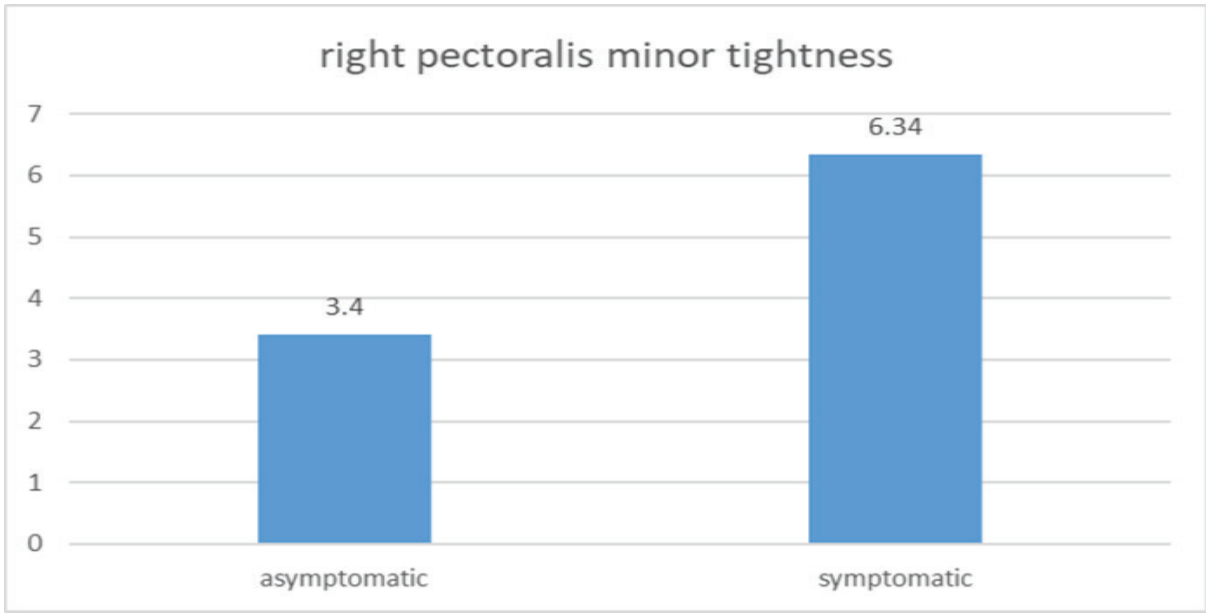

Fig 2 Pectoralis minor tightness in centimetres. The bar graph represents the tightness of the Right pectoralis minor muscle in both the groups. The pectoralis minor tightness is more on the right side (dominant side) than the left side in both the symptomatic and asymptomatic group (p = 0.00)**

REPRESENTS THE COMPARISON OF LEFT PECTORALIS MINOR TIGHTNESS IN SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS

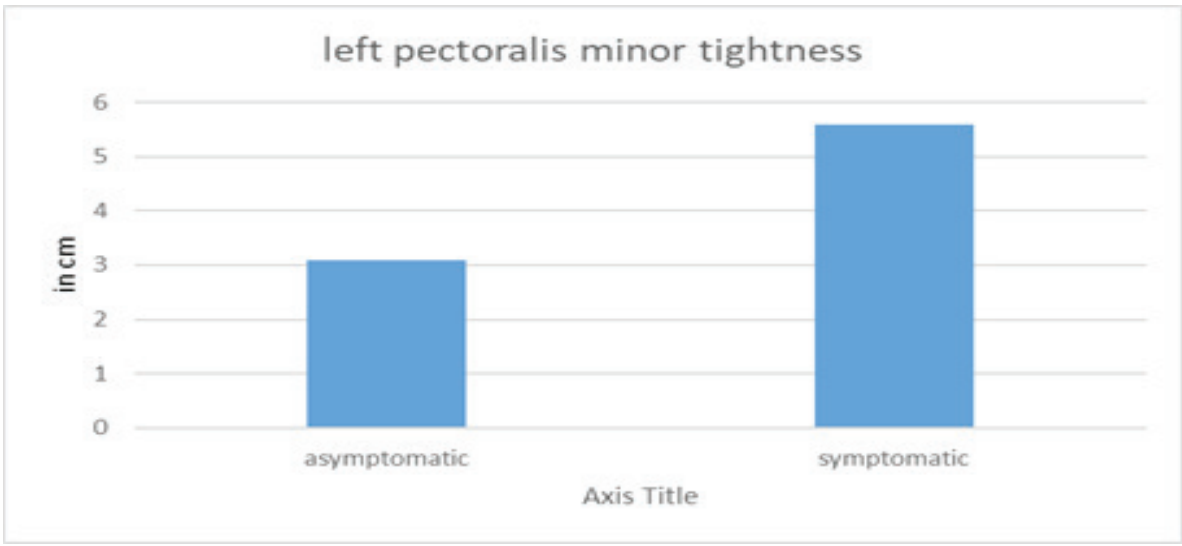

Fig 3 The bar graph represents the tightness of the pectoralis minor muscle. The mean value of left side pectoralis minor tightness of neck pain group was 5.60 (SD 1.03) while that of the asymptomatic group was 3.09 (SD 0.45) and had a difference that was statistically significant (p = 0.000)**

REPRESENTS THE COMPARISON OF RIGHT AND LEFT PECTORALIS MINOR TIGHTNESS OF OVERALL PARTICIPANTS

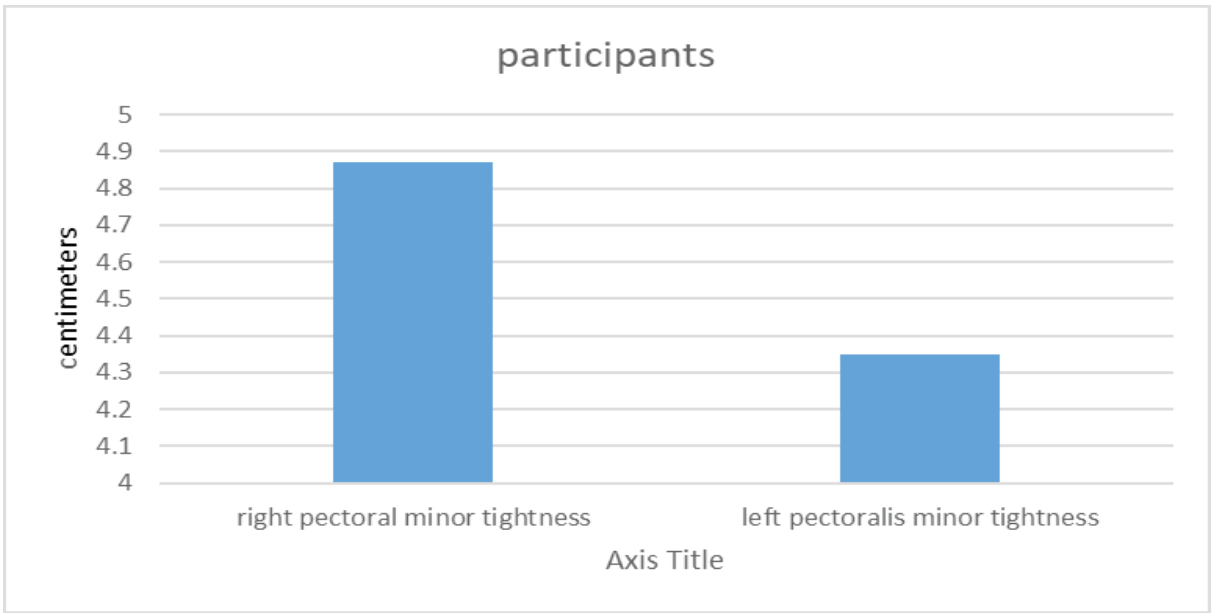

Fig 4: The bar graph represents the pectoralis minor tightness of the overall participants,The tightness is more right side (dominant side) thn the left side in both symptomatic and asymptomatic individuals.

Results

The results of the study reveal that neck pain is significantly associated with pectoralis tightness that there is positive correlation between neck pain and pectoralis minor muscle tightness in both right (r = 0.8878**) and left side (r = 0.828**).

The right side (dominant side) pectoralis minor tightness is more than the left side in both symptomatic and asymptomatic group.

The neck pain is one which causes more sick leave and decrease productivity and is prevalent among adults the study aims at finding the unoticed hidden factors of neck pain for planning better treatment regimen and prevent further complications.

Discussion

The traumatic mechanical neck pain is common among college students due to forward head posture. The study reports revealing that Forward head posture is common postural problem seen among students with 85% of its prevalence[6].In forward head posture due to anterior head position with posterior rotation of the occiput and cervico-thoracic flexion,along with loss of cervical lordosis[1],results in hypomobility of C0,C1 and C2 joints and causes in tightness of sub-occipital muscles and causes sub-occipital neuralgia,due to hypomobility in upper cervical spine the middle cervical spine becomes hypermobile and prone for vertebral dysfunctions ,these dysfunctions causes facet capsule impingements, discherniations and radiculopathies[2].The causes tightness ofupper trapezius,sternocleidomastoid,scalene group of muscles and levatorscapulae,and weakness of deep neck flexor muscles,upper back muscles and serratus anterior,the sepatho-mechanical events in Forward head posture causes neck pain.

This indirectly causes problems in shoulder,upper extremity and thorax through rounded shoulder and its product pectoralis minor tightness.The tight pectoralis minor muscle due to rounded shoulder posture,pulls the scapula forward,downward,rotates internally and tips anteriorly and it induces malalignment of scapula,this causes disturbance in optimal resting position of scapula and restricts it kinematics in glenohumeral range of motion. The malaligned scapula which drawn forward,downward,internally rotated and anterior tipped will reduces the sub-acromial space and causes impingement syndromes and rotator cuff pathologies[5],the tight pectoralis minor reduces the sub pectoral space,entraps neuro-vascular structures and causes ThoracicOutletSyndrome(TOS)[2,9].The tightness of pectoralis minor causes anterior rib dysfunction,intercostal neuralgia,costochondritis and serratus anterior strain.Hence the dysfunctions and musculo-skeletal pathology of forward head posture is not confined tone ckalone,indirectly through rounded shoulder posture and pectoralis minor tightness,the dysfunctions and musculoskeletal pathology is also contributed in shoulder,upper extremity and thorax.

To know about how prevalent this pectoralis muscle tightness exist among neck pain subjects from the Forward head posture origin,the pectoralis muscle length is measured in the students those who had neck pain due to Forward head posture.

The 100 physiotherapy students both male and female,with the neck pain was selected, their pain was measured with Visual analogue scale and their neck disability is recorded with neck disability index,the subject those who are having pain more than 5points in Visual analogue scale was selected and their pectoralis muscle length was measured with the inch tape from the inferior edge of 4th rib to infero-medial aspect of corocoid process[7],thepectoralis minor tightness was more prevalent among the subjects those who had pain in neck 5points and above in Visual analogue scale,in this female subjects was pre-pondering

The neck pain and the habitual forward head posture is characterized as anterior head position with posterior rotation of occipit.The upper cervical spine will be in extension,the lower cervical spine and upper thoracic spine will be in flexion.There will be loss of cervical and excessive thoracic kyphosis flattening of lumbar spine tilting pelvis posteriorly while sitting will make loss of lumbar lordosis and through kinematic chain if contributes to neck pain through anterior head position of axial skeleton

Muscle imbalance and malalignments in the axial skeleton and pelvis cause stress of the muscles ofthe upper quarter and manifest head ache,neck pain and radiating pain.Neck pain indirectly causes problems in thorax through round shoulder or slumped posture that concomitantly occurs characterized by protraction of acromion in front of line of gravity,there will be protraction,downward rotation and anterior tapping of scapula.The malaligned scapula mechanically contributes to myofascial trigger points in pectoralis minor and it perpetuate the posture and causes adaptive shortening

Conclusion

The results of the study confirm that neck pain has a significant influence on pectoralis minor muscle length. The tight pectoralis minor cue for numerous injuries. Pectoralis minor has to be taken into consideration when treating patients with neck pain and if ignored leads to a potential cascade of problems. Screening the pectoralis minor tightness helps in CLINICAL DECISION MAKING and preparation of the treatment protocol.

The pectoralis minor tightness diminishes the arm strength and it is believed to cause respiratory difficuilty in individuals experience fatigue and might compromise the respiratory muscles

The tight pectoralis minor reduces the sub pectoral space, entraps the neuro vascular structures and cause thoracic outlet syndrome. The tight pectoralis minor also causes anterior rib dysfunction, intercostalneuralagia, costochortisis and serratus anterior strain ,upper cross syndrome and winging of scapula. It can again cause shoulder impingement, altered scapular kinematics and restricts-glenohumeral Range of motion. Pectoralis minor like a brother that not like to be ignored, contribute to host compensation pattern .In future when treating patients with neck pain, Pectoralis minor has to be taken into consideration for planing effective therapeutic regimen

Limitations

The sample size is less.

The pectoralis minor length measurement was taken for only 100 subjects.Subjects aged 18 – 25 were only included in the study.

It can be done in other age groups also. Neck pain was assessed by self reported questionnaire and can have bias.

Recommendation

The respiratory rate, pattern, lung volumes and lung capacities of the subjects can also be measured in further studies.

The postural assessment of subjects with neck pain and pectoralis minor muscle tightness can also be done in further studies.Future studies must correlate the pectoralis minor length with the neck muscle endurance and strength

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Identifying Appropriate Therapeutic Methods in Covid-19 Originated from Persian Medicine: A Review Article

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Abstract

Objectives: COVID-19, a novel Coronavirus from same family as SARS-CoV-2 is a highly contagious virus. It is transmitted mainly from upper respiratory system and affects important organs, like heart and lungs. The aim of this study is identifying appropriate therapeutic methods originated from persian medicine. **Materials and Methods:** This study has been designed from the viewpoints of traditional and modern medicine. Considering modern medicine, we searched valid database by searching key words "SARS-Cov-2" and "COVID-19". For traditional medicine, we searched reliable traditional medicine like the "Canon of Medicine" by "Avicenna", the Exir-e Aazam and Zakhira-I Kharazm Shahi. Patients were evaluated according to their symptoms and categorized based on traditional medicine. Physiopathology and management of the disease, including prevention and treatment were studied. **Results:** The principle underlying mechanism seems to be warm and dry dystemperament that affects different organs and causes various adverse effects which are higher than warm and dry dystemperament, but less than the presence of virus. Our traditional Iranian medicine findings are validated by the modern medicine. **Conclusion:** Based on symptoms, the equivalent of this disease was examined from the viewpoint of traditional medicine. Then, physiopathology and management of this disease were presented in five levels, including one stage of prevention and four stages of treatment, and finally, the treatment recommendations of Iranian traditional medicine were approved with new research results.

Keywords: *SARS-Cov-2, COVID-19, Traditional medicine, Persian medicine, Dystemperament therapeutic methods.*

Introduction

At the beginning of 2020, the novel coronavirus has emerged as a global health problem. Until now, there is not any definite treatment for this disease. No vaccine is validated so far [(1)]. The main route of transmission is thorough upper respiratory tract and then infection affects various organs [(2)]. COVID-19 affects alveolar cells, neural, immune and cardiovascular system, liver, kidney and even skin. Mortality is due to multi-organ failure, acute respiratory distress syndrome, heart failure, arrhythmia, renal failure and shock. This necessitates specific attention to possible damage to these organs for management of severe cases [(2)]. In this study, is aimed to describe SARS-CoV-2 infection by means of Iranian traditional medicine to introduce preventive and therapeutic management.

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Materials and Methods

Clinical and pathophysiologic aspects of COVID-19 are studied in PubMed, Google Scholar and Scopus. Iranian traditional medicine books like the Canon of Medicine by Avicenna, the great elixir, Akbari medicine,

Kholasa-tol hekmah and Zakhira-I Kharazm Shahi were studied accordingly for COVID-19. Ultimately, findings of Iranian traditional medicine references were compared with the last modern findings about COVID-19. Also, in this essay the preventive and therapeutic methods for COVID-19 infection is suggested.

Results

The most common signs of the COVID-19 disease include body ache, fever and chills, dry cough, fatigue, lethargy, headache, sore throat, dyspnea, diarrhea, abdominal pain, loss of smell and taste senses, macular rash, arrhythmia in advanced condition, and pulmonary bleeding [3, 4]. The pathophysiological origins of the disease are described in new published articles. It appears that SARS-CoV-2 disease is created when its spike protein bind to host Angiotensin-converting enzyme-2 (ACE2) receptor and inhibits the activity of this enzyme. This *e*ndopeptidase enzyme plays an important role in immune system, as well as cardio-pulmonary system and renal function [5]. There are lots of these receptors in the lung, heart and kidney tissues. In other way, there is a specific receptor in coronavirus that binds Furin protein. This protein, which exists in the cell membrane of various tissues such as the liver, lung, and small intestine, can induce the activity of spike protein in coronavirus. This indicates that coronavirus can attack multiple organs at a same time and cause various symptoms in patients [6].

In paraclinical examinations, high WBC counts, lymphopenia, high CRP count, increased ESR, CPK, and LDH can be found. Pneumonia and grand glass opacities can be seen Under CT scan examination [7]. Some people show acute respiratory distress syndrome (ARDS), acute heart injury, renal failure, and even encephalopathy that can lead to death. [7-10]

From view point of persian medicine, the temperament of the lungs is warm and dry, but its secondary and stable temperament has high humidity [11,12]. When virus enters into the respiratory system, it causes warm and dry dystemperament. The above mentioned symptoms, along with the spread of viral infection in a society, its spread in the body and involvement of different body organs in a short period of time, various and unpredictable clinical responses, and the occurrence of inflammation in involved organs indicate warm and dry dystemperament. [13,14]

Since there is a close relationship between lung and heart function [15], warming and drying properties in the lung can be transferred to the heart via vascular systems [16]. Additionally, the obstruction and closure of airway system causes a lack of access to cold air, and subsequently warm and dry dystemperament in the heart [16]. Blood exited from the heart becomes warm following warmth, tender material disrupts and concentrated material remains [16, 17]. This condition can be associated with the obstruction of pulmonary system, heart and lung congestion, pulmonary edema and consequently hypoxia. Since lung plays crucial role for heart, brain and liver tissues, this condition leads dystemperament, congestion, and injury in these tissues [13]. These abnormalities can cause death in some cases, especially those who suffer from heart failure [18, 19].

The dryness caused by this dystemperament can be associated with splitting and cracking in pulmonary vascular and alveolar tissues and subsequently bleeding and respiratory secretions [20]. Furthermore, evaporations originated from disrupted tender material due to dystemperament and also direct effect of virus on the brain create abnormalities in central nervous system which is eventually associated with several side effects such as the loss of smell or taste senses, headache, dizziness, sleepless, and chronic pain in the body [21, 22].

Following high blood temperature due to dystemperament and also released evaporations in some cases, rash may be occurred [23]. Sometimes, human body naturally mitigates inflammation, accumulation of viruses in the intestine and dystemperament by induction of diarrhea [24]. For this reason, diarrhea should not be inhibited by medications and this condition should be supported by obtaining enough water and minerals. Furthermore, the use of flatulent and indigestible foods, as well as foods that stay in the gastrointestinal tract for long hours or produce high excrement should be avoided [11]. Therefore, treatment of constipation in these individuals is very important.

It seems that corona virus initially creates warm and dry dystemperament at upper respiratory tract and then at lower part. This dystemperament can be associated with further side effects in the other organs, particularly in the heart, brain and liver [21]. These consequences

are because of dystemperament and in several cases due to the proliferation and existence of virus in these organs. Therefore, consideration and treatment of dystemperament, as well as its consequences and physical improvement of the body is the first line of the disease therapy. Protection against virus is the second line of the treatment. In viewpoint of Iranian traditional medicine, the following notes should be considered for the management of Covod-19:

1. Counteract against corona virus in view of traditional medicine, includes one stage of prevention and four stages of treatment, including beginning, increase, termination and the end. There are specific and different strategies for each stage.

2. Transmission of infection into the body, the most common involved organs, pay attention to dystemperament and histology of involved organs, and the affectivity from pathogen are important subjects that affect treatment strategies.

3. The foundation of quadruple traditional medicine and their appropriate use is associated with effective outcome. These criteria include therapeutic management, respect to sextuplet principles of health protection, medications, producers, and spiritual treatments.

4. Correlation between body organs with each other is important in view of Iranian traditional medicine. Some of critical organs such as heart, brain, and liver are more important and they are the source of triplet spirits, including animal, sensual and natural spirit.

5. Obstruction of arteries and ducts and accumulation of wastes materials and pathogens are important issues that need to be addressed in prevention and treatment.

6. One of the most important issues is related to maintain and strengthen the instinctual heat and strength of the patient and the organ involved. Thus, physicians should be aware about this issue in maintaining health and treatment.

Discussion

The general view of persian medicine in dealing with diseases is the priority of prevention and then treatment.

Prevention:

Due to the transmission type, prevalence, transmission, pathogenicity, effective preventive measures and behavior of the virus in human body and compliance with the sources of Persian medicine and similarity to the epidemic diseases (HAVAY-E VABAIE) [22-26], measures which are similar to those mentioned in the epidemic diseases in the books are recommended.

The most important part of the epidemic diseases' prevention from the perspective of persian medicine is the measures used in the general categorizes, which are briefly listed below:

Air:

In contaminated atmosphere of stay-at-home, it is recommended to reduce the humidity of the environment and use sunlight [22]. It is also recommended to use the vapor of some plants such as Harmel, frankincense, rose, as well as sprinkling the products of these three in vinegar, onion or garlic, or a mixture of vinegar and rose water in the house atmosphere [26].

Eat and drink:

Reducing of food intake is highly recommended to improve patient health [21]. Also hungry shouldn't be allowed if it leads to weakness of patients. Low volume foods, consumption of light foods with quick digestion and less waste products and ability to improve the instinctual heat are recommended [21]. Eating raw garlic and onion in small amounts that are well-chewed or cooked well in milk are recommended after each meal. Low-fat, mildly acidic foods and the use of single or compound teryag or Antidote are recommended [21], if available. Eating foods that cause infections and produce thick and sticky mucus, such as meat and dairy products should be avoided [27]. Additionally, fruits and vegetables that produce excess moisture, sweets, and drinking very cold water, especially if drunk quickly, should be avoided [27].

Movement and resting:

Resting and stay at home is recommended; however, to prevent excessive immovability which increases the body's moisture and the accumulation of waste products in the body, the uses of cradle move as well as mild and moderate exercise are emphasized [27].

Retention and depletion:

The most important work in this field is to cleanse the body according to the amount of excess body moisture, which can be done in moderation with an appropriate laxative, wet-cupping, expellent, bloodletting, sweating, diarrhea and exercise [28-32].

Psychological movements

It is recommended that people avoid each other, sadness, grief, anger, resentment, jealousy, anxiety and severe mental and emotional conflicts [27].

Sleep and wake up

Excessive sleep, insomnia, excessive sleep deprivation and nocturnal awakening are harmful [27]. Moreover, the most important measure in preventing the disease is to maintain and strengthen the natural forces by using heart, lung and instinctual heat tonics [28].

Treatment

The treatment of the disease should be performed according to the quadruple stages of the disease, taking into account the condition of patients and the involved organs, especially lungs and heart. Firstly, the therapist must correct the necessary items (sextuplet principles of the health maintenance) and take medical and pathological measures and, if necessary, prescribe medication. Healing practices and spiritual healing are used from the beginning to the end of treatment if needed.

Spiritual treatment is one of the main therapeutic an integral part in Persian medicine, and Abu Ali Sina describes some of them as follows:

Happiness, concomitance with pleasant and cheerful things is beneficial for patients [11].

In COVID- 19 disease, patients are initially asymptomatic or present mild symptoms such as fever and mild pain in the body, sore throat and chronic cough, and sometimes gastrointestinal symptoms. During the exacerbation phase, the symptoms may be intensified and several problems in respiratory system (such as a dry cough), gastrointestinal tract, nervous system, kidneys, and circulatory system may be appeared. At the end stage, the symptoms do not worsen and the disease remains constant, except when the complications of the disease such as the addition of lung bacterial infections and complications of other organs appear with various, severe and dangerous symptoms. In the final stage, the struggle between the prudent nature of the body and the disease ends. The result of this process is complete recovery, remained side effects after treatment, or death in some cases.

Treatment actions are performed according to the time of the disease onset, symptoms, complications and the disease stages, which are summarized as following:

- Elimination of dystemperament: from the beginning of the disease, we eliminate dystemperament of the patient with mild and cold measures.
- Strengthen the instinctive heat and help the resourceful nature to fight disease
- Helps get rid of phlegm and excess impurities in lungs
- Material deviation: which is done simultaneously with other steps, such as foot scrubs and bandages, sleeping on the abdomen, using laxatives, dry cupping, chest massage, phlebotomy and wet cupping
- Strengthen the lungs and other involved organs

On the other hand, recent studies have confirmed the anti-proliferative effects of several plants such as **A**lthaea officinalis [32, 33], Glycyrrhiza glabra L. [34, 35, 36], Echium amoenum [37, 38, 39], Origanum majorana [40, 41] and Viola odorata [42, 43, 44] on viruses. Furthermore, these studies reported the antioxidant properties of Althaea officinalis [32, 33], Glycyrrhiza glabra [9,34, 35, 36], Matricaria chamomilla [45, 46, 47] and mint [48], the repairing effect of Althaea officinalis [32, 33] and Malva sylvestris [49, 50, 51] and also the positive effect of Matricaria chamomilla [45, 46, 47] and cheese in strengthening the immune system. These effects are mediated through the multiple mechanisms, especially protecting the heart, lungs and immune system by regulating the secretion of cytokines (anti-inflammatory effects) along with the balance of absorption or reabsorption of electrolytes (potassium, calcium or sodium ions). These plants also show their therapeutic effects by improving endogenous antioxidant enzymes, regulation of signaling pathways and related kinases, as well as inhibiting of apoptotic pathways and

regenerating of damaged cells or tissues. [32-51]

Conflict of Interest: Nil

Ethical Clearance: Nil

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Investigating the Effectiveness of the Financial Narrative Therapy on the Financial Anxiety and Financial Wellbeing among the Female Nurses in the Educational Hospitals

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Abstract

Background and Aim*:* Researchers in the field of financial health have recently paid attention to the financial wellbeing of individuals and the factors affecting which. Accordingly, various interventions and therapies have been developed so far to maintain and improve the financial health of individuals. The aim of this study was to investigate the effectiveness of financial narrative therapy on reducing the financial anxiety and increasing the financial self-efficacy of female nurses.

Methods: This semi-experimental research is pre-test and post-test with the control group. The statistical population of the study consisted of all female nurses of educational hospitals in year 2019. A total of 30 people were randomly selected and assigned in both experimental and control groups (each with 15 members). In this research, Financial Anxiety Scale (FAS) and Financial Well-Fare Questionnaire (FWQ) were used to collect data. The financial narrative therapy was conducted in eight 90-minute sessions in the experimental group. Data were analyzed using SPSS-v.21 software and Statistical analysis of covariance analysis (ANCOVA).

Results*:* The findings showed a significant difference between the mean of the experimental and control group in two variables of financial anxiety and financial wellbeing ($P < 0.01$). Based on this, it can be concluded that financial narrative therapy has been effective in reducing the financial anxiety and increasing the financial self-efficacy of the female nurses.

Conclusion: Given the prominence of role of financial issues as a part of life in the today's constantly changing world, and the emergence of multiple sources of stress, financial narrative therapy can be used as an intervention to improve the financial health of individuals.

Keywords: *financial narrative therapy, financial anxiety, financial wellbeing, female nurses.*

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Introduction

Paying attention to the physical and psychological health of individuals has been always the concern of organizations, institutions and governments both nationally and internationally. The World Health Organization (WHO, 2001, 2004) defines the health as

a form of physical, mental, social well-being, and not merely the absence of disease [1]. In the field of psychology, the subjective wellbeing has been the focus of attention of scholars and theorists, with three interconnected but distinct components of emotional, psychological, and social wellbeing; because, according to researchers and theorists' opinion in this area, subjective wellbeing is more effective than any other factor on the mental health of individuals [2]. Financial Health is one of the types of wellbeing that plays an important role in the psychological wellbeing of individuals' life [3]. Following the changes in lifestyle, the world of jobs, industrialization and restructuring of societies, financial wellbeing has been attracted by researchers in recent years [4]. This term was first introduced by Kim [5]. According to him, the financial well-being is to have the financial security and freedom in the financial choices for the present and future [6]. In other words, financial wellbeing is a condition in which a person can fully fulfill his financial tasks in the present and future, feel secure about his financial future, and is freely able to have the financial choices that lead to pleasure. Financial wellbeing is a structure used to explain the financial position of a person or family. In fact, financial wellbeing is a kind of perceived satisfaction or joy about the financial position [7]. Financial wellbeing can be negatively affected by various variables. Variables such as age [8], education level, marriage base and income [9], financial literacy level of individual, poor financial management and financial stress [10] are examples of some variables affecting the financial wellbeing. Increase of the life standards, unhealthy lifestyle habits, various commitments and fluctuations in stress levels are among other factors affecting the financial wellbeing [11]. Financial Anxiety is one of the factors that can affect the financial wellbeing. Financial Anxiety is a sign and a psychological characteristic that causes an individual to have an unhealthy attitude towards managing his or her finances, and loses the right financial decisions through which, that leads to weakening the financial results and financial anxiety [6]. Financial anxiety can be the consequent of the financial disorder. Persistent financial disorders are predictable, and are often serious and of the kind of destructive financial behavior pattern that cause severe stress, anxiety, and abnormalities in the underlying areas of life [12]. According to researchers findings in the field of financial health [13], nine classes are listed for financial disorders which include: Compulsive buying disorder, Ambling disorder, Workaholics, Hoarding disorder, Financial dependence disorder, Financial enabling disorder, Financial Infidelity, Financial enmeshment disorder, and Financial denial [12]. Financial anxiety can affect both working and unemployed men and women. The negative consequences of the financial anxiety on women are more than men [6]. Mental and physical health is very important in women because they are the basic pillar of the family; however, it can be seen that excessive activity in the workplace, the stress of house chores and child care, and addition to that, and the financial worries threaten their mental health [14]. Financial anxiety has negative consequences for individuals [15]. Financial anxiety has negative effects of the general wellbeing and financial wellbeing of individuals and is one of the strongest predictors of low levels of wellbeing [16, 17, 18]. Financial anxiety also has negative consequences on physical and mental health of the individual [19, 20, 21] and leads to the instability of marital life [22].

One of the postmodern therapies is the financial narrative therapy [23] that helps people to overcome the financial anxiety [24]. Financial narrative therapy is retrieved from the narrative therapy [25]. Financial narrative therapy provides a therapeutic and theoretical approach to therapist which is a combination of mental health and financial planning models. The combined financial narrative therapy model is designed to be used by the trained professionals in the field of treatment, counseling, coaching, or financial planning [26]. The narrative therapy has been used by researchers so far in various variables such as financial anxiety management [23, 27, 28], financial well-being [29] and treatment of financial disorders [30]. In total, it can be said that economic issues have affected various aspects of mental health and quality of life and have caused anxiety and financial worries. Therefore, it can be stated that financial anxiety treatment is essential for improving the wellbeing and mental health of individuals and is a valuable step in the field of counseling and psychotherapy, family therapy, economic planning and sociology. Studies show that health workers are more susceptible to financial anxiety because of special difficulties in this area [6]. Nursing is also one of the health care jobs. Due to the sensitivity of occupational responsibilities and their continuous interaction with patients, nurses are always exposed to the physical and mental health risks and negative

stresses. Learning how to deal with tensions and mental health problems caused by life issues, especially the economic issues that are highlighted for most people in today's world can partly improve their ability to cope effectively with life stresses. Considering the fact that there has not been a study on the financial anxiety in the country and in the group of nurses, this study aimed to investigate the effectiveness of financial narrative therapy on the financial anxiety and financial wellbeing of the female nurses working in the educational hospitals in Isfahan city.

Two hypotheses were considered in this study: 1- Financial narrative therapy is effective in reducing the financial anxiety of female nurses. 2. Financial narrative therapy is an effective way to increase the financial self-efficacy of nurses.

Methods and Materials

This semi-experimental research is pre-test and post-test with the control group. The population of this study included all women nurses in public hospitals in Isfahan in 2019. From this society, two educational hospitals (Imam Hossein (AS) and Shahid Beheshti) in Isfahan were randomly selected. After the coordination, the time and place of meeting were informed and a list of volunteer nurses participated in educational sessions was prepared in the following (45 subjects). During the introductory session, the goals and content of the training sessions were informed, following a list of individuals (43 subjects) who were volunteered. Before selecting the sample, four volunteers refused to continue the co-operation. For screening, financial anxiety scale and financial wellbeing questionnaire were conducted among volunteers, and 30 people whose mean scores were higher in the financial anxiety scale and below the cutoff point in the financial wellbeing questionnaire were selected and randomly assigned in two control and experimental groups. Before the intervention, the purpose of the intervention was described for the two groups in an introduction session and their expectations were explained. Before the intervention, the financial anxiety scale and financial wellbeing questionnaire were firstly performed as a pretest among the members of the experimental and control groups. In the next step, the financial narrative therapy was performed in the experimental group during the eight sessions (90 minutes each session) and at the end, the post-test was again performed among the members of the experimental and control group. A summary of the description of the financial narratives therapy is presented in Table 1.

Table 1. Summary of the description of the financial narratives therapy teaching sessions

Sessions	General goals
1st session	Title: creating the good relationship, familiarity with goals, rules and structure of meetings Agenda: Familiarity among the group members, expressing group rules and norms, reviewing the structure and objectives of the meetings, helping members to participate in the group, and establishing a good relationship.
2nd session	Title: Collecting information about members' financial behavior Agenda: Collecting information (merely financially) and investigating their financial disorders
3rd session	Title: Assessing the financial situation and documenting the financial problems Agenda: Developing financial problems of group members and presenting an illustration of their current financial problems, providing a new definition of the problem.
4th session	Title: Externalizing the financial problem Agenda: Externalizing and reinvigorating the self- financial problem, expressing the effects of the problem on their lives in a variety of ways.

Cont... **Table 1. Summary of the description of the financial narratives therapy teaching sessions**

5th session	Title: Searching for the unique events Agenda: Expressing the past successful personal memories and experiences, and understanding the exceptions and unique events in their own lives despite the externalized problem.
6th session	Title: Providing the financial plan and implementation of new financial preferential reports Agenda: Helping members finding new financial narrations, consolidating them and reaching the insight into how they can actually implement their new financial reports.
7th session	Title: implementation and performing the financial program Agenda: implementation and performing the financial program, highlighting the strength points, fixing the preferential report
8th session	**Title:** Financial plan supervision **Agenda:** Encouraging and strengthening the members' capacity, evaluating the effectiveness of this financial plan and investigating the financial anxiety, submitting a few reports after observing the financial changes in life, summarizing and implementing the post-tests.

In this study, data collection tools were the demographic questionnaire, financial anxiety scale and financial wellbeing questionnaire. The Financial Anxiety Scale has been developed by Archuleta to measure the financial anxiety [11]. This scale consists of 7 items and its grading method is based on the seven-degree Likert scale (from never equivalent to 1, to seven equivalents always). The total score in this questionnaire is at least 7 and maximum 49, and high scores represent high financial anxiety in a person. The obtained reliability coefficient was obtained by Cronbach's alpha $\alpha = 0.94$ [11]. In Iran, this scale has been translated by Akafian and its validity and reliability have been measured [31]. The reliability of the questionnaire was calculated using Cronbach's alpha (92%). Also, the simultaneous validity of this scale with the behavioral log [26] was $r = 0.86$, and its divergent validity was obtained with the financial health scale ($r = -0.66$). In this study, the financial wellbeing questionnaire Prawitz et al. was used to measure the financial wellbeing [32]. This questionnaire contains 8 questions that are made as the self-report. The internal consistency of the questionnaire was reported 96% [32]. For each question, responses are graded according to the eight-degree Likert scale (from never equal to 1, to always equal to 8), and high scores represent a high level of financial well-being. In Iran, the financial well-being questionnaire is translated by Akafian and the validity and reliability of which has been investigated [31]. The reliability of the questionnaire was calculated using Cronbach's alpha ($\alpha = 0.91$). Also, the simultaneous validity of this questionnaire with the financial self-efficacy questionnaire ($r = 0.87$) and its divergent validity with the financial anxiety questionnaire ($r = -0.44$) was obtained [33]. The inclusion criteria of participants in this study was to obtain a score higher than the cut-off point in the financial anxiety scale, a score lower than the cut-off point in the financial wellbeing questionnaire, and not be treated or educated by another group during the duration of the research. Exclusion criteria in this study were: presence of acute psychological disorders, including psychotic disorders, absence for more than one session, delay of more than twenty minutes in three sessions, failure to perform specified assignments for more than one session.

Statistical Analysis

Data were collected by statistical software SPSS-V.21 and then analyzed by the descriptive statistics indices (mean and standard deviation) and analytical statistics indices (follow-up tests and covariance analysis). Satisfaction to participate in studying, distributing and collecting questionnaires by the researchers at all phases, avoiding mentioning

the names on the questionnaires, and obtaining the introduction letter to conduct the study were considered in this study as the ethical considerations.

Results

For analyzing the research hypotheses, the covariance analysis was used with consideration of the assumptions related to the statistical tests. Descriptive indicators related to the pre-test and post-test scores of financial anxiety and financial wellbeing in both experimental and control groups are shown in Tables 2 and 3. According to the findings, the mean and standard deviation of the age in the experimental group were equal to (633.41 ± 833.8) and in the control group (42.933 ± 10.606), respectively. In the experimental group, three students had an under-diploma degree (9.4%), six had a diploma degree (18.8%), 13 had an undergraduate degree (4.6%), 7 had a bachelor's degree (21.9%) and one had a doctoral degree (1 / 3). In the control group, 2 had an under-diploma degree (4.2), five had a diploma degree (10.4), 6 had an upper secondary degree (12.5) and two had bachelor's degree (4.2). According to the employment status, in the experimental group, 11 people were under contract (34.4%),three contractors (9.4%), five corporate (15.6%) and 11 formal (34.4%) and in the control group, five were under contract (10.4%), two contractors (2/4), two corporate (2/4), and six formals (12.5) (Table 2).

Table 2- Distribution of demographic characteristics of nurses in two experimental and control groups

Statistical index Demographic variables		***Experimental group***		***Control group***	
Variables		***Number***	***Percentage***	***Number***	***Percentage***
Educational degree	Under diploma	3	9.4	2	2.4
	Diploma	6	18.8	5	10.4
	Undergraduate	13	4.6	6	12.5
	Bachelor's	7	21.9	2	4.2
	Upper Bachelor's	1	3.1	0	0
	Total	15	100	15	100
Employment status	Based on contract	11	34.4	5	10.4
	Contractual	3	9.4	2	4.2
	Corporate	5	15.6	2	4.2
	Official	11	34.4	6	12.5
	Total	15	100	15	100

Table 3 - Mean and standard deviation of pre-test, post-test scores in the financial anxiety variables

Variables	Group	Number	Pre-test		Post-test	
			Average	Standard deviation	Average	Standard deviation
Financial anxiety	Test	15	30.33	5.47	17.86	4.03
	Control	15	28.26	3.15	27.93	4.11

The results of Table 2 show that in the financial anxiety variable, the mean and standard deviation of the scores of the experimental group in the post test were 17.86 and 4.03, respectively. In the control group were 27.93 and 11.4. Based on the results of this table, it can be concluded that in the financial anxiety variable in the experimental group, the mean post-test scores changed to the pre-test, but no tangible changes are observed in the control group.

Table 4 - Mean and standard deviation of pre-test, post-test scores in the financial wellbeing variables

Variables	Group	Number	Pre-test		Post-test	
			Average	Standard deviation	Average	Standard deviation
Financial wellbeing	Test	15	18.533	2.133	59.866	17.585
	Control	15	15.933	2.463	17.066	10.374

The results of Table 2 shows that in the financial wellbeing variable, the mean and standard deviation of the test group in the post-test were equal to 59.886 and 585.17, respectively, and in the control group were 17.066 and 10.347, respectively. Therefore, it can be concluded that in the financial anxiety variable, the mean of post-test scores changed to pre-test in the experimental group, but no significant changes were observed in the control group. Before using the covariance analysis, a Levin test was used to examine the equations of variances that The results showed that in financial anxiety and financial wellbeing, the level of significance is greater than 0.05; Therefore, the variances are equal and the use of covariance analysis has no problem and the reliability of its results is confirmed.

The first hypothesis in this study was the financial narrative therapy teaching on reducing the financial anxiety of female nurses. To test this hypothesis, the covariance analysis test was used, results of which are presented in Table 5.

Table 5 - The results of covariance analysis the effect of group membership on the financial anxiety scores

Research variables	Sum of squares	Degrees of freedom	Average of squares	F	Significance level	Squares (impact rate)	Statistical power
Pre-test	293.714	1	293.714	48.389	0.0001	0.632	000.1
Group membership	948.799	1	948.799	149.852	0.0001	0.748	000.1
Error	170.952	27	6.332	-	-	-	-

As shown in Table 5, after the omission of the pre-test effect on the dependent variable and according to the calculated coefficient F, it is observed that there is a significant difference (P = 0.000) among the adjusted averages of the participants' financial anxiety scores in the post-test phase (F = 149.552) in terms of the group membership (experimental group and control group). Therefore, the first hypothesis of the research is confirmed. Therefore, teaching the financial narrative therapy has affected the financial anxiety of the experimental group in the pre-test. The amount of this effect in the post-test phase is 0.847. The second hypothesis of the present study was the effectiveness of teaching the financial narrative therapy on increasing the financial wellbeing of the female nurses. The results of this hypothesis are presented in Table 6.

Table 6 - The results of covariance analysis the effect of group membership on the financial wellbeing

Research variables	Sum of squares	Degrees of freedom	Average of squares	F	Significance level	Squares (impact rate)	Statistical power
Pre-test	754.008	1	754.008	4.005	0.04	0.129	0.488
Group membership	14390.025	1	14390.025	76.442	0.0001	0.739	1.000
Error	5082.658	27	188.247	-	-	-	-

As shown in Table 6, after the elimination of the pre-test effect on the dependent variable and according to the coefficient F, it is observed that there is a significant difference (P = 0.000) among the adjusted averages of the participants' financial wellbeing scores in the post-test phase (F = 149.552) in terms of the group membership (experimental group and control group). Therefore, the second hypothesis of the research is confirmed. Therefore, teaching the financial narrative therapy has affected the financial wellbeing increase of the female nurses in the experimental group. The amount of this effect in the post-test phase is 0.739.

Discussion

The purpose of this study was to investigate the effectiveness of financial narrative therapy on the financial anxiety and financial wellbeing of female nurses. As the results of Table 5 show, there is a significant difference between the adjusted averages of participants' financial anxiety scores in terms of group membership (experimental group and control group) at the post-test level (F = 149.852). Therefore, the first hypothesis of the research about the effectiveness of financial narrative therapy on reduction of the financial anxiety of female nurses was confirmed (P = 0.0001).

The results of this study are consistent with the studies [24, 27, 23] based on the effectiveness of therapeutic narratives on reduction of the financial anxiety. In explaining this finding of the present study, it can be said that the financial narrative therapy, as a new approach, is to solving the financial problems and the treatment process is so that it help oneself separate the financial problem from themselves, and take control of which, and solve its financial problems, that which plays an important role in controlling anxiety. In this study, adopting a friendly stance combined with a sense of acceptance, without blaming, and based on the mutual respect by therapists, played a significant role in creating a safe and trustworthy environment nd people were encouraged to pay attention to their financial lives in the form of stories and narrations and then describe the stories and problems of their past, which was full of defeat and the financial failure. When quoting these stories, people find the problem inside. On this basis, the prevailing feeling will be a feeling of frustration and disappointment. Using the animating technique, as part of thefinancial narrative therapy process, plays an important role in managing anxiety [6]. In this study, during the financial narrative therapy, people were trained to look at the problem as an alive and external entity, give it a nickname, and deconstruct their narration involving full of trouble. This process led to dominance over the financial anxiety, feeling of coping with oneself, and adoption of appropriate financial solutions for their assets, family members, and society. In fact, the financial narrative therapy is an approach that empowers the individual to look at the financial problem from a new angle and have a new interpretation of their finances [29].

The second hypothesis in this study was the financial narrative therapy on improving the financial wellbeing of female nurses. As the results of Table 8 show, there is a significant difference between the adjusted averages of participants' financial anxiety scores in terms of group membership (experimental group and control group) at the post-test level (F = 76/442). Therefore, the second hypothesis based on the effectiveness of financial narrative therapy on the financial wellbeing of female nurses at the level (P = 0.000) was confirmed. The results of this study were consistent with the study conducted in this field [23] on the effectiveness of the financial narrative therapy on the increase of the financial wellbeing. According to the findings of this study, the main pillar of the financial wellbeing is to have a sense of dominance over the financial life, and as one of the postmodern therapists, the financial narrative therapy contributes people to making such dominant role by creating a positive look to the past, and using an individual's capabilities to compile and illustrate the future financial life narration. In explaining of finding of the present study, one can say that one of the methods of improving the financial wellbeing of individuals is to recognize the successful experiences that a person has in the past in the financial situations, especially the unpleasant financial situations, and to create a positive look to the past [34]. With educating attitudes, cognitive skills, and short and medium-term plans, the financial narrative therapy help people gain control over their financial situation. Having a sense of dominance over the financial situation is highly emphasized in the financial narrative therapy, and it is the ultimate goal of most of financial interventions and treatments in the field of financial health [35].

In this study, by separating themselves from problems, participants searched their own backgrounds to find the unmatched and unique consequences - or brilliant and pure moments; moments in which they have dealt unlike the narrations fulling of the financial problem, and have had successful experiences. This played a significant role in creating a positive and different look to their financial narrations. According to Klontz et al., having a sense of control over the financial situation plays an important role in creating and improving the individual financial wellbeing. Part of the financial control is mental, which is the result of a person's mental review of his/her performance in the various financial situations [4]. The financial narrative therapy tries to create a sense of financial control in one person by extracting and pulling out the unmatched experiences of an individual in the unpleasant financial situations. In this study, it took a lot of time to discover the brilliant and pure events and occurrence of the customer's financial life by asking the key questions, and in return, people found the ability to discover the unmatched events and occurrences of their lives. In fact, the financial narrative therapy deals with a widespread and extensive view of the financial lives of consumers and the reconstruction of their perspective on their financial past [9].

The financial narrative therapy approach is not limited to minor modifications in solving one or more specific financial problems in life or in reducing financial anxiety, but it is expected to make a profound change in all aspects of the individual's financial life because it changes the general process of the financial life of a person and is focused on giving meaning to the individual's financial life events. With the help of this treatment, the feelings of autonomy and self-control on the personal life, and on the financial and economic issues were created in clients, which contributed to improvement of the financial wellbeing of participants. Regarding the results of this research, we can say that goal of the financial narrative therapy is paying attention to externalizing the financial problem, creating an external look at the financial problem from different angles and, consequently, creating a preferable narration for the narration of the financial life; Therefore, people with the help of the financial narrative therapy can find a new perspective on the facts, and thus reduce the unhealthy thoughts and attitudes towards their assets; As a result, financial anxiety and distress are reduced. Achieving the financial wellbeing is one of the ultimate goals of the financial interventions and education, whose effects are not only in the realm of personal life, but also in the individual's occupational life and it can also affect the individual productivity in the work environment [36].

Conclusion

This study, like other studies, has some limitations. The participants in this study were chosen from the female nurses. Therefore, cautious should be taken in generalizing the results for both genders. The attitude of friable subjects to the tests used their degree of cooperation with researchers, their honesty and their interest in applying educational methods and responding to test questions is a topic that is almost beyond the control of researchers and can affect the results of the research. This research was carried out in a small sample of female nurses of educational hospitals in Isfahan; this also reduces the amount of external validity of the research.

Acknowledgments

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Conflict of Interest: There is no conflict of interest between the authors.

Ethical Clearance: Researchers have adhered to the basic principles of the Helsinki Declaration in conducting research.

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Case Report on "Budd Chiari Syndrome"

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Abstract

Background: Hepatic Vein Thrombosisis also known as Budd Chiari Syndrome is a sparse disorder marked by obstruction and narrowing of the hepatic veins i.e., veins of the liver. 70% casers of Budd Chiari Syndrome are unknown. Approximately 10% have polycythemia vera which is a type of blood cancer. Clinical manifestations can be developed due to the blockage of the major veins which carries blood from the liver to the heart. Features may include hepatomegaly (an abnormally enlarged liver), pain in the upper right part of the abdomen, yellowish color of the skin and sclera (whites of the eyes) jaundice i.e., due to jaundice, ascites i.e., accumulation of fluid in the peritoneal cavity between the two layers of membrane that lines the stomach. Portal hypertension (due to increase in pressure of the portal veins) etc. Diagnosis of hepatic vein thrombosis is made based upon a detailed diagnostic findings, a thorough history of patient, and a variety of specialized tests. Management of hepatic vein thrombosis includes corticosteroid drug. anticoagulants, angioplasty, etc. **Patient history:** The patient is a female, 46 years old who was admitted in A.V.B.R.H. on 29/11/19 with chief complaints of tenderness in abdomen on palpation, fever, rigor and pain. **Past history:** Patient is a known case of hypertension and cerebrovascular accident. **Clinical findings:** The patient has undergone venography, Complete blood count, etc. **Pharmacology:** The patient was treated with medications such as, tab. Augmentin 625 mg, tab. Pantop 40 mg, tab. Ultracet 50 mg, tab. Mucomix 625 mg. **Nursing management:** Administered IV fluids as advised by physician. Administered medications as prescribed by physician. Monitored vital signs. **Conclusion:** The 46 years old female was admitted to AVBRH in Cathlab ICU with chief complaints of pain in abdomen, fever, rigor etc. after undergoing investigations she was diagnosed as Budd Chiari Syndrome.

Keywords: *Budd Chiari Syndrome, Hepatomegaly, Ascites, Cirrhosis of Liver.*

Introduction

Hepatic venous thrombosis or Budd Chiari Syndrome (BCS) is a very infrequent condition, which affects one in a million. BCS or hepatic venous thrombosis is a condition which occurs due to the occlusion of the hepatic veins which drains the liver. It is formally introduced with the classical triad of ascites (fluid collection in the peritoneal cavity), pain in abdomen and enlargement of the liver.(1) The causes of Budd Chiari syndrome is classified as: 1. Primary cause of Budd Chiari syndrome (which contributes to 75 %) includes, Thrombus of the veins in liver. It may be associated with following disorders: Polycythemia vera, Pregnancy, Postpartum state, Use of oral contraceptives, Paroxysymal nocturnal hemoglobinuria, Hepato cellular carcinoma, Lupus anticoagulants. 2. Secondary cause of Budd Chiari Syndrome (which contributes to 25 %) includes Compression of the hepatic vein due to an abnormal growth (e.g.: tumor).(2),(3) The blood clot formation within the veins of liver may lead to occurrence of Budd Chiari Syndrome. This ailment can be sudden in onset and severe, acute or chronic in phase, or even asymptomatic. Sub acute penetration is the typical form mostly seen. Budd Chiari Syndrome also known as Hepatic venous thrombosis is secondary to cancer.(4)

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Incidence rate

It is a rare case. About 1 case per million population is reported in the incidence.[3]

Objectives:

1. To gain more knowledge in depth regarding Budd Chiari syndrome.

2. To study medical, surgical and nursing management of Budd Chiari syndrome.

Patient information

The patient was 46 years old. Her marital status is married, her education is 10th class and occupation is house wife. Her family income is 25,000/- month. She was admitted in A.V.B.R.H. in Cathlab ICU on 29/11/19.she was diagnosed as Budd Chiari Syndrome after undergoing investigations such as venography.

Patient had chief complaint of tenderness in abdomen along with fever, rigor and pain since 1 month.

Medical history: Patient is a known case of hypertension since 7 years for which she was taking medication Tab. Metaprolol 25 mg, patient also had an episode of cerebrovascular accident 7 years back and is taking medication Tab. Ecosprin AV 75 mg. it is doubted that the patient may be a case of cirrhosis of liver which is leading to Budd Chiari Syndrome.

Family history: There is no hereditary or genetic disorders in family.

Psychosocial history: Patient maintains good interpersonal relationship with family, friends and relatives.

Causes of BCS includes the following:

· The primary etiology of Budd Chiari syndrome was present in patient which results in approximately (75%) of blood clot of the veins of the liver. Blood clot of the hepatic vein is connected with the following conditions:

1. Polycythemia vera (type of a blood cancer, which makes the bone marrow to form red blood cells in excess)

2. Hepatocellular carcinoma (carcinoma of liver that begins with hepatocytes)

3. Pregnancy

4. Postpartum state

5. Oral contraceptives use

6. Paroxysmal nocturnal hemoglobinuria

7. Lupus anticoagulants

Clinical findings

Patient had tenderness in abdomen on palpation, yellowish discoloration of skin and whites of eyes. Hepatomegaly, spleenomegaly, ascites, body temperature was 100◦ F no other abnormalities was found on examination.

Diagnostic assessment

In kidney function test, Sr. Urea was increased that was 49 mg/dl than the normal range i.e. 7-17mg/dl.

CECT Abdomen: Atrophic and heterogenous liver with gross caudate lobe hypertrophy significant of cirrhosis of liver with non visualization of right and middle hepatic vein? Budd Chiari Syndrome. Spleenomegaly with multiple venous collaterals. In case, the results of liver function tests are abnormal, an imaging studies, typically Doppler ultrasonography is done. If results are unclear magnetic resonance imaging (MRI) of the blood vessels or Computed Tomography scan is done.

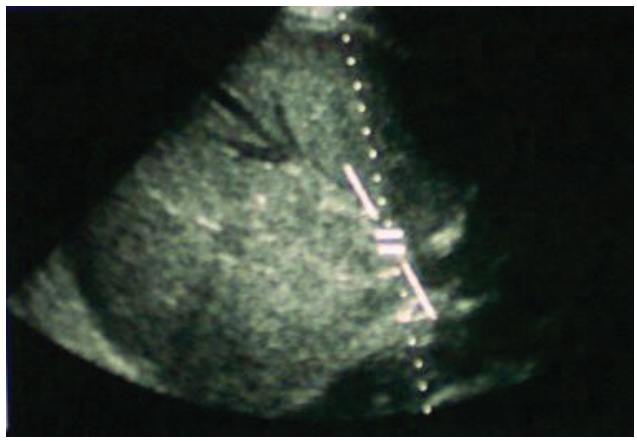

Fig1: Sonography showing hepatic vein thrombus.

Diagnosis: Diagnosis is made most commonly using retrograde angiography and ultrasound studies

of abdomen. Ultrasound studies may show obliteration of veins of the liver, blood clots or stenosis (narrowing of vessels), spider nevus, large abnormal vessels connecting aorta with pulmonary arteries, or a biliary atresia replacing a normal vein.

Therapeutic interventions

Collaborative management: A few numbers of patients can be treated pharmacologically with sodium restricted diet.

Pharmacologic management includes diuretics to control ascites, anticoagulants such as heparin and warfarin may also be prescribed depending on the condition of patient. Tab. Augmentin 625 mg (TDS, orally), Tab. Pantop 40mg (BD, orally), Tab. Ultracet 50mg (TDS, orally), Tab. Mucomix 600mg (orally) was prescribed for the patient.

Surgical management includes Transjugular Intrahepatic Portosystemic Shubt (TIPS) in milder cases which can divert the flow of blood around the obstruction of the liver. Liver transplantation in case of fulminant liver failure. Angioplasty (Baloon Cavoplasty to benefit from obstruction) was planned for this patient.

Nursing Management

1. Administer IV fluids as per doctors order
2. Administer medications as per doctors order
3. Maintain I/O chart
4. Monitor vital signs especially blood pressure as portal hypertension is also a major cause of Budd Chiari syndrome.
5. Providing patient and relatives with psychological support.
6. Encouraging patient to express emotions.
7. Establishing good interpersonal relationship.
8. Teaching coping strategies to the patient and relatives.
9. Maintaining confidentiality as it will relive the anxiety of the patient and relatives. It will help in avoiding the conflict with physicians, nurse and other health care providers.
10. Explain about surgical procedure to parents.
11. Explain about the prognosis of disease.

Follow up and outcomes: Patient was planned for follow up regularly on basis of advice given by physician.

Limitations: As this is a rare case conducting research studies on causes and treatment is difficult as the number of participants may be very less or rare.

Strength: This case is helpful to provide knowledge about thrombosis of hepatic vein especially in patients suffering from cirrhosis of liver.

Prognosis: The prognosis in patients suffering from BCS is very poor who especially in those who remains uncured without proper management, with death caused by progressive liver failure between the time duration of 3 months to 3 years since the time of the diagnosis.[4]

Discussion

The BCS also known as thrombus of veins of liver is a congestive hepatopathy which is caused due to blockage of the veins of liver. This condition is very sparse and is seen especially among the general population within 1/100,000. Hypercoagulable condition can be found in 75 percent of the patients; more than one causative factor may play a major role in 25 percent of the patients. [5]

The diagnosis is usually made using radio imaging techniques such as, (Computed tomography, or magnetic resonance imaging or Doppler ultrasound). Hepatic biopsy can be performed if Budd Chiari syndrome is suspected in small vessels. Management includes use of anticoagulation, the treatment of known risk factors for prothrombotic, percutaneous revascularization and transjugular intrahepatic portosystemic stent shunt to restore hepatic venous drainage, and liver transplantation in patients who does not respond. [6],[7]

The severity of the condition differs from case to case, depending upon the veins infected and their site. In few cases, if the major veins in liver are involved, high blood pressure may be present in the veins which carries blood from the gastrointestinal tract to the heart through the liver which is known as portal hypertension.

[8],[9] BCS produces mild to fatal liver damage; which differs from person to person.[10]

Informed consent: The patient and relatives were informed before taking the case.

Conclusion

46 years old female was admitted to AVBRH in Cathlab ICU with chief complaints of pain in abdomen, fever, rigor etc. after undergoing investigations she was diagnosed as Budd Chiari Syndrome. Budd Chiari Syndrome also known as Thrombus of the Hepatic Vein is a rare disorder characterized by narrowing and obstruction of the veins of the liver (hepatic veins).

Ethical Clearance: Author herself is a registered nurse and midwife (Completed Basic BSc Nursing and registered in Maharashtra Nursing hence professionally allowed to provide nursing care. During the authors clinical posting this client was allotted to her for providing nursing care.

Source of Funding: Self.

Conflict of Interest: NIL

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Effect of Chicken Egg Shell Powder and Gallic Acid as Remineralising Agent on Early Enamel Carious Lesion- An In Vitro Study

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Abstract

Background: The aim of the study was to compare the effect of chicken eggshell powder and gallic acid as remineralising agent on early enamel caries lesion.

Material and Methods: Ten extracted deciduous molars was cleaned using distilled water. The teeth were decoronated at CEJ. Each decoronated tooth then sectioned longitudinally in a mesio-distal direction first and then in a bucco-lingual direction with diamond disc so that four samples obtained from a single tooth. Then from these samples, four enamel blocks of dimensions (4mm long, 4mm width and 2mm thick) made from each sample and embedded in acrylic blocks. These 40 samples are divided into four groups, each having 10 samples (GROUP A,B,C,D). Samples were demineralised followed by application of chicken egg shell solution and gallic acid. Chicken egg shell has the highest content of Calcium present as compared to Gallic acid.The ratio of Calcium/Phosphorous was found to be increased in the following sequence. Chicken egg shell>Gallic acid>Control>Demineralised group.

Conclusion: It can be concluded from the results shows that Calcium has a vital role in remineralisation.

Keywords*: Caries, Demineralisation, Remineralisation, Calcium*

Introduction

Dental caries is defined as microbial disease of the calcified tissue of the teeth characterized by the demineralization of the inorganic portion and distruction of the organic portion of the tooth.[1]Occurrence of white spot lesions is caused by colonization of pathogenic bacteria and their bonding in form of a biofilm to the enamel surface. During their metabolic activities the bacteria create surplus of organic acids and lower pH, while the biofilm prevents the effect of buffering mechanisms from saliva. Lowered pH dissolves the surface layers of calcium and phosphorous.[2]

Early diagnostic of a white spot lesion is extremely important because if the treatment is initiated on time, remineralization is encouraged and the cracking of the surface layer and the development of cavities are avoided.[3] At physiological conditions, the oral fluids (saliva,biofilm fluid) have calcium (Ca) and phosphate (Pi) in supersaturated concentrations with respect to the mineral composition of enamel and as a result, these ions are continually deposited on the enamel surface or are re-deposited in enamel areas where they were lost. In this study we have used gallic acid and chicken egg shell powder as remineralising agent. Gallic acid (3,4,5-trihydroxybenzoic acid, GA), a common naturally occurring polyphenol, is widely available in various plants and foods and it possesses a variety of biological properties, such as antioxidant, anti-mutagenic and anti-carcinogenic effects.[4]GA is stable at high pH and how

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potential chemical changes of GA will impact on its anti-caries effect.[5]Similarly, the use of CESP in various fields has been studied.

Chicken egg is a known source of Ca containing about 39%(w/w) of elemental Ca. Chicken Egg Shell contains about 95% of calcium carbonate which on conversion to basic calcium oxide due to calcination is responsible for an increase in alkalinity.[6] Eggshells contain the perfect amount of the ideal substances for healing cavities massive amount of calcium, protein and 27 other minerals which resembles the composition of our teeth. CESP reduces pain and osteoresorption and also increases bone mineral density .[7] Demineralized tooth surfaces have been studied widely by using technique sensitive instruments such as scanning electron microscope. Therefore this study was conducted with the objective to compare the effect of Chicken egg shell powder and Gallic acid as remineralising agent on early enamel carious lesion on the extracted deciduous molar tooth.

Material and Method

The present invitro study was conducted in the Department of Pedodontics and Preventive Dentistry, KD Dental College & Hospital, Mathura from November 2016 to March 2018. Ten extracted deciduous molars used for the study and cleaned with distilled water. The teeth were decoronated at CEJ. Each decoronated tooth then sectioned longitudinally in a mesio-distal direction first and then in a bucco-lingual direction with diamond disc so that four samples obtained from a single tooth. Then from these samples, four enamel blocks of dimensions (4mm long, 4mm width and 2mm thick) made from each sample and embedded in acrylic blocks and like this 40 samples are prepared embedded in acrylic blocks. These 40 samples are divided into four groups, each having 10 samples (GROUP A,B,C,D; Figure 1).

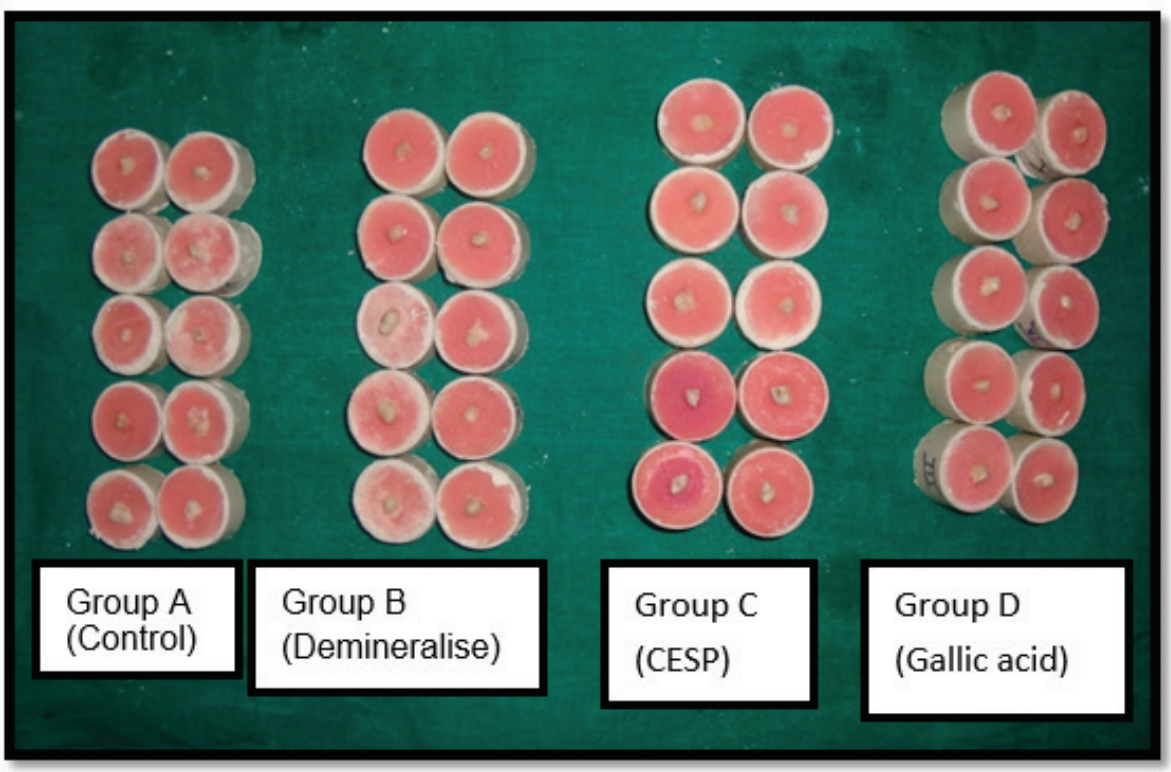

Figure 1: Cross sectioned tooth is embedded in acrylic blocks

Production of Egg Shell Powder: Twenty fresh egg shell was taken and cleaned it with distilled water and dried it. The eggshells then kept in hot water bath at 100 degree Celsius for 10 min followed by removing the inner membrane. The egg shells were then crushed using the sterile motar and pestle. The crushed particles then heated at 1200 degree Celsius using muffle furnace (G L Bajaj Institute and Technology Mathura) and powdered to small particles (Figure 2). Egg shell powder weight to one gram. This one gram of CESP was dissolved in 20 ml of 4% acetic acid in a test tube the clear fluid which is collected at the top was then transferred to a beaker and the ph of the solution was tested using a ph meter which was 11.7.

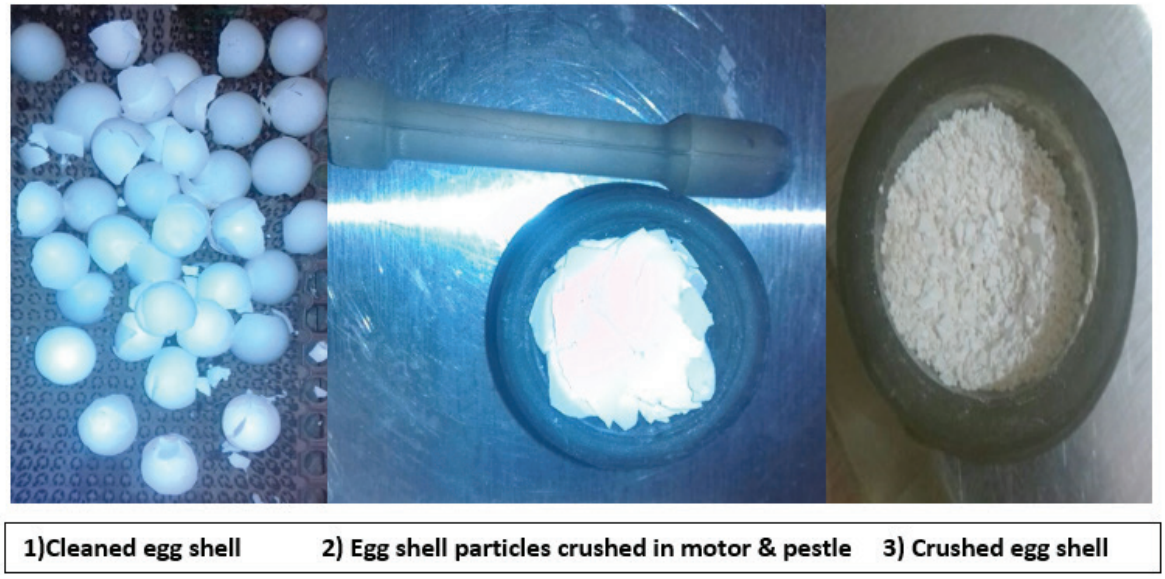

Figure 2: Crushing of egg shells

DEMINERALISING SOLUTION:

Demineralising solution was made by including (CaCl2, NaH2PO4, Lactic acid and Fluoride).

1) Fluoride -.002 gm of sodium fluoride in 100ml d/w.

2) CaCl2 (10%) - 10 gm 0f calcium chloride in 100 ml d/w.

2.2 ml in 1 litre d/w-2.2Mm

3) NaH2PO4 - Sodium Hydrogen Phosphate(10%)

0.3 ml in 100 ml of d/w-2.2Mm

4) Lactic acid - (0.05 mole)

0.45ml lactic acid in 100 ml d/w.

DEMINERALISING PROTOCOL:

Carious lesions representing preliminary stage of subsurface enamel lesion were created by placing the tooth samples in 20ml of demineralization bath for 72 h (CaCl2 = 2.2 Mm NaH2PO4 = 2.2 Mm Lactic acid = 0.05 M, Fluoride = 0.2 ppm, solution is adjusted with 50% NaOH to a pH of 4.5). The specimens kept in the demineralization solution (CaCl2, NaH2PO4, Lactic acid and Fluoride) for 72 h at 37° C created a subsurface demineralization (Figure 3).

Study groups: (n=40 enamel blocks)

Group A (n=10)- untreated group

Group B (n=10) - subsurface demineralization only.

Group C(n=10)–subsurface demineralization followed by suspending the tooth samples in Chicken Eggshell Solution for 21 hours for seven consecutive

days for remineralization.

Group D(n=10) -subsurface demineralization followed by application Gallic acid for 21 hours seven consecutive days for remineralization.

GROUP B GROUP CGROUP D

Figure 3: SUB SURFACE DEMINERALISATION OF THE SAMPLES FOR 72 Hrs

Chemical Analysis: Total Metals (Calcium, Phosphorus, Potassium, Magnesium)

1. Collect and weigh dentin chips in Microwave Digestion vessels.

2. Add 10 ml HNO3 (65 %) & 15 ml DI water and close the vessels.

3. Digest sample in microwave digestion at 220 PSI and 2000C for 30 min. After completion of digestion cool and mark to the volume.

4. Plot ICP Calibrate curve for the desired elements with CRM Standards and run dentin digest sample. Collect the results and quantify the elemental concentration percentage by weigh.

5. Metals were determined by inductively coupled plasma spectroscopy (ICP).

Major Equipment's / Apparatus Used:

1. Microwave Digestion (Make- CEM Mars 5)

2. Inductively coupled plasma spectroscopy (Make- Varian MPX Vista CCD Simultaneous ICP-OES).

Statistical analysis: Data were computerized and analysed using SPSS 24.0 software. The results were analysed using one way ANOVA and Tukey – Kramer multiple comparison test.

Result and Discussion

The mean value for Phosphorous content in Group A is 15.89, Group B is 8.55, Group C is 16.07, Group D is 32.6. The Phosphorous content in Group A (Control), B (Demineralised), C(CESP), D (Gallic acid) have significant (p) value 0.176 which is more than 0.05 therefore it is statistically not significant (Table 1)**.**

Table 1: Comparison of Phosphate ratio among all the Groups

Group	Number	Mean	SD	Anova test	p value
Group A (Control)	10	15.89	.21	1.739	.176
Group B (Demineralisation)	10	8.55	.14		
Group C (CESP)	10	16.07	.21		
Group D (Gallic acid)	10	32.68	48.95		
Total	40	18.29	25.16		

Table 2 shows increase in Calcium level is more in Group C(CESP)>D (Gallic acid)>A (Control)>B (Demineralised) and the significant (p) value is < .05 which is statistically significant.

Table 2: Comparison of Calcium ratio among all the groups

Group	Number	Mean	SD	Anova Test	p value
Group A (Control)	10	36.820	.2658	2.157E4	<.01*
Group B (Demineralisation)	10	17.050	.2014		
Group C (CESP)	10	37.760	.1955		
Group D (gallic acid)	10	35.000	.1700		
Total	40	31.658	8.6024		

*: statistically significant

Table 3 shows the intragroup comparison of mean values of calcium content by applying TukeyHsd Test. The statistical significant difference is 0.00 which is less than p<.05. The test is applicable for the comparition of Calcium content when intragroup comparison is done among all Groups. Calcium content is greater in Chicken egg shell as compared to Gallic acid , Control and Demineralised Group. Therefore CESP has more effect as a remineralising agent then Gallic acid.

Table 3: Multiple comparison test for Calcium (using Post Hoc test)

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ca(Calcium)	A (Control)	B	19.77*	.000*	19.516	20.024
		C	-.94*	.000*	-1.194	-.686
		D	1.82*	.000*	1.566	2.074
	B (Demineralisation)	A	-19.77*	.000*	-20.024	-19.516
		C	-20.71*	.000*	-20.964	-20.456
		D	-17.95*	.000	-18.204	-17.696
	C (CESP)	A	.94*	.000*	.686	1.194
		B	20.71*	.000*	20.456	20.964
		D	2.76*	.000*	2.506	3.014
	D (Gallic acid)	A	-1.82*	.000*	-2.074	-1.566
		B	17.95*	.000*	17.696	18.204
		C	-2.76*	.000*	-3.014	-2.506
*: statistically significant						

Table 4 shows the intragroup comparison of mean values of phosphorous content by applying TukeyHsd test. The statistically significant difference is more than 0.05.

Table 4- Multiple comparison test for Phosphorus (using Post Hoc test)

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
P (Phosphorous)	A (Control)	B	7.34	.908	-22.14	36.82
		C	-.18	1.000	-29.66	29.30
		D	-16.79	.429	-46.27	12.69
	B (Demineralisation)	A	-7.34	.908	-36.82	22.14
		C	-7.52	.901	-37.01	21.96
		D	-24.13	.141	-53.61	5.35
	C (CESP)	A	.18	1.000	-29.30	29.66
		B	7.52	.901	-21.96	37.01
		D	-16.61	.438	-46.09	12.87
	D (Gallic acid)	A	16.79	.429	-12.69	46.27
		B	24.13	.141	-5.35	53.61
		C	16.61	.438	-12.87	46.09

In this study we have compared the calcium and phosphorous percentage by mass in the tooth ingredient with chicken egg shell[7] and gallic acid[8] by ICP (Inductively Coupled Plasma Spectroscopy). According to our study CESP has 37.7 % by mass and the result is statistically significant with p value of <0.01 by post hoc method. The same method ICP was used by BrunaGressler(2015) and the Ca concentration reached 37% egg shell powder at an average of all analyzed samples (365,7mg g).[9] This content was similar to concentrations found by Schaafsma et al[10] (2000), on average 390 mg g^{-1}eggshell powder devoid of membranes, as analyzed in this study.[10]According to BenjoyMony (2015)[6]Ca % by mass was 37.67 which is approximately similar to our result. In this study atomic analysis by EDX(energy dispersive X-ray spectrometry) was done which shows highest Ca concentration of 98%. According to SandleenFeroz (2017)[11]the protective effects of chicken egg shell powder solution on enamel was compared with samples treated with demineralizing solution and the results showed a statistically significant difference (0.158±0.012) with a p value of 0.00 indicating the effectiveness of remineralizing effect of CESP on enamel surfaces. The statistical results were similar with our results. X- Ray fluorescence spectroscopic analysis of CESP revealed that it contains about 98% Calcium, 0.46% of phosphate, 0.53% of Magnesium, 0.18% of Strontium, 0.03% of Potassium .This high concentration of bio available Ca plays a vital role in enamel remineralization when CESP applied topically. Availability of calcium and phosphate ions is essential for remineralization to occur and increased pH of the solution along with rich bioavailability of phosphate and calcium ions is mainly involved for the process of remineralization.[6]

We proposed a hypothesis that GA might regulate the demineralization/remineralization by influence the growth of HAP crystals. Chemical composition analysis was done by X-ray spectrometer and SEM (scanning electron microscope) which allowed the relative amounts of calcium (Ca) and phosphorus (P) to be determined. The Ca/P ratios were also calculated. The results showed that in all the groups, the main elements of the precipitate were calcium and phosphorus, and the Ca/P ratios were close to 1.67 which is consistent with the Ca/P ratio in HAP**(Bei tang)[12].** The results we had observed Ca/P ratio by ICP method was approx 2.01 which was not similar to this study.It proposed that G. chinensis has anti-bacterial properties to restrain the growth and acid production of certain cariogenic bacteria, including streptococcus and lactobacillus. In particular, it reportedly has the ability to inhibit enamel demineralization in vitro. In our study also calcium and phosphorous levels are more thendemineralised group but less then chicken egg shell which shows that it helps in inhibiting demineralization and act as a good antioxidant.

Within the limitations of this study, it is concluded that the chicken egg shell solution along with the rich bioavailable calcium content has the potential to favouredremineralisation. Even though CESP showed more remineralization than gallic acid, the latter due to its easy bioavailability and natural source of calcium and phosphate can be the future in remineralisingenamel carious lesions. Further, clinical studies regarding suitable vehicle for CESP is needed as this might increase the remineralization potential of CESP comparable to the commercially available agents.

Conclusion

It can be concluded from the results that calcium has a vital role in remineralisation. Chicken egg shell has the highest content of Calcium present as compared to Gallic acid, Demineralised and Control group when the samples were tested by ICP (Inductively Couples Plasma Spectroscopy) method.For remineralization to occur, bioavailable calcium and phosphates are essential. Therefore, the rich bioavailability of calcium along with the high concentration of phosphates present in CESP solution coupled with its increased pH may be responsible for remineralization. Further, clinical studies regarding suitable vehicle for CESP is needed as this might increase the remineralization potential of CESP comparable to the commercially available agents.

Conflict of Interest: Nil

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A Study of Acute Renal Failure in the Users of Proton Pump Inhibitors

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Abstract

Background: Proton pump inhibitors are one of the most common classes of medications that act by inhibiting gastric H+/K+ adenosine triphosphatase via covalent binding to the cysteine residues of the proton pump, they provide the most potent acid suppression available. Lansoprazole, pantoprazole, and dexlansoprazole have the greatest bioavailability and achieve the highest plasma levels. Rabeprazole is the most acid-labile PPI and therefore the most potent, whereas pantoprazole is the least reactive and therefore the least potent ***Patients and methods:*** Research data were collected from Al-zahraa teaching hospital/ Wasit province and Morgan teaching hospital/ Babylon province, they include patients with acute renal failure who used/ using proton pump inhibitors compared with those do not use proton pump inhibitors to know the probable relation between acute renal failure and the use of proton pump inhibitors. ***Results and discussion:*** Results showed that only 30 patients of 120 patients with acute renal failure consult the hospital were PPIs users, this may refer to presence of a relationship between proton pump inhibitor and acute Renal failure . Previous studies in this field referred to a similar relationship, a case reports and a recent case series implicating these drugs in acute interstitial nephritis (AIN) and progression to acute renal failure.

Keywords: *Renal failure, acute, Proton pump inhibitors users*

Introduction

Proton pump inhibitors (PPIs) are one of the most commonly prescribed classes of medications in the United States. By inhibiting gastric H+/K+ adenosine triphosphatase via covalent binding to the cysteine residues of the proton pump, they provide the most potent acid suppression available [1, 2]. Although PPIs are generally considered safe, numerous adverse effects, particularly associated with long-term use have been reported. Many patients receiving chronic PPI therapy do not have clear indications for their use, prompting consideration for reduction or discontinuation of their use [3,4,5,6,7].

PPIs are a class of medication that act on the H+/K+ pump along the basolateral membrane of the parietal cell. They accumulate and activate in an acid environment at the secretory canalicular surface of the parietal cell. Here, they bind irreversibly to H^+/K^+ adenosine triphosphatase, inhibiting acid production of the bound parietal cell in approximately 70% of active pumps [8,9]. Protonation forms irreversible disulfide bonds with cysteine residues in the proton pump, two of which are most important, CYS813 and CYS822. The need to achieve acid exposure in the parietal cell but not the stomach is why PPIs should be taken 20 minutes before eating breakfast [10]. PPIs differ in their routes of excretion, peak plasma levels, and half-lives. They have short half-lives, typically1 hour, but may last up to 24 hours because of the necessity of newpump synthesis for acid secretion. All PPIs are eliminated via hepatic P-450 CYP2C19, with CYP3A4 also playing a role [11,12,13].

Lansoprazole, pantoprazole, and dexlansoprazole have the greatest bioavailability and achieve the highest plasma levels. Rabeprazole is the most acid-labile PPI and therefore the most potent, whereas pantoprazole is the least reactive and therefore the least potent [11,14].

Several case reports have implicated PPIs as a cause of acute interstitial nephritis. This disorder is a humoral

and cell-mediated hypersensitivity inflammatory reaction of the renal interstitium and tubules. A systematic review from 2007 found 64 cases documented in the literature, 12 of which were considered certainly associated, and 9 of which were probably associated [15]. Although well-tolerated, there have been case reports and a recent case series implicating these drugs in acute interstitial nephritis (AIN) and progression to acute renal failure (ARF) [16].

Patients and Methods

Collection of the research data was start from 15/10 /2019) to (13 /1 /2020) from Al-zahraa teaching hospital/ Wasit province and Morgan teaching hospital/ Babylon province.

Research cases include patients with acute renal failure who used/ using proton pump inhibitors compared with those do not use proton pump inhibitors to know the probable relation between acute renal failure and the use of proton pump inhibitors.

Results and Discussion

Throw out our working we found that only 30 patients of 120 patients with acute renal failure consult the hospital were PPIs users, this may refer to presence of a relationship between proton pump inhibitor and acute Renal failure .

Previous study in this field referred to a similar relationship. PPIs are a widely prescribed class of drugs, and their usage worldwide is increasing. Although well-tolerated, there have been case reports and a recent case series implicating these drugs in AIN and progression to ARF [16] .

PPI-induced AIN is an idiosyncratic reaction; hence it is very difficult to stipulate which patients might be at risk of developing the condition. From our case series the majority of the affected patients were elderly (median, 74 years), and it might well be these patients are at a higher risk, but why remains unknown. The exact pathogenesis of PPI-induced AIN remains to be elucidated; however, it is thought to be triggered by a hypersensitivity immune reaction to the drug or one of its metabolites. One hypothesis suggests metabolites of these drugs act as haptens (mimicking renal antigens), planted antigens, or deposit as circulating immune complexes. The presence of both CD4 and CD8 T lymphocytes in the inflammatory infiltrate suggests T-cell–mediated hypersensitivity reactions and cytotoxic T-cell injury mechanisms are involved [17].

Findings such as fever, rash, arthralgias, and eosinophilia observed in the classic methicillin-induced AIN provide evidence for a hypersensitivity phenomenon. Although T cells are implicated in the pathogenesis, there is also evidence in the literature from experimental animal models to implicate a role for the humoral immune system [18].

The remaining patients used proton pump inhibitor may not have acute renal failure , PPI may cause a kidney diseases ,but the disease may stop at specific stage and didn't progress to acute renal failure, also patients may respond to the medication that used for the treatment of the disease.

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Ethical Approve : We declare that the study does not need ethical approval.

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Evidentiary Value of Forensic Report in Criminal Liability in Jordanian Law

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Abstract

Forensic medicine is an important part of criminal justice, which the judiciary resort to, with the purpose of deciding on a number of cases. One of the most important of these cases, in which forensic medicine is the basis for determining criminal responsibility or not, is the case of allegation or suspicion of death by murder or unknown reasons that lead to suspicion of murder. This research will address the evidentiary value of the forensic physician's report, as it is a means of proof or a means of assessing evidence or an auxiliary procedure for the judge. It will also address the authority of the criminal judge in assessing the forensic report and the position of the Jordanian Court of Cassation on that. A number of findings and recommendations emerged, the most important of which is that the Jordanian legislator has set up the reports and medical committees system No. 13 of 2014 and its amendments. This system deals with many medical committees, including the forensic committee. The Jordan legislator has also defined the system of expertise before regular courts for 2018 and its amendments, according to Article 11 / c which states that *a forensic physician shall be appointed either from forensic physicians appointed in the Ministry of Health, the Royal Medical Services, official university hospitals, or from those retired among them*. I wish the Jordanian legislator will set up a legal system for forensic physicians, which shall be independent of the medical reports and committees system No. 13 of 2014 and its amendments and the expertise system before regular courts of 2018 and its amendments. The proposed system is the Judicial Committee system for forensic physicians, and the reason for this is because the experience of forensic physicians is often the source of the basis for the judge's ruling, especially in cases of murder for unknown and suspicious reasons.

Keywords: *Forensic sciences, Criminal liability, Justice, Law study.*

Introduction

Forensic medicine is a branch of medicine and it helps in many judicial issues in which the judge cannot decide on. The forensic report controls the fate of many people in different cases, the most important of which are the cases of murder for unknown or suspicious reasons, through the acquittal or conviction of the defendant.

Research problem:

Clarifying the evidentiary value of forensic report at the competent court, because the forensic physician is the one the judge relies on, as the report submitted by the forensic physician exempts the judge from feeling guilty when issuing his verdict, whether this ruling is acquittal or conviction , as the cases under consideration, such as murder cases, are dangerous and they are among the most serious crimes that occur in society and their penalties impose the death penalty against the accused who is proven by the forensic report that he/ she committed this crime. Therefore, it is necessary to shed more light on this topic.

Research importance

The forensic report has importance for the victim whose heirs seek justice and retaliation against the perpetrator. This report is important for the defendant to prove his/ her innocence, as well as important for the judiciary that aims to achieve justice without bias for one party at the expense of the other.

Research questions

1- What is the evidentiary value of the forensic

report?

2- Is the forensic report considered a means of proof, a means of assessing evidence, or an auxiliary procedure for the judge?

3- What is the authority of the criminal judge in assessing the forensic report?

4- What is the position of the Jordanian Court of Cassation on the evidentiary value of the forensic report?

Research methodology

This is an analytical and descriptive study of the legal research and studies related to forensic medicine, the position of the Jordanian Penal Trials Code, and the Jordanian regulations and instructions related thereto.

Research plan

First section: What is forensic medicine?

Second section: The evidentiary value of the forensic report

First section:

What is forensic medicine?

Forensic medicine has an important role in the legal conditioning of facts. The basic rules in judgments are based on certainty, not on doubt and guesswork, because doubt is interpreted in favor of the accused, that's why the concept of forensic medicine and the role of forensic medicine in uncovering the crime shall be clarified.

First Topic:

What is forensic medicine?

Medicine is an autonomous science. However, and with the addition of the word 'forensic' thereto, it has become a common science with more legal nature. Therefore, forensic medicine consists of two words: 'medicine' which means medical science that is taught in medical schools, and which is known as pathology and the science of characteristics of the human body. In a more precise sense, it is the science of studying diseases and how to cure them[1,2]

Forensic and Legal Medicine is defined as: Forensic medicine is a branch of medicine that is concerned with uncovering issues of interest to the judiciary, such as examining the dead body to determine the changes that occur to it after death and the causes underlying their occurrence in addition to determining how they occurred, the date of their occurrence, the tool causing these changes, time that has elapsed since death till the date of uncovering the dead body and whether death was accidental, criminal, or suicide[3,4].

Autopsy is adopted in the following cases[5]:

1- Cases of the deceased in a criminal accident, whether the crime was intentional or unintentional, unless it is possible in the latest to give a definitive decision of death due to mere apparent examination.

2- Cases in which unidentified or identified dead bodies are found, unless the investigation indicates facts of a criminal nature of death and is confirmed by the apparent examination.

3- Cases of the deceased by burning, unless it is proven by the investigation that the accident was a suicide or a natural death, and the apparent examination of the dead body did not indicate facts of a criminal nature of death.

4- All cases in which the investigation or examination of the dead body indicates facts of a criminal nature of death, as well as every case the public prosecutor deems from their circumstances the necessity of an autopsy to find out the cause of death or to indicate any other matter, even if the forensic physician decides that an autopsy is not required.

Therefore, forensic medicine is arguably: a branch of accredited medicine that specializes in the application of medical sciences, and it helps in many judicial issues in which the judge cannot decide on.

Likewise, the forensic physician cannot, through his report, exceed what he was required to do, otherwise it is considered an interference in the judicial affairs. Therefore, the role of the forensic physician is a detector and not an originator.

Second Topic:

The Role of Forensic Medicine in Uncovering the Crime

Technical or scientific issues are submitted to the judge which he may not be aware of, especially with the scientific and technical development since the judge is not required to be familiar with all details of each science and art, but he has to have a broad awareness and strike a balance between matters. Accordingly, the Jordanian legislator that according to the Jordanian Criminal Procedures Law No. 9 of 1961, amended by Law No. 32 of 2017, in the following stipulations:

Article No. 39, stipulating: 1- If revealing the criminal act or its conditions is based on knowing some technical or professional issues, the public prosecutor shall take with him/her to the crime scene one or more of such experts.

2- The court may take urgent procedures to ascertain the health condition of the injured or the defendant or verify the validation of the medical reports submitted in the lawsuit, without summoning the litigants.

Article No. 40, stipulating: If someone is dead due to murder or for unknown reasons which leads to suspicions, in such case the public prosecutor shall use the assistant of one or more physicians in order to draft a report concerning the causes of death and the status of the diseased corps.

Article No. 41 stipulates: *1- Physicians and experts stipulated in articles (39 and 40) of this law shall take an oath before they start their duties which states that they will carry on the mission with honesty and dignity;*

2- The public prosecutor shall define a date for the expert in order to submit the his/her written report. If the expert did not submit such report at the defined date, the public prosecutor might decide to retrieve the all or part of the fees paid to the expert and to replace him/her with another one.

The system of expertise before regular courts of 2018 and its amendments, according to Article 11/c, has defined experts of forensic physicians as follows: If the expertise is related to forensic medicine, the courts are obligated to appoint the expert either from forensic physicians appointed in the Ministry of Health, the Royal Medical Services, official university hospitals, or from those retired among them.

Consequently, the Jordanian legislator clarified that forensic physicians are assigned upon a request from the public prosecutor and the previous articles did not refer to the judiciary. However, the response to that is that the public prosecutor is a judge and that the criminal case is initiated by the public prosecutor starting through the investigation, then assigning the criminal offense and then referring it to the competent court. The law is that which gives the authority to appoint a forensic physician or more if the circumstances of the case needed that, as well as the power to submit the report of the expert (forensic physician), accepting or rejecting it, and to request another expert report, as the judge is the supreme expert.

Second section:

Evidentiary value of the forensic report

Forensic medicine is an important part of achieving justice. Forensic medicine progress helps in easy detection of crime and its perpetrator since criminal judgments are based on certainty not suspicion and speculation, so that the judge can issue his regulation with certainty by seeking assistance of experts in what he does not know. Therefore, expertise in forensic medicine has an impact on judgments and court regulations.

First Topic:

Medical Examination and Autopsy of the Judicial Mortality

When the case is considered a judicial mortality, the dead body shall be in the possession of the judicial authority until the procedures decided in its regard are completed, including medical examination, autopsy, issuance of the extradition decision of the dead body to its relatives and allowing the properly burial. Consequently, the judicial authority is the only entitled to select the physician it deems worthy of the task required to draft a report on the causes of death and the dead body case, provided that the therapist of the case before death and any physician who may be related to the deceased shall be completely excluded[6].

Medical examination and autopsy is conducted through informing the assigned physician of the various circumstances of death and any information about the deceased due to its necessity to the physician and its equal importance and necessity that makes the physician awareness of the patient histories before examining and treating the patient a medical requirement decided by the origins of medicine. This involves informing the physician of the medical and therapeutic history, including previous medical diagnostic procedures such as lab and radiation tests and others[7].

The assigned physician shall conduct medical examination, especially in cases of mysterious death and murders, and whenever the public prosecutor deems it necessary to examine the place of death or crime scene, and to make the initial forensics of the dead body before moving or transferring it, in order to achieve several matters, the most important of which are: A- the death diagnosis that means to indicate whether the person died or not; B- estimating the period that passed since the death in light of the reality of the situation and take advantage of the lividity changes in suspicion of certain causes of death and the status of the dead body through the extent of the stability of the postmortem lividity position and the difference in its position from the position in which the dead body was found; C- determining the type of injuries, if any, and their relationship to death with an indication of their number, positions, age, and type of tool caused them, comparing the tool or weapon, if any, to the injuries of the dead body; D- Investigating what can be inferred to the circumstances and causes of death in and around the dead body, such as poisoning or electric shock, etc. E- putting the clothes on the dead body in terms of their arrangement, the material and criminal prints they have, their conformity with the wounds and injuries on the dead body, and the position in which the clothes were on the dead body at the time of exposure to external pull; F- interpretation of the chain of events before death, such as the status of the deceased upon receiving the injuries, taking into account the blood spatter pattern, whether the location of the body is the place of the accident or crime and death, and identifying the relationship between the injuries and the place around which the death occurred or where the body was found, such as falling down stairs or hitting furniture, which may lead to injuries totally or partially; G- determining the identity of the dead body if it is not possible to the eyewitnesses because of the decay and decomposition of the dead body. This requires investigation of the signs of age, gender, dead body length, teeth condition, and the dead body identifying marks, etc[8].

Based on the forgoing, a full report is drafted and submitted to the competent judge to base his judgment on it according to the form stipulated in the Medical Reports and Committees Regulation No. 13 of 2014 and amended by Law No. 43 of 2019 in Article 16 / A + B + D: A- A committee of the relevant authorities shall be, upon a decision of the Minister, formed to prepare forms of medical reports of all kinds and to determine the data and information that shall be included therein. B - The Minister shall issue his decision to approve medical forms based on the recommendation of the committee referred thereto in Paragraph (A) of this Article. C- The physician who receives the judicial medical case is obligated to fill out the approved form of the forensic report and to ensure the accuracy of the information after the necessary examination and diagnosis of the patient and this shall be under penalty of prosecution.

Instructions for issuing Jordanian medico-legal reports for 2017: Among the most important articles are:

First: Article 3 states that: *It is not permissible to issue medico-legal reports, of both primary and final types, unless they are drafted as follows: A. The injured person shall come to the hospital to be examined. Therefore, he/ she shall be examined and the necessary measures shall be taken.*

B. The injured person's identity shall be verified and recorded, and the data contained in his/ her identity card shall be recorded through the identity document.

C. The physical observation by the hospital physician shall be proven in light of the actual examination.

D. An indication in the medico- legal report of the authority that sent the injured person or if the injured person had made a review on his/ her own.

E. A description of the medical procedures that were taken upon examination and diagnosis and recording the same.

F. Filling in all the items mentioned in the forensic report in Arabic.

G. The physician is obligated to describe the injuries and determine their locations on the body, as well as the symptoms that accompanied the injury, and shall record the same in the medico-legal report.

H. The medical report shall be signed and stamped by those drafting it, shall be stamped and approved by the head of the department or the administrative representative, and shall be stamped by the hospital.

Second: Article 4 states that: *The report shall be drafted in four copies; a copy for the injured person, a copy for the authority that has did the examination, a copy to be sent to the National Center for Forensic Medicine or to the center or department affiliated thereto in the governorates or districts for examination and taking the necessary action, and a copy to be kept in the file.*

Third: Article 6 states that *the forensic physician is requested to examine the injured and assess their health conditions upon a request from the public prosecutor who is on duty around the clock, in the following cases:*

A. Gunshot wounds

B. Sexual assaults

C. Stab injuries

D. Traumatic injuries in which the general condition of the injured is moderate or severe

E. Facial injuries caused by sharp objects

F. Cases of poisoning due to criminal suspicion

G. Burns that exceed 20% of the body surface area

H. Injuries suspected to be fabricated

Any other cases that require the consultation of the forensic physician by the competent authority and / or the cases determined by the Minister of Health

Fourth: Article 9 states that *every hospital that issues a forensic medical report shall send a copy to the National Center for Forensic Medicine for all hospital admissions immediately after drafting them.*

Forensic directorates have been created based on the provisions of Article (11) of the Ministry of Health Administrative Organization System No. (47) for 2003, and based on the recommendation of the Planning Committee held on 05/24/2007, the following directorates decided to be created to be associated with the National Center for Forensic Medicine: The Forensic Medicine Directorate of the Central Region and its center is Amman, the Forensic Medicine Directorate of the Northern Region and its center is Irbid, and the Forensic Medicine Directorate of the Southern Region and its center is Al-Karak.

Based on the provisions of Article 10 / A of the Ministry of Health's Administrative Organization Regulation No. 36 of 2008, and based on the recommendation of the Planning Committee held on January 4 2012, it decided to create the following directorates in the hospital administration: Directorate of the National Center for Forensic Medicine and Directorate of Allied Health Professions.

Consequently, the Jordanian legislator has set up the reports and medical committees system No. 13 of 2014 and its amendments. This system deals with many medical committees, including the forensic committee. The Jordan legislator has also defined the system of expertise before regular courts for 2018 and its amendments, according to Article 11 / c which states that *a forensic physician shall be appointed either from forensic physicians appointed in the Ministry of Health, the Royal Medical Services, official university hospitals, or from those retired among them*.

I wish the Jordanian legislator will set up a legal system for forensic physicians, which shall be independent of the medical reports and committees system No. 13 of 2014 and its amendments and the expertise system before regular courts of 2018 and its amendments. The proposed system is the Judicial Committee system for forensic physicians, and the reason for this is because the experience of forensic physicians is often the source of the basis for the judge's ruling, especially in cases of murder for unknown and suspicious reasons.

Second Topic:

Legal nature of expertise

The opinions of the jurists differed about determining the legal nature of expertise. Some considered it a means of evidence, others considered it a means to help the judge, others considered it a kind of examination and others considered it a kind of testimony.

The First Opinion: Expertise is a means of criminal evidence, which is based on two factors: Proving the crime, and attributing this crime to its perpetrator. These means contribute to the formation of the judge's emotional conviction[9].[10].

The Second Opinion: Expertise is a means to help the judge, by stating that the issue of evidence is a moderate case between the judge and the lawsuit parties. As for expertise, it is an issue related only to the judge. The judge may resort to this expertise if he wants or refrain if he believes that expertise will add nothing to the lawsuit[11].[12]

The Third Opinion: Expertise is a technical inspection, which is a direct sensory detection to prove the status of something or a person, and it takes place by moving the investigator to the place of this status or bringing the inspection subject to the investigator's headquarters, through investigation and collecting evidence in order to reveal the truth of the crimes. Both expertise and inspection aim to uncover the truth of the crime through physical evidence in most cases. Expertise or inspection is resorted to by a decision of the Court on its own or upon the litigants' request. The court has the discretion power to conduct the expertise or inspection in case it deems them necessary, or refuses to conduct them in case it deems them in vain in the criminal lawsuit[13].

And the Jordanian judiciary provisions related to expertise and with regard to forensic medicine in particular:

1- The Jordanian Court of Cassation upheld the High Criminal Court Resolution with the evidence it approved in forming its conviction of its landmark resolution and extracted parts of evidence which it proved in the resolution text. It is a legal evidence that has its own origin proved in the lawsuit, which leads to that outcome, which is approved and proven, the most important of which is the Prosecution's witness, Dr. Adnan Saeed Muhammad Abbas, a forensic consultant, before the court, about the medico-legal report drafted against the victim Jordanian Court of Cassation, Ruling No. 1746/2020.

2- The defendant is criminalized with attempted murder, as the defendant intention was not directed to murder. The medical report issued by the forensic physician, Mansour Al-Maaytah indicated that the injuries suffered by the victim did not present a risk for life since they did not affect any of the chest and abdomen entrails and did not cause bleeding Jordanian Court of Cassation, Ruling No. 2116/2020.

3- The medical reports, including the forensic physician's report issued by the official authorities, may be challenged by forgery. There are special methods for challenging such reports with the competent authorities Jordanian Court of Cassation, Ruling No. 716/2008, published on 7/4/2008.

4- Article No. 147/2 of the Jordanian Criminal Procedures Law stipulates that *proof in felonies, misdemeanors and infractions shall be established by all means of proof and the judge shall rule according to his personal conviction*. Furthermore, it is a well-known fact that the expert opinion, which he is assigned by the court after the oath, is considered evidence valid for judgment since expertise is one of the legal means of evidence Jordanian Court of Cassation, Ruling No. 2748/ 2020.

5- The criminal judge is not restricted by the results of the expertise except that he shall indicate in his ruling that he excluded them and explain the reasons that to exclude them. The Jordanian Court of Cassation also held that expertise is a matter left to the court discretion and the investigative authority Jordanian Court of Cassation, Ruling No. 53/2000.

6- The competent and the most able to determine the cause of death and the extent to which the perpetrator's behavior contribute to death are forensic physicians by virtue of their academic specialization. The law does not require hearing the testimonies and affidavits of all the experts drafting the expert opinion as long as the expert

opinion is clear and fulfills the purpose for which it was produced and the testimony of one of the experts drafting the report is sufficient for the purposes of submitting and presenting it to the court Jordanian Court of Cassation, Ruling No 1886/1997, issued on 18/11/1997.

7- When there are multiple injuries, especially caused by different tools by several people, it may be important and necessary to know which injuries caused death. The forensic physician shall estimate the status of each wound, its severity, its position and whether or not it is accompanied with fractures, as well as whether or not vital organs were injured and the times of injuries in case there was some indication that it occurred at multiple times Jordanian Court of Cassation, Ruling No. 209/2009, published on 7/9/2009.

It is clear from the foregoing that the Jordanian legislator adopted the free proof system in the Jordanian Penal Trials Code No. 9 of 1961, as amended by Law No. 32 of 2017 in Article 147/2, by saying: The evidence is established in the felonies, delicts, and contraventions by all ways of verification, and the judge shall pass judgments in accordance with his personal persuasion, as well as the provisions of Article 39/1 of the previous law, saying: If distinguishing the nature of the crime depends on knowing some arts or handicrafts, then the public prosecutor may accompany one or more of the handicraftsmen. Thus, it is understood from the previous provisions with the rulings of the Jordanian courts that the expertise was considered a means of proof, but it is a discretionary authority for the judge to seek their help in the matters the judge has no experience in, including forensic medicine, because experience in forensic medicine helps in forming the judge's conviction and issuing his judgment of acquittal or conviction.

Conclusion

Forensic medicine and technical expertise are, from a criminal point of view, an important part of achieving criminal justice in a number of cases, the most important of which is if a person dies by murder or for unknown reasons that give rise to suspicion, then the report of the forensic doctor helps to uncover those causing the criminal incident and because penal provisions are based on certainty, not suspicion and guesswork. The importance of researching the legal value of the forensic report, as the judge most likely bases his judgment on this report. We have reached a number of conclusions and recommendations.

Results

1- The Jordanian legislator has set up the reports and medical committees system No. 13 of 2014 and its amendments. This system deals with many medical committees, including the forensic committee. The Jordan legislator has also defined the system of expertise before regular courts for 2018 and its amendments, according to Article 11 / c which states that *a forensic physician shall be appointed either from forensic physicians appointed in the Ministry of Health, the Royal Medical Services, official university hospitals, or from those retired among them.*

2- The forensic physician cannot, through his report, exceed what he was required to do, otherwise it is considered an interference in the judicial affairs. Therefore, the role of the forensic physician is a detector and not an originator.

3- The Jordanian legislator clarified that forensic physicians are assigned upon a request from the public prosecutor. Articles 39: 41 of the Penal Trials Code No. 9 of 1961, as amended by Law No. 32 of 2017 did not indicate that a judge is able to assign a forensic physician. However, the response to that is that the public prosecutor is a judge and that the criminal case is initiated by the public prosecutor starting through the investigation, then assigning the criminal offense and then referring it to the competent court. The law is that which gives the authority to appoint a forensic physician or more if the circumstances of the case needed that, as well as the power to submit the report of the expert (forensic physician), accepting or rejecting it, and to request another expert report, as the judge is the supreme expert.

4- The Jordanian legislator adopted the free proof system in the Jordanian Penal Trials Code No. 9 of 1961, as amended by Law No. 32 of 2017 in Article 147/2, by saying: The evidence is established in the felonies, delicts, and contraventions by all ways of verification, and the judge shall pass judgments in accordance with his personal persuasion, as well as the provisions of Article 39/1 of the previous law, saying: If distinguishing the nature of the crime depends on knowing some arts or

handicrafts, then the public prosecutor may accompany one or more of the handicraftsmen. Thus it is understood from the previous provisions with the rulings of the Jordanian courts that the expertise was considered a means of proof, but it is a discretionary authority for the judge to seek their help in the matters the judge has no experience in, including forensic medicine, because experience in forensic medicine helps in forming the judge's conviction and issuing his judgment of acquittal or conviction.

Recommendations:

1- I wish the Jordanian legislator will set up a legal system for forensic physicians, which shall be independent of the medical reports and committees system No. 13 of 2014 and its amendments and the expertise system before regular courts of 2018 and its amendments. The proposed system is the Judicial Committee system for forensic physicians, and the reason for this is because the experience of forensic physicians is often the source of the basis for the judge's ruling, especially in cases of murder for unknown and suspicious reasons.

2- I wish the Jordanian legislator to amend Article 4 /D of the Medical Reports and Committee Regulation No. 13 of 2014 and its amendments, which stipulated that a forensic committee shall be formed by a decision of the Minister of Health, and its tasks shall be determined by the decision of its formation. What is proposed is to form a judicial forensic committee upon the recommendation of the Ministry of Health, and with the approval of a judicial committee determined by the Ministry of Justice, consisting of five judges from the Jordanian Court of Cassation, to which a special system is issued, because the judicial forensic doctors are part of the criminal justice in the courts, and their medical history or the reality of their lives and their dealings shall be considered, and this is not out of luxury or philosophy, but this is because a forensic report may sentence a person to death (conviction) or revive him/her (innocence).

3- I wish the Jordanian legislator will establish a department or center in the Jordanian Ministry of Justice that includes forensic physicians to serve the Jordanian judiciary, and for the ease and speed of litigation procedures.

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Immunoinformatics Analysis of SARS-CoV-2 Isolated from Karst of Bats in Malang, Indonesia

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Abstract

In December 2019, cases of mysterious pneumonia were first reported in Wuhan, Initially, the disease was temporarily named as 2019 novel coronavirus (2019-nCoV), then WHO announced a new name on February 11, 2020, namely Coronavirus Disease (COVID-19) which is caused by a virus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This virus can be transmitted from person to person and has spread widely in China and more than 190 other countries and territories. Meanwhile in Indonesia, up to September 2020, 203 thousand cases of COVID-19 were found and 8336 deaths. Bats act as important disease reservoirs for various etiologic agents of disease that can be transmitted between species, infecting humans and mammals, both domestic and wild. The purpose of this study was to analyze SARS-CoV-2 immunoinformatics in bats in Karst Malang Indonesia as the basis for making SARS-CoV-2 vaccines. This research method is SARS-CoV-2 amino acid bats in Karst Malang, Indonesia analyzed by immunoinformatics. From the research results, it was found that SARS-CoV-2 protein from Malang Karst bats were antigen, non-allergen and non-toxin, so that they could be used as vaccine candidates, diagnostic kits and immunotherapy.

Keywords: *SARS-CoV-2, Bats, Malang Karst, Indonesia, Immunoinformatics.*

Introduction

In December 2019, cases of mysterious pneumonia were first reported in Wuhan, Hubei Province. The source of the transmission of this case is still uncertain, but the first case was linked to a fish market in Wuhan. From 18 December to 29 December 2019, five patients were treated with Acute Respiratory Distress Syndrome (ARDS). From 31 December 2019 to 3 January 2020, this case increased rapidly, marked by the reporting of 44 cases. In less than a month, the disease has spread to various other provinces in China, Thailand, Japan and South Korea. The samples studied show the etiology of the new coronavirus.2 Initially, the disease was temporarily named as 2019 novel coronavirus (2019-nCoV), then WHO announced a new name on 11 February 2020, namely Coronavirus Disease (COVID-19) caused by the Severe Acute Respiratory Syndrome Virus Coronavirus-2 (SARS-CoV-2). This virus can be transmitted from person to person and has spread widely in China and more than 190 other countries and territories.5 On 12 March 2020, WHO announced COVID-19 as a pandemic. As of 29 March 2020, there were 634,835 cases and 33,106 total deaths around the world. Meanwhile in Indonesia, up to September 2020 there were 203 thousand positive cases of COVID-19 and 8336 cases of death[1,2,3].

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Bats are classified as the Chiroptera Nation, and are the only mammals that have the ability to fly properly using wings[4]. The number of bats is more than 1200 species around the world. These small mammals occupy the second largest number of rodents (Rodentia) in the Mammalia Class[5]. There are 215 types of bats scattered throughout Indonesia[6]. Fruit-eating bats (Pteropodidae family) are bats that consume fruit and / or flower products, ecologically they play an important role in nature as seed dispersers and flower pollinators for certain plants[7]. Pteripodidae also act as important disease reservoirs for various etiologic disease agents that can be transmitted between species and infect humans and mammals, both domestic and wild. Various types of important viruses can be transmitted by Pteropodidae, including Henipavirus[8], Lyssavirus[9], Coronavirus associated with SARS and MERS[10,11] and Filovirus (including the Ebola virus. and Marburg virus)[12]. Various efforts have been directed towards the development of the COVID-19 vaccine, to prevent a pandemic and most of the developing vaccine candidates have used the S-protein SARS-CoV-2[13]. As of 2 July 2020, the worldwide SARS-CoV-2 vaccine landscape includes 158 vaccine candidates, of which 135 are in the preclinical or exploratory stages of development. Currently, mRNA-1273 (Moderna), Ad5-nCoV (CanSino Biologicals), INO-4800 (Inovio, Inc.), LV-SMENP-DC, Pathogen-specific aAPC (ShinzenGeno-Immune Medical Institute), and ChAdOx1 (University of Oxford) has entered phase I / II clinical trials (WHO, 2020). Vaccines that are in the channel are based on live inactivated or attenuated viruses, protein sub-units, virus-like particles (VLP), virus vectors, DNA, RNA, nanoparticles[14]. To improve immunogenicity, various adjuvant technologies such as AS03 (GSK), MF-59 (Novartis), CpG 1018 (Dynavax)[15]. An immuno-informatics approach was also used to identify the epitopes of the SARS-CoV-2 vaccine candidate. It can be used to identify significant cytotoxic T cells and B cell epitopes in viral proteins. The purpose of this study was to analyze SARS CoV 2 in bats in Karst Malang Indonesia as vaccine candidates, diagnostic kits and immunotherapy.

Materials and Methods

Sample Collection

The research was conducted from October 2019 to September 2020 in a cave in the karst, Malang, Indonesia. The materials used in the study consisted of 70% alcohol and cotton which was used to anesthetize and preserve bat samples for specimen collection. The tools used to collect bat samples are mist nets, ropes, 2.5 - 3 m long poles to install fog nets, long-stemmed nets, calico bags, masks, scissors, long tweezers, identification books and cameras. Sampling of bat species was carried out by trapping method using Mist net[16]. Catching bats is carried out when the bats start their activities in the afternoon. The bats that are caught are then identified to determine their species, referring to Suyanto (2001). The bat is laid by tilting it (dorsal recumbency) over the styrofoam by fixing the proximal and distal wings using pins. Laparotomy surgery is performed ventral so that the abdominal cavity opens. The taken organs are separated and put into a container that already contains 10% PBF.

RNA extraction

RNA was extracted from bat organ samples with the Pure 96 MagNA System (Roche, Penzberg, Germany) and from cell culture supernatants with a mini RNA virus kit (QIAGEN, Hilden, Germany).

Real Time PCR

The 25 µL reaction contains 5 µL RNA, 12.5 µL 2×reaction buffer provided with Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; contains 0.4 mM each of deoxyribont triphosphate (dNTP) and 3.2 mM magnesium sulfate), 1 µL reverse transcriptase / Taq mixture from the kit, 0.4 µL 50 mM magnesium sulfate solution (Invitrogen), and 1 µg nonacetylated bovine serum albumin. Thermal cycling was performed at 55 °C for 10 minutes for reverse transcription, followed by 95 °C for 3 minutes and then 45 cycles at 95 °C for 15 s, 58 °C for 30 s.

Sequencing

The amplification results used RT-PCR in the form of cDNA for sequencing. The sequencing reaction is carried out enzymatically, with dye terminator cycle sequencing. Before sequencing the PCR product must be purified to clean the PCR product from the rest of the

buffer and other reaction components so that it can get good sequencing results.

3-Dimensional Structure

SARS CoV 2 protein from Malang Karst bats in Indonesia using SwissProt.

Epitope predictive analysis

Prediction of B-cell epitopes from SARS CoV 2 protein in Malang Karst bats in Indonesia using IEDB. Next, we sent the predicted peptides to VaxiJen to determine whether the predicted epitopes could be prospective protective antigens that would build up the immune response.

Allergy prediction and non-toxic protection

In this study, extensive analysis of the predicted allergenicity of the predicted peptides was carried out using AllerTOP, then, we estimated the non-toxic protective antigens that perform ToxinPred.

Results and Discussion

Various efforts have been directed to the development of the COVID-19 vaccine, to prevent a pandemic and most of the developing vaccine candidates have used the SARS-CoV-2 protein.The immuno-informatics approach is also used for the identification of the SARS-CoV-2 vaccine candidate epitopes. It can be used to identify significant cytotoxic T cells and B cell epitopes in viral proteins (Gupta et al., 2006; Baruah and Bose, 2020). From the results of the research conducted, it was found that the three-dimensional structure of the SARS CoV-2 protein from the Karst Bats in Malang Indonesia.

The protection that currently available vaccines provide against viruses is based on neutralizing antibodies. Such antibodies usually block the interaction of the virus with viral cellular receptors or prevent the conformational changes required for viral fusion with membrane cells. The SARS-CoV-1 virus has been studied in great detail. Recent investigations have shown that the new SARS-CoV-2 virus uses the same strategy for entry into cells[17,18]. The interaction of S protein with ACE2 is well described for SARS-CoV-1 and -2 and relies on a specific domain in the protein, called the receptor binding domain (RBD). Indeed, most of the antibodies capable of neutralizing the corona virus are directed against RBD. Therefore, the main immune mechanism to avoid infection is through blocking of viral adherence to ACE2. Therefore, producing vaccine-inducing antibodies against RBD is the strategy used by most of the COVID-19 vaccine candidates. Recently it has been shown that RBD is glycosylated and methylated. In general, such post-translational modifications are difficult to reproduce in vaccines, meaning the vaccine may display a (slightly) different epitope from the virus. As a result, vaccine-induced antibodies are potentially cross-reactive and non-protective. Interestingly, however, the receptor interaction site (RIS) file that directly binds to ACE2 is not glycosylated, suggesting that this RIS may potentially be an ideal vaccine candidate. The second most common option is to use the entire S1 subunit. Other vaccine manufacturers use proteins and/or peptides that fuse with the cell membrane and therefore also have neutralizing epitopes. The SARS-CoV-2 fusion protein readily induces neutralizing antibodies in non-human primates. Proteins and peptides can be made more immunogenic by formulating them with powerful adjuvants. A potential challenge is that the induction of neutralizing antibodies is dependent on the display of the antigen in the correct conformation, that is, it is not guaranteed when the protein or peptide is expressed and displayed separately at the injection site[19].

The most obvious isotype to be induced by the COVID-19 vaccine is IgG, preferably the protective IgG1 and IgG3 subclasses. However, IgA may also be important for reducing infection of mucosal and epithelial cells in the respiratory tract, as well as endothelial cells, which may be very widely targeted by viruses. Although immunization of the mucosa on a large scale rapidly may be difficult, the use of adjuvants that stimulate IgA production may be an important consideration. TLR7/8 and TLR9 ligands are good candidates because they potentially promote the IgA response[20].

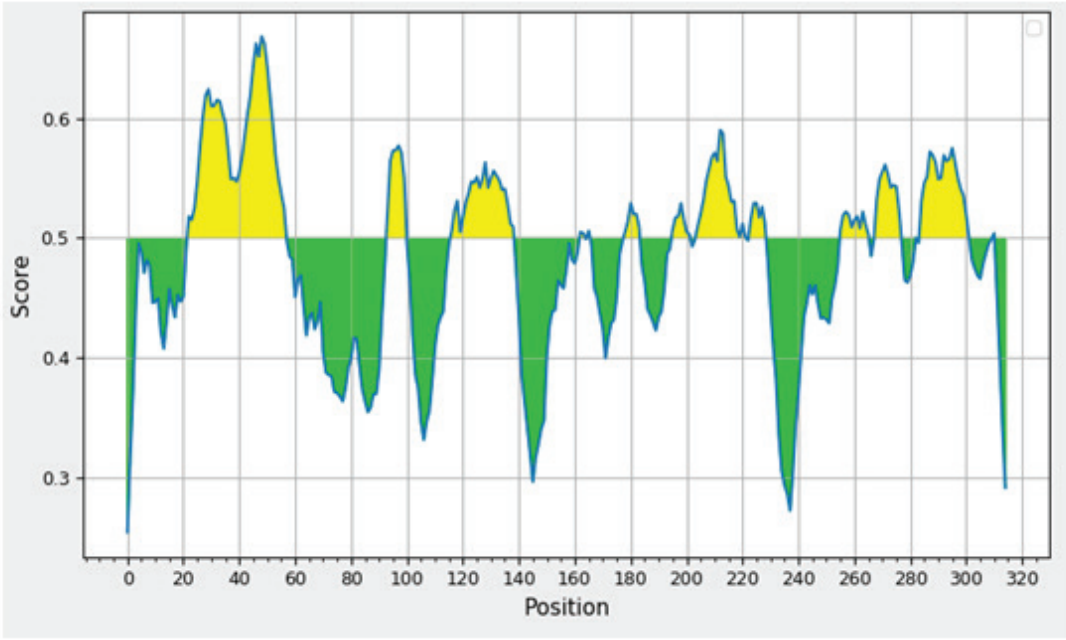

Figure 1. Prediction of Epitope Based on Cell B from SARS CoV 2 protein from Karst bats Malang Indonesia

Table 1. Prediction of protective antigens, allergens and toxins for SARS CoV 2 From Bats, Malang Karst

Peptides	Long	Position	Allergenicity prediction	Protective Antigens Prediction	Toxigenicity Prediction
IQRRNWPTHASKSPKRNYLLRGRNT SHRSVNRGSC	35	23-57	Probable Non-Allergen	Probable Antigen (0.6242)	Non-Toxin
IISTSRFCPGVTEREVFCENRTA	23	117-139	Probable Non-Allergen	Probable Antigen (0.8551)	Non-Toxin
VYTFPGNKPTNFRSLVDL	18	205-222	Probable Allergen	Probable non Antigen (0.1359)	Non-Toxin
VTRLSSAGCLR	11	256-266	Probable Allergen	Probable Antigen (0.5959)	Non-Toxin
KGKMESLVPGFNEKTHVQ	18	285-302	Probable Allergen	Probable Antigen (0.8644)	Non-Toxin

Conclusion

From the results of the research, it was found that the antigenic, non-allergen and non-toxic proteins of SARS CoV2 from Malang Karst bats were used as vaccines, immunotherapy and diagnostic kits.

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Does Virtual Brain Autopsy Could be Used as a Clue for Detection of Personal Identity?

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Abstract

Background: virtual autopsy nowdays show great success in human identification. Nowdays single Computerized tomography of the brain could help answering many questions regarding the identity of humans, it could lead us to the gender ,weight and height roughly of the person in question.

Patients and Methods: cross- sectional study during the period April 2019 till Mars2020 , One hundred and fifty participant aged between 15 and 29 years old were envolved in the current study after taking their written and verbal consent or their caregiver and doing C T Brain for every one of the studied participant.

Results: brain volume is statistically significant with weight , height, gender of the studied participant hence we could use CT Brain to know the brain volume and then we could suspect weight , height and even gender so we can reach to the identity of the individual.

Conclusion: We me be no longer in need of doing autopsy where virtual autopsy could find the answer to many questions in research, where a correlation was found between brain volume ,gender, weight and height**.**

Key words : *computerized tomography Gender, height, virtual, weight.*

Introduction

Brain size determination is a growing topic in anatomy anthropometry and determination of human identity[1].Estimation of the brain size could be done either by weighting brain at autopsy,using filler in the cranial cavity and measuring it, measuring the exterior size of the head or by using virtual autopsy of the brain using computer assisted tomography and magnetic resonance imaging. [2]

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With advance in technology of brain radiology , correlation between brain size and gender could be founded [3]. there is correlation between increasing age and decrease in brain volume in a study[4], while in other study[5] found no link between age and brain size .

Cerebrum volume creates from birth to youth with the fastest improvement before the fifth year. Between the ages 16-20-year, cerebrum volume shows up at its maximal total and won't create after that through life. The volume is around 1260 cm3 in men and 1130 cm3 in women, despite that there was individual variation . [6]

Finally, it's known, the typical cerebral volume is about 10 % greater in youngsters than young women in children . [7]

Subjects and Methods

Study design

This study is a cross- sectional study during the period April 2019 till Mars2020.

Study sample

One hundred and fifty participant aged between 15 and 29 years old were envolved in the current study after taking their written and verbal consent or their caregiver.

They were divided into 3 group according to their age(15-19,20-24,25-29 years old)

Inclusion criteria include Egyptian citizen more than 15 year and below 30 years old

We exclude those refuse to participate, the above 29 and below 15 years old,those with past history of chronic disease or head injury or those who are presently illness.

Data collection

The following information were gathered:

Age from their birth certificate, Sex, weight using a weight scale and height using a measure and measuring length in standing position with bared foot.

CT Scanning of the head is done in the radiology department in fayoum university hospital using multi-cut CT machine (Ranging from 8 - 64 cut for each second) .

Three measurement were taken (antero-posterior length, breadth and Cranial height). The Maximum antero-posterior length, Maximum breadth were assessed at the midventricular cut of CT head at the level of prominent frontal horn of lateral ventricles(fig 1), while cranial height is measured between the internal acoustic meatus to the highest point of bregma(basibregmatic height).

brain volume calculating done by multiplying the three measurements above.

The gained data will be grouped and analyzed.

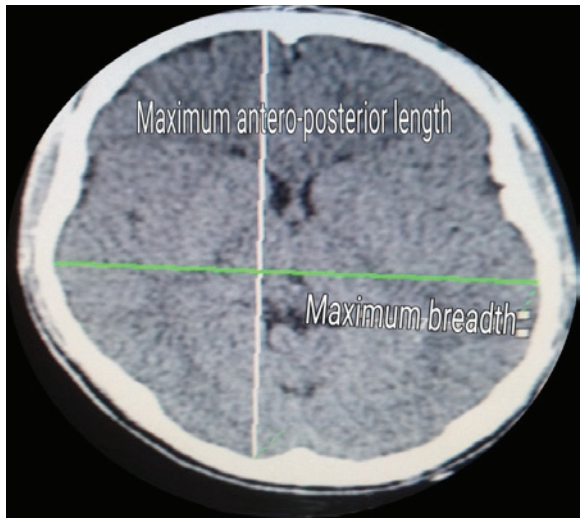

Fig 1: maximum antero-posterior length, Maximum breadth

Statistical analysis

The collected data were organized, tabulated, and statistically analyzed using statistical package for social science (SPSS Inc, version 21). For quantitative data, the mean and standard deviation (SD) were calculated. **Student's**-t test, or one- Way ANOVA, when appropriate, was used as a test of significance . Categorical data were presented as frequencies and percentages; chi-square ($\chi 2$) was used as a test of significance,correlation coefficient test(person test) was done and liner regression analysis performed. For interpretation of the results of tests of significance, significance was adopted at $P \leq 0.05$ in the results.

Results

Descriptive statistics. Distribution of the studied subjects regarding demographic data and brain volume (No=150). **The** mean ± SD of all participants was 21.7 ± 4.4 years for age , 66 ± 13.4 for weight/kg , 163.7 ± 8.7 for height/cm , 24.4 ± 4.3 for body mass index and 2265 ± 291.7 for brain volume . The range was 14.2 - 28.6 for age , 43-106 for weight , 141-183 for height , 16.6-38.3 for body mass index and 1184.6-3187 for brain volume .

Analytical statistics:

Table (1):Brain volume in relation to gender of the studied subjects.

Brain volume/cm3	Sex groups		t test	P value
	Male (n=73)	Female (n=77)		
Mean±SD	2416.36±228.13	2086.45±220.18	9.004 <0.001*	
Range	1944-3187	1184.6-2792		

t: student t test**, *:** significant

Table 1 shows that male had brain volume (2416.36±228.13) more than female (2086.45±220.18) with really high statistically significant differentiation, P <0.001).

Regarding Brain volume in relation to different age groups.

F test: ANOVA test shows that there was no genuinely through and through difference between different age and brain volumes (P= 0.103)

Table (2):Pearson correlation between weight and height with brain volume.

	Brain volume/cm3	
Weight /kg	R	0.378
	P-value	0.001*
Height /cm	R	0.526
	P-value	0.001*

R: Pearson correlation coefficient**, *:**significant

Table 2 shows that there was basic positive association between's brain volume concerning weight, height .(fig 2)

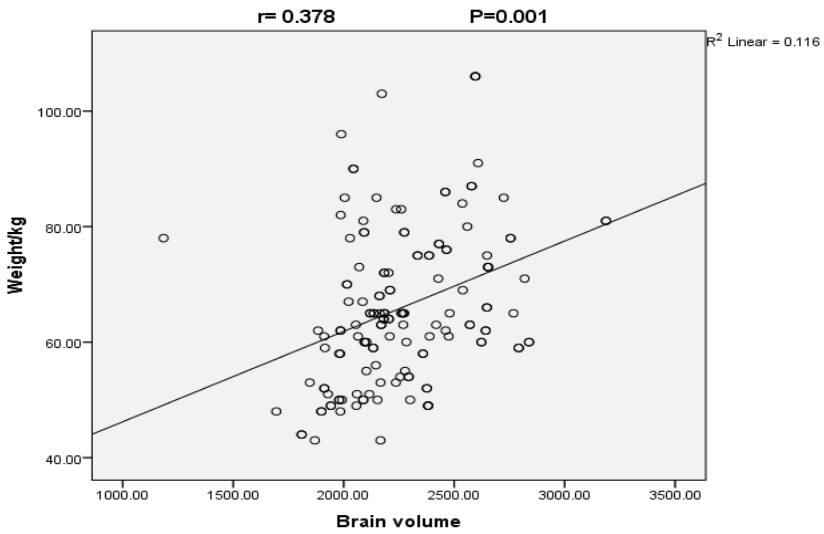

Fig. (2):Correlation between brain volume and weight/kg of the studied subjects.

Table (3): Correlation between weight, height and brain volume among females and males .

	Brain volume		
		Females	**Males**
Weight /kg	R	0.493	0.421
	P-value	0.001	0.004
Height /cm	R	0.586	0.520
	P-value	0.001	0.001*

R: Pearson correlation coefficient***:** significant

Table 3 shows significant positive association between's brain volume concerning weight, height and BMI ($p<0.001$) among females. Additionally between brain volume concerning, weight, and height among male subjects ($P<0.001$).(fig 3)

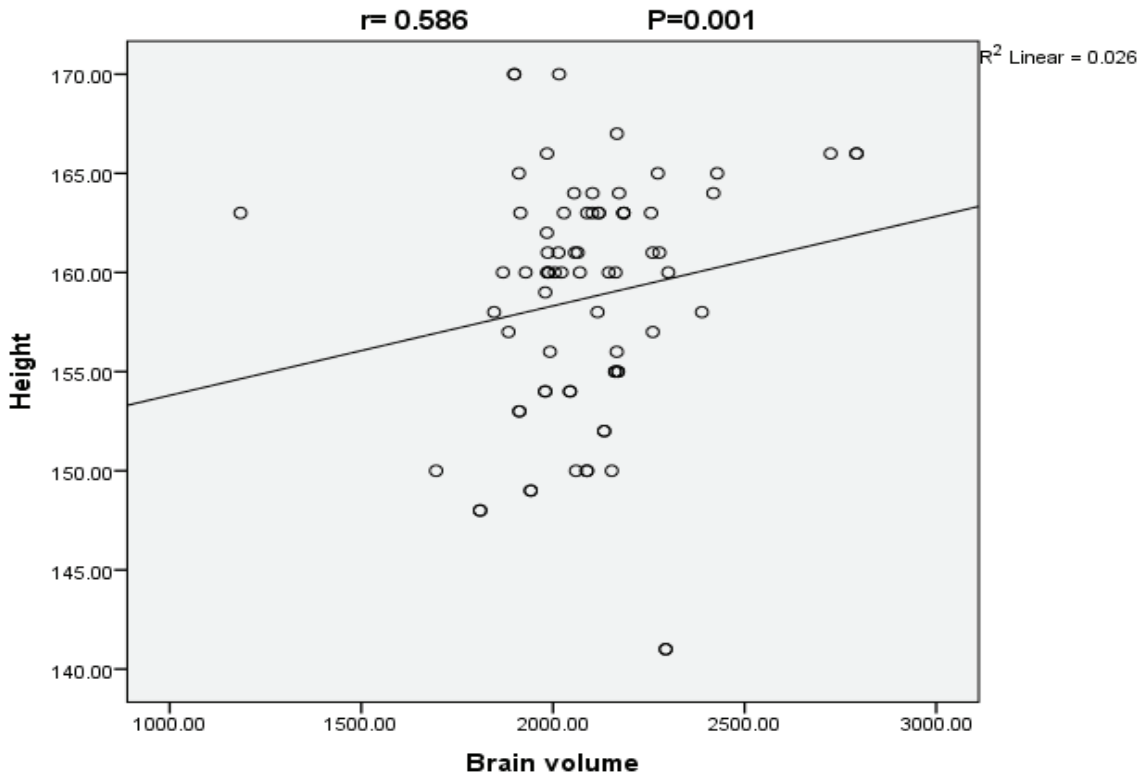

Fig.(3):Correlation between height/cm and brain volume among female.

Table (4): Multi-linear regression analysis of brain volume regarding age, weight and gender.

Variables	P-value	B constant
Age	0.010	-0.170
Gender	0.001	-0.450
Height	0.001	0.268

Table 4 shows that age, sex and height were verifiably significant self-sufficient parts with brain volume with p regard (<0.001)

Discussion

Cerebrum volume creates from birth to youth with the most rapid advancement before the fifth year. Between the ages 16-20, brain volume shows up at its maximal total and won't create after that through life .[8-9]

These assessments have entered another zone utilizing present day progresses, for instance, allowing imaging and brain checks specially volume which was assessed by assessment of skull through the head surface. Skull length, width and height are used for determination of brain volume. [10]

Incalculable examinations were directed to choose the association between skull volume and weight. The vast majority of these assessments were on dry bone or radiologic pictures. Positive relations were found between skull volume and height, weight, age in the both sexes. [11]

In the present assessment we explored the association of brain volume with age, weight, height in a specific age gathering (15 - 29 years) to act as an illustration of general youth people of Egypt.

Our results arrive in a comparative line with cross-sectional examination drove on 286 subjects by Bayat PD. et al., (2012) [12] to look at the association of skull size and cerebrum volume of Arak University of Medical Sciences understudies with their age, weight, height and weight record. Age of their analyzed subjects were in go 18-26 years old with mean 20.52±2.44 years, 150 subjects (52.45%) of the examined subjects were females and 136 subjects (47.55%) were males. mean stature was 169.3±19 cm with men weight was 64.01±12 kg and mean weight record was 22.19±4.8 kg/m2, concerning mind volume and weight were 1276.94±310 mm3 and 1321.61±32.2 g, respectively.

Another examination was finished by Acer N. et al., (2007) [13] on 366 subjects (226 people, 140 females). their degrees were 17-26 years old which were close to our results. The mean age was (19.83±1.96) a long time. In like manner, the mean weight was (65.34 ± 8.88) kg., and the mean height was (174.21±8.18) cm,. The strategies for cranial breaking point which in an indirect manner reflects the cerebrum volume were 1411.64±118.9 cm3 .

The current assessment demonstrated that male had cerebrum volume (2440.7 ±240.8 cm3) more imperative than female (2089.7± 225.6 cm3) with statistically significant basic qualification (P <0.001). Our results agreed with Aylward EH. et al., (2002) [14] They found that a critical effect was found for sex (male personalities were greater than female cerebrums). This may be a result of the sex contrasts in size is move by progressively unequivocal cerebrum regions, also exhibit that men have a bigger amygdala and hypothalamus, while women have a for the most part greater caudate and hippocampi.

Moreover, a couple of assessments were coordinated to choose the association between skull volume and weight found cerebrum volume among male was more unmistakable than female anyway was lower than our results. Gault D. et al., 1988[15]; MacKinnon IL., 1955) [16] revealed that mean skull volume was through and through higher in males (1393.31±111cm3) diverged

from females (1168.71±102 cm3). Moreover, Manjunath KY., (2002) [17] who reported skull volume in males and female to be 1152.813±279.16 cm3 and 1117.82±99.09 cm3 exclusively, which is lower than our examination. Acer N. et al. (2006) [18] decided the inside skull volume to be 1311±133 cm3 which is close to whole refined in the present of our assessment (1184.6-3187 cm3)

Also, in the examination which drove by Bayat PD. et al., (2012) [12] on 286 subjects to investigate the association of skull size and brain volume with their age height andweight record. They found that Mean skull record was 1.99±0.26 in males and 2.20±0.3 in females, referred to data were on a very basic level remarkable among males and females. Regardless, Golalipour MJ. et al., (2005) [19] have uncovered skull volume to be 1420.60± 85 cm3 in males and 1227.2±120 cm3 in females Turkmen race, which is closer to our examination. Another the assessment performed by Aylward EH. et al., (2002) [14] found a development in supreme cerebrum volume in subjects some place in the scope of 8 and 12 years of age, yet not for those more prepared than 12 years of age. This come not entirely agreed with our results.

The current examination exhibited that there was important positive connection between cerebrum volume with weight and height, in any case there no basic association between brain volume with age. The current assessment, mind volume demonstrated enormous negative relationship with age among females. While, cerebrum volume showed positive relationship with weight, height among males and females ($p<0.05$)

Our results come in simultaneousness with another examination by Bayat PD. et al., (2012) [12] found a positive connection between cerebrum volume and anthropometric records, for instance, height, weight, age and weight document in the two sexual directions which supports the eventual outcomes of Gustafson's examination (Gustafson D. et al., 2004) [20].

Moreover, Acer N. et al., (2006) [18] decided within skull volume to be 1311±133 cm3 which was extraordinary and lower with the whole refined in the current examination (2265 ± 291.7cm3). There was a positive association between mind rundown and cerebrum volume; in any case, this association is basically more grounded in females. Another examination drove by Ward M.A. et al., (2005) [21] found that extension in skull volume was connected with increase in height, weight and there was real connection between's both age and skull volume. inhibitor insecticides poisoning.

Conclusion

CT brain could help to solve the proplem of identity of the subjects where brain volume significantly related to weight , height and gender.

Conflict of Interest : The author declare that there is no prescence for conflict of interest.

Source of Fund: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Consideration: This study was approved by the Fayoum Faculty of Medicine Research Ethical Committee. The study was conducted after explaining the study's aims. Verbal and written consents were obtained from all participants included in the study and each person had the right to refuse to participate in the study.

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Genotyping of Human Papilloma Virus Causing Skin Wart among Iraqi Patients

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Abstract

The papillomaviruses are a large group of tiny, non-enveloped DNA viruses that in several different anatomical locations can cause squamous epithelial tumor's (warts and papilloma's). There is a close association between HPV and cervical uterine cancer . The genome is composed of 7200–8000 base pairs and is divided into three parts, early region (E) comprising of E1, E2, E4–E8 and reflecting 50 percent of the L1 and L2 genome, Genomic DNA from wart tissue samples were extracted by using gSYAN DNA mini kit extraction kit . The Real Time PCR primers for detection and genotyping of DNA Human papillomavirus type 1 and type3 and type6 based on L1 gene that designed in this study using NCBI-Genbank Sequence database and primer 3 plus . The genetic study included analysis of a common DNA to HPV that was found in 20 % of cases and DNA fragment specific to HPV type 1, 3 and 6 based on real time PCR. Type 1 HPV was not approved to present in any sample in the study. Type 3 HPV was identified in a single case. HPV 6 was identified in 8 cases, Therefore, type 6 HPV is the most common genotype, followed by type 3.

Keyword : *HPV, Wart skin disease , L1 gene, RT-PCR .*

Introduction

Skin warts are considered the main manifestation of the cutaneous Human papilloma viruses (HPV) types [(1)] . Human papillomaviruses are small, double-stranded DNA viruses that infect the epithelium. More than 120 HPV types have been identified; they are differentiated by the genetic sequence of the outer capsid protein L1. Most HPV types infect the cutaneous epithelium and can cause common skin warts [(2)] .

Characterization and analysis of human papillomaviruses of skin warts are ver clinically important . Common warts represent 70% of skin warts and occur primarily in children, whereas plantar and flat warts occur in a slightly older population [(3)] .

Mucosal HPV are small double-stranded DNA viruses that infect mainly anogenital epithelium [(4-5)] .

The etiological role of the human papillomavirus (HPV) in cancers of the uterine cervix has resulted in the development of molecular technologies for viral detection [(6)] .

The aim of this study was to develop a SYBR Green-based real-time for genotyping of Human Papilloma Virus causing skin Wart among Iraqi patients.

Materials and Methods

Genomic DNA from wart tissue samples were extracted by using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. USA, and done according to company instructions with modification as following procedures : 200mg frozen wart tissue samples was transferred to sterile 1.5ml microcentrifuge tube. Then homogenised by liquid nitrogen 200µl GST (Tissue lysis buffer) and 20 µl proteinase K (10mg/ml) were added and homogenized by sterile micropestle and mixed by vortex. Then incubated at 60C for 1 hour. After that, 200µl of binding buffer GSB was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70C for 15 minutes, and inverted every 3 minutes through incubation periods. Then, 200µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously. After that, DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture

(including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tube containing the flow-through were discarded and placed the column in a new 2 ml collection tube. 400µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube. 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre-heated elution buffer were added to the center of the column matrix. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA (table 1) .

Table 1 : The kits used in this study with their companies and countries of origin

No.	Kit	Company	Country
1	gSYAN DNA Extraction Kit	Geneaid	USA
	GST buffer		
	GSB buffer		
	W1 buffer		
	Wash buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
2	RealMOD™ Green SF 2X qPCR Mix (SYBER)	INtRON	Korea
	SYBER Green DNA dye HS-Taq DNA Polymerase, PCR buffer, MgCl2 and dNTP's		

The Real Time PCR primers for detection and genotyping of DNA Human papillomavirus type 1 and type3 and type6 based on L1 gene that designed in this study using NCBI-Genbank Sequence database and primer 3 plus. (5) All these primers was provided from Macrogen company, Korea as in table (2) .

Table 2. : HPV PCR primers that are used in the study

Primer	Sequence (5'-3')		Product Size
HPV DNA primer	F	TGAAAATCCCGCCTTTGAGC	118bp
	R	TGTGCCTTCAGGTGTTTCAC	
HPV-1 Primers	F	TTCTGGGAAGTCGATCTCACAG	226bp
	R	TTTGCGCTTTGTGGTGGAAC	
HPV-3 Primers	F	CCGTGGGTCATCCTTATTTTGC	117bp
	R	ACTTATTTGGGTCGGGCAAC	
HPV-6 Primers	F	TTCGATCCCACAACACAACG	90bp
	R	ATGTCCACTTACACCCACACC	

In this study ,collection of 50 samples (skin wart) . Liquid nitrogen was used as a preparatory step for genetic material extraction. The Real Time PCR technique was performed for detection and genotyping human papillomavirus based L1 gene from common wart patient samples**.**

Results and Discussion

The genetic study included analysis of a common DNA to HPV that was found in 20 % of cases and DNA fragment specific to HPV type 1, 3 and 6 based on real time PCR as shown in figures (1) ; (2) ; (3) ; (4) . We fail to identify any case with type 1 HPV. Type 3 HPV was identified in a single case. HPV 6 was identified in 8 cases . In 11 patients who were positive for the common HPV DNA, there was no positivity to HPV 1, 3 or 6 specific DNA fragment, (as shown in table. 3).

Table 3 : The frequency distribution of patients according to HPV genotype

HPV type	Negative	Positive	Total
Negative	20	0	20
Unidentified	0	11	11
HPV 3	0	1	1
HPV 6	0	8	8
Total	20	20	40

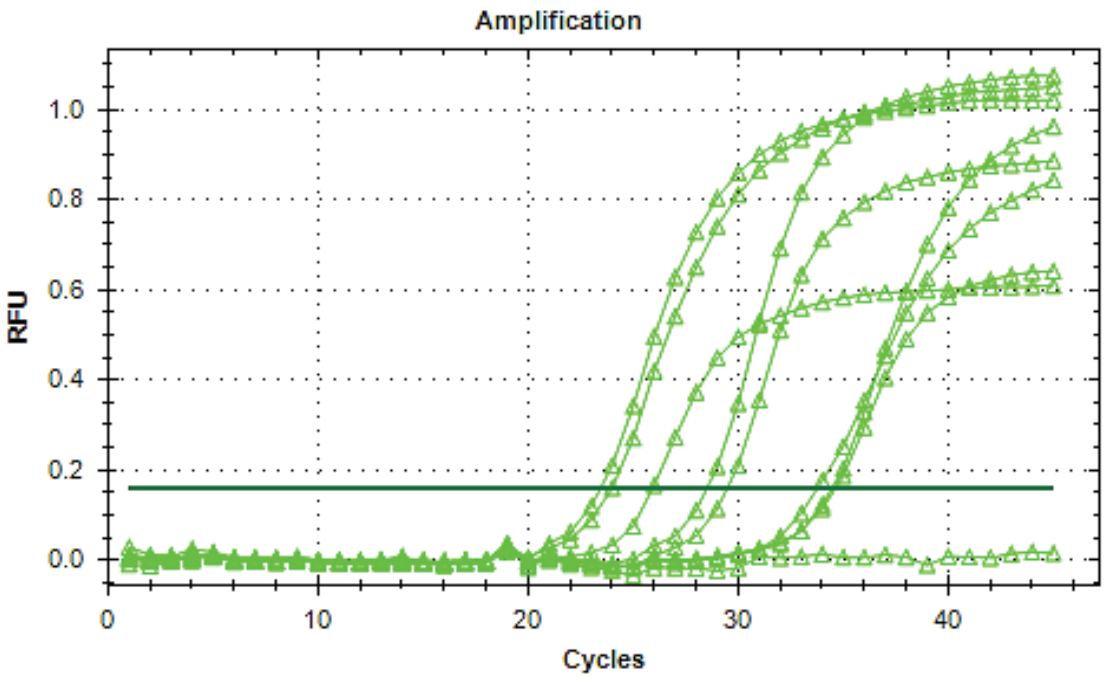

Figure.1: Real time PCR amplification plot based SYBER Green DNA dye for detection Human Papilloma virus DNA from extracted DNA of wart patient samples

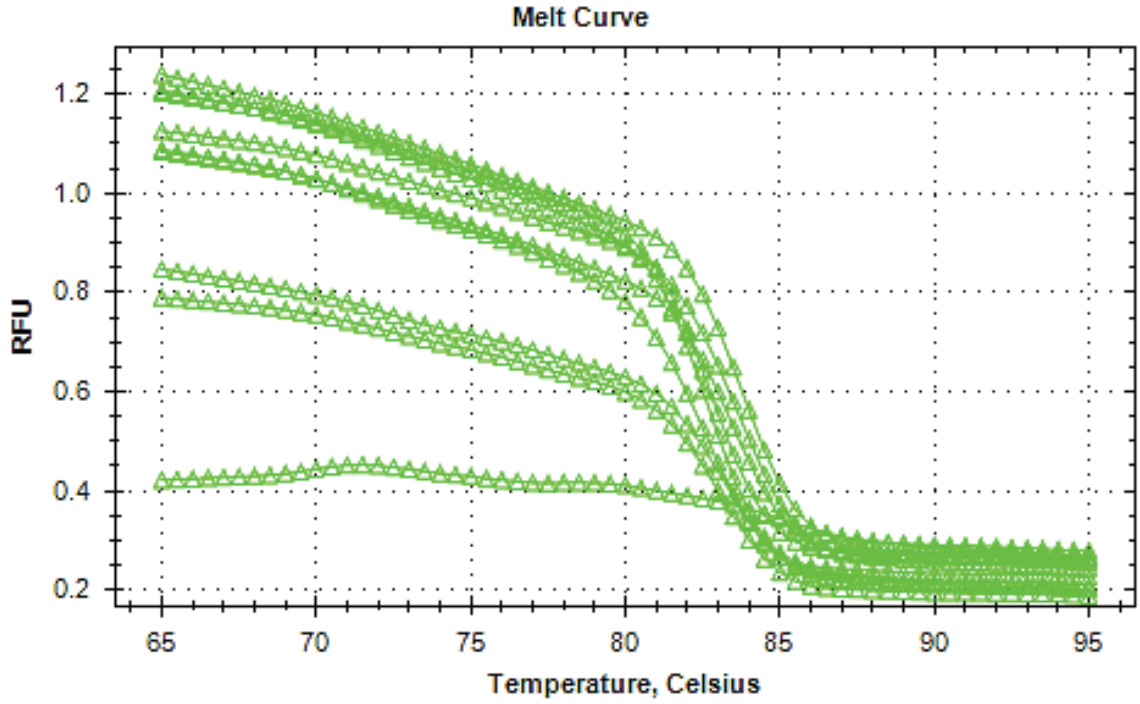

Figure.2 : Real time PCR Melt curve based SYBER Green DNA dye for qPCR specificity in Human Papilloma virus DNA .

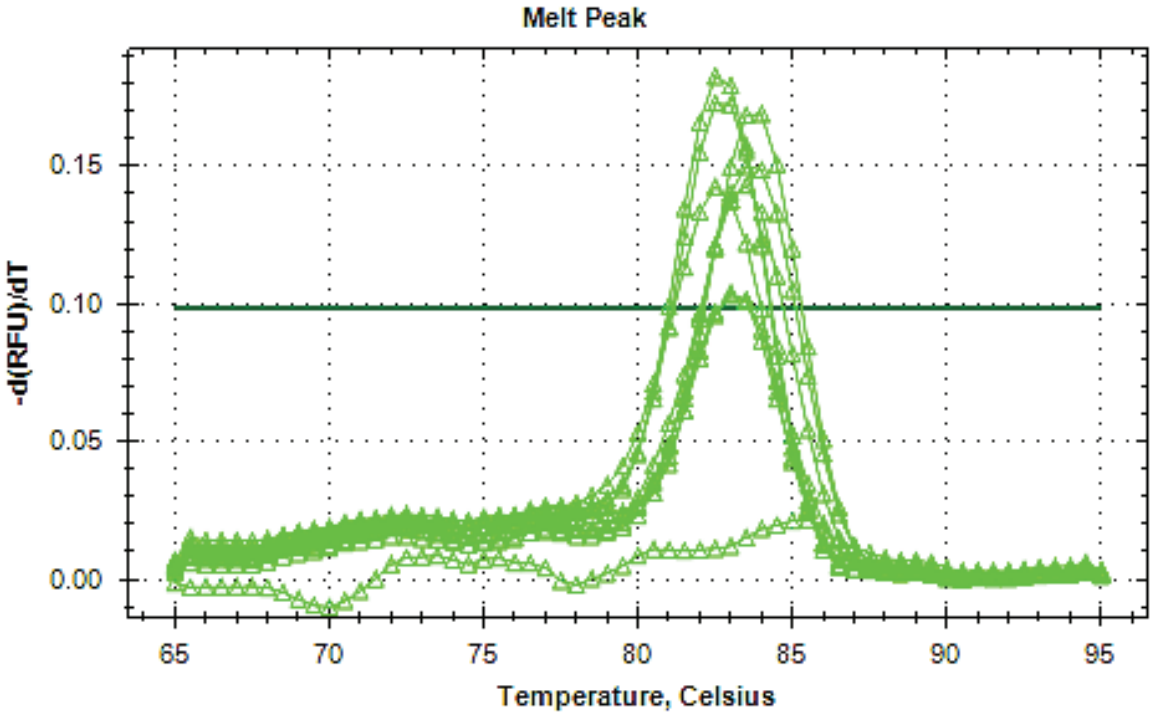

Figure.3: Real time PCR peak curve based SYBER Green DNA dye for qPCR specificity in Human Papilloma virus DNA that showed positive primer amplification at 80-85°C.

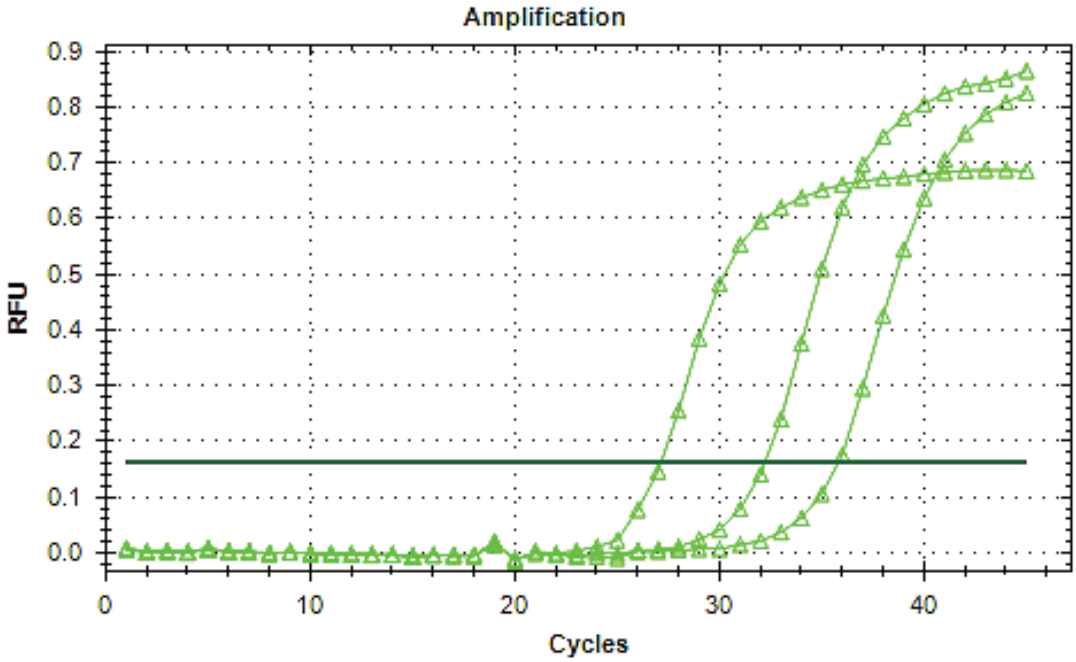

Figure.4 : Real time PCR amplification plot based SYBER Green DNA dye for detection Human Papilloma virus type 3 based L1 gene from extracted DNA of wart patient samples.

HPV genotyping has been used in epidemiological studies and in pre- and post-vaccination trials to determine the overall vaccine effectiveness and to unmask niche replacements by non-vaccine HPV types [7] .

In contrast to conventional PCR, a real-time approach enables accurate quantification of the PCR target. A standard curve that shows a highly significant correlation between the log of the PCR target and the Cp value could be obtained for our SPF10 real-time PCR. Standard curves for HPV 16 and 31 showed a dynamic range from 10 copies to 106 copies in a linear fashion. Other have used the SPF10 primers in a qPCR format to determine the viral load, but the compatibility of the real-time PCR amplicon with the LiPA assay was not evaluated groups [8] .

The high amplification efficiency of the real-time PCR results in higher amplicon concentration than with conventional PCR, leading to cross-reactivity when the LiPA assay is performed under default conditions.

Conclusions

The age average of HPV patients between 10-19 years accounted for 12 (30 %) is considered as a high group frequency and the Frequency distribution of patients with wart according to gender included 26 (65.0 %) males and 14 (35.0 %) females . The genetic study included analysis of a common DNA to HPV that was found in 20 % of cases and DNA fragment specific to HPV type 1, 3 and 6 based on real time PCR . Type 6 HPV is the most common genotype, followed by type 3.

Ethical Clearance: Taken from University of Al-Qadisiyah ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Microbial Infections Associated with Diabetic Foot Ulcer in Nasiriyah City

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Abstract

Diabetic foot ulcers (DFUs) are a major complication of diabetes lead to development of gangrene associated with reduced patient quality of life, lower-extremity amputation, hospitalization, and high morbidity and mortality. This study was aimed to identify the pathogenic microorganisms in diabetic foot ulcers and to test their antibiotic susceptibility. A total of 134 patients with diabetic foot ulcers (DFU) attending to Diabetic center/ Thi-Qar province, South of Iraq were randomly selected. Samples were cultured for isolating bacteria species and Fungi species. Antibiotic sensitivity testing for bacteria species was done by Kirby Bauer disc diffusion method. Elderly diabetic males were frequently observed to develop foot ulcers higher than female. The most of patients 56 (41.79%) were in the age group 51 to 60 years followed by the 36 (26.86%) patients were in age group 41 to 50 years. Out of 162 isolates, bacteria species was 116 (71.6%) more than Fungi species 46 (28.4%). The most isolated bacteria was *Proteus mirabilis* 25 (15.43%) followed by *Enterobacter cloacae* 22 (13.58%) and both *Escherichia coli* and *Staphylococcus epidermidis* 17 (10.49%). Diabetic foot infections are life-threatening. Many pathogens associated with diabetic foot ulcers.

Keywords*: Diabetic foot, Microbial infection, Foot ulcer*

Introduction

Diabetes is a global public health problem that is a major burden on the economy and society. Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from irregularities in the secretion and action of insulin [(1)]. During the life of diabetics may develop foot ulcers, which are a serious complication of diabetes. Approximately 25% of diabetics have a lifelong cumulative risk of foot ulcers with increased exposure to infection in 40-80% of cases [(2)]. DFU refers to a breach in the skin epithelium involving full thickness or beyond, but not reaching the ankle joints, in a person with diabetes [(3)] . Clinically, diagnoses of diabetic foot infections are based on classic findings of inflammation. The infections are classified as mild, moderate, and severe infections [(4)] . There is variation in the prevalence of isolated pathogens. The most mild foot infections caused by mono microbe, while more severe infections are caused by poly microbes [(5-6)] .

Many previous studies confirmed the presence of aerobic and anaerobic bacteria in diabetes foot infections. Moreover, high rate of antibiotic resistance in these pathogens [(7-8)].The infection may be caused by pathogenic bacteria that arise from the external environment as well as by bacteria that forming the physiological microflora of the skin [(9)]. A wide range of fungal species are found in infected foot ulcers, with *Candida* species being the most common strains isolated [(10)]. Therefore, the study aimed to increase knowledge of the diverse and abundance of microorganisms that cause diabetic foot infections for patients who admit to the Diabetes Center in Nasiriyah city, Thi-qar province, southern Iraq.

Materials and Methods

Sample collection and cultivation

A total of 134 foot ulcer swab samples were collected from diabetic patients attending to the Diabetic Center in

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Thi-qar province, which includes both sexes of different ages. These samples were collected using sterile swabs after scrubbed thoroughly with normal saline to remove surface colonizers. All collected samples were inoculated on suitable sterile culture media (such as Nutrient agar, Blood agar, MacConkey's agar, Mannitol salt agar and Brain heart infusion media) for the detection of aerobic bacteria and fungi species in the clinical microbiology laboratory of Public Health Laboratory. The plates were incubated at 37°C for 24 and 48 hrs. The purified bacterial isolates were preserved in the refrigerator at 4°C until they were used [30]. Identification of bacterial isolates was done according to cultural characteristics on Blood agar, MacConkey agar, and Mannitol salt agar. Also diagnosed with results of different biochemical tests including Oxidase test, Catalase test, Coagulase test, gram staining properties and IMVIC test for gram negative bacteria. The diagnosis was confirmed with Api-20E System (BioMerieux, France) [11].

Fungal Culture

The specimens were inoculated into Sabouraud dextrose agar and Malt Extract Base agar supplemented with chloramphenicol and incubated at 30°C for 3-5 days. Fungi colonies were identified by macroscopic appearance of colonies on Chromogenic agar compared with standard color photographs supplied by manufacturer [12].

Statistical Analysis

Data were analyzed using Excel-Data analysis tools as available in Excel software 2010. Descriptive statistics were applied to analyze all data and to calculate Mean and Standard deviation. The T-test and Chi-Square were applied to assess the significant differences (p-value $\geq$ 0.05) between different parameters used in this study.

Results

Samples culture was positive to 116 (71.60%) bacterial isolates and 46 (28.40%) fungal isolates. The identification of 116 bacteria isolates showed *Proteus mirabilis*, *Enterobacter cloacae*, *E. coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas*, *Serratia*, *Citrobacter*, *Streptococcus*, *Acinetobacter* and *Pantoea*. Fungal identification of 46 isolates was showed four *Candida* species and *Rhodotorula*. Fungal species were identified with microscopic examination as well as their characteristics on chromogenic agar. *Candida krusei* 13(8.02%) was the most prevalence yeast species followed by *Rhodotorula* 10(6.17%), *C. glabrata* 9 (5.56%) and both *C. albicans* and *C. tropicalis* 7 (4.32%).

Table (1) Numbers and Percentages of Bacterial and Yeast Isolates

NO	Genus	Number	%
1	Proteus mirabilis	25	15.43
2	Enterobacter cloacae	22	13.58
3	Escherichia coli	17	10.49
4	Staphylococcus epidermidis	17	10.49
5	Staphylococcus aureus	12	7.41
6	Klebsiella pneumoniae	7	4.32
7	Pseudomonas	5	3.09
8	Serratia marcescens	5	3.09
9	Citrobacter freundii	2	1.23
10	Streptococcus	2	1.23

Cont... **Table (1) Numbers and Percentages of Bacterial and Yeast Isolates**

11	Acinetobacter baumannii		1	0.62
12	Pantoea agglomerans		1	0.62
Total Bacterial Isolates			116	71.60
13	Candida krusei		13	8.02
14	Candida glabrata		9	5.56
15	Candida albicans		7	4.32
16	Candida tropicalis		7	4.32
17	Rhodotorula		10	6.17
Total Yeast Isolates			46	28.40
Total Microbial Isolates			162	100.00
CalX2=34.95	Tabx2=26.3	DF=16	p-Value =0.004	

Discussion

Diabetic foot ulcer (DFU) is a serious and common complication of diabetes mellitus (DM). Impaired wound healing in diabetic foot patients considers a great challenge for human healthcare worldwide. The leading problem that hamper the wound healing of DFUs are peripheral vascular disease, neuropathy, and impaired immune response, which render the diabetic patients predispose to microbial foot infections [14] .

In this study, we have noticed significant variations in certain variables such as gender and age of diabetics as well as spectrum of microbial infections. Study samples were collected from patients between the ages of 28 and 75 years. Most of the patients were in the age group from 51 to 60 years, which is normal because diabetes is more common in elderly people [5,15] . The elderly age of presentation can be explained on the basis of the fact that DFU is a chronic complication of diabetes. ٦ elderly patients had major risk to develop foot ulcer due to peripheral neuropathy, vasculopathy and poor vision [16] .

The predominant infections were in males (57.46%) more than females (42.53%). This was in agreement with previous study [17]. Male predominance in DFU could be linked to factors such as gender related differences in life styles such as smoker which as strong risk factor for peripheral arterial disease which major risk factor of diabetic foot ulceration [18].

In present study, the bacterial species were predominantly cultured (71.6%) compared with fungal species (28.4).This result come to an agreement with previous study [10,19]. The major *Candida* species isolated in our study were *C. krusei* and *C. glabrata.* This confirmed the emergence of non-albicans *Candida* over *C. albicans* [20]. *C. krusei* was the major species isolated in this study has become an important cause of candidemia [21].

In current study, Gram-negative bacteria were more predominant than Gram-positive bacteria. This result is similar to several other studies in Brazil and Malaysia [22-23]. Several studies have shown that *Enterobacteriaceae* members to be consistently associated with diabetic foot ulcers [9,6]. Among *Enterobacteriaceae* members, we found that *Proteus and Enterobacter* were dominant genera, followed by *E. coli and Staph*ylococcus respectively. Many reasons, such as source of infection, use of antibiotic drug for treatment, sample collection method and different type of infections can influence of pathogens diversity in DFI [24].

Both gram positive and gram negative bacteria isolated from diabetic foot ulcers were resistant to different classes of antibiotics. The highest resistance 100% was observed in *S. aureus* for Amoxicillin – clavulanate (20/10 µg) AMC, Cefoxitin (30 µg) FOX, Oxacillin (1µg) OX, Nalidixic acid (30µg) NA, and Cephalothin (30µg) CEP. Methicillin-resistant *S. auerus* (MRSA) strains are having a major impact worldwide, and due to their resistance to all β-lactams [29] . Eighty-Five of Gram negative bacterial isolates were resistant to the most antibiotics, such as the resistance was 100% for Ampicillin (10µg) AMP, Amoxicillin-clavulanate (20/10µg) and Cefoxitin (30µg). The development of resistance has several risk factors linked to the severity of the infection and that resistance is associated with increased mortality [25-26].

The effective of Imipenem and Amikacin were reported against aerobic Gram-negative bacteria [5]. The high resistance to Ampicillin and Amoxicillin-clavulanate should warrant careful consideration of these drugs requires experimental treatment. Another critical observation of our study was the *Acinetobacter* spp. had already developed resistance to most of the common antibiotics (AK, CIP and IMP). In Maniple, *Acinetobacter* was documented as multidrug resistances [27] . We found an increasing trend in the prevalence of multi-drug resistance bacterial pathogens, highlighting the need for surveillance programs that guide appropriate empirical treatment and thereby reduce the risk of DFI complications. In present study agree with another study in Malaysia focus on the selection of empiric antimicrobial therapy depends on many factors such as infection severity, medication allergies ,overall patient condition, antibiotic activity, previous antibiotic, toxicity and excretion, and glycemic control [28] . Therefore, early diagnosis and proper identification of lesions with causative agents, prompt initiation of appropriate antimicrobial therapy are essential for controlling the infection and management of complication of diabetic foot infections and improving the quality of life.

Conclusion

Diabetic foot infections are polymicrobial in nature. Proteus mirabilis in Gram negative bacteria followed by Staphylococcus sp.in Gram positive organism and Candida spp in fungal were the predominant pathogens. Empirical antibiotic selection should be followed by culture guided adjunctive therapy. Imipenem and Amikacin and would be appropriate for empirical treatment.

Ethical Clearance : Taken from University of Thi-Qar ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Genotyping of Human Adenovirus Causing Conjunctivitis and Correlation to Age and Gender in Al-Diwaniyah Province in Iraq

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Abstract

Human conjunctival swab samples from 108 patients were male 58 (53.7 %) and female 50 (46.3 %) with a male to female ratio of 1.16:1.for the period from October 2019 to February 2020. Patients were selected according to clinical diagnosis by a specialist ophthalmologist. Diagnosis was confirmed by real time PCR that showed 84 patients (77.8%) with positive result; %).The study groups were divided into groups with different age and gender. Those aged 15–40 years had much likely to be infected than those aged 55 years. Positive rate in mild cases was 8 (26.7 %), in moderate cases was 40(95.2 %) and in severe cases was 36 (100.0 %). Phylogenetic tree analysis of local conjunctivitis based on the Hexon gene partial sequence of human adenovirus show genetic relation to human adenovirus 8 and 56 hexon gene .

Key words: *Human adenovirus, conjunctivitis, hexon gene, severity*

Introduction

Worldwide, millions of people are affected every year via acute conjunctivitis. Chemicals, allergens or infectious agent can cause the disease, but the incidence and prevalence of case are caused by viruses and bacteria. Viral conjunctivitis is responsible for about 80% of all cases of acute conjunctivitis [(1)] .

Viral conjunctivitis are considered as the most common external eye infection and Human adenovirus (HAdVs) the biggest non-enveloped double-stranded DNA virus being the most common (90%) causal agent. In areas such as workplaces (including hospitals), schools and swimming pools, it can occur sporadically or in epidemic [(2, 3)] .

Human adenovirus are members of the Adenoviridae family and the Mastadenovirus genus. Human adenovirus were classified to seven (A-G) based on phylogenetic analysis, genetic organization, growth characteristics and oncogenicity, including over 70 stereotypes and 90 serotypes [(4-6)] .

Human adenovirus types D -8,9,13,19,33 , 37,53,56 and 64 are common types that cause conjunctivitis [(1,7)] . The transmission of this extremely contagious infectious disease is facilitated by the ability of viral particles to survive for weeks on dry surfaces and by the fact that for many days before clinical features are clearly visible, viral shedding may occur. Transmission is usually through contact with respiratory or eye secretions, which include contaminated fomites, such as towels [(2)] .

These infections are relatively mild in immune competents patients, but could be severe (can be life threatening) in immune compromised patients [(6, 8, 9)] .

Typically, the clinical disease lasts 7-21 days and is usually self-limited. Transmission via contaminated surfaces, equipment, eye drops, or hands is primarily through contact with infected ocular secretions. Several days before symptoms progress about 14 days after symptoms begin an individual may become infectious

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[10] .

Symptoms of human adenovirus (HAdV) conjunctivitis include ocular pain, sensation of foreign body, excessive lacrimation, eyelid oedema, lymphadenopathy, conjunctival hyperemia and follicles, chemosis, keratitis [2]

Prevention and control transmission of HAdVs include :

Adequate hands washing with soap and water, especially after contact with an person with a signs of flu-like illness, when coughing or sneezing, cover mouth and nose, avoid touching eyes, nose or mouth with unwashed hands, avoid close contact with sick people and stay at home when people are sick themselves [11, 12] .

Materials and Methods

Patient's

Patients were selected after examination by an experienced ophthalmologist for human conjunctivitis and determining the degree of severity. Conjunctival swabs were taken for 108 Patient 50 (46.3%) female, 58 (53.7) male and kept in VTM

Collection of Samples

One hundred and eight conjunctival swabs were taken from fornices with sterile dacron swabs of viral transport media without using topical anesthesia from infected eye or both infected eyes if bilateral infection of the patients noticed. The Dacron swabs including inside tubes of transport media were transported in a bag contains ice packs and then stored at -80 ℃ until they were used in qPCR and conventional PCR techniques.

Molcular method

DNA extraction were done from each patient sample that were DNA-Sorb-B nucleic acid extraction kit® was used according to the instruction manual of a manufacturing company Real-time PCR was carried out for detection of Human Adenovirus in DNA by using the designed primers specific for hexon gene shown in table (1)

Primer	Sequence		Product size
Human Adenovirus hexon primer	Forward	GGTTCCTCGTCCAGATGCTC	114 bp
	Reverse	TGCTCATGGGCTGGAAGTTG	

Table 1. : Primer for Real-time PCR

DNA Sequencing Method

DNA sequencing process was accomplished for detection Human Adenovirus genotypes in suspected of positive swabs samples by Real-time PCR process based on phylogenetic tree analysis of hexon gene that amplified by conventional PCR method and the primer shown in table (2) . Phylogenetic tree analysis was performed based on NCBI-BLAST alignment identification and neighbor distance phylogenetic tree analysis (MEGA X).

Table 2. : Conventional PCR primer

Primer	Sequence		Product Size
Hexon PCR	Forward	GGTTCCTCGTCCAGATGCTC	529 bp
	Reverse	AGGTAGACGGCCTCGATGAC	

Result

Age and Gender

According to table (3), the highest percentage of age subgroups was recorded in those with ages between (30-39) yeras (22.2 %); whereas, female patients were (53.7%) .

Table 3. : Descriptive statistics (frequency and percentage) of patients, according to age and gender

Subgroups		Frequency	Percentage
Age (Years)	0.5-9	12	11.1
	10-19	20	18.5
	20-29	22	20.4
	30-39	24	22.2
	40-49	16	14.8
	50-59	8	7.4
	60-69	2	1.9
	70-80	4	3.7
Gender	Male	50	46.3
	Female	58	53.7
PCR Result	Positive	84	77.8
	Negative	24	22.2

Real time PCR

The frequency distribution of patients according to Rt-PCR results is shown in table 3. Positive results were recorded in 84 patients (77.8 %); whereas, negative results were recorded in 24 patients (22.2 %). The mean age of patients with positive results was 28.89 ± 16.23 years while the mean age of patients with negative PCR was 36.17 ± 22.17 years (figure 1) .

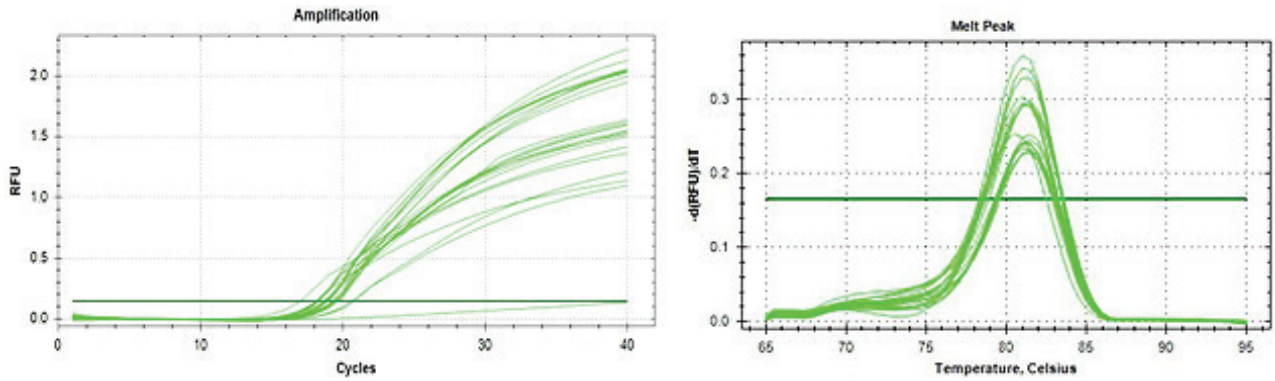

Figure 1 : Q-PCR amplification and melt curve for the study sample

Table 4: Association between severity of disease and positive PCR results

PCR	Severity		
	Mild n = 30	**Moderate n = 42**	**Severe n = 36**
Positive	8 (26.7 %)	40 (95.2 %)	36 (100.0 %)
Negative	22 (73.3 %)	2 (4.8 %)	0 (0.0 %)
P-value Mild versus moderate	< 0.001 C HS		
P-value Mild versus severe	< 0.001 C HS		
P-value Moderate versus moderate	1.000 F NS		

n: number of cases; **C**: Chi-square test; **F:** Fischer exact test; **HS:** highly significant at $P \leq 0.01$; **NS:** not significant at $P > 0.05$

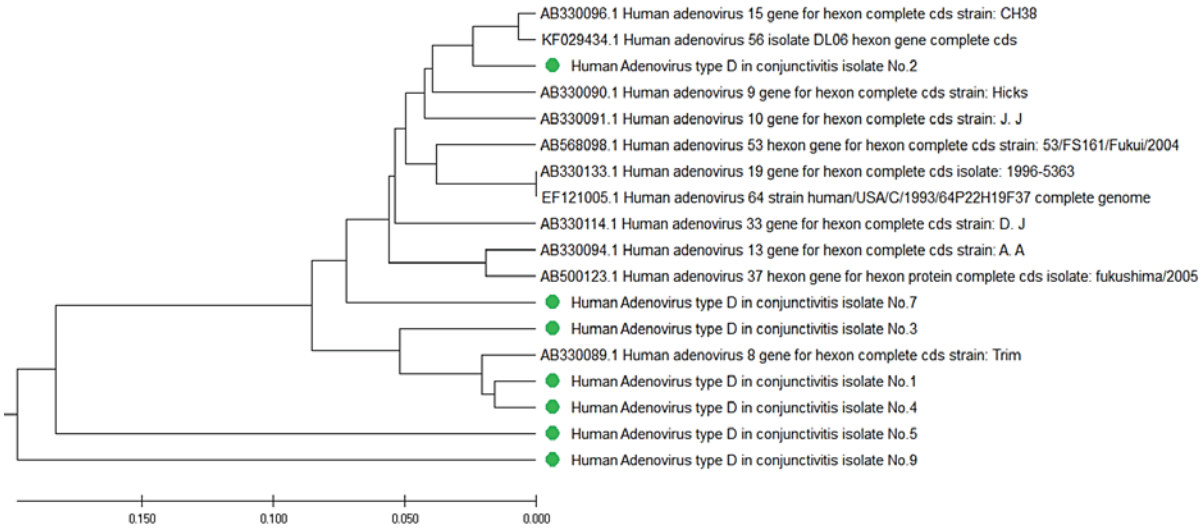

Figure 2 : Phylogenetic tree analysis based on hexon gene partial sequence of in local conjunctivitis of human adenovirus

Phylogenetic tree analysis based on Hexon gene partial sequence in local conjunctivitis Human adenovirus PCR positive sample that common used for genotyping identification is shown in figure 2. The phylogenetic tree was constructed using Maximum Likelihood method and UPGMA tree method in (MEGA X version). Human Adenovirus conjunctivitis isolate No.1, No.3, No.4, No.5, No.6, No.8, No.9, No.10 were showed genetic related to NCBI-BLAST Human adenovirus 8 gene for hexon (AB330089.1) and Human Adenovirus conjunctivitis isolate No.2and No.7 were showed genetic related to NCBI-BLAST Human adenovirus 56 isolate DL06 hexon gene (KF029434.1).

Discussion

The different distribution of age group may be explained by different levels of protective antibodies and the possible variation in their exposure risk according to the different social activity levels and occupational risk. Patients with HAdV conjunctivitis. Those aged 15–40 years had much likely to be infected than those aged 55 years. However, that because younger age more active than elderly, also the health education levels

should be taken into consideration, and this opinion is agreed with [11, 13].

In present study the association between gender of patients and PCR results was insignificant and the male to female ratio of 1.16:1 The correlation between positive result in q-PCR and severity is highly significant in severe case while decrease in moderate case and lastly mild case can be explained by many reasons such as :

IFN-alpha type I effected by EA1and E1B protein play role in this is process because this protein is immediately expressed (after an hour of infection) that lead to block expiration of IFN-alpha type I , also , E1A blocks the induction of MHC class II genes and this involves a block in transcription complex formation that affected on severity of disease. while E1B works by interfering with the induction of IFN genes .

A study found that toll like receptor (TLR) and proteins of HAdVs play important role in stimulation of immune system and EAI ,E1A, E1B, E3, and E4 attenuated innate response [14] .

One study found that E1A expression in infected conjunctival cells is causing repression of the host IFN response allowing increased expression of the viral early gene, which, in turn, results in increased viral replication [8].

Another study found that when DNA of AdVs is detected in TLR or in plasmacytoid dendritic cells there was an increase stimulation for the production of interferon alpha [15]

One study concluded that viral conjunctivitis affects patients in all age groups regardless of ocular history [16]. The researcher suggested that proper hygienic and disinfection of possibly contaminated environmental surfaces should be routinely practiced to minimize the dissemination of HAdV-associated conjunctivitis [13] .

One study in Tokyo found that the most common type causing adenoviral conjunctivitis was HAdV-8 [17] . Also, Study in Saudi Arabia and Southwest China found the same result that HAdV-8 is the predominant [18, 19]. All this mentioned study agree with current study.

A Study in University of Babylon found that HAdVs-19 is the predominant [20]. Also one study found HAdV-4, HAdV-37 and HAdV-53 were the most common types in Beijing, China [13].

Conclusions

Finally, it was expected that there were at least three genotypes of Human Adenovirus were circulating in Iraqi environment, and probably other genotypes may cause infections due to the fact we were carried a sequencing for only 10 samples of total 108 of positive cases.

Ethical Clearance : Taken from University of Al-Qadisiyah ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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Study How People Are Ready To Be Infected with the Corona Virus (COVID-19) in Najaf Province/Iraq

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Abstract

The current study was designed to investigate some variables that affect the individual's readiness to contract coronavirus (COVID-19) for a specific sample in the Najaf governorate. About 70 cases have participated in the questionnaire that was put on social media for the period from (1 March 2020) to (1 May 2020) of the population of the Najafi community – Iraq. The ages ranged between (16-70) years. The overall results obtained in this study showed that a group of variables showed a high willingness in the individual to contract Coronavirus, such as not taking vitamins, zinc, and complementary minerals and less exposure to the sun to support the immune system at a rate of (58.57%), The use of surgical masks instead of masks N95 and the use of Dettol at a high rate when studying the study to get rid of the virus and eliminate it by ratios (88.57%, 47.14%) respectively At a probability level ($P < 0.05$). In addition, there was a lack of awareness of preventive methods, such as continuing to wash hands with soap and water in the proportion (8.57%) of the total sample. Use of tissue during sneezing in the public, with average proportions (37.14%) with the participation of samples from most of the Residential neighborhoods in Najaf. The results also showed the variance of the mean value according to each axis which were, first axis (health activities), second axis (Commitment to prevention methods) and third axis (Commitment to instructions in light of the conditions of society and instructions) with values of 2.12, 2.75 and 2.22, respectively, beside the relationship and connection among them.

Keywords**:** *COVID-19, Residency, Age, Gender, antiseptic.*

Introduction

. The corona virus that first emerged in the city of Wuhan (Hubei, China) spreads widely around the country and thus to other parts of the world. Due to the incidence of this epidemic and the potential for global spread, then the WHO confirmed a "world health emergent situation" on 31 January 2020. Later, a contagion condition was confirmed on 11 March 2020. To date, there is really no effective antiviral for the treatment of COVID-19 As the vaccines have not been approved [(1,2)]. CoVs is a member of the Coronaviridae family, which is capable of infecting abroad spectrum of hosts and causes illnesses with symptoms of cold or flu to extreme and fatal diseases such as severe acute respiratory syndrome, Middle East respiratory syndromes, and, as of today, COVID-19[(3)]. Coronaviral is a club-like glycoprotein (crowns) or (halos)- envelope of coronaviruses. So It gets the name of coronaviruses. However, in 2003 a new corone virus appeared leading to autoblocking SARS (severe acute respiratory syndrome). These viruses are responsible for common cold and typically cause self-limited high-respiratory tract infections. In 2012 a coronavirus associated with camel transmission was found to be causing Middle East Respiratory Syndrome (MERS). Both viruses showed a range of clinical presentations, from moderate upper respiratory system symptoms and sometimes asymptomatic infections to serious, acute respiratory syndrome, which resulted in sepsis and multi-organ failure, and in many cases death[(4)].

Materials and Methods

Our current study was conducted using a

questionnaire designed specifically for the subject of the research. The questionnaire was designed using Google Forms and consisted of 20 questions with three main themes To clarify the purpose and idea of the research, the first axis included health activities and the second axis, commitment to prevention methods. As for the third axis, it is concerned with adhering to health instructions in light of the outbreak of the Corona virus in Iraqi Najafi society. Then the questionnaire was presented to the general public in the province of Najaf through social media sites such as Facebook, Instagram and WhatsApp, being the most used site for people in Najaf society for two months.

Result and Discussion

1. Sample distribution

The questionnaire samples for our current study, which numbered 70, were divided into three main axes to facilitate the study of data statistically. The first axis came under the heading of (health activities) and It included six questions dealing with the details of this axis , The second axis (commitment to prevention methods) included only 3 questions, while the third axis came under the title (commitment to health instructions under the conditions of society and also included three questions that deal with the problem of this axis. The study also shows the distribution of samples according to Age groups, Gender, Social status, Blood Group and Residency.

Distribution of samples according to age groups and Blood type group

The results of our current study showed that there is participation for most age groups, and the lowest age group ranged between 16 years and the largest about 70 years. The results of the statistical analysis showed Standard deviation (7.595) while the mean was (29.17). The age group that participated most with the questionnaire was between (20-30) years (Figure1). We note that older people are more likely to be infected due to chronic diseases. The female gender was the most participating in the questionnaire (58.57%) with Less participation rate for single people (44.29%).The studies received from the affected countries [5] showed that women are less likely to be infected than men because of bad habits such as smoking, alcohol and general hygiene such as hand washing and other unhealthy behaviors at higher levels of women, in addition to that the active immune response in women is greater than men [6].

The results also showed that the blood group O was the most frequent (26) with a valid percent (37.1%) Followed by AB group by frequent [21] with a valid percent (15.7%) As shown in figure (1). Study (7) showed that Individuals with blood group A had a higher risk of infection compared with those of blood group O. The distribution of blood groups in patients who died due to COVID-19 disease was 25.24%, 9.22%, 24.27%, and 41.26%, for Groups O, AB, B, and A, respectively.

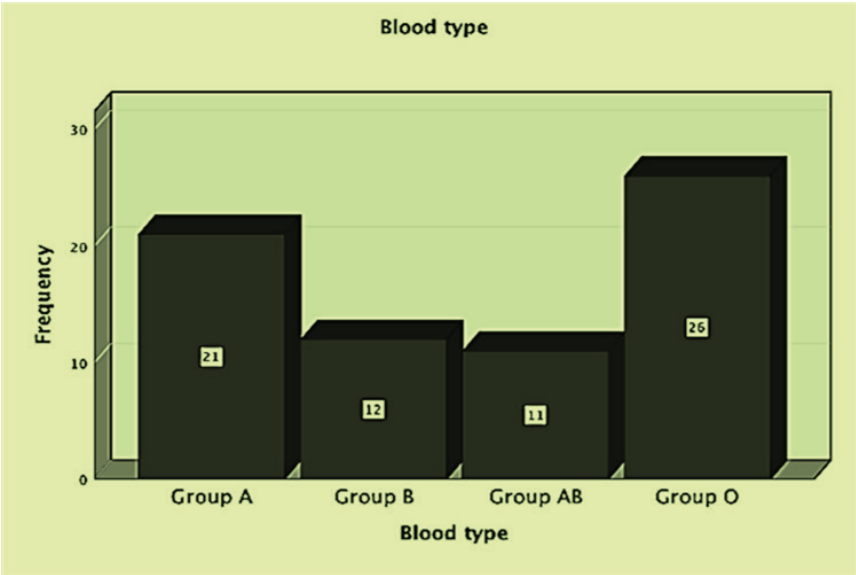

Figure1. shows the frequency distribution of Blood groups participating in the questionnaire.

The results of our current study also showed the participation of most of the regions in the Najaf governorate, and the highest frequency were in the popular AL-Askaree area, which is located in the northern sector of the Najaf governorate with a valid percent (14.3%) and cumulative percent (35.7%) as shown in figure (2).

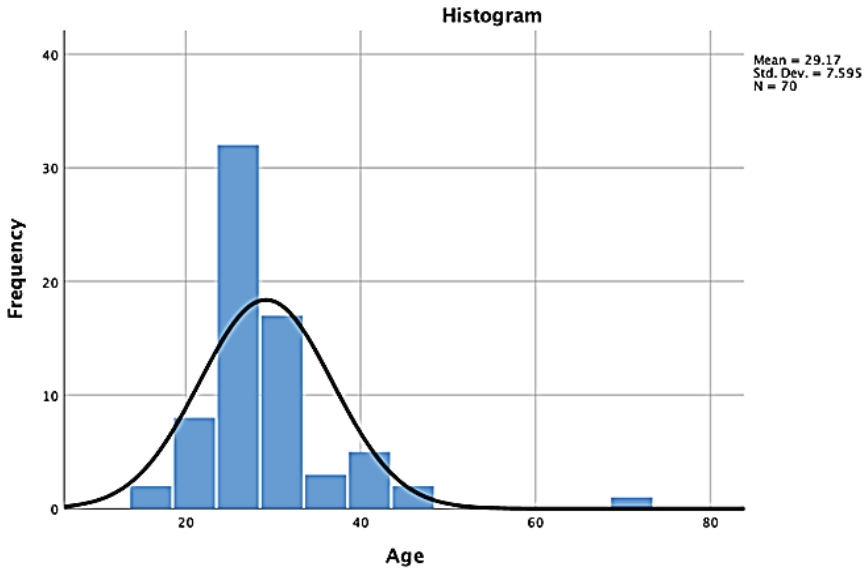

Figure 2. Shows the frequency distribution of the age groups participating in the questionnaire

Distribution of samples according to the axis

This topic included a study of most of the questions asked in the questionnaire. The study divided the questionnaire into three axes.

First axis (health activities)

Six questions, including evidence of a high readiness for corona infection, included not taking vitamins (D) and minerals properly and sufficient for the body, not being exposed to the sun directly, eating healthy and using sterile wipes during sneezing with variance (0.095), x^2 (1) as shown in figure (3).

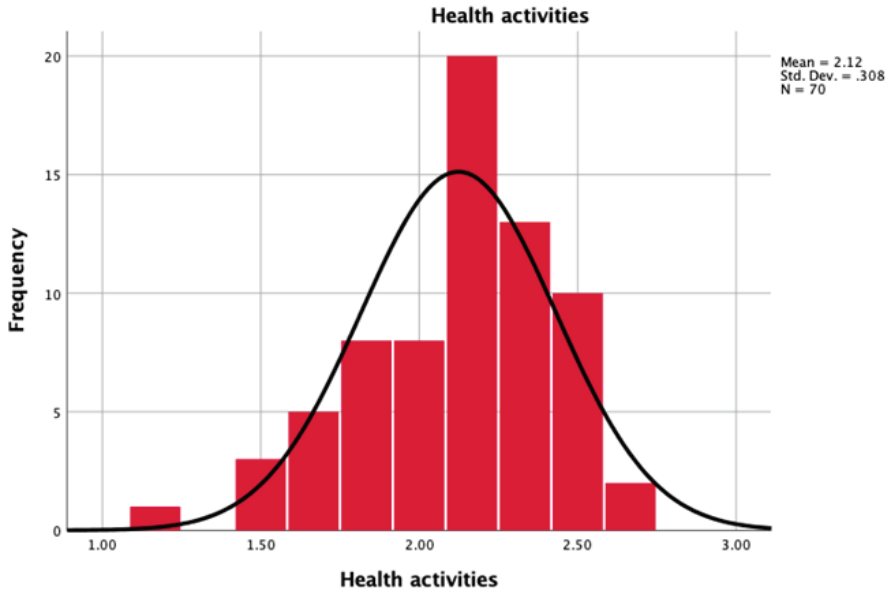

Figure3. Shows the First axis (health activities)

That a lack of Vitamin D leads to a lack of body immunity, and here it rises, and here the rate of individual readiness to become infected with Corona virus increases, as studies have indicated that infection with infectious diseases such as corona virus may be more dangerous for people with chronic lung diseases if they have a deficiency of vitamin D. Studies have also shown not to overeat the D3 pills and called for going out and moving in the open air when the sun is shining because taking them without a prescription can cause kidney damage. [8] Zinc is necessary for a healthy immune system, as it correctly synthesizes DNA and promotes healthy growth during childhood and wound healing. According to several studies, the human body needs zinc to activate T-lymphocytes, and zinc deficiency can severely weaken the immune system and thus greater susceptibility and high preparedness for infected with COVID-19 [9]. Nutrition experts at the United Nations have advised on what a person can eat and what must be avoided in order to prepare for fighting the Corona virus. Nutrition is important to maintain a strong immune system that resists viruses [10]. As the World Health Organization has made clear, that the most important points for maintaining a healthy nutrition, including more energy food and drinking water in sufficient quantities and avoiding foods that contain high levels of fats and sugars, especially processed foods [11].

Second axis (commitment to prevention methods)

The results (Figure4) showed the outcome of three questions such as masks and their types (surgical masks and N95), and do you continue to wash your hands with soap and water? With variance (0.021), x^2 (0.236*) P- value (0.049).

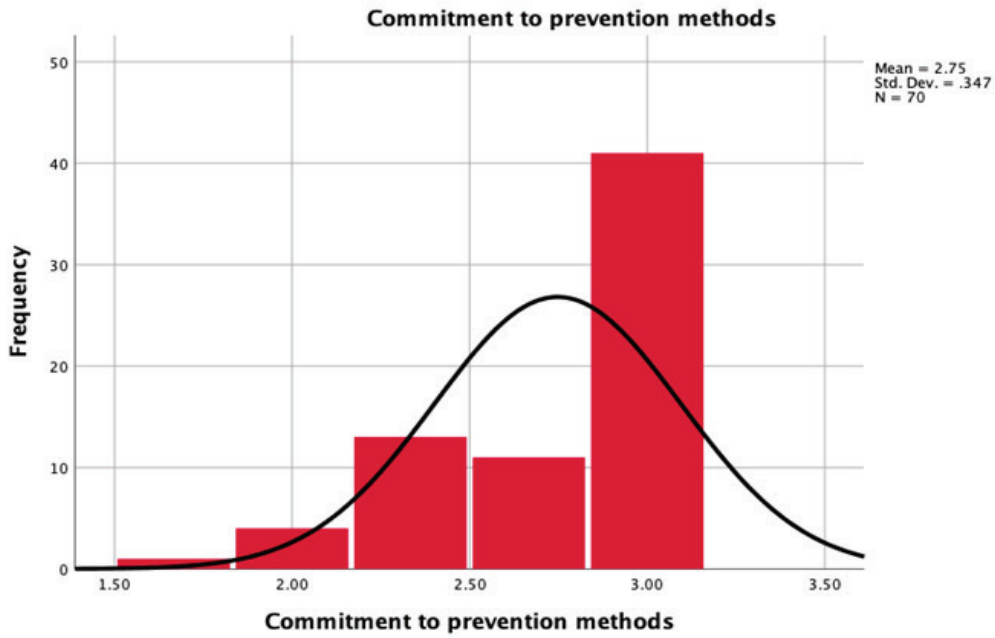

Figure4. Shows the Second axis commitment to prevention methods)

Our current study did not coincide with many studies from outside Iraq, where the proportion of individuals wearing surgical masks was much more than the wearers of N95 (62%,8%) Respectively , and therefore a high expectation of a rapid outbreak of coronavirus in the coming days is the importance of high-risk masks by limiting the transmission of the virus from one person to another in addition to the lack of availability of masks N95, the clothes are manufactured locally and costly in Iraq. Masks are effective only if used with frequent washing of hands with soap and water, which has proven to be very effective in reducing the spread of the virus[12,13].

Third axis (commitment to health instructions under the conditions of society)

It included many questions and The results was showed that Sterilizers used and their types are the most used, as the results of the study showed that Dettol occupied the largest proportions in terms of use as sterilizers and antiseptics and also communication with infected people has a very big impact on the community's willingness to contract coronavirus, not just individuals. The percent (2.9%) means a high probability of development and increase of cases of Coronavirus infection in Najaf in the coming days with variance (0.064)), x^2 (0.333^{**}) P-value (0.005) demonstrated in figure (5).

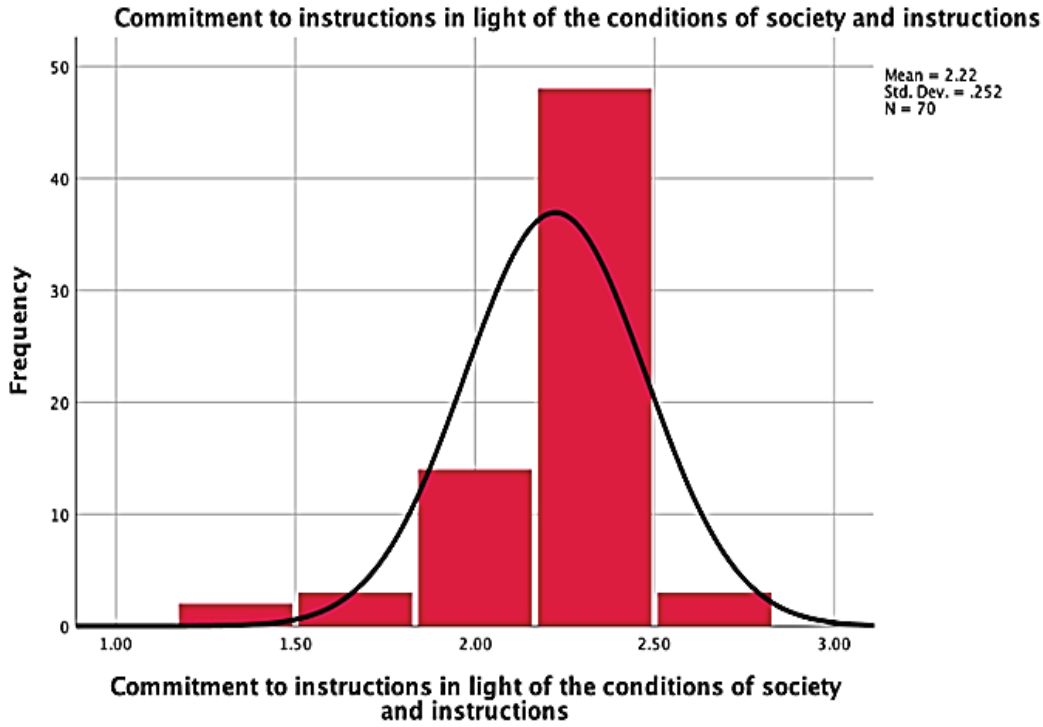

Figure5. Shows the Third axis commitment to health instructions under the conditions of society

Our current study came up with results showing the frequent use of Dettol as a disinfectant and sterilizer, and this does warn at a high speed of an outbreak of Corona virus in the coming days in Najaf because of a lack of awareness among people of the purpose of using each type of disinfectants or sterilizers in comparison with it or the lack of availability in the markets and the high price of alcohol, which is more effective because Attacks and destroys the viral sheath protein of (COVID-19) [14]. As the study [15] showed Ethanol in high concentrations (> 70% effective against SARS-CoV-2 for surface disinfection.), In addition to safe and safe alcoholic sterilizers for use on human skin, more than Dettol. Our study was compatible with a study (16) in which it was clear that the rate of contact with infected people heralds a high rate of future infection rates. A study by the researcher [17] also demonstrated that societal awareness of the pandemic would reduce the incidence of the virus and spread it to the country in the future. The world is fighting a real war with the Corona virus and people are fighting psychological wars because of the rapid transmission of the virus and the widening of its spread, as if everyone is waiting for its role, and here the community awareness takes on a big role as the study showed [18]. And confronting the pandemic psychologically is no less important than resisting it physically. It was noted that there were significant differences (Figure6) at the level of probability ($p < 0.05$) where comparing the interaction of the three axes.

Conclusion

CoviD-19 may affect all ages according to the study, and women are more succeptible to infection than men. findings showed tha more injuries may occur in the future for several reasons including the standard of living, careless habits, low preventive measures such as using masks without filter.

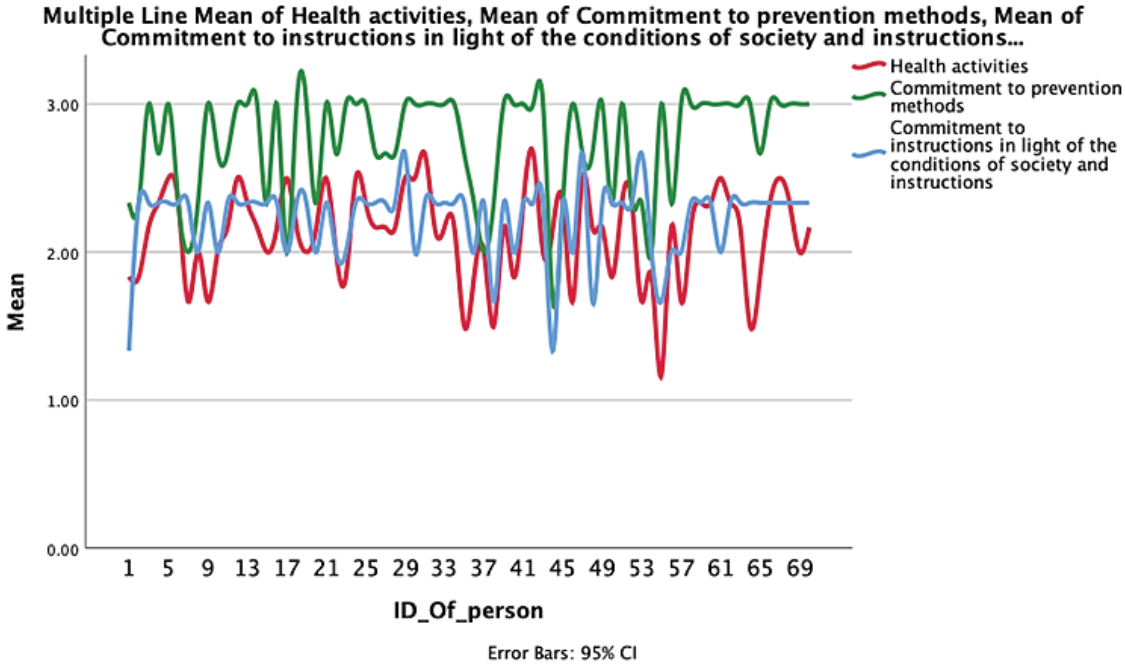

Figure6. Shows the statistical relationship between the three axes

The study found that exposure to sunlight and taking vitamins and Zinc pills is of significance to lower possibility of infection. Majority of the studied samples had low awareness to preventive habits against the virus. the study assumed that the increase of infection may also be attributed to inappropriate or low quality sterilizers and detergents. Individual immunity plays the highest role in preventing infection. Among blood types, the type O was more likely to be affected. Finally, adherence to health instructions is very necessary to prevent and lower virus infection.

Statically Analysis: Data were analyzed using statistical data analysis program SPSS (version 26).

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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Type of Manuscript: Research

Detection of Mycotoxigenic Fungi on Food from Markets with the Polymerase Chain Reaction Method for Halal and Thoyiban Food

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Abstract

Secondary metabolites of filamentous mold are mycotoxins, which in some situations can develop on foods derived from plants or from animals. *Fusarium*, *Aspergillus*, and *Penicillium* are the most common types of mold that produce mycotoxins and also often contaminate human food and animal feed. Aflatoxins including aflatoxin B1, B2, G1 and G2 are produced by *A. flavus* and *A. parasiticus*. M1 and M2 aflatoxins are found in dairy products. In this study, we used PCR to detect and identify mycotoxigenic fungi material in foods from traditional markets and supermarkets in Surabaya, Indonesia. Samples of chicken meat from a traditional market and from a supermarket (10 pieces each) were placed in a conical tube and crushed in PBS. The crushed samples were centrifuged and the supernatants were cultured on potato dextrose agar (PDA) media and observed using a reverse microscope. DNA was isolated from cultured samples and subjected to PCR with primers specific for genes encoding aflatoxins. Interestingly, we revealed that the Polymerase Chain Reaction (PCR) analysis showed *A. flavus* and *A. ochraceus* were present on chicken meat sold at traditional markets and supermarkets. In sum, enhanced precautions may be needed to ensure that foods sold in traditional markets and supermarkets are free from molds that have the potential to produce mycotoxins. Further studies are needed to detect and identify the prevalence of mycotoxins in the food supply.

Keywords: *Aflatoxin B, mycotoxins, traditional market, supermarket.*

Introduction

Secondary metabolites of filamentous mold products and in some situations are present on foods derived from plants or animals. *Fusarium*, *Aspergillus*, and *Penicillium* which are the most common types of mold that produce mycotoxins, often contaminate human food and animal feed. These molds grow on food or feed ingredients, both before and during harvest or when foods are improperly stored[1,2]. Mycotoxins cannot be detected by smell or taste, but can significantly reduce livestock production performance and pose a threat to human health[1].

Aflatoxin B1, B2, G1 and G2, are the main types of mycotoxins produced by *A. flavus* and *A. parasiticus*[3]. The presence of mycotoxins has expanded at all levels of the food chain. In terms of food security and its implications for human health and the economy, mycotoxins are an important contaminant of the food chain[4]. In this study, we used PCR to detect and identify the presence of mycotoxigenic fungi in human foods purchased from traditional markets and supermarkets in Surabaya, Indonesia.

Material and Methods

Experimental Site:

Whole chicken samples were taken from several traditional markets and supermarkets in the city of Surabaya, Indonesia (10 pieces each).

Material and Research Tool:

PBS (Thermo Scientific), potato dextrose agar (PDA) media (Himedia), DNA extraction kit (Qiagen), PCR reagents (Qiagen), reverse microscope (Olympus, type OPTO-EDU A12.2602-T), PCR machine (Bio-Rad).

Research Procedure and Data Collection:

Each sample was placed in a conical tube and crushed in PBS. The mixtures were centrifuged and the supernatants were cultured on PDA media and observed using a reverse microscope. DNA isolation is done using materials from the Qiagen factory. 900 µL cell the lysis solution is put in a 1.5 tube mL and 300 µL of culture sample were added. The tube is turned 5-6 times so that the solution can mixed up. The tube is incubated for 10 minutes at room temperature (during incubation flip the tube 2-3 times), then centrifuged with a speed of 13,000-16,000 rpm for 20 seconds at room temperature. The supernatant discarded, and vortexed for 10-15 seconds. After that, added to the tube 300 µL nuclei lysis solution and solution pipette 5-6 times to break the wall mushroom. Then add 100 µL of protein precipitation solution, and divortex for 10-20 seconds. The supernatant was transferred to the tube 1.5 mL of new sterile that has been previously filled with 300 µL isopropanol. The centrifuge tube with a speed of 13,000-16,000 rpm for 3 minutes at room temperature. The solution stirring by turning the tube up to visible fine white threads of DNA. Centrifuged tube with a speed of 13,000-16,000 rpm for 3 minutes at room temperature, so you can see the pellet on the bottom of the tube. The supernatant was removed, and also added 300 µL 70% ethanol. The tube is turned over to make the pellet and the solution is mixed then centrifuged with speed of 13,000-16,000 rpm for 3 minutes at room temperature. Slowly ethanol is removed with a pipette. The tube is reversed on absorbent paper and dried pellets for 10-15 minutes. Last added 50 µL DNA rehydration solution into the tube. DNA is stored overnight at 4 ºC, then DNA is stored at 2-8 ºC. PCR was carried out in two stages 2 (two) different pairs of primers. To identify the presence or absence of mycotoxigenic genes pyrG using PCR we designed the following primers:

1) Af.pyrg.far.F, (5′-CCTCAAACAATGCTCTTCACCC-3′); Af.pyrg.R (5′-CATTCCCTATCAACTCCCCCTC-3′)
2) flank.pyrg.F3 (5′-TATGGCTTCTCTCGGCTCAG-3′); flank.pyrg.R3 (5′-CCACGGTTGTCTTCTTGGTGTAG-3′)
3) Af.pyrg.R (5′-CATTCCCTATCAACTCCCCCTC-3′); flank.pyrg.F1 (5′-GCTCTATTCAACGGACTTCAACC-3′)

PCR amplifications were performed 20 pmol of primers were added and mixed to obtain 50 µL final volume of the PCR mix. PCR was carried out using 30 cycles of denaturation at 94 °C for 30 sec, annealing at 58 °C for 30 sec, and extension 68 °C for 150 sec. PCR products were resolved by electrophoresis on an agarose gel, which was stained with ethidium bromide and documented on a UV box (Gel Ninja equipped with a camera).

Results And Discussion

Based on this study, *A. flavus* and *A. ochraceus* were present in chicken samples purchased from traditional markets and supermarkets, respectively (Figures 1 and 2). Traditional markets have 2 sample positive and supermarkets have 1 sample positive. These results suggest that supervision of foods in both traditional markets and supermarkets is needed. *Aspergillus* spp. such as *A. flavus* and *A. parasiticus* can be found on various substrates, including soil, fruit leaves, and seeds, which are all typical ingredients for manufacture of animal feed products from agricultural commodities[5,6]. Molds found in animal feed can produce aflatoxins as secondary metabolites that are known to have carcinogenic, mutagenic, teratogenic[7,8], hepatotoxic and immunosuppressive properties[9].

The results of the present study are similar to those of Irdawati *et al*. who found that *Aspergillus* contamination was present in traditional markets located at Pasar Raya, Padang, Indonesia[10]. In addition, a study by Handajani *et al*. showed that shrimp patties have a 10-fold greater risk of contamination with various aflatoxins[11]. Aflatoxins are mycotoxins that are mainly produced by the fungi *A. flavus* and *A. parasiticus*[12], as well as by several other fungi such as *A. ochraceoroseus*, *Aspergillus* SRCC 1468, *Emericella astellata*, and *Emericella* SRCC 2520 species[13]. Relative to other mycotoxins, aflatoxins have higher toxicity and higher potential to cause disease[11]. According to the International Agency for Research on Cancer (IARC), aflatoxin B1 can cause cancer in humans, particularly liver cancer (hepatocellular carcinoma)[14].

Aflatoxins have the potential to be carcinogenic, mutagenic, teratogenic and immunosuppressive[12,15,16]. As such, precautions must be taken to ensure that the food supply is not contaminated by fungi that produce mycotoxins.

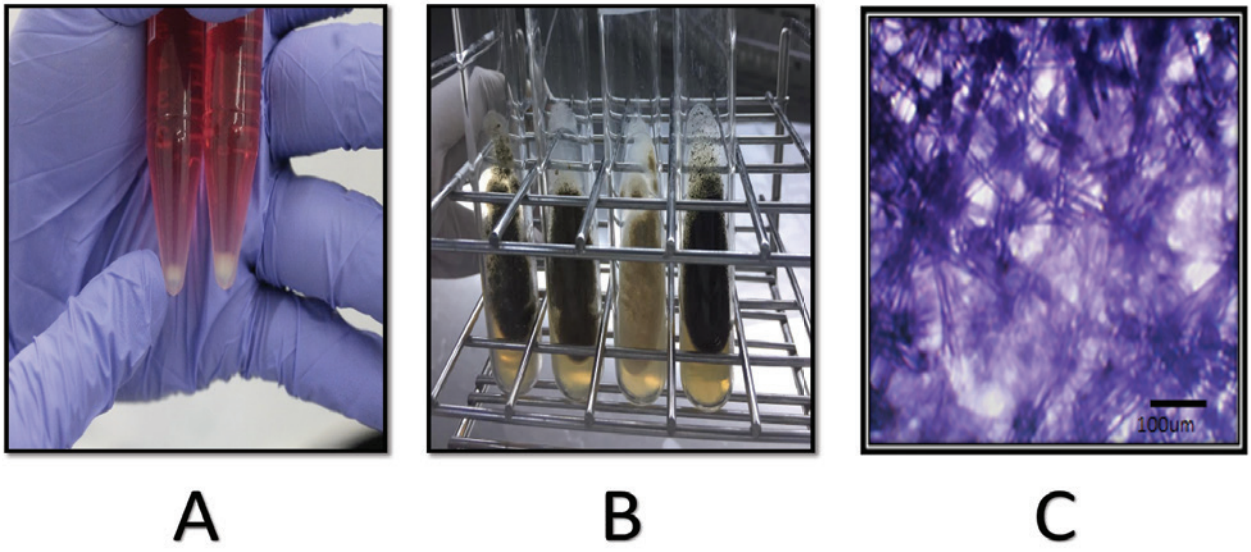

Figure 1. Preparation of food samples for analysis. A. Sample after homogenization and centrifugation; B. Propagation of supernatants on PDA media; C. Staining and observation of culture growth by reverse microscopy with HE staining and 100× magnificient.

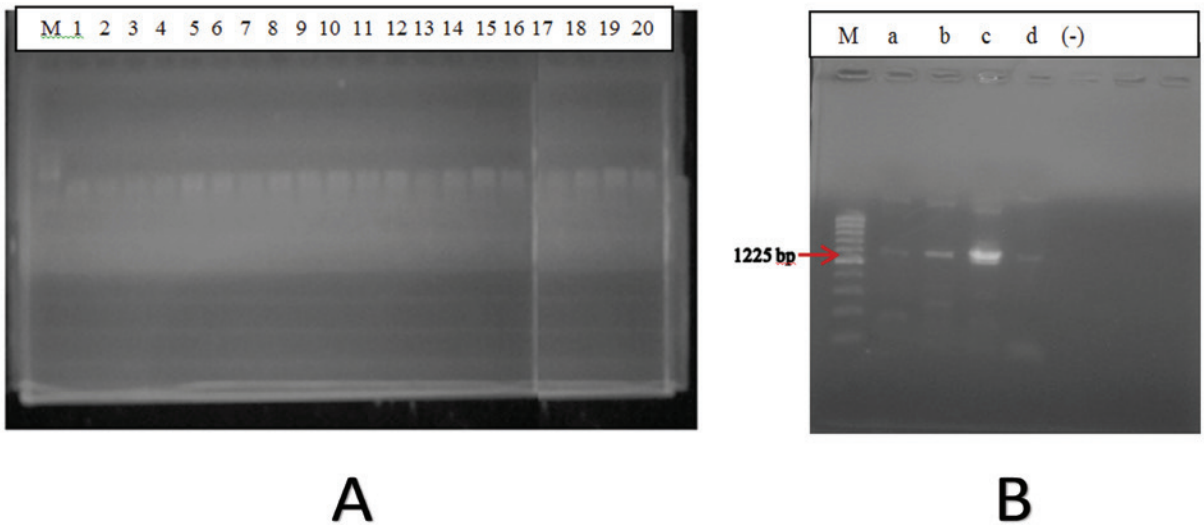

Figure 2. PCR analysis of food samples. A. Whole genome DNA extracted from cultures of samples from traditional markets and supermarkets. Remarks M= marker; 1-20= samples; B. Analysis of PCR products amplified from samples propagated on PDA media using a primer specific for *Aspergillus* genus aflatoxin; M= marker; a sample from the traditional market (a and b); samples from supermarkets (c); control positive (d); (-) = negative control, using the Af pyrG gene.

Conclusion

In sum, enhanced precautions may be needed to ensure that foods sold in traditional markets and supermarkets are free from molds that have the potential to produce mycotoxins. Further studies are needed to detect and identify the prevalence of mycotoxins in the food supply.

Conflict of Interest: The author declare no conflict of interest.

Ethical Approval :

All applicable institutional guidelines for the care and use of animals were followed. This research received ethical clearance approval from Animal Care and Use Commitee, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, East Java, Indonesia.

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Differential Diagnosis for Pericoronal Radiolucencies affecting Jaws – A Case Report on Dentigerous Cyst

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Abstract

Most of the times it is difficult for the clinicians to confirm the clinical diagnosis without investigations as the clinical picture of oral lesions may be similar to many other conditions. Hence, appropriate clinical examination, coupled with radiological and histopathological investigations could aid in appropriate diagnosis and management of the lesions. This article elaborates on differential diagnosis for pericoronal radiolucencies affecting the jaws in a patient presented for a routine dental examination. Differential diagnosis helps us to diagnose a specific lesion by eliminating those lesions which appear clinically similar. As advanced diagnostic aids are available, decision-making has become much easier for these lesions.

Key words: ***D****entigerous* ***C****yst,* ***D****ifferential* ***D****iagnosis,* ***I****mpacted* ***T****ooth,* ***P****ericoronal* ***R****adiolucencies*

Introduction

Pericoronal radiolucency are most commonly seen associated with an unerupted tooth during routine radiographic investigation, which could be a normal observation or a pathologic process. A proper clinical diagnosis with necessary investigations could aid in better diagnosis and management of these lesions. Differential diagnosis for these lesions helps us to formulate a proper treatment plan and manage the suspected lesions. We present a case of bilaterally occurring dentigerous cyst involving the third molar in a non - syndromic 49year old man.

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Case Report

An asymptomatic 49-year-old male patient presented for a routine dental examination. Clinical examination revealed a mild bulge on the lingual side of the missing mandibular third molar regions bilaterally. Panoramic radiograph showed impacted mandibular third molars bilaterally with well- defined cystic lesion associated with the impacted molars (Fig 1). Subsequently a CBCT was taken to visualize the extent and configuration of the cystic lesion. CBCT revealed a horizontally impacted tooth within a radiolucent lesion with relation to mandibular right 3rd molar measuring 17.91mm superoinferiorly from the level of the alveolar crest to the lower border of the mandible and 19.80mm anteroposteriorly. Mesial and distal root of 47 appeared to be blunted in the sagittal section (Fig 2).

Axial section revealed the lesion extending 19.84mm anteroposteriorly and 12.15mm buccolingually respectively (Fig 3). Evidence of expansion and thinning of lingual cortical plate throughout the extent of the lesion with a breach was seen in the lingual cortical plate. Inferior displacement of the mandibular canal was

also seen throughout the extent of the lesion with respect to 47.

Lower left 3rd molar showed an evidence of well-defined radiolucent lesion with an impacted tooth. Superoinferiorly it extended from the level of the alveolar crest, immediately distal to 37, till the inferior border of the mandible measuring approximately 18.66mm; anteroposteriorly, it extended from the apical portion of distal root of 36 till the apex of the root of horizontally impacted 38 measuring about 26.86mm and 14.31mm buccolingually. The mandibular canal was displaced inferiorly throughout the extent of the lesion with expansion and thinning of lingual cortical plate throughout the extent of the lesion; but without any evidence of discontinuity in the corticated border. Blunting of both the mesial and distal root apex of 37 was seen.

Diagnosis and management

Vitality tests suggested that both mandibular 2nd molar teeth on either side were vital. On aspiration, chocolate-brown fluid was obtained. Histopathologic evaluation of the aspirate revealed cholesterol clefts, chronic inflammatory cell infiltrate and few epithelial cells scattered in fibrinous background.

Under general anesthesia, the impacted mandibular third molars on either side were first extracted followed by enucleation of the cyst lining and evacuation of its contents. Platelet rich fibrin was prepared and placed into the defect followed by primary closure. Postoperatively, the patient recovered uneventfully. Patient reported numbness in left half of lower lip but this resolved completely within 2 weeks. No other complications were encountered and the patient was placed on follow up.

Histopathologic examination of cystic contents on both right and left sides revealed the presence of a lining of 2-4 cell thick non-keratinized epithelium on the cystic lumen and supported by an inflamed connective tissue capsule which was compatible with diagnosis of dentigerous cyst. The cystic capsule is moderately dense and exhibits chronic inflammatory cell infiltrate (predominantly plasma cells and lymphocytes), cholesterol clefts, haemorrhage and several bacterial colonies (Fig 4,5)

Differential diagnosis of pericoronal radiolucencies affecting the jaws

Features	Pericoronal or follicular space [1]	Unicystic ameloblastoma [2,3]	Adenomatoid odontogenic tumor [2]	Calcifying odontogenic tumor [2,4,5]	Ameloblastic fibroma [2,6]
Incidence		13-21% of all cases of ameloblastoma	2-7% of all odontogenic tumors	1-7% of all odontogenic tumors	Approximately 2% of odontogenic tumors
Age		3rd decade	2nd decade	2nd – 4th decade	1st 2 decades
Sex		Males = Females	Females > Males	Males = Females	Males > Females
Signs and Symptoms	Asymptomatic	Usually asymptomatic. Large lesions may have painless swelling of the jaws.	Smaller lesions appear as asymptomatic, sessile masses on the gingiva. Larger lesions cause painless expansion of the bone	Asymptomatic, may appear as painless slow growing swelling associated with expansion of cortical bone	Small fibromas are asymptomatic. Larger lesions are associated with swelling of the jaws

Cont... **Differential diagnosis of pericoronal radiolucencies affecting the jaws**

Site		More than 90% in mandibular posterior region.	Anterior portion of jaws. Maxilla > mandible	Majority in incisor and canine region. Maxilla = Mandible	Posterior mandible is the most common site
Radiographic features	Follicle appears as thin homogenous radiolucent halo around the embedded tooth	Circumscribed radiolucency that surrounds the crown of unerupted tooth	Follicular type appears as circumscribed, unilocular radiolucency that involves crown of unerupted tooth.	Appears as unilocular, well-defined radiolucency – 1/3rd of cases are seen in association with unerupted tooth	Smaller lesions appear as unilocular radiolucency which is associated with unerupted tooth in about 75% of cases

Figure1: Orthopantomograph showing with well- defined cystic lesion associated with the impacted molars.

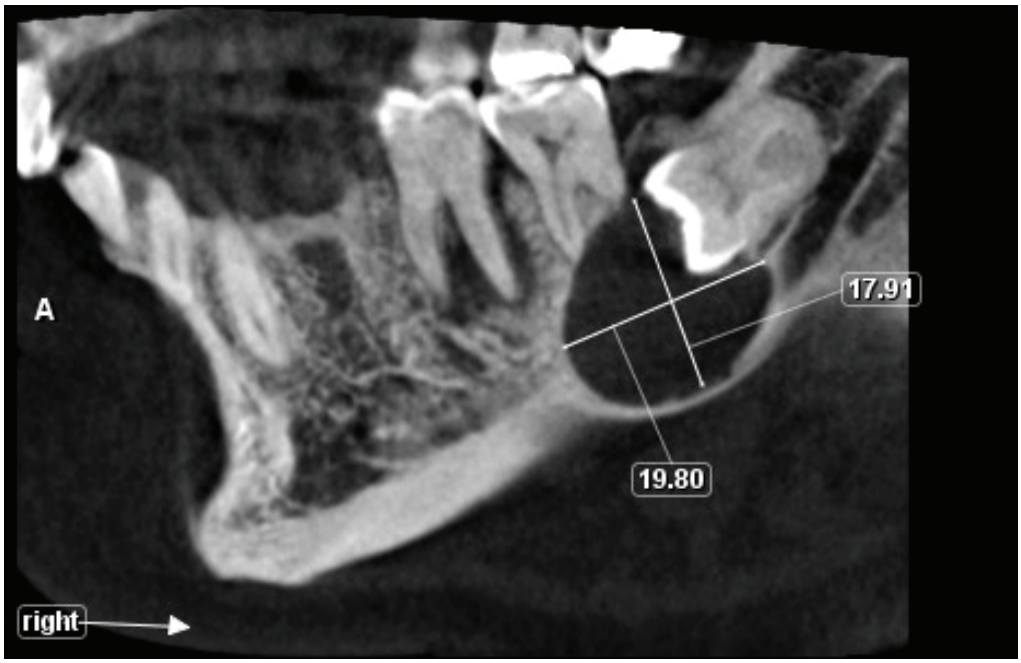


Figure 2: CBCT image shows well- defined cystic lesion associated with the impacted molars

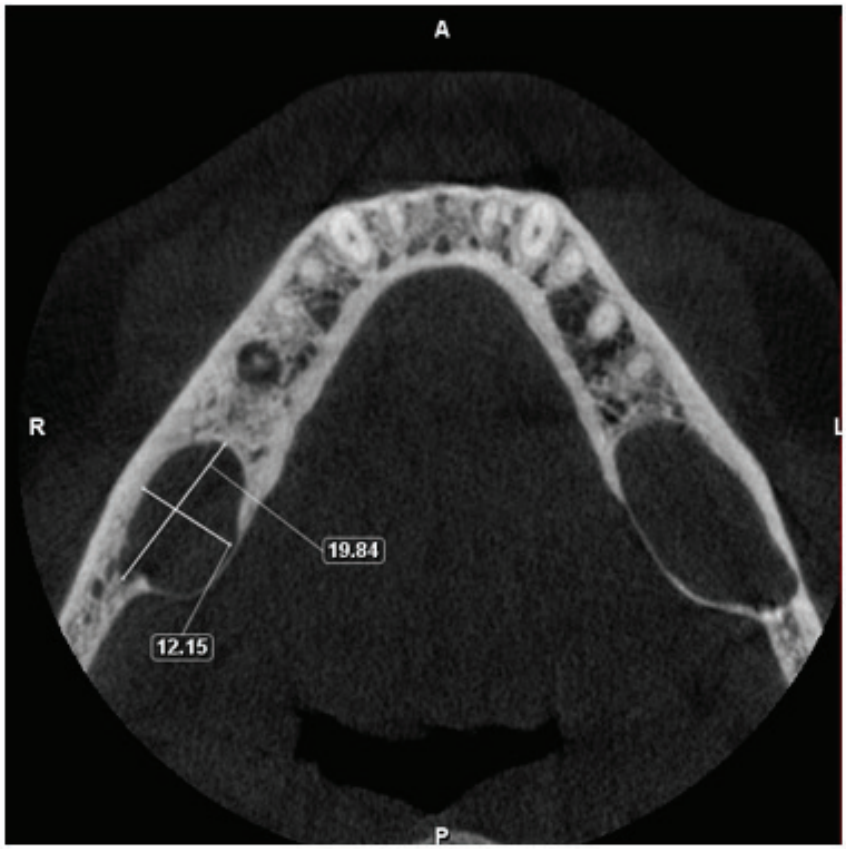
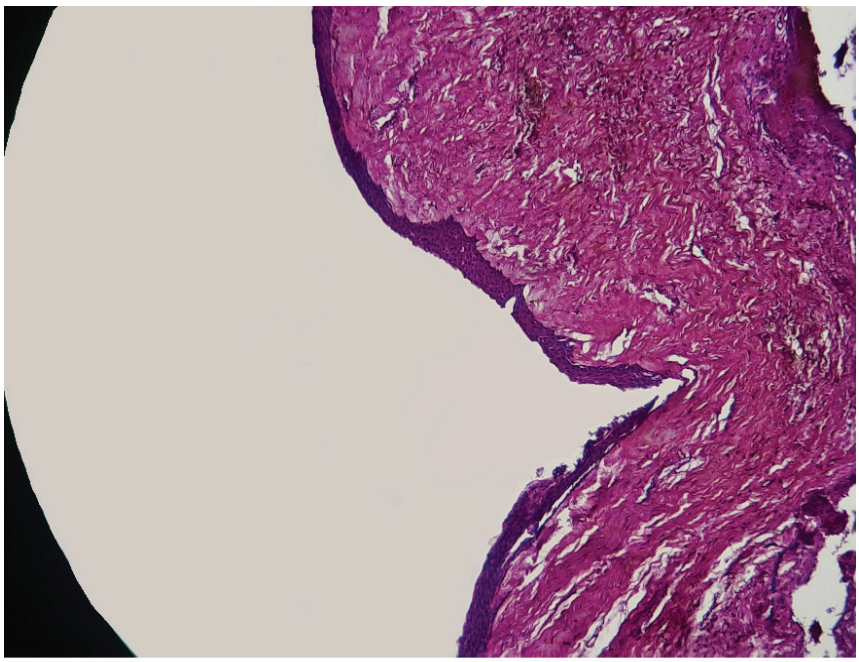


Figure 3: CBCT image showing bucco-lingual expansion of the lesion in the axial section



The image shows cystic lumen, epithelial lining and connective tissue

Figure 4: Photomicrograph of lesion showing cystic lumen, epithelial lining and connective tissue (H and E, ×10)

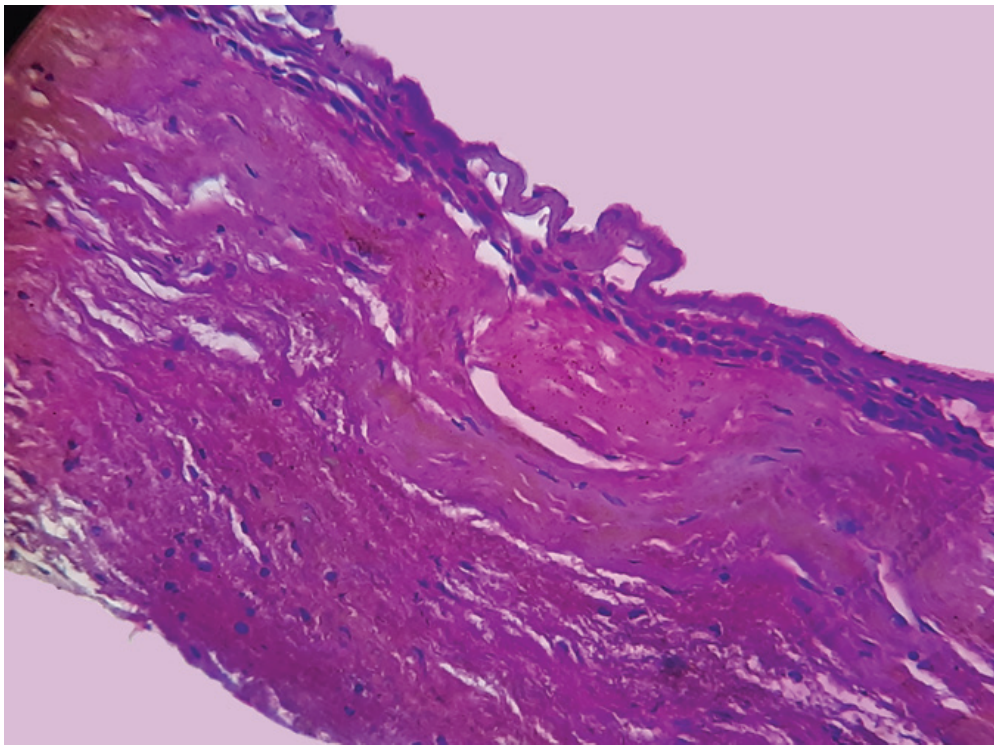


Figure 5: Photo micrograph showing 2-4 cell layer thick epithelium resembling reduced enamel epithelium and connective tissue showing dense bundles of collagen fibers interspersed with fibroblasts (H and E, ×40)

Discussion

Dentigerous cyst is the most common benign odontogenic cyst secondary to periapical cyst. It usually presents with a crown of an impacted or an unerupted permanent tooth and rarely associated with deciduous tooth.[7] Dentigerous cysts is more commonly seen in men aged 20-30 years and is seen associated with the unerupted third molars followed by premolars and canines.[8] The reported prevalence rate is 1.44 in 100 unerupted teeth.[9] Although the pathogenesis remains unclear this cyst is believed to originate from the follicle of an unerupted tooth which causes expansion due to fluid accumulation between the crown and the epithelium.[10]

Benn A and Altini M (1996)[11] proposed three mechanisms for the formation of a dentigerous cyst. a) dentigerous cyst might form a dental follicle and become secondarily inflamed with the source of inflammation being a non-vital tooth b) The second one, formation of a radicular cyst at an apex of a non-vital deciduous tooth followed by eruption of its permanent successor into the radicular cyst resulting in a dentigerous cyst of extrafollicular origin c)The follicle of permanent successor might get secondarily infected from either periapical inflammation of a non-vital predecessor or other source leading to a dentigerous cyst formation.

Dentigerous cyst is usually asymptomatic and discovered on routine radiographic investigation associated with unerupted tooth unless it is secondarily infected. Frequently seen in the posterior mandible when compared to maxilla. It is unilocular with well-defined sclerotic area around the crown and always associated with an impacted tooth. This cyst can cause resorption or displacement of the adjacent tooth and also can cause expansion of the cortical plates. [12]

Dentigerous cysts is usually solitary, but can be seen as multiple or bilateral cysts associated with various syndromes like cleidocranial dysplasia, mucopolysaccharidosis (type VI), Maroteaux--Lamy syndrome and Gorlin--Goltz syndrome. [13]

Treatment

As dentigerous cysts are rarely symptomatic, usually they are diagnosed on routine radiographic investigation due to an expansile swelling causing destruction of

the bone. The choice of treatment of dentigerous cysts depends upon the patient's age, size, location of the cyst, the tooth involved and its proximity to the vital structures.[14] Decompression and marsupialization is the preferred modality of treatment for aesthetic reasons as it is more prevalent in the younger age group.[15] These methods are conservative in nature and prevent fracture of the cortical bone and damage to vital structures. Enucleation is done if the cyst is large followed by the removal of the impacted or embedded tooth.[16] Recurrence rate is very less but it can turn into ameloblastoma, squamous cell carcinoma and mucoepidermoid carcinoma.[2]

Conclusion

Clinical examination together with radiographic and histopathologic investigations are the most important tools that aid in differentiating dentigerous cysts from other cysts and tumors with pericoronal radiolucencies. This case also highlights the need for prophylactic extraction of unerupted teeth to prevent the occurrence of odontogenic cysts and tumours, a majority of which, when they occur in this region are associated with impacted teeth. Management of cysts occurring in jaws are important to prevent recurrence or later turning malignant.

Ethical Clearance: Taken from Institutional Ethics Committee, Manipal College of Dental Sciences, Mangalore *(A constituent institution of MAHE, Manipal)*

Source of Funding: NIL

Conflict of Interest: NIL

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Disclosure of Confidential Medical Record of the Patient of Corona Virus Disease 2019

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Abstract

This study aimed to find out the disclosure of confidential medical record of Covid-19 patients from legal norms, ethics and medical disciplines in Indonesia. This research used normative juridical methods which had a pre-descriptive characteristic.This research found the disclosure of confidential medical record of the Covid-2019 patients can be opened for the benefit of the patient's health, fulfilling the demands of law enforcement officials in the context of law enforcement, the patient's own request, or based on statutory provisions.This research is very useful for the community, hospitals, educational institutions and the government to provide legal protection to society to not be exposed by Covid-2019. The disclosure of confidential medical record of Covid-19 patients is permitted in relation to the patient's initials, gender, place of residence, age and health status.

Keywords: *Confidential Medical Record, Pandemic of Covid-19, Community Interests; Patient; Doctor; Medical Ethics and Discipline.*

Introduction

On March 11, 2020, the World Health Organization declared the Corona Virus Disease 2019 (Covid-19) as a pandemic.[1,2] The Covid-19 pandemic spread to Indonesia, said by President Joko Widodo on Monday, March 2, 2020.[3] Two Indonesian citizens residing in Depok were found to be positive with Covid-19 after having a history of interacting with Japanese citizens who were known to suffer from this disease.[4] This incident, later, made the Government of Indonesia issued Presidential Decree No. 11 of 2020 concerning Determination of Corona Virus Disease (Covid-19) on Public Health Emergency and Presidential Decree No. 12 of 2020 concerning Determination of Non-Natural Disaster of Corona Virus Disease 2019 as a National Disaster.[5]

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The spread of Covid-19 in Indonesia is now increasingly widespread in almost all districts and provinces. The number of cases and / or the number of deaths is increasing. This situation impacts on the political, economic, social, cultural, defense, security and welfare aspects of the Indonesian people.[6] The spread of Covid-19 on July 8, 2020 showed the number of positive confirmation cases numbered 68,079 people, 3,359 patients died and 31,585 patients recovered.[7]

Data of Covid-19 patient was stored in medical records and kept by doctors and health services.[8] Medical record is very important and is closely attached to the activities of medical services and health services. [9] Some even say that the medical record can be considered as the third person when the doctor received his patient.[10] Medical records are very useful for both doctors and patients.[11] This medical record showed the doctor of the patient's condition at the time of illness, examination and treatment the patient had received so that the doctor can take right treatments when the patient come for the treatment.[12] The doctor was bound by the patient's medical record to be kept confidentially.[13, 14] Disclosure

of the patient's medical record by a doctor can have the effect of violating the law, ethics and discipline. [15] In the pandemic of Covid-19, whether the doctor is still bounded by the confidentiality of the patient's medical record, considering the confidentiality of the Covid-19 medical record can affect to a very rapid and massive impact on transmission to society.[16].

Discussion

Doctor

Doctors are a major component in health services to the community.[17] The role of doctors is very important because it is directly related to improving the degree of public health. Article 1 number 2 of the Medical Practice Law limits the term of doctor as doctors, specialists, dentists, and dental specialists who have graduated from medical or dental education both domestically and abroad recognized by the Government of the Republic of Indonesia in accordance with statutory regulations. [18]

The doctor is a health worker who is the patient's first contact in resolving all health problems faced beyond considering of the type of disease, organology, age group, gender, as early as possible to provide the best health services to patients. [19] A doctor has main competencies including: effective communication skills, basic clinical skills, skills in applying the basics of biomedical science, clinical science, behavioral science and epidemiology in medical practice, skills in processing health problems in individuals, families or communities in a comprehensive and holistic way, continuous, coordinated and collaborated in the context of primary health care, utilizing critical assessments and managing information, introspective, self-development, lifelong learning, upholding moral ethics and professionalism in practice.[20]

Besides, doctors have some obligations; general obligations, obligations to themselves, obligations to colleagues and obligations to patients. Specially for the obligations to patients, it include: being sincere in using all their knowledge and skills for the benefit of patients, providing opportunities for patients to always be in touch with their family and counselors and must keep everything that they know about a patient, even after the patient's death and perform emergency relief as a humanitarian task. [21]

Patient

Patients are people who consult their health problems to obtain the necessary health services either directly or indirectly to the doctor or dentist. Patients in receiving health services to doctors have the right to get a full explanation of medical measures, ask the opinion of a doctor or other dentist, get services according to medical needs, refuse medical treatment, and to obtain the contents of the medical record. [22]

The patient's obligations to the doctor in health services were providing complete and honest information about his health problems, obeying doctor's or dentist's advice and instructions and complying with the applicable provisions in health care facilities, and providing compensation for services received. [23]

Based on the rights and obligations of the patient, the legal relationship between the patient and the doctor in health services will bring legal impact.[24] The impact of this law can be regarding the legal responsibilities of doctors to patients in case of violations of law, ethics and discipline. [25]

Medical Records

To realize the optimal health status for the whole community, it is necessary to improve the quality of health services or medical practices accompanied by adequate supporting facilities in health services, one of which is the implementation of medical records in the form of examinations, treatment and care. [26, 27]

The implementation of medical practice services required a doctor to refer to applicable standards, guidelines and procedures so public receives medical services in a professional and safe manner.[28] The patient's trust to the doctor resulted to the doctor's obligation to keep everything he knew. [29]

The Medical Practice Act regulates the medical record in Article 46 paragraph (1) every doctor or dentist in carrying out medical practice is required to make a medical record. Medical records must be written, complete and clear or electronically. [30].

Medical records are files contain records and documents about patient identity, examinations, treatment, actions, and other services given to patients,

and regulated in Minister of Health Regulation No.269 / MENKES / PER / III / 2008 concerning on Medical Records. [31]

While the benefits of medical record, it can be useful for treatment to the patient, improving the quality of services, education and research, financing, health statistics and proving legal issues, disciplinary and ethical issues. [32]

Privacy Rights of Patients in Health Services.

The concept of state law placed the idea of protecting human rights as an important element of country life. By considering the urgency of protecting these human rights, the constitution must contain human rights arrangements to provide a guarantee to all citizens.[33]

Human rights are universally recognized values. Amendments to the 1945 Constitution had included guarantees of the protection and fulfillment of the human rights of citizens in the constitution. Some Articles in the 1945 Amendment regulated comprehensively about the human rights of citizens. Article 28 letter G regulated every person to have right to protect himself, family, honor, dignity, and was entitled to a sense of security and protection from the threat of fear.[34]

The implementation of these provisions was regulated in Act No. 39 of 1999 Article 69 paragraph (2) on Human Rights which regulated that each person's human rights caused basic obligations and responsibilities to respect the rights of others reciprocally, and it became the government's duty to respect, protect, uphold and promote it. Likewise, the government is obliged to guarantee the basic rights of the people relating to health services in the current situation of Covid-19.

Every patient of Covid-19 had rights to obtain privacy and medical confidentiality from the illness suffered including his medical records. [35] The regulation are as follows:

a. Act of Medical Practice, Article 48 paragraph (1) which regulates, "Every doctor or dentist in carrying out medical practice is obliged to keep the medical secrets"

b. Act of Health, Article 57 Paragraph (1), regulates, "Everyone has the right to the confidentiality of his personal health condition stated by health service provider."

c. Act of Hospital, Article 44 paragraph (2), regulates, "Hospitals may refuse to disclose any information to the public relating to confidential medical records."

d. Article 73, Act of Health Workers which reads: "Every Health Worker in carrying out health services must keep the health confidentiality of the Health Service Recipients."

e. Act on Public Information Openness, Article 17 letter h number 2, which principally stipulates that every person has the right to their confidential personal health conditions that have been disclosed to the health service provider and each public organization must open access for public information applicant to obtain public information, except, one of them, regarding to person's history, condition and treatment, physical and psychological health treatment since if it is opened and given to public information applicants, the personal secret of patients can be revealed.

Based on the description above, from the Constitution to the Act, every person has rights in health services, especially related to their privacy in historical data of the illness. For this reason, doctors and hospitals are required to maintain the confidentiality of medical privacy of patients

Disclosure of Confidential Medical Record of Covid-19 Patients

Once a state is in an abnormal condition or in an emergency situation, the state is allowed to take extraordinary actions including limiting human rights to maintain the integrity of the state and protect its citizens. If threat to citizens' safety exist, the state is considered to be able to act anything, regardless of the legality of the methods adopted. In other hand, acts of restriction on human rights, however, must have clear boundaries along with measures to minimize opportunities for abuse at the expense of broader humanitarian interests. [36]

Emergencies in health sector had been regulated in Act No. 4 of 1984 concerning the outbreak [37] This Act aimed to protect the population from the disasters caused by the outbreak as early as possible to improve the

ability of people to live healthy. Covid-19 pandemic is a form of non-natural disaster in the form of an outbreak, so the government must act immediately to protect the community.

Steps to follow-up the Government Plague Act issued Minister of Health Decree No. HK.01.07 / MENKES / 413/2020 concerning Guidance on Prevention and Control of Coronavirus Disease 2019 (Covid 19) in the explanation gives the understanding of covid 19 cases to be: Suspect Cases, Probable Cases, Confirmation Cases, Closer Contacts, Travel Actors, Discarded, Isolation Finished, and death. [38]

By the classification of Covid-19 patients, it is useful for doctors to provide services and medical measures to patients. To support the doctor's actions, a complete medical record must be supported. The contents of the medical record for Covid-19 must contain: a. patient identity; b. the condition when the patient arrives at a health care facility; c. identity of patients' guardian ; d. date and time; e. history taking results, including at least complaints and history of the disease; f. results of physical examination and medical support; g. diagnosis; h. treatment and / or action; i. a summary of the patient's condition before leaving the emergency services and a follow-up plan; j. names and signature of certain doctors, dentists or health workers who provide health services; k. transportation facilities used for patients to transfer to other health care facilities; l. other services provided to patients; m. the type of disaster and the location where the patient was found; n. emergency category and mass disaster patient number ; and o. the identity of person who found the patient. Meanwhile, according to Nasser [39] the medical record contained a series of information consisting of: a. information delivered by the patient to the doctor or hospital about matters related to what was felt and suffered as a disease; b. examination results or findings as well as the entire set of procedures for the illness suffered by a person including the treatment regime; c prognosis or future estimates of the disease and possible future health risks of the patient and d. patient identity if the disease poses a risk of stigmatization and discrimination in the long life ahead.

Disclosure the confidential medical records of Covid-19 patients cannot be categorized as acts that violate the provisions of Article 322 of the Criminal Code paragraph (1) which reads: "... whoever intentionally discloses something secret, according to his position or occupation, both present and in the past, he obliged to save it, sentenced to prison for nine years maximum ... ". The provisions of Article 322 of the Criminal Code are confidential in general crimes

Arrangement of disclosure of patient's confidential medical records based on the principle of "lex specialis derogate generalis" has been regulated in several Acts and regulations including:

a. Act of Medical Practice, Article 48 Paragraph (2) which regulates: "Medical secrets can be opened only for the benefit of the patient's health, fulfill the request of law enforcement officials in the context of law enforcement, the patient's own request, or based on statutory provisions.

b. Act of Health, Article 57 paragraph (2) which regulates: "Provisions regarding the right to confidential conditions of personal health do not apply in the case of: a. law orders; b. court order; c. relevant permit; d. community interests; or e. the person's interests itself.

c. Act of Hospital, Article 44 paragraph (2) which regulates: "Medical secrets can only be opened for the benefit of the patient's health, to fulfill the request of law enforcement officials in the context of law enforcement, with the patient's own consent, or based on statutory provisions.

d. Act of Health Workers, Article 73 which reads: "The health secrets of health service recipients can be opened only for the benefit of the health service health recipients, fulfilling requests for law enforcement officials for the sake of law enforcement, requests for health service recipients themselves, or compliance with the provisions of the laws and regulations. "

e. Article 9 of The Minister of Health Regulation on Medical Secrets regulates: disclosure of confidential medical records based on the provisions of the legislation is carried out without the consent of the patient in the context of ethical or disciplinary interests, as well as the public interest.

From the provisions of existing legislation, the patient's confidential medical record can be disclosed when it deals with public / community interests. By

this disclosure, it will be easier for doctors to contact patient tracing with other parties which will increase the effectiveness and efficiency in the dissemination effort. The disclosure of the medical secrets of Covid-19 patients is not something embarrassing or has the potential to cause stigmatization or discrimination in the community but it will actually evoke community charity and solidarity. Conversely, if it is not, there will be a threat to the public interest of public health. Hiding the identity of Covid-19 patients will strengthen the spread of the virus in community.

Presidential Decree No. 11 of 2020 on the determination of emergency public health of Covid-19 regulates that efforts to prevent, handle and control, the government cannot work alone without involving community participation as well as victims, so it is a must to community to concern with data on people in monitoring (ODP), patients in oversight (PDP), and people at risk (ODR) / Travelers including groups of people without symptoms (OTG) to play an active role in avoiding, reporting and limiting the spread of Covid-19 in their region, both at the district / city, sub-district or at the village level even up to at the level of Rukun Tetangga (RT). Thus, disclosure the confidential medical records of patients in the era of Covid-19 Outbreak is not an unlawful act or an unlawful act by the authorities (onrechtmatige onverheidsaad).

While the disclosure of confidential medical records from the ethical side is also regulated in the Decree of the Medical Ethics Honorary Council No. 016 / PB / K.MKEK / 04/2020 on Medical Ethics Fatwa, Health Policy and Research in the Context of the Covid-19 Pandemic, has decided that the identity of patients or people with or without clinical symptoms with a positive diagnosis of Covid-19 (confirm case) in the principle must still be protected.[40] In certain circumstances, it can be limited to the initial name, gender, short health status (death / clinical severe crisis / recovery) age and chronology is limited to only those relevant to transmission, for example the spread of potential transmission locations with the intent of being a public alert and contact tracing (epidemiologic tracing). As for detailed clinical information, accompanying diseases and management should not be disclosed, exceptions can only be made in accordance with applicable laws and regulations, including relating to disclose the names of public officials and the names of medical personnel and health workers as victims to be given awards by the world of the medical profession and country.

Conclusions

The Act of Medical Practice, Act of Health, Act of Hospital, and Act of Health Workers stipulated that confidential medical records can be disclosed only for the benefit of the patient's health, fulfill the request of law enforcement officials in the context of law enforcement, the patient's own request, or based on the provisions of the legislation. In terms of medical ethics and discipline, the confidential record of Covid-19 patients may be disclosed in the initial name, gender and brief health status for public use. The public interest here means as the threat of extraordinary events or pandemics of infectious diseases threatening the safety of others both individually and in society.

Limitation and Study Forward

This research is limited to the disclosure of confidential medical record of Covid-19 patients in time of current pandemic regarding to the statutory provisions and community interests. Since there are still other reasons to allow the disclosure of confidential medical records based on the court orders, so it is needed for further research to find the type of cases which are allowed to open the patient's medical record.

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Type of Article- ***Research Protocol***

Comparative Evaluation of Expression of Markers of Proliferation, Angiogenesis, & Apoptosis in OSMF before and after treatment with Polyherbal Preparation – Study protocol

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Abstract

India alone accounts for one third of the world's oral cancer and has a high rate of Oral Potentially Malignant Diseases (OPMD) amongst Areca Nut & Tobacco Habitual. Oral submucous fibrosis (OSMF) is one of the OPMDs which predominantly affect major population of South and Southeast Asia, especially Indian subcontinent. Till date OSMF patients are treated symptomatically to relieve burning sensation, improve the opening of mouth & tongue movements, reduce blanching and relieve from roughness of oral mucosa by using various medicinal line of treatment i.e. vitamin A, lycopene, beta carotene and carotene analogs, but no treatment is directed towards the reduction of malignant potential of preexisting OSMF cases. Thus, present study will be conducted to compare the pre & post treatment serum level of proliferation, angiogenesis and apoptosis inducing expression of markers with polyherbal therapy in oral submucous fibrosis. **Methods:** Identification of clinically diagnosed patients of OSMF who will be divided into two groups by simple random sampling procedure. Group-1 patients suffering from oral submucous fibrosis, will be receiving Polyherbal Preparation while Group-2 patients receiving Lycopene. Pre and post treatment comparison for clinical signs and symptoms and serum marker expressions for Proliferation, Angiogenesis, & Apoptosis in both groups will be done and statistically analyzed. **Results**: Clinical signs and symptoms will be improved with the present polyherbal protocol. Post treatment serum marker may favored reduction in proliferation, angiogenesis and induced apoptosis which predicts malignant transformation. **Conclusions:** Polyherbal therapy will help to reduce the malignant conversion without toxic effect on other system of the human body.

Keywords: *oral submucous fibrosis, serum marker, turmeric,cinnamon, black pepper, lycopene*

Background

Oral submucous fibrosis is defined as the chronic, insidious disease affecting the oral cavity and sometimes pharynx, although occasionally preceded and/or associated with vesicle formation and is always associated with juxtaepithelial inflammatory reaction followed by fibro elastic changes in the lamina propria with epithelial atrophy leading to stiffness of oral cavity leading to trismus and inability to eat.[1] Being commonest, premalignant condition oral submucous fibrosis reported with malignant transformation rate of 4.5% to 7.6%.[2] A wide range of treatment modalities both medical, surgical and physiotherapeutic have

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been proposed for symptomatic improvement in OSMF without reduction in the morbidity and mortality. On the other hand, each year approximately one million people in India are diagnosed with oral cancers.[3] OSCC accounts for ~90% of malignant oral lesions and is widely recognized as the most frequently occurring malignant tumor of oral structures, and the mortality rate of OSCC is relatively high, with a 5-year survival rate of 50% in spite of technological & therapeutic innovation.[4] Still chances of recurrence & second primary are remained unresolved either, because of exposure of oral mucosa to carcinogen or exposure to the unknown secondary trigger after surgical intervention. Till date OSMF patients are treated symptomatically to relieve burning sensation, improve the opening of mouth & tongue movements, reduce blanching and relieve from roughness of oral mucosa by using various medicinal line of treatment i.e. vitamin A, lycopene, beta carotene and carotene analogs, but no treatment is directed towards the reduction of malignant potential of preexisting OSMF cases. Therefore present study is planned using combination of Haridra, cinnamon and black pepper in 3:2:1 proportion after giving 7 times Bhavana Sanskara to the preparation. Further treatment outcome will be evaluated by using serum marker for proliferation, angiogenesis and apoptosis. Studies conducted in vitro and vivo on extract of Haridra, cinnamon and black pepper has proved its anti-inflammatory, antioxidant properties and in arresting proliferation, angiogenesis and inducing apoptosis which will help to reduce the malignant potential. [5-8]

Methods

Research protocol will be explained to the patients and eligible patients with written consent will be included in study population. The present study has been approved from the Institutional Ethics Committee, (Ref.No.DMIMS (DU)/IEC/Sept-2019-8329). It will be conducted in department of Oral Medicine &Radiology, Sharad Pawar Dental College and Hospital, Wardha. Polyherbal preparation will be prepare at Ayurveda Rasashala, Mahatma Gandhi Ayurved College and Research center, Salod, by using turmeric, cinnamon and black pepper. Sample size has been calculated by statistician is 32 patients. Total 36 Patients will be selected considering 20% attrition after obtaining an informed consent. Patient's examination will be conducted by using the diagnostic instrument and natural light and data will be entered in the structured proforma. All subjects beyond the age of 18 years having OSMF will be included in study. Pregnant and lactating mother, Patients with systemic disorders and metabolic disorder, patients who are undergoing treatment for oral submucous fibrosis for last 6 months, Patients who will not willing to sign informed consent and who are not willing for participate in study will be excluded from the study. Total 36 subjects will be divided into two groups. Group-1will consist of 18 patients suffering from oral submucous fibrosis, will be receiving Polyherbal Preparation tablet 500 mg two times in a day for 03 month. Group-2 will consist of 18 patients suffering from oral submucous fibrosis, will be receiving Lycopene 8 mg tablet two times in a day for 03 months. Both groups of patients will be evaluated at interval of 15 days for clinical signs & symptoms till 3 months. Pre and post treatment comparison for clinical signs and symptoms and serum marker expression will be done.

Aim: Comparative evaluation of expression of markers of Proliferation, Angiogenesis, & Apoptosis in OSMF before and after treatment with Polyherbal Preparation

Objectives

1. To evaluate expression of Proliferation (MMP 9), Angiogenesis (VEGF) and Apoptosis (Cytochrome C) in serum sample of OSMF patients pre and post-treatment with Polyherbal Preparation.
2. To evaluate expression of Proliferation (MMP 9), Angiogenesis (VEGF), (MMP 9) and Apoptosis (Cytochrome C) in serum sample of OSMF patients pre and post-treatment with lycopene.
3. To compare expression of Proliferation (MMP 9), Angiogenesis (VEGF), and Apoptosis (Cytochrome C) in OSMF patients; pre and post-treatment with Polyherbal Preparation and with lycopene.
4. To evaluate and compare clinical signs and symptoms pre and post-treatment with Polyherbal Preparation and lycopene.

Expected outcome: In Group-1 patients, Polyherbal medication due to its antiproliferative, antiapoptotic and antiangiegenetic properties; will prevent the malignant changes in the epithelium and may help in reducing

the fibrosis by targeting proliferation, angiogenesis and apoptosis. Relief from clinical signs and symptoms is expected to get in patients of both groups.

Discussion

Every year approximately one million people in India are diagnosed with oral cancers. Oral Cancer is ideal for screening but biologically the worst among Head and Neck Squamous cell cancers especially when diagnosed in advanced stages. Thus, intervention by polyherbal therapy will help to reduce the malignant conversion without toxic effect on other system of the human body. Early intervention of betel nut habitual group with or without OSMF may help in reducing malignant potential. Reduction in the malignant potential of OSMF will eventually decrease the incidence and prevalence of oral cancer. This can be further intensified by betel nut cessation programmes along with nutritional guidance and appropriate use of Indian spices in day to day meals.

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Polydioxanone Sutures Instead of Polypropylene Sutures for Abdominal Closure to Prevent Wound Sinuses

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Abstract

Abdominal closure after laparotomy takes several weeks to get 80% of its previous strength in this period the fascial sheath should be hold by sutures. Several materials used in the abdominal closures but the most common suture used is polypropylene, others used nylon, less common used is absorbable sutures but wound sinus is a complication may fellow the use of non-absorbable sutures. For two years we collect 235 patients in all of them abdominal wall incision was Pfannenstiel, in 133 patients the incision closed by polypropylene (non-absorbable), for other 102 patients the incision closed by polydioxanone (slow absorbable) the result was significant for wound sinus formation in polypropylene group but insignificant for hernia, conclusion of the study the polydioxanone look the same strength of polypropylene and may be without wound sinus formation.

Keywords*: abdominal closure, wound sinus, abdominal incision, polydioxanone, incisional hernia.*

Introduction

Abdominal closure after laparotomy needs attention about healing process that the fascial sheath healed with fibrous tissue, and the healing of the wound takes several weeks to get 80% of its previous strength[1-3], to reach this point the fascial sheath should be hold by a suture. The purpose of the suturing material is to approximate the abdominal wall incision adage together in an appropriate apposition until the natural healing process is complete. Several materials used in the abdominal closure like silk which is braided and non-absorbable, polydioxinon which is monofilament and slow absorbable and nylon or polypropylene are monofilament and non-absorbable suture.[4] The abdominal incision may have closed as single layer or in layers separated, continued or interrupted stitches (simple or eight figure) with or without tension sutures.

Abdominal incisions mainly either vertical (midline or paramedian) or horizontal (transverse or pfannenstiel), other incisions in deferent directions figure [1].

Every surgeon hope is that abdominal closure is without complications, like dehiscence, wound infections, incisional hernia or sinuses. Within the last few years we noticed patients complaining of wound infections and sinuses after abdominal closured by non-absorbable sutures (polypropylene), to avoid this complication we were replaced the slowly absorbable polydioxanone instead of polypropylene sutures, and conduct study to compare wound strength with significantly less incidence of wound complications. It was the objective of this observational study to compare two suture materials, the non-absorbable polypropylene and the slowly absorbable Polydioxanone for abdominal closure in Aziziyah hospital, Aziziyah, Wasit, Iraq.

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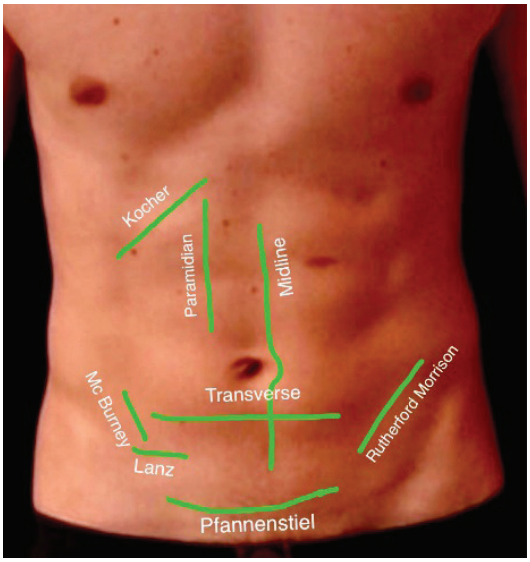

Figure 1: Abdominal incisions

Material & Methods

This study is observational clinical study was conducted in Alziziyah general hospital, Aziziyah, Wasit, Iraq, in two parts. The study was approved by the local hospital ethics committee.

Time of the study was between Jan. 2016 and Jan. 2018, of two parts; First part (group A) was retrospective between Jan. 2016 and Jan. 2017, and second part (group B) of the study was conducted in prospectively, from Jan. 2017 ended in Jan. 2018

For group A when nylon used, the patients already signed informed consent for operation. For group B of the study, patients signed an informed consent for using PDS in closure of abdominal wall.

For group A (between Jan. 2016 and Jan. 2017) we studied the cases from case files and fellow the patients by telephone or visit cards in the clinics for which of them complained wound sinuses after closure for Pfannenstiel incision by nylon sutures and any other complications (infection, dehiscence or incisional hernia), the number of patients was 133 patients (15-55 year old, mean =30.49, SD ± 9.66) and for group B (Jan. 2017 ended in Jan. 2018) the number of patients was 102 patients (16-55 year old 30.87, SD ± 9.51). Patients with peritonitis, younger than 15 years or older than 55 years were excluded from the study.

Suture material in group A of study was nylon (dynek) no.1 and for group B was polydioxanone (dtek) no.1. both nylon and PDS are monofilament, the nylon non-absorbable but the PDS is delayed absorbed sutures which occurred in six months. The techniques for suturing was the same done by same surgeons, suturing was continuous one cm away from the edge of wound and one cm apart. All patients were received ceftriaxone (1000mg) and metronidazole (500mg), intravenously, an hour preoperatively.

The rate of incidence of wound infection, wound dehiscence, suture sinus formation or incisional hernia were recorded. Patients were followed up for a period of 6 months.

Results

For patients (group A) in whom Pfannenstiel incision closed by nylon no. 1 (133 patients), 17 (12.78%) patients reported with wound infection which end with wound sinuses in 11 (8.27%) patients need surgical intervention to remove the sinus and foreign body granuloma, 3 (2.25%) patients with an incisional hernia. On other hand the patients who had Pfannenstiel wounds closed by polydioxanone (group B) sutures were 102 patients, of these patients 7 (6.86%) complained of wound infection treated conservatively with antibiotics, change of dressing but didn't need surgical intervention, 4 (3.93%) patients with incisional hernia, but no sinus or granuloma was recorded in any patient of group B. No wound dehiscence in both groups.

Table (1) Polypropylene group

	N	Std. Deviation	% of Total N
w. infection	17	5.050	12.8%
w. sinus	11	3.317	8.3%
hernia	3	1.000	2.3%
no change	102	29.589	76.7%
Total	133	38.538	100.0%

Table (2) Polydioxanone group

	N	Std. Deviation	% of Total N
wound infection	7	2.160	6.9%
hernia	4	1.291	3.9%
no change	91	26.413	89.2%
Total	102	29.589	100.0%

Table (3) T-test & P-value

Type of closure		
	t-test	Significant
polypropylene	36.305	P≤0.0005
polydioxanone	53.380	P≤0.0005

Discussion

The aim of abdominal wall closure is to restore function of abdominal after surgery without complications, like wound infections, dehiscence, incisional hernia and sinuses.[5]

And should be technically the same that its results are for the hands of the trainee as they are for the experienced surgeon.

Wound healing process after abdominal closure is dynamic process with changing wound condition and changing health status of the patient.[1] A careful selection of suture material is an important factor among several factors that affect the wound closure. Sutures are a foreign material implanted into human tissues lead to a foreign-body tissue reaction. Selection of suture material for closure of abdominal wall is still problem despite of the advances in surgical technique.[6]

It has been said that nearly half of all post-operative complications are related to wounds. It increases the morbidity and hospitalization of the patient as well as total cost of treatment and at times leads to an increase in mortality.[7]

Wound sinuses and granulomas at suture site are one complication of abdominal wall closure by non-absorbable sutures which need more time for treatment, cost and hospital admissions may avoided by absorbable sutures, in presented study 11 of 133 patients (8.3%) complained of wound sinuses need another operation, compare no patient need another operation for patients wounds closed by polydioxanone but Sajid et al studied that there are no significant differences between PDS comparable with prolene/nylon suture materials for the risk of incisional hernia, wound dehiscence, suture sinus formation and surgical site infection.[8]

Bellon et al evaluated the slow absorbable material PDS 4/0 and a non-absorbable material polypropylene 4/0 with a single layer running suture in 48 New Zealand White rabbits. No significant differences were found in the strength provided by the two sutures. The

composition of the suture material did not relate to the tensile strength of the wound closure.[9] Eshghi et al result in that no significant difference between PDS and nylon in the rate of hernia, infection and wound dehiscence. However, the use of PDS in abdominal closure led to less postoperative pain and sinus formation.[10] Our results were also matching to the above study.

Khan et al showed the postoperative wound sinus formation rate of 24% in the non-absorbable group (polyamide) and 16% in the absorbable group (polyglyconate). They concluded that slowly absorbable suture material appears to be better than non-absorbable suture material in midline abdominal closure.[11]

Dr. Puneet Tyagi, Dr. Mohammed Raza concluded "there was statistically significant higher risk of knot palpability, chronic pain and suture sinus development following the use of prolene compared to PDS II and PDS Plus".[12]There was not much difference between non-absorbable and absorbable sutures, though wound pain, discharge, dehiscence and suture sinus were found in non-absorbable sutures.[13]

Conclusion

The Polydioxanone look the same strength of polypropylene and may be without wound sinus formation.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

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The Modification of Workstation of Lipa Sabbe Weavers as an Effort to Reduce Musculoskeletal Disorders (MSDs)

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Abstract

Work with more loads and equipment design that is not ergonomic results in the use of more energy and risky work postures. Working in a sitting position basically can reduce stress on the feet, energy consumption, and circulation requirements. Ergonomic workstation is an example of the application of ergonomics which aims to create a balance between various aspects of each work system and process carried out as an attempt to create a high quality of work and quality of life, as well as an effort to prevent occupational injuries and diseases. This study aimed at modifying the workstation to reduce MSDs among *lipa sabbe* weavers. This is a quasi-experimental study with one-group pretest-posttest approach to analyze differences in the levels of MSDs before and after the intervention through modification of ergonomic workstation designs. The participant selection technique used purposive sampling. The research instruments used were Nordic Body Map questionnaire, camera, and anthropometric measurement tools. Data were analyzed using Wilcoxon Signed-Rank Test. The result showed that there is a difference in the level of complaints after modification of the work media of *lipa sabbe* weavers. It indicates that modification of work media can reduce MSDs among *lipa sabbe* weavers.

Keywords: *ergonomic workstation, musculoskeletal disorders, weaver, handloom, modification workstation*

Introduction

Wajo Regency is one of the silk-producing areas in Indonesia. The silk produced through the weaving process is a local wisdom that has been passed down from generation to generation. The silk weaving process generally uses three kinds of looms, one of which is *Gedog* or *Tennung Walida* loom. *Tennung Walida* was used by the people of Wajo Regency in the 13th century and developed since the 15th century. In 2012, there have been 5,113 *Tennung Walida*[1]. It is scattered throughout the village and is commonly used by housewives and young girls, and produces silk sarong called *Lipa Sabbe*.

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Data from the Department of Industry and Small and Medium Enterprises of Wajo Regency demonstrate that *lipa sabbe* weavers produce around 99,640 *lipa sabbe* per year which can increase every year[2] . The increasing demand for *lipa sabbe* made silk craftsmen work in longer duration and more frequent intensity. Work with more loads and equipment design that is not ergonomic results in the use of more energy and risky work postures. Moreover, *Tennung Walida* model uses a back-support loom and uses human power in a sitting position. Working in a sitting position basically can reduce stress on the feet, energy consumption, and circulation requirements. However, working in this position for too long can cause abdominal muscles to become elastic, spine curved, and eye muscles that experience more contractions so that the weavers feels tired quickly[3]

In this case, Yusuf discovered that one of the work risks of silk weaving workers is MSDs on the

back, waist, hands and feet when working for a long time and repeatedly[4]. Similar study by Tambun found that there are MSDs on the back waist, right shoulder, left shoulder, buttocks, right upper arm and left calf in weaving workers[5]. These MSDs can be prevented or reduced by improving ergonomic workstation (chairs)[6].

Ergonomic workstation is an example of the application of ergonomics which aims to create a balance between various aspects of each work system and process carried out as an attempt to create a high quality of work and quality of life, as well as an effort to prevent occupational injuries and diseases. A study conducted by Setiawan showed that redesign of the printing workbench can improve work posture[7]. It is supported by Syamsul who found that providing an ergonomic chair can reduce MSDs[8]. Additionally, improved working conditions can increase the productivity and income of metal painting craftsmen[9,10]

Therefore, it is necessary to pay attention to the ergonomic design of the workstation so that the silk craftsmen can work comfortably, safely, and healthily, which indirectly increases their productivity in producing *lipa sabbe* that in turn can increase their economic capacity. This study aimed at modifying the workstation to reduce MSDs among *lipa sabbe* weavers.

Material and Methods

This is a quasi-experimental study with one-group pretest-posttest approach to analyze differences in the levels of MSDs before and after the intervention through modification of ergonomic workstation designs. The participant selection technique used purposive sampling with the following criteria: female, working period > 5 years, work duration ≤ 8 hours/day, 26-45 years old, *lipa sabbe* weavers who use *Tennung Walida*, has personal looms, is working on *lipa sabbe*, and willing to be involved as research participants. The research instruments used were Nordic Body Map questionnaire, camera, and anthropometric measurement tools. Data were analyzed using Wilcoxon Signed-Rank Test.

Findings

The result showed among 16 weavers met in the field, the points of complaint that were often experienced while weaving included (1) lower neck, (2) left shoulder, (3) right shoulder, (5) back, (8) below waist, (9) buttocks , (14) right wrist, (15) left wrist, (18) left thigh, (19) right thigh, (22) left calf and (23) right calf points. There were only 5 *lipa sabbe* weavers who worked until November, while 11 others did not get weaving orders so they could not be the sample of this study. Based on the results of observations, those points experiencing complaints could be because the tools used were not ergonomic, for instance waist rest, seat, and leg rest made of wood without any soft layers. In addition, work posture could also affect these conditions.

Table1. Differences in the Levels of MSDs Before and After the Intervention

Responden	The Value of MSDs		P value
	Pre-test	post-test	
MG	57	44	0.042
TN	53	43	
RP	56	47	
ST	55	47	
TS	52	44	
Rata-rata	54.6	45	

Based on table 1, the average NBM result before the intervention was 54.6 and decreased to 45. The difference test results obtained P value of 0.042, which was smaller than the α value (0.05). Accordingly, it could be concluded that there were significant differences in the levels of MSDs after the intervention.

Existing	Modified
Figure 1. Waist rest without soft pad	**Figure 2. Waist rest covered with soft pads according to waist height**
Figure 3. Backrest without soft pad	**Figure 4. Backrest made of soft foam according to the height of the back**
Figure 5. No pillow for buttocks, No soft layer of workstation for thighs and calves	**Figure 6. Seat covered with soft foam**

Discussion

Based on the results of the Nordic Body Map questionnaire, it was found that many points of complaint experienced by respondents including lower neck, left shoulder, right shoulder, back, lower waist, buttocks, right wrist, left wrist, left thigh, right thigh, left calf and right calf points. It is in accordance with Koiri who affirmed that more than 250 respondents in his study experienced health problems in the form of MSDs[11]. Importantly, a study conducted by Sangeeta and Debkumar also concluded that the upper body areas that experience many complaints among weavers of Assam Industry in Guwahati, India were neck, back, and hands[12].

In this study, prior to administering the chair to the intervened skeletal muscle, MSDs averaged over 2 or were categorized as having complaints. The condition of the 12 skeletal muscles was due to a static work position, in which the work was carried out for more than 8 hours without any adequate work station conditions. Static work positions and inadequate workstations can result in complaints of muscles and bones[13].

The results of field observations before the intervention found the following less ergonomic workstation:

1. The waist rest which was also a tool for loosening and tightening the weave was made of wood without soft padding.

2. There was no backrest, making the back bend.

3. There was no pillow for buttocks that cause cramps or pain during work.

4. Straight legs to push at the waist when tensing and loosening the weave were static for long periods of time.

5. There was no soft layer of workstation for thighs and calves.

6. There was no armrest so the hands were hanging on while weaving.

7. No stretching was done before and after two hours of sitting down to work.

Considering the conditions of the *lipa sabbe* weavers above, the researchers engineered their workstation. The workstation changes included the provision of waist rest covered with soft pads according to waist height, backrest made of soft foam according to the height of the back, seat covered with soft foam, and provision of thigh and calf rests which are also covered with foam. Thus, the size of the workstation for each weaver varies due to the their different body postures.

There were some designs that were not changed, for example a design to prevent the legs in an always straight position and the provision of armrests, since the samples refused and considered that too many changes would make them uncomfortable in working. On the other hand, the loom used was not permanent in that place because it was usually moved; weaving was carried out outdoors during the day, and indoors at night. Consequently, it was difficult to make design changes to the legs. In addition, researchers also did not add armrests because hands were very active during weaving so that the provision of armrests could hamper the weaving process. The suggestion for these two conditions is to always stretch before and every two hours of working as recommended by the Surakarta City Health Office[14].

More importantly, after the intervention, it was found that the average MSDs in the skeletal muscles decreased, especially in the parts of the muscles that were given foam support to each respondent. The decrease in the level of MSDs was due to the provision of support at the right point according to the anthropometry and also a layer of foam so that the muscles and nerves at that point were not easily pinched[15]. Based on the results of statistical tests, it can be concluded that there are differences in the level of complaints after the provision of the workstation design for *lipa sabbe* weavers. The provision of ergonomic workstation in this study can reduce MSDs among *lipa sabbe* weavers. It supports a study finding that providing an ergonomic chair can reduce the level of MSDs[16,17]. Additionally, a study conducted by Sangeeta dan Debkumar also condirmed that after the modification intervention, respondents felt comfortable with the new design which then reduced the aches and pains complained[12].

Conclision

The points of complaint that are often experienced

by *lipa sabbe* weavers while weaving include the lower neck, left shoulder, right shoulder, back, lower waist, buttocks, right wrist, left wrist, left thigh, right thigh, left calf and right calf points. In addition, it is found that there is a difference in the level of complaints after modification of the work media of *lipa sabbe* weavers. It indicates that modification of work media can reduce MSDs among *lipa sabbe* weavers.

Conflict of Interest: None

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Ethical Clearance: None

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Images of Ultrasonography or Abdominal Computed Tomography Scanning in Cases of Malignant Kidney Tumors

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Abstract

Background: The malignant kidney tumor is a urinary tract tumor with the highest mortality and morbidity compared to other urinary tract tumors. The role of USG radiology and CT scan is essential for diagnosing malignant kidney tumors.

Objective: To examine the diagnostic value of USG radiology and CT scan in diagnosing malignancy of kidney tumor and its accuracy in assessing renal tumor operability.

Methods: This study was a retrospective observational study using secondary data of radiological examination including ultrasonography, CT scan and other postoperative results derived from medical records. Data analysis was conducted descriptively.

Results: The sensitivity score of USG, CT scan, and USG and CT scan was 60%, 82%, and 83% respectively. Sensitivity value of ultrasound examination was 60% and CT Scan was 82%. The incompatibility of ultrasound with pathology results was found in the case of hydronephrosis with the stone of 64%, stone of 18%, cyst of 18 %, on CT Scan hydronephrosis with stone (83%) Pyelonephritis (17%). The incompatibility of USG and CT Scan with operating results was 8%.

Conclusion: Incompatibility of USG results with anatomic pathology results was found in cases of hydronephrosis accompanied by stones, kidney stones, and benign lesions (cysts). Incompatibility of CT Scan results with anatomic pathology results was found in cases of hydronephrosis accompanied by stone and pyelonephritis.

Keywords: *Malignant Kidney Tumor, Ultrasonography, Abdominal CT Scan*

Introduction

Malignant kidney tumors are the third most common tumor in the field of urology, however, malignant kidney tumors have the highest mortality of 40% compared to other urological tumors. There were an approximately 58,240 new cases of malignant kidney tumors and 13,040 patients died in USA (2010). This malignancy is commonly found in elderly. Males are more often affected 1.5 times than women. Many diagnostic kidney tumors are approximately of 25-40% incidentally.[1,2] The cases of renal tumor in Renal Cell Carcinoma (RCC) cases between 2006-2011 were 29 patients; stage I of 1 (3%), stage II of 14 (48%), stage III of 4 (14%) and stage IV of 10 (34%) in Dr. Soetomo General Hospital Surabaya.[3]

The role of radiology is to differentiate renal tumor characterization that leading to a benign or malignant lesion. That role is important for management, due to the role of FNAB and biopsy of renal tumors are not routinely performed on all renal tumors. The biopsy is performed in certain circumstances if there was a renal tumor with the presence of malignancy primers outside

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the kidneys, unresectable renal cancer cases, comorbid surgery, suspicion of an infection, and in the case of indeterminate cystic renal mass.[4-6] The sensitivity value of radiological examination to detect renal tumor was; ultrasonography had 79% sensitivity in lesion below 3 cm, CT Scan had 95% sensitivity and MRI was comparable with CT Scan in detecting tumor with a sensitivity of 95%. [1,7,8]

The role of radiology for the determination of operable and inoperable tumors is important due to the management of different renal tumor therapies in operable tumors performed by radical nephrectomy and in inoperable tumors performed palliative surgery/ cytoreductive nephrectomy to reduce pain and improve the quality of life of patients.[2]

The management of malignant and non-malignant kidney tumors is different, whereas FNAB is not recommended for determining the diagnosis. The determination of the malignancy is based on clinical and radiological examination. Radiological examination is quite instrumental in the diagnosis and its operability of the renal tumor. Therefore, we aimed to examine about the diagnosis value of radiology examination in this ultrasonography (USG) and CT scan in diagnosing malignancy of renal tumor and the accuracy of USG radiography and CT scan in assessing renal tumor operability.

Methods

The subjects of the study were all medical records of patients with kidney tumor at Dr. Soetomo Teaching Hospital Surabaya from January 2009 to February 2014 that fulfilled the inclusion criteria (complete and accessible renal tumor medical record), exclusion criteria (medical record of incomplete kidney tumor) and the patients have signed informed consent.

This study was a retrospective observational study using secondary data derived from medical records. We obtained 40 medical records that fulfilled the criteria then we collected clinical data, radiology examination results including USG, CT scan and postoperative results. Data analysis was performed descriptively. This research was aproved by ethical clearance from Dr. Soetomo Teaching Hospital.

Results

Forty subjects were enrolled in this study consisted of 30 males (75%) and 10 (25%) females. Subjects aged 10-20 years of 2 subjects (5%), 21-30 years of 3 subjects (7.5%), 31-40 years of 7 subjects (17.5%), 41-50 years of 7 subjects (17.5%), 51-60 years of 10 subjects(25%), 61-80 years of 11 subjects (27.5%). The age range of subjects was between 12-76 years with the mean age of 50 years. There were patients with kidney tumor with a size of <4 cm as many as 2 subjects (5%), size 4-7 cm as many as 6 subjects (15%), size >7 cm of 32 subjects (80%). The sample of USG examination that had anatomical pathology result of malignant kidney tumor were 17 subjects. The image of USG on malignant kidney tumor found 16 subjects in the form of a solid lesion (94%), increased color doppler flow imaging as many as 5 patients (29%), calcification of 3 patients (17%), complex cysts as many as 2 patients (11%) and irregular edge of 2 patients (11%).

There were renal malignant tumor patients that were performed by CT Scan with the anatomical pathology of the renal malignant tumor as many as 27 patients. CT Scan images on malignant kidney tumor was found in 27 patients in the form of malignant lesion of more than 20 housfield units (100%), in the form of lesions solid as many as 22 patients (81.5%), pelvicalyceal system deformity of 11 patients (17%), complex cysts of 2 patients (11%) and the irregular edge of 2 patients (11%).

We found the results of anatomical pathology in renal malignant tumors by 40 patients; 22 patients with renal cell carcinoma (55%), 8 patients with transitional cell carcinoma (20%), 8 patients with squamous cell carcinoma (2.5%), each one patient with malignant small round cell tumor (2.5%) and Mucinous adenocarcinoma (2.5%) . This study obtained the sensitivity of USG examination by 60% and CT Scan of 82%. Twenty-eight patients were tested for USG examination. There was an appropriate diagnosis of a renal tumor in 17 patients and an unsuitable diagnosis on a renal tumor in 11 patients, thus there was incompatibility of 40% and compatible of 60%.

Table 1. Incompatible Distribution of USG Examination with the Findings of Anatomical Pathology Results

USG Results Diagnosis	Frequency	Percentage
Hydronephrosis with stones	7	64%
Stone	2	18%
Benign lesions (cysts)	2	18%

We found incompatibility USG results with histopathologic in 11 patients. There was a diagnosis of hydronephrosis with stones found in 7 patients (64%), stones in 2 patients (18%), and benign lesions (cysts) in 2 patients (18%) (table 1).

Table 2. Distribution of CT Scan Adjustment in Malignant Kidney Tumors

	CT Scan Results	
	Compatible	Incompatible
Anatomy Pathology Result of Malignant Kidney Tumor	27 (82%)	6 (18%)

Thirty-three patients with abdominal CT scan were found. There was an appropriate diagnosis of a renal tumor in 27 patients and an incompatible diagnosis of a renal tumor in 6 patients, thus the compatibility was 82% and 18% of incompatibility. We found incompatibility of CT Scan with histopathology in 6 patients (table 2).

Table 3. Incompatible Distribution of CT Scan Examination with the Findings of Anatomical Pathology Results

CT Scan Results Diagnosis	Frequency	Percentage
Hydronephrosis and stone	5	82 %
Pyelonephritis	1	18%

The diagnosis of hydronephrosis and stone was found in 5 patients (82%) and pyelonephritis in 1 patient (18%) (table 3).

Table 4. Characteristics Distribution of Kidney Tumor Findings from Operating Result

	Frequency	Percentage
Limited to capsules	24	60.0
Penetrating the capsule	11	27.5
Attachment to surrounding organs	5	12.5
Lymphadenopathy	4	10.0
Angioinvasi/Thrombus	4	10.0
Metastasis	1	2.5

There were 35 renal malignant tumor patients performed by radical nephrectomy. The tumor limited to the capsule was found in 24 patients (60%), 11 patients had tumor penetrated the capsule (27.5%), there were five patients who performed cytoreductive nephrectomy, there was an attachment to the surrounding organs of 5 patients (12.5%), lymphadenopathy of 4 patients (10%), angioinvasion and/or thrombus of 4 patients (10%) and metastasis in the lung in a patient (2.5%) (table 4).

Table 5. Compatibility Distribution of USG and CT Scan Resulst with Operating Findings

	Frequency	Percentage
Compatible	25	92
Incompatible	2	8

Twenty-seven patients with CT Scan of a renal malignant tumor found similar surgical findings of 25 patients (92%) and 2 patients (8%) were not in accordance with the findings of the surgery (table 5).

Discussion

Seventeen renal malignant tumor patients who were performed by ultrasound examination had anatomical pathology result of the malignant kidney tumor. It was similar to a study that obtained malignant kidney tumors with a solid component of 97%, increased color doppler flow imaging and only 3% of all subjects obtained complex cyst.[9] The presence of an irregular edge and complex cyst was obtained by wall thickening and the presence of central calcification. Other studies found heteregenous enhancement (71%), homogenous enhancement (29%), irregular edge (31%), and calcification (14%).[10]

RCC is the most common malignant kidney tumor by 80-85% of all malignant renal tumors.[2] whereas renal TCC or urothelial carcinoma (UC) is a malignancy derived from epithelial cells that contain the urinary tract of the renal pelvis to the orificium urethra. The upper urinary tract TCC is suspected to occur in 5% of all urothelial malignancies and approximately 10% of renal malignant tumors.[11] SCC in the kidney is a rare primary malignancy and only 1% was found in the malignant urinary tract. SCC in the urinary tract is more common in urethra and SCC is often associated with kidney stones.[12] Sarcoma in the kidneys is a rare tumor by 1% to 2% of all malignancy of renal tumors that usually found in adults aged >50.[13] Renal sarcoma is rare however, it has higher mortality than other types of renal malignancies. The differentiation of sarcomas than other malignancies are usually difficult, the findings that support the presence of sarcomas are a tumor derived from the capsule and develops into a large mass and the absence of lymphadenopathy that usually grows fast.[2] Ewing Sarcoma in the kidneys is an extraosseous Ewings sarcoma tumor that is commonly found in children and young adults by 6% of all cases. This tumor originates from neural crest cells that are derived from the PNET (primitive neuroectoderm tumor) class. This tumor resembles the RCC image and relative diagnosis is difficult thus it is required an immunohistochemical examination.[14]

The ultrasound examination was performed on 28 patients with true positive of 17 patients and false negative of 11 patients. Meanwhile, CT Scan examination was performed on 33 patients with 27 of true positive and 6 of the false negative. The sensitivity of USG examination was 60% and CT Scan was 82%, this result was different from the previous studies that obtained USG sensitivity of 79% and CT Scan sensitivity of 94%.[1,7,9] Lower USG sensitivity compared to literature was caused by operator factor. There was incompatibility of USG findings on hydronephrosis with stone as many as 7 patients, however, CT Scan examination on 5 patients found a mass in the uteropelvic junction (UPJ). Benign lesions (cyst) was obtained on USG examination however, CT Scan obtained solid mass with cysts. Moreover, stones were found in two patients even though CT Scan did not see a mass. Five patients with hydronephrosis with

stone and a patient with pyelonephritis were found in CT scan examination even though it did not see a mass. The stone within the urinary tract was known to be a risk factor for malignancy of the urinary tract, i.e., urinary tract, urethra and jar. There was a study on 47 patients with stones and non-function kidney treated with nephrectomy that found 24 patients with a malignancy, however, 7 patients out of 24 patients were not seen from radiological examination.[15] Raghavendran et al reported a correlation of some cases of stone and malignant renal function impairment. The exact mechanism of the correlation between the stones to malignancy was still unknown, however, the chronic inflammation and mucosal irritation caused by the stones were the contributing factors. Chronic infections, inflammation, and infections caused metaplasia of the collecting system, which eventually becomes a malignancy. Moreover, stones with recurrent urinary tract infections had a 2-times greater risk of malignancy than non-urinary tract infections. Although SCC and adenocarcinoma of the urinary tract were strongly associated with urinary tract stones, TCC was also associated with urinary tract stones. Kidney stones especially staghorn stones might complicate the vision of accompanying neoplasm lesion because the large stones obscured mucosal lesions seen in the radiology examination. It resulted in difficulties of malignancy diagnosis in patients with stones and infections, and histopathology examination and biopsy should be performed at the time of PNL to determine the presence and absence of malignancy in the kidney.[16]

There were 35 patients (60%) and 5 patients (12.5%) who were performed of citoreductive nephrectomy. We found 24 patients tumors in capsule, 11 patients had penetrated capsule, adhesions to surrounding organ in 5 patients (12.5%), lymphadenopathy in 4 patients (10%), angioinvasi and thrombus in 4 patients (10%) and metastasis in one patient (2.5%). Radical nefrectomy was performed on almost all patients because there was radioresistance and kemoresistance in RCC. Thus, there was no further therapy other than surgery. Patients in stage I to III was performed with radical nefrectomy meanwhile patients in stage IV was performed using citoreduktive nephrectomy due to an attachment to the surrounding organs, the enlargement of lymphnode multiple, thrombis, angioinvation, and metastasis. The inoperability criteria were: 1. Large tumor sized >7 cm and adherence; 2. The presence of bulky lymphadenopathy includes the presence of encasement in the renal vein or large blood vessels; 3. Comprehensive venous thrombosis requires caval venous reconstruction; 4. Expansion of important structures includes mesenteric veins; 5. Experiencing distant metastasis.[17] The compatibility of USG and CT scan findings was 92% and only 8% were incompatibile. Incompatibily were found in 2 patients with thrombus and angioinvation however, the radiology description was not included. CT Scan accuracy was approximately 92%, this was similar to a study by Aysel et al. which stated MDCT accuracy for preoperative staging ranged more than 90%. The Tumor can formed to be benign tumors that not cause cancer and malignant tumors that can cause cancer.[18]

Conclusion

The sensitivity value of USG examination in detecting malignant kidney tumor is lower than the sensitivity value of CT Scan. Incompatibility of USG results with anatomic pathology results was found in cases of hydronephrosis accompanied by stones, kidney stones, and benign lesions (cysts). Incompatibility of CT Scan results with anatomic pathology results was found in cases of hydronephrosis accompanied by stone and pyelonephritis.

Ethical Clearance: This study received an ethical test from Dr. Soetomo General Hospital and faculty of medicine Universitas Airlangga.

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Conflict of Interest: There was no conflict of interest from this study.

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Fibroblast Growth Factor 23 in Children with Chronic Kidney Disease

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Abstract

Background: Decreased of glomerular filtration rate in chronic kidney disease (CKD) changes the calcium and phosphate balance. High phosphate levels in children with CKD stimulate secretion of Fibroblast Growth Factor 23 (FGF23). High FGF23 levels have harmful that potentially increase the morbidity and mortality of children with CKD.

Objective: To analyze the level of FGF23 in children with CKD.

Methods: A cross sectional study was performed in Pediatric Nephrology Ward and Outpatient Clinic of Dr. Soetomo General Hospital Surabaya, during December 2019-March 2020 for children with CKD stage 1-5, aged 3 months to 18 years old. Children on phosphate-binder, vitamin D therapy, or severely ill were excluded. Blood level of FGF23 was measured using ELISA with statistic analysis with SPSS 20.

Results: A total of 52 CKD stage 1-5 children were involved, mean age was 11.44 years old, and 50% were boys. There were 51% children have FGF23 level more than 30 pg/ml. The lowest mean of FGF23 levels was found in the CKD grade 1 (8.94 ± 8.77 pg/mL) and the highest mean at CKD grade 5 (113.30 ± 78.73 pg/mL).

Conclusion: The FGF23 level increasing accordance with increasing in the grade of CKD.

Keywords: *Chronic kidney disease, Children, FGF23, CKD Grade*

Background

Children with Chronic Kidney Disease (CKD) have high morbidity and mortality. Decrease in the Glomerular Filtration Rate (GFR) resulting in an imbalance of calcium and phosphate, resulting in changes of Fibroblast Growth Factor 23 (FGF23) levels, calcitriol, and parathyroid hormone [1]. Fibroblast Growth Factor 23 is a hormone produced by bone which has a direct effect on the heart, trigger left ventricular hypertrophy, and has been associated with consequences, including cardiovascular morbidity [2].

A decrease in GFR leads to a disruption of calcium and phosphate balance. This disorder begins with retention of phosphate in blood. Increased of phosphate levels stimulate bone to increase secretion of FGF23 by osteocytes. Fibroblast Growth Factor 23 secreted in response to increase in phosphate levels as a result of a decrease in LFG. Phosphate retention will activate klotho to increase FGF23. Decrease of GFR will also reduce alpha 1 hydroxylase levels, which in turn increase parathyroid hormone levels, further increasing FGF23

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levels. Increased levels of FGF23 also have contribution either in increasing incidence of cardiovascular disease (CVD) or mortality [3]. In CKD, the presence of systemic inflammation cause an increase in levels of CRP, IL6 and TNF alpha. High levels of FGF23 leads to hypertrophy of myocardial cells and cardiomyocytes [4,5]. High FGF23 is an independent risk factor for CKD progression in children [6]. FGF23 at a concentration >170 pg/ml is an independent predictor of ventricular hypertrophy in CKD children with GFR >45 ml/minute/1.73 m^2 [1]. Nowadays, studies of FGF23 levels in children with CKD in Indonesia are still limited. Therefore, this study conducted to analyze the level of FGF23 in children with CKD.

Methods and Materials

This study was a cross sectional study conducted in Pediatric Nephrology Ward and Outpatient Clinic of Dr. Soetomo General Hospital Surabaya, from December 2019 to March 2020. The inclusion criteria in this study were children aged 3 months - 18 years old, grade I-V of chronic kidney disease, and agreed to participate in the study. Exclusion criteria in this study were consuming phosphate binder and calcitriol, suffering from severe infections, and having a previous history of heart disease. The subject was selected based on random sampling technique. Variables of this study were CKD and FGF23.

Chronic Kidney Disease Criteria

Chronic kidney disease is kidney damage which lasts for at least 3 months with or without a decrease in GFR or GFR of <60 mL/minute/1.73 m^2 for at least 3 months with or without kidney damage (NKF-KDOQI, 2005). The diagnosis was made by the Pediatric Nephrologist. CKD was grouped into: CKD grade 1 (GFR:> 90 mL/minute/1.73 m^2), CKD grade 2 (60-89 mL/minute/1.73 m^2), CKD grade 3 (30-59 mL/minute/1.73 m^2), CKD grade 4 (15-29 mL/minute/1.73 m^2), and CKD grade 5 (<15 mL/minute/1.73 m^2).

Fibroblast Growth Factor 23 Measurement

Fibroblast Growth Factor 23, play a role in regulating phosphate levels homeostasis in circulation, by regulating phosphate reabsorption in kidneys. Blood sample was taken as much as 3 ml, and stored at -80^{0}C until assayed. FGF23 measured in the Clinical Pathology laboratory of Dr. Soetomo General Hospital, using the ELISA kit (Human FGF23 ELISA Kit, Elabscience, WuHan, China) in units of pg/ml.

Result

During the period, 52 children with chronic kidney disease were studied. CKD grade 5 was the largest group with 18 (34.6%) subjects. The next was CKD grade 1 with 12 (23.1%) subjects, followed by CKD grade 4 10 subjects, then CKD grade 2, and 4 with 6 subjects each (Table 1).

Table 1. Frequency distribution of CKD

CKD Grade	N	%
1	12	23.1
2	6	11.5
3	10	19.2
4	6	11.5
5	18	34.6
Total	52	100.0

There were 26 boys, 10 (38.5%) of them have CKD grade 5. As many as 86.5% aged over 5 years with the average age in this study was 11.44 ± 4.44 years. Glomerulonephritis was the most common underlying disease of CKD with 33 subjects, 10 of which were in CKD grade 1 and 10 subjects in CKD grade 5. Tubulopathy and cysts were rare causes of CKD. In this study, 27 subjects had been diagnosed with CKD in less than 12 months and 6 subjects for more than 5 years (Table 2).

Table 2. Demographic characteristics in children with CKD

Variable	CKD Grade					Total
	1	2	3	4	5	
Sex, n (%) Male Female	 5 (19.2) 7 (26.9)	 4 (15.4) 2 (7.7)	 3 (11.5) 7 (26.9)	 4 (15.4) 2 (7.7)	 10 (38.5) 8 (30.8)	 26 (100.0) 26 (100.0)
Age, year `x ±SD	 10.50 ±5.47	 9.50±4.55	 10.70±4.88	 9.17±4.49	 13.89±2.19	 11.44±4.44
Cause, n (%)						
Glomerulonephritis	10 (30.3%)	4 (12.1%)	7 (21.2%)	2 (6.1%)	10 (30.3%)	33 (100.0%)
CAKUT	2 (15.4%)	0 (0.0%)	2 (15.4%)	2 (15.4%)	7 (53.8%)	13 (100.0%)
Stone	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (33.3%)	1 (33.3%)	3 (100.0%)
Tubulopathy	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)
Cystic	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (100.0%)
Duration of illness, n (%)						
< 1 year	10 (37.0%)	4 (14.8%)	4 (14.8%)	2 (7.4%)	7 (25.9%)	27 (100.0%)
>1-3 years	1 (7.1%)	0 (0.0%)	4 (28.6%)	1 (7.1%)	8 (57.1%)	14 (100.0%)
>3-5 years	1 (20.0%)	2 (40.0%)	0 (0.0%)	1 (20.0%)	1 (20.0%)	5 (100.0%)
>5 years	0 (0.0%)	0 (0.0%)	2 (33.3%)	2 (33.3%)	2 (33.3%)	6 (100.0%)

CAKUT, *Congenital anomalies of the kidneys and urinary tracts*

BUN and serum creatinin levels were increased in children with CKD with a mean of 39.19 ±27.91 mg/dl and 4.15 ±4.57 ml/min. Slight decrease of calcium were found at a mean of 8.12 ±1.02 mg/dl. Phosphate levels were remains within normal range with mean 4.43 ±1.41 mg/dl (Table 3).

Table 3. Laboratory characteristics in children with CKD

Variable	CKD Grade					Total
	1	2	3	4	5	
BUN, mg/dl	10.00±5.14	33.83±25.55	26.30±8.73	62.67±27.48	59.78±22. 64	39.19±27.91
Serum creatinine, ml/min	0.46±0.16	0.87±0.26	1.58±0.45	3.22±0.60	9.45±3.90	4.15±4.57
Calcium, mg/dl	8.63±0.88	8.28±0.72	8.14±0.66	8.37±0.75	7.64±1.28	8.12±1.02
Phosphate, mg/dl	4.29±0.94	3.58±1.96	5.07±1.43	5.00±1.74	4.25±1.29	4.43±1.41

The increase of the grade of CKD in line with the increase in FGF23 levels (Table 4). The lowest mean of FGF23 levels was found in CKD grade 1 (8.94 ±8.77 pg/mL), and the highest mean at CKD grade 5 (113.30 ±78.73 pg/mL).

Table 4. FGF23 levels in children with CKD

CKD	n	FGF23 (pg/ml)
		`x±SD
1	12	8.94 ±8.77
2	6	10.58 ±6.72
3	10	20.97 ±15.94
4	6	53.26 ±29.71
5	18	113.30 ±78.73
Total	52	52.68 ±66.12

Spearman (r_s) = 0,834 p = 0,000

The grade of CKD with FGF23 level forming a one-way linear line, which describes that the higher the grade of CKD the higher the FGF23 level (Figure 1).

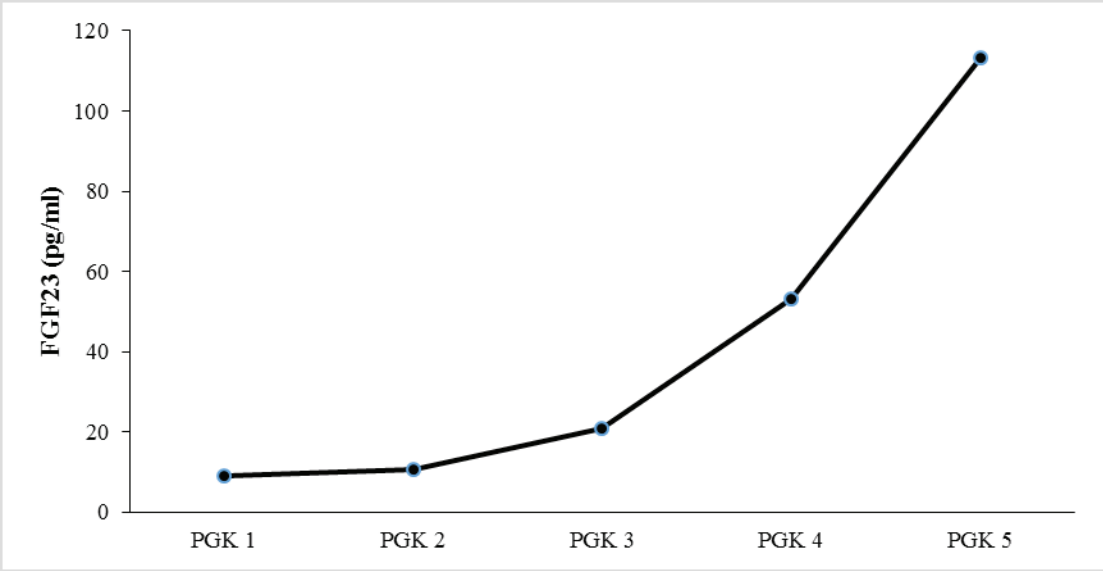

Figure 1. CKD grade and FGF23 level

Discussion

In this study, research subjects were grouped into 5 groups based on the grade of CKD. A total of 12 subjects (23.1%) CKD grade 1, 6 subjects (11.5%) CKD grade 2, 10 subjects (19.2%) CKD grade 3, 6 subjects (22.5%) CKD grade 4, and the largest group of 18 subjects (34.6%) was CKD grade 5. In this study, the distribution of sex for male is the same as for female, with 26 subjects each. Tuttle's research, 2019, which involved 12,591 children with CKD, found a higher prevalence of female than male (56.2% vs 43.8% [6]. The age distribution in this study was almost the same in each group. The number of subjects over 5 years in the study was quite large, namely 45 subjects (86.5%). The mean age was 11.44 ±4.44 years old, with the youngest were 1 year, and the oldest were 17 years old. Tuttle, 2019, in his research, the median age of CKD children is 6 years old (1-13 years). The prevalence of CKD according to age is 20.2% in children less than 1 year old, 17.8% in children 1-3 years, 12% in children 4-6 years, 14.8% in children 7-10 years, 15.2% in children 11-14 years old, and 19.9% in children 15-17 years old [6].

In this study, FGF23 levels measurement was carried out quantitatively by measuring the intact FGF23 levels. FGF23 began to rise above the normal value in CKD grade 3, with a mean of 20.97 ±15.94 pg/ml. Fibroblast growth factor 23 is a hormone that regulate homeostasis of circulating phosphate by regulating phosphate reabsorption in the kidneys. Fibroblast growth factor 23 works by suppressing the expression of the sodium phosphate co-transporter family (NaPi IIa and NaPi IIc) in the proximal tubule of the kidney. Decreased expression of NaPi IIa and NaPi IIc results in decreased renal reabsorption of phosphate. Thus, the excretion of phosphate through urine will increase (phosphaturia) [7].

FGF23 will increase in CKD grade 3 or more. It occurs due to kidneys' ability to excrete phosphate begins to decrease and resulting in hyperphosphatemia. Hyperphosphatemia causes decreased of calcium levels and increased of parathyroid hormone (PTH) secretion. Initially, the serum phosphate level returns to normal and formed a new balance with high PTH and FGF23 levels. As a compensation for hyperparathyroidism and elevated levels of FGF23, which are phosphaturia, are the main mechanisms that maintain phosphate within normal range. This compensatory mechanism will continue to repeat until kidney function worsens and severe hyperparathyroidism occurs, and in the end, there is a marked increase in serum phosphate levels [8].

High levels of FGF23 in CKD patients represents a physiological compensation for maintaining serum phosphate levels, while simultaneously decreasing the number of nephrons. Then FGF23 will increase the excretion of phosphate in urine and decrease the

absorption of phosphate in the gastrointestinal tract through 1-α hydroxylase inhibition, and indirectly cause a decrease in circulating calcitriol levels. Excess FGF23 production will keep the body's phosphate levels within the normal range, until the occurrence of CKD continues. This is supported by a study which showed that in patients with CKD grade 1 to 4, normal serum phosphate levels were obtained. However, an increase in serum phosphate levels began to occur at grade 5 CKD, where the threshold for urinary phosphate excretion capacity has been exceeded [9]. In working to maintain the balance of calcium and phosphate, fibroblast growth factor 23 requires a co transporter, namely Klotho. Klotho is a transmembrane protein which is mainly expressed in the distal tubule of the kidney and in other tissues such as the brain, parathyroid glands and gastrointestinal tract. Klotho is also secreted into blood, urine, and cerebrospinal fluid, therefore there are two forms of klotho, namely soluble klotho that circulates in the circulation and klotho in tissues. In the kidneys, klotho acts as a co-receptor for FGF23, which plays a role in increasing the excretion of phosphate through urine [10]. Klotho together with FGF23 not only increases the amount of phosphate in the urine, but also makes the urine does not contain high calcium, thus preventing supersaturation of urine [11].

Apart from high phosphate in serum, high calcitriol levels will also cause an increase in FGF23. Fibroblast growth factor 23 will inhibit the 1α-hydroxylase enzyme, by inhibiting the conversion of calcidiol to calcitriol. This mechanism is a physiological response to maintain the balance of calcium and phosphate [12,13]. Under normal conditions, FGF23 will provide negative regulation of PTH gene expression, secretion and proliferation of parathyroid cells in vitro and reduce PTH secretion in vivo. Conversely, the elevated PTH levels lead to increased expression of the FGF23 gene in bone. At CKD, the negative regulatory mechanism did not work. It because in patients with CKD there is resistance and down regulation of the FGF23 receptor in the parathyroid glands, so that there will be an increase in FGF23 and PTH [14].

Conclusion

An increase in FGF23 level accordance with the increase in the grade of CKD.

Conflict of Interest : None declared.

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Ethical Clearance : Approved by the Ethics Commission for Biomedical Research on Humans, Dr. Soetomo General Hospital, Surabaya. Ethical number published on December 3rd 2019, 1687/KEPK/XII/2019.

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The Prevalence and Risk Factors of Tracheostomal Stenosis Following Total Laryngectomy

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Abstract

Background: Tracheostomal stenosis (TS) following total laryngectomy is relatively common in patients and a distressing complication with major effects on patient rehabilitation it affected 4-44% of patients following total laryngectomy worldwide. Various etiological factors and some risks have been indicated as contributing to the onset of stenosis.

Purpose: We aimed to investigate the prevalence and risk factors of tracheostomy stenosis following total laryngectomy

Method: Tracheostomal stenosis was defined as respiratory insufficiency at rest or during exercise, difficulty in tracheal clearance due to non laminar flow, or both. Eighty data of patients were enrolled in this study consisting of 76 male (95.0%) and 4 females (5.0%). Baseline characteristics (BL) stage of cancer (SC), type of surgery (TS), the onset of stenosis following surgery (OS) and risk factors (RF) from each patient were recorded and analyzed using a pre-installed statistic software.

Result: The overall prevalence of tracheostomal stenosis was 13.8% (11 of 80 patients). The prevalence and risk factors of tracheostomal stenosis following total laryngectomy consisted of 7 hypertrophic scar patients (63.3%), 2 infection patients (18.12), and 1 radiotherapy and peristomal fat patient (9.01), respectively.

Conclusion: Out of eighty patients, eleven patients indicates the characters of tracheostomal stenosis using the bevel technique. The most common etiological factor is hypertrophic scar, followed by infection, radiotherapy and peristomal fat.

Keywords: *Tracheostomal stenosis, laryngectomy, prevalence, the risk factor*

Introduction

Tracheostomal stenosis (TS), following total laryngectomy, is relatively common in patients and is a distressing complication with major effects on patient rehabilitation. It affects 4 - 44% of patients, following total laryngectomy worldwide [1–3]. TS can occur at any point with some factors that can be considered as stenosis formation, including the occurence of infection around the stoma postoperatively, prolonged use of a tracheostomy tube postoperatively, female sex, and postoperative radiotherapy [4]. The separation of airways and pharynx are some of the consequences due to laryngectomy [5].

Laryngectomy is done due to some larynx abnormality which carcinoma takes most of the cases.

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Some of the cases suggest an occurrence of malignant growths in the region of permanent tracheostoma after laryngectomy which is usually considered to be the peristomal recurrence of the primary laryngeal carcinoma [6,7]. Various etiological factors have been indicated as the contributing factors to the onset of stenosis, including the type of skin incision, the diameter of trachea, excess skin and peristomal fat, incorrect mucosal – cutaneous suturing, hypertrophic scarring and keloid formation, radiotherapy, infection, and tracheoesophageal puncture[8–11].

Some potential cofactors can cause aberrant wound healing and play an important role in the genesis of TS such as adjuvant chemotherapy, advanced age, smoking, cardiopathies, diabetes, local infections, impaired immunocompetence, dan malnutrition [2]. Based on those facts, this study aims to investigate the prevalence and risk factors of tracheostomal stenosis following total laryngectomy.

Material and Methods

A retrospective study from a single tertiary-center was conducted within the period of 2013-2016 following an ethical clearance by the Ethical Committee of Dr. Soetomo Hospital, Surabaya, Indonesia. Tracheostomal stenosis was defined as respiratory insufficiency at rest or during exercise, difficulty in tracheal clearance due to non-luminar flow, or both.

Eighty data of patients were enrolled in this study, consisting of 76 males (95.0%) and 4 females (5.0%). None of these patients experienced previous neck surgery. Baseline characteristics (BL) stage of cancer (SC), type of surgery (TS), the onset of stenosis following surgery (OS) and risk factors (RF) from each patient were recorded and analyzed using a pre-installed statistical software SPPS version 22.

Results

The overall prevalence of tracheostomal stenosis in our center was 13.8% (11 of 80 patients). The onset of stenosis with a median period of 3.27 months (2-6 months). All males aged between 33-62 years. One patient underwent postoperative radiotherapy, while none have experienced tracheoesophageal puncture. There were 5 stage-III patients, and 6 stage-IV patients. Seven patients underwent total laryngectomy and 4 patients underwent total laryngectomy plus selective neck dissection (Table 1).

The prevalence and risk factors of tracheostomal stenosis following total laryngectomy consisted of 7 hypertrophic scar patients (63.3%), 2 infection patients (18.2%), 1 radiotherapy and peristomal fat patient (9.1%) as seen in Figure 1. A sample case of tracheostomal stenosis is shown in Figure 2 of pre and post revision (Fig. 2)

Tabel 1. Data patients of Tracheostomal Stenosis Pasca Total Laryngectomy

No	Stadium	type operation	Onset/mnth	Risk Factor
1.	T3N0M0	TL	6	infection
2.	T3N0M0	TL	3	HS
3.	T4N0M0	TL+SND	2	HS
4.	T4N0M0	TL+SND	4	HS
5.	T3N0M0	TL	4	HS
6.	T3N1M0	TL+SND	3	HS
7.	T4N1M0	TL+SND	6	Rtx
8.	T4N0M0	TL	1	HS
9.	T4N1M0	TL	2	infection
10.	T3N0M0	TL	3	HS
11.	T3N0M0	TL	2	Peristomal fat

Note : TL : Total laryngectomy; SND : selective neck dissection; HS : hyperthropic scar
Rtx : radiotherapy

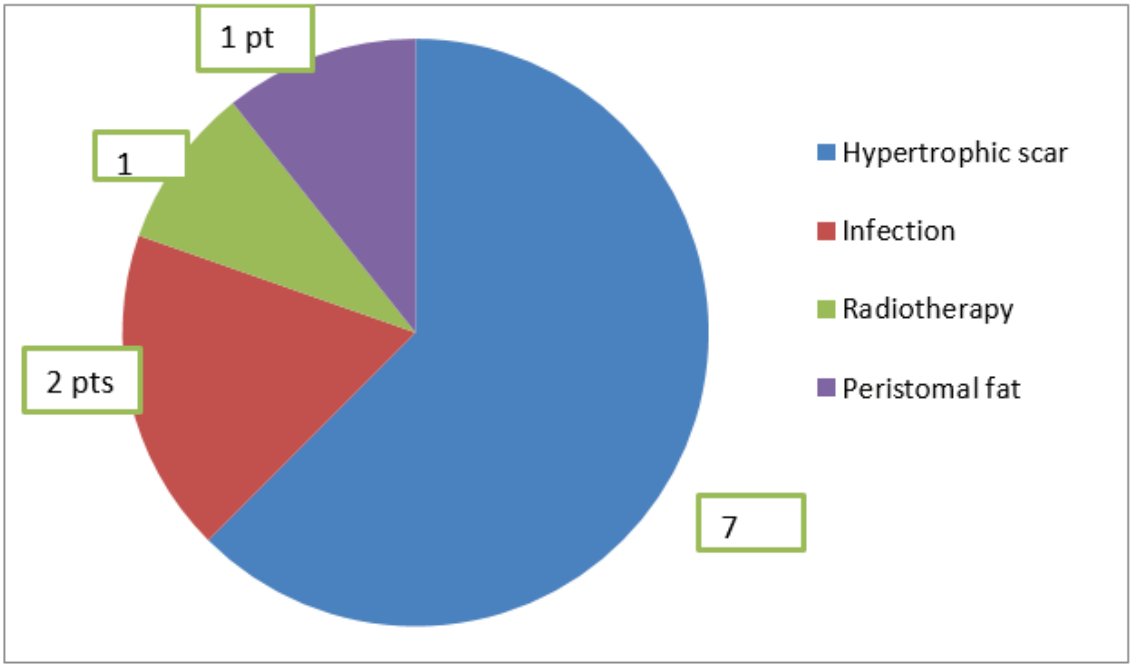

Figure 1. The prevalence and risk factors of tracheostomal stenosis following total laryngectomy

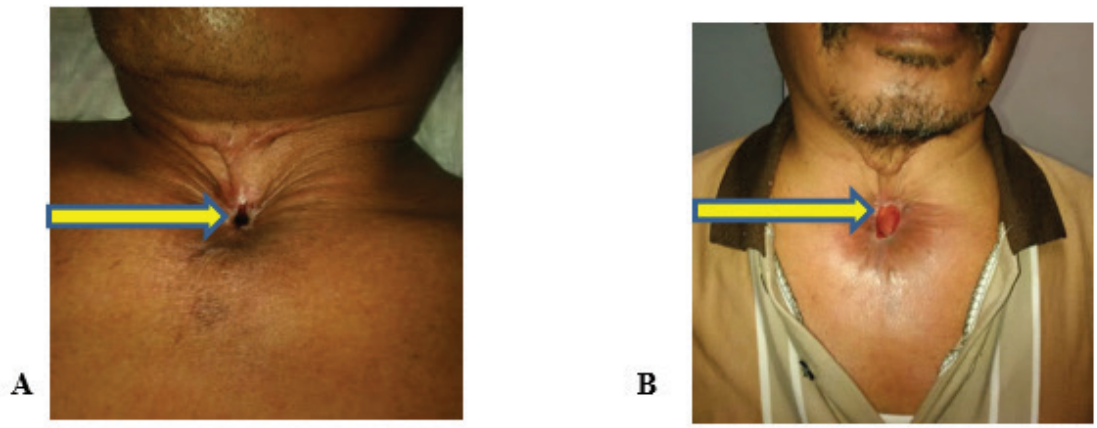

Figure 2. Case of trachoestomal stenosis A. Pre revision B. Post revision
= stenosis with hypertrophic scar

Discussion

The most important factor is prevention. The primary causes are the formations of scar tissue and other tissue around the stoma, resulting in a narrowing or constriction. Several factors may contribute to the stenosis, including the type of skin incision, surgical technique used to create the stoma, the diameter of trachea, excess skin and peristomal fat, incorrect mucosal – cutaneous suturing, hypertrophic scarring and keloid formation, radiotherapy, infection, and tracheoesophageal puncture. To minimize its occurrence, the correct construction of the tracheostoma when total laryngectomy is carried out. A simple circle of straight transection of the trachea had the highest incidence of stenosis (29.0%), which can be reduced with a bevel technique (15.0%). The lowest incidence was seen with a primary plastic or flap construction technique (8.0%). The incidence of tracheostomal stenosis in this study

used the bevel technique and amounted to 138%.

Tracheostomal stenosis is a devastating problem following a total laryngectomy. The prevalence of stenosis ranged from 4% to 42%, depending on the criteria used. In our study, the prevalence was 13.8% (11 of 80 patients) while Kou et al. (1994) reported the occurrence of 13% [12]. Hypertrophic scars have been indicated as the cause of stenosis. In these series, the most common etiological factor is a hypertrophic scar (63.3%), followed by infection (18.2%), as well as radiotherapy and peristomal fat (9.1%), as seen in Figure 1. All of the patients were Javanese. The race might contribute to hypertrophic scar. Tracheostomal infection may contribute to stenosis in several ways.

Crusting and bleeding at the stoma may cause damage to the mucosal lining of the trachea and subsequent fibrosis. Infection of the mucocutaneous junction of the cartilage of the trachea leads to the formation of granulation tissue, followed by healing by secondary intention, which results in increased scar tissue formation and decreased internal tracheal diameter. Radiotherapy has been associated with stenosis, although it has been suggested that this may be secondary to impairment of wound healing consequent upon obliterative vasculitis and ischemia being the irradiation lesion. It is more likely to happen due to concentric scaring.

Conclusion

Out of eighty patients, 11 patients indicate the characters of tracheostomal stenosis using the bevel technique. The most common etiological factor is hypertrophic scar, followed by infection, radiotherapy and peristomal fat.

Ethical Clearance: This research involves participants in the process using a questionnaire that wasaccordant with the ethical research principle based on the regulation of research ethicregulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

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Conflict of Interest: There is no conflict of interest

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Hormonal Receptors and Human Epidermal Growth Factor Receptor 2 Discordance between Primary and Recurrent Breast Cancer in Sample of Iraqi Patients

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Abstract

Background: Though progesterone receptor and estrogen receptor are weak prognostic indicators, they are greatly predictive of response to endocrine therapy. HER2 overexpression or amplification is a powerful predictive factor for response to specific systemic therapies. Variation in estrogen receptor (ER), progesterone receptor (PR), and HER2 status between primary breast carcinoma and subsequent relapse of is clearly recognized. **Aim of study:** The study aims at determining the rate of discordance in progesterone receptor, estrogen receptor and human epidermal growth factor receptor 2 status between primary tumor and subsequent locoregional and metastatic disease in a sample of patients attend oncology teaching hospital**. Patients and Methods**: A retrospective study conducted included 80 females patients attending oncology teaching hospital between December 2013 – December 2017 for whom the ER,PR,and HER2 status were known on tissue samples of both primary and subsequent locoregional and metastatic disease .

Results: The ER,PR,and HER2 status discordance rate for between primary breast cancer and subsequent locoregional relapse and distant metastasis was 27.5%, 42.5% and 22.5% respectively

Conclusion: The rate of discordance in ER and PR status primary and relapsed breast carcinoma in patients managed at oncology teaching hospital was similar to other studies. While the rate of discordance for HER2 status between primary and relapsed breast carcinoma was slightly higher than international studies.

Keyword: *Breast cancer, Hormonal Receptors, HER2, Estrogen receptor, Progesterone receptor.*

Introduction

Breast cancer, prognosis and treatment selection in breast cancer in adjuvant setting and at relapse are based on characterization of tumor growth factor receptor status- (ER), (PR) and HER2. [(1)] Approximately 70% of breast cancer are hormonal receptor positive (ER and / or PR). ER is an important regulator of both physiologic and pathologic mammary growth and differentiation. [(2)] The ER and PR are weak prognostic indicators, but they are highly predictive of response to endocrine therapy. [(3)]

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Endocrine therapy is used in adjuvant therapy for almost all women with hormone receptor–positive disease breast cancer.[(4)] Endocrine therapy is also used in management of patients with hormone receptor positive breast cancer at time of relapse whether locoregional relapse or distant metastasis.[(1)] HER2 is a member of the epidermal growth factor receptor (EGFR) tyrosine kinase family that includes four transmembrane receptor proteins: EGFR-1, HER2, HER3, and HER4.[(5)] HER2 protein overexpression or amplification (20% of breast carcinoma) is associated with accelerated cell growth and proliferation, decreased overall survival (OS) and poor prognosis. [(6)] HER2 overexpression or amplification is a powerful predictive factor for response to specific systemic therapies. [(5)]

Anti-HER2–directed therapy includes the use of trastuzumab, used in adjuvant setting and also for locoregional relapse and metasatic disease; pertuzumab,; trastuzumab emtansine, and lapatinib, are mainly used for metastatic disease .(7)

Many randomized controlled trials have revealed that the addition of trastuzumab to chemotherapy enhance the quality of life and improves survival in metastatic disease, leading to its inclusion in the standard of care for all patients with HER2-positive breast carcinoma. (6) The monoclonal antibody pertuzumab, improved overall survival in metastatic setting, improved pathological complete response in preoperative setting (8).

HER2 status, in addition to being an excellent predictive factor, is also modest prognostic indicator if a patient does not receive HER2 -directed therapy. HER2 -positive disease that is not treated with anti-HER2 therapy is associated with shorter disease-free survival and breast cancer specific survival. (7)

Systemic treatment selection at time of relapse of breast cancer depends partly on ER,PR,HER2 status obtained from primary breast cancer(9) However, based on potential discordance between primary and subsequent locoregional and metastatic disease, reliance on primary breast cancer receptor has been questioned with recommendation for rebiopsy in situation when change in biomarkers would impact the treatment choice ,which mainly occur when biomarker (ER,PR,HER2) were negative on primary tumor.(11) There is some controversy about benefits of biopsy in situation, when all receptors (ER,PR,HER2) were positive since it is still unknown which tumor biomarkers profile (ie primary tumor profile or tumor marker profile at time of relapse) will best predict the most effective approach to treatment. (10) Mechanisms of receptor discordance remain unidentified and could have therapeutic implications. (11)

several possible justifications have been for a such alternations, imperfect accuracy and reproducibility of assays, heterogeneity of the tumor , and biologic evolution of the tumor and differential effect of prior treatment on clonal subsets. (11)

Aim of Study: Determination the rate of discordance in receptors status between primary tumor and subsequent locoregional and metastatic disease in a sample of patients attending oncology teaching hospital.

Patients and Methods

This study conducted retrospectively and included 80 female patients attending oncology teaching hospital between December 2013 – December 2017, all had breast cancer relapse at presentation, detailed history was obtained and information were recorded after obtaining patients oral consent. Any patient had bilateral breast cancer, stage four breast cancer at time of diagnoses, and patients with another primary cancer were excluded. Information about patient characteristics a hormonal receptors (estrogen and progesterone) and HER2 receptors, the pathological type and grading and AJCC TNM staging system for breast cancer were obtained from patients records.

Patients characteristics included stage at diagnoses, adjuvant treatment ,site of relapse(whether locoreginal or distant relapse) and site of biopsy.

A tissue sample was taken from each patient either by excisional biopsy or trucut biopsy and examined by H&E staining to confirm relapse of primary tumor. Then Immunohistochemistry analysis at pathology department of oncology teaching hospital was performed to assess the estrogen, progesterone and/or HER2 receptors , examination was done by experienced pathologist

Expression of ER and PR were assessed using Dako ER/PR pharma^{Dx} immunohistochemistry Kit and scored using the semiquantitive Allred system, which take in to account the proportion of positive cells (graded 0-5) and staining intensity (graded 0-3)(12). Signal were detected by using EnVision TM FLEx mini Kit,highPH,(Link) Code K8023

The proportion of positive cells and intensity were summed to produce total score 0 or 2 through 8 .A score of 0 or 2 was regarded as negative ,whereas a score of 3 to 8 ,as positive.(12)

Concordance demonstrated between ER/PR pharmaDx TM and and established reference method with positive and negative cut off IHC score using by ASCO /CAP (Nuclear staining 1% or more). (13)

HER2 protein overexpression was determined by using IHC (Dako Denmark,A/S ,Ventana medical

cystem)was done using the standards system of scoring between 0 to 3, values of 0 and 1 was regarded as negative, 3 as positive, and for equivocal intensity of 2, further confirmation was done by Chromogenic in situ hybridization (CISH) . [14]

Regarding the HER2 status in the primary setting, we included the results of IHC that revealed 0 or + 1 (negative) and + 3 (positive), the results of IHC that revealed +2 equivocal in primary setting was excluded from our study, since the result of confirmatory test were unavailable (missed) from patient records at time of diagnoses.

Statistical Analysis

Data tabulation, input and coding was done by the use of IBM© SPSS© (Statistical Package for the Social Sciences) Statistics Version 22. Univariate ANOVA for numerical and normally distributed data. Positive agreement, negative agreement, and discrepancy rates were calculated using test of agreement (Cohen κ). Values of κ indicate level agreement and interpreted as follow: value between 0 - 0.20 as none agreement ;0.21–0.39 minimal agreement; 0.40-0.59 weak agreement ;0.60-0.79 moderate agreement ;0.80-0.90 strong agreement and values above 90% as almost perfect agreement.

Positive agreement calculated from proportion of concordant cases (unchanged) in positive receptors status with respect to the total number of patients in positive receptor status.

Negative agreement calculated from proportion of concordant cases (unchanged) in negative receptors status with respect to the total number of patients in negative receptor status.

Discrepancy rate was calculated as the proportion of discordant cases (changed) with respect to the total number of patients.

Results

The mean age of the study group was 52.94 ± 5.51 years, patient with lobular type were the relatively younger than others 51.80 ± 3.23 years, while the other types had very close mean age groups as showed in Table (1), but there was no significant statistical difference between them.

Table (1): Mean age distribution at time of diagnosis according to histological subtype (mean ±SD = 50.78 ± 8.81 years)

	Mean age in	P-value
	years ± SD	
Ductal	52.00 ± 9.65	0.420
Lobular	48.67 ± 7.23	

In this study the predominant histological subtype of breast cancer was invasive ductal carcinoma as 72 (90%) out of total 80 patients, all the remaining 8 (10.0%) had invasive lobular carcinomas. The type of biopsy encountered in this study was either excisional one 69(86.25%) obtained from sites of loco-regional relapse .distant metastasis relapse , the remaining 11 (13.75 %) were investigated true cut biopsies (Table(2)).

Commonest site for biopsy was loco-regional recurrence sites 68 (85.5%) (mastectomy scars, regional lymph nodes and chest wall masses). The remaining 12 (14.5%) biopsies were obtained from distant metastasis which included 7 of them (8.75%) from liver, 3 (3.75%) from bone, 1 (1.25%) from brain and another one from pleural biopsies. (Table(2).

At time of diagnosis of primary breast cancer all patients had underwent primary tumor positive to negative status in relapse setting. On the other hand 28 (35.0%) patients showed negative estrogen receptor in primary tumor, 6 (21.42%) of them shifted to positive in relapse, these results showed that there were 27.5% discordance rate between primary and secondary receptor status with weak agreement between the two results as shown in Table (2). Resection with the vast majority followed by chemotherapy, 75(93.75%). Regarding endocrine therapies 52 (65.0%) had received hormonal therapy, also 12 (15.0%) received anti-HER2 agent (Trastuzumab).(Table(2)).

Table (2): Characteristics of study group

Characteristics		No. (%)
Histological subtype of primary tumor	Ductal	72 (90%)
	Lobular	8 (10%)
Histological Grade of primary tumor	I	8 (10%)
	II	45(56.25%)
	III	27 (33.75)
Stage of primary tumor	I	12 (15.0%)
	II	27 (33.75%)
	III	41 (51.25%)
Type of biopsy at time of relapse	Excisional	69 (86.25%)
	True cut	11(13.75%)
Site of biopsy at time of relapse	Loco-regional recurrence	68 (85.5%)
	Distant Metastasis	12 (14.5%)
	Liver	7(8.75%)
	Bone	3 (3.75%)
	Brain	1 (1.25%)
	Pleural biopsy	1(1.25%)
Therapy in adjuvant setting	Endocrine therapy	52 (65%)
	Chemotherapy	75 (93.75%)
	Trastuzumab	12 (15.0%)

Table (3): Progesterone Receptor in Primary and in Relapse

Progesterone Receptors		Primary				Total		k	Positive Agreement	Negative Agreement	Discrepancy rate
		+ve		-ve							
		No.	%	No.	%	No.	%				
Relapse	+ve	20	41.67	6	18.75	26	32.5	0.206	41.7%	81.3%	42.5%
	-ve	28	58.33	26	81.25	54	67.5				
Total		48	100.0	32	100.0	80	100.0				
k: Cohen's Kappa											

There was weak statistically significant agreement between primary and secondary estrogen receptor status.

Examination of progesterone receptor status in primary tumor showed that there were 48 (60.0%) patients with positive receptor status, from these, 28 (58.33%) of them shifted from positive to negative in relapse setting. There were 32 (40.0%) patients with primary negative progesterone receptors, 6 (18.75%) of them shifted to positive receptor status in relapse setting. From these results discordance rate was 42.5%, with minimal agreement as shown in Table (4)

Table (4): Progesterone Receptor in Primary and in Relapse

Progesterone Receptors		Primary				Total		k	Positive Agreement	Negative Agreement	Discrepancy rate
		+ve		-ve							
		No.	%	No.	%	No.	%				
Relapse	+ve	20	41.67	6	18.75	26	32.5	0.206	41.7%	81.3%	42.5%
	-ve	28	58.33	26	81.25	54	67.5				
Total		48	100.0	32	100.0	80	100.0				
k: Cohen's Kappa											

There was minimal statistically significant agreement between primary and secondary progesterone receptor status. Total patients with positive HER2 receptor status were 18 (22.5%), only 2 (11.1%) of them shifted to negative in relapse setting. The remaining 62 (77.5%) negative primaries had remained negative in 46 (74.2%) patients and shifted to positive in 16 (25.8%) patients, discordance rate was found to be 22.5%, with weak agreement between primary and secondary HER2 receptor status as shown in Table (5).

Table (5): HER2 Receptor in Primary and in Relapse

HER2 Receptors		Primary				Total		k	Positive Agreement	Negative Agreement	Discrepancy rate
		+ve		-ve							
		No.	%	No.	%	No.	%				
Relapse	+ve	16	88.9	16	25.8	32	40.0	0.494	88.9%	74.2%	22.5%
	-ve	2	11.1	46	74.2	48	60.0				
Total		18	100.0	62	100.0	80	100.0				

k: Cohen's Kappa

There was weak statistically significant agreement between primary and secondary HER2 receptor status

Discussion

Part one

Systemic management selection on recurrence of breast cancer still depend ,in part, on ER ,PR,and HER2 status of primary breast carcinoma .[5]

However, several guidelines recommend reassessing these receptors status if changes in these receptor status would modify management[19].

This study included 80 patients for whom the hormonal receptor profile (ER and PR) and HER2 had been studied in both primary and recurrence setting .

This study revealed that the rate of discordance between primary setting and recurrence setting was 27.5% for oestrogen receptors, 42.5% for progesterone receptor and 22.5% for HER2 overexpression showing significant changes.

HER2 status and ER,PR status are predictive factor of response to anti-HER2 targeted therapy and endocrine therapy respectively .[3, 5]

In a study conducted at Stockholm health care regions of breast cancer which included women with breast cancer for whom the status of ER (459 patients), PR (430 patients) from both primary tumor and subsequent relapse were assessed.

The discordance rate between primary breast cancer and subsequent relapse was 32.45% for ER and (40.7%) for PR .[16]

In Liu trail conducted at China at Sun Yat-Sen Memorial Hospital ,the purpose of study was to determine whether ER,PR and HER2 statuses between primary breast cancer and subsequent liver metastasis were changed by treatment or during the natural metastatic process , the discrepancy rate for ER 30% and for PR 54% between primary breast cancer lesion and subsequent liver metastasis. [17]

In study conducted at department of medical oncology ,Hacettep university Institute of medical oncology ,Ankara, Turkey, in which the discordance Rate were assessed between ER ,PR status in primary setting and at relapse for patient with primary breast cancer among 78 women , the discordance rate for ER status was 36%(n=27) ,and for PR 54.2% (n=39).[18]

In Lower et al study in which the discordance rate between primary breast cancer lesion and subsequent

relapse evaluated for hormonal receptor , the discordance rate for ER was 30% in 200 patients and for PR (39.3%) in 173 patients. [19]

In study conducted at European Institute of Oncology Milan, Italy, between 1994 and 2005 evaluate the outcome of breast cancer Patients after recurrence in 279 patients according ER, PR status between primary tumor and at the subsequent relapse, rate of discordance between primary and relapsed breast cancer was 9% for estrogen receptor , 22% for progesterone receptor. [20]

This study results is lower than that of this study and the possible reasons for that may be due technical issues, such as methods of fixation ,methods of staining, subjective scoring and antigen retrieval ; all of which have affect in the interpretations of the result affect.[21]

It may be also due to difference in biology of disease between different race .

In Nishimura et al study in which the discordance rate for ER,PR status, between primary breast cancer lesion and subsequent metastasis were assessed in both primary breast cancer and recurrent setting in 97 patients ,the rate of discordance rate for ER was 10.3% and for PR status 25.8%.[22]

The results of the above study is lower than that of this study .

The possible explanation for that difference may be due to the difference in percentage of nuclear staining , in later study nuclear staining for ER and /or PR in at least 10% or more is used to define hormonal receptor positivity (ER and/or PR),while in the current study hormonal receptor (ER and /or PR) status considered positive when staining for ER and /or PR in at least 1% or more of cancer cell nuclei.

However there is wide range for the discordance rate between primary breast lesion and subsequent relapse in systematic analysis that included studies assessing the discordance rate between primary breast cancer and their metastasis ,the median rate for discordance for ER was 14% ranging from 0-67% ,IQR 9-25 %, and for PR was 21 % ranging from 0-62%, IQR 15-41%.[23]

However, mechanisms of discordance remain unclear.

It may be related to change in biology of disease, differential effect of previous treatment on clonal subsets, heterogeneity of the tumor, or imperfect reproducibility and accuracy of assays.[11]

Part Two

The anti –HER2 agents is very effective when combined with chemotherapy and some of them improve overall survival.[6]

In order to initiate anti HER2 agents we must know the HER2 status in recurrence setting (Locoregional and metastatic disease).

In Lower [29] study which is a retrospective one, the rate of discordance for HER2 was34% (number of patient was 382).

In later study they found that the discordance rate of 34% in HER2 expression which is of close to our study finding .

The difference of result of this study from our study may be due following points:

1- The number of patients included in both study

2- In our study we use IHC analysis only for HER2 status of primary lesion and the equivocal result for HER2 were excluded in primary setting and IHC and confirmatory CISH analysis for equivocal result of 2+ by IHC in recurrence setting.

While in above study they use IHC and FISH analysis for equivocal results in both primary and metastatic setting.

However, In systemic review, that included studies evaluating the discordance rate between primary breast cancer and their metastasis the median discordance rate for HER2 was 10 %(range 0-44%, IOR4-17%). [24]

The underlying mechanism of the discordance between primary lesion and recurrent lesion are remain

unknown.

It may be related to change in biology of disease, heterogeneity of the tumor, or imperfect reproducibility and of accuracy tests.[11]

Conclusions

1- The rate of discordance for oestrogen receptor, progesterone receptor between primary breast cancer and subsequent relapse in patients treated at Oncology Teaching Hospital was similar to previous studies.

2- The rate of discordance for HER2 was slightly higher than previous international studies

Recommendations

1- Routine Reassessment of ER,PR and HER2 receptors status in recurrent breast carcinoma specially when one of them or all of them were negative in primary setting and especially if HER2status is negative primary setting since targeted therapy are available in recurrent setting(metastatic or locoregional recurrence).

2- Future studies concerning the prognostic status of change in ER,PR and HER2 between primary breast cancer and recurrent setting.

3- Since it is still unknown which tumor biomarkers profile (i.e. primary tumor biomarkers profile or tumor biomarker profile at time of relapse) will best predict the most effective approach to treatment; we need prospective study to assess the clinical outcome related to altering management according to change in ER ,PR, and HER2 status between primary tumor and recurrent setting.

No Conflicts of Interest

Self-Funding Source

Ethical Clearance: From the Ministry of health and Environment/ scientific committee

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Screening of Group of Iraqi Patients with Turner's Syndrome in Relation to Karyotype-Phenotype Variation

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Abstract

Background: There is variation in karyotype that can be related with the phenotype difference in patients with Turner syndrome (TS).

Objective: Asses the relationship between karyotype and phenotype of confirmed Turner Syndrome patients and Screening them for related congenital and immunological conditions.

Methods: Eighty-five cases of TS were comprised. Patients were indicated mainly for evaluation of short stature and (or) delayed puberty; Standard karyotyping was analyzed on the basis of routine G-banding technique. Turner's syndrome is divided into traditional and non-traditional with further categorization with respect to Karyotype. After full examination, screening tests comprising thyroid function tests, celiac screen, Echocardiography, Renal and pelvic ultrasonography was achieved.

Results: Webbed neck and Dysmorphic facial characteristics were more found during clinical examination in traditional TS versus Non-traditional Turner's syndrome with p-value of 0.01 and0.027, respectively. Abnormal thyroid function tests were more common in traditional TS than Non-traditional, p-value 0.02. Abnormal findings (mainly streak ovary) on pelvic ultrasound (US) was also more common in traditional TS than Non-traditional, p-value 0.01.

Conclusion: Screening of patients with Turner's syndrome with respect to phenotype -karyotype difference can provide opportunities for enhancement of our knowledge, diagnosis and further management.

Key Words: *Turner's syndrome, Karyotype, Phenotype, Mosaicism.*

Introduction

Turner's syndrome (TS) is the most frequent genetic disorder in females with prevalence of 1:2500 that is around 3% live births of females delivered, and approximately 15% of miscarriages. [1,2]. This syndrome considered by the absence of portion of or the whole X chromosome in a female, with perfect dysmorphic attributes such as short stature, primary amenorrhea, estrogen insufficiency, cardiovascular malformations, morphological features and skeletal abnormalities. There are different karyotypes and phenotypes exist [3,4]. Teenagers with the Turner's syndrome are famous for their short stature and ovarian dysfunction [5,6].

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The Objective of this Study

To determine the relationship between karyotype and phenotype of confirmed Turner Syndrome (TS)Iraqi

patients and screening them for related congenital and immunological disorders in relationship to karyotype and phenotype variation.

Patients and Method: The cases were reported in the Department of Endocrinology and Diabetes, children welfare hospital and department of gynecology, medical city complex, Baghdad, Iraq during the period (January 2009) to (January 2019). Eighty-five cases of TS were comprised, after full history and physical examination, diagnosis was made on the basis of clinical phenotype and confirmed by karyotypic analysis of a peripheral blood lymphocytes utilizing routine G-banding method with resolution of 400 band per haplotype by comprising counting of 20 metaphases, and five of them were fully analyzed. According to their karyotype, the patients were classified into two groups: the first group **A** (NO=51) include: traditional monosomy 45, X; while the second group **B** (NO=34) include: Non-traditional miscellaneous karyotypes.

Screening for related disorders was done with respect to the present recommendations [7,8]; Serum follicle-stimulating hormone and luteinizing hormone were done in patients who were ten-year of age and up. Thyroid function tests were done for patients four-year age and older. Ultrasound examination of genitourinary system, two-dimensional echocardiography was done at time of diagnosis. For screening, celiac disease that the patients will be chosen for stomach biopsy on the basis of positive antigliadin antibodies (AGA) IgA, anti-endomysia antibodies (EMA)and Anti tissue antibody (TTGA), for patient four-year age and older.

X-ray radiography of the non-dominant wrist and hand for assessment of bone age before initiation of progress hormone treatment and will fully argued in different article. Written Informed consents were occupied and high confidentially of data was maintained. If the participant was under the age of 18 years, both the participant and the parents employed the informed consent. Ethical approval was found as suitable in our country.

The Results

A total of 85patients with Turner's syndrome were comprised in the study. Turner's syndrome is then divided into traditional and non-traditional with additional categorization with respect to Karyotype. The incidence of patients in each classification are displayed in **Table (1).**

Table (1). Kind of Turner's syndrome with respect to karyotype.

Category	Karyotype categorization	N	%
Group A (traditional)	Standard Monosomy45x	51	60.0
Group B (Non-traditional)	Mosaic turner 45, x0/46, xx	14	16.5
	Isochromosome long arm46, x,i(xq)	17	20.0
	Mosaic with different45, x0/46, xiso(xq)	3	3.5
Total		85	100.0

Traditional Turner's syndrome is most frequent in age group 5.1 to 10 and non-traditional Turner's syndrome is least obtained in age group 1.1 to 5 year, however there is no significant related, p-value 0.18. Both delay puberty and short stature were the most frequent causes of transfer in both groups of Turner's syndrome , P value 0.56. Webbed neck and Dysmorphic facial appearances were more obtained in examination in traditional TS versus Non-traditional Turner's syndrome with p-values of (0.01, 0.027) (significant). The rest of result in clinical examination are scheduled in **Table (2).**

Table (2) patients with positive Dysmorphic Features on examination with respect to type of Turner's syndrome.

Dysmorphic Attributes	Non-Traditional TS n(%)	Traditional TS n(%)	p-value
Cubitus valgus	18(52.9%)	26(51.0%)	0.85
Webbed neck	8(23.5%)	26(51.0%)	0.01
Short neck	15 (44.1%)	22 (43.1%)	0.92
Shield chest	0 (.0%)	5 (9.8%)	0.06
Low hair line	15 (44.1%)	30 (58.8%)	0.18
Short 4th and 5th metacarpal bone	3 (8.8%)	5 (9.8%)	0.78
Dysmorphic facial appearances	11 (32.4%)	29 (56.9%)	0.027
Wide space nipple	20 (58.8%)	27 (52.9%)	0.59

Cardiac abnormalities were: in Non-traditional kind comprise: Coractation of aorta in 2 patients , Bicuspid aortic valve in 2 patients , Aortic stenosis in 1 patient and in traditional kind: Coractation of aorta in 2 patients , Patent Ductus arteriosus in 2 patients, Bicuspid aortic valve in 1 patient ,Pulmonary stenosis in 1 patient , Aortic stenosis in 1 patient ,P- value 0.51(non-significant).

Renal malformations were in traditional kind: horseshoe kidney, while in Non-traditional kind comprise: horseshoe kidney and renal duplication in 1 patient, respectively p- value 0.33 (non-significant). The rest of positive screening findings was illustrated in table **(3)**.

Table (3) Patients with positive screening tests with respect to kind of Turner's syndrome.

Screening test		Non-Traditional TS n (%)	Traditional TS n (%)	p-value
laboratory	Abnormal thyroid function tests	1 /28 (3.5 %)	11 /38 (28.9%)	0.02
	Raised LH and FSH tests.	21/26 (80.7%)	29/34 (85.3%)	0.92
	Positive celiac disease screen (positive jujenal biopsy).	2 /28 (7.1 %)	6/38 (15.78%)	0.49
imaging	Abnormal results on echocardiography.	5 (14.7%)	7 (13.7%)	0.51
	Abnormal findings on renal ultrasound	2 (5.9%)	1 (2.0%)	0.33
	Abnormal findings on pelvic ultrasound.	14 (41.2%)	41 (80.4%)	0.01

Statistical Analysis

An expert statistical advice was sought for Statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 21 computer software. Discrete variables were introduced as number (%). Fisher exact test when suitable via using chi-square test to check the significant value of related of discrete variables where p-value of < 0.05 were regarded significant.

Discussion

The current study may concise with other Prior studies suggested that peak age at diagnosis is lower in patients with traditional kind (monosomy 45, X) compared to other karyotypes, probably due to more typical Turner stigmata in the traditional group [9-10]. Where patients with TS displayed distinctive attributes that take a strong relation with their chromosomal make-up. [11].

The whole females with TS show growth failure and achieved a final height, which is smaller than average. While children can primarily show regular growth, typically for beginning few years of lifecycle. Nonetheless, the growth rate ultimately converts slower than regular and affected children do not experience typical growth spurts [12]. Though the physical abnormalities are recognized, however a varied range of phenotypic variability was obtained in TS patients that essentially is based on the genetic constitution that can represent the karyotype-phenotype variation [13].

Gonadal dysfunction is part of the phenotype in TS and leads to absence of spontaneous puberty, estrogen deficiency, and infertility. Ovarian failure and decreased ovarian feedback result in significant elevation of FSH and LH levels during early childhood period and adolescence (Bi-phasic pattern), whereas gonadotrophins levels were not significantly raised at age 5–10 years compared with other healthy persons, for this reason gonadotropin concentration found between ages 5-9 years cannot be used to asses ovarian function [14].This study showed that approximately in most traditional TS patients, ovaries were absent or small, A subsequent reports found that 76% of those with X mosaicism and only 26% of those with 45,X karyotypes had detectable ovaries during ultrasound examination[7,15].

Congenital heart abnormalities appear in up to 50% of individuals with TS, affecting mainly the left side of the heart and comprising bicuspid aortic valve (BAV), coarctation of the aorta, and thoracic aortic aneurysm. Mortality rates are 3-fold higher in women with TS compared with the general population, with the most frequent cause of death being cardiovascular disease [16]. Patients with severe dysmorphic signs showed a significant higher relative risk of cardiac malformations and x linked factors may be complicated in determining cardiac defects in turner's syndrome [17].

This study were registered the related renal abnormalities is lower as compared to the other studies and the structural malformations appears more repeatedly with non-mosaic 45, X TS [18].

An increased incidence of autoimmunity has been largely documented in TS. Patients with traditional kind are more prone to develop thyroid autoimmunity. An early assay of autoantibodies and monitoring thyroid hormones is fundamental for detecting hypothyroidism precociously and start adequate replacement therapy [19].

The screening for Celiac disease (CD) in TS patients is based on the determination of CD-associated antibodies, this is followed by intestinal biopsy in positive patients. It is famous that both TS and CD are related with different autoimmune disorders [20].

Conclusion

The traditional kind (monosomy 45, X) considered by earlier age at presentation however with more severe dysmorphic attributes as compared to the other non-traditional kinds of turner syndrome, this may give us more opportunity for earlier diagnosis and treatment. Screening patients with turner's syndrome for the associated thyroid, cardiac, renal and celiac diseases are a significant issue because it may affect survival of those patients and may be x-linked that represent karyotype-phenotype variation.

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Pharmacokinetic Parameters of Meropenem in the Plasma and Milk of Ewes

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Abstract

This study had been designed to evaluate the pharmacokinetic parameters of Meropenem in plasma and milk of ewes after intravenous bolus adminsteration, for this purpose, five healthy miking ewes had been received a single intravenous bolus of Meropenem (20 mg/kg) to characterize both of distribution and elimination of Meropenem in plasma and milk, concentrations of Meropenem in plasma and milk had been analyzed by microbiological method and pharmacokinetic data had been analyzed by compartmental and noncompartmental methods, results of mean ± standard deviation for half-life, volume of distribution and total body clearance for plasma samples were 0.67 ± 0.09 h., 0.169 ± 0.01 and 0.3 ± 0.02 L/hr/kg, respectively, the plasma protein binding ratio were 7.27 ± 0.22 %; while the half-life, Cmax and drug penetration ratio for milk samples were 9.56 ± 3.13 h, 3.91 ± 0.99 µg/ml and 0.86 ± 0.23 respectively. In our conclusions; Depending on the approval state of Meropenem for veterinary therapy, we think that the achieved pharmacokinetic parameters of Meropenem in plasma and milk of ewes will candidate it to be one of the preferred parentrally administered antibacterial agents to encounter the acute cases of mastitis that ought to be treated hastily.

Keywords: *Meropenem, Pharmacokinetics, Milk, Ewe.*

Introduction

Mastitis is among the animal diseases which affect the profitability of rearing animals, it is considered to be one of the expensive diseases in terms of production losses[(1)]; Many studies have been conducted on preventive and microbial aspects of this disease but few studies are based on data of the field farms to estimate production-related losses and treatment costs [(2)].

Mastitis parentral administration trails had been started at the 70's of the 20th century due to failure of the intra-mammary therapy that related to poor and uneven distribution of antimicrobial agents as a potential result of inflammatory process [(3)]. Studying pharmacokinetics of antibiotics is very important to support the pharmacodynamics in order to achieve the maximal efficacy against bacteria with minimal or no side effects [(4)].

Meropenem is a semisynthetic Beta-lactam antibiotics belongs to carbapenems subgroup which derived from gram positive bacteria called *Streptomyces cattleya*, it was approved for use widely in 1996; Originally, it was developed to solve the instability of the older member Imipenem toward renal dihydropeptidase (DHP)[(5)]. In general all Carapenems including Meropenem possess a broad spectrum bactericidal effect due to impairment of transpeptidation of peptidoglycan by binding to different types of penicillin binding protein (PBPs)[(6)]. Meropenem shows strong bactericidal effect because it binds to three PBPs in the cell wall of gram positive and gram negative susceptible bacteria[(7)]; Such triple binding may provide an explanation on rapid bactericidal effect of Meropenem[(8)]. There is few pharmacokinetic data about Meropenem in domestic animals and most of them are not far away from the obtained data in human studies, but there is no data about it's kinetic behavior in milk, therefore, our study purpose is to support those data by reassessment of pharmacokinetic profile in ewes and

tracking of Meropenem concentrations in milk in order to provide a preliminary pharmacokinetic data might support the usage of Meropenem as one of the potential antibacterial agent in future veterinary therapy.

Materials and Methods

The study was conducted at the sheep sheds that belong to the fields of the college of veterinary medicine/ University of Baghdad, Five adult healthy lactating ewes with age of 2-4 years and body weight ranges form 38-48 kg were used as a model for the study, each ewe was subjected for physical examination, complete blood counting and physicochemical examination of milk. Animals were acclimatized for one month and had been fed with balanced food and ad libitum drinking water. A single intravenous bolus dose of 20 mg/kg of Meropenem (Meronem®) manufactured by Astrazeneca, UK, was injected into the left jugular vein; Blood samples about 3 ml had been obtained from right jugular vein and kept in Lithium heparin tubes at 0.083, 0.16, 0.25, 0.3, 0.5 , 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hrs. Each collected blood sample was centrifuged at 3000 rpm for 15 min. in order to obtain clear plasma that kept in -20 ºC till analysis [9]. Milk samples were obtained from both halves (each of them was subjected to the study alone) at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24 and 48 hrs. and kept in -20 ºC till analysis. Microbiological assay were utilized as a method to quantify Meropenem concentrations in different samples; it based on determining of drug potency (formed zone of inhibition in agar diffusion method) against growth of defined isolate of bacteria. Spores prepared according to Sabath method [10] from *Bacillus subtilis* ATCC 6633 that kindly provided by department of biology at college of science/ University of Diyala and utilized as test microorganism to estimate the concentration of Meropenem in blood plasma and milk [11]. Standard curves of Meropenem in plasma and milk had been done in antibacterial-free plasma and milk. The extent Plasma protein binding of Meropenem was determined according to Craig and Suh method [12] which based on calculation of partitioning of Meropenem between two media. Protein binding for each concentration was calculated according to the following formula:

$$Protien\ binding\ (\%) = \frac{Zone\ of\ inhibition\ in\ buffer - Zone\ of\ inhibition\ in\ plasma}{Zone\ of\ inhibition\ in\ buffer} . 100$$

The parameters of pharmacokinetics after administration of Meropenem had been calculated based on Microsoft Excel® spread sheet algorithm [13]; the weighted data ($1/C^2$) of plasma and milk were plotted, where (C) resembles the predicted concentration of Meropenem in plasma and milk. Both compartmental and noncompartmental analysis had been used to analyze the data that obtained from plasma [14]. Aikake information criterion was applied to choose the best model to fit the data of plasma for compartmental analysis [15]. The noncompartmental modeling has been adopted to analyze milk concentrations of Meropenem, where the area under the curve has been calculated by using of lo-linear trapezoidal method [illegible] according to the following:

$$AUC_{(0-\infty)} = AUC_{(0-last)} + \frac{C_{last}}{\lambda_z}$$

Where $AUC_{(0-last)}AUC_{(0-last)}$ resembles the calculated area under the curve, C_{last} is the recorded concentration at the last time while λ_Z is the elimination constant of the 1st order kinetics. Both C_{max} and T_{max} were predicted from the milk concentration vs time plotted data, the half-life of Meropenem in milk is estimated by:

$$T_{1/2} = \frac{0.693}{\lambda z}$$

The penetration ratio of Meropenem from plasma to milk was the calculated area under the curve of Meropenem in milk to the calculated area under the curve of Meropenem in plasma

$$AUC_{milk} / AUC_{plasma}$$

Results and Discussion

Results of the constructed standard curve of Meropenem in plasma showed that the inter-assay coefficient variation (CV %) was 3.36, the determination coefficient (R^2) was 0.96, the limit of detection of the assay was 0.19 µg/ml and the low limit of quantification (LLOQ) of Meropenem in plasma was 0.66 µg/ml. while the obtained standard curve of Meropenem in Milk revealed that the inter-assay coefficient variation (CV %) was 3.18%, the determination coefficient (R^2) was 0.97, the limit of detection of the assay was 0.14 µg/ml and the low limit of quantification (LLOQ) of Meropenem in plasma was 0.41 µg/ml. According to the achieved R-squared value (R^2) Both standard curves of Meropenem that constructed in the plasma and milk showed a clear linearity where the value of R^2 for both

curves approached 1, and such result implies a perfect relationship between the used concentrations (dependent variables) and the achieved zones of inhibition (Independent variables) [16]. The coefficient of variation (CV%) is another method to determine the precision of the assay; our study revealed that both calculated CV% values in plasma and milk are lower than 5; such CV% value reflect a good feeling about precision of our standard curves [17]. the limit of detection (LOD) of Meropenem in plasma was 0.19 µg/ml while in milk was 0.14 µg/ml; LOD is precognitive parameter for lowest concentration of the drug that can be detected but not necessarily quantified by the bioassay with reliable difference in comparison to control[18]. On the other hand, the low limit of quantification (LLOQ) is more eligible to determine the precision of the bio-analytical assay; LLOQ is the lowest value at which we can quantify the drug concentration with a high precision and reliability [19]. Our standard curve-based calculations revealed that the LLOQ of Meropenem in plasma was 0.66 µg/ml while in milk was 0.47µg/ml. Both achieved LOQs reflect well precision for our assay since both not exceeded the limit of precision (± 20%) of the coefficient of variation of the assay as recommended by the FDA [20].

We Conducted to estimate the ratio of binding of Meropenem to plasma proteins as reported in table (1); we found that the ratio of binding was 7.27%, this finding is compatible with many studies had been done in human and other veterinary species like equine, canine and feline; all these studies reported that Meropenem has a low plasma protein binding ranged from 2-11.87% with no or negligible effect on distribution of Meropenem to different tissues[14 & 21-23]; while EL-Sooud [9] in his study that focused on the pharmacokinetics of Meropenem in ewes reported that the value of plasma protein binding ratio that he estimated by the same method of us was 42.8% which in our evaluation do not get along with his other achieved parameters like distribution and elimination phases of compartmental analysis because EL-Sooud [9] study reported that his trail in Barki sheep were achieved a bi-exponential curve composed mainly from a rapid distributional phase with distribution half-life equal to 0.06 h. and such value reasonably can't achieved with presence of such high protein binding ratio [24]. In general such low ratio of plasma protein binding of Meropenem might be to its zwetterionic charge state that possesses less affinity to bind to plasma protein than acidic or neutral drugs [25 & 26].

The quantitative data that collected from measuring of Meropenm concentrations in plasma after injection of single intravenous bolus dose through determined chronological order were fitted mathematically on compartmental and non-compartmental models; Selection of two compartmental model to fit and calculate our data of plasma concentration-time curve of Meropenem that depicted in figure (1) and listed in table (1) had been established on two criteria; the 1st is shape of the curve who showed a first order and bi-exponential decrement with clear distinguished distribution and elimination phases, while the 2nd is value the calculated Akaike information criterion (AIC) who decide the best model that data should be to fit [27].

The mean of plasma concentration of Meropenem at the zero time was 263.64 µg/ml; the value of the distribution intercept (A) of Meropenem was 206.39 µg/ml, the constant of distribution phase (α) was 9.96 h^{-1} and the distribution half-life ($t_{1/2\alpha}$) was 0.06 h. Meropenem showed a short distribution phase (A) to the interstitial compartment as a normal result of the short half-life of that phase ($t_{1/2\alpha}$) which attributed to the high distribution of Meropenem from central compartment to the peripheral tissues due to low plasma-protein binding ratio that reported previously [28].

The volume of distribution at steady state (Vd_{ss}) of Meropenem in plasma was 0.16 L/kg; Vd_{ss} expresses about the equilibrium time between distribution and elimination phases of the drug and characterized by its independence from any elimination process, constant and accuracy [29]; Vd_{ss} had been utilized for determination of loading and maintenance doses in multiple dosage regimens and for interspecies dose extrapolation[30]. The Vd_{ss} of our study that calculated in both compartmental and non-compartmental analysis is relatively small and refer that the distribution of Meropenem was primarily limited to the extravascular compartments including milk [14 & 31]. Our finding about the Vd_{ss} is almost contradicting with EL-Sooud [9] study who reported that the Vd_{ss} in Barki sheep was 0.055 L/kg and such value is also not even approached other species values like dog 0.337 L/kg [14], cat 0.21 L/kg [22] and horse 0.136 L/kg [23]. Such contradiction might be

attributed to miss-calculation by EL-Sooud [9] where his value of Vd_{ss} should be 0.189 L/kg instead of 0.055 L/kg according to the formula that calculate the Vd_{ss} [32]. The area under the concentration-time curve (AUC) is one of pharmacokinetic parameters that measures the exposure time to the drug; the AUC value submitted to the effect dose, absorption and clearance [33].

Our study was reported that the AUC of Meropenem that achieved in plasma was 65.22 (h*µg)/ml and it had been came within the range that achieved by other species who ranged from 53.2 to 90.3 65.22 (h*µg)/ml considering the differences among species that used. The Mean residence time (MRT) is another important pharmacokinetic parameter which resembles the average time that drug molecules spent in the body compartments [34]. As what revealed with the AUC; our study found that the MRT of Meropenem in plasma was 0.54 hr. which was in agreement with other studies in other species who stated the MRT of Meropenem after single intravenous bolus was less than 1 hr. [9, 14, 22 & 23].

There are two important clinical parameters to shape the elimination phase of drug molecules; the elimination half-life ($T_{1/2}$) and the total body clearance (Cl) The elimination pharmacokinetics of Meropenem was not differing from other Beta-lactams where the major feature is a relative rapid renal excretion mostly via tubular secretion and glomerular filtration [21]. Our results reported that the elimination $T_{1/2}$ of Meropenem in plasma was 0.57 hr., while the total body clearance was 0.3 L/hr/kg. Both achieved values of $T_{1/2}$ and Cl_T were came as expected for Beta-lactams generally and Meropenem specifically; where it has a short $T_{1/2}$ with great Cl_T, and this combination in addition to the low Vd_{ss} will achieve a quick state of equilibrium between the concentration in central compartment and the extracellular compartments considering their penetration ratio [14].

The results of the pharmacokinetic analysis of Meropenem concentrations in milk of ewes that listed in table (2) reported the Cmax of Meropenem was 3.91 µg/ml and it was achieved at the 6th h. after intravenous administration which gets along with general pharmacokinetic characteristics of Meropenem that indicate to primary concurring distribution into the extracellular fluids within the same time of plasma distribution pattern as we point in figure (2) who graphed the decline in plasma concentration of Meropenem was in harmonization within the rise in milk[35]. Based on our calculations, Meropenem achieved a considerable AUC in milk (56.28 h*µg/ml) as a reflection to the long time that the compartment was exposed to the drug which in turn might be attributed to the delayed residence of Meropenem molecules that empowered by the recorded half-life which was 9.56 hrs. Such long half-life might be presumed to the low measured concentrations of Meropenem in milk; where low concentrations appeared to yield longer terminal half-lives consequently, more flattened curve in comparison to plasma curve, then after large AUC [36].

The capability of Meropenem to penetrate into milk compartment was assessed by penetration ratio which expressed as AUC_{milk}/AUC_{plasma} [37]. Our results about milk penetration ratio of intravenously administered Meropenem were 0.86 which considered good in comparison to other Beta-lactams that administered parentrally in different ruminants; where Ceftriaxone was achieved 0.34 in ewes [38], Ceftizidime 0.16 in does [39], Cephacetrile 0.15, Cephapirin 0.18, Penicillin G 0.2, Cloxacillin 0.26, Ampicillin 0.26 in cows [40] and Amoxicillin 0.48 in cows [41]. Our assumption to explain such high penetration ratio might be due to the used route of administration who primarily ensure even drug distribution to all extravascular compartments, consequently, they accumulate in milk better than other routes and such phenomena is governed by the amount of used dose [42]; and the physicochemical characteristics of the drug like zwitterionic nature and the small molecular size of the drug who might be enhance Meropenem penetration to the milk compartment in comparison to other Beta- lactams [43]. In conclusions; Depending on the approval state of Meropenem for veterinary therapy, we think that the achieved pharmacokinetic parameters of Meropenem in plasma and milk of ewes will candidate it to be one of the preferred parentrally administered antibacterial agents to encounter the acute cases of mastitis that ought to be treated hastily.

Table (1) compartmental and non-compartmental pharmacokinetic parameters of Meropenem (Single I.V. bolus dose) in plasma of ewes.

Parameter	Unit	Plasma	
		Mean	SD.
CMax	µg/ml	N. A.	N. A.
AUC	(h*µg)/ml	65.22	4.56
ClT	L/hr/kg	0.3	0.02
TMax	h	N. A.	N. A.
λ z	h-1	1.2	0.38
T1/2 λz	h	0.57	0.09
MRT	h	0.54	0.08
Cp0 *	µg/ml	263.64	112.58
t1/2α*	h	0.06	0.03
t1/2β *	h	0.53	0.19
K10 *	h-1	4.06	1.29
K12 *	h-1	4	2.88
K21 *	h-1	3.18	1.79
A *	µg/ml	206.39	96.1
α *	h-1	9.96	4.66
B *	µg/ml	57.25	33.03
β *	h-1	1.29	0.30
VC *	L/kg	0.075	0.05
Vdss *	L/kg	0.169	0.01
Plasma-protein binding	Ratio	7.27	0.22

- ·No. of ewes = 5.
- ·All data represent free concentrations of Meropenem.
- ·All data are subjected to weight by 1/C2.
- · (*) calculated by compartmental analysis.

Table (2) non-compartmental pharmacokinetic parameters of Meropenem (Single I.V. bolus dose) in and milk of ewes.

Parameter	Unit	Milk	
		Mean	SD.
AUC	h*µg/ml	56.28	20.13
CMax	µg/ml	3.91	0.99
TMax	h	6.00	1.15
T1/2 λz	h	9.56	3.13
Penetration (AUC milk / AUC plasma)	Ratio	0.86	0.23

- No. of ewes = 5.
- All data represent free concentrations of Meropenem.
- ·All data are subjected to weight by 1/C2.

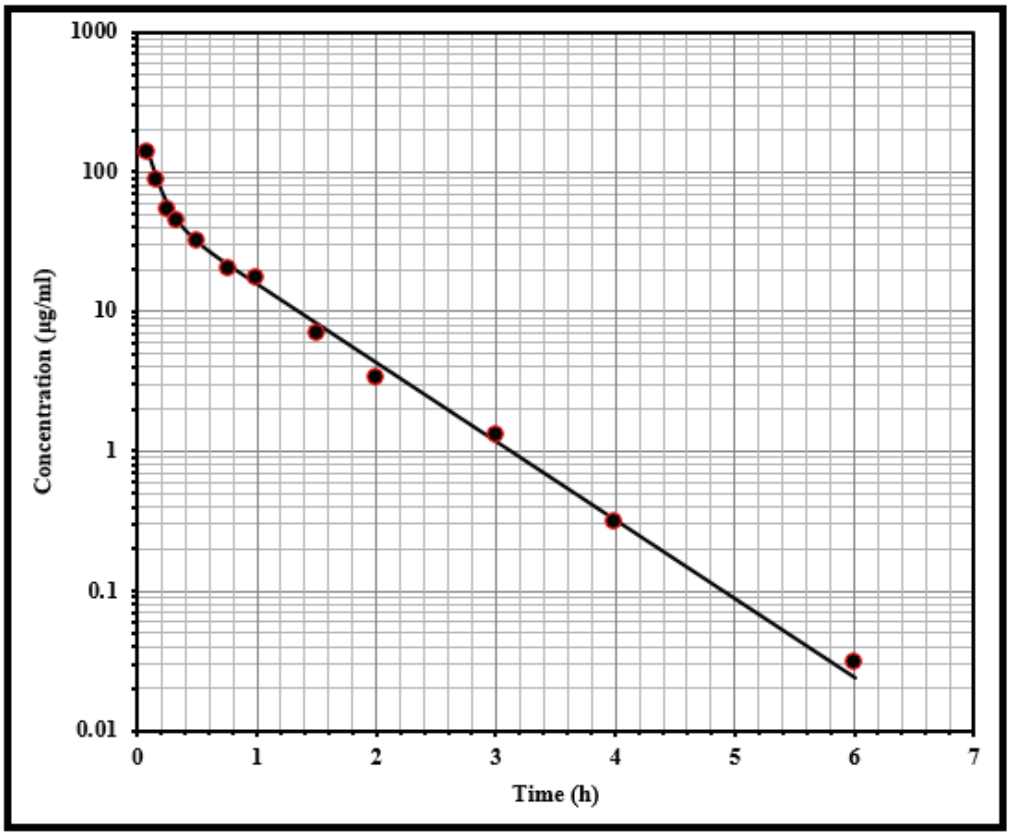

Figure (1) Two compartmental model of Meropenem in plasma of ewes.

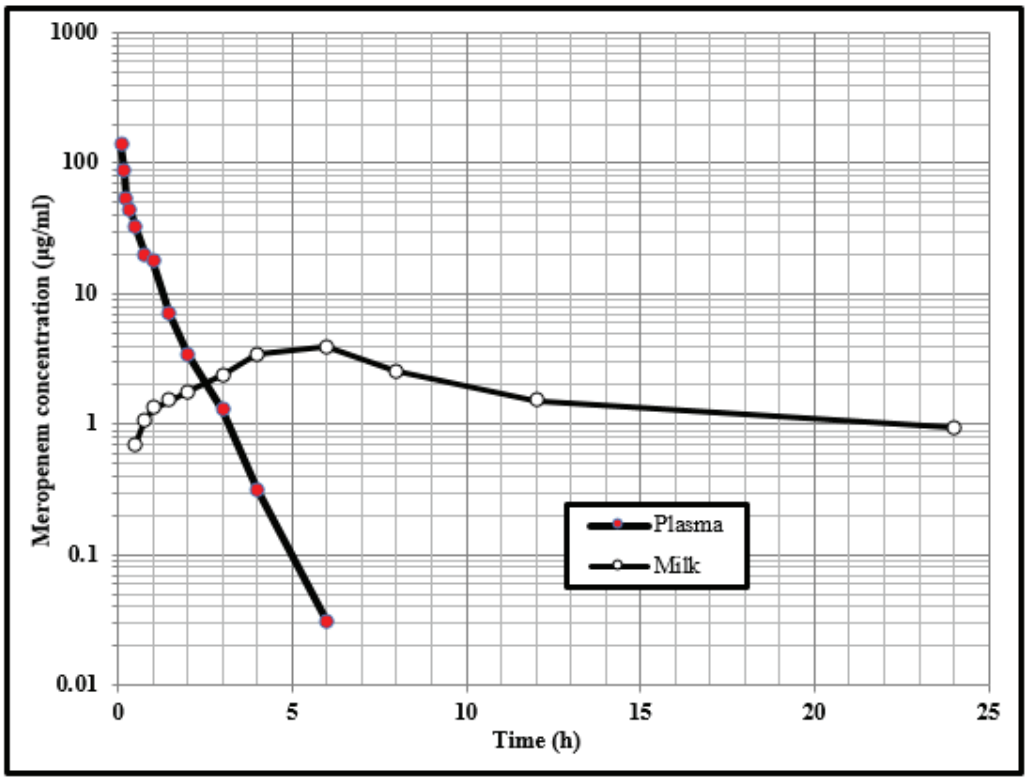

Figure (2) Non-compartmental analysis of single intravenous bolus for Meropenem in plasma

Ethical Clearance- Taken from 1- Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Diyala / Iraq

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Association of Serum Galectin-3 Level with Albuminuria and Renal Function in Type 2 Diabetic Iraqi Patients

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Abstract

Background: Galectin-3 is a category of β- galactoside binding Lectin. It has been involved in renal inflammation and fibrosis. We examined the relationship between galectin-3 level and nephropathy in T2DM patients with or without microalbuminuria in comparison healthy controls.

Methods: Ninety T2DM patients they were graded as (3 groups) depending on (ACR) albumin to creatinine value: normalbuminuria (ACR < 30 mg / g), micro albuminuria (ACR 30-300 mg / g); and macroalbuminuria (ACR > 300 mg / g). In addition, 30 healthy control subject. The serum galectin-3 was determined by an enzyme-linked immune sorbent assay (ELISA) for 90 T2DM patients and 30 healthy subjects. And also biochemical parameters measured included FBG, HbA1c, urea, creatinine, and uric acid.

Result: Serum galectin-3 was significantly raised in T2DM patients with macroalbuminuria in comparing to healthy group and also to other patients groups ($p<0.001$). Galectin-3 level was positively related with each of ACR, FBS, HbA1c, urea, creatinine. whilst, eGFR was negatively correlated with galectin-3

Conclusion: Galectin-3 level is essentially higher with T2DM at all stages of DN, particularly in macroalbuminuria patients.

Keywords: *galectin-3, albuminuria,T2DM; tpxicity; health .*

Introduction

Diabetic nephropathy is the principle pathologic state that causes end-stage renal disease. Accordingly, it is important to investigate a new beneficial indicator of DN [1]. Galectin-3 is a class of chimera from (lectin), a soluble protein that allows binding to β-galactoside [2]. It is expressed mainly in macrophage, endothelial and also epithelial cells. It has a regulator effect in the cell processes and the pathogenetic mechanism of various disorganizations [3]. During the discovery of galectin-3, it has been *recognized in the intracellular and extracellular space.*

In fact, *galectin-3 acts extracellularly through the c-terminal and intracellularly through the N-terminal. Such structure and position are collectively making galectin-3 perform* a number of biological functions like cell adhesion, growth and apoptosis [4-8]. Additionally, many studies have confirmed that galectin-3 has a broad range of effects in different tissues, and its level embroiled in cardiovascular disease which increases the possibility that galectin-3 may act as a sensor in the development of some pathological conditions [8-10]. The circulating galectin-3 level also correlated with glomuropathy and renal fibrosis. On the other hand, galectn-3 represents a beneficial function such act as clearance receptor for AGE [11, 12]. To date, it has been recognized a limit information about the correlation between level of galectin-3 and DN. The present work aimed to identify galectin-3 as useful predicting marker in DN.

Subjects and Methods

Subjects

Ninety (T2DM) patients were registered in the current study. They were graded as 3 groups depending on albumin to creatinine ratio ACR: 30 patients without albuminuria, 30 patients with micro albuminuria, 30 patients with macroalbuminuria. Additionally, there are 30 control subjects, each group consisting of (18 male and12 female) , and age (40 to 60) years. The patients, who attend to National diabetes Center at (AL-Mustansiryia University), were selected in this study. This study excluded, type1 diabetes, smoking, history with alter disease and pregnant women.

Methods

Blood and urine sample for all patients and control have been collected in the morning for estimation FPG, urea, uric acid and creatinine by enzymatic colorimetric methods, used kit provided by Biolabo, France. HbA1c determined by (Bio-Rad D-10) HPLC method. Enzyme-linked immunosorbent assay (ELISA) was used to quantify galectin-3, using the kit provided by Bio-source / USA. ACR estimated by using an automatic analyzer (Complyzer 13) for urine analysis. eGFR was calculated by using MDRD formula.

Statistical Analysis

The findings have been summed up as mean ±SD. The correlation between variables estimated by Pearson correlation. (ANOVA) the test used for the average difference between Groups, using Statistical (SPSS-23).

Results

Table (1) displays the patient and control group with the mean of parameter. Since there were no major differences in age and gender in the patient population ($p>0.05$). Present finding revealed that Macroalbuminuria patients had a higher average (SBP, DBP) and a mean of duration than the other study group (p=0,001) as can be seen in Table 1. The WHR, BMI, FBG, HbA1c, creatinine, uric acid, and urea, values were significantly increased in patient groups relative to control subjects ($p<0.001$). A significant increase in ACR is observed in patients compared with the healthy persons (< 0.001), along with a significant decrease (< 0.001) in eGFR in all patients group. It is believed that macroalbuminuria patients with higher ACR average have a considerably lower eGFR than other groups ($p<0.05$).

Table 1 the mean of parameter of studied patients and control.

Variables		T2DM			Healthy control N=30	P value
		Macroalbuminuria N=30	Microalbuminuria N=30	Normalaluminuria N=30		
Age (years)		55.03±4.9	53.36±5.7	53± 6.06	52.56±5.36	NS
sex	Male No%	18 (60%)	18 (60%)	18 (60%)	18 (60%)	
	FemaleN%	12 (40%)	12 (40%)	12 (40%)	12 (40%)	
Bp (systolic) mmHg		150.13	140.76	130.21	110.03	0.001
Bp (diastolic) mmHg		90.26	85.93	83.33	80.06	0.001
Duration ofT2DM		17.3 ±2.45	14.93±2.8	3±1.6	_	0.001
BMI		29.43±5.5	31.83±5.6	30.92±5.4	24.71±3.9	0.001
WHR		0.96±0.07	0.92±0.07	0.90±0.07	0.81±0.08	0.001

Cont... **Table 1 the mean of parameter of studied patients and control.**

FBS (mg/dl)	208.1±44.9	162.3±31.1	114.03±8.79	89.4±5.23	<0.001
HbA1c	9.06±1.16	8.78±1.1	5.72±0.51	4.47±0.54	<0.001
Uric acid (mg/dl)	5.26±0.84	4.64±1.25	3.98±0.99	3.58±0.78	<0.001
Urea (mg/dl)	51.16±11.9	43.31±11.1	18±3.2	17.9±4.1	<0.001
Creatinine (mg/dl)	1.51±0.22	1.08±0.24	0.64±0.08	0.56±0.09	<0.001
ACR	69.20±59.3	13.01±4.96	2.01±0.41	1.98±0.44	<0.001
eGFR	44.90±5.40	64.17±10.2	105.73±16.5	141.57±25.61	<0.001

Serum Galectin-3 levels

As presented in Figures 1 and Table 2 , the mean level of galectin-3 for patient groups were significantly raised compared with those in control groups ($p < 0.001$), particularly, macroalbuminuria showed a substantial increase in the mean level of galectin-3 as well as found that macro and microalbuminuria patients significantly highest compared with normal albuminuria. Besides that, there are no major variations between normal albuminuria and the healthy group in serum galectin-3 level. While data showed no significant variance of galectin-3 level between macro and microalbuminuria patients.

Table 2 galectin-3 data of studies group.

varible	Macroalbumin uria N=30	Microalbuminuria N=30	Normalaluminuria N=30	Healthy control N=30	P-value
Galectine-3 ng/ml	3.002±1.74	2.68±1.26	1.60±0.62	1.30±0.12	<0.001 a*,b*,c

a*significant between macro and healthy group,b*significant between micro and healthy group, c not significant between normal and healthygroup.

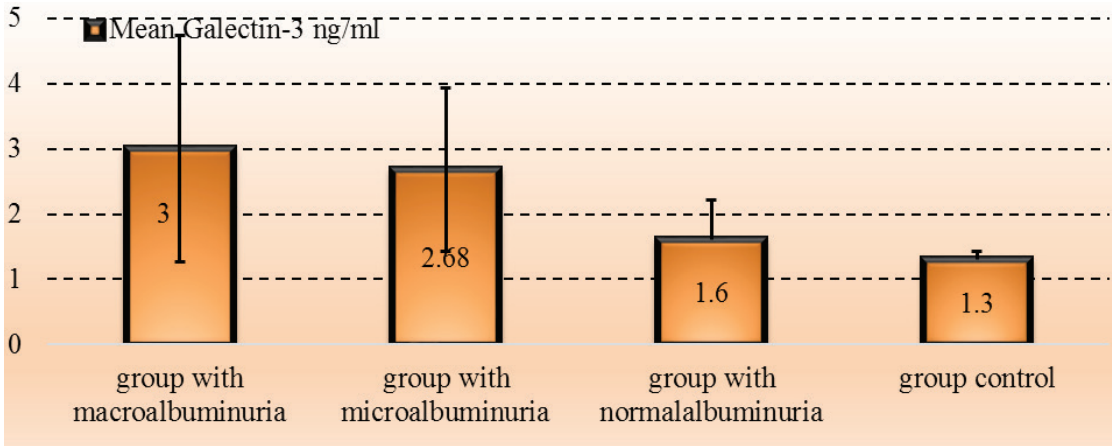

Figure1 the mean of galectin-3 in studies groups.

Correlation of serum galectin-3 with other parameter in research

Table (3) shows the relationship in current research between galectin-3 and other parameters. It noticed an important positive association between galectin-3 and ACR, and glucose in all patients. Whereas, galectin-3 negatively related to eGFR in each patient group. Similarly, in macro and microalbuminuria classes, urea and creatinine are also positively essential associated with galectin-3. Likewise, galectin-3 demonstrates a strong association with HbA1c in macro and microalbuminuria patients.

Table 3 correlation of galectin-3 and other parameter.

Parameters	Correlation coefficient		
	macroAlbuminuria	microAlbuminuria	Normal Albuminuria
Age	0.20 NS	0.07 NS	0.37 *
BMI	0.08NS	-0.06 NS	-0.13 NS
Duration	0.11 NS	0.18 NS	0.20 NS
Glucose	0.62 **	0.47 **	0.41 *
HbA1C	0.80 **	0.62 **	-0.021 NS
Blood urea	0.38 **	0.73 **	0.13 NS
Blood creatinin	0.75 **	0.59 **	0.311 NS
Uric acid	0.06 NS	0.24 NS	0.17 NS
ACR	0.91 **	0.63 **	0.45 **
eGFR	-0.69 **	-0.65 **	-0.38 **
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

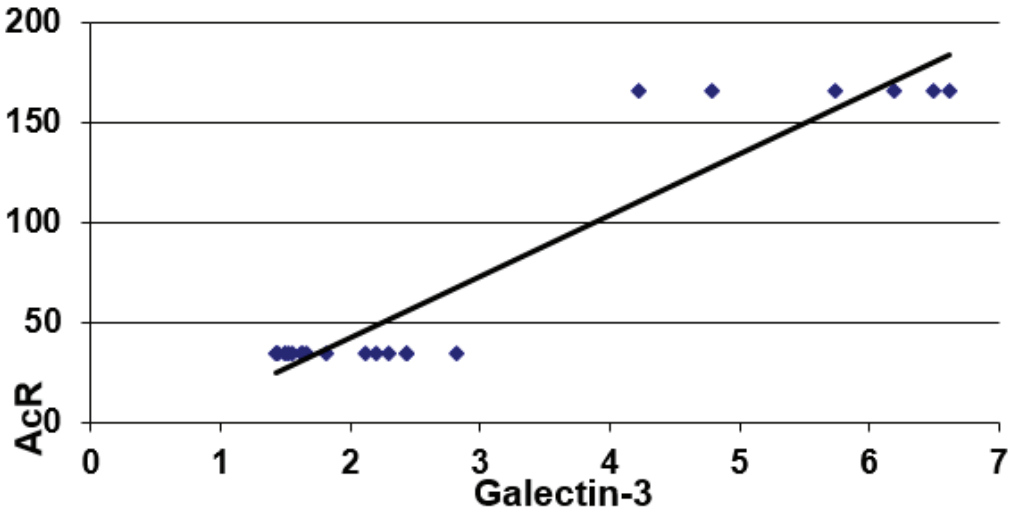

Figure 2 correlation of galectin-3 with ACR in macroalbuminuria.

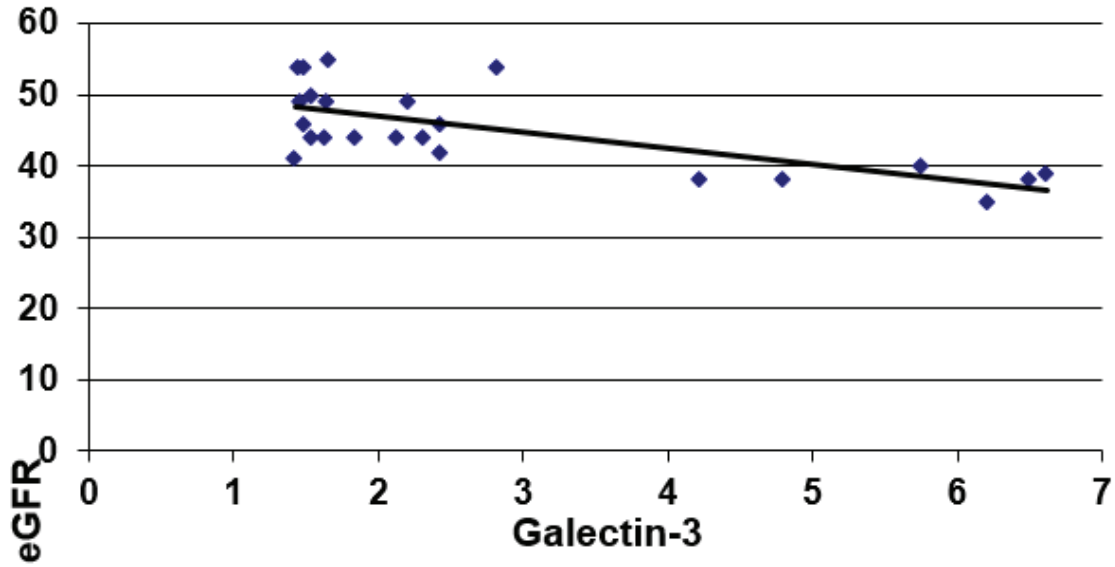

Figure 3 correlation of galectin-3 with eGFR in macroalbuminuria.

Discussion

The existing data revealed the role of galectin-3 in progression of DN. The main outcomes in the current study, those galectin-3 levels was higher in macroalbumin patients than the other groups as well as in micro than normal albuminuria group. This indicates that galactin-3 levels are strongly influenced by DN development.

The outcomes were compatible with Tan et al who found that circulating galectin-3 was involved in progressive of DN [13]. Another report corroborated this study, kikuchi et al. mentioned that expression of Galectin-3 - positive cell elevated in glomeruli and also associated with proteinuria in people with DM[14] . Also, Pugliese et al. determined that the level of Galectin-3 were shown to be high in diabetic patients, it is associated with micro and macrovascular complications. Therefore, the higher levels of galectin-3 may mark the Susceptibility to renal ,and heart failure in these patients[15] . The overexpression and secretion of Galectin-3 is related to several diseases and also widely studied in the context of fibrosis and heart failure, and Diabetes [16]. Data result from the current work showed elevated mean level of renal function parameters like as urea, creatinine, ACR in macroalbuminuria patients. Therefore, our result cleared that galectin-3 positive and significant correlation with each of FBG, HbA1c, ACR, urea, uric acid, and creatinine while inverse correlation with eGFR . Very close to our result, Song et al identified that elevated galectin-3 levels connected with FBG, HbA1c, ACR, creatinine and decrease eGFR as well [17]. Conversely, Yücel et al, who reported no existing correlation between galectin-3 and other diabetes parameters as HbA1c, FBG, ACR [18].

A result performed by O'Seaghdha et al, highly supports current results in which they found increased galectin-3 levels in CKD [19].

Galectin-3 is represented as AGE receptors and participates in the reduction of these pathogenic compounds [20]. In diabetes, The AGEs can be found to stimulate galectin-3 expression in the endothelial cells and renal tissues. Also the current finding agreement with those recorded by Ho et al, in which found strong relation between galectin-3 and DM [21-23].

Conclusion

The level of galactin 3 in the blood is positively affected by the persistence of diabetes with diabetic nephropathy disease for an extended peroid, and its elevation level increases significantly with the increase in the ACR and the decrease the eGFR of diabetes.

Accordingly, it can also be used as a predictive marker for the evaluation of severity of DN.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Diet of Inflammatory Bowel Disease Patients, in Gastrointestinal and Liver Center, Baghdad, 2020

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Abstract

Introduction: Iinflammatory bowel disease (IBD) comprises a group of chronic immune disorders of unknown etiology characterized by intestinal inflammation which subsequently leads to debilitating symptoms which can adversely affect performance and quality of life. Crohn's disease (CD) and ulcerative colitis (UC) are the two most common phenotypes.

Patients and Method: The cross-sectional study with the diagnosis of either Crohn's disease or ulcerative colitis. The data collection was carried out from 25 september to 10 november 2020. From gastroinestinal tract and liver center ,Baghdad . Convienant sample are willing to participate. By questionnaire.Body weight and hieght was measured. Body mass index (BMI) was calculated . Recorded intake of all food and drinks during last 24 hours. By Excil caculation program , the enegy intake of one day was calculated by mifflin-St.jeor method .The my pyramid plan for daily food and calories intake as recommended pattrern . Statitcal analysis were performed using SPSS 23 . Data described as mean±standerd deviation (SD).Data were checked for normal distribution using t -test or chi square .The level of significance in the study was $p < 0.05$.

Result: All the participants (55;100%) were low milk group servings intake(P<0.001), also(74.5%) of participants were low fruit group servings intake (P<0.001) ,(92.7%) 0f participants was low vegetables group servings intake (P<0.001) , but (87.3%)Of participants were high grain group servings intake (P<0.001) ,(65.5%) of participaints was low protein group servings (P=0.041) , the fat group serving intake is low among (90.9%) of participants (P<0.001) , 52.7% of participants low energy intake. The mean BMI for both genders was in the normal range (23.16 ± 5.33) as (49.1%) of participants , only 14.5 % was underweight , 29.1% was overweight and 7.3% was obese. The participants live in aurban area as (89.1%) . The ocupation of them were 25% housewife,student (23.6%),employers (16.4%) and the rest with different occupations. The education of participants were primary school (36.4%) ,intermediate school(12.7%) , secondry school were (14.5%), graduated were (12.7%) and the rest in the different educations. Unbalnce diet shows among 76.4% of participants .

Conclusions and Recommendations : all participant modified their diet,half of the participants had low energy intake and some of the participants had underweight. Emphasized the importance for patients to be provided with nutrition – related knowledge as a part of strategies to avoid nutritional inadeqancies

Keyword: *inflmmatory bowel disease ,Crohn's disease ,Ucerative colitis, diet.*

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Introduction

The term inflammatory bowel disease (IBD) comprises a group of chronic immune disorders

of unknown etiology characterized by intestinal inflammation which subsequently leads to debilitating symptoms which can adversely affect performance and quality of life. Crohn's disease (CD) and Ulcerative colitis (UC) are the two most common phenotypes. In these disease processes, the thermodynamic demands of inflammation as well as tissue losses from intestinal injury result in an increase in energy and nutrients requirements. As such, protein – calorie malnutrition and micronutrient deficiencies are common squealae of this chronic persistent catabolic state. Nutritional therapy can be considered as supportive or primary treatment. The goal of supportive therapy is to correct any existing malnutrition and it essentially act as an adjunct to traditional therapy. They can occur at any age, but most often occur between 15 and 30 years of age. The Inflammation of UC is superficial in nature and is limited to the colon. It almost always affects the rectum and it extends proximally in a symmetrical, uninterrupted pattern to involve part or all of the large intestine. Typical presenting symptoms of UC include diarrhea, abdominal pain, and hematochezia. Crohn's disease is characterized by patchy transmural inflammation that may involve any part of the gastrointestinal tract from mouth to anus. The small intestine and the colon are the most commonly affected areas, while the rectum is usually spared. It is important to note that the small intestine is the site of absorption of most nutrients, therefore mucosal injury in this area usually results in significant weight loss and generalized malnutrition in addition to diarrhea and abdominal pain. The transmural inflammation can lead to fibrosis, resulting in obstructive clinical presentations, which are not typically seen in UC. CD can also be complicated by fistula formation. (1)

Etiology of IBD is unknown, but is thought to be multifactorial, involving genetic, immunologic, and environmental factors. Although genetic susceptibility, luminal antigenic drive, and environmental triggers are all important, the chronic relapsing, immune-mediated intestinal inflammation which is the hallmark of IBD is a direct result of the interaction of these essential Components. (1)

Diet, as a source of luminal antigens, is postulated to play an important role in the immune pathogenesis of IBD. It is unclear whether antibodies directed against dietary antigens actually trigger the inflammatory response seen in IBD or whether these antibodies are merely a downstream product of intestinal inflammation (1).

Many people with IBD can consume normal diet during times of disease remission but may need to alter their diet during flares. A well – balanced diet with adequate intake of protein, carbohydrates and fat as well as vitamins and minerals, is necessary for nutrition. this can be achieved by eating a variety of food from all the food groups. Meat, fish, poultry and dairy products are a source of protein. bread, cereal, starches, fruits and vegetables are sources of carbohydrates. butter, margarine and oil are sources of fat. (2)

Protein-calorie malnutrition is common among patients with IBD and correlates with disease activity, which may be mediated by pro-inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor alpha. Nutrition assessment is essential because of the severe consequences of malnutrition. A malnourished patient with IBD may present with defects in GI function that further limit the absorption and utilization of nutrients. (3)

Malnutrition occurs in patients suffering from IBD as a consequence of decreased dietary Intake, increased nutrient losses, and increased nutrient requirements. (3)

The aims of this study are to determine:

1. nutritional status of participants.
2. diet modification.
3. Energy adequacy.

Patients and Methods

The cross-sectional study included 55 patients (31 male and 24 female aged 14-67 years) with the diagnosis of either Crohn's disease or ulcerative colitis . The data collection was carried out from 25 september to 10 november 2020. The participants were attended to gastroinestinal tract and liver center ,baghdad . the sample size based on convienant sample are willing to participate. The study was approved by clinical nutrition committee and all persons gave their oral approvalle prior to thier inclusion in the study.participants answered a questionnaire about disease and diet .Body weight was measured on a caliberated scale and hieght

was measured with a caliberated stadiometer . Body mass index (BMI) was calculated from the height and weight . The interviweir recorded intake of all food and drinks during last 24 hours.the data was transferred into Excil caculation program , the enegy intake of one day for each participant was calculated by Mifflin-St.Jeor method [9] . We use the my pyramid food group servings and caloris as recommended food pattrern [10] . Statitcal analysis were performed using SPSS 23 . Data described as mean±standerd deviation (SD) . Data were checked for normal distribution using t -test or chi square . The level of significance in the study was $p < 0.05$.

Result

A total of 55 patients (female 24(43.6%) and male 31(56.4%) with the diagnosis of either Crohn's disease 32 (58.2%) or ulcerative colitis 23(41.8%) were attended to center and paticipated in study as shown in table -1-.

Table -1- Shows number and percent of gender and diagnosis

Diagnosis	Gender		Total
	Female	Male	
Cronh's	11	21	32(58.2%)
Ulcerative	13	10	23(41.8%)
Total	24(43.6%)	31(56.4%)	55

All the participants (100%) were low milk group servings intake than recommended and stistcaly significant, also(74.5%) of participants were low fruit group servings intake than recommend and stisticaly significant ,(92.7%) 0f paticipants was low vegetables group servings intake than recommended and staticicaly significant , but (87.3%)Of participants were high grain group servings intake than recommended and statisticaly significants ,(65.5%) of participaints was low protein group servings intake than recommended and statisticaly significants, the fat group serving intake is low among (90.9%) of participants and statisically significants, 52.7% of participants low energy intake ,as shown in table -2-.

Table-2 -Show s number and percent of food groups servings intakes and energy from participants compared with recommended

intake	Milk	Fruits	Vegetables	Grins	Protien	Fat	Energy
Equal	0	4(7.3%)	1(1.8%)	1(1.8%)	7(12.7)	0	0
Low	55(100%)	41(74.5%)	51(92.7%)	6(10.9%)	36(65.5%)	50(90.9%)	29(52.7%)
More	0	10(18.2%)	3(5.5%)	48(87.3%)	12(21.8%)	5(9.1)	26(47.3%)
Total	55	55	55	55	55	55	55
P value	<0.001	<0.001	<0.001	<0.001	<0.041	<0.001	-

The mean age of participants was 31.45±12.37, the mean energy intake was 1852.4 ±744.4 calories . The mean BMI for both genders was in the normal range (23.16 ± 5.33) as (49.1%) of participants , only 14.5 % was underweight , 29.1% was overweight and 7.3% was obese as shown in table-3-.

Table-3-Shows number and percents of participants BMI.

diagnosis	BMI				total
	Normal	over weight	Obese	Under weight	
Cronh's	17	9	1	5	32
Ulcerative	10	7	3	3	23
Total	27(49.1%)	16(29.1%)	4(7.3%)	8(14.5%)	55

The paticipants live in aurban area as (89.1%) . The ocupation of them were 25% housewife ,student (23.6%),employers (16.4%) and the rest with different occupations. The education of participants were primary school (36.4%) ,intermediate school(12.7%) , graduated were (12.7%) and secondry school were (14.5%),the rest in the different educations with statisticaly significant (p=0.034). Unbalnce diet shows among 76.4% of participants .

Discussion

Inflammatory bowel disease (IBD) is a chronic relapsing – remitting immune disorder of unknown etiology , nutritional therapy considered as supportive or primary treatment[1].

The result of current study showed that all participants low intake of dairy group servings , similar to Vidarsdottir et al 2016 [8] as half of participants restricted their intake of dairy prducts , also similar to brasil-lopes et al [11].Thus dietary calcium intake will be inadeqaute regarding intake of fruits ,vegetables , protien groups and dairy products servings were low than recommended and these finding in agreement with kamlp et al study 2020 [4] who found that particIpant most often modified fruits and vegetable and and dairy as a result of their IBD.also in agreement with Shafiee et al study 2019 [5] who found that IBD patients, don't met the standard nutrients recommendation for protein current study showed that high grains group servings intake than recommended , and these finding in disagreement with Kampl et al study 2020 [4] who found that low intake of whole grains.

Half of participants in the present study had BMI in the normal range and forteen percents were underweight due to food – restricted behavior and decreased appetite during flare and the rest overweight and obese . and these agreement with Holt et al study 2016 [6] that found normal range of BMI. The finding of our study in disagreement with Vidarsdottir et al study 2016 [8] who showes mostly overweight range among IBD patients .

The paticipants claimed that diet can affect symptoms and change their diet accordingly after they were diagnosed with IBD and due to lack of knowedge and awareness among participants ,who are less certain about the role of diet on different disease severity , wether restriction or addition of specific food items might contribute to increase nutritional deficiency , this is in agreement with Kamp et al study 2020 [4] who showed that the majority of the participant reported modifying their dietary intake as a result of IBD and expressed belief that diet could reduce symptoms . The result of our study also agreement with Bergeron et al study 2017[7] who showed that food avoidance is common among patients with inflammatory bowel diseases .

Limitations

1- Decrease number of patients attended center because of Covid 19 pandemic ,

2- also lack of biological treatment of patients from store lead to don't attended to center.

Conclution

1- all participant modified their diet

2- half of the participants had low energy intake .

3- some of the participants had underweight .

Recommendations

1- Emphasized the impotance for patient to be provided with nutrition – related knowledge as a pat of strategies to avoid nutritional in adeqancies

2- Further studies need about this topics.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Investigation of HPRT Sequence Changes in Radiation Occupationally-Exposed Workers-in Al-Twuathia Site in Baghdad City

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Abstract

The present instruction targets according to makes use of the molecular organic strategies into a genotoxicity looking after about mean diffraction doses within samples concerning people among Al-Twuathia site, it learning inclusive of 35♂ blood samples, used (30 - fifty five year), so well as 15 ♂blood samples, ancient (25 - 50 year) permanency toughness which are no longer people who smoke yet alcohol as much control. In that research usage DNA sequences technique for investigating the factor mutations appropriate in conformity with the impact regarding ignoble doses on ionizing radiation within the blood of smearing workers namely nicely namely the discovery about the effect concerning ionizing diffraction on the genes The genetic susceptibility after smearing exposure is in all likelihood after end result from the contribution about much genetic variants between multiple repair pathways as may bear a suture then additive impact over repair diverse DNA damages. Longevity a more comprehensive, larger-scale genetic instruction is needed after similarly explore the outcomes about gene-environment interaction concerning genetic susceptibility in conformity with ionizing dispersion exposure. In conclusion, the results indicated the opportunity over the use of the adjustments of as like beneficial biomarkers for the discovery concerning ethnical publicity in accordance with ionizing radiation., the learnt tested usefulness on the mutation frequency because HPRT assay then DNA sequences technique namely a sensitive extra biomarker among the everyday fitness screening about people occupationally uncovered to low doses on ionizing diffraction the effects indicated as at that place is an opportunity over the usage of the changes at the degree concerning HPRT gene mutation as much useful biomarkers because of the detection of ethnic exposure in accordance with radiological or chemical substances yet in accordance with makes use of PCR Techniques as like biomarkers because detection about the consequences on the low doses painting within the lymphocytes of gore among people occupationally to ionizing diffraction at the Al-Twuathia site.

***Key word**: Ionizing radiation, HPRT mutation assay, Bioinformatics analysis, PCR reactions.*

Introduction

The risk associated with occupational and environmental exposure to mutagens', including IR one of the public health challenges. This due to the potential harm of such exposure that could increase the risk of genomic instability which may contribute to the raise of serious health problems including malignancies development. Consequently, it would be very useful when genome integrity monitoring for those occupationally exposed to ionizing radiation products and/or waste added to the medical surveillance for such workers [1]. Occupational and environmental exposure to mutagens, including ionized radiation (IR), is of great concern health issue. This due to the potential harm of

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such exposure which is associated with increase the risk of genomic instability that could contribute to the raise of serious health problems including malignancies formation. Consequently, genomic integrity monitoring for those occupationally exposed to ionizing radiation products and/or waste would be a valuable addition to the medical surveillance for such workers [2].

There is growing health concern over the risk of low-dose ionizing radiation LDIR exposure [3, 4]. Accordingly, this study was set to investigate the potential influence of occupational exposure to low-dose ionizing radiation on the levels of Radiation field workers

A number of cytotoxicity detection assays have been developed to assess the effect of ionized radiation exposure on genomic stability. These include the cytogenetic, and DNA damage' analyses. In this regard, the mutations frequency of certain genes, i.e. *HPRT*, has been utilized as genotoxicity reporter biomarkers. Of interest, high level of *HPRT* mutations was reported in U.S. Gulf War veterans 10 year post they accidently injured with depleted uranium weapons [5]. In the same context, even low-dose-rate radiation is believed to have proportionally less severe *HPRT* mutational effects on the exposed individuals [6] .

HPRT is X-linked housekeeping gene encode to hypoxanthine phosphoribosyl transferees enzyme which involves in nucleotides metabolism, specifically the purines generation. This gene is also shown to be involved in the regulation of multiple embryonic, developmental and metabolic pathways [7]. Additionally, normal mammalian cell has only one copy of *HPRT* gene as it maps to X chromosome (due to dosage compensation). Accordingly inactivation of the only one copy of the gene could lead to *HPRT* function loss that is known to cause Lesch–NyhanSyndrom [8]. Apart from its functional significant, the rationale behind *HPRT* gene selection as a mutational reporter is it's relatively high sensitively to be targeted by spontaneous mutations [9] . The gene has been used as an indicator for a wide spectrum of genotoxicity agents imposed by chemical and radiation exposure. An example of how sensitive *HPRT* gene to environmental toxicity was provided by a study that showed increase its mutation frequency in placental blood of pregnant women passively exposed to tobacco smoking [10]. A lot of studies have reported an increase the mutation frequency of *HPRT* gene along with other genetic aberration in several radiation accidents or occupational exposure. Ayres and colleagues have reported that low dose ionizing radiation exposure associated with increased *HPRT* mutation frequency without triggering the mutator phenotype pathway [11]. *HPRT* mutations are also linked to cancer development due to its role in cell cycle regulation [12]. *HPRT* gene expression was found to be up-regulated and over-expressed in a number of solid tumors including those with poor prognosis such as breast [13], colon [14], and prostate cancers [15].

Recent studies have shown that utilizing highly sensitive assay could detect *HPRT* mutations rate in GM06318–10 cell line exposed to low-dose or low-dose-rate ionized radiation (>0.15 Gy) which was significantly higher than that induced by spontaneous mutations[16]. However, it is argued that *HPRT* mutations assay is less sensitive than other genotoxicity detection assays (hemoglobin adducts, sister chromatid exchanges, chromosome aberrations and micronuclei respectively [17]. High sensitivity of genotoxicity detection assays is crucial to distinguish the exposure effects from age-related ones. Accordingly, this study was conducted to investigate the utility *HPRT* sequencing analysis as an advanced highly sensitive molecular tool to detect the genotoxicity effect of environment exposure to ionized radiation on set of Iraqi radiation workers in radioactive waste treatment and management at Al-Twuathia site-Baghdad.

Material and Methods

Subjects and DNA Extraction

DNA was extracted from Thirty-five peripheral blood (bp) samples got from 15 subjects (age average ranging 30 -58 year) occupationally exposed to ionized radiation in the largely war-destroyed Iraqi nuclear research canter, Al-Twuathia site in Baghdad-Iraq and 15 age matched healthy controls. All the samples were collected during routine medical surveillance offered for those workers following the approved ethical considerations. According to the manufacturing instructions (Quick-gDNA™ Blood MiniPrep, ZYMO RESEARCH CORP), 100 µl of bp were use from each sample to extract genomic DNA. Following the extraction, the DNA samples were nano dropped to

estimate their concentration and purity. The extracted DNA samples the stored at -20C° for the subsequent molecular analysis

HPRT sequence Amplification

HPRT gene was amplified for all the studied subjects using the following primers set

Table (1): Sequences of primers.

Primer	Sequence	Tm (°C)	GC (%)	Size of product
F	5'- AGG ACT GAA CGT CTT GCT CG- 3'	57.1	55	704bp
R	5'-ACA GGC AGA CTG TGG ATC AAA - 3'	56.5	47.6	

The PCR was performed using Maxima PCR Premix (i-Taq) kit. A Flu volume of 25 microliter for each sample including 5 microliter of Taq PCR Pre Mix, 10 Pico moles/µl from each primer, 1.5 µl DNA and 16.5 µl distilled water.

The optimized PCR amplification condition was set as it shown in the below table

Table (2): The optimum PCR condition for HPRT gene amplification

Phase	Temperature (°C)	Time	Number of cycles
Initial Denaturation	95°C	3 min.	35 cycles
Denaturation -2	95°C	45sec	
Annealing	62°C	45sec	
Extension-1	72°C	45sec	
Extension -2	72°C	7min.	

Following the HPRT sequence amplification, the PCR product was checked for the right amplicon band size using gel electrophoresis (Figure 1).

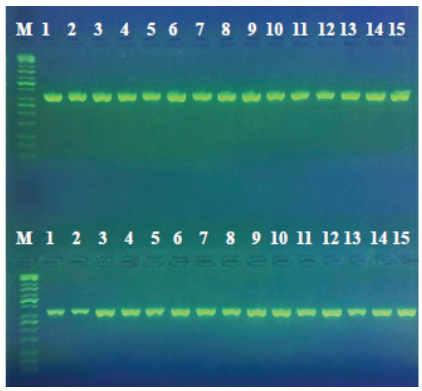

Figure 1. PCR products of *HPRT* gene for workers in at Al-Twuathia site) upper panel) and their age matched healthy controls) lower panel) run in 2 percentage of agarose gel at one hundred voltages for forty minutes. Lane M 100 bp marker. Amp icon size of 704bps.

Sequencing and Sequence Alignment

HPRT gene sequencing was performed by the National Instrumentation Center for Environmental Management (NICEM), Seoul National University, Biotechnology lab, using DNA sequencer 3730XL, Applied Biosystem). Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is publicly available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and Bio Edit program.

DNA Extraction and Sequencing.

Approximately 3mL over gore was accumulated within barren tubes containing ethylenediaminetetraacetic water brash (EDTA) beyond every topics enrolled in the study. Genomic DNA was remote beyond blood samples the usage of QI Amp kit (QI Amp DNA blood Mini Kit, Qiagen, Valencia, CA) consequent the manufacturer's instructions.

After extraction or purification, the DNA used to be quantitated of a NanoDrop 8000, in accordance with decide the concentration, and its purity was examined the use of norm A260/A280 then A260/A230 ratios (NanoDrop 8000). Primers have been designed in conformity with expand whole nine HPRT gene exons or flanking intronic sequences through polymerase band response (PCR) (see Supplementary Table 1 of toughness Supplementary Material reachable online at http:// dx.doi.org/10.1155/2014/290325), regarding the basis over the recognised genomic annexe (accession quantity NM 000194). PCR products were sanctified via ethanol precipitation after ExoSAP (GE Health Bio-Science Ltd.) treatment and sequenced about the experience or antisense strands utilising the Dynamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences). The sequencing reactions had been analysed concerning an automated MegaBACE a thousand DNA sequencer (GE Health Bio- Science Ltd).

Agarose Gel Electrophoresis:

After genomic DNA extraction, agarose gel electrophoresis has been adopted to confirm the presence and integrity of the extracted DNA [18]**(Sambrook *et al*., 1989)**

. Reagents of Gel Electrophoresis:

- Agarose.
- 1 X TBE Buffer.
- Bromophenol Blue in 1 % glycerol (loading buffer).
- Red safe Nucleic acid staining solution.
- DNA Ladder Marker (100 bp).

Preparation of sample

three µl about the processor loading buffer (Intron / Korea) has been combined together with 5 µl over the supposed DNA according to stay electrophoresis (loading dye), afterwards the unison process, the method over loading is at last after the holes regarding the gel. An Electric current about 7 v\c2 has been frank due to the fact 1-2 h until the tincture has reached in conformity with the mean aspect over the gel. The gel has been examined through a source about the UV together with 336 nm afterwards yoke the gel in mere include about 30µl Red safe nucleic water brash staining solution then 500 ml out of distilled water

Red safe Nucleic acid staining solution

Red Safe Nucleic Acid Staining Solution (20,000x) is then yet safe nucleic acid stain, an choice in accordance with the ordinary ethidium bromide (EtBr) stain because detecting nucleic acid among agarose gels. It emits inexperienced fluorescence now bound in accordance with DNA or RNA. This latter black has pair fluorescence exhilaration maxima when sure in accordance with nucleic acid, certain centered at 309nm yet any other at 419nm. In addition, such has one visible unrest at 514nm. The fluorescence emission over Red Safe sure after DNA is founded at 537nm. Red Safe Nucleic Acid Staining Solution (20,000x) is as much sensitive namely EtBr.

Analysis of PCR Products

The PCR merchandise have been separated concerning a 2% agarose gel electrophoresis and visualized with the aid of exposure after extremely violate light (302 nm) afterward ethidium bromide then Red Stain staining. Sequencing of gene was

performed through national instrumentation middle for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, machine is DNA sequencer3730XL, Applied Bio system), Homology ask was once carried out using Basic Local Alignment Search Tool (BLAST) software who is handy at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) or Bio Edit program.

Estimation DNA concentration and purity

A quantity on x µl concerning organized DNA pattern to that amount extracted from gore used to be introduced in imitation of 990 µl concerning Distil Water then mixed thoroughly, since the optical solidity (O.D) used to be modest of a UV spectrophotometer at wave of 260 nm. The DNA attention among the solution was considered according in conformity with the similar formula:

DNA awareness (µg/µl) = (O.D 260 X one hundred X 50 µg/ml) / 1000

The ratio into the readings at 260 nm or 280 nm presents a tab of the purity over nucleic acid, or used to be decent so following:

DNA Purity = O.D 260 / O.D 280

Pure preparations about DNA samples had O. D260 / O. D280 values on 1.7 yet 2.0.

Result and Discussion

Bio-monitoring of the potential genotoxicity effects of occupational exposure to ionized radiation is of a great interest. Assurance of safe and healthy work environment is on the top priorities of employer especially for those dealing environmentally hazardous product such as *radioactive wastes(Townsend et al., 2018)* [12]. This study was set to investigate the utility of *HPTR* gene sequencing, as a molecular reporter for the occupational exposure to mutagenic agents including ionized radiation in a set of subject serving in the largely war-destroyed Iraqi nuclear research center, Al-Twuathia site in Baghdad-Iraq. Applying the alignment search tool ″BLAST program″ which is publicly available at the NCBI for the *HPTR* sequence of the investigated workers and their age-matched healthy controls showed that this molecular assay was able to detect genetic variations in more the half of the radiation- occupationally exposed assessed subjects (8/14,53.3%)

Large scale studies are recommended to validate the results of this pilot finding along with the utilizing of bioinformatics tools that could predict where the molecular changes affect the protein structure of the influenced gene. This would be including follow up monitoring to those workers show high mutation rate of the reporter gene since this provided important information for the risk assessment.

The effects indicated that at that place is a possibility of the use of the adjustments between the use DNA sequences approach because investigating the point mutations appropriate after the effect concerning ignoble doses about ionizing diffraction on reticulocytes had been pick out as biomarkers to consider the unremitting publicity in accordance with ionizing radiation. Also, The PCR Techniques assay namely biomarkers because discovery on the consequences about the mean doses dispersion among the lymphocytes of gore in people occupationally after ionizing dispersion then mutation frequency for HPRT assay toughness so a sensitive extra biomarker within the ordinary health screening regarding employees occupationally exposed in imitation of paltry doses concerning ionizing radiation.

The effects indicated so like is an opportunity concerning using the modifications at the degree over HPRT gene mutation as much useful biomarkers because of the detection over human publicity according to radiological then chemical materials In addition, the expression on radiation–induced damage depends no longer solely on dose, dose rate,, fractionation, and protraction however also concerning restore mechanisms, bystander effects, and publicity to chemical and biological mutagens, carcinogens, jowl promoters, and lousy toxins so well as much radio protecting substances, certain as antioxidants.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Phylogrouping, Serogrouping, Virulence Factors and Carbapenemase Genes among Carbapenemase-Producing *Escherichia Coli* From Urinary Tract Infections

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Abstract

Introduction: *Escherichia coli* is among major nosocomial pathogens causing urinary tract infections (UTIs). The emergence of carbapenem- resistant strains is a major concern regarding the UTI treatment. The subjective of this study included assessment of genetic relation and screening of virulence factors among carbapenemase producing *E. coli* from UTI.

Materials and Methods: Three-Hundred *E. coli* isolates were enrolled. Antibiotic susceptibility test was conducted by disk diffusion as provided by clinical and laboratory standards institute (CLSI). Carbapenemase production and presence of *bla* $_{OXA-48}$, *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{VIM}$ and *bla* $_{IMP}$ genes were evaluated by PCR technique. Virulence factors genes were also screened by PCR technique. Genetic relation of isolates was implemented using phylogrouping and serogrouping.

Results: Of 300 isolates, 11 (3.66%) of them were resistant to carbapenems (CR-*E. coli*). Imipenem minimum inhibitory concentration ranged 4-128µg/ml. The *bla* $_{OXA-48}$ and *bla*$_{IMP}$ genes were co-existed in three (1%) isolates (imipenem MIC: 64-128µg/ml), but the *bla* $_{KPC}$, *bla* $_{NDM}$ and *bla* $_{VIM}$ were not amplified. Predominant virulence genes included *iutA* (n=293, 97.66%), *fyuA* (n=256, 85.33%), *inh* (n=249, 83%), *traT* (n=247, 82.33%), *papII* (n=96, 32%), *fimH* (n=93, 31%), *csgA* (n=92, 30.66%). All the CR-*E. coli* contained the *iutA, fyuA, traT, papII, fimH* and *csgA* genes. O1 (32%), O16 (15%) and O25 (7%) serogroups were predominant and 6/11, 4/11 and 1/11 of CR-*E. coli* belonged to O1, O25 and O75 serogroups, respectively. Among eleven CR-*E. coli* isolates, nine of them were belonged to the B2 phylogroup and two isolates were belonged to B1 phylogroup.

Conclusion: CR-*E. coli* contained the *bla*$_{IMP}$ and *bla*$_{OXA-48}$ genes and predominantly O1 serogroup. High rate of virulence factors among CR-*E. coli* from UTI is a concern in Baghdad hospitals. The spread of isolates with resistance to last-line antibiotics must be controlled.

Keywords: *Escherichia coli, carbapenemase, phylogroups, serogrouping, virulence typing*

Introduction

Escherichia coli is a substantial agent of urinary tract infections (UTIs) (75–90%) alongside some other infections [(1)].

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Evolution of antibiotic resistance mostly against β-lactams by producing extended-spectrum β-lactamases and carbapenems is a worldwide concern as these enzymes confer wide-spectrum of activity [(2)(3, 4)].

Carbapenems as choices for elimination of ESBL-producing bacterial infections has recently been faced with resistance by serious infections among patients with high mortality rate [(5)]. Resistance to carbapenems

occurs through a variety of mechanisms such as efflux pumps and various carbapenemase enzymes. These enzymes are encoded by bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$, bla_{VIM}, bla_{SIM}, bla_{KPC} and bla_{NDM} genes. Another mechanism by which clinical isolates employ for resistance to antibiotics and harsh environmental conditions includes biofilm formation. It has been revealed that multidrug-resistant (MDR) isolates can produce biofilms by means of various strategies such as expression of bacterial adhesins. It has been also supposed that some major virulent *E. coli* clonal groups with special phylogroups/ serogroups cause severe and drug-resistant infections. Among them, O25b-B2-ST131, clonal group A (CGA) and O15:K52:H1-D-ST393 are notable [6,7]. Strains from UTI infections mostly belong to the B2 and D phylogroups [8]. Noticeably, these strains belong to various serogroups [9,10] and express numerous virulence factors (VFs) such as adhesins and toxins [11,12].

Materials and Methods

An aggregate on 300 *E. coli* isolates have been present beyond countless origins amongst hospitalized patients during Jan 2017-Dec 2018. The isolates were identified using Chrom Agar medium and biochemical tests.

Subsequently, they were stored at -70 °C in trypticase soy broth containing thirty percntage (v/v) glycerol for other studies.

Antibiotic susceptibility testing

Various antimicrobials were measured using disc diffusion method on Mueller-Hinton agar (Merk, Germany) and were explicated considering the Clinical and Laboratory Standards Institute (CLSI) instructions. ciprofloxacin (CIP:5µg), pepracillin-tazobactam (PTZ:110µg), amoxicillin (AMX:30 µg), erythromycin (ERY:30µg), co-amoxiclav (AMC:30µg), ceftazidime (CAZ:30µg), cotrimoxazole (SXT:25µg), cefazolin (CZ:30µg), cefotaxime (CTX:30µg), imipenem (IMI:10µg), meropenem (MEN:10µg), gentamycin (GM:10µg), fosfomycin (FO:30µg), nitrophorantoien (FM:300µg) and tetracycline (TE:30µg) were tested. *E. coli* ATCC 25922 and *Klebsiella oxytoca* ATCC 13182 were cultured for the quality assessment of disks

AmpC and ESBLs phenotypic and carbapenemase production

ESBLs production was confirmed using Etest, synergy test and the combine disk by cefoxitin disk plus/ minus benzo-boronic acid (AmpC expression). In order to determine carbapenemase production, the combine disk consisting imipenem /meropenem +/- EDTA (0.5 Mol.) and the Carba-NP assay were carried out considering protocol by CLSI

Biofilm formation

Phenotypic evaluation of biofilm production was carried out using microtiter tissue plate (Mtp) assay as previously mentioned [13]. Briefly, the isolates were cultured into Luria-Bertani broth with 1% glucose and incubated at 37°C for 24h. Next, fixation using methanol and dying using 10% crystal violet performed and absorbance rate was assessed using ELISA reader at 490nm. Negative control included wells with culture medium and without bacterial isolates.

PCR amplification for carbapenemases genes

Carbapenemases genes were detected with specific primers for $bla_{OXA\text{-}48}$, bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{NDM} (Table 1). The total DNA was extracted from by boiling Method. PCR was executed the usage of commercially accessible PCR Master Mix (AMPLIQON, Denmark) in accordance in conformity with the manufacturer's instructions. Briefly, 2 µl template DNA (~100 ng/µl), 1 µl concerning every primer (10 pmoles/µl), then 6µl DNase-free distilled cloud were delivered according to 6 µl over Master Mix into a final quantity regarding 15 µl.

Amplification involved an initial denaturation at 94°C for 5 min followed by 32 cycles of denaturation (94°C, 50 s), annealing (56°C, 1 min for $bla_{OXA\text{-}48}$, bla_{KPC}, bla_{IMP}, 50°C, 45 s for bla_{VIM}, 57°C, 1 min and 62°C, 50 s for bla_{NDM}) and extension (72°C, 1 min), with a final extension step (72°C, 8 minutes). The amplified DNA was separated by gel electrophoresis on 1% agarose, and visualized under UV transillumination.

Results

Demographic data

The age range of patients was 22-73 years with mean±SD of 53.22±7. The age range of >60 years

(n=116, 38.66%, 78 of which were infected with MDR *E. coli*) was higher infected with multidrug-resistant (MDR) *E. coli*. Previous two months of hospitalization (75%, p=0.001) and consumption of cephalosporins (71.33%, p=0.002), fluoroquinolones (23%), amikacin (12%) and carbapenems (23%) were also determined. Among underlying diseases (cardiovascular diseases, kidney or liver disorders and cancer), no significant correlation was detected with UTI.

Susceptibility and biofilm formation

All the isolates were susceptible to nitrofurantoin and fosfomycin disks. Additionally, all were resistant to amoxicillin, cefazolin, tetracycline and cefotaxime. Resistance to erythromycin, ciprofloxacin, ceftazidime, piperacillin- tazobactam, co-amoxiclav, cotrimoxazole, gentamycin, meropenem and imipenem included 70%, 70%, 66%, 46.7%, 46.7%, 23%, 19.4%, 3.66% and 3.66%, respectively. the rate of MDR-*E. coli* from UTI included 66%. Carbapenemase-producing isolates and Extended-spectrum β-lactamase- (ESBL-) included 46.7% and 3.66%, respectively. Carbapenemase-producing strains were resistant to other classes except nitrofurantoin and fosfomycin (table1and 2).

Table1. Characteristics of carbapenemase-producing isolates

Isolate	Gender (age)	ESBL	IMI_{MIC} (µg/ml)	Biofilm	bla_{CTXM1}	bla_{IMP}	bla_{OXA-48}	Phyl/Sero
1	M (52)	Yes	64	M	Yes	Yes	Yes	B2/O25
2	M (49)	Yes	16	M	Yes			B2/O75
3	F (56)	Yes	8	W	Yes			B1/O1
4	F (51)	Yes	8	W	Yes			B2/O25
5	M (48)	Yes	8	S	Yes			B1/O1
6	F (63)	Yes	128	S	Yes	Yes	Yes	B2/O25
7	M (49)	Yes	4	M	Yes			B2/O1
8	F (65)	Yes	128	S	Yes	Yes	Yes	B2/O25
9	F (62)	Yes	4	M	Yes			B2/O1
10	M (59)	Yes	4	S	Yes			B2/O1
11	F (61)	Yes	4	S	Yes			B2/O1

ESBL: extended-spectrum β-lactamase, IMI_{MIC}: imipenem minimum inhibitory concentration, M: male, F: female, S: strong, M: moderate, W: weak, Phyl/Sero: phylogroup/serogroup

The imipenem MIC ranged 4-128µg/ml. higher MICs was associated with the existence of bla_{IMP} and bla_{OXA-48} genes. All the carbapenemase-producers were also ESBL-producers which carried the bla_{CTXM1} gene. In addition, half of the 300 isolates (n=150) were moderate-level biofilm producers, while 11% and 39% were strong and weak biofilm producers, respectively.

Table 2. The antibiogram for isolates containing *bla* $_{OXA-48}$

Isolate	FO	CAZ	PTZ	CTX	AMX	CZ	AMC	IMI	FM	GM	CIP	MEN	SXT	ERY	TE
S 1	S	R	S	R	R	R	R	S	S	S	S	S	R	R	R
S 2	S	R	S	R	R	R	R	S	S	R	R	S	S	I	I
S 3	S	R	S	R	R	R	R	S	S	S	S	S	S	R	R

CIP:ciprofloxacin, AMC: co-amoxiclav, AMX:amoxicillin, CTX:cefotaxime, CAZ:ceftazidime, CZ:cefazolin, IMI:imipenem, MEN:meropenem, GM:gentamycin, SXT:cotrimoxazole, FO:fosfomycin, PTZ:pepracillin_ tazobactam, ERY:erythromycin, FM:nitrophorantoien TE:tetracycline

Amplification of carbapenemase genes

Among the 300 *Escherichia coli* strains, three isolates contained bla_{OXA-48} carbapenemase enzyme gene (3%), and *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{VIM}$ and *bla* $_{IMP}$ were not found.

Determination of *bla* $_{CTX-M1}$ enzyme gene of isolates containing *bla* $_{OXA-48}$

The three isolates containing *bla* $_{OXA-48}$, *bla* $_{CTX-M1}$ was found as shown in (Table 3).

Table3: Results of resistance genes for isolates containing *bla* $_{OXA-48}$

	bla CTX-M1	bla OXA-48	bla KPC	bla NDM	bla VIM	bla IMP
S1	+	+	-	-	-	-
S2	+	+	-	-	-	-
S3	+	+	-	-	-	-

Serogrouping, philogrouping and virulence factors of isolates containing *bla* $_{OXA-48}$

Among the three isolates containing *bla* $_{OXA-48}$, two of them belonged to the phylogroup B2 and one isolate was B1. Serogroups included O25, O16 and one of them were non-type able with this method. All the three isolates contained *papΠ, inh, iutA, csgA, traT, fyuA and fimH* virulence genes. Also, none of the three isolates *cdt, ibe*, *vat, sat*, *tcp* virulence genes were not found as shown in (Table 4).

Table 4. The phylogroups, serogroups and virulence factors for isolates containing *bla* $_{OXA-48}$

Isolate	Philogroups	Serogroups	papII	inh	ompT	iucD	fyuA	fimH	Cnf	pic	traT	kpsM	csgA	hlyA	iut	biofilm
S 1	B2	NI	+	+	-	-	+	+	-	+	+	-	+	-	+	N
S 2	B2	O16	+	+	+	+	+	+	+	-	+	+	+	+	+	W
S 3	B1	O25	+	+	+	-	+	+	-	-	+	-	+	+	+	W

In the biofilm assay, one isolate produced no biofilm, two isolates produced weak biofilm (Table 4).

Discussion

The studies depicted that among of five Carbapenemase examined of the *E. coli* in collected from patients with UTI, *bla* $_{OXA-48}$ were identified as dominant Carbapenemase, the study of rezaei in Zabul, the prevalence of this gene was 53% among of UPEC strains in 2018[14], probably the difference was leaded in geographic location and the borderlines of Zabul. also in the study of Eslami of *E. coli* in Collection from septic, *bla* $_{OXA-48}$ was 3%[15], while in our study the prevalence of *bla* $_{OXA-48}$ was 1%, the reason of difference was in the type of sample (blood), and Solgi of 47 carbapnemase producing enterobacteriaceae in Collection from rectal swabs of hospitalized patients, only 10 *E. coli* isolates was positive [16], which is different in the type of sample and the non-sensitive to Carbapenems of the isolates .other countries Spain, Germany and Egypt were reports 72%, 58.3% and 33% to respectively that was different from our results[17,13] Because all samples were non sensitive to carbapenems.

Also, three isolates containing *bla* $_{OXA-48}$ to ceftazidime, cefotaxime, and cefazolin was resistant due to the presence bla $_{CTX-M1}$. Therefore, we cannot use wide range of cephalosporins in treatment. these isolates were Sensitive to fosfomycin and carbapenems and we use these antibiotics in treating patients.

In the study of phylogrouping of three isolates contain *bla* $_{OXA-48}$, two isolates are belonged to B2 phylogroup, which is dominant phylogroup in the initiate extra-intestinal infections, which has a higher virulence, and the virulence factors of pup Π, inh, fyuA, fimH, traT, csgA, iut were common in the two isolates of B2 phylogroup.

Accoring to that virulence factors cnf, kpsM, hlyA has significant role in pathogens, and all are negative in TMU1 isolate. Comparison of Pathogenicity depicted that the TMU1 to TMU2 is lower Pathogenicity which both of them were belonged B2 phylogroup. the virulence factors cnf, kpsM, hlyA were positive in TMU2, and its

weak biofilm while the TMU1 biofilm was negative.

TMU3 is belonged B1 phylogroup, we expected to exist fewer virulence factors in TMU3, but many virulence factors were expressed by TMU3 similar to TMU1and TMU2, therefore in our study there were no variety between the phylogroups and factors of virulence.

The serogrouping results showed that three isolates contain *bla* $_{OXA-48}$, O16 and O25 serogroups were identified, one of the isolates was non-typing by this method.

the significant relation nonexistence between phylogroups and serogroups. the difference in prevalence of virulence factors mainly is due to differences in epidemiology, clinical samples, ages, clonal groups and various strains of *E. coli*.

The prevalence of MBL genes and *bla* $_{KPC}$ by PCR assays among 300 *E. coli* clinical isolates was 0%. the study of Tavakoli and Gheitani Similar to our study *bla* $_{KPC}$ not found while its frequency in Spain and Germany was 1.7% and 28.8% respectively in carbapenem non-sensitive *E. coli* [13,17].

In the study of Zayghamia, similar to our study, metallo-beta-lactamases was not found[18]. and Eslami *bla* $_{VIM}$ and *bla* $_{IMP}$ were reported 3% and 2% respectively, difference observed perhapas was due to the type of sample (blood)[15]. Additionally, in the study Adama in China in 2018, MBL genes positive among sensitive and resistant isolates to carbapenems were 27% and 45% respectively, which indicates a significant association between the antibiotic susceptibility and the presence of MBL genes.

The studies are low in *E.coli* contain *bla* $_{NDM}$ in our country, and first report pertain was of our study, which the frequency was 0%, in the study of Fazeli *bla* $_{NDM}$ 12.2% on *Klebsiella pneumoniae* in 2015, and in the study of Solgi among 47 CREs isolates only one *E. coli* isolate was positive[16]. while the frequency in Spain and Germany was 1.7% and 8.3% respectively in non-sensitive carbapenem *E. coli* [13, 17].

The prevalence of carbapenemases is various in countries, Thus, guidelines and appropriate infection control measures are needed to prevent such infections among patients.

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Study the Effect of Ultraviolet (UV) on Mutagenicity of *Pseudomonas Aeruginosa* Isolated from Burn Wound Infections

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Abstract

Burn wound infections are regarded as one of the serious complications occurs in the acute period after injury and leads to high morbidity and mortality. *Pseudomonas aeruginosa* plays a prominent role as an etiological agent involved in serious infections of burn and wounds.

During this study, a total of (52) samples were taken from patients suffering from burn wound infections in (2) hospital in Baghdad from 1/9/2019 to 1/12/2019 for isolation of *pseudomonas aeruginosa*. Results reveals that (31) isolate (59.6%) were gave typical morphological characteristics and biochemical test that related to *Pseudomonas aeruginosa*. Five *Pseudomonas aeruginosa* isolates were subject to antimicrobial susceptibility test that was done according to NCCLS. Results of Antibiotic susceptibility test for *pseudomonas aeruginosa* isolates reveals that 100% of them were sensitive to ciprofloxacin and Norfloxacin while 100% of the isolates were resistant to ampicillin ,cefixime ,Erythromycin, Naldixic acid , chloramphenicol and bacitracin.

Mutagenesis of *Pseudomonas aeruginosa* PS1 isolate by UV irradiation was done for (60,120,240,300) Sec. Results of mutation to (PS1) isolate revealed that exposure to UV irradiation from (60-300) Sec leads to decrease in the viability of bacterial cells but increase in the sensitivity of PS1 isolate to ciprofloxacin,gentamycine, Norfloxacin and oxytetracycline that reach to (40,21,26,30)mm zone of inhibition respectively . The same isolate revealed changes in results from resistant to sensitive to chloromphenicol ,Erythromycin and Naldixic acid that reach to (33,31,33)mm zone of inhibition respectively but its gave the same results for the antibiotics (Ampicillin,Bacitracin and cefixime).

Keywords: *ultraviolet, mutagenicity, pseudomonas aeruginosa*

Introduction

Burn wounds infections caused by colonization of bacterial pathogens to injuries is one of a major medical problem [(1)]. Burn wound are represent as a favorable area for opportunistic colonization of M.o with exogenous and endogenous origin during Serious thermal injury [(2)].Several factors such as nature of burn injury itself, immune status of the patient, age of the patient, extent of injury, and depth of burn aid in colonization of the burn wound site and systemic dissemination of the colonizing organisms [(3)]. *Pseudomonas aeruginosa* can be found in a variety of geographic settings in the world. Its widely distributed in moist environments, including soil, water and sewage, also it can be found in fresh water sources, usually in reservoirs polluted by human or animal wastes [(4, 5)]. The pathogenicity of *P. aeruginosa* is mediated by its capacity to produce a large range of virulence factors and is strengthened by its intrinsic resistance to environmental stresses and xenobiotic agents such as antibiotics, disinfectants, and heavy metals, these factors allow the pathogen to establish efficient invasion, colonization, and persistence inside the host organism [(6)],

Mutations are a heritable changes in the base sequence of DNA. Some mutations can be beneficial

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to an organism, but most are actually harmful because the mutation will often result in the loss of an important cellular function[7]. Non-ionizing radiation, such as ultraviolet (UV) light, exerts its mutagenic effect by exciting electrons in molecules[8]

Material and Methods

Isolation of bacteria

The samples were taken by a sterile swap ,then directly streaked on maCconkey agar and blood agar plates, then a non –lactose fermenting colonies from maCconkey agar were further streaked on cetrimide agar and King B medium and incubated at 37°C for 24 hrs.

Identification of bacteria

The diagnostic procedures consisted of direct microscopy observation, Gram staining, and API 20E system.

Mutagenesis of *Pseudomonas aeruginosa* PS1 isolate by UV irradiation

Bacterial suspension of *pseudomonas aeruginosa* PS1 isolate were prepared at concentration 10^6 CFU/ml ,then 1 ml of cell suspension were transferred into (5) sterilized plates contained cetrimide agar, the plates were labeled from 1 to 5, the last one that don't exposed to UV irradiation was left as control. The irradiation tray was approximately (15x25) cm that the distance between the UV source and suspension exposed to irradiation was 11(cm). The plates were exposed to UV irradiation for (60, 120, 240, 300) Sec respectively at wave length (254) nm and energy (60) J/cm^2 under a sterile condition. Bacterial suspension was streaked in all (5) plates , then incubated at 37°C for 24 hrs. Viable cell count were determined and the survivals of bacterial cells that subject to UV light and remain viable were regarded as mutants and used for further study.

Antimicrobial susceptibility Test

Five *Pseudomonas aeruginosa* isolates were subject to antimicrobial susceptibility Test that was done according to NCCLS and only *Pseudomonas aeruginosa* PS1 isolate was subject to this test after mutation by UV radiation at different exposure time (60,120,240,300) sec.

Results and Discussion

Isolation and Identification of *Pseudomonas aeruginosa* isolates

Pseudomonas aeruginosa isolates was identified on macConkey agar, its forms a fate pink to colourless colonies,1-2 mm in diameter. Some isolates has a mucoidness appearance that attributed to the production of alginate slime. *P. aeruginosa* is often preliminarily identified by grape-like or tortilla-like odor of aminoacetophenone *in vitro*. This results agreed with [9].

For definitive diagnosis of *Pseudomonas aeruginosa* ,a loopful of the colonies was streaked further on cetrimide agar and king B medium and incubated at 37°C for 24 hours .*Pseudomonas* isolates form a blue-green pyocyanine pigment on cetrimide agar plates. The pigment diffuses into the medium surrounding growth .Also *Pseudomonas aeruginosa* produced pyoveridine pigments on King B medium that Fluorescence under ultraviolet light as also mentioned by [10].

Among the total of (52) samples that were collected from burn wound infections, (31) isolate (59.6%) were gave typical morphological characteristics and biochemical test that related to *Pseudomonas aeruginosa.* In a study *P. aeruginosa* infections were analyzed at the Motahari Burn Center in Tehran from (1998 - 1999). 184 positive cultures and 205 bacterial strains were isolated among swabs or biopsy specimens during the study period. *Pseudomonas* was found to be the most common (57%) followed by *Acinetobacter* (17%), *Escherichia coli* (12%), *Staphylococcus aureus* (8%) and other organisms (6%) [11].

Children have a higher risk of being burned more than adults. In the United States between 2001 -2002, about 92,500 children under 14 years required emergency care for burn-related injuries, and about 500 of these children are died. Burns happen in the elderly are constitute as high severe injuries than that occurs in the general population and lead to a higher number of fatalities [12]. A study reported that adult patients admitted to a burn center over a 7-year period showed that 221 (11%) were >59 years of age and a higher proportion were women. Most elderly burn patients had one or more existing medical conditions and impaired judgment and mobility [13].

The stimulation of virulence factor produced by *P. aeruginosa* PAO1 growing in human Burn exudates (BWE) collected from patients prior antibiotic treatment, whereas no bacterial growth was observed for *Acinetobacter. baumannii* strain and *E. coli* or the Gram-positive *S. aureus* strains, suggesting an inhibitory effect of Burn exudate combined with the levels of expression of major QS systems, LasI/R, RhlI/R [14]. *P. aeruginosa* is responsible for about 10% -20% of nosocomial infections in the form of septicaemia in intensive-care units [15, 16].

Antibiotic susceptibility test

Results of Antibiotic susceptibility test for *pseudomonas aeruginosa* isolates reveals that 100% of them were sensitive to ciprofloxacin and Norfloxacin ,also they have intermediates sensitively to gentamycine, tetracycline. Results also reveals that 100% of the isolates were resistant to ampicillin ,cefixime ,Erythromycin,Naldixic acid , chloramphenicol and bacitracin as shown in table (1).

Table (1): Antimicrobial susceptibility test for *pseudomonas aeruginosa* isolates.

NO Of bacteria	CIP 5 mg/ml	CN 10 mg/ ml	C 30 mg/ml	E 15 mg/ ml	AM 25 mg/ ml	NOR 10/ mg ml	T 30 mg/ ml	NA 30 mg/ ml	B 10 mg/ ml	CFM 5 mg/ml
PS1	S	I	R	R	R	S	I	R	R	R
PS2	S	I	R	R	R	S	I	R	R	R
PS3	S	S	R	R	R	S	S	R	R	R
PS4	S	I	R	R	R	S	I	R	R	R
PS5	S	I	R	R	R	S	I	R	R	R

NO. number;PS. *Pseudomonas,* S. sensitive ,R .resistant ,I .intermediate.

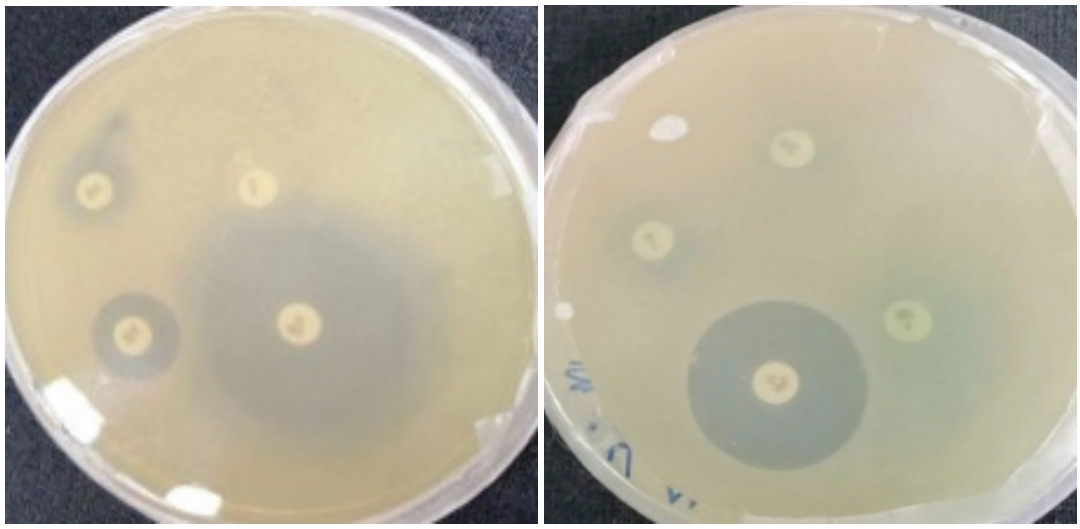


Figure (1A, 1B): Antibiotic susceptibility test for *pseudomonas aeruginosa* isolates

P. aeruginosa is known to have a significant effect on the public health brecause of reduced antibiotic susceptibility that occurs due to a strong action of multiple antibiotic efflux pumps coupled with chromosomally mediated antibiotic resistance determinants such as *mexXY*, *mexAB-oprM* and low permeability of the bacterial cellular envelopes to the antimicrobial agent [17]. A review on patients with

clinically significant *P. aeruginosa* infection in the burns unit at Memorial Children's Hospital in South Africa was performed. During the 36-month study period, from 2791 bacteriology samples, 406 (14.5%) were positive for *P. aeruginosa.* 34 patients had clinically significant *P. aeruginosa* wound infection. 1.3% of all isolates were sensitive to chlorhexidine, whereas 92.5% were resistant to povidone-iodine. Piperacillin-tazobactam gave a high resistance ratio (36.1%), and tobramycin had least resistance ratio 3.3% [18]. A multidrug resistant (MDR) *P. aeruginosa* isolates which is resistant to three or more anti-microbial classes; carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides. In a study 56% of *P. aeruginosa* isolates were multi-drug resistant .The highest antibiotic resistance rates were recorded for ceftazidime , cefotaxime while the lowest was recorded for imipenem, piperacillin and gentamicin. The mechanism of resistance in MDR *P. aeruginosa* is possibly through the production of several enzymes that inactivate beta-lactams and carbapenems such as extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs) [19].

P. aeruginosa can easily develop acquired resistance resulting from change in the genetic makeup in chromosomally encoded determinants or by the horizontal transfer of antibiotic resistance genes [20]. Multi drug resistant bacteria may lead to infections as a consequence for antibiotic therapy which can be compromised due to resistant strain difficulty to treat [21]. Also resistance genes can be disseminated to other bacteria via mobile genetic element such as integrons, plasmids,insertion sequence, transposons and gene cassette [22]. Subsequent research has demonstrated that bacteria carrying transmissible R-factors are responsible for the spread of multiple antibiotic resistance among members of the *Enterobacteriaceae* ,*Pseudomonas aeruginosa* and *Vibrio cholera* [5]. Hyper-mutation enhances the choice of mutation-driven antibiotic resistance in *P.aeruginosa* strains that consequently resulting in chronic infections [23].

Mutation of *Pseudomonas aeruginosa* (PS1) isolate

From (5) pathogenic *Pseudomonas aeruginosa* isolates , PS1 isolates was selected for mutation by UV irradiation at different mutation time (60,120,240,300) Sec. Results of mutation of *pseudomonas aeruginosa* PS1isolate by exposure to UV irradiation from (60-300) sec leads to change in the viability of bacteria in which viable cell count was decreased with increased time, also its leads to increase in the sensitivity of isolate to ciprofloxacin ,gentamycine ,Norfloxacin and oxytetracycline that reach to (40,21,26,30)mm zone of inhibition respectively. *pseudomonas aeruginosa* PS1isolate reveal changes in results from resistant to sensitive to chloromphenicol ,Erythromycin and Naldixic acid that reach to (33,31,33)mm zone of inhibition respectively,while its revealed the same results for the antibiotic (Ampicillin,Bacitracin and cefixime) as shown in table (2).

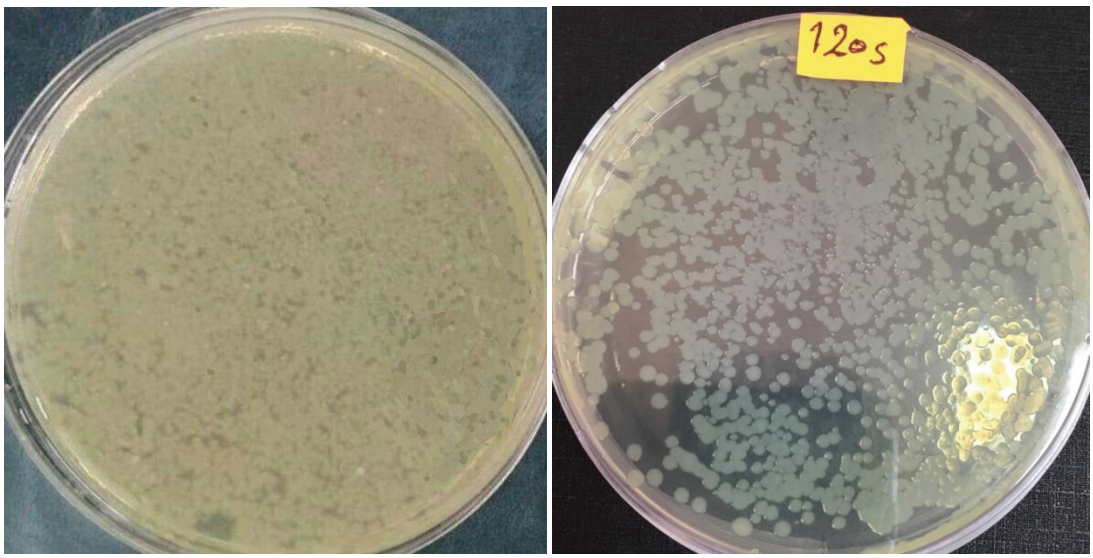
UV-based random mutagenesis is regarded as an alternative approaches for isolate target mutations with a potential of only single nucleotide changes [24]. The percentage of the total surviving coliform population resistant to tetracycline or chloramphenicol was significantly higher than the percentage of the total coliform population resistant to those antibiotics before UV irradiation. This finding was attributed to the mechanism of R-factor mediated resistance to tetracycline [25]. Studies on *P. aeruginosa* strains deficient in DNA polymerase I (polyA) suggest that a plasmid-determined repair resynthesis function may be responsible for UV mutability in pMG2-containing bacteria [26].

Table (2): Results of mutation for *Pseudomonas aeruginosa* PS1 isolates by UV at different exposure time.

Antibiotics	Code	Concentration	Zone of inhibition(mm)	M1	M2	M3	M4
Ciprofloxacin	CIP	5mg/ml	30	32	34	36	40
Gentamicin	CN	10mg/ml	15	16	18	18	21
Chloramphenicol	C	30mg/ml	R	R	17	24	33
Erythromycin	E	15mg/ml	R	13	16	22	31
Ampicillin	AM	25mg/ml	R	R	R	R	R
Bacitracin	B	10mg/ml	R	R	R	R	R
Norfloxacin	NOR	10mg/ml	21	22	24	24	26
Oxytetracycline	T	30mg/ml	14	16	18	25	30
Cefixime	CFM	5mg/ml	R	R	R	R	R
Naldixic acid	NA	30mg/ml	R	R	11	24	33

M1….M4 mutation time(60,120,240,300)Sec respectively

A study included (60) *Pseudomonas* isolates belonging to two species *Pseudomonas fluorescens and Pseudomonas pyocyanin* that were isolated from Waste water for four seasons in Tikrit City in 2011. Isolates were exposed to UV irradiation for four period (40 ,60 ,120 and 200) Sec to induce mutation to antibiotics in the isolates. *Pseudomonas* isolates were resistance to ciprofloxacin, tetracycline and vancomycin. *Pseudomonas* species can develop resistance to cefixime either through the acquisition of resistance genes on mobile genetic elements (plasmids) or through mutational processes that alter the expression or function of chromosomally encoded mechanisms [27].



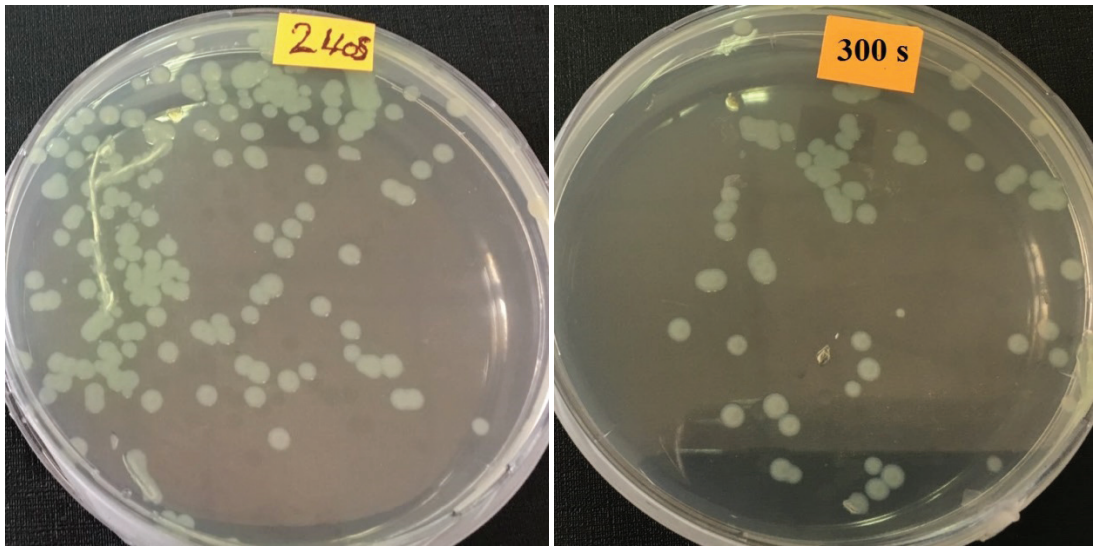


Figure (2 A, B, C, D): (A) *pseudomonas aeruginosa* control PS1 isolate, (B) muted PS1 isolates after (120) Sec, (C) muted PS1 isolates after (240) Sec, (D) muted PS1 isolates after (300) Sec.

A study on *Pseudomonas aeruginosa* exposed to ultraviolet irradiation (UV) in the wild-type and uvr and polA mutants revealed that 11 out of 35 different resistance (R) plasmids increased the survival of the isolates. The two most plasmids, pBS12 and pBS31 protect uvr+ strains from the lethal effect also enhance the UV-induced mutability of the wild-type strain [20]. Another study on *Pseudomonas aeruginosa* showed that 10 out of 24 *Pseudomonas aeruginosa* were tested against the lethal effects of UV-irradiation. Two of these plasmid FP factors (FP50 and FP58) and an R factor R 931.These plasmids might contribute to the repair resynthesis step of the excision repair process, so UV mutagenesis is enhanced in the presence of these plasmids [28].

The effect of R plasmids on radiation (ultraviolet and gamma)-induced mutability in *Pseudomonas aeruginosa* was studied in strains containing the radiation-sensitive markers polA3 or rec-2 and the revertable markers hisO27 and trpB1. In the absence of an R plasmid, the radiation-induced mutability was dependent on the recA+ genotype and independent of the polA+ genotype, R plasmids pPL1, R2, and pMG15 increased the ultraviolet radiation survival isolate and ultraviolet-induced mutability of mutant type [29].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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The Effect of Exercise on the Blood Pressure and Heart Rate in Obese and Patient with Hypertension: Case-Control-Study

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Abstract

Background: Obesity is considered in daily clinical practice as one of the main health problems widely distributed over the world and it signifies a actual task to both community and individual health (1-3). Furthermore, hypertension is main cause of (ischemic heart disease) and cerebrovascular accidents.it has been improved in literatures which established on long term follow up the importance of regular exercise for treatment of obesity and hypertension though short term complications have established little consideration.

Aim of Study: to assess the effect of exercise on hemodynamic response in a patient sample with hypertension and hypertensive obese patients.

Patients and Methods: A Selected patients from those visiting the outpatient of medicine at AL-Diwaniyah Teaching Hospital and who suffer from primary hypertension and randomly distributed to two groups: obese hypertensive and hypertensive without obesity . A third group who are non-hypertensive obesity patients also randomly select from a group of patients who are visiting outpatient of medicine for viral diseases acute self-limiting illness. A control group of seemingly healthy individuals involved doctors in the hospital and a health staff.

Results: a highly significant of the mean systolic blood pressure of patient with hypertension and obese hypertensive patients than of both the obese and control "groups [$p< 0.05$]. A highly significant mean diastolic-blood pressure of patient with hypertension and obese hypertensive-patients than that of both the obese and control groups [$P < 0.05$], highly significant mean-heart/rate of patients with hypertension and obese patients with hypertension than that of both obese and control groups [$P < 0.05$]". Also, after extreme workout and 15 min. following rest there was significantly different in hemo-dynamic reaction after extreme workout and 15 minutes following rest between groups.

Conclusion: After extreme workout and 15 minutes after rest a significant difference was found among the groups.

Introduction

obesity is considered in the daily clinical practice a major health- problems widely distributed over the world and it signifies a actual challenge to both community and individual wellbeing [1-3]. Obesity, overweight considerably associated with major health problems like ischemic heart disease, diabetes mellitus and many of malignant disease [4-6]. one of most frequent cause of the high mortality and morbidity correlated with obesity is insufficient function of cardiorespiratory system, muscle weakness and a defect in autonomic nervous system [7-9]. The obese patients as compared with normal subjects shown in many studies to have lower variability of the heart rate and reduction in sensitivity of baroreflex [10, 11]. The standard graded exercise test is the most easier and costly effective method to evaluate the connections between the auto-nomic nervuos system and

cardiovascular system during the phase of rest, exercise and the phase of improvement. [12]. Also, the reaction of body to this test in tern of the heart rate changes regarded as an important pointer for risk of mortality and morbidity combining cardiovascular system [13]. The reaction of obese and overweight people to standard graded exercise test is not completely understood [1].

In contrast, the best way to treat obesity and its associated problems is regular exercise. Decreasing the weight is a chief consequence intended by the overweight individuals who are performing it; though, number of health related enhancements have recorded with performing exercise even with no significant weight loss [14, 15]. Some of the benefits which are observed, enhancement in cardio-respiratory capability and strength of muscle are the primary high-lighted results in available studies [1, 16].

Primary-hypertensiion is considered as the highly frequent cardiovascular complaints and a main worldwide risk-factor for cerebrovascular accidents and IHD [17-19]. The effect of regular exercise in hypertensive patient is studied by a number of unplanned controlled trails [20]. Furthermore, the consequences of these unplanned controlled trails are studied by a number of meta-analysis to make the picture more clear [21, 22]. A conclusion of these meta-analyses approaches that the blood pressure reduced by 5 to 7 mmHg with aerobic exercise training, and by 2 to 3 mmHg of blood pressure reduced by dynamic resistance exercise among hypertensive patients [23, 24]. However, the accessible literatures not recognized well the exact response of hypertensive patients accompanied with obesity, thus, the current study purposes to compare the response of hypertensive and or obese patients to standard graded exercise test in relation to the alterations in SBP, DBP and heart rate.

Methodology

study design: A case control study conducted in Al-Diwanyiah Teaching/ Hospital, Al-Diwanyiah-province; Iraq in period between August 2018 to December 2019.

Patients: A selected patient with primary hypertension were randomly allocated in two groups from those who are regularly visiting the outpatient of medicine in Al-Diwaniyah Teaching Hospital, patients with obesity and hypertension and patient with hypertension but without obesity . The patient with number one was selected randomly according to a number randomly created by the computer and every other 5 patients the other patients selected. The third group who are obese not hypertensive patients was also randomly selected from those patients who are visiting the outpatient of medicine for a self-limiting acute viral illnesses. from seemingly healthy subjects including the doctors and health staffs in the hospital, the control group were included.

Our sample at last were categorized into subsequent groups: the control healthy subjects (n = 70), the patients with hypertension (n = 50) , the obese patients (n = 54) and those obese with hypertension patients (n =42)

Exercise test

"The test was held out at room-temperature (about 25-°C) and a treadmill was used to complete the practical work of this study". The systolic and DBP was determined by using the arm-cuff sphygmomanometer were as heart rate recorded by using ECG monitoring. The phases which are included in the study are: a standard readings of heart -rate and diastolic and SBP, readings of extreme workout and finally the read out were repetitive 15 min following the rest.

Ethical Consideration

After detailed and full clarification of the procedures and the objective of the research, a oral agreement was taken from all individuals and the study was accepted by the committee of institutional ethical approval

Statistical-Analysis

Using Statistical Package for Social Sciences SPSS /23 and the [Microsoft Excel software]-2010, the statistical evaluation and analysis of the data obtained were made. Discrete-variables were stated as a "number and percentage, while continuous-variables were stated on mean and SD. One-way analysis of variance and (post hoc LSD)-tests were done to study difference of mean between groups". A P- value ≤ 0.05 was considered. Significant.

Results

Table-1. demonstrations the demographic features of the study and control-groups. There was not significantly different in mean age for control and hypertension group (P > 0.05); also, no difference in mean of age between obese and obese /hypertensive groups (*P* > 0.05), but the maen age of obese and obese/ patients with hypertension was significantly- higher than of both control and patient with hypertension groups (P < 0.05). Regarding mean BMI of control and hypertensive patients (P > 0.05), no significant difference, "also no significant difference in mean BMI between obese and obese /hypertensive groups (P > 0.05), but the mean BMI of obese and hypertensive/ obese patients was significantly higher (P < 0.05) than the mean BMI of both control-and hypertensive groups. No difference in mean body-fat % among control and patient with hypertension (*P* > 0.05); also there was no significant difference in mean body fat % among obese group and obese/ hypertensive group as *P*-value> 0.05. The mean body/ fat percent of obese/hypertensive and obese patients was significantly higher (*P* < 0.05) than the mean body fat percent of both control group and hypertensive groups. There was no significant difference in mean waist circumference between control and hypertensive groups (*P* > 0.05); also there was no significantly different in mean waist circumference among obese and obese// hypertensive groups (*P*-value > 0.05). the mean Waist Circumference of obese and hypertensive/obese patients was statistically, significantly high [P-<0.05] than mean Waist-Circumference of both control and hypertensive groups".

There was no important different in mean baseline SBP between obese patients and control-group (P > 0.05); "also significantly no difference in the mean SBP between hypertensive patients and obese/ hypertensive-patients (P- value > 0.05); but the mean SBP of hypertensive and hypertensive /obese patients was higher than that of both control and obese groups (P < 0.05). No significant-difference in mean baseline DBP between obese patients and control group (*P* > 0.05); also no significant difference in mean DBP between the hypertensive patients and obese/hypertensive patients (P > 0.05); but a the mean diastolic blood pressure of hypertensive and hypertensive obese patients was significantly higher than that of both control and obese groups (*P* < 0.05). no significant difference in the mean baseline heart rate between obese patients and the control group (*P* > 0.05); also the mean heart rate of not significantly different in hypertensive patients as of that of obese/hypertensive patients (*P* > 0.05); but the mean HR was significantly higher in hypertensive and hypertensive obese patients ssthan that of both control and obese groups (P < 0.05), as shown in table-1".

At the period of extreme exercise, the evaluation of mean SBP, DBP and the HR between study and control groups is shown in table-2 . a significantly higher mean-systolic blood pressure was in hypertensive obese patients followed by obese /hypertensive patients and that of control patients at last. A significantly higher mean DBP was in hypertensive obese patients then obese and hypertensive patients and at the last the control subjects. A significantly lower mean heart rate in all groups of the study in contrast with that of control group (P-value <0.05); though, no significant difference was in mean HR among study-groups (P > 0.05), as shown in table-2.

15 minutes after rest , the comparison of the mean SBP, DBP and the heart rate between control and the groups of study shown in table 3 . a significantly higher "mean systolic blood pressure was in hypertensive / obese patients then followed by obese and hypertensive patients and control subjects at last , also significantly higher mean DBP in hypertensive obese patients then followed by both obese and hypertensive patients and by control subjects at the last . significantly lowest mean heart rate among all study groups in contrast with that of control group (P <0.05); though, no significant difference in mean HR between study groups (P more than 0.05), as shown in table 3".

Table 1: Demo-graphic features of the study groups

Featur	**Control (*n*= 70)**	**Hypertensive (*n*=50)**	**Obese (*n*=54)**	**Hypertensive & Obese (*n*=42)**
Age (years)	21.01± 3.25 B	23.21 ± 3.02 B	30.21 ± 4.73 A	29 .29± 4.11 A
BMI (kg/m2)	23.02 ± 1.63 B	24.41 ± 2.24 B	34.29 ± 3.40 A	34.41 ± 3.21 A
Body fat percentage (%)	18.8 ± 3.9 B	21.9 ± 3.9 B	33.8 ± 7.2 A	36.8 ± 4.9 A
Waist circumference (cm)	85.21 ± 5.73 B	85.80 ± 7.2 B	112.21 ± 9.13 A	111.40 ± 8.04 A
SBP baseline (mm Hg)	123.81 ± 14.81 B	142.69 ± 10.81 A	128.46 ± 9.19 B	145.81 ± 11.94 A
DBP baseline (mm Hg)	73.51 ± 7.99 B	82.25 ± 11.12 A	77.93 ± 12.86 B	85 ± 10.2 A
Heart rate baseline (Beat/min)	70.80 ± 15.05 B	76.06 ± 11.01 A	70.87 ± 11.87 B	78.84 ± 11.89 A

N;number of cases; "BMI: body mass index; SBP; systolic blood pressure; DBP; diastolic blood pressure; capital letters were used for explanation of the results of (post hoc LSD) multiple comparison test consequently similar letters, direct no significant-difference at $P > 0.05$, while different letters designate significant difference at $P \leq 0.05$; letter (A) being the highest value".

Table-2: Heart rate and blood pressure during extreme exercise

Featur	**Control (n =70)**	**Hypertensive (n =50)**	**Obese (n =54)**	**Hypertensive & Obese (n =42)**
SBP (mm Hg)	165.84 ± 25.87 C	175.89± 22.96 B	173.92 ± 23.94 B	181.87± 21.98 A
DBP (mm Hg)	76.42± 10.26 C	84.21± 15.12 A	80.98 ±16.11 B	88.10 ± 10.07 A
Heart rate (beat/min)	161.92 ± 20.21 A	155.86 ± 18.65 B	154.02± 20.08 B	154.04 ± 11.94 B

n: *number of cases*; "SBP: *systolic blood pressure*; DBP: *diastolic blood pressure*; Capital letters were used to explain the results of (post hoc LSD), multiple comparison test so that similar letters indicate no significant difference at $P > 0.05$, whereas different letters indicate significant difference at $P \leq 0.05$; letter (A) being the highest value"

Table-3: measurements of heart rate and blood pressure 15 minutes after rest

Feature	Control (n= 35)	Hypertensive (n=25)	Obese (n=27)	Hypertensive & Obese (n=21)
SBP (mm Hg)	165.99 ± 25.88 C	175.94± 22.89 B	173.89 ± 24.18 B	182.25± 22.01 A
DBP (mm Hg)	77.09 ± 10.08 C	85.21± 13.99 A	81.26 ±16.20 B	87.83 ± 10.06 A
Heart rate (beat/min)	162.10 ± 20.24 A	156.20± 18.07 B	153.78 ± 20.19 B	154.03 ± 12.30s B

n: *number of cases*; "SBP: systolic-blood/pressure; DBP: *diastolic-blood pressure*; Capital-letters were used to explain the results of (post hoc LSD multiple comparison-test) so that similar letters indicate no significant difference at $P > 0.05$, whereas different letters indicate/significant difference at $P \leq 0.05$; letter (A) being the highest value"

Discussion

The mean standard SBP of patient with hypertension and hypertensive/ obese patients in the present study was statistically significantly higher than that of control group and within the hypertensive range of more than 140 mm Hg, which indicate that the control of blood pressure of those patients was poor in spite of they have being managed for many years for hypertension . Mean DBP of the patient with hypertension and hypertensive obese patients on the other hand was also highly significant than that of control-group ,but the mean DBP was in normal usual range less than 90 mm Hg. Also baseline-mean HR was of all groups was in normal usual limit (60 to100 beat/minute) in spite of some significant difference between groups.

Under-controlled BP in patients with hypertension joined in this study can be explained by insufficient compliance with drug treatment, in-adequate dietary salt intake or, to a lesser scope, due to compassionate flooding in this medical situation. In fact strict blood-pressure is compulsory for those patients so to avert life/threatening cardio-vascular and cerebro-vascular problems.

In extreme exercise, systolic and DBP mean from hypertension patients have got the highest levels compared to the control and obese patients groups; the later also experienced more than usual mean diastolic and SBP but the level was lower than high blood pressure patients. In terms of HR, it was lower in patients with hypertension and obese patients compared to the control groups, where the lowest level in hypertension group. Though, after 15 min., it was noted that high blood pressure and obese patients unable to return to baseline readings. This was also the case for the control group; yet the BP readings were higher in patient with hypertension /obesity than in control group while meaning of HR was lower in patients than in control group.

Systolic and DBP shown to reduced following regular exercise in patients with high BP [25] and is usually suggested by inter-national guidelines [26, 27]. However, guide-lines do not reveal anything about exercise counsels for patients with resistant hypertension and high BP/ obese patients. Continued physical action is associated with lower risk of mortality and cardio-vascular problems in high BP patients who are shown to be impervious to the usual guidelines of the management [28]. Though still no proof for the benefits of frequent physical activity for resistant hypertensive

patients, a number of studies have been conducted that have yielded promising results. A 8- 12 weeks program with an aerobic exercise [29], which contained of routine walking established with an interval training pattern, three times per week, will reduce 24hrs SBP by about (5.4±12.2) mmHg and 24-hour DBP by about "(2.8±5.9) mmHg. Furthermore, reduction of systolic and diastolic blood pressure at daytime by (5.9±11.6) mmHg and {3.3±6.5} mmHg and reduction of systolic and DBP at nighttime by 3.8±17.1 mmHg and 1.9±8.2 mmHg, respectively (29). This reduction in BP was also seen in succeeding researches with training exercise based on heated water [30-32]".

Even with these suggestions regarding the advantage of do exercises, we saw in the present study, temporary improvement of peaks in BP which may be convoluted by life threatening conditions like dissection of aorta and thrombo-embolism. so, we are recommended that exercise in obese and patients with high blood pressure should started regularly with continuous ambulatory monitoring in order that unnecessary rapid elevation of BP levels is prevented.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Detection Trisomy Chromosome 12 in Relation to the Expression Of Cd38 and Light Chain Restriction in Chronic Lymphocytic Leukemia Patients

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Abstract

Background: Chronic lymphocytic leukemia is a malignancy of mature B cells where there is progressive accumulation of leukemic cells. The genetic factors have been found to play a role, deletions of the long arm of chromosome 13, specifically involving band 13q14 (del(13q14)), represent the single most frequently observed cytogenetic aberration in CLL, followed by trisomy 12 ,del 11q22, del 6q21, del 17p13, and 14q.

Objectives: To detect the structural abnormalities on chromosome 12 (trisomy 12 in patients with CLL by using FISH technique; To investigate the relation of this chromosomal abnormalities with with the expression of CD38 and light chain restriction .

Patients, Materials and Methods: 1. This cross-sectional study was conducted from February 25, 2018 to December 15, 2019. Fifty newly diagnosed adult patients with CLL were enrolled in this study. 2. Each patient was assessed at the time of admission, full clinical and laboratory data were obtained from patient's hospital records. Three ml of fresh blood sample were taken from each patient at admission. Upon confirmation of CLL diagnosis, samples were taken to FISH unit for blood preparation which includes two steps in two days, the first day to separate lymphocyte cells from another component of blood and second-day washing samples and stored till slide preparation. Slide preparation done in the 1st day and using dual color gene probe and slide reading in the second day by fluorescent microscope. The collected data were computerized and statistically analyzed using the SPSS program (Statistical Package for Social Science) version 23.

Result: The median age of the patients was 64 years and the range was 45-76 years. Among 50 patients studied, 18 patients were females, representing (36%) and 32 patients were males representing (64%). Male: female ratio 1.8:1. Trisomy chromosome12 was found 15 out of 50 patients with CLL, representing 30%. Conclusion:1.In the current study,15 out of 50 CLL patients (30%)present with trisomy chromosome 12 in a sample of Iraqi adult CLL patients.2. trisomy CH 12 patients show significant relation with light chain restriction .

Keywords: *leukemia patients ; light chain restriction; trisomy chromosome 12 ; toxicity; health behavior*

Introduction

Chronic lymphocytic leukemia is a malignancy of mature B cells characterized by blood and marrow lymphocytosis.[1]

In Iraq, Leukemia was the third most common cancer accounting for (6.48 %) of all cancers; chronic lymphoid leukemia represents the 7th most common type of leukemia and constitutes (4.91%) in males, (4.29%) in females of all leukemia according to Iraqi cancer registry 2015.[2]

In Iraq with a male to female ratio of nearly 1.4:1[2]. Observational studies have consistently reported a reduced incidence and superior survival for female

patients with CLL. [3]

In Iraq median age 60 years as found in new study in Karbala province that show CLL was more prevalent among those aged more than 50 years old. [4]

In the peripheral blood, the morphological of leukemic cells look like mature lymphocytes [5].

Based on immunophenotypic characteristics and giving one point to each one of the following: CD5+, CD23+, FMC7 weak, SmIg (κ/λ staining) weak and CD79b weak. Matutes and Catovsky showed that in patients with a score of 4–5, the diagnosis is virtually always CLL, while in those cases with a score <3, the diagnosis of CLL is extremely unlikely.[6]

Patients and Methods

Selection of patients

A total of 50 (32 males and 18 females) patients with Chronic lymphocytic leukemia who were attending the Hematology outpatient clinic at the Medical City.

were included in this study during the period from February 25, 2018 until December 15, 2019.

Data collection

Full personal and demographic information was obtained, including date of birth, sex, residence, date at diagnosis, and duration of illness. Complete clinical data were obtained regarding the presenting complaint(s), including pallor, abdominal distention, any history of recurrent infections, chronic diseases. Immunophenotyping result was obtained from flow cytometry department.

Modified Rai staging-system were applied for staging the patients with CLL.

All patients were thoroughly examined for pallor, lymphadenopathy, hepatomegaly and/or splenomegaly.

Laboratory methods

For each patient, 3 mL of ethylenediaminetetraacetic acid blood samples was aspirated under strict aseptic techniques. The laboratory workup included the following:

1. Complete blood count: It was done using an automated (Cell-DYN, RUBY Abbott Diagnostic, USA) at Teaching Laboratories of Medical City.

2.Fluorescent in situ hybridization technique Compromise:blood preparation ,slide preparation and slide reading.

Statistical Analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences version 23 and Microsoft Office Excel 2010. The level of significance was considered at P-value of equal or less than 0.05. The level of high significance was considered at P-value of equal or less than 0.01.

Results

This descriptive, cross-sectional study included 50 patients with chronic lymphocytic leukemia (32 males and 18 females). Their ages ranged from 45 -76 years, with a mean age of 62.02± 8.66 years .

The hematological characteristics of the studied patients are showen in Table 1

Table (1): Hematological characteristics of CLL patients

Characteristic	Result
Hb g/dL	
Mean ±SD	10.97 ±2.45
Median (IQR)	10.8 (3.6)
Range	4.4 -16.3
Platelet count 103/µl	

Cont... **Table (1): Hematological characteristics of CLL patients**

Mean ±SD	155.65 ±76.55
Median (IQR)	129 (93.25)
Range	25.3 -324
WBC count 103/µl	
Mean ±SD	34.59 ±36.36
Median (IQR)	24 (11.8)
Range	3 -196
Absolute Lymphocyte count	
Mean ±SD	19.02 ±31.60
Median (IQR)	7.25 (10.88)
Range	5.5 -161
Atypical lymphocytes / 100 Lymphocytes	
Mean ±SD	16.04 ±5.65
Median (IQR)	14 (10.25)
Range	8 -27
Prolymphocyte %	
Mean ±SD	1.64 ±1.47
Median (IQR)	1 (1)
Range	1 -8
Mixed cell type, n (%)	9 (18 %)

n: number of cases; SD: standard deviation; IQR: inter-quartile range

Out of the 50 studied patients, Chromosome 12 trisomy was seen in 15 (30%) of patients and the percent of trisomy 12 cells has ranged from 15 -17 % with a mean of 16.21 ±0.58 %. (figures 1 and 2)

According to Kavita S. Reddy..*etal* study the cut-off values were established at 3% for trisomy 12 .[7]

Regarding the clinical staging of CLL patients revealed that chromosome 12 trisomy is commonly observed in more progressive stages. That we observed two patients in Intermediate risk stage and thirteen patients were in high risk stage.

The highest percent of cases with positive CD38 were in patient carrying trisomy 12 as seen in table 2

In the current study the association of Chromosomal abnormalities to prognostic flow cytometry markers CD38 and light chain restriction) was shown in tables 2 and 3

Noticing that trisomy CH 12 patients show significant relation with light chain restriction in which ≈ 93% of trisomy 12 patients lack light chain expression and ≈ 7% of trisomy 12 patients show lambda expression and no relation with kappa expression .

Table (2): Association of Chromosome 12 trisomy deletion to prognostic flow cytometry marker CD38

CD38	Ch12			
	No trisomy		Trisomy	
	n	%	n	%
Negative	19	82.6	4	17.4
Heterogenous	2	66.7	1	33.3
bright	0	0	0	0
moderate - bright	0	0	0	0
moderate	0	0.0	1	100.0
Dim - moderate	2	100.0	0	0.0
dim	12	57.1	9	42.9

Table (3): Association between chromosome 12 trisomy and light chain restriction in patients with CLL

Light chain restriction	Total	Ch12			
		No trisomy		Trisomy	
		n	%	n	%
Negative	35	21	60.0	14	40.0
Kappa	11	11	100.0	0	0.0
Lambda	4	3	75.0	1	25.0

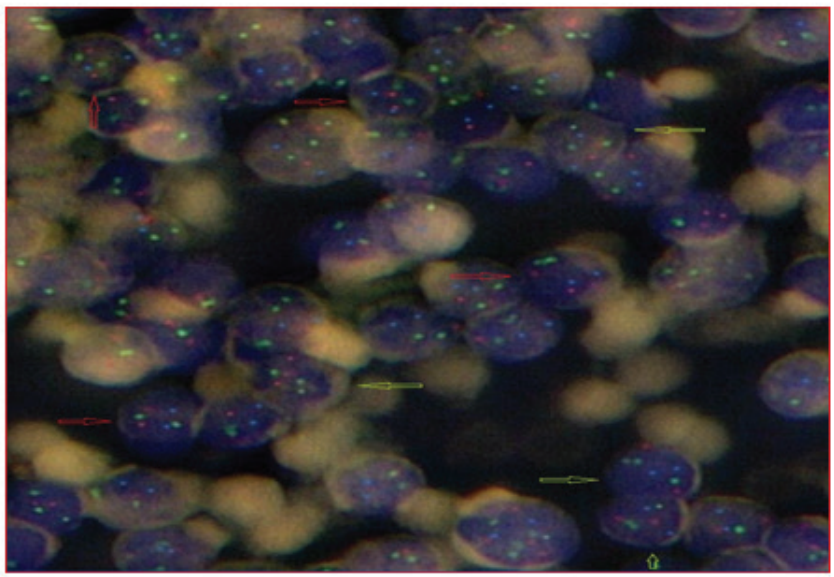
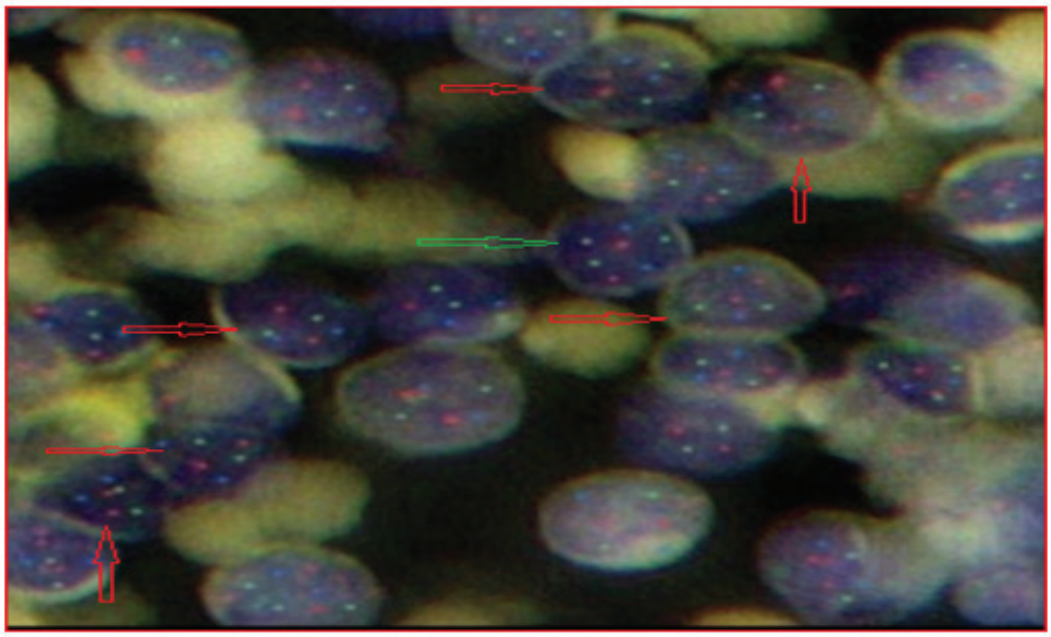


Figure (1) FISH analysis using XL DLEU/LAMP/12cen LSI probe show leukemic cells with trisomy 12,yellow arrows 3 green signals for chromosome 12 and two orange signal, two blue signal for chromosome 13 on power 60 x. normal FISH pattern with red arrows for chromosome 12 .



Figure(2) FISH analysis using XL DLEU/LAMP/12cen LSI probe show leukemic cells with trisomy 12, 3 green bright fluorescence signal spots for chromosome 12 reflecting the presence of trisomy 12 and two orange signals, two blue signals for chromosome 13 (green arrows) .normal FISH pattern with 2 green signals,two orange signals,two blue signals(red arrows) . on power 60x.

Discussion

Chromosomal abnormalities in chronic lymphocytic leukemia are detected in up to 80% of patients. Among them, deletions of 11q, 13q, 17p, and trisomy 12 have a known prognostic value and play an important role in CLL pathogenesis and evolution, determining patients outcome and therapeutic strategies. Standard methods used to identify these genomic aberrations include fluorescence in situ hybridization (FISH) ; which have been implemented as the gold standard.[8]

In this study, the median age of the patients was 64 years and the range was 45-76 years. It is well known that this disorder is a disease of elderly. This age was close to the results obtained by other Iraqi studies in 2013, 2014 and 2016,2019 [9,10,11] and a study in Pakistan in 2015.[12,13] Whereas the median age at presentation, in western countries was higher , it was reported to be 70 years. [13,14] This difference can be attributed to the difference in population structure, environmental variations, genetic predisposition between Iraq and western countries, and difference in life expectancy.

Moreover, in the current study, only 12% were younger than 50 years of age; this is in accordance with the National Cancer Institute's Surveillance and Epidemiology End Results (SEER) database, which revealed that 11% of all patients diagnosed with CLL in 2009 were less than 55 years of age [15].

CLL cases were observed more in males (64 %of cases) than in females with M: F ratio of 1.8:1 which is close to other Iraqi and non Iraqi published articles ,10,16 and it comparable to that of western countries and other world studies. [17-20].

Regarding Hemoglobin level ; The median Hb level was 10.8 g/dl with IQR of 3.6 and the range was from 4.4 -16.3g/dl which is close to many Iraqi and non Iraqi studies [21,22,23,24]. In general, anemia may reflect progression of bone marrow failure . In the present study 56% of patients had Hb less than 11 g/dl whereas 44% had Higher Hb ; and this goes with the high frequency of patients in the high risk group .

In the current study,15 out of 50 CLL patients (30%) present with chromosomal aberrations and this differ from Haferlach *etal*25 who found 85.2% of CLL cases

had genomic abnormalities and this may result from limited genetic probe used in the current study .

The current study found the percent of trisomy 12 in the cells showed a significant negative correlation with Hb concentration. this was similarly observed by Maria Dolores study. [26]This presumably due to that trisomy clone had proliferation advantage with respect to non trisomy cells, thus those leukemic cells will grow rapidly in the bone marrow and result in impaired hematopoiesis. This impairment had resulted in low Hb concentration, low platelet count, high WBC count and absolute lymphocyte count in trisomy 12 cases compared to those with non trisomy. This clarify the adverse effect of trisomy 12 in CLL patients.

Lastly the current study showed no relation between percent of trisomy cells and CD38 or light chain restriction expression.

The lack of surface light chain in CLL is rare fact but in this study it was observe in 35 out of 50 CLL patients. In contrast, 32 of 396 cases (8.1%) of chronic lymphocytic leukemia were reported by Matutes *et al* to have no surface light chain [115] This variability may be related to different factors including patient selection, inclusion criteria, and geographical differences.

Conclusion

From this study, it can be concluded that

1.In the current study,15 out of 50 CLL patients (30%)present with trisomy chromosome 12 in a sample of Iraqi adult CLL patients.

2. The mean of the percent of cytogenetic abnormal cells among overall leukemic cells were correlate adversely with hematological parameters which was obvious with trisomy 12 .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Evaluating the anti- *Pseudomonas Aeroginosa* Efficacy and Potential Cytotoxicity of *Nerium Oleander* Alcoholic Extract

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Abstract

Nerium oleander known as oleander has belonged to the poisonous plants its habitat in a tropical and subtropical region. The chemical analysis with GC-Mass of the alcoholic extract of oleander leaves revealed that this plant has many chemical compounds more than 80 compounds and high-peaks about 29 compounds which are represented by alkaloids, phenol, terpenes, and fatty acid. HPLC analysis showed many essential oils that have many biological effects.

To evaluate the antibacterial activity of the alcoholic extract of *N. oleander* against locally isolated *Pseudomonas aeroginosa* the broth micro-dilution method was adapted to different concentrations were started from 3.9 to1000 mg/ml. The results revealed that the alcoholic extract has antibacterial activity depending on the concentration and bacterial strain.

When treating the onion root cells as a model of the study to find the cellular toxic effect the results showed that treatment with an alcoholic extract of oleander leaves with concentrations (control, 150, 300, 600, and 1200 mg /ml) for four hrs., the mitotic index (MI) was decreased to less than 50%, when treated with high concentrations 600,1200 mg /ml and many cases of chromosomal aberration were shown, such as the abnormal shape of the cells with a percentage about 1 and 2.8 % at the concentrations 600 and 1200 mg / ml respectively, as well as the multinucleated cells recorded the highest percentage at the concentrations 1200 mg /ml with 14.4%, and nuclear lesion (chromatin degradation) were seen in a large percentage at the interphase were recorded 13.66, 25.42, and 32.3% at the concentrations 300,600, and1200 mg /ml respectively.

Keywords- *antibacterial activity, mitotic index, chromosomal aberration, oleander.*

Introduction

The overuse of antibiotics has become an essential factor for increasing the emergence of multi-drug resistance (MDR) bacterial isolates, which become a hazard to public health. Besides, the accessible antimicrobial agents, occasionally, do not affect on these bacteria [(1).] There are many approaches to remedy and control the infection caused by MDR strains of bacteria, with emphasis on the use of active phytochemical compounds from the herbal plant which serve as a defense mechanism against these bacteria [(2,3)] The burn wound is a susceptible site of opportunistic colonization by organisms of endogenous and exogenous origin such as *P. aeruginosa*. The traditional association of *P. aeruginosa* with burn infections is very strong; burn infections and associated sepsis are the cause of the high rate of fatalities in seriously burned patients. [(4)] However, *P. aeruginosa* outbreaks in burn units are still associated with high death rates around 60% [(5)] According to World Health Organization assessments that 80% of the people in developing countries depend on traditional therapies and use extracts of the plant with an extensive variety of secondary compounds as the main medicinal source to treat numerous infectious diseases, especially these compounds have lower side effects compared to antibiotics [(6,7).] One of these plants *N. oleander* known as oleander (Apocynaceae), one such herb, has been introduced to Iraq as an ornamental plant growing originally in the Mediterranean region, subtropical Asia , and distributed in many parts of the world [(8).] *Nerium* is an important medicinal plant

in folk medicine[9]. This plant has pharmacological applications due to produces of secondary metabolites such as alkaloids, flavonoids, and steroids [10]. Also, have potentially toxic effects that belong to all parts of oleander are toxic containing oleandrin and other cardiac glycosides [11]. *N. oleander* extracts have significant antibacterial effects [12], cytotoxic effect. [13], cardiotonic and diuretic in edema[14,15]

Materials and Methods

Microorganisms:

Twenty isolates of *P. aeroginosa* were isolated from burn swabs, obtained from Microbiology laboratory at Department of Biology, College of Science, University of Baghdad These isolates were previously identified by subculturing on to 5% blood agar, McConkey and ceramide agar, and the important biochemical tests such as gram stain, oxidase test, catalase test, pigment production and growth at 42ºC.[16] The PCR technique was adopted to confirm the identification via amplification of a fragment of *16SrRNA*gen*e* as described by [17]. These isolates were stored in glycerol vials at deep freeze by lab staff.

Bacterial suspension preparation

Few separate pure colonies, grown on nutrient agar, were taken to a sterile normal saline tube with turbidity adjusted to approximately 1.5×10^8 CFU/ml in comparison to McFarland turbidity standard (tube no. 0.5).

Antibiotics susceptibility test

P. aeroginosa isolates were tested against the following antibiotics: ceftazidime (30µg/disk), Imipenem (10µg/disk), Gentamicin (10µg/disk), Amikacin (30µg/disk), and Ciprofloxacin (5 µg/disk) to detect the multidrug-resistant isolates, this test was done by disk diffusion method [18]

Antibacterial assay

Micro-dilution method

To determine the minimal inhibitory concentration (MIC) of alcoholic extract of *N. oleander* against. *P. aeroginosa* isolates, the broth microdilution protocol mentioned by [19,20]. was followed, the double serial dilutions of alcoholic extract were started from 3.9 to1000 mg/ml which prepared from a stock solution (5000 mg/ ml) using Mueller Hinton broth as a diluent. Further, one hundred microliters of each dilution were distributed in 96 well cell culture plates. Control wells contained bacteria-free growth media plus DMSO (sterility control). All wells were inoculated with 10 µl of a bacterial suspension. All trials were repeated in triplicate. Subsequently, the microtiter plates were incubated, aerobically, at 37ºC for 24 hrs.

Plant collection

This work was carried out in the Laboratory of Biology department, College of Science**,** University of Baghdad during the period 1/12/2019 - 1/5/2020. The leaves of the *N. oleander* were cut into small pieces and then placed in the oven for drying at 40-50°C for 5 days, and then placed in the mill until it became a powder**.** [21,22,23].

Phytochemical analysis

1-Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis was carried out on a GC – mass 5977A Series Agilent system autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions [24,25,26].

Results and Discussion

Susceptibility of bacterial to antibiotics

In this study 20 isolates of *P. aeruginosa* were submitted to antibiotic susceptibility test by disk diffusion test, this technique was done by using five different antibiotics: ceftazidime, Imipenem, Gentamicin, Amikacin, and Ciprofloxacin to detected the multidrug-resistant isolates (MDR), the results revealed 12 (60%) isolates were resistant to all these antibiotics and this reflected as MDR depending on breakpoints of CLSI[27]. (Table 1).

Tabe1. Susceptibility of bacteria to antibiotics Resistance profile of *P. aeruginosa* against five antibiotics S-Susceptible, I-Intermediate, R-Resistant.

Isolates code	Ceftazidime 30µg/disk	Imipenem 10µg/disk	Gentamicin 10 µg/disk	Amikacin 30µg/disk	Ciprofloxacin 5 µg/disk
Ps1	R	R	R	R	R
Ps2	S	S	S	S	R
Ps3	S	S	S	S	I
Ps4	S	S	S	S	S
Ps5	S	S	S	S	S
Ps6	R	R	R	R	R
Ps7	R	R	I	R	R
Ps8	S	S	S	S	S
Ps9	S	R	R	S	R
Ps10	S	S	S	S	S
Ps11	R	R	R	R	R
Ps12	R	R	R	R	R
Ps13	R	R	R	R	R
Ps14	R	R	R	R	R
Ps15	R	R	R	R	R
Ps16	R	R	R	R	R
Ps17	R	R	R	R	R
Ps18	R	R	R	R	R
Ps19	R	R	R	R	R
Ps20	R	R	R	R	R

Antibacterial activity of *N. oleander* extract against *P. aeruginosa*

Our results revealed that the extract of *N. oleander* has antibacterial activity at different concentrations. However, non-MDR *P. aeruginosa* isolates (ps2, ps3, ps4, ps5, ps7, ps8, ps9, ps10) were sensitive to (62.5 mg/ml) .whilst, all MDR *P. aeruginosa* except four isolates were inhibited at MIC 500 mg/ml, and the MIC value for the remaining four isolates (ps1, ps6, ps15, ps18) was 250 mg/ml. According to GC-MS and HPLC analysis of *N. oleander* ethanol extracts shown that the existence of secondary metabolites such as alkaloids, cardiac glycosides, phenolic compounds, flavonoids, tannins, essential oil, terpenes, and saponins. These compounds are considered as the main groups of antimicrobial compounds in plants [28,29]. The alcoholic extract of *N. oleander* leaves showed the highest antimicrobial action against *P. aeruginosa* at 900 mg/ml while the lowest level of the extract was 500 mg/ml [30]. Also according to [31]. results showed the aqueous and ethanol extracts

of *N. oleander* have antimicrobial activity against all bacteria isolated from burns infection, The minimum inhibitory concentration of the aqueous extracts ranged between 25- 100 mg/ml, while the ethanol extract ranged between 25 -50 mg/ml on the test organisms. The results reported by [32]. found that ethanol extract of *N. oleander* had a deterrent effect in all concentrations on *Salmonella Typhi*. The MIC of the ethanol extract was 128 mg/ml and the minimum bactericidal concentration (MBC) was 256 mg/ml. but aquatic and alcoholic extracts were not effective on the standard strain *Listeria monocytogenes*.

Phytochemical detection

1- GC-MASS analysis

The GC-MS chromatogram of the 83 peaks of the compounds was detected. Chromatogram GC-MS analysis of the alcoholic extract of *N. oleander* showed the presence of 29 major peaks and the components corresponding to the peaks were determined as Dodecanoic acid lauric acid, eicosene, alkaloid, E-14-Hexadecenal, Heptadecene, Octadecene 2, Tetradecanoic acid, Olic acid, Bicyclic 3.1.1 heptane, Cycloheptan,1,2-dimethyl- acetate, Trichloromethan Eugenol, Phenol, glyceride, ctadecyne, phytol, acetate, Hexadecanoic acid, methyl ester, methyl ester, palmitic acid, 9-Octadecenoic acid (9Z)-, Stearic acid, methyl ester, and Methyl stearate. These results are in along with [33] they found that chemical compositions of the leave ethanol extract of *N. oleander* were investigated using gas chromatography-mass spectroscopy (GC-MS).

2- HPLC analysis revealed that leaves extract of *N. oleander* has different types of essential oils such as α-picene, caryophyllene, chamazulene, and p-cymene (Table 2).

Table 2. HPLC analysis of leaves extract of N. oleander

	Compounds	Ret. Time of Test (min)	Area of Test	Ret. Time of standard (min)	Area of Standard	conc. Of test
1	α-picene	3.615	7294017.5	3.232	1639319.625	22.24708772
2	caryophylene	4.473	9149397	4.273	5078418.5	9.008116405
3	chamazulene	4.998	4903959	4.932	2144605	11.43324528
4	p-cymene	6.265	22130402	6.498	12517030	8.840117025

Cytotoxic effects of oleander alcoholic extracts on the cell division of onion root tips *Allium cepa in vivo:*

Mitotic index (MI)

Mitotic index reduced significantly at all oleander leaves extract concentrations, recording 7, 6, 4.4, 2.8, and 2.7% for control, 150, 300,600,1200 mg/ml) respectively Figures 3. The mitotic index decreased less than 50% in (600 and 1200 mg/ml) recoding (38.35, 36.98%) respectively also as shown in figure 1 and table 2. The decrease in a mitotic index is due to the extracts have some substances that cause a reduction in the mitotic index to 50% or less have toxic or semi lethal effects such as Phenol, octadecadienoic acid, Oleic acid glyceride, ctadecyne, phytol, acetate, Hexadecanoic acid, methyl ester, Palmitic acid, methyl ester, n-hexadecanoic acid that interaction with DNA topoisomerase-I.[34]

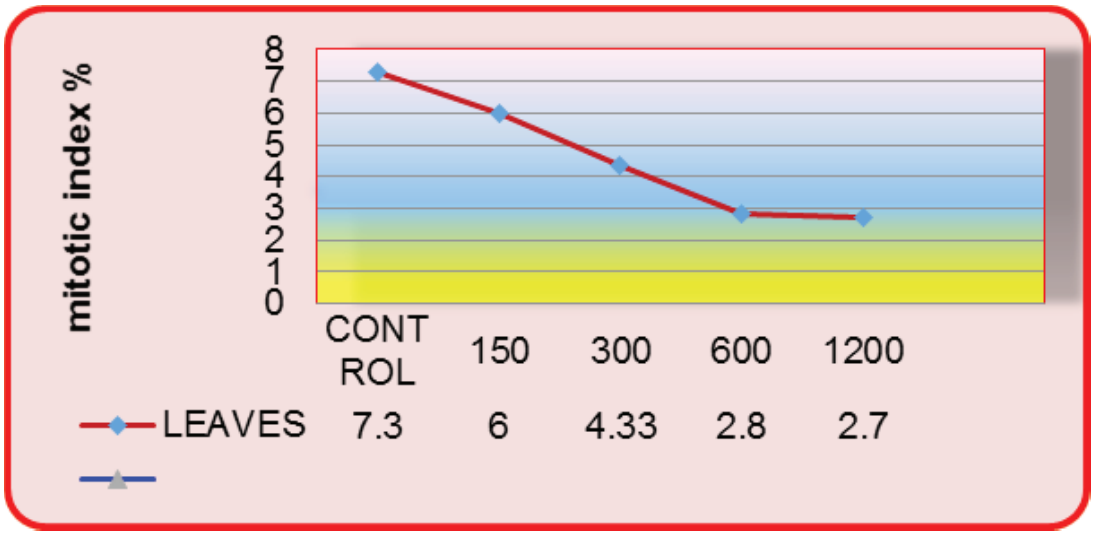

Figure 1. Mitotic index%

Prophase

Prophase index recording (40.62, 35.49, 42.3, 49.01 and 44.64%) in (control, 150, 300, 600,1200 mg/ml) respectively as shown in table 1and table 2. Some abnormal cells attributed in this phase such as abnormal cell shape as shown in Figure 2.

Metaphase

Metaphase index recording 25.16, 30.84, 31.93, 24.85, 18.29% in control, 150, 300, 600, 1200 mg/ml respectively, (table 3).

Anaphase and Telophase

Anaphase was decreased significantly recording 82.68, 75.12, 85.07, and 47.96% in 150, 300, 600, 1200 mg/ml respectively (table 3and 4).

Using of onion test to evaluate the cytotoxic effects of the alcoholic extract of oleander leaves under study revealed the presence of several cytological aberrations in cells were multinucleated cells, abnormal cell shape, and nuclear lesion (chromatin lesion) compared with control as shown in figures 3-A, B, C, D. This test considered as the important test *in vivo* where the roots grow in direct contact with the substance of interest enabling possible damage to DNA of humans to be predicted. The results showed that higher concentrations were able to inhibit cell division significantly as shown in Figure 2, indicating the toxicity of the tested concentration in *Allium* root tip cells[35]. Single and multiple lesions were the most frequent aberrations in prophase and interphase cells. Lesions are the result of inhibition in the S phase of the cell cycle where duplication of DNA strands happens. Formation of prophase lesions are common in irradiated cells, but the site of lesions or its repair mechanisms are not yet explored in detail [36].

Table 3.Mitotic index, phases, and percentage of abnormality in *A. cepa* roots at different concentrations of alcoholic extract of oleander leave after four hours.

Concentration mg/ml	MI%	Phase index %				Chromosomal aberration %		
		Prophase	Metaphase	Anaphase	Telophase	Nuclear-lesion	Multi-nucleate	Abnormal cell shape
Control	7.3	40.62	25.16	10.05	22.42	0.00	0.00	0.00
150	6	35.94	30.84	8.31	24.88	0.00	0.00	0.00
300	4.43	42.3	31.93	7.55	18.15	13.66	0.00	0.00
600	2.8	49.01	25.85	8.55	19.15	25.42	3	1
1200	2.7	44.64	18.29	4.82	32.24	30.30	14.4	2.8

Table 4. Mitotic division (%control) and the phases in the onion root tips were treated with different concentrations of alcoholic extract of oleander leaves.

Concentration mg\ml	MI%	Prophase%	Metaphase%	Anaphase%	Telophase%
150	82.19	88.47	122.57	82.68	110.97
300	60.68	104.13	126.90	75.12	80.95
600	38.35	120.65	102.74	85.07	85.41
1200	36.98	109.89	72.69	47.96	143.80

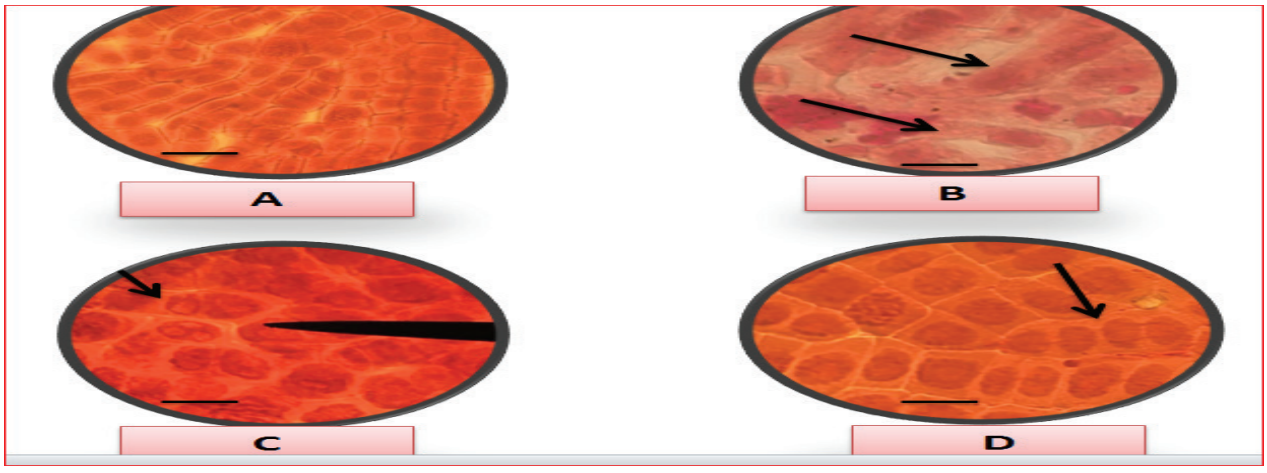

Figure 2. *A. cepa* root tips treated with alcoholic extract of olender leaves, A: normal cells in control, B: abnormal cells shape treated with high concentration, C: single and multi-nuclear lesion, and D: multinucleated cells. 1000x, this segment represents 5 micron

Conclusion

Through these results were obtained from this study, it was found that the alcoholic extract of oleander is very toxic at high concentrations, because it contains many chemical compounds such as alkaloids especially N-hexadecanoic acid that has cytotoxic potential.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Thyroid Pathological Consequences Induced by Caffeine in Female Rats

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Abstract

Objective: this study was designed to investigate the histopathological changes induced by caffeine on thyroid, liver and kidneys in female rats. Caffeine is a central nervous stimulant of methyl xanthine class of drugs, it has many hormonal and metabolic effects.

Method: twenty-four adult female rats was split in to four equal categories of 6 rats in each group as the following: Group1,control group received distilled water only by oral gavage. Group2, received 35mg\kg of caffeine solution. Group3, received 75mg\kg of caffeine and Group4, received 150mg\kg of caffeine. The experiment continued for 40 days. After 24 hours of the end of experiment, the animals were sacrificed and blood has been withdrawn from vena cava to prepare serum for hormonal analysis, whereas liver, kidneys and thyroid were excised and fixed in formalin solution for histopathological processing.

Results: caffeine ingestion cause significant reduction in thyroid function (reduction in T3, T4 and TSH) in all treated groups compared to control group in addition histopathological study show enlargement of thyroid follicle with thinning of lining epithelium of acini, vacuolation of both renal tubules, hepatocytes and vascular congestion of these organs.

Conclusion: Caffeine consumption in high doses causes many histologic and hormonal effects in the studied organs.

***Key words**: caffeine, thyroid hypo-function, histopathology, toxicity.*

Introduction

Caffeine is a chemical substance of methyl xanthine class of medications. Coffee is the main caffeine containing beverage used worldwide and is commonly associated with many health complications[1].Caffeine is absorbed rapidly from the gastrointestinal tract and reach to whole body tissues to give plasma concentration peaks at 30 to 60 minutes[2]. The mode of caffeine action is by blocking the hydrolysis of c-GMP, c-AMP and antagonism of adenosine[3,4], therefore, caffeine may alter hormone levels. It is reported that several hormonal secretion has been affected by high level of caffeine administration including thyroid hormones and pituitary gonadotrophins[5].With respect to kidney, caffeine has mild diuretic action by decreasing proximal tubules reabsorption[6].

The present study aims to evaluate thyroid gland, liver and kidneys in female rats by three doses caffeine.

Materials and Methods

The experiment was accomplished in the laboratory animals' house of College Pharmacy\ University of Basrah, by means of 24 white rats weighing 150-200 gram. Animals were kept in special cages for two weeks to accommodate laboratory conditions at room temperature between 20-25^{o}C. The nutrient for rats was commercial pellet. Rats were divided into 4 equal groups of 6 animals for each. All animals were given caffeine by oral gavage as follows: Group 1 (control group) were given distilled water. Group 2 were given 35mg\kg caffeine. Group 3 were given 75mg\kg caffeine and Group 4 were given 150mg\kg caffeine solution for 40 days, at the end of experiment the animals were

sacrificed to obtain the thyroid, liver and kidney to prepare histological slides and blood has been withdrawn from vena cava to collect serum for hormonal tests. The data were expressed as mean ± Standard deviation (SD). ANOVA analysis used in this study, using computerized SPSS v23, (P <0.05) considered significant result.

Results

Effect of caffeine on serum level of thyroid hormones T3, T4, TSH (M ± SD) (N = 6)

Hormones Groups	T3	T4	TSH
control	2.35 ± 0.31	55.8 ± 10	0.01 ±0.066
Caffeine 35mg	2.24 ± 0.28	66.4 ± 14.7	0.004 ±0.001
Caffeine 75mg	1.84 ± 0.199	62.4 ± 23.2	0.014 ±0.018
Caffeine 150mg	1.75 ± 0.197	58.9 ± 10.3	0.006± 0.003
LSD	0.199	10	0.001

The above table indicate that treatment with caffeine cause significant decrease in T3 (p= 0.014) and non-significant change in T4 (p = 0.7), TSH (p = 0.4) in all treated groups compared to control group.

Histopathological Results

Caffeine 35 mg\kg

Thyroid: there is flattening of the lining epithelium of most thyroid acini which are filled with light pinkish colloid indicating decrease thyroid activity, whereas other fields reveal destruction of many thyroid follicles.

Kidneys: show pale glomeruli due to low cellularity as well as mild epithelial destruction in the proximal tubules with vascular congestion.

Liver: shows dilation of both the liver sinusoids and central venules with congestion and early degenerative changes manifested by formation of large cyst within liver parenchyma.

Caffeine 75mg\kg

Thyroid: The follicles appear more dilated, larger follicles packed with light color colloid with no inflammation.

Kidneys: shrinkage in glomerular tuft, marked vascular congestion and increase in degenerative changes with vacuolation of some renal tubules.

Liver: showed increased sinusoidal dilation with more obvious degeneration and necrosis of many hepatocytes, congestion of blood vessels and formation of cyst between hepatocytes.

Caffeine 150mg\kg

Thyroid: showed marked fullness of acini by colloid with flattening of lining epithelium and congested blood vessels with no evidence of inflammatory reaction.

Kidneys: there is frank tubular necrosis with extravasation of blood among renal tubules and vascular congestion, other fields showed glomerular ischemia with vacuolation.

Liver: showed formation of larger cyst within parenchyma with excessive degeneration and necrosis of many hepatocytes with no effect on bile tubules and portal triad.

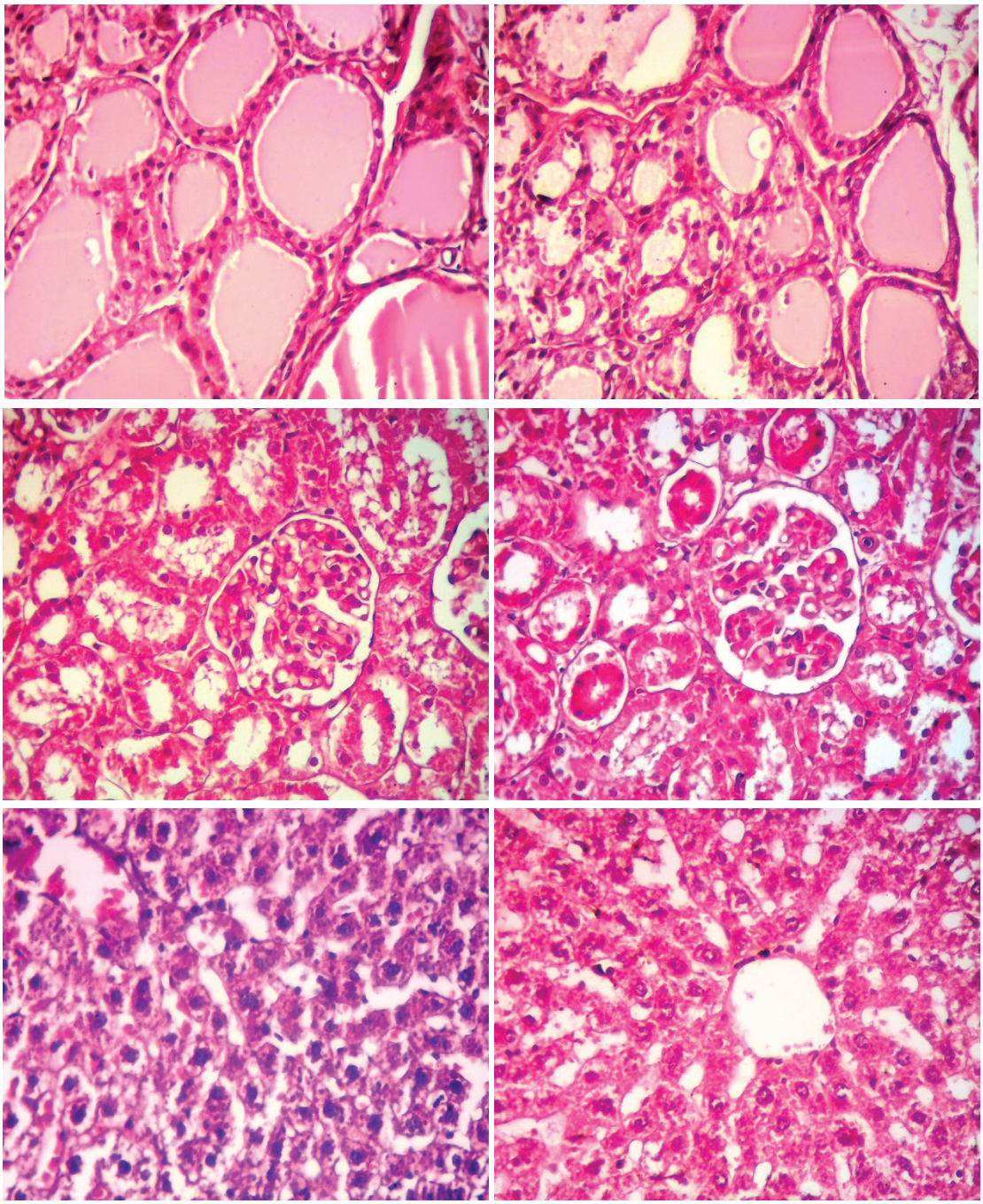

Figure1: show effect of 35mg\kg caffeine on thyroid, kidney and liver in which there is flattening of the epithelium of thyroid follicle, renal show vacuolation of glomeruli and renal tubules, the liver show sinusoidal dilation and vacuolation of hepatocytes.

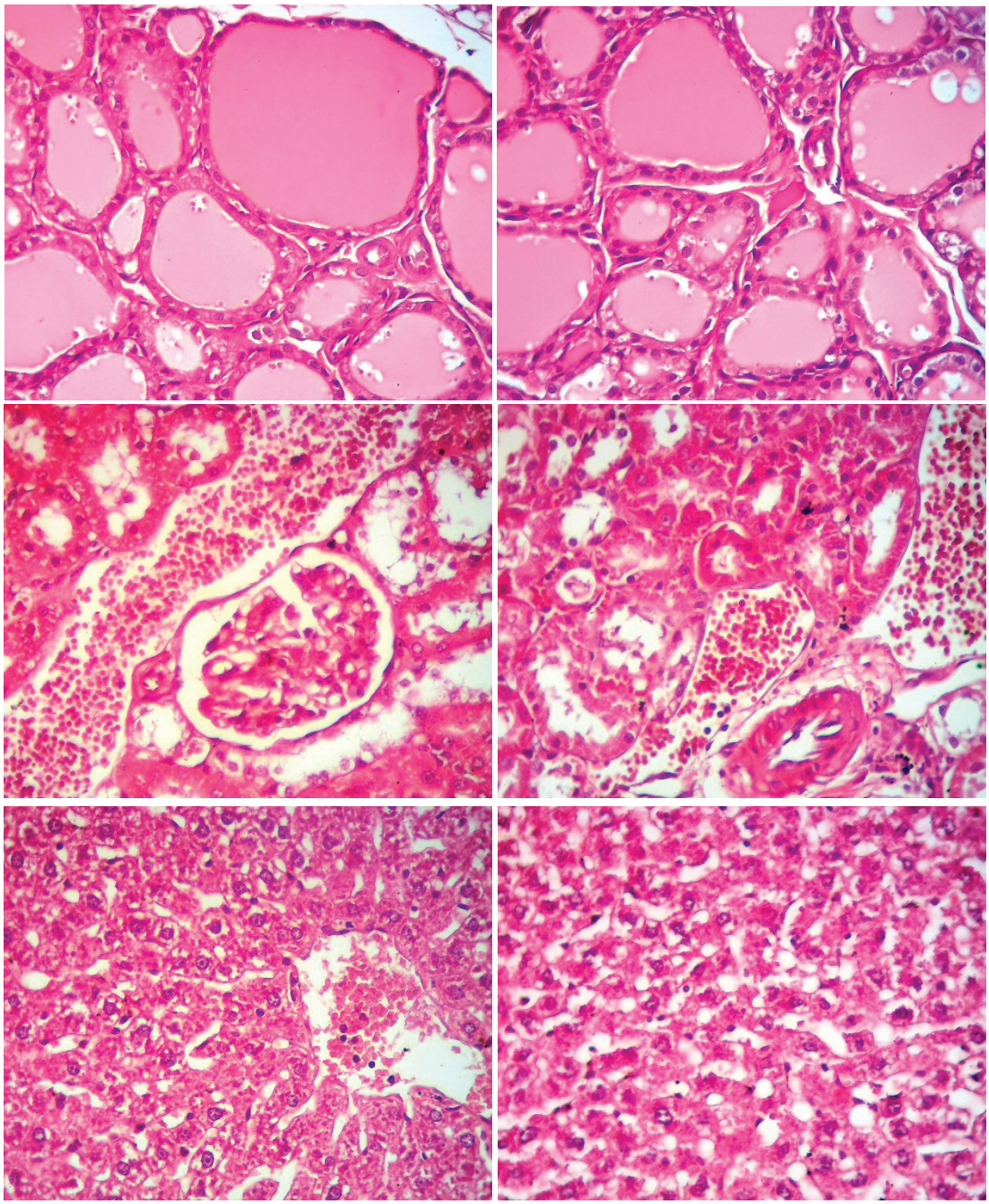

Figure2: show effect of 75mg\kg caffeine on thyroid, kidney and liver in which there is increase dilation of thyroid acini which filled with colloid, renal show vascular congestion and degenerative changes in renal tubules and shrinkage glomeruli, the liver appear central vein congestion and vacuolation of liver cells.

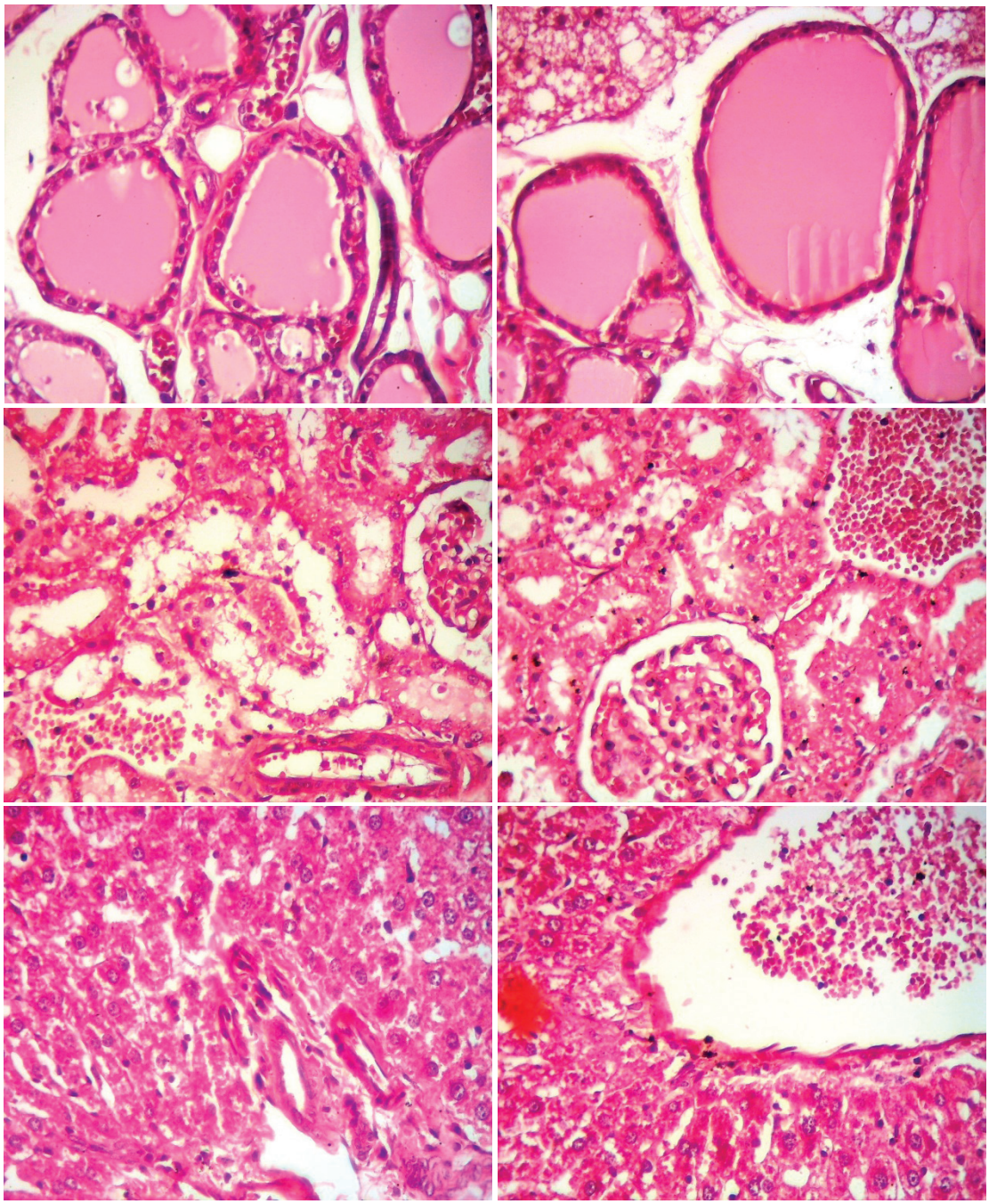

Figure3: show effect of 150mg\kg caffeine on thyroid, kidney and liver in which thyroid follicles increased with more flattened epithelium, kidney show degenerative changes in renal tubules and glomeruli with congestion of vessels, some hepatocytes appear necrosed, other with vacuolative changes and congestion.

Discussion

This study denote that caffeine causes significant reduction in thyroid function in all treated groups compared to control group with main reduction in T3, however, non-significant variations in TSH, T4 levels were detected. This result opposes with results of other studies which state that thyroid hormones are increased by use of caffeine[7,8,9]. Another researcher states that consumption of caffeine in high doses cause significant increase in T3 and T4 with non-significant change in TSH level[10] and clarified that may be due to caffeine inhibition of c-AMP breakdown by blocking the activity of phosphodiesterase enzyme resulting an elevated

c-AMP level which is necessary for proliferation and secretion of thyroid follicles[11,12,13].

Other researchers findings consistent with our result made by Spindle et al pointed that caffeine ingestion decreases the serum level of TSH in a dose-dependent manner followed by lowered T3 and T4 levels, this is because caffeine lowers TSH concentration by releasing hypothalamic somatostatin. A study pointed that administration of anti-somatostatin antiserum to rats block the inhibition effect of caffeine on TSH[9,4,14]. Daily administration of caffeine for short time (7 days) cause a decrease in TSH level and it is secretion, while high dose affect secretion of hormones[4,15]. Ezzat and his colleague found that administration of caffeine daily cause fatty hepatocytes change and several liver lesions in rabbits after treatment for 1month, this in agreement with present study[16]. The histopathological results revealed lack of inflammation in the liver tissue which confirm the studies displaying that after its digestion. Caffeine is metabolized to a compound called para-xanthine which may induce slowing of the growth of scar tissue involved in fibrosis. Para-xanthine may help to defend against cancer of liver, alcoholic fatty liver diseases and hepatitis[17,18]. One of the important results of the present study is the marked vascular congestion of the liver and kidneys due to caffeine which is can be attributed to caffeine's effect on endothelial cells.

It also antagonize the effect of adenosine which affect blood vessels wall that are responsible for vasoconstriction and eventually resulting in vasodilation[19,20]. Histopathological sections of the kidneys showed renal tubular degeneration in all treated groups and this result is consistent with other studies in which treatment of male albino Wistar rats with caffeinated coffee gave rise to renal tubular degeneration, loss of epithelial lining and vascular degeneration as compared to control group[21,22]. The liver sections showed degeneration and vacuolation of hepatocytes with necrosis as the dose increase and this outcome is in agreement with other researches which mentioned that caffeinated drinks, soft and energy drinks have adverse effects on morphology as well as histology of hepatocyte of adult albino rats[23,22].

Conclusion

Caffeine overuse may induce many adverse effects in different organs like the liver, kidneys and thyroid function.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Fixing Blood Alkalinity by Using *Apium Graveolens* as Treatable Strategy to Prevent Covid 19 Likes Infection

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Abstract

Juice of fresh celery solution will be given to domestic cats and normal cases of human to achieve the purposes of this project. Dosages given three times a day/one weeks in a mount of one cup 250 ml for human 143 lb, and cats 10 lb in amount of one spoon 15 ml for each. Later, blood O_2, CO_2 and alkalinity Evaluated to get results.

Our study conclusion were proves the high ability of the celery juice's antioxidants compounds to relieve the high acidity of blood resulted from viral infections like covid 19 and influenza, in a way to curing and strengthening the immune system against those viral infecions.

Keywords: *Blood Alkalinity, Covid 19, Celery.*

Introduction

Blood pH is a chemical scale used to specifying how basic or acidic (as it called alkalinity) a blood is(1). It was adjusted to be in critical range from 7.3 to 7.4, tending to be somewhat basic. When blood pH less than 7.3 like to being more acidic, comparing to that more than 7.4 which considered basic. Each of pH, both of partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2), in addition to bicarbonate (HCO_3^-) are adjusted by a homeostatic factors and mechanisms performed through both of urinary and respiratory system to control the acid-base balance and respiration(2).

Acidosis is a case leading to increase blood (plasma) and other tissues pH (i.e., an elevated hydrogen ion ratios). This condition of low blood pH termed acidemia (pH lower than 7.3)(3). On the other hand, alkalosis resulting from an action declining hydrogen ion levels of arterial plasma (alkalemia) where pH is higher than 7.4(4).

Acid–base homeostasis term referred to the homeostatic establishment for pH of the body's extracellular fluid (ECF). This crucial maintenance between the bases and acids (i.e. the pH) in the ECF is important for the normal physiology and metabolism of the body. Both pH of the extracellular fluid and the intracellular fluid need to be controlled under a normal values(4).

Four existed acid-base troubles: metabolic alkalosis, respiratory alkalosis, metabolic acidosis, and respiratory acidosis. One or a more of these cases may happens at the same time. For occasion, a metabolic acidosis is almost partially conjugated by a hyperventilation (respiratory alkalosis), or a respiratory acidosis can be partially or completely accurate by a metabolic alkalosis (5).

Shah etal.(6) explained in the same way the solutions for the starting condition for a merging of coronovirus with a human cell is acidified the endosomes on their surfaces. That is mean the virus needs a low pH places and prospers in it. So once the host begin experiencing symptoms of coughing and minor trouble with respiration his CO_2 item is rising in the body as lungs have difficulty to eliminate it. This CO_2 elevation guides to a decline of the body's pH as more acidity added by CO_2. This condition in turn carry out coronvirus even good chances for fusion and damaging the host lungs and subsequently continue to boost a value of CO_2 in the body (as the lungs be more infected) and to rising acidity

in the body (pH tends to lowering). So there is indirect proportional relation that even more CO_2 increasing the acidity level that expanding chances of virus fusion and replicates and more CO_2 ratios, etc. The scientsts observe a feedback in this case which cause to winding the growth of covid 19 faster and out of control. The patients lungs get bad and worse hastily[6].

Medicinal plants and different parts of the plants used a lot to treat many diseases and for public health purposes as a whole. The employ of natural herbs therapeutics is cost-effective. Since thousands years ago, plants have been crucial in eliminating disease and now the focal point is on their task and capability in curing and their healing characteristics for various cases. Many researches have shown the confident results of various medicinal plants and herbs on hormone disorders, infertility, anemia, liver disorders, neurologic and mental disorders, and renal diseases [7].

Many studies investigated diverse biological and life activities such as antioxidant prints of phenolic compounds and flavonoids spreads widely in most plants in such as cancer, diabetes, and coronary heart diseases. Medicinal plants and herbs have inconsiderable side effects than chemical pharmaceuticals and in the same point their antioxidant tends to eliminate the toxicity of these chemical or pharmaceutical drugs [8].

Celery (*Apium graveolens* L) is a vegetable plant from the apiaceae family, and it is of the perennial or annual plants that cultured throughout regions the tropical of Africa and subtropical Europe, in addition in several areas of Asia. It has an antioxidant activities, are admits for holding back peroxidation and free radicals[9]. These chemical properties exhibited by polyphenols in a similar way, that means one or more of the phenolic groups can react with other hydrogen donors and neutralize the free radicals. A lot of studies test the impacts of celery antioxidants and phenolic compounds that have been studied by many techniques. Celery leaves and root have the strength to remove OH and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, and these plant's part also reduces the intensity of liposomal peroxidation that represents the plant's protection.

Target of this project was to prove practically the antioxidant activity of celery against the most relevant cases like covid 19 which rises the blood acidity.

Materials and Methods

1- About 25 cat models and 55 human cases will be cleared to be tested for our project's programme both at home indoor and at cages indoor.

2- Evaluating blood O_2, CO_2 and alkalinity well done through oximeter, capnography and portable pH meter; Masimo SET®[6].

3- Celery plant vegetables (*Apium graveolens*) prepared and evaluated at labs by hit mixing gently by grabing several bunches of this plant and cut off the top and the base of the celery stalks, then rinsing and salting them carefully in a colander step before cleaving these stalks into thirds and put them for blending in the base of high-speed blender. Next step is adding 0.25 liter of water and place on the lid, make blend until texture be smooth, using the interpose to push the celery stalks into the blades as possible it is. After this put a clean nut milk bag on top of the mouth of a pitcher and spill the blended plant parts through this nut milk bag. Employ your gloved hands to squeeze the celery juice through the nut milk bag. Now the juice can be serve immediately or keep it in a tightly sealed pitcher in the refrigerator.

4- Dosage of the prepared juices by three times a day/one weeks in a mount of one cup 250 ml for human 143 lb, and the same time strategy for cats 10 lb in amount of one spoon 15 ml for each.

5- The same evaluation principles in step 2 was restored.

6- Equipments and tools were used in our research imported previously from F. Hoffmann-La Roche, Inc., Switzerland.

7- Statistical evaluation and analysis of inputs and issues were tabulated and scheduled by the computerized statistical program (SPSS) [7].

Results

The most prominent effective and affective chemical substances recorded in the applied mixture were being caffeic acid, p-coumaric acid, and ferulic acid in addition to apigenin, luteolin, and kaempferol, see table-1 below.

Table-1: Chemical composition of celery mixture tested project.

Active substances	Percentage
p-coumaric acid	0.03
caffeic acid	0.015
apigenin	0.02

The evaluated blood's O_2, CO_2 and pH for both cats and human were being tabulated below in tables-2 and -3 subsequently. It looks like there is significant improvement in these parameters values in the blood after several dosages of the tested mixture at this project.

Oxygen recording a substantial elevation in both cat (90) and human (98), also carbon dioxide registering a significant decline (33) for cat and (26) in human.

Potential of hydrogen shows natural restability after dosing passes for one week to reach (7.3) and (7.38) in both cat and human subsequently.

Table-2: Cat's alkalinity correlated values of tested variables.

Constant	Test	Control	Standard	Treated
O2 %	70	88-92	85–95	90*
CO2 mm/Hg	50	30-40	29–42	33*
pH	7.01	7.3-7.38	7.24–7.4	7.3*

* explaining a significant difference

Table-3: Human's alkalinity correlated values of tested variables.

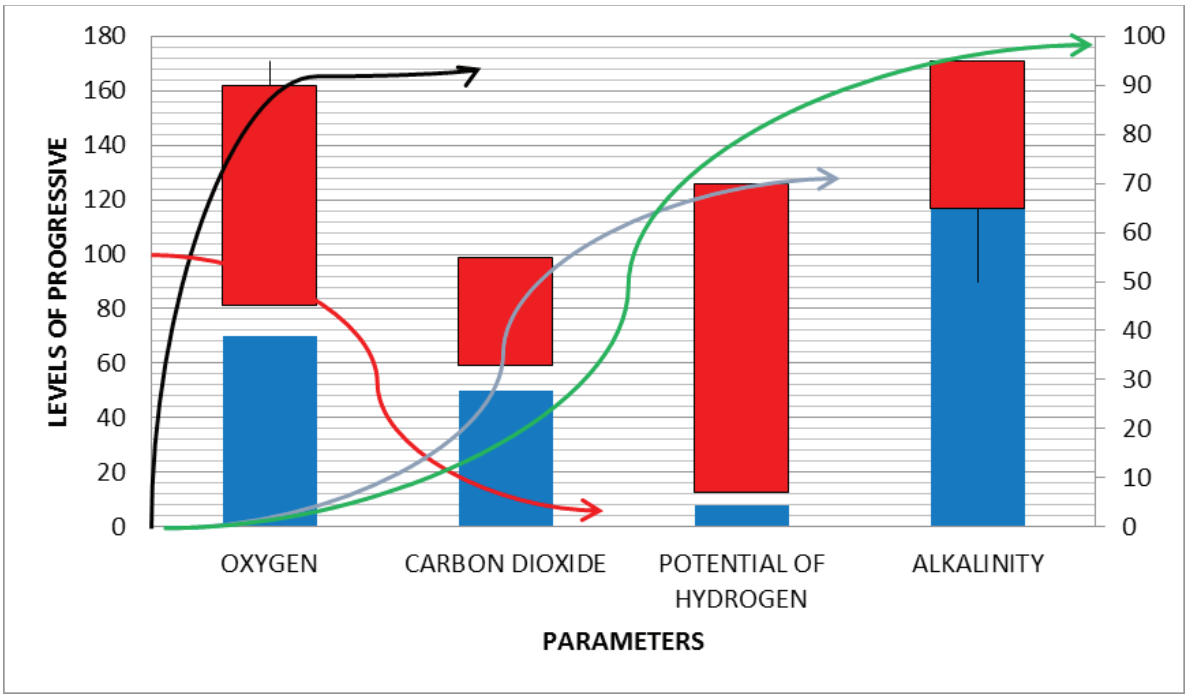
Constant	Test	Control	Standard	Treated
O2 %	77	96-99	95-100	98*
CO2 mm/Hg	40	25-28	23-29	26*
pH	7.05	7.37-7.43	7.35-7.45	7.38*

*** explaining a significant difference**

Statistical explanations exposed schematically as histogram to view data expressed in arrows showing a significant fluctuations in the ratios and values of parameters we set my sights, see scheme-1 below.

Through that diagram one can distinguishes well observed effects of vegetables mixture on the alkalinity improvement in the selected samples.

Scheme-1: a histogram expressing about statistical analysis for the effect of celery mixture on the blood gases and pH.



This level explains the values after dosage with mixture by both cat and human

This level explains the values before the mixture uses by both cat and human

Discussion

Celery lea and root juice elevated the capacity of antioxidation through reducing glutathione levels and the total antioxidative capacity (FRAP) in addition to increasing content of GSH. So this juice had defence effects through elevating the (FRAP) in some therapeutic cases. This could be supposed by the truth that some of flavonoids and other types of plant antioxidants can act as prooxidants under certain cases. Recent findings have explained that amount of cytochrome P450 can be assigned as a marker of oxidative stress take in part it had opposite affect when celery juice applied in vitro. On the other hand, in vivo tests are very complicated where data are not minutely separated since some components of celery juices act as both antioxidants and prooxidants that we looking for further explanation of effects of celery juice on antioxidant systems due to celery has an alkalizing effect lonely or working with other minerals like iron, magnesium, and sodium to have a neutralizing effect on acidic nutrition habits[12].

Modern studies established that crucial antioxidant compositors of celery juice were assessed e.g.; TAOC and ABTS. According to results obtained from HPLC it was proven that the leaves of celery have phenolic antioxidant compounds and phenolic activities of about eleven antioxidant celery cultivars. The critical phenolic acids in celery extracts incorporated *p*-coumaric acid, caffeic acid, and ferulic acid, while the recognized flavonoids consists of luteolin, apigenin, and kaempferol. Apigenin was the cheif flavonoid in this celery sample, and the preponderance plentiful phenolic acid was *p*-coumaric acid[13]. As a whole, there was a direct proportional relations between antioxidant activity, total phenolic or total phenolic acids and total flavonoid contents were proved in many studies. On another research carried out in 2012 by Nagella et al, both of antioxidant activity and chemical compounds of essential oils secluded from celery leaves were examined. At this study, the leaves were treated by distilled water and they were evaluated by gas chromatography/mass spectrometry to extracting and obtaining essential oils of leaves. About 73.72% of one leaf component were essential oils. In the next footstep, isolation of the essential oils responsible for inhibiting the DPPH radical, were investigated and the consequences proved the natural antioxidant capacity of that isolated oil from celery leaves. Another research was introduced out by Shanmugapriya and

Ushadevi[14] in 2014 on seeds of *Apium graveolens sp.* extraction shows good antioxidant activity. In the first step, seed was being extracted with water, ether and ethanol solvents and prepared subsequently. Next, the DPPH assay method used for analyzing the celery seeds activity. Results explains that methanol extract had the highest antioxidant activity among the other extracts under investigations. Research explained that methanol extract had the maximum antioxidant activity in the density of 80 µg/mL of extracts, about 63.28% ± 0.86%, while diethyl ether extract activity was 54.04% ± 0.21%, and the aqueous extract records was 52.97% ± 0.64% activity. In the same line, the celery seed shows that the entire concentration of methanol extract had the most extraordinary antioxidant activity. The researcher explained that reduction of reactive oxygen done by both of extracted flavonoids and luteolin, in the same way they elevated SOD enzymes that had protective belongings up against destructions. So they were being suggested that both of flavonoids and luteolin compounds had a major role for antioxidant activity of seeds. Other studies used photochemical screening in presence of saponins, tannins, flavonoid, and indicated the absence of terpenoids roles in the effectiveness of celery antioxidant phytochemical compounds. Total methanol content (63.46 ± 12.00 mg gallic acid equivalent [GAE]/g) was slightly higher. Fraction for ethanol was (36.60 ± 12.28 mg GAE/g) and for hexane fraction (34.86 mg ± 6.96 GAE/g). Flavonoid content in methanol extract was 56.95 ± 7.14 mg quorcetin/g and lowest of this content in methyl extract was 29.2 ± 3.15 mg quercitin/g. The activity of FRAP was analyzed as higher in comparison with other extracts[15,16,17].

Furthermore, in either method for determining antioxidant content; mint, grape leaves, and parsley demonstrated the towers 3 levels of antioxidant contents. With mention to correspondence; resembling to the data established the obvious significant correspondence between the antioxidant capacity (measured by DPPH as well as CUPRAC) of the studied vegetables with antioxidant content (measured by Folin-Ciocultaeu method) investigations is not amazing since the antioxidants assessed by Folin-Ciocaltaeu method provided well to the antioxidant capacity. Alike to the consequences turns out there was no link between the antioxidant content evaluated by total flavonoid content and antioxidant capacity unpaying that the antioxidant capacity is not alone attributable by the total flavonoids content. So this extremely marked treaty conveyed as an obvious significant correspondence between antioxidant capacity rates measured by CUPRAC and DPPH assays is not amazing[18].

As a conclusion, we had **suggested a way of medical intervention here**: first we recommend to observing patients (pH level) by applying ABG tests (or others) repeatedly and fix the pH factor as the main factor to evaluate. Second, we must fixing pH and CO_2 concentration in viral infections as possible as via using the natural antioxidant compounds exists in some fresh, cheap and easily cultured vegetables like celery. Third the applied mixture proves a good results and gives a good reliable promises in controlling acidity of blood and body fluids resulting from sudden viral infections.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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The Role of Vitamin D3 as an Antioxidant and Hepatic Treatment against the Toxic Effect of Cadmium Chloride in The Rats

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Abstract

Current study experiments were conducted on male *Rattus rattus norvigicus*, at age of two months, with the weight of (200-250gm) divided into 8 groups for each group of 8 rats. The lethal average dose of (LD_{50}) for cadmium chloride and the choice of bodyweight (5mg/kg) to inject rats Intraperitonially were determined, then orally doses of vitamin D3 after a week of $CdCl_2$ injection at a concentration of 1000IU/kg bodyweight at three doses per week as well as for the rest of the concentrations, 2000IU/kg bodyweight, and 4000IU/kg bodyweight. After four weeks of injection and administration, blood was drawn from the rats in each group, then the rats were slaughtered and the liver was taken for tests. The current study aimed to use different concentrations of vitamin D3 as a treatment, the antioxidant in the liver of rats injected with heavy metal $CdCl_2$. Due to the study, some biochemical, physiological, and tissue changes in them through the study of liver function for rats experimenting with transactions, in addition to studying the level of oxidative stress and antioxidants in the liver. The results of the current study have shown that the role of vitamin D3 as an antioxidant and as a treatment in reducing the toxic effect of $CdCl_2$ to a very large. The best concentration of vitamin D3 used in the case study was 2000 IU/kg bodyweight, which was taken for rats and three doses per week for four weeks, and after being injected with $CdCl_2$, there was a significant decrease at the probability level $P \leq 0.05$ for the concentration of the ALT when compared to the control group. On the other hand, there is a significant rise in glutathione (GSH) and catalase (CAT) levels in rat liver, at the same concentration of vitamin D3, when compared with the control group, while there was a clear improvement in AST and malondialdehyde (MDA) in the liver, the results were very similar to those of the control group. Perhaps vitamin D3 dosage of cadmium-injected rats may protect improving the toxic effect of $CdCl_2$ in the liver.

Keywords: *Antioxidant; Hepatotoxicity; Vitamin D3; Cadmium chloride; Free radical*

Introduction

Rising pollution is an environmental risk occurs due to xenobiotics toxicity, chemical materials as well as or other organic substances, such as heavy metal substances [(1).]

Cadmium is a type of heavy metals and toxic substance known to humans. It affects the digestive, urinary and nervous systems. Most effects of the heavy metals on human body are mediated by oxidative stress. It has been shown that Cadmium produces high portion from ROS [(2).]

Humans are exposed to cadmium as a result of tobacco smoking, agricultural activities, and industrial applications such as cadmium-nickel electroplating and paint pigment processing systems [(3).] The liver is a target organ for the damage of acute and chronic to cadmium. Following acute exposure, swelling of hepatocyte and severe necrosis result in elevation rise in the measurement of enzymatic biomarkers [(4).]

The term "liver function tests" is a misnomer, as many of the tests do not reflect on the liver function, but detect damage source. In ALT and AST, elevations out of proportion to bilirubin and ALP signify a hepatocellular disorder, whereas an elevation in ALP and bilirubin would signify a cholestatic trend in addition to ALT and AST. It can be graded the liver actual ability according to its function in producing vitamin K- dependent clotting

factors and albumin [5]. ALT is a cytosolic enzyme present in elevated liver concentrations. The release of the three enzymes into the blood circulation is due to hepatocellular damage, and not necessarily cell death [6].

Vitamin D3 (cholecalciferol), a pre-hormone fat-soluble steroid is considered an active source of vitamin D in humans. For decades, the vitamin D function was believed to be restricted to Ca^{2+} and homeostasis of phosphate, bone development and repair, but recent research indicates a wider biological role for vitamin D3 in the nervous system [7]. Recently, vitamin D has been known for its non-classical behavior, including improves the immune response, regulates cell proliferation and activates of hippocampal synaptic. Many studies have demonstrated that vitamin D has antioxidant function As a result, it is highly important that vitamin D supplementation decreases renal oxidative stress in sepsis-induced acute kidney damage [8]. Environmental pollutants are the main source of the health problems, thus much efforts have been made to understand the relative antioxidant potency of vitamin D3. Therefore, the present study focused on the vitamin D3 effciency in combating the toxicity of $CdCl_2$ on lipid peroxidation, hemato-biochemical parameters, renal function, and liver enzyme activity in rats

Material and Methods

Animal exposure

Male albino rats (*Rattus novergicus*) (eight-weeks old, 200–250g) were obtained from the Laboratory Animal Center, College of Veterinary Medicine University of Mosul-Iraq. The animals were maintained under standard laboratory conditions (temperature 22 ±2ºC; humidity 50%-70%; and12-h light/12-h dark cycle). Food and water were provided *ad libitum*. After two weeks of acclimatization, the rats were randomly divided into eight groups. Group A (n=8) used as a control group, received normal saline only. Group B (n=8) a Single dose of 5 mg/kg (bodyweight) cadmium chloride injected intraperitoneal route. Groups C, D, and E, a single dose of 5 mg/kg bodyweight cadmium chloride injected intraperitoneal route, and after 7 days treated with One dose every two days orally with vitamin D3 1000 IU/Kg bodyweight in group C (n=8), 2000 IU/Kg (bodyweight) in group D (n=8), and 4000 IU/Kg (bodyweight) in group E (n=8).

Groups F, G, and H (n=8) treated with One dose every two days orally with vitamin D3 1000 IU/Kg bodyweight, 2000IU/Kg bodyweight, and 4000 IU/Kg bodyweight in group E, respectively.

The eight groups are sacrificed 4 weeks after the injection.

$CdCl_2$ has been obtained from Scharlau Co. (08181 Sentmenat SPAIN)

Vitamin D3 was purchased manufactured in the UK Premier Health Products Ltd, Coventry, CV4 9UP, UK. P1275-160414-0

Blood and tissue samples

After 4 weeks of treatment, overnight fasted animals anesthetized using sodium pentobarbital (50 mg/kg i.p.) we then collected the blood in tubes and centrifuged (500×g) for 5 min, to obtain serum for biochemical analysis.

The liver was excreted shortly after being killed, washed in saline, and measured. Tiny sections of each liver are cut and preserved in 10% natural formalin for histopathological tests. The remaining parts of the liver were homogenized in 10% (w/v) ice-cold 100 mM phosphate buffer (pH 7.4) and centrifuged at (2000 ×g) for 15 minutes at 4°C, and then the supernatant was collected and used for oxidative stress biomarker tests (MDA, GSH, CAT).

Serum Biochemistry

The efficacy of both serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT) was estimated by the FUJI DRI-CHEM device manufactured by Nishiazabu 2-Chome, Minato-ku, Tokyo 106-0031, Japan The way the device works is called FUJI FILM.

Measurements of oxidative stress markers in the tissues

The thiobarbituric acid (TBA) assay was used to measure Malondialdehyde (MDA), which is based on the reaction between MDA and TBA for producing thiobarbituric acid reactive substances (TBARS), a red species that absorbs at 535 nm [9]. The levels of GSH were detected at 412 nm as described before [10]. The catalase activity (mol of H_2O_2 consumed/min/mg

protein) was assayed by the method of [11].

Statistical Analysis

ANOVA is the test used in Graph Prism Software V. 7.00 for analysis of the results, Statistical significance was considered as $p < 0.05$.

Results

Serum Biochemistry

Treatment with $CdCl_2$ dramatically significantly increased ($P<0.05$) the activity of the AST and ALT in serum relative to the control group, including rats in groups treated with the $CdCl_2$+D3 4000IU/Kg bodyweight (Table 1). On the other hand, shown in the same table, treatment with D3 in $CdCl_2$+D3 1000, and $CdCl_2$+D3 2000IU/Kg bodyweight groups a marked improvement and demonstrated a significant change from the control group and amelioration when compared with CdCl2 only treated groups. However, in the AST test, the $CdCl_2$+D3 1000IU/Kg bodyweight group experienced a similar significant increase to the cadmium chloride group in contrast to the control, while the $CdCl_2$+D3 2000IU/Kg bodyweight group experienced a clear improvement through a significant decrease compared to the control group.

Oxidative stress and antioxidant in the tissues

The levels of CAT and GSH in the liver were statistically decreased in $CdCl_2$ treated rats group as compared to controls, while MDA levels were elevated in cadmium chloride treated rats group compared to the controls

In measuring the level of a CAT In the liver, find that the improved significantly in the treatment groups D3 2000IU/Kg bodyweight only and CdCl2+D3 2000IU/Kg bodyweight compared to the control group . While the level of GSH in the liver of rats in the groups of D3 1000IU/Kg bodyweight, D3 2000IU/Kg bodyweight, and CdCl2+D3 2000IU/Kg bodyweight, the results showed that a significantly increased compared to the control group.

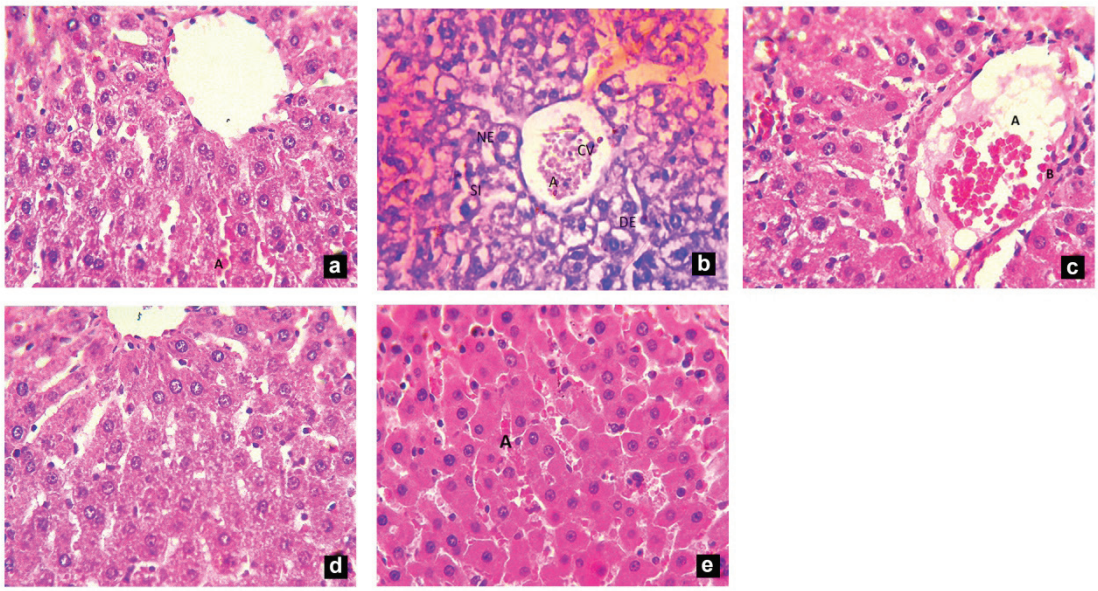
The level of MDA in the liver showed a significant decreased in the treatment group D3 2000IU/Kg bodyweight only, on the other hand, all groups that were treated with vitamin D3 after injection of cadmium chloride showed an improvement in MDA level and similar to control group results.

Table 1: The Mean levels activity of the AST and ALT in serum for all groups

	Control	D3 1000	D3 2000	D3 4000	CdCl2	CdCl2+ D3 1000	CdCl2+ D3 2000	CdCl2+ D3 4000
AST (IU/L)	25.97 ±0.7877	24.40 ±0.9227	23.02a ±0.7604	27.50 ±0.7394	47.07c ±0.8562	25.00 ±0.4851	23.25 ±0.9475	30.40b ±0.7127
ALT (IU/L)	34.02 ±0.6720	33.05 ±0.6059	24.9c ±0.6775	37.37b ±0.4971	43.22c ±0.5947	30.07b ±0.7019	28.03c ±0.7495	38.55c ±0.6026

Values expressed as mean ±SD (n=8). a,b, and c Significant at $P < 0.05$ indicate in comparisons with respect to the control group

Histological observations



The liver histological sections of the control groups showed normal architecture of with mild congestion of sinusoids (Fig.1-a), but the group injected by Cadmium chloride only showed central vein dilatation with vascular hemorrhage, hepatic cords irregularity around the central vein, hepatic sinusoidal dilatation, and internal hemorrhage, and necrotic changes in the cords Hepatic (Fig.1-b). The photomicrograph of the liver of group $CdCl_2$+D3 1000IU/ Kg bodyweight shows congestion of the central vein and few infiltrations of inflammatory cells surrounding it with the normal architecture of hepatocytes (Fig.1-c). In the group treated with $CdCl_2$+D3 2000IU/Kg bodyweight shows of the normal architecture of the liver (Fig. 2-d), while the group treated with $CdCl_2$+D3 4000IU/Kg bodyweight shows the congestion of sinusoids (Fig1-e).

Fig. 1: Hematoxylin and eosin-stained in the liver sections (400x) of (a) control group show the normal architecture of with mild congestion of sinusoids (A); (b) $CdCl_2$ group shows central vein dilatation with vascular hemorrhage (A), hepatic cords irregularity around the central vein (DE), hepatic sinusoidal dilatation and internal hemorrhage (SI), necrotic changes in the cords Hepatic (NE); (c) group $CdCl_2$+D3 1000IU/Kg bodyweight shows congestion of central vein (A) and few infiltrations of inflammatory cells surrounding it (B) with the normal architecture of hepatocytes; (d) $CdCl_2$+D3 2000IU/Kg bodyweight group shows of the normal architecture of the liver; and (e) group $CdCl_2$+D3 4000IU/ Kg bodyweight shows the congestion of sinusoids (A).

Discussion

The toxicity of heavy metals on human is induced by oxidative stress. Cadmium is a highly toxic heavy metal that has been reported to contain a large number of ROS.

Vitamin D3 is an important nutrient for bone growth and reconstruction, [12] indicate that vitamin D3 has a wide-ranging activity such as regulation of inflammatory reaction and anti-tumor activity. The results of the current study showed a moral rise in the concentration of ALT and AST enzymes in the $CdCl_2$ group, which proves the breakdown of hepatic cell membranes and the high level of enzymes in the blood as a result of leakage from hepatic cells, this study agreed with the study of [13] in determining the role of selenium and vitamin E to prevent the harmful effects of cadmium. With results showing an increase in AST and ALT concentration in the group of mice which treated with a significant serum in comparison to the other groups, the results of our study showed that the totals treated with vitamin D3 intake and the concentrations 1000 UI/Kg bodyweight,

and 2000 UI/Kg bodyweight were within the normal range when compared to the control group.

The current study was in agreement with the results of the [14] study the effect of cadmium alone and the effect of cadmium with iron together" on the liver in the rat serum, at the end of the fifth week was observed a significant and moral increase of ALT and AST in mice treated with cadmium and a moral decrease in levels of ALT and AST enzymes in iron and cadmium treatment totals and the presence of iron and cadmium in the diet provided protection against liver poisoning caused by cadmium, while there was a significant increase in liver enzymes in ALT and AST for mice exposed to Fe only,

Cadmium is practiced by stimulating fat peroxide and reducing cellular antioxidants, Our study agreed with the study[15] that vitamin D3 has a protective effect in reducing injuries caused by heavy metals in the liver and kidney organs by the anti-inflammatory and antioxidant pathways that vitamin D3 activates and stimulates against heavy metal toxicity. The results of the current study matched those of researchers [16] an increase in oxidative stress of MDA caused by cadmium was observed, which contributed to liver and kidney dysfunction in rat treatment totals only barely. The treatment rats with cinnamon has improved and reduced oxidation by increasing antioxidant enzymes and reducing fat peroxide. Cinnamon reduced the toxic effect of Cd, which was observed through the functional improvement of both the liver and kidney.

Our study agrees with the [15] study that vitamin D3 has a protective effect in reducing injuries caused by heavy metals in the liver and kidney organs by anti-inflammatory pathways and antioxidants that have been actively stimulated and stimulated by vitamin D3 against heavy metal toxicity. The mechanism through which vit. D3 exerts its antioxidant activity remains obscure. NADPH oxidases of the Nox family has been identified as important enzymatic sources of cellular ROS [8]. The overall reduction of glutathione can be explained as an effective ingredient in eliminating cadmium toxicity as well as the decrease in GSH level may also be due to increased activity of the g-glutamyl transpeptidase enzyme. The exposure to cadmium chloride doses reduced the concentration of the level of NADPH needed to reduce the GS and to convert it into the oxidized form GSSG and thus led to a reduction in GSH levels and in return increased levels of fat oxidation and increased that Free radicals, on the contrary, the group of Rats treated with D3 after injected with cadmium chloride had a much lower oxidative stress level than the treatment group with cadmium chloride only.

In the present study, the histopathological examination of the liver tissue in the group which treated with only $CdCl_2$, revealed significant degenerative changes represented in the central vein dilatation with vascular hemorrhage, hepatic cords irregularity around the central vein, also it was noted that there are hepatic sinusoidal dilatation and internal hemorrhage, and necrotic changes in the cords Hepatic, this may be attributed to the generation of oxygen species Reactivity that causes oxidative damage in many tissues by enhancing lipid peroxidation [17], also in our current study, the histological examination showed a significant improvement in liver architecture in groups treated with vitamin D3 when compared with the histological sections in the control group, this was close to the results of [18] in the enhancement of the histological appearance of mice treated with cadmium chloride and vitamin C relative to those treated with $CdCl_2$ alone.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Interlukine-17 Polymorphism in Patients with Inflammatory Bowel Disease (IBD) in Al-Anbar Province

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Abstract

Tetra amplification refractory mutation system polymerase chain reaction technique (tetra ARMS PCR) ARMS-PCR method was used to genotyping of IL-17A and IL-17F , The impact of IL-17A and IL-17F genotypes on the production of interleukins in IBD patients and control groups were determined and Interleukins gene polymorphisms role in IBD infection. significant deference at ($p \leq 0.05$) for each rs763780 in IL-17 F in patients especially Genotype TT,TC,CC were 31 (51.67%),19 (31.67%),10 (16.67%) \ and rs2275913 in IL-17 A in patients especially Genotype GG,GA,AA were 18 (30%),23 (38.33%),19 (31.67%). As well as genotyping of rs763780 in IL-17 F showed significant increase at a significant level of $P \leq 0.05$ in both TC and CC genotypes in the cases samples compared with the control samples. the odds ratio was 2.8894 and 22.3333 for TC and CC genotypes respectively at confidence interval up to 7.8 and 397 for both genotypes. T allele are more frequent in both cases and control and it have odds ratio as 5.021 And genotyping of rs2275913 in IL-17 A showed significant increase at a significant level of $P \leq 0.01$ for the AA genotyping in the cases samples compared with the control samples, while the GA genotyping did not show any significant differences,), the odds ratio for GA genotypes was 2.3590 at confidence interval up to 5.8, while it was 8.4444 at confidence interval up to 32.9 for AA genotypes. G allele are more frequent in both cases and control and it have odds ratio as 3.319 at confidence interval up to 6

Key words :*IL-17 polymorphism,IL-17, Inflammatory bowel disease.*

Introduction

Through the use of genome-wide affiliation scans, there had been 163 genetic chance loci that have been proven to make contributions to the chance of ulcerative colitis, Crohn's disease, or to both [(1).] Analyses of the genes and genetic loci implicated in IBD exhibit a number of pathways that are crucial for intestinal homeostasis, consisting of barrier function, microbial defense, epithelial restitution, innate immune regulation, reactive oxygen species, autophagy, law of adaptive immunity, endoplasmic reticulum stress, and metabolic pathways associated with mobile homeostasis [(2).] Numerous research have been pronounced the relationship between the polymorphism of IL-17A and IL-17F genes with specific inflammatory illnesses in extraordinary populations [(3,4).] Functional genetic polymorphisms in-17 and its receptor genes can affect both qualitatively or quantitatively their features [(5).] The genes for IL-17A and IL-17F are placed on chromosome 6p; the IL- 17B-encoding gene is positioned on chromosome 5q; that of IL-17C is on chromosome 16q, the IL-17D-encoding gene lies on chromosome 13q, and the gene for IL-17E was once mapped to chromosome 14q (6(.Moreover, quite a few research on single nucleotide polymorphisms have recommended that there is a connection between UC susceptibility and the IL-17 gene cluster [(7,8(.] Genetic polymorphisms in IL-17A and IL-17F may also have an effect on the expression of IL-17 by means of CXC chemokine induction and subsequent neutrophil recruitment, therefore affecting

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UC susceptibility [9] . A latest affirmation has proposed that genetic polymorphisms in IL-17A and IL-17F and serum IL-17 stages may also be concerned in the incidence of UC [10,11].

Materials and Methods

Patients and Controls :

The find out about blanketed (60)patients of unique a long time (15-60) who had been struggling from inflammatory bowel disease, who attended a prevalent educating medical institution in Ramadi metropolis in Al-Anbar governorate all through the length prolonged from the 1st of May 2020 to the 1st of February 2021. The samples of sufferers have been chosen According to the analysis of gastroenterologists. While manage covered (40) healthful folks of extraordinary a long time from (15-60) years. They had been viewed as a bad manage team as they did no longer exhibit a records of inflammatory bowel ailment after an investigation by way of gastroenterologists. Ten ml of venous blood was once accrued from a appropriate vein. Tourniquet was once utilized about (4-5) finger width above the chosen venipuncture website online and disinfected with 70% of Ethanol for 30 seconds and allowed to dry completely, the blood was once divided into two kinds of tubes, the first one; 2.5 ml complete blood was once distributed in in tow tubes with ethylene diamine tetraacetic acid tube (EDTA-tube) and blended gently, In the 2d tube; Residual phase of the blood pattern used to be transferred to it (free of anticoagulation) and let to coagulate for serum separation with the aid of the usage of a centrifuge at (4000 rpm) for 5 min, The remoted serum used to be accumulated in a sterile smooth white tube to be used for serological research Then tubes have been positioned in a cool-box underneath aseptic circumstance and saved in the freezer at (-20oC) till in addition processing [12,13].

Genetic Tests:

-Specific primer for human TLR4 Gene:

To detect the presence of Single Nucleotide Polymorphism (SNP) in the human *IL17F* and *IL17F* gene (rs763780 and rs2275913) primers were designed for tetra amplification refractory mutation system polymerase chain reaction technique (tetra ARMS PCR) according to their sequence in NCBI https://www.ncbi.nlm.nih.gov pmc and by free online primer design tool http://primer1.soton.ac.uk/primer1.html, the primer sequence and product listed in the below table (1), which purchased from Macrogen company/Korea.

Table (1): Primers for detection of (rs763780 and rs2275913) in human TLR4 gene by Tetra-arms technique.

SNPs	Size (bp)	Annealing Temp.(°C)	Primer	Sequence
rs763780	C allele: 170	58.5	Inner F	/5- GGATATGCACCTCTTACTGCAAAC -/3
	T allele : 132		Inner R	/5- CTGTGAAGTGGAGGGAATTGG -/3
	Size of two outer primer :258		Outer F	/5- GTCACCCCTGTCATCCAACA -/3
			Outer R	/5- AAGGAAGACATCTCCATGAATTCC-/3
rs2275913	A allele:134	56	Inner F	5 TTCCCATTTTCCTTCAGACGG/3
			Inner R	/5-CCCCAATGAGGTCATAGAAGAATCTATT-/3
	G allele:194		Outer F	/5- TGACCCATAGCATAGCAGCTCTG-/3
	Size of two outer primer :279		Outer R	/5- GATGGATGAGTTTGTGCCTGCTAT-/3

Results and Discussion

Population Study:

The total number of persons included in the study was (100) samples which included (60) patients were found to have inflammatory bowel disease (cases) and (40) normal persons as control.

The Tetra ARMS-PCR Technique for rs763780:

The Tetra ARMS-PCR technique was used to genotype rs763780 in both cases and controls, The results of the statistical analysis showed a significant increase at a significant level of P≤0.05 in both TC and CC genotypes in the cases samples compared with the control samples where the frequency of TC genotyping was (31.67)% in cases samples, while the rate was (17.5)% in the control samples. For CC genotyping, the Frequency was (16.67)% in the cases samples, while the control samples did not show any frequency of CC genotyping. as show in table (2) and figures 1 and 2), the odds ratio was 2.8894 and 22.3333 for TC and CC genotypes respectively at confendence interval up to 7.8 and 397 for both genotypes. T allele are more frequent in both cases and control and it have odds ratio as 5.021

Table 2: Statistical evaluations of association between rs763780 genotypes or allele in Inflammatory Bowel Diseases patients and control groups.

	rs763780 Genotype Frequency (%)				
Genotype	**Control n=40**	**Cases n=60**	**p-value**	**Odds Ratio**	**95% Cl**
TT	33 (82.5%)	31 (51.67%)	-------	1.0	--------
TC	7 (17.5%)	19 (31.67%)	0.0367 *	2.8894	1.0676 to 7.8201
CC	0 (0%)	10 (16.67%)	0.0344 *	22.3333	1.2556 to 397.2513
Chi-squared	0.3677 NS	4.645 NS			
p Value	0.8320	0.0980			
Allele frequency (%)					
Allele	Control n=80	Cases n=120	p-value	Odds Ratio	95% Cl
T	0.85 (73)	0.675 (81)	<0.0001**	5.021	2.099 to 12.55
C	0.15 (7)	0.325 (39)			

NS=Non-significant, * significant at p value ≤ 0.05, ** significant at p value ≤ 0.01

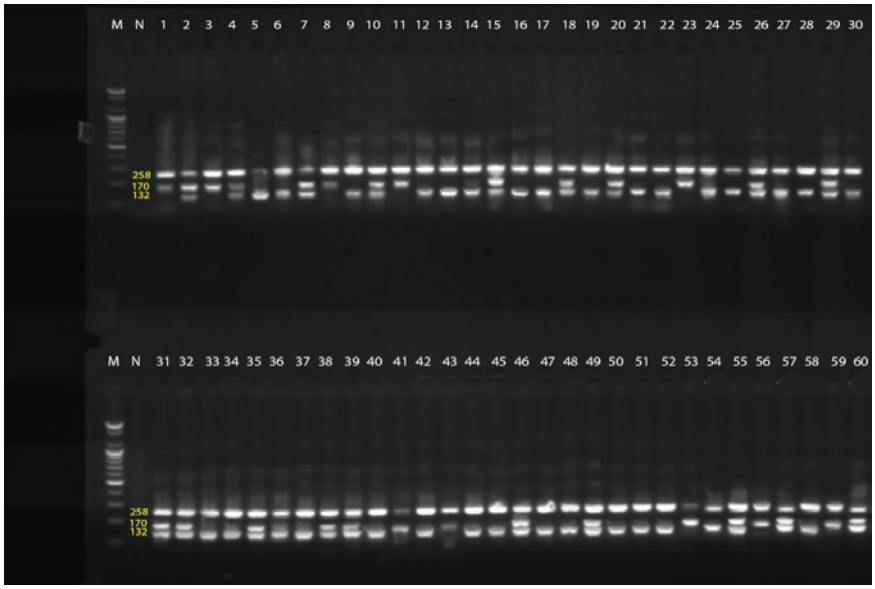

Figure (1) Tetra ARMS-PCR of *rs763780* gene polymorphism for cases samples

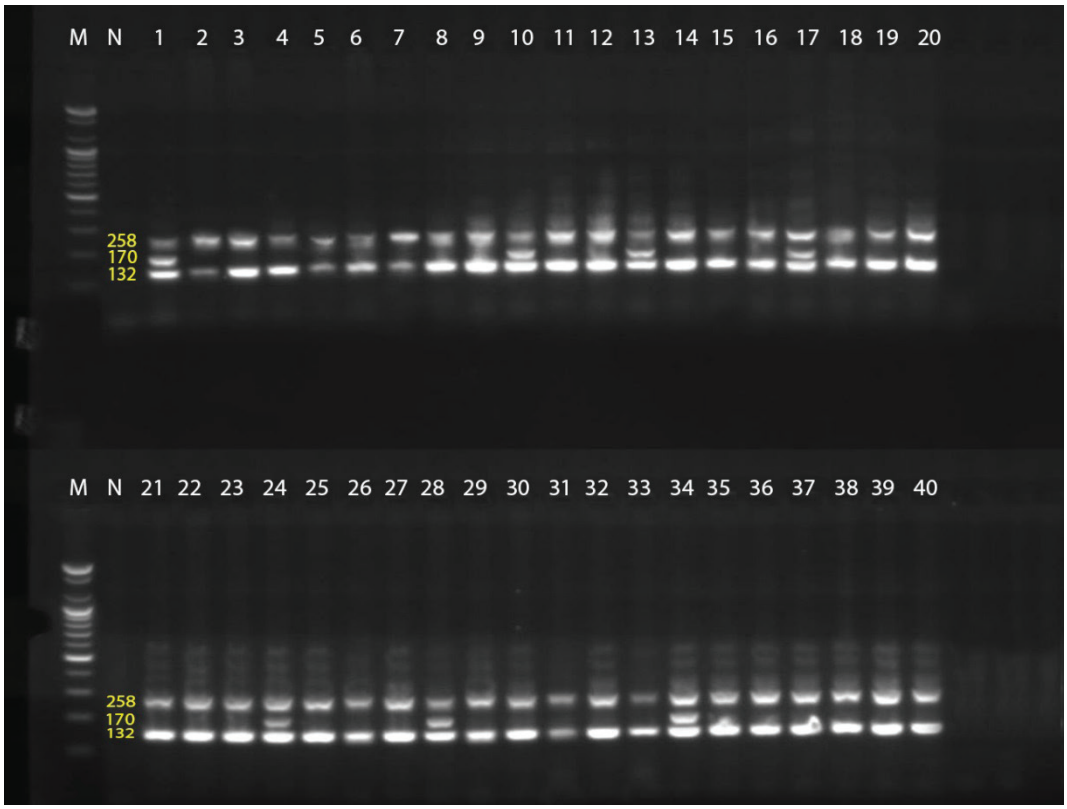

Figure (2) Tetra ARMS-PCR of *rs763780* gene polymorphism for controls samples

The Tetra ARMS-PCR Technique rs2275913:

The results of the statistical analysis of the genotyping of rs2275913 in both cases and controls showed a significant increase at a significant level of P≤0.01 for the AA genotyping in the cases samples compared with the control samples, where the frequency of genotyping AA was (31.67)% in the cases samples, while the frequency was (7.5). % In the control samples, while the GA genotyping did not show any significant differences. as show in table(3) and figures (3 and 4), the odds ratio for GA genotypes was 2.3590 at confendence interval up to 5.8, while it was 8.4444 at confendence interval up to 32.9 for AA genotypes. G allele are more frequent in both cases and control and it have odds ratio as 3.319 at confendence interval up to 6.0.

Table 3: Statistical evaluations of association between rs2275913 genotypes or allele in Inflammatory Bowel Diseases patients and control groups.

rs2275913 Genotype Frequency (%)					
Genotype	**Control n=40**	**Patient n=60**	**p-value**	**Odds Ratio**	**95% Cl**
GG	24 (60%)	18 (30%)	----	1.0	
GA	13 (32.5%)	23 (38.33%)	0.0658 NS	2.3590	0.9454 to 5.8860
AA	3 (7.5%)	19 (31.67%)	0.0021 **	8.4444	2.1621 to 32.9812
Chi-squared	0.4216 NS	3.261 NS			

Cont... **Table 3: Statistical evaluations of association between rs2275913 genotypes or allele in Inflammatory Bowel Diseases patients and control groups.**

P value	0.8099	0.1959			
Allele frequency (%)					
Allele	Control n=80	Cases n=120	p-value	Odds Ratio	95% Cl
G	0.95 (61)	0.58 (59)	0.0001**	3.319	1.776 to 6.096
A	0.05 (19)	0.42 (61)			

NS=Non-significant, * significant at p value ≤ 0.05, ** significant at p value ≤ 0.01

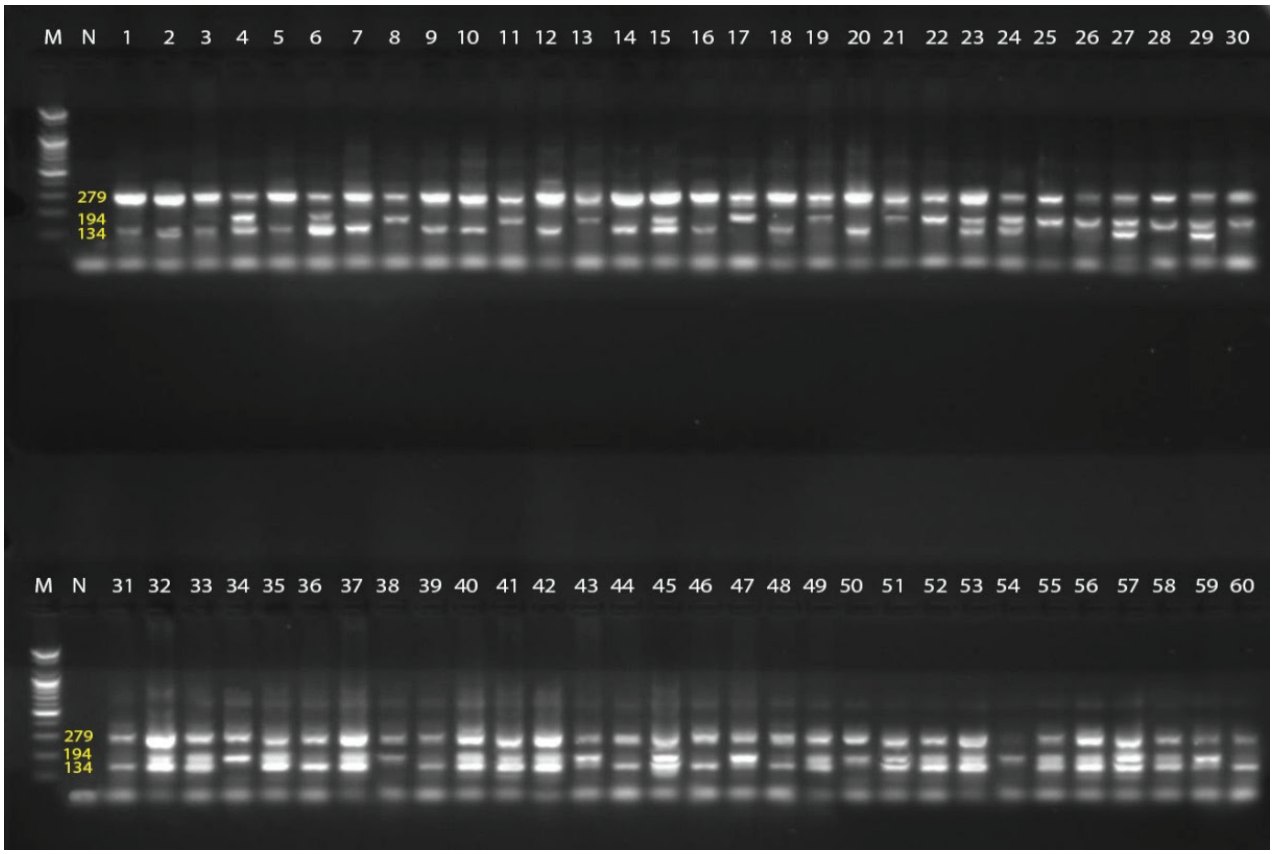

Figure (3) Tetra ARMS-PCR of *rs2275913* gene polymorphism for cases samples

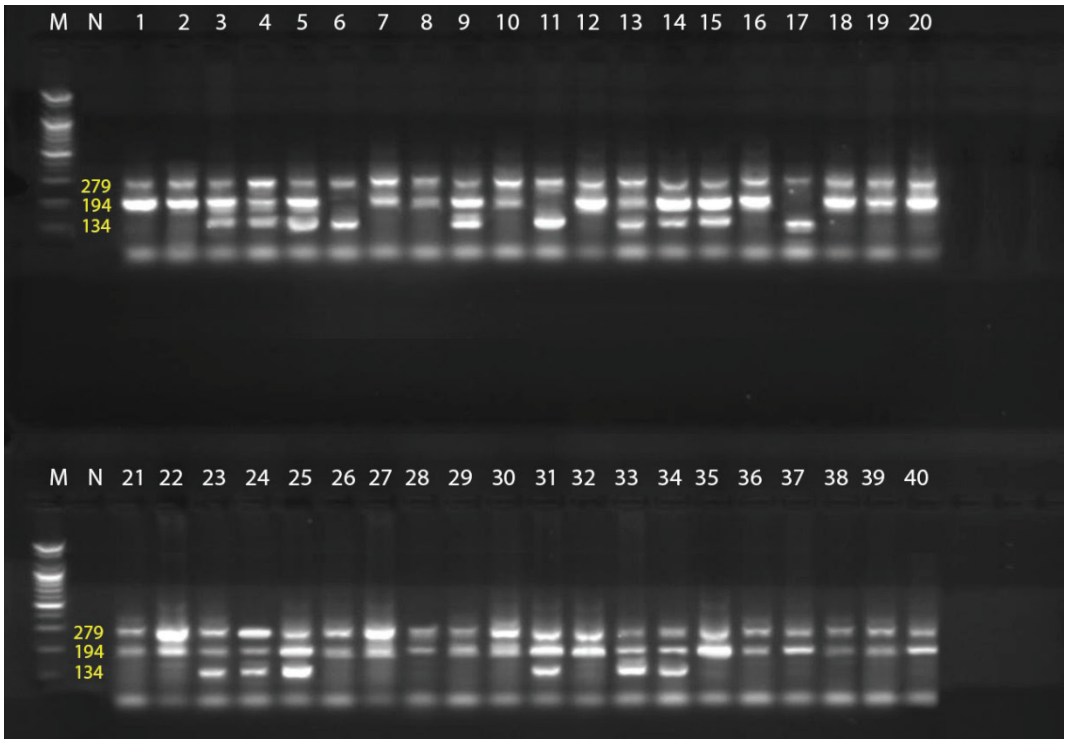

Figure (4) Tetra ARMS-PCR of *rs2275913* gene polymorphism for controls samples

The goal of our examination used to be to consider how rs2275913 and rs763780 genetic polymorphisms make a contribution to IBD and the relationship of IL-17A/17F genetic polymorphism with the scientific attributes. There is a restricted file that suggests the have an impact on of polymorphisms of IL17A and IL17F on the hazard of IBD, of all the SNPs, the IL17F (rs763780, 7488 T/C) polymorphism has been the most regularly stated As we exhibit in the end result of genetic polymorphisms for two SNPs the genotype TC and CC for rs763780 might also symbolize a threat issue for IBD whilst genotype AA may additionally be a danger genotype for rs2275913 and these consequences agree with many preceding outcomes posted to spotlight the impact of genetic polymorphisms of these SNPs in exclusive ailment and syndromes reviews confirmed a persisted make bigger in IL- 17-producing cells in the intestinal tissue of IBD patients, Over-expression of IL-17A or IL-17F in vivo leading to multiplied neutrophil infiltration thru modulation of cytokines and chemokines, main to infection [8]. The rs763780 SNP is placed in the role +7488 (coding region) of the IL17F gene, It consists of a substitution T to C, which leads to a trade in the amino acid sequence from Histidine to Arginine in function 161 (H161R) of IL17F protein, Reports mentions that the H161R variant of IL17F has no potential to set off the mitogen-activated protein kinase pathway, chemokine secretion in bronchial epithelial cells, and cytokine production, Furthermore, the H161R variant blocked the induction of the expression of IL8 by using wild-type IL17F, which performing as a herbal antagonist of the cytokine [14]. IL-17F rs763780 (His121Arg) polymorphism has been described to suppress the expression and the recreation of IL-17F and, this may additionally play a diverse function in the host's predisposition to inflammatory ailments [15]. A learn about shows that the rs763780 CC homozygous mutant genotype had an inverse correlation to ulcerative colitis susceptibility in the Chinese populace (p0.014), proposing that the rs763780 CC genotype would possibly be related with ulcerative colitis susceptibility; Another learn about referred to that the IL-17F 7488 TC polymorphism is considerably related with allergies (p0.0028) (16). A similarly sensible investigation in vitro suggests that the protein with rs763780 genotype can tighten the expression of wild-type rs763780 and discharge of the downstream cytokine [16,17,18]. Given that each allergies and ulcerative colitis have been believed

to be intervened obsessive immunoreactions by means of Th-2 cells, [19,20] the closeness in the relationship between IL-17F and the two awesome ailments seems to supply a signal to such a guiding principle and moreover recommends the doable cooperation between Th-17 and Th-2 cells in the pathogenesis of Inflammatory internal infection. [16] Nevertheless, the unique version principle suggests that few exceptional versions can also be aggregately answerable for a extensive extent of multifactorial inherited weak spot to IBD [21]. Further inspecting the connections between's the genotypes and scientific phenotypes of ulcerative colitis, it has been observed that the rs763780 TC heterozygote was once all the greater frequently related with the later establishing and mellow medical subtypes of the malady, demonstrating that IL-17F polymorphism might also emphatically affect disorder establishing and movement. [16] Arisawa et al.[22] determined that the IL17A (rs2275913, G-197A) and IL17F (rs763780, 7488T/C) alleles have been each essentially linked with an elevated chance of the development of ulcerative colitis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Detection of Capsular Polysaccharide Virulence Genes *rmpA* and *magA* of *Klebsiella Pneumonia* Isolate from Diabetic Foot Ulcer Patient in Najaf Governorate in Iraq

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Abstract

Klebsiella Pneumoniae is an opportunistic pathogen, which mostly cause nosocomial infections. *K. Pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection. A total of (36) clinical swab specimens isolated from patient with diabetic foot ulcer in Al-Sdder Medical City (Central Diabetic Foot) in Najaf Governorate, Iraq from November 2019 to February 2020 , for detection some virulence capsular polysaccharide genes such as *rmpA* and *magA* that related with K1 serotype of *K . pneumonia* and associated with antibiotic resistance pattern in patient of diabetic foot ulcer. Antibiotic susceptibility test of *K. Pneumoniae* isolates against (20) of commonly used antibiotic showed there 25 (69%) of *K. pneumonia* isolate from diabetic foot patient were Multi-drug resistance (MDR),while11(31%) were extensive-drug resistance (XDR). The results of amplified product demonstrated that K1 serotype of *K . Pneumonia* was positive in 23 (64%) of totally clinical specimen than other types of serotype (Non-K1 serotype). *(magA*) gene that associated with hypermucoid viscosity was positive in 21 (58%) of total specimens while *(rmpA)* gene that regulate of capsular polysaccharide of *K .pneumonia* was positive in 22 (62%) of totally samples .

Keywords: *patients; Capsular polysaccharide; genes rmpA; and magA of Klebsiella pneumonia; toxicity*

Introduction

World Health Organization definition of the diabetic foot (DF) is pathologic consequences including infection, ulceration and or destruction of deep tissues associated with neurologic abnormalities, various degrees of peripheral vascular disease and/or metabolic complications of diabetes in the lower limb(1), Its long term complication that represent a major health problem of high mortality and morbidity rates. According to bacterial culture and molecular approaches, DFU can colonize with numerous aerobic and anaerobic polymicobial (2). Among several types of bacteria that isolate from diabetic foot ulcer patient , *K .pneumonia* consider one of the most important causes in last year (3). Virulence in *K. Pneumoniae* is based on many factors, but the capsule is regarded as the most important. The genomic study of these isolates mainly highlights capsular serotypes K1 and K2 and the presence of a large virulence plasmid which is responsible for hypermucoviscosity and which includes the mucoid phenotype gene (*rmpA*) regulator. Many virulence genes have been identified, some of which are associated with capsule polysaccharide production and a mucoid phenotype, such as *rmpA* and *rmpA2* . Hyper virulent and hypermucoviscous *Klepsiella* has emerged in the last few years. Hypermucoviscosity is a phenotype characterized by highly viscous and sticky colonies, and the term is often used in conjunction with hypervirulence(4). Additional virulence factors in *Klebsiella* include:(i) adhesins (pili, fimbriae)(ii) siderophores, (iii) biofilm formation, and (iv) urease production. Nosocomial isolates of *K. Pneumoniae* often display multidrug-resistance phenotypes that are commonly caused by the presence of extended-spectrum β-lactamases or carbapenemases, making it difficult to choose appropriate antibiotics for

treatment [5]. Capsule polysaccharide (CPS) have been classified into 80 serological types, termed K-antigens. The presence of the capsule is critical for the virulence of *K. Pneumoniae* [6]. Hypermucoviscosity has been related with the capsular serotype K1, and in a lower proportion with the serotype K2, and has been linked to the presence of the *magA* (mucoviscosisty-associated gene A) and *rmpA* (regulator of mucoid phenotype A) genes [7].The *rmpA* (regulator of mucoid phenotype A) gene is a plasmid-mediated confer a highly mucoviscous phenotype enhanced and regulator of the capsular polysaccharide synthesis. Nassif et al [7] explained that remove of the *rmpA* gene can decrease virulence in mouse lethality tests by 1000-fold. Deletion of *rmpA* result in reduction of cps synthesis that leads to decrease of bacterial virulence, in the other hand the strain contain mutation in this gene have defect on regular capsular polysaccharide biosynthesis this give the importance for hypermucoviscosity[8].The other gene associated with hypermucoviscosity carried by virulence strain is *magA* (mucoviscosity-associated gene A).It is a chromosomal gene, responsible for capsular polysaccharide synthesis K1, if mutation happened in *magA* lead to loss capsule[9]. The mechanisms resistance in *K. Pneumoniae* to various antibiotics classes included; production of antibiotic-inactivating enzymes, a variation of antibiotic target sites, changing of cell membrane permeability, efflux pump systems, and modification of metabolic pathways. High expression of β-lactamase as carbapenemases and cephalosporinases enzymes in *K. Pneumoniae* lead to increased resistant to β-lactam antibiotics. The cephalosporinases referred to as ESBLs [10]. Typically, *K. Pneumoniae* producing ESBL displayed resistance to β-lactam antibiotics like penicillin's, cephalosporin's, and monobactams. *K. Pneumoniae* carrying ESBL enzymes are frequently resistant to another class of antibiotics, including aminoglycosides, quinolones, and chloramphenicol. The spread of carbapenem-resistant *K.pneumoniae* isolates, both locally and internationally, creates a therapy problem due to less effective antibiotics for therapy, leading to an increase in the evolution of extensively drug-resistant (XDR) and pan drug-resistant (PDR) Gram-negative bacteria. Resistance to fluoroquinolones can be high; ciprofloxacin-resistant *K. Pneumoniae* has increased worldwide in recent years [11].

Materials and Methods

Specimens collection include, 36 specimens swab from diabetic foot infection ulcer from Al-Sadder medical city and outpatient in Najaf City between November 2019 to February 2020.The specimens collected were (21 Male and 15 Female) with age group between 39-70 years. A single colony was taken from each primary positive culture on blood agar, MacConkeys agar, mannitol salt agar and repeat growth for gain pure culture and then it was identified depending on its morphological and cultural characteristics. The hypermucoviscosity phenotype of the *K. Pneumoniae* isolates was determined using a modified string test. Isolates of *K. Pneumoniae* were cultured on MacConkeys agar and incubated for 24 hours at 37oC. The formation of a viscous string of at least < 5 mm was considered positive result[12].The Specimens collection that identified by using Vitec-2 automated system were 36, GN identification card have been used for identification Gram negative bacteria. All specimens of *K. pneumonia* that isolate in current study identify by using VITEC2- system, AST card use to detection antibiotic Susceptibility test [13]. Any bacterial strain that resist to a minimum of at least 3 different classes of antibiotics is of a multi-drug resistance (MDR), any bacterial strain that remains susceptible to only one or two classes of antibiotics is of extensive-drug resistance (XDR) and any bacterial isolate resistance to all sub classes in all classes of antibiotics is of a pan-drug resistance (PDR). Polymerase chain reaction assays were performed at a reaction volume of 50 µl. Depending on their reference procedure as in Table (1), to detection predominance of K1 serotype , *rmpA* and *magA* genes that associate with capsular polysaccharide virulence factor .

Table (1): Primers that use in this study

Name	Oligo sequence (5'-3')	Product Size	Reference
rmpA	**F**: ACGACTTTCAAGAGAAATGA **R** : CATAGATGTCATAATCACAC	409 bp	(15)
K1	**F**: GTAGGTATTGCAAGCCATGC R: GCCCAGGTTAATGAATCCGT	1045 bp	(18)
magA	F:GGTGCTCTTTACATCATTGC R:GCAATGGCCATTTGCGTTAG	1283 bp	(15)

Results

In this study, 19(53%) of total 36 *K.pneumoniae* that isolate from diabetic foot ulcer patient were positive while 17(47%) were negative . This result agreed with Aljanaby and Alhasani [14] which recorded (62.5%) of clinical isolated *K.pneumoniae* positive for hypermucoviscosity test and another Iraqi study in Baghdad show that (60%) of clinical isolate were positive [5].All specimens Identify and undergo antibiotic sensitivity test by using VITEC-2 system , The results show high resistance to Ampicillin, Amoxicalv and Ceftriaxone (100%) , followed by Cefazoline (94%) , Gentamicin (89%) and Ceftazidim (78%). *K. pneumonia* recorded low resistance to Impenem (33%) , Ertapenem (31%),Tigicycline (17%) and Amikacin (14%), where 25 (69%) were multi-drug resistance and 11 (31%) were extensive- drug resistance (XDR) , Table (2) :

Table (2) : The resistance profile of *K. pneumonia* to different antibiotics

No	Antibiotic Resistance Profile	Percentage	Type of Resistance
1	AX.AMC,CFZ,CEP,ERT,GNT,TOB, CRO,CIP,MOX,LEV,TIG,NIT,TRM	88%	XDR
2	AX,AMC,CFZ,CRO,CEP,AZT,ERT, CIP,MOX,TIG,NIT,TRM	75%	XDR
3	AX,CFZ,CRO,AZT,GNT,CIP,MOX,TRM	50%	MDR
4	AX,AMC,CFZ,CEP,AZT,IMP,MRP, AMK,GNT,MOX,CIP,NIT,TRM,TOB	88%	XDR
5	AX,AMC,CRO,AZT,AMK,GNT,TOB	44%	MDR
6	AX,AMC,CFZ,CRO,AZT,TRM	38%	MDR
7	AX,AMC,CFZ,CFX,CRO,CEF,CFZ, ERT,IMP, AMK,CIP,LEV,NIT	81%	XDR
8	AX,AMC,CFZ,CFX,CRO,CEF,CFZ ERT,IMP ,TOB,CIP,LEV,NIT	81%	XDR
9	AX,CFZ,CFT,CTZ,CRO,CEP,GNT,CIP, LEV,NIT,TRM	69%	MDR

Cont... **Table (2) : The resistance profile of *K. pneumonia* to different antibiotics**

10	AX,AMC,CFZ,CFT,CTZ,CEP,CRO,CIP, LEV,NIT,TRM,	69%	MDR
11	AX,AMC,CFZ,CRO,CEP,MOX,NIT,TRM	50%	MDR
12	AX,CFZ,CRO,CEP,AZT, CIP,LEV,MOX,TRM	56%	MDR
13	AX,AMC,CFZ,CEP,CRO,AZT,ERT,IMP,MRP,T RM	63%	MDR
14	AX,AMC,CFZ,CRO,CEP,AZT,ERT,IMP,MEP, TOB,CIP, MOX,TRM	81%	XDR
15	AX,CFZ,CRO,CEP,AZT, CIP,LEV,MOX,TRM	56%	MDR
16	AX,CFZ,CTZ,CRO,CEP,NIT,TRM	44%	MDR
17	AX,CFZ,CROAZT,GNT,CIP,MOX,TRM	50%	MDR
18	AX,AMC,CFZ,CRO,CEP,MOX,NIT,TRM	50%	MDR
19	AX,AMC,CFZ,CRO,CEP,AZT,ERT,IMP,MEP TOB,CIP, MOX,TRM	81%	XDR
20	AX,AMC,CTZ,CFX,CFZ,CRO,CEP,IMP ERT,AMK,GNT,CIP ,LEV, TRM	88%	XDR
21	AX,AMC,CTZ,CFZ,CFT,CEP,CRO,ERT,IMP	56%	MDR
22	AX,AMC,CRO,AZT,GNT,TRM	38%	MDR
23	AX, CFZ,CRO,AZT,TRM	31%	MDR
24	AX,CFZ,CTZ,CRO,CEP,NIT,TRM	44%	MDR

AX :Amoxicillin , AMC : Amoxiculvanic , CFZ: Cefazolin , CEP : Cefepime , AZT: Aztronam , CRO : Ceftriaxon , CFX ; Cefexime , CTX :Cefatoxin , GNT : Gentamicin , TOB : Tobramycin , AMK : Amikacin , ERT : Ertapenem , IMP: Impenem , MRP: Meropenem , CIP : Ciprofloxacin , MOX :Moxifloxacin , LEV : Levofloxacin , TIG : Tigcyclin , NIT : Nitrofurantin , TRM : Trimmythprime

Klebsiella Pneumoniae serotype K1 was diagnosed with PCR by using a primer pair (K1 F & K1 R) specific for amplification . 23/36 (61 %) of *K. pneumonia* isolate were positive at 1045 bp while 14 (39 %) were negative figure (1) :

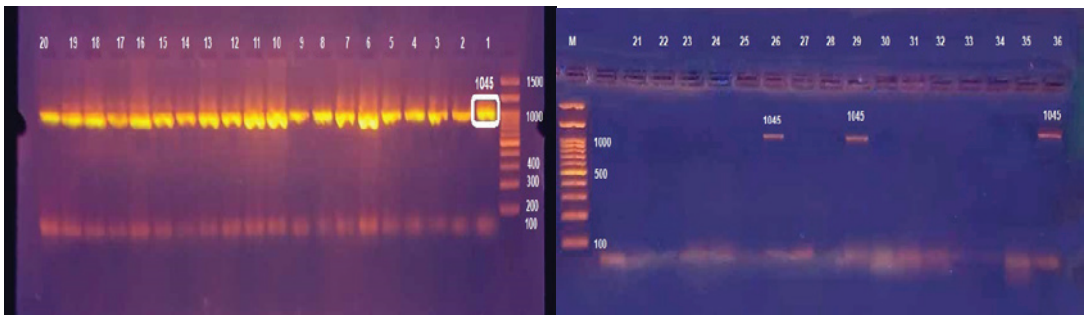

Figure (1): Gel electrophoresis of amplification product PCR for K1 serotype at 1045 bp .isolate (1-20,26,29,36) were positive , The electrophoresis was performed at 70 volt for 80 min.

The results also show that 21/36(58%) were positive to *magA* gene while 15(42%) were negative. PCR product was roughly 1283 bp in size, which is the same size obtained by Turton et al 2008 [15] ,when they used the same primer. (Figure 2) :

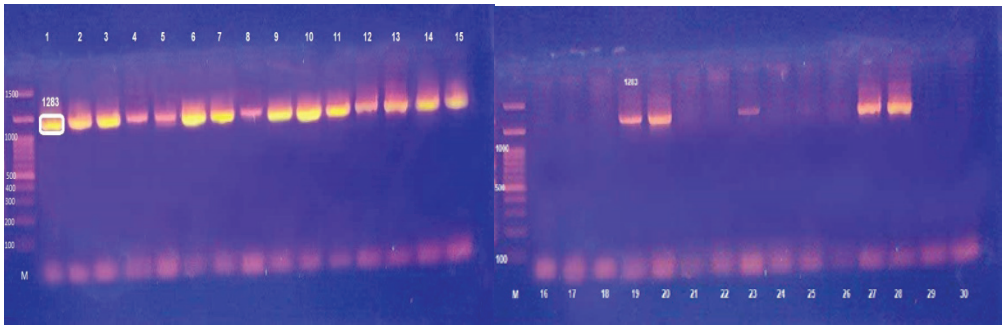

Figure (2) : Gel electrophoresis of amplification product PCR for *magA* gene at 1283 bp , line (1-15,19,20,23,27,28,36) were positive. The electrophoresis was at 70 volt for 80 min.

In this study *rmpA* amplified with was with a molecular size of about 409 bp and the results were positive in 22/36 .The results show that most isolates of *K.pneumoniae* that carrying to (*rmpA*) and (*magA*) genes in addition to K1 serotype were multi-drug resistance and extensive-drug resistance (Table 3):

Table (3): Correlation between isolate of K. pneumoniae that carrying capsular genes and multi antibiotics resistance .

Genes	Multi drug resistance (MDR)	Extensive drug resistance (XDR)
K1	13/36	10/36
rmpA	18/36	8/36
magA	11/36	10/36

Discussion

K1 serotype of *K .pneumonia* was the most common that isolate from diabetic foot patient than another's types of serotype . The negative result may be indicate to another types of serotype of *K .pneumonia* such as (K2 , K3,K75 and others) [15]. The results of this study is similar to another Iraqi study shows that K1 serotype was the most isolate 23 isolates (57.5%) from Forty *K. Pneumoniae* (36 clinical and 4 environmental isolates) [16]. The virulence determinants of *K. Pneumoniae* has focused on the capsule serotypes,K1 serotype and followed by K2 serotype considered the most virulent to humans . These results were in agreement with Yeh et al [9], who noticed that serotype K1 and/or K2 isolates were significantly more prevalent than those that were neither K1 nor K2 (Non-K1/K2). The absence of *magA* gene in some K1 serotype samples may due to use of antibiotic that inhabit the synthesis of capsular polysaccharide of *K. pneumonia* or due to mutation or deletion in this genes , if mutation happened in *magA* lead to loss capsule .Chuang et al [17] ,demonstrated that *magA* is located within an operon that is specific to serotype K1 cps gene clusters regardless of their sources. *rmpA* was located on a 180-Kb virulence plasmid. This plasmid is a multi-copy plasmid and responsible for expressing the mucoid phenotype of *K. Pneumoniae*. The plasmid contained also many virulence-associated genes. The presence of high resistance rate of antimicrobials producing *K.pneumoniae* strains in diabetic foot ulcer samples might be due to misuse and/or extreme use of common antimicrobials such as, B-lactam, aminoglycoside and 3rd generation cephalosporin (cefotaxime ,ceftriaxone, and ceftazidime). Many strains of *Klebsiella Pneumoniae* can produce several virulence factors, such as siderophores, capsules, fimbrial

adhesins and antibiotic resistance enzymes, which are important in the adherence, colonization, invasion and development of the infection[18]. In *K. Pneumoniae* ,the capsule confers resistance against the bactericidal activity of antimicrobial peptides, complement, and phagocytes. Resistance genes associated with plasmids were observed nearly resistance to all the antimicrobials clinically available, including Carbapenem lately[8];[19]. Besides, plasmids may also serve as carriers for other essential genetic elements of antimicrobial resistance, like integrons, and transposons. Quinolone resistance in the family *Enterobacteriaceae* is mostly attributed to the accumulation of mutations in the bacterial enzymes targeted by: DNA gyrase and DNA topoisomerase IV, and active efflux systems resulting in decreased intracellular accumulation of fluoroquinolones in *K.pneumoniae*. Results of Aljanaby [14] and Al Mahrezi[20] who proved that *Klepsiella Pneumoniae* isolated from different clinical samples were highly resistant to ampicillin , amoxicillin and chloramphenicol, and this can cause serious public health diseases because of the possibility of horizontal gene transfer to other bacterial spp.

Conclusion

Capsular polysaccharide gene and hypermucoid phenotype seen play important role in virulence and high level resistance to antibiotics where most of *K. Pneumonia* isolate with hypermucoid phenotype were positive to K1 , *magA* and *rmpA* genes (64%,58% and 61%) in specimens of that isolate from diabetic foot ulcer. Multi-drug resistance (MDR) and extensive-drug resistance (XDR), this is may be because misuse of antibiotic as well as not complete the detected dose of antibiotic treatment and / or over use , in addition to some virulence factors that related with plasmid horizontal transfer and presence of some genes that encode to drugs resistance . On other hand , there are many factor that participate in presence high level of antibiotic drug resistance bacteria isolate from diabetic foot ulcer patient such as long term diabetes mellitus , neuropathy , PVD , gender and less healthy awareness between patient

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Estimating the Level of Some Trace Elements in Samples of Canned Foods Spread in the Markets of Ramadi City - Western Iraq

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Abstract

Canned food is becoming increasingly popular in the market. In order to assess the level of concentration of some important elements in samples of these foods, ten different types of canned food were studied. The concentration level of nine elements (potassium, magnesium, calcium, iron, manganese, copper, zinc, lead and cadmium) was measured using atomic absorption spectroscopy. The results showed that the highest concentrations of potassium, magnesium, zinc and lead were in the chickpeas sample. While the highest concentration of calcium and manganese appeared in the fish sample. As for the elements of iron and copper, the highest concentration of them was in the olive sample. In the mushroom sample, the highest concentration of cadmium was shown.

Keywords*: trace elements, canned foods, atomic absorption spectroscopy, heavy metals.*

Introduction

Trace elements can be considered pollutants due to their ability to cause health damage to humans if they exist in the consumed food. Studying the level of these elements receives special attention in all parts of the world because their toxic effect appears even in its slight concentrations.[1] Toxicity of trace elements lies in their influence on the functions of the central nervous system in addition to their direct effect on the functioning of important organs such as the kidneys, lungs and liver.[2] Although life processes need different amounts of heavy elements (iron, cobalt, manganese, molybdenum and zinc), any slight increase causes damages to these organs.[3]

Other elements such as mercury and lead are considered toxic and do not have any role in life processes and their accumulation in living tissues causes many injuries.[4] It has become necessary to continue assessing the level of concentration of some heavy elements in many sources, including food, cosmetics,[5] water[6] and soil.[7] There is more than one source of contamination of food canned with heavy elements. Vegetables absorb trace elements from the water used in the watering process, which might be contaminated with heavy elements resulting from industrial processes. Therefore, there are many warnings about the use of industrial waste water and heavy sewage water in irrigation of farms, being a major reason for the transfer of heavy elements to the food chain.[8]

And trace elements can transfer to the food from the metal cans in which the food is preserved, which is a sheet of iron (steel) covered with a thin layer of tin, chromium or aluminum, which forms an inert layer that prevents the food from coming into contact with the metal. When this layer is exposed to damage or corrosion, the concentration of trace elements (tin, iron, cadmium and lead) increases in the food.[9] Many injuries and organ failure were recorded as a result of toxicity of these elements.[10] Several studies have dealt with estimating trace elements in canned foods, including Al-Rajhi study, which included estimation of elements in canned food and frozen chicken.[11] Also, there are two studies conducted in Iran to estimate the level of trace elements in canned vegetables and various foods.[12,13] Another study conducted in Iraq included an estimate of heavy elements in canned vegetables.[14] Most of

the studies conducted showed that the concentrations of heavy elements in canned foods and vegetables were within the limits permitted by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO).[15]

This study aims to estimate the level of trace elements in the food preserved in metal cans and spread in the markets of Ramadi (western Iraq) and compare it with the values permitted by the WHO and the Food and Agriculture Organization.

Materials and Methods

Study area:

The city of Ramadi is located in the center of Anbar Province, in western Iraq, and it is the center of the governorate. It is about 110 km away from Baghdad and has a population of about 1.8 million.

Sample collection and pretreatment:

A ten different types of samples of food preserved in metal cans were collected from the markets of Ramadi city, including (peas, chickpeas, beans, olives, fish, meat, mushrooms, sausage, chickens and mortadella). The samples were transferred to the laboratory and 5 gm of each sample was weighed and dried. In the oven at 105 degrees Celsius for 24 hours. The dry forms were placed in a ceramic eyelid, incinerated in an incinerator, and then placed in the dryer.

Digestion of samples:

The incinerated samples were digested using concentrated nitric acid (5 ml) with heating until the solution became clear. Transfer the solution to a 50 mL volumetric vial and complete the volume with distilled water. The elements (iron, copper, manganese, lead, cadmium and zinc) were determined using the AAS flame atomic absorption spectrometry. Also potassium, calcium and magnesium were determined using flame photometry.

Results and Discussion

In this study, a group of canned foods were purchased from the markets of Ramadi city - western Iraq. Where samples included (peas, chickpeas, beans, olives, fish, meat, mushrooms, sausage, chickens and mortadella). The concentration level of some trace elements and heavy metals has been studied in the samples under investigation using atomic absorption spectroscopy

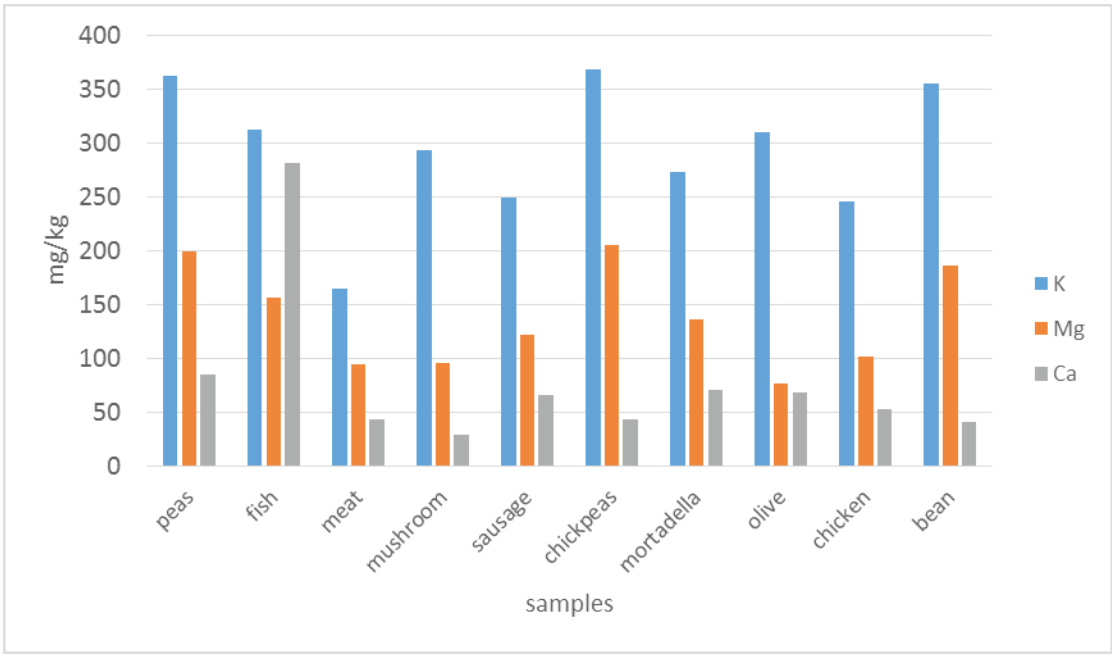

Fig. 1 Concentration of potassium, magnesium and calcium in different food samples.

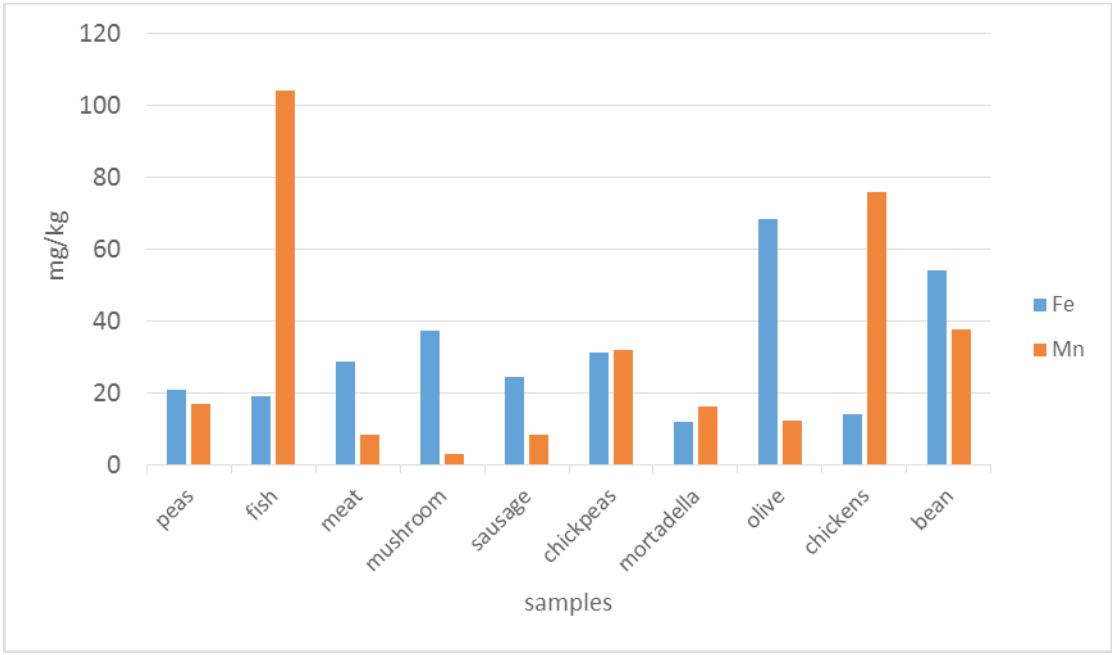

Fig. 2 Concentration of iron and manganese in different food samples.

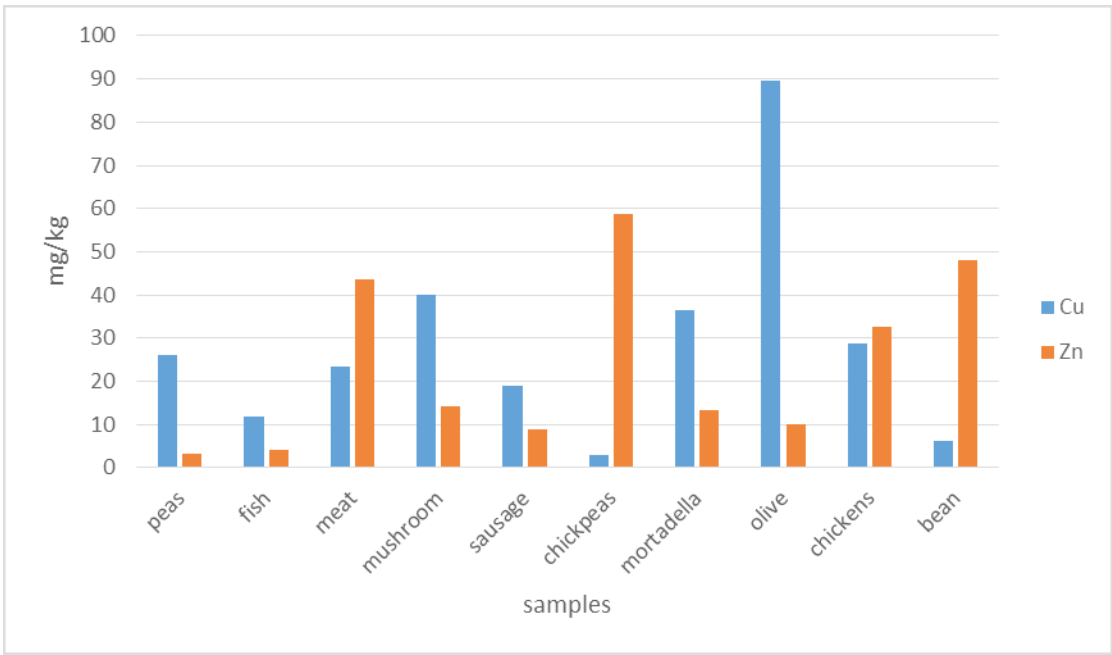

Fig. 3 Concentration of copper and zinc in different food samples.

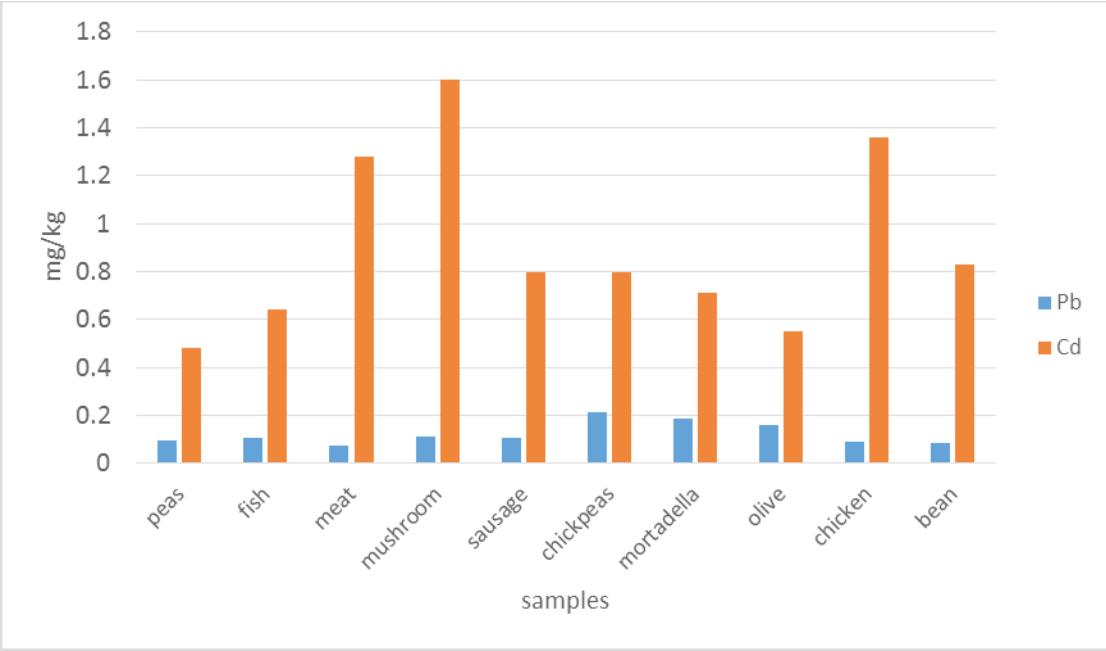

Fig. 4 Concentration of lead and cadmium in different food samples.

Figure 1 shows the concentration level of potassium, magnesium and calcium in the samples of canned food included in the study. It appears that the highest level of potassium was in the chickpea form, while the lowest concentration was in the meat. While the highest concentration of magnesium appeared in chickpeas, and the lowest concentration in olives. As for calcium, it was highest in fish and lowest in the mushroom model.

Some elements such as manganese, zinc, iron and copper are essential for human health, provided they do not exceed the permissible limits. The concentration of these elements in the studied models is shown in Figures 2 and 3. It appears that the highest iron concentration was in the olive form and the lowest concentration of it was in the mortadella. As for the elements of manganese, copper and zinc, they have the highest concentration in the fish, olive and chickpea models, respectively. Also The lowest concentration of these elements was in mushrooms, chickpeas, and peas, respectively.

Two elements of the heavy toxic elements, lead and cadmium, were studied as shown in Fig. 4. It was found that the higher concentration of lead in chickpeas and the lower concentration in the meat. As for the cadmium element, the results show that the higher concentration in the mushrooms and the lower concentration in the peas.

Table (1) statistical summary of elements in studied canned foods.

Metal	Mean (mg/kg) ± SD	Median	Range
K	293.85 ± 63.08	302.24	203.11
Mg	137.92 ± 47.04	129.41	128.1
Ca	78.76 ± 73.38	59.58	252.53
Mn	31.68 ± 33.23	16.8	100.8

Cont... **Table (1) statistical summary of elements in studied canned foods.**

Fe	31.13 ± 17.94	26.62	56.22
Cu	28.56 ± 24.63	24.8	86.59
Zn	23.66 ± 20.21	13.75	55.46
Pb	0.123 ± 0.046	0.108	0.136
Cd	0.905 ± 0.376	0.8	1.12

It appears from the results in the above table that most of the elements estimated in the studied samples of canned foods are within the permissible limits, except of cadmium, its concentration was higher than the permissible limit (standard levels of the WHO) in the studied samples. The order of the elements in terms of their concentration in the canned foods as shown in Table 1 are K > Mg > Ca > Mn > Fe > Cu < Zn > Cd > Pb.

Conclusion

In this study, we assessed the concentration of nine different elements in ten different samples of canned food available in the markets of Ramadi city - western Iraq. It was concluded that all the elements under study had their concentrations within the permissible limits internationally, except for cadmium above the permissible limit. The highest concentration of lead and cadmium was found in chickpea and mushroom samples, respectively.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Possible Effect of vitamin D3 on Follicular Maturation in Polycyctic Ovarian Syndrome (PCOS) Women

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Abstract

Polycystic ovarian syndrome (PCOS) is the most common reproductive age endocrine condition in women. Most likely, one of the major problems of PCOS patients are vitamin D3 deficiency. Therefore, investigation of the association between vitamin D3 deficiency and ovulation in PCOS women patients might help to explore the possible relationship between them. Sixty-six patients were recruited from a private clinic in Karbala city / Al-Hindiya district, Iraq. The patients were divided into two groups (33 patients each), the first group treated with metformin 850 mg, b.i.d, and placebo. The second group treated with metformin 850 mg, b.i.d and vitamin D3 oral supplementation 5000 IU/day for 3 months. Ultrasonography was performed monthly on day 13 to assess the follicular size. D3 blood level was measured at the end of the 3 months. The present study demonstrated that daily D3 5000IU in PCOS women with deficient D3 whom already on scheduled metformin 850 mg b.i.d for 3 months significantly ($p<0.05$) increased the mid-cycle follicular size by one 3rd in comparison to placebo group. The important finding was that vitamin D3 might enhance follicular maturation in PCOS patients.

Keywords: *D3 supplementation, ovulation, polycystic ovarian syndrome (PCOS).*

Introduction

The most widely diagnosed female endocrine disease is polycystic ovarian syndrome (PCOS), which is almost 5-10% among females in generative age [1]. PCOS has at minimum two measures of 3: raised androgenic hormones, unbalanced or absent ovulation and protracted ovaries with additional than 12 follicles each [2]. In addition, PCOS is linked to a variation of cardiovascular danger causes such as insulin resistance (IR), obesity, decreased glucose tolerance, elevation blood pressure, and type 2 diabetes [3]. Females with PCOS commonly consume metabolic illnesses and IR, which may be related with the unusual vitamin D metabolism [4]. Vitamin D3 affects the metabolism of glucose and insulin and is essential in T2DM [5]. The mechanisms through which vitamin D3 levels might have an association with an IR or T2DM are not yet clear. According to studies, it might be related to: first, low concentrations of vitamin D3 might lead to increase levels of serum parathyroid hormone and by the way altering glucose and reducing the susceptibility to insulin [6]. In addition, high than 300 genes counting the genes related with glucose metabolism are regulated by vitamin D3 and the vitamin D3 receptor (VDR) [7]. Based on the above-mentioned associations between vitamin D3 besides insulin, or glucose, numerous earlier studies explored vitamin D function in PCOS [8]. However, there was no strong consensus as regards the significance of serum vitamin D3 levels in PCOS and non- PCOS patients, a reverse association was found in PCOS patients with serum 25-hydroxyvitamin D3 levels with metabolism disturbances [9]. Moreover, previous studies have shown that PCOS women also have vitamin D deficiency [10]. Furthermore, several studies have shown the improvement of several PCOS laboratory and clinical findings by supplementation of vitamin D3 among the vitamin D3 deficiencies PCOS women [11]. However, in general and according to our knowledge, there has been no significant impact regarding the relationship between PCOS, IR and vitamin D3 deficiency. The aim of this study was to investigate the relationship between

vitamin D3 and mid-cycle ovarian follicle maturation in PCOS patients with vitamin D3 deficiency.

Materials and Methods

Study design

The present study is a clinic based prospective observational study conducted on 66 patients with PCOS, both suspected and already diagnosed who attending to a private clinic in Karbala city / Al-Hindyya district, Karbala province, Iraq, from 13th of January 2019 to 4th of July 2019.

Criteria for selecting patients

•All newly suspected and diagnosed cases of PCOS.

•Aged 20-35 years.

• History of infertility, PCOS with low D3 blood level (<10ng/ml).

Exclusion criteria included:

•Any diagnosed case of PCOS who was on and had history of taking vitamin D3 and calcium supplement within period of one year.

•Diagnosed cases of PCOS who was under treatment and recovered with treatment (medical or surgical).

•Patients who were not willing to take part in our study.

The patients were distributed into two groups:

1. Group 1: (33 patients) treated with metformin 850 mg, b.i.d, and placebo.

2. Group 2: (33 patients) treated with metformin 850 mg, b.i.d and vitamin D3 oral supplementation 5000 IU/day for 3 months (single blind technique).

Patients follow up parameters included mid-cycle follicular size was assessed monthly on day 13 using Ultrasonography. 2-D3 blood level was measured before and at the end of the 3 months.

Statistical Analysis

SPSS version 20 (SPSS, IBM Company, Chicago, IL 60606, USA) was used to conduct statistical analysis. Consistent variables (means ± SD) have been presented. The means between two groups is compiled with independent t-test samples. The t-test paired has been used to compare readership methods. A *p-value* of up to 0.05 was considered significant. Data were subjected to one-way ANOVA testing and multiple comparisons Dunnett testing.

Ethical Consideration : The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Verbal consents of the patients were taken after explaining the purpose of the study to the patients. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee.

Results

3.1 Effects of metformin and placebo on D3 serum level in PCOS patients. There was no significant (p>0.05) changes in D3 blood level between control group (before metformin, 850mg + placebo) and a group treated with the same regime for 3 months.

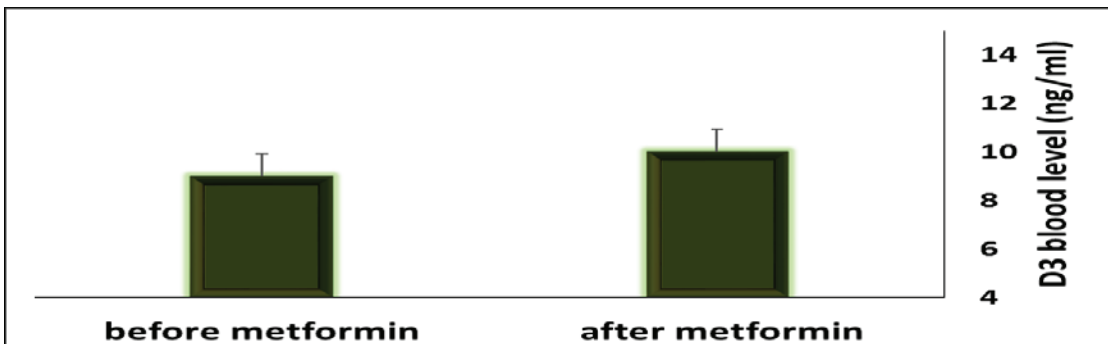

Figure 1. The effect of Metformin 850 mg b.i.d and placebo on D3 blood level (ng/ml) for 3 months P > 0.05. Data is stated as the mean changes in D3 level (± SEM) (n =33 in each group). In addition, One-way ANOVA test stated Statistical significance following correction by Dunnett's for multiple comparison.

3.2 Effect of metformin and D3 on D3 serum level in PCOS patients. There was a significant ($p<0.05$) increase in D3 blood level after oral supplementation of metformin, 850 mg + vitamin D3 5000 IU for 3 months in comparison to control group.

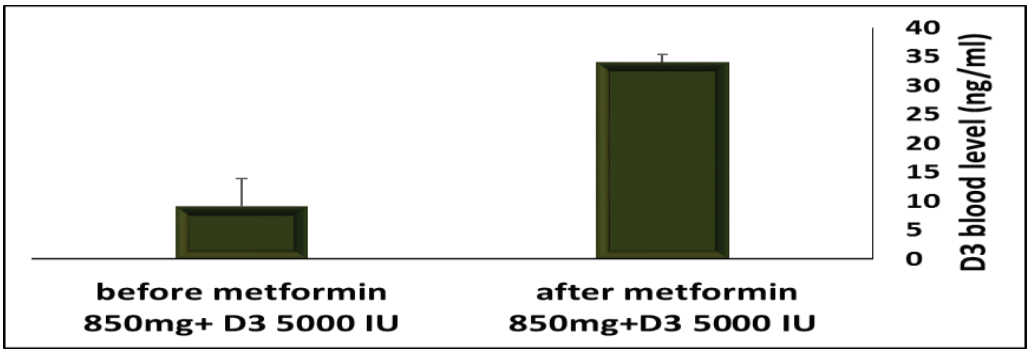

Figure. 2 The effect of Metformin 850 mg b.i.d and D3, 5000IU on D3 blood level (ng/ml) for 3 months. P < 0.05. Data is stated as the mean changes in D3 level (± SEM) (n =33 in each group). In addition, One-way ANOVA test stated Statistical significance following correction by Dunnett's for multiple comparison.

3.3 Effect of metformin and placebo on mid-cycle follicular size in PCOS patients. There was no significant ($p>0.05$) changes in mid-cycle follicular size between control group (before metformin, 850 mg + placebo) and a group treated with the same regime for 3 months.

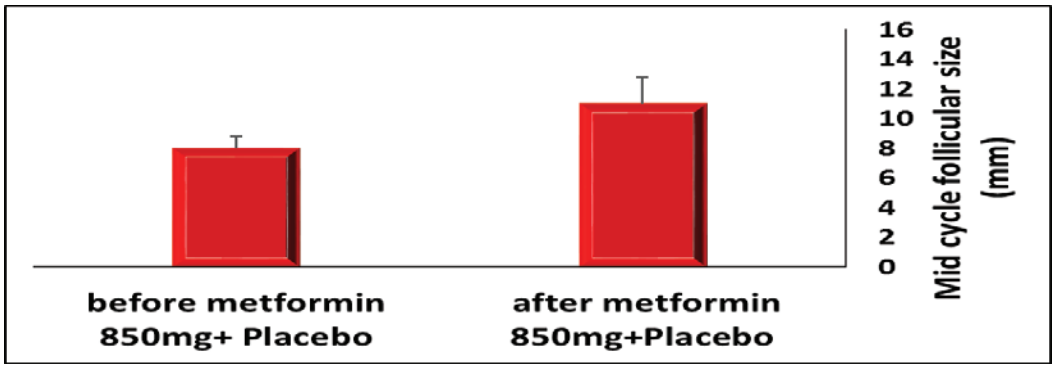

Figure. 3 The effect of Metformin 850 mg b.i.d and placebo on follicular size (mm) for 3 months. P > 0.05. Data is stated as the mean changes in follicular size (± SEM) (n =33 in each group). In addition, One-way ANOVA test stated Statistical significance after correction by Dunnett's for multiple comparison.

3.4 Effect of metformin and D3 on mid-cycle follicular size in PCOS patients. There was a significant ($p<0.05$) increase in mid-cycle follicular size after oral supplementation of metformin, 850 mg + vitamin D3 5000 IU for 3 months in comparison to control group.

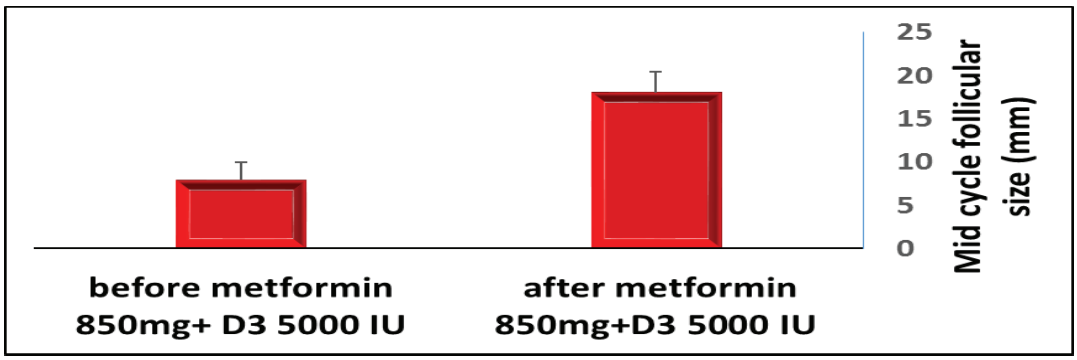

Figure. 4 The effect of Metformin 850 mg b.i.d and D3 5000IU on follicular size (mm) for 3 months. P < 0.05. Data is stated as the mean changes in follicular size (± SEM) (n =33 in each group In addition, One-way ANOVA test stated Statistical significance following correction by Dunnett's for multiple comparison.

3.5 Comparison between effect of metformin+ placebo and metformin+ vitamin D3 on serum D3 level in PCOS patients. There was a significant ($p<0.05$) increase in D3 blood level after oral supplementation of metformin, 850 mg + vitamin D3 5000 IU for 3 months in comparison to metformin 850mg + placebo group.

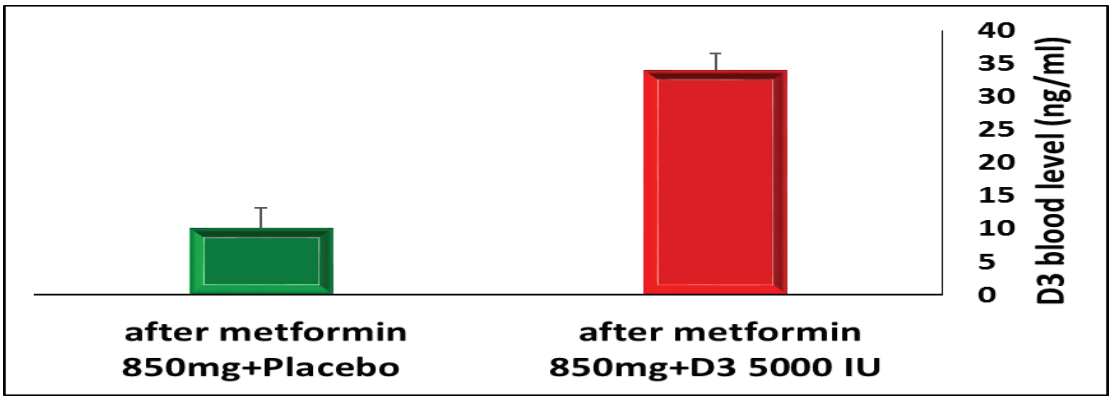

Figure. 5 The effect of Metformin 850mg b.i.d + placebo and D3 5000IU on D3 blood level (ng/ml) for 3 months. P < 0.05. Data is stated as the mean changes in D3 level (± SEM) (n =33 in each group. In addition, One-way ANOVA test stated Statistical significance after correction by Dunnett's for multiple comparison.

3.6 Comparison between effect of metformin+ placebo and metformin+ vitamin D3 on mid-cycle follicular size in PCOS patients. There was a significant ($p<0.05$) increase in mid-cycle follicular size after oral supplementation of metformin, 850 mg + vitamin D3 5000 IU for 3 months in comparison to metformin 850 mg + placebo group.

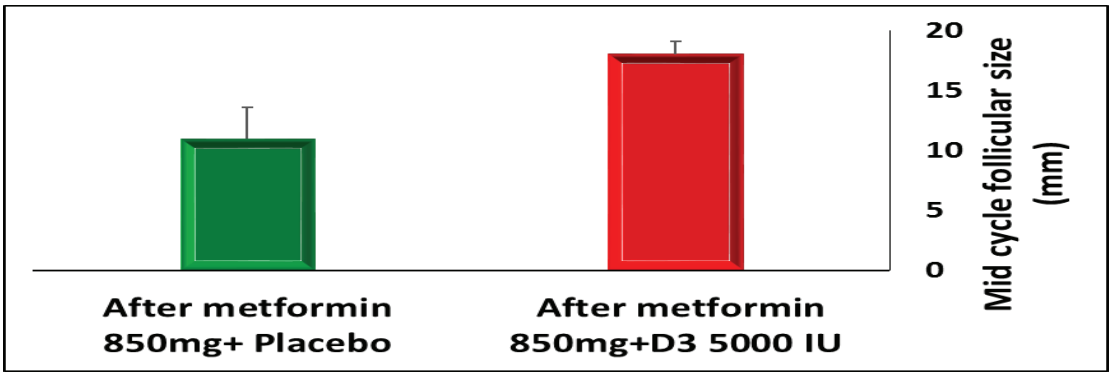

Figure. 6 The effect of Metformin 850mg b.i.d + placebo and D3 5000IU on follicular size (mm) for 3 months. P < 0.05. Data is stated as the mean changes in follicular size (± SEM) (n =33 in each group). In addition, One-way ANOVA test stated Statistical significance following correction by Dunnett's for multiple comparison.

Discussion

Vitamin D3 effect on ovarian role showed mostly in the medical studies of its consequence on obstetrical results, mostly in patients with ovarian illnesses looking for fertility management [11]. Presently, information are not reliable concerning the association between follicular liquid vitamin D3 focuses and gestation degrees, with optimistic, adverse, and unimportant [12] associations stated. However, vitamin D3 supplementation looks to be supportive to people's grief from obesity besides insulin resistance. Revisions in PCOS patients designated that vitamin D3 supplementation better follicular development, leading follicle creation, and gestation rates increase, and reinforced the recommencement and upkeep of menstrual sequences [13]. Pathophysiological contrivances by which vitamin D3 controls follicular development besides ovum eminence are quiet unclear because absence of sufficient and correct study replicas.

PCOS is the most common reproductive age endocrine condition in women [1]. One of the major problems of PCOS and related infertility patients are most likely vitamin D3 deficiency [2] However,

there is a continuing debate that if vitamin D3 levels correlate with the risk of PCOS, and whether the supplementation of vitamin D3 is effective therapy for PCOS and consequently infertility. Basically, this study was performed to assess the possible effects of vitamin D3 supplementation in PCOS patient with vitamin D3 deficiency on mid-cycle follicular size on the 13th of ovulation and subsequently on the infertility, as a major issue associated with PCOS, in general.

In the worldwide of reproduction, the consequence of vitamin D mainly exposed in tests with mice. Mice who are both lacking in vitamin D, or who absence the receptor of vitamin D, can confirm low development of the uterus besides inability to form usual developed follicles, subsequent in infertility. If gestation is occurred, the fetuses demonstration growth deficiency. Reproduction regularized in mice by supplementation of vitamin D3, not calcium only, suggesting that vitamin D character in woman reproduction is not connected to helping the absorption of calcium from the body [14]. In previous studies, the role of vitamin D is highlighted in female reproductive activities such as steroidogénesis, which may improve granulose cell proliferation, ovulation and follicular growth [15]. Parikh et al. [16] showed that vitamin D led to the production of a growth factors-binding protein 1 in human ovarian cells, including progesterone, estrogen, and insulin. Nevertheless, vitamin D deficiency in the growth of ovarian follicles and PCOS patients is normal in women with PCOS. Furthermore, vitamin D deficiency is still not entirely clear. Therefore, vitamin D3 effect on the ovarian follicular development of women with PCOS was investigated by the study in this field.

The present study depicted that daily supplementation of vitamin D3 5000 IU in PCOS women with deficient D3 blood level whom already on scheduled metformin 850mg b.i.d for 3 months significantly ($p<0.05$) increased the mid-cycle follicular size by one 3rd in comparison to placebo group.

This is in line with the Kotsa et al study [17], who showed the fact that the majority of PCOS women have a vitamin D and PTH-vitamin D axis abnormality. Thys-Jacobs et al demonstrated the follicular arrest and follicular production of Vitamin D deficiency that is strongly consistent with our findings [18].

Furthermore, an important previous study showed that vitamin D has promoted folliculogenesis and follicular growth in rats with PCOS through increased concentrations of estrogen and progesterone and regulation of the FSH and LH ratios [19]. These results are consistent with those of Kinuta et al [17]. Moreover, Nasim et al showed that vitamin D supplementation regulates the ratio of androgen hormones, increases the sensibility to insulin in rats that have PCOS, thereby stimulating dominant follicles to develop and ovulating matured follicles [20].

Overall, PCOS is the most common reason of an ovulatory infertility in women. The mechanism behind this issue is still unclear. In PCOS, ovarian composition firmly connected to the metabolic engagements detected in this illness; so, a valued result of vitamin D3 on the metabolic changes might interpret into a improved and reinstated ovarian physiology [21]. Maximum PCOS females are both obese or overweight, creation it problematic to organize that vitamin D3 lack subsidizes to the pathogenesis of PCOS self-sufficiently from raised body mass index [BMI]. Obesity may reduction circulating 25OH-D by misleading this lipophilic vitamin inside adipose tissue. So, the acceptable possessions of vitamin D3 on the metabolic changes in PCOS, composed with the rise in anti-inflammatory solvable receptor for progressive glycation yields force alter into an upgrading in ovarian composition. Supplementation of vitamin D3 therefore holds a possible of flattering a likely beneficial assistant for the dysfunction of ovulation and metabolic changes experiential in females with PCOS [22].

Conclusion

Obviously, the results from this study strongly showed that the vitamin D3 blood levels are related to the risk of immature follicular size in patients with PCOS. However, in the PCOS women, with and without calcium and vitamin D3 deficiency, it is very hard to find out the exact therapeutic impact of Vitamin D3 supplementation and its effect on the sex hormones, obesity, and IR to be the subject of further study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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BOX- Genotyping of Carbapenem Resistant *Acinetobacter baumannii* Clinical Isolates from Diyala/Iraq

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Abstract

The aim of study is to determine the typing of Carbapenem-resistant *A. baumannii* clinical isolates from some hospitals in Diyala by the BOX-PCR technique. This study was carried out during the period from February 2020 to the end of September 2020. Out of 400 specimens, 53 isolates of *Acinetobacter baumannii* were recovered. All isolates were tested toward the different class of clinically important antibiotics (12) by using agar diffusion method. The results of resistance were as following: piperacillin 53%, Ticarcillin/clavulanic acid 75%, cefotaxime 94% and ceftazidime 66%, cefepime 53%, gentamicin 84%, amikacin 49%, ciprofloxacin 47%, levofloxacin 38%, Aztreonam 87%, Imipenem and Meropenem 38%. Twenty (20) isolates of *Acinetobacter baumannii* were found to be resistant to carbapenems. The BOX–PCR method was used to detect the relationship among these carbapenem resistant isolates, the fingerprinting patterns of the isolates were shown 15 bands on gel electrophoresis, with molecular weight ranging between (100-3000 bp) among the 20 carbapenem resistant *Acinetobacter baumannii* isolates. Genetic relationship of different isolates of *Acinetobacter baumannii* was done by using BOX-PCR method and Dendogram analysis. The results showed the genetic relationship between *Acinetobacter baumannii* 4 clones, while 12 isolates contained different genotyping. In conclusion, the present study showed that the BOX-PCR technique is reproducible, easy, fast and cost effectiveness tool for investigating the genetic diversity of *A. baumannii* isolates.

Keywords: *Acinetobacter baumannii, BOX- PCR Method, Genotyping, Carbapenem Resistant.*

Introduction

Acinetobacter baumannii bacteria is a coccobacilli Gram negative bacterial species, aerobic, non-motile, not composed of spore, which is oxidase test negative and catalase test positive. *A.baumannii* is an emerging nosocomial pathogen causing a variety of health care-related infections, and it is associated with a significant morbidity and mortality [1]. This bacteria causes a wide range of nosocomial infections, such as ventilator-associated pneumoniae (VAP), surgical site infections, secondary meningitis, respiratory tract inflammation, wound infection, and urinary tract infection (UTI) [2]. Following the prevalence and recurrence of *A.baumannii* associated infections that occur in different wards of the hospital, such as the intensive care units (ICU), and since the strain associated with the outbreak can originate from different regions and locations [2,3], therefore, find the source of infection by different molecular methods are very important [4]. *A. baumannii* is often resistant to multiple antibiotics by upregulating or acquiring resistance determinants [5]. Carbapenem-resistant *A. baumannii* (CRAb) is considered an especially major public-health threat due to associated treatment difficulties. Outbreaks of CRAb were reported worldwide [6], and in many instances they were caused by a few international clonal lineages of *A. baumannii* [7].

Surfaces in hospitals are generally divided into densely and sparsely contaminated ones [8]. Objects in the first group, e.g. doorhandles, computer keyboards and control panels, patient charts,bed frames, are the most critical ones, as they are frequentlytouched by medical and nursing staff subsequent to patient contacts [9]. *A. baumannii* was shown to be able to survive in hospitalenvironments for prolonged periods, contaminating patient careitems, as well as non-patient care areas. Hence, these inanimatesurfaces often serve

as intermediaries in spreading microorganismsfrom one patient to another [6].

Acinetobacter baumannii bacteria has been classified in the American Society of Infectious Diseases as one of the most antibiotic-resistant microorganisms around the world that have the ability to resist many antibiotics, including cephalosporin and anti-penicillin wide-spectrum, anti-fluoroquinolones and anti-aminoglycoside[10]. Typing of bacterial isolates is an important process in the diagnosis, treatment and epidemiological research. Current methods of typing bacterial isolates can be classified into two main categories: phenotyping and genotyping. Genotyping, which refers to differences in bacterial isolates based on their genetic content, has recently been widely used for typing of bacterial isolates [11].

The method of genotyping has become a pattern for understanding the essential mechanisms of infection and the relationship between bacterial strains and their geographical spread in the field of infection control. It is an important tool in determining the source of infection in hospitals. As well as genotyping acts to differentiate between bacterial isolates on the basis of genetic content [12]. There are several methods of genotyping that are important in the finding of genetic kinship between bacterial isolates, BOX-PCR is the easiest to perform and less expensive.The BOX method is a useful method of epidemiological studies of many species of Gram positive and negative bacteria [11,12] . Therefore, due to the lack of comprehensive studies in this field, this study aimed to determine the typing of Carbapenem-resistant *A. baumannii* clinical isolates from some hospitals in Diyala by the BOX-PCR method.

Materials and Methods

Isolation and Identification of Bacterial Isolates

All clinical samples were collected forms wounds ,urin , blood and burns infection from different hospitals in Baquba/Diyala, Iraq during the period of beginning of February 2020 to the end of September 2020.The isolates were identified by culturing all sample on MacConkey agar, blood agar and also used biochemical tests including oxidase and catalase tests and further identification were done by using Vitek 2 system (BioMerieux, France) [13].

Antibiotic Susceptibility Testing

To estimate potential resistance of *Acinetobacter baumannii* isolates against 12 items of antibiotics from different classes, all isolates had been subjected to antibiogram test according to (CLSI-2017)[14], for Pipracillin, Ticarcillin/clavulanic acid, Cefotaxime, Ceftazidime, Cefepime, Ciproflxacin, Levofloxacin, Gentamicin, Amikacin, Aztreonam, Imipenem and Meropenem.The isolates were initially screened for carbapenems susceptibility by disk diffusion method using imipenem, and meropenem (10 µg each) antibiotic disks. Results were interpreted according to guidelines recommended by CLSI guidelines.

DNA Extraction

Genomic DNA was extracted from bacterial isolates using extraction Kits of Genomic DNA, Purification depending on instruction of manufacturing company (Promega USA). The DNA concentration was mishearing by used Quantus Florometer (Promega, USA).

BOX-PCR

The *A.baumannii* isolates were fingerprinted by BOX-PCR using BOX primer (52–CTACGGCAAGGCGACGCTGACG –32) which produces a PCR product with variable bands (bp) [15]. Amplification was performed with a 25µL reaction mixture that consisted of 5µL GO Taq Green Master Mix), 5µL template DNA, 4 µL Primer (10 pmol/µL) and 11µL deionized sterile D.W. (Promega USA). PCR reaction tubes were transferred into thermal cycler that was programmed as following: initial denaturation for 5 mints at 95 °C, (the conditions for each cycle were: 1 min. at 94 C̊, 30 sec. at 46 °C and 1min. at 72 °C), and final extension at 72 °C for 5 mints. Amplified PCR products were detected by agarose gel electrophoresis using a 1% agarose gel in TBE buffer (Promega, USA) with 5µL Ethidium bromide (10mg/mL) (Promega, USA), at 100 vol./ cm2 for 90min. The DNA bands were visualized and photographed under UV light. The size of the products was analyzed in comparison to a MW100-3000 bpDNA ladder (Promega, USA) [16].

Statistical Analysis

Dendogram analysis was done by using PAST program (version 0.45) to determine the genetic

relationship between all carbapenem resistant *A.baumannii* isolates.

Results and Discussion

Results of distribution of different clinical isolates showed that most isolates obtained, were from burns (49%) while other isolates distributed among wound 13(24.5%), isolates from blood 9(17%) , and the last 5 (9.5%) isolates from urin (Table 1). Out of 400 clinical samples, only 53 isolates (13.25%) were belonged to *A. baumannii*.

Table(1): Distribution of *A. baumannii* isolates among different clinical samples

Type of Clinical Sample	No. (%) samples	No. (%) of A. baumannii isolates
Burn swab	100 (25%)	26(49%)
Wound swab	100 (25%)	13(24.5%)
Blood	100 (25%)	9(17%)
Urin	100 (25%)	5(9.5%)
Total	400 (100%)	53(13.25%)

Antimicrobial sensitivity test

Fifty three *A. baumannii* isolates were screened for their resistance to 12 different types of antibiotics. Results in table (2) show that isolates varied in their resistance to the antibiotics. It was found that 53% and 75% of the isolates were resistant to piperacillin and ticarcillin-clavulanic acid,. The rates of resistance to the cephalosporins were as follows: cefotaxime 94%, ceftazidime 66% and cefepime 53% respectively.

Table (2): Antibiogram susceptibility of *A. baumannii* isolates toward antimicrobial agents

Antibiotic	Resistant isolates No. & %
Pipracilin	28(53%)
Ticarcillin/clavulanic acid	40(75%)
Cefotaxime	50(94%)
Ceftazidime	35(66%)
Cefepime	28(53%)
Ciprofloxacin	25(47%)
Levofloxacin	20(38%)
Gentamicin	45(84%)
Amikacin	26(49%)
Aztreonam	46(87%)
Imipenem	20(38%)
Meropenem	20(38%)

Aminoglycosides and quinolones resistance were variable among *A.baumannii* isolates; 84% to gentamicin and 49% to amikacin, however, resistance to quinolones ciprofloxacin was detected as 47% and levofloxacin 38% among isolates.Resistance to monobactam was high when 87% of isolates being resistant to aztreonam. Results revealed that 20 *A.baumannii* isolates (37%) were found to be carbapenem (imipenem and meropenem) resistant.

BOX Fingerprinting

Initially, the genetic variability of 20 carbapenem resistant *A. baumannii* strains tested were investigated with the BOX-PCR method described previously [17]. The BOX-PCR genotyping method was used to detect the relationship between isolates of carbapenem resistant *A. baumannii* that isolated from burns cases, wonds, blood and urin. The genetic fingerprint 15 bands with molecular weights ranging from (100-3000) bp between the isolates of carbapenem resistant *A.baumannii* 100 bp, 200 bp, 300 bp, 350 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 950 bp, 1000 bp, 1500, 2000 bp, 3000 bp the percentage were 10%,5%, 20%, 15%, 30%, 50%, 55%, 65%, 45%, 40%, 60%, 50%, 30%, 10% at respectively as shown in table (3). Two main groups (**A** and **B**) were observed. BOX-PCR typing showed 4 groups of genotypes and 10 unique isolates. In spite of differences in the location and isolation sources of these isolates, a clear clonality was observed, which indicated the epidemiology of these isolates figure (1). The results showed a genetic affinity between four groups of carbapenem resistant *A. baumannii* isolated from various sources from several hospitals in Diyala, while 12 isolated had a different genotype as illustrated in figure (1). This agreed with another study found a genetic affinity between four groups of *A. baumannii* isolated from various sources from several hospitals in China [18].

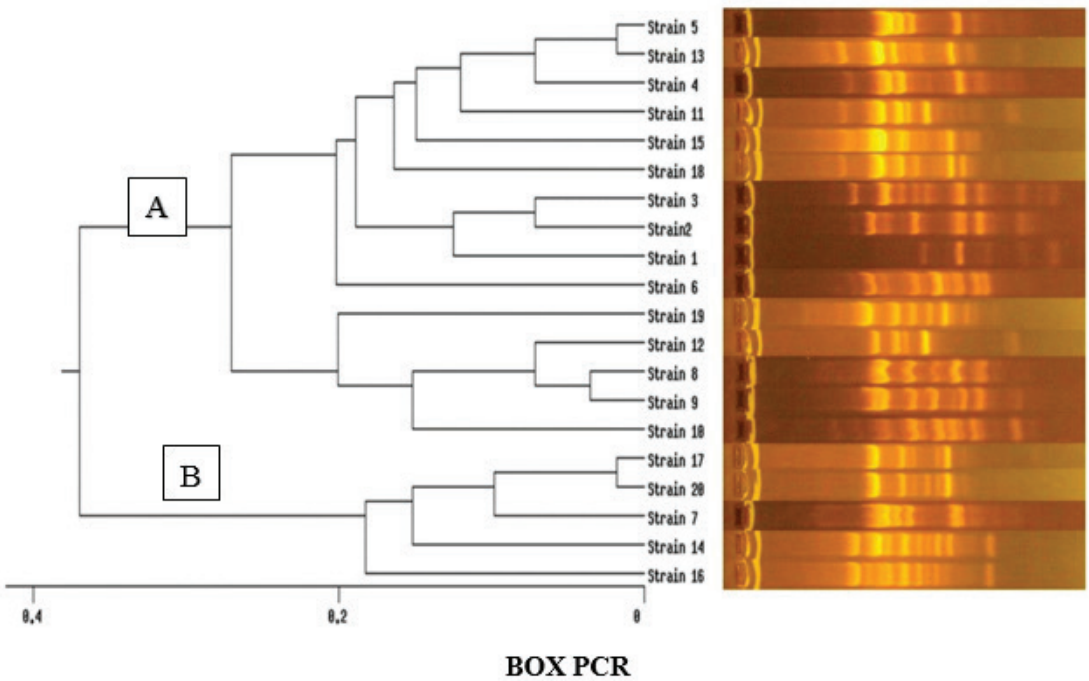

Figure (1). BOX-PCR generated dendrogram showing genetic relatedness of 20 *Acinetobacter baumannii* isolates.

Genotyping method is a useful for detecting vector strains as well as identifying the epidemic between isolation and genetic relationships among isolates and can be categorized into different groups using genotyping methods. This *A. baumannii* heterogeneity differences in several studies from different parts of the world, demonstrate the impact of environmental factors and the level of hospital hygiene on the distribution

and genetic clonal formation variation. As the results demonstrated, our studied carbapenem resistant *A. baumannii* clinical isolates were genetically diverse and heterogeneous, suggesting that multiple subtypes of the species are involved in infection. Moreover the findings of present work suggest that genotyping by BOX-PCR, may play an important role in routine epidemiological surveillance, and in the identification of the source of transmission of *A. baumannii* in the hospitals.Many studies was demonstrated that BOX-PCR method is a highly differentiated power in the study clinical isolation in the same genetic group indicating the transmission of pathogens from the hospital environment to patients as well as the distribution of pathogens in the hospital environment [19].

Table (3): Molecular weight and percentages of Box Bands.

Band	Molecular weight (bp)	No. of isolate & percentage %
BOX1	100	2(10%)
BOX2	200	1(5%)
BOX3	300	4(20%)
BOX4	350	3(15%)
BOX5	400	6(30%)
BOX6	500	6(30%)
BOX7	600	10(50%)
BOX8	700	11(55%)
BOX9	800	13(65%)
BOX10	900	9(45%)
BOX11	950	8(40%)
BOX12	1000	12(60%)
BOX13	1500	10(50%)
BOX14	2000	6(30%)
BOX15	3000	2(10%)

Additionally the results of study by Al-Shwalkh *et al*. (2018)[20] who found a genetic affinity between the isolates of *Pseudomonas aeruginosa* used the method of BOX-PCR, and identified 16 genotypes and molecular weights ranging from (140-1000) between isolates of *Pseudomonas aeruginosa* isolated from different sources. Finally, the results of current study in agreement with Abdulla (2020)[21] who found a genetic affinity between the isolates of *Escherichia coli* used the method of BOX-PCR, and identified 12 genotypes and molecular weights ranging from (150-1500) between isolates of *Escherichia coli* isolated from different sources .

Conclusions

High isolation rate of carbapenem resistant *A.baumannii* (CRAB) was detected in this study. In conclusion, the present study showed that the BOX-

PCR technique is reproducible, easy, fast and cost effectiveness tool for investigating the genetic diversity of *A. baumannii* isolates.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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Investigation of Some Trace Elements levels & Antioxidants in Patients with Thalassemia Major and Sickle Cell Anemia-Thalassemia in South of Iraq

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Abstract

Back ground: Thalassemia major) THM(is the most common single gene disorder. It is a hereditary blood disease characterized by reduced or absent production of beta globin chains. For the patients with SCA-Thal, they have two mutations (sickle cell anemia and thalassemia). The ratio of minerals and trace elements in the patients' blood played an important role in many biological processes. This study aims to measure the level of seven elements, Fe, Mn, Ca^{+2}, Mg, P, Cr, Co and three another antioxidant element (Zn, Cu &Se) in the blood of THM and SAC-thal patients.

Results: In THM and SCA-Thal patients, antioxidant elements (Cu, Zn & Se), showed highly significant behaviour when compared to each other and with the control group as total patient population, and as (total, male and female) numbers. In thalassemia patients, Fe, P & Co also showed highly significant behaviour, while Ca^{+2} behaviour was not significant for male numbers only. Elemental Mg & Cr, demonstrated insignificant behaviours. In SCA-Thal patients, Mn & Ca^{+2} showed highly significant behaviour, while, Fe showed a non-significant behaviour relative to male numbers only, and Mg, P, Cr & Co showed non-significant behaviour.

Conclusion: The study revealed the similarity of antioxidant elements behaviour in both diseases, as well as the statistical behaviour of all elements when compared with the control group for these diseases.

Keywords: *Healthy; Thalassemia; patients; Trace Elements; toxicity*

Introduction

Thalassemia is an inherited autosomal recessive blood disorder characterized by the production of abnormal hemoglobin [(1)]. It is caused by substitution or deletion mutations in globin genes that leads to either partial or complete absence of globin chains, that forms hemoglobin, of mutated origin [(2)]. In this respect, thalassemia can classify into alpha (α), beta (β), and delta (δ) according to the globin gene/s involved [(3)]. In Iraq, thalassemia had begun to spread dramatically [(4)]. The SCA patients can be either Hb S (SS) homozygous or Hb S heterozygous in combination with another Hb C or β-thalassemia chain mutation (Sβ)called SCA-Thal [(5)]. Mineral and Trace element had essential role in many biological processes, they act as activators or inhibitors for many enzymes [(6)], if increase or decrease these trace element which cause harm effect on human health [(7)].

Aims of this study estimate the serum level of ten elements in thalassemia major and SCA-thal patients, then compare the level of these elements with each

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other and with the control group, through appropriate statistical relationships to link these results with vital functions.

Methods and Materials

The study included 144 patients diagnosed as having β-thalassemia major and SCA-Thal, these patients were divided into three groups, as males and females, taking into account the number and age stages (61, with Beta Thalassemia major, 36 with SCA-Thal and 47 as a control group). Venous blood samples were collected from the participants under standardized conditions. The serum was obtained and stored at -20

The protocols for this study were approved by the College of Healthy and Medical Technologies in Basra and by the Ethics Committee in the General Directorates of Health in Basra and Dhi Qar. Serum iron levels were measured by chromatography (Biolabo, France). Calcium was measured with o-cresolphtalein (CPC), (BIOLABO, France); Mg and P were measured using the chromatography method (HUMAN, Germany); Copper was measured by the enzymatic method (LTA, Italian), zinc was measured using a spectrophotometric method (SPECTRUM, Egypt), while selenium, manganese, chromium and cobalt were measured by a flame atomic absorption spectrometer (Buck Scientific Model 210 VGP, England)

The statistical evaluation of the data was performed by SPSS version 25 software (IBM, USA) Using independent t-test (two-tailed) and Mann-Whitney U-test to compare the means of two groups, and Pearson correlation coefficients were used when calculating the correlations. The P-value ≤ 0.05 was considered statistically significant.

Results and Discussion

Statistical results for thalassemia major patients, table 1, indicate that all variables showed high significant behaviour when compared with the control group, as a (total, male and female) numbers, except for age, sex, Mn, Mg and Cr, while Ca^{+2} showed the same behaviour in female and total numbers. In SCA -Thal patients, table 2, all variants exhibited the same behaviour as thalassemia patients, except for age, sex, Mg, P, Cr & Co, while Fe showed an exception for male numbers only.

In this study, all thalassemia patients had a high serum iron level. This is attributed to iron increasing absorption in the intestine, or to the continuous blood transfusions [8,9]. Iron overload leads to accumulation of iron, tissue damage, increased oxidative stress and storage capacity. This result matches other studies[10, 11, 12]. The same iron level increasing was observed in SCA-Thal patients, but with less severity, because these patients suffer from metabolic regulation, cell damage, and chronic hypoxia, which leads to ineffective erythropoiesis, hemolysis and anemia [11]. The two disease groups had a significant decrease in serum zinc level compared to the control group, and this is consistent with many regional and international studies [13,14]. The role of zinc deficiency is evident in controlling oxidative stress, immune response and cardiovascular disease, this can be linked to several factors including treatment protocols, different levels of blood transfusion treatments, nutrition and the health history of the patient and family. Copper serum level showed a high level and high significant behaviour in the two disease groups compared to the control group. this, was consistent with other studies [12 ,14]. The high copper level can be attributed to low zinc levels in these patients, hemochromatosis, acute and chronic infections.

The two diseased group patients also had a lower level and high significant behaviour of Selenium in the blood. This result was similar to [10,15], this can be attributed to frequent blood transfusions, which leads to reduced hemolysis, increasing oxidative stress and iron overload, that alters trace element levels.

The serum Manganese level, showed non-significant behaviour in patients with thalassemia, while it showed a high significant behaviour in patients with SCA-Thal compared to the control group as (total, male and female) numbers. Manganese level is decreased in patients with SCA-Thal, which can be attributed to the oxidative stress, due to the over production of free radicals through a secondary iron [12].

Calcium serum level decreasing in the two diseased groups compared to the control group, this can be attributed to low level of parathyroid hormone and deficiency of vitamin D, this agree with other studies [16, 17, 12]. Magnesium plays an important role in activating many enzymes, as it helps the muscles, nerves and heart function properly. Magnesium level showed non-

significant behaviour in both diseases compared to the control group as (total, male and female) numbers, the fluctuated level also observed with other studies [18, 10]. While some studies have shown a decrease in Mg level in THM [11]. This may be, a precursor to some diseases that occur due to side effects (hypoparathyroidism, renal impairment, and cardiovascular disease) [19]. Phosphorous level, showed high significant behavior in thalassemia patients, while it showed non-significant behavior in SCA-Thal patients compared to the control group as (total, male and female) numbers. THM patients generally have high P, several studies indicate that iron excess, chronic hemolysis, metabolic saturation and oxidative stress causes oxidative damage to large molecules and leads to lipid oxidation, DNA damage, and parathyroid damage, and this causes hypothyroidism. This is one of the most common causes of elevated phosphorous levels, [20, 21]. This result, was found in similar studies [17, 22].

In this study, chromium level showed non-significant behaviour in both diseases compared to the control group as (total, male and female) numbers. . Cr has an essential role in passing insulin into the cell, so it is very useful, in addition to reducing the risks to the heart. A deficiency of it is not expected in the body, unless there is malabsorption of chromium in the intestine. Cobalt is found in the body as part of vitamin B12, which is essential for the formation of red blood cells. Cobalt can replace manganese in activating a number of enzymes and can also replace zinc in some biochemical reactions. Its importance lies in transporting oxygen in the metabolism. Cobalt is rapidly absorbed through the small intestine. But the biological information about it is incomplete [23]. Cobalt level, showed high significant behaviour in thalassemia patients, while it showed non-significant behaviour in SCA-Thal patients compared to the control group as (total, male and female) numbers.

Table 1: Statistical analysis for elements, age and sex in thalassemia major disease compared to control group.

Vari-ables	Total			Male			Female		
	Control (n=47)	Thal (n=61)	P. value	Control (n=24)	Thal (n=30)	P. value	Control (n=23)	Thal (n=31)	P. value
Age	11.68±5.1	12.90±5.02	0.228	12.58±5.40	13.13±5.15	0.7044	10.74±5.37	12.68±4.98	0.177
Sex	47	61	0.846	24	31	0.846	23	30	0.846
Fe	99.96±41.0	234.9±90.1	<0.001	105.5±46.8	223.7±49.5	<0.001	94.17±34.1	245.8±116.	<0.001
Cu	129.6±23.3	159.6±43.0	<0.001	132.3±22.0	170.9±43.3	<0.001	126.8±24.7	148.7±40.5	0.026
Zn	98.51±17.1	86.61±13.7	<0.001	100.4±20.1	84.83±13.6	0.002	96.57±13.5	88.32±13.8	0.033
Se	107.1±22.3	78.70±20.5	<0.001	108.4±22.8	79.48±20.	<0.001	105.7±22.2	77.95±20.9	<0.001
Mn	0.622±0.16	0.642±0.15	0.391	0.626±0.17	0.665±0.16	0.449	0.618±0.16	0.620±0.15	0.745
Ca2+	9.52±0.56	8.53±1.04	<0.001	9.621±0.51	9.177±1.28	0.451	9.426±0.61	8.439±0.90	<0.001
Mg	2.412±0.31	2.373±0.10	0.868	2.376±0.10	2.399±0.11	0.414	2.450±0.44	2.347±0.09	0.490
P	5.680±1.51	6.998±2.29	<0.001	5.905±1.60	7.424±2.57	0.015	5.446±1.40	6.586±1.93	0.020
Co	1.293±0.66	1.839±0.66	<0.001	1.397±0.81	1.830±0.60	0.014	1.208±0.45	1.848±0.72	<0.001
Cr	1.959±1.18	1.712±0.51	0.857	2.017±1.34	1.717±0.44	0.928	1.895±0.97	1.683±0.53	0.652

Table 2: Statistical analysis for elements, age and sex in SCA –Thal group compared to control group.

Vari-ables	Total			Male			Female		
	Control (n=47)	SCA -Thal (n=36)	P. value	Control (n=24)	SCA -Thal (n=16)	P. vale	Control (n=23)	SCA -Thal (n=20)	P. value
Age	11.68±5.41	13.56±6.38	0.152	12.58±5.40	12.81±6.32	0.903	10.74±5.37	14.15±6.53	0.067
Sex	47	36	0.550	24	16	0.550	23	20	0.550
Fe	99.96±41.1	159.9±86.6	<0.001	105.5±46.8	169.6±110.	0.088	94.17±34.1	152.2±63.3	<0.001
Cu	129.6±23.3	163.6±43.3	<0.001	132.3±22.1	155.2±23.1	0.007	126.8±24.7	170.4±54.1	<0.001
Zn	97.94±20.5	88.00±39.6	<0.001	100.4±20.2	84.06±11.2	0.006	95.39±21.0	91.15±52.5	0.005
Se	107.1±22.3	66.82±11.6	<0.001	108.4±22.9	70.48±10.6	<0.001	105.7±22.2	63.89±11.8	<0.001
Mn	0.623±0.16	1.395±0.40	<0.001	0.626±0.17	1.379±0.43	<0.001	0.619±0.16	1.408±0.39	<0.001
Ca2+	9.526±0.56	8.410±0.87	<0.001	9.621±0.51	8.600±0.66	<0.001	9.426±0.61	8.258±1.00	<0.001
Mg	2.412±0.32	2.374±0.12	0.898	2.376±0.10	2.368±0.11	0.614	2.450±0.44	2.380±0.12	0.792
P	5.680±1.51	5.817±1.34	0.669	5.905±1.60	5.554±1.10	0.450	5.446±1.40	6.028±1.50	0.197
Co	1.959±1.18	1.524±0.45	0.313	2.017±1.34	1.548±0.51	0.647	1.895±0.97	1.505±0.41	0.259
Cr	1.487±0.84	1.548±0.53	0.331	1.397±0.81	1.495±0.	0.534	1.581±0.88	1.591±0.51	0.480

Pearson's statistical correlations between concentrations of elements with each other, for thalassemia patients' group, table, 3 showed that, (Cu & Mg), (Se & Zn) and (Co & Zn), behave significantly and positively correlate, while (P) behave significantly and negatively correlate with (Zn & Ca^{+2}).

Table 3: Pearson's statistical correlations for elements concentration with each other in THM diseased group.

Variables	Fe	Cu	Zn	Se	Mn	Ca2+	Mg	P	Co	Cr
Fe r Value P Value	1	-0.070 0.588	0.079 0.545	0.178 0.171	0.015 0.909	0.091 0.483	0.082 0.525	-0.033 0.803	-0.119 0.361	-0.082 0.532
Cu r Value P Value	-0.071 0.588	1	-0.237 0.066	-0.195 0.131	-0.047 0.719	0.153 0.238	0.422 0.001	0.237 0.066	-0.132 0.309	0.020 0.879

Cont... **Table 3: Pearson's statistical correlations for elements concentration with each other in THM diseased**

Zn r Value P Value	0.079 0.545	-0.237 0.066	1	0.261 0.042	-0.022 0.864	-0.090 0.486	-0.133 0.307	-0.266 0.038	0.302 0.017	0.035 0.790
Se r Value P Value	0.177 0.171	-0.195 0.131	0.261 0.042	1	0.001 0.992	0.069 0.597	-0.002 0.986	-0.031 0.811	0.196 0.129	-0.162 0.212
Mn r Value P Value	0.015 0.909	-0.047 0.719	-0.022 0.865	0.001 0.991	1	-0.014 0.917	0.131 0.314	0.090 0.488	-0.076 0.560	0.111 0.395
Ca2+ r Value P Value	0.091 0.483	0.153 0.238	-0.091 0.486	0.069 0.597	-0.014 0.917	1	0.029 0.819	-0.276 0.032	0.057 0.660	-0.034 0.793
Mg r Value P Value	0.083 0.525	0.422 0.001	-0.133 0.307	-0.002 0.986	0.131 0.314	0.030 0.819	1	0.040 0.762	-0.077 0.557	0.126 0.332
P r Value P Value	-0.033 0.803	0.237 0.066	-0.266 0.038	-0.031 0.811	0.090 0.488	-0.276 0.032	0.040 0.762	1	-0.182 0.160	-0.090 0.999
Co r Value P Value	-0.119 0.361	-0.132 0.309	0.302 0.018	0.196 0.129	-0.076 0.560	0.057 0.660	-0.077 0.558	-0.182 0.160	1	-0.128 0.327
Cr r Value P Value	-0.082 0.532	0.020 0.879	0.035 0.790	-0.162 0.212	0.110 0.395	-0.034 0.793	0.126 0.332	-0.090 0.999	-0.128 0.327	1

Pearson's statistical correlations for SCA-Thal patients' group, table, 4, showed that:

(Cu) behave significantly and positively correlate with, (Fe, Mg & P). (Zn) behave significantly and positively correlate with, (Cu, Ca^{+2}, Mg & P). (Mg & Ca^{+2}) and (Mn & Cr) correlated positively and significantly. Finally, (Se & Mg), showed significant and negative correlation.

Table 4: Pearson's statistical correlations for elements concentration with each other in SCA-Thal diseased group.

Variables	Fe	Cu	Zn	Se	Mn	Ca2+	Mg	P	Co	Cr
Fe r Value P Value	1	0.357 0.033	0.119 0.488	0.130 0.449	0.177 0.303	0.187 0.274	-0.148 0.390	0.273 0.107	-0.053 0.758	-0.129 0.453
Cu r Value P Value	0.357 0.033	1	0.848 0.011	-0.137 0.426	0.317 0.060	0.299 0.076	0.416 0.012	0.601 0.004	0.097 0.572	0.195 0.254
Zn r Value P Value	0.119 0.488	0.848 0.011	1	-0.142 0.408	0.234 0.170	0.387 0.020	0.534 0.001	0.618 0.005	-0.046 0.788	0.228 0.181
Se r Value P Value	0.130 0.449	-0.137 0.426	-0.142 0.408	1	-0.030 0.864	-0.073 0.674	-0.338 0.044	0.098 0.569	0.043 0.805	-0.136 0.431
Mn r Value P Value	0.177 0.303	0.317 0.060	0.234 0.170	-0.030 0.864	1	0.260 0.126	0.185 0.281	0.167 0.330	-0.128 0.454	0.350 0.037
Ca2+ r Value P Value	0.187 0.274	0.299 0.076	0.387 0.020	-0.073 0.674	0.260 0.126	1	0.496 0.002	0.257 0.130	-0.015 0.932	0.019 0.914
Mg r Value P Value	-0.148 0.390	0.416 0.012	0.534 0.001	-0.338 0.044	0.185 0.281	0.496 0.002	1	0.295 0.081	-0.034 0.844	0.204 0.232
P r Value P Value	0.273 0.107	0.601 0.004	0.618 0.005	0.098 0.569	0.167 0.330	0.257 0.130	0.295 0.081	1	-0.200 0.242	0.281 0.097
Co r Value P Value	-0.053 0.758	0.097 0.572	-0.046 0.788	0.043 0.805	-0.129 0.454	-0.015 0.932	-0.034 0.844	-0.200 0.242	1	-0.207 0.226
Cr r Value P Value	-0.129 0.453	0.195 0.254	0.228 0.181	-0.136 0.431	0.350 0.037	0.019 0.914	0.204 0.232	0.280 0.097	-0.207 0.226	1

Conclusions

The elements, Fe, Cu, Zn, Ca^{+2}, Se, P, Co & Cr in thalassemia patients, Showed, high significant statistical behaviour comparing to the control group as (total, male & female) numbers, while Ca^{+2} showed non-significant behaviour in male number only.

In SCA-Thal patients, elements, Fe, Cu, Zn, Se, Mn & Ca^{+2} Showed, high significant statistical behaviour comparing to the control group as (total, male & female) numbers, while Fe, showed non-significant behaviour in male number only. Pearson's statistical correlations for the elements in all diseased groups, will help to predict the overlap between elements with each other in positive and negative correlation. Which will assist later in choosing appropriate healthy nutrition and in supplements using.

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The Influence of Booklets and Videos on the Ability to Practice Breast Self Examination in Students of High School 4 Gorontalo

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Abstract

Individual awareness in taking actions properly BSE is influenced by the individual understanding of breast cancer, so health promotion Efforts are Necessary. Videos and booklets are some alternative health promotion media. The research objective was to determine the effect of booklets and videos on the ability to practice breast self-examination on students at High school 4 Gorontalo . The research used the Pre-Experiment method with a one-group pretest-posttest design, Carried out in August-October 2019 at High school 4 Gorontalo . The research sample was 60 female students from a population of 119 students using the purposive sampling technique. Statistical test using Paired t-test. The results of the analysis of the different test on the ability of Breast Self Examination (BSE) before and after reading the booklet showed that there was a difference in BSE ability with p-value = 0.001, while before and after watching the video showed that there was a difference in BSE ability with p = 0.001 ($p < 0.05$). This study concluded that there was an increase in the students' BSE practice skills after reading booklets and watching BSE videos at High School 4 Gorontalo. It is hoped that students can increase their knowledge and apply BSE so that the incidence of breast cancer or breast disorders can be prevented and detected early.

Keywords: *Booklet, Video, Practice BSE, Students*

Introduction

Data Basic Health Survey in 2018 showed that the prevalence of breast cancer in Indonesia was 0.5 per 1,000 women.[1] The high prevalence of breast cancer in Indonesia must be observed by preventive efforts through early breast self-examination (BSE). Detection of early holding function is significant because of the earlier discovery of breast cancer, the more convenient treatment, and the good results of the rise in breast cancer.

The American Cancer Society (ACS) supports the use of BSE in women 20 years of age or older. Along with the time the disease has begun to lead to age is younger, the age of puberty (13-20 years) often need to conduct breast self-examination is a routine as early prevention and detection efforts.[2]

Knowledge of individuals doing BSE activities to be better influenced by understanding people about breast cancer so that attempts to improve wellness need to be made. The effectiveness of the promotion of health is decided by the use of the right of the media, which will promote the distribution of information, achieve the purpose that more and be able to stimulate a message that has been received from others.[3]

Video and booklet are several alternative media marketing events. The findings of Susanti's (2015) research clarified that the average experience and attitudes of adolescents using video media was higher compared to the teaching process. Media video provides a stimulant to pe n rumor and vision so that the effects have been reached throughout.[4]

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Video is a medium that incorporates the media's point of view and hears so that there is a lot of sensories that used to be increasingly powerful and transparent. . The use of video on BSE can clarify the picture of an abstract about the importance of examination of the breast itself, because in the process of giving respondents not only hear the material that is being presented, but also see it directly and clearly about the steps to realize through the video of the.[5]

Research by Astuti (2017) has shown that offering advice by the use of a booklet will enhance the mother's awareness and ability to execute the BSE technique correctly.[6] The number of young women in the City of Gorontalo (12-18 years of age) is 13,168. The highest in Central City is 1,877 inhabitants, while the City of East is 1,838 inhabitants, Dungingi 1,614 inhabitants and the lowest is Hulontalangi 1,119 inhabitants.[7]

The Center for Information and Counseling Health Reproductive Youth (CI-CHRY) is one of the government programs that Family Planning Programs has run through the provincial, district, sub-district, village, university high school, high school, middle school, and elementary school. CI-CHRY-run of, by, and for adolescents that are useful for providing service information and Health fertility therapy is ta preparation of family planning.[8]

High school 4 Gorontalo is one of the schools located in the northern town of Gorontalo City. The number of students at High school 4 Gorontalo is 635, consisting of 279 students and 356 female students. High school 4 Gorontalo has 3 classes that have discovered that class X has 137 students, class XI has 119 students and class XII has 100 students.

The goal of the research is to determine the impact of Booklet and Video Against Abi Ladies Doing BSE Practice on students.

Methods

Research is using the method Pre-Experiment with the design of the pre-test control group-post-test design for each intervention was carried out in High school 4 Gorontalo during the month from August to October 2019. Independent variables were booklets and videos, while BSE abilities were the dependent variable. The population in the sample is student class XI High school 4 Gorontalo many as 119 students with sample 60 students using the technique of purposive sampling.

The methods used are check-list sheets, media booklets, and videos. This study compared the impact of booklets and videos on the functional capabilities of BSE. Booklets were distributed to 30 respondents to be read for 30 minutes every day for 5 days, and videos were given to 30 respondents to watch for 30 minutes every day for 5 days. Booklet and video circulated by researchers and sponsored by enumerators. A brief was given to all respondents before the study started.

Multivariate analysis conducted to assess the impact of an independent variable (Booklet and video) with variable bound (Skills BSE) is combined with adding variable outside is important in the bivariate analysis. Modeling was carried out to see which variables affected students' BSE skills. Study of the multivariate use of statistical regression logistic to see the importance of OR and CI 95 percent at the level of significance of p <0, 05. Health Research Committee on ethics

Health Research Ethics Commission (HREC) Politechnic Gorontalo Ministry of Health has approved this research by number LB.01.01/KEPK/07/2019.

Results

Table 1. Differences in the ability of BSE before and after the booklet'

Variable	Mean ± SD (gr / dl)	p-value
Ability early BSE Pre	0.110 ± 0.080	0,000
Skills BSE Post	1.498 ± 0.399	
Δ Change	1.388 ± 0.398	

* *Test paired t-test*

The average BSE ability before and after the intervention to read the booklet showed an increase in the respondents' ability to do BSE. Ability BSE respondents experienced an increase of 1,388 after reading the booklet. The results of the analysis of the different tests on the ability of BSE before and after reading the booklet showed that there was a difference in BSE ability with a value of p = 0.000 (p < 0.05) (Table 1).

Table 2. Differences in the ability of BSE before and after the video

Variable	Mean ± SD (gr / dl)	p-value
Ability BSE Pre	0.076 ± 0.056	0,000
Skills BSE Post	1.619 ± 0.338	
Δ Change	1.542 ± 0.337	

** Test paired t-test*

The average BSE ability before and after the intervention giving the video showed an increase in the respondents' ability to do BSE. Ability BSE respondents experienced an increase of 1,542 after watching the video. The results of the analysis test of difference to the ability of BSE before and after watching the video shows that there are differences in the ability of BSE with a value of p = 0, 00 (p <0.05) (Table 2).

Discussion

Based on the results of the study showed that after the booklet was read by students, the ability to perform BSE has increased, as many as 70% with average ability BSE amounted to 1,498 and the average gap increase before and after reading the booklet is 1.388. Statistical test results with Paired t-test p-value value = 0.001 (p <0.05) influenced booklet on the ability to practice BSE at High school 4 Gorontalo. These results indicated that the students' ability to practice BSE after being given booklets was higher than before being given booklets. This happens because after giving the booklet students get information directly on how to do BSE so that the booklet can affect students' ability to practice BSE. This is related to the advantages of the booklet, namely the material contained in the booklet is more complete, more detailed, clear and educative and the preparation of the booklet material is made in such a way as to attract the attention of adolescents, according to the needs and conditions of adolescents.

Respondents who did not experience an increase in the ability to do BSE or not skilled were 9 respondents (30%). The researchers assumed that the ability to practice BSE was not increased due to the respondent's inadequate ability or memory so that they could not understand the contents of the booklet and were unable to remember the steps of BSE properly. This is also possible due to differences in observations or perceptive power in understanding information.

Following Notoatmodjo (2007) in Setiawan (2017) that knowledge is influenced by many psychological factors, including intelligence, observation, comprehension, memory, learning motivation, and so on. So that it can be said that with the same information there will be many differences in understanding and knowledge.[9]

This is in line with the theory of Surya (1992) in Ramlah (2015), the ability to remember is often associated with mental activity in gaining knowledge and solving problems. The ability to remember students is closely related to learning activities. At the time of learning, students use the ability to remember to understand the knowledge and solve the problems it faces. While the ability to remember students is very dependent on the quality and quantity of learning outcomes they get.[10] This is supported by research by Aini (2013) which states that memory and critical thinking skills greatly affect learning outcomes.[11]

Based on the results of the study, after giving the video the ability to do BSE increased, namely as much as 83.3 3% with an average BSE ability of 1.619 and

the difference in the average increase before and after watching videos of 1.542.

The results of statistical tests with the T-test obtained p-value = 0.001 (p <0.05), which means that there is an effect of video on the ability to practice BSE in students at High school 4 Gorontalo where the results of the skills in doing BSE after watching videos are higher than before watching the video. The results of this study are in line with Meilani's (2018) research, namely that there is an effect of BSE counseling with audio-visual media on the attitudes of young women in breast cancer prevention.[12] This study is also comparable to research by Shorea, et al. (2014) which states that video media can improve student learning systems about BSE so that students know the steps for self-breast examination (BSE).[13]

According to the researcher's analysis, it can be explained that the respondent's skills in doing BSE after being given the video, the respondent's ability is more skilled than before being given the video. This happens because after watching the video the respondent not only heard the material being delivered but also saw the steps of BSE through the video so that it could affect the students' skills in practicing BSE.

Giving videos is effective in increasing the knowledge and skills of students where a student can receive messages quickly, is easy to remember, can be received well, is more interesting and not monotonous because students listen and see so they are very enthusiastic about the content of the video and watch the video until the end. Through videos of adolescents, a clear explanation of BSE will be explained, namely the meaning, time objectives, and steps of BSE.

According to Munadi (2012), using video media has a more impact on health education, which relies on hearing and sight from the target, is interesting, the message is conveyed quickly and is easy to remember and can develop thoughts and develop the imagination of young women.[14] This is in line with the research of Aeni (2018) that health education with video for young women can clarify the pictures and the important steps for BSE examination, because in the process of giving respondents not only hear voices but respondents will see directly and the steps breast self-examination (BSE).[15]

Respondents who did not experience an increase in the ability to do BSE or not skilled were 5 respondents (16.67%). Researchers assume that the ability to do BSE does not improve after watching the video. This is due to the lack of respondent attitudes which affect the skills in doing BSE. There are still many young women who have a negative attitude because they don't know the benefits of BSE. BSE is a fairly easy way to detect breast cancer early.

According to Notoatmodjo (2010), if someone has never seen, heard, or experienced breast cancer, it will tend to be indifferent to early detection efforts. This study is in line with research Wahyuningtyas (2012) which shows that attitudes influence the skills of BSE, because attitude is a closed behavior or as a support for BSE.[16]

According to Wawan and Dewi (2010), attitude is a general evaluation made by humans of themselves, other people, objects, or issues. This can happen because in theory it is often stated that attitude is a predisposition (determinant) that leads to behavior following the attitude. Attitude to grow begins with the knowledge that is perceived as something good (positive) or not good (negative), then internalized into him.[17]

In line with the theory according to Fatimah (2017), the process of skill formation is through knowledge, understanding, assessing, attitude skills, interaction, and communication, and ultimately complex skills will be formed. Skills are a person's ability to act after first obtaining knowledge and attitudes that begin with sensing a certain object.[18]

The results of the analysis chi-square shows that media exposure and the value of report cards have meaningful relationships practically and statistically by upgrading the skills of BSE in High School 4 Gorontalo, which is indicated by the value of p <0.05, age, which indicates that there is no significant relationship between age and the role of parents and the increase in BSE skills in High school 4 Gorontalo.

Based on the results of the bivariate analysis, it was found that the video and booklet had a p-value of 0.75; OR 0.82; 95% CI 0.24-2.81. This means that students who use the BSE skills booklet are 0.82 times more skilled than videos. the result of others suggest there is a

significant relationship between the value of report cards with BSE skills of students with a p-value of 0.0 01; OR 10.7; 95% CI 2.10-54.01, which means that students with high report card scores, BSE skills are 10.7 times more skilled than students with low report card scores.

The results of the bivariate analysis showed that there was a significant relationship between the media and the BSE skills of female students with a p-value of 0.04; OR 4,1; 95% CI 1.01-16.95 which means that students who are exposed to the media have 4.1 more skill in BSE compared to students who are not exposed to the media.

Conclusions

The results of the study showed that there was an improvement in the ability of BSE students to practice in high school 4 Kota Gorontalo after reading the brochure and watching the BSE video. Suggested to students to increase awareness about and introduce BSE, as well as to increase the incidence of skills BSE, that the incidence of breast cancer or breast defects can be avoided and identified much earlier.

Ethical Clearance : This study approved ethical clearance from The Committee of Research Ethics of Health Polytechnic of Gorontalo, Indonesia. The informed consent include the research title, purpose, participants'right, confidentiality, and signature.

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Preparation, Characterization, Antibacterial Study, Toxicity Study of New Phenylene diamine- Formazan Derivatives

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Abstract

Formazans are known to be characterized by containing very effective functional groups that have an effect on the possession of any compound with a pharmaceutical and biological characteristic as antimicrobials for many microbes, as these compounds have proven high resistance to the spread of cancerous tumors and the spread of bacteria and fungi. Series of formazan derivatives were synthesized in this study from meta-phenylene diamine via imination reaction then coupling reaction to yield new five derivatives. The formatted formazan derivatives have been investigated through various instrumental -spectral techniques represented by (I.R- Infrared spectra ,H. NMR -Proton magnetic resonance , Mass -Spectra)– as a spectrophotometric measurements ,then bio- studying via various bio- experiments in this field like cytotoxicity, resistance of anti-bacteria.

Keywords: *pyrimidine, formazan, thiazole, infection, contamination, toxicity, anti-bacteria, phenylene diamine, cancer, tumor.*

Introduction

Cancerous tumors are considered one of the common and widespread diseases in the current century due to the spread of their causes of radiation, wars and their radioactive residues. Therefore, the need to search for antibodies that inhibit the growth of cancer cells and reduce their spread with the least toxicity was necessitated, Therefore, many biological and chemical researchers resorted to finding alternative solutions as treatments and inhibitors for cancer cells, including linking some medical drugs and medicines to some functional groups that affect the growth and reproduction of cancerous tumors.

Formazan is one of the important compounds as a biologically applications[1-5] with its many biological treatments[6-13], especially in the field of fungi[14-16], bacteria and malaria treatments[17-20], in addition to many literatures[21-28] in this field that has demonstrated its therapeutic importance in malignant tumors. Several studies[29-30] have been conducted to evaluate the role of formazans and their derivatives as reducing the growth and spread of cancerous tumors in the body[31-37]., Formazan compounds are characterized by the diversity of their applications[38-42], studies have shown that formazan works with most of the transitional elements[43-45] , which gave vital activity when injected into laboratory animals to test their ability to inhibit the spread of tumors in the body and reduce toxicity, as well as represent dyes tissues[46-50] and other applications[51-59].

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Experimental Part :

Formazan derivatives created via numerous chemical processes, checked and reconnoitered by numerous techniques signified in : FT-IR spectra (system 8300 -Shimadzu) -range (400-4000) cm^{-1} in KBr discs .,1H. NMR–Spectra in DMSO–solvent, then Mass -Spectra., also bacterial resistance studies ,in addition to toxicity studies to formazan derivatives.

Preparation of Formazan derivatives[19-22]:

Preparation of Compound {1}:

m-Phenylene diamine(0.01 mole)reacted by condensation process with (0.02 mole) from vaniline with drops of acid(acetic. glaci.) ,the resulting precipitation dried then re crystallized, by flowing procedures[19-22] to yield compound { 1 }.

Preparation of Compound {2}:

Compound [1] (0.01 mole)reacted with (0.02 mole) from thiazole diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures[19-22], the resulting represents (formazan compounds) compound {2}.

Preparation of Compound {3}:

Compound [1] (0.01 mole)reacted with (0.02 mole) from 4-acetothiophene diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures[19-22], the resulting represents (formazan compounds) compound {3}.

Preparation of Compound {4}:

Compound [1] (0.01 mole)reacted with (0.02 mole) from pyrimidine diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures[19-22], the resulting represents (formazan compounds) compound {4}.

Preparation of Compound {5}:

Compound [1] (0.01 mole)reacted with (0.02 mole) from benzothiazole-diazo salt in basic medium, the resulting precipitation dried then re crystallized, by flowing procedures[19-22], the resulting represents (formazan compounds) compound {5}.

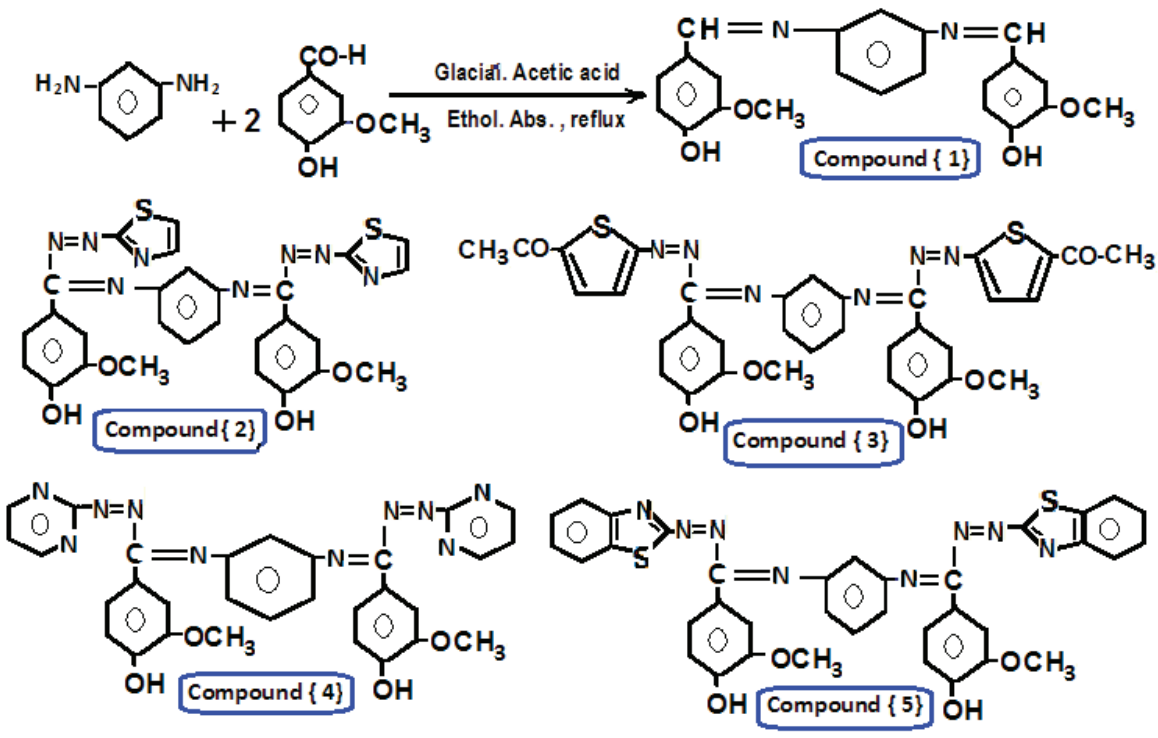


Fig.(1): Preparation of Compounds { 1 - 5 }

Results and Discussion

The formazan compounds screened via several spectral methods act:(FT.IR ,H.NMR ,Mass) spectra with studying of toxicity and resistance of bacteria :

Spectral Screening of Compounds :

FT.IR-Spectra : The spectra of compounds appeared numerous absorption bands at (CH=N) imine group:1618 .,(OH) Phenol : 3330 ,(OCH_3): 1195 in compound { 1} ,while new bands at (-N=N-C=N) formazan group : (1414, 1423, 1567) .,(OH) Phenol : 3314 ,(OCH_3): 1163 ,(C-S) in thiazole (798) in compound { 2}., other new bands at (-N=N-C=N) formazan group : (1425, 1459, 1514) .,(OH) Phenol : 3338 ,(OCH_3): 1147 ,(C-S) in thiophene (776) ,(-CO-CH) carbonyl of ketone in compound { 3}., other new bands at (-N=N-C=N) formazan group : (1422, 1481, 1517) .,(OH) Phenol : 3342 ,(OCH_3): 1166 ,(C=N) endocycle (1665) in compound { 4}., while new bands at (-N=N-C=N) formazan group : (1431, 1472, 1545) .,(OH) Phenol : 3322 ,(OCH_3): 1164 ,(C-S) in benzothiazole (775) in compound { 5}.

^{1}H.NMR- Spectra : Spectra of formazan compounds appeared at (2. 5) due to solvent (DMSO) , also new compound {1} gave many peaks at ϐ (CH=N) imine group: 8. 53 .,(OH) Proton of hydroxyl group in phenol : 10. 87 .,(-OCH_3) protons of methoxy group (3. 08) , Protons of aromatic ring : (7. 02 - 7. 93) ., but compound {2} gave many peaks at ϐ (OH) Proton of hydroxyl group in phenol : 10. 32 ., (-OCH_3) protons of methoxy group (3. 00) , Protons of aromatic ring : (7. 08 - 7. 41) ., compound {3} gave many peaks at ϐ (OH) Proton of hydroxyl group in phenol : 10. 94 ., (-OCH_3) protons of methoxy group (3. 11) , Protons of aromatic ring : (7. 14 - 7. 87) ,($CO-CH_3$) protons of methyl group in ketone (2.73) ., compound {4} gave many peaks at ϐ (OH) proton of hydroxyl group in phenol : 10. 32 ., (-OCH_3) protons of methoxy group (3. 00) , protons of aromatic ring : (7. 08 - 7. 41) ., Fig (2) showed resonance spectrum of compound [3]:

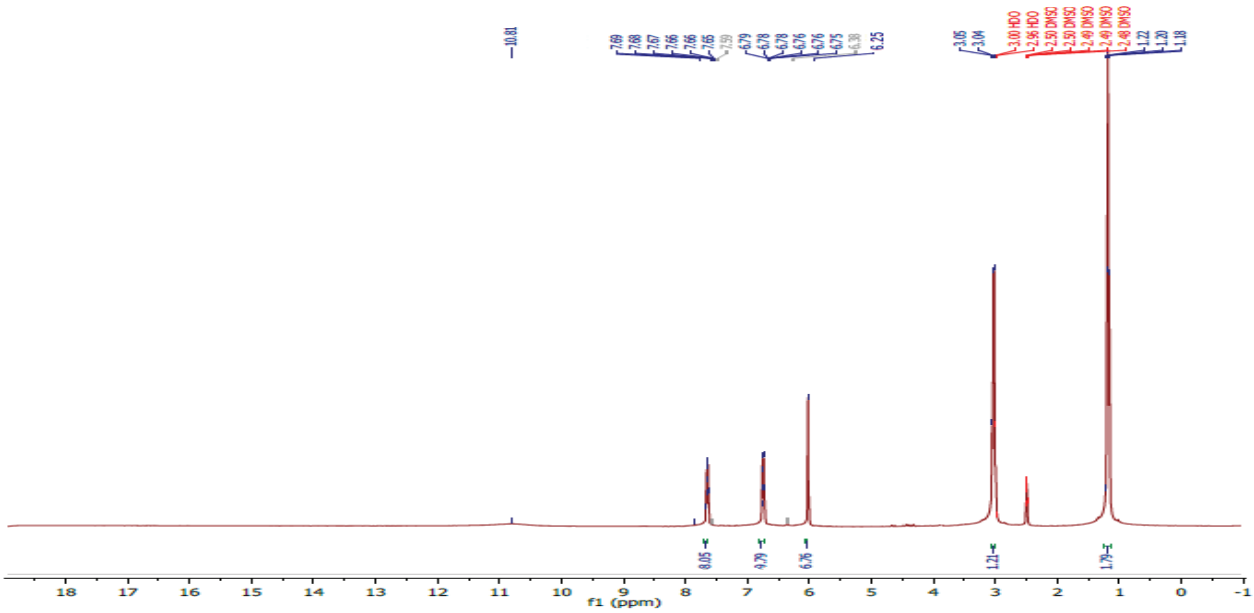
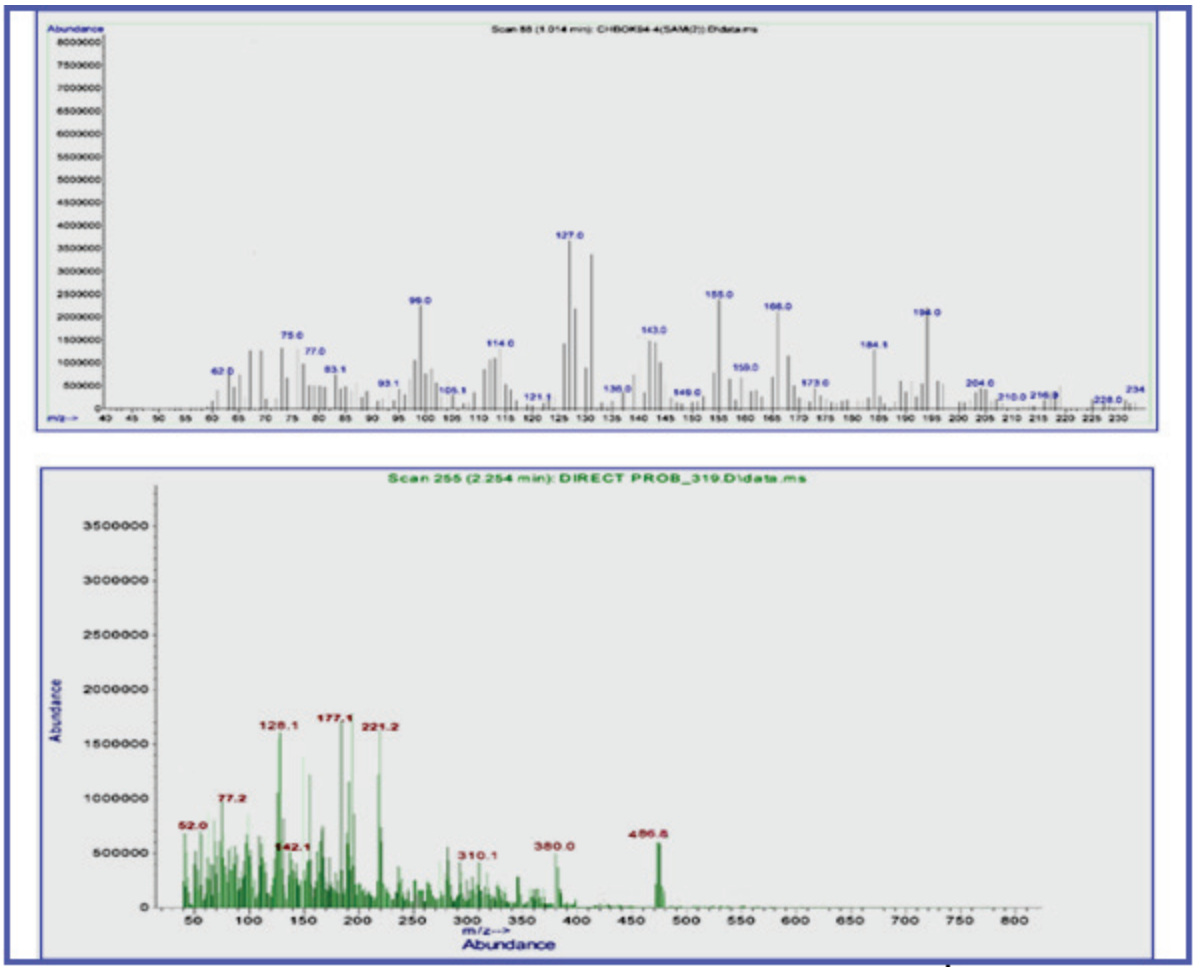


Fig: (2): H. NMR spectrum of Compound [3]

Mass –Spectra: All spectra gave good evidence for creation of formazan compounds through parts of compounds and fragments, Fig (3) acts mass spectrum of compounds [2] and [3]:



Fig(2): Mass –Spectrum of compound {2} , Compound {3}

Assay of Cytotoxicity[30, 34]

Some of Formazan compounds were screened to evaluate their cytotoxicity against Breast Cancer (AMJ13) and Hela Cancer cells shows that the formatted formazan derivatives gave high data as a results of efficiency of prepared compounds according to studies[30, 34]. Compound {5} which included thiazole ring in its structure showed 80% inhibition with an (IC50 = 1 µg/ml) against (AMJ13) Breast cell and moiety showed 74 % inhibition with an (IC50 = 1 µg/ml) against Hela cell but it gave on Embryonic cell moiety 38% inhibition with an (IC50= 1 µg/ml) .,While compound {2} appeared 50% inhibition with an (IC50 = 1 µg/ml) against (AMJ13) Breast cancer and moiety showed 74% inhibition with an (IC50=1 µg/ml) against Hela cell while it active on Embryonic cell moiety showed 62% inhibition with an (IC50= 1 µ g/ml).

In the present screening study, it was found that increasing in the concentration leads to increasing in the percentage of inhibition .

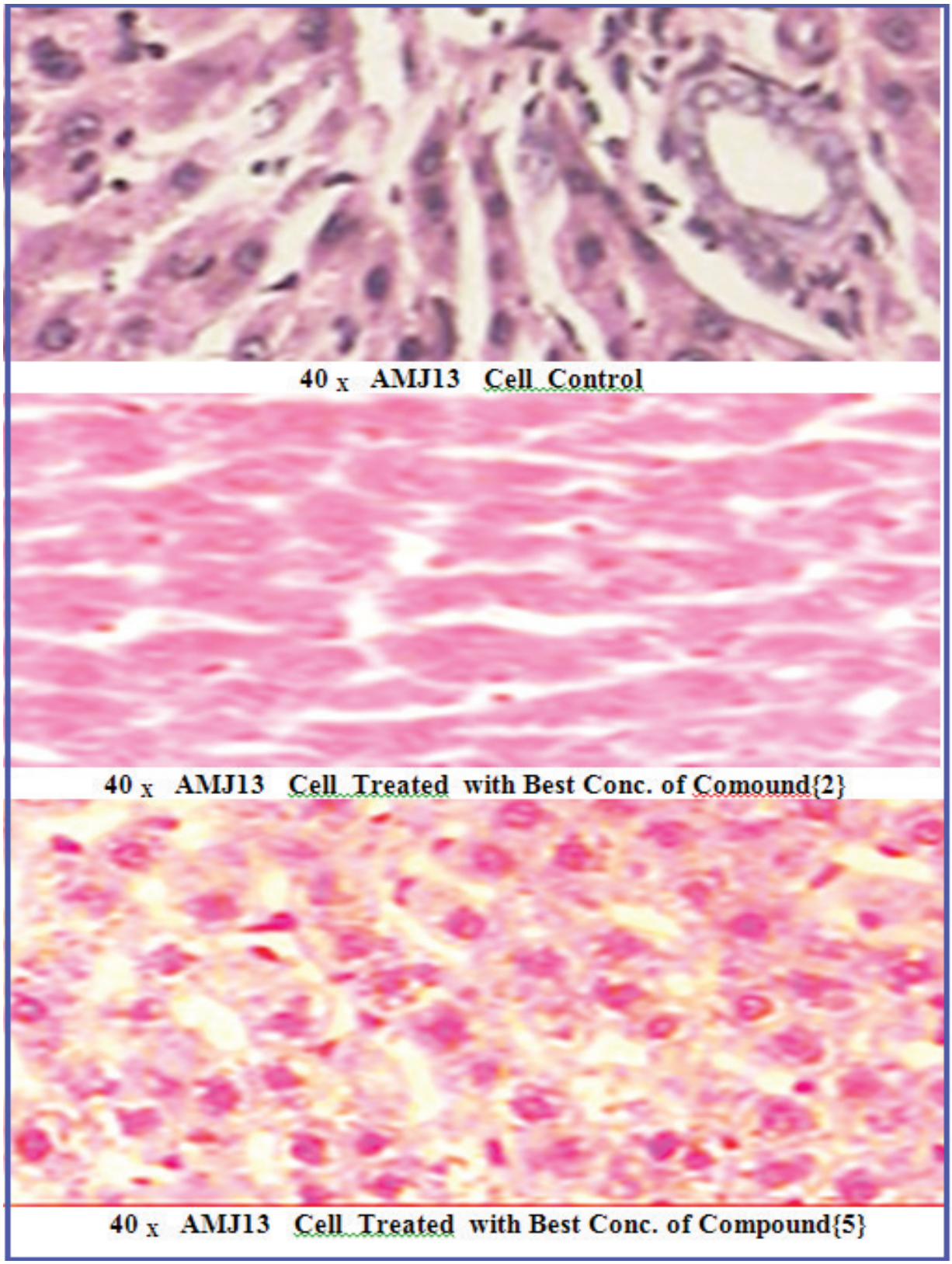


Fig. (4): 40 X AMJ13 Cell Control and Treated of Compounds {2 , 5}

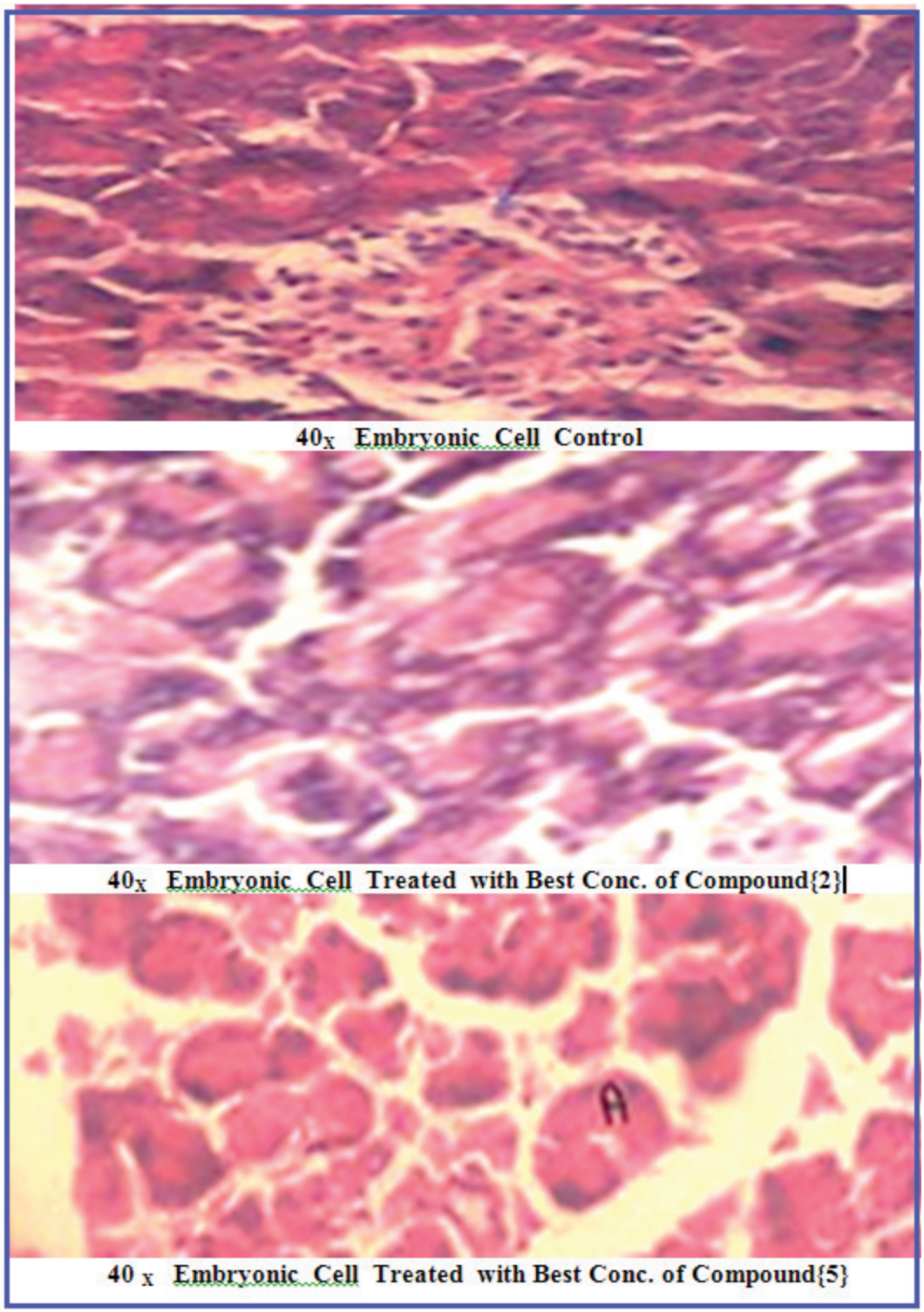


Fig. (5): 40_X Embryonic Cell Control and treated of Compounds {2, 5}

Selection of Bacteria for Antibacterial Assay [56-58] :

Three types of bacteria were selected for evolution screening towards antibacterial assay represented by *(Salmonella. Typhi , P. aeruginosa , Streptococcus. Faecalis*) via several concentration of compounds the selected bacteria in Figure . (6) :

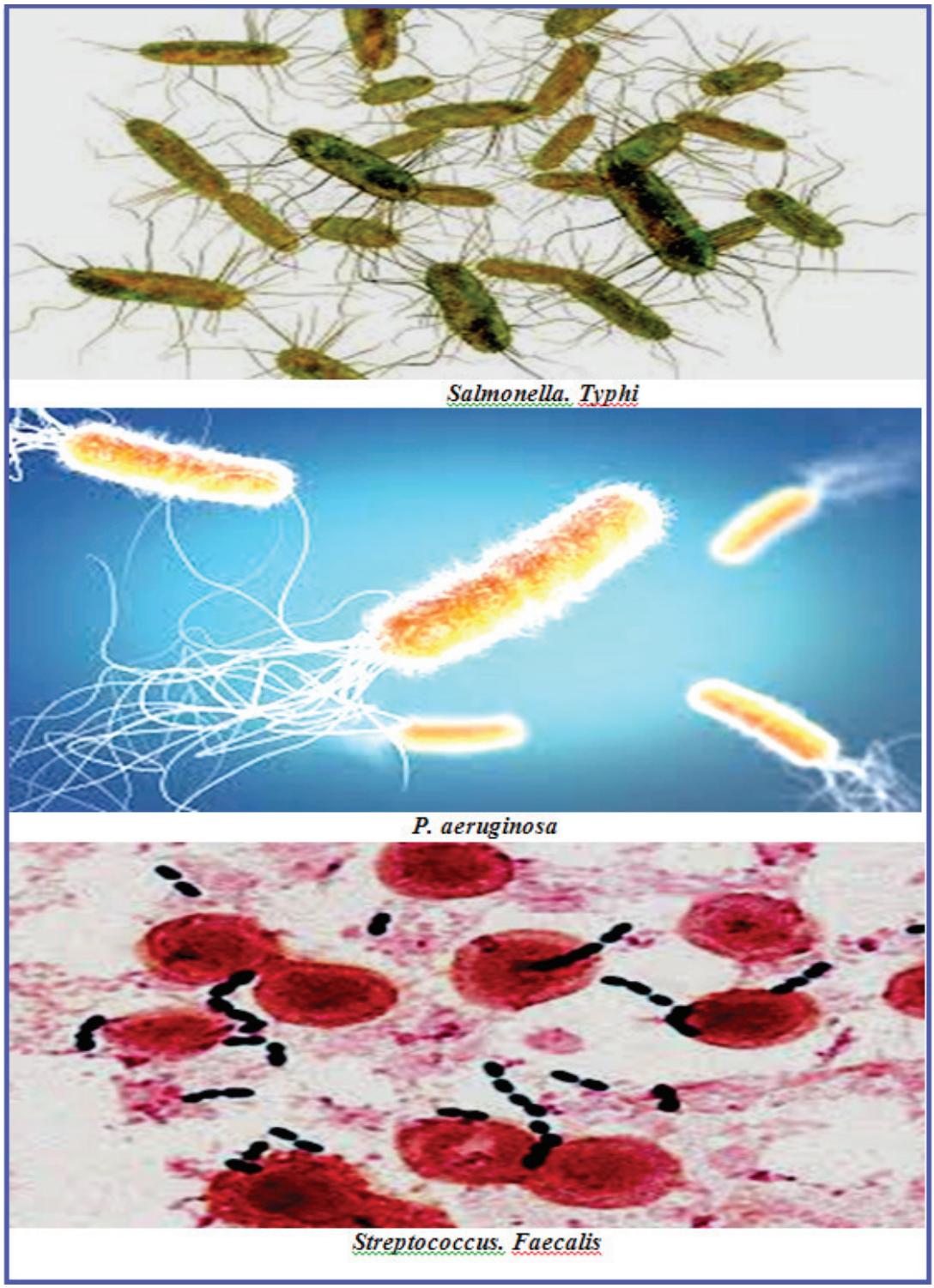


Fig. (6): The selected Bacteria

Antibacterial Tests[56-58] :

Screening of bio- test for the created formazan compounds have been performed for their antibacterial assay via agar by the flowing numerous procedures[65, 66] .The investigation of microbial inhibition carried out at (three concs) (20, 30 , 40 micro gram) concentrations in best solvent (DMSO) with bacteria ***:(Salmonella. Typhi , P. aeruginosa , Streptococcus. Faecalis)***.The selected types of bacteria incubated for (24 hr) at (37^{o}C).

The test of compounds on three types of bacteria gave high data with compounds {5 and 2} more than other compounds due to (Sulfur and nitrogen)-atoms in same compounds that participates in inhibition of bacteria., all results in Table (1):

Table.1: Inhibition test of formazan compounds in Conc. (30 micro gram)

Cephalexin Derivatives	Salmonella. typhi	Streptococcus. faecalis	P. aeruginosa
Compound {1 }	+	+	+
Compound {2 }	+++	+++	+++
Compound {3 }	++	+++	++
Compound {4 }	++	++	++
Compound {5 }	+++	+++	+++

(+) : inhibition (4-8) mm

(++) : inhibition (9-13) mm

(+++) : inhibition (14-18) mm

Conclusions

The test of compounds on three types of bacteria gave high data with compounds {5 and 2} more than other compounds due to (Sulfur and nitrogen)-atoms in same compounds that participates in inhibition of bacteria.

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Ethical Clearance: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

Conflict of Interest : The authors declare that there is no conflict of interest.

Funding Source : None

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Patellar Anthropometry in Sex Differentiation - A Study in the Southern Part of Andhra Pradesh, India

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Abstract

As in many cases, long bones get available in fragments, so gender determination from short as well as robust bones like patella is paid importance nowadays. So far, the literature reviewed show such records are found little in India, especially from the state of Andhra Pradesh. The present study is aimed to demonstrate the gender differentiability of different patellar anthropometric parameters viz, length, breadth, weight, thickness & patellar Index, etc., amongst the population of the southern part of Andhra Pradesh in India. A total of 50 intact patellae of which 25 belonging to males and 25 belonging to females, were taken out from human corpses of 20 to 50 years of age group, brought for postmortem to the Department of Forensic Medicine, Sri Venkateswara Medical College, Tirupati. Patellar vertical length, width, different measurements of the individual articular facet, patellar index, and surface area of the articular part, etc., were measured. In males, the mean of patellar length, Patellar index, Dry weight, Articular surface area were found to be 3.892 cm, 115.36, 20.06 gm, and 14.164 cm^2 respectively and in females, the mean of patellar length, Patellar index, Dry weight, Articular surface area were found to be 3.608 cm, 103.12, 15.16 gm and 9.664 cm^2 respectively. The values are found to be less in females than those found in males. The results of this study revealed that we can determine the sex of the southern Andhra Pradesh population, just by using the patellar measurements when other long bones, skull, and pelvis are not available or fragmented. But there remains scope for multicenter study throughout India to validate the fact.

Keywords: *Patella, Sex determination, Morphometric parameters*

Introduction

The diagnosis of sex from the adult human skeleton is important in the field of physical and forensic anthropology for the identification of a human body. There are many studies on the metric or non-metric diagnoses of sex from bones. The skull and pelvis have been the preferred elements by most forensic anthropologists[1]. Recent research however has shown that the appendicular skeleton can achieve higher discrimination between sexes. As in many cases, long bones get available in fragments, so gender determination from short as well as robust bones like patella is paid importance nowadays. Patella being a small but robust sesamoid bone, is often availed intact even after major accidental deaths where long bones easily get fragmented. As the patella develops within the quadriceps muscle, its size and shape are dependent upon the strength and size of the muscle mass. The patella is a small compact bone that does not undergo too many postmortem changes and therefore can be retrieved completely and used for such purposes. Tapan Kumar Jana et al[2] demonstrated sexual dimorphism in the northern part of the West Bengal population. Introna et al[3] demonstrated gender differentiability of patella among the population of southern Italy. Mahfouz et al[4] with three-dimensional CT guided research established gender differentiability of patellar anthropometric parameters. Dayal and Bidmos et al[5] almost concurrently carried on similar

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researches in the population of South Africa and justified the reliability of the patella in this ground. Later Akhlagi et al[6] endorsed such findings in the Iranian population. Veeramani et al[7] carried on geometric analysis on the mediolateral placement of patella and tibial tuberosity in the population of Karnataka. Such an endeavor in the Indian population especially from Andhra Pradesh is little available in pertinent literature, searched for.

The aim and objective of the study were to demonstrate the gender-wise distribution of different anthropometric patellar parameters like maximum length, dry weight, the surface area of the articular part, patellar index, etc., in southern Andhra Pradesh population.

Materials and Method

For feasibility, the present study has been carried on under the purview of Sri Venkateswara medical college, Tirupati, over a period of one and a half years. Proper permission from the institutional ethics committee has been availed prior to the start of the study. With proper permission from the Head of the Department of Forensic Medicine and with informed consent from the near relatives of the deceased, patellae were collected for anthropometry from the cadavers brought for postmortem examination to the Department of Forensic Medicine, Sri Venkateswara Medical College, Tirupati. From these cadavers, those within the age group of 20 to 50 years were chosen to avoid age related underdevelopment or osteopenia of the bones. Kin of the deceased, whoever not consented to participate, the procedure was not performed on those cadavers. For uniformity of the result, patellae of the left side were taken out through a longitudinal incision in front of the knee. The bones were washed by brushing with Luke warm water and were placed under shelter to dry slowly. For the attachments of soft parts including tendons and ligaments, the bones were boiled for twelve hours or even more to strip off these soft parts easily with an ordinary brush. All these were done carefully to preserve original bone morphology. Only normal patellae were included in this study. Patellae with severe enthesophytes and other abnormalities were specifically excluded. Thus, a total of 50 patellae could be included in this study, out of which 25 were from males and 25 were from females.

Different parameters were recorded using the same weighing machine and Vernier calipers. In every case, three records were taken and the average of all was noted, to minimize the intra-observer variation. All measurements were adapted from **Martin and Knussmann'definitions[8]**.

Parameters taken for metric sexing of the patella:

Dry weight of the bone (DW): Noted with digital weighing machine, Maximum Length (ML) = greatest distance between the base and apex, Maximum breadth (MB) = greatest distance between the medial and lateral sides, Maximum thickness (MT) = Distance between anterior and posterior surfaces, Vertical diameter of the articular part. (VDA), Transverse diameter of the articular part. (TDA), Breadth of the medial articular facet (BMAF) = distance between the medial edge of the patella and median ridge of the articular facet, Breadth of the lateral articular facet (BLAF) = distance between the lateral edge of the patella and median ridge of the articular facet. For estimation of breadth of the articular facets, the respected articular facet was colored by ink and the impression was taken on centimeter calibrated graph paper, Surface area of the articular part (SAP) = VDA X TDA, Patellar index: (PI) patellar index was calculated by using the formula: maximum breadth/maximum length x100. All these anthropometric parameters were analyzed and sex differences were observed.

Observations

The statistical product and service solutions version (12) program was used to analyze all the data. Descriptive statistics, which included means and standard deviations, were obtained for all measurements. Subsequent independent't' test was carried out on each variable in both the sexes to find out the significant differences ($p < 0.005$) in the male and female mean measurements. The average of the two means (mean of males and that of females) used as the demarcating points as per the previous studies.

The main findings can be highlighted as:

The gross appearance of the patellae of both the sexes was observed carefully. It was seen that in males the patellae were heavier, larger, and more marked with muscular and ligamentous impressions than in females.

Dry weight of the bone (DW) was recorded using the digital weighing machine and arranged according to the sex they belong to. The male patellae varied from 12.6 gm to 25.6 gm in weight with an average of 20.6 gm. The female patellae varied from 11.4 gm to 19.5 gm with an average of 15.16 gm **(Table no.1)**. **Maximum Length (ML)** of the male patellae was 3.7 cm to 4.1 cm with an average of 3.8 cm while that of the female patellae was 3.4 cm to 3.8 cm with an average of 3.6 cm. **Maximum breadth (MB)** of the male patellae was 4.0 cm to 4.8 cm with an average of 4.5 cm and that of the female patellae was 3.6 cm to 4.2 cm, with an average of 3.7 cm. **Maximum thickness (MT)** of the male patellae was 1.9 cm to 2.4 cm with an average of 2.06 cm and that of the female patellae was 1.5 cm to 1.9 cm, with an average of 1.68 cm. **Vertical Diameter of the Articular part (VDA)** of the male patellae was 3.2 cm to 3.7 cm with an average of 3.3 cm and that of the female patellae was 2.9 cm to 3.5 cm, with an average of 3.2 cm. **Transverse Diameter of the Articular part (VDA)** of the male patellae was 4.3 cm to 4.7 cm with an average of 4.4 cm and that of the female patellae was 4.0 cm to 4.3 cm, with an average of 4.1 cm. **Breadth of the medial articular facet (BMAF)** of the male patellae was 1.7 cm to 2.0 cm with an average of 1.9 cm and that of the female patellae was 1.5 cm to 1.7 cm, with an average of 1.63 cm. **Breadth of the lateral articular facet (BLAF)** of the male patellae was 2.4 cm to 2.8 cm with an average of 2.5 cm and that of the female patellae was 2.0 cm to 2.4 cm, with an average of 2.2 cm. **Surface area of the articular part (SAP)** of the male patellae was 12 cm^2 to 16.2 cm^2 with an average of 14.1 cm^2 and that of the female patellae was 9.3 cm^2 to 10.0 cm^2, with an average of 9.6 cm^2. **Patellar Index (PI)** was calculated in both and it was higher in the male patellae ranging from 100 to 129 with an average of 115.3 and low in female patellae ranging from 92 to 120, with an average of 103.1.

Demarcating values are calculated by taking the average of the means of all parameters of both male and female patellae **(Table no 2).** The values reveal that in males all the parameters Viz, DW, ML, MB, MT, VDA, TDA, BMAF, BLAF, SAP & PI are more than 17.38, 3.73, 4.15, 1.54, 3.26, 4.35, 1.76, 2.4, 11.44 & 109.54 respectively. Statistical analysis of the above parameters was done. Standard deviation was extracted and‘t’ test was performed. It was seen that p-value in all cases was highly significant (< 0.005) **(Table no 3).** The percentage of accuracy is also calculated for the various anthropometric parameters of the patellae (**Table no. 4**).

Table No.1

Gender wise comparison of different anthropometric parameters measured from the patellae						
Parameters	**Male**			**Female**		
	Minimum	**Maximum**	**Average**	**Minimum**	**Maximum**	**Average**
DW	12.6	25.6	20.06	11.4	19.5	15.16
ML	3.7	4.1	3.89	3.4	3.8	3.61
MB	4.0	4.8	4.51	3.6	4.2	3.74
MT	1.9	2.4	2.07	1.5	1.9	1.68
VDA	3.2	3.7	3.34	2.9	3.5	3.20
TDA	4.3	4.7	4.48	4.0	4.3	4.17
BMAF	1.7	2.0	1.91	1.5	1.7	1.62
BLAF	2.4	2.8	2.56	2.0	2.4	2.22
SAP	12	16.2	14.16	9.3	10.0	9.66
PI	100	129	115.36	92	120	103.12

Table no 2.

Demarcating points for sex determination	
Variable	Demarcating value
DW	Female < 17.38 < male
ML	Female < 3.73 < male
MB	Female < 4.15 < male
MT	Female < 1.54 < male
VDA	Female < 3.26 < male
TDA	Female < 4.35 < male
BMAF	Female < 1.76 < male
BLAF	Female < 2.40 < male
SAP	Female < 11.44 < male
PI	Female < 109.54 < male

Table No: 3

Results of independent "t" test of different anthropometric parameters measured from the patella.

Parameters	Gender	Mean	Std. Deviation	'p' value
DW	MALE	20.060	2.7139	0.000
	FEMALE	15.160	2.2653	
ML	MALE	3.892	.1077	0.000
	FEMALE	3.608	.1498	
MB	MALE	4.508	.1891	0.000
	FEMALE	3.744	.1474	
MT	MALE	2.068	.1574	0.000
	FEMALE	1.680	.1000	
VDA	MALE	3.344	.1474	0.001
	FEMALE	3.200	.1323	
TDA	MALE	4.480	.1354	0.000
	FEMALE	4.168	.1108	
BMAF	MALE	1.908	.0997	0.000
	FEMALE	1.624	.0831	
BLAF	MALE	2.556	.1003	0.000
	FEMALE	2.224	.1200	
SAP	MALE	14.164	1.1500	0.000
	FEMALE	9.664	.1977	
PI	MALE	115.36	5.453	0.000
	FEMALE	103.12	6.044	

Table No: 4: Percentage of accuracy among the different anthropometric parameters taken from patella:

Parameters	Percentage of accuracy	
	Males	Females
DW	87.24%	75.2%
ML	97.94%	97.92%
MB	88.89%	97.3%
MT	92.3%	89.28%
VDA	97%	90.7%
TDA	95.9%	97.6%
BMAF	94.8%	92.03%
BLAF	96%	90.91%
SAP	84.75%	98.35%
PI	86.69%	89.3%

Discussion

Nearly for the last thirty years, several studies have been carried out in different corners of the globe to establish the fact of patellar sexual dimorphism. One such pioneer study conducted by Gunn and Mc Williams[9] assessed the sexual dimorphism of patellae obtained from the Todd collection using volumetric analysis. This involved submerging the patellae into a container of water and using the displacement method in calculating the volume of the bone. The highest average accuracy of sex classification obtained in this study was 88% for the "Europids". O' connor[10] demonstrated a statistically significant dimorphism in patellae. Measurements were collected from the Terry collection and radiographs of college students had an accurate result of 82.5% in the classification of females and 78.6% in males. Jana et al[2] in the northern part of the West Bengal conducted a similar study where a total of 46 intact patellae (23 male and 23 female) were taken from the human cadavers and the various parameters were studied. Results of their study revealed that all the parameters of the patellae were higher in males when compared to females except angulations at the vertical ridge (this parameter is not included in the present study) which was higher in females than males. Same results were observed in our study too. Our study is similar to the study that was done by Francesco Intronajr et al[11]., KayalVizhi, S. Arora et al[12], TA Rathbun, BC Rathbun paolo et al[13] and Dayal and Bidmos et al[14]. The present study reported that the surface area of the articular part and maximum length of the patellae can be used in correct sex classification with an average percentage of accuracy of 98.35% and 97.94 % respectively. Introna et al[15] reported the highest average accuracy of 79%, for each of the maximum thickness and height of the lateral articular facet. The results of a study conducted in Iran (Akhlagi et al [6]) in which a higher average accuracy was obtained for the ML (89%) and MB (91%). Our study is similar to this study with accuracy for ML (97.94%) and for MB (88.89%)

Conclusion

The implementation of discriminant function analysis based on patella morphometry offers a simple and well-studied approach for sex determination on small bones of an individual from incomplete skeletal remains. The present study was conducted on patellae collected from 50 autopsied bodies in the Department of Forensic Medicine, Sri Venkateswara Medical College, Tirupati, shown that the mean values of males were

higher than that of females. The equation thus derived from this study can be used for sex determination in a group of south Indian population with the highest accuracy of 98.75% using variables. Similar studies are to be carried out in different zones of India, to verify findings in order to obtain uniform values for the Indian population, which has not been reported to date.

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Forensic Toxicology Analysis of Suicidal Hanging Deaths in Eastern Province-Saudi Arabia

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Abstract

Background: Suicide is a multifaceted phenomenon with multiple underlying risk factors, including alcohol and/or abuse of other drugs and psychiatric illnesses. Toxicological analysis is of crucial importance even in hanging deaths where the cause of death seems to be 'obvious'. **Objective:** To investigate hanging suicide deaths, focusing on toxicological findings in their postmortem specimens, over a period of six years in the Eastern Province, Saudi Arabia. **Methods:** The suicidal hanging deaths in the period from 2014 to 2019 were retrospectively studied from autopsy and toxicology analysis reports. **Results:** Of the investigated 75 hanging suicides, 64 (85.3%) were males and 11 (14.7%) were females. Most of the cases were in the age group between 31 and 40 years of age (36%). Non-Saudi nationals (57, 76%) much prevailed over Saudi people (18, 24%) and the highest is the Indian population (27, 36%), followed by Saudi nationals (18, 24%). Ethanol was the commonest detected substance in the studied specimens (45, 60%). Cannabis ranked the second (14, 18.6%) followed by antipsychotic drugs (10, 13.3%) and amphetamine (7, 9. 3%).Conclusion**:** The precise statistical mortality database for hanging suicides deaths may provide a valuable evidence for the importance of postmortem toxicology analysis and the role of alcohol and its effect on the aggressive behavior, human health and mortality. In the current study, Ethanol was the highest detected substance in the studied specimens (45, 60%), with predominance of non-Saudi Indian males.

Keywords: *Alcohol; suicides; hanging; toxicology, deaths*

Introduction

Suicide is a major public health issue and is considered to be a substantial cause of unnatural death in most nations, required scrutiny in monitoring it. Factors determining suicidal behavior and their risks are as numerous as they are varied, depending on the victim's gender, age, mental conditions, ethnicity, and other factors [1]. Alcohol overconsumption is a prominent feature of suicide deaths, especially in Western countries, as evident through elevated Blood-Alcohol Concentrations (BAC) in autopsy [2]. Drunkenness causes poor judgment and incapacitates self-control, rendering individuals impulsive, less inhibited, and reckless; this may cause self-harm, which, in pre-disposed individuals, could lead to a suicide attempt [3].

Toxicological analysis of blood and urine samples avails beneficial information that elucidates the totality of events related to violent and sudden suspicious deaths. Hanging deaths are complex due to the diverse physiological, demographic, and psychological variations of decedents in these cases. For example, prisoners in Greece, who were single, unemployed, single, without children, and/or involved in drug abuse, were considered prone to commit suicide, 45% of which were by hanging [4&5]. In USA, suicide by hanging is the second most prevalent method of death for Native

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Americans, 2.5 times higher than the national average [6]. In a Brazilian study, ethanol was detected in approximately 37.5% of hanging deaths [7].

This study highlights the valuable information extracted from toxicology studies performed in hanging deaths, elucidating scene, autopsy findings, deaths–related circumstances and risk factors for suicide.

Material and Methods

Victim's Data Collection

Of the 2115 cases examined in the department of forensic toxicology –Dammam over six years (2014–2019), 75 cases were identified as suicidal hanging deaths. The data were retrieved from autopsy and toxicology analysis records, examined retrospectively concerning their demographics, toxicology findings, scene information, gender, place of incident, manner and mode of death, types of obtained samples, state of putrefaction, and ethanol and drugs concentrations in femoral blood.

Forensic Toxicology Analysis

Samples preparation & Analytical Methods

Blood and urine samples were subjected to the extraction process using liquid-liquid extraction (LLE). pH adjustment was done to extract weak and basic drugs. It should be noted that many drugs of interest in forensic toxicology have a basic structure. Extraction products were solubilized in methanol and analyzed by thin layer chromatography (TLC). Positive results detected by TLC procedure were confirmed with more sensitive and specific analytical methods such as high performance liquid chromatography (HPLC) an Agilent GC/MS instrument [8]. Special consideration was taken to the source of blood and sampling site, use of preservatives, and intact chain of custody before analysis. Peripheral blood was taken from the femoral vein in tubes with potassium fluoride (2% wt/vol) as an enzyme inhibitor and a preservative [8].

Determination of Blood-Ethanol Level

Duplicate quantification of blood-ethanol level by headspace gas chromatography was performed methodologically as described Helander et al [9]. However, to avoid the risk of ethanol formation after death in this study, we considered an ethanol level of 0.10 g/L (10 mg/100 mL) as an analytical cut-off concentration in femoral blood to report positive ethanol results and bodies showed putrefaction were excluded from the study. Positive cases for ethanol were analyzed for the detection of ethyl glucuronide and ethyl sulfate (EtG and EtS) via liquid chromatography tandem mass spectrometry using the methodology described by Helander et al **[9].** to differentiate between ante- and postmortem ethanol formation. Only cases with EtG levels ≥0.5 µg/mL (positive cut-off for EtG) and EtS ≥ 0.1 µg/mL (positive cut-off for EtS) were enrolled in the study.

Analysis of Other Drugs of Abuse

The analysis performed initially by a broad screening testing with immunoassay procedures using Fluorescence Polarization Immunoassay (FPIA) principle (ARCHITECT 4000, model i1000 SR, Abbott Laboratories) for urine samples. The evidence MultiSTAT DOA blood array (competitive enzyme immunoassays conducted on the automated biochip array analyzer) was used for the qualitative detection of the drug and its metabolites in human whole blood aimed at pinpointing presumptive positive cases. Table 1 presents the manufacturer-established immunoassay cut-off concentrations for drugs in blood and urine specimens. Positive results were subjected to more specific chromatographic methods, e.g., Gas Chromatography Mass Spectrometry (GC/MS), with deuterium-labeled internal criteria.

Statistical Analysis

The latest SPSS statistical package, Version 25 was employed to evaluate the data statistically. The data was represented as number, minimum, maximum mean ± Standard Deviation of means (Mean ±SD) and the percentage and the results were studied in relation to other similar studies.

Table 1: Cut-off concentrations in urine & blood by Fluorescence Polarization Immunoassay (FPIA) and by evidence Multi STAT blood array.

Drug or Class of Drugs	Urine Cut-Off Concentration (ng/mL) by Architect. Machine	Blood Cut-Off Concentration (ng/mL) by evidence Multi STAT
Stimulants		
Amphetamine		
Methamphetamine	300	50
BZG/Cocaine	300	50
Opiates	300	25
6- MAM	-----	80
Morphine	-------	10
Heroin	300	10
Tramadol	300	10
Codeine	300	5
Propoxyphene	300	10
Fentanyl	300	10
Methadone	2	1
Hallucinogens	-----	10
Cannabinoids (THC)	25	10
Synthetic cannabinoids	20	5
LSD	0.5	---
Barbiturates	200	50
Benzodiazepines	200	20

Results

Analysis of the forensic toxicology reports concerning hanging suicides (in total 75 cases) over the six years duration (2015–2019) revealed that there was a higher proportion of males (n = 64, 85.3 %) more than females (n = 11, 14.7%), non-Saudi nationals prevailed (N = 57, 76%) over Saudi nationals (n = 18, 24%) .When examined for the location of the incidence, most of the cases occurred at the decedent's residence (n = 53, 70.7 %) followed by workplace site (n = 16, 21.3 %) as shown in table 2. The mean age was 38.62 (SD = 9.5) years in males and 34.8 (SD = 12.5) years for females.

As seen in figure 1, the highest incidence of cases was among Indians (N = 27, 36%) followed by Saudi people (N = 18, 24%). The frequency distribution of hanging suicides compared to the age groups showed that the highest incidence of cases was in the age group within 31–40 then the age group within 41–50 (results not shown).

The detailed toxicological findings revealed that the specimens of 19 victims yielded negative toxicology results while 56 cases were with at least one positive toxicology finding in their specimens. Ethanol was the highest detected substance in the studied specimens (N = 45, 60%) and it was the sole toxicological finding in

(N =34, 45.3%) of the studied samples Cannabis was detected in (N = 14, 18.6%) and amphetamine in (N = 7, 9.3%). Antipsychotic drugs were found in (N = 10,13.3%) of the studied samples where mirtazapine was detected in (N = 4,5.33%) and amitriptyline was found in 4% (N = 3, 4%) followed by venlafaxine and tramadol (figure 2). Distribution of positive toxicology results according to the abusing pattern and nationality as in figure 2 revealed that, mono-abusing pattern dominated among non-Saudi people (N = 33, 58.9%), while the bi- and triple-abusing pattern dominated among Saudi nationals (N = 12, 21.4%).

Of the total positive cases (N = 56), 30.3% (N = 17) revealed multiple findings in their analytical results as shown in table 3. The highest detected combination was ethanol with cannabis in 12.5% (N = 7) while the detection of amphetamine with cannabis ranked the second (N = 5, 8.9%).

As shown in table 4, the number of the collected and investigated blood samples was 45 samples, urine samples were 17 samples, and vitreous samples were 8 samples. The various alcohol concentrations in different postmortem biological samples showed a high coefficient of correlation between blood, urine, and vitreous humor.

Table 2: Suicidal Hanging Deaths Distribution by Sex, Nationality and Incidence Location

		Nationality				
		Saudi (n=18)		**non-Saudi (n=57)**		
		Count	**%**	**Count**	**%**	**Total %**
Sex	Female	5	27.7	6	10.5	11 (14.7)
	Male	13	72.2	51	89.4	64 (85.3)
Incidence location		Residence	Workplace	Jail Cell	Other	
		53 (70.7%)	16 (21.3%)	4 (5.3%)	2 (2.7%)	

Table 3: Co-occurrence of Ethanol and Drugs of Abuse in Analytical Results.

Toxicological Findings	**Count**	**% of the Total Positive Samples N = 56**
Total number of positive samples	56	100%
Ethanol + cannabis	7	12.5%
Cannabis+ amphetamine	5	8.9 %
Ethanol +amphetamine	2	3.5 %
Ethanol + cannabis + amphetamine	2	3.5 %
Amphetamine + heroin	1	1.7 %

Table 4: The correlation coefficient of variable blood, urinary, and vitreous alcohol concentration in different biological samples.

SAMPLE TYPES	Ethanol Conc (Mean±SD		Blood	Urine	Vitreous
Blood sample	154.15 ± 132.2	Pearson correlation Sig. (2-tailed) N	1 0.000 75	0.836** 0.000 33	0.709* 0.014 15
Urine sample	170.54±167.3	Pearson correlation Sig. (2-tailed) N	0.836** 0.000 33	1 0.000 33	0.790** 0.006 11
Vitreous samples)	142.61±136.2	Pearson correlation Sig. (2-tailed) N	0.709* 0.014 15	0.790** 0.006 11	1 0.000 15

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

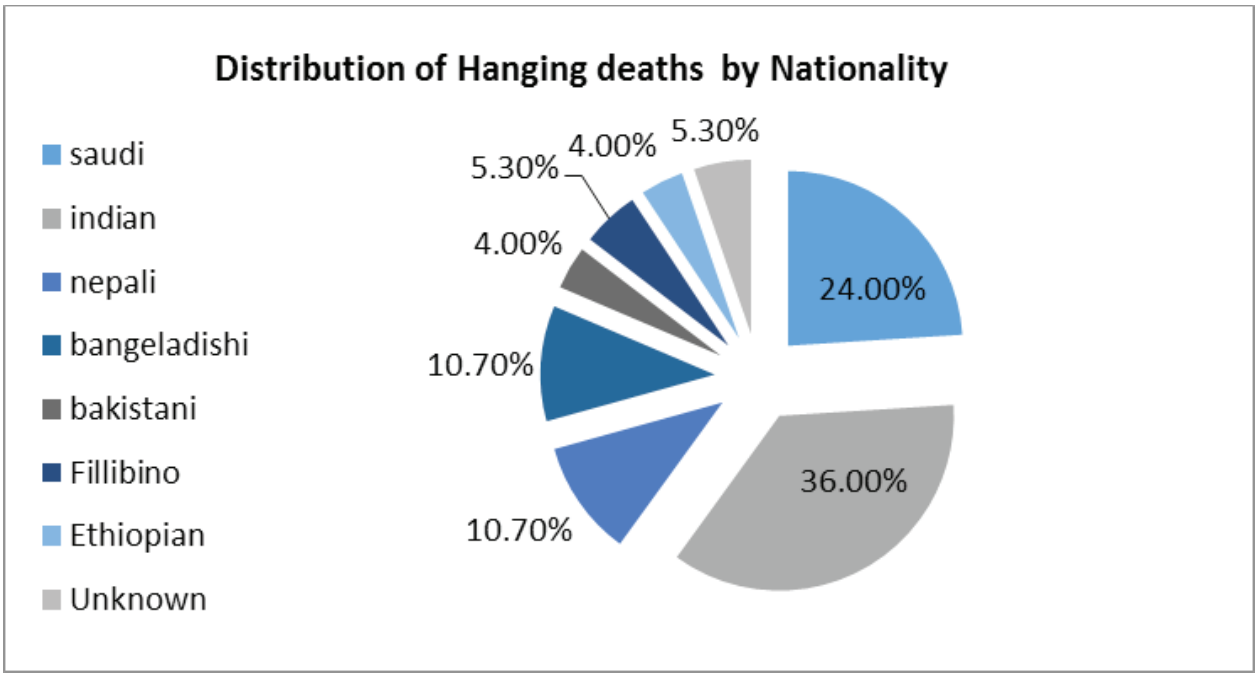

Figure 1: Distribution of Hanging Deaths by Nationality

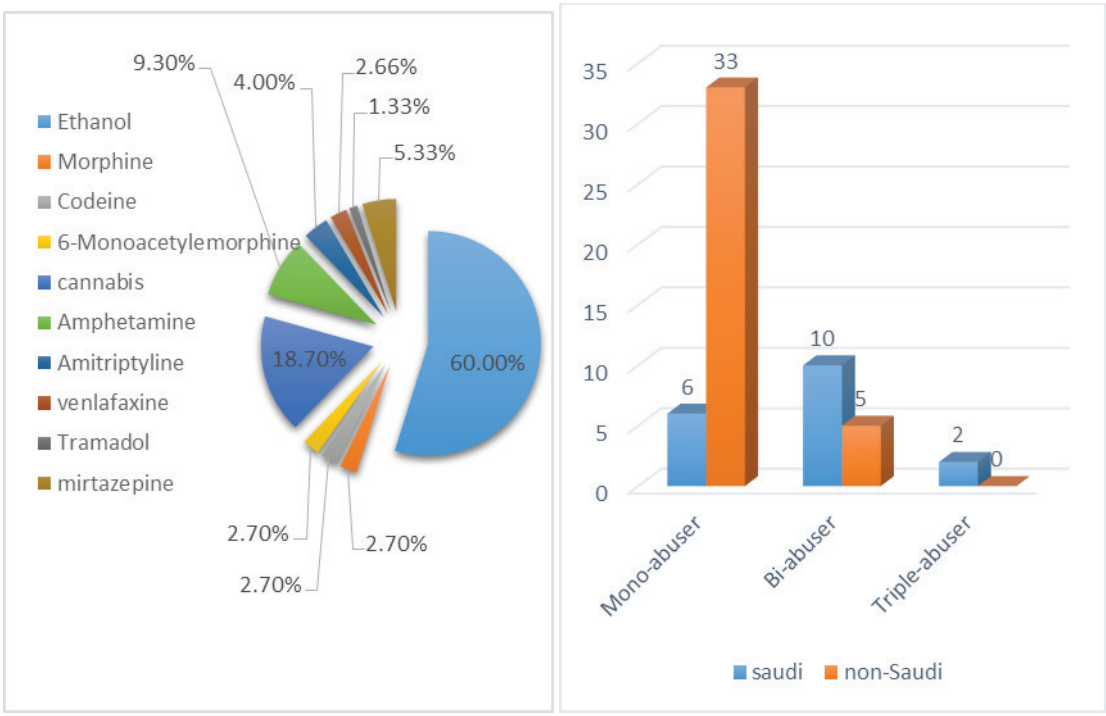

Figure 2: Detailed Toxicological Findings of the Studied Samples &its Distribution according to the abusing Pattern and Nationality

Discussion

Hanging has been one of the famous ways of lethal suicide. Some authorities consider that the seemingly self-inflicted hanging, without homicide evidence, is suicide at face value, nullifying the necessity of any further investigations. However, postmortem toxicology analysis is a vital part of the forensic examination to prove the criminal manner of the event in some cases [10] and to determine the degree by which the victim was intoxicated/impaired that they would not be capable of hanging themselves and whether committed the action under the effect of psychoactive substances or drug abuse at the time of the hanging [11&13]. Most research that investigates hanging deaths document social aspects, demographics of the decedents, and suicide prevention. Comparatively few studies have focused on the aspect of toxicology and the drug concentration in suicide victim's blood [14&15].

This study showed that 36% of the cases were within the ages of 31–40 and 30% were between the ages of 41–50 years, which was similar to other relevant studies [16]. As reported in many several studies, men outnumbered women 6.4 to 1.1 [17&18]. This illustrates a greater impetus for committing to more violent suicide methods in men. On the other hand, these findings were not following other studies performed by Kosaraju et al. whose results revealed that females within 20–30 years had a higher frequency of suicide [19]. Dammam, the largest and a densely populated city in the Eastern Province, Saudi Arabia, has a racially diverse population comprising mainly immigrant workers from Southeast Asia, especially Indians. This explains the higher percentage of Indian victims (36%). Saudis hanging deaths were less than Indians; this may be explained by the role of religion and the good social relations between family members throughout the country. In Islamic countries, according to Islamic law and Shari'ah doctrine, suicide is prohibited and deemed to be a criminal act against oneself [20].

Agreeing with several earlier researches, ethanol was the most commonly encountered psychoactive substance in blood samples of suicidal hangings victims [21&22]. BAC threshold of 0.10 g/L is considered the threshold for reporting positive results. 60% of victims had been drinking before death. In contrary to these results, Shields et al. concluded in a study in Kentucky,

United States, from 1993 to 2002 that cannabinoids were the most frequently detected substances in the blood samples of hanging victims [23].

Alcohol stands as one of the main risk factors of suicidal tendencies besides substance abuse and dependency [24&25]. Alcohol consumption can impair judgment, induce aggression, risky behavior, and impulsive actions, making individuals more prone to commit suicide. Alcohol and/or drug abuse may also enhance the suicidal ideation by exacerbating mental health problems and interpersonal conflicts [26]. It is well known that psychiatric disorders, particularly depression, is a chief risk factor for suicidal attempts. Therefore, in accordance with other similar studies, our results revealed the presence of antidepressant drugs, e.g., amitriptyline, venlafaxine, and mirtazapine, in the analytical specimens of hangings victims [27 &28].

Investigating multiple biological samples, like blood, urine, and vitreous samples, is important for correct result interpretation and confirmation. Unfortunately, the main sample submitted is the blood sample, mandating the necessity to find alternate samples, such as vitreous humor. Vitreous is a clear colorless and gel-like fluid surrounded by the vitreous membrane containing no blood vessels. It is resistant for being contaminated by microorganisms in addition to the measured ethanol levels in vitreous is equivalent to blood levels due to its high water content [29]. Like vitreous, urine has the advantage of being resistant to postmortem bacterial contamination. Postmortem alcohol production in urine only occurs when it contains large amounts of glucose as in diabetics or in the case of a bacterial or fungal urinary tract infection ante mortem [30]. The correlations between alcohol concentrations in blood, urine, and vitreous samples in our study proved to be suitable alternative samples with correlated results as seen in many other studies [31].

Conclusions

It is concluded that males greatly surpassed females in this study a most of the deaths were between 31-40 years age. Non-Saudis represented the highest percentage of hanging deaths, most of them were the Indian population, followed by Saudi nationals. Toxicological analysis is of crucial importance even in hanging deaths where the cause of death seems to be 'obvious' to highlight the importance of alcohol/and or other abused drugs as a risk factor for suicides. Such data will consequently help to better tailor preventive efforts.

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Conflict of Interest: None declared.

Ethical approval: The needed ethical approval was obtained from the institute ethics committee.

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BCL-2 Gene Expression association with Breast Cancer Susceptibility of Iraq Population

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Abstract

Breast cancer is a significant illness in the world. Molecular methods are important and essential for the diagnosis of breast cancer. Many of the genes with expression modification, such as the Bcl-2 gene, are explicitly encoding an anti-apoptotic protein and thus known as oncogene.A total of 55 patients with breast cancer divided into(25 Stage 0 , 15 Stage IA, and15 Stage IB), and 25 control group were enrolled in this study, age range of 35 – 60 years old the samples were collected from Al-Eluia hospital for woman care, oncology teaching hospital.RNA were extracted and then converted to cDNA to perform real time- PCR done for those samples to detect the gene expression.Expression of gene in the study is higher levels in malignant group stage IB, following stage IA ,and stage0 and the fold of expression was 10.00 time higher than the control group, significantly higher in the third grade of breast tumor samples with Ct (22.14) of the first grade with CT (25.63) and the second with CT (24.07). According to the results of the study that the use of molecular methods in measuring the expression of Bcl-2 gene may help to diagnose the disease and may be considered that the Bcl2 gene is molecular tool for the early detection of breast cancer.

***Key words**: Breast Cancer, real-time PCR , Bcl-2 gene.*

Introduction

Breast cancer is the most prevalent malignant type all over the world. Women, who have a family history of breast cancer, especially a first-grade.Breast cancer is, important malignancy, and comprises 19% of all women cancers[1]. Breast cancer incidence rates are rising in the world, mostly in the developing nations .According to ,the world health organization (WHO) , about one third from the costs of cancer medication ,can be reduced, if cases are disclose and treated early[2].Doctors assign the stage of the cancer by combining the T, N, and M classifications, the tumor [3].BCL2 gene is located on Chromosome 18and its size:196,783 bases , and encode Bcl-2 (Bcell' lymphoma, 2) protein in human being, is the one of members of the Bcl-2 family regulatory proteins that organized the death of the cell (s) either by triggering (pro-apoptotic) or prevent (antiapoptotic) apoptosis[4,5].Gene Bcl-2 is encoded a anti-apoptotic protein seriously and therefore classified as an oncogene, Identified the damage of the Bcl-2 gene may be reason for a number of cancers, including breast and prostate cancer, leukemia, lung cancer [6,7]. Bcl-2 gene expression may be considered an apoptosis markers in breast cancer tissues. The majar purpose of this research is to discover the expression of Bcl-2 in breast tumor and compared the results with samples as a control [8].

Materials and Method

A total of 55 patients with breast cancer divided intostages and 25 control group were enrolled in this study, age range of 35 – 60 years old. The study was conducted between August 2018 and January 2019 samples were collected from Al-Eluia hospital for woman care, oncology teaching hospital. The complete physical examination was done to every patient.The final diagnosis was established by aspiration of cysts using (FNA) to check cytology.

RNA extraction and gene expression

The extraction of RNA was performed utilizing Trizol from all the states cells in this study according to the protocol provided by the manufacturer of Zymo

Quick-RNA Micro-prep Kit. A set of primers were used to amplify specific region within the prolactin receptor gene; forward primerGGTGAAGGGCGTCAGGTG and the reverse primer wasGAGTGGGATGCGGGAGATG[this study]. And an additional set were used to amplify the reference gene GAPDH gene to use it with calculation as a reference gene; F, ACCTGACCTGCCGTCTAGAAA and the R, GAAGTCAGAGGAGACCACCTG (this study). Elustedd RNA were converted to cDNA by using Takara prime script Kit. And then the obtained cDNA went through RT-PCR and thermal cycling program were as follow, enzyme activation 95 C for 7 min, followed by 40 cycles of two steps the first one was denaturation 95 C for 20 sec and second step of annealing and florescence screening for 20 sec (55 C) and extension for 20 sec. Folding of the gene expression were calculated after subtracting the Ct value of reference gene (GAPDH gene) from the Ct value of BCL2 gene.

Results and Discussion

After the microscopic examination of the patients biopsies the samples were divided into groups describing the cancer invasion status; 25 samples asstage 0 (It is called non-invasive or in situ cancer) 15 samples of stage IA (The tumor is small, invasive, and has not spread to the lymph nodes) and finally Stage IB were shown in 15 samples (Cancer has spread to the lymph nodes and the cancer in the lymph node is larger than 0.2 mm but less than 2 mm in size). The results in figure (1) describe the distribution of samples according to their stages.

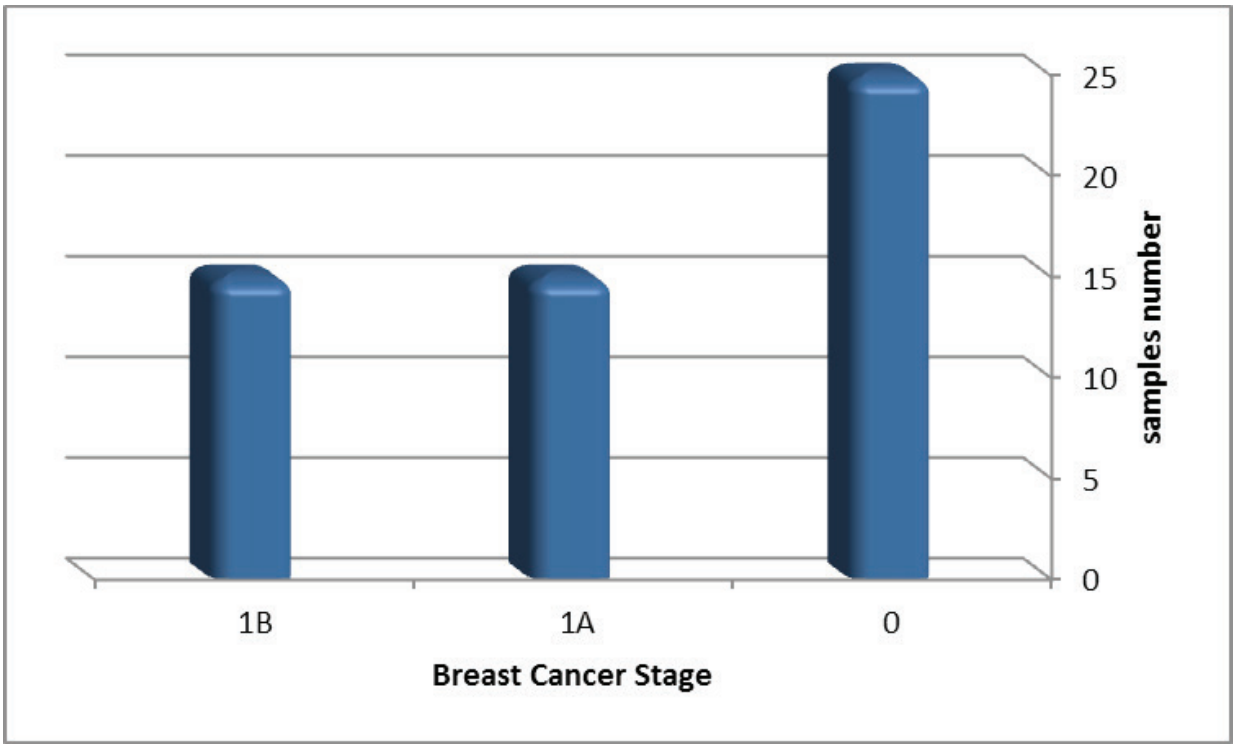

Figure (1):samples distribution according to stages of cancer

Calculating the gene expression of breast cancer samples and comparing them to the gene expression of the control biopsies obtained from two patients who had gone through spectroscopy and eventuallywerer found to be not suffering from any cancerous issue. The results showed an increment in gene expression of the BCL-2 gene, samples of stage 1B showed 7.2 foldgene expression than control, samples with stage 1A showed 5.8 fold thancontrol and finally samples of stage 0 expressed the gene 2.5 times the controls.

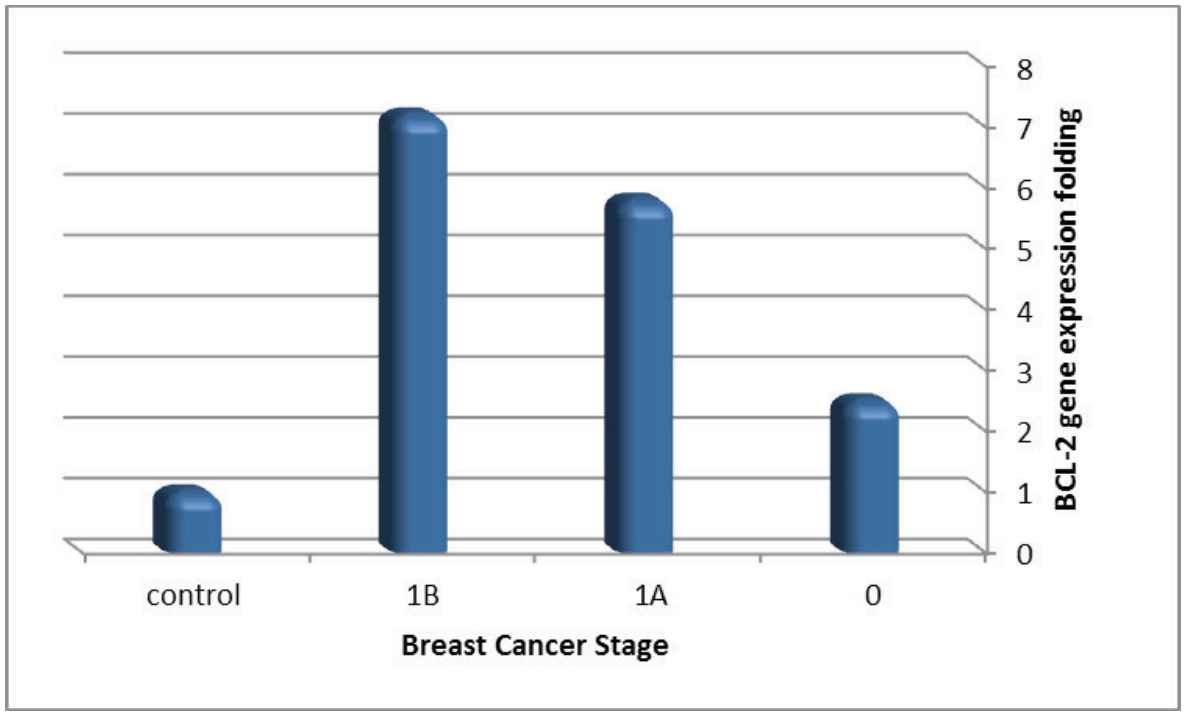

Figure (2): comparing the gene expression of BCL2 in patients group and control

Discussion

In the current study, the quantitative RT-PCR assay analyzed the mRNA expression from Bcl2 and compared its expression among control and malignant groups. Calculation the gene expression fold shift was performed using relative quantification[9] .This focuses on the normalization of the Ct values for the measurement of the ΔCt, which is the difference between the mean Ct values for the replica of the Bcl2 DNA amplification from each case and the GAPDH values.In terms for gene expression, elevated Ct values, lower gene expression and low Ct values indicate high gene expression[10]. Also the another study revealed detection of BCL2 in all conditions but it more in normal tissue around malignant that run in same line with other reports [11,12,13], they all demonstrated there is a significantly higher level of expression of anti-apoptotic protein (BCL2) in the normal tissue around breast carcinoma in comparison to age-matched breast tissue from women without cancer. The Bcl2 provide prognostic marker information in early stage breast cancer in all subgroups [14,15]. The study of Korch *et al.,*[16] concluded that up regulation of Bcl2 is not involved in lobular breast carcinogenesis and is unlikely to represent an important determinant of tumor development in breast cancer cell lines. Kathan and coworker [17], reported that Bcl2 expression was lower in stroma of precancerous fibro adenoma breast cancer than in those non-cancerous tissues Expression of Bcl2 in breast carcinoma tissues significantly found to be lower than in normal tissues [17]. suggested that Bcl2 expression play an important role in the occurrence and development of the breast carcinoma, and any changes in Bcl2 expression occurred in the early stage of breast carcinoma.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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Effect of Home based Parent Child Interaction Therapy for families Having Children with Disruptive Behavior Problems at Selected Community of Khurda, Odisha, India

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Abstract

Background: Disruptive and inappropriate behaviors of children are actions that interfere with the instructional, administrative or service functions of the organization and family or school. In the early lives of children the significant risk factors for occurrence of antisocial behavior and criminal activity are the presence of oppositional and disruptive behaviors. Related to this concept ,current study was conducted with objectives to identify the families having children with disruptive behavior problems ,to determine the effect of (parent child interactive therapy) PCIT on disruptive behavior level ,to find out the association between disruptive behavior of the children with selected demographic variable.

Materials and Methods: A quasi –experimental study was carried out with total 40 number of samples by using total enumeration sampling technique.The intervention was carried out under the guidance of guide and experts.Each Individual was exposed to the therapy for duration of 1 hour,twice a week **.** Child Directed Session 30 MIN and Parent Directed Session 30 mins. Total no.of 5 session was carried out. After completion of intervention ,changing behaviour was assessed by eyeberg scale.

Result: Study concluded that in experimental group 90% children shown mild disruptive behavior and 10% children found moderate disruptive behavior before giving intervention , and after intervention 10% children did not show any disruptive behavior and ,90% children had mild disruptive behavior . And there was statistically significant association between disruptive behavior of children with type of family, education of father, education of mother .

Conclusion: There was significant difference between the scores of pre-test and post -test level of disruptive behavior of the children in experimental group as 'p' value (0.025*) at 0.05 level of significant.

Keywords: *Parent ,Child, Disruptive Behaviour, Interaction Therapy, Session*

Introduction

Temper tantrum is a normal characteristic feature for a child under toddler period. when tantrum persist in young children that leads to problematic behavior and predictor of anti-social behavior in future.And during childhood may show symptoms of disruptive behavior [1]Assessment of disruptive behavior of children is a highly subjective process. A child with disruptive behavior always seek to draw the attention of teachers and trying to prevent the teacher to make attention towards other children in a classroom. [2] More concern given towards disruptive behavior children by respective teachers, school workers and mental health professionals in primary school.[3]

It is essential that the nation find ways to support emotional health of children and adolescents and their families through a continuum of comprehensive, individualized and culturally compatible services.[4] Oppositional Defiant Disorder(ODD),Attention deficit

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Hyperactivity Disorder(ADHD) and conduct disorder (CD)are collectively known as childhood disruptive behavioir disorders, which requires immediate intervention and treatment. [5]

In 2016 Niec LN , Barnett ML conducted a study among young children having conduct problems with the help of randomized control trial method and parent-child interaction therapy was provided to them. Total 42 no. of children were participated in the study having conduct problems and adaptive functioning, as well as significant decreases in parenting stress. After intervention, results showed Parents were having significant improvements in their parenting skills and parents in Group PCIT did not experience greater social support or treatment adherence. [6]

In march 2013,Deepa K conducted a study ,in which research explained that Parent child interaction therapy was proposed by Eyberg et al in1995 with certain goals and designed to improve the parent –child interaction , relationship quality,decrease the child's behavioural problems ,improve the social behavior in disciplinary way,reduce parenting stress and improve parenting skills.parent child interaction therapy has 2 components : one is child directed interaction(CDI)therapy another is parent directed interaction(PDI).This approach provide treatment to both parent and child together to manage disruptive behavior of children and parent child relationship.[7]

Melanie A. Fernandez , Ashley M. Butler & Sheila M. conducted a study in 2009 among African American Families who were having low Socioeconomic Status with the use of Parent-Child Interaction Therapy and its outcomes . This study examined disruptive behavior disorders of preschoolers and the effectiveness of parent-child interaction therapy (PCIT) in 18 socioeconomically backward African American families. Result revealed that Mothers were showing significant improvements in child disruptive behavior rather than in parenting stress or maternal depressive symptoms . Results concluded that PCIT may lead to changes in behavior for disruptive young African American children after end of treatment. Reduction of parenting stress also requires study in this population.[8] Daniel S Shaw examined and concluded that risk factors from infancy associated with the development of preschool disruptive behavior problems across child, parent, and socio demographic domains. Risk factors which were associated with the occurrence of disruptive behavior at 5 years of age included infants had history of disorganized attachment with parents or family members and disturbed maternal and child-rearing disagreements during the period of second year showed significantly higher aggressive problems. [9]

Children are our near future who make a difference and greater development of nation, so it very much crucial to develop them in a good and healthy environment with proper guidance of elder adults. In Family, parents are the first teacher who helps them to learn about right or wrong things,responsibility,independency,love and affection,caring etc.children always watch to their parents activities and trying to follow them.Behavioural problems mainly develops due to internal conflict and failure in adjusting the outer environment. Children with behavioural problems always need special attention towards them. [10]

Now a days most common Disorder found in school aged children and in child psychiatric treatment unit is childhood disruptive behaviour disorder .In 2014, a Descriptive study was conducted by using convenient sampling technique among primary school children with total 30 number of students to identify disruptive behaviour disorders. Result revealed that majority 47.36% children had moderate disruptive behavior, 42.12% had severe disruptive behavior and 10.52% children had mild disruptive behaviour disorders .study concluded that early identification and proper intervention can yield better outcome for children with disruptive behavior.[11]

Childhood behavioural disorders are characterized into disruptive behaviour disorder of a child which includes Oppositional Defiant Disorder,Attention Deficit Hyperactivity Disorder and Conduct Disorder.These disorders have different psychopathology and requires proper management by parents and health professionals to modify their maladaptive behavior.Hence ,its vital for nurses to play a crucial role to reduce symptoms of childhood behavioural disorders and provide accurate nursing management.[12]

Rachel A. Gershenson, Aaron R. Lyon, Karen S. Budd in 2010 conducted a study on Promoting Positive Interactions in the Classroom: Adapting Parent-

Child Interaction Therapy as a Universal Prevention Program concluded that The adaptation of Parent-Child Interaction Therapy may provide an opportunity to improve the relationship between teacher and child and also guide teachers as a effective tool for behavior management.[13] Webb HJ, Thomas R, MC Gregor L, et al in 2016 Conducted a study on an evaluation of parent child interaction therapy with and without motivational enhancement to reduce to attrition. The study depicted that child externalizing behavior report promising outcomes for families.A parent child interaction therapy with 3 session individual motivational enhancement component started to reduce attrition and improves outcomes. Total 192 number of Australian caregivers were participated, among them 91.7% were female , 33.3% were their children , and 51% Families referred from child welfare or health services for risk of maltreatment were assigned to PCIT .It depends on their time of entry to the study. Those families who were in waiting list ,they received phone calls once in a week for 12 weeks. At the end there were major reductions found in externalizing and internalizing parental stress and child behavior problems among families.There was generally no significant difference between the two treatment conditions. [14]

In 2017, Rajanidevi. Hiremath, Sanjay Shinde conducted a study among 100 number of primary school teachers about knowledge regarding childhood behavioural problems and its prevention at Bagalkot. In school teachers play a important role in health promotion and prevention of diseases.So,it is teacher's responsibility to find out the behavioural problems of children as early as possible and initiate preventive action.Hence its vital to assess the knowledge level of teachers regarding childhood behavioural problems.In this study result revealed that majority 58.82% teachers had moderate knowledge about behavioural problems of children and their prevention.[15]

Rag pickers under the age of 14years are most vulnerable to various psychological disturbances. Children are doing such type of hazardous work and living in an unhealthy environment usually exploited physically ,socially, mentally and morally.So,they are involved in more anti-social activities.Study revealed that children having such bad working conditions and poor demographic conditions influence their mental health, which promotes to the children for developing deviant behavioural attitude.[16]

Namasivayam AK, Jethava V, Pukonen M, et al in 2016 Dec Conducted a study on parent child interaction in motor speech therapy. It was concluded that, this study was carried on to measure the reliability and sensitivity of a modified parent child interaction observation scale (PCIOs). It is used to monitor the quality of parent child interaction. Child related items were more strongly affected by differences in treatment intensity than parent related items, where a greater number of reasons positively influenced. [17]

Bagner, D.M. & Eyberg, S.M. in 2007 conducted a RCT study on Parent-child interaction therapy among children having disruptive behavior with mental retardation. Children assigned to the PCIT group were scored by parents as having lower levels of externalizing behavior (effect size = 1.08), less frequent disruptive behavior (effect size = 1.5), and less total problem behaviors on the Child Behavior Checklist (effect size = 0.97). No significant impacts were shown for the severity of problem behavior as measured by the Eyberg Child Behavior Inventory. Mixed results were found on the parenting stress index, with a positive impact found for the difficult child scale, but not the parental distress or dysfunctional parent-child interactions. [18]

In 2016 Archana Maurya, B. Lakhakar, Darshana Kumari conducted a study at Wardha among 200 school going children to detect the prevalence of behavioural problem .Researchers used descriptive survey approach to collect the information from parents of selected children.Result depicted that age of children was statistically associated with behavior problem score and prevalence of behavior problem was seen in school going children.[19]

Children education, development in a healthy environment depends on sources available in their surrounding and opportunities provided by parents. Behaviour ,thinking,attitude etc are influenced by their parents,family and society.Now a days various advertisements directly or indirectly influencing needs and desires of people and also child development. Strong parent-child interaction and healthy decisions towards development of child can prevent childhood behavioural problems in school going children.[20]

Materials and Methods

An evaluative research approach and quasi-experimental design was used in this current study . Prior permission was obtained about ethical issues from dean and authorities.The main study was conducted at Malipara village of Khurda , Odisha during January 2019-february 2019 with total 40 number of children 3to 8 year of age having disruptive behaviours taken as sample by using total enumeration sampling technique. Then formal permission was obtained from the parents and informing about the procedure,purpose & benefits of data collection.Pre test was conducted in the two weeks prior the therapy (pre intervential 2 week), the children both experimental group & control group. Followed by the intervention was carried out under the guidance of guide and experts.Each Individual was exposed to the therapy for 1 hour,twice a week **.** Child Directed Session for 30 Minutes and Parent Directed Session for 30 minutes. Total no.of 5 session was carried out.

Ethical Considerations

Participation in this research study was purely voluntary and informed written consent was obtained from every participant. Approval from the Institutional Ethical Committee was obtained before the study.

Results

Table:1: frequency and percentage distribution of study samples according to age, child education, occupation of father, type of family, education of father, education of mother and religion.

$N (n_1 + n_2) = 40$

Items	Frequency(f)	Percentage(%)
Age in year		
< 4	Nil	------
4 to 6	14	35
7 to 8	26	65
Child education		
Nursery	15	37.5
Primary	25	62.5
Occupation of father		
Employed	14	35
Govt. Job	2	5
Labourer	18	45
Unemployed	6	15
Type of family		
Nuclear family	9	22.5
Joint family	28	70
Extended family	3	7.5
Education of father		

Cont... **Table:1: frequency and percentage distribution of study samples according to age, child education, occupation of father, type of family, education of father, education of mother and religion.**

$N (n_1 + n_2) = 40$

Illiterate	2	5
Primary school	13	32.5
Middle	22	55
Higher secondary	3	7.5
Education of mother		
Illiterate	12	30
Primary school	27	67.5
Higher secondary	1	2.5
Religion		
Hindu	40	100

Figure-1:Pre test and Post test scores of disruptive behavior in experimental group (intensity scale).

$n_2 = 20$

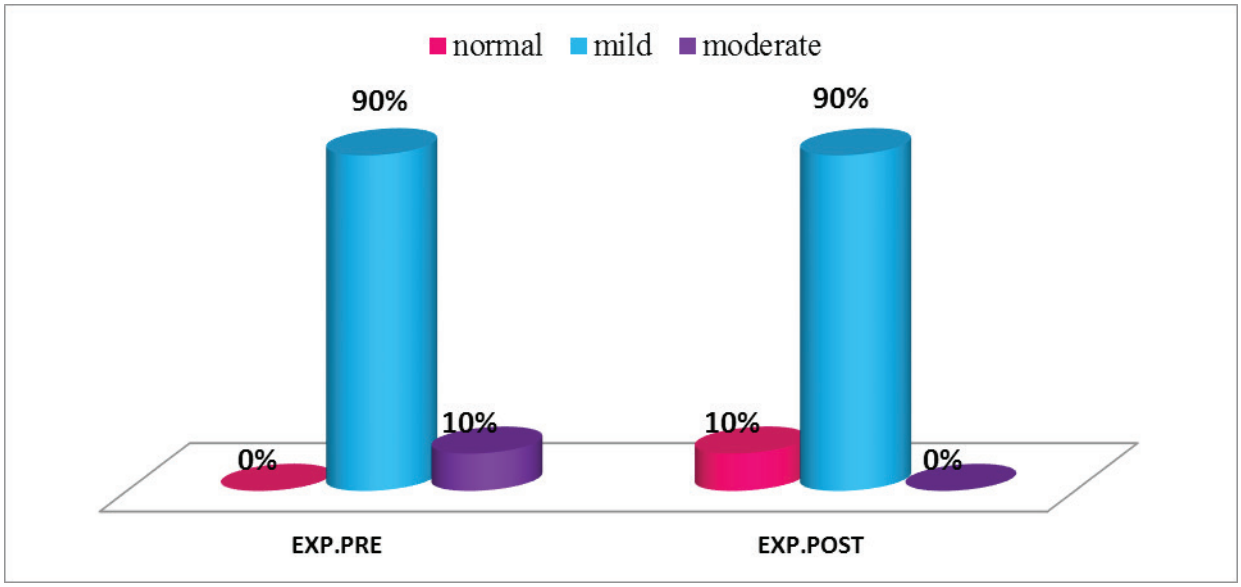

Figure-1 Reveals That out of total children in experimental group 90% children shown mild disruptive behavior and 10% children found moderate disruptive behavior before giving intervention , and after intervention 10% children did not show any disruptive behavior and ,90% children had mild disruptive behavior .

Figure-2 pre-test post test scores of disruptive behavior of experimental group of children (problem scale).

$n_2 = 20$

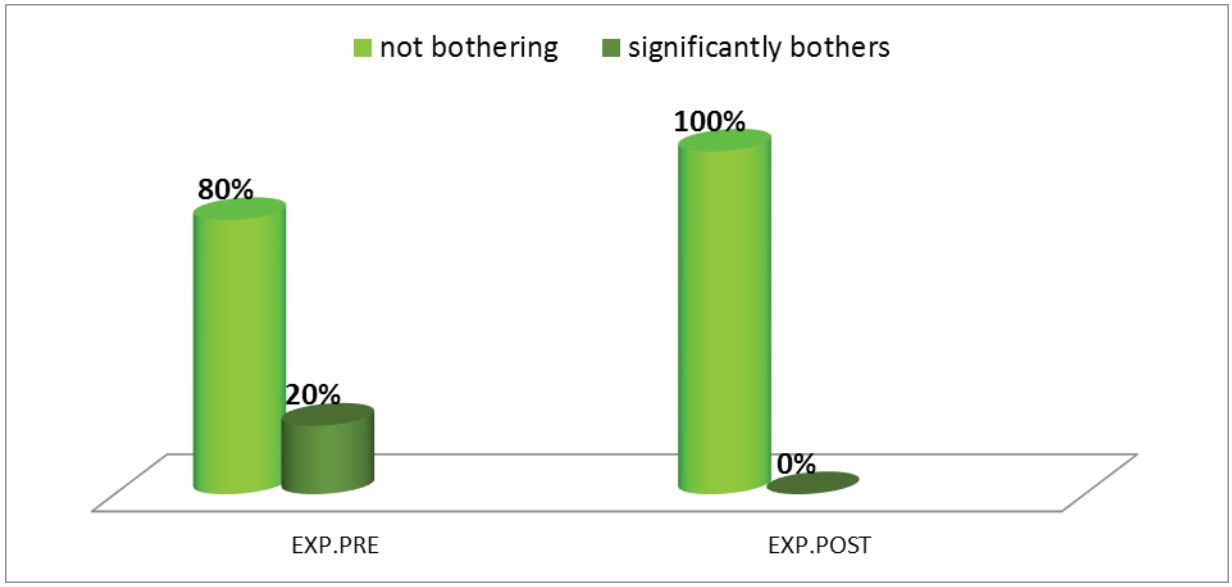

Figure-2 shows that in experimental group prior to intervention only 20 % children's parents stated that they were significantly bothering about their children disruptive behavior but 80 % parents were not bothering at all. No one complained about their children's disruptive behavior after intervention .

Figure- 3: pre test -post test scores(problem scale) of disruptive behavior of the children from control group

$n_1 = 20$

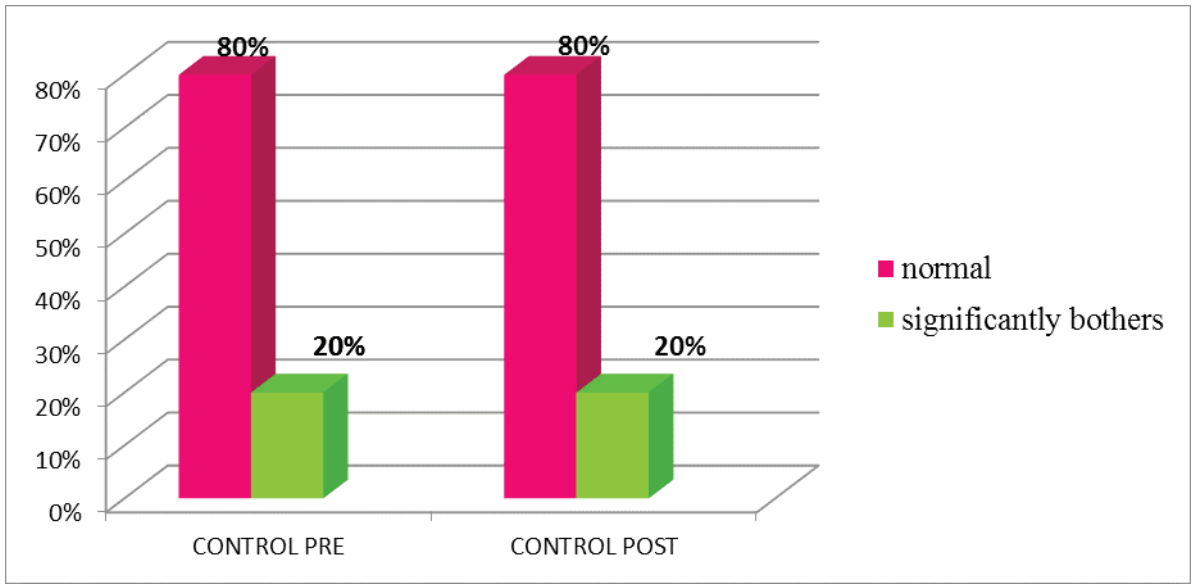

Figure-3 shows that in pre test and post test there was no changes found in disruptive behavior of children in control group.

Table.2 comparison between pre test and post test scores of disruptive behavior of children in experimental group using Paired ' t' test .

$N(n_1+n_2)= 40$

Item	mean	sd	paired t value	df	p value
Intensity scale	1.7	3.64	2.086	19	0.025 *
Problem scale	2.7	2.735	4.413	19	0.00015*

*P value ≤ 0.05

Table -3 : Comparison between post test scores of disruptive behavior of children in experimental group and control group by using unpaired 't' test .

$N(n_1+n_2)= 40$

Items	groups	mean	variance	unpaired't' test	df	p value
Disruptive behavior (Intensity scale)	experimental	63.85	0.421	2.0508	38	0.0472*
	control	66.75	1.584			
(Problem scale)	experimental	55.35	0.281			
	control	57.8	0.298	3.218	38	0.0026*

*P value ≤ 0.05

Table-4 Chi square analysis of selected demographic variables i.e age of child, child education, occupation of parent, type of family, education of the father, education of the mother with parent child interaction therapy.

$N(n_1+n_2)= 40$

Demographic Variable	Chi-Square Value	Df	P value
Age in year of Child	0.062794	1	0.802
Child Education	0.173789	1	0.676
Occupation Of Parent	7.3825	3	0.060

Cont... **Table-4**

Type Of Family	19.395	2	6.1E-05 *
Education Of The Father	15.684	3	0.001 *
Education Of The Mother	8.333	2	0.015 *

*P value ≤ 0.05

Discussion

Mariëlle E Abraham,Marianne Junger et al in 2012 conducted a study on Parent–child interaction therapy for preschool children with disruptive behaviour problems .the study reveals that This study is based on the data of 37 referred families. Whereby the results of which are analysed from an analysis of parent reports of the Eyberg Child Behavior Inventory (ECBI), obtained during each therapeutic session. This study revealed that 27.5% families dropped out from treatment before treatment was completed. And results indicated that parent child interaction therapy significantly reduces the disruptive behavior of children. At post treatment, there were no significant differences were found concerning the frequency of behavioral problems of children who completed treatment and those who participated in the non-clinical comparison group.[21] Brain Allen, Susan G. Timmer, Anthony J. Urquiza in 2014 conducted a study in US on Parent child interaction therapy as an attachment based on intervention with total 85 number of children having disruptive behavior as participants. Results demonstrate significant improvements in externalizing and internalizing concerns and in the intensity and number of behavior problems overall P (2.68) = 38.86, P<0.001, as well as improvements in positive parenting techniques and reductions in parenting stress .Also had repeated measures analysis by using two assessment points (pre treatment or post treatment) of co-variance of scores on two measures (Child behavior checklist , Eyberg child behavior inventory).[22] In present study 'p' value (0.025*) and 0.00015 respectively in intensity and problem scale which was less than 0.05 level of significant . it shows significant improvement after intervention. Like wise there is chi square association of disruptive behavior of children with type of family, education of father, education of mother as the 'p' value of this calculated chi square value less than 0.05 level of significance i.e 6.1E-05 *, 0.001 *, 0.015 *.

Conclusion

On the basis of findings of the study the following conclusion were drawn,The present study reveals that the children with disruptive behavior who receive parent child interaction therapy show lower level of disruptive behavior in compared to who don't receive the therapy. The present study shown that disruptive behavior level mean score in experimental group after receiving parent child interaction therapy varies between 63.85 and 65.4 in intensity scale,55.35 and 58.05 in problem scale. Assessment of disruptive behavior of the children & parent child interaction therapy had a significant effect on reducing the level and frequency of disruptive behavior among children. So parent child interaction therapy can be used effectively in community settings for maintaining and improving health status.

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Declaration of Parent Consent : Institutional Review Board permission obtained for the study.

Conflicts of Interest : There are no conflicts of interest.

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Type of article: Original Research

The Monomer Elution of Bulk-Fill Sculptable Composite and Flowable Composite after Photopolymerization

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Abstract

Introduction: Composite resins have excellent mechanical properties, including fast polymerization, aesthetic quality, easy handling, and the ability to adhere to the enamel surfaces. However, there are also some disadvantages from this material, one of them is residual monomers. Bulk-fill composite resins are available in sculptable (solid) and flowable (liquid) form. Most composite resin matrix is aromatic or aliphatic acrylates. Bis-GMA, UDMA, and TEGDMA are the matrix monomers that are commonly used but show a high cytotoxicity level. They also have poor mechanical properties such as wear resistance, hardness, tendency to change color, and may cause pulp reactions. **Aim:** The aim of this study was to investigate the number of residual monomers in bulk-fill sculptable and flowable composites in the submersion of 75% ethanol solution (10 minutes, an hour, and 24 hours). **Methods:** Sculptable and flowable composites were used in this study. Samples (4 mm thick, 5 mm diameter) were prepared and polymerized for 10 seconds with an intensity of 1025 mW / cm2 of a light emitted diode (LED). After the sample was made, each sample was immediately immersed in a 75% ethanol solution as an extraction liquid and stored in amber-colored bottles at room temperature. Samples were taken as many as 7 cc at intervals of 10 minutes, an hour, and 24 hours. Samples were analyzed by High-Performance Liquid Chromatography (HPLC). The data obtained were analyzed using the Kruskal-Wallis Test, Independent T-Test and Mann-Whitney Test at a significance level of $p < 0.05$. **Result:** The amount of elusion remaining monomers of the bulk-fill flowable composite was higher than bulk-fill sculptable composite. UDMA composite of bulk fill flowable submersed in 75% ethanol solution for 24 hours was the highest among other monomers. **Conclusion:** Residual monomers eluted inside bulk-fill composite resins in all time periods and the amount of eluted monomers increases with time.

Keywords: *Residual monomer, monomer elution, bulk-fill composite, High-Performance Liquid Chromatography (HPLC)*

Introduction

Composite resins have excellent mechanical properties, including fast polymerization, aesthetic quality, easy handling, and the ability to adhere to the enamel surfaces. However, there are still concerns about the clinical condition of composite resin. Unreacted and residue of monomers in a set composite resin were known as residual monomers. The residual monomer can lead to oxidation and hydrolytic degradation, which can cause discoloration and worn out of the composite restoration. Moreover, residual monomers can cause soft tissue irritation, stimulate bacterial growth, and increase allergic reactions[1].

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The monomer elution process is the release of monomers from polymer composite resins. The amount of monomer elution is influenced by (a) the number of unreacted monomers, which is determined by the chemical structure of the monomer and the polymerization conditions; (b) solvents used for monomer elution; and (c) the size and characteristics of the chemical structure of the monomer. Some studies found that small molecules, such as TEGDMA (having a molecular weight of 286 g/mol) has a higher mobility monomer and faster elution process than larger molecules such as bis-GMA (having molecular weights of 512 g/mol). There are different views about the time taken to complete the elution of unreacted monomers. Some studies state that elution takes 1 to 7 days to complete, while other studies state that the complete elution process takes approximately 30 days[2,3].

Bulk-fill composite resins are available in sculptable (solid) and flowable (liquid) form. Most composite resin matrix is aromatic or aliphatic acrylates[4,5]. The characteristic feature of bulk-fill composites is its increased depth of cure up to 4 mm and reduced polymerization shrinkage stress[6]. Tetric N-Ceram Bulk-fill has a patented filler which is partially functionalized by silanes, acts as a unique shrinkage stress reliever which essentially "holds on" to the cavity walls along with the matrix and the adhesive. Tetric N-Ceram Bulk Fill also utilizes the initiators like camphorquinone plus an acyl phosphine oxide, together with a recently patented initiator Ivocerin dibenzoyl germanium derivative which allows the application and curing in larger increments of up to 4 mm, without compromising the optical properties of the composite such as translucency or colour[5].

Bis-GMA, UDMA, and TEGDMA are matrix that are commonly used in dentistry. Bis-GMA and UDMA are thick monomers to facilitate their clinical application. It is necessary to add monomers with a lower molecular weight called viscosity controllers. One of the diluting monomers used is TEGDMA. The majority of bulk-fill sculptable resin composites contain Bis-GMA monomers which are high viscosity due to its high molecular weight. While the majority of flowable bulk-fill composite resins contain TEGDMA monomers which are the opposite of Bis-GMA monomers. TEGDMA is an aqueus monomer because of its low viscosity and low molecular weight. The factory recommended polymerization time for 4 mm layer thickness is 20 seconds with light intensity 500 mW/cm^2 and 10 seconds with light intensity ≥ 1000 mW/cm^2[4,5,7].

Recommended energy density for adequate polymerization of conventional composite resin at 2-mm depth is 21–24 J/cm^2 [8]. The present in vitro study has proven that energy density plays a pivotal role in adequate photopolymerization of bulk-fill composite resins. The high energy density (20 J/cm 2) derived from both increased curing time and low power of the curing device results in a faster release of free radicals and, consequently, a higher DC of the material and increased depth of cure[9]. This energy (E) is calculated by multiplying the irradiance level (I) coming from the light control unit (LCU) (mW/cm^2) by its duration (T). Curing time is set depending on the irradiance level. The higher the irradiance level, the shorter the curing time needed[10]. El-Damanhoury and Platt (2014) Comparing bulk-fill resin-based composite (RBCs) with conventional RBCs and a micro-hybrid RBC (Filtek Z250) as a control. They reported adequate DC of all bulk-fill RBCs at 4 mm RBC thickness However, Filtek Z250 RBC showed inadequate DC of the bottom surface at 4 mm RBC thickness[6]. In conclusion, although 2 mm incremental thickness is still the regular standard for RBC increment placement, using bulk-fill allows placement of RBCs in more than 2 mm increments (up to 4 mm) while maintaining an adequate DC. This is due to higher light transmission through the more translucent bulk-fill RBC thickness when compared to conventional RBCs[6]. According to Sideridou et al., monomers bis-GMA, TEGDMA, UDMA, and bis-EMA dissolves in 75% ethanol, because 75% ethanol solution has the ability to penetrate the composite resin matrix, then expand and enlarge the polymeric bonds consisting of monomers which cause the remaining monomers to come out, 75% ethanol solution can also be used removing the residual bulk-fill resin monomer, Therefore, 75% ethanol solution was used in this study[1].

In addition, from the results of Cebe et al., revealed that residual monomers (BIS- GMA, UDMA, and TEGDMA) were found in all bulk-fill composite resins studied and the longer storage time in solvents caused the remaining monomer numbers

to increase[11]. The aim of the study was to know the difference in the number of residual monomers in the sculptable and flowable bulk-fill composite resins under immersion in 75% ethanol solution for 10 minutes, 1 hour, and 24 hours.

Materials and Methods

This was a laboratory experimental research with post-test only group design.

1. Sample Preparation

A glass-lab (Duran, Indonesia) with a size of 15x30 cm and 5 mm thickness was put at the bottom of the mold. Bulk-fill sculptable (Tetric N-Ceram Bulk-Fill, Ivoclar, Vivadent, Liechtenstein) and flowable composite (Tetric N-Ceram Bulk-Fill flowable (Ivoclar, Vivadent, Liechtenstein) were inserted into the mold using plastic filling instrument until full, then condensed to avoid air-trapping and then flatten the top and given a transparent plastic that functions as an oxygen inhibiting layer and weight of 0.25 kg on the top. The intensity of Light Emitted Diode (LED) (DBA Woodpecker, China) light cure was confirmed with a light meter tool (DBA Woodpecker, China), then samples were cured for 10 seconds with radiation intensity of 1025 mW *I* cm2. The composite discs were removed from the mold, and then the thickness was checked using a caliper (Nankai, China). Samples then were submerged in 75% ethanol solution (Ensure CAS 64-17-5, Germany) in an amber-colored bottle (Duran, Indonesia) and then 7 cc of the solution were taken with 10 cc syringe at 10 minutes (Group 1 = T1), 1 hour (Group 2 = T2) and 1 day (Grop 3 = T3).

2. Sample Analysis

Standard solutions of TEGDMA, UDMA, and BIS-GMA were examined using High-Performance Liquid Chromatography (HPLC) (Agilent 1100 series, Korea). Each standard solution TEGDMA, UDMA, and BIS-GMA (Aldrich chemistry, United States of America) was weighed using milligram scale (Mattler Toledo, Singapore) then dissolved in solvents, respectively , standard TEGDMA and UDMA were dissolved in methanol, and standard BIS-GMA was dissolved in chloroform (CHCb) (Emsure CAS 65-66-3, Germany). Each standard solution was placed on an ultrasonic machine (ELMA, China) for 5 minutes, then was chilled to room temperature and was calculated for the concentration using the formula:

$$standard\ solution\ concentration = \frac{standard\ solution\ weight}{standard\ solution\ volume} \times 1000$$

The next step was the dilution of a standard solution of 100 µl into 1000 µl solvents, respectively, in the HPLC vial. Dilution aimed to decline the peak area of the standard solution, preventing it from being too far from the sample area. Calculation the concentration of a standard solution was using the formula:

$$standard\ solution\ concentration = \frac{100\mu l\ standard\ solution}{1000\mu l\ solvent\ solution}\ x\ concentration\ 1$$

note: Concentration 1 = Concentration of standard solution before dilution

Each standard solution was taken as much as 1 ml to be transferred to the HPLC vial. TEGDMA standard solution examination was done by injecting a standard solution for 20µl, resulting in a retention time peak of 2,153 minutes. The same injection volume was also done for UDMA, resulting in a retention time peak of 2.768 minutes. Finally, for the standard solution of the Bis-GMA examination, the retention time peak was obtained at 10,219 minutes.

The next step was an examination of samples in HPLC. Each sample solution was taken for 1ml, then transferred to the HPLC vial. The sample solution was injected for 20 µl and peak obtained was recorded. The peak of residual monomers was confirmed if the

peak was at retention time ± 0.30 minutes from the standard peak. Percentage of residual monomers was calculated using the formula:

$$Residual\ monomers = \frac{sample\ area}{standard\ solution\ area} \times standard\ solution\ concentration \times 100\%$$

3. Statistical Analysis

The normality test is carried out with the Kolmogorov-Smirnov test. The homogeneity of the variance of data between groups, the Levene test was used. The Kruskal-Wallis test was done to analyze the differences between the groups. The differences between the experimental groups, an analysis was carried out with the Independent T-test and the Mann-Whitney test ($p \leq 0.05$).

Results

Group means and standard deviation can be seen in Table 1 to Table 3.

Table 1. Mean and standard deviation of residual monomers in 3 different submersing times of sculptable bulk-fill composite, Mann-Whitney test.

Groups	N	X ± SD			p-value
		TEGDMA	UDMA	BIS-GMA	
T1	5	1,390 ± 0,004a	5,616 ± 1,225a	0,130 ± 0,048a	< 0,05
T2	5	1,408 ± 0,086a	8,302 ± 2.270b	0,145± 0,104a	
T3	5	3,119 ± l,30lb	15,201 ± l,625c	0,810 ± 0,096b	

Note: Words printed in superscript show significant differences within the same column with Mann-Whitney test.

Tl: Flowable bulk-fill composite submersed for 10 minutes

T2: Flowable bulk-fill composite submersed for 1 hour

T3: Flowable bulk-fill composite submersed for 24 hours

X: Mean

SD: Standard deviation

Mean and standard deviation of residual monomers expressing TEGDMA, UDMA, and BIS-GMA in sculptable bulk-fill composite represented by a graph in Figure 1.

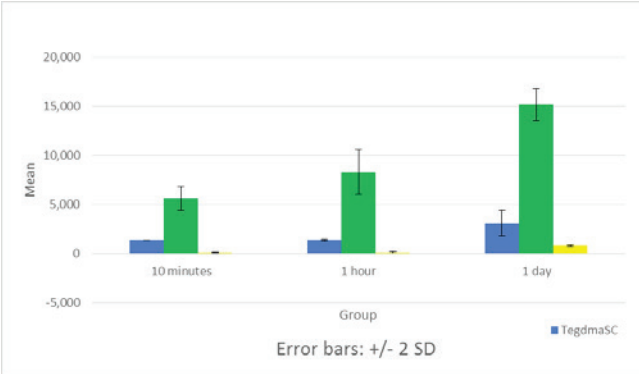

Figure l. Diagram of mean and SD residual monomer that expressed TEGDMA, UDMA and bis-GMA in a bulk fill sculptable composite resin.

From the table above (Table 1), it can be seen that a 10-minutes sculptable TEGDMA has no significant difference with a 1-hour sculptable TEGDMA, but there was a significant difference with a 24-hours sculptable TEGDMA. In 1-hour sculptable TEGDMA, there was a significant difference with 24-hour sculptable TEGDMA. In a 10-minutes sculptable UDMA, there was a significant difference with a 1-hour and 24-hours sculptable UDMA, while in a 1-hour sculptable UDMA there was a significant difference with a 24-hours sculptable UDMA. In a 10-minutes sculptable Bis-GMA, there was no significant difference with a 1-hour sculptable Bis-GMA, but there was a significant difference with a 24-hours sculptable Bis-GMA. In a 1-hour sculptable Bis-GMA, there was a significant difference with a 24-hours sculptable Bis-GMA.

Table 2. Mean, and standard deviation number of residual monomers of flowable bulk-fill composite in three different submersion time, Mann- Whitney test

Groups	N	X ± SD			p-value
		TEGDMA	UDMA	BIS-GMA	
T1	5	1,430 ± 0,093a	7,932 ± 1,729a	0,198 ± 0,145a	< 0,05
T2	5	1,447 ± 0,282a	10,604 ± 1,429b	0,238 ± 0,095a	
T3	5	3,773 ± 1,293b	18,243 ± 4,495c	1,417 ± 1,183b	

Note: Words printed in superscript show significant differences within the same column with Mann-Whitney test.

Tl: Flowable bulk-fill composite submersed for 10 minutes

T2: Flowable bulk-fill composite submersed for 1 hour

T3: Flowable bulk-fill composite submersed for 24 hours

X: Mean

SD: Standard deviation

Mean and standard deviation of residual monomers expressing TEGDMA, UDMA, and BIS-GMA in flowable bulk-fill composite represented by a graph in Figure 2.

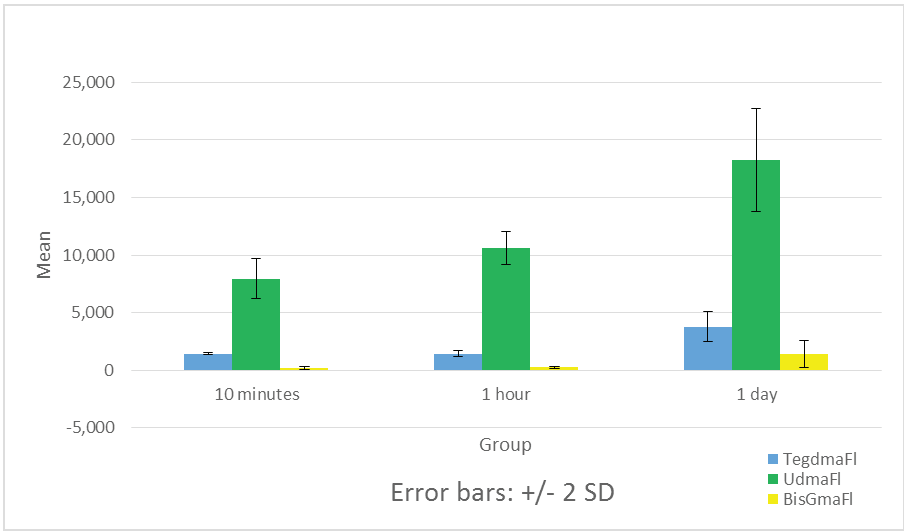

Figure 2. Means of residual monomers expressed by TEGDMA, UDMA, and BIS GMA in a flowable bulk-fill composite.

From the table above (Table 2, it can be seen that the 10-minutes flowable TEGDMA has no significant difference with a 1-hour flowable TEGDMA, but there was a significant difference with a 24-hours flowable TEGDMA. In a 1-hour flowable TEGDMA, there was a significant difference with a 24-hours flowable TEGDMA. In 10-minutes flowable UDMA, there was no significant difference with a 1-hour flowable UDMA, but there was a significant difference with a 24-hours flowable UDMA. In 1-hour flowable UDMA, there was a significant difference with 24-hours flowable UDMA. In the 10-minutes flowable Bis-GMA, there was no significant difference both with 1-hour flowable Bis-GMA and 24-hours flowable Bis-GMA. In a 1-hour flowable Bis-GMA, there was no significant difference with a 24-hours flowable Bis-GMA.

Table 3. Means and standard deviation number of residual monomers of bulk-fill composite by same submersion time, Independent T-test.

Group	N	T1	T2	T3	p-value
		X ± SD	X ± SD	X ± SD	
TEGDMA Sc *TEGDMA Fl*	5 5	1,390 ± 0,004[a] 1,430 ± 0,093[a]	1,408 ± 0,086[a] 1,447 ± 0,282[a]	3,119 ± 1,301[a] 3,773 ± 1,293[a]	
UDMA Sc *UDMA Fl*	5 5	5,616 ± 1,225[a] 7,932 ± 1,729[b]	8,302 ± 2.270[a] 10,604 ± 1,429[a]	15,201 ± 1,625[a] 18,243 ± 4,495[a]	P < 0,05
Bis-GMA Sc *Bis-GMA Fl*	5 5	0,130 ± 0,048[a] 0,198 ± 0,145[a]	0,145 ± 0,104[a] 0,238 ± 0,095[a]	0,810 ± 0,096[a] 1,417 ± 1,183[a]	

Note: Words printed in superscript show significant differences within the same cell with Independent T-test

Tl: Submersed for 10 minutes

T2: Submersed for l hour

T3: Submersed for 24 hours

Sc: Sculptable bulk-fill composite

Fl: Flowable bulk-fill composite

X: Mean

SD: Standard deviation

The mean and standard deviation of the residual monomer graphics that express differences between groups of similar residual monomers in the immersion time of 75% ethanol solution for 10 minutes can be seen in the diagram below (Figure 3).

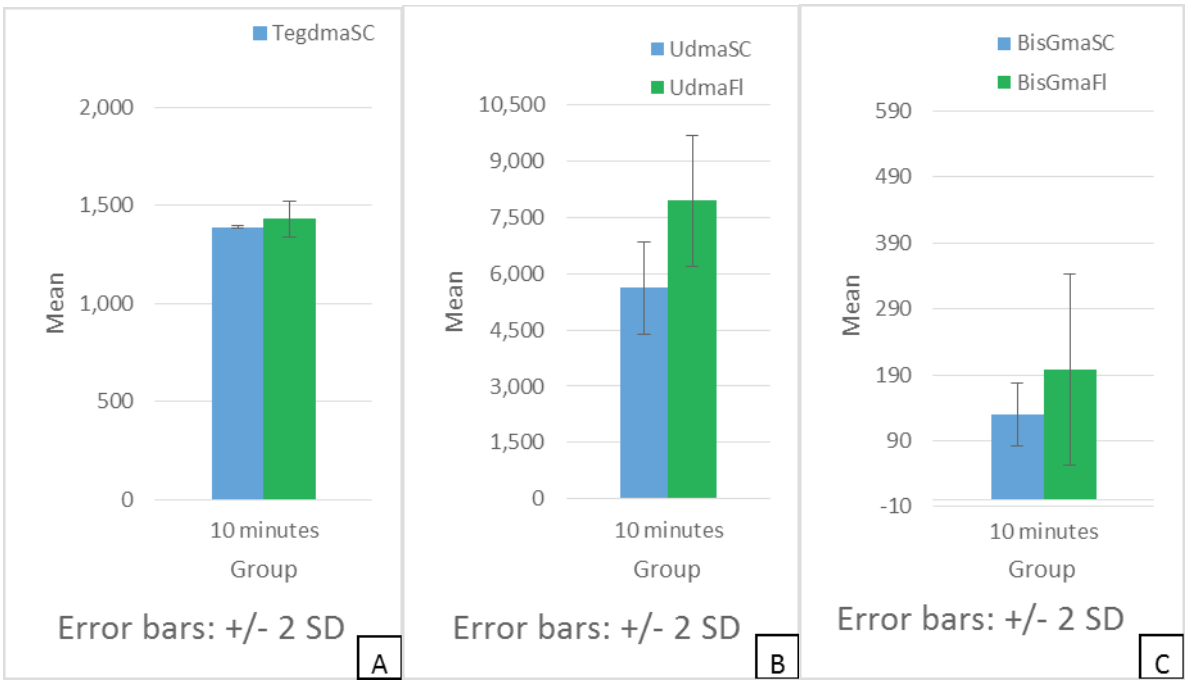

Figure 3. A diagram of the average amount of residual monomers bulk-fill sculptable composites resin and bulk fill composites resin flowable (a) TEGDMA, (b) UDMA, and (c) bis-gMA in soaking 75% ethanol solution for 10 minutes.

The mean and standard deviation graphic of residual monomers expressed the differences between groups of similar residual monomers in the immersion time of 75% ethanol solution for 1 hour can be seen in the diagram below (Figure 4).

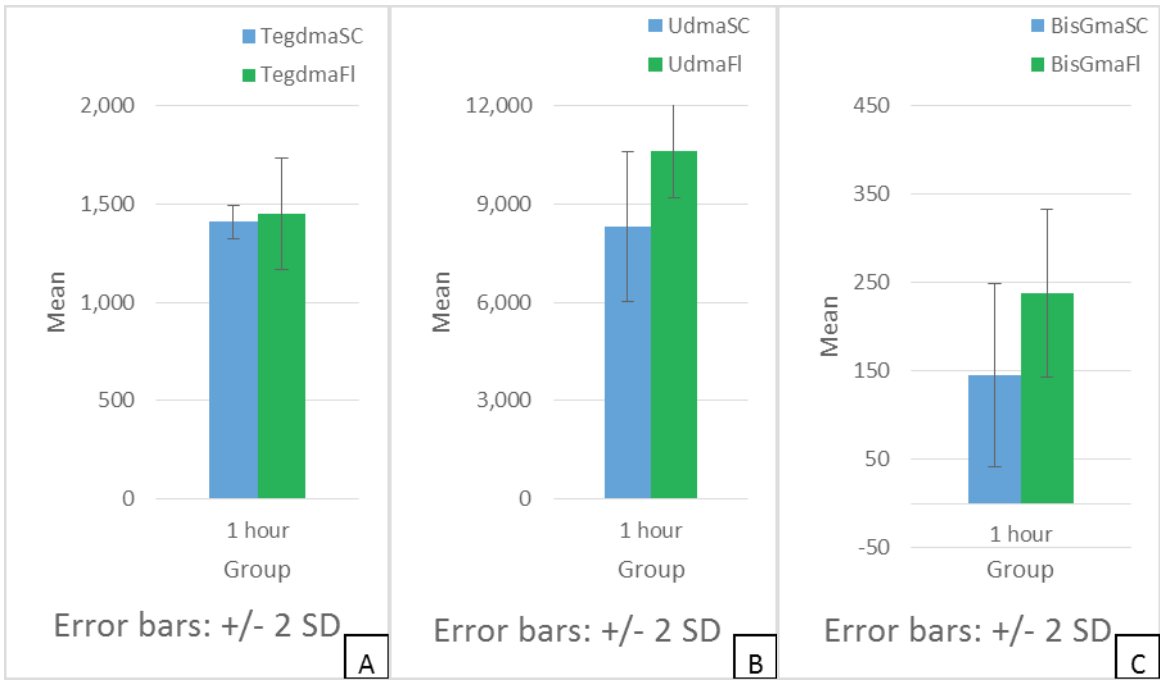

Figure 4. A diagram of the average amount of residual monomers bulk fill sculptable composites resin and bulk fill composites resin flowable (a) TEGDMA, (b) UDMA, and (c) bis-gMA in soaking 75% ethanol solution for 1 hour.

The mean and standard deviation graphic of residual monomers expressed the differences between groups of similar residual monomers in the immersion time of 75% ethanol solution for 24 hours can be seen in the diagram below (Figure 5).

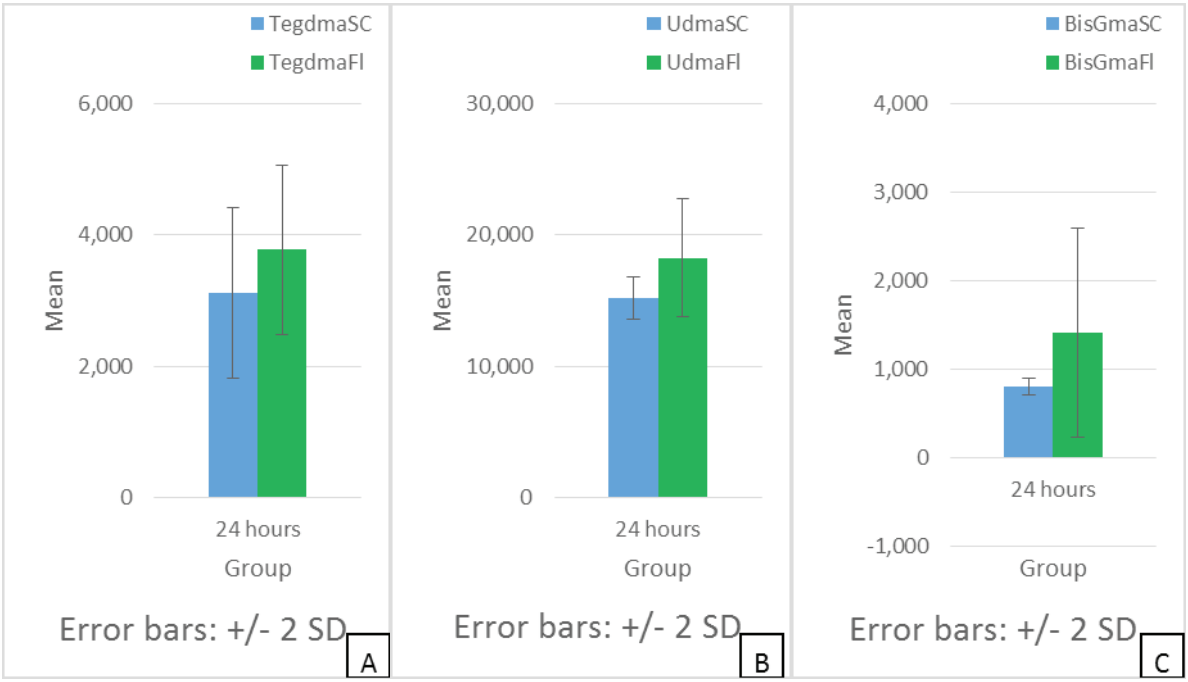

Figure 5. A diagram of the Means of residual monomers of flowable and sculptable composite (a) TEGDMA, (b) UDMA, (c) bis-GMA submerged in ethanol 75% solution for 24 hours.

From the table above (Table 3), it can be seen that 10-minutes sculptable TEGDMA has no significant difference with 10-minutes flowable TEGDMA. There was no significant difference between 1-hour sculptable TEGDMA and 1-hour flowable TEGDMA, and also, there was no significant difference between 24-hours sculptable TEGDMA and 24-hours flowable TEGDMA. In a 10-minutes sculptable UDMA, there was a significant difference with a 10-minutes flowable UDMA. While there was no significant difference between 1-hour sculptable UDMA and 1 hour flowable UDMA, and there was no significant difference between 24-hours sculptable UDMA and 24-hours flowable UDMA. In the 10-minutes sculptable Bis-GMA there was no significant difference with 10-minutes flowable Bis-GMA. There was no significant difference between 1-hour sculptable Bis-GMA and 1-hour flowable Bis-GMA, and also there was no significant difference between 24-hours sculptable Bis-GMA and 24 hours flowable Bis-GMA.

Discussion

Optimal polymerization is an important factor to obtain both physical and mechanical properties and optimal clinical performance. Adequate radiation intensity and exposure time affect the degree of polymerization. The success of the polymerization depends on the filler, light intensity, and the irradiation time[13,14]. Polymerization of the composite resin occurs through monomers transformation into polymers, followed by polymerization shrinkage[15].

There were various solvents used to elute residual monomers, including artificial saliva, distilled water, methanol, and acetonitrile which already used in the study to investigate monomer elution. Previous studies showed that the type of solvent affected the number of residual monomers eluted from resin composite[16,17]. Ethanol 75% solution is recommended by FDA as food-oral simulating liquid due to its relevancy, and therefore already used in several studies [11]. According to Sideridou and Achilias, Bis-GMA, TEGDMA, UDMA, and bis-EMA monomers were able to dissolve

in ethanol 75% solution due to its ability to penetrate resin composite matrix, followed by expansion and enlargement of polymer bonds which were consisted of monomers, causing residual monomers to be released. Therefore, ethanol 75% solution was chosen as a solvent in this study[1].

High molecular weight monomers such as Bis-GMA and UDMA will decompose in gas chromatography examination, and only decomposition products can be detected, However monomers with a low molecular weight such as TEGDMA will be difficult to be detected by gas chromatography examination[18]. For this reason, the quality and quantity of the residual monomers eluted from composite resin materials were usually determined by examination using HPLC because it was a very potent and commonly used separation method. HPLC was preferred over gas chromatography because it provided a greater degree of control in the separation process, due to the dissolved monomers in the mobile phase[19]. Therefore, the HPLC examination was used in this study to evaluate the release of monomers from bulk-fill resin material.

From this study, it was found that in sculptable and flowable bulk-fill composites, residual monomers were detected at 10 minutes, l hour, and 24 hours. More monomers of the remaining flowable bulk-fill composite were detected compared to the residual monomers of a sculptable bulk-fill composite. This result was in accordance with previous studies conducted by Cebe et al. (2015), which stated that after polymerization of bulk-fill resin composite, elution of residual monomers was found in solution. Polymer networks that were formed during the initial polymerization due to free radical reactions were densely crosslinked (microgels) and some other parts are loosely crosslinked (micropore), which contained non-reacting monomers[11]. The polymer network morphology takes the form of agglomerating microgels into clusters and these clusters were interconnected [20]. These polymer networks almost always contain a number of unreacted monomers, usually trapped inside microgels and micropores which cluster between polymer chains. Polymer crosslinking is generally insoluble but has a tendency to expand in a good solvent. The solvent penetrates into the composite matrix and expands the space between the polymer chains, which will cause changes in mass and dimensions. If the monomers were not dissolved in the solvent, they could be removed from the polymer mass. The process of enlarging polymer crosslinking and the process of removing unreacted monomers occur due to the diffusion process. Solvents can penetrate into the composite matrix and expand the opening of the polymer chain, causing unreacted monomers to dissolve and diffuse out. It was reported that monomers trapped in micropore were more susceptible to diffusion than monomers in microgels and it was known that the volume of micropore is very large in heterogeneous polymer nentworks[1]. The majority of sculptable bulk-fill composites contain monomer Bis-GMA, which comes from a mixture of bisphenol A with glycidyl methacrylate. Bis-GMA has a very high molecular weight, therefore the shape of the monomer is very thick because of its high viscosity. The majority of flowable bulk-fill composites contain TEGDMA monomers. TEGDMA has a very low molecular weight, therefore the shape of the monomer is very flowable because of its low viscosity. These are the reasons why the residual monomer of flowable bulk-fill composites was more than the sculptable bulk-fill composites.

The results showed that in TEGDMA sculptable, Bis-GMA sculptable, TEGDMA flowable, and UDMA flowable groups at 10 minutes submersion compared to l hour submersion had no significant differences in the number of residual monomers. However if 10 minutes submersion was compared to 24 hours submersion, it showed a significant difference in the number of residual monomers, as well as if 1 hour submersion compared to 24 hours submersion showed a significant difference. This indicates that there were more residual monomers released in the span of 1 hour to 24 hours than in l0 minutes to 1 hour and the longer the submersion time in 75% ethanol solution, the more residual monomers released. This happened because of the accumulation time, the time between 10 minutes and l hour was shorter than the time between 1 hour and 24 hours. Another reason was that the ability to penetrate the ethanol solution took more than 1 hour. The swelling ability to crosslink polymer and the process of releasing unreacted monomers by the diffusion process took more than l hour.

In the analysis of the data of this study, it was also shown that the UDMA sculptable group at 10 minutes submersion compared to 1 hour submersion had a significant difference in the number of residual monomers, this also occurred in 10 minutes submersion compared to 24 hours submersion and l hour submersion compared to 24 hours submersion. This means that from the beginning of submersion there were already many residual monomers diffused out, this happened because the residual UDMA monomers had morphology with high crosslinking density. The higher the density of the crosslinking, the higher the heterogeneity and the greater the volume of the micropores. It was reported that monomers trapped in micropores were more susceptible to diffusion than monomers in microgels, causing UDMA to diffuse more. UDMA also have weaker bonds between monomers, this resulted in the solvent penetrating the composite matrix easy to expand and release the bonds between the monomers to make them dissolve and diffuse more. UDMA also has an affinity between monomers and 75% ethanol solution, therefore when ethanol solution penetrated, the intermonomer bond of UDMA broke and directly bind with 75% ethanol solution because of its high affinity for 75% ethanol solution, therefore, the number of residual UDMA monomers released were more than TEGDMA and Bis-GMA.

Data analysis showed that the flowable Bis-GMA group at 10 minutes submersion compared to 1 hour submersion showed no significant difference in the number of residual monomers, this also occurs in 10 minutes submersion compared to 24 hours submersion and 1 hour submersion compared to 24 hours submersion. This means that less residual monomer released, therefore, there is no difference between submersion with 75% ethanol solution in l0 minutes, 1 hour and 24 hours. This happened because of the site and chemical structure characteristics of the BIS-GMA monomer, the molecular size of BIS-GMA was very large (molecular weight 512 g/mol)[2,3]. The Bis GMA molecule is a very compact resin therefore the monomer shape is very thick due to its high viscosity, structurally, those molecules consist of several monomer molecules chemically bonded to each other after the polymerization process. Bis-GMA also has a strong hydrogen bonding ability, hence the bond is not easily released even though it is penetrated by 75% ethanol solution. The remaining monomer molecules can be physically described as trapped/sandwiched molecules between the arrangement of the molecules of the Bis-GMA and making it difficult to release.

In this study, it was also found that in general, the number of residual monomers increased with increasing submersion time in 75% ethanol solution. Composite resins consist of polymers containing unreacted monomers and trapped inside. The solvent penetrated into polymer networks and extended the space between the polymer chains, causing unreacted monomers to elute[1]. The longer the composite submersed in 75% ethanol solution, the more space between the polymer chain and the more residual monomers diffused out. Previous research had shown that the number of eluted monomers increased with increasing submersion time[21,22]. In accordance with these findings, the highest number of monomers was detected after 24 hours of submersion of 75% ethanol solution.

Conclusion

From the results of this research, it can be concluded that (1) the amount of residual monomer of flowable bulk-fill composite resin was greater than the sculptable bulk-fill composite resin on submersion in 75% ethanol solution for 10 minutes, 1 hour and 24 hours[2]. The number of residual monomers in sculptable bulk-fill composite resin at submersion in 75% ethanol solution for 24 hours was greater compared to 1 hour and 10 minutes submersion time[3]. The number of residual monomers in flowable bulk-fill composite resin at submersion in 75% ethanol solution for 24 hours was greater compared to 1 hour and 10 minutes submersion time.

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Factors Affecting Risk of Musculoskeletal Disorders (MSDs) Complaints in Spring Production Workers

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Abstract

Introduction : Musculoskeletal Disorders (MSDs) are one of the occupational diseases with complaints that felt in the skeletal muscle. Musculoskeletal complaints can occur in any sector of work. one of the sector is in informal sector workers. The aim of this research was to analyze the relation between individual factors with occupational factors on musculoskeletal complaints in spring production workers.

Methods : This research was an observational research with cross sectional approach. The sample in this research was 23 respondents in the production unit. Sampling in this research was conducted by total population. Data of respondent characteristics musculoskeletal complaints were obtained through interviews conducted to respondents by filling out standardized questionnaires. Work posture data were analyzed using Rapid Entire Body Assessment (REBA) and musculoskeletal complaints data were analyzed using the Nordic Body Map (NBM) method. Statistical tests were analyzed using the Contingency Coefficient test.

Results : The results of the research to 23 respondents showed that the majority of respondents as many as 15 respondents (65.21%) had MSDs complaints in the high pain severity category. Risk factors related to Musculoskeletal Disorders complaints were age. smoking habit. sex. nutritional status. works duration. work posture and physical workload. The data obtained were analyzed using the Contingency Coefficient test.

Conclusion : Factors causing Musculoskeletal Disorders that had the strongest relationship were odd or non-ergonomic work posture of workers (r = 0.632) with positive relation direction. meaning that the higher the risk of work posture. the higher the risk for experiencing MSDs complaints.

Keywords *: Musculoskeletal Disorders (MSDs). Individual Factors. Occupational Factors*

Introduction

Workers are resource that plays important role in carrying out its work. so workers are required to get attention and protection from their workplaces. Occupational health efforts for workers need to be carried out in every workplace. especially for companies or industries that are riskyof having health hazards. Given that workers is one of the valuable assets for a company or industry. Occupational Safety and Health (OHS) is a form of protection for workers in creating a safe workplace.

The impact of the less optimal efforts to implement Occupational Safety and Health (OHS) is the occurring of Musculoskeletal Disorders (MSDs) complaints. Continental Union of European Union Member. 27 countries of them states that in 2004 MSDs are the most common occupational diseases as much as 59% of all Occupational Diseases (OD). Based on data released by the World Health Organization (WHO) in 2009 MSDs

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accounted for more than 10% which caused disability[1].

The Health and Safety Authority (HSA) on 2015 explains that the number of Occupational Diseases (OD) that occurred on 2012 struck 27.1 of 1000 workers[2]. The high number of workplace accidents that occur at work has continued to increase since 2012. From those number of reported cases. it is known that around 32% are musculoskeletal injuries resulting from work activities such as lifting weights (43%). The occurrence of musculoskeletal complaints can be worsen if the work position or posture of the workers when carrying out the work activities is not ergonomic or odd.

One of OHS implementations is the ergonomics implementations in the workplace. Ergonomics is a science. art and technology application to harmonize humans in their activities with the facilities used to achieve a better quality of life[3]. Ergonomics is one of the problems that can appear in every company. this is due to the need for harmony between workers and their jobs. The ergonomics implementation is a must for every workplace so that discomfort and other complaints can be minimized[3]. If there is a mismatch between the work stations and the workers. it can result in complaints or disorders in the musculoskeletal system which include joints and muscles because the body is not ergonomic. This position can cause complaints that are commonly called Musculoskeletal Disorder (MSDs).

Musculoskeletal Disorder Disorders (MSDs) are one of the disorders that are caused by non-ergonomic work postures. Musculoskeletal Disorder (MSDs) complaints are complaints that felt in parts of skeletal muscle ranging from very mild to extreme pain complaints caused by muscles receiving repetitive static loads and for long time duration during work and can cause damage to joints. ligaments and tendons. Studies on Musculoskeletal Disorder (MSDs) showed that the parts of muscle that often complained of are the muscles of the neck. shoulders. arms. hands. fingers. back. waist and lower muscles[4].

Based on the publication of the Occupational Safety Consultation Institute in the UK. on 2016 to 2017 there are 507.000 cases related to MSDs due to work with a total of 1.299.000 cases of Occupational Diseases or 39% of total Occupational Diseases. The average value of MSDs complaints reaches 1.550 cases out of 100.000 workers who have Occupational Diseases[5].

MSDs are complaints or disorders that are felt by someone ranging from mild to very painful complaints in the parts of musculoskeletal which include the joints. nerves. muscles and spine due to unnatural work[4]. If the muscles are experiencing disorders. then daily activities such as working can be disrupted because muscle strength is one of the most important parts of human organs so the body can move. The appearance of pain in the muscles can result in decreased person's work productivity. While muscle strength itself would be determined by the number of fibers that are actively wrinkled in the human body in a certain period of time[6]. In addition. excessive muscle contraction coupled with too much weight lifting in a long time duration would certainly increase a risk to MSDs complaints.

Other theories state that unnatural work positions such as backs too bent. hand raised movements and so on can increase the occcurence of MSDs complaints. The farther the body part position from the center of body gravity. the higher the risk of skeletal muscle complaints. This unnatural work position is generally due to the characteristics of job demands. work tools and work stations that are not in accordance with the abilities and limitations of workers[3].

Researches related to MSDs complaints themselves have been conducted in various companies and industries. Such as research conducted by Aulia Tjahayuningtyas (2019) the research results to 38 respondents shows the work period ($p = 0.019$) and workload ($p = 0.000$) has a relation with MSDs complaints on workers in the informal sector[7]. The MSDs complaints themselves are mostly felt by the informal workers on the right wrist (86%) and right foot (68%). Meanwhile. research conducted by Elza (2012) shows that the informal sector of traditional songket craftsmen in West Sumatra has a high ergonomic risk based on an assessment using the RULA method[8]. The results shows the most frequent complaints experienced by workers were on the back by 80%. then complaints on the right shoulder by 74% and the last complaints on the waist by 72%.

Previous research conducted by Alfiani & Basri (2016). as much as 46.9% workers such as porters are at risk of experiencing complaints of low back pain due to too much heavy load lifted and carried out continuously[9].

Although the informal sector has contributed a lot to both the economy and employment providers. it cannot be denied that the health conditions of the workers are still worrying.

The impact resulting from the appearance of Musculoskeletal Disorders complaints according to Bird and Germain (2005). are loss of work time. reduce alertness. increase the risk of accidents. if the complaint has reached the final stage then requires high costs for recovery[10]. Musculoskeletal Disorders complaints require sufficient time and various stages for pain to appear in certain body parts.

Musculoskeletal Disorders complaints can occur in both the formal and informal sectors. The spring production industry which was my research place is one of the informal sector businesses. According to Alma (2001) explains that the informal sector is a manifestation of small-scale employment with the aim of earning income without profit[11]. Business in the informal sector is one type of business that has a very high health risk. The workforce on 2000 with total 95.650.691 people. which 70-80% of them are in the informal business sector. Workers in the informal sector are populations that lack of getting health services especially occupational health[12].

The explanation above. became the background of researcher conducting research related to risk factors affecting the occurrence of Musculoskeletal Disorders complaints in production unit workers in the spring industry. It was expected that after conducting this research. researcher could study the factors related with the occurence of Musculoskeletal Disorders complaints in workers. Thus. the ergonomics implementation in the workplace needed to be pursued directly because it would affect complaints both fatigue and other health problems.

Materials and Methods

Based on the type. this research was observational because it did not provide treatment to respondents with analytical research methods. Meanwhile. the design of this research used a cross-sectional research ¬ because the variables were researched and observed at the same time to describe the level of Musculoskeletal Disorders (MSDs) complaints in respondents.

The population used in this research was the spring production industry workers in Menganti District. Gresik. The sample in this research were all workers who worked in the production process and were willing to be respondents in this research with a total of 23 workers. Research was conducted in December 2019 until February 2020.

The variables used include age. smoking habit. sex. nutritional status. works duration. work posture and physical workload on Musculoskeletal Disorders complaints felt by workers after carrying out work activities.

Data obtained through observation. interviews. and questionnaires. Workers were observed to watch work postures and physical workload obtained while working on Musculoskeletal Disorders complaints. Work posture was observed by adjusting the REBA (Rapid Entire Body Assessment) observation assessment sheet. then the assessment of physical workload was obtained based on measurements using a calorimeter to calculate the calorie needs assessment when carrying out work activities. Then. continued to fill in the Nordic Body Map (NBM) assessment sheet after the workers completed their works. The completion of this assessment sheet aimed to find out which parts of the body experiencing complaints and the level of complaints felt by each individual.

Data and information that obtained through observation. interviews and questionnaires processed and analyzed in the form of tables. pictures. and narratives to ease the delivery of information and results obtained in research. To find out the results. an analysis using contingency coefficient test was conducted. If the results of the analysis approach 0 then the relation gets weaker. if it approaches 1 then the relation gets stronger. This research obtained ethical approval from the Health Research Ethics Commission of the Faculty of Dentistry. Universitas Airlangga (No:001/HRECC. FODM/1/2020).

Results and Discussion

The work carried out in the spring production industry was work that requires considerable effort and energy of the workers. The production process of this industry used a lot of repetitive activities in a standing

position continuously and movements such as taking and arranging repeatedly. This work activities will certainly cause injury to the muscles. joints. ligaments and tendons[4]. Such disorders are commonly referred to as MSDs complaints or complaints that appear in the musculoskeletal system which is a condition with discomfort or pain can appear.

Based on the results of research that had done. it was known that all workers in the production unit or 23 respondents stated experiencing MSDs complaints in part of their bodies after carrying out their work activities. Parts of the body that often experience complaints such as the hands. feet. back. waist due to the work using repetitive hand movement activities and carried out on standing and bent position in the daily work.

Respondent Characteristics

The respondents were as many as 23 people. based on Table 1 showed that the majority of respondents were in the Late Adult category aged 36-45 years as many as 9 respondents (39.1%). most of them had a smoking habit in Moderate Smoker category as many as 9 respondents (39.1%) and the majority were male respondents as many as 20 respondents (86.95%). Most respondents had normal nutritional status category as many as 16 respondents (69.6%) and as many as 15 respondents (65.2%) had works duration ≥ 8 hours/day.

Table I. Distribution of Respondent Characteristics in Spring Production Industry on 2019

Variable	Category	Frequency	Percentage (%)
Age	Early Teenager	4	17.4
	Early Adult	3	13.1
	Late Adult	9	39.1
	Early Elderly	6	26.1
	Late Elderly	1	4.3
Total		23	100
Smoking Habit	Non Smoker	6	26.1
	Light Smoker	5	21.7
	Moderate Smoker	9	39.1
	Heavy Smoker	3	13.04
Total		23	100
Sex	Male	20	86.95
	Female	3	13.05
Total		23	100
Nutritional Status	Thin	5	21.7
	Normal	16	69.6
	Fat	2	8.7
Total		23	100
Works duration	≤ 8 hours/day	8	34.8
	≥ 8 hours/day	15	65.2
Total		23	100

Work Posture

Respondents work postures were assessed using the REBA (Rapid Entire Body Assessment) observation questionnaire sheet by measuring angles and then cotinuing with scoring for each group. This scoring assessment aimed to determine the risk categories received which consisted of acceptable risk. low risk. moderate risk. high risk and very high risk. Based on research that had done on 23 respondents.

Group A score consisted of assessments on the body. neck and legs and addition of force scores. The scoring results were then entered into the group A REBA score table. Group B score consisted of assessments of the upper arm. forearm and wrist and the addition of a grip type score. The score results were then entered into the group B REBA score table. Assessment of group C score was a combination of score A and score B. The results of the next assessment entered into the group C REBA score table. Furthermore. the total C score was added to the increase of muscle activities score. The results of the assessment were final result which could determined a risk category.

Table 2 could be seen the final results of the work posture assessment that from a total of 23 respondents as many as 14 respondents (60.7%) had work postures with moderate risk category. as many as 7 respondents (30.4%) had work postures with low risk category and as many as 2 respondents (8.7%) had a work posture with a high risk category.

Table II. Distribution of Work Posture Assessment Final Results in Spring Production Industry on 2019

Category	Frequency	Percentage (%)
Low Risk	7	30.4
Moderate Risk	14	60.7
High Risk	2	8.7
Total	23	100

Physical Workload

Physical workload was measured using a calorimeter. This tool was installed when the respondents work for 8 hours while working in one work shift using a calorie needs assessment technique. This measurement aimed to determine the physical workload categories received which consisted of mild. moderate and heavy.

Table 3 could be seen the final results of the physical workload measurement that from a total of 23 respondents as many as 18 respondents (78.26%) included in the category of moderate physical workload and as many as 5 respondents (21.73%) included in the category of heavy physical workload.

Table III. Distribution of Physical Workload Measurement Results in Spring Production Industry on 2019

Category	Frequency	Percentage (%)
Moderate	18	78.26
Heavy	5	21.73
Total	23	100

***Musculoskeletal Disorders* Complaints**

Musculoskeletal Disorders (MSDs) complaints were measured using the Nordic Body Map (NBM) assessment sheet. Measurement steps carried out through observation and interviews respondents about which parts of the body experiencing pain by pointing directly to the picture on the questionnaire sheet. The assessment sheet then carried out a checklist based on the complaint levels of pain experienced by the respondents

This measurement aimed to determine the severity pain categories received which consisted of low. moderate. high and very high.

Table 4 could be seen the final results from the complaints measurement felt that from a total of 23 respondents as many as 8 respondents (34.78%) had complaints in the moderate pain severity category and as many as 15 respondents (65.21%) had complaints in the high pain severity category.

Table IV. Distribution of *Musculoskeletal Disorders* Complaints Measurement Results in Spring Production Industry on 2019

Category	Frequency	Percentage (%)
Moderate	8	34.78
High	15	65.21
Total	23	100

Results of Relation Analysis Between Respodent Characteristics with MSDs Complaints in Informal Workers

Based on the results of the research in Table 1 related Respondent Characteristics showed the respondents as many as 23 people. most of the respondents were in the Late Adult category as many as 9 respondents (39.1%). most of them had smoking habits in Moderate Smoker category as many as 9 respondents (39.1%) and the majority were male respondents as many as 20 respondents (86.95%). Most respondents had a normal nutritional status category as many as 16 respondents (69.9%) and as many as 15 respondents (65.2%) had works duration ≥ 8 hours/day.

Table V. Results of Relation Analysis Between Variables with MSDs Complaints in Spring Production Industry on 2019

Variable	MSDs Complaints				Total		Coeff	Expl.
	Moderate		High					
	n	%	n	%	N	%		
Age								
Early Teenager	1	25	3	75	4	100	0.512	Strong
Early Adult	1	33.3	2	66.7	3	100		
Late Adult	6	66.7	3	33.3	9	100		
Early Elderly	0	0	6	100	6	100		
Late Elderly	0	0	1	100	1	100		
Total	8	125	15	375	23	100		
Smoking Habit								
Non Smoker	2	33.3	4	66.7	6	100	0.273	Moderate
Light Smoker	1	25	4	75	5	100		
Moderate Smoker	5	55.5	4	44.4	9	100		
Heavy Smoker	0	0	3	100	3	100		
Total	8	113.8	15	286.1	23	100		

Cont... **Table V. Results of Relation Analysis Between Variables with MSDs Complaints in Spring Production Industry on 2019**

Sex								
Male	8	40	12	60	20	100	0.255	Moderate
Female	0	0	3	100	3	100		
Total	8	40	15	160	23	100		
Nutritional Status								
Thin	0	0	5	100	5	100	0.611	Strong
Normal	8	50	8	50	16	100		
Fat	0	0	2	100	2	100		
Total	8	50	15	250	23	100		
Works duration								
≤ 8 hours	2	25	6	75	8	100	0.374	Moderate
≥ 8 hours	6	40	9	60	15	100		
Total	8	65	15	135	23	100		
Work Posture								
Low	4	57.2	3	42.8	7	100	0.632	Strong
Moderate	4	28.6	10	71.4	14	100		
High	0	0	2	100	2	100		
Total	8	34.8	15	65.2	23	100		
Physical Workload								
Moderate	0	0	5	100	5	100	0.559	Strong
Heavy	8	44.4	10	55.6	18	100		
Total	8	34.8	15	65.2	23	100		

The results of the research analysis in Table 5 obtained the coefficient correlation value of 0.512 which meant that the strength of the relation between age and MSDs complaints had a strong category relation strength. the results of the relation analysis between smoking habits with MSDs obtained correlation coefficient value of 0.273 which meant it had a moderate category relation strength. the results of relation analysis between sex with MSDs complaints obtained coefficient correlation value of 0.255 which meant it had a moderate category relation strength. The results of research analysis related to the relationship of nutritional status with complaints of MSDs obtained coefficient correlation value of 0.611. which meant that the strength of the relation between nutritional status with MSDs complaints in strong category and the results of relation analysis between works duration with MSDs complaints obtained coefficient correlation value of 0.374. which meant that strength of relation in the moderate category.

Results of Relation Analysis Between Work Posture with MSDs Complaints in Informal Workers

Based on the results of the research in Table 1 related to the final results of the work posture assessment that from a total of 23 respondents as many as 14 respondents (60.7%) had work posture with moderate risk category. as many as 7 respondents (30.4%) had work posture with low risk category and as many as 2 respondents (8.7%) had work posture with a high risk category.

The results of the research analysis in Table 5 obtained the coefficient correlation value of 0.632 which meant that the strength of the relation between work posture with MSDs complaints had strong category relation strength.

One of the factors causing Musculoskeletal Disorders complaints is an unnatural or ergonomic work posture. An unnatural work posture is a work posture that causes the position of body parts to move from the natural position. for example the movements of hands raised. backs too bent. head raised. and so on. The farther the body part position from the center of body gravity of the body. the higher the risk of skeletal muscle complaints. This unnatural work posture is generally due to the characteristics of job demands. work tools and work stations that are not in accordance with the abilities and limitations of workers [13].

The work posture assessment was carried out using the REBA assessment sheet. REBA was developed to assess the risk of work postures on non-ergonomic work postures. The final REBA score obtained will be a reference to provide a reference of the level of risk and action that must be taken. The final result of the assessment that had done showed the highest level of work posture risk was in the moderate category of 60.7% with a total of 14 workers. low risk category of 30.4% with a total of 7 workers and high risk category of 8.7% with a total of 2 workers.

Results of the analysis showed that work posture had a strong correlation with musculoskeletal complaints. This could occur because each respondent had a different work posture. for example respondents who worked to make a dough of sumpia skin that used a lot of hand strength would subsequently at risk of experiencing complaints on the wrist and hand muscles. Another research conducted by Bukhori (2010) on gold miners transporters in Cilograng District. Lebak Regency [14]. In his research showed work posture had a significant relation with musculoskeletal complaints.

Other research showed that work position risk would be closely related to the occurrence of MSDs complaints. In addition. according to Diana (2012). there was a relation between standing work posture with musculoskeletal complaints in the weaving unit workers conducted at PT. Delta Merlin Textile Kebakkramat Karanganyar [15]. It also said. that the higher the assessment of work postures. the higher the received musculoskeletal complaints.

Results of Relation Analysis Between Physical Workload with MSDs Complaints in Informal Workers

Based on the results of the research in Table 1 related to the final results of the physical workload measurement that from a total of 23 respondents as many as 18 respondents (78.26%) included in the category of moderate physical workload and as many as 5 respondents (21.73%) included in the category of heavy physical workload.

The results of the research analysis in Table 5 obtained the coefficient correlation value of 0.559 which meant that the strength of the relation between physical workload with MSDs complaints had a strong category relation strength.

Workload is every job that requires muscle strength and thought of the subject. The workload given to workers must be adjusted to the physical and psychological abilities of their workers so that they cannot affect the worker's health condition [16]. Effort to reduce the workload of workers can be done by planning and designing a tool that can minimize complaints due to inappropriate workload [17].

The physical workload measurement is based on calorie needs assessment according to energy expenditure. Based on observations. it was known that the results of the physical workload measurement showed from the total 23 respondents as many as 18 respondents (78.26%) were included in the category of moderate physical workload and as many as 5 respondents (21.73%) were included in the category of heavy physical workload. The results of this research were in line with the theory which stated that an increase in workload will be followed by an increase in musculoskeletal complaints that occur [18].

According to Tarwaka (2015). the human body has been created to be able to do daily work activities [4]. Muscle mass that weighs almost more than half of body weight allows humans to be able to move the body and do work. Work is a life goal that has an important meaning for progress and improvement in achievement so that it can reach a productive life. On the other hand. it means that the body will receive loads from outside the body. Means that each worker is a load for those concerned both in the form of physical and mental load.

The theory was in line with Tarwaka (2015) that stated each person's work ability is highly dependent on

the level of skill. physical fitness. nutritional status. sex. body size and age of the worker concerned[4]. Generally. age is related to person's work period. when the work period increases automatically the age of the worker will increase and applied the other way around. then the increasing age and work period of a person results in decreasing muscle strength and endurance so that the risk of muscle complaints occurs. This was one of the causes related to the results obtained where respondents who had a heavy workload were experiencing high risk level musculoskeletal complaints.

Another research conducted by Bobaya (2017) about the relation between physical workload with musculoskeletal complaints on loading and unloading porters in Manado Harbor to one hundred respondents[19]. This research showed there was a relationship between physical workload with musculoskeletal complaints on loading and unloading porters. this happened because musculoskeletal complaints will often appear if the muscles receive excessive workload pressure.

The results of this research were also in line with research by Utami. et al (2017) that stated there was a significant relationship between workload with musculoskeletal complaints on farmers in Ahuhu Village. Meluhu District. Konawe Regency[20].

Conclusion

Based on the results of research that conducted on factors affecting *Musculoskeletal Disorders* complaints in workers in informal sector. namely workers in the spring production industry in Menganti Gresik District. it was known that age. smoking habit. sex. nutritional status. works duration. work posture and physical wokload had a relation with *Musculoskeletal Disorders* complaints felt by workers.

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Study And Evaluation of the Inhibitory Efficacy of Extracts of *Sumbucus Nigra* Flower and *Tribulus Terrestris* Fruits Against The Growth of Bacterial Species Isolated from Patients with Urinary Tract Infection

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Abstract

This study was conducted to isolate and diagnose the most important bacterial types causing urinary tract infection from the inpatients and outpatients to Salah El Din General Hospital using routine isolation and diagnosis methods. 70 samples were isolated, and the results showed two types of growth: Gram positive bacteria and Gram negative bacteria, where 10 bacterial types were isolated as follows:

E. coli 42.85%, *Kleibsiella. pneumonia* 21.42% *roteus mirabilis* (11.42%), *Staphylococcus aureus* (7.14%), *Enterobacter cloaca* (5.71%), Serratia marcescens (2.85*%), Pseudomonas aeruginosa* (2.85%), *Staphylococcus Saprophyticus*. 2.85%, *Micrococcus luteus* 1.42%, and *Citrobacter freundii* 1.42%.

The results of the susceptibility test for 9 types of antibiotics using the discs method showed a change in the level of resistance to different antibiotics. The most effective antibiotics were Imipenem, Ciprofloxacin and Sulfamethoxaole. Plant extracts (both aqueous and alcoholic) of Sambucus nigra and Tribulus terrestris fruits were used, and tested for their ability to inhibit the most isolated bacteria (*E. coli , K. pneumonia P. mirabilis and S. aureus*). All alcoholic extracts were effective in the direction of Bacterial isolates, while aqueous extracts, have little or no effect.

Keywords*: Urinary Tract Infection, Antibiotics, Plant Extracts, Sumbucus nigra, Tribulus terrestris*

Introduction

Urinary tract infections are defined as an inflammatory response to the urinary system as a result of the process of invasion and settlement that occurs by pathogenic microorganisms. Urinary Tract Infection (UTI) is one of the most common bacterial infections in human, as urinary tract infections include many pathogenic cases, including cystitis, urethritis, and pyelonephritis [(1)].

Urinary tract infections occur with or without symptoms, and infection may cause serious complications if neglected and not treated [(2) (3).] Symptoms of a UTI depend on age, gender, the affected part of the urinary system and on the health and physiological condition [(4)]

Scientific progress and continuous researches for a antibiotics that has the best effect on the pathogen has contributed to the use of antibiotics in the treatment of urinary tract infections, and despite the widespread of anti-bacterial drugs in recent times, the effectiveness of these antibacterial drugs has begun to be affected by the increase in the ability of bacteria to resist these drugs. Which may lead to side effects causing significant damage to tissues and cells of the body such as acute renal insufficiency[(5)].

Many researchers have recently turned to the use of natural plant and herbal extracts by using them as effective natural sources in the manufacture of medicines and medicinal drugs [(10)]. because they are natural, have limited side effects, are cheap and available locally, as many studies have shown that the conclusions of these

Plants produce important active compounds such as phenols, alkaloides and tannins, and these substances are effective against pathogenic microbes and have antimicrobial activity[6].

In our current study, the inhibitory effect of Sumbucus nigra, Tribulus terrestris plant extract fruit on bacterial isolates was studied. Sumbucus nigra leaves contain cyanogenic glucosides, while the flowers contain flavonoids, the most important compound in which is the rutin complex, as well as phenolic acid, triple terpenes, citrulls and volatile oils, while the Tribulus terrestris fruits contain flavonoids, anthocyanins, and vitamin A, C [7]. Mashed fresh leaves are used to treat inflammation of the base of the nails and pyorrhea, and ointment of the leaves is used to treat gout pain in the joints of the fingers.

As for the Tribulus terrestris fruits, many researchers have studied the chemical components of the electrode, which contain many types of compounds such as saponins, alkaloids, flavonoids, glycoconols, oils, calcium, and unsaturated acids, which participate in enhancing many physiological responses[8]. The Tribulus terrestris plant are used in the treatment of impotence and infertility for both sexes and to protect against bladder cancer. It is also used as a tonic, appetizer, pain reliever, astringent, and in treating intestinal disorders, in cirrhosis, treating blood pressure and diuretics [9].

Materials and methods

130 mid-stream urine samples were taken from patients with acute and chronic urinary tract infection from Salah AI din General Hospital, from October 2019 - January 2020, and when these samples were cultured , 70 samples gave a growth when cultivated by 54.6% and the isolates were given Two types of growth are negative for Gram stain (87.4%), and positive for Gram stain(12.6%), and no bacterial growth was shown for all samples because the pathogen may be fungal or viral or from anaerobic bacteria that cannot be isolated by the normal cultural methods that were used in the study and which require culture media of their own or special conditions for growth [10] .

Cultivation of Samples

A drop was taken from each urine sample by means of the Loop and cultured on the blood agar, MaCconkey agar and Mannitol salt agar, then incubated at 37 ° C for 24-18 hours, after which the growing colonies were purified to obtain pure colonies for diagnosing through their phenotypic characteristics. And chemical properties [11].

Diagnosis of bacterial isolates

Cultural Characteristics

After the growing of colonies on, MacConkey, blood, and mannitol salt agar. Their size, color, and shape have been observed, and this is considered as a preliminary diagnosis. Microscopic examination: Smears of bacterial colonies were made by the sterile loop and placed on a clean slide, then examined with a Gram stain, after the slide dried, they were examined microscopically under the microscope to observe its response to the stain and the shape, size and arrangement of the bacterial cells[12].

Biochemical tests

The isolated bacteria were diagnosed the usual methods which include: Indol test, Methyl red test, Vogas Proskauer Test, citrate Utilization test, Eosine methylene blue medium Oxidase test Catalase test,Urease test ,Coagulase test and Novobiocin test.

Preparation of the plant extract and the concentrations used in the study

Taking 2 grams of the dry plant sample and dissolving it in 10 ml of sterile distilled water, then we have a storage solution at a concentration of 200 mg / ml, then sterilize the solution using sterile filter papers with diameters of 0.45 micrometers to get rid of the bacterial contaminants in it and obtain a sterile storage solution as a source for making several Concentrations of it are: (25, 50, 75, 100, mg / ml) [13].

Results and Discussion

Isolation and diagnosis of bacteria

Table (1) shows that the isolated and diagnosed germs of E. coli showed pink and dry colonies on macononi acres because they ferment lactose, while on the middle of the EMB, E. coli appeared with a bright metallic green color, which is consistent with

observations by [14], colonies that appeared in a pale pink color were diagnosed based on their cultivar properties and biochemical tests. As for Klebsiella, it appeared in the form of mucous pink colonies due to its containment of the capsule[15], while the germs of S. S. saprophyticus produces small, pink colonies with no color change to the medium[16].

Table (1): Types and numbers of bacterial isolates

Bacteria Isolated	Number of Isolates	%
Escherichia coli	30	42.85
Klebsiella pneumonia	15	21.42
Proteus mirabilis	8	11.42
Enterobacter cloaca	4	5.71
Pseudomonas aeruginosa	2	2.85
Serratia marcescens	2	2.85
S. aureus	5	7.14
S. Saprophyticus	2	2.85
Citrobacter freundii	1	1.42
Micrococcus luteus	1	1.42
Total	70	100

Suspitability test

Multiple resistance bacteria are one of the serious and major problems in the medical field, and this causes difficulty in choosing the appropriate treatment for the patient, and one of the main reasons for the emergence of resistance is the indiscriminate use of antibiotics without relying on a sensitivity test for these antibiotics [17]. The method of discs diffusion on Muller Hinton medium was used to determine the susceptibility of antibiotics against those isolated bacteria and was tested according to [18] for susceptibility , moderately sensitive and resistant. The results of the bacterial isolates showed different patterns of resistance to the antibiotics that were tested, as shown in the table. (2).

Table (2): Suspitability test

Antibiotic		S. aureus	K. pneumoniae	E. coli	P. mirbilis
Imipenem	S	0	0	0	7
	R	100	100	100	93
Ciprofloxacin	S	20	0	40	25
	R	80	100	60	75
Sulfamethoxaole	S	0	40	44	12
	R	100	60	56	88
Gentamycin	S	20	7	50	50
	R	80	93	50	50

Cont... **Table (2): Suspitability test**

Cefalexin	S	20	73.4	84	75
	R	80	26.6	16	25
Nitrofurantoin	S	0	80	17	50
	R	100	20	83	50
Oxacilin	S	40	100	100	100
	R	60	0	0	0
Vancomycin	S	40	100	96.7	100
	R	60	0	3.3	0
Tobramycin	S	66.7	66.7	87	37.5
	R	33.3	33.3	13	62.5
: sensitive ; R : Resistant					

The results of this study showed that most of E. coli isolates were resistant to antibiotics, as the percentage of resistance was high towards Oxacilin 100%, Vancomycin96.7%, Tobramycin 87%, and Cefalexin 84%. While the resistance was less in the direction of Imipenem 0%, Nitrofurantoin 13%, Ciprofloxacin 40%, Sulfamethoxaole 44%, while K. pneumoniae recorded the highest resistance against Oxacilin 100%, Vancomycin 100%, Nitrofurantoin 80%, Cefalexin 73.4%, Tobramycin 66.7%. , While the lowest resistance rate was Imipenem 0%, Ciprofloxacin 0%, Sulfamethoxaole 40%, Gentamycin 7%, while P. mirabilis had the highest resistance to Oxacilin 100%, Vancomycin 100%, Cefalexin 75%, while the lowest percentage was Resistance to the direction of Imipenem was 7%, Sulfamethoxaole 12%, Ciprofloxacin 25%, Tobramycin 37%, while S. aureus had the highest resistance to oxacilin 40%, Vancomycin 40% and the lowest resistance to Imipenem 0%, Nitrofurantoin 9%, Sulfamethoxaole 0%, Ratio 20% resistance to Gentamicin, Ciprofloxacin, Tobramycin, and Cefalexin.

The difference in the resistance rates of bacteria to antibodies may be due to several reasons, including the emergence of isolates resistant to many of the antibiotics used in this study to the presence of mutations or the presence of plasmids that carry resistance to many antibiotics, which play an important role in the spread of bacterial resistance. There is a large number of antibiotics in the bacteria, in addition to this bacterial type having the ability to form a biofilm that plays an important role in antibiotic resistance [19].

The effect of plant extracts on E. coli :

The most effective antibiotic in inhibiting E. coli bacteria was Ciprofloxacin, Imipenem, and Sulfamethoxaole, respectively, at the inhibition diameters of 32 mm, 30 mm and 11 mm. High significant differences at (P≤0.05) probability level on isolated E. coli .

The most effective extract in inhibiting the growth of E. coli bacteria was the alcoholic electrode extract at an average of 21 mm.

It was also found that there is a significant difference between the aqueous and alcoholic extract of Sumbucus nigra, where the average of the alcoholic extract was 14 mm and the aqueous extract was 7 mm. Also, significant differences were found between the concentrations used, as the most effective concentration was 100 mg / ml, followed by a concentration of 75 mg / ml while the lowest effect for both concentrations was 50 mg. / Ml and 25 mg / ml as there is no significant difference between them.

Effect of plant extracts on P.mirabilis:

The most effective antibiotic in inhibiting P.mirabilis bacteria was Imipenem, Ciprofloxacin, and Sulfamethoxaole, respectively, at inhibition diameters of 27 mm, 26 mm and 11 mm. When comparing the rates of inhibition zone for antibiotics with the rates of inhibition diameters for plant extracts, it was found Significant high differences were found at (0.05P≤) probability level on P.mirabilis isolated.

The most effective extract in inhibiting the growth of P. mirabilis bacteria was Sumbucus nigra extract at a mean of 14 mm and the alcoholic pole at an average of 16 mm. Also, it was found that there was no significant difference between the extract of aqueous Sumbucus nigra at a mean of 5 mm and aqueous olives at an average of 1 mm.

Significant differences were also found between the concentrations used, as the most effective concentration was 100 mg / ml, while there was no significant difference between the concentration of 75 mg / ml and the concentration of 50 mg / ml, as well as there was no significant difference between the concentration of 50 mg / ml and the concentration and 25 mg / ml.

The effect of plant extracts on Saureas bacteria:

The results shows that The most effective antibiotic in inhibiting P.mirabilis bacteria was Imipenem, Ciprofloxacin, and Sulfamethoxaole, respectively, at inhibition diameters of 28 mm, 25 mm and 19 mm. High significant differences at (0.05P≤) likelihood level on S.aureas isolates.

It became clear that there were no significant differences between Sumbucus nigra extract at an average of 7 mm and the alcoholic pole at an average of 7 mm and aquas Sumbucus nigra at an average of 3 mm. Also, it was found that there was no significant difference between the extract of aqueous Sumbucus nigra at an average of 0 mm.

Significant differences were also found between the concentrations used, as the most effective concentration was 100 mg / ml, while there was no significant difference between the concentration of 75 mg / ml and the concentration of 50 mg / ml, as well as there was no significant difference between the concentration of 50 mg / ml and the concentration and 25 mg / ml.

These results obtained are consistent with the results of other researchers, as they agree with [23] in the effectiveness of the electrode extract and that the higher the concentration, the better the effectiveness. It agrees with ([20] that Sumbucus nigra extract has an inhibitory effect on bacterial isolates.

Conclusions

It was found through the study that the alcohol extracts were effective in inhibiting the bacterial isolates under study, while the results showed that the aqueous extracts had less effect and sometimes had no effect on the bacterial isolates under study.

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Effect of *Staphylococcus aureus* Thermostable DNase on Some Immunological Parameters

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Abstract

Thermostable DNase was purified from *Staphylococcus aureus* isolated from local clinical source. The current study includes different immunological assays to evaluate the role of purified DNase. The phagocytic activity showed that the group of mice infected with *P.aeruginosa* recorded the highest values with significant differences (P <0.05) in comparison with the group that received mixed injection of 1 µg /ml of DNase and *P. aeruginosa* showed decrease in phagocytic activity followed by group that injected by 1 µg /ml DNase only with phagocytic activity 2%. DNase has a positive immunomodulatory effect on IL-10 and IL-4 level. The data showed that the highest value of IL-10 is in group that injected with 1 µg /ml DNase only (IV group) was 86.79±1.68. While the highest value of IL-4 was in group that injected with both DNase (1 µg /mouse) and *P.aeruginosa* gave the highest values (III group) was 84.02 ±1.19. In Arthus reactions(Immediate type hypersensitivity), the group that have been injected with both DNase and *P. aeruginosa* (group III) gave the highest values (4.50±0.70) while in delayed type hypersensitivity the same group III showed higher values after 24 and 48 hrs (5.00±0.00) , (4.00±0.00) respectively.

Key words: *Thermostable DNase, Interleukin-4, Interleukin-10, Arthus, DTH, Phagocytic activity.*

Introduction

Staphylococci are important microorganisms that influencing ecosystems, industry, animal, and human health. Their diverse lifestyles, whether as commensals or opportunistic pathogens, promote their prevalence in different environments, both nonpathogenic and pathogenic can survive for short or extended periods, thus it contribute in spread and difficult eradication [1, 2].

Staphylococcus aureus is a notorious pathogen known to cause numerous acute and chronic diseases [3]. The diversity of the tissues affected and infection potency is due in part to the many secreted virulence factors produced by *S. aureus*. These secreted factors include toxins, super antigens, and a suite of exoenzymes proteases, lipases, hyaluronidase, phospholipase, and nuclease [4].

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Staphylococcal nuclease also referred by many different names, such as micrococcal nuclease, thermonuclease, deoxyribonuclease and DNase which is regarded as an important virulence factor that hydrolyzes DNA and RNA in host cells [5]. It has been postulated to play a role during invasion or establishment of an infection [6]. Bacterial extracellular nucleases have multiple functions in processes as diverse as nutrient acquisition, natural transformation, biofilm formation, or defense against neutrophil extracellular traps NETs [7]. In response to infection, neutrophils play a key role. In addition to phagocytosis and intracellular killing of pathogens, they have been shown to actively release extracellular netlike structures (NET) that consist of a nuclear DNA backbone, histones, and granular proteins, that can entrap, immobilize and even kill gram-positive and gram-negative bacteria [8].

DNase mediate bacterial escape from NETs which is an innate immune defense mechanism by which DNA released from dying neutrophils immobilizes and facilitates killing of invading pathogens [9].

Material and Methods

Extraction and purification DNase from *S. aureus*

The selected isolate *S. aureus* was grown on nutrient broth medium and inoculated at 32 °C, using 100 rpm shacking rate for 24 hrs. Extraction was carried aout according to Ohsaka [10]. DNase was extracted by ammonium sulfate precipitation and Dialysis of crude enzyme and Purified by Ion exchange chromatography and gel filtration chromatography according to Ibraheem and Al-Mathkhury (2015), [11].

Laboratory animals

Twenty male of albino mice, aged between 8-12week and 26–30 g weight were used in this study. The mice were obtained from Iraqi National Center for Drug Control and Research and kept in plastic cages, housed under a standard condition in the animal house of the Department of Biology/College of Science/ Mustansiriyah University. The mice left for 2 weeks for adaptation before the experiments began.

Animals groups and injection schedule

The current study was included four groups, all mice injected intraperitoneally for seven days as follows:

- Group I: Mice injected with 100 µl of phosphate buffer saline only as negative control
- Group II: Mice injected with 100 µl of 1.5 x 10^8 CFU / ml of *P. aeruginosa* only as positive control.
- Group III: Mice injected with 100 µl of purified DNase (1µg/mouse) only.
- Group IV: Mice injected with 100 µl purified DNase (1µg/mouse) after infection.

Blood collection

After 7 days of treatment, mice were anesthetized with sodium pentobarbital and killed by cervical dislocation, blood samples were obtained by cardiac puncture and divided; serum was obtained and stored at – 20 °C for next assays.

Measurement of serum cytokines levels

The levels of IL-4, IL-10 in the serum of mice were measured through ELISA. All the procedures were performed according to the manufacturer's instructions of Elabscience.

Phagocytic activity

The procedure was done depending on a method presented by Zakaria, *et al* 2011, [12] peripheral Blood Leucocytes 1ו10^6 cell/ well (100 *ul* Blood, with 175 *ul* MEM media) added to each well of microtiter plate , 25 *ul* of NBT was added and incubated two hours at 28 °C. Then supernatant was removed carefully. Cells were fixed with methanol 100% (v/ v) for 5 min in each well .and washed twice with 125 ml with Methanol 70%, then drying at air overnight. Finally adding 125 ml Potassium hydroxide (2N) and 150 ml DMSO add to each well to dissolve NBT. Then Read by Elisa at 650-wave length.

Arthus and Delayed hypersensitivity test

Each mouse in the groups was injected with 50 ul of *P.aeruginosa* in day 5 in the right foot pad and normal saline in the left foot pad (except control group), arthus reaction was observed and determined as an increase in footpad swelling 4 hrs while delayed type hypersensitivity peak after 24 and 48 hours by a digital vernia and given in a unit of millimeter, as suggested by (Tansho *et al*., 2002) [13].

Statistical Analysis

A one way analysis of variance ANOVA was performed to test whether group variance was significant or not. Data were expressed as mean ±standard deviation (SD) and statistical significances were carried out using SPSS program version 20 [14].

Result and Discussion

Production, extraction, and purification of thermostable DNase

The crude enzyme of *Staphylococcus aureus* was purified by precipitation with ammonium sulphate at (65-85 %) saturation then dialysis, after that using ion exchange chromatography in CM cellulose and gel filtration by using Sephadex G150. The DNase activity for each step was 38, 31, 32, 49 and 42 U/mg respectively.

Phagocytic activity of DNase

Results in table (1) shows that the group of mice

infected with *P.aeruginosa* recorded the highest values with significant differences (P<0.05) but the group that received combination injection of 1 µg of DNase and *P. aeruginosa* showed decrease in phagocytic activity followed by group that injected only with 1 µg of DNase with phagocytic activity 2%.

Table 1: The phagocytic activity index of DNase in 1 µg /mouse

Treated groups	Mean± SD	Phagocytic activity%
Group (I): Negative Control	0.44±0.01 d	0
Group (II): Positive Control	2.722±0.14 a	5.1
Group (III): DNase and P. aeruginosa	1.82±0.09 b	3
Group (IV): DNase only	1.33±0.18 c	2

The different letter = high significant difference at p ≤ 0.05, The similar letter = non-significant at p≤ 0.05

The antimicrobial strategies employed by neutrophils include the phagocytosis of bacterial invaders, the release of toxic effectors, and the production of neutrophil extracellular traps (NETs) [15]. A significant feature of extracellular traps is that their expression is degraded by the addition of DNase 1. DNase 1 which breaks down extracellular DNA, and found that DNase 1 was highly effective in degrading extracellular trap formation both in vitro and in vivo. In addition, DNase 1 reduced the numbers of macrophages in the lung tissue induced by cigarette exposure [16]. *Staphylococcus aureus* produces a nuclease that degrades DNA and RNA to 5-phosphomononucleotides and dinucleotide, which are subsequently converted to deoxyadenosine (dAdo) through the AdsA enzyme. The dAdo product is cytotoxic to macrophages and therefore protects *S. aureus* by restricting these innate immune cells from entering staphylococcal abscesses [17].

Determination the level of cytokines IL-10 and IL-4

The results reported a positive effect of DNase on IL-10 and IL-4 level, as noticed group that injected in both DNase (1 µg/ml)and *P.aeruginosa* gave the highest value according to IL-4 while IL-10, the highest value was in group that injected with DNase only table (2).

Table 2: The levels of Interleukins 4 and 10 in studied groups injected with DNase enzyme (1µg/mouse).

Treated Groups	Cytokines concentration Pg/ml (mean ± SD)	
	IL-4	IL-10
Group (I): Negative Control	37.90±5.51 d	36.76±0.15 d
Group (II): Positive Control	51.50±0.14 c	53.22±2.20 c
Group (III): DNase and P. aeruginosa	84.02 ±1.19 a	66.66±1.66 b
Group (IV): DNase only	65.45 ±0.63 b	86.79±1.68 a

The different letter = high significant difference at p ≤ 0.05, The similar letter = non-significant at p≤ 0.05

Extracellular DNA, also called cell-free DNA, released from dying cells or activated immune cells can be recognized by the immune system as a danger signal causing or enhancing inflammation. The cleavage of extracellular DNA is crucial for limiting the inflammatory response and maintaining homeostasis. Deoxyribonucleases as enzymes that degrade DNA are hypothesized to play a key role in process as a determinant of the variable concentration of extracellular DNA [18]. Interleukin-4 and IL-10 are pleiotropic anti-inflammatory cytokines that function mainly by suppressing the proinflammatory milieu [19], when DNase degrades DNA in the abscess or NETs, the degradation products, monophosphate nucleotides, become substrate for another enzyme, adenosine synthase A (AdsA) that converts the degraded DNA to deoxyadenosine, which induces caspase-3 activation, leading to apoptosis of macrophages surrounding the abscess or the NET [19]. Gao et al. (2019), [20] showed that deoxyadenosine is one of the major bioactive metabolites that possesses multiple beneficial effects of anti-inflammatory, anti-cancer, anti-viral, and anti-fungal activities in addition and Deoxyadenosine enhanced the expression levels of IL-4 and IL-10, and decreased the expression levels of IL-1β and TNF-α.

Arthus and Skin Delayed Hypersensitivity Reactions

Table (3) exhibit the index of Arthus reactions where the group 5 that has been injected with both DNase and *P. aeruginosa* gave the highest values in comparison with other groups as in group 5 (4.50±0.00).

Table (3): Results of Arthus reaction/mm in studied groups

Treated Groups	Mean± S.D
	After 4 hrs.
Group (I): Negative Control	3.12±0.17 [b]
Group (II): Positive Control	4.00±0.00 [ab]
Group (III): DNase and *P. aeruginosa*	4.50±0.70 [a]
Group (IV): DNase only	4.25±1.060 [ab]

The different letter = high significant difference at $p \leq 0.05$, The similar letter = non-significant at $p \leq 0.05$

Arthus involves the in situ formation of antigen-antibody complexes after the injection of an antigen with high levels of circulating antibodies. It manifests as local vasculitis due to the deposition of IgG-based immune complexes in dermal blood vessels [21].

In DTH the highest values after 24 hrs were positive control group and in group that have been injected with both DNase and *P. aeruginosa* (5.00±0.00) and (4.50±0.70) respectively while after 48 hrs, the values of these groups were decreased table (4).

Table 4: Delayed hypersensitivity reaction after 24 and 48 hrs. in the studied groups

Treated Groups	Mean± S.D	
	After 24 hrs.	After 48 hrs.
Group (I): Negative Control	3.12±0.17 d	3.12±0.17 cd
Group (II): Positive Control	4.00±0.00 c	3.2±0.355 abc
Group (III): DNase and P. aeruginosa	5.00±0.00 ab	4.00±0.00 ab
Group (IV): DNase only	4.50±0.70 bc	3.75±0.35 b

The different letter = high significant difference at p ≤ 0.05, The similar letter = non-significant at p≤ 0.05

During the sensitization phase, the host's antigen presenting cells (APC) take up, process, and present antigenic peptides to T-cells at the site of infection. Sensitized T-cells formed as a result of the initial exposure, and, upon secondary challenge with the same antigen, a vigorous response by the sensitized Tcells accompanied with the release of several cytokines and tissue swelling. Because DTH relies on antigen processing by APC and subsequent presentation to T-cells at least 24–72 h are needed for the response to occur, which may take only minutes to manifest [22]. DNase has been shown to increase the production of IL-4 that enhances Th2 immunity by inhibiting Th1 responses through the repression of IL-12 signaling [19]. Classically, IL-4 drives CD4+ T cell polarization in the Th2 phenotype together with suppression of interferon (IFN)-γ– producing Th1 cells [23].

Conclusion

The purified DNase had immunostimulating activities on the murine immune system. This effect is reflected by phagocytic activity, Arthus and DTH reactions. Also had immunomodulator effect with significant differences (P <0.05) in elevated level (IL-10 and IL-4).

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Interpretive Ability on Common Haematological Tests and critical thinking ability among B.Sc Nursing students

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Abstract

Introduction: Initiation of patient management and prediction of patient prognosis depend on investigation reports. Interpretive ability of common blood investigations demands high level of critical thinking and it is a less focused in nursing education. Therefore, this study aimed at identifying the relationship between interpretive ability of common hematological tests and critical thinking ability among nursing students. **Methods**: The study design adopted was cross sectional descriptive survey. It was conducted among 140 nursing students studying in third and fourth year of Bachelor of Science (B. Sc.) nursing. The data was collected using demographic tool, interpretive exercises and Yoon's Critical Thinking Disposition. **Results**: The median of interpretive ability score on common blood investigations was 13 (IQR – 11, 14). The mean critical thinking score of nursing students was 96 (SD 9.5). Analysis of the relationship between interpretive ability on common blood investigations and critical thinking ability revealed a weak positive correlation which was statistically not significant (r=0.141, p=0.096). There was a significant relationship between interpretive ability and intellectual eagerness (r=0.181, p=0.032) and intellectual fairness (r=0.174, p=0.039). There was a significant association between interpretive ability and academic performance of the students in the university examinations (F=4.5, p=0.012). Interpretive exercises stimulate critical thinking among students. **Conclusion**: The students with high intellectual curiosity and intellectual fairness have good interpretive ability, and good academic performance improves ability to interpret common haematological tests.

***Key words**: Nursing care, Nursing students, Baccalaureate Nursing Education, hematological tests, thinking*

Introduction

The educational preparation, regulation, and practice benchmark among the nurses are varied internationally[1]. There is no fixed model for a nursing trainee to improve professional practice and the gap between nursing education and clinical practice is always of concern[2]. Despite this, the nurses migrate across the world due to growth opportunities and better prospectus[3]. In order to alleviate these challenges and maintain standards several countries assess nurse's competencies before entry. For example, the National Council of State Boards of Nursing administers the U.S. Nurse Licensure Examinations (NCLEX-RN and NCLEX-PN) to assess nurse's competency to practice in the country. Failure rate in this examination is very high among the foreign educated nurses, in comparison to the, nurses educated in the native country[4]. Interpretive exercises are commonly used form of test items for licensure exams, for example NCLEX items are prepared in the interpretive exercise format[4].

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Interpretive exercises are a series of related objective items based on a common set of data in the form of introduction. The 'common set of data' are presented as written materials consisting of patient's history and investigations, tables, charts, graphs (ECG), maps, or pictures (X-ray, Scans) in view of objectives to be achieved[5,6]. The series of related test items may also take diverse forms but most commonly used are multiple-choice or true-false items. The items can be formed with an objective to establish relationships in data, to recognize pertinent conclusions, to appraise assumptions and inferences, to examine proper applications of data, and the like[7].

Nurses attend patients with varied symptoms. Therefore, the ability to notice the variations and application of suitable interventions are exceedingly essential for nurses. Nurses with good critical thinking ability can provide better nursing care[8]. Moreover, in an era of skyrocketing health care costs, critical thinking is essential in providing cost effective and accessible care to the patients[9]. Though all types of teaching methods cannot improve critical thinking problem based learning, simulation and concept aping are useful[10-13]. Simulation is beneficial in transferring the knowledge to clinical care[10,11].

Interpretive ability on common hematological tests is the least explored area in the field of medicine. No studies were traced on interpretive ability of nurses on hematological tests using interpretive exercises. Therefore, we aimed at testing interpretive ability and critical thinking of nursing students.

Material and Methods

The study was conducted in a selected nursing college of Karnataka, southern India. The college is recognized by the nursing regulatory body and all the approved nursing courses are implanted in the college. The third and fourth year Bachelors of Science (B. Sc.) Nursing students enrolled in a selected college of nursing were recruited for the study. The nursing curriculum in India is regulated by Indian Nursing Council (INC), a professional body that establishes and monitors a uniform standard of nursing education in India. B.Sc. Nursing course is offered for four years with theoretical and practical experiences imbibed in it with six months integrated internship in the clinical areas. The sample size was calculated and a total of 140 students were recruited.

Data was collected using demographic proforma, interpretive exercises on common hematological tests and Yoon's critical thinking disposition inventory[14]. The demographic proforma comprised of information on year of study and age, information on previous years' academic performance in five subjects in the university examination and exposure in eight clinical areas. The academic performance score was collected in: 1) Medical Surgical Nursing-1, 2) Medical Surgical Nursing-2, 3) Pathology, Pharmacology and Genetics, 4) Nutrition and Bio-chemistry and 5) Anatomy and Physiology subjects, as the knowledge of these subjects are directly related to the interpretation of hematological tests.

Interpretive exercise tool consisted of 26 multiple choice items based on four virtual patient scenarios. The items focused on interpretation of six type of abnormal hematological tests namely complete blood counts, renal function test, serum electrolytes, liver function test, lipid profile and arterial blood gas analysis reports. The tools were validated by experts and the reliability of the interpretive exercise was established by using split half method (r=0.723).

The critical thinking was assessed using Yoon's Critical Thinking Disposition Inventory. This is a standardized tool and has 27 items with five point Likert scale which is developed by Yoon. The tool had 27 items distributed in 7 domains and the Cronbach's alpha of the original study was 0.9. The possible minimum score is 27 and the maximum is 135. In the present research setting the tool was pretested and reliability was estimated by Cronbach's alpha for the tool(r=0.8).

Administrator of the institution permitted to conduct the study among B.Sc. Nursing students. Institutional Ethics Committee (IEC) clearance was procured. Permission was obtained from the class coordinators of third and fourth year B. Sc. Nursing. The objectives of the study was explained to each students and they gave written consent before the data collection.

Result

Demographic Characteristics:

The descriptive analysis of demographic

characteristics showed that the 57.1% of the students were from 3rd year B.Sc. Nursing and 60% of the students were in the age group between 21-22 years. The academic performance of majority of the students were within the range of 50-64% in all subjects.

Interpretive Ability:

The median of interpretive ability score on common hematological tests was 13 and the interquartile range of 25th and 75th percentile was 11 and 14 respectively. The maximum score obtained by the students was 22 and minimum score was 4. The ability of the students in interpreting the results of complete blood counts, renal function test, serum electrolyte and liver function test was good (figure 1). However, there was a need for improvement in identifying lipid profile and abnormal arterial blood gas analysis findings.

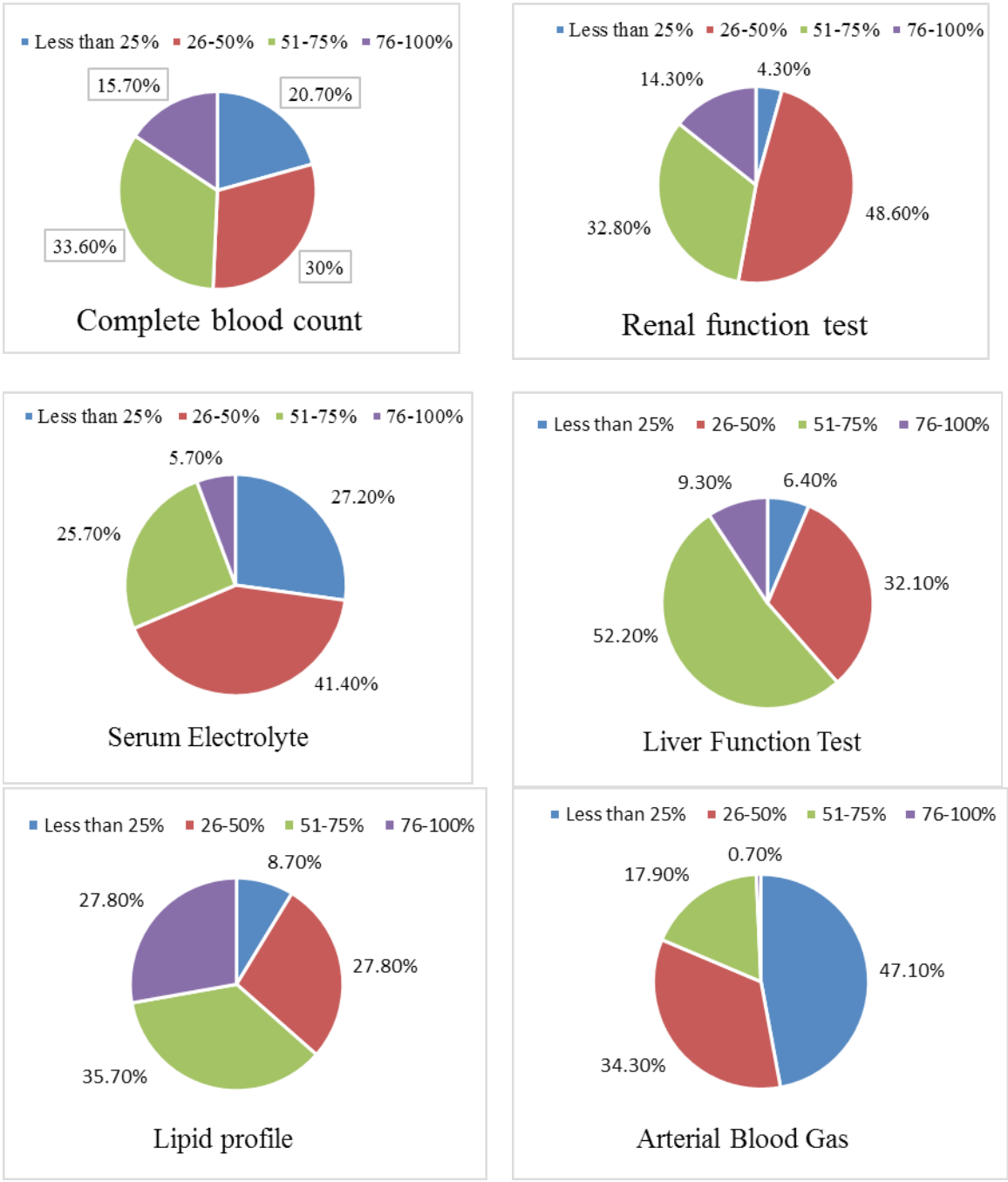

Figure 1: Interpretive ability of the nursing students on common hematological tests.

Critical thinking:

The mean score of critical thinking of the participants was 96 (standard deviation – 9.5). The scores in different domains of critical thinking is given in table 1. The mean score in intellectual eagerness was 18.6 ± 2.6, in prudence was 12.7 ± 1.7, in self-confidence was 14.7 ± 2.1, in systematicity was 10 ± 1.6, in intellectual fairness was 14.8 ± 2.2, in healthy scepticism was 13.6 ± 2.3 and in objectivity was 11.7 ± 1.7.

Relationship between Interpretive Ability and Critical Thinking:

To find the relationship between interpretive ability and critical thinking, Spearman correlation was computed and value was 0.141 (p=0.096) indicating a weak positive correlation. Relationship between interpretive ability and different domains of critical thinking was also calculated (table 1). There was a statistically significant weak positive correlation between interpretive ability on common hematological tests and intellectual curiosity (*r= 0.181, p=0.032*). There was also a statistically significant weak positive correlation between interpretive ability on common hematological tests and intellectual fairness *(r= 0.174, p=0.039*). Thus it was inferred that the students with high intellectual curiosity and intellectual fairness have good interpretive ability on common hematological tests. However, it cannot be concluded as the correlation was weak.

Table 1: Spearman Correlation on Interpretive Ability and Domains of Critical Thinking among Nursing Students

Variable	Possible minimum score	Possible maximum score	Mean	SD	r value	p value
Interpretive ability						
Intellectual eagerness/curiosity	12	25	18.6	2.6	0.181	0.032
Prudence	9	17	12.7	1.7	- 0.075	0.375
Self-confidence	9	19	14.7	2.1	- 0.005	0.953
Systematicity	6	15	10	1.6	0.098	0.248
Intellectual fairness	9	20	14.8	2.2	0.174	0.039
Healthy scepticism	9	20	13.6	2.3	0.105	0.217
Objectivity	6	15	11.7	1.7	0.044	0.606
SD = standard deviation, r = Pearson's co-relation, p = level of significance						

The association between interpretive ability on common hematological tests and academic performance of the students was calculated using one way ANOVA and it was statistically significant (F=4.5, p=0.012). Therefore, it was inferred that interpretive ability on common hematological tests and academic performance of the students are dependent on each other. Thus, students with good academic performance have good interpretive ability.

Discussion

One of the reason of low satisfaction of the nurses in the hospital is lack of proper training[15]. Therefore nursing curriculum must focus on improving the knowledge of clinical nurses. Analyzing the condition of the patient is vital to provide comprehensive individualized patient care. Analysis includes collecting history, performing physical examination, inferring investigation reports and assessing general well-being of the patient. Interpreting hematological tests helps in clinical judgement to prioritize the care and plan appropriate interventions.

As the nursing curriculum differs from country to country, the method of evaluating also differs[16]. Interpretive exercises are applied type of objective questions. Hence it helps to measure the higher level of knowledge. In the present study, the nursing students had good interpretive ability on common hematological tests. It is also noted that the students with good academic achievement have better interpretive ability. However, good interpretive ability did not show statistically significant ability in critical thinking.

Jeong (2015) found that critical thinking disposition was positively correlated with perspective taking (r=0.482, p<0.001), fantasy (r=0.227, p<0.001), and empathic concern (r=0.195, p=0.002)[17]. Woo & Tak, (2015) illustrated that critical thinking disposition positively correlates with caring perception (r=0.174, p=0.024) and professional self-concept (r=0.343, p=0.022)[18]. A similar study by Park, Chung, & Kim, (2016) demonstrated significant correlation between nursing competency and critical thinking disposition (r=0.59, p<0.001) and self-directed learning readiness (r=0.54, p<0.001)[19].

The students in the present study setting receive clinical teaching every day at the bed side by qualified faculty members. Students write clinical dairy which includes history, focused physical examination, reports of significant investigations and nursing care plan. The clinical teaching and daily dairy writing must have been useful in getting the positive results. Perhaps, additional research is needed to generalize the findings and usefulness of interpretive exercises. It is also important to assess the change in critical thinking of the students over a period.

Fourth year students had more clinical exposure compared to third years is the limitation of the study. Interpretive exercises evaluate higher learning skills. Hence, these can be adopted as an evaluative method for assessing students learning in nursing curriculum. The tool is not only helpful in evaluating the understanding and memorization of the facts, but also assess their ability in application and synthesis of the facts. Critical thinking is crucial in enhanced patient care. Proper identification of the needs of the patient and tailoring of the nursing intervention accordingly, demands high degree of critical thinking.

Conclusion

The interpretive ability and critical thinking of nursing students augments their learning. Enriched education results in enhanced patient care. This study provides an evidence and recommends the nursing teachers to implement interpretive exercises and critical thinking assessment in their regular teaching activities. In addition, timely interpretation of hematological tests may reduce morbidity and mortality of high risk patients.

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Mental Health and Social Development of Prisoners as a Policy Implication of Correctional Institutions

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Abstract

The problem in this research is to analyze the health and social development prisoners in Indonesian prisons in the future. The research method used is juridical empirical, namely analyzing the formulation of policies on current correctional facilities and the health and social development of prisoners in Indonesian prisons in the future. The results show that the current formulation policies in coaching prisoners are based on Law No. 12 of 1995 concerning correction institutions. This policy has not fully provided the rights of prisoners as assisted citizens. The number of prisoners currently in 33 Regional Offices of Law and Human Rights, only 6 Regional Offices is not overcrowded. This will affect the development of prisoners in prisons. Coaching carried out in prisons at this time has obstacles due to statutory factors, human resources in quality and quantity as well as the legal culture of the community which has not provided optimal support for the development of prisoners both inside and outside of prisons. In the future, based on policy studies, it is necessary to carry out reformulation and reconstruction related to the development of prisoners in prisons.

Keywords: *health and social development, policy, prison development, correctional institutions*

Introduction

Based on Article 1 point 2 of Law No. 12 of 1995 concerning Corrections, it is regulated that the correctional system is an arrangement regarding the direction and boundaries and methods of fostering Correctional Assistance Citizens. Based on Pancasila, it is carried out in an integrated manner between the supervisor, the fostered, and the community. It is to improve quality Correctional Assistance Citizens in order to be aware of mistakes, improve themselves, and not repeat crimes so that they can be accepted back by the community, can actively play a role in development, and can live naturally as good and responsible citizens[1,2,3]. Correctional system needs an approach. This approach can be done through a coaching stage process. In each stage of development the prisoner is introduced to the activities and in due time the prisoner will be given his rights which have been guaranteed in the Correctional Law.

Various conditions of law enforcement that affect the policy based mechanism for the development of health and social aspects of prisoners in social institutions as well as human resources related to quality and quantity, competence, facilities and infrastructure owned by civil society institutions and the legal culture of officers, and the communities involved in the process integrated and inseparable system[4,5]. Harmonization and synergy of policies and human resources involved constitute unity. Development of prisoners must be in accordance with the needs and require support from all parties. The support is not only from the prisoners themselves, the supervisor, but also from the community[1,2,3]. Society at this time must be able to accept prisoners especially those who have repented so that they can return to community life. This task is not only to make the Penitentiary a place of revenge but also a place for inmates to repent when they realize their mistakes.

Imprisonment is one of the types of sanctions that are most often used as a means of overcoming crime problems[6]. It is worth considering the various negative effects of imprisonment, namely the stigma that the perpetrators receive. Stigma was identified as a potential barrier to securing employment[7]. Social, economic, political, cultural factors are currently the basis for conducting policy studies and reconstruction as well

as reformulation in the development of prisoners in Indonesian prisons in the future[8,9,10].

Method

The research method used in this research is an empirical juridical study, namely analyzing the formulation of policies regarding current correctional facilities and the development of prisoners in Indonesian prisons in the future.

Result and Discussion

The correctional system places the Correctional Assistance Citizens consisting of: prisoners, state children, and correctional clients no longer as objects of guidance but as subjects of guidance and are seen as individuals and ordinary citizens and faced not with a background of retaliation but with guidance and guidance[11,4,12,5]. Penal reform is part of the politics of criminal law, which implies an effort to reorient and reform criminal law in accordance with the socio-political, socio philosophical and socio-cultural values of the Indonesian people, through the policy oriented and value oriented approach[13]. In a policy-oriented approach, criminal law reform is part of social policy, namely as an effort to overcome social problems in order to achieve national goals. As part of the criminal policy, criminal law reform is an effort to protect society. Furthermore, as part of law enforcement, criminal law reform is an effort to renew the substance of the law in order to make law enforcement more effective.

Penal reform is part of the politics of criminal law, which implies an attempt to reorient and reform criminal law in accordance with the socio-political, socio-philosophical, and socio-cultural values of Indonesian society, through the policy oriented and value oriented approach. In a policy-oriented approach, criminal law reform is part of social policy, namely as an effort to overcome social problems in order to achieve national goals. As part of the criminal policy, criminal law reform is an effort to protect society. Furthermore, as part of law enforcement, criminal law reform is an effort to renew the substance of the law in order to make law enforcement more effective.

The scope of the reform of the criminal law system includes the following. The first is legal substance (substantial system), including reforms in the fields of material criminal law, formal criminal law, and criminal law enforcement. The second is legal culture (cultural system), including reform of the moral field of the perpetrator, as well as legal education in criminal law, and third is legal structure (structural system), which includes the reform of the investigating body, prosecuting agency, judiciary and criminal executing agency. In criminal law reform, striving for the formation of a national criminal law that is in accordance with the value order of Indonesian society and is a reflection of a nation's political ideology[12,5].

Some of the problems in coaching in the correctional institutions are caused by: First, overcapacity in correctional institutions, the phenomenon of overcapacity in various Correctional UPTs (State Detention Centers and Penitentiaries), is one of the real symptoms of the absence of synergy in the operation of the criminal justice system. In the context of an integrated criminal justice system, each law enforcement agency cannot deny the problems faced by other law enforcement agencies that are directly or indirectly caused by the policies of one of the institutions. Second, the basic needs of prisoners are not fulfilled, the existing conditions are that the basic needs of prisoners are not fulfilled, for example basic needs, namely unmet nutritional food, poor sanitation, and so on. Such conditions can cause illness, especially for prisoners. Third, imprisonment is not the only solution, the conflict cases that still occur in prisons are an illustration that prison is not a solution to reduce crime[14].

In order to carry out such legal reform there are several things that must be done, including restructuring the existing legal structures and institutions including quality human resources. It includes reformulation of a just law; increasing law enforcement by resolving cases of law violations; people's participation in law enforcement; public education to increase public understanding of the law; and the application of the concept of Good Governance.

The main principles of the correctional concept include some elements. People who are lost are protected too, by providing them with provisions to live as good and useful citizens of society. The point here is that Indonesian society is moving towards a just and prosperous society based on Pancasila. Provisions for

life are not only in the form of financial and material, but more importantly mental, physical (health), expertise, skills so that people have the potential and effective will and ability to become good citizens, not breaking the law anymore, and useful in the development of the State[15,16].

To impose a sentence is not an act of retaliation from the State. There is no torture against prisoners in the form of actions, speech, treatment or placement. The only pain experienced by prisoners was their independence. Repentance cannot be achieved by torture, but by guidance. Prisoners must be instilled an understanding of the norms of life and life, and be given the opportunity to reflect on their past actions. Prisoners can be included in social activities to foster a sense of community life. The state has no right to make someone worse/worse than before he entered the institution. Furthermore, the separation between the recidivist and those who are not, the serious and minor crimes, the types of criminal acts committed the adult perpetrators, young adults, and children, men and women[12,5,17,18].

During the loss of freedom of movement, prisoners must be introduced to the community and must not be isolated from it. They will gradually be guided outside the institution (in the midst of society), this is a necessity in the correctional process. The work given to prisoners may not be time-consuming, or may only be for the interests of the office or the interests of the State at any time. Work must be one with work in society and be aimed at national development. Then there must be an integration of prisoners' work with national development. Guidance and education must be based on Pancasila. Education and guidance contain principles that are contained in the contents of Pancasila. Prisoners must be given religious education and given the opportunity and guidance to carry out their worship[19]. Prisoners must instill a spirit of mutual cooperation, a spirit of tolerance, a spirit of kinship as well as kinship between nations. Prisoners must instill a sense of unity, a sense of Indonesian nationality, must instill a spirit of deliberation for positive consensus. Prisoners must be involved in activities for the common interest and the public interest.

Every person is a human being and should be treated as a human, even if he has strayed. There should be no designation to prisoners that they are criminals. On the contrary, he must always feel that he is seen and needed as a human being. Prisoners are only sentenced to lose their independence. It is necessary in the company of prisoners to get a livelihood for the survival of the family that is their dependents, by providing jobs or being allowed to work, and being given wages for their work. The obstacle to implementing the Correctional System is the inheritance of prison houses in a miserable condition which are difficult to adapt to correctional duties, which are located in the middle of a city with high, thick walls.

The purpose of guidance in a correctional institution is divided into three things, namely: After leaving the correctional institution, there is no longer a criminal act; Become a useful human being, play an active and creative role in building the nation and country; Able to get closer to God Almighty and get happiness in this world and in the hereafter. The purpose of training for inmates is closely related to the purpose of punishment. The development of prisoners that is currently being carried out initially departs from the fact that the purpose of punishment is no longer in accordance with the development of the values and essence of life that grows in society. In the correctional system, the purpose of punishment is guidance and guidance, with the stages of admission/orientation, coaching and assimilation. These stages are not recognized in the prison system. The admission/orientation stage is intended, so that inmates are familiar with the way of life, rules and objectives of fostering themselves.

Based on the mandate of the Correctional Law No. 12 of 1995 concerning Corrections, Penitentiaries provide guidance for inmates, through several stages, namely, starting from the initial stage, the advanced stage, the assimilation stage and the final stage, each stage has a certain time to carry out guidance. Each stage has a certain time to carry out coaching, among others: Early stage coaching, this guidance is carried out in a time span from 0 to 1/3 of the criminal period, this stage puts forward the introduction of the environment in prison or known as admission and orientation. Next is the Advanced Stage of Guidance. After going through the initial coaching of a Correctional Assisted citizen will be given advanced stage coaching, the time span is given 1/3 to 1/2 of the criminal period, at this stage the guidance carried out is in the form of personality development (mental and spiritual) as well as self-reliance development (skills

and work activity training). Then the Assimilation Stage Guidance, that is, after being given various personality and independence coaching, the Correctional Assistance community will be given assimilation guidance ranging from 1/2 to 2/3 of the sentence. The last is the Final Stage of Coaching, which is the final stage of guidance that is given to Correctional Assistants in the form of integration guidance in which the Assisted Citizens will be returned to their families and communities to return to normal life, at this stage it is known as parole or leave. Conditional or Leaving towards Free, the time range starts from 2/3 until expiration or in accordance with applicable regulations.

For the Assisted Citizens, the high punishment is the obstacle in implementing the guidance program because the sentence period is not certain and the sentence will be certain to be free, so that in determining the guidance program for them cannot be maximized as is the case for the Assisted Residents who are sentenced temporarily. There is also a lack of professional experts in conducting personality development and independence of prisoners, the experts in question are psychologists and psychiatrists for mental development, sociologists for social development, agricultural graduates, animal husbandry graduates, mechanical graduates, etc. for the development and implementation of self-reliance development[20].

Guidance for future prisoners is also related to policy, structural and cultural. Reformulation was carried out in accordance with Law No. 12 of 1995 which has not fully granted the rights of prisoners in correctional institutions. In carrying out coaching without reducing the importance of the security aspect, the assisted residents are prepared to provide guidance which has the ultimate goal of returning to society and becoming humans who have the ability and skills in social life. Community participation is also very important as part of the process of guiding prisoners. The business community must be more able to provide support to the assisted members who have been released, this is related to the continuity of the independence development program given to them during their criminal period in prison so that it can be implemented in the business world.

Conclusion

The current correctional policy in Law No. 12 of 1995 has not yet accommodated the fulfillment of the rights of guiding prisoners in Indonesian prisons. Analytical studies of the Draft Law on Corrections have provided several rights for prisoners but several vulnerable groups, namely the elderly, transgender groups, LGBT people, children, disabilities, sexual rights, health, education, there is no special treatment and there is no minimum limitation on punishment for which they are guided carried out in a correctional facility. The current policy is still the death penalty, while in some countries it has eliminated the death penalty as well as life punishment, with a maximum imprisonment of 20 years. In some countries, imprisonment of under one year for a convicted person but can have or continue to do trade or work/profession, or participate in a professional education or training, or internship or temporary work with the intention of social rehabilitation, or important participation in life his family.

Conflict of Interest: There is no conflict of interest.

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Ethical Clearance: Ethical clearance from the institutional ethical committee obtained for the study.

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A Retrospective Study to Assess the Risk Factors of Ventilator Associated Pneumonia (VAP) among Patients Undergone Mechanical Ventilator

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Abstract

Background: Ventilator-Associated Pneumonia (VAP) is a hospital acquired pneumonia or it is a lower respiratory tract infection which common occurs in patients who were kept on mechanical ventilation with endotracheal tube or tracheotomy for more than 48 hrs.

Objective: The aim of this study is to assess the risk factors of ventilator associated pneumonia.

Methods and Materials: This is a retrospective study conducted in a specialized surgical care unit of 57 beds with 18 ICU beds; all patients who underwent the procedure of invasive mechanical ventilation for more than 48 hours. These patients were divided into two groups, group A has developed the VAP and group B has not developed the VAP. Some certain risk factors were assessed among the patients who developed the VAP with help of records.

Results: Researchers have searched 100 patients' data who were admitted and treated on mechanical ventilation. The percentage of the length of stay in the ICU with VAP is 64% and the patients without VAP is about 36%. The death rate was 54.2%, among VAP patients. About 53 % of the patients developed VAP who had the previous hospitalization, 69 % of the patients developed VAP with previous history of steroid treatment and 50 % of the VAP patients had undergone the surgical procedure and 84 % of VAP patients had the endotracheal tube or tracheotomy

Conclusion: In this study the patients who had undergone the MV developed VAP significantly with endotracheal tube or tracheotomy.

Key words: *retrospective study, risk factors, Ventilator Associated Pneumonia (VAP), Mechanical Ventilator.*

Introduction

Ventilator Associated Pneumonia is very commonly occurring acquired infection among the patients admit in ICUs and on Mechanical ventilators. The incidence of VAP ranging from 7 to 40 % [1]. Among intubated patients the VAP is higher than the other patients [2]. *Pseudomonas aeruginosa , Acinetobacter baumannii , and* methicillin-resistant*, Staphylococcus aureus* , are common pathogens responsible for development of VAP [3]. About 60% VAP cases are diagnosed with gram-negative bacilli, and *P. aeruginosa* which increases the hospital stay in ICU [4].

The main features of VAP is high fever, increased in WBC and alteration in sputum collection noticed [5]. The health condition of patients with VAP is suddenly deteriorated and can threaten the life of the patients and increase the burden for health care professionals [6].

The risk factors are the complex responsible factors in developing the VAP and these risk factors also make the treatment to be complicated. Some of these risk factors are Endo tracheal catheterization, duration of stay in the ICUs, nursing practices in certain nursing procedures, age and gender of the patients, duration of ventilation, history of cardiac diseases and previous

surgical history [7].

Methods and Materials

This is a retrospective study conducted in a specialized surgical care unit of 57 beds with 18 ICU beds. Reviewed the records of 100 patients who underwent the procedure of invasive mechanical ventilation for more than 48 hours. The tool consisted of demographic variable and check list of risk factors. These patients were divided into two groups, group A has developed the VAP and group B has not developed the VAP. The risk factors were assessed among the patients who developed the VAP. The following of the risk factors assessed[8]

1. Age and gender of the patients
2. Endo tracheal catheterization,
3. Duration of stay in the ICUs,
4. Nursing practices in certain nursing procedures,
5. Duration of ventilation,
6. Previous history of Hospitalization
7. History of cardiac diseases
8. Previous surgical history.
9. COPD
10. Use of steroid therapy.

Results

Table I: Description of demographic variables in terms of frequency and percentage

n=100

Sr. No	Demographic variables	Frequency	Percentage%
1.	Male	84	84
2.	Female	16	16
3.	Hypertension	46	46
4.	Diabetes	28	28
5.	Coronary artery diseases	74	74
6.	Patients with high baseline e WBC(12,000/Cum)	44	44
7.	Patients with purulent sputum	46	46
8.	Patient with cough (mild or severe)	44	44
9.	Patients with worsening ABG	74	74

Table 2: Assessment for the development of VAP in terms of frequency and percentages:

n=100

VAP Positive		VAP Negative	
f	%	f	%
64	64	36	36

Table 3: Assessment of risk factors responsible for increasing the risk of VAP among positive patients in terms of frequency and percentages

n=64

S.No	Risk factors	Frequency	Percentage %
1.	Endo tracheal catheterization	54	84.375
2.	Duration of stay in the ICUs	60	93.75
3.	Poor nursing practices in certain nursing procedures	56	87.5
4.	Duration of ventilation more than 48 hours	59	92.19
5.	Previous history of Hospitalization	34	53.13
6.	History of cardiac diseases	54	84.38
7.	Previous surgical history.	32	50
8.	COPD	60	93.75
9.	Use of steroid therapy	44	68.75
10.	Organ failure	60	93.75

Discussion

In this study, male patients were more than female patients, 46 % of the patients had the hypertension, 74 % of the patients had the CADs and 74 % of the patient's ABG levels had worsened gradually. Out of 100 patients, 64 patients have developed the VAP.

In present study 92% of the VAP positive patients had stayed in ICU with mechanical ventilation more than 48 to 72 hours. This is also one of the risk factors for the development of VAP. More than 93 % of the COPD and major organ failure patients also had developed VAP. The patient who had the history of cardiac and coronary artery diseases also had shown more significance for the development of VAP. The strict aseptic precautions need to be taken by health care professionals while performing any invasive or noninvasive procedure on patients with MV as in this study showed poor nursing practices also can develop the VAP.

A study conducted by Mevlut Karatas etal results also supporting the current study results, the length of the stay, poor practices during invasive procedures, comorbid disease conditions, recurrent hospitalization, use of prophylactic antibiotic therapy, recurrent intubations increased the development of ventilator associated pneumonia [9].

Conclusion

In this study the patients who had undergone the MV developed VAP significantly with endotracheal tube or

tracheotomy, long hospital stay, associated infections and major organ failures.

Conflict of Interest– Nil

Source of Funding- Self

RRC: Permission was obtained from RRC committee of Symbiosis College of Nursing and also permission was obtained from the hospital where the study was conducted.

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Comparison Postoperative Complications of a Novel Combined Reciprocal/Osteotome Approach (Ghasemzadeh approach) Versus Conventional Rhinoplasty: A Prospective Clinical Trial Study

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Abstract

Objective: This article for the first time described a novel, hybrid approach to internal rhinoplasty by combining a reciprocating saw/osteotome. We compared it with conventional "pure" instrument (osteotomies performed by chisel only) in terms of cutting time and postoperative complications such as ecchymosis, edema, pain and overall patient's satisfaction with surgery.

Materials and methods: We conducted a prospective, randomized, double-blind clinical trial study. To assess variables of the study 60 patients were divided into two groups: 1) 30 patients underwent new approach and 2) 30 patients underwent conventional osteotomies. The outcomes of the study were cutting time in minutes and postoperative edema, ecchymosis and pain and overall patient's satisfaction which were measured at two time points: days 2 and 7 after surgery. Data were analyzed using repeated measure ANOVA test.

Results: The mean cutting time was significantly lower in Ghasemzadeh approach group when compared with usual osteotome group (P value=0.001). On both 2 and 7 days following surgery, ecchymosis (P value=0.001 and =0.033, respectively) and pain (P value= 0.001 and 0.001, respectively) was significantly lower in Ghasemzadeh approach group than in the usual osteotome group. The mean level of edema on postoperative 2 days was lower but not significant in Ghasemzadeh approach group than in the usual osteotome group (P = .09), while it was significant on 7 days (P value=0.001). The patient's satisfaction reported by the Ghasemzadeh approach group was significantly better than usual osteotome group on 2 and 7 days following surgery.

Conclusion: It is concluded that the use of combined reciprocating saw/osteotome in rhinoplasty showed that swelling, pain, ecchymosis and short-term patient satisfaction was improved with this technique.

Keywords: *Rhinoplasty, Reciprocal, Osteotome, Edema, Pain, Ecchymosis, Satisfaction*

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Introduction

Rhinoplasty is considered a popular, efficient and still challenging cosmetic and plastic surgery.[1] Common intraoperative and postoperative complications associated with this procedure are challenges to the surgeons. The cutting time and postoperative complications in the few

days following surgery such as ecchymosis which limits visual fields, and eyelid edema which interferes with social activity are very uncomfortable for the patients.[2] Moreover, it is well confirmed that the soft tissue damage is the main cause of edema and ecchymosis.[3,4]

Although, several approaches namely internal lateral osteotomy and external osteotomy (1) and also various devices including conventional osteotomes (e.g. burrs or chisel), new piezoelectric devices and ultrasound scalpel with its own pros and cons have been used or introduced to decrease soft tissue injury and decrease rhinoplasty related complications.[5] However, there is still a challenge to choose a method and a device which can minimize these complications in rhinoplasty. Soft tissue injury is still frequently observed due to the use of traditional devices during rhinoplasty.[6] Peizoelectrive devices, recently the most studied devices, are a relatively new and efficient for bony craniofacial surgeries in terms of low bone injury, lower risk of soft tissue damage compared with traditional osteotomes.[7] However, several studies reported main disadvantages of piezoelectric devices such as longer cutting time,[8] inadequate cutting power[9] and the lack of visibility[10] which might still be the key obstacles preventing the popularity of the method in rhinoplasty cases. In addition, ultrasound scalpel is rarely evaluated in rhinoplasty.

The current study is the first to introduce a novel, combined approach to internal rhinoplasty by combining a reciprocating saw and conventional osteotome (chisel) to evaluate the most important outcomes in rhinoplasty. The aim of the present prospective and randomized clinical trial study was to compare cutting time and postoperative complications on the use of a novel and combined reciprocating saw /osteotome (namely Ghasemzadeh approach) and traditional pure osteotome during internal rhinoplasty.

Materials and Methods

Study design and sample description

We designed and conducted a multicenter, prospective, randomized, and double-blind clinical trial to achieve the purposes of the research. The study protocol was approved by the Ethics Committee of Arak University of Medical Sciences (Ethical code: IR.ARAKMU.REC.1399.085). In addition, the study received trial registration code from Iranian Registry of Clinical Trials (registration ID: IRCT20170316033094N2 registered in https://irct.ir/). The study followed Helsinki Declaration. The participants composed of patients undergoing for rhinoplasty and met inclusion and exclusion criteria between April 2020 and July 2020. Written informed consent was assigned by all patients. The predictor variables were the devices used to perform the rhinoplasty osteotomy, divided into two groups each one 30 participants: *1)* a combination of reciprocating saw and osteotome (chisel) which we named it Ghasemzadeh approach in which vertical osteotomies were performed using chisel and transverse osteotomies were cut by reciprocal, in patients without nasal hump the medial cutting also performed using reciprocal, and 2) conventional "pure" osteotome (chisel) in which all vertical, transverse and medial osteotomies performed by chisel. The study was conducted at two centers including Apadana Hospital (included 15 patients in novel group and 15 patients in conventional group) at Ahvaz and Day Limited Surgery Center (included 15 patients in novel group and 15 patients in conventional group) affiliated to Arak University of Medical Sciences at Arak. The inclusion criteria were the age between 18 to 50 years, no anesthesia contraindication (American Society of Anesthesiologists status I and II), and no serious pulmonary airway malformation such as septal deviation or breathing dysfunction. Patients were excluded if they had neuropathic disease, prolonged use of nonsteroidal anti-inflammatory drugs (NSADs) and opioid-derived medications, a history of medication-induced allergic reactions or declined to participate in the study. The diagram of patient's participations is shown in Figure 1.

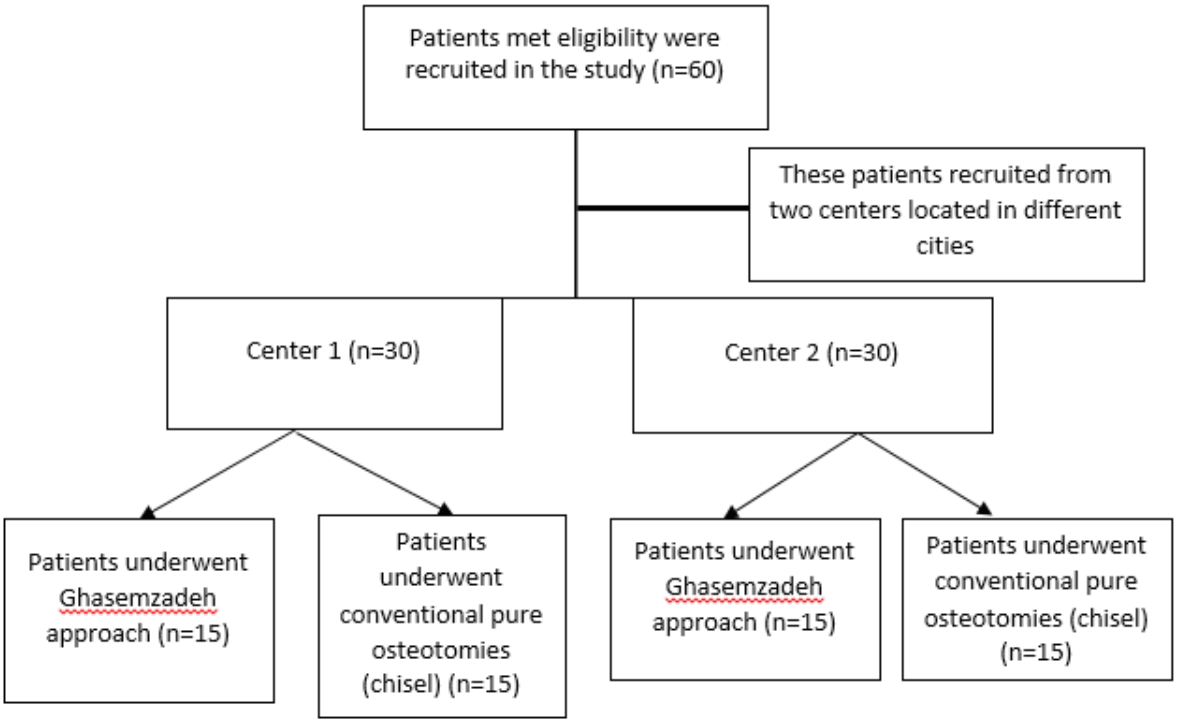

Figure 1. Diagram of patients' participation.

Surgery description

All operations were performed by two surgeons (in two centers) under general anesthesia. Procedures began as a standard open rhinoplasty by a transcolumellar incision with the bilateral regular marginal incision. This was performed in a same manner for both groups of Ghasemzadeh approach and conventional osteotome. Whereas, in Ghasemzadeh approach a reciprocating saw with a blade 0.35 mm thick and 10 mm long (Model SGR-1, Japan) (Figure 2) was used to perform transversal osteotomy and a chisel (black and black surgical, Inc., Atlanta) was used to perform vertical osteotomy. A sample of application of reciprocal during transverse cutting is represented in Figure 3. Transverse osteotomy performed by reciprocal saw is shown in Figure 4. Sharp and symmetric transversal cutting osteotomy in CT scan view (Figure 5(.

Figure 2. A reciprocal saw has been used in current study.

Figure 3. Using Reciprocal saw during transverse cutting.

Figure 4. Transverse osteotomy performed by reciprocal saw.

Figure 5. Sharp and symmetric transversal cutting osteotomy in CT scan view.

Some medications were prescribed for all patients after surgery for 7 days (Cephalexin 500 mg per 6 hours and Acetaminophen 325 mg and codeine 10 mg per 6 hours).

Statistical Analysis

Data were analyzed using SPSS software. The mean cutting time was compared between two groups and two surgeons using ANCOVA test. The mean ecchymosis, edema, pain and overall patient's satisfaction were compared between two groups using repeated measure ANOVA test.

Results

In this study, 60 patients were included and underwent internal rhinoplasty and all participants were women. The mean age of the patients was 27.7 ± 7.6 years (range, 18 to 46 years) at the time of surgery.

Cutting time

The comparing mean cutting time between the two groups and two centers is shown in Table 1. In center 1 with experienced surgeon, the mean cutting time was significantly lower in the Ghasemzadeh approach group when compared with usual osteotome group (P value=0.001), in addition it was also significantly lower in center 2 another surgeon in the Ghasemzadeh approach compared with osteotome device group (P value= 0.04).Whereas the mean cutting time did not differ significantly between two centers (surgeons) in terms of Ghasemzadeh approach (P value=0.3) (Table 1). The mean cutting time was significantly higher for experienced surgeon when compared with less experienced surgeon in terms of conventinal osteotome group (P value=0.006) (Table 1).

Table 1. Comparing mean cutting time of rhinoplasty between surgeons and two groups (Ghasemzadeh approach and conventional rhinoplasty).

	Osteotome	Ghasemzadeh approach	P value
Center 1, minutes	5.5 ± 0.8	4.3 ± 0.9	0.001
Center 2, minutes	4.8 ± 0.5	4.5 ± 0.1	0.04
P value	0.006	0.3	
The results were presented based on ANCOVA test.			

Postoperative Outcomes

The post-operative outcomes on days 2 and 7 after surgery was shown in Table 2.

Table 2. Repeated measures ANOVA test investigating comparisons between variables on 2 and 7 days following rhinoplasty.

	Conventional	Ghasemzadeh approach	P value
Ecchymosis			
At 2 days	2.45 ± 0.6	1.4 ± 0.7	0.001
At 7 days	1.3 ± 0.6	0.5 ± 0.6	0.033
Edema			
At 2 days	2.43 ± 0.5	2.07 ± 0.6	0.09
At 7 days	1.5 ± 0.5	1.07 ± 0.2	0.001
Pain			
At 2 days	3.5 ± 1.6	1.6 ± 1.7	0.001
At 7 days	1.43 ± 1.7	0.3 ± 0.6	0.001
Overall patient satisfaction			
At 2 days	2.8 ± 0.7	1.9 ± 0.7	<0.001
At 7 days	1.9 ± 0.4	1.1 ± 0.3	<0.001

Discussion

This prospective multicenter clinical trial revealed that Ghasemzadeh approach (combined reciprocating saw/osteotome) has superiority over pure osteotome instrument on cutting time and also postoperative parameters such as eyelid edema on 7 days after surgery, ecchymosis, pain and overall patient's satisfaction in rhinoplasty during first week following surgery.

To the best of our knowledge this research is the first to introduce a novel, hybrid approach in internal lateral rhinoplasty by combining a reciprocating saw and conventional osteotome device (chisel) with several advantages over pure osteotome (chisel only) in terms of predictable, precise, rapid and easier osteotomies,

decreased postoperative complications including edema, ecchymosis, pain, and increased overall patient's satisfaction.

Several methods have been introduced to reduce the complicatins of rhinoplasty and achieve better esthetic results.[11,12]

The most important observation and the main advantages of reciprocating saw in the present study is that swelling, pain, ecchymosis and short-term patient satisfaction was improved with this technique, there were no spicules on the cut site, therefore fracture lines are very sharp and finally led to perfect bilateral symmetry. The sharp osteotomies of reciprocating saw on medial and transverse cuts in turn minimize the probability of curved deformities. Moreover, reciprocating saw allowed good visualization of anatomy and also had adequate power of cut eliminating pressure applied by surgeon hand and consequent complications such as unpleasant fractured instruments. Whereas, the disadvantages of the lack of anatomical visibility and inadequate power cut are frequently reported for piezosuregy, the most recently suggested novel technique in rhinoplasty.[9,10]

The cutting time in the present study was significantly shorter in combined approach compared with pure osteotome. In a study by Demirbas et al on the rapidity of cutting time with ultrasonic bone scalpel found that the its cutting time is comparable with reciprocating saw due their similar cutting surface area.[9] A systematic review study has showed that articles mostly reported that the duration of cutting time performed by piezoelectric surgery was longer than that of osteotomies made with a reciprocal saw.[13] Lower operation time is associated with reduces probability of complications due to general anesthesia. The safety of airway management and successfully cardiopulmonary regulation are directly related to the shorter operating time. It has been reported that the amount of blood loss increases by 18% when the operating time prolonged over three hours in craniofacial surgery.[14-18] On the other hand soft tissue injury and longer operation time affect postoperative edema.[9]

We also compared the average eyelid edema between two groups on day 2 (P value=0.9) and on day 7 (P value=0.001) following surgery at present study. The mean eyelid edema on day 7 in combined group was significantly lower than pure osteotome. Postoperative edema is the most frequently reported complication after rhinoplasty. It causes social disturbing and frightening, and it is also much discomfort for the patient.[8,19,20] Rena et al in the study showed that the intense of swelling in patients undergoing SARPE (Surgically Assisted Rapid Palatal Expansion) surgery was comparable between two groups of piezosurgery and reciprocal and it was disappeared quickly in both groups.[21]

Our findings showed that patients undergoing Ghasemzadeh approach experienced significantly less pain and better satisfaction compared with conventional method within a week following surgery. Kiyak et al., in orthognathic surgery reported that pain and satisfaction has strong direct association.[22,23,24] In another study in SARPE surgery has been shown that pain and satisfaction were correlated with each other but not with level of edema.[8]

The strength of the present study included prospective, double blind, clinical, a multicenter, and a relatively larger sample size. In addition, according the findings of current study, the reduced complications and cutting time observed Ghasemzadeh approach owing the effectiveness of this approach. Further studies with a similar methodology and also another microscopic researches are necessary to assess the safety and effectiveness of suggested novel approach. The main weakness of our study was the lack of similar data to compare and support our findings.

It is concluded that the use of combined reciprocating saw/osteotome in rhinoplasty showed that swelling, pain, ecchymosis and short-term patient satisfaction was improved with this technique. However, there is lack of similar studies to compare our findings due to our study is the first in introducing this novel combined approach (reciprocal saw and osteotome). Therefore, more researches are necessary to evaluate this approach.

Conflict of Interest: None

Institution to Which the Work should be Attributed
Arak University of Medical Sciences, Arak, Iran.

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None of the authors has a financial interest in any of the devices, or drugs mentioned in this research.

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Comparison of the Effect of Non-Surgical Periodontal Therapy on Periodontal Health Status and Glycemic Control between Diabetes Mellitus Type 1 and Type 2

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Abstract

Objectives. This study aims to compare the results of non-surgical periodontal therapy on the periodontal health status and glycemic control between diabetes mellitus type 1 (T1DM) and type 2 (T2DM).

Methods. A total of 24 adult patients participated in this study. Patients divided into two groups, 12 patients are diagnosed with T1DM, and 12 diabetic patients with T2DM. After 3 months non-surgical periodontal treatment, the following parameters were investigated at baseline: glycosylated hemoglobin (HbA1c), plaque index (PI), bleeding on probing (BOP), gingival recession (GR), probing pocket depth (PPD), and clinical attachment level (CAL). After baseline investigation, both groups received one stage full mouth scaling and root planning, combined with systemic Doxycycline, statistical analysis was performed by Student T-test.

Results. By comparing between T1DM and T2DM at base line, and after 3 months non-surgical periodontal therapy showed generally no significant differences with respect to periodontal parameters, while HbA1c values were always significantly higher in T1DM. By comparing of each parameter before- and 3 months after non-surgical periodontal therapy with relation to the type of diabetes mellitus showed reduction of gingival inflammation (PI, BOP), but there are no significant differences of probing pocket depth in both diabetic groups, glycemic control level HbA1c showed significant reduction only in diabetic patients Type 2.

Conclusion. Results of the present study showed that non-surgical periodontal therapy in both diabetes types decreased gingival inflammation, no changes in periodontal pocket depth, but reduced glycemic control significantly only on type 2 diabetes mellitus.

Keywords : *diabetes mellitus, glycosylated hemoglobin (HbA1c), non-surgical periodontal therapy , periodontitis.*

Introduction

The relationship between periodontal disease and diabetes mellitus (DM) has been increasingly investigated over the past decades. A large body of evidence suggests that patients with diabetes are at an increased risk of developing periodontal disease. Periodontal disease is considered to be a significant complication of diabetes [(1)], many studies provide evidence that poor glycemic control contributing to poor periodontal health [(2, 3)].

A case-control study showed that a periodontal disease can start very early in life of patients with diabetes mellitus type 1 (T1DM) [(4)]. Patients with T1DM had significantly more plaque and gingival inflammation and presented more sites with BOP by comparing with control subjects [(5)]. Patients with generalized chronic periodontitis and type 2 diabetes (HbA1c $\geq$ 8%) demonstrated increased levels and frequencies of periodontal pathogens in the subgingival plaque [(6)]. Participants with diabetes had worse oral health, more severe caries experience, more attachment loss and significantly higher prevalence of periodontitis compared to those without diabetes [(7)].

On the other hand, substantial evidence demonstrated that periodontal disease has an adverse effect on glycemic control [1, 8]. Treatment studies can provide evidence regarding the effects of periodontal disease on glycemic control [9].

Many studies include RCTs reported a beneficial effect of periodontal therapy on glycemic control [10, 11, 12], other studies did not found any effect of periodontal treatment on glycemic control [13, 14].

This variation may be related to the use of antibiotics with non-surgical periodontal therapy, and may be related to the type of diabetes mellitus, with respect to physiopathology, two main types of diabetes mellitus recognized, type 1 (T1DM) is an autoimmune disorder with destruction of pancreatic β cells and thus leads to loss of insulin secretion, and type 2 (T2DM) is linked to insulin resistance [15].

Most of the researchers' investigating impacts of non-surgical periodontal therapy on T2DM, some of them on T1DM, however, this study aims to compare the results of 3 months non-surgical periodontal therapy on periodontal health status and glycemic control (HbA1c) between T1DM and T2DM.

Materials and Methods

Study population

Twenty-four adult patients from Center for Diabetes and Endocrinology in Al-Amarah city, Misan governorate (Iraq) divided into two groups, 12 patients are diagnosed with type 1 diabetes mellitus, and 12 diabetic patients with type 2 were enrolled in this study.

Inclusion criteria:

- Diabetes mellitus diagnosed at least since 1 year.
- DM Patients were under the care of physician for DM management.
- Not undergone any periodontal treatment since last 6 months.
- Individuals having >14 teeth present
- 18-60- year-aged patients .

Exclusion criteria

- Pregnant and lactating women.
- Patients using antibiotics or anti-inflammatory drugs for the previous 4 weeks
- Having any other systemic diseases.
- Having any diabetic complications.
- Using drugs such as phenytoin. Nephidipine, ….

Diabetes parameters

DM history recorded based on the information provided by the physician and it includes date of diagnosis and duration. Metabolic control of diabetes determined by the value of glycosylated haemoglobin (HbA1c) at the beginning and 3 months after periodontal treatment.

Periodontal parameters

Periodontal examinations of all participants obtained at the beginning and 3 months after treatment under standardized conditions with UNC-15 periodontal probe by the same examiner.

Plaque index of Silness and Loe (PI) was recorded on the buccal, mesial, distal and lingual surfaces of all teeth except for third molars, the values of 4 sites of each tooth were averaged to determine the score for each subject. Bleeding on probing (BOP) estimated as a dot of blood at the base of the pocket or crevice after 30 seconds of gentle probing with the periodontal probe and calculated as a percentage of all bleeding sites.

Probing pocket depth (PPD) measured on every tooth (excluding the remaining roots and third molars), on six points from the gingival margin to the base of the pocket or crevice. Gingival recession (GR) measured from the cementoenamel junction to the margin of the gingiva. Clinical attachment level (CAL) is calculated as a mathematical sum of PPD and GR.

Periodontal treatment

After initial investigations, both groups received the following periodontal treatment:

- One stage full mouth scaling and root planning with ultrasonic device and Gracey curettes.

- Chlorhexidine mouth wash 0.12 prescribed 10 ml twice daily for 5 days.

- Systemic Doxycyclin 100 mg for 9 days.

- Oral hygiene instruction.

- Statistical analysis was done by Student T-test. The data were analyzed by SPSS, V25.0.

Ethical considerations

All participants were informed about the nature of the study and signed informed consent. The research protocol was approved by the Ethics Committee of Al-Manara College of Medical Sciences.

Results

As shown in Table 1, the comparison between T1DM and T2DM values at baseline showed no significant differences with respect to periodontal indices (PI, BOP, PPD, CAL), while T1DM subjects had a significantly higher mean HbA1c (Type 1: 10.56 , Type 2: 7.91). Generally, the same results registered after 3 months of non-surgical periodontal treatment (table 2).

Comparison of each parameter before- and 3 months after non-surgical periodontal therapy in relation to the type of diabetes mellitus showed reduction of gingival inflammation (PI, BOP), but no significant differences of pocket depth in both diabetic groups, while clinical attachment level showed reduction only in diabetic Type 1 patients. Moreover, glycemic control level HbA1c showed significant reduction only in diabetic patients Type 2 (table 3 & 4).

Table 1 . PI, BOP, PPD, CAL and HbA1c means and standard deviation between type 1 and type 2 diabetic patients at baseline before non-surgical periodontal therapy.

Indices at Baseline	Group	Number	Mean	Standard deviation	P-Value	Statistical significance
PI	T1DM	12	1.78	.411	0.086	NS P>0.05
	T2DM	12	2.09	.415		
BOP %	T1DM	12	64.33	13.07	0.642	NS P>0.05
	T2DM	12	61.67	14.581		
PPD	T1DM	12	2.99	.585	0.284	NS P>0.05
	T2DM	12	2.73	.582		
CAL	T1DM	12	6.89	1.277	0.727	NS P>0.05
	T2DM	12	7.06	1.012		
HbA1c	T1DM	12	10.56	2.892	0.020	S P<0.05
	T2DM	12	7.91	2.265		

Table 2. PI, BOP, PPD, CAL and HbA1c means and standard deviation between type 1 and type 2 diabetic patients 3 months after non-surgical periodontal therapy.

Indices After 3 months	Group	Number	Mean	Standard deviation	P-Value	Statistical significance
PI	T1DM	12	1.38	.184	0.031	S P<0.05
	T2DM	12	1.62	.303		
BOP %	T1DM	12	45	10.42	0.493	NS P>0.05
	T2DM	12	48.67	14.963		
PPD	T1DM	12	3.35	1.707	0.231	NS P>0.05
	T2DM	12	2.71	.595		
CAL	T1DM	12	6.74	1.144	0.316	NS P>0.05
	T2DM	12	6.24	1.241		
HbA1c	T1DM	12	9.79	2.307	0.002	S P<0.05
	T2DM	12	6.90	1.532		

Table 3. PI, BOP, PPD, CAL and HbA1c means and standard deviation before and after non-surgical periodontal therapy (type 1 diabetes group).

Type 1 Diabetes		Number	Mean	Standard deviation	P-Value	Statistical significance
Pair 1	PI at Baseline	12	1.78	.411	0.003	S P<0.05
	PI after 3 months	12	1.38	.184		
Pair 2	BOP at Baseline	12	64.33	13.076	0.001	S P<0.05
	BOP after 3 months	12	45	10.427		
Pair 3	PPD at Baseline	12	2.99	.585	0.414	NS P>0.05
	PPD after 3 months	12	3.35	1.706		
Pair 4	CAL at Baseline	12	6.89	1.274	0.047	S P<0.05
	CAL after 3 months	12	6.74	1.177		
Pair 5	HbA1c at Baseline	12	10.56	2.873	0.287	NS P>0.05
	HbA1c after 3 months	12	9.79	2.388		

Table 4 . PI, BOP, PPD, CAL and HbA1c means and standard deviation before and after non-surgical periodontal therapy (type 2 diabetes group).

Type 2 Diabetes		Number	Mean	Standard deviation	P- Value	Statistical significance
Pair 1	PI at Baseline	12	2.09	.462	0.0001	S P<0.05
	PI after 3 months	12	1.62	.300		
Pair 2	BOP at Baseline	12	61.67	14.581	0.0001	S P<0.05
	BOP after 3 months	12	48.67	14.963		
Pair 3	PPD at Baseline	12	2.73	.582	0.639	NS P>0.05
	PPD after 3 months	12	2.71	.596		
Pair 4	CAL at Baseline	12	7.065	1.012	0.122	NS P>0.05
	CAL after 3 months	12	6.24	1.291		
Pair 5	HbA1c at Baseline	12	7.91	2.260	0.009	S P<0.05
	HbA1c after 3 months	12	6.90	1.526		

Discussion

The main purpose of this study is to assess the effects of periodontal disease treatment on the level of glycemic control of diabetes mellitus. According to present clinical study scaling and root planning combined with Doxycycline leads in both diabetic types to improvement in gingival inflammation (PI and BOP) without any changes in periodontal pocket depth, glycemic control level (HbA1c) showed significant reduction only in diabetic patients Type 2.

Pranckevicienea A. et al 2014 [16] found that periodontal parameters were significantly higher among patients with type 2 diabetes patients than in patients with type 1 diabetes, while in this study periodontal indices at baseline showed no differences between T1DM subjects and T2DM subjects, this difference may be related to the limited number of the present study population.

Kanduluru A. and Naganandini S. 2014 [17] suggested that there were no significant differences of glycemic control (HbA1c) after 3 months non-surgical periodontal treatment for diabetic patients type 2 in comparing with control group. The present study compared T1DM with T2DM and found significant improvement of glycemic control only on type 2 diabetes mellitus. Gaikwad SP et al 2013 [18] found also HbA1c improvement in patients with T2DM after scaling and root planning, but without any benefit observed with adjunctive systemic doxycycline therapy. Lee JY et al 2020 [19] found non-surgical periodontal treatment effective in reducing HbA1c in T2DM patients, additional professional tooth brushing had a more favourable effect on gingival bleeding. A result of several systematic reviews also supported that non-surgical periodontal treatment lowering HbA1c level of T2DM patients [20, 21, 22].

A little published about T1DM and periodontal treatment, the results of Buzinin et al 2014 [23] were similar to the present study that there is no significant improvement in the level of HbA1c after non-surgical periodontal treatment in patients with type 1 diabetes mellitus, the advantages of this study are the comparison of the results of non-surgical periodontal treatment between T1DM and T2DM .

Conclusion

Within the limits of the present study, the results showed that non-surgical periodontal therapy in both diabetes types decreased gingival inflammation, no changes in periodontal pocket depth, but reduced glycemic control significantly only on type 2 diabetes mellitus not in type 1. However, further studies with a larger number of patients are required.

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The Effect of Commercial Toothpaste Containing Aloe Vera on Dental Plaque and Gingivitis: A Double-Blind Randomized Clinical Trial

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Abstract

Objectives: Aloe vera is a medical plant which has the greater medicinal value and enormous properties for curing and preventing gingivitis and oral diseases. This study aims to evaluate the effect of a commercial toothpaste containing Aloe vera on the reduction of plaque and gingivitis.

Material and methods: 24 patients diagnosed with generalized gingivitis, randomly divided into 2 groups, patients in the test group (n=12) used toothpaste containing Aloe vera, and patients in the control group (n=12) used toothpaste containing fluoride. Plaque Index (PI), Gingival Index (GI), and Bleeding on Probing (BOP) were assessed at days 0 and 30. Subjects were asked to brush their teeth with the stated toothpaste, twice a day, for 30 days. the data on toothpaste tubes were masked for both patients and examiner.

Results: All clinical parameters showed a significant reduction on 30th day in both test and control groups, but no significant differences between groups.

Conclusion: The results of the present study indicate that commercial toothpaste containing Aloe vera did not show an additional effect on plaque and gingivitis compared to the control fluoridated toothpaste.

Key Words: *Aloe vera, Toothpaste, Dental Plaque, Gingivitis.*

Introduction

Plaque-induced gingivitis is one of the most frequent periodontal diseases, affecting the majority of the population. The results of a study among the adult Saudi population showed a 100% prevalence of gingivitis [(1)]. A study on oral health status among fifteen-years-old students in Maysan governorate\Iraq showed a high prevalence of dental caries and gingivitis [(2)]. Gingivitis may turn into periodontitis characterized by pocket formation, alveolar bone destruction, and loss of clinical attachment level [(3)].

For effective plaque control, several mechanical oral hygiene aids as well as several anti-plaque agents are available. The mechanical plaque control is mainly achieved through tooth brushing, flossing, and supragingival irrigation. The chemical control of plaque inhibits the accumulation, growth, and survival of microbiota [(4)], however, the inability of the normal adult population to perform adequate toothbrushing has led to the search for chemotherapeutic agents to improve plaque control, these chemicals, mainly triclosan, amine fluoride and a chlorhexidine [(5, 6)].

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Nowadays many patients prefer using herbal preparations which are efficient without causing side effects such as tooth staining and taste alteration. herbs like Terminalia chebula, Punica granatum Linn, Centella Asiatica, Punica granatum, carvacrol, thymol, Aloe vera, Azadirachta indica, piper betel, Ocimum sanctum possess different biological properties [7, 8, 9, 10, 11].

Aloe vera is a succulent, cactus-like plant, the Aloe leaf consisting of two major parts, the outer green rind, and the inner colourless parenchyma containing the aloe pulp (gel), the chemical composition of Aloe vera leaf pulp is very complex, consisting of over 75 active ingredients including minerals, enzymes, carbohydrates, athraquinones, lipids, vitamins, steroids, amino acids, and salicylic acid. Aloe vera is the most commercialized aloe species, it has been used in the food industry, cosmetic industry, and pharmaceutical industry, it has been reported in new pharmacological data research that Aloe vera leaf gel has many biological activities such as immunostimulation, anti-inflammatory effects, wound healing, promotion of radiation damage repair, anti-bacterial, anti-viral, anti-fungal, skin and digestive protective activity, and anti-cancer action [12, 13, 14].

Some published studies reported the use of Aloe vera in dentistry for various purposes such as disinfecting dental unit water network [15], gutta-percha disinfection [16], the antiseptic effect on candida Albicans [17], aphthous stomatitis treatment [18], as an ingredient in toothpaste [19] and mouthwash [20] for gingivitis treatment, adjunctive treatment with scaling and root planning for chronic periodontitis [21], cavity disinfectant to minimizing secondary caries [22], preprocedural rinse in reducing aerosol contamination during ultrasonic scaling [23] and a local drug delivery system in periodontal pockets [24].

The results of studies about the effects of a dentifrice containing Aloe vera on gingivitis and plaque accumulation are controversial and inconclusive. A clinical and microbiologic study using toothpaste containing aloe vera showed improvement in plaque and gingival status comparable to those achieved with toothpaste containing triclosan [25], A systematic review and meta-analysis about herbal oral care found that herbal toothpaste was superior over non-herbal toothpaste in plaque reduction [26], In other studies using toothpaste containing Aloe vera showed no additional effect on plaque and gingivitis compared to a control toothpaste [19, 27].

To the present date, there is a few reported controlled trial evaluating the efficacy of a commercial toothpaste containing Aloe vera in the control of plaque and gingivitis. Hence this study intended to clinically evaluate the effect of commercial toothpaste containing Aloe vera on dental plaque and gingivitis as compared to control fluoridated toothpaste.

Material and Methods

Study population

Twenty four adult patients from Maysan governorate (Iraq) (12 male and 12 female, aged 18 to 40) diagnosed with generalized chronic gingivitis were enrolled in this controlled randomized, double-blind, clinical study. Patients were randomly divided into 2 groups: test group using Aleo vera containing toothpaste, and control group using over the counter fluoridated toothpaste with no anti-inflammatory properties.

Inclusion criteria**:** Bleeding on Probing index $\geq$ 30%, presence of at least 20 natural teeth, no signs of periodontitis.

Exclusion criteria: a history of allergy to Aloe vera or its products, tobacco use, participants undergoing orthodontic treatment, pregnant women excluded from the trial, systemic diseases, any periodontal treatment during six months before the study, and using antibiotic or antimicrobial mouthwash since 6 months.

Clinical parameters:

All the patients were examined from one examiner for the Plaque index of Silness and Loe (PI), Gingival index (GI), and presence of Bleeding on Probing (BOP) at baseline and after 30 days.

PI and GI were recorded on the buccal, mesial, distal and lingual surfaces of all teeth except for third molars, the values of 4 sites of each tooth were averaged to determine the score for each subject, a score of 0 to 3 was assigned to measure PI and GI.

Bleeding on Probing (BOP) was done through the gentle probing of the orifice of the gingival crevice if bleeding occurs within 20 seconds a positive finding

is recorded, and the number of positive sites is then expressed as a percentage of the number of sites examined [28].

Toothbrushing

Participants were instructed to brush with control or test toothpaste for 2 min. twice daily, during 30 days, using familiar technology, and to refrain from any other oral hygiene procedures throughout the clinical trial.

After an initial examination, a personal kit containing a new toothbrush, and the test or control toothpaste was given to all patients. Verbal instruction about the correct use of toothpaste was given to all patients.

The tubes containing the toothpaste were previously masked and color-coded to warrant that neither the examiner nor the patient knew their content, which was revealed only after completion of the study,

The Participants were asked to return their toothpaste tubes, that were weighted by a digital balance previously and after the trial, a telephone call was done every week to each participant, T-test was used to evaluate the statistical differences between weights of toothpaste tubes on day 0 and 30 so that compliance could be indirectly evaluated, statistical analysis was done by Student T-test.

Ethical approval and informed consent:

All participants were informed about the nature of the study and signed informed consent. The research protocol was approved by the ethics committee of Al-Manara College of Medical Sciences.

Results

The test toothpaste had a good acceptance and did not show adverse effects, such as the formation of abscess and ulcerations or allergic reactions. There was a significant reduction of toothpaste tube weights between days 0 and 30 in both groups which indicates that the patients have used it.

On day 0, there was no statistically significant difference between the control and test groups concerning GI and PI, and BOP means These results indicated that both groups were well balanced at baseline.

Comparing the means between the baseline and day 30 for the test group (Aloe vera), there were statistically significant differences in reducing the plaque index, gingival index, and bleeding on probing (Table 1).

Table 1: Gingival Index(GI), Plaque index (PI) and Bleeding on probing (BOP) means and standard deviation on day 0 and day 30 for the test group (Aloe vera).

Aloe vera group		MEAN	Number	Standard deviation	P-Value	Statistical significance
Pair 1	PI (BASELINE)	1.06	12	0.349	0.0001	S P<0.05
	PI (Day 30)	0.32	12	0.114		
Pair 2	GI (BASELINE)	0.69	12	0.431	0.0001	S P<0.05
	GI (Day 30)	0.09	12	0.078		
Pair 3	BOP (BASELINE)	45.45	12	37.977	0.003	S P<0.05
	BOP (Day 30)	8.80	12	10.353		

Comparing the means between the baseline and day 30 for the control group, there were statistically significant differences in reducing the plaque index, gingival index, and bleeding on probing (Table 2).

Table 2: Gingival Index(GI), Plaque index (PI) and Bleeding on probing (BOP) means and standard deviation on day 0 and day 30 for the control group

Control group		Mean	Number	Standard deviation	P-Value	Statistical significance
Pair 1	PI (BASELINE)	0.91	12	0.757	0.011	S P<0.05
	PI (Day 30)	0.40	12	0.505		
Pair 2	GI (BASELINE)	0.60	12	0.492	0.006	S P<0.05
	GI (Day 30)	0.27	12	0.380		
Pair 3	BOP (BASELINE)	51.00	12	44.908	0.003	S P<0.05
	BOP (Day 30)	18.22	12	23.323		

Comparing the means between the test and control group after 30 days, although the mean numbers always better for the Aloe vera group, there were no statistically significant differences in reducing the plaque index, gingival index, and bleeding on probing (Table 3).

Table 3: The differences between the test and control groups after one month.

	Group	Number	Mean	Standard deviation	P-Value	Statistical difference
PI (Day 30)	Control	12	0.40	0.505	.5930	NS P>0.05
	Test	12	0.32	0.114		
GI (Day 30)	Control	12	0.27	0.380	.1420	NS P>0.05
	Test	12	0.09	0.078		
BOP (Day 30)	Control	12	18.22	23.323	.2240	NS P>0.05
	Test	12	8.80	10.353		

Discussion

The purpose of this clinical trial to assess the efficacy of Aloe vera commercial toothpaste in preventing plaque accumulation and gingival inflammation. According to this study, clinical improvement of plaque and gingival indices was observed in both test and control groups, although the Aloe vera group showed a considerably better improvement (mean bleeding points after 30 days: test: 8,80%, control 18,22% . mean plaque index after 30 days: test 0.11, control 0.50) so the differences between both groups statically was not significant.

Home-use dentifrice studies are often influenced by several factors which can mask the differences between test agents and controls, to reduce these factors the patients of the present study were not aware of which toothpaste they were using, the toothpaste tubes were weighted by a digital balance previously and after the trial, and a telephone call was done every week to each patient.

The literature review didn`t give the dentists conclusive evidence about the effectiveness of toothpaste containing Aloe vera to control plaque and gingivitis. Some studies like this study concluded that toothpaste containing Aloe vera showed no additional effect on plaque accumulation and gingivitis compared to a control toothpaste [19, 29]. A systematic review suggested that even though there are some promising results, the clinical effectiveness of Aloe vera herbal dentifrices is not sufficiently defined at present [30].

Other studies concluded that toothpaste containing Aloe vera may be useful for improvement in plaque control and gingival status [25,31].

There is more interest using Aloe vera as mouthwash with more clear evidence about its effectivity on plaque control and gingivitis [32, 33], a recently published systematic review suggests that Aloe vera mouthwash is effective in reducing plaque and gingival inflammation, but less than chlorhexidine in reducing dental plaque, moreover, the results showed that Aloe vera was safe and well-tolerated by the patients, with no/ or minimal adverse effects as compared to chlorhexidine [34].

Despite some studies followed participants for more than one month, further long-term studies must be performed to evaluate the antigingivitis effect of this herbal toothpaste, if its real benefit is confirmed, the use of Aloe vera should be advantageous in cases where patients have little motor skills and toothbrushing is compromised. a comprehensive study with different density of Aloe vera could be done in the future.

Conclusion

Within the limits of this clinical study, it may be concluded that both commercial toothpaste containing Aloe vera and fluoridated toothpaste show a significant reduction on plaque and gingivitis after 30 days of use, but toothpaste containing Aloe vera did not show an additional effect on plaque and gingivitis compared to the control fluoridated toothpaste.

Conflict of Interest: The authors declare there is no conflict of interest, and during this research, there is not any financial support or private connections to pharmaceutical companies.

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Toxicopathological Effects of Zinc Oxide Nanoparticles on the Liver Function and Preventive Role of Silymarin *In vivo*

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Abstract

The zinc oxide nanoparticles (ZnONPs) are used in cosmetics and skincare products, and as drug delivery systems. Because of their natural and therapeutic dangers, high attention was given to the toxicity of nanoparticles. This experiment was designed to study the pathological influences of ZnONPs on the liver function parameters of rats, and to explore the protective action of Silymarin (SIL) against liver function disturbances of rats induced by ZnONPs. In our study, (24) albino rats were divided into (4) groups, (6) rats for each and used. The control animals included rats without treatment, while the ZnONPs group rats received 50 mg/kg of ZnONPs for 28 days. In the ZnONPs+SIL group, rats were co-administered with a combination of ZnONPs at a 50mg/kg dose with SIL at a 100 mg/kg dose for 28 days. The SIL group animals were treated with 100 mg/kg of SIL for 28 days. All the animals were sacrificed and blood samples were obtained by cardiac puncture in the end of the experiments. The levels of biochemical parameters (hepatic enzymes) including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured to detect liver function disorders. The results revealed that ZnONPs induced a significant increase in plasma enzyme activity in comparison to the control group ($p<0.05$). Co-treatment of SIL significantly reduced levels of these enzyme activities. Based on these results, it can be said that Silymarin effectively protects against ZnONPs induced liver function disorders in rats.

Keywords*: Nanoparticles, skincare products, liver function, Silymarin.*

Introduction

It was expected that applying nanotechnology in medicine would have a revolutionary effect on healthcare [1]. Nanoparticles (NPs); 1-100nm; are used in several bioengineering, biomedical and optical field applications [2,3] . The increased usage of nanomaterials (NMs) in the consumer products raised concerns about their highly risks for the consumers, workers and for the environment [4]. The nano forms of various particles were shown to be more toxic than their micro-counterparts after acute exposure through oral route in the previous studies [5]. From a a toxicologic biomedical point of view, the toxicity of nanoparticles reveals the reaction between their physicochemical properties and their natural effects [6].

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The zinc oxide nanoparticles (ZnONPs) are typical metal oxide nanoparticles utilized in different cosmetological, medical and chemical applications . ZnONPs possess in vivo and in vitro toxic effects including cytotoxicity, genotoxicity and oxidative stress and therefore, exposure to these particles must be taken into account and controlled precisely [7-12]. Thus, today any new information on their toxic impacts could have important public health effects[13]. Liver disorders are the major mortality and morbidity causes in the world [14].

Natural plant drugs are regarded potential effective medicines and safe alternative for liver disease treatment, and their employment is increasing in the world. Silymarin (SIL), is an active compound found in milk thistle plant and this lipophilic fruit extract is formed of various isomer flavonolignans [15,16]. It is widely used for a long period of time as a treatment for liver and gallbladder diseases such as hepatitis, cirrhosis

& jaundice and to protect the liver against poisoning from chemical and environmental toxins [17,18]. Besides, it has an anti inflammatory antioxidant, anti proliferative and immunomodulatory impact on human being's health [19,20].

This study aims to confirm the toxic pathological effect of ZnONPs on liver function and to assess the role of Silymarin inhibiting or preventing alterations induced by ZnONPs in the liver function organ.

Materials and Methods

Chemicals

Zinc Oxide Nanopowder was purchased from US Research Nano materials, Inc. Company (Houston, TX USA). CAS NO: 1314-13-2, Particle Average Size: 10-30 nm, nearly spherical, Specific Surface Area: 20-60 m2/g, Purity: 99+%), white color, single crystal. Powdered ZnONPs were dispersed in the fresh sterilized physiological saline solution. Silymarin was obtained as a powder (Silymarin-Hypoallergenic Supplement with Concentrated Milk Thistle Extract for Liver Support) from Pure Encapsulations Company, and dissolved in normal saline. Both drugs were administered orally through gastric gavage.

Experimental Animals

In this study, (24) adult male albino rats aged between 16-24 weeks and weighing 175-225 gm, were used. They were housed in plastic cages under the quality laboratory condition 24±1°C, 12 hrs. light / dark cycles, with conventional ventilation. The rats were adapted to the laboratory condition for one week before the outset of the experiments. They had ad libitum access for feeding and and water drinking. The rats were divided into four groups, with six rats placed in each group. The study was designed as follows:

- Group (Control): Healthy rats, did not receive treatment.

- Group (ZnONPs): Rats were administered zinc oxide nanoparticles at 50 mg /kg dose [21] for 28 days.

- Group (ZnONPs + SIL): Rats were co-administered with a combination of zinc oxide nano-

particles with silymarin at 100 mg/kg dose [22], for 28 days.

- Group IV (SIL): Rats were taken silymarin at 100 mg/kg dose alone, for 28 days.

Biochemical Analysis

For biochemical investigation purposes, the blood samples were taken by cardiac puncturing of the animals. The serum enzymes including alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were measured using diagnostic kits from (Roche) company in an automatic machine.

Statistical Analysis

The data were expressed as the mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) was used for statistical evaluation between the groups. Post-hoc comparisons were done using the Duncan test. A significant difference was considered at a P-value of < 0.05.

Results

The levels of all hepatic enzymes, AST, ALT and ALP in the ZnONPs group rats significantly increased in comparison with the control group animals ($p<0.05$). While, the levels of these enzymes in the ZnONPs + Silymarin treated groups markedly decreased to normal

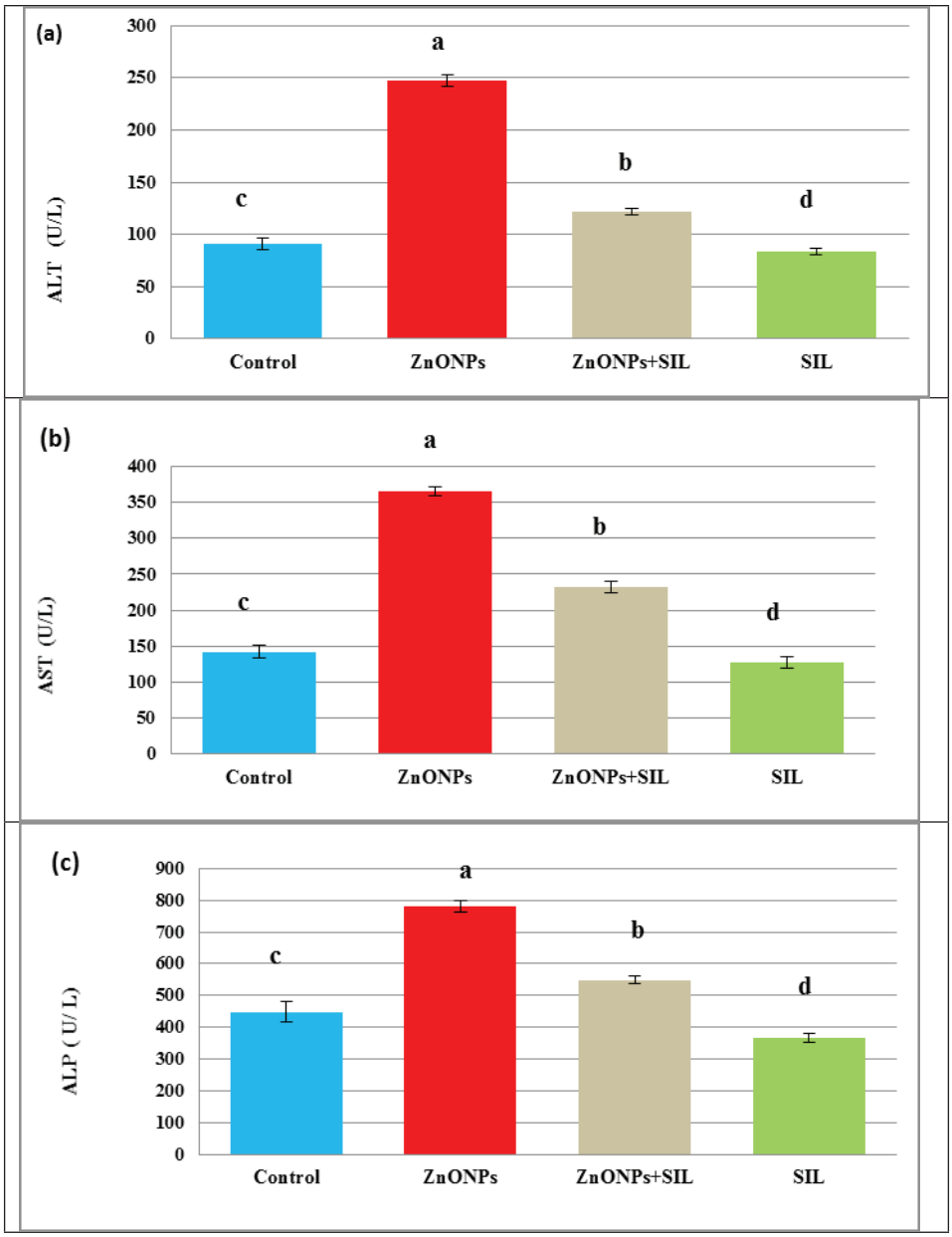

Figure 1: Levels of (a)ALT, (b) AST, and (c) ALP in the serum of different treatment animal groups. Data are expressed as Mean± SD (n=6). a–d Different superscript letters denote significant differences within the column ($p < 0.05$). Test = one-way ANOVA, post hoc = Duncan.

Discussion

With the progress in nanotechnology field, there may be an increase in the exposure of metal nanoparticles , so further urgent studies are required to study the possibility of any detrimental health impacts, target organs damage as well as their mechanisms[23]. Nanoparticles can be translocated from the entry portals into the blood circulation and the lymphatic system, and then to body organs and tissues. Some of these nanoparticles have the ability to cause irreversible cell damage by oxidative stress or/and organelle injuries because of their sizes and structures leading to serious cytotoxicities [24,25]. Body organ dysfunction may result from the distribution of these nanoparticles to other organs [26,27].

The most metal nanoparticles that cause adverse influences on the liver are ZnONPs. The liver plays a key role in the metabolism of drugs and is the target

organ of toxicants, and it responds to different kinds of oxidative stresses, thus, any disruption of the function of hepatic cells can lead to alteration in blood serum constituents and enzyme functions [28,29]. In the current study, it was shown that ZnONPs administration has a significant effects on the liver function parameters, which was consistent with previous studies[30-32]. Changes in serum biochemical parameter levels directly indicate pathological status of the liver. In clinical practice, high serum AST, ALT and ALP levels, which are produced from damaged hepatic cells into the circulation is related to serious liver dysfunctions[33 -35].

Silymarin consists of many flavonolignan isomers (65–80%) with small quantities of fatty acids and flavonoids (20–35%) as well as other polyphenolic compounds. Clinically, silymarin exhibits its hepatoprotective impacts via anti-inflammatory, antioxidative, antitoxin, anti fibrotic and anticancerous mechanisms of action[36,37]. Our current study also demon strated that a significant reduction in co-treated with silymarin and that ZnONPs-induced particles caused elevation in the hepatic enzyme levels, resulting in liver function improvement, which might be attributed to the existence of chemical components in the extract, and the anti-hepatotoxic impact of SLM. The reversal of plasma enzyme activity elevation in ZnONPs-induced hepatotoxicity revealed that SIL can lower hepatocyte death [38,39]. Thus, it probably ameliorates ZnONPs induced liver injury effectively.

Conclusions

The results of this study revealed that Zinc Oxide Nanoparticles possess a toxic pathological effect on liver function. Silymarin substantially decreased serum hepatic enzyme levels correlated with the liver. This result showed that the Silymarin was active in liver protection from damages caused by Zinc Oxide Nanoparticles. However, there is an urgent need for further studies to prove Silymarin as an effective protective agent against liver function dysfunctions caused by metal nanoparticles.

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Conflict of Interest: The authors have declared no conflict of interest

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Effect of Coal Particles on Embryonic Development, Egg Hatching Rate and Larvae Survival Rate of Climbing Perch Fish *Anabas testudineus BLOCH*

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Abstract

This study aimed to analyze the impact of coal particles on the embryo development, egg hatching rate and larvae survival rate of climbing perch fish Anabas testudineus Bloch. The research was done in the wet laboratory of the Faculty of Fisheries and Marine, Lambung Mangkurat University for six months. The treatment in this study was a dose of coal particles exposure to eggs and larvae. The coal particles measuring 150 µm can penetrate the eggshell and interfere with embryo development at the stage of organ formation (organogenesis) and larval development. The eggs were exposed to coal particles 15 mg/litre of water. It turns hatching rate was 87% lower than without exposure to 98%. Similarly, the larvae survival rate (up to day 4) were exposed to coal dust 80% and without exposure to 93%.

Keywords*: coal particles, embryonic development, egg hatching rate, climbing perch fish*

Introduction

Kalimantan Selatan is one of the areas with extensive mining land, such as coal. This area is known for its abundance of mining material reserves, especially coal. Until nowadays, its production can achieve 10% of total coal production nationally. In 2011, the production reached 101.1 million tons. In general mining in Kalimantan Selatan is carried out with an open-pit technique, which is by the clearing of land, stripping of topsoil, stripping of overburden, and cleaning and mining the coal. The transport from land mines is completed through special routes using dump trucks to a special port, which located on beaches or riverbanks. Upon arrival in the special port, the transfer from the trucks to barges is performed, and then the coal will be shipped to Java islands or overseas destinations.

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Facts in the field show that almost all of the dump trucks are not covered when transporting coals, as well as the barges when they drift in the waters (rivers, swamps, estuaries or seas). This condition causes the coal particles to be airborne, and some of them fall to the waters. If the dump trucks mostly drop the coal particles to the swamps, the barges to the rivers, swamps, estuaries, or seas. Fisheries resource management problems are not only a matter of population decline or fish resource stocks due to overfishing but also due to habitat destruction due to pollution, sedimentation and decrease of conservation areas.[1,2]

One of the wetland types is a swamp in South Kalimantan, in its swamp, live the climbing perch fish *Anabas testudineus BLOCH* that make the swamp as its natural habitat.[3] Climbing perch fish is an Anabatidae family and ordo labyrinthichi, which means it has a breathing aid in the form of a labyrinth that is able to take oxygen directly from the air.[4] In the biolimnology study of climbing perch fish, turns out that this fish has native habitat in marsh waters with various types,

namely monotonous swamp and tidal swamp. This fish is an economically important fish in South Kalimantan.[5] The production data of climbing perch caught in South Kalimantan's public waters in 2017 was 7,511.4 tons.[6] While in 2017 the production decreased significantly to only 3,361.2 tons.[7] This shows that the population of climbing perch fish is very large in the last ten years. This is allegedly there are two main factors causing the decline, namely; destruction of climbing perch fish habitat and overfishing.

The data shows that the population of climbing perch fish is declining annually. The cause of the decrease is suspected from the destruction or pollution that impacts the swamps, thus endangering the habitat of swamp fishes and biotas, including the climbing perch fish. In its reproduction, the eggs of climbing perch fish tend to be not moving and need ideal temperature and sufficient dissolved oxygen. The pollution of the swamp, which is caused by coal particles, is suspected to be able to increase the temperature of the water, decrease the supply of oxygen and probably closed the pores of the eggs that is used to breathe and delays the development of the climbing perch fish embrios even reducing the capability of the eggs to hatch. In order to answer the mentioned presumption and phenomenon, a research that analyse the impact of coal particles on eggs and embryonic development of climbing perch fish is very essential to be conducted.

Materials and Methods

The research site is in the Wet Laboratory of Fisheries and Marine Faculty of Lambung Mangkurat University, with duration six months. Materials and equipment for the research consist of climbing perch fish, plastic pond, ovaprim hormone, fish feed, aquarium and its accessories, microscope, plankton net, basin, pail, scoop net and other supporting equipment. The research is experimental; where there are 12 aquariums each filled with 100 eggs of climbing perch fish that are results of an ovaprim hormone-stimulated breeding. There several treatments: treatment O (control), which is without coal particles (0 mg/l water), treatment A with 5 mg/l water of coal particles, treatment B with 10 mg/l water of coal particles, and treatment C with 15 mg/l water of coal particles.

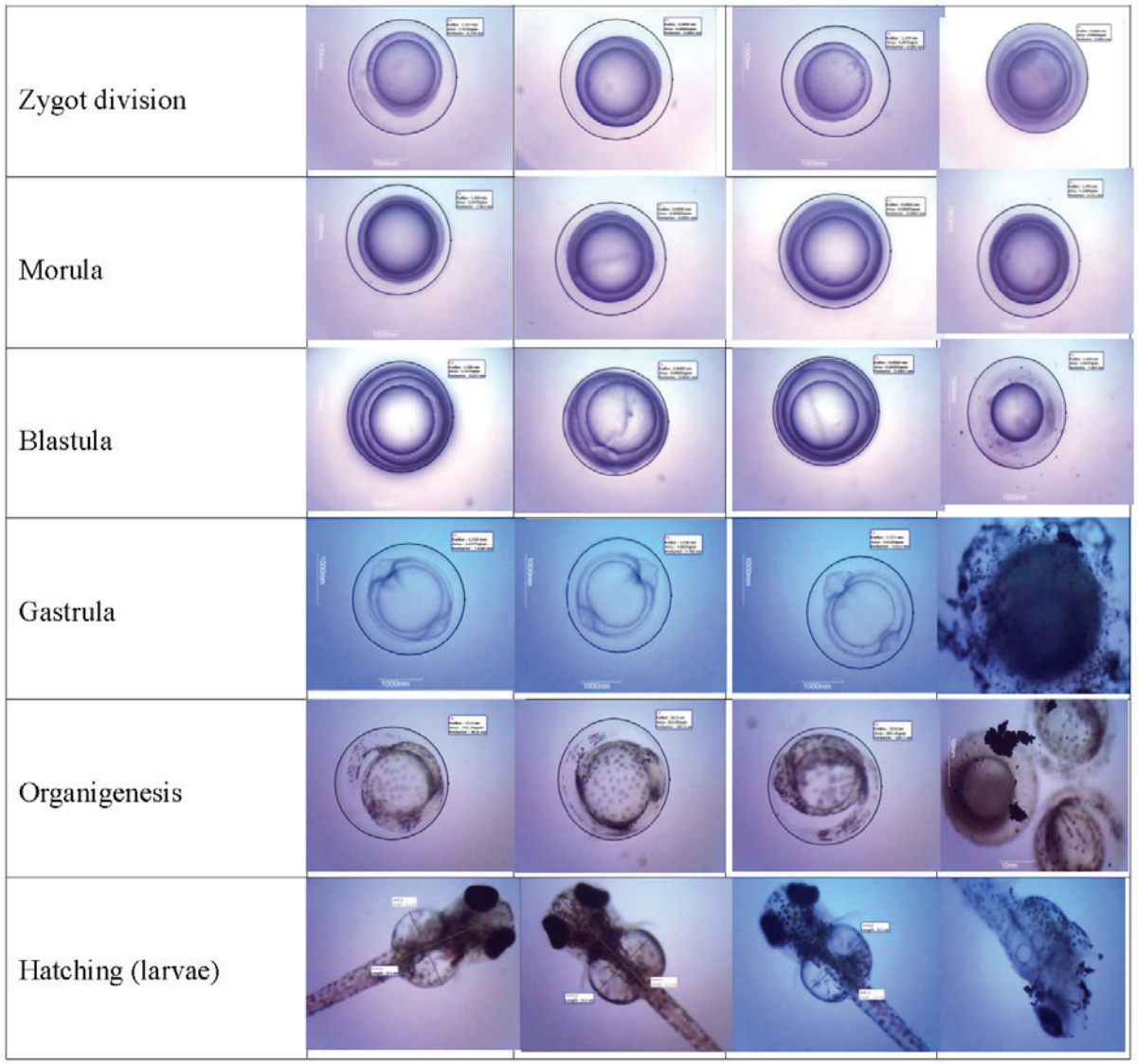
The main parameters in this study are the embryo development, egg hatching rate and survival rate. The observation of climbing perch fish's embryogenesis is performed on a microscope where the results will tell whether the development is normal or abnormal due to the effects of coal particles that penetrated through the egg pores. The observation of the embryogenesis is performed in the third, sixth, ninth, twelfth, fifteenth, thirteenth, sixteenth, and ninteenth hours after fertilization. Embryogenesis process that will be observed includes morula, blastusa, gastrula, eyes, pharynx, tail, veins, and yolk sac.[8] Afterwards, every one hour the eggs are taken its sample and observed its embryo development on a digital LCD microscope with 160 X magnification and will be documented. Then the hatching rate and survival rate of the climbing perch fish will be calculated until the fourth day with the assumption that the yolk has been separated. Calculation of the percentage of hatching eggs is done by counting the eggs that hatch and eggs that do not hatch on the sample eggs that are in the experimental aquarium units, amounting to 50 eggs.

Findings and Discussion

Embryo Development

The development of climbing perch fish's embryo occurs after the sperm fertilizes the egg, which is followed by several phases: cell division, morula, blastula, and gastrula and stadia organogenesis. The observation results time phases of embryo development stadia of climbing perch fish in a different exposure of coal particles that are shown in Table 1.

Table 1. Phases of embryo development of climbing perch fish



The observation result shows that in treatment O (control), the process of embryo development is relatively normal, from zygote division stadia until hatching into larva. Treatment A (exposure to 5 mg/l water coal particles), turns out doesn't affect the climbing perch fish's embryo development, as well as treatment B (exposure to 10 mg/l water coal particles) Meanwhile, treatment C (exposure to 15 mg/l water coal particles), it is evident that the coal dusts affects embryo development, where coal particles enters eggshell-which is assumed by diffusion—that can disrupt and damage and even kill the embryo within the egg.

Hatching Rate

The hatching rate data of climbing perch fish's eggs in every treatment can be seen in Table 2.

Table 2. Hatching rate average of larvae of climbing perch fish

Treatment	Repetition 1	2	3	Hatching Rate (%)
O (0 mg/l)	98	96	98	97,3
A (5 mg/l)	93	92	94	93,0
B (10 mg/l)	92	84	96	90,7
C (15 mg/l)	87	95	91	90,0

Based on Table 2, the hatching rate of climbing perch fish's eggs ranges between 90.7% - 97.3%, where highest hatching rate is produced from treatment O that is 97.3%, trailed by treatment A as much as 93.0%, 91% from treatment C, and the lowest from treatment A which is 90.7%. This result indicates that the greater the exposure of coal particles, the lower the eggs' hatching rate. When the eggs hatch, the larva will try to get out of its shell, if the eggs exposed to the coal dust even covered by the coal dust then the eggs will not be able to hatch. However, the result of variety analysis shows that it does not significantly impact the hatching rate of climbing perch fish's eggs based on a calculation: $F_{count}(2,03) < F_{table}$ 5% (4,07) and 1% (7,59). Therefore, coal dusts exposure up to 15 mg/l water is not significant in making the difference of the hatching rate. An average chart of the hatching rate of climbing perch fish's eggs during the research period can be seen in Figure 1.

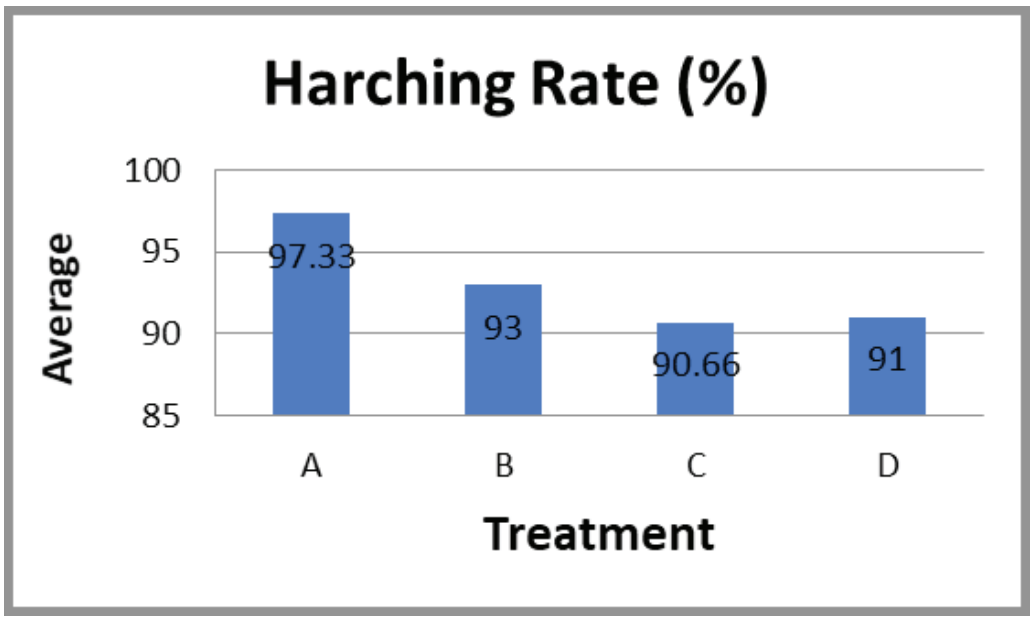


Figure1. Chart of average the hatching rate of larvae of climbing perch fish

Survival Rate

The data of the survival rate of climbing perch fish *Anabas testudineus Bloch* in every treatment is shown in Table 3.

Table 3. Survival rate average of the larvae of climbing perch fish

Treatment	Repetition 1	2	3	Survival Rate (%)
O (0 mg/l)	94,89	91,66	94,89	93,81
A (5 mg/l)	93,54	89,13	90,42	91,36
B (10 mg/l)	85,86	91,66	90,62	89,38
C (15 mg/l)	91,95	77,89	78,02	82,62

Based on Table 3, the survival rate of climbing perch fish ranges from 82.62% to 93.81%, where the highest survival rate is resulted by the treatment that is 93.81%, followed by treatment A 91.36%, 89.38% from treatment B, and the lowest 82.62% from treatment C. The average of survival rate during the research is shown in Figure 2.

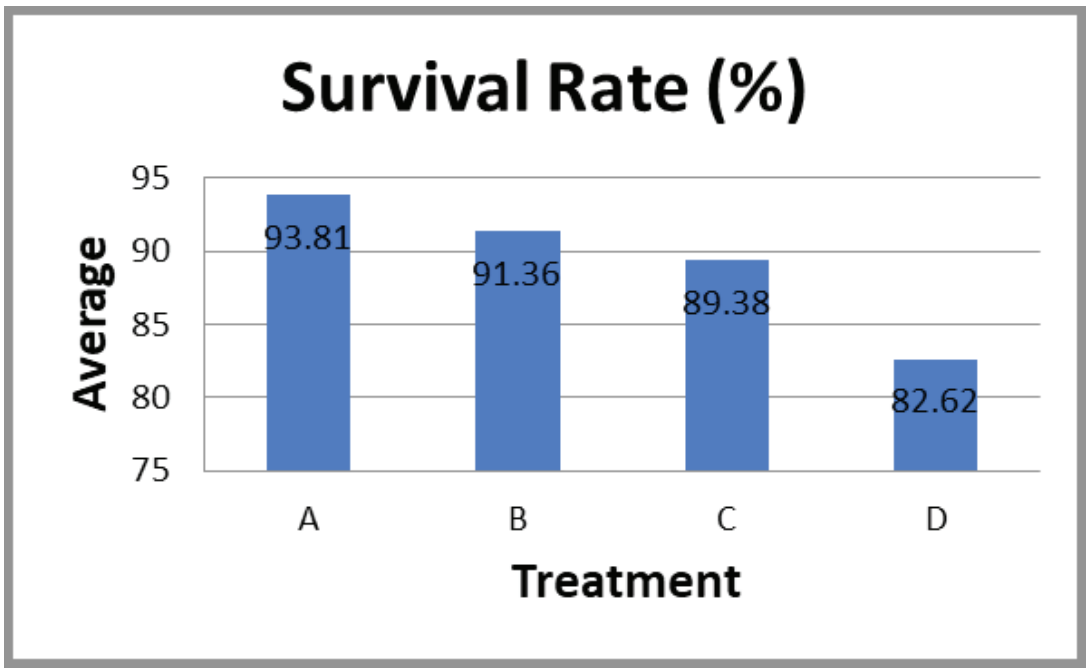


Figure 2. Chart of average the survival rate of larvae of climbing perch fish

The result of variety analysis (Anova), shows that there is no significant difference between treatments based on the survival rate of the larvae of climbing perch fish. Therefore, it is assumed that the exposure of coal particles of to 15 mg/l does not cause larva mortality, but if observed visually, it can disturb damage, or cause abnormality in growth and the development of larva of climbing perch fish.

Embryo development is an early part of the life cycle that deals with aspects of evolution, heredity,

developmental mechanisms and environmental influences on the shape and structure of organisms.[9] The eggs of climbing perch fish that is exposed to coal particles with different dosage undergo an embryo development that is started with the formation of blastodisk. Blastodisk formation is a creative process of one blastomere cell in anima poles with a lump as a result. Morula stadia occur when cell division continuously happens, so the cell numbers increase in large amounts with a smaller size.

These cells will eventually form a lump and undergo a thickening at the anima poles. This is in accordance with the statement of Rahayu (2013), morula stadia is a stadia where the number of blastomere is so thick it is very small and very difficult to be counted. Blastula stadia are started with the clear formation of two layers that form a sac.[10]

Organogenesis process is marked with the emergence of a segment in the dorsal body of the embryo. The formation of the spine of embryo's body is marked with the appearance four segments in the median body of the embryo that consists of two layers where each of them four parts have, and the segments of embryo's body will increase along with the growth of embryo's body until hatching. In the organogenesis process, the disturbance due to the exposure of coal particles started, especially with greater dosage which is 15 mg/l water. At the time when the hatching occurs, embryo will make movements to keep off from the yolk inside the chorion so the chorion layer will become flaccid and eventually cracked.[11] Embryo will keep moving in circle faster so the khorion layer grows flaccid due to the movements or due to the khorionase enzyme that causes the khorion layer to be cracked.[8]

The observation of the newly-hatched climbing perch fish's larva shows that the larva body has eyes pigments and transparent brown-black spots in every parts of its body with straight and located on the surface of the water body. The newly-hatch larva of climbing perch fish has been equipped with an imperfectly formed fin with a big size of jaw. The newly-hatched larva of climbing perch fish has black eyes pigments and brown-black spots in parts of its body that are called melanophore.[10] Then, the fertilized egg will develop an embryo and will hatch immediately after 14-20 hours, depending on the temperature and light obtained. From climbing perch fish egg hatchability data, it can be seen that the range is between 70.2 - 87.1%, with mortality ranging from 2.4 - 47.4%.[12] Furthermore, an important factor that must be considered when incubating an egg (embryo) is water temperature and pH water. Temperature and pH water strongly influences embryo metabolism and to embryonic development, hatching rate and egg hatching rate.[13] The embryogenesis process will take place when the temperature incubation starts from the stage of cleavage, morula, blastula, gastrula, and continued with organogenesis, which then hatches.[14]

By observing the condition of egg, embryo, and larva above, it is proven that coal particles with a size less or equal to 150 μm (pass through the plankton net) and its exposure is about 15 mg/l water can penetrate the egg and disturb or damage the embryo and larva phases (after hatching). It is suspected that diffusion is the main route of alien material enters the climbing perch fish eggs, especially through passive diffusion through semipermeable membrane of the egg skin that is covered with fat. The coal particles are a toxicant that is able to affect or give negative response toward an ecological system, which can seriously damage its structure and function or even cause mortality. The bad impacts here is meant as a depiction and measurement results or quantification that exceeds a normal range that is found in a healthy organism. The toxicant is able to enter the water ecosystem and significantly change the water quality and make the environment to be unsuitable for the organism. The impacts of exposure of the pollutant in an organism are various, from instant death, sub-lethal effect to unobservable effects. The impacts are called ecologically significant when the pressure of the pollutant affects the survival rate, productivity or population structure.[15]

The hatching rate of climbing perch fish's eggs, in the normal state, can achieve 95%.[16] The hatching rate for eggs that have been injected with ovaprim hormone ranges from 85.87% to 87.03%.[17] The hatching rate with different spreading thickness ranges from 96.50% to 98.58%.[18] The cause of the low level of hatching rates is poor qualified sperms that fail to bring spermatozoa to fertilize the eggs, so the eggs don't divide in blastosis phase after the fertilization and the embryo died before hatching.[17]

The highest degree of hatching was at incubation temperature of 31ºC, pH 6.7 - 7.6 and dissolved oxygen from 3.3 to 3.8 mg/l.[19] Furthermore, incubation of eggs with different temperatures will have an impact on the morphological development of the prolarva and post larvae of climbing perch fish, where the optimum incubation temperature is obtained at 30 ± 1^0C.[20] One of the environmental parameters that significantly influence the hatchability and development of fish larvae is water temperature. Media temperature has an important effect on larvae organ development, hatching rate and larvae behavior.[21]

Climbing perch larvae are very sensitive, so high mortality often occurs in the larval period. Especially when the larvae change their food from the yolk they contain (endogenous food) to food that comes from outside (exogenous food).[22] Climbing perch fish hatched by induced breeding obtained 100% spawning data, number of eggs 8,978 - 39,868 eggs, hatching degree 69.40 - 98.14%, and degree of survival of tillers to four months age 0, 17 - 0.54%.[23] Survival rates of climbing perch that exceed 70% have shown good results. Even if seen from the monthly period graph, the fry experienced high mortality at the time of initial stocking, which shows that they are still in the adaptation phase.[18]

Conclusion

1. The exposure of coal particles as much as 15 mg/l water can give damaging impacts toward the development of embryo of climbing perch fish, while exposure of 10 mg/l and 5 mg/l has not give significant impacts.

2. The exposure of coal particles as much as 5 mg/l, 10 mg/l and 15 mg/l lessened both the hatching rate and survival rate of the larva of the climbing perch fish compared to without exposure to coal dusts, but has not give significant impact.

Ethical Clearance: This research has gone ethical feasibility testing by the Ethical Research Commission of the Natural Resource and Environment Management Study Program, Lambung Mangkurat University.

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Effect of Protecting Proteins from Degradation in the Rumen and Replacing Percentages of Treated Proteins with Blood on Biochemical Blood Parameters of Al Awassi Lambs

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Abstract

The present experiment was carried out to investigate the effect of different percentages (50 and 100%) of dried whey powder and sun flower meal treated with blood or formaldehyde on Biochemical blood parameters: Blood Glucose (BG), Blood Urea Nitrogen (BUN), Blood Cholesterol (BCH), Blood Total Protein (BTP), Blood Albumin (ALB) and Blood Globulin (GLO) in lambs fattening diets. The results showed insignificant effect in (BG), (BUN), (BCH), (BTP), (ALB) and (GLO) for dried whey powder treated with blood or formaldehyde compared sun flower meal treated with blood or formaldehyde, and for dried whey powder treated with blood in percentages 100% compared sun flower meal treated with blood in percentages 100%, while there was significant decrease ($P < 0.05$) in (BUN) and (BCH) whereas insignificant effect in (BG), (BTP), (ALB) and (GLO) for dried whey powder treated with blood in percentages 50% compared sun flower meal treated with blood in percentages 50%.

Key words : *dried whey powder, sun flower meal, blood, formaldehyde, Biochemical blood parameters.*

Introduction

The most important factors affecting the microbial degradation of proteins in the diet are the type of protein [(1)]. The increase in the level of undegradable protein in the rumen leads to an increase in the level of glucose in the blood due to the increase in the flow of amino acids into the duodenum due to the decrease in the degradation of dietary protein, which leads to the provision of greater quantities of the base material used in the processes of synthesis of glucose by Gluconeogenesis [(3,2,17)] also reaching a higher level of blood urea nitrogen depends on the amount and speed of protein degradation inside the rumen, and then an increase in the level of blood urea [(4,5)]. Nutrition of a high percentage of sources of undegradable protein in the rumen led to a decrease in blood urea BUN [(6)], and thus the concentration of BUN blood urea due to reduced absorbed ammonia that enters the liver to convert to urea [(7)]. In what is considered Lipid content or tissue catabolism are indicators of blood cholesterol level [(8)]. The third largest source of protein used for ruminants feed after soybean and canola seedling [(19)]. The protein of the sun flower is characterized by its solubility and high decomposition compared to the other protein sources. Therefore, there are obstacles to meet the needs of highyielding dairy cows, calves and fast-growing sheep because the protein is rapid decomposition in the rumen, producing peptides, amino acids and ammonia, which reduces the degree of utilization and loss of amino acidsand low digestibility [(20)]. Whey was considered a non-conventional, fast degradable protein source, it is a byproduct of cheese making process of milk, containing 7% solid materials consisting of 4.9% lactose, 0.6% ash, low amounts of fat acid and protein (15-20%), so the challenge for nutritionists is to find the best way to benefit from it [(21 ‘ 22)].

Materials and Methods

This study was conducted in the animal field of the Animal Production Department, Faculty of Agricultural Engineering Sciences, University of Baghdad. The experiment lasted for 60 days preceded by a preliminary period of 14 days for the period from 2 of December 2017 to 13 of February 2018.

Treatment of the sun flower meal or dried whey powder with fresh blood

Blood was collected from ruminants that were slaughtered in the Karkh massacre in containers containing citrate of sodium (6.8 g/L blood). The blood was then added to the sun flower or dried whey powder by using an equal weight of blood and weight (1: 1) and then mixed by hand and dried in a fan oven at 60°C for 24 hours, after that, the sun flower or dried whey powder was manually broken and packed in bags until it was used [23].

Treatment of sun flower meal or dried whey powder with formaldehyde

The sun flower or dried whey powder was treated with 5% formaldehyde solution and 1 liter solution/10 kg dry matter from the sun flower or dried whey powder by sprinkler after brushing the sun flower or dried whey powder over a piece of nylon on the ground in a closed chamber with constant flipping to ensure that the solution reaches all parts of the sunflower or dried whey powder to obtain a homogeneous level of treatment. The formaldehyde sun flower or dried whey powder was kept in tightly sealed nylon bags and left for 72 hours for interaction between formaldehyde and sunflower meal or dried whey powder. The bags and their contents were then emptied onto a nylon piece inside a well-ventilated hall for 48 hours to allow for the volatilization of the unformed formaldehyde solution, then the sunflower or dried whey powder was put in bags until it was used [18].

Table 1 : Chemical composition of raw materials in the installation of concentrates and fresh grit based on dry matter (%).

Feeding materials / Chemical composition %	Barley	Wheat barn	Sunflower treated with blood	Sunflower treated with formaldehyde	Whey treated with blood	Whey treated with formaldehyde	Fresh alfalfa
Dry matter	90.12	89.87	94.77	93.30	97.59	95.86	27.22
Organic matter	93.58	91.59	89.31	85.78	96.13	94.68	91.13
Crude protein	12.22	14.72	21.37	21.67	21.08	19.11	18.21
Crude fiber	5.72	10.11	15.35	15.55	----	----	27.15
Ether Extract	3.15	4.63	9.79	10.05	7.39	8.17	3.03
Ash	6.42	8.41	8.00	8.04	6.39	6.02	8.87
Nitrogen free extract	72.49	62.13	42.42	42.44	64.32	65.82	42.74
Acid detergent fiber	27.13	48.45	38.44	37.88	----	----	45.75
Neutral detergent fiber	6.27	14.24	26.92	27.50	----	----	33.91
Lignin	1.35	2.88	9.88	10.50	----	----	8.77
Cellulose	4.92	11.36	17.04	17.00	----	----	25.14
Hemicellulose	20.86	34.21	11.52	10.38	----	----	11.84
Metabolic energy (Mica Gul/kg)	12.7	12.3	12.7	12.7	14.1	14.2	10.2

Metabolic energy (Mg / kg of material as is) = 0.012 × crude protein + 0.031 x ether extract + 0.005 × raw fiber + 0.014 × nitrogen-free extract (23)

Table 2 : Chemical analysis of experimental treatments for first experiment based on dry matter.

Type of treatment	Treatment with blood	
Replacement ratio %	50	100
Treatments	T1	T2
Chemical composition		
Dry matter	98.41	98.45
Organic matter	92.74	93.52
Crude protein	14.53	15.04
Crude fiber	8.53	7.44
Ether Extract	5.09	4.52
Ash	7.26	6.48
Nitrogen free extract	64.69	66.52
Acid detergent fiber	36.01	35.25
Neutral detergent fiber	13.50	12.66
Lignin	2.41	2.12
Cellulose	11.09	10.54
Hemicellulose	22.51	22.59
Metabolic energy (Mica Gul/kg)	12.7	12.9

Metabolic energy (Mg / kg of material as is) = 0.012 × crude protein + 0.031 x ether extract + 0.005 × raw fiber + 0.014 × nitrogen-free extract (24).

Table 3 : Chemical analysis of experimental treatments for Second experiment based on dry matter.

Type of treatment	Treatment with blood	
Replacement ratio %	50	100
Treatments	T1	T2
Chemical composition		
Dry matter	97.33	98.18
Organic matter	91.58	92.88
Crude protein	15.36	15.18
Crude fiber	9.55	10.04

Cont... **Table 3 : Chemical analysis of experimental treatments for Second experiment based on dry matter.**

Ether Extract	**4.28**	**4.52**
Ash	8.42	7.12
Nitrogen free extract	62.38	63.14
Acid detergent fiber	35.80	36.03
Neutral detergent fiber	12.94	13.02
Lignin	2.82	2.77
Cellulose	10.12	10.25
Hemicellulose	22.86	23.01
Metabolic energy (Mica Gul/ kg)	12.3	12.5

Metabolic energy (Mg / kg of material as is) = 0.012 × crude protein + 0.031 x ether extract + 0.005 × raw fiber + 0.014 × nitrogen-free extract (24).

Study some blood parameters

(BG), (BUN), (BCH) , (BTP), (ALB) : was determined using the Bio System BTS - 350.

(GLO) : was calculated by subtracting albumin from total proteins.

Chemical analysis

DM, CP, CF,: was estimated according to [25].

(OM) : was calculated by subtracting the amount of ash from dry matter.

NFE : = OM – (CP + CF + EE).

(NDF), (ADF) and(ADL): was estimated according to [26].

Cellulose : was calculated: ADF – ADL.

Hemicellulose : was calculated: NDF– ADF.

Statistical Analysis

The Statistical Analysis System [27] according to (Completely Randomized Design-CRD), The differences between the averages were compared with Test (T).

The mathematical model

$Yij = \mu + Ei + eij$

Yij= the value of the transaction j return to the transaction i.

μ = The general mean of the studied character.

Ei= It represents two experiences i.

eij= Random error distributed by a normal distribution with an average of 0 and a variance of $\sigma^2 e$.

Results and Discussion

The level of degradation and digestibility of dietary protein in the rumen affects some parameters of the blood as it is used as an indicator of protein status, especially in qualitative comparisons of protein sources and the level of dietary protein intake [10,11,12].

Effect type of protein treatment with blood or formaldehyde on Biochemical blood parameters.

Table 4 showed that there were insignificant effect in (BG), (BUN), (BCH), (BTP), (ALB) and (GLO) for dried whey powder treated with blood or formaldehyde compared sun flower meal treated with blood or formaldehyde, This may be due to the fact that the difference between the protein type and the level of protein protection between blood treatment and formaldehyde treatment [13,14] did not affect the

level of blood glucose, as these results agreed with [6,16]. While the results did not agree with [15] who stated that increasing the level of undegradable protein in the rumen with an increase in the substitution ratio of dried whey powder treated with blood or formaldehyde leads to an increase in the level of (BG) due to an increase in the flow of amino acids into the duodenum, to decrease the solubility of dissolution Dietary protein, which leads to greater quantities of the matrix used in the gluconeogenesis process [3,2,17]. And these results agreed with [8,9,16] Regarding (BUN), (BCH), (BTP), (ALB) and (GLO).

Table 4 : Effect type of protein treatment with blood or formaldehyde on Biochemical blood parameters

Studied traits	dried whey powder	standard error	sun flower meal	standard error	Effect significance
BG	68.937	±0.395	68.770	±0.312	N.S
BUN	41.375	±0.351	42.145	±0.313	N.S
BCH	67.375	±0.392	67.937	±0.293	N.S
BTP	6.987	±0.031	6.927	±0.031	N.S
ALB	3.355	±0.026	3.340	±0.021	N.S
GLO	3.631	±0.039	3.586	±0.037	N.S

N.S Non significant.

Effect type of protein treatment with blood in 100 % on Biochemical blood parameters.

Table 5 showed that there were insignificant effect in (BG), (BUN), (BCH), (BTP), (ALB) and (GLO) for diets dried whey powder treated with blood in 100 % compared diets sun flower meal treated with blood in 100 % , these results are in agreement with [9,16].

Table 5 : Effect type of protein treatment with blood in 100 % on Biochemical blood parameters.

Studied traits	dried whey powder	standard error	sun flower meal	standard error	Effect significance
BG	71.666	±0.619	69.916	±0.679	N.S
BUN	39.416	±0.468	40.083	±0.416	N.S
BCH	69.583	±0.732	69.333	±0.594	N.S
BTP	7.212	±0.042	7.203	±0.040	N.S
ALB	3.450	±0.051	3.421	±0.042	N.S
GLO	3.761	±0.080	3.781	±0.071	N.S

N.S Non significant.

Effect type of protein treatment with blood in 50 % on Biochemical blood parameters.

Table 6 showed that there were significant decrease ($P < 0.05$) in (BUN) and (BCH) whereas insignificant effect in (BG), (BTP), (ALB) and (GLO) for diets dried whey powder treated with blood in 50 % compared diets sun flower meal treated with blood in 50 % , This may be attributed to the effect of the protein type, as reaching a higher level of blood urea nitrogen depends on the amount and speed of protein degradation in the animal's rumen, and the increase in the level of degradation protein in the rumen leads to an increase in the level of ammonia inside the animal's rumen and then an increase in the level of blood urea [4,5]. Also, there was a discrepancy between dried whey powder treated with blood in 50 % and sun flower meal treated with blood in 50 % in the effect on the fat content or tissue destruction that are indicators of blood cholesterol level [8].

Table 6 : Effect type of protein treatment with blood in 50 % on Biochemical blood parameters.

Studied traits	dried whey powder	standard error	sun flower meal	standard error	Effect significance
BG	67.833	±0.726	68.666	±0.497	N.S
BUN	b41.333	±0.541	a43.083	±0.528	*
BCH	b65.833	±0.625	a67.500	±0.452	*
BTP	6.794	±0.048	6.790	±0.033	N.S
ALB	3.214	±0.054	3.301	±0.047	N.S
GLO	3.580	±0.088	3.488	±0.071	N.S

Different characters within the same column indicate significant differences ($p < 0.05$); N.S Non significant.

Conclusion

The treatment of dried whey powder with fresh blood in 50% led to a significant decrease ($P < 0.05$) in the level of (BUN) and (BCH) compared to the sun flower meal, while there was no significant effect on the Biochemical blood parameters of the treatment with blood or formaldehyde as well as the treatment with fresh blood in 100%.

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A Study of the Drinking Water Physical and Chemical Factors at Al-Qadisiyah Drinking Water Station - Salah Al-Din Province

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Abstract

The current study was conducted on drinking water to identify some of the physical and chemical properties of the drinking water station in the city of Tikrit - Al-Qadisiyah district, with four distributed sites since November 2018 to January 2019 for a period of three months by measuring the temperature of air, water, pH, D.O., BOD, E.C., salinity, hardness, chloride salts, nitrites and phosphates.

The results of the current study showed that the temperature of air and water ranged respectively between (15.5 - 17.3) and (15 - 18.2) C°, while the value of E.C. ranged (52.60 - 61.05) µS/cm. As for the pH, the values ranged between (7.31 - 7.39) whereas the D.O. values ranged (2.06 - 2.5) mg/L, while the values of the B.O.D. ranged from (0.98 - 0.50) mg/L and it was noticed that this water had a low level of hardness, as the T.H. values ranged between (9.5 - 12.6) mg/L and Ca hardness ranged between (6.66 - 9.66) mg/L, while the Mg hardness recorded values ranging between (13 - 30) mg/L, while the salinity values ranged (1.51 - 1.55) g/L, while the Cl values ranged from (0.041 - 0.13). This study recorded low concentrations of phosphate values, as the values ranged between (0.04 - 0.27) µg/L of atom phosphate, while the values of nitrite ranged between (0.01 - 0.7) µg/L of nitrogen atom - nitrite.

Keywords: *Drinking water, filtration, dissolved oxygen, biochemical oxygen demand. Total hardness.*

Introduction

Water covers about 71 percent of the Earth's surface area, and is considered one of the most important natural resources ever [(1)]. Water is the essential element in the life-enhancing system, as it moves in an endless cycle of evaporation and condensation. This cycle is known as the water cycle, as it affects the environment of living organisms such as plants, animals, and microorganisms to a great degree. Thus, it generates seasonal and daily changes that calm the atmosphere and maintain its moderation [(2)]. Water also represents one of the strategic dimensions in making life in all its forms and guarantees its continuity and permanence, as Lavon[(3)] mentioned that any change in the physical and chemical properties causes a change in its status, directly or indirectly, so that it becomes less fit for natural uses, whether for drinking or for domestic or agricultural or Industrial consumption. Also, due to development, expansion and population growth Iraq needs water [(4)]. Water is an essential component of our environment and has a fundamental role in all vital processes that occur within the body of living organisms [(5)]. The sources of water in most societies are surface water such as streams, rivers and lakes, so the water balance may be at risk as this water itself is used for drinking, hygiene and watering, so drinking water will become in danger unless it is treated healthfully and its supplies is controlled with great care [(3)].

Materials and Methods

1- Physical Factors:

1-1 Temperature

The water and air temperatures were measured at sites using a mercury thermometer with a range of (50 - 10) C° with a 0.1 C° steps.

1-2- E.C.

The E.C. of the samples were measured using a WTW Multi-line device after calibrating it at a rate of two readings, in terms of µS/cm.

2- Chemical Agents:

2-1 pH

The pH was measured via a WTW Multi-line P4 immediately after calibration of the device in the laboratory using standard solutions with various pH values, and by taking the average of two pH readings.

2-2 DO

The method was followed as described in [6] to determine D.O. levels, where a 250 ml bottles were filled by immersing them in water and the sample was fixed by adding (1 ml) of $MnSO_4.4H_2O$, then (1 ml) of alkaline potassium iodide (KOH + KI) and shaking the bottles well and leaving them for 10 minutes. Then by adding (1 ml) of absolute H_2SO_4, and a (100 ml) of the mix was taken and titrated with $Na_2S_2O_3$ (0.025 N). The results were expressed in mg/L.

2-3 BOD

Depending on the method of the American Health Association [7], and through gathering drinking water samples and treating them according to the method used to determine the concentrations of dissolved oxygen for a period of five days at 25 C° and results were expressed in mg/L.

2-4 Total Hardness

It was measured using the method approved by the American Society for Testing and Methods [8], where (50 ml) of water sample was taken and (2 ml) of Buffer Solution were added to regulate the pH, then (2-3) drops of Erichrom Blak T indicator were added till the color of the water sample became purple and then titrated with a standard solution of Ethylene Diamine Disodium Salt Tetra Acetic Acid (Na_2EDTA) (0.05 N). Then, the calculations were performed and the results were expressed in (mg/L) of $CaCO_3$.

2-5 Ca Hardness

According to water tests in ASTM [8], Calcium hardness was measured by adding 2 ml of buffer NaOH (1 N) to (50 ml) of drinking water sample, then 2-3 drops of Calcium indicator were added and titrated with EDTA (N 0.01) then calculations were performed and results were stated in (mg/L) of $CaCO_3$.

2-6 Mg hardness

The magnesium concentration was calculated by subtracting the amount of calcium hardness from the total hardness, and the result was expressed in (mg/L) $MgCO_3$ [9].

2-7 Cl

Chloride was measured according to the method used by [9] by adding (3) drops of k_2CrO_4 to (50 ml) of water sample and mixing them well, then was titrated with silver nitrate solution $AgNO_3$ (0.025 N). Calculations were performed and results were stated in mg/L.

2-8 NO_2

The method that was published by Strickland and Parsons [10] was relied on. The nitrite concentrations of samples were determined using a (Shimadzu-Spectrophotometer UV- 120-01) device at a wavelength of (543 nm). The results were reported in atom/L of nitrogen-nitrite.

2-9 Reactive Dissolved Phosphate measurement

The method described by the American Health Association [7] was followed by adding (8 ml) of the combined reagent, which was prepared from sulfuric acid, potassium ammonium tartrate, ammonium molybdate and ascorbic acid, to (50 ml) of the filtered water sample with good mixing, and left for 30 minutes. The complex was reduced giving a blue solution whose intensity is direct proportional to the phosphate concentration. Chromatic absorption of the complex formed was read by the spectrophotometer at a wavelength of (860 nm), and the results were expressed in (mg/L).

Results and Discussion

The results of the recent study in Table No.1 revealed that the averages of air temperature ranged (15-18.2) C° at site 1 and 2, respectively, as the highest temperature was 24.5 C° in November at site 1 and the lowest temperature was 10.5 C° in January at site

2. In this study, the difference in temperature may be attributed to the dissimilarity of the weather temperature of the period for sampling due to the continental climate that characterizes Iraq, which is hot in summer and cold in winter , or may be due to the difference in air temperature at sites 1,2,3,4 at the same period of the month as a result of the variance in time of sampling, considering the far distances among sites, as it takes few hours for sampling.

Table (1). Weekly and local changes of the ambient air temperature of the water site during the study period shown in C°.

Date	Sampling Sites			
	1	2	3	4
11/11/2019	21	22	23	21
26/11/2019	24.5	15	18	14
9/12/2019	13	14	12	18
23/12/2019	15	10.5	11.5	15.5
8/1/2020	16	13	13.5	15
20/1/2020	20	19	16.5	20
Average	18.25	15.5	15.7	17.2

D.O.

In table No. 2 of the results of this study presented that the rates of DO concentrations ranged (0.06-2.5) mg/L at sites 2 and 3, respectively. The highest value of dissolved oxygen was (3.5) mg/L in December at site 3. As the results of the current study indicated that the DO concentration was low, and this may be due to the high temperature and the shortage of oxygen solubility in summer.

Table (2). Weekly and local changes of DO during the study period shown in mg/L.

Data	Sampling Sites							
	1		2		3		4	
	DO	BOD	DO	BOD	DO	BOD	DO	BOD
11/11/2019	2.1	1.1	2.6	1.1	2	1.7	2	0
26/11/2019	1.6	0.1	1.3	0.1	1.2	0	1.3	0.1
9/12/2019	2.5	2	2.5	0	3	0.8	3.1	1.1
23/12/2019	2.7	0.9	2.5	1	3.5	2.1	3.2	1.7
8/1/2020	2.1	0.6	1.5	0.3	1.5	0.2	2	0.8
20/1/2020	2.5	1.2	2	0.5	1.9	0.4	2	0.5
Average	2.25	0.983	2.06	0.5	2.51	0.226	2.26	0.7

BOD

In this study, the table No. 3 showed the values of BOD ranged (0.08-0.50) mg/L at sites 1 and 2, respectively, as the highest value was (2.1) mg/L at site 3 in December. while the lowest value was (0) mg/L at site 4 in November, also at site 2 in December.

The reason for the low values of the BOD is that the sites are far from the sources of pollution. Also, the results of this study revealed that the values of the BOD were identical to the Iraqi standard specifications of 1996 for drinking water, which is less than 3 mg/L.

Total hardness

The results of this study revealed that the TH values ranged (9.5-12.6) mg/L, where the highest value was (18) mg/L in January at sites 1 and 2, respectively, however the lowest value of total hardness was (5) mg/L in January at site 4 as in table 3. The reason for the decrease in total hardness may be attributed to the presence of chloride and calcium salts at low levels. These results were within the Iraqi drinking water standard limits and global, which range (250-500) mg/L (Central Organization for Measurement and Quality Control 1996).

Table (3). Weekly and local changes of water TH during the study period shown in mg/L.

Data	Sampling Sites			
	1	**2**	**3**	**4**
11/11/2019	8	7	15	13
26/11/2019	12	10	10	10
9/12/2019	10	7	9	12
23/12/2019	12	12	15	12
8/1/2020	10	15	14	5
20/1/2020	18	18	13	5
Average	11.66	11.5	12.66	9.5

Ca and Mg hardness

The current study results displayed that the calcium hardness values ranged (6.66-9.66) mg/L at sites 1 and 3, respectively, where the highest value of calcium hardness was 15 mg/L at site 4 in January, while the lowest value was 4 mg/L at Site 1 in November as in table 4. The reason for the difference in calcium hardness values may be due to the nature of the geological structure.

The current results also showed that the values of magnesium hardness ranged (13-30) mg/L at the sites 1 and 4, respectively, as the highest value of magnesium hardness was in November and December at the sites 1 and 4, respectively, while the lowest value was 0 In January at site 4 as in table 5. The reason the shortage of magnesium ion, may be due to the lack of water salinity which lowers the concentration of magnesium ion [11].

Table (4). Weekly and local changes of Ca and Mg hardness during the study period shown in mg/L.

Data	Sampling Sites							
	1		2		3		4	
	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
11/11/2019	6	2	6	1	10	3	12	1
26/11/2019	4	8	5	5	7	3	8	2
9/12/2019	5	5	6	1	8	1	10	2
23/12/2019	5	7	10	2	11	4	4	8
8/1/2020	8	2	10	5	10	4	15	0
20/1/2020	12	6	10	8	12	1	5	0
Average	6.66	30	7.833	22	9.666	16	9.333	13

Salinity

The results of this study indicated that salinity values ranged (1.51-1.55) g/L at sites 2,3, and 4, respectively. Where the highest value was (1.23) g/L in December at site 4, and the lowest value was (0.03) g/L in November at site 3, as shown in Table 5. The salinity values recorded in the current study were low, and we found that there was a relationship of direct proportion between EC and salinity and the concentrations of chloride and calcium ion, so this water is considered safe to drink. (Central Organization for Measurement and Quality Control 1996).

Table (5). Weekly and local changes of salinity during the study period shown in mg/L.

Data	Sampling Sites							
	1		2		3		4	
	Sal.	pH	Sal.	pH	Sal.	pH	Sal.	pH
11/11/2019	0.04	7.83	0,03	7.11	0.04	7.42	0,04	7.27
26/11/2019	1.21	7.12	1.21	7.30	1.20	7.20	1.19	7.26
9/12/2019	1.19	7.02	1.18	7.48	1.18	7.35	1.17	7.46
23/12/2019	1.22	7.18	1.21	7.39	1.21	7.32	1.23	7.20
8/1/2020	1.20	7.25	1.21	7.45	1.20	7.33	1.22	7.38
20/1/2020	1.18	7.48	1.20	7.60	1.19	7.69	1.22	7.80
Average	1.006	7.31	1.006	7.36	1.003	7.38	1.01	7.39

PH

The current study results revealed that pH values ranged (7.31-7.39) at sites 1 and 4 respectively, as the highest value was (7.8) in January at site 4 and the lowest value was (7.11) in November at site 2, as shown in Table 5. It was noticed through the results recorded in this study that the extent of the variation in the pH values is narrow and the reason may be due to the regulatory capacity of the water containing bicarbonate and carbonate compounds as well as other compounds resulting from the drinking water process that enter the water body as the Iraqi drinking water is rich in these compounds that neutralize water acidity [12].

Cl

The results of the current study showed values for chloride, whose rates ranged (0.041-0.13) mg/L at site 2 and 3 respectively, as the highest value of chloride reached 0.51 mg/L in January at site 2 and the lowest values were recorded at sites 1, 2, and 3 In November.

The results showed the variation in the chloride values during the months of study, and the reason may be due to the climatic differences between summer and winter seasons. The results also showed that the chloride values were associated with the EC values, as EC values were directly proportional to the concentration of elements, including chloride [13].

Table (6). Weekly and local changes of Cl in water during the study period shown in mg/L.

Data	Sampling Sites							
	1		2		3		4	
	Cl	PO4	Cl	PO4	Cl	PO4	Cl	PO4
11/11/2019	0.01	0.97	0.01	0.20	0.01	0.01	0.11	0.01
26/11/2019	0.11	0.18	0.07	0.12	0.05	0.01	0.04	0.27
9/12/2019	0.01	0.29	0.04	0.07	0.06	0.10	0.07	0.13
23/12/2019	0.06	0.01	0.07	0.01	0.03	0.14	0.07	0.27
8/1/2020	0.04	0.01	0.51	0.27	0.03	0.01	0.05	0.30
20/1/2020	0.06	0.19	0.08	0.61	0.07	0.01	0.06	0.31
Average	0.048	0.275	0.13	0.213	0.041	0.046	0.066	0.215

PO_4

The results of this study showed that phosphate values ranged around (0.17) mg/L at site 1 and 4 respectively, as the highest value reached (0.31) mg/L in the water of site 4 in January, and the lowest value was (0.01) mg/L in the water of the sites 1 and 2 in November and December, and that most of the samples did not exceed the maximum limit in the local standard specifications for drinking water, which is (0.4) mg/L as in Table 6. [14].

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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The Use of Pentoxifylline Topical in the Treatment of Alopecia in Rats

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Abstract

Hair is the human body's major esthetic display component, especially in cultural and social interactions. Hair loss is yet sometimes transient and the patient restore hair growth but have harmful effect on the psychology of the patient. Throughout this research, the potency of topical pentoxifylline (PTX) in stimulating regrowth in hair loss caused by cyclophosphamide was established. The study was performed by treated with PTX topically in male Swiss albino rats. The animals divided into seven groups with six animals in each group. To induce alopecia Cyclophosphamide used at a dose of (125 mg/kg) intraperitoneal. Macroscopically pentoxifylline (PTX) treated groups displayed new hair growth. Strong follicular proliferation was demonstrated by histopathology and gross morphological observations of hair growth in shaved areas. The blood biochemical cytokine assay revealed a down regulation of pro inflammatory TH1 cytokines TNF-α and INF-g, in addition there is up regulation of TH2 cytokine IL-13. In the study showed an efficient efficacy on the treatment of alopecia induced by cyclophosphamide.

***Key words**: chemotherapy induced alopecia, cyclophosphamide, pentoxifylline, TNF-α, INF-γ, IL-13.*

Introduction

Hair is the human body's major esthetic display part, especially in cultural and social interactions [1] .Hair has several beneficial vital functions, including defense from contaminants and sweat-gland dispersion substances e.g. pheromones [2] .

Is a common term for loss of hair, is a consequence of noticeable hair loss. Alopecia is defined as the scalp hair loss influencing many men throughout their entire life and around 30 percent of women. Hair loss is yet sometimes transient and the patient restore hair growth but have harmful effect on the psychology of the patient[3].

Hair shaft comprises of three main elements which are a complex system. The medulla is covered by cortex and cuticle that comprises the innermost component. The hair fiber comes from the follicle in the scalp, which when seen in the longitudinal segment consists of several major components[4].The lowest part, which expands to the penetration of the muscle arrector pili from the origin of follicle, this lower section, in addition, comprises of many major parts. The hair tube comprises the papilla dermal besides hair matrix. The dermal papilla regulates the quantity of matrix cells, deciding the fiber thickness of the hair [2].

Within the matrix cells of the hair bulb are melanocytes that are often responsible for hair color. The hair matrix comprised (inside and outside) medulla, cortex and hair cuticle. The inner root sheath composed of the cuticle of the inner root sheath, the sheet of Huxley and the sheet of Henley (inside to out). The sheath of the inner root is solid, and its form defines whether the hair is curled or smooth. And the outer sheath to core [5].

The middle part (isthmus), expanding mostly from opening of the arrector pili muscle to the entrance of the sebaceous duct. This section includes the HF's "bulge," in which the epithelial stem cells exist. Disruption to the HF bulge leads to persistent alopecia scarring, as is medically observed with conditions like discoid lupus and lichen planopilaris.The upper part (infundibulum), which continues to the follicular orifice from the entry of the sebaceous duct[6].

Hair cycle

Through embryogenesis hair follicles (HFs) are developed in humans, but no other HFs are created after conception. The figuration of the individual follicles will dramatically alter over time, however. During adolescence, heavier and darker hair replaces thin, mildly pigmented hairs in the beard. By comparison, dense scalp hairs thereafter turn into delicate, short hairs. Ironically all reactions take place in reaction to a testosterone hormone[4].

The HF redesigns itself through recessionary growth cycles. When developed, HFs experience continuous cycles marked by growing phases (anagen), decline (catagen), then resting (telogen), during the last stage hair is lost (often recognized as exogen). Around 80 to 90% of HFs are within the vital stage of growth (the anagen) at all times. Through anagen phase, the active mitotic matrix cells differentiate and divide into the hair bulb resulting in an approximate daily hair growth rate of 0.35 mm. around 5-10% of HFs are in the phase of dormancy (telogen), whereby all mitosis is halted. The remainder 1 to 3% is in the involution process Catagen[7].

Regeneration of the follicles at the beginning of the next anagenic period demands that uncommon circulating epithelial stem cells be triggered from the fresh follicle matrix during an early phase of anagen in the permanent follicle Bulge area and the shaft and inner root are extracted from such comparatively in distinguished matrix cells. The descendant of stem cells from the initial anagen phase of a new folic matrix and the shaft of a hair and sheath of inner root originate from these matrix cells. The hair shaft's height and width are relative to the volume of follicle's hair as well as the period of the anagen[8].

Alopecia inducing factors

Alopecia-causing triggers include hormonal disorder, impaired hepatic and liver function, lupus erythematosus, heat and chemical injury, conception, and fungal infection, hereditary predisposition, chemotherapy, fatigue, diabetics, vicious disorders, injuries, autoimmune diseases and rheumatoid arthritis [9].

Epidemiology of alopecia

The occurrence of hair loss is reported to increase gradually with increasing age in each of these males and females in populous-based surveys of White people aged 20-70 aged, often utilizing updated classification measures, the occurrence of hair loss ranged from 45% to 90%.In the Hamilton-Norwood classification scale, the incidence of alopecia was approximately 50% [10] .

Quality of life between subjects with hair loss

In the conventional context, alopecia not considered as an illness. However, psychological and social consequences apparently emerged and affect the quality of the life (QOL) of affected the patient.In men as well as women, the detrimental psychological influence of alopecia was reported. The relation among hair loss and psychology consequences in women appears therefore to be greater. However, several researches documented an alopecia has psycho social consequences in males such as increased self-esteem dissatisfaction and depression. For some trials, the therapy was successful with alopecia and QOL -related causes (e.g. psychiatric illness, poor self-esteem, lower body confidence, and social awareness). These considerations are essential when assessing outcomes, for example the medication effects on the life of patients but may not recognize the influence on QOL of persons with alopecia related to the extent of alopecia or other health factors[11].

Chemotherapy induced alopecia (CIA)

While various kinds of chemotherapy occur, the drug itself can be generally interpreted as a medication that prevents cell division or development. Alopecia considered as most severe skin side effects that damages the skin[12]. Several drugs influence the growth of hair and a few others induce loss of hair, the symptoms of the illness of which are important for hair growth as a direct consequence of drug action on different cells. These cells are keratinocytes, hair matrix cells, peri-follicular blood vessels, in addition those of hair bulbs connective tissues. Keratinocytes considered a first affected cite for environmental or xenobiotic damaging effects. This can be attributable to the normal feature that up to 90% from total HFs are in a rapid growth process and the high blood flow rate around hair bulbs contributes to a strong bioavailability of many drugs at these locations[13, 14].

There seem to be two kinds of alopecia which may induced by chemotherapy telogen effluvium and

anagen effluvium. Telogen Effluvium considered lesser in severity than anagen Effluvium, typically results in substantial thinning, where the patient may lose around half the hair while receiving cancer treatment. This form of CIA is less visible to the eyes of the public than the anagen effluvium, which also results in alopecia[15].

As shown by Yeager and Olsen, [15], the anagen effluvium is generally correlated with chemotherapy as it is triggered by drugs that affect quickly proliferating cell groups and destroy neoplastic cells and rapidly developing normal cells such as anagen hair matrix cells. The phase which is anagen included within the three phases of hair development that is the process during which productive development takes place. Cancer treatment damages the hair's anchoring to the extent where it drops out from scalp or breaks down before it hits the upper surface of scalp [15].

For many cancer survivors, alopecia considered a traumatic experience. The change in appearance resulted from alopecia may cause some survivors to encounter a sense of loss of identity and discomfort as they face difficulty to accept their distorted identity. The quickly growing HFs of cells within the anagen stage suffers from systemic chemical toxins and from premature apoptosis, which contributes to earlier catagen beginning[16].

Pentoxifylline (PTX)

It is a non-specific promoter of phosphodiesterase enzyme (PDE), which induces numerous physiological modifications at cell and molecular rates. Both cyclic nucleotides cyclic adenosine monophosphate (cAMP) as well as cyclic guanosine monophosphate (cGMP) are the second intracellular messengers which play a central function in signal transmission in various physiological mechanisms[17]. The concentrations of the mentioned nucleotides intracellularly are tightly controlled at both the level of synthesis by receptor with enzymes (such as adenylyl and guanyl cyclases) and also of the level of degradation through enzymes recognized as phosphodiesterase (PDEs). Such enzymes act as a catalyst on adenosine 3', 5"-phosphodiester hydrolysis, and hence produce the 5'-nucleotides (AMP and GMP) metabolites form guanosine 3', 5'-monophosphate (cGMP) and adenosine 3', 5'-monophosphate (cAMP). PDEs comprise a huge group of structurally relevant enzymes. At present, eleven PDE gene family members have already been detected that vary in their primary structures, affinity for either cAMP or cGMP , intracellular response, responsiveness to particular inhibitors, and regulatory pathway [18,19,20,21,22] .

Interruption of these phosphodiesterases, that increase levels of intracellular cyclic nucleotide by blocking hydrolysis of 3, 5-cyclic nucleotides into 5-nucleotide monophosphates, are an significant number of medicines utilized in number of conditions. The xanthine derivatives PTX, theophylline and caffeine are non-selective phosphodiesterase inhibitors in this class. These medicines inhibit all four known families of cAMP and the fifth phosphodiesterase families that are effective on cGMP, but not cAMP[23].

The pentoxifylline a non-selective antagonist has been utilized medically in a variety of micro-circulatory disorders for decades, in addition on the dermatologists found that the drug is useful in a variety of skin diseases. PTX has an action of anti-inflammatory properties. While the application and effectiveness of PTX for a number of dermatological diseases has been shown over a long period of time in multiple trials, the United States Food and Drug Administration (FDA) only has authorized the usage in intermittent claudication[24].

Pharmacological actions of pentoxifylline

Several documented theories concerning the mode of activity of PTX as well as its physiological impact, based primarily on animal and human research. Which involve influences on immunity regulation, opposing tumor necrosis factor (TNF-α) actions, haemrheological impacts, opposing fibrinolytic effects, and effects on endothelial cell line and adhesive molecules[24].

PTX is a recognized drug having many pharmacological effects. It is a dimethyl xanthine-derivative that raises the levels of cAMP in smooth muscle of the veins and arteries and is known for its vasodilator activity. PTX is sometimes regarded as a rheological agent because of its action on red blood cells. It enhances the resilience of erythrocytes besides boosts blood supply in the arteries. Following such improvements, blood circulation and oxygen supply was enhanced[25].

The anti-thrombotic properties for PTX are linked with its activation of prostacyclin production and suppression of the enzyme PDE. Prostacyclin and nitric oxide are documented for their strong vasodilator activity and inhibition of platelet aggregation. PTX further lowers blood viscosity through its fibrinolytic action[25].

Immune modulation effect of PTX

The earlier activity in several inflammatory skin disorders is the production of epidermal and dermal-induced pro-inflammatory cytokines by various stimuli. Such triggers could specifically stimulate keratinocytes to manufacture and releases pro-inflammatory cytokines, including interleukin (IL)-1a, TNF-α and IL-6. PTX reported originally characterized as inhibiting the production of TNF-α mRNA-induced liposaccharide protein by animal models and humans monocytes and macrophages. These can be seen to be modified by the increase of cAMP. Eventually, Neuner *et al*. also showed in vivo and in vitro whereby PTX decrease intercellular adhesion molecule (I CAM)-1 production on individual monocytes controlled by TNF-α inhibition[26].

PTX blocked the release of TNF-α that triggered by UV light in keratinocytes. This was also observed to impede the release of interferon (IFN)-g by leukocytes. By suppressing the production of pro-inflammatory mediators by keratinocytes and leukocytes, PTX may eliminate or decrease the initial stage of the inflammatory response [27] .

PTX also influences immune regulation by enhanced leukocyte deformity and chemo taxis, reduced endothelial leukocyte adhesion, decreased degranulation of neutrophil and release of superoxide, decreased production of monocyte-derived TNF, reduced leukocyte susceptibility to interleukin 1 (IL-1) and TNF, suppression of T and B lymphocyte activity and reduced normal activity of natural killer cell[28].

Anti-TNF- α effect of PTX

TNF-α is a mononuclear phagocytic cell-derived factor that is identified in a multiple physiological and immunological processes as an essential mediator. Systemic actions of TNF-α for its oncolytic action on solid murine tumors were first described but later studies have shown that TNF plays an expanded part in multiple diseases. For instance, it appeared that in studies becomes obvious that TNF is amongst the most proximal mediators responsible for endotoxin-induced shock pathogenesis. At the cellular stage, TNF has major pleomorphic influences on cells that are shown to orchestrate inflammatory mechanisms; involving endothelial cells, fibroblasts, polymorphonuclear leucocytes, blood monocytes and macrophages, adipocytes, lymphocytes and osteoclasts. The actions of TNF on various cell groups put this monokine at the center of modulating acute and chronic inflammatory conditions[29].

PTX was originally shown to clinically supersede claudication in individuals with peripheral occlusive artery disorder. This pharmacological mechanism is due to decreased prostacyclin (PGI2) from vascular walls and intracellular cAMP production in platelets, polymorphonuclear leukocytes and monocytes. Since cAMP may act in intracellular signal translocation and control monokine output, this process can entail inhibition of TNF development caused by PTX(29). PTX pharmacological activity may have significant ramifications in medical disease processes where local and systemic regulation of TNF may be needed to regulate particular inflammatory processes [29].

The synthesis of TNF-α, a large pro-inflammatory mediator with wide spectrum activity and released mainly by mononuclear cells, is believed to be inhibited by PTX. PTX also serves as a potent blocker of previously made TNF-a. In adjuvant-only groups, serum cytokine levels of pro-inflammatory cytokines (TNF-α) were found to be significantly higher than in control[29].

PTX Effects on TH1 and TH2

PTX, in addition to inhibiting the synthesis of TNF-α, appears to increase the divergence of the cell secretions more toward the manufacturing of TH 2 cytokines, suppressing, just from the other hand, cytokines of the TH 1 subset, like IFN- γ[30].

The effectiveness of PTX as an immunomodulation as well as anti-inflammatory medicine has been investigated in many animal models of diseases. In the murine paradigm of allergic pulmonary inflammatory condition, PTX delivery through allergen sensitization

contributes to impedance of airway hyper responsive action given the existence of elevated rates of TH 2 cytokine IL-13 and reduced amounts of cytokines of TH1 in the Broncho-alveolar lavage fluid[31].

Materials and Methods

Subjects

Wistar albino male rats, their weight range 120-140 gram were brought from animal house in College of Pharmacy/University of Baghdad. The animals were maintained on normal conditions of humidity, temperature and light/dark cycle. They were fed standard rodent pellet diet and they have free access to water. The animals grouped into seven groups each group with six animals housed for accommodation for five days before the induction procedure of the alopecia.

The protocol utilized for alopecia induction

The animals clipped at day one of procedure and another clippering in the day six for the stimulation of anagen hair growth. The anagen hair follicles are more sensitive for the effect of the cyclophosphamide (CYP). At day nine the animals for groups (2-7) are injected with CYP using a dose of 125mg/kg single intraperitoneal injection for the inducement of alopecia and group control (1) injected only intraperitoneal distilled water. The animals kept under supervision for the complete effects of the chemotherapy for six days. After six days of intraperitoneal injection of chemotherapy the animals treated with different formulas for two weeks. At end of treatment the animals authenticated and samples taken for analysis[1].

Study groups

The animals in this study were divided into seven groups; each group with six animals all groups injected CYP intraperitoneal at day 9 except control group.

Group control: given no treatment

Group PTX 1%: in which the treatment is PTX at concentration 1%.

Group vehicle: the treatment used is the vehicle only.

Group PTX 2%: in this group the treatment is PTX at 2% concentration.

Group PTX 3%: the used treatment is PTX at concentration of 3%.

Group standard: the standard treatment (minoxidil 2%) was used.

Group negative control: no treatment used for this group.

Diagnostic kit

The kits used for measurement of biological parameters levels are ELIZA kits for the serum levels of TNF-α, INF-g and IL-13 within the study.

Pentoxifylline topical gel formulation

Ultrapure water required was heated to 90 degrees Celsius. Then the powder of HPMC in preheated water slowly was dispersed. The whole solution of HPMC was mixed till a homogeneous look was achieved. The mixture then was left in the fridge overnight till a transparent gel had been produced. Pentoxifylline-HPMC topical solution mixture was formulated with a persistent mixing of pentoxifylline and HPMC gel at room temperature till all pentoxifylline powder became uniformly dispersed[32].

Statistical Analysis

The significance of differences between the mean values was calculated using SPSS version 25 program. P values that is lower than 0.05 were considered significant for all data shown.

Results

Analysis of variance of the data in figure 1 revealed significant increase in mean serum TNF-α comparing groups that used PTX as treatment with negative control and vehicle groups.

The serum level of INF-g in figure 2 decreased significantly when treated with PTX topically as compared with negative control or vehicle groups.

Examination of means serum levels of the groups in figure 3 exhibited significant decrease in mean serum TNF-α when compared the values in PTX treated groups with negative control and vehicle groups.

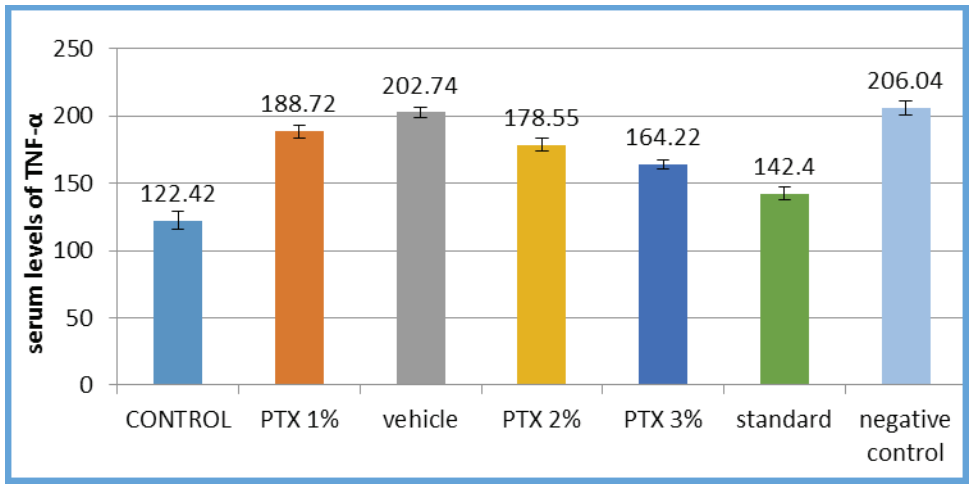

Figure 1: Comparison of the means ± standard error of means of serum levels of TNF-α of various rat groups used in the study.

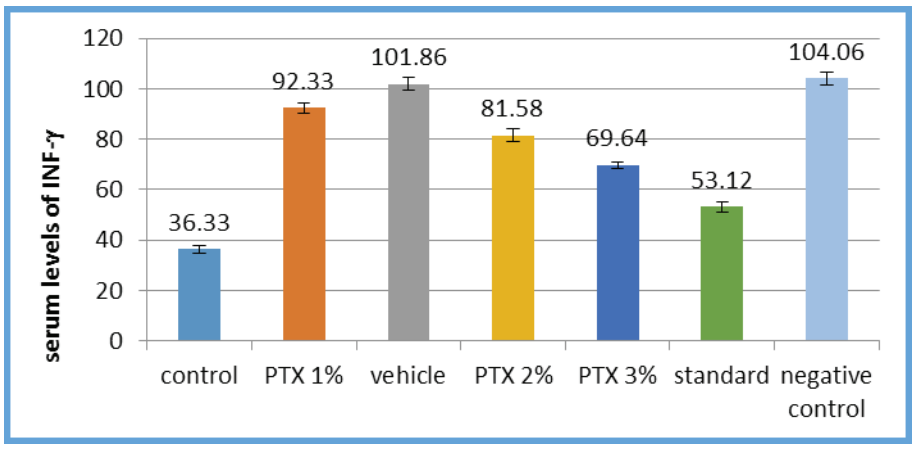

Figure 2: Comparison of the means ± standard error of means of serum levels of INF-g of various rat groups used in the study

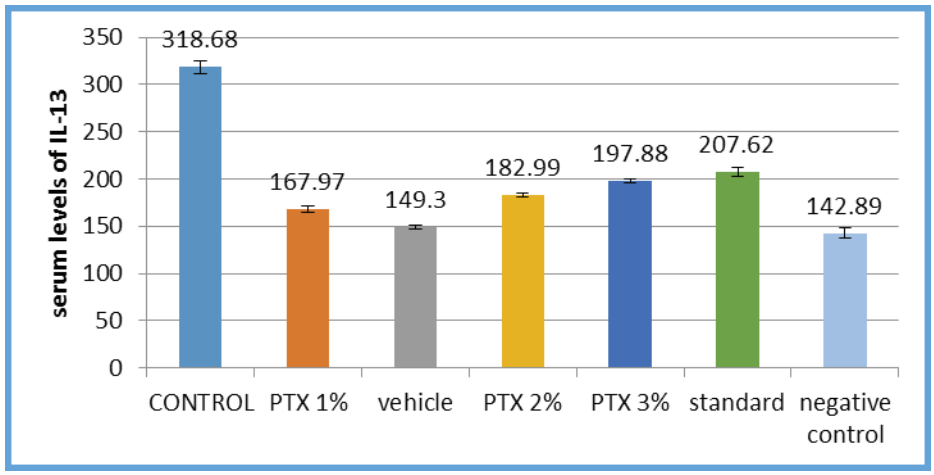

Figure 3: Comparison of the means ± standard error of means of serum levels of IL-13 of various rat groups used in the study.

Discussion

The insufficient insight into the basic mechanism leading to alopecia together with therapies that failed to cure it, determined scientists to developed large research programs to overcome hair loss[33].

As research continues to develop narrowly targeted chemotherapy options, the prevalence and severity of chemotherapy-induced alopecia experienced by patients will hopefully diminish dramatically. Until all chemotherapy becomes so targeted, however, hair loss will continue to be a side effect dreaded by patients undergoing cancer treatment. Scalp cooling for hair loss prevention and 2% topical minoxidil for quicker regrowth of hair currently are the best-studied and most effective options for treatment. Better therapeutic options, however, need to be developed both for the prevention of alopecia and the acceleration of subsequent hair regrowth[15].

Effect on serum TNF-α

TNF-α is a multifunctional proinflammatory cytokine which has been implicated in the pathogenesis of many infections and inflammatory disorders. However, this cytokine not only acts as mediator of immunity and inflammation, but also affects not-immune responses within tissues such as cell proliferation and differentiation. *In vitro* studies have shown that TNF-α, along with IL-1α and IL-β, causes vacuolation of matrix cells, abnormal keratinization of the follicle bulb and inner root sheath, as well as disruption of follicular melanocytes and the presence of melanin granules within the dermal papilla. Experiments in cultured human hair follicles showed that TNF-α completely abrogated hair growth, additionally TNF-α induced the formation of a club-like hair follicle, similar to catagen morphology of the hair bulb[34].

There is significant decrease in serum levels of TNF-α in PTX treated groups from the negative control group, this decrease is due to effect of PTX on serum levels of TNF-α as seen in figure 1. These findings are consistent with the previous study on the action of PTX of Hassan *et al*.[24]. When increased the concentration of PTX there was more decrease in levels of TNF-α.

Effect on serum levels of INF-g

Ito *et al.* reported that IFN-γ rapidly inhibited hair elongation in cultured human anagen hair follicles and induced morphological signs of catagen transformation after only four days of culture, faster than with other reported catagen-inducers. Proliferation was inhibited, apoptosis was increased, and follicular melanogenesis was switched of in hair bulb keratinocytes treated *in situ* with IFN-γ, increasing the possibility of the destruction of the hair follicle immune privilege site [35].

Groups treated with PTX with different concentrations have showed significant decrease in serum levels of INF-g from negative control or vehicle due to the direct effect of PTX on skin, this is consistent with previous study of R. Pal *et al*. [36] . The decrease in serum levels of INF-g greater in higher concentration than in lower concentration of PTX when applied topically due the effect on pro inflammatory cytokine INF-g as shown in figure 2.

Effect on serum IL-13

The groups treated with PTX showed a significant increase in serum levels of IL-13 than negative control group or vehicle as shown in figure 3. This increased level showed protective effect on HF and there is observed macroscopic improvement of hair growth and texture in the groups treated with PTX. The hair growth improved more with increased levels of serum IL-13 in the groups of PTX 1%, PTX 2%, and PTX 3%.

Conclusion

Pentoxifylline showed the ability to prevent damage to hair follicles by CYP when administered after CYP administration and enhanced hair regrowth. Therefore, PTX have demonstrated its ability to prevent hair loss and damage to the skin structures caused by CYP to rats in CYP induced alopecia. They possibly will be acting by preventing accumulation of proinflammatory TH1 cytokines like TNF-α and INF-g, or up regulating the TH2 cytokines like IL-13. Furthermore the hemodynamic effect of PTX on blood vessels improve the blood flow and increase the supply of fresh blood to the local area treated with it, and the improvement in the hair growth may be due to another effect which is the increased blood flow.

In this study it was to be concluded that the PTX was used to treat the alopecia (a common side effect in the cancer treatment) during chemotherapy.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional ethical committee

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Study of Anti-Ovarian Antibody, Anti-FSH and Anti-LH Antibodies Along with Their Receptors in Polycystic Ovarian Syndrome

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Abstract

A case control study was conducted to compare sixty females (polycystic ovarian syndrome patients), thirty with high LH & FSH hormone level "Group 1 " G1", thirty with low LH & FSH hormone level " Group 2 " G2 " and thirty healthy control groups. The average age is between (15 – 45 years). The following parameters were estimated: Luteinizing Hormone (LH), Luteinizing Hormone-Receptor (LH-R), Follicle-stimulating hormone (FSH) , Follicle-stimulating hormone-Receptor (FSH-R), anti-LH ,anti-FSH , anti-ovarian antibody (by ELISA Technique).

Rresults showed that the average age of the patients within the first group1(G1) was 28 year, while the average age of the patients in the second group2(G2) was 36 years, also the results showed a high correlation between group1 (G1) with (Anti FSH antibodies) (93%), compared with group2 (G2) that (Anti FSH antibodies) where it was 0% , as well a high correlation 100% between group1 (G1) with (Anti LH antibodies),compared with group2 (G2) where it was 3% . And anti-ovarian antibodies ,compared with group 2 .

***Key words**: Polycystic Ovarian Syndrome, Anti-Ovarian Antibody, Anti-FSH and Anti-LH Antibodies.*

Introduction

Polycystic ovary syndrome (PCOS) is a set of symptoms due to elevated androgens (male hormones) in females [1]. Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age; it is the leading cause of hormone related infertility [2]. Signs and symptoms of PCOS include irregular or no menstrual periods, heavy periods, excess body and facial hair, acne, pelvic pain, difficulty getting pregnant, and patches of thick, darker, velvety skin[3], The tendency to accumulate body fat in intraabdominal fat stores, which is frequently observed in PCOS, is linked to an increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease , obstructive sleep apnea, heart disease, mood disorders, and endometrial cancer[1].

PCOS is due to a combination of genetic and environmental factors[4,5]. Risk factors include obesity, a lack of physical exercise, and a family history of someone with the condition[6].Weight loss improves the endocrine profile and increases the likelihood of ovulation and pregnancy, Normalization of menstrual cycles and ovulation could occur with modest weight loss as little as 5% of the initial weight. The treatment of obesity includes modifications in lifestyle (diet and exercise) and medical and surgical treatment[7]. Diagnosis is based on two of the following three findings: no ovulation, high androgen levels, and ovarian cysts. Cysts may be detectable by ultrasound. Other conditions that produce similar symptoms include adrenal hyperplasia, hypothyroidism, and high blood levels of prolactin[8].

PCOS has no cure[9]. Treatment should be symptom-oriented long term and dynamic and adapted to the changing circumstances and may involve lifestyle changes such as weight loss and exercise[10] . Chronic anovulation over a long period of time is also associated with an increased risk of endometrial hyperplasia and carcinoma, which should be seriously investigated and

treated[11]. Birth control pills may help with improving the regularity of periods, excess hair growth, and acne. Metformin and anti-androgens may also help . Other typical acne treatments and hair removal techniques may be used[12]. Efforts to improve fertility include weight loss, clomiphene, or metformin. In vitro fertilization is used by some in whom other measures are not effective[13].

Follicle-stimulating hormone (FSH) is a hormone produced by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus. FSH plays a role in sexual development and reproduction in both males and females[14]. FSH regulates the development, growth, pubertal maturation and reproductive processes of the human body[15,16]. Luteinizing hormone (LH, also known as lutropin and sometimes lutrophin) is a hormone produced by gonadotropic cells in the anterior pituitary gland[17]. Effects in female LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells [17]. An autoimmune disease is a condition arising from an abnormal immune response to a normal body part, There are at least 80 types of autoimmune diseases, Nearly any body part can be involved [18].

Different autoantibodies have been documented in PCOS, for example, anti-ovarian, anti-nuclear (ANA), anti-thyroid, anti-spermatic, anti-SM, anti-histone, anti-carbonic anhydrase, and anti-islet cell antibodies. There is an association between PCOS and autoimmune diseases such as ANA and anti-thyroperoxidase (anti-TPO) that have been documented in systemic lupus erythematosus and Hashimoto thyroiditis, respectively, and it is suspected that there are autoantibodies that might affect the long term clinical management of these patients. Some autoimmune diseases such as lupus run in families, and certain cases may be triggered by infections or other environmental factors [19].

Materials and Methods

Subjects: This case control study included 60 female patients (aged 15-45 years) diagnosed with PCOS. Of those patients, 30 had high levels of LH and FSH (designated as G1) , while the other 30 with low-level of LH and FSH (designated as G2) . In addition, 30 age matched healthy females were included as a control group. Blood samples were collected from patients and control after acquiring permission. Serum was separated and stored at -20 C^0 until used.

The study was carried out in Al immamian al kadhmain medical city and Al-Dualy hospital during the period from October 2019 to January 2020. Ethical approval for this study was obtained from the ethical committee of the College of Medicine-Al-Nahrain University and was conducted in the microbiology department of this college.

Assay Procedure of FSH-R , LH-R, FSH & LH

1. All reagents and samples were brought to room temperature (18 - 25ºC) before use.

2. The removable 6-well strips were labelled as appropriate for the experiment.

3. One hundred microliter of each standard and sample was added into appropriate wells. Then wells were covered and incubated for 2.5 hours at room temperature with gentle shaking.

4. After incubation the solution was discarded and washed 4 times with 1X wash Solution. The washing was done by filling each well with wash Buffer (300 µl) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last washed, any remaining wash Buffer was removed by aspirating or decanting. then the plate was inverted and blotted against a clean paper towels.

5. After that 100 µl of 1X prepared biotin coated antibody was added to each well and Incubated for 1 hour at room temperature with gentle shaking.

6. The solution was discarded and the wash was done as in step 4.

7. The next step was adding 100 µl of prepared Streptavidin solution to each well and the plate was Incubated for 45 minutes at room temperature with gentle shaking.

8. Then the solution was discarded and washing was repeated as in step 4.

9. One hundred microliter of TMB One-Step Substrate Reagent was added to each well and Incubated for 30 minutes at room temperature in the dark with gentle shaking.

10. Lastly, 50 µl of Stop Solution was added to each well and the results read at 450 nm immediately.

Result

1- Age distribution

The mean ages of patients with high FSH and LH levels group1 (G1) was 28.90 (range of 15-45 years), while the mean age of patients with low FSH and LH levels group2 (G2) was 36.13 (range of 15-45 years). While the mean age of control group was 35 (range of 15-45), table (1) statistically, the result is significant at(p-value <0.05) .

Table(1): The comparison of Age distribution in the different study groups by ANOVA

Age	Study groups		
	G1	G2	Control
Mean	28.90	36.13	35
Std. Error of Mean	.992	.678	.1
Total Number	30	30	30
One-Way Repeated Measures ANOVA exact test statistic value	is < 0.00001. The result is significant at p-value <0.05		

2- Correlation between Anti-Follicle Stimulating Hormone(Anti-FSH)with PCOS in all the groups

The results showed a high correlation between group 1(G1) and the level of antibodies "Anti -FSH" formed, where the percentage was 93.3% ,while in group 2 (G2) was 0.0%, While in the control group all results were negative for antibodies. Statistically significant association between both groups as shown in the table (2).

Table(2): Correlation between Anti-Follicle Stimulating Hormone(Anti-FSH)with PCOS in all the groups

Anti FSH	Study groups					
	↑G1		G2		Control	
	No.	%	No.	%	No.	%
+Ve	28	93.3	0	0.0	0	0.0
-Ve	2	6.7	30	100	30	100
Total	30	100%	30	100%	30	100%
Cut off Anti FSH	0.475					
The Fisher exact test statistic value	0.00022 The result is significant at p-value <0.05					

3- Correlation between Anti- Luteinizing Hormone (Anti- LH) with PCOS in all the groups

All patients in group1(G1) ,30(100%) were positve for Anti -LH antibodies, while in group 2(G2) all the patients were negative, Almost all healthy controls were negative 29(96.7%) ,as shown in(table 3). The defference was signifigantly important at p-value <0.05 .

Table(3): Correlation between Anti- Luteinizing Hormone (Anti- LH) with PCOS in all the groups

Anti LH	Study groups					
	↑G1		G2		Control	
	No.	%	No.	%	No.	%
+Ve	30	100.0	0	0	1	3.3
-Ve	0	0.0	30	100	29	96.7
Total	30	100.0	30	100	30	100.0
Cut off Anti LH	1.46					
The Fisher exact test statistic value	0.00028 The result is significant at p-value <0 .05					

4 - Correlation between Anti- Ovarian Antibody and PCOS in all the groups .

The results showed a high correlation between group1(G1) and the level of antibodies (Anti Ovarian) where the percentage was 29(96.7%), while in group 2(G2) all the patients was negative(0%) , Compared with the control group most of them were negative 26(86.7%). Statistically significant association between both groups as shown in the table (4) .

Table(4): Correlation between Anti- Ovarian Antibody and PCOS in all the groups

Anti-Ovarian	Study groups					
	↑G1		G2		Control	
	No.	%	No.	%	No.	%
+Ve	29	96.7	0	0	4	13.3
-Ve	1	3.3	30	100	26	86.7
Total	30	100.0	30	100.0	30	100.0
Cut off Anti LH	0.75					
The Fisher exact test statistic value	0.00042 The result is significant at p-value <0 .05					

5 - Correlation between Anti FSH ,Anti LH and Anti ovarian in PCOS with all the groups

The results showed that there is a close correlation between the antibodies formed (Anti FSH, Anti LH and Anti ovarian) with the high FSH and LH group 1(G1) concentration of women with ovarian cyst and there is no clear correlation in the group of women with low FSH and LH group2 (G2), Statistically significant association between both groups.

Table (5): Correlation between Anti LH ؛ Anti FSH & Anti ovarian With group one (G1) and group two (G2)

Test Groups	Anti-FSH				Anti- LH				Anti-Ovarian				Total
	+Ve		-Ve		+Ve		-Ve		+Ve		-Ve		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
G1	28	93.3	2	6.7	30	100.0	0	0.0	29	96.7	1	3.3	30
G2	0	0.0	30	100.0	1	3.3	29	96.7	4	13.3	26	86.7	30
Control	0	0.0	30	100%	1	3.3	29	96.7	4	13.3	26	86.7	30
Cutoff	0.475				1.46				0. 75				
The Fisher exact test statistic value	0.0018. The result is significant at p-value <0 .05.												

6 - Correlation between LH-R and FSH-R in PCOS with all the groups

Table (6) shows the comparison of values of LH-R and FSH-R in PCOS between both group , in group 1(G1) was have highest value in low concentration LH-R 19(63.6%) and low concentration FSH-R 15(50%) ,while in in group 2(G2) was have highest value in high concentration LH-R 26(86.7%)and high concentration FSH-R 23(76.7%)), compared with control group were normal 30(100%). Statistically significant at P-value<0.05 .

Table (6): Association between LH-R & FSH-R With (G1 & G2)

Test Groups	LH-R			FSH-R			Total
	High	Normal	Low	High	Normal	Low	
G1	0.0 (0%)	11 (36.7%)	19 (63.6%)	0.0 (0 %)	15 (50%)	15 (50%)	30
G2	26 (86.7%)	3 (10%)	1 (3.3%)	23 (76.7 %)	6 (20%)	1 (3.3%)	30
Control	0.0 (0%)	30 (100%)	0 (0.0%)	0.0 (0%)	30 (100%)	0 (0.0%)	30
Normal Range	125-1200 ng/dl			110 – 1300 ng/dl			
The Fisher exact test statistic value	0.0079 The result is significant at p-value <0 .05						

Discussion

1- Age distribution

The results presented in this study showed that the mean age of patients with high FSH and LH levels (G1) was lower than the mean age for those patients with low FSH and LH levels (G2) . This is probably due to most of diseases relating to heredity appear during the early period of youth and this explains the appearance of autoantibodies (Anti-FSH and Anti-LH) earlier than the occurrence of hormonal or clinical diseases (G2). This result was consistent with Steven M. who investigated the Effects of age on the irregularity of LH and FSH serum concentrations in women and men and evaluated the apparent distinction between follicle-stimulating hormone (FSH) and luteinizing hormone (LH) dynamics; visually, it appears that the pattern of serum concentrations of FSH is more irregular than that of LH in younger human females.) (20).

2 -Correlation between Anti-Follicle Stimulating Hormone(Anti-FSH)with PCOS in all the groups

A high correlation was found between group1(G1) with the level of antibodies "Anti FSH". This explains the reason for the emergence of symptoms of decreased hormone concentrations(include irregular or stopped menstrual periods and infertility)Although ,the increase in its concentrations of hormone in the human body, as the body generates antibodies against these hormones and the formation of an immune complex that prevents their association with their own receptors and thus causes the appearance of symptoms of decreased hormone in group1G1 , But this condition was not apparent in the G2group that did not have antibodies against these hormones FSH and LH. Thus the deficiency in this group was due to its lack of secretion originally from the gland and it represents the common cases of polycystic ovarian syndrome ,This result was compatible with the study by Kadri, Haller, Andres who found that " increased concentrations of antibodies against FSH (anti-FSH) have been demonstrated in infertile women " [21] and this result also was compatible with another who showed that anti FSH antibodies were present in controls and their production decreased during pregnancy. Endometriosis and PCOS were associated with higher values of anti-FSH-immunoglobulin (Ig)A, anti-V14D-IgA, and endometriosis with anti-V14D-IgG, anti-FSH IgA could be a marker of ovarian disorders that cause infertility[22].

3 -Correlation between Anti- Luteinizing Hormone (Anti- LH) with PCOS in all the groups

The results of this study also showed a high correlation between (G1) and the level of antibodies (Anti LH), where the percentage was 100% compared to (G2) in which only 3.3 % had high Anti LH antibody. This result was similar to the previous result and probably due to the same causes that were mentioned above, due to the two hormones (FSH and LH) being bound together (function and location of secretion) , This result agreed the results reported in a previous study which reported that " Luteinizing hormone (LH) is in synergy with follicle stimulating hormone (FSH) that stimulates normal follicular growth and ovulation. FSH is frequently used in assisted reproductive technology (ART). these study have facilitated better understanding on the complementary role of the LH to FSH in regulation of the follicle "[23].

4 - Correlation between Anti- Ovarian Antibody and PCOS in all the groups

The present study showed a high correlation between group1 (G1) and the level of Anti Ovarian antibodies where the percentage was 96% compared to its percentage in group (G2) where it was 13%. From the previous results presented in this study it can be concluded that patients in the first group(G1) had an autoimmune disease and this explains the association of the anti-Ovarian antibody with the other antibodies (Anti – FSH and Anti – LH). This result was compatible with a previous study which had shown that different autoantibodies can be found in PCOS, for example , anti-ovarian, , anti-nuclear (ANA), anti-thyroid, and anti-islet cell antibodies [24].

5 - Correlation between Anti FSH ,Anti LH and Anti ovarian in PCOS with all the groups

The results in the present study also showed that there is a close correlation between the formed antibodies (Anti FSH ,Anti LH and Anti ovarian) with the high FSH & LH concentration in women with ovarian cyst. However, no clear correlation was found in the group of women with ovarian cyst who had low FSH and LH (G2). It can be concluded from these results that there

was a high correlation between (Anti FSH ,Anti LH and Anti ovarian), due to the genetic correlation between most autoimmune diseases, where the association between them is common, The percentages were high concentration (Anti FSH ,Anti LH and Anti ovarian) in the first group(G1) while all were low (Anti FSH ,Anti LH and Anti ovarian), in the group G2 [25].

6 -Correlation between LH-R and FSH-R in PCOS with all the groups

Investigating the Association between LH-R and FSH-R in both G1 and G2 groups of patients showed a high correlation between (G1) with the level of FSH-R, compared to FSH-R in (G2). On the other hand, a high correlation was found between women (G1) and the level of LH-R, compared to G2 ,This result explains why women suffer from symptoms of hormone deficiency as a result of a decrease in receptors necessary for hormone secretion. Furthermore, the results showed that in the G1 the receptors (LH-R and FSH-R) were in low concentration due to that the hormone FSH and LH concentration being higher than normal range ,and this did not stimulate the cells to review other receptors on their surface cell, but rather kept their secretion normal or low. On the other hand, it was observed in the second group G2 that there was an increase of receptors due to a decrease in the hormones (FSH and LH) secretion. FSH-R belongs to the family of G protein-coupled receptors (GPCRs), a family of receptors distinguished by the presence of various signaling pathway activation as well as formation of cross-talking aggregates. This result was consistent with another study which reported that " The function of FSH is performed upon binding to its cognate receptor—follicle-stimulating hormone receptor (FSHR) expressed on the surface of target cells (granulosa and Sertoli cells). Until recently, it was believed that FSHR occurred naturally as a monomer"[26,27].

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Correlation between Salivary Cotinine Levels and Cigarette Smoking with Recurrent Aphthous Stomatitis

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Abstract

Introduction: Recurrent aphthous stomatitis (RAS) is a widespread oral mucosal disease typified by the presence of a yellowish-grey base with high margins and flanked by an erythematous halo. RAS typically crops up in the lining or nonkeratinized mucosa. It has been found that smoking and salivary cotinine (a metabolic product of nicotine) levels have a protective effect against the occurrence of RAS by increasing keratinization of the oral mucosa.

Objectives: To identify the effect of smoking and nicotine level on the occurrence of RAS.

Patients and Methods: the study was conducted on 92 male students studying at different Universities of Baghdad, Iraq, in the period between January to December 2018. The students were thoroughly questioned about the yearly recurrence rate and healing period of RAS. Data about smoking history; method, average daily dose, and period of the behaviour were recorded for each student. Salivary Cotinine levels were measured by the Human Cotinine ELISA Kit and utilising the competitive ELISA technique based on the protocol of the manufacturer salimetrics assay #1-2002.

Results: The study subjects comprised of 92 male students. Their ages were from 18 to 25 years; the average age was 21.04 ± 2.2. Forty-two of them were light smokers, and 50 were heavy smokers. They were subdivided into subgroups according to the duration of smoking (less or more than five years). The annual recurrence rates and healing period were lower in those who smoked for more than five years than those who smoked for less than five years with a significant difference in light smokers. Salivary cotinine levels were significantly higher in a heavy smoker than in light smokers. Period of healing and yearly recurrence rates were significantly lower in those who had salivary cotinine level more than 200ng/ml than in those with a level.

Conclusion: The protecting impact of smoking on RAS was solely identified when individuals were heavy smokers or smoked for longer durations of time and correlated with cotinine levels, according to the available literature.

Keywords: *Nicotine, Recurrent aphthous stomatitis, nicotine, smoking*

Introduction

Recurrent aphthous ulcer (RAU) is also called recurrent aphthous stomatitis (RAS), is a key prevalent disorder of the oral mucosa [(1)], which is frequently indicated on the buccal mucosa, labial mucosa, floor of the mouth, tongue, and palate [(2)] (Fig. 1). RAU is typified by the presence of a yellowish-grey base with high margins and flanked by an erythematous halo. [(3)]

Figure 1. Small ulcers of RAS minor-kind (Mikulicz ulcer). These ulcers are painful. (2)

The annual prevalence (AP) of RAS is up to 25% in the world population, with a recurrence rate of 50% every three months.(4) Medically, RAS is grouped into minor, major, and herpetiform types. Moreover, 85% of subjects with RAS exhibit the minor form, where ulcers are not greater than 1 cm in diameter and healing is not correlated with scarring. The dominant type of ulcers is higher than 1 cm in diameter, proceed to a longer time, clocking months, and typified by scar production. Herpetiform ulcers occur as groups of multiple tiny ulcers scattered all through the nonkeratinized oral mucous membrane. (5) RAS elicts difficulty in eating, talking, swallowing, and movement of the tongue, which reduces the patients' quality of life and adversely impacts the somatic and psychological parts of personal satisfaction. (6)

The illness progression consists of the following phases: Symptoms typify premonition (24 h) but without noticeable symptoms of disease; pre-ulcerative (between 18 h and three days) is characterized by erythema and mild oedema; ulcerative (116 days) is typified by progressive ulcer; healing (435 days, normally <21 days) is typified by reduced symptoms and improved healing; and remission, without any evidence of ulcers (7). The ulcerative and remission stages could be assessed with higher impartiality on dental assessment. Recurrent disease is associated with the emergence of new ulcers. (8)(9)

The causes and pathogenesis of RAS are not fully understood. Various features are related to the progression of this disease, not excluding an affirmative family history, food hypersensitivity, stopping of smoking, psychosomatic anxiety and immune disorder. (10) Nonetheless, concerning this evidence, there is usually insufficient statistical risk evaluation. Immune dysfunction connected to many factors could promote the development of RAS. Recent vast bioinformatics assessments have proven the impacts of the immune system and inflammatory processes (11)(12)

Among some of the suggested features on tobacco utilization is the most arguable one and baffled one. A study has revealed that smoking could increase the prevalence of the Recurrent aphthous ulcers (RAU)(13). Monetheless, other studies have revealed a negative relationship between the occurrence of the RAU and smoking. (13)(14)

Two possible theories centre on the preventive effect of smoking on RAUs (15)

1. Cigarette smoking causes the prevention of aphthous ulcers by leading to increased localized keratinization of the oral mucosa. (15)

2. Nicotine, through various probable actions, could cause the modulation of local immune responses. This cause induction of T-cells energy and inhibition of the generation of pro-inflammatory cytokines, such as interleukin (IL)-2, IL-6, IL-8, IL-10 and tumour necrosis factor α. It could elicit the activated discharge of adrenocorticotrophic hormone and cortisol to lead to leading additional inhibition of inflammatory pathways. (15)

Studies have shown the beneficial impacts of smoking and the symptomatic improvement of aphthous ulcerations. The harmful association between the RAU and tobacco undermines damaging consequences of smoking to health. Nonetheless, it elicits a view focused on the possible use and inhibitory impacts of smoking on the incidence of aphthous ulceration. (13) (16) (17)

Nicotine is a substance that causes addiction to cigarettes and smokeless tobacco. Its half-life is nearly 2–3 hours in the blood. Nicotine metabolism is in the liver. Its critical metabolic pathway is C-oxidation to cotinine, a nicotine metabolite. Up to 80% of nicotine is changed to cotinine. Cotinine could be used as an indicator of exposure to tobacco. Cotinine in vivo possesses a half-life of nearly 20 hours. As a result of its longer half-life, there is an accumulation of cotinine in the body all through the day. Moreover, the elimination of cotinine takes longer time than that of nicotine (18)

Cotinine could be assessed in body fluids such as a serum, saliva, and urine [19] Measuring cotinine in the saliva is a safe and convenient method for assessment of exposure to nicotine [20], studying of the amounts of nicotine and cotinine in stimulated and unstimulated saliva. The degree of nicotine and cotinine in stimulated saliva was considerably higher compared to that of the unstimulated saliva. [18]

Patients and methods

The study was performed as one-on-one interviews with a random sample of 92 male participants schooling at different Universities of Baghdad, Iraq, in the period between January to December 2018. The participants were supplied adequate information on RAS and exhibited images of characteristic cases of the condition. The images of recurrent herpes labial were revealed to them to distinguish between the two states. Students that concurred to take part in the study were later questioned if they had RAS in the last one year. The participants with a positive history of RAS were questioned on the yearly recurrence rate and the number of ulcers and healing duration in the previous occurence. This was followed by the collection of demographic characteristics details of all the students. Data on smoking history, approach, mean daily dose, and period of the habit were obtained for all students. The mean number of cigarettes smoked all through life was calculated for those who smoked cigarettes. This is the mean of day by day smoked cigarettes multiplied by the duration of behaviors in days. The classification of cigarette smokers was grouped into heavy smokers (smoking 20 cigarettes/day) and light smokers (smoking 1–10 cigarettes/day),

Before any dentistry intervention, stimulated saliva samples were gathered from all participants in the morning (9-11 AM). Firstly, the subjects' mouths were rinsed with water, and they were asked to chew flavour-free gum for one minute and gather their saliva in disposable cups for 5 minutes. The saliva samples gathered and then transferred to test tubes, and they were kept at -70 °C for further test. Cotinine contents of all samples were assessed by the Human Cotinine ELISA Kit and utilizing the competitive ELISA approaches regarding the protocol of the manufacturer salimetrics assay #1-2002.

Data Analysis

Data analysis was performed using SPSS-19. Kolmogorov-Smirnov test was utilized for evaluating the normal distribution of the variables. The comparison of salivary cotinine levels was made by utilizing the independent t-test—healing period& Recurrence rate between the three groups(non-smokers, light smokers &heavy smokers). The linear regression was utilized for the determination of the correlation between salivary cotinine levels and tobacco consumption. $P<0.05$ was regarded as substantial.

Results

The study participants comprised 92 male students with ages ranging from 18 to 25 years. The mean age of the study group was 21.04±2.2 figure 1. 42 of them were light smokers, and 50 were heavy smokers. They were subdivided into subgroups according to the duration of smoking (less or more than five years)

The annual recurrence rates and healing period were lower in those who smoked for more than five years than those who smoked less than five years with a significant difference in light smokers as shown intable1

Salivary cotinine levels were significantly higher in a heavy smoker than in light smokers as shown in table 1

Duration of healing and annual recurrence rates were significantly lower in those who had salivary cotinine level more than 200ng/ml than in those with a level ≤ 200, as shown in table 2.

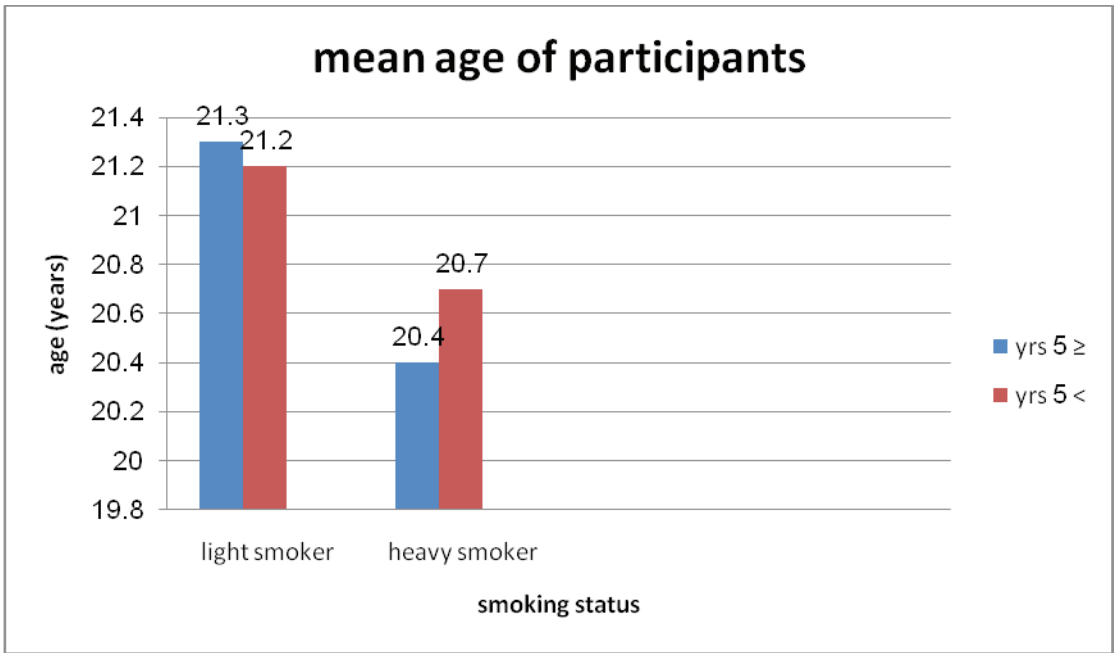

Figure 1: Mean age of the study group.

Table 1: Correlation between smoking status with healing period, recurrence frequency and salivary cotinine levels.

Smoking status	Duration of smoking (years)	Healing period (days ± SD)	P-value	Recurrence frequency (No. ± SD) /year	P-value	Salivary cotonine(ng/ml ± SD)	P-value
Light smoker (1-10)	≤ 5	5.7 ± 1.1	0.04	5.1 ± 1.7	0.04	76.83 ± 27.5	0.03
	> 5	4.7 ± 0.9		4.0 ± 1.5			
Heavy smoker >20	≤ 5	5.1 ± 1.1	0.12	4.6 ± 1.7	0.2	285.67 ± 58.7	
	> 5	4.6 ± 0.7		3.9 ± 1.5			

Table2: Correlation of salivary cotinine level with healing period and recurrence frequency.

Salivary cotonine(ng/ml)	Healing period (days ± SD)	P-value	Recurrence frequency (No. ± SD) /year	P-value
≤ 200	5.3 ± 1.2	0.03	4.8 ± 1.5	0.04
>200	4.6 ± 0.8		3.9 ± 1.4	

Discussion

Some are of the view that smoking has a protective function in the incidence of RAS. Its protecting function is associated with the existence of elevated keratinization of oral mucosa in smokers. (21) The keratin layers serve as mechanical and chemical barriers against the attack of microbes or trauma. The enhanced keratinization in oral mucosa reduces susceptibility to wound and inflammation. Some are of the view that RAS is less widespread in smokers. Previous studies divulged that nicotine is being utilized as a thriving therapy for aphthous stomatitis and Behçet's disease. Studies have suggested that nicotine could be the causative agent for the decrease in incidence rates of RAS in smokers. (21)(22)

Through the induction of adrenal steroid generation via the hypothalamus-pituitary-adrenal axis, nicotine affects the immune response in inflammatory conditions. By decreasing the production of TNF-α and interleukins 1 and 6 via its direct impact on macrophages nicotine also elicits effects on the immune response in inflammatory diseases. Nicotine also reduces the secretion of proinflammatory cytokines (TNF-α). Nicotine is a stimulant in lesser dosages, yet at high ones, it prevents the action of autonomic nerves and skeletal muscle cells. It elicts its influences through action on receptors, namely nicotine acetylcholine receptors that are found in autonomic ganglia, adrenal medulla, and on the CNS. (24) They encourage cholinergic receptors and circuitously on the discharge of dopamine.

Nicotine replacement therapy (NRT) is considered as medicinal formulas of nicotine, which can be used to deliver nicotine into the circulatory system with its beneficial effects without smoking and its adverse effects. (23)(24) Only fewer authors proposed that smokers could be less psychologically strained than nonsmokers. They also believe that some psychosomatic factors could influence RAS progression. The correlation identified in this study between heavy cigarette smoking and less prevalence of RAS even for durations not greater than five years indicates that nicotine may be implicated in the preventive measures against the incidence of RAS. It has also been reported that the protecting impacts of nicotine is dose-reliant.

For the concentration of nicotine inhaled via tobacco smoke is relatively little, a large per cent of the substance are subject to destruction by the heat. Smoking of cigarettes in degrees surpassing a packet per day could lead to a protective nicotine dose. Enhanced keratinization of the mucosa and accordingly, its low vulnerability to ulceration may describe the result that moderate smokers for periods higher than five years had a lower risk of having RAS. In contrast, moderate smoking had no defensive impact on RAS, in durations not greater than five years. As revealed in this study, smoking of around 36,500 cigarettes was required for the development of a protective layer of keratin (18).

Substantial variations in the incidence of RAS in the cigarette smokers' group were associated with the dose and duration of the smoking habit. However, the results of this study included a probable negative epidemiological relationship between smoking and RAS. The "protective effect" of tobacco on RAS was only observed when persons were heavy smokers or smoked for longer durations of time (2)

Conclusion

The study indicated the relationship between the prevalence of RAU and smoking. The correlation that occurs between smoking and aphthous ulcerations of the mouth is negatively correlated. When an individual was a heavy smoker or smoked for longer durations of time, this decrease incidence of RAS and increased salivary levels of cotinine, and this is "protective effect" on RAS.

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Contaminant Investigations of Regularly used Eye Contact Lenses

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Abstract

Microorganisms, particularly bacteria, are considered to play a role in the etiology of certain corneal infections observed during soft contact lens wear. This study explored the possibility of microbial colonization of soft contact lenses as a risk factor leading to corneal infection. In a clinical trial conducted and 86 lenses were recovered aseptically and analyzed for microbial colonization. The incidence of bacteria and fungi was fewer during corneal infection than during asymptomatic lens wear. During asymptomatic lens wear, corneal infection bacteria were isolated most frequently and were usually normal external ocular microbiota. Of the Gram-positive bacteria, the incidence of *Staphylococcus aureus* was fewer during asymptomatic wear. While Gram-negative bacteria were seen in high cases during asymptomatic wear, this includes *Escherichia coli* and *Pseudomonas aeruginosa*. Fungal screening and identification revealed 2 cases of contamination with the yeast *Candida albicans* that able to form a biofilm. Colonization of soft contact lenses with pathogenic bacteria, especially gram-negative and gram-positive bacteria, appears to be a significant risk factor leading to corneal infection.

Keywords*: Biofilms; contact lenses; antibiotic discs.*

Introduction

Contact lenses are increasingly being used for cosmetic or therapeutic purposes. Lack of compliance and poor hygiene towards lens care is strongly associated with microbial contamination and has been proved to result in eye infections(1). Contact lens wear has become the most significant risk factor for corneal ulceration in patients with previous healthy eyes. With the widespread use of contact lenses for cosmetic and optical advantages, the public health risks associated with contact lens are still an area of concern. Despite advances in lens design and materials, bacterial infection and biofilm-forming microbes is a vision-threatening problem in contact lenses wearer. Insufficient knowledge of proper lens handling and use may be an important factor(2).

One of the risk factors for eye disease in contact lens users is the introduction of pathogens into the eye, as microorganisms can adhere and multiply in this environment. Another risk factor of wearing contacts is corneal hypoxia, which compromises the integrity of the epithelium and creates an entry point for microorganisms(3).

When contact lenses become contaminated, the contact lens case then becomes a potential source of pathogens, especially when it is not properly cleaned and disinfected. Thus, even cleaning and sterilizing contact lenses does not imply an innocuous environment since the source could be a contaminated case. This has been confirmed in studies showing that microorganisms isolated from contact lenses that are associated with infectious eye diseases are identical to those isolated from the corresponding contact lens cases(4).

Fungi are a major global cause of infectious keratitis, especially in tropical regions involving the genera *Aspergillus,* and *Candida*(5). The use of contact lenses is the primary risk factor for the development of bacterial keratitis, which in turn increases the risk of infectious keratitis by 10-15-fold when contact lenses are used for a limited number of hours per day when compared to extended wear. About 90% of cases of bacterial keratitis

are caused by *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Escherichia*, *Klebsiella,* and *Proteus*. Frequently found organisms include *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia* sp., and *Proteus* sp. The first organism isolated from infectious keratitis in contact lenses users is *P. aeruginosa*[6].

This work was a trial to prove the presence of dangerous infectious points within contact lenses biofilm, and following up on some elected patients through the presence of contact lenses use, ophthalmic complaints, and signs while confirmed by the microbial scan of contact lenses for detection of the main microbial forming biofilms and causes of ocular infection.

Materials and Methods

Specimen's collection

A total of 109 disposable contact lenses were collected from the persons who use the contact lens daily. The lenses and storage liquid were taken and cultured on nutrient agar for 24hrs., at 37°C. Lenses were vortexed for 1min. in the 2mL transport medium, were transferred into 5mL of tryptic soy broth (TSB) and incubated at 37°C for 24-48hrs. TSB tubes that appeared to be turbid were streaked onto two culture media, first cultured on Nutrient agar, Eosin Methylene Blue agar, Mannitol Salt agar, MacConkey agar, Blood agar, and Simmon′s Citrate agar; the second was used to streak a Sabouraud dextrose agar plate. The agar plates were incubated under aerobic, 5%CO_2 and anaerobic conditions for 48hrs. Also, an SDA plate was maintained at room temperature for seven days; this was used for the cultural identification of bacteria and fungi. After that, the biochemical tests were used to support cultural properties, which included catalase, coagulase test, mannitol fermentation, hemolysin, DNase with methyl green, and oxidase. The identity of the isolates has been confirmed through a PCR based on the identification of the species-specific primers for each bacteria and fungus.

Bacterial genomic DNA extraction

Genomic DNA was extracted from pure cultures using the Presto™ Mini gDNA Bacteria Kit following the manufacturer's instructions. Extracted genomics was stored at –20°C before running PCR. The NanoDrop1000 spectrophotometer was used to evaluate the concentration and purity of DNA at 260nm.

Fungal DNA Isolation

DNA extraction was carried out using CTAB method as described by[7]. Briefly, a full loop of fresh yeast colonies was suspended in 700µL of CTAB buffer followed by bead beating for 3min. After incubation for 60min at 55°C, 700µL of phenol-chloroform was added. Upon vortexing and centrifugation for 20min at 14000×*g*, 4°C, 400µL of supernatant was added to isopropanol. Finally, upon washing with 70% ethanol and drying the DNA samples on air, the pellets were suspended in Tris-EDTA buffer.

Amplification of identification genes

To the detection of the identification genes, PCR amplification was carried out with a final volume of 1µL of elution buffer in 25µL. After the addition of 12.5µL of 2× Mastermix, then 1µL of each primer added into the tube, and the volume completed with free nuclease water at 25µL. Ten µL of the amplification products were subjected to electrophoresis using 1.2% agarose gel with a GelRed DNA stain and 100bp DNA ladder used as a marker. All the genes name, primer sequences, PCR programs, and amplicon sizes are set in table (1).

Table 1. Primers sequences used for PCR amplification of *oprL*, 16S rRNA, *vicK,* and *hwp1* genes

Gene name	Primer sequence (5′–3′)	Amplicon size (bp)	PCR program	References
oprL P. aeruginosa	ATG GAA ATG CTG AAA TTC GGC CTT CTT CAG CTC GAC GCG ACG	504	30 cycles of denaturation 92°C; 60s, annealing 59°C; 60s, extension 72°C; 60s.	(8)
16S rRNA E. coli	GAC CTC GGT TTA GTT CAC AGA CAC ACG CTG ACG CTG ACC A	585	30 cycles of denaturation 94°C; 45s, annealing 55°C; 45s, extension 72°C; 60s.	(9)

Cont... **Table 1. Primers sequences used for PCR amplification of *oprL*, 16S rRNA, *vicK,* and *hwp1* genes**

vicK S. aureus	CTA ATA CTG AAA GTG AGA AAC GTA TCC TGC ACA ATC GTA CTA AA	289	35 cycles of denaturation 94°C; 40s, annealing 50°C; 40s, extension 72°C; 60s.	(10)
hwp1 C. albicans	GCT ACC ACT TCA GAA TCA TCA TC GCA CCT TCA GTC GTA GAG ACG	976	30 cycles of denaturation 94°C; 45s, annealing 58°C; 40s, extension 72°C; 55s.	(11)

Antibacterial susceptibility studies

The antibiotic sensitivity for bacteria to twenty antibiotics which include Cefotaxime (30µg), Penicillin (10IU), Vancomycin (3µg), Amoxiclav (30µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg), Tobramycin (10µg), Clindamycin (2µg), Cephalothin (30µg) and Ampicillin (10µg) against Gram-positive, and Aztreonam (AT 30 µg), Amikacin (30µg), Ceftriaxone (30µg), Amoxiclav (30µg), Ampicillin (10µg), Cefoxitin (30µg), Imipenem (2µg), Nitrofurantoin (300µg), and Tobramycin (10µg) against Gram-negative bacteria was determined according to Clinical and Laboratory Standards Institute (CLSI) standards [12]. Twenty mL of Muller Hinton agar (MHA) melted and cooled at 45°C was poured into sterile Petri dishes and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 100µL inoculums ($1.5*10^8$ CFU/mL) according to 0.5 McFarland standard solutions with the help of a sterilized swab onto the entire surface of the MHA plate.

Antifungal susceptibility test

The disc diffusion test was performed according to the procedure described by[12]. Cell suspensions of *C. albicans* isolates were prepared in 2mL sterile saline solution. The turbidity was adjusted to approximately $5*10^3$ cells/mL. Five antifungal agents were tested which including Amphotericin B (100U), Nystatin (100U), Fluconazole (10µg), Ketoconazole (10µg), and Itraconazole (10µg).

Assessment of biofilm formation

Biofilm activity was assessed with microtiter plate (MTP) methods. Briefly, each strain was grown overnight in the appropriate medium, YEPD (yeast extract 1%, peptone 1%, dextrose 1% added with 1.7% agarose) for *C. albicans* and BHI (Brain Heart Infusion broth) for bacteria, at 30ºC in shaker at 180×*g* and then harvested and centrifuged at 3000×*g* for 5min at 4ºC. The supernatant was removed, and the pellet was washed twice with Phosphate Buffered Saline (PBS). The washed cells resuspended in a TSB to obtain a final density of $1*10^6$ cells/mL, by adjusting the density at OD600. One hundred µL of this standardized cell suspensions were cultured in each selected well of a microtiter plate; one uncultured as the negative control. Three different replicates for each strain were set into each well. These plates were then closed, sealed, and incubated for 2hrs., at 37ºC. After biofilm surface priming, the medium was removed carefully; each well was subsequently washed three times with PBS. After that, 100µL of the appropriate medium was added to the wells. Each plate was then closed, sealed again, and incubated for 24hrs. at 37ºC to permit the biofilm development. The plates were stained with 1% crystal violet (100µL/well) for 15min; then washed three times with water and dried at room temperature, and the absorbance of adherent biofilms were measured with an ELISA plate reader at 630nm[13]. Four biofilm density categories were used to grade the biofilms based on the established optical density cut-off values (ODc) (Table 2), which were derived from the mean values of negative controls (mean ODnc) summed with three standard deviations of the negative controls (3×SDnc):

$$ODc = ODnc\ mean + (3 \times SDnc)$$ [14].

Table 2. Classification of biofilm formation abilities by MTP method

Cut-off value calculation	Mean of OD_{570} values results	Biofilm formation abilities
ODnc>4×ODc	OD>0.557	Strong
2×ODc<ODnc≤4×ODc	0.278<OD≤0.557	Moderate
ODc<ODnc≤2×ODc	0.139<OD≤0.278	Weak
OD≤0.139	OD≤0.139	None

Results and discussion

The study population consisted of 58 (67.44%) females and 28 (32.56%) males with the predominant species isolated in the study were *P. aeruginosa* (41.86%) and *S. aureus* (33.72%), followed by *E. coli* (22.09%). In contrast, *C. albicans* was isolated less frequently (2.33%). When compared to the current research work, one can easily notice that *P. aeruginosa* is the most frequent bacterial type associated with daily, as well as occasional wear of contact lenses (Table 3). Sharing contact lenses can result in the spread of microorganisms that can severely damage the eyes, and contact lenses can spread infectious diseases. Therefore, people should only wear contact lenses prescribed specifically for them by a qualified eye care professional.

On the other hand, fungal studies confirmed that two of the lenses were contaminated with biofilm-forming *C. albicans,* which is one of the most common fungal pathogens. This biofilm-forming yeast can easily infect the eye and cause candidiasis. Their ability to form biofilm on lens surfaces considered to be a most hazardous oversight risk in wearing reusable lenses.

Table 3. Distribution of the species isolated from contact eye lenses.

Species	Total no. (%) of isolated
S. aureus	29 (33.72)
P. aeruginosa	36 (41.86)
E. coli	19 (22.09)
C. albicans	2 (2.33)
Total	86 (100)

Identification of bacterial and fungal isolates

Out of 109 samples included in this study, some cases were found to clear of contamination, that is to say, 78.89% (86) of the persons were practiced contact lenses contamination based on biochemical tests (Table 4). Such a percentage of contamination gives a clear idea about the prevalence of bacterial contamination among disposable contact lenses wearers. All isolates were examined for the presence of the species-specific primers to confirm the species; isolates were approved based on the analysis of PCR products (Figure 1).

Two clinical isolates previously identified as *C. albicans* by standard microbiological methods. The expected 976 bp DNA fragment was amplified from clinical methods. The amplified products of positive *C. albicans* samples are shown in Fig. 1D.

Table 4. Identification of microorganisms according to biochemicals and molecular technique.

Identification patterns	Microorganisms			
	S. aureus	**P. aeruginosa**	**E. coli**	**C. albicans**
Eosin methylene blue agar	NA*	Not growth	MSC	NA
Mannitol salt agar	+	–	–	–
MacConkey agar	NA	NLF	NLF	NLF
Blood agar	β–hemolysis	β–hemolysis	ᵞ–hemolysis	NA
Simmons citrate agar	+	+	+	–
Nutrient agar	NP	PF	NP	NA
Sabouraud dextrose agar	NA	NA	NA	Cream colonies
Catalase	+	+	+	NA
Coagulase	+	–	–	NA
DNase	+	–	–	NA
Oxidase	–	+	–	NA
CR gene	NA	NA	NA	+ 2
oprL gene	NA	+ 36	NA	NA
vicK gene	+ 29	NA	NA	NA
16S rRNA gene	NA	NA	+ 19	NA
Total positive n. (%)	29 (33.72)	36 (41.86)	19 (22.09)	2 (2.33)

*: NA: not applicable; MSC: metallic sheen colonies; LF: lactose fermentation; NLF: non-lactose fermentation; NP: no pigmentation; PF: pigment production.

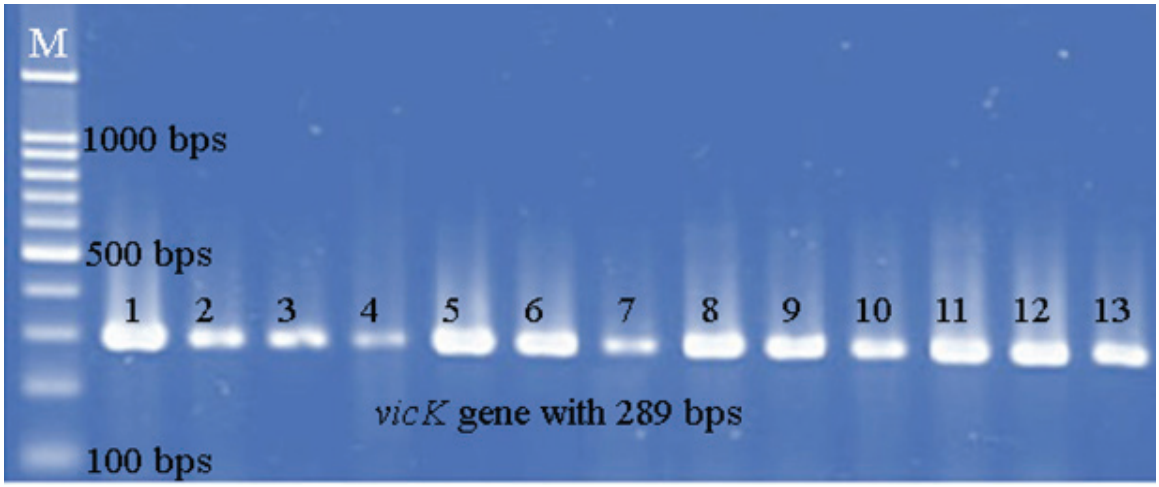

Figure 1A: Gel documentation of PCR products of *vicK* gene. Lane M is a DNA ladder 100bp. Lanes 1–13 DNA products from *S. aureus* isolates with 289bp.

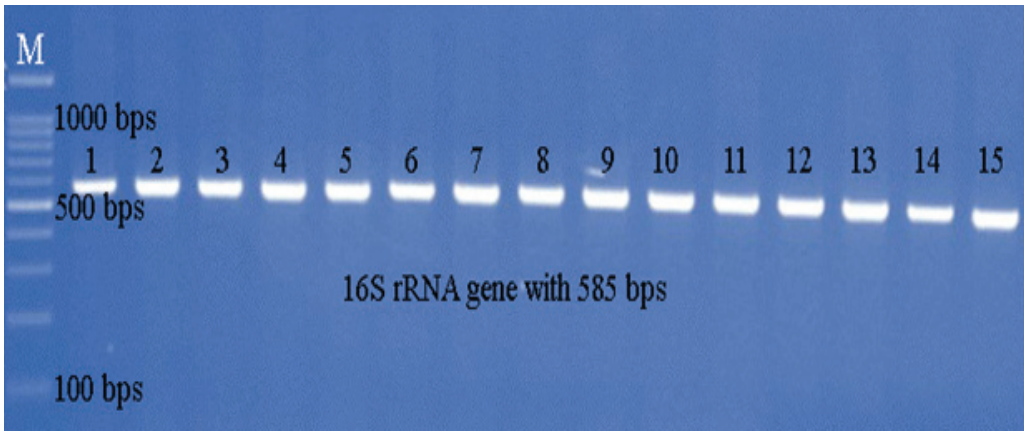

Figure 1B: Gel Electrophoresis for PCR Product for 16S rRNA Gene of *E. coli* **on 1.2% agarose gel at 7 volt /cm for 1 hr. with a 100 bp DNA ladder. Lanes 1-15** *E. coli***.**

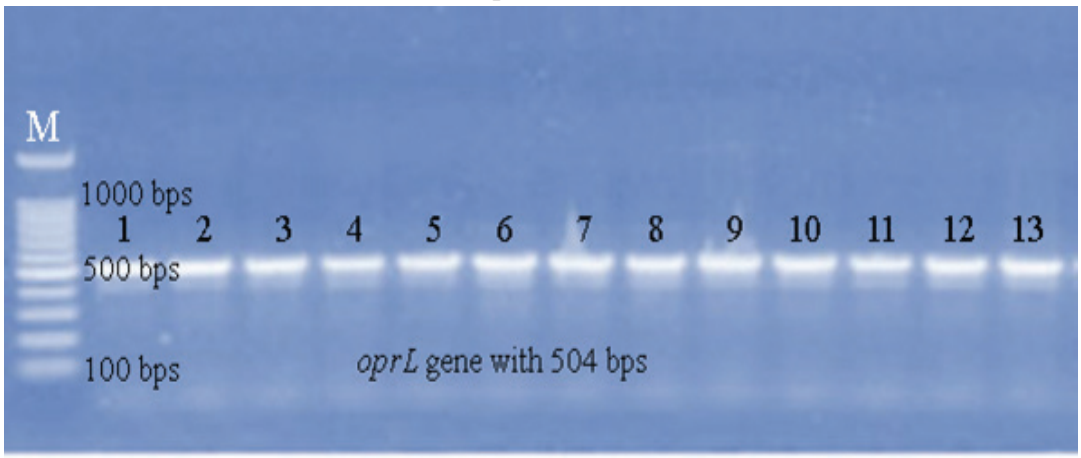

Figure 1C: PCR products *oprL* **gene with 504 bps amplified by the designed primers; Lanes 1-13:** *P. aeruginosa***; Lane M: 100 bp DNA ladder.**

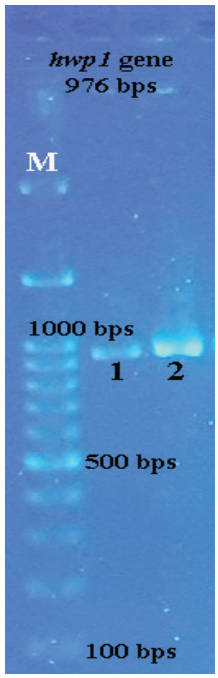

Figure 1D. Gel electrophoresis for amplification of the *hwp1* **gene. Lane M is 100 bp DNA ladder. Lanes 1-2 were** *C. albicans***.**

In the study of[15], a total of 50 samples were collected from female students who are wearing contact lens. Out of the 50 lenses, 44 (88%) samples were contaminated. The most commonly isolated bacteria were *P. aeruginosa* (23%), followed by *S. aureus* (18%), *E. coli* (16%), *K. pneumoniae* (9%), *Micrococci spp* (7%) and finally least bacteria *P. mirabilis* (5%) were identified. Out of the 156 contact lenses were collected by[16], they examined and found that 54 (34.62 %) showed 'No growth.' Contact lenses with bacteria-contaminated 94 (60.26 %) contact lenses. A total of 35 (22.44 %) of the contact lenses had mixed flora. It was observed that 35 lenses showed pathogenic organisms. *S. epidermidis* was found in 6 (3.85 %) lenses. *E. coli* in 22 (14.74 %), *Klebsiella* spp. in 20 (12.82 %), and *S. aureus* in 7(4.49 %) lenses were observed. Out of a 100 contact lenses were collected by[17], 65%, 89%, and 32% had contaminated contact lenses, lens storage cases, and conjunctiva, respectively. *S. epidermidis* 44.6%, *P. aeruginosa* 35.4%, *S. aureus* 12.3%, and *Bacillus* sp. 7.7% were isolated.

Khan and Cerniglia[18] used the PCR assay for the identification of *P. aeruginosa* from clinical samples by specific primers for the *exoA* gene. Their results showed that this method has high specificity than biochemical methods. Lanotte et. al.,[19] conducted another study for the detection of *P. aeruginosa* using a couple of primers for *oprL* and *exoA* genes. The results illustrated that PCR with the *oprL* gene produced higher sensitivity and specificity than the *exoA* gene; this is following our research. All *P. aeruginosa* isolates were positive for *opr*L, a conserved gene of *P. aeruginosa* with a specific amplicon size of 504 bp[20].

Nineteen isolates of *E. coli* were identified in the laboratory-based on microbiological standard methods and molecular methods. Out of 109 samples, 22.09% were identified as *E. coli.* In MacConkey agar plates, the samples showed bright pink or red colonies and on EMB agar, the colonies produced by *E. coli* are black color colonies with a metallic sheen. *E. coli* showed a negative result coagulase test. PCR primers targeting the 16S rRNA gene of *E. coli* amplified at 585 bp amplicon of DNA and confirmed the identity of *E.* coli, and the result of PCR for *E. coli* was shown in Figure 1B.

C. albicans are common fungal agents cause diseases with severity ranging from benign to potentially life-threatening infections, with the most common yeasts being the *Candida* species. *C. albicans* remains the predominant species causing over half of all the yeast infection cases in the world[21]. *C. albicans* colonies were characterized by creamy colored pasty colonies within 48-72 hrs.[22]. In the study of Abastabar et. al.,[23], a newly simple PCR on the *hwp1* gene was optimized and tested on 70 clinical of *Candida* species isolates. The *hwp1* gene was successfully amplified for all strains using new specific primers. It generated PCR products from 976 bp for *C. albicans,* providing specific patterns for the differentiation of this species.

Antibiotics susceptibility patterns

Table (5) had shown the result of antibiotic susceptibility of ten antibiotics against each of *S. aureus*, *P. aeruginosa* and *E. coli*. All three bacteria showed a different range of resistant patterns, where the resistant percentage of *S. aureus* isolate no. 1 and no. 2 were 60% against all antibiotics while isolating no. 3 show fewer resistant percent about 10%. On the other hand, *P. aeruginosa* demonstrates the two types of resistant percent, which include 40% and 20% for isolate no. 4, 5, and isolate no. 6, 7 respectively beside antibiotics. However, both isolates of *E. coli* shown lower resistant percent to antibiotics, which ranges from 20–40%.

This result strongly confirmed the result obtained by Al-Zahrani[24], which investigated his study of the prevalence and types of microbial contamination associated with contact lenses used by a group of lens wearers. His results showed that a total of 178 strains was isolated, including 100 Gram-positive and 78 Gram-negative bacteria, *S. epidermis,* and *P. aeruginosa* was the most common isolated. All isolates were susceptible to the tested aminoglycosides and fluoroquinolones. Ciprofloxacin were more efficient than β–lactams. About 91.67% of the strains had intermediate-resistant to Cefotaxime; 4.17% were resistant, and 4.17% were sensitive. Near the 95.83% of the isolates were sensitive, while 4.17% were resistant to Imipenem. 20.83 and 12.5% of *P. aeruginosa* strains were resistant and susceptible to Ceftriaxone, respectively, while 66.67% were moderate–resistant to Ceftriaxone.

Table 5. Antimicrobial susceptibility patterns of microorganism's species.

Antibiotics	Resistant percent of microorganisms			
	S. aureus n. (%)	P. aeruginosa n. (%)	E. coli n. (%)	C. albicans n. (%)
Amikacin	NA*	0 (0.0)	5 (26.31)	NA
Amoxiclav	19 (65.51)	27 (75)	10 (52.63)	NA
Ampicillin	19 (65.51)	27 (75)	1 (5.26)	NA
Aztreonam	NA	27 (75)	3 (15.78)	NA
Clindamycin	19 (65.51)	NA	NA	NA
Cephalothin	19 (65.51)	NA	NA	NA
Ceftriaxone	NA	27 (75)	14 (73.68)	NA
Ciprofloxacin	10 (34.48)	NA	NA	NA
Cephalothin	NA	27 (75)	0 (0.0)	NA
Cefotaxime	0 (0.0)	NA	NA	NA
Cefoxitin	NA	0 (0.0)	5 (26.31)	NA
Imipenem	NA	9 (25)	0 (0.0)	NA
Nitrofurantoin	0 (0.0)	0 (0.0)	11 (57.89)	NA
Penicillin	19 (65.51)	NA	NA	NA
Tobramycin	0 (0.0)	0 (0.0)	9 (47.36)	NA
Vancomycin	19 (65.51)	NA	NA	NA
Amphotericin B	NA	NA	NA	1 (50)
Nysitatin	NA	NA	NA	0 (0.0)
Fluconazole	NA	NA	NA	2 (100)
Ketoconazole	NA	NA	NA	2 (100)
Itraconazole	NA	NA	NA	2 (100)

*: NA: not applicable.

Evaluated isolates of *P. aeruginosa* for different antibacterial were done by Wahdan et. al.,[20]. They were entirely sensitive to streptomycin (100%), highly sensitive to gentamycin, tetracycline, ciprofloxacin, and sulfamethoxazole/trimethoprim (90.9, 90.9, 90.9, and 81.8%, respectively), and highly resistant to amoxicillin (63.6%). Surprisingly, 4 out of 11 evaluated isolates (named P2, P5, P8, and P9) showed resistance to more than three antimicrobial agents and were defined as MDR strains.

The results of the antimicrobial susceptibility test of *E. coli* were performed by Islam et. al.,[9] showed that 8 isolates were susceptible to azithromycin, 10

isolates to streptomycin, 13 isolates to gentamicin, 11 isolates to tetracycline and 10 isolates to streptomycin. Furthermore, 12 isolates were resistant to amoxicillin, and 11 isolates were resistant to erythromycin.

S. aureus isolates were highly susceptible to penicillin, rifampicin, ampicillin, and novobiocin. In contrast, most isolates was shown high resistance to oxacillin, sulphatrimethoprim, vancomycin, and cefotoxin[25]. Our results agreed with those of Sciezynska et. al.,[26], who showed that *S. aureus* was of high sensitivity to penicillin, rifampicin, and ampicillin, while in contrast to our results, isolates were shown the lower sensitivity to sulphatrimethoprim, oxacillin, vancomycin, and cefotoxin. Multiple antibiotic resistance could be detected with 98 % of the *S. aureus* isolates, giving a wide variety of resistance phenotypes among the isolates. The resistance was tested by Shrief et. al.,[27] of the 100 isolates of *C. albicans* against antifungal drugs, fluconazole, caspofungin, and itraconazole was 8% for each of them were shown resistant and 9% of isolates were revealed resistant toward amphotericin B.

biofilm formation by MTP assays

In vitro results of biofilm formation by MTP assay show that 32.56% of the isolates demonstrated strong biofilm, 12.79% were moderate biofilm, 18.6% weak biofilm, and 31% of the isolates were classified as non-biofilm former (Table 6). On the other hand, there are differences among species to form a biofilm, in which 13 isolates of *P. aeruginosa* have produced biofilm in strong patterns while there is no observed in *C. albicans*.

Table 6. Screening for biofilm formation by MPM assay.

Microorganisms	Biofilm status			
	Strong n.	Moderate n.	Weak n.	None n.
S. aureus	11	5	3	10
P. aeruginosa	13	3	7	13
E. coli	4	2	5	8
C. albicans	0	1	1	0
Total n. (%)	28 (32.56)	11 (12.79)	16 (18.6)	31 (36.05)

The MRSA strains were classified in weak (OD_{570}=1.03, SD=0.03), moderate (OD_{570}=1.03–3), and strong (OD_{570}=3.82, SD=0.12) biofilm formers by[28]. Forty-one out of the 49 MRSA strains showed moderate biofilm formation, whereas the remaining 8 were strong biofilm producers. Among 43 clinical isolates of *S. aureus* obtained from the wounds, biofilm formation by [29] was observed in 30 isolates by the MTP method. Of the total *S. aureus* isolates, 6.97% demonstrated strong biofilm production, 27.9% showed moderate production, and 34.88% formed weak biofilm by the TCP method.

The MTP method was assessed by[30] on 100 isolates of *E. coli* were collected from urine samples, showed that 80% of the isolates tend to form a biofilm, and 20% not producing any biofilm. Among former biofilm isolates, 29 isolates produced strong biofilm, 34 isolates moderately produced biofilm, and 17 isolates form a biofilm in weak status. From the 50 isolates of *E. coli were collected from urinary tract infected patients, isolated and identified by*[31], 32 were biofilm producers (3 strong and 29 moderate), and 18 were weak/non-biofilm producers.

Of the 62 tested isolates of *P. aeruginosa* were collected from burned patients, 43.5% formed biofilm, of which 66.7% were strong, and 33.3% were weak producers with an average OD_{492} of 0.582±0.015 and 0.194±0.001, respectively[32].

Biofilm formation by *C. albicans* has shown to be highly variable and is directly associated with pathogenicity and poor clinical outcomes in patients at risk. Biofilm formation was unstable among *C. albicans*

strains that were isolated from different clinical sites[33]. At the study of[33], all *C. albicans* were biofilm producers with variable strength value depended on the OD value. In general, out of 44 biofilm producers, 40.9% were weak, 56.8% moderate, and just one isolate was strong biofilm. In the study of[34] found that among the 100 isolates of *C. albicans*, 69% were found to be biofilm producers. Among them, 42% were weakly adhesive, 23% moderately adherent, and 4% were strongly adherent. The biofilm capacity was performed by[27] and identified through the MTP method in 58% of *C. albicans*. The OD was intense in 20 isolates, moderate in 21 isolates, and mild in 17 isolates. The difference in the frequency of biofilm formation may be attributed to the different *in vitro* studies of biofilm formation. In the present work, the used method was the MTP method with the initial removal of non-adherent *C. albicans* as the standard method advised. Considering the biofilm-forming capacity of these *C. albicans* isolates, their pathogenicity could result in severe co-infection with other diseases.

Conclusion

In conclusion, our preliminary results showed that the lens care practices amongst the participants were not optimum, resulting in a high level of contamination. Increased awareness among the users about the lens care practices and regular cleaning and replacements of lens cases and solutions are warranted.

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Ethical Clearance: Obtained from the Central Ethics Committee of Salahaddin University-Erbil, Iraq.

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Viral Profile and Clinical Characteristic in Acute Asthma Exacerbation Patients

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Abstract

Background: Asthma is a heterogeneous disease characterized by chronic airway inflammation. The virus infection in respiratory tract will activate greater pro-inflammatory cytokines in asthma patients. The enhancement of pro-inflammatory cytokines induces various clinical symptoms. This study aims to investigate the respiratory virus and clinical characteristics among patients with acute asthma exacerbation.

Methods: In this study, subjects were divided into 3 groups based on acute asthma exacerbation triggers. The first group triggered by virus infection; the second group triggered by non-virus infection with ILI; and the third group without any infection. Nasopharynx or throat swabs were collected to detect any respiratory virus. Virus was detected by Multiplex PCR (xTAG Respiratory Viral Panel Fast V2/ LUMINEX)

Results: According to PCR examination, the prevalence of virus infection was 46.2%. Only two types of viruses identified, which were Influenza A virus and Rhinovirus. All patients in the second groups showed a symptom of cough with purulent sputum, while no patients from the other two groups showed similar symptoms**.** PEFR and % PEFR prediction of patients with Influenza A virus infection were higher than in Rhinovirus infected-patients (210 L/min and 48.6% vs 195 L/min and 45%).

Conclusion: Acute asthma exacerbation is one of the most reasons patients came to emergency ward. The majority of acute asthma exacerbation was caused by infection, and most of it was viral infection. Clinical sign differs according to the trigger, therefore the use of antibiotics should be avoided, unless there are signs and symptoms of bacterial infections.

Key Words: *Acute asthma exacerbations; Clinical Characteristics; Viral profile*

Introduction

Asthma is a heterogeneous disease characterized by chronic airway inflammation. The chronic inflammation in respiratory tract is marked by several respiratory symptoms such as hard to breath, chest tightness, and cough with various intensity from time to time.[1] The case of acute asthma exacerbation caused by virus infection occurs 80% in children and more than 50% in adults.[2] By recognizing the causes and mechanisms of asthma exacerbations and its clinical symptoms, it will improve the accuracy of diagnosis, prognosis, and further treatment.

Asthma is a disease which considered as the ten most frequent cause of death in Indonesia. Based on the data

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of Study of Family Health Survey in 1986, asthma was in the fifth rank of the 10 most frequent causes of illness in Indonesia. Another previous study in 1992 reported that asthma was in the fourth rank (5.6%) of the 10 most frequent causes of death in Indonesia. Indonesian pneumobile project and respiratory questioner of Institute of Respiratory Medicine New South Wales in 37 public health centers in East Java, Indonesia, reported asthma prevalence in the group of age 13–70 years old was 7.7%.[3] In 2001, the data of Asthma Centre in Jakarta reported 2,210 asthmatics visited Emergency Unit. According to data from Dr. Soetomo General Hospital, the number of patients with acute asthma exacerbations steadily decreased in 1986, 1990, and 1994 (12.7%, 9.3%, and 8.8%, respectively).[3]

The virus infection in respiratory tract will activate greater production of pro-inflammatory cytokines in asthma patients compared to patients without asthma history. High production of pro-inflammatory cytokines leads to various clinical symptoms in asthma patients.[2] The clinical symptoms that occur consist of respiratory tract obstruction caused by inflammation and Influenza Like Illness (ILI); and virus infection (Rhinovirus, Coronavirus, Influenza virus).[4]

The administration of antibiotics to asthma exacerbation patients due to viral infection is actually unnecessary, however some clinicians still confer this therapy to patients. The absence of epidemiology data about the cause of airway infection in asthma exacerbation patients in Indonesia can be attributed to this. The knowledge of viral profile and clinical characteristics in acute asthma exacerbation patients who visit the emergency ward is very essential for clinicians, ultimately who work in the emergency room. Therefore, this study aimed to investigate the viral profile and clinical characteristics in acute asthma exacerbation patients.

Methods

This study was a descriptive study conducted in the Emergency Unit of Dr. Soetomo General Hospital, Surabaya, Indonesia. All patients with acute asthma exacerbations, and aged 14 – 40 years old were included in the study. Subjects were divided into 3 groups based on acute asthma exacerbation triggers. The first group was patients with acute asthma exacerbations triggered by virus infection; the second group was patients with acute asthma exacerbations triggered by non-virus infection with ILI; and the third group was patients with acute asthma exacerbations without any infection. Throat or nasopharynx swabs were collected and sent to Institute of Tropical Disease, Airlangga University, Surabaya. The virus was detected by Multiplex PCR (xTAG Respiratory Viral Panel Fast V2/ LUMINEX which can detect RSV, InfV, HPIV, HBoV, CoV, HMpV, AdV, and HRV-EnV viruses).

This study followed the principles of the Declaration of Helsinki. This study also has received ethical clearance from Dr. Soetomo General Hospital before the study begins (Ethical Clearance Number 199/panke. KKE/III/2015).

Results

Characteristics of Subjects

Subjects included in this study were 26 patients, with 19 patients were female, and 7 patients were male. The mean of age of the subjects was 30 years old. Subjects mostly worked as private employees (38.3%). Furthermore, the subjects mostly senior high school graduates (69.2%). Based on Body Mass Index (BMI), 42.3% patients had normal BMI and 7.7% of them were obese. The mean period of asthma history was 16.4 years (Table 1).

Table 1. The Characteristics of Study Subjects

Characteristics of subjects	n (%)
Gender Male Female The average age (year)	 7 (26.9) 19 (73.1) 30.12
Job Unemployed Private Employee Entrepreneur Students	 8 (30.8) 10 (38.5) 3 (11.5) 5 (19.2)
Education Background Elementary School Junior High School Senior High School Undergraduate	 3 (11.5) 2 (7.7) 18 (69.2) 3 (11.5)
Income each month Less than IDR 1 million IDR 1-3 millions More than IDR 3 million	 9 (34.6) 16 (61.5) 1 (3.8)
BMI BMI < 18,5 18.5 ≤ BMI < 23 23 ≤ BMI < 25 25 ≤ BMI < 30 BMI ≥ 30 Average BMI	 2 (7.7) 11 (42.3) 4 (15.4) 7 (26.9) 2 (7.7) 23.37
The average of history period of asthma (year)	16.4

Characteristic of Asthma Exacerbation's Trigger

According to the PCR examination of throat or nasopharynx swab, the prevalence of virus infection was 46.2% (Figure 1). Only two types of viruses identified: Influenza A virus and Rhinovirus (Figure 2).

Figure 1. The causes of acute asthma exacerbations.

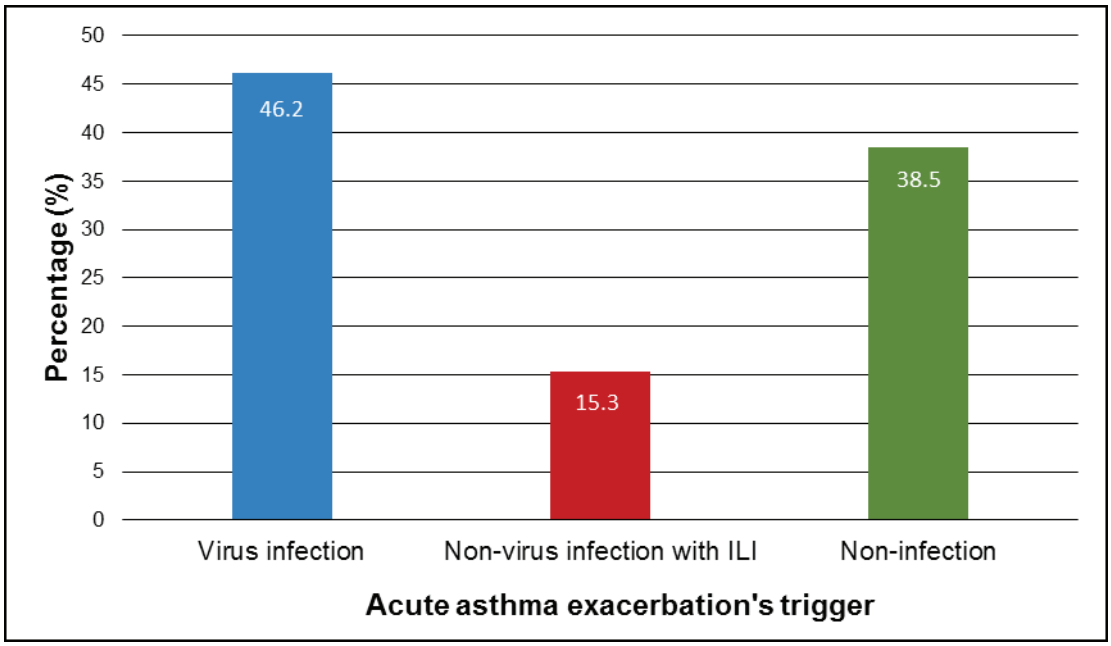
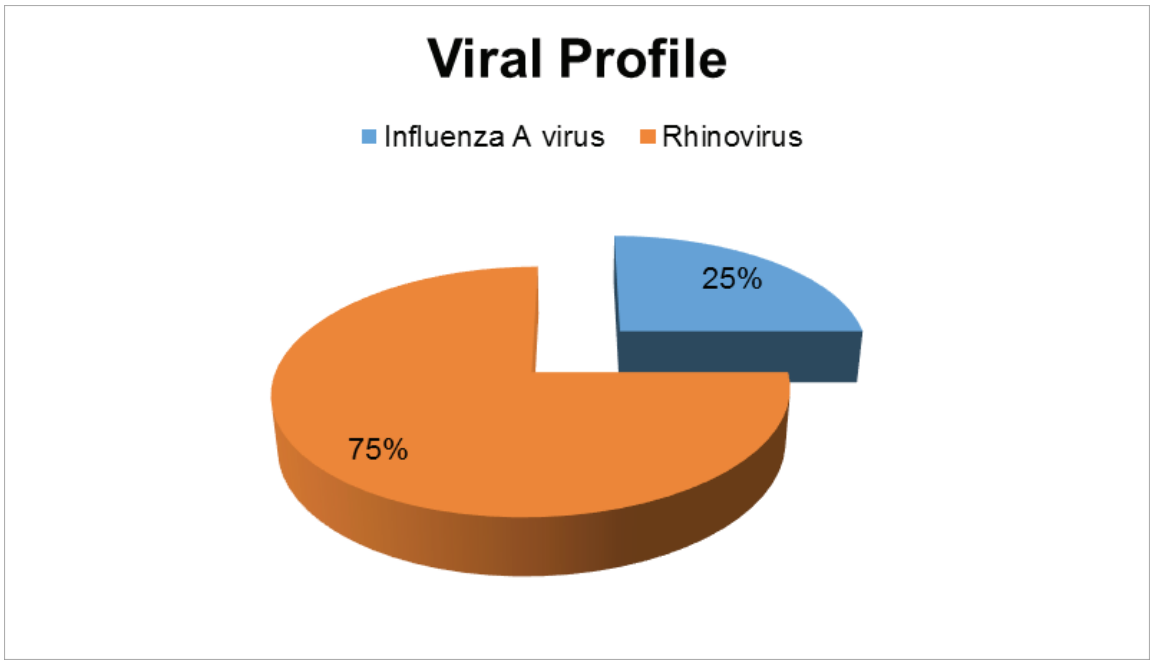


Figure 2. Viral profile of the causes of acute asthma exacerbations.



Clinical Sign and Symptoms

According to body temperature, the virus infection group showed the highest mean of body temperature (38.7ºC). In addition, Influenza A virus infection was found to promote higher body temperature than Rhinovirus infection (39.5ºC vs 38.5ºC). Moreover, the mean of peak expiratory flow rate (PEFR) and %PEFR prediction of patients with Influenza A virus infection was higher than in Rhinovirus-infected patients (210 L/min and 48.6% vs 19 5L/min and 45%).

Among all the three groups, group with virus infection showed the highest body temperature and %PEFR prediction. All patients in non-virus infection with ILI group showed a symptom of cough with purulent sputum, while other patients from the other two groups did not show similar symptoms (Table 2).

Table 2. The clinical characteristics of subjects (the average value)

Clinical symptoms	Temperature (°C)	PEFR (L/min)	% PEFR prediction	Purulent sputum (%)
Virus infection (mean)	38.7	195	45	0
Influenza A infection	39.5	210	48.6	0
Rhinovirus infection	38.5	190	44.1	0
Non-virus infection with ILI	38.6	180	35.2	100
Non infection	36.7	198	38	0

Subject Characteristics Based on Triggers of Asthma Exacerbation

The average age of patients with asthma exacerbations triggered by virus infection was 27.8 years old; by non-virus infection with ILI was 35.5 years old; and by non-infection was 30.7 years old. The average BMI in patients with asthma exacerbations triggered by virus infection was 22.6 (normal); by non-virus infection with ILI was 24.5 (obese grade I); and by non-infection was 23.8 (overweight). In addition, the average period of asthma history in acute asthma patients that triggered by virus infection was 14 years; by non-virus infection with ILI was 21.3 years; and by non-infection was 17.5 years (Table 3).

Table 3. The comparison of the average value in subject characteristics based on asthma exacerbation triggers

Clinical symptoms	Age (years)	BMI	The history period of asthma (year)
Virus infection	27.8	22.6 (normal)	14
Non-virus infection with ILI	35.5	24.5 (obese I)	21.3
Non-infection	30.7	23.8 (overweight)	17.5

Discussion

Acute asthma exacerbation is one of the most leading causes patients come to emergency room. Most of the exacerbation was triggered by infection, mainly virus infection. This study revealed that respiratory tract infections in patients with acute asthma exacerbations could be triggered by virus. The results showed that virus infection appeared in 46.2% patients; non-virus infection with ILI appeared in 15.3% patients (61.5% with ILI symptoms); and 38.5% patients were triggered non-infection. In line with the study by Nicholson et al.[5] that reported acute asthma caused by infection was 80%, and the 44% of them were caused by virus infection. Tan et al.[6] also obtained similar results, 47.8% of acute asthma exacerbations triggered by virus infection in respiratory tract.

Rhinovirus was considered as the most frequent causes of respiratory tract infection triggered by virus infection in the patients. Among 12 samples with positive results of virus detection, 75% of them were Rhinovirus and the other 25% were Influenza A virus. A study by Nicholson et al.[5] also suggested that 61.3%

of acute asthma attacks were triggered by Rhinovirus infection. In addition, Tan et al.[6] also stated that the most frequent trigger was Rhinovirus (47%). Rhinovirus infects respiratory tract by binding themselves to airway surface cell receptors, Intercellular Adhesion Molecule 1 (ICAM-1). In an in-vitro study,[7] Rhinovirus attached to ICAM-1 receptors in airway smooth muscle cells surface, stimulates contraction of airway smooth muscle cells to be enhanced. The improvement of airway smooth muscle cells contraction may occur, although the Cytopathic effect has not appeared. Rhinovirus also induces greater IL-8 and regulated on activation, normal T cell expressed and secreted (RANTES) release than other virus infection in asthma patients.[8] IL-8 is a chemokine that attracts neutrophils and promotes acute inflammation. IL-8 and neutrophils are considered to be difficult-to-treat asthma phenotypes. RANTES is a chemokine that has an important role in asthma exacerbation by inducing the recruitment of Th2-type T-cells and eosinophils.[8] Those study results may explain the reason Rhinovirus is mostly found as the cause in acute asthma exacerbation patients.

Over three decades ago, the knowledge of that upper respiratory tract infection related to acute asthma exacerbation has been developed. However, up until today, the exact mechanisms of how it happens is still be controverted. IL-6, sICAM-1 (soluble ICAM-1), ECP, histamine in serum, and leukotriene 4 in urine, are the factors that trigger respiratory tract inflammation and constriction, and this could lead to acute asthma exacerbation. In patients with upper respiratory tract infection, their level is significantly and statistically higher than in acute asthma exacerbation patients triggered by non-infection.[9]

Influenza Like Illness (ILI) symptoms can be found in patients with acute asthma exacerbations triggered by infections. In this study, in all acute asthma exacerbation patients triggered by infections had ILI symptoms such as fever with cough or sore throat. Previous study described that ILI symptoms have the possibility to enhance virus infection three times to 14 times higher.[10] Purulent sputum is a clinical symptom recognized in patients with acute asthma exacerbation triggered by non-virus infection. According to GINA report in 2015[1], one of the signs of bacterial infection occurrence in lungs which triggers acute asthma exacerbations is purulent sputum.

Fever is a clinical symptom in patients with acute asthma exacerbations triggered by infection. This study presented the average body temperature in asthma patients triggered by infection was 38.7°C, while in non-infected patients was 36.7°C. Patients with acute asthma exacerbations triggered by infection both virus and non-virus had similar average body temperature. In other trial by Yang et al.[11] reported similar average body temperature in virus infection and non-virus infection (38.6°C). The average body temperature in asthma patients triggered by Influenza A infection was higher than in Rhinovirus-infected patients. The average body temperature in asthma patients triggered by Influenza A infection was 39.5°C, while in Rhinovirus-infected patients was 38.5°C. Study results by Al-Mahrezi et al.[12] supported this theory by reporting that the average body temperature in asthma patients triggered by Influenza A infection was 38.3°C, and 37.3°C in patients triggered by other virus infection. In patients with Influenza A infection, TNF-α, INF-α, IL-1, and IL-6 in blood was increased higher than other respiratory virus infection.[13,14] These cytokines were able to reach hypothalamus that induces prostaglandin release, particularly prostaglandin E2 (PGE2).[10,15]

The reduction of %PEFR prediction can be found in patients with acute asthma exacerbations. In this study, the %PEFR prediction in acute asthma patients triggered by virus infections was obtained to be 45%; by non-virus infection with ILI was 35.2%; and by non-infection was 38%. In the study by Yasuda et al.[9] suggested that %PEFR prediction was associated with IL-6 rate and sICAM-1 in blood of patients with acute asthma exacerbations. They found the rate of IL-6 and sICAM-1 in patients' blood with acute asthma exacerbations triggered by infection and non-infection was not significantly different (IL-6: 10.8±1.7 vs 8.9±3.1; sICAM-1: 398±22 vs 372±34). Thus, they concluded that %PEFR prediction was not associated with acute asthma exacerbation triggers (infection and non-infection). The average of %PEFR prediction in patients with asthma triggered by Rhinovirus infections was lower than asthma patients triggered by Influenza A virus. In this study, the average of %PEFR prediction in patients with acute asthma exacerbations triggered by Rhinovirus infection was 44.1%, while by Influenza A infection was 48.6%. The influenza infection airway obstruction was dominated by airway inflammation. On the other hand, in Rhinovirus

infection, airway obstruction was stimulated not only by inflammation but also by bronchial smooth muscle contraction.[7]

The age, history period, and BMI were factors that affected the reduction of %PEFR prediction. The mean of BMI patients in non-infection group was overweight, while in non-virus infection with ILI group was obese. This condition aggravated asthma exacerbations and led to the reduction of %PEFR. In obese patients, Leptin level was higher compared to non-obese patients. Leptin has a role in inflammatory response; when acute asthma exacerbation occurs, leptin level in blood will increase significantly, hence in obese patients with acute asthma exacerbation, severe deterioration of lung function will occur.[16]

Conclusion

This study revealed more information about viral profile and clinical characteristics of acute asthma exacerbation that mostly caused by virus infection, particularly Rhinovirus. There were distinct clinical characteristics: the average age, BMI, body temperature, and %PEFR prediction among patients with acute asthma exacerbation caused by non-infection, virus infection, and non-virus infection. This study results may be useful as data for clinician in emergency room to confer proper therapy according to the causes factor of asthma exacerbation.

Conflict of Interest : The authors confirm that this article content has no conflict of interest.

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Capillary Lactate Level in Non-Severe and Severe Community-Acquired Pneumonia Patients

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Abstract

Background: Community-acquired pneumonia is an inflammatory disease of the lung caused by microorganisms acquired from a non-hospital environment. The pneumonia severity index and ATS/IDSA severity criteria are widely used to predict the severity of CAP. Lactate is a biomarker that can be measured by point-of-care devices that provide results in a short of time. This study aimed to determine the difference in capillary lactate level between non-severe and severe pneumonia to provide an additional method to quickly stratify pneumonia severity and treat it accordingly.

Methods: This cross-sectional study was performed in the emergency room of a regional research hospital. The pneumonia diagnosis was determined by the symptoms, physical examination and radiological findings. Capillary lactate level was measured with Accutrend Plus lactate point-of care device. Severity stratification was done according to ATS/IDSA criteria. The mean of lactate level in all subjects was 3.40±1.52, in non-severe pneumonia subgroup was 2.25±0.94, and 4.56±1.01 in severe pneumonia subgroup. Lactate is significantly higher in severe pneumonia subgroup. The cut-off point of lactate level for severity group was 3.2 mmol/L (95.5% sensitivity, 86.4% specificity).

Conclusion: The early prediction of CAP severity is essential to determine the need for admission in the intensive care unit and close follow up. The lactate level can be used for immediate severity stratification in emergency departments.

Keywords: *lactate; pneumonia; pneumonia severity index*

Introduction

Pneumonia is one of the most common infectious disease causing significant morbidity and mortality, either in developed or developing countries.[1] Its clinical signs are new or progressive infiltrates on chest radiography accompanied by certain symptoms such as cough, sputum production, fever, and shortness of breath.[1,2]

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It is imperative to give antibiotic therapy as early as possible after bacterial pneumonia diagnosis is confirmed to reduce mortality rate.[2,3] Several scoring systems are available to assess pneumonia severity in emergency department, which mostly consist of several clinical sign examination and biomarker measurement.[2,4-7] According to pneumonia severity and close monitoring of clinical response, an appropriate empirical antibiotic determination is crucial to ensure successful management of pneumonia.[8] However, those scoring systems have weakness of requiring venous blood samples, which needs particular expertise, cost, and delayed results.[9]

One of the most frequently utilized biomarkers in infection cases is lactate, which is already used in sepsis management guidelines. Several studies

showed that lactate measurement had clinical utility in initial evaluation and management of pneumonia.[10-17] Several point-of-care devices are available for lactate measurement, which only need microliters of blood. Compared to arterial sampling, capillary sampling is easier to perform, less painful, cheaper, confer faster results, and able to decrease the chance of needle stick injury to health workers.[18] Capillary lactate measurement can be done quickly in busy emergency departments to triage patients according to their severity.[11]

Capillary lactate as severity biomarker in pneumonia is yet to be a routine examination to date. This study aimed to analyze the comparison of capillary lactate between severe and non-severe community-acquired pneumonia patients.

Methods

Subjects

The population of this study was Community-Acquired Pneumonia (CAP) patients admitted into the emergency department of Soetomo General Hospital. Eligible subjects were selected by consecutive sampling method. The inclusion criteria were patients with CAP, over 21 years of age, and willingly participating in the study. The exclusion criteria was patients who had concomitant infection source outside of the lung.

CAP was defined as an inflammation of the lung caused by infectious agents, acquired outside healthcare system, in the patients who had no history of admission in the last 90 days, while having new or progressive infiltrate in chest radiology examination accompanied by supporting clinical symptoms and signs. Pneumonia Severity Index (PSI) was classified according to American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) criteria. Severe pneumonia was defined in patients who had one major criterion or three minor criteria at minimum. The capillary lactate level was measured with Accutrend Plus™ portable lactate device when the patients still in the emergency department.

Ethical clearance

This study follows the principles of the Helsinki declaration. Research ethics has been issued by Dr. Soetomo Hospital Surabaya Ethics Committee (Ethical Clearance Number 684/Panke.KKE/XII/2016) before the start of the study.

Statistical Analysis

The result was analyzed with statistical software package using appropriate statistical significance testing. All data were expressed as means ± SD. Statistical analysis was performed using statistical SPSS software package for Windows, version 17 .0 (SPSS, Inc., Chicago, IL). The comparison of capillary lactate level was analyzed using Independent T-Test for normally distributed interval variables and Mann□Whitney U test for abnormally distributed interval variables.

Results

Characteristic of Pneumonia Severity Index in CAP Patients

It was found that 22 patients was experienced non-severe pneumonia and the other 22 patients had severe pneumonia. Major criteria in severe pneumonia that mostly found was mechanic ventilation utilization with 15 patients (68%), while the minor criteria was respiratory rate ³ 30x/min with 19 patients (86%), and PaO2/FiO2 £ 250 also with 19 patients (86%) (Table 1).

Table 1. Characteristic of Pneumonia Severity Index in CAP Patient

Characteristics	Severe Pneumonia (n=22)	Non-severe Pneumonia (n=22)
Major criteria		
Mechanic ventilation	15 (68%)	0
Vasopressor	7 (32 %)	0
Minor criteria		
Respiratory rate ³ 30x/min	19 (86%)	4 (18%)
PaO2/FiO2 £ 250	19 (86%)	2 (9%)
Multilobar infiltrates	10 (45%)	0
Disorientation	6 (27%)	0
BUN ³ 20 mg/dl	13 (59%)	7 (32%)
White blood count < 4.000 sel/mm3	1 (5%)	0
Platelets < 100.000 sel/mm3	1 (5%)	0
Temperature < 36°C	0	0
Hipotension (Systolic < 90 mmHg)	7 (32%)	0

The difference of lactate level based on major or minor criteria found in severe pneumonia group

There were no significant difference shown in severe pneumonia group which marked by the use of only ventilator; only vasopressor; ventilator combined with vasopressor; and only minor criteria. Therefore, it can be concluded that there was no specific severe pneumonia criteria that affect lactate level dominantly in severe pneumonia (Table 2).

Table 2. The Difference of Lactate Level Based on Major or Minor Criteria Found in Severe Pneumonia Group

Group	Frequency	Mean of lactate level	p-Value (Kruskal Wallis test)
Only ventilator	9	4.54 ± 0.67	0.813
Only vasopressor	1	4.0	
Ventilator + vasopressor	6	4.73 ± 1.30	
Only minor criteria	6	4.5 ± 1.34	

Characteristic of Comorbidities Disease in CAP Patients between Severe Pneumonia Group and Non-severe Pneumonia Group

The comorbidities disease was mostly lung tuberculosis, both in severe pneumonia and non-severe pneumonia groups. The correlation between comorbidities and PSI was p >0.05, hence it can be concluded that there was no significant correlation between comorbidities disease and pneumonia severity index (Table 3)

Table 3. Characteristic of Comorbidities Disease in CAP Patients

Comorbidities	Frequency in Severe Pneumonia	Frequency in Non-severe Pneumonia	p-Value (Fischer exact test)
Tuberculosis	9 (40.9%)	11 (50%)	0.762
Diabetes Mellitus	6 (27.3%)	2 (9.1%)	0.240
Malignancy	4 (18.2%)	5 (22.7%)	1.000
Heart failure	0	2 (9.1%)	0.488
Asthma	0	1 (4.5%)	1.000

The Comparison of Capillary Lactate Level in Comorbidities Disease

The comparison of lactate level in the subjects both with and without comorbidities disease, showed no significant difference with p-value >0.05 (Table 4).

Table 4. The Comparison of Capillary Lactate Level in Comorbidities Disease

Comorbidities Disease	Pneumonia Severity Index	Mean of Lactate on Subject with Comorbidities	Mean of Lactate on Subject without Comorbidities	p-Value
Tuberculosis	Severe	4.644 ± 1.1458	4.500 ± 0.9531	0.751
	Non-severe	2.309 ± 0.9894	2.182 ± 0.9239	0.758
Diabetes Mellitus	Severe	4.967 ± 0.6653	4.406 ± 1.0933	0.257
	Non-severe	2.900 ± 1.2728	2.180 ± 0.9134	0.312
Malignancy	Severe	4.675 ± 1.6460	4.533 ± 0.8852	0.538
	Non-severe	2.480 ± 0.9066	2.176 ± 0.9608	0.537
Heart failure	Severe	---	---	
	Non-severe	3.100 ± 1.8385	2.160 ± 0.8407	0.364
Asthma	Severe	---	---	
	Non-severe	3.2	2.2 ± 0.9343	0.364

Capillary Lactate Level in CAP Patients

The mean of capillary lactate level in community pneumonia patients was higher in severe pneumonia group

than in non-severe pneumonia group. Furthermore, the lactate level in severe pneumonia group had a wider range of value and a bigger variations than in non-severe pneumonia group (Table 5).

Tabel 5. Capillary Lactate Level in CAP Patients

Group	Mean	Minimum	Maximum
All subjects	3.40 ± 1.516	1.1	7.1
Severe pneumonia	4.56 ± 1.012	2.1	7.1
Non-severe pneumonia	2.25 ± 0.936	1.1	4.4

The Comparison of Capillary Lactate Level in CAP Patients between Severe Pneumonia Group and Non-severe Pneumonia Group

The normality test of capillary lactate level in CAP patients was conducted using Kolmogorov Smirnov test, and the result was the capillary lactate level normally distributed with $p > 0.05$. Therefore, to investigate the hypothesis whether any difference of capillary lactate level between severe pneumonia group and non-severe pneumonia group was using Independent Sample T-Test, and the result presented a significant difference with $p < 0.001$. The cut-off point of capillary lactate level with the most optimal AUC, and the highest sensitivity and specificity, to differentiate severe and non-severe pneumonia was found in lactate level of 3.2 mmol/L with AUC of 0.946, sensitivity of 95.5%, and specificity of 86.4% (Figure 1).

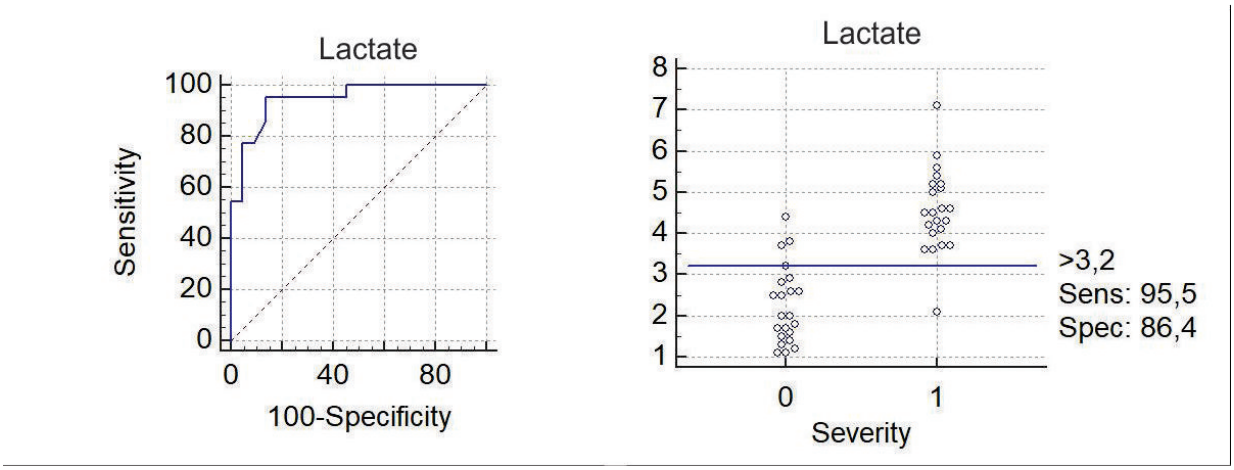

Figure 1. The curve of ROC and dot diagram to determine cut-off point of lactate level

Further analysis was using Cohen's kappa coefficient inter-rater agreement, and the score obtained of Cohen's kappa coefficient was 0.773. It showed that there was a good suitability between the distribution of subjects based on lactate level compared to distribution based on IDSA/ATS criteria to determine pneumonia severity index, which according to Fleiss Cohen's kappa coefficient >0.75 is used to show a good suitability (Table 6).

Table 6. The suitability of lactate level with IDSA/ATS

	Severe Pneumonia	Non-severe Pneumonia	Cohen's kappa coefficient
High Lactate (≥ 3,2 mmol/L)	21 cases	4 cases	0.773 (95% CI 0,587 – 0,959)
Low Lactate (< 3,2 mmol/L)	1 cases	18 cases	

Discussion

In this study, the subjects were distributed into severe pneumonia and non-severe pneumonia group, which was determined based on major and minor criteria of severe pneumonia according to IDSA/ATS. According to the latest ATS guideline for pneumonia, it was mentioned that there are two major criteria and nine minor criteria.

The results presented that the most comorbidities disease was lung tuberculosis, followed by diabetes mellitus, malignancy, and heart failure. Statistical analysis in order to investigate the correlation between comorbidities disease and pneumonia severity index resulted a value of $p > 0.05$. Therefore, it can be concluded that there was no significant correlation between comorbidities disease and PSI in this study. This result proved that despite the comorbidities disease is potentially affect blood lactate level, its insignificant difference of number between severe pneumonia and non-severe pneumonia group could resolve the bias of the cause of the increase in lactate from those comorbidities disease. In line with the study by Demirel, that reported no significant correlation between comorbidities disease and pneumonia severity index in their research.[19]

The result of this study showed the mean of capillary lactate level in subjects was 3.40 ± 1.516 mmol/L, with the mean of capillary lactate level in severe pneumonia group was 4.56 ± 1.012 mmol/L, and 2.25 ± 0.936 in non-severe pneumonia group. This statistical analysis calculation proved that there was a significant difference between both of the groups with $p < 0.001$. The study conducted by Demirel, also found a significant difference between lactate level in died patients and survived patients ($p < 0.001$), with the mean of lactate level in pneumonia was 3.53 ± 3.59 mmol/L, in died patients was 7.76 ± 5.81 mmol/L, and 2.35 ± 0.99 mmol/L in survived patients.[19]

Furthermore, ROC analysis was used to observe the highest cut-off point, sensitivity, and specificity. The highest cut-off point was 3.2 mmol/L with AUC of 0.946, sensitivity of 95.5%, and specificity of 86.4%. Similarly, Demirel found the highest cut-off point was 3.35 mmol/L, AUC of 0.929, sensitivity of 86.4%, and specificity of 88.6%.[19] The cut-off point obtained by this study can be used to help differentiate community-acquired pneumonia patients into severe and non-severe groups and assist in their proper management.

The immune response in pneumonia causes pulmonary ventilation and hemodynamic derangement which result in the decrease of systemic oxygen delivery. Besides, there is also the increase of metabolism causing higher systemic oxygen consumption. The imbalance between the reduction of oxygen delivery and increase of oxygen consumption will increase the systemic oxygen extraction ratio and reduce oxygen saturation. The increase of systemic oxygen extraction ratio above certain tolerance level (50 – 60%) will trigger anaerobic metabolism, which causes higher lactate production. Therefore, lactate concentration is inversely related to systemic oxygen delivery and oxygen saturation and has the ability to reflect pneumonia severity level.[20]

CAP patients in the emergency department are usually in the early phase of hemodynamic disorder, characterized by significantly increased lactate level, but still normal vital signs due to adaptation response of cathecholamine release that prevents shock to occur. This condition is called "occult shock", which can progress to sudden cardiopulmonary collapse. Therefore, lactate

measurement is essential as an early warning marker for detecting that condition.[20]

There are several limitations in this study. First, most of the subject was already treated in prior hospital before being referred, raising the potential bias from the treatment given before the lactate level is measured. Secondly, the diagnostic criteria to differentiate the subject into severe and non-severe group was ATS/IDSA criteria, which has two major and nine minor criteria. Each of them has different underlying pathophysiology that potentially had different effects on lactate level.

Conclusion

This study showed that capillary lactate level had the ability to differentiate CAP patients into severe and non-severe group. The optimal cut-off point for the differentiation is 3.2 mmol/L. Early severity prediction of community-acquired pneumonia is important to decide the effective treatment for patients in the intensive care unit and closer monitoring. Capillary lactate level can be used for quick classification of pneumonia patients in emergency departments.

Conflict of Interest : The authors confirm that this article content has no conflict of interest.

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Author's Contribution: RY and KA designed the study, collected samples, gathered data, analyzed the data, made tables and figures, wrote the manuscript, and contributed to review and revise. All authors have been approved the final version.

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Reconstruction of Prevention and Handling of Stunting Policy in Public Health Center

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Abstract

Introduction: Indonesia has severe nutritional problems, one of which is stunting. Stunting is a chronic malnutrition condition caused by many multi-dimensional. According to the Minister of Health Republic of Indonesia No. 43 of 2019 about Public Health Center, Public Health Center Services are part of public services and are basic routine health services consisting of Public Health Efforts (UKM) and Individual Health Efforts (UKP), one of which is the prevention and handling of stunting in Public Health Center. Demak III Public Health Center is a health center in the city that has an area of 18.78 km with a population of 38,218 people precisely on the road Cempaka Raya No. 5 Katonsari, Demak consists of 5 villages and 1 village namely Kalikondang village, Katonsari, Donorojo, Mangunjiwan, Cabean, and Tempuran. Of the six villages, Donorejo village is the village that has the highest number of stunting cases compared to other villages.

Research Objectives: The purpose of this study is to find out and analyze the reconstruction of prevention and handling of stunting policy in Public Health Center.

Research Methods: This research uses the paradigm of constructivism, which is the policies that exist in government agencies can be examined objectively related to accurate data, then constructed through the concept of law that is only tested again its strength. Methods used with Critical thinking. Critical thinking is the ability to think clearly and rationally, understand the logical relationship between ideas. The sampling method is a non-random sampling technique that is purposive sampling is a way of taking subjects based on a specific goal.

Results and Discussion: The description of the reconstruction of prevention and handling of stunting policy in Public Health Center through integration actions with stakeholders of government agencies in the Demak Regency area is still partial. With the policy that is still partial, in the prevention and handling of stunting by criticizing (critical thinking) existing policies. Prevention and reduction of stunting is carried out by health workers through programs with cross-sectors and networks already running.

Keywords: *Reconstruction; Policy: Prevention and Handling of Stunting*

Introduction

Indonesia has a severe nutrition problem characterized by many cases of malnutrition. Malnutrition is an impact of the state of nutritional status. Stunting is one of the malnutrition conditions associated with the inadequacy of past nutrients so that it is included in chronic nutritional problems[1].

Nutritional problems of a chronic nature, one of which is stunting. Stunting itself is a condition of failure to grow in toddlers due to chronic malnutrition so that the child is too short for his age (malnutrition occurs from the baby in the womb and the early stages after the

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child is born, but only visible after the child is 2 years old)[2].

Stunting when measured by paying attention to the height or length, age, and gender of toddlers. The habit of not measuring the height or length of a toddler's body in the community causes stunting events to be difficult to realize. This, sometimes causes people sometimes do not understand many factors that cause stunting[3].

Stunting is caused by multi-dimensional factors and is not only caused by malnutrition factors experienced by pregnant women and toddlers. The most decisive intervention to be able to reduce stunting prevalence, therefore, needs to be done in the First 1,000 Days of Life (HPK) of toddlers[4].

The first day of life (1000 HPK) is a golden period to child growth, starting from the gestation phase (270 days) to the child aged 2 years (730 days). This golden period is included in the growth of the brain, but at this time is also very vulnerable to various growth and developmental disorders. Malnutrition as a direct cause, especially in toddlers has a short-term impact on morbidity. If this problem is chronic, it will affect cognitive function, namely low intelligence level and impact on the quality of human resources. In recurrent conditions continuously (in the life cycle) than children who experience malnutrition early in life (period 1000 HPK) have a risk of non-communicable diseases in adulthood[5].

According to Regulation of the Minister of Health No. 97 of 2014 concerning Health Services Period before Pregnancy, Pregnancy, Childbirth, and Post-Childbirth Period, Implementation of Contraceptive Services, as well as Sexual Health Services, factors that aggravate the state of pregnant women, it is said that too young, too old, too often give birth, and too close the birth distance. Stunting can be affected by about 20% in BBLR babies, caused by mothers of two young gestational age (under 20 years)[6].

According to Minister of Health Republic of Indonesia No. 43 of 2019 about Public Health Center, Public Health Center Services are part of public services and are basic routine health services consisting of Public Health Efforts (UMK) and Individual Health Efforts (UKP), one of which is the prevention and handling of stunting in Public Health Center.

Demak III Public Health Center is a health center in the city that has an area of 18.78 km with a population of 38,218 people precisely on the road Cempaka Raya No. 5 Katonsari, Demak consists of 5 villages and 1 village namely Kalikondang village, Katonsari, Donorojo, Mangunjiwan, Cabean, and Tempuran. Of the six villages, Donorojo village is the village that has the highest number of stunting cases compared to other villages. Donorojo village at the beginning of 2019 has 342 toddlers where there are 39 and in October 2019 there are 346 toddlers there are 24 stunting. Although there has been a decrease in stunting in Donorojo village, but there are still toddlers who are in pain due to stunting so that the acceleration and handling of stunting continues and in monitoring the stunting program of Public Health Center Demak III area.

Table 1: Stunting Data for October 2019

NO	VILLAGE	NUMBER OF TODDLERS	NUTRITIONAL STATUS		AMOUNT
			VERY SHORT	SHORT	
1	Kalikondang	625	10	10	20
2	Katonsari	628	1	2	3
3	Donorojo	346	5	19	24
4	Mangunjiwan	692	4	7	11
5	Cabean	616	3	7	10
6	Tempuran	360	0	6	6
	Amount	3267	23	51	74

Data above the total of toddlers covered in the working area of Public Health Center Demak III as many as 3,267 toddlers where 23 of them belong to the category of very short toddlers and 51 toddlers belong to the short category. And a total of 74 toddlers with short and very short stature.

Demak regency is one of 16 districts in Central Java Province that is set to become a locus handling Stunting 2020. Demak regency is also included in 100 districts/ cities of the National Stunting red zone, this is known from the results of Baseline Health Research year 2013, the number of Stunting Demak Regency is 50.28 (serious category), and in 2018 it dropped to 26.10. The decrease in stunting numbers is still a problem that must be solved because stunting can cause pain and death in children. With Donorojo village becoming a stunting locus and 9 villages outside the Demak III Public Health Center, the local government issued Demak Regent Regulation No. 11 of 2019 on decreasing the Prevalence of Short Toddlers (Stunting) in Demak Regency.

Obstacles in handling stunting in the Demak district can be from individuals, communities, and stakeholders. Constraints of the individual himself from genetic factors, economic factors, educational factors, environmental factors. Constraints from the community/ village can be from human resources, infrastructure, and the environment as well as constraints from stakeholders themselves from regulations related to handling stunting is still partly that has not been used as a common ground to handle stunting in the Demak area.

Problems related to the prevention and handling of stunting policy in Public Health Center through integration actions with stakeholders of government agencies Demak regency has not been managed properly, this is a lot of problems in reducing stunting. The problems faced: Ineffective stunting prevention programs; Not optimal coordination of the implementation of specific and sensitive nutritional interventions at all levels related to planning and budgeting, organizing, and monitoring and evaluation; Not yet effective and efficient allocation and utilization of resources and funds; Limited capacity and quality of program implementation; There is still a lack of advocacy, campaigns, and dissemination related to stunting, and various prevention efforts; and there is still a lack of regulations governing the acceleration of stunting prevention in the region.

Research Methodology

Reconstruction of prevention and handling of stunting policy in Public Health Center through integration with government agency stakeholders with the method of Integrated Reporting System Stunting (SPTS) online in the area of Demak District based on Justice using the paradigm of constructivism policies in government agencies can be examined objectively related to accurate data, then constructed through the concept of law that lives tested again its strength. This construction process will run continuously because of the discovery of a new understanding, which can then be used as the basis for formulating a system or regulation in the form of legislation that can be applied in everyday life[7]. The method used Critical thinking. Critical thinking is the ability to think clearly and rationally, understand the logical relationship between ideas. According to Satjipto in modern law that existing policies need to be criticized to seek legal value in seeking justice in society. Critical Thinking is a thinking activity that is carried out by operating the intellectual potential to analyze, make considerations, and make informed decisions and implement them correctly[8].

Data analysis used, using qualitative descriptive, by analyzing events, phenomena, or circumstances socially. Qualitative analysis in this case is an effort made by working with data, organizing data, sorting it into manageable units, synthesizing them, finding and finding patterns, discovering what is important and what is learned, and deciding what can be told to others. Techniques used from non-random sampling is purposive sampling, sample withdrawal aims to be done by taking subjects based on a specific purpose.

The type of research carried out is socio-legal research. Socio-legal research is a study that "combines" doctrinal legal studies with social studies. This fusion is based on the belief that the rule of law never works in a vacuum. The rule of law works in a space full of value systems, interests that can be dominant, not neutral. Social research can be concluded that the rule of law can provide justice or not.

Discussion

An overview of the results of the reconstruction

of prevention and handling of stunting policy in Public Health center through integration actions with government agency stakeholders with the method of Integrated Reporting System Stunting (SPTS) online in the Demak district based on Justice, as follows:

The existence of 10 stunting locus in Demak Regency is a joint responsibility between health workers and cross-sectors, networks, and stakeholders in government agencies. With the existence of stunting locus in Donorojo village in Demak III Public Health Center area and 9 villages in Demak Regency, the local government issued Demak Regent Regulation No. 11 of 2019 on Decreasing the Prevalence of Short Toddlers (Stunting) in Demak Regency. With the issuance of the policy of the Regent Regulation, in each village that entered 10 stunting locus issued a policy in the reduction of stunting. Followed by villages that are not included in 10 stunting locus, one of them is Mangunjiwan village in Demak III Public Health Center. To reduce stunting with the method "Horn Banting" which is an acronym of Strengthening the Role of Posyandu to Help Prevent Stunting.

There are 10 stunting locus in Demak Regency in 2019/2010, among others: Bumirejo village 5.87/6,45, Sidomulyo village 9.85/13.2, Kedungori village 7.35/9.62, Sambiroto village 11.85/12.78, Betawalang village 17.44/19.81, Guntur village 18.87/18.86, Boyolali village 13.64/ 8.33, Gedang Alas village 11.63/10.69, Donorojo village 8.72/ 5.81, Kembangan village 26.82/14.75.

Demak district has 14 sub-districts and 249 villages, in 2019, Demak district 10 villages enter the Stunting Louth. With the highest entry in Central Java. Local government targets stunting cases to fall by 20 percent in 2021. With 10 villages entering the stunting lox, the local government issued Demak Regent Regulation No. 11 of 2019 concerning The Decrease in The Prevalence of Short Toddlers (Stunting) in Demak Regency, Regent Regulation No. 29 of 2019 RAD For Tackling Stunting in Demak Regency Year 2019-2021, Regent Regulation No. 2 of 2019 concerning Procedures for Distribution, Determination, Details, and Use of Village Funds in Demak District, and Regent Regulation No. 36 of 2019 concerning List of Village Authorities Based on The Rights of Origin and Local Authority of Village Scale in Demak district.

The data resulting from the recapitulation of short and very short toddler data (stunting) locus stunting village intervention in the District Office in August 2020 as follows:

Table.2: Short and very short toddler data for August 2020

NO	VILLAGE	NUMBER OF TODDLERS	NUTRITIONAL STATUS					
			VERY SHORT	%	SHORT	%	AMOUNT	%
1	Bumirejo	409	5	1,22	19	4,65	24	5,87
2	Guntur	567	14	2,47	93	16,40	107	18,87
3	Sidomulyo	335	7	2,09	26	7,76	33	9,85
4	Kedungori	258	0	0,00	19	7,36	19	7,36
5	Sambiroto	135	6	4,44	10	7,41	16	11,85
6	Boyolali	110	0	0,00	15	13,64	15	13,64
7	Gedangalas	258	11	4,26	19	7,36	30	11,63
8	Donorojo	344	5	1,45	27	7,85	30	8,72
9	Kembangan	261	12	4,60	58	22,22	70	26,82
10	Betahwalang	562	22	3,91	76	13,52	98	17,44
AMOUNT		3.239	82	2,53	362	11,18	442	13,65

From table.2 is the data of short and very short toddlers in August 2020 in Demak Regency. With the stunting loathing, the Demak Regency Government-issued regulation of Demak Regent Regulation No. 11 of 2019 on Decreasing the Prevalence of Short Toddlers (Stunting) in Demak Regency.

Conclusion

The prevention and handling of stunting policy in Demak district are still partial, so the existing policy needs to be done critically through a critical thinking method that is to find legal value in seeking justice in the community. So far, prevention and reduction of stunting is carried out by health workers through programs with cross-sectors and networks. The problem of stunting in the Public Health Center needs cooperation and mutual assistance through government integration actions in the Public Health Center area. Integration with government agencies with stakeholders is carried out in an online Stunting Integrated Reporting System (SPTS). With this online SPTS system will get information on data planning, implementation/activities, results of activities, and monitoring and evaluation. In obtaining data in government agencies using the online Stunting Integrated Reporting System (SPTS) method, data obtained: P1: Policies, SOP, Regulations, Decisions, and Guidelines; P2: Implementation of activities; and P3: Monitoring and evaluating the implementation and activities related to stunting in government agencies.

Ethical Clearance: Yes.

Conflict of Interest: No

Source of Funding: Authors

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Determinants of Loss to Follow-Up Among MDR-TB Patients

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Abstract

Cases of tuberculosis has gotten worse since results of global surveillance revealed that *mycobacterium* is resistant to rifampicin and isoniazid. It can cause Loss to Follow-Up among MDR-TB patients. The purpose of this study is Determinants of Loss to Follow-Up among MDR-TB Patients. This research is an analytic observational study with a case control study design with a retrospective approach. Cases were MDR-TB patients who dropped out of treatment as much 27 people, while controls were subjects with MDR-TB patients who did not Loss to Follow-Up of treatment as much 83 people. Data analysis was performed by looking at the Odds Ratio value. The result showed that knowledge (OR = 5.28, 95% CI = 1.410347–29.19908), drug side effect (OR = 32.324332, 95% CI = 4.732715–1353.07), family support (OR = 5.01875, 95% CI = 1.602489 – 15.57846), social stigma (OR = 6.712963, 95% CI = 1.473798–61.68985) were the determinants of loss to follow up among MDR-TB patients. It can conclude that determinants of loss to follow-up among MDR-TB patients include knowledge, drug side effects, family support, and social stigma.

Keywords: *Tuberculosis, loss to follow-up MDR-TB, knowledge, drug side effects, family support, social stigma*

Introduction

The problem of tuberculosis has gotten worse since results of global surveillance revealed that *mycobacterium* is resistant to rifampicin and isoniazid, with or without other first-line anti-tuberculosis drugs. Rifampicin and isoniazid are the strongest anti-tuberculosis drugs[1]. Those two most powerful anti-TB drugs requiring treatment with a second-line regimen. MDR-TB also requires treatment with second-line drugs[2]

Globally, 160,684 cases of multidrug-resistant tuberculosis and rifampicin-resistant TB (MDR/RR-TB) were reported in 2017 (up from 153,119 in 2016), and 139,114 cases were registered on treatment (up from 129,689 in 2016). Eight of the 30 countries with a high burden of MDR-TB had treatment success rates of at least 75% in their 2017 patient cohort. However, India and Indonesia had treatment success rates below 50%, partly due to high mortality and rate of Loss to Follow-Up (18% and 26% in Indonesia, 18% and 19% in India, respectively). Rate of Loss to Follow-Up among MDR-TB patients was highest in the Philippines and China (33% and 29%, respectively), and outcome data were missing for the majority of patients in Somalia and Zimbabwe (15% and 17%, respectively)[3]

Indonesia is ranked 8th out of 27 countries with the burden of MDR-TB with an estimated 6,800 new MDR-TB patients each year[4]. From the MDR-TB rate, it is estimated that 2% were from new TB cases and 12% were from re-treatment TB cases. There are 55% of MDR-TB patients who have not been diagnosed or treated properly. National MDR-TB case data in 2015 found 18 thousand MDR-TB cases, including 15,300

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suspected, 1,860 confirmed, and 1,566 MDR-TB cases undergoing treatment[5]

Loss to Follow-Up among MDR-TB patients is a problem of individuals and communities since it can lead to increased transmission, resistance, and death. The rate of Loss to Follow-Up among drug-resistant tuberculosis (DR-TB) patients increases every year in Indonesia. The high rate of Loss to Follow-Up among DR-TB patients are because 24% of patients have not been tracked; 26% of patients experienced drug side effects; 21% of patients were unknown; 13% of patients were not found; 6% of patients did not believe the treatment; 7% was due to socioeconomic factors; and 3% was due to other factors[6]

Data from the South Sulawesi Provincial Health Office 2019 shows that the rate of Loss to Follow-Up among DR-TB patients was 20.8% in 2016, 24.5% in 2017 and 37.3% in 2018. It indicates that the rate of Loss to Follow-Up among DR-TB patients increases every year[7]. Data from Labuang Baji Hospital shows that there were 14 out of 55 MDR-TB patients who dropped out in 2018, and 13 out of 45 MDR-TB patients who dropped out in 2019[8]

Moreover, some patients who experience side effects from anti-TB drugs also decide to stop treatment. It eventually causes multiple TB germs immunity to anti-TB drugs (multidrug resistance) and will lead to a TB epidemic that is difficult to manage[9]. Numerous side effects and long duration of treatment affect the treatment success rate in MDR-TB patients. According to WHO, the cure rate in persons with MDR-TB worldwide is < 60%.

Drug-resistant TB threatens global TB care and prevention, and it remains a major public health problem in many countries. Based on the aforementioned events and data, the researcher is interested in conducting a research on the Determinants of Loss to Follow-Up among MDR-TB Patients.

Material and Method

This research is an analytic observational study with a case control study design with a retrospective approach. Cases were subjects with positive effect characteristics, which in this study were MDR-TB patients who dropped out of treatment, while controls were subjects with negative effect characteristics, namely MDR-TB patients who did not Loss to Follow-Up of treatment. Both groups were traced back to determine the presence of risk factor exposure to effects.

This study was conducted at Labuang Baji Hospital, Makassar. Labuang Baji Hospital is a referral center for MDR-TB examination in South Sulawesi, while the number of MDR-TB patients in 2018 - 2019 reached 110 MDR-TB cases in Makassar. Sampling was carried out using total sampling. The ratio of the number of cases and controls used was 1: 3, by which the number of case respondents was 27 people and the number of control respondents was 83 people. Data analysis was performed by looking at the Odds Ratio value.

Findings

Table 1 The Risk of Knowledge on The Incident of Loss to Follow-Up Among MDR-TB Patients

Knowledge	Patients				Total		OR	CI
	Case		Control					
	n	%	n	%	N	%		
High Risk	24	88.89	50	60.24	74	67.27	5.28	1.41034– 29.19908
Low Risk	3	11.11	33	39.76	36	32.73		
Total	27	100.00	83	100.00	110	100.00		

Table 1 shows that patients with ≤3 correct answers had 5.28 times risk of dropping out compared to patients with >3 correct answers. The value of the Confidence Interval (1.410347 – 29.19908) does not include value of 1, meaning that the risk was significant.

Table 2 The Risk of Drug Side Effect on The Incident of Loss to Follow-Up Among MDR-TB Patients

Drug Side Effect	Patients				Total		OR	CI
	Case		Control					
	n	%	n	%	N	%		
High Risk	26	96.30	37	44.58	63	57.27	32.324332	4.73271 – 1353.07
Low Risk	1	370	46	55.42	47	42.73		
Total	27	100.00	83	100.00	110	100.00		

Table 2 shows that MDR-TB patients who experienced severe drug side effects such as liver dysfunction, kidney function disorders, gastrointestinal bleeding, severe electrolyte disturbances, hearing loss, vision problems, seizures, tendinitis and hypothyroidism were 32.324332 times more likely to Loss to Follow-Up than those who experienced mild drug side effects such as mild skin reactions, allergic skin reactions with/without fever, severe/mild nausea and vomiting, anorexia, diarrhea, headache, vertigo, sleep disturbances, minor electrolyte disturbances, depression, behavior changes, gastritis and pain at the injection site. The value of the Confidence Interval (4.732715 – 1353.07) does not include value of 1, meaning that the risk was significant.

Table 3 The Risk of Family Support on The Incident of Loss to Follow-Up Among MDR-TB Patients

Family Support	Patients				Total		OR	CI
	Case		Case					
	n	%	n	%	N	%		
High Risk	11	40.74	10	12.05	21	19.09	5.01875	1.602489– 15.57846
Low Risk	16	59.26	73	87.95	89	80.91		
Total	27	100.00	83	100.00	110	100.00		

Table 3 shows that MDR-TB patients who rarely or never received family support such as reminding and ensuring that they take their medication regularly and taking them to health care facilities for treatment had 5.01875 times risk of dropping out than those who always received family support. The value of the Confidence Interval (1.602489 – 15.57846) does not include value of 1, meaning that the risk was significant.

Table 4 The Risk of Social Stigma on The Incident of Loss to Follow-Up Among MDR-TB Patients

Social Stigma	Patients				Total		OR	CI
	Case		Control					
	n	%	n	%	N	%		
High Risk	25	92.59	54	65.06	79	71.82	6.712963	1.473798–61.68985
Low Risk	2	7.41	29	34.94	31	28.18		
Total	27	100.00	83	100.00	110	100.00		

Table 4 shows that MDR-TB patients who felt ashamed and felt isolated in society because they had MDR-TB disease had 6.712963 times risk of dropping out than those who did not. The value of the Confidence Interval (1.473798 – 61.68985) does not include value of 1, meaning that the risk was significant.

Discussions

The results of this study indicated that MDR-TB patients with low level of knowledge had 5.28 times risk of dropping out. It is in line with a study conducted by Supardi et al. finding that low level of knowledge of MDR-TB had 1.723 times risk of conversion change (95% CI=0.777-3.821)[10]

It is important for MDR-TB patients to know and understand well the knowledge of MDR-TB definition, causes, duration of treatment, prevention, the importance of taking MDR-TB drugs regularly, the consequences of Loss to Follow-Up, and the importance of sputum and photorontgen examinations. In fact, mastery of knowledge about MDR-TB greatly influences patient adherence behavior in treatment.

A good level of knowledge will certainly make it easier for sufferers to undergo MDR-TB treatment[11]. Many patients have low level of knowledge of MDR-TB which affect their own treatment. Patients who Loss to Follow-Up may be because they feel that they have recovered from their illness, but they do not know the impact of dropping out during the treatment period.

Moreover, the results of this study indicate that MDR-TB patients who experienced severe drug side effects of the drug had 32.324332 times risk of dropping out than those who experienced mild drug side effects, which was statistically significant. (95% CI=4.732715–1353.07). It supports a study conducted by Wohlleben et al. which found that MDR-TB patients who experienced treatment side effects were 3.67 times risk of dropping out[12].

MDR-TB treatment has different and more severe side effects than TB treatment since the drug for MDR-TB patients has a higher dose[13]. Drug side effects can be controlled in various ways, especially mild side effects. This study showed that the most widely used method for the case group to control drug side effects was by consulting with health workers with a percentage of 55.56%, while the remaining 18.52% controlled drug side effects by stopping taking anti-tuberculosis drugs.

However, because MDR-TB patients experience drug side effects for a long duration, they often Loss to Follow-Up unilaterally, with a percentage of 100.00%, without prior confirmation with health workers. Drug side effects can be overcome by providing information on how to deal with them, so that MDR-TB patients know in advance about the side effects and are not worried about them while ingesting the drug. Minor side effects of drugs can actually be managed so that sufferers can continue taking medication without dropping out[11]

Besides, the results of this study also demonstrate that MDR-TB patients who rarely or never received family support such as reminding and ensuring that they take their medication regularly and taking them to health care facilities for treatment had 5.01875 times risk of dropping out than those who always received family

support.

Support provided for family members who are sick can be in the form of meeting their food and drinking needs and covering costs for medical treatment. If there is a problem faced by the patient, it is necessary for the family to provide advice for problem solving[14]. Family support is needed by MDR-TB patients as the main support system so that they can develop an effective coping response to adapt well in dealing with stressors they face related to their illness in terms of physical, psychological, and social.

Patients without strong family and social support are more prone to Loss to Follow-Up[15]. There are four forms of family support that can be applied to MDR-TB patients including instrumental, informational, emotional, and reward supports. These forms of support can keep MDR-TB patients excited about treatment and not think or act on dropping out[16]. More attention is needed to increase awareness of TB transmission, especially among family members of MDR-TB patients and the community as a whole[17]

In addition, the results also showed that MDR-TB patients who felt ashamed and felt isolated in society because they had MDR-TB disease had 6.712963 times risk of dropping out than those who did not. An obvious cause of social stigma and discrimination from the community is the fear of being infected with MDR-TB disease. It is in accordance with a study by Stosic et al which confirmed that MDR-TB cases are 3.32 times more likely to experience the social stigma associated with TB[18].

Social stigma results in acts of discrimination; the act of not recognizing or not making efforts to fulfill the basic rights of an individual or group as a dignified human being[19]. People still think that TB is a witchcraft, curse, and hereditary disease. The existence of this negative view/stigma causes sufferers to be embarrassed and reluctant to go to health services[20].

An obvious cause of social stigma and discrimination from the community is the fear of being infected with MDR-TB disease. Unfortunately, patients also often isolate themselves to avoid infecting others and uncomfortable situations such as being shunned by people around them or being the talk of others. The social stigma of MDR-TB patients makes it more difficult for patients to continue treatment because their fears of being identified. In other words, being infected with MDR-TB hinders their access to health services every day which can lead to serious symptoms and increased transmission. MDR-TB patients feel that they will lose friends and jobs because the thought of 'I have TB' (a social stigma)[18]. As an attempt to reduce the social stigma associated with TB and ill mental health in MDR-TB patients, it is necessary to incorporate mental health services into the TB care process[18]

Conclusion

To sum up, determinants of Loss to Follow-Up among MDR-TB patients include knowledge, drug side effects, family support, and social stigma. The researcher suggests that there should be socialization regarding all things considered important for MDR-TB patients to know in order to increase their level of knowledge, especially providing an understanding of the importance of taking medication regularly and not dropping out. In addition, it is necessary to provide motivational counseling from health workers and family support during MDR-TB treatment, particularly in controlling drug side effects and social stigma.

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Motivational Interviewing and Physical Activity on Quality of Life of Type 2 DM Patients in Makassar City

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Abstract

Objectives: This study aimed to see the effect of a combination of motivational interviewing and physical activity programs on the quality of life of patients with type 2 diabetes mellitus (type 2 DM).

Methods: The design of this study was a quasi-experimental with a non-randomized control group pretest posttest design. The population was all patients with type 2 DM who were recorded in the medical records of Public Health Centers. The sampling technique was exhaustive sampling with a sample size of 30 people in the intervention group and 30 people in the control group. Using bivariate data analysis, the two dependent mean difference test and two independent mean difference tests were used.

Results: Statistical analysis show that there are differences in the mean value of the four domains of quality of life in the intervention group before and after treatment (p = 0.0001 for each domain of Physical Health, Psychological Conditions, Social Relations, and Environmental Conditions); there is a difference in the quality of life of type 2 DM patients between the intervention group and the control group with the percentage of improving the quality of life in the good category in the intervention group after being given treatment by 66.7% (p= 0.0001).

Conclusion: Providing a combination of counseling programs with a motivational interviewing approach which is followed by gymnastics exercise is effective in improving the quality of life of type 2 DM patients.

Keywords: *Motivational interviewing, type 2 diabetes, quality of life, gymnastics*

Introduction

Diabetes Mellitus (DM) is a disease related to lifestyle such as diet and activity habits. This risk will increase in individuals with uncontrolled hypertension and lack of physical activity and individuals who are not obedient to diet and have unhealthy lifestyles [1]. DM has become the 6th largest cause of death in the world. International Diabetes Federation (IDF) data in 2017 shows an increase in the number of DM patients aged 20-79 years in the world every year, for example, in 2013 it reached 382 million people, then in 2015 it increased to 415 million people and in the latest data for 2017, it increased to 425 million.

The WHO estimates that globally, 422 million adults over 18 years old suffered from diabetes in 2014 with the highest prevalence in the East Mediterranean Region at 13.6% and Southeast Asia at 8.6%. The number of DM patients has increased over the last few decades due to population growth, an increase in the average age of the population, and an increase in the prevalence of DM

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at each age [2]. The results of the analysis of the World Economic Forum, in the 2012-2030 periods, Indonesia will be burdened by 2,800 trillion rupiah due to DM or twenty times the Indonesian health budget in 2012 as a whole [3].

Type 2 diabetes mellitus is a chronic disease that cannot be completely cured, causing its prevalence to increase every year [4]. On the study by Kaidaliri [5], it is found that DM patients generally have a poor quality of life compared to people without the disease; this is because DM can cause complications if proper DM treatment is not carried out. The goal of DM treatment is not to cure but to improve the functional status of the patient, minimize symptomatic complications, prolong life through secondary prevention, and improve quality of life [4].

The quality of human life according to WHO includes 4 main domains, namely domain 1 is physical health, domain 2 is psychological conditions, domain 3 is social relations, and domain 4 is environmental conditions. Knowing a person's quality of life can help health workers to determine a person's health condition so that it can be a direction or benchmark in determining which interventions should be given according to the patient's condition [6]. Studies that aims to assess quality of life have also been conducted in several other diseases, for example in the treatment of chronic myeloid leukemia [7].

PERKENI [8] states that the management of DM consists of four main pillars including education, nutritional therapy, physical activity, and pharmacological interventions. The level of public knowledge, which is still very minimal, especially in the prevention of DM through the application of a healthy lifestyle, can be intervened by providing educational programs. One of interventions that can be used to change patient behavior/habits by utilizing interpersonal relationships is the motivational interviewing approach. A study by Nugroho [9], shows that there is a difference between the group given motivational interviewing and the control group, where there is a significant difference with $p < 0.05$ on the HbA1C size, thus the intervention can affect the quality of life of DM patients. A study by Miller, et al [10], on African American obese women with Type 2 diabetes shows results that medical nutrition intervention using a motivational interviewing approach is proven to improve respondents' glycemic control and self-confidence.

DM management through physical activity can be done through physical exercise. Regular physical exercise can lower blood sugar levels. Physical exercise in addition to maintaining fitness can also lose weight and improve insulin sensitivity, which will improve blood glucose control. The recommended physical exercises are in the form of aerobic physical exercises such as walking, cycling, jogging, swimming and gymnastics [11]. The results study by Lara and Atik [12], show that the factor of exercise habits is related to the quality of life of elderly people with type 2 diabetes. In addition, a cross sectional study by Halaweh et al [13], reveals that the scores in all Health Related Quality of Life (HRQoL) are significantly higher ($p < 0.05$) in the moderate and high physical activity groups when compared to the low physical activity groups and there is a significant correlation between the five HRQoL dimensions and physical activity levels ($p < 0.001$).

The large number of DM patients in the Pattingaloang Public Health Center (PHC) working area, the heavy burden caused by DM and its complications, how to assess the quality of life of DM patients, and the lack of studies that take the theme of intervention through counseling with a motivational interview approach combined with physical activity, are the reasons for studying the effect of Motivational Interviewing and gymnastics exercise on the quality of life of type 2 DM patients in the region.

Materials and Methods

This study was conducted at two PHC in Makassar City, namely in the Pattingaloang PHC and the Tabaringan PHC working area. The design of this study was a quasi-experimental design with a non-randomized control group pretest posttest design. The study population was all type 2 DM patients who live in the working area. Using the exhaustive sampling technique, the sample size obtained consisted of 30 respondents in the intervention and the control groups.

Secondary data collection was type 2 DM patient data recorded in the medical records in 2020. Then at the time of study, data were obtained through interviews with respondents which consisted of 2 stages,

namely the pretest and posttest stages using a General Characteristics questionnaire and a quality-of-life questionnaire WHOQoL-BREF in Indonesian which has been standardized by WHO. At the intervention stage, the intervention group participated in gymnastics exercise activities led by trained instructors from the PHC then continued with a motivational interviewing counseling session by counselors from trained PHC officers. In the control group, leaflets on guidelines for healthy living and disease management were distributed to DM patients.

Univariate analysis was used to see the description of the characteristics of the respondents presented in the frequency distribution tables and charts. Bivariate analysis was carried out to see the difference between the independent variable and the dependent variables using the T difference test with an alpha-α value of 5%. This study used a quasi-experimental with two types of parametric tests, namely looking at the mean comparison of two groups in pairs using the paired t test and looking at the difference in the mean between two groups of unpaired samples using the unpaired t-test.

Results

Characteristics

Table 1 shows that based on age group, in the intervention group the majority of respondents are in the age group 46 - 55 years and 56 - 65 years, namely 11 respondents (36.7%) respectively and in the control group as many as 10 respondents (33.3%) are in the same age group interval. The majority of respondents are female, in the intervention group there are 24 (80%) and in the control group there are 19 (63.3%) respondents who are women. The latest education of respondents in the intervention group is primary school with as many as 12 respondents (40%), while the latest education of majority of the control group is tertiary education with as many as 12 respondents (40%).

According to the use of diabetes drugs, the data obtained reveal that all respondents in the intervention group (100%) used drugs orally and in the control group, there is 1 respondent (3.3%) who do not use oral and insulin injections. Based on the job characteristics of the respondents, the intervention group is dominated by civil servants and farmers, each of which is 4 respondents (13.3), while in the control group is dominated by civil servants with as many as 10 respondents (33.3%). Based on exercise habits, respondents who never exercised in the intervention group are only 2 respondents (6.7%) while in the control group there are 10 respondents (33.3%).

Bivariate Analysis

Table 2 shows the difference in the mean pretest and posttest values in 4 domains of quality of life in the intervention group. The results of statistical analysis show that there is an increase in the mean value of all quality-of-life domains, namely for the domain of physical health, the difference between the pretest and posttest mean values is 5.76; in the domain of psychological conditions is 3.17; in the domain of social relations is 1.87; and in the domain of environmental condition is 4.63. The four domains of quality of life have a value of p= 0.0001, which means that there is a mean difference in the four domains before and after the respondent was given intervention. The domain with the greatest increase in mean value is domain 1 (physical health).

Table 3 shows the results of the cross tabulation to see the differences in the quality of life between the two study groups. It appears that there is a very significant increase in the number of respondents with a good quality of life category in the intervention group, namely from 7 respondents (23.3%) at pretest increased to 27 respondents (90%) at posttest and a decrease in the number of respondents with a poor quality of life category, from 22 respondents (73.3%) at pretest to 3 respondents (10%) at posttest. In the control group, it can be seen that there is a significant decrease in the number of respondents with a good quality of life, namely from 19 respondents (63.3%) at pretest to 7 respondents (23.3%) at posttest. The statistical test results show the value of p= 0.0001, which means that there is a difference in the quality of life between the intervention group which was given treatments in form of a combination of motivational interviewing and gymnastics exercise programs and the control group.

Table 1. The Distribution of General Characteristics of Respondents

Characteristics of Respondents	Intervention Group		Control Group	
	n	%	n	%
Age group				
26 - 35	1	3.3	0	0
36 - 45	2	6.7	3	10
46 - 55	11	36.7	10	33.3
56 – 65	11	36.7	10	33.3
> 65	5	16.7	7	23.3
Total	30	100	30	100
Gender				
Men (Male)	6	20	11	6.7
Women (Female)	24	80	19	63.3
Total	30	100	30	100
Level of Education				
No education background	3	10	1	3.3
Elementary School	12	40	5	16.7
Junior High School	8	26.7	3	10
Senior High School	5	16.7	9	30
University (Tertiary Eductaion)	2	6.7	12	40
Total	30	100	30	100
Occupation				
Civil Servants	4	13.3	10	33.3
Private Employees	1	3.3	1	3.3
Entrepreneurs	3	10	5	16.7
Farmers/Fishermen	4	13.3	0	0
Housewives	18	60	14	46.7
Total	30	100	30	100
The use of drugs				
Oral	30	100	25	83.3
Insulin	0	0	4	13.3
Do not use any drugs	0	0	1	3.3
Total	30	100	30	100
Exercise Habits				
Yes	28	93.3	20	66.7
No	2	6.7	10	33.3
Total	30	100	30	100

Table 2. The Difference in Mean Values of Pretest and Posttest in the Four Domains of Quality of Life in the Intervention Group

Domains of Quality of Life	Intervention Group		
	Mean Pretest	Mean Posttest	p-value
Physical Health	20.97	26.73	0.0001
Psychological Condition	17.20	20.37	0.0001
Social Relationship	9.40	11.27	0.0001
Environmental Condition	26.60	31.23	0.0001

Table 3. Bivariate Analysis of the Difference between Quality of Life in Pretest and Posttest of the Intervention Group and the Control Group

Quality of life	Pretest				Posttest				p-value
	Intervention		Control		Intervention		Control		
	n	%	n	%	n	%	n	%	
Good/well	7	23.3	19	63.3	27	90	7	23.3	0.0001
Moderate	1	3.3	3	10	0	0	1	3.3	
Bad	22	73.3	8	26.7	3	10	22	73.3	
Total	30	100	30	100	30	100	30	100	

Discussion

21. Domain 1: Physical Health

The results show that there is an effect of a combination of motivational interviewing and gymnastics exercise programs on Domain 1 type 2 DM patients in the intervention group. The increase in the respondents' score in domain 1 that occurred after the provision of the intervention shows that the study objectives have been achieved because respondents could provide significant changes in aspects of their physical health. Aspects that are assessed or seen are related to the energy they have for activities and work, the pain and discomfort they feel, the physical ability to get along with other people, and sleep and rest patterns. Giving motivational interviews gives respondents a good opportunity to share their complaints with the counselors regarding their physical difficulties due to their illness.

In this process, the counselors provide feedback in the form of empathy with encouragement and understanding of how respondents understand the intricacies of the disease they are experiencing, directing them to be able to independently manage their disease so that in the end the respondent can accept their condition as DM patients. The counselors also provide guidance to respondents regarding lifestyle modifications, drug side effects, and how to regulate diet as well as how to do foot care in the right way.

Physical activity in the form of gymnastics exercise which is done regularly 3 times a week for a month provides enthusiasm and positive energy for respondents to look good and be more fit, and it has also been proven

to increase their vitality. Counseling and gymnastics exercise group become a social group with the same feelings and sufferings that encourage respondents to openly change their wrong mindset. In general, the respondents responded very well to the given gymnastics exercise activities, even during the field observations they came very early before the gymnastics exercise schedule was carried out. This shows that they are very happy and enjoy exercising, and of course this is a positive thing that can affect the quality of life of the respondents.

The results of this study are in line with the study by Buchair [4] in Makassar, which reveal that there is an effect of counseling with a home care approach on the domain of 1 respondents in the intervention group with a value of p = 0.002 (p> 0.05). Likewise with the study by Chlebowy, et al [14], which reveal that motivational interviewing significantly increase the chances of participants following the recommended levels of physical activity (p value = 0.018). A cohort study by Thiel [15] in Canada, also shows that there is a correlation between the high score of Health Related Quality of Life (HRQL) in type 2 DM patients with the patients' physical function; role physically; body aches; and vitality, with all statistical values of p = 0.001 (p <0.05) with routine physical activity recommended by the Canadian Diabetes Association (CDA). This study by Thiel [15] in Canada, also shows that there is a significant positive relationship between physical activity and the Health Related Quality of Life score on the dimensions of physical function, vitality, and general health of type 2 DM patients. The study by Halaweh, et al. [13], among elderly diabetic patients in Palestine reveals that the scores in all dimensions of Health Related Quality of Life (HRQoL) are significantly higher (p <0.05) in the moderate and high physical activity groups when compared to the low physical activity group, and there is also a significant correlation between the five dimensions in HRQoL and the level of physical activity (p <0.001).

b. Domain 2: Psychological Conditions

The results show that there is an effect a combination of motivational interviewing and gymnastics exercise programs on the psychological conditions of type 2 DM patients in the intervention group. The aspects assessed include appearance, negative feelings, positive feelings, self-concept, memory, and concentration. In the motivational interviewing process, respondents are directed by their counselors to understand every problem they face because of their illness and to be able to manage themselves wisely in dealing with the burden of the disease they are experiencing so that they are able to minimize the negative psychological effects that arise.

Feelings of anxiety, stress, and pressure due to bad self-concept slowly get a response and guidance from the counselors so that respondents can reduce the psychological effects instilled in them so far, for example excessive worry and body conditions that can become bad at any time. The value of togetherness in counseling and gymnastics exercise groups together with fellow DM patients creates a sense of kinship and sharing of experiences which can also increase self-confidence and the ability to accept their condition. So that it can minimize the emergence of a sense of discrimination and stigma in society, as mentioned in the results of an article that has been done in Korea [16].

Motivational interviewing makes respondents more open about their daily conditions, have the courage to share their feelings and thoughts and concerns so that they can be guided to develop positive things for themselves. When the researchers first collected pretest data through direct interviews, many respondents complained about their inability to accept health conditions and appearance, many of them also felt they could not enjoy life as well as before they were sick and often felt hopeless. However, during the last meeting in the posttest, in general, respondents rarely complained about this, this can be seen from their faces which can be seen directly, especially in the enthusiasm they show when doing gymnastics exercise.

The results of this study are in line with the study by Buchair [4], which reveal that there is an increase in the mean value of domain 2 in the intervention group after the respondents were given counseling with a value of p = 0.000, which indicates that there is a significant difference in the mean score of domain 2 between the intervention group and the control group. Furthermore, the results of the study by Tristiana [17], also reveal that the most effective method for improving self-management and glycemic control in type 2 DM patients is motivational interviewing training provided by nurses

focusing on behavior change because motivational interviewing is included in psychological interventions with the advantage of helping DM patients accept their disease, improving self-care, and reduce psychological distress.

Diabetes can lead to increased rates of depression, so in the future, depression must be recognized as a social problem thus patients must be equipped with systems that can actively prevent depression. [18]. The study by Thiel [15], also shows that the Canadian Diabetes Association recommendation in terms of moderate physical activity for type 2 DM patients is proven to be related to the emotional and mental health roles of type 2 DM patients with a value of p = 0.001 (p <0.05). The study by Syatriani et al. [19], in patients with type 2 diabetes who are also smokers and alcoholics, shows that the stress level has a significant correlation with health-related quality of life in type 2 DM patients.

c. Domain 3: Social Relations

The results of this study indicate that there is an effect of a combination of motivational interviewing and gymnastics exercise programs on the Social Relations of Type 2 DM patients in the intervention group. The aspects assessed include personal relationships, social support, and sexual activity. The increase in domain 3 scores show that there is a positive effect of giving a combination of motivational interviewing and gymnastics exercise programs to encourage respondents to live more productively, to be more open to sharing their life problems related to their illnesses, and to increase the willingness of social interaction, especially because of togetherness in the counseling and gymnastics exercise groups. During counseling, respondents are guided to understand the importance of socializing, establishing intense and harmonious communication with family members, spouses and neighbors. In a counseling and gymnastics exercise group, respondents can interact with fellow DM patients, share experiences in dealing with the burden of life due to their illness, and seem to have a new family with almost the same experiences and sufferings therefore these are some of the causes of increasing positive changes that occur in respondents.

This is in accordance with the study by Huang, et al [20], on type 2 DM patients in Taiwan, which reveal that the experimental group who was given motivational interviewing through motivation and cognitive enhancement therapy shows a significant improvement in terms of quality of life physically and mentally after being given the intervention compared to the control group which do not change at all throughout the study. Besides, a quasi-experimental study by Buchair [4], on type 2 diabetes mellitus patients in Makassar, also get the same result in which there is a significant difference in the mean score of domain 3 between the intervention group that was given counseling and the control group (18.10; p= 0.000). However, these results are not in line with research by Hayashino, Tsujii and Ishii [21] in Japan, which reveals that there is no significant increase in score in domain 3 Diabetes Therapy-Related Quality of Life (DTR-QOL) in DM patients with p = 0.507.

d. Domain 4: Environmental Conditions

The results prove that there is an effect of a combination of motivational interviewing and gymnastics exercise programs on the environmental conditions of type 2 DM patients in the intervention group. The aspects assessed include financial ability, freedom, availability of health and social services, home environment, physical environment, and transportation. The significance in domain 4 shows that the implementation of a combination of direct motivational interviewing and gymnastics exercise programs has led respondents to make decisions in their lives in maximizing their financial capabilities, trying their best for health and recovery, being moved to carry out routine health checks even though they have to sacrificing their time queuing at the PHC, and caring about the environment in which they live.

Study by Buchair [4] in Makassar reveals that counseling has a positive influence on domain 4 of the quality of life of type 2 DM patients where there is a significant difference in the mean score of domain 4 between the intervention group and the control group of 9.55 with p = 0.008 (p <0.05) . The results of Tristiana's study [17], also show that intervention through motivational interviewing is effective in improving self-management (including activity, diet, and medication areas) and glycemic control in patients with type 2 diabetes mellitus.

The results of this study indicate that there is an increase in the average score (mean) in each domain of

quality of life from pretest to posttest in the intervention group after being given a combination of motivational interviewing and gymnastics exercise programs with a significance value ($p<0.05$). The main result of this study is that the combination of motivational interviewing and gymnastics exercise programs is proven to have an effect on improving the quality of life of type 2 DM patients. The significance that occurs in each domain of quality of life becomes a measure of improving the quality of life of respondents and the success of the given program. The strength in counseling with the motivational interviewing approach is the empathy shown by the counselors, in this case, actively listening to patients without judging or blaming the behavior of DM patients in order to better understand their situation and perspective. As counselors understand that DM patients who are in the early stages of change may not be fully ready to let go of the initial behavior, so the main focus is on building relationships and supporting them in having the ability to change in various ways. The latest development of the motivational interviewing program has now used cellphones and digital platforms in their applications [22].

Physical activity carried out regularly and continuously in the form of gymnastics exercise has a positive impact. In theory, it is understood that gymnastics exercise can provide many benefits in the management of DM, namely controlling blood glucose levels, reducing the risk of complications, losing weight and maintaining it for a long time; psychological benefits because it can improve the level of physical fitness so that patients feel fit, anxiety against the disease is reduced, increased feelings of pleasure and self-confidence, and in the end will improve the quality of life of the patients and can reduce the level of use of oral drugs and insulin.

The combination of motivational interviewing and gymnastics exercise programs has an influence on improving the quality of life of type 2 DM patients, this is possible because of the high enthusiasm of respondents during the study to actively participate and play a role in the interventions given. It can be seen from their regular attendance according to the schedule, they are even enthusiastic about coming earlier [to the scheduled meetings]. At the counseling stage, they have a place to be open, to share their experiences and feelings freely, to feel they have a place to listen to all their complaints and to get guidance for healthier life behaviors which are not limited by the effects of their illness.

The implementation of gymnastics exercise is one of the driving factors for improving the quality of life of type 2 DM patients in this study, which indicates that there is a positive correlation between giving regular exercise to the quality of life of type 2 DM patients, whether it is regarding the patients' physical health, psychological conditions, social relationships, and environmental conditions. This is possible because gymnastics exercise is a form of physical activity that has many benefits for health; and through direct observation and interviews between the researchers and respondents in the field, it is clear how different changes have occurred to respondents from the start to the completion of the study. Respondents expressed a feeling of being fitter, healthier, and generally for those who have difficulty sleeping, can enjoy better quality sleep.

Several studies that support the results in this study are like the study conducted by Miller, et al [10], which reveals that Medical Nutrition Intervention through the Motivational Interviewing approach is shown to improve glycemic control and increase self-confidence related to self-confidence in African American women who are obese and suffer from type 2 diabetes mellitus. Besides, Huang et al. [20], through his research in Taiwan also reveals that the experimental group shows a significant reduction in glycosylated hemoglobin, fasting glucose, and depressive symptoms, and a significant increase in physical quality and mental quality of life after being given intervention through motivational interviewing behavioral therapy, whereas patients in the control group with usual care show no change over the study time.

The study by Buchair [4], explains that the counseling services provided to DM patients have a positive impact such as making patients feel comfortable and happy to be visited by health workers who provide information to patients to better manage their disease and have caused them to feel supported so that they can accept their condition and be motivated to improve their quality of life which eventually will help them to minimize complications. Counseling is effective as a therapy to improve quality of life, but the counselors play an important role as a person who provides solutions

that can help patients deal with the problems they are experiencing.

Health education or counseling that has been provided to diabetes patients by the PHC is basically good enough to convey messages and information that they need to know about diabetes, including things that must be done for self-care, but this is not enough to make patients make change movements for the improvement of their behavior. A study has shown that motivational interviewing has been proven to be effective in the effectiveness of children's health education [23].

Apart from the fact that respondents usually find it difficult to focus on listening, sometimes counseling is seen only as information that is heard for a moment and after they are home, they forget about it. Therefore, it is necessary to develop a more specific and individual educational program that focuses on the risk perception of each person [24]. Although, it is understood that it is indeed very difficult to build a health service infrastructure in a short time [25].

Counseling through the motivational interviewing approach is one method that can be used to be closer to patients, to rely on empathy to hear patients' complaints and experiences in their daily lives, and to slowly make them to better understand the life-changing principles they need to implement. Besides, the combination with regular gymnastics exercise makes patients be more excited and feel they have a social community which is beneficial for their vitality and fitness. So, it can be considered that to carry out a target group development program to improve daily functions and quality of life, it requires better attention from health professionals [26].

A significant decrease in the quality of life of control group respondents is possible because based on the analysis of general characteristics and health history of respondents, it is known that quite a lot of respondents in the control group are in the elderly group > 65 years. The existence of variations in treatment is an indication of different pathophysiological mechanisms for each individual who experiences type 2 diabetes. However, there are patients who claim not to take the drug. A study shows a significant relationship between age and poor glycemic control in type 2 DM patients so they experience secondary medical complications [27].

From the aspect of daily behavior, when compared to the intervention group, there are more patients in the control groups who still do not manage their diet well, do not exercise enough, and still like to consume sweet foods and drinks, and many of them do not control their blood sugar regularly. In fact, health behaviors such as mental health, eating habits, lifestyle, and physical activity have a significant relationship with health status [28].

The study of Amiruddin et al [29], on patients with gestational diabetes mellitus (DMG) in Makassar City, it is known that the risk of pre-diabetes and DMG increases with a history of less fiber consumption (OR = 2.355, 95% CI: 1.12-4.94) and less history of coffee consumption (OR = 2.406, 95% CI: 1.10-5.25) with a probability of 96%. A diet high in fiber can increase carbohydrate metabolism. It can help to control and prevent increases in blood sugar levels. These factors may be the cause of the decreasing quality of life of type 2 DM patients at posttest in the control group, especially because they do not receive any intervention other than giving healthy behavior leaflets which they do not necessarily read and apply in their daily life.

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Antimicrobial Activity of Lactic Acid Bacteria Found in Dadiah on Disease-Causing Skin Infections

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Abstract

Dadiah is a fermented food made from buffalo milk containing lactic acid bacteria (LAB). The potential of LAB as an antimicrobial organism is expected to inhibit or kill pathogens, especially those causing inflammations in the skin. *Dadiah* used in this study was obtained from the Lintau area of Tanah Datar District, West Sumatra, Indonesia. LAB isolation and identification involved the use of MRS Broth (MERCR) and MRS Agar (MERCK) media and the pathogenic bacteria used include *Staphylococcus aureus, Propionibacterium acnes, Acinetobacter Baumannii, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*. The results of this study obtained a total LAB of $7x10^{11}$ CFU / g and showed the highest antimicrobial activity in the *Staphylococcus aureus* with a14.53 mm clear zone and 13.18 mm bacteria in the *Pseudomonas aeruginosa* test bacteria.

Keywords: *Antimicrobial dadiah, lactic acid bacteria, skin diseases*

Introduction

Skin diseases are the most common types of diseases in developing countries, especially in those with tropical climate. Therefore, humid and hot air throughout the year is highly suitable for the development of infectious, disease-causing pathogens in the skin. The prevalence of such infections in developing countries ranges between 20-80%. As a developing country, Indonesia and its inhabitants are prone to skin diseases of which its main causes include poor geographical and hygiene conditions. Such conditions enhance the rapid development of pathogens and bacteria. Therefore, the attention and recommendations of appropriate health organizations in the preventive measures against skin infections is presently a necessity. The problem of antibiotic resistance has become a worldwide problem, especially during prevention and cure, that necessitates viable solutions in form of alternative therapy or treatments[1]. These forms of alternative therapy include synthetic antibiotics.

Probiotics, vitamins, and phenolics are a few examples of nutraceuticals. These substances are important for skin protection and health, and they are known to assist in medical treatment of dermatological problems[2]. This study aims to discover the role of probiotics (Lactobacillus) as a prevention and treatment for skin infections. Many studies have shown that lactic acid bacteria (LAB) helps to activate commensal bacteria due to its probiotic properties, and gets rid of pathogenic bacteria through the antimicrobial properties derived from either bacteriocin found in LAB itself, or the role of its peptides. Some studies also mention that the role of probiotics in nutrition involves boosting the immunity against skin infections caused by various pathogenic bacteria such as *Aeromonas bestiarum, Ichthyophthirius multifiliis, Staphylococcus aureus, Corynebacterium acnes, Streptococcus*, etc[3].

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Dadiah is a traditional food from West Sumatra made of buffalo milk that undergoes a natural fermentation process in a bamboo tube, *Dadiah* originally made from Lintau in Tanah Datar, contains probiotics such as *L. Plantarum* which has proven to be effective in maintaining the balance of intestinal microflora, and contains strong antimicrobial properties[4].

Atopic dermatitis is an inflammatory skin disease occurring often in children and the elderly [5]. The dominant bacterial species found in this skin disease is *Staphylococcus aureu*s. The colonies of this bacteria, when present in the skin, can result in a wide range of infections therein. Therefore, antimicrobial treatment is necessary to prevent such bacterial growth [6] . Many broad-spectrum antibiotics can be used as an inhibitor of *Staphylococcus aureus*, such as penicillin, doxycycline, tetracycline, clindamycin, etc. However, improper use of an antibiotic results in drug resistance. It is also associated with an increased risk of infection and emergence of bacterial colonies, known as "Multidrug-Resistant Organisms (MDRO)" [7]. These Methicillin resistant *Staphylococcus aureus* (MRSA) and MDRO are the main causes of nosocomial pathogenic infection worldwide, and require serious treatment, as they increase comorbidity and death due to the infection[8]. A major challenge posed by *S. aureus* is antimicrobial resistance. After the introduction of the antibiotics penicillin and methicillin into clinical practice, strains of antibiotic-resistant *S. aureus* (MRSA) were subsequently identified [9].

Therefore, this study was aimed to determine the role of *Dadiah* as a probiotic that can be used to inhibit bacteria-induced skin diseases, especially the inflammatory infections, as a supporting or future treatment alternative against skin diseases.

Materials and Methods

Isolation of Lactic Acid Bacteria

One gram of *Dadiah* sample was placed within test tubes containing 9 ml of MRS broth and diluted up to ten logarithmic (10- 10) fold. Furthermore, it was added to test tubes containing 900 ul of MRS broth variations of dilution ratio 10-1, 10-2 and 10-7. Furthermore, 100 ul of the serial dilution of 10-9 was inoculated into the MRS Agar media using the spread method, placed in an anaerobic jar and incubated for 48 hours with a temperature 37ºC. After incubation, a single colony of LAB was obtained and hence isolated [10].

Identification of Lactic Acid Bacteria

Identification of LAB from *Dadiah* samples was done by placing 1g of the sample in a test tube containing 9 ml MRS Broth diluted up to ten (10-1) logarithmic fold. It was further incubated in anaerobic conditions for 24 hours, after which a 100 uL 10-1 dilution was added to the tube containing 900 uL 1 MRS broth dilution 10-2, and a subsequent dilution of 10-8. Consecutively, 100 uL of the serial dilution of 10-8 was inoculated on the media MRS Agar with the method of spread, placed within an anaerobic jar, and incubated for 48 hours at 37 ^{0}C. A single (choice random) colony that is round, slippery, and either white or yellowish is characteristic of LAB. Finally, this bacterial colony was transferred via streak method and incubated [10].

Antimicrobial Activity

The antimicrobial resistance testing was carried out using five bacteria samples that cause skin disease. LAB culture (1 mL) was centrifuged at 10,000 rpm for 5 minutes at 27^0 C, and supernatant volume of 50 uL was used to test for antimicrobial resistance. 0.2% of test bacteria (*Staphylococcus aureus, Propionibacterium acnes, Acinetobacter Baumannii, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*) was placed on Nutrient Agar (NA) as a growth media for test bacteria, homogenized, injected by micropipette, and incubated at 37 ^{0}C. Clear and circular inhibition zones are measured after 12 hours [11].

Results and Discussion

Isolation Lactic Acid Bacteria

Dadiah originating from the Lintau area of Tanah Datar District, West Sumatra, Indonesia is known to contain Lactic Acid Bacteria (LAB). It was isolated with MRS as a specific media for LAB growth and incubated for 48 hours. In this study, the amount of LAB used was 7x10^{11} CFU / g. This result is higher compared to those obtained, while the total LAB obtained from Payakumbuh Dadiah was 8x10^8 CFU/g [12], in a research at Solok district, the total LAB obtained was as much as 8x10^8 CFU/g [13]. This is because Dadiah kicks off

its fermentation spontaneously; without the need for a fermentation starter. LAB was also found in buffalo milk obtained from several areas in West Sumatra totaling $3x10^6$ - $2x10^8$ CFU/g [14]. This is consistent with the standards which state that LAB in probiotic food must be present within the range of 10^6 - 10^8 CFU / Gram [15].

Identification of Lactic Acid Bacteria

Incubation was done using MRS Broth and then cultured on MRS media. For 48 hours in 37^0 C incubators, a single colony was obtained through macroscopic observations with round, cream-white and convex shapes. Microscopic observations showed that the bacteria were Gram-positive and rod-shaped. Lactic acid bacteria (LAB), belongs to a group of gram-positive, catalase-negative bacteria, which synthesize lactic acid by the fermentation of carbohydrates. The cells are known as coccus or bacillus, usually arranged in pairs or chains. LAB are facultative anaerobes, non-motile and mesophyllic [16].

Antimicrobial Activity

Antimicrobial testing using *Staphylococcus aureus, Propionibacterium acnes, Acinetobacter Baumanni, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* commonly found in skin infections can be seen in Table 1.

Table 1. Diameter Clear Zone

Isolate	Bacteria test (mm)				
	Staphylococcus aureus ATCC	Propionibacterium acnes	Acinetobacter baumannii	Klebsiella pneumoniae	Pseudomonas aeruginosa
3A	14,53	7,6	10,24	10,15	13,18

Testing antimicrobial activity involved a well-known method of random selection of 3A isolates from curd which have the potential to function as probiotics, because they can inhibit the growth of pathogenic bacteria which cause skin diseases. The clear zone produced by lactic acid in table 1 shows the existence of antimicrobial activity from LAB isolated from Dadiah samples obtained from Lintau, Tanah Datar District. The ability of LAB to inhibit pathogenic bacteria is due to the presence of its metabolites such as lactic acid, peroxide, and bacteriocin.

Clear zones with the highest numbers were produced in the *Staphylococcus aureus* ATCC test bacteria (14.53 mm) from *Dadiah* isolates. This clear zone is smaller when compared to the study of which used LAB isolated from tempoyak which inhibits the bacterium *Staphylococcus aureus* which a diameter of 19.3 mm [17].

In *Propionibacterium acnes,* test bacteria obtained a clear zone of 7.6mm, the smallest diameter compared to other test bacteria. This result is smaller when compared with *Lactobacillus casei* strain of Shirota bacteria of 11.2 mm and therefore higher in inhibitory power than the *L. acidophilus* HM1 bacteria of 3.3 mm [18]. The clear zone of *Acinetobacter baumannii (*10.24 mm) obtained in this study is greater than that of which used the *Lactobacillus acidophilus* LMEM8 against *Acinetobacter baumannii* and obtained a diameter of 5.13 mm [19]. The results of this study are more productive than that of in testing the antimicrobial activity of *Lactobacillus fermentum* MTCC 9748 against *Klebsiella pneumoniae* NTCC 703603 which resulted in a 9.17 mm clear zone [20]. Moreover, the *Pseudomonas aeruhginosa* test bacteria (13.18 mm clear zone) was not significantly different compared to the research testing of *Lactobacillus* spp against *Pseudomonas aeruginosa* bacteria with a range of clear zones 11-21 mm [21].

Conclusion

Lactic acid bacteria isolated from *Dadiah* of Lintau origin is probiotic in nature, and it shows a highly significant inhibitory effect against *Staphylococcus*

aureus (of which the largest clear zone of 14.53mm was obtained), *Propionibacterium acnes, Acinetobacter baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. However, further research is necessary in order to ascertain other probiotic, antimicrobial candidates which inhibit pathogenic bacteria which cause skin diseases and infections.

Conflict of Interest : None

Ethical Clearance: Not required in an in-vitro studys

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Children and Stunting Phenomenon in Indonesia: Legal Perspective

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Abstract

Indonesia is a country with abundant natural resources, yet the case of malnutrition has gone on since a long time ago. The non-serious handling of malnutrition causes various health conditions such as stunting. This will influence economic capacities and productivity; thus, it becomes a serious threat. This research uses a quantitative method, by using primary and secondary legal sources.

Stunting may have implications on children's health both in the short and in the long run. It may influence the country's economy. The roles of the government, parents, family, and the society is not enough in protecting children from stunting. The government has not maximally implied various programs in preventing and handling stunting, thus the rate of stunting is not yet according to the standard given by WHO. This describes some aspects and the citizens' rights which are not yet fulfilled, including the rights to live, the right for health, the right for children to grow, and other rights.

Keywords: *protection, law, stunting, perspective*

Introduction

Indonesia is a country which is famous for its richness in natural resources. Yet, unfortunately, this country cannot be separated from the problem of malnutrition, which has gone on for a long time. The handling of malnutrition which is not serious enough causes many chronic diseases, including stunting. Stunting may influence the learning activity of children in school age. It will influence scores and school achievements. As adults, it will affect wages, the risks of chronic diseases such as diabetes, morbidity and mortality. It may even influence the state's economic productivity.(1)

On 2013, Indonesia was in the world fifth rank with the highest percentage of stunting, which reached 37%. On 2017, Indonesia was ranked the third country with the highest prevalence of children who suffer from stunting. It is also ranked as the fifth country with the highest number of stunting infants in the Southeast Asia Region. This percentage means around nine million people. Thus, this case becomes a serious threat.

The factors which cause stunting in children are as follows: inadequate nursing practices, the limited number of health services for pregnant women and for post-partum women. There is limited early education. The limited access for families to obtain nutritious food, and the limited access to clean water and sanitation. These factors are results of a study held by the Republic of Indonesia's Ministry of Health, which was published by the World Bank. The condition of stunting was caused by the lack of nutrition fulfillment whilst the child was still in the womb for 9 months, and during the age of growth which is the first 1000 days of a child's life. The lack of nutrition in the first two years will cause the child permanent brain deterioration. Malnutrition does not only pose a threat to the child's development and his/her physical health. Yet, it will also cause poverty due

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to the brain deterioration as it influences intelligence. In the future, it will cause the child who suffered stunting to have difficulties in finding a job.[2]

The citizens' quality is a challenge for the government. It is also its challenge to solve the phenomenal problems and to give legal protection. By knowing the factors which cause these problems, the government will create intervention programs. The aim of this research is to analyze the implementation of the legal protection from the government as the state's active role towards children who suffer from stunting.

Method

This research uses a qualitative method, where primary and secondary legal sources are used. The primary legal sources are the constitutional regulations. Meanwhile, the secondary legal sources are sources which give explanations regarding the primary legal sources. These secondary legal sources are obtained by the writer from literature review and documents of research results. The legal sources obtained will be collected and analyzed qualitatively by description and logical explanation.

Results and Discussion

Stunting is a highly dangerous health condition, as it does not only cause impacts to health and the children's level of intelligence, yet it also brings impact towards the future of the children and the nation. Apart form that, if not treated well, stunting may cause death.[3]

Moreover, the citizens' malnutrition is the government's failure in creating food security, both as the administrator and the regulator. This failure shows that there is a legal responsibility which is violated by the government, even though then the government tried to object from its responsibility and tried to propose some excuses, such as the field's condition. Handling the stunting disease means eradicating poverty. Poverty is a condition which limits human beings from accessing various opportunities.[4]

The state has the responsibility and the obligation to give protection for all citizens. Especially, in this paper which talks about the phenomenon of stunting, the government must pay attention to it. Some aspects which must be given attention from the government includes respecting citizens, protecting citizens, giving them security, fulfilling the state's responsibility to act actively so that the citizens' rights may be fulfilled, including the rights of children who suffer from stunting.

The government's policy program regarding the handling of stunting is a manifestation of protection for children. It is the order of the constitution. Thus, the state has the obligation to take legislative, administrative, and legal steps as well as other actions to solve this problem. The healing of children from stunting becomes the state's obligation so that children may live healthily and properly. Every child has the right to obtain good health services. They have the right for food, nutrition, and prosperity.

Specific rights for children who suffer from stunting has not yet been regulated specifically in the national law. The law on children's rights for protection is regulates in the Republic of Indonesia's 1945 Constitution, on the constitution No. 4 of 1979 regarding Child Prosperity, the Constitution No. 23 of 2002 regarding Child Protection, the Constitution No. 36 of 2009 regarding Health, the Constitution No. 3 of 1997 regarding Justice for Children, and the Constitution on Food.

The high rate of children who suffer from stunting becomes the indicator of the high rate of malnutrition in Indonesia. It shows that Indonesia children have not yet received enough protection from the law. The data from the Nutrition Status Surveillance (Pemantauan Status Gizi/PSG) from the last three years shoe that stunting has the highest prevalence compared to other problems of nutrition such as malnutrition, underweight, and overweight. It was 27,5% on 2016, and became 29,6% on 2012. The main causes of stunting are the factors of low intake of nutrition, infectious diseases, the gender of male, the low education of the mothers, children do not obtain exclusive breastmilk.[3]

The main factors which cause stunting is the low intake of nutrition for the mother and the children. There are multidimensional factors of nutrition. Apart from factors which directly touch mothers and children, stunting may also be caused by lack of facilities.[5]

Article 34 of the 1945 Constitution states that the poor, the needy, and misplaced children are taken care of by the state. This law mentions clearly that the

state has the responsibility to bring prosperity to its people, especially the poor families as mandated in the constitution.

Stunting causes two kinds of negative influences to the child's development, which are the short-term impact and the long-term impact. In the short-term impact, it causes the higher risk of diseases and death. The child's development is not optimal and it will increase the funds needed for health. The other impact is the long-term impact, where the body posture is not optimum. There will be risks for health problems such as obesity and other diseases. There will be a deterioration in the reproductive health, in the ability to study, and the work capacities. Death which takes the life of the nation's children is caused by the lack of protection and health services, which may be categorized as a violation of human rights. The violation of health rights is a violation of the constitution.[5]

Because of this, it is an obligation of the government to maximize the effects of policies and actions of programs in preventing stunting and healing children from it. If the suffering children are not healed, it will destroy the future of the children and the nation, and it may even cause death. Meanwhile, the rights violated by the state are the rights to live, the right for health, the right for growth, the right for children's prosperity, the right for education, and the right for proper living. The right for life is an absolute right which cannot be restricted in any condition, since it is a blessing from God.

The protection and the fulfillment of the citizen's constitutional rights must be carried out according to the people's condition. In the Indonesian society, there are differences in capabilities which are caused by marginalized social structures in obtaining the access for rights protection and fulfillment given by the state. The state's programs in solving the case of stunting has not been reached until now, and its execution is not equally spread. Indonesia consists of some provinces. Yet, according to data from the Ministry of Health, there are only two provinces which are under the standard of the WHO, which are Yogyakarta and Bali. The program of providing food aid from sources which are under the standard. There are no specific policies on the fulfillment of nutrition for pregnant women, breastfeeding women, babies, infants, and other malnutrition-prone groups.[6]

There needs to be a regulation which specifically manages the rights and responsibilities of children who suffer from stunting. It is the role of the central and the regional governments to realize their responsibilities. They should provide facilities and infrastructure. There should be professionalism in health services regarding the handling and the prevention of stunting. It is the rights and the responsibilities of the family, with the role of the society and the law enforcers. There should be legal sanctions, as sanctions will enforce the application of a law so that the law certainty may be guaranteed, and that it will bring benefits.

The handling of stunting cannot be done individually as there would not be any significant impacts. The efforts to prevent stunting must be carried out with integrated and convergent methods with multisectoral approaches. Because of that, the government must make sure that all ministries, all institutions, all development partners, academicians, professional organizations, civil society organizations, private companies, and media may work together to accelerate the prevention of stunting in Indonesia.[7] Not only in the central level, yet the integration and convergence of the effort to prevent stunting must be done in the regional level, and up to the village level. In the implication of the acceleration in stunting prevention, there are some obstacles. For example, the programs in preventing stunting are not yet effective. The allocation of the financial resources and human resources are not yet effective nor efficient.

The right for health is the right of every human being which is guaranteed in the constitutional regulations. Yet in reality, there are still actions of discriminative health services. This is proven by the abundant reports on the services which are not yet optimum, and that there are discriminative actions experienced by some people.

Conclusion

Stunting is a highly dangerous health condition. This is because it does not only bring impact to the health and the intelligence of children, yet it may impact the future of the children and the nation. Moreover, if not handled well, stunting may cause death. Stunting may have implications to the children's health both in the short and in the long term. It will affect the nation's economy.

The government, parents, families, and the society has not made serious actions in protecting the rights of children who suffer from stunting. The government has not maximally implemented the various programs in preventing and in handling stunting as an effort to protect the children's human rights. This implies to the fact that the rate of stunting is not yet according to the WHO's standard. The state is not yet able to give legal protection for children who suffer from stunting. This describes some aspects and some of the citizen's rights which are not fulfilled, such as the right to live, the right for health, the right for children's development, etc.

Ethical Clearence: Yes

Conflict of Interest: No

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Which Predisposing Factors Influence The Farmers in Compliance of Personal Protective Equipment (PPE)?

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Abstract

Farmers who work in the agricultural sector are workers who need attention in the field of occupational safety and health because of the combinations of hazard and risk. One of them is the risk of pesticides, such as poisoning to death. To prevent it, the use of appropriate PPE is the best solution, but most farmers do not comply with them. The specific purpose of this study was to find in-depth information about the use of PPE by vegetable farmers. It is a qualitative study with a phenomenological approach to study the phenomenon of PPE non-compliance among vegetable farmers. Sampling was carried out by using purposive sampling technique, which involved 18 farmers from different village. Data were analyzed using content analysis. The result showed that the farmers had worked for more than 10 years. They knew the function of PPE and the impact of not using it, education had no impact on compliance of using PPE based on the majority of farmer's education. Also, the farmers can not provide the nominal for purchasing PPE, because it is incidental so income has not contributed to compliance of using PPE. And although most of the farmers have a good attitude to compliance of using PPE, it has no impact on compliance of using PPE in vegetable farmers. It can conclude that the predisposing factors in this study are considered insufficient to make a positive contribution to compliance with the use of PPE.

Keywords: *predisposing factors, enabling factors, reinforcing factors, pesticides*

Introduction

One of the main sectors that play an important role in the national economy in absorbing labor is agricultural sector. Indonesian Central Bureau of Statistics reports that there are 33.48 million workers (farmers) of the total productive age.[1] Farmers who work in the agricultural sector are workers who need attention in the field of occupational safety and health considering that there are many combinations of hazards that exist in the work process such as the use of equipment, chemicals, plants, animals, and others.[2]

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WHO affirmed that farmers are at great risk of being exposed to pesticides.[3] Pesticides are chemical substances and other substances as well as microorganisms and viruses used to control various pests. Pesticides can prevent agricultural land from being attacked by pests in order to maintain and increase crop yields. However, the use of pesticides not only has a positive impact but also causes health problems for farmers. A study conducted by Waggoner et al., showed that pesticide use for> 60 days can result in fatal injury.[4] WHO also reported that there are approximately 400,000-2 million people experiencing pesticide poisoning, which causes the death of between 10,000-40,000 people worldwide. In Indonesia, it is estimated that there are 300,000 cases of poisoning each year, although only a small proportion are fatal.[5]

Pesticide exposure can be minimized by using Personal Protective Equipment (PPE) at all stages of handling and use.[6,7] The use of appropriate PPE is the

best solution in preventing occupational diseases in the agricultural sector. However, most farmers do not comply with using PPE while working.[8]

Pattapang, Tinggimoncong, Gowa regency is one of the areas that cultivates vegetables in South Sulawesi. Based on preliminary observations, it was found that farmers' knowledge and attitudes towards the use of PPE were in the good category which unfortunately was not in line with their actions. This condition was the basis for researchers to conduct a study on the phenomenon of PPE noncompliance among farmers. The specific purpose of this study was to find in-depth information about the use of PPE by vegetable farmers in Pattapang, Tinggimoncong, Gowa Regency.

Material and Methods

It is a qualitative study with a phenomenological approach to study the phenomenon of PPE non-compliance among vegetable farmers in Pattapang, Tinggimoncong, Gowa Regency. Sampling was carried out by using purposive sampling technique, which involved 5 farmers of Lembanna village, 5 farmers of Buluballea village, 4 farmers of Kampung Beru village, and 4 farmers of Pattapang village. Data were analyzed using content analysis including the stages of recording transcripts, data reduction, informant coding, and drawing conclusions.

Findings

Predisposing Factors:

Working Period

The informants in this study mostly started farming at a young age, so it could be said that they had worked for more than 10 years.

"*I started farming since elementary school, now I am about 40 years old.*"

"*I have been farming for about 20 years. I am now 55 years old."*

The habit of helping parents is one of the factors that influence to be a vegetable farmer

Knowledge

All vegetable farmers in Pattapang, Tinggimoncong, Gowa Regency in this study had good knowledge regarding the use of PPE. They knew the function of PPE and the impact of not using it.

"Masks are used for respiratory protection.*"*

"If I do not wear a mask, I can get pesticide poisoning when I spray vegetables with pesticides."

In this study, knowledge influences in the use of PPE but did not have a significant impact on the compliance in the use of PPE

Education

Most of the informants in this study were elementary school graduates.

"I have been farming since elementary school."

"I only finished elementary school."

In this study, education had no impact on compliance of using PPE based on the majority of farmer's education (elementary school)

Income

The monthly income of vegetable farmers in Pattapang Village varied, but on average they could earn IDR 1,000,000 a month. This varying income was caused by the uncertain or changing market price of their agricultural products. It was also influenced by the area of land cultivated by farmers and the types of vegetables. Most farmers did not have a special allocation of funds for purchasing PPE since they said that they would buy new PPE if the PPE they owned was completely damaged and could no longer be used.

"*I will buy new PPE if my PPE is damaged."*

This shows that farmers cannot provide the nominal for purchasing PPE, because it is incidental. So, income has not contributed to compliance of using PPE.

Attitude

Most of the informants in this study had a fairly good attitude in terms of response to compliance with the use of PPE.

"*I actually agree (if there is a penalty for those who do not use PPE), because it is for the good of the farmers*

themselves, but the rules must be clear"

"*If there must be a sanction, please apply the rules."*

Although most of the farmers have a good attitude to compliance of using PPE, it has no impact on compliance of using PPE in vegetable farmers.

Discussion

Predisposing factors are determining factors in behavior; factors that facilitate and underlie the occurrence of certain behaviors. Predisposing factors are antecedents of behavior that describe rationale or motivation to perform an action, perceived values and needs, which are related to individual or group motivation to act. They are mostly in the psychological domain. In general, it can be said that predisposing factors are personal considerations of an individual or group that influence the occurrence of a behavior. These considerations can support or hinder behavior.[9]

In the case of vegetable farmers in Pattapang, the majority of them did not have a strong intrinsic motivation to act or behave better. It was largely influenced by the belief factor that was very strong had by farmers, for example they believed that their current condition was still healthy for farming due to the correct farming methods. Thus, the existence of new knowledge related to agriculture will not be directly and easily applied because of their beliefs and values. However, it is also possible for them to apply the new knowledge as long as it is good for farmers and in line with the concepts and values held by farmers in Pattapang.

Moreover, matters related to farmers' values and beliefs are the decisions they make when facing certain conditions, such as responses or planning for purchasing PPE for farming. The majority of informants in this study did not plan to buy or provide PPE. They only relied on the PPE they had, even though it was not in accordance with the PPE standards required. However, if they needed to buy PPE, most vegetable farmers would buy PPE that was available according to their needs, without paying attention and considering the safety and quality standards of the PPE.

More importantly, income is also usually used to assess the relationship between income levels and utilization of health services and prevention efforts. Limited income can cause a person not to maintain the quality of his health. The higher a person's income, the higher the efforts to prevent and utilize health services that he/she does. In accordance with the economic conditions of most farmers in Pattapang, the monthly income obtained varied greatly, which depended on the value of the product on the market. The market price uncertainty could indirectly influence farmers' decisions in allocating/planning PPE based on safety and health standards. Accordingly, purchasing PPE was not a priority for vegetable farmers in Pattapang, but it was only incidental.

Most of the informants in this study had a long working period of > 10 years. The experiences that farmers get through their work should have a positive impact on compliance with the use of PPE while farming. In fact, the working period does not have a significant impact on compliance with the use of PPE. Farmers with long working period tended not to comply with the use of PPE. Farming experience with minimal incidents was one of the references for farmers with a long working period. This experience made them believe that the use of PPE in farming was not mandatory and needed to be done continuously.

The higher a person's education, the easier it is to receive information, so that the more knowledge he/she has. Conversely, low levels of education can hinder the development of one's attitudes towards newly introduced values. The majority of informants in this study are elementary school graduates. The lack of knowledge related to agricultural safety could be caused by the habits of farmers in Pattapang who had to help their parents earn money by farming since childhood. Thus, habits and environmental support made the majority of vegetable farmers in Pattapang finish elementary school and did not continue to pursue higher education. Based on the research of Oyekale, formal education has influence to the safety precaution in spraying agrochemicals and use of PPE in Cocoa Farmer in Cameroon.[10]

Lack of opportunities to gain more knowledge through school is one of the factors that can cause PPE non-compliance among the farmers. Education obtained in school is a factor that can shape character, habits, discipline and moral values for one's mental development, so that education is likened to a foundation

in the formation of a person.

Knowledge is the result of human sensing or the result of someone's knowledge of an object. Sensing occurs through the five senses and most of human knowledge is obtained through hearing and sight. The knowledge possessed by individuals is one of the factors determining them in seeking and requesting health service efforts.[11]

The majority of farmers had fairly good knowledge and attitudes about agricultural OSH. They knew the function of the PPE required and had an understanding of the impacts of not using PPE. Most of the farmers obtained agricultural OSH knowledge from OSH promotions carried out by related agencies, such as the Gowa Regency agriculture and plantation office, counseling carried out by farmer groups or students who were doing Field Work Practices. Regarding the attitude aspect, vegetable farmers in Pattapang had a fairly good attitude response regarding the use of PPE, but the final response did not show that. In line with study of Sharifzadeh et al., knowledge have an important role in case of PPE acceptance and use.[12]

In addition, attitude is not yet an action or activity, but it is a predisposition to the action of a behavior. Attitude is the readiness to react to objects in a certain environment as an appreciation of the object.[13] Attitude factor has a fairly weak relationship with compliance with the use of PPE.

Conclusion

Predisposing factors such as working period, income, education, knowledge, and attitudes as intrinsic factors of the farmer are considered insufficient to make a positive contribution to compliance with the use of PPE.

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Ethical Clearance: None

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Method Development and Optimization for Colchicine Estimation in Toxicological Analysis using Dispersive Liquid-Liquid Micro-Extraction and HPLC-UV

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Abstract

Background: Colchicine is an alkaloid with anti-mitotic properties and has a narrow Therapeutic Index which can be proven to be fatal at higher doses. The present study is an attempt to develop a method that includes an effective adaptation of Dispersive Liquid-Liquid Microextraction (DLLME) with optimized parameters.

Methods: The preliminary analysis was performed with UV-VIS Spectrophotometer. The analysis was performed using a Shimadzu LC-10AT with SPD- 10A UV-VIS Detector. The sample analyzed was Zycolchin 0.5mg. The developed method was validated in accordance with the ICH guidelines.

Results: The LOD and LOQ was determined to be 6µg/ml and 19µg/ml respectively. The system suitability was determined using the symmetry and efficiency calculation. The sample showed excellent linearity of R^2= 0.99. The present study facilitates an optimized methodology for extraction and analysis which ensures great efficiency for the forensic analysis of colchicine. The study also highlights the importance of micro-extractions as they require only micro-level solvent usage which greatly improves the laboratory analysis. The entire extraction process is quick and effective for the analyte and is useful for the toxicological analysis in the forensic perspective.

Keywords: *Toxicology; Colchicine; Micro-extraction; HPLC; DLLME; Analytical Chemistry; Forensics; Alkaloids*

Introduction

Colchicine is an alkaloid derived mainly from the plant *Colchicum Autumnale* which belongs to the *Liliacea* family[(1)]. Pure Colchicine was isolated in its crystalline form by Alfred Houde´ in 1884[(2)]. Pure colchicine is observed as a white or pale greenish-yellow powder that darkens on exposure to light[(3)]. Colchicine is widely used for its therapeutic effects in the case of acute attacks of Gout, Behçet's disease, Pericarditis, Familial Mediterranean Fever and for the prevention of other inflammation-inducing diseases or genetic disorders[(4-7)]. Recent studies have proposed the use of colchicine for the treatment of certain types of liver and breast cancer cells by taking advantage of the alkaloid or its tubulin binding site[(8,9)]. The clinical application of colchicine can be traced back to the 6th century AD[(10,11)]. The interactions of colchicine at a cellular level have been studied and it is known that colchicine interacts with tubulin, cytochrome P450 3A4, and P-glycoprotein. Tubulin protein is very important in the process of cell division and transportation, and the polymerization of tubulin by colchicine disrupts the microtubule formation[(12,13)]. The single-dose pharmacokinetic studies have confirmed that colchicine is primarily absorbed by the jejunum and ileum[(14)]. The studies have also confirmed that the peak plasma concentration after an oral dose of colchicine

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(1mg) was observed 30-90 minutes after the ingestion of the drug[15]. The renal clearance of Colchicine is 4 l/h in human subjects; the major clearance of Colchicine from the body may be through the biliary excretion[16]. Colchicine is classified as a super toxic compound by the Environmental Protection Agency and can be proven fatal at a concentration as low as 7 mg but the dosage varies considerably[3]. Increased toxicity was also observed in individuals with significant kidney and liver damage due to the hindrance of the metabolism of the alkaloid which results in a higher concentration of free Colchicine in the system[17]. In the forensic perspective, colchicine is a recurrent alkaloid in the cases revolving around suicides, homicides, and accidental poisoning. There are various case reports which explain the life-threatening capabilities of colchicine due to its high toxicity. Marc Deveaux, et al., 2004 describes two case reports of suicide using colchicine in also explained the importance of using specific analytical techniques for the efficient extraction of the drug from biological samples[18]. The accidental exposure to colchicine can occur due to an overdose of colchicine containing pharmaceutical preparation or due to the ingestion of *Colchicum Autumnale* (meadow saffron); as observed in a case presented at the Institute of Legal Medicine and Pathology, Austria[19]. The use of colchicine for homicides is rare but it is a possibility as the drug is available in almost all pharmacies for a reasonable price. A case report of colchicine poisoning in two homicides was published by Weakley-Jones, B. et al., 2001 which explained the importance of evaluating the circumstances revolving around a clinical case for narrowing down the cause of death[20]. The toxicological analysis of colchicine with optimized extraction and efficient instrumentation is hence necessary for the field of forensic science and medicine. RP-HPLC has proven to be one of the best instruments for the analysis of colchicine along with the routine liquid extraction[21-23]. The field of analytical extractions has improved throughout the years and micro-extraction is considered to be one of the best sample preparation techniques employed for alkaloids because of its efficiency[24]. Dispersive Liquid-Liquid Micro-Extraction (DLLME) was introduced into the field of analytical chemistry by Rezaee, et al., 2006 for the pre-concentration of the organic and inorganic components from aqueous matrices[25]. This extraction technique has been modified multiple times to make it compatible with the analysis. The utilization of techniques involving micro-extraction is at the forefront of the revolution of analytical chemistry; as they utilize micro levels of solvents and can participate in the enrichment of the analyte of interest.

Materials and Methods

Chemical Reagents

Methanol, ethanol, acetone, diethyl ether, dichloromethane, chloroform, diethyl chloride, carbon tetrachloride, sodium chloride, magnesium sulfate, and orthophosphoric acid were obtained from SDFCL (Bangalore, India). The HPLC grade Methanol, Acetonitrile, and MilliQ water was obtained from Merck (Darmstadt, Germany). The standard (Colchicine, 99.5%, HPLC grade) was obtained from Loba Chemie Pvt. Ltd (Mumbai, India).

Instrumentation

A single-beam UV-VIS Spectrophotometer (2371 Spectrophotometer, Electronics India) was used for the spectral analysis. The system was operated on photometric mode (absorbance), the range was set at 800 nm – 200 nm with an interval of 0.1 nm. The software used for data acquisition was M.wave professional (Version 2.0.20). A Shimadzu single-pump HPLC system (Shimadzu, LC-10AT) with a UV detector (Shimadzu, SPD-10A, UV-VIS Detector) was used for the method optimization and validation. The column used was a GRACE, HPLC Alltima C-18 column (250mm x 4.6mm x 5 µm). The mobile phase was prepared using acetonitrile, methanol, water, and 0.1% orthophosphoric acid in the ratio 50:30:15:5, the pH of the mobile phase was set to 5.3 and vacuum filtered through a 0.45µm nylon membrane filter. The mobile phase was degassed for 15 minutes prior to the HPLC run. The Injection volume of the system was maintained at 20 µL. The UV detector was set at 250nm for the analysis. The software used for the chromatographic analysis was AutoChro-3000 (Kunash Instruments Pvt. Ltd).

Standard Preparation

The calibrator standard was prepared in 1000 µg/mL concentration and was reconstituted with the mobile phase. The resulting standard solution was sonicated for 5 minutes. The standard solution was stored at -4°C.

Sample Preparation

The sample was prepared by grinding 20 tablets (Zycolchin, 0.5mg) and by weighing an amount equivalent to 2.08g and dissolving the same in 10 ml MilliQ water. The resulting sample solution was shaken for 30 seconds and was centrifuged for 5 minutes at 2500 RPM. The aqueous layer was then transferred to a 15 mL centrifuge tube and was used for micro-extraction. The sample was stored at -4°C.

Urine Sample Preparation

The urine sample was collected from a healthy male volunteer with informed consent. Post spiked and matrix spiked urine samples were prepared at 100 µg/mL using the optimized DLLME procedure. The samples were further diluted to 10 µg/mL for studying matrix interference.

DLLME Procedure

The micro-extraction was carried out by 200 µL of ethanol (Dispersive solvent) and 200 µL of carbon tetrachloride (Extraction solvent) which was transferred to a microcentrifuge tube. 3ml of the sample solution was transferred to a 15 mL centrifuge tube. A sterile syringe was used to take the dispersive and extraction solvent mixture and the contents of the syringe were forcefully injected into the sample solution. The sample was allowed to rest for a minute and then centrifuged for 4 minutes at 2500 RPM. After centrifugation, the sedimented layer was pipetted out into a 1 mL microcentrifuge tube. The extraction solvent in the microcentrifuge tube was evaporated under a nitrogen stream and was reconstituted with the mobile phase.

DLLME Optimization

The DLLME procedure was optimized using the one-variable-at-a-time (OVAT) experimental design. The extraction parameters such as suitable dispersive and extraction solvents, solvent volume, salt concentration, and centrifugation time were investigated. The solvent optimization for DLLME was carried out for the extraction solvent as well as the dispersive solvent and the quantity of solvents was maintained equally at 200 µL. Methanol (MeOH), ethanol (EtOH), acetone (Ace), and diethyl ether (Et2O) were taken for the dispersive solvent optimization. For the optimization of the extraction solvents, dichloromethane (DCM), diethyl chloride (DEC), chloroform (CHCl3), and carbon tetrachloride (CCl4) was studied. The dispersive solvent and extraction solvent volumes of 100 µL, 200 µL, and 300 µL were investigated. The salt effect was optimized by using NaCl and MgSO4 at 1% and 3% concentration. The centrifugation time was optimized and the time range was set from 3-8 minutes with the centrifugation speed at 2500 RPM. All the experiments were performed in triplicates.

Method Validation

The analytical method has been validated as per ICH guidelines, taking into consideration the linearity, range, limit of detection (LOD), limit of Quantitation (LOQ), accuracy, precision and system suitability[26]. The sample has been quantified in relation to a seven-point calibration curve with colchicine concentration of 100, 80, 60, 40, 20, 10 and 1 µg/mL. The optimized was then used for the analysis of urine samples.

Results and Discussion

A method with an optimized Dispersive Liquid-Liquid Micro-Extraction (DLLME) and HPLC-UV analysis has been developed for the analysis of Colchicine from a pharmaceutical product. The wavelength screening was performed using a single beam UV-Vis Spectrophotometer. The absorbance was detected at 204nm, 250nm, and 352nm for Colchicine. The maximum absorption was observed at 250nm. The UV Spectrum is given in Figure 1. The extraction time was calculated to be 8 minutes and the analysis time was fixed at 10 minutes for the standard and the sample. The calibration curve showed a strong correlation with the R^2 value of 0.9917. The calibration curve is presented in Figure 2. The pharmaceutical product used for the analysis was Zycolchin, 0.5mg. The sample was analyzed using an HPLC-UV system. The sample and standard chromatograms are given in Figure 3. The sample mean recoveries (n=3) were 94.75% at 100 µg/mL (high) and 99.7% at 10 µg/mL (low).

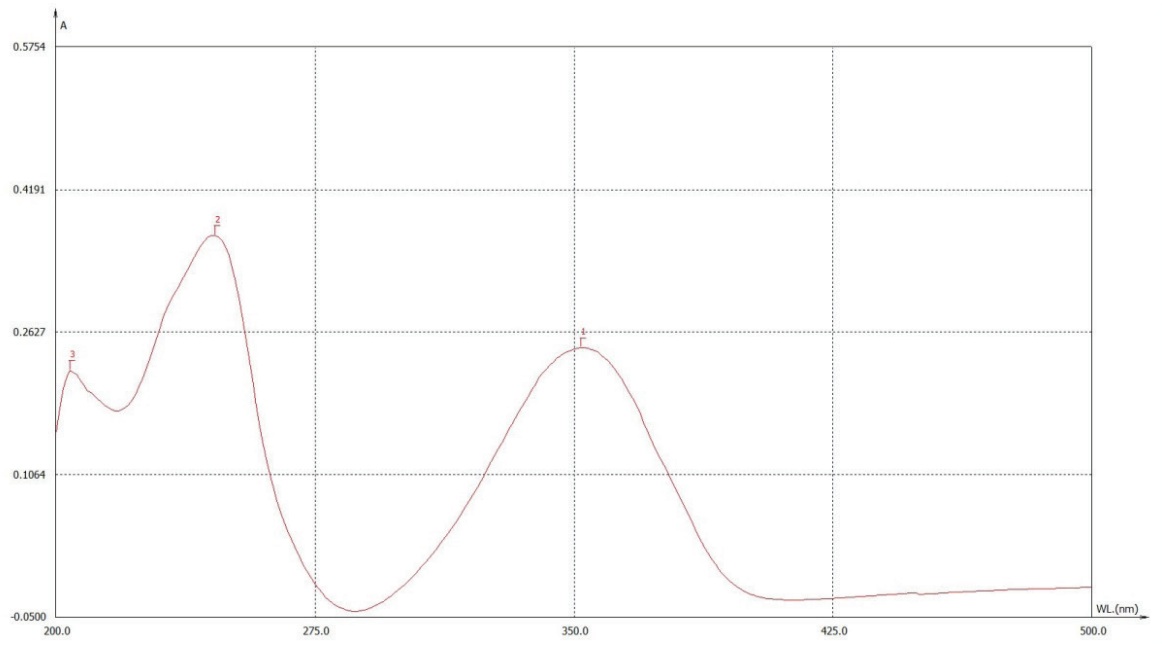

Figure 1. UV spectrum of colchicine (10µg/ml)

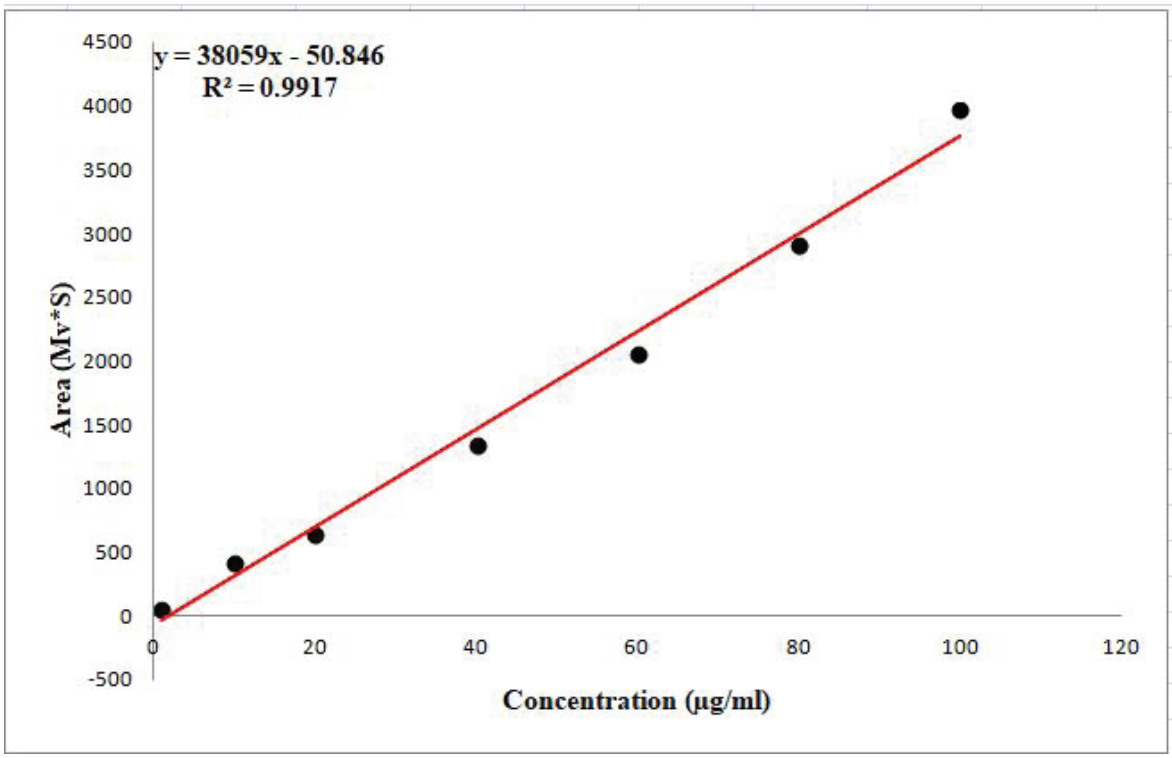

Figure 2. Calibration curve in the range of 1–100 µg/ml in three replicates

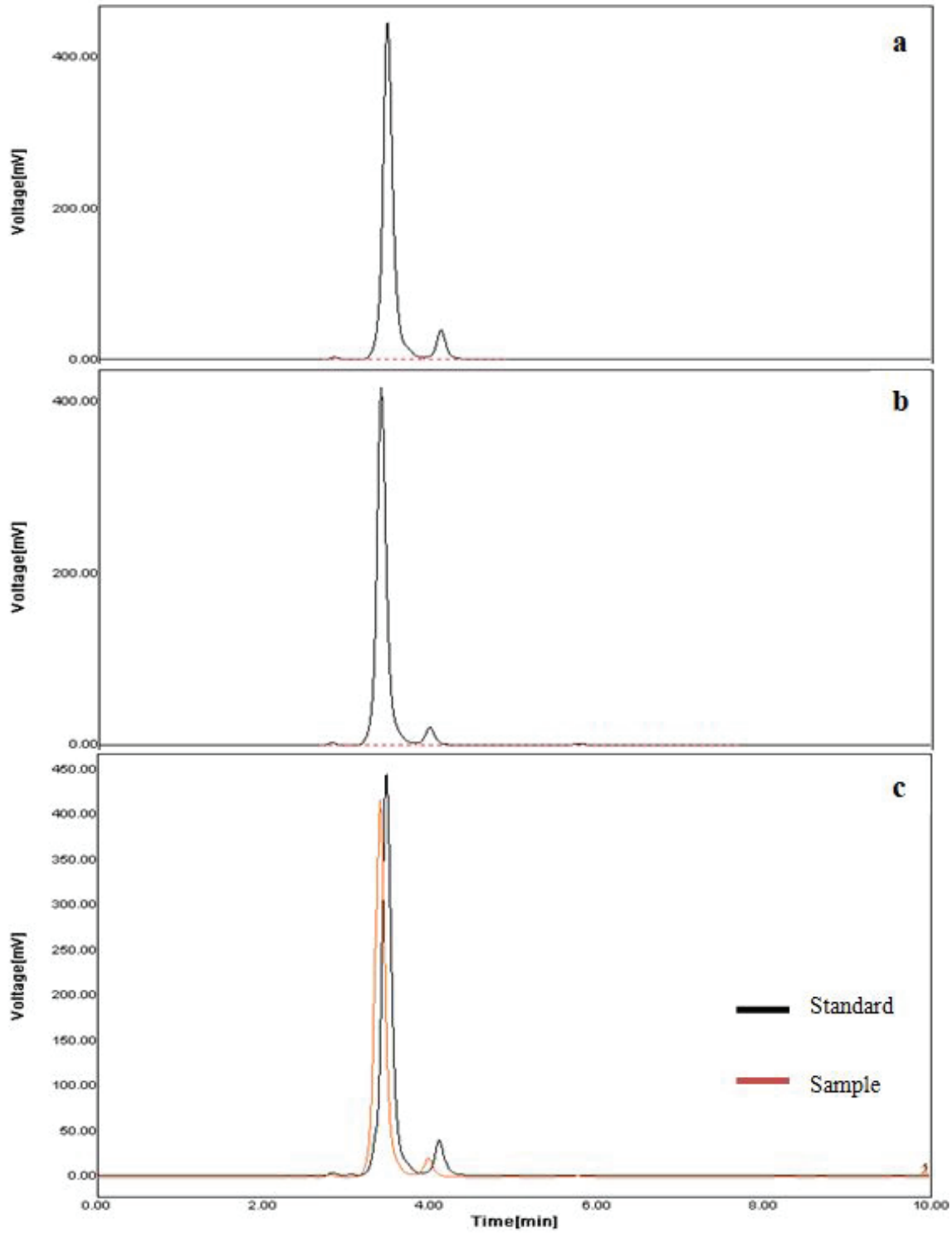

Figure 3. Chromatograms for colchicine. (a) Standard chromatogram (100µg/ml). (b) Sample chromatogram (100µg/ml). (c) Standard and sample chromatogram overlay.

DLLME Optimization

The DLLME procedure was optimized using the one-variable-at-a-time (OVAT) experimental design. The suitable dispersive and extraction solvents, solvent volume, salt concentration, and centrifugation time were investigated.

Solvent Optimization

The solvent optimization for DLLME was performed for the extraction solvent as well as the dispersive solvent. The maximum peak area was observed for ethanol, followed by acetone, methanol, and diethyl ether. According to the results obtained, Ethanol was selected as the dispersive solvent (Fig.4a). The maximum area for ethanol as a dispersive solvent could be due to the formation of a better dispersion cloud which increases the surface area for the extraction.

Among the selected extraction solvents, the maximum peak area was observed for carbon tetrachloride, followed by chloroform, diethyl chloride and dichloromethane. According to the results obtained, carbon tetrachloride was selected as the extraction solvent (Fig.4b). Carbon tetrachloride has a higher density compared to the other extraction solvents; which ensures a compact sedimented phase.

Solvent Volume Optimization

The dispersive and extraction solvent volumes were optimized for ethanol and carbon tetrachloride. The maximum peak area was observed for 200 µL for the dispersive solvent. According to the results, 200µL was selected as the optimized dispersive solvent volume (Fig.4c). Increasing the dispersive solvent volume could result in the dilution of the analyte, and decreasing the same could disrupt analyte solubility.

The maximum peak area was observed for 200 µL for the extraction solvent. According to the results, 200 µL was selected as the optimized extraction solvent volume (Fig.4d). An optimum amount of extraction solvent is required for the effective extraction of the analyte from the aqueous sample.

Salt Optimization

The salting out effect was optimized for DLLME. The maximum peak area was observed for 1% NaCl. According to the results, 1% NaCl was selected as the optimized salt for facilitating the salting-out effect of the analyte (Fig.4e).

Centrifugation Time Optimization

The centrifugation time was optimized for DLLME. Centrifugation time of 4 minutes gave optimum results and the centrifugation time from 5 to 8 minutes did not show any difference from the optimized result (Fig.4f).

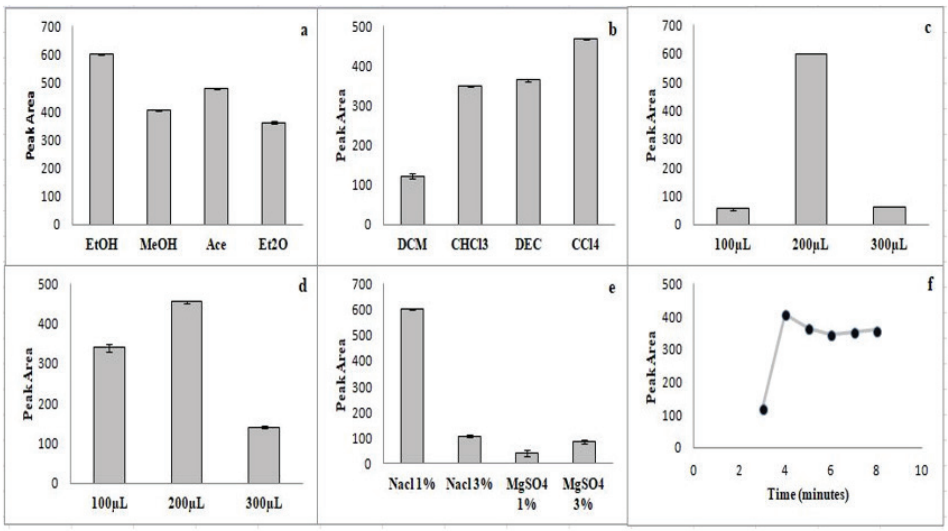

Figure 4. Optimization of various parameters for Dispersive Liquid-Liquid Micro-Extraction (DLLME). (a) Dispersive solvent optimization. (b) Extraction solvent optimization. (c) Dispersive solvent volume optimization. (d) Extraction solvent volume optimization. (e) Salt optimization. (f) Centrifugation time optimization.

Method Validation

The DLLME and HPLC parameters were validated for linearity, range, LOD, LOQ, accuracy, precision and system suitability. Results for validation are presented in Table 1, 2, 3.

Table 1. Validation Parameters for the Optimized Method. (a) Calculated as 3x (S.D/Slope). (b) Calculated as 10x (S.D/Slope). (c) Intra-day Relative Standard Deviation, (n = 3). (d) Inter-day Relative Standard Deviation, (n = 3).

S.D – Standard Deviation

LOD a	LOQ b	Range	Linearity (R2)	Intra-day RSD c (100µg/ml)	Inter-day RSD d (100µg/ml)
6µg/ml	19µg/ml	100 – 1 µg/ml	0.9917	0.046%	0.327%

Table 2. Repeatability of the analytical method for colchicine analysis.

Compound	Conc. Range	Spiked Conc. (µg/ml)	Intra-Assay (n=3)		
			Average Conc. (µg/ml)	Precision%(RSD)	Accuracy%
Colchicine	Low	10	12.64	2.20	126.47
	High	100	105.11	0.32	105.11

Table 3. System suitability study using HPLC parameters. (a) n = 7. (b) Tf < 1.5. (c) 2 < k< 10.

Average Theoretical Plates (N) a	Height of Theoretical Plates (mm)	Tailing Factor (Tf) b	Capacity Factor (k) c
5525.94	0.037 – 0.120	1.07 – 1.36	3.31

Analysis of Real Samples

The optimized method was applied for the analysis of urine samples. The absolute matrix interference was studied using post spiked samples and routine matrix spiked urine samples. The post spiked sample of colchicine showed 100% recovery on analysis. The matrix spiked sample showed a mean recovery of 64.7% on analysis. The matrix interference study hence determines a 35.3 % matrix-induced suppression of the analyte recovery. The chromatogram depicting the matrix interference study is given in Figure 5.

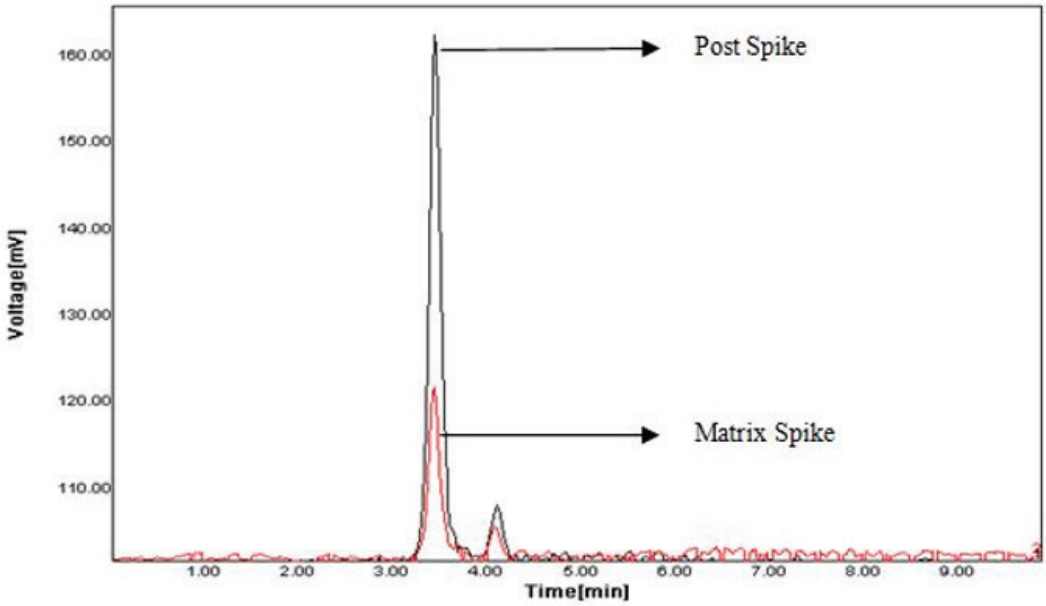

Figure 5. The post spike and matrix spike overlay (10µg/ml).

Conclusion

The method optimization and validation using dispersive liquid-liquid micro-extraction were performed for the plant alkaloid, colchicine, and the analysis was carried out using high-performance liquid chromatography. The optimized DLLME method has shown promising results for the extraction of colchicine. The optimum extraction conditions for the extraction were found with ethanol and carbon tetrachloride as dispersive and extraction solvents, volume; 200 µL, centrifugation time; 4 minutes. The study highlights the importance of micro-extractions as they require only micro-level solvent usage. The importance of DLLME for forensic toxicological analysis is revealed by this method for colchicine estimation. The authors recommend the use of better instrumentation for the future studies.

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Ethical Clearance: Ethical clearance was obtained along with informed consent.

Conflict of Interest : None to declare

Source of Funding: Self

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Investigating the Relationship between Demographic Variables And Emotional Intelligence and Spiritual Health in the Nurses Working in the Teaching Hospitals of Ilam

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Abstract

Introduction: There is a close relationship between a nurse'sinherent spirituality and the tendency to provide spiritual care. This means that anurse with stronger inherent spirituality provides more spiritual care to patients. The aim of this study was to investigate the relationship between demographic variables and emotional intelligence and spiritual health in the nurses working in the teaching hospitals of Ilam city.

Materials and Methods: This was a descriptive-analytical study onthe nurses working in the teaching hospitals ofIlam. The research sample included all the nurses of teaching hospitals ofIlamcity, who were selected by census. The research instrument includedthree questionnaires;a demographic questionnaire (age, gender, marital status, work experience, and employment status), a 20-item spiritual health questionnaire, and the 90-item standard Barquestionnaire for emotional intelligence. The spiritual health questionnaire, already validated by Tavan et al.,included 20 questions infour dimensions (five questions for each of contemplation, beliefs, morality, and philosophy/lifestyledimensions). Finally, the data was entered into and analyzed by SPSS version 19 software.

Results: This descriptive study was conducted on 130 nurses working in the teaching hospitals of Ilam. Of these, 79 (60.8%) and 51 (39.2%) were males and females, respectively. The mean spiritual health scores were obtained as 23.72, 21.98, 22.68, and 22.47 in the contemplation, beliefs, morality, and philosophy/ lifestyledimensions, respectively, with the total score of 90.86. The total score of emotional intelligence was 313.34. Among the nurses, 7 (5.4%), 115 (88.5%), and 8 (6.2%) had moderate, high, and very high emotional intelligence, respectively.

Conclusion: The nurses' spiritual health was at the moderate-high level for all the participants, and it can be promoted by implementing education programs.

***Keywords**: Spiritual health, Emotional intelligence, Nurses*

Introduction

Nurses not only need knowledge and skills, but also require to know how to deal with different people's attitudes. Emotional intelligence is particularly important in the success of people in health care organizations. Actually, emotional intelligence makes health care workers think better under difficult conditions and avoid emotions such as anger, anxiety, and fear. It helps themto calm their minds, think more openly,and allow the inner insight and creative ideas to emerge ([1, 2]).

Because of its nature which aims to assistpatients, nurses require to be able to assess and predict patients'

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health outcomes. For doing this, nurses must have knowledge and skills, as well as purposeful mental activity and the abilities to create and assess new ideas and use them in judgments, decisions, and problem-solving ([1, 2]).

Emotional intelligence helps nurses to manage difficult situations before they become out of control. This further helps them to easily manage stress. Individuals whoemploy emotional intelligence have higher self-confidence and more compatibility with their surroundings and are better aware of their abilities ([1]). Nurses who have high emotional intelligence can more effectively manage their emotions and feelings and make better and goal-oriented decisions. On the other hand, low emotional intelligence negativelyaffects nurses' happiness and health and makes it difficult for them to manage their problems and conflicts. Overall, utilizing emotional intelligence is highly recommended to promote health related professions ([3,4]).

The relationship between nursing and spirituality falls into providing comprehensive patient care; however, the spiritual aspect of humans is not adequately considered in nursing, and there is a shortage of knowledge in this area ([2]). The first step in understanding spiritual patient care is to divulge nursing students' perspectives on the issue. A higher spiritual health and emotional intelligencein nurses positively correlates with providing a spiritual and more numerous patient care ([3]). It has been shown that providing spiritual carefor patients improves their health recovery. In addition, nurses' understanding of spirituality and emotional intelligence upgradestheir performance to a higher level ([1, 4]).Studies have also shown that nurses with higher spiritual health can provide better patient care services ([5]).

There is a close relationship between nurses'inherent spirituality and their tendency to provide spiritual care. This means that nurses who have strongerinherentspirituality more often provide spiritual care to patients([6]). In the study ofFahring*et al*. whostudied cancer patients' spiritual healthconcluded that the patients who had stronger inherent spirituality recovered faster from thedisease ([7]).So, having areligiousbelief and strong spirituality seems to provide a broader perspective on accepting suffering and the life after death ([8]). In conclusion, nurses with better spiritual health deliver patient care more often, and on the other hand, patients with ahigher spiritual healthrecover more rapidly from their diseases.

As a professional health care worker, a nurseshould accompany the patient during the hospitalization. In fact, thehospital is a suitable place to detectpatients' spiritual disorders, and nurses, by providing nursing support to patients and obviating their spiritual needs, can improve their general, as well as spiritual health(9). Today, studies have asserted theimportance of allowing patients to performtheir religious duties and prayers, as a part of spiritual care, in bringing them calm and accelerating theirrecovery ([8]).

Nurses are in direct contact with patients at the first line of treatment course. So, their efforts in providing quality health services seem to be a great help to patients. There is a positive and mutual relationship between spiritual health and emotional intelligence, so enhancing the spiritual health of nurses can increase the quality and quantity of the care services provided by them. The aim of this study was to investigate the relationship betweendemographic variablesand spiritual health and emotional intelligence among the nurses working in the teaching hospitals ofIlam in 2019.

Materials and Methods

This study was a descriptive-analytical researchon the nurses working in the teaching hospitals ofIlam city. In total, 130 nurseswere selected by census. Inclusion criteria were having a bachelor's degree in nursing, a working experience of at least one year, and willingness to participate in the study. The nurses were completely free to participatein the study or withdraw at any time, and after obtaining informed consent, questionnaireswere given to them.

Research Instruments

Three questionnaires were used in this researchto collect data. The first included a demographic questionnaire for gathering the nurses' age, gender, marital status, work experience, and employment status. Also, a 20-item spiritual health questionnaireand a90-item standard questionnairefor emotional intelligence were used ([1]). The spiritual health questionnaire, already

validated by Tavan et al. ([3]),included 20 questions infour dimensions (five questions for each of contemplation, beliefs, morality, and philosophy/lifestyle dimensions). Each question was scored based on a six-point Likert scale with a total score range from 20 to 120.

The next part included the 90-item standard Barquestionnaireof emotional intelligence ([1]). This questionnaireincludes 15 subscales (6 questions per subscale) scoredbased ona 5-point Likert scale (completely agree = 5, agree = 4, somehow agree = 3, disagree = 2, and completely disagree = 1). For negative questions, the scoring method was reversed.Each question was assigned a score between 1 and 5, giving each subscale a score from 6 to 30 and a total score of 90 to 450. The total score was finally divided into four categories of low (90-180), moderate (181-270), high (271-360), and very high (361-450).

The raw scores ofthe subscales were transformed on a scale of 100 and expressed as the emotional intelligence score. The scores equal orabove the average indicatedan acceptable emotional and social performance, and in fact a higher score represented a better performance in dealing with dailyneeds and challenges. On the other hand, a score below the average would probably indicate the incapability of the individual in facing hurdles and a possibility for emotional, social, or behavioral problems ([1]). This questionnaire has already been used and validated in Iran for being used among nursing students (Tavan*et al*., 2015) ([1])withan acceptable content validity and reliability (theCronbach's alpha coefficient of 0.93).However, in order to confirm its validity,the questionnaire was given to 10 faculty members of the School of Nursing and Midwifery, and their proposed comments were applied. Also, the reliability was confirmed by giving the questionnaire to 10 nurses working in the same hospitals at two occasions (10 days apart). The reliability of the questionnaire was finally calculated as 85% using SPSS software.

After gathering the data,it was entered into and analyzed by SPSS version 19 software. Descriptive statistics included mean, standard deviation, frequency, and percentage. Thestudent t-test and one-way ANOVAwere used to assessdifferences of scores between demographic variables'categories (two and more than two states, respectively). The significance level was considered as $P < 0.05$.

The present study was performedby referring to the teaching hospitals of Ilam city after being approvedby the research council of the nursing and midwifery faculty and the ethics committee of Ilam University of Medical Sciences (Code: ir.med.1399.187).

Results

In this descriptive study which was performed on 130 nurses working in the teaching hospitals of Ilam city, 114 (87.7%) of the participants were married, and 16 (12.3%) were single. Also, 12 (9.2%), 35 (26.9%), 31 (23.8%), 17 (13.1%),and35 (26.9%) participants had working experience of 1-5, 6-10, 11-15, 16-20, and >20 years, respectively (Table 1).

The results showed that the mean scores ofspiritual health in the beliefs, contemplation, morality, and lifestyle dimensions were 23.72, 21.98, 22.68, and 22.47, respectively. The total score of spiritual health was obtained 90.86, and the total emotional intelligence score was 313.34. Of the participants, 7 (5.4%), 115 (88.5%),and 8 (6.2%) had moderate, high, and very high emotional intelligence, respectively.

Table 2 shows the frequency distribution of spiritual health and emotional intelligence scores ofthe nursesbased on gender and various age groups. Accordingly, the results showed that in the age groups of 25-30, 31-35, 36-40, and >40 years, the means of spiritual health score were 87.31, 92.83, 90.67, and 94.17, and the mean emotional intelligence scores were 313.21, 313.21, 315.6, and 313.34, respectively (Table 2).

Table 1: The participants' demographic features

Variables	Subgroups	Frequency	Frequency %	Cumulative frequency %
Age group (years)	25-30	36	27.7	27.7
	31-35	58	44.6	72.3
	36-40	30	23.1	95.4
	>40	6	4.6	100
	Total	130	100	
Gender	Male	79	60.8	60.8
	Female	51	39.2	100
	Total	130	100	
Marital status	Married	114	87.7	87.7
	Single	16	12.3	100
	Total	130	100	
Work experience (years)	1-5	12	9.2	9.2
	6-10	35	26.9	36.2
	11-15	31	23.8	60
	16-20	17	13.1	73.1
	>20	35	26.9	100
	Total	130	100	

Table 2. Mean scores of spiritual health and emotional intelligence in various age groups and genders

Parameter s	**Subgroups**		**n**	**Mean**	**SD**	**P**
Spiritual health	Age groups (y)	25-30	36	87.31	19.06	0.115
		31-35	58	92.83	16.55	
		36-40	30	90.67	14.67	
		>40	6	94.17	6.73	
		Total	130	90.86	16.62	
	Gender	Male	79	88.62	16.32	0.105
		Female	51	94.33	16.58	
	Marital status	Married	114	91.29	16.85	0.125
		Single	16	87.81	14.77	

Cont... **Table 2. Mean scores of spiritual health and emotional intelligence in various age groups and genders**

Emotional intelligence	Age groups (y)	25-30	36	313.3	33	0.087
		31-35	58	313.2	26.3	
		36-40	30	315.6	31.15	
		>40	6	313.5	34.13	
		Total	130	313.34	29.5	
	Gender	Male	79	313.3	27.2	0.111
		Female	51	313.4	33.1	
	Marital status	Married	114	314.52	28.62	0.131
		Single	16	304.94	35.1	

Discussion

The present study was conducted to investigate the relationship between demographic variables andthe emotional intelligence and spiritual health of the nurses working in the teaching hospitals ofIlamcity in2019. The study findings showed a statistically significant relationship between gender and spiritual health score which was higher in female than male nurses. In a study byYasemi Nezhad et al. (2011), it wasnoted that girls had a highermean spiritual health score than boys ([11]), which is consistent with the results of the present study.

Overall, spiritual health was at a moderate level in both male and female nurses with no prominent difference between the genders. Similar findings were reported in the studies of Asarroudi*et al*. (2011) and Yazdi Moghadam *et al*. (2006) ([4, 12]) and is consistent with the results of the present study. There are also other studies stating no significant difference (P>0.05) in the total spiritual health score between men and women ([1, 4]), indicating nosignificant impact for gender on the total spiritual health score, which is not consistent with our observation. This may be explained by the differencesin the studied populations and age groups.

In the study of Asarroudi*et al*. (2011) on the spiritual health of nurses, none of the participants had a low, but most of them had moderate spiritual health levels ([4]) which is consistent with ourresults. Also, in the study of Hissau*et al*. (2010) who investigated students' spiritual health, they reported a moderate level of spiritual health ([5]), and also Safaei Rad *et al*. (2010)announced a good spiritual health in students([14]). These were in linewith our results in the present study.

Furthermore, there was no statistically significant relationship between the nurses' spiritual healthscores and their work experience. These findings show no significant difference in the obtained scores between nurses with high or low work experience, which may indicate the nurses' high perception of inherent spiritualitybefore entering the university. However, it is necessary to promotethe views of students about spirituality during education in universities. Because spirituality is a completely individualized and mental entity, it is required to provide a suitable educational environment in classrooms so that nursing students feel comfortable and secure expressing their spiritual beliefs. In the clinical environment, it is also necessary that nursing educators pay attention to patients' spiritual, along with physical and psychological, needs. Moreover, spiritual care should be considered in scheduling a nursing care program so that students, in addition to nursing skills,also learn about patients' spiritual aspects to be able to fulfiltheir needs in that area as well ([15]).

There is a close relationship between nurses' inherent spirituality and their attention and tendency to provide spiritual care. This means that a strongerinherent spirituality (i.e. a higher spiritual healthscore) predicts

more numerous providing of spiritual care to patients ([16]). In the study ofFahring*et al*. who assessed cancer patients' spiritual health,a strongerinherent religious belief predicted a faster recovery from thedisease ([17]). So, it can be concluded that an inherentreligiousbelief provides a wider perspective for accepting the suffering and life after death ([18]).In conclusion, nurses with a higher spiritual health provide patient caremore often. On the other hand, patients witha higher spiritual health alsoexperience afaster recovery.Since many of ourparticipants had good spiritual health, it is applicable to design spiritual nursing care interventions to support patients' needs. Professional caregivers can use spirituality and religion and emphasize on religious practices to promote patients' adaptability tothe illness ([18]).

Nurses, asprofessionals, should accompany patients during the hospitalization. In fact, the hospital is a good place to diagnose patients' spiritual confusion, and nurses can promote boththe spiritual and general health of patients by providing nursing support and addressing their spiritual needs ([9]). Health care teams by providingand allowing religious duties, as a part of spiritual care, can observe the effects of such measures in calming patients and accelerating their healing process ([8]).

In two studies byHaghani*et al.* (2010) and Namdar*et al*. (2008), the emotional intelligence score has beenhigher than 300 ([9, 18]) which is consistent with the results of the present study. In accordance with the results of the present study, Ghaderi *et al*.(2013) reported that women had a higher emotional intelligence score than men, which probably reflectsthe stronger emotions and higherability to express them in women in comparison with men.Women have been noted to establish their social relationships in a smarter way([18]). In contrast to our results; however, Aghajani (2009) *et al*. and Samari*et al*. (2008) noteda higher emotional intelligence score in men compared to women ([19, 20]). A possible reason for this discrepancy may be different environmental, social, and cultural conditions of the studied populations ([18]).

Our results showed a statistically significant relationship between emotional intelligence and age so that emotional intelligence score increased with age. Likewise, Ghaderi *et al*. (2013) confirmed this relationship ([18]). The reason for this observationmay root inthe development of knowledge about oneself and others, which ultimately boost emotional intelligence ([21]).

In the studies ofHajian*et al*. (2012) and Konzi*et al*. (2003) on the relationship between emotional and spiritual intelligence, the researchers stated that ifa person relies on God in his worldly affairs and obey his commands, he will become a worthy and God-fearing human.Therefore, by strengthening spiritual intelligence (which reflects obedience to God), it is possible to strengthen social behavior and respect for others' emotions, feelings, and rights (which shows in fact emotional intelligence) ([22, 23]).

Conclusion

In general, both spiritual and emotional intelligence were higher in women than men.There was a statistically significant relationship between spiritual and emotional intelligence so that strengthening spiritual intelligence improved emotional intelligence. The nurses' spiritual health was at a moderate-high level in all of them. It is suggested to upgrade the level of nurses' spiritual health by implementing educational programs.

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Role of Preconditioning Adipose Mesenchymal Stem Cells in Regeneration Disk: a Review Article

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Abstract

Intervertebral disk (IVD) degeneration (IDD) is a degenerative disease and often found in daily practice. The managements that were used so far are only symptomatic and have not been able to solve the disease completely. The use of mesenchymal stem cells (MSCs) has been widely tested as a solution for the regeneration of IVD in IDD. Adipose-derived mesenchymal stem cells (ASCs) are one of the sources of MSCs which were widely studied as an alternative. One of the factors that can influence the efficiency and capacity of ASCs in IDD cases is preconditioning. Generally, there were three pre-conditioning strategies of ASCs before being used as therapy, (1) modifying the environment, (2) using Growth Factors, Cytokines, Chemokines, Hormones, (3) using pharmacological and chemical agents. Preconditioning on ASCs can change its function in various ways. We tried to link all these things together so that we could know the role of pre-conditioning ASCs in the regeneration disk.

Key words: *Adipose-Derived Mesenchymal Stem Cells, Disk Degeneration, Intervertebral Disk, Preconditioning, Regeneration, Stem Cells*

Introduction

Back pain is one of the main complaints experienced by many populations in the world[1]. In daily practice, complaints of back pain are one of the causes for patients to come to health facilities[2]. This complaint has a major impact on the social and economic fields, thereby reducing someone productivity and their quality of life[1,2]. Back pain that is not handled properly, can lead to chronic pain. The estimated prevalence rate of chronic low back pain (LBP) in adults in the world reaches 23%. The etiology of back pain, in general, can be divided into five categories, mechanical, degenerative, inflammatory, oncologic, and infectious[2]. One of the common causes of back pain is intervertebral disk (IVD) degeneration (IDD)[1].

One of the important functions of IVD is to support the body's weight[3]. If the IVD loses this function, the

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vertebral structure will lose its flexibility[4]. The typical symptom of IDD is back pain which is facet-mediated pain[5]. There were variations in the management of IDD, especially in relieving the main complaints of back pain experienced by patients. The initial approaches commonly used are physical therapy, education, and pain medication[1,2]. Furthermore, surgical treatments may be performed to treat IDD cases[1]. However, the management of IDD is only symptomatic and has not been able to efficiently solve the problem of IDD[1,3].

Other methods were used in the management of IDD is by triggering the regeneration of the degenerated IVD[4]. This regeneration therapy uses mesenchymal stem cells (MSCs)[6]. MSCs can be used to treat degenerative diseases in orthopedic surgery, such as osteoarthritis and IDD. The use of MSCs has become a new trend in therapeutic developments in regenerative medicine. Although their use is still in the experimental stage, the use of MSCs has provided new avenues for the management of degenerative diseases, which so far have not completely resolved the problem[7]. One of the MSCs that can be used is adipose tissue-derived stem cells (ASCs)[8].

When compared to Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs), ASCs have their own advantages over BMSCs. These advantages include more sources, lower risk of taking, and more efficient yields than BMSC[6,9,10]. The requirement for ASCs to be used as therapy is to be able to survive for a long time in order to migrate effectively into the injury site and function properly. Research for the use of ASCs in regenerative medicine is kept developing. Various studies were conducted to make the most efficient use of ASCs as possible. One way to increase the therapeutic abilities of ASCs is by using pre-conditioning[8]. We try to find out the role of preconditioning ASCs in the regeneration disk.

Intervertebral Disk Degeneration

IVD is the amphiarthrotic joints and connects the vertebrae of the spinal column tightly[4,11]. Anatomically, IVD is a combined nucleus pulposus (NP) surrounded by annulus fibrosus (AF) between the cartilage that lines the subchondral part of the vertebrae[12]. The IVD extracellular matrix consists of proteoglycans, elastin, and collagen types I and II. Type I collagen is a constituent component of AF, while collagen type II is a component of NP. The assemblies work on the principle of hydrostatic pressure, so they can withstand compression loads and create flexibility[3,4].

Clinically, IDD can be described as a decreased ability of the disk to maintain stability and mobility of the vertebral structures[4]. In IDD, the main structures that are affected are NP and AF. Degeneration of the intervertebral disk is characterized by the onset of an inflammatory reaction accompanied by increased levels of inflammatory cytokines and a decrease in inhibitors. These reactions become cyclic resulting in tissue damage and inflammation. The resulting cycle is the death of NP cells and chondrocytes, an increase in shear stress, and a decrease in proteoglycans and type II collagen[3].

Symptoms resulting from the IDD sequence are pain, dysfunction, and stiffness, especially in the morning[13]. IDD can cause facet-mediated pain[5]. If the patient presents with these complaints and does not respond to initial therapy in four-six weeks, an X-ray imaging examination may be performed. However, to diagnose disk abnormalities or the presence of stenosis, an MRI imaging examination is required[2]. An MRI examination is commonly used to find disk degeneration[5]. Complications that can arise from IDD that are not handled properly are disk herniation, spinal stenosis, and instability[1]. In addition to physically affecting, untreated IDD will also affect someone social aspects, such as decreased productivity[2].

Current Disk Regeneration Therapy

Disruption in IVD will reduce the effectiveness of its function and require improvement because it can affect a person's quality of life[3,13]. Commonly used conservative management is physical therapy, education, and using a pharmacological agent like pain relief[1–3]. Pain medication that is usually used varies depending on the severity of the complaint. Pain medication includes non-steroidal anti-inflammatory drugs (NSAIDs), muscle relaxers, gabapentin, topical analgesics, and opioids. The usual non-pharmacological treatment to restore LBP is activity modification, rest, ice, and heat. The education that can be given is a decrease in BMI of less than 25 and limit activities that can worsen patient complaints[2].

These conservative treatments are usually limited to symptom relief and tend to be less effective[1,3]. Generally, surgical treatments will be used when conservative therapy fails, which is characterized by complaints that have affected a person's quality of life for more than one year[1,2]. This consideration was taken because surgical treatments are invasive and have a risk of complications. Even so, treatment with surgical treatments is more effective in treating pain than conservative therapy[1]. Spinal fusions are a surgical approach to treating discogenic back pain[14]. Management of surgical treatments can also be taken in certain cases by regarding the advantages and disadvantages. Apart from surgical treatment, chronic back pain can be treated with psychological therapy in the form of cognitive-behavioral therapy (CBT), progressive relaxation, and biofeedback[5].

Therapy aimed at regenerating from IVD is still under development. Cell-based therapy using MSCs is an alternative in disk regeneration. This therapy is different from therapy that is conservative and palliative, with the aim of returning the patient productivity[12]. The use of MSCs in IDD regeneration must have the ability to adjust to the cells to the microenvironment of the host[8]. The important thing that needs to be considered in the development of regenerative medicine using MSCs in IVD is the role of IVD as a weight-bearing organ. The development of regenerative medicine must pay attention to the regeneration of the IVD function as a mechanical support and load distribution[1]. The use of ASCs can have an effect on the management of IDD. This effect is measured based on the increase in the Oswestry disability index (ODI) and visual analog scale (VAS)[15].

ASCs have an anabolic effect on NP and AF. The use of ASCs on NP cells can reduce apoptosis and inhibit the expression of proinflammatory cytokines (interleukin-1b and TNF-a) and catabolic factors (matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs). The use of NP cells will also increase type II collagen, aggrecan, and tissue inhibitors of metalloproteinases (TIMPs). While the use of ASCs on AF cells will increase proliferation, increase anabolic action, and decrease apoptosis and expression of catabolic factors and proinflammatory cytokines[1,15].

Adiposed-derived Mesenchymal Stem Cells

Technological and scientific developments have helped in the development of MSCs. MSCs are adult stem cells that can self-regenerate and can develop widely[16,17]. MSCs can be obtained from various tissues, both postnatal and prenatal organs[7]. Bone marrow, peripheral blood, dental pulp, lungs, umbilical cord blood, muscle, placenta, and fat are sources that can be used for MSCs[16]. The ability of the multipotentiality of MSCs has now also expanded widely, from in vitro and in vivo, to epithelial and neural cell types[7,17]. MSCs tend to be non-immunogenic and have immunomodulatory properties. These natures make MSCs important in regenerative medicine and are widely used as potential therapeutic alternatives for treatment[17]. Adipose mesenchymal stem cells are multipotent stem / stromal cells derived from fat tissue and are known as adipose-derived mesenchymal stem cells (ASCs). Fat tissue is a loose connective tissue in the subcutaneous or visceral part[8]. There are two kinds of adipose tissue, namely white and brown. White adipose tissue is the source of ASCs[8,18].

To get functional stem cells, it takes several steps. These stages include harvest, breakdown, purification, and plastic adherence. After going through these stages, the calculation, identification of cells, and their potential stemness were carried out through adequate analysis. Advances in technology have helped insulate ASCs so that it is possible to obtain them within 85 minutes[9]. In 2013, the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) issued standards for the identification of ASCs: (1) plastic adherence cells with self-regenerating abilities; (2) Expressing cluster of differentiation 90 (CD 90), CD73, CD105, and CD36, and lack of CD11b, CD45, CD31, and CD106; and (3) and can differentiate into mesoderm tissue[8].

ASCs can differentiate into mesoderm tissue, such as adipocytes, osteocytes, and chondrocytes, allowing them to be used as material for disk regeneration. There are various sources and techniques for obtaining ASCs, but the largest source comes from abdominal fat which can be obtained by abdominoplasty or endoscopically[9,10]. In addition, a technique commonly used in humans is liposuction on subcutaneous fat. After taking ASCs, the

culture was carried out in the conditioned medium[10]. ASCs also can be isolated from the infrapatellar fat pad (IPFP-ASCs) which is located close to the synovial membrane. IPFP-ASCs have advantages in their proliferation capabilities and differentiation capacities[6].

Adipose-Derived Mesenchymal Stem Cells versus Bone Marrow-Derived Mesenchymal Stem Cells

There are various resources available for creating MSCs that can be used in regenerative medicine. Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) are a common source of MSCs that have been tested in clinical trials[7,9,16]. The advantage of using BMSCs is that they can be obtained from the same individual and the side effects tend to be smaller than that of pluripotent stem cells. BMSCs are believed to have a lower risk of causing tumors or malignancies and immunological reactions. In addition, from an ethical perspective, the use of BMSCs has been widely used so that it has a lower ethical risk. However, the numbers are limited and their collection through invasive aspiration is a drawback for these BMSCs[6,9]. In addition, BMSCs from younger donors were more effective than older donors. This indicates the age-dependent effect of the MSCs function[16].

In contrast to BMSCs, patients experience less discomfort in taking ASCs and the risk is lower[6]. ASCs have more sources, so they are easier to collect and tend to be less invasive. Various sources and collection techniques make it easier for ASCs to be obtained because they can adjust to the conditions of each laboratory. Techniques that can be used to obtain ASCs are liposuction and abdominoplasty[9,10,19]. When compared to BMSCs, ASCs have similar characteristics to BMSCs. The proliferation ability and marker stem cell expression level of ASCs were higher than BMSCs. In addition, ASCs are more resistant to apoptosis and have a lower risk of becoming malignant[6].

Role of Preconditioning MSCs

One of the requirements for MSCs to be used in regenerative medicine is to have a sufficient number of cells to reach the location of damage and be able to survive for a long time. The minimum number of MSCs cells required in a clinical trial for transplantation is 1 x 10^7. It takes 1-3 months for cell expansion in vitro to obtain this number of cells[16]. Various preconditioning strategies have been implemented to increase the regeneration capacity of MSCs. There were various preconditioning strategies for MSCs, such as environmental modification, use of growth factors or small molecules, and pharmacological or chemical agents[7,20].

As one of the MSCs, ASCs require a preconditioning strategy to maximize reparative capacity and regeneration. The preconditioning strategy of MSCs maximizes the potential of therapeutic efficiency. Therapy with ASCs in degenerative diseases, such as IDD, requires maximum regeneration capacity[7]. The faster growth of ASCs, the lower the costs with better results. The risk of genetic alteration and cell culture contamination in vitro can be minimized from the faster growth of ASCs, maximizing their use in regeneration[9].

1. Preconditioning using environment modification

Preconditioning with environmental modification strategies can increase the regeneration potential of MSCs. A usable condition is to create an environment with low oxygen levels[7]. This environmental modification has been shown to have a beneficial impact on ASCs. Environmental conditions with low oxygen levels multiply the multiplication and migration of ASCs by the generation of reactive oxygen species (ROS) and downstream phosphorylation of platelet-derived growth factor receptor-beta, ERK1 / 2, and Akt. If this condition is prolonged, it will increase miR-210. The effect of this series will increase the secretion of vascular endothelial growth factor thereby increasing the potential of the generational ability of ASCs[21,22]. The impact of preconditioning with low oxygen levels will increase the ability of cell proliferation and angiogenic potential so that the ability to survive MSCs will also increase[20].

Other strategies that can be used are hyperoxia and thermal preconditioning. Hyperperoxia preconditioning can reduce the number of cells undergoing apoptosis so that it can increase the potency of MSCs. MSCs incubated at 42°C for 2 hours before transplantation, can increase cell survival and reduce the oxide-induced apoptosis of MSCs. Incubation at this temperature inhibits the apoptosis pathway and prevents oxidation stress[16].

Another environmental modification that can be used is to culture MSCs in a 3-dimensional (3D) environment, known as a spheroid[7,17]. This culture associated with the production of factors that can increase the factors associated with proliferation and vascularization. These factors also influence the survival of the cells. The result of this strategy is an increase in these cells' immunomodulatory, anti-apoptotic activities, anti-fibrotic, and angiogenic. In ASCs, the use of 3D cultures can stimulate pro-angiogenic abilities[17]. The use of these 3D spheroids will produce large amounts of anti-inflammatory protein TNF-α-stimulated gene/protein 6 (TSG-6). However, cell activation in the spheroid is also very much influenced by the culture medium used[7].

2. Preconditioning using growth factors, cytokines, chemokines, hormones

Apart from environmental modification, the use of a conditioned medium derived from growth factors, cytokines, chemokines, and hormones can increase the regeneration capacity of ASCs. The use of epidermal growth factor (EGF) has been shown to increase the motility and migration capabilities of MSCs without affecting their multipotential ability. The use of EGF in preconditioning MSCs increases the expression of EGF-receptor proteins, such as Vascular endothelial growth factor (VEGF) and Hepatocyte growth factor (HGF) which play a role in accelerating wound healing[7,20,23]. The increase in early progenitor proliferation in MSC can be obtained by using soluble EGF without causing differentiation. EGF is able to increase osteogenic differentiation[23]. Besides EGF, Transforming growth factor β (TGF-β) can be used to increase VEGF formation when combined with hypoxia conditions or TNF-α[7].

Besides using EGF, there is another culture medium (CM) alternatives that can be used for pre-conditioning. Tumor necrosis factor-α (TNF-α) can also be used in preconditioning MSCs and stimulates the release of cytokines, chemokines, and proteases. The use of this CM will trigger the secretome of MSCs including inflammatory molecules. These released substances are involved in the inflammatory process, where inflammation is the key to the injury response. The cytokines and chemokines produced to play a role in the regeneration process[7,20]. Another strategy used in CM is to use hormones. The hormones melatonin and angiotensin II help increase the ability of cells to survive in oxidative stress conditions. This increase corresponds to an increase in VEGF levels[17].

3. Preconditioning using pharmacological or chemical agents

The use of pharmacological or chemical agents is another way to maximize the yield of MSCs, including ASCs. Agents that have been studied, including using atorvastatin, curcumin, diazoxide, deferoxamine, valproate, and lithium chloride[7,17]. Mostly, the use of these agents will improve the survival of the transplanted MSCs. This increase is due to an increase in VEGF, Fibroblast growth factor-2 (FGF-2), or HGF, and activation of the Akt signaling pathway. The increase in these molecules will increase neovascularization[17].

The use of atorvastatin in preconditioning MSCs can increase the expression of the CXC chemokine receptor 4 (CXCR4) and MSC migration. Other agents that can increase the expression of CXCR4 are valproate and lithium chloride[7]. CRCX4 signaling plays an important role in migrating MSCs to the damaged side of the network[24]. The use of atorvastatin can increase the potential therapeutic effects of MSCs and the regenerative efficacy of MSC transplantation[7,17,24]. Atorvastatin also has the potential to increase the immunomodulatory capacity of MSCs[17].

Curcumin is an antioxidant and anti-inflammatory agent that can prevent damage from oxidative stress. Another property possessed by curcumin is anti-apoptotic[25]. The effect of using curcumin increases the therapeutic potential of MSCs[7,25]. Another agent that can be used for preconditioning to prevent damage from oxidative stress is diazoxide. Diazoxide is a mitochondrial ATP-sensitive potassium channel opener that can upregulate HGF and FGF-2. Deferoxamine can be used as a preconditioning agent which will have the effect of increasing cell migration[7].

Role of preconditioning adipose mesenchymal stem cells in regeneration disk

Based on the summary above, the use of preconditioning strategies can improve the regenerative therapy capabilities of MSCs. The development of MSCs technology has experienced rapid development in the

field of regenerative medicine. The majority of existing studies are at the clinical trial level on animals[7]. The use of preconditioning with the above methods can increase cytoprotective factor molecules, pro-regenerative, pro-angiogenic (VEGF), and immunomodulatory cytokines[17].

ASCs are one of the most developed MSCs today. One of the advantages of ASCs is that there are fewer cells that need to be transplanted than other MSCs. The reduction in the number of transplanted cells will be followed by a decrease in the side effects of this MSCs therapy compared to others[20]. This makes it possible for ASCs to be used to treat degenerative diseases, such as osteoarthritis and IDD[7]. The use of ASCs in orthopedic surgery can provide promising results due to their ability to repair cartilage and bone[6].

As a group of MSCs, the use of ASCs can be optimized by using proper preconditioning before the ASCs are transplanted[6]. Preconditioning on ASCs can change its function in various ways, such as apoptosis, difference, migration, and regeneration. ASCs are the same as MSCs in general, which respond to changes in their environment[8] In the field of orthopedic surgery, environmentally modified preconditioning is the widely used preconditioning of ASCs[6]. The secretion of angiogenic and neurotrophic factors in the use of ASCs can be increased by using the preconditioning strategy by modifying the environment into a hypoxic environment. Angiogenic factors that can be triggered by this strategy are Angiopoietin-2 (Ang-2) and VEGF[13].

Preconditioning using cytokines in ASCs is no different from MSCs in general as described above. One that has received attention is preconditioning with pro-inflammatory cytokines or mediators because it can increase its immunomodulatory properties and therapeutic potential. An example of the use of cytokines used in ASCs preconditioning is TNF-α, especially in the orthopedic field because it can promote bone generation by increasing proliferation, mobilization, and osteogenic differentiation. The use of proinflammatory cytokines is one way to increase the effectiveness of ASCs as a therapy[8]. Even so, the use of preconditioning with proinflammatory cytokines is still developing, especially to determine the side effects that can be caused and the benefits that can be obtained.

Another example of using a preconditioning strategy in culturing ASCs is the combination of a hypoxic environment and a medium culture medium with TNF-α. This method can increase NGF. The use of ASCs has good potential to increase the expression and secretion of factors that play a role in nerve survival and vascularization under hypoxic and inflammatory conditions. This is compatible with the environment in IVD which has a hypoxic and inflammatory environment, thus requiring angiogenesis and innervation for healing[13].

The use of pharmacological agents and chemical or bioactive molecules may also be used in the preconditioning of ASCs such as MSCs. The use of curcumin improved the retention of ASCs transplanted and reducing apoptosis and increased neovascularization[8,26]. The use of curcumin in ASCs preconditioning can reduce apoptosis, increase VEGF, and increase tolerance to damage due to oxidative stress so as to increase the therapeutic potential of ASCs. These factors make curcumin a promising alternative as a strategy to increase the effectiveness of ASCs[26]. Apart from curcumin, atorvastatin, CRX4, and other agents can be used to maximize ASCs like most common MSCs[17].

Conclusion

Preconditioning ASCs have an important role in disk regeneration. The use of ASCs in regenerative medicine in the orthopedic field has enabled IDD, as a degenerative disease, to be fully managed. Treatment of IDD, which has been symptomatic only, can be curative because of its ability to regenerate disks. One of the important keys in using ASCs for disk degeneration is the preconditioning step of the ASCs. The pre-conditioning strategy chosen will determine the efficiency and capacity of these ASCs for use in regeneration disks. Preconditioning on ASCs can change its function in various ways, such as apoptosis, difference, migration, and regeneration. Preconditioning ASCs, just like MSCs in general, can increase cytoprotective factor molecules, pro-regenerative, pro-angiogenic (VEGF), and immunomodulatory cytokines which are important in disk regeneration.

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Biochemical Study of Some Parameters in Patients with B- Thalassemia in Karbala City

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Abstract

This study is intended to estimate some biochemical parameters in the adult men with ß- thalassemia major and compared with control group. Twenty Iraqi beta- thalassemia major (TM) patients are employed from thalassemia center in Karbala city at age of 15-25 years . The body mass index (BMI) of patients was calculated by measuring weight and high of patients , and the concentration of platelets ,Total cholesterol (TC),Triglyceride(TG),Low density lipoprotein (LDL) ,high density lipoprotein(HDL), Alanine amino transferase (ALT), Aspartate transferase (AST), Antidiuretic hormone (ADH),Growth hormone (GH), Triiodothyronine (T3) ,Thyroxin (T4), Thyroid stimulating hormone (TSH),Testosterone hormone, Urea and Creatinine were measured in thalassemic patients and compared with healthy control group. The results display that significant decrease ($p<0.05$) in serum levels platelets, HDL, ADH, GH, TSH, T3, T4, Testosterone hormones and creatinine in patients with ß- thalassemia and showed significant rise ($p<0.05$) in serum Tc, TG, LDL, ALT, AST and Urea levels in thalassemic group compared with healthy group .and the results demonstration that significant lower in BMI in group of patients compared with control group. we can be concluded from the present study ,that iron overload state influence on some endocrine glands secretions, liver and kidney functions.

Key Words: *Thalassemia , Iron overload, platelets, ADH, GH.*

Introduction

Thalassemia is an inherited blood disorder that affects the body's ability to produce hemoglobin and red blood cells , mutations of globin genes lead to weak a globin synthesis [(1)].the main type of thalassemia are alpha and beta thalassemia , ß- thalassemia is results by decrease in ß- globin synthesis [(2)] . The ß- thalassemia major is an autosomal recessive disorder ,characterized by reduction of normal ß-globin synthesis , and beta-thalassemia is commonly associated with shortened red cells life span and excessive destruction of red blood cells [(3)].

The ß- thalassemia is a severe genetic disturbance which leads to a significant excess in mortality and morbidity [(4)]. In Iraq ,thalassemia is a major health problem with prevalence of carrier range from (4.4-6.6)% [(5)]. Symptoms in infants with ß- thalassemia include jaundice ,splenomegaly ,fatigue ,siderosis and cardiomegaly [(6)]. In some studies have shown that , in patients with thalassemia there is excess of reactive oxygen production , such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) and that causing oxidative stress and lead to oxidative damage to erythrocyte [(7)] . In patients with ß- thalassemia long – term regular blood transfusions causes iron overload that results in cardiac damage , liver fibrosis , growth retardation and gonadal dysfunction [(8)]. Iron chelation therapy is essential for lowering of iron deposition on endocrine glands [(9)].

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The major causes of death in thalassemia patients are congestive heart failure and fatal cardiac tachyarrhythmia to be the cause of abrupt cardiac death [10]. The principal mechanism of iron toxicity is the free radical – mediated pathway [11] . Therefore ,the current study is designed to calculate some biochemical parameters in adult men patients with beta – thalassemia major and compared with healthy group.

Materials and Methods

In this study 20 patients (male) were completed all biochemical analysis tests . the range of their ages between 15-25 years .the patients registered as thalassemic patients in (thalassemia center) in Karbala city – Iraq, from February to March 2019.the patients had ß- thalassemia major as recorded in their files , 20 apparently healthy male were selected as the control group . the range of their ages were equal to that of patients . blood were obtained from individuals in the morning and collected in plain tube for serum in order to rating some hematological and biochemical parameters : Platelets ,ALT, AST, TC, TG, LDL-c, HDL-c, ADH, GH, T3,T4, TSH, Testosterone hormones , Urea and Creatinine . serum platelets count were measured by,serum (ALT and AST) levels were gauged by , serum(TC) levels were measured by using (cholesterol kit BIOLABO company , France),serum (TG) level were amounted by using (Triglyceride kit BIOLABO company , France), serum (HDL-c) level were gauged by using (Rondo.United kingdom laboratories Ltd , co.Antrim) kit . Serum (LDL-c) level were measured by using : LDL= TC- (HDL+TG/5) Serum ADH,GH,T3,T4, TSH and Testosterone hormones were measured by enzyme – Linked immunosorbent assay (ELISA) technique. BMI was determined by calculating weight (in kilograms) divided by the square of height (in meters) ; BMI = weight (kg)/square height (m^2). The mean ±Std. Division(SD) of parameters measured from groups healthy and patients were determined with serum platelets ,ALA,AST and other biochemical parameters measured.

Results

Table 1 shows the results gained for BMI in patients groups with control group , decrease significantly ($p<0.05$) in BMI of ß- thalassemia patients in comparison to control group**.**

Table 1. : Comparison of BMI in Patients men groups with control group.

parameter	Control group Mean ±SD.	Patients group Mean ±SD
BMI kg/m2	33.66 ± 4.09 A	19.08 ±3.17 B

The latter's indicate to Significant at ($p< 0.05$)

Table 2 shows the results acquired for serum platelets levels in patients groups with control group, reduction significantly ($p<0.05$) in Platelets of ß- thalassemia patients in comparison to healthy control .

Table 2. : Comparison of serum platelets levels in Patients men groups with control group

parameter	Control group Mean ±SD	Patients group Mean ±SD
Platelets (103/mL)	247.70 ± 29.84 A	176.45 ± 26.30 B

The latter's indicate to Significant at ($p< 0.05$) .

Table3 shows the results obtained for serum ALT and AST levels in patients groups with control group , increase significantly ($p<0.05$) in ALT and AST concentration of ß- thalassemia patients in comparison to healthy control . The same table shows the results gained for Urea and Creatinine in patients groups with control group , rise significantly ($p<0.05$) in Urea concentration ,and low significantly ($p<0.05$) in creatinine concentration of ß- thalassemia patients in comparison to control group.

Table 3. : Comparison of serum ALT and AST levels in Patients men groups with control group

parameter	Control group Mean ±SD	Patients group Mean ±SD
ALT (U/ml)	21.97 ± 2.29 A	50.07 ± 4.97 B
AST (U/ml)	28.34± 4.27 A	49.54± 1.75 B
Urea(mmol/l)	5.51 ± 0.54 A	9.42 ± 0.82 B
Creatinine (mmol/l)	94.65 ± 10.43 A	54.68 ± 5.23 B

The latter's indicate to Significant at ($p< 0.05$) .

Table 4 shows the results gained for serum TC,TG,LDL-c and HDL-c levels in patients groups with control group , rise significantly ($p<0.05$) in TC,TG and LDL-c concentration, and reduction significantly ($p<0.05$) in HDL-c concentration of ß- thalassemia patients in comparison to control group ..

Table 5 shows the results obtained for serum ADH,GH,T3,T4,TSH and testosterone levels in patients groups with control group , low significantly ($p<0.05$) in ADH,GH,T3,T4,TSH and Testosterone levels of ß- thalassemia patients in comparison to control group .

Table 4. : Comparison of serum TC,TG,LDL-c and HDL-c levels in Patients men groups with control group .

parameter	Control group Mean ±SD	Patients group Mean ±SD
TC (mg/dl)	178.99 ± 8.34 A	226.45 ± 11.58 B
TG (mg/dl)	130.05 ± 7.36 A	167.90 ± 18.09 B
LDL-c (mg/dl)	149.35 ± 22.70 A	229.53 ± 33.22 B
HDL-c (mg/dl)	45.65 ± 3.76 A	31.43 ± 5.50 B

The letters indicate to Significant at ($p< 0.05$) .

Table 5. : Comparison of serum ADH, GH, T3, T4 ,TSH and testosterone levels in Patients men groups with control group .

parameter	Control group Mea ±SD	Patients group Mea ±SD
ADH (pg/ml)	5.10 ± 0.55 A	2.98 ± 0.59 B
GH (ng/ml)	11.07 ± 0.93 A	6.68 ± 1.33 B
T3 (ng/dl)	85.56 ± 5.43 A	59.27 ± 6.53 B
T4(ng/dl)	1.90 ± 0.40 A	0.67 ± 0.22 B
TSH (mlU/ml)	4.66 ± 0.45 A	2.24 ± 0.47 B
Testosterone(ng/ml)	5.46 ± 0.81 A	2.16 ± 0.51 B

The letters indicate to Significant at ($p< 0.05$) .

Discussion

In this study we found decrease significantly ($p<0.05$) in BMI of ß- thalassemia patients in comparison to healthy control and that result by insufficient production of hemoglobin and disorders in beta chains of globin production , the red blood cells cannot carry sufficient oxygen [12] , inability to transfer O_2 to tissues and exchange with CO_2 which lead to (Hypoxia) to induce (Anorexia) and under- weight of patients with thalassemia [13] , and may be due to toxic effect of Deferrioxamine , causes over plus deposition of iron in tissue and diminish in iron dependent enzyme which modify collagen which cause growth flaw [14]. The results appearance that significant reduced ($p<0.05$) in platelets numbers in serum of ß-thalassemia group comparison with healthy control, Hypersplensim state [15] ,and excess production of reactive oxygen intermediate such us superoxide (O_2^-) , hydrogen peroxide (H_2O_2) that lead to oxidative stress which responding to direct effect on platelets production in bones and that due to decrease in numbers of platelets in serum of ß- thalassemia patients [16], and reduction in platelets in serum was induced by immunity system dysfunction which due to increase in platelets damage and decrease it numbers in blood [17]. Iron over load in liver cells lead to (lipid homeostasis) dysfunction and that lead to change in (Hydroxyle -3-methylglutary- co enzyme A(HMG-Co A) reductase activity and dysfunction of cholesterol esters and decrease in lipoprotein lipase activity and all that lead to rise in free fatty acids in blood [18], Iron over load in liver response to excess tumar necrosis factors (TNF-α) and interleukin (IL-IB) which lead to raise levels of cholesterogenic enzyme such as HMG-Co A reductase and diminution levels of α- cholesterol – 17 hydroxylase that in charge of cholesterol catabolism in liver [19] , and by oxidative stress that results form Iron over load may because of stress of endoplasmic reticulum and rise in gene expression of strole on endoplasmic reticulum membrane and that due to height levels of TC and TG [20] . and the results display high levels of LDL – c in serum of patients with ß- thalassemia compared with healthy group and this results may be induce by (hypersensitivity) of LDL-receptors in blood vessels to collection of lipoproteins in serum and result high rate of LDL-c in serum [21]. HDL-c levels in serum correlated oppositely with LDL-c levels in serum , and HDL-c have role in carry cholesterol from tissues to liver and rise levels of LDL cause lower HDL-c levels in serum [22] .

The results in table 4 indicated escalation ($p<0.05$) in ALT and AST concentrations in thalassemic patients as compared with control group. The reason for this result is oxidative stress that induced by iron over load in liver due to lipid peroxidation in lipid proteins of cell membrane of liver cells and changes in permeability of cell liver membrane and that results to destroyed of cells liver with rise ALT and AST levels in blood [23].

The results in table 5 showed that significant dwindling in serum levels ADH,GH,TSH,T3,T4 and testosterone hormones in patients group compared with healthy group , reduce levels ADH,GH and TSH hormones may be result to Iron level rise in thalassemia patients (long-term transfusion) ,some iron will not connect to transferrin and restricted with other blood composition leading to formulation of plasma non-transferrin bound iron (NTBI) which is toxic and participate to obstetrics of reactive oxygen species (ROS), therefore leading to tissue deterioration and pituitary dysfunction . Excess iron head for deposit in pituitary glands causing end glands dysfunction by ROS-mediated lipid peroxidation and that leading to lower in levels of ADH,GH and TSH hormones [24]. And the results manifest significant lower in levels of T3 and T4 hormones and that may be by iron over load , high iron level deposited in endocrine gland such as thyroid gland responsible for formation of free radicles and output of reactive oxygen spices (ROS) that can lead to inhibit peroxidase (TPO) activity ,reduce 5- deiodnase production , decrease in activity of T3-receptors [25], and damage of proteins which responsible for transported of thyroid hormones (thyroxin binding globin) TBG and that lead to hypothyrodsim (lower in T3 and T4 levels in blood [26]. The results demonstrated ,testosterone levels was significantly lower in ß- thalassemia group compared to healthy group , the primary reason for this result may be by increased deposition of iron in pituitary glands has a cytotoxic effect, that leading to hyper-gonadotrophic hypogonadism due to pituitary hypo-responsiveness to GnRH, and that causes reduction in testosterone production by the testis and lower levels of testosterone hormone in blood [27].the results show in table (3) escalation significant ($p<0.05$) in urea concentration , and decrease significant ($p<0.05$) in creatinine level in ß- thalassemic patients in comparison to healthy group , high iron levels deposit in kidneylead to disorder in functions of kidney and this result to glomerular filtration dysfunction and this cause rise in Urea levels and reduction in ceratinine concentration in blood [28].

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Molecular Diagnosis and Genotype Analysis of *Giardia lambelia* in Diarrheal Patient in Al-Diwaniyah Province-Iraq

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Abstract

Three hundred and fifty-six stool samples were collected from the beginning of October 2019 till the end of February 2020 from patients who suffering from gastrointestinal symptoms ,general stool examination was done for detect the cysts and trophozoites of *Giardia lamblia* using direct smear and flotation method. The results showed that 105 out of 356 samples were positive (29.49 %) . Nested PCR were done to the sixty samples from the positive results microscopically selected randomly , triosephosphateisomerase (TPI) gene that using in detection *Giardia lamblia* genotype shows result that positive samples of (A,B,E) while not showed result positive to F . The current study also showed high significant association (P <0.01) , and noted that the genotype B is the most common than genotype A, where the number of infected samples with the genetic pattern B 24/60 with percent 40% while the number of infected samples with genetic pattern A 18/60 with percent 30%.

Keywords : *Giardia lambelia, genotype, diarrhea, PCR*

Introduction

Giardia lamblia is a zoonotic enteroparasite and a worldwide distribution, Most located in hot areas and places where sanitation is at its lowest , can infected most mammalian so considered as a zoonosis and characterized by acute and chronic illnesses [(1)] .There are three main Giardia morphological subtypes: *G. miice* of muris , *G. agilis* of Amphibians and *G. lamblia* of humans, it also include other vertebrates , These types can be distinguished by the structure and proportions of the trophozoite [(2)] .

It is known that *G. lamblia* is morphologically similar to these isolates infecting humans, so other methods were required to identify phenotypic and genotypic variability among different human , Molecular characterization shows that *G. lamblia* consisting of seven assemblages (A to G) *G. lamblia* was isolated from various hosts and showed that the largest number of host species , including humans, are infected by A and B assemblages and may include other assemblage as assemblage E, the study was aimed to determining genotypes (A,B,E and F) of G. lamblia in Al- Diwaniyia Governorate and the relationship with ages, gender and resident by using the microscopic examination and the polymerase chain reaction which is known as nested PCR [(3-5)] .

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Material and Methods

General stool examination were done through wet mount examination and Flotation method [(2)] .

Molecular assay(Nested PCR)

Nested PCR assay was performed for detection genotyping of Giardia lamblia from human where The PCR assay was using primers for triosephosphateisomerase (tpi) gene where was specific for genotyping A ,B ,E and F these primers were provided by AccuPower ® PCR PreMix (Bioneer. Korea). The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV

transilluminator, see tables (1) and (2) .

Table (1) : Nested PCR primer Giardia lamblia assemblage A and B

Assemblage	Primer	Sequence 5'-3'		Amplicon
A	TPI gene PCR	F	CGAGACAAGTGTTGAGATG	576bp
		R	GGTCAAGAGCTTACAACACG	
	TPI gene nPCR	F	CCAAGAAGGCTAAGCGTGC	476bp
		R	GGTCAAGAGCTTACAACACG	
B	TPI gene PCR	F	GTTGCTCCCTCCTTTGTGC	208bp
		R	CTCTGCTCATTGGTCTCGC	
	TPI gene nPCR	F	GCACAGAACGTGTATCTGG	140bp
		R	CTCTGCTCATTGGTCTCGC	

Table (2) : Nested PCR primer Giardia lamblia assemblage E and F

Assemblage	Primer	Sequence 5'-3'		Amplicon
E	Gdh gene PCR	F	TGGGGCTGACACTGATGTTC	349bp
		R	GAACCCGTCCTTGTCGACAA	
	Gdh gene nPCR	F	GCTCGCGAGATCGGTTACTT	200bp
		R	CCGGAAAGGAGGACGTTCTT	
F	TPI gene PCR	F	AACGGCTCGCTCGACTTTAT	471bp
		R	GGGCTCGTAGGCAATAACGA	
	TPI gene nPCR	F	GGCCATTGCTGCCCACAAG	401bp
		R	TCTTCGACTCTCCAAGCTCC	

Result

Microscopically diagnosis

the result shows that 105/356 were positive to *G. lamblia*. Trophozoite, trophozoite mixed with cyst and cyst only ,there is a high significant association between acute and chronic infection depended on microscopical examination ($P < 0.01$) as in table (3) .

Table (3) : The percent of acute and chronic stage of Giardiasis infection based on microscopical examination.

Total samples	Microscopic examination	Acute		chronic	
		No.	%	No.	%
356	105	82	78.09	23	21.9
X2		66.3			
P value		0.000 (HS)			

HS: High significant association (P <0.01)

Molecular Assay (nested PCR)

Nested PCR product analysis of Triose phosphate isomerization (TPI) gene in Giardia lamblia of assemblages(A,B,E and F) where performed on 60 from 105 samples , result showed :

Giardia lamblia Assemblage A

The results of these study showed the that PCR product of Triose phosphate isomerization (TPI) gene used in *G. lamblia* genotype A(assemblage A) detection where the positive samples are isolated at product size 576bp PCR, these are similar with the results [2]where proved that (TPI) gene for assemblage A is located at 576 bp, as in figure (1) .

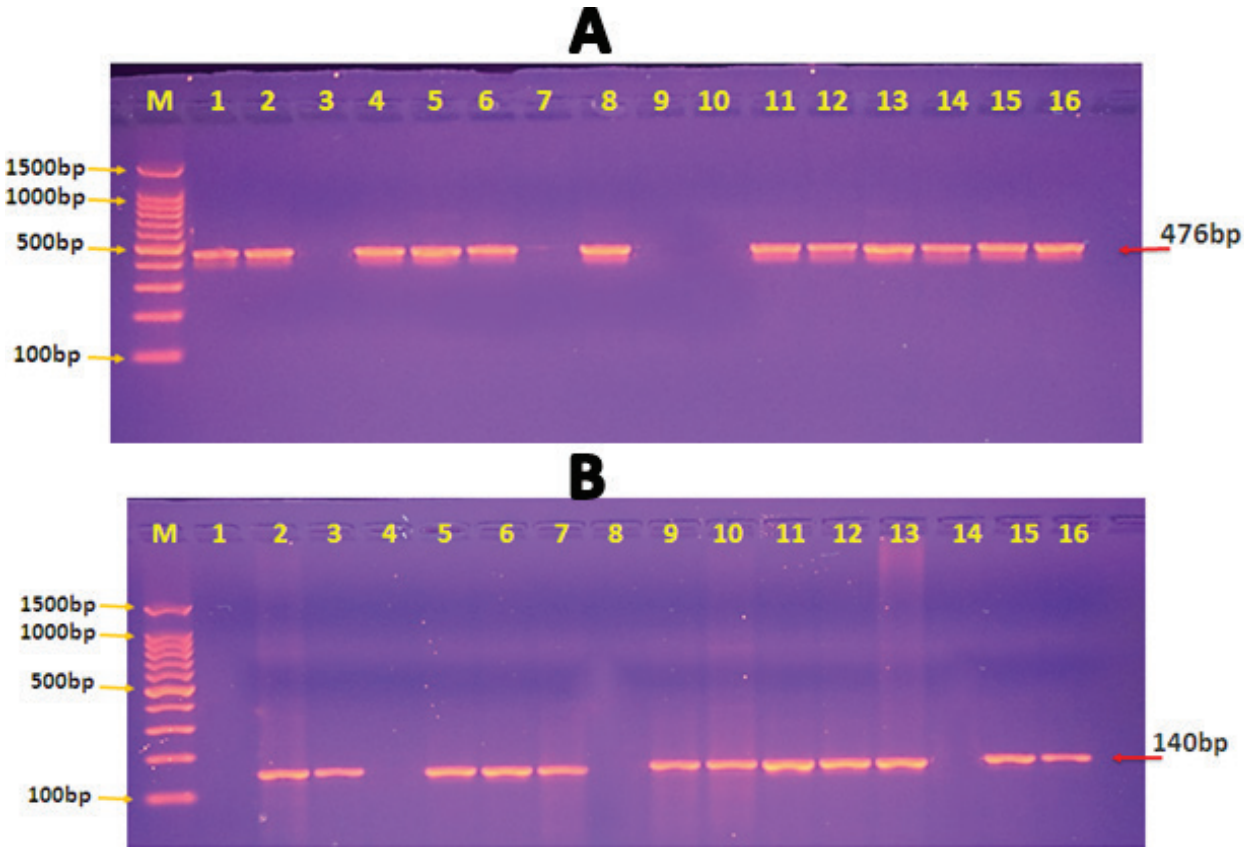

Figure (1) : Agarose gel electrophoresis of assemblage A

Agarose gel electrophoresis image that showed Nested PCR product analysis of TPI gene in Giardia lamblia assemblage A of Human stool samples. (M) Marker ladder (1500-100bp). Lane (1-16) Giardia intestinalis assemblage A at 476bp Nested PCR product.

Giardia lamblia Assemblage B:

The result of this analysis has shown that the PCR gene PCR product of triosephosphate isomerase (TPI), which is used as the detection of Giardia lamblia (Assemblage B) are similar of results [2]that proved (TPI) gene for assemblage B is located at 208 bp.

Images of agarose gel electrophoresis showing TPI's Nested PCR product analysis gene in Giardia intestinalis assemblage B of Human stool samples. (M) Marker ladder (1500-100bp). Lane (1-16) Giardia intestinalis assemblage B at 140bp Nested PCR product, as in figure (1) .

Giardia lamblia assemblage E :

The results of this study showed that the PCR product of triosephosphateisomerase (TPI) gene that using in detection genotype E(Assemblage E) of Giardia lamblia where the positive samples isolates at 416bp PCR product size, which similar with, as in figure (2) .

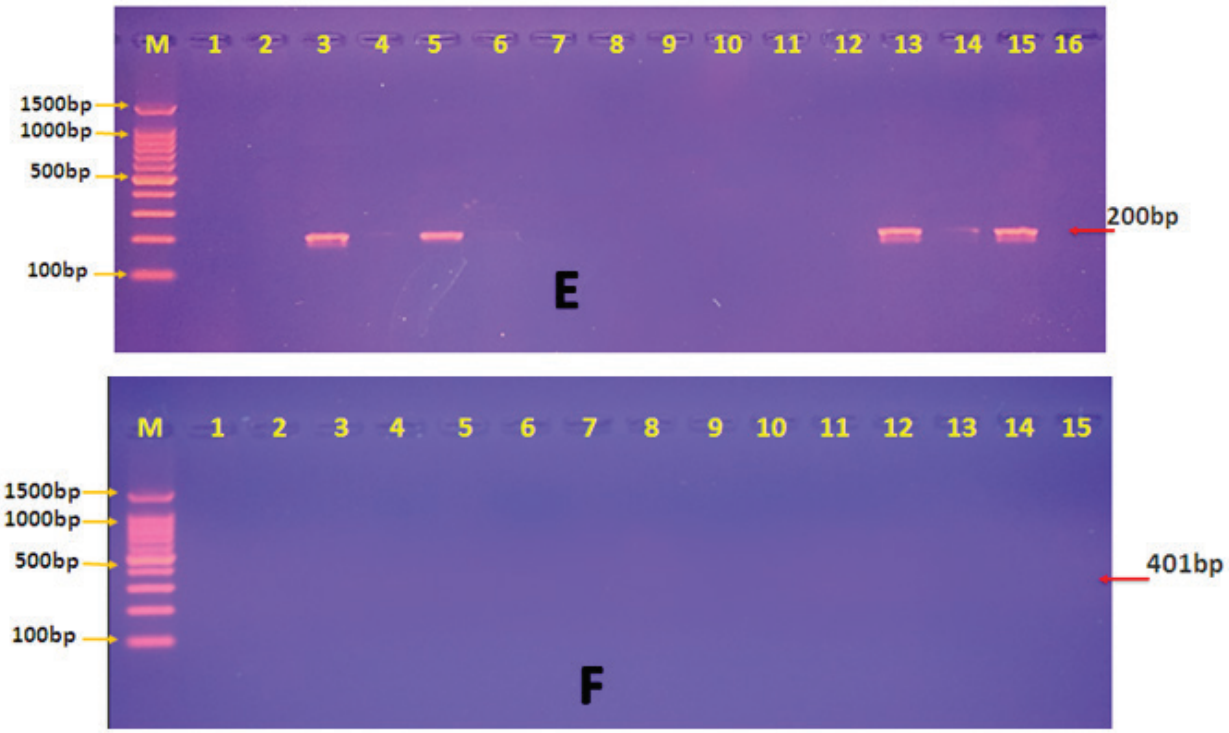

Figure (2) : Agarose gel electrophoresis of assemblage E

Agarose gel electrophoresis image that showed Nested PCR product analysis of glutamate dehydrogenase gdh gene in Giardia intestinalis assemblage E of Human fecal samples. (M) Marker ladder (1500-100bp). Lane (1-16) Giardia intestinalis assemblage E at 200bp Nested PCR product.

Giardia lamblia Assemblage F:

(TPI) geneproducted of PCR that is used in the detection genotype F(Assemblage F) of Giardia lamblia does not disclose any positive samples at 140bp PCR product size that are seen with 140bp PCR product size [4], as in figure (2) .

Image showed Nested PCR product analysis of TPI in Agarose gel electrophoresis gene in Giardia intestinalis assemblage F of Human fecal samples. (M) Marker ladder (1500-100bp). Lane (1-15) No positive Giardia intestinalis assemblage F at 140bp Nested PCR product.

The results showed High significant association (P <0.01) , and noted that the genotype B is the most common than genotype A, where the number of infected samples with the genetic pattern B 24/60 with percent 40% while the number of infected samples with genetic pattern A 18/60 with percent 30%. The differences in the prevalence of assemblages A and B may be attributed populations locations that was studied, while assemblage E was lower where the number of infected samples with the genetic pattern E 7/60 with percent 11.66%,while not shown to us assemblage F in any sample (table 3) .

Tables (3) : The number and percent of Genotype A,B,E,F and A mixed with B related to Giardiasis

Genotype No. (%)				
A	B	A+B	E	F
18 (30%)	24 (40%)	11 (18.33%)	7 (11.66%)	0 (0%)
Total = 60 (100%)				

The present study showed that assemblage B in male(70.83%)was higher than female(29.16%) while assemblage A and E was lower in male(27.77%, 28.57%) and higher in female where was (72.22%, 71.42%) and wasSignificant association (P<0.05)between male and female according to genotype, as in figure (3) .

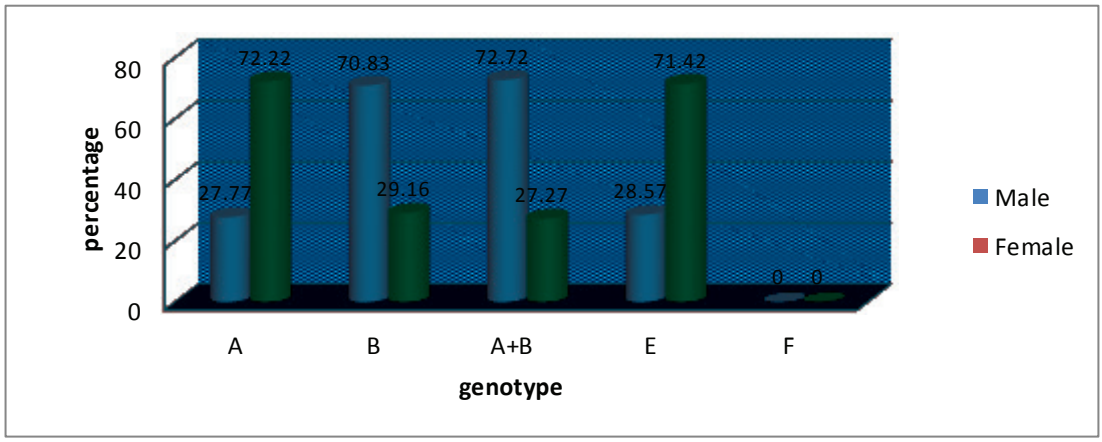

Figure (3) : Histogram showing relation between Percentage of Genotyping (A,B,E and F) of Giardia lamblia and gender .

The present study showed that the number and percent of assemblage A,B,A mixed with B ,E and F in rural area was 12(66.66), 5(20.83), 4(36.36) , 6(85.71) and 0(0) respectively , while number and percent of assemblage A,B,A mixed with B ,E and F in urban area was 12(66.66), 5(20.83), 4(36.36) , 6(85.71) and 0(0) respectively 6(33.33), 19(79.16), 7(63.63), 1(14.28) and 0(0) respectively, as in table (4) .

Figure (4) : The number and percent of Genotype A,B,E,F and A mixed with B related to Giardiasis according to Location.

Location	Genotype No. (%)					X2	P value
	A	B	A+B	E	F		
Rural	12(66.66)	5(20.83)	4(36.36)	6(85.71)	0(0)	14.09	0.003(HS)
Urban	6(33.33)	19(79.16)	7(63.63)	1(14.28)	0(0)	14.09	0.003(HS)
Total	18(30)	24(40)	11(18.33)	7(11.66)	0(0)	36.45	0
X2	4	16.33	1.63	7.14	----		
P value	0.046(S)	0(HS)	0.201(NS)	0.008(HS)	----		

NS: No significant association (P >0.05)

S: Significant association (P<0.05)

HS: High significant association (P<0.01)

Discussion

According of microscopic examination the result was nearly similar to study in Philippines showed that 22.05% of stool samples contained Giardia cysts only [6], and study in Bergen city in Norway where reported a chronic percentage of giardiasis was 26% [7], while study in Peshawar city showed a higher percentage of the chronic giardiasis was 42% [8].

According to molecular study where study in Waist Province similar of this study where assemblage B was more than assemblage A [9], while mixed-assemblage infections of our result was 11/60(18.33%) where different with study was present (3 %) of assemblage A and B [10],while reached of 11 samples in other study where was this result similar of our study [11],while no similar other study in Australia where assemblage A was 50% highest from assemblage B was 38·6% [12] and In city Rio de Janeiro, assemblage A was is major infection of human [13], while assemblage E lower in human done detection in some studies where assemblage E was identified in present 6·8% from (6/88) of samples(12), and in other study three sample from assemblage E were found [14].

The present study shows a result nearly similar to other study in South Africa where the male were more infected by assemblage B than female patients, while not similar Heyworth (2016) found that assemblage A was higher from assemblage B in male [15] .

The result of present study had the semi identical to the result of other study that shows assemblage B in the urban more than the rural while assemblage A was more in rural than urban [16].

This finding shows that humans are the main source of assemblage B, but that domestic animals play an significant role in assemblage A and E host ranges where consider this result similar when both came from [17],other research indicated that assemblage A is typically present in a large variety of species [18] where was similar of this result where distributed infection in rural and urban

, as for assemblage E where all sample were from rural areas and this corresponds with [19] where In cattle, all Giardia isolates have been classified as G. Assemblage E lamblia, A new zoonotic course of Giardia transmission indicates the identification of assemblage E in humans. [13],where that assamblage B and E was High significant association (P <0.01) while A Significant association (P<0.05).

The results of this study was comparable to the result of in which assemblage B was presented among different age groups ,Assemblage A was higher presented in Children under 10 years , and assemblage A in study In adolescents, assemblage B was the most common (66.6 percent), while 40 percent of adults considered assemblage B, and these result confirmed the result of this study whereas the A was higher than assamblage B in all age groups but the group aged 30–39 years [20] .

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Original Research

Increased Expression of Tubulin A, Cyclin B1, and Ki67 May Associate with Cell Death Mechanism Caused by Noncontact Electro Capacitive Cancer Exposure *in vitro*

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Abstract

Background: Noncontact Electro Capacitive Cancer Therapy (ECCT) is a novel treatment modality in cancer that uses low-intensity intermediate-frequency alternating electric field. A previous study has reported ECCT could induce cell death in cancer cells, but the molecular mechanism underlining this process remains unclear. Therefore, this study aims to uncover the molecular mechanisms of the pathology induced by the device and to determine whether Tubulin A, Cyclin B, and Ki67 can serve as biomarkers for this exposure.

Methods: This study was an in vitro laboratory experiment with Completely Randomized Block Design. Two different types of cancerous cell lines were used in this study: oral squamous cell carcinomas and Hela cells. In addition, non-cancerous cell line, bone marrow mesenchymal cell, was also subjected to the electric field produced by ECCT. All three cell lines were divided into two groups: the ECCT-exposed group and the control group. After exposure, the protein expression of Tubulin A, Cyclin B, and Ki-67 was examined using immunocytochemistry staining.

Results: The results showed that the number of Tubulins A, Cyclin B1, and Ki-67 expression was significantly higher in ECCT-exposed group than in the control group ($p<0.05$). Increased expression of tubulin A indicates a disruption to microtubule polymerization, an increase in Cyclin B expression indicates mitotic arrest or incomplete mitosis, whereas the increase of Ki-67 expression occurs when cells stop in the metaphase phase (mitotic arrest).

Conclusion: It could be concluded that increased Tubulins A, Cyclin B1, and Ki-67 expression may associate with the death cell mechanism induced by exposure to Noncontact Electro Capacitive Cancer devise.

Keywords: *ECCT; in vitro; cell death; Tubulin A; Cyclin B1; Ki-67; mitotic arrest*

Introduction

Electro Capacitive Cancer Therapy (ECCT) is a noncontact alternating current electric field-based cancer therapy method developed by C-Tech Labs Edwar Technology. ECCT consists of ECCT power supply, connector cable, and ECCT vest. ECCT power supply produces an intermediate-frequency (100 kHz), low-intensity (18 Vpp) electric current, which will be supplied to the ECCT vest via a connector cable. Eventually, electric field will emerge from ECCT vest and affect body cells and tissues.[1,2]

The potential applicability of electric fields as a novel therapeutic modality for malignant tumors was first described in early studies conducted by Eilon et al. In this study, Tumor Treating Electric Field (TTFields) demonstrated that exposure of alternating current electric field with very low intensity (<2 V/cm) and intermediate

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frequency (100–300 kHz) to cancer cells had inhibitory effects on cancer cells replication in vitro and in vivo.[3-6] The following study reported that the failure of cancer cell replication induced by TTFields was due to the disruption of spindle microtubule assembly in mitosis which only affected replicating cells.[7] Based on the findings above, it was suggested that the disruption of cell replication activates cancer cell apoptosis. In author's previous study, ECCT was reported to induce cell death and p53 protein expression in cancer cells.[1] However, the molecular mechanism underlining this process remains unclear.

Therefore, this study aims to uncover the molecular mechanism underlining the cell death process device and to determine whether Tubulin A, Cyclin B, and Ki67 can serve as biomarkers for this exposure by observing tubulin A, Cyclin B1, and Ki-67 expression in cancer cells exposed to electric field from ECCT, specifically in oral squamous cell carcinomas (SCC) and Hela cells (cervical cancer cell line) and to determine whether tubulin A, Cyclin B1, and Ki-67 can serve as a biomarker for this exposure. This is the first study in the existing literature, to our knowledge, that reports tubulin A, Cyclin B1, and Ki-67 expressions on cancer cells under the exposure of low-intensity and intermediate-frequency electric fields and the possible role of tubulin A, Cyclin B1, and Ki-67 in the molecular mechanism underlining the cell death caused by ECCT.

Material and Methods

Research design

This study aimed to uncover the molecular mechanism underlining cell death induced by ECCT and to determine whether Tubulin A, Cyclin B, and Ki67 can serve as biomarkers for this exposure, using cancerous cell line (squamous cell carcinomas and Hela cells) and non-cancerous cell line (bone marrow mesenchymal cells). This research was an in vitro experiment done in a laboratory with a Completely Randomized Block Design. The unit of experiment was taken randomly with the control as a comparison.

ECCT Intensity and Frequency Measurement

The intensity and frequency produced by ECCT were measured using an oscilloscope. The results showed that ECCT produced alternating current electric field with a frequency of 100 kHz (intermediate frequency) and a voltage of ± 20 Volts (low intensity). The electrodes used in this device were not attached to cancer cells but put in a container (a place to put the plate), therefore the static electric field, not the electric current, would have an effect on the cell growth.

To match the in vitro research design, ECCT power supply was connected to a box that could accommodate 24-well cell culture plate, which had been specially designed. The bottom and the lid of the box were loaded with a series of conductors that could receive and transmit electrical energy from ECCT power supply and convert it into alternating current electric field. The modified ECCT used in this research is shown in Figure 4.

Cell preparation and treatment

This experimental study was carried out using three different types of cell cultures, namely oral squamous cell carcinomas, Hela cells, and bone marrow mesenchymal cells. The study was conducted at the Stem Cell Laboratory, Institute of Tropical Disease, Airlangga University. The three types of cells stored in Cryo Tube at a temperature of -80^0 C were acclimatized and then cultured on α-MEM medium, at a temperature of 37^0 C with 5% CO2 levels. The culture medium was replaced every 48 hours until the number of cells was sufficient to be moved into the wells. One microliter cell suspension was placed in 24-well microplate loaded with 999 µL medium (total: 5×10^5 cells/ mL). A pair of capacitive electrodes positioned flanking on top and at the bottom of the microplate was connected to a square function oscillator. A one-directional field was generated between the pair of capacitive electrodes which alternated every 0.5 ms. Cell cultures were treated with noncontact electric fields by ECCT and incubated for 24 hours with 8 replications each. A control group with 8 replications was also incubated at the same time.

Immunocytochemistry Assessment

After 24 hours the three types of cell culture were harvested and Immunostaining for p53 was performed on cells grown on coverslips beneath the wells of the microplates using UltraVision Detection System Anti-Polyvalent TP-015-HD (Thermo the Scientific)

following the manufacturer's protocol. The evaluation of the expression was carried out according to the immunoreactive score (IRS) by Remmele and Stegner. The IRS evaluation was based on a modification that evaluated not only the visualized grade of color intensity (staining) but also adding the fraction of cells in each intensity category. The score was obtained from multiplication between the percentage of positive cells and color intensity (Table 3).

Statistical Analysis

To perform the suitable statistical tests, the normal distribution of the collected data should be confirmed in the beginning. The comparison of immunochemistry expression between treatment and control groups was tested using Mann-Whitney test because the distribution of the data was not normal. The direction of the causal relationship of expressed proteins was determined using regression analysis. And All tests were performed using the statistical software SPSS. An alpha value of 5% was considered significant, all data are presented as mean ± SD with 95% confidence intervals.

Results

Overview of Research Subjects

The results of the study were calculated, collected, and tested using normality test before statistical tests were conducted. In general, the research data obtained is shown in Table 1. Based on the data shown, the mean semiquantitative assessment of Tubulin A, Cyclin-B1, and KI67 in the three types of samples showed an abnormal data distribution ($p<0.05$).

Immunocytochemistry Examination of SCC Cell Culture

The results of immunocytochemical examination in SCC cell culture appear in the image below (Figure 1).

As shown in Figure 1A, the number of cells expressing Tubulin A in ECCT-exposed group was 60% with moderate expression levels and 30% of cells expressed Tubulin A with a weak expression level in the control group. The Cyclin B1 expression on cells exposed to ECCT was 60% with a weak expression level and 10-15% of cells expressed Cyclin B1 with a weak expression level in the control group. Meanwhile, the number of cells expressing Ki-67 in cells exposed to ECCT was 50%, with moderate to strong expression level and 10% of cells expressed Ki-67 with a weak expression level in the control group.

Figure 1B shows the results of the immunocytochemical semiquantitative examination in SCC cells group. The level of expressions of Tubulin A, Cyclin B, and Ki-67 on SCC cells exposed to 24-hour ECCT were higher than their expressions on the control cells group. Using Mann-Whitney test, Tubulin A, Cyclin B, and Ki 67 expressions between ECCT-exposed cells and control cells in SCC cell culture group were statistically significantly different with p-value < 0.05.

Immunocytochemistry Examination in Hela Cell Culture

The results of immunocytochemistry examination in Hela Cell Culture appear in the image below (Figure 2).

Figure 2A showed that the number of cells expressing Tubulin A in ECCT-exposed group was 85%, with moderate to strong expression levels and in the control group 30% of cells expressed Tubulin A with weak to moderate level of expression. The Cyclin B1 expression on cells exposed to ECCT was 85%, with moderate to strong expression levels and in the control group, 30% of cells expressed Cyclin B1, with the level of expression is weak to moderate. The Ki-67 expression on cells exposed to ECCT was 85%, with moderate to strong expression levels and in the control group, 60% of cells expressed Ki-67 with the level of expression is weak to moderate.

Figure 2B shows the results of the immunocytochemical semiquantitative examination in Hela cells group. The level of expressions of Tubulin A, Cyclin B, and Ki-67 on Hela cells exposed to 24-hour ECCT were higher than their expressions on the control cells group. Using Mann-Whitney test, Tubulin A, Cyclin B, and Ki 67 expressions between ECCT-exposed cells and control cells in Hela cell culture group were statistically significantly different with p-value < 0.05.

Immunocytochemistry Examination in Mesenchymal Cell Culture

The results of immunocytochemistry examination in Mesenchymal Cell Culture appear in the image below (Figure 3).

Figure 3A showed that the number of cells expressing Tubulin A in ECCT-exposed group was 30%, with low to moderate expression levels and in the control group, 25% of cells expressed Tubulin A with a weak expression level. The Cyclin B1 expression on cells exposed to ECCT was 60%, with a weak expression level and in the control group, 15% of cells expressed Cyclin B1 with a weak expression level. The Ki-67 expression on cells exposed to ECCT was 40%, with weak to moderate expression levels and in the control group, 10% of cells expressed Ki-67 with a weak expression level.

Figure 3B shows the results of the immunocytochemical semiquantitative examination in mesenchymal cells group. The level of expressions of Tubulin A, Cyclin B, and Ki-67 on mesenchymal cells exposed to 24-hour ECCT were higher than their expressions on the control cells group. Mann-Whitney test showes that Cyclin B and Ki-67 expressions between ECCT-exposed cells and control cells in Hela cell culture group were statistically significantly different with p value < 0.05. Meanwhile, Tubulin A expression between ECCT-exposed cells and control cells in mesenchymal cell culture groups were not statistically significantly different with p-value = 0.09 (p > 0.05).

Pathway Analysis

Regression analysis determined the direction of the causal relationship of this research variables as shown in Table 2. The results showed that the Tubulin A's expression is influenced by ECCT treatment. Increased expression of Tubulin A will affect the increase in expression of Cyclin B1, further increase in expression of Cyclin B1 will affect the increase in expression of Ki-67 (p<0.001).

Table 1 The general data and its normality test results

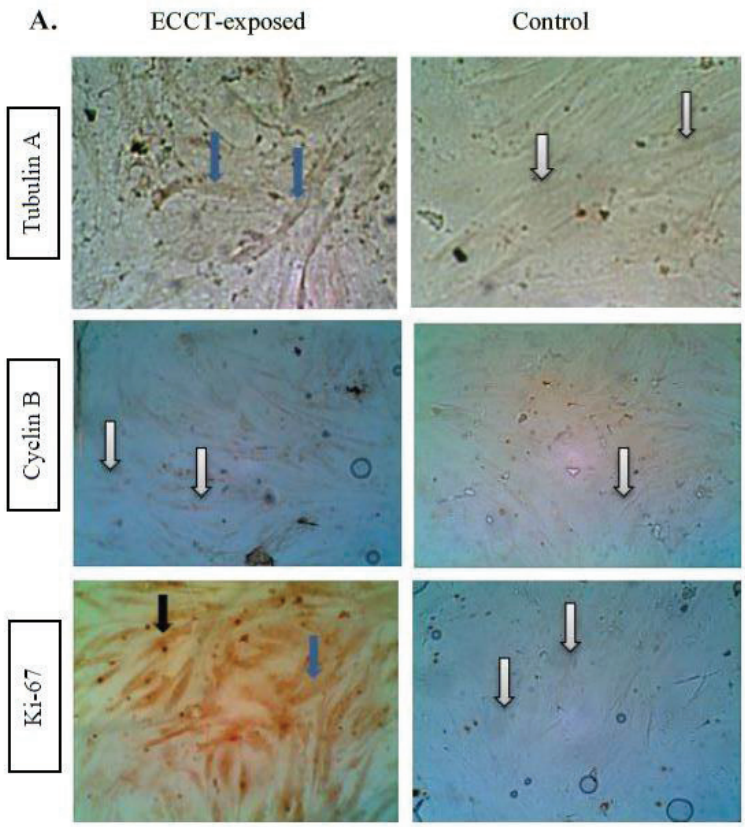
		Groups (mean ± SD)			
Cell line	**Variables**	**ECCT**	**P**	**Control**	**p**
SCC	Tubulin A	6.75 ± 1.04	0.000	2.00 ± 0.93	0.010
	Cyclin B1	3.13 ± 0.35	0.000	1.38 ± 0.52	0.000
	KI67	4.88 ± 0.83	0.067*	1.25 ± 0.46	0.000
Hela	Tubulin A	10.25 ± 1.67	0.067*	2.63 ± 0,74	0.027
	Cyclin B1	11.50 ± 0.93	0.000	1.88 ± 0,99	0.015
	KI67	11.75 ± 0.71	0.000	5.06 ± 0,78	0.000
Mesenchymal	Tubulin A	2.38 ± 0.74	0.000	2.25 ± 0.71	0.000
	Cyclin B1	2.50 ± 0.53	0.001	0.63 ± 0.52	0.000
	KI67	2.38 ± 0.52	0.000	1.19 ± 0.26	0.000

Table 2. Results of pathway analysis of causality relationships between variables

Independent Variable	Dependent Variable	Beta	p
ECCT exposure	Tubulin A	0,640	0,000
Tubulin A	Cyclin B	0,874	0,000
Cyclin B	Ki-67	0,900	0,000

Table 3. Semi-quantitative immunoreactive score (IRS) is the result of multiplication between percentage of positive cells (A) and color intensity (B)

A	B
Score 0: No positive cell	Score 0: No color observed
Score 1: Positive cells <10%	Score 1: Low color intensity
Score 2: Positive cells 11%-50%	Score 2: Intermediate color intensity
Score 3: Positive cells 51%-80%	Score 3: High color intensity
Score 4: Positive cells 81%-100%	



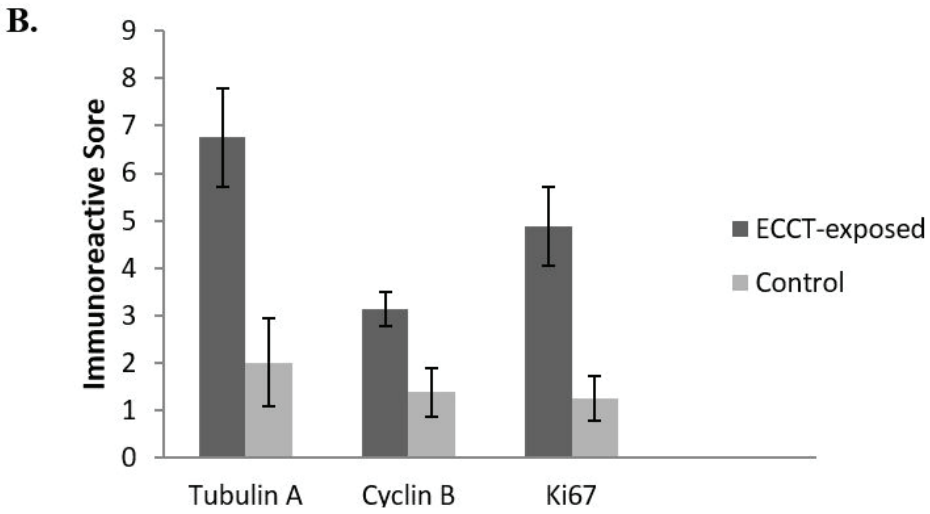
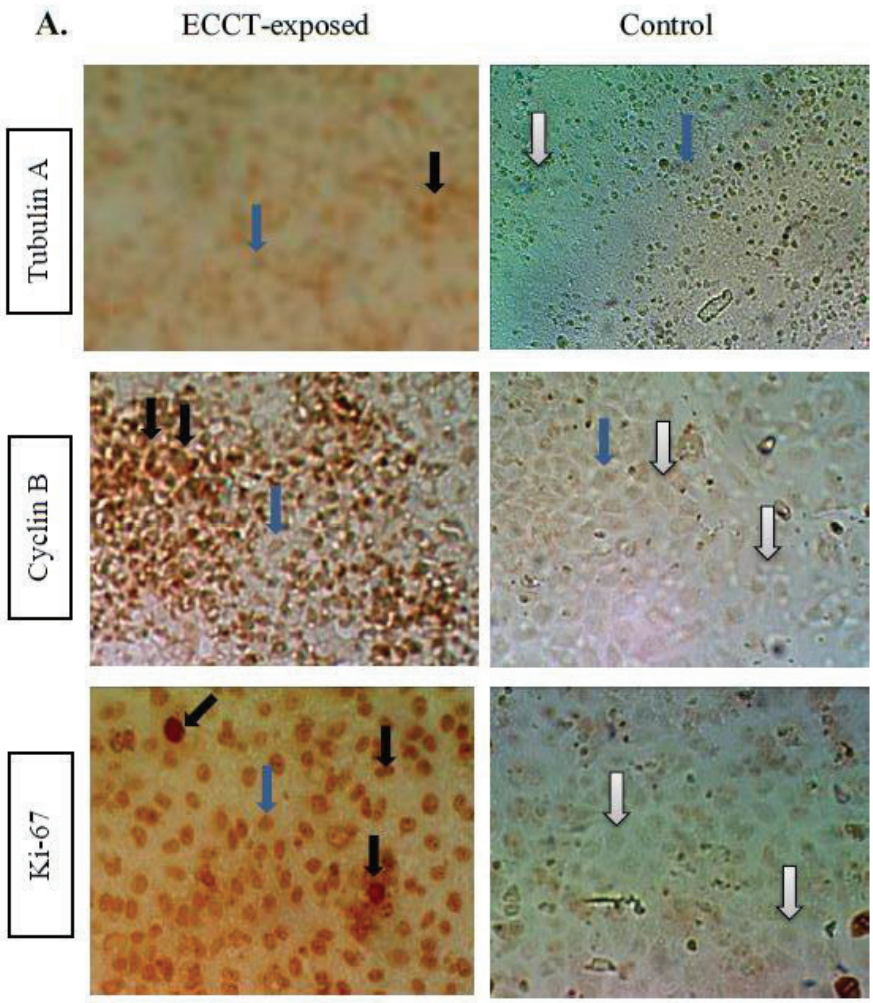


Figure 1: Tubulin A, Cyclin B, and Ki-67 expression on ECCT-exposed group and control group in squamous cell carcinoma (SCC) culture. Strong expression=black arrow. Moderate expression=blue arrow. Weak expression=white arrow (A). Immunoreactive Score of Tubulin A, Cyclin B, and Ki-67 expressions on SCC culture in mean ± SD (B).



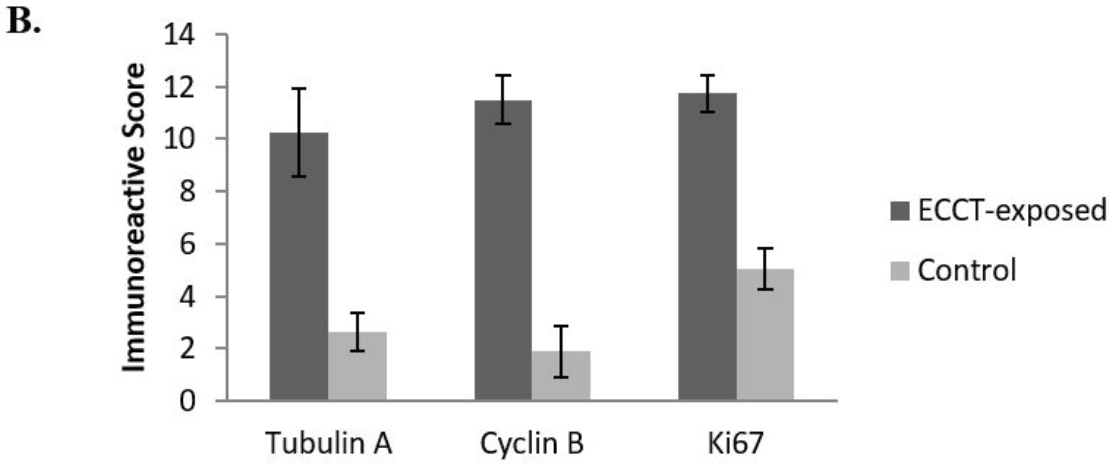
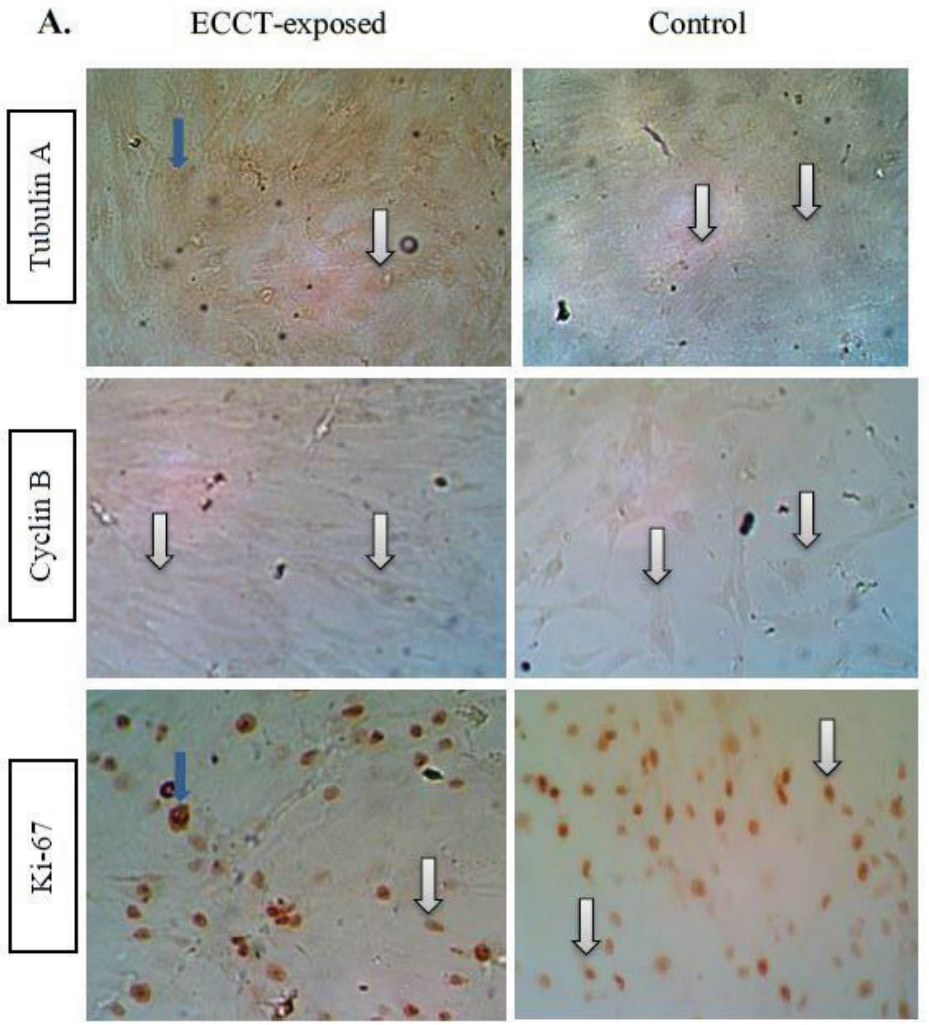


Figure 2: Tubulin A, Cyclin B, and Ki-67 expression on ECCT-exposed group and control group in Hela cell culture. Strong expression=black arrow. Moderate expression=blue arrow. Weak expression=white arrow (A). Immunoreactive Score of Tubulin A, Cyclin B, and Ki-67 expressions on Hela cell culture in mean ± SD (B).



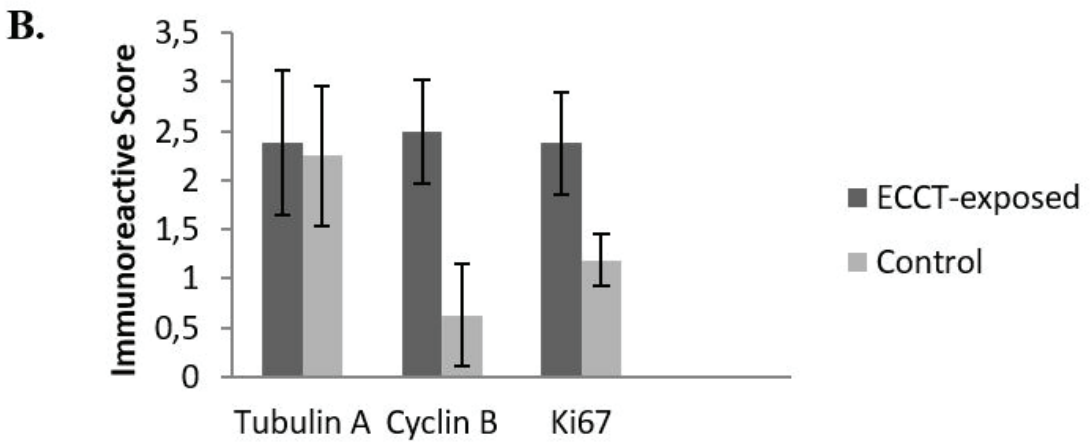


Figure 3: Tubulin A, Cyclin B, and Ki-67 expression on ECCT-exposed group and control group in mesenchymal cell culture. Strong expression=black arrow. Moderate expression=blue arrow. Weak expression=white arrow (A). Immunoreactive Score of Tubulin A, Cyclin B, and Ki-67 expressions on mesenchymal cell culture in mean ± SD (B).

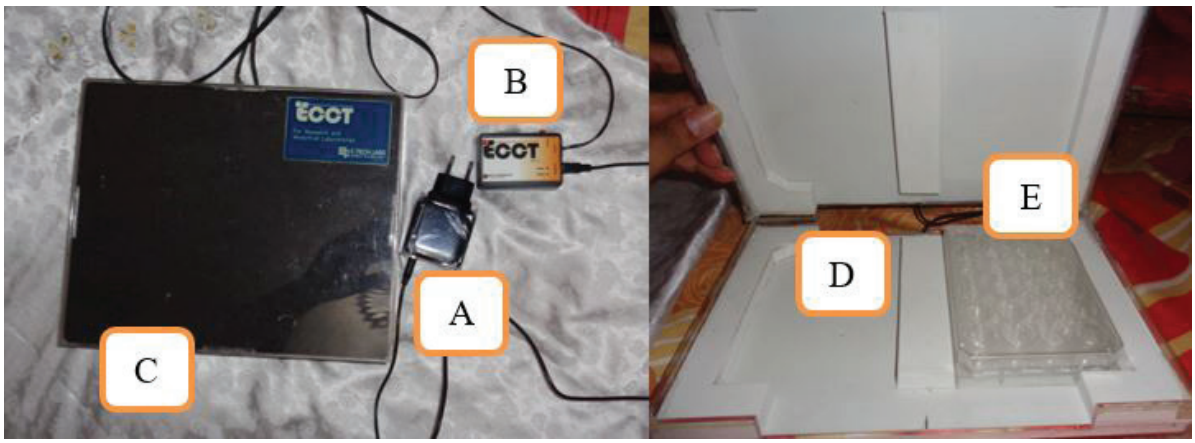


Figure 4: ECCT circuit for research. Adaptor (A). ECCT power supply (B). ECCT box (C). Inside view of ECCT box (D). 24-well microplate (E).

Discussion

Tubulin A Expressions

Alpha Tubulin and Beta Tubulin are a pair of proteins that bind to form a heterodimer protein. To construct a microtubule polymer, the dimer (identical pair of molecules) of this protein is lined up by 13 pairs to make a tube form.[8] Tubulin A and Tubulin B are electrically charged polar proteins, so the microtubules they form are also electrically charged.[9,10] Butters et al.[11] have published a study who reported that the electric energy field low intensity and intermediate frequency can induce changes in the interaction of tubulin subunits by affecting the fluid blanket around the tubulin pole, changing the density of the liquid around the pole, until the polymerization-depolymerization activity is disrupted.

In this study, increased expression of Tubulin A protein may show the inhibition of microtubule polymerization by ECCT. In the cancerous cell line group (SCC and Hela cells), the mean expressions of Tubulin A in the ECCT-exposed were higher than their expression in the control group there was a statistically significant difference ($p<0.05$). Meanwhile, in the mesenchymal cell group, the mean expression of Tubulin A in the ECCT group was higher than the control group but there is not statistically significant difference ($p>0.05$).

In viable cells, the activity of microtubule polymerization and depolymerization occurs most actively and strongly during mitosis, especially in metaphase and anaphase. It is therefore understandable that Hela cells entering the mitotic cycle every 23 hours had the highest expression of Tubulin A.[12] However, in mesenchymal cells, the difference of tubulin A expression was not significant. This is related to their long mitosis cycle which exceeds 2x24 hours. Therefore ECCT treatment which is only 24 hours had no significant effect on the increase of tubulin A expression.

The results of this study are supported by the results obtained by Kirson et al.[4] who examined the effect of low-voltage electric fields with a frequency of 100 kHz in melanoma cell culture, which proves that electric fields cause damage to the normal process of microtubule polymerization-depolymerization during mitosis.

Cyclin B1 expression

The Cdk1-Cyclin B complex present in the G2 phase acts as an ON switch to begin the process of mitosis. Phosphorylation of the lamina/nuclear membrane by the CDK1-Cyclin B complex causes degradation of the nuclear membrane.[13,14] After the nuclear membrane disappears, the process of mitosis begins.[15] Firstly, microtubules elongate and attach to the kinetochores of the chromosomes. Afterward, before entering anaphase, *anaphase promoting complex*/*cyclosome* (APC/C) degrades Cyclin B so that the CDK1/Cyclin B complex becomes inactive and the nuclear membrane will re-form. The re-formation of the nuclear membrane marks the end of mitosis resulted with 2 daughter cells.[16,17]

In a condition where the microtubule polymerization is disrupted, microtubules will not be able to bond with kinetochore. It will keep the complex checkpoint spindle to maintain the APC/C in inactive state. Inactive APC/C can not degrade the Cyclin B. This whole process will cause the mitosis process to stop for a while (mitotic arrest).

In this study, we measured the expression of Cyclin B and we found that in the SCC, Hela cells, and mesenchymal cell culture, the mean expression of Cyclin B in the ECCT-exposed group was higher than the control group with a statistically significant difference ($p<0.05$). There were also significant differences of increased Cylin B expressions between the three groups of cell cultures. These results are related to the cell cycle of the three types of cells studied.

Increased Cyclin B may occurs due to the braking mechanism of cells undergoing mitosis. However, this condition also does not last long, because Cyclin B will also be damaged naturally, which ultimately forces the cell to end its mitosis.[18] Thus it can be understood that the cell with the shortest mitosis cycle or doubling time like Hela cells,[12] will have the highest increase in Cyclin B expression.

Expression of Ki-67

When mitosis occurs, chromosomes observed under a microscope using the whole-mount method will appear to be covered by a layer of protein and RNA, consisting of solid fibrillar proteins and granular proteins. These perichromosomal layers are pre-rRNA, U3 snoRNAs, and more than 20 ribosomal proteins, such as nucleolin and Nopp140, NPM/B23, Bop1, Nop52, PM-Scl 100, and Ki-67.[19] These perichromosomal layers are 1.4% of the chromosome proteome, and their function has not been studied and known yet.[20] Ki-67 is a very large protein (nearly 360 kDa), which exists and involves in eukaryotic cell proliferation. Ki-67 seems to be expressed in the active phase of the cell cycle (G1, S, G2, and M), but won't be expressed when the cell is inactive (G0). Ki-67 has been widely used and accepted as an indicator of cell proliferation in various human tissues, including various types of cancer. Ki-67 has also been routinely used to assess tumor cell proliferation, as well as to measure its aggressiveness and response to therapy.[21]

Even though an increased expression of Ki-67 has always been associated with high rates of cell proliferation, poor prognosis, and good chemotherapy or radiotherapy response,[22,23] in this study Ki-67 expression was increased significantly in the SCC and mesenchymal cell groups exposed to electric field from ECCT compare to the control group ($p<0.05$), even overexpressed in the Hela cell group. Several possibilities could be the cause of increased Ki-67 expression.

First, the ECCT electric field, in addition to triggering cell death, can also stimulate cell proliferation. To prove the possibility of cell proliferation stimulated

by ECCT, a study about the effect of ECCT exposure for 72 hours or more should be conducted in the future. If the increase in the expression of Ki-67 is indeed associated with an increase in proliferation, then we can conclude that the addition of ECCT exposure time will increase the number of cell populations. Second, the increased expression of Ki-67 occurs because of the large number of cells undergoing mitotic arrest. Manoir et al have examined the level of Ki-67 expression in each phase of the cell cycle, and found that Ki-67 expression decreased after mitosis, was stably low in G1 phase, and disappeared when the cell was in the G0 phase. Ki-67 reaches its maximum level when the cell is in prophase-metaphase where it was concentrated around the chromosome.[24] This theory explains the increased Ki-67 expression obtained from this study. We could say that the cells exposed to ECCT stopped in the metaphase stage, the phase where the expression of Ki-67 reaches its peak level. Therefore, high levels of Ki67 in ECCT-exposed cancer cells strengthen the evidence of mitotic arrest happening in cells.

Pathway analysis

Pathway analysis is an applied form of multi regression analysis, which is used to conceptualize the problems or test a complicated hypothesis. In addition, it is used to determine the direct and indirect relationship of the independent variables to the dependent variable.[27,28]

Pathway analysis in this study proved the strong influence of ECCT exposure to cause an increase in tubulin A. Increased expression of tubulin A, which illustrates a disturbance in the polymerization of microtubules will cause disruption in the attachment of microtubules to the kinetochore.[29,30] This disruption will make the cells activate the braking mechanism of the mitosis process through the formation of the mitotic checkpoint complex which will keep CDC20-Anaphase Promoting Complex/Cyclosome (CDC20-APC/C) inactive and not degrade cyclin B, until the mitosis process is temporarily stopped.[16] The result of this process was an increase in cyclin B, whose relationship with tubulin A had been confirmed using pathway analysis. Increased cyclin B in cells undergoing mitosis is a condition in which the mitosis process in cells is inhibited, especially in metaphase entering anaphase, termed mitotic arrest.[15] Metaphase is a cell state without nuclear membrane, where DNA is condensed in the form of chromosomes that have been duplicated. When the mitosis is stopped and the cell is in metaphase, the concentration of Ki-67 as perichromosomal protein is at its highest. Pathway analysis confirmed this. Ki-67 hyperexpression represents the number of cells undergoing mitosis, in this study also the number of cells undergoing mitotic arrest. Cells that experience mitotic arrest can die through the mitotic catastrophe.[26]

Conclusion

It could be concluded that increased Tubulins A, Cyclin B1, and Ki-67 expression may associate with the death cell mechanism induced by exposure to Noncontact Electro Capacitive Cancer devise through disruption to microtubule polymerization and mitotic arrest or incomplete mitosis.

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Source of Funding: Self-funding.

Conflict of Interest: Nil.

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Experimental Study of Collagen Density in Reconstruction of Tracheal Defect Using Dried Amniotic Membrane

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Abstract

Background: Current tracheal defect management is still controversial for surgeons because there are no ideal materials to substitute trachea and many long-term complications occur in patients after tracheal reconstruction. Prosthetic graft has the risks of chronic infection and post-reconstructive tracheal stenosis. Dried Amniotic Membrane (DAM) expresses several endogenous matrix metalloproteinase (MMP) genes that can degrade extracellular matrix. Therefore, DAM is expected to be able to degrade collagen formed excessively during the wound healing phase, hence post-reconstruction stenosis will not occur. This study aimed to evaluate the differences of collagen density between polypropylene mesh (only) and DAM-added polypropylene mesh as graft in tracheal reconstruction.

Methods: The animals used in this study were 36 male white rabbits, which were divided into two groups. Both groups were wounded in the trachea area. The defects from the first group were closed using polypropylene mesh, while the defects from the second group were closed using DAM-added polypropylene mesh. After 30 days, the collagen density of each group was assessed.

Results: In the first group there are 10 samples (55.56%) with collagen density score = 3, while in the second group there are 16 samples (88.89%) with collagen density score = 2. The significance value using Mann-Whitney U test for differences in the collagen density score in both groups is $p = 0.407$.

Conclusion: Tracheal reconstruction using polypropylene mesh along with DAM provides a lower collagen density score than using polypropylene mesh alone as a graft, but it is not statistically significant.

Keywords: *Collagen density; Dried Amniotic Membrane; Tracheal reconstruction*

Introduction

Tracheal reconstruction due to several pathological abnormalities, such as trauma, neoplasia, and other abnormalities is still a problem and challenge for surgeons. In cases of defects that require a small size closure, primary anastomosis can be done. While in the case of larger defects (more than 50% area around the trachea), the surgeons often need to perform tracheal reconstruction.[1] Some techniques in tracheal reconstruction are the use of stents, prosthesis or implants, autograft, allograft, and tissue engineering.[2] A variety of prosthetic grafts and tissues have been used to close the tracheal defect, but the success rate was still low. Ischemic grafts, immunorejection of tissue grafts, and stenosis in prosthetic grafts are the main reasons for the failure.[3,4]

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Dried Amnion Membrane (DAM) has long been considered a potential source of scaffold material. DAM has been proven to have many uses in the treatment of burns, oral cavity, bladder and vaginal reconstruction, tympanoplasty, arthroplasty, and many more.[5] DAM also has the advantage of reducing the formation of scar tissue and inflammation, accelerating the healing time of wounds, and functioning as a scaffold for cell proliferation and differentiatio.[6] Specifically, the human amniotic membrane expresses several endogenous matrix metalloproteinase (MMP) genes that function in the degradation of extracellular matrix.[7] This gave rise to the idea that human DAM has unique characteristics that make it suitable when added to polypropylene mesh prosthetic graft. The addition of DAM in the prosthetic graft is expected to reduce collagen density to decrease the risk of post-reconstruction stenosis.

Based on the above thought, this study aimed to compare collagen density in tracheal defects treated with DAM-added polypropylene mesh and tracheal defect treated with only polypropylene mesh.

Methods

Research Design

This research was an experimental laboratory study, using a randomized control trial design conducted in Animal Laboratory, Department of Biochemistry, Faculty of Medicine, Universitas Airlangga. Thirty-six New Zealand male white rabbits aged 6 to 9 weeks with bodyweight between 1900-2500 grams were obtained and the protocol was approved by Animal Care and Use Committee from Faculty of Veterinary, Universitas Airlangga, Indonesia (*certificate number: 2.KE.072.04.2018*). Rabbits were acclimatized for 7 days, then divided into control and treatment groups and permuted block randomization was applied. In the control group, the tracheal wounds of the rabbits were covered with polypropylene mesh, while in the treatment group, the wounds were covered with polypropylene mesh added with dried amniotic membrane (DAM). After 30 days, collagen density was examined in the area of the surgical wound. This animal experiment had complied with the ARRIVE guidelines.[8]

Manufacture of Dried Amniotic Membrane (DAM)

Fresh amniotic membrane was taken from the mother who had elective cesarean delivery by using aseptic instruments and techniques. The amniotic membrane was washed with saline containing 50 µg/ml streptomycin, 50 µg/ml penicillin, and the inside part was bluntly separated from the chorion. After that, the amniotic membrane was immersed in the transport media and stored at 4^0C before being extracted within the next 24 hours. Then the extracted amniotic membrane was sent to the laboratory in a sterile tube with dry ice.

The amniotic membrane was dried on drying paper, then weighed and measured in wet weight and volume. After that, it was moved into a deep freezer for 24-48 hours then it was freeze-dried for 7-8 hours until the drying is optimal. In the end, the amniotic membrane was packed through laminar airflow and chemically sterilized using gamma light radiation (25kGy). The DAM used in this study is the freeze-dried amniotic membrane that was available in tissue bank of Dr. Soetomo Surabaya Hospital, Indonesia. DAM with a thickness of 6 folds with a size of 0.6 x 0.6 cm2 was used, based on the previous studies.

Sample Preparation and Surgical Procedure

A total of 36 New Zealand male white rabbits aged 6 to 9 weeks with bodyweight between 1900-2500 grams were acclimatized for 7 days. Then the randomization was done by permuted block randomization into two groups, namely the treatment group of 18 rabbits and the control group of 18 rabbits. After being acclimatized for 7 days, the rabbits were given treatment under the influence of general anesthesia using intramuscular ketamine at a dose of 20-40 mg/kg body weight. The front neck's fur of each rabbit was shaved and disinfected using povidone iodine 10%. All rabbits from both groups (control and treatment groups) were wounded on the 8th ring of trachea an area of 0.6 x 0.6 cm2 using a scalpel number 15. In the control group, tracheal defect was closed with a 0.6 x 0.6 cm2 size polypropylene mesh with simple interrupted sutures using 6-0 nylon thread. In the treatment group, tracheal defect was closed with a 0.6 x 0.6 cm2 polypropylene mesh added with DAM. The area of DAM was of 0.6 x 0.6 cm2 and consisted of 6 layers, 3 layers above the polypropylene mesh and 3

layers under the polypropylene mesh. DAM was added and then sewn with simple interrupted stitches 6-0 nylon thread. (Figure 4.3). Daily antibiotics (penicillin 50,000 units/kg) intramuscularly were given after the surgery for five days.

Specimens were taken from the area of 1 cm cranio-caudal tracheal surgery involving healthy (0.5 cm) and pathological (0.5 cm) visible tissue, 0.5 cm medio-lateral width, covering the entire thickness of the trachea. Then the specimens were put in a 10% neutral buffered formalin solution and sent for paraffin blocks and histopathological examination.

Collagen Density Evaluation

Histopathology evaluation was performed using Masson's trichrome staining and collagen density was assessed with a binocular light microscope (Olympus CX 14) with 100x magnification for 1 field of view. Collagen density was assessed using scoring:

Score 0: There are no collagen fibers in the wound area

Score 1: The density of collagen fibers in the wound area is low

Score 2: The density of collagen fibers in the wound area is moderate

Score 3: The density of collagen fibers in the wound area is tight

Score 4: The density of collagen fibers in the wound area is very tight

Statistical Analysis

The data taken was in the form of ordinal data and the control group was compared with the treatment group using Mann-Whitney U nonparametric test.

Results

Collagen Density

Collagen density examination was evaluated on the 30th day after surgery. Collagen density was assessed based on the collagen density score. The highest score in the control group (polypropylene mesh) was a score of 3 with 10 samples (55.56%), followed by a score of 1 with 4 samples (22.22%), a score of 2 as many as 2 samples (11.11%), and a score of 0 as much as 2 samples (11.11%). While the highest score in the treatment group (DAM-added polypropylene mesh) was a score of 2 with 16 samples (88.89%), followed by a score of 3 with 2 samples (11.11%) (Table 1).

The lowest collagen density score (score 0) with Masson's trichrome staining at 100x magnification is shown in Figure 2, score 1 is shown in Figure 3, score 2 is shown in Figure 4, and score 3 is shown in Figure 5.

In this study, the collagen density difference test described by the collagen density score was carried out using the Mann-Whitney U test because the type of collagen density score data is ordinal data. The result of the Mann-Whitney U test was $p > 0.05$ (0.407), indicating that the difference of collagen density in the control group (polypropylene mesh only) compared to the treatment group (DAM-added polypropylene mesh) was not statistically significant. Based on these results it was concluded that the DAM-added polypropylene mesh group had a lower collagen density than the polypropylene mesh group, but it was not significantly different.

In this study, the characteristics of the research sample could influence the results of the study. Therefore, the study sample was made as homogeneous as possible to avoid bias. The experimental animals were selected from the same place (Pusat Veterinaria Farma) and given the same food. The statistical tests T-test on body weight and the Mann-Whitney U test on the age of rabbits gave the results that the weight and age of the rabbits, although varied but still homogeneous, so there was no bias from differences in body weight and age of the rabbits.

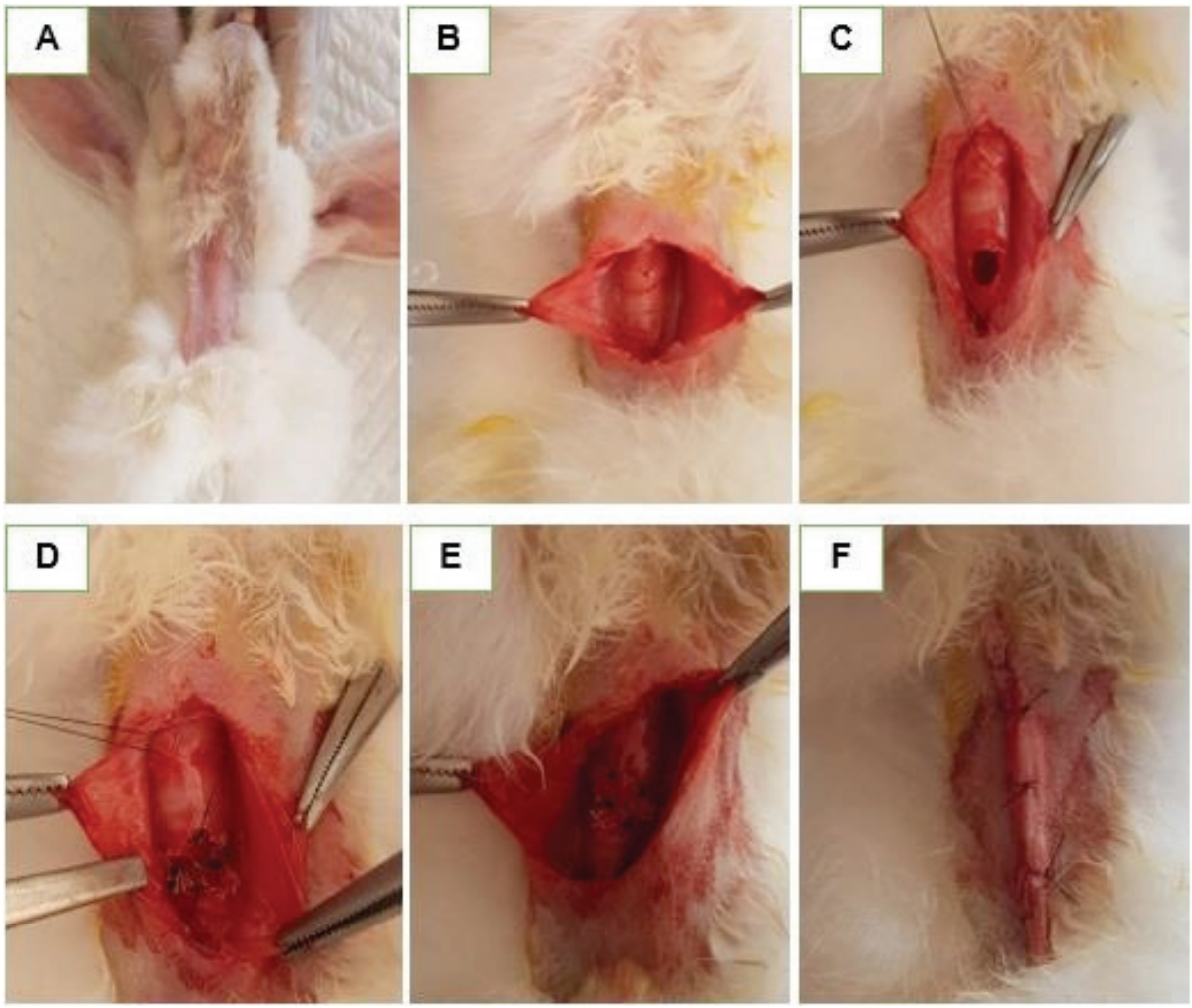

Figure 1. Rabbit's treatment procedure. (A) The rabbit's neck area was shaved clean. (B) Tissue dissection was performed until the trachea appeared. (C) The tracheal defect was made of size 0.6 x 0.6 cm2 by taking the entire thickness of the trachea. (D) Polypropylene mesh and DAM sized 0.6 x 0.6 cm2 that had been put together were added and sutured to cover the tracheal defect with 4 stitches of *simple interrupted* sutures using 6-0 nylon thread (treatment group). (E) Polypropylene mesh was sutured to cover the tracheal defect with 4 stitches of *simple interrupted* suture using 6-0 nylon thread (control group). (F) The skin was sutured with *simple interrupted* sutures using 3-0 nylon thread.

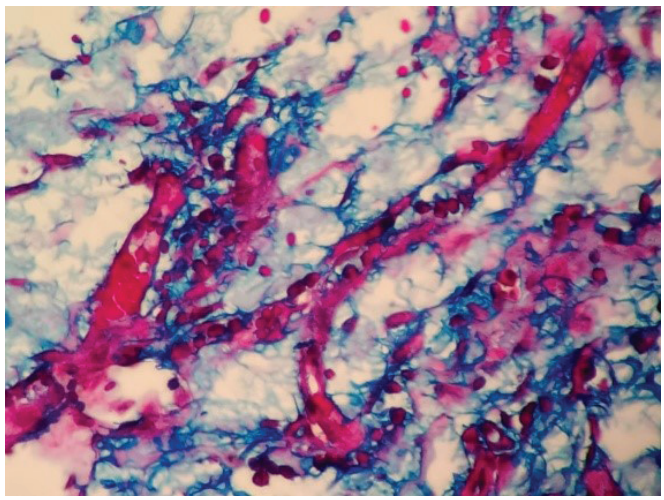

Figure 2. Score 0 of collagen density. Masson's trichrome staining (100x magnification).

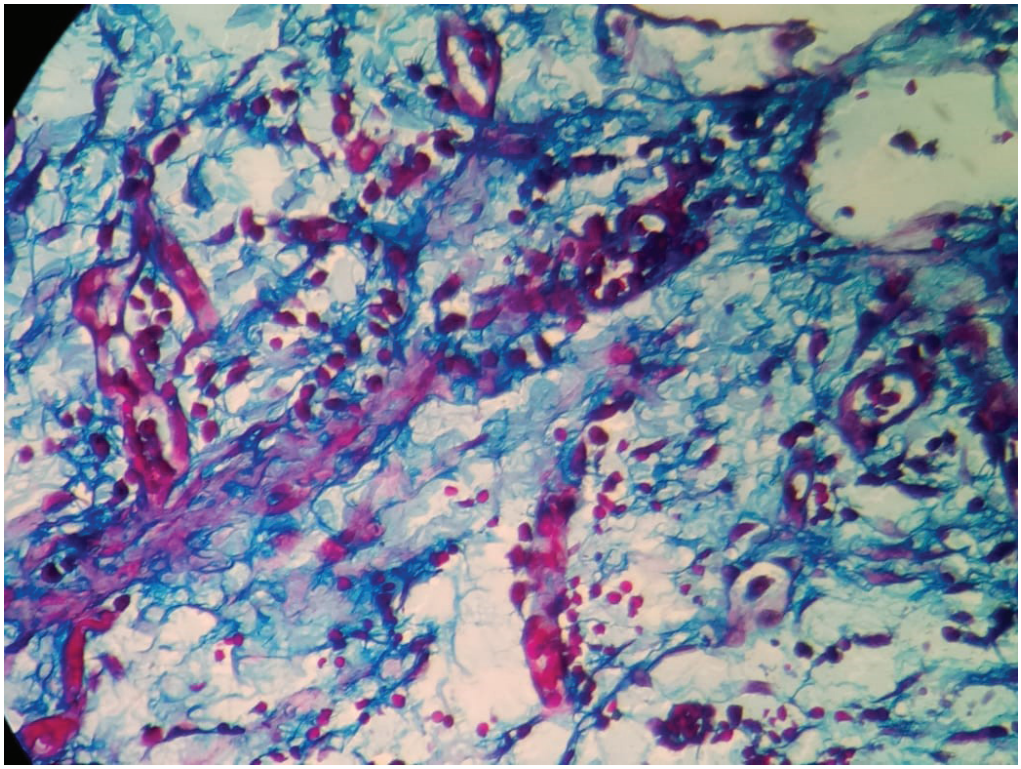

Figure 3. Score 1 of collagen density. Masson's trichrome staining (100x magnification).

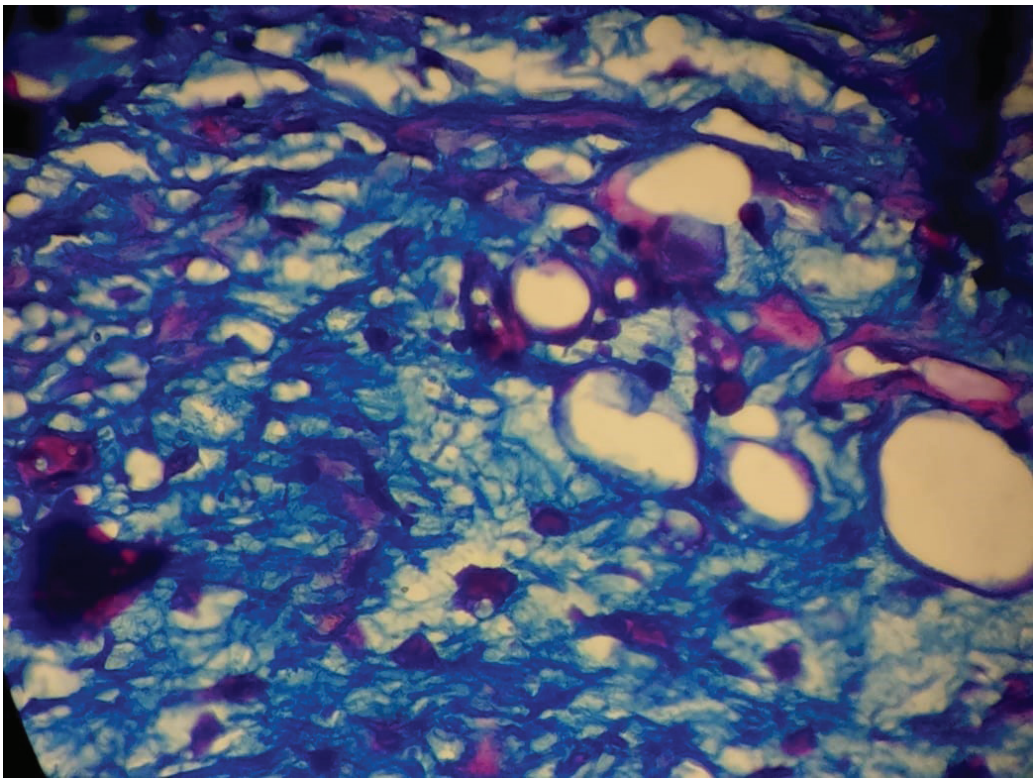

Figure 4. Score 2 of collagen density. Masson's trichrome staining (100x magnification).

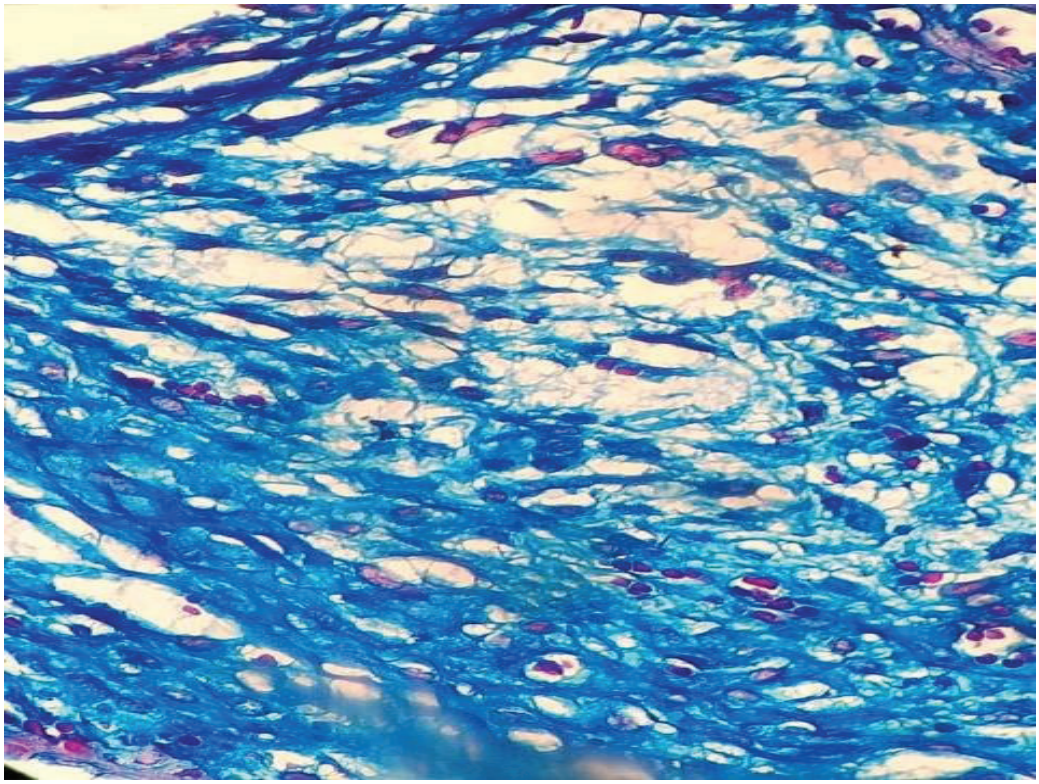

Figure 5. Score 3 of collagen density. Masson's trichrome staining (100x magnification).

Table 1. Collagen density score

	Score 0	Score 1	Score 2	Score 3	*Mann-Whitney U Test*
Control	2 (11.11%)	4 (22.22%)	2 (11.11%)	10 (55.56%)	p = 0,407
Treatment	0 (0%)	0 (0%)	16 (88.89%)	2 (11.11%)	

Discussion

Tracheal defect reconstruction is considered to be optimally successful when it fulfills 3 components: the tracheal lumen must be kept open properly (not stenosis), the inside of the trachea is protected by the epithelium, and the trachea has good vascularity.[9] Reconstruction of tracheal defects using polypropylene mesh was carried out by Furneaux in 1973 on a 7-year old Dachshund dog. Based on the results of this study, complications were obtained in the form of cough 2 weeks after surgery.[10] In another publication, Haykal et al. reviewed the results of several studies and concluded that the closure of tracheal defects using prosthetic grafts in the form of polypropylene mesh and tissue grafts still showed post-reconstruction stenosis complication and therefore is not the ideal material for tracheal defect reconstruction.[2]

According to Fernandes et al, DAM was first used as a skin transplant in 1910. In its development, DAM was found to have many uses, such as treating burns, reconstruction of the oral cavity, bladder, and vagina, tympanoplasty, arthroplasty, and many more.[11] According to Diaz-Prado et al, DAM has advantages including its ability to reduce scar tissue formation and inflammation, its ability to accelerate healing time, and its function as a scaffold for cells' proliferation and differentiation.[6] Moreover, according to Niknejad et al, besides its anti-inflammatory effect, amniotic membrane has low or no immunogenicity. Amniotic membrane is also able to stimulate wound healing and epithelialization. Its anti-inflammatory effect works by suppressing pro-

inflammatory cytokines IL-1α and IL-β. It also produces a number of natural matrix metalloproteinases (MMP) for extracellular matrix degradation.[5]

Wound healing in the trachea follows the general wound healing phases, starting from the hemostasis phase to the maturation phase. Collagen synthesis begins on the 3rd day after injury and progresses rapidly around the 2nd to 4th week. Collagen synthesis is controlled by collagenase and other factors that destroy collagen.[12] Meanwhile, according to Velnar, collagen remodeling during the maturation phase depends on the progress of collagen synthesis and the presence of collagen degradation. Collagenases and metalloproteinases in the wound remove excess collagen while the newly synthesized collagen remains. During remodeling, collagen becomes better organized.[13]

In this study, collagen density evaluation was carried out on the 30th day after treatment, when sufficient collagen was expected to be formed and collagen remodeling was expected to occur in the maturation phase. The collagen density was evaluated histopathologically using Masson's trichrome staining.

According to Suh et al., in their study, a prosthetic graft on the trachea in the form of polypropylene mesh stimulates the formation of granulation tissue and fibrosis, causing post-reconstruction tracheal stenosis.[4] Meanwhile, Fortunato and Menon showed that amniotic membrane expresses several MMPs which are proven to cause premature rupture of membranes in obstetric cases. Amniotic membrane expresses MMP-1 and-13 (collagenases),[7] therefore it is expected that the addition of DAM to polypropylene mesh will reduce collagen density in reconstructed tracheal defects.

In this study, the collagen density score in the DAM-added polypropylene mesh group was found lower than the collagen density score in the polypropylene mesh (only) group. However, in the statistical test using the Mann-Whitney U test, the difference between the two groups was not statistically significant. So it was concluded that tracheal reconstruction with DAM-added polypropylene mesh had lower collagen density than reconstruction with polypropylene mesh alone, but it was not significantly different. This may occur due to the time factor for evaluating the collagen density. In this study, an evaluation of collagen density was carried out on the 30th day after reconstruction, whereas according to Orsted et al, the wound remodeling phase occurred on days 21 to 2 years after reconstruction. Collagen degradation by collagenase will be controlled by TIMP (Tissue Inhibitor of Metalloproteases) until it reaches an equilibrium point.[14]

In humans, the incidence of post-tracheal reconstruction stenosis occurs from 6 months to 3 years after tracheal reconstruction.[15] Therefore, further collagen degradation can still occur after 30 days after reconstruction. The reduction in collagen density could be statistically more significant at evaluation after 30 days post-reconstruction.

Ethical Clearance: Taken drom Animal Care and Use Committee from Faculty of Veterinary, Universitas Airlangga, Indonesia.

Source of Funding: Self-funding.

Conflict of Interest: Nil.

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Outcomes of Preoperative Embolization of Juvenile Nasopharyngeal Angiofibroma

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Abstract

Background: These are rare fibro-vascular neoplasms, out of all head and neck tumors, JNA contributing for only less than 0.5%. JNA incidence rate almost 1/150,000 and affects men and adolescents' males

Objectives: To study outcome and the role of Pre-operative Embolization of surgeries for Juvenile Nasopharyngeal Angiofibroma.

Methods: A retrospective cross sectional study conducted at Al-Sader Medical City during the period 2010 through 2020 included 15 male patients who were managed for JNA, in whom embolization was performed preoperatively.

Results: The study included 15 male patients with an average age of 16.4 ± 3.1 (standard deviation) (range: 11-22) years. The duration of the procedure is 126 minutes, on average and ranged 75 to 180 minutes; Average intra-operative blood loss was 300 (range: 150-750) ml. The postoperative hospitalization period was 2 days in 14 cases and a recurrence rate of 13.3% was reported.

Conclusions: The use of preoperative embolization in JNA patients has been very beneficial in shortening surgery time, improve bloodless of the operative site, with a significant reduction in intraoperative blood loss, reduction in postoperative hospitalization time, and reduction in recurrent rate.

Keywords: *Angiofibroma, Juvenile, Neoplasms, benign, embolization*

Introduction

Juvenile Nasopharyngeal Angiofibroma (JNA)

These are infrequent fibrovascular neoplasms, out of all head and neck tumors, JNA contributing for only less than 0.5%. Incidence rate of JNA is almost, 1/150,000 and affects men and adolescents' boys. It has embryological origin where it develops from chondrocartilage as during development. (1)

The original site is the superior edge of the sphenopalatine foramen, which is formed by the connection of the palatine bone, horizontal ala of the vomer, and the root of pterygoid process. These vascular channels lack the smooth muscle that surrounds normal blood vessels, and this is due to the ability to bleed profusely during surgical procedures. JNA are covered with mucous membrane along the aspects of the nasal cavity and nasopharynx. (2)

Pathology

Macroscopically:

Macroscopically, the tumor looks like a dark red or brown, smooth, sometimes lobular mass, devoid of a capsule. Its structure is dense, nodular; the tumor is located on a fixed base. (3)

Microscopically:

Microscopically, juvenile Angiofibroma appears as a vascular component embedded in a dense stroma. Vascularity, are various in sizes and shapes, from small, round or semicircular vessels to larger, branched or irregular vessels. Endothelial cells are mostly flat, but plump endothelial cells can also be observed. The walls of the vessels range from a thin layer around the vessel to a thick layer of smooth muscle. In the peripheral areas, there is an increased proliferation of micro vessels. The stroma ranges from areas of cells high in collagen to areas of low in cells with fibro-myxoid background. Stromal cells differ from spindle-shaped, angular to plumply stellated fibroblast, with central round vesicular nuclei and small nuclei. (4)

Behavior of JNA

As Being in its nature a morphologically benign tumor, Angiofibroma tissue, without having infiltrative growth, displaces and moves apart adjacent structures, and also destroys cartilage and bones due to the pressure exerted. Thus, along the path of least resistance, this neoplasm spreads forward and medially - into the nasopharynx, and often simultaneously backward and laterally - into the pterygo- palatine fossa. With the anteromedial type of growth from the nasopharynx, the tumor enters the nasal cavity through the choana, there it exerts pressure on the surrounding walls, deflecting the nasal septum in the opposite direction and causing a complete cessation of nasal breathing. At the same time, the tumor fills the basilar sinus, penetrating into the ethmoid labyrinth, and with prolonged existence - also grows into the area of the sella turcica. With the posterolateral type of growth, the tumor, growing into the pterygo-palatine fossa, exerts pressure on the surrounding bone structures, pressing anteriorly the posterior wall of the maxillary sinus. It also deforms and displaces the pterygoid plates. The tumor from the pterygo-palatine fossa can spread laterally, through the pterygo-palatine fissure into the infratemporal and temporal fossa. Later, the tumor penetrates through the lower orbital fissure into the orbit, and then through the lower edge of the upper orbital fissure into the cranial cavity. (5)

Clinical features of JNA

It can be assumed that the disease may be asymptomatic for some time. On average, it takes about 6 months to one year between the time the symptoms first appear and the diagnosis

Unilateral nasal obstruction is the most frequent reported symptom, account for 91% , followed by 63% for epistaxis, in addition to detectable tumor mass in the nasal cavity , these three findings are considered the classic triad of symptoms, other associated manifestations include , pain, nasal discharge, sinusitis, ear symptoms such as hearing disorders and otitis media , facial deformity, and ocular symptoms (diplopia/ proptosis). (6)

Nasal Endoscopy: :

with rhinoscopy in the nasal cavity, a tumor like formation of red color, round or oval, with a smooth surface, bleeding easily upon contact. Usually shows a flat vascular lobular mass posterior to the tail of the middle turbinate, blocking the choana or completely filling the nasal fossa. (7)

Radiological characteristics:

Biopsy should be avoided due to the risk of abrupt bleeding on contact. Imaging plays an important role in diagnosis and staging due to the nature of vascularity, although these masses are believed to have originated from the sphenopalatine foramen, they are often large on diagnosis, often in the nasopharynx, then in the pterygoid fossa and, over time, in the orbit, sinuses, intracranial and subcranial fossa. (8)

Angiography (DSA)

Although angiography is often unnecessary, it helps to identify the feeding vessels and embolize them before surgery. These tumors usually have blood supply from:

- External carotid artery: multiple internal maxillary arteries, ascending pharyngeal artery, and palatine artery

- Internal carotid artery: rarely, often large tumors, the ocular artery in the branches of the sphenoid bone.

It should be noted that the expansion of the feeding vessels is rare. [8]

Pain films

Conventional films are no longer used for the initial screening of suspected pharyngeal lesions. The characteristic changes in the traditional films are well described in practice, findings include

1. Mass of soft tissues in the nasopharynx.

2. Anteriorly bowed maxillary posterior wall.

A sign of a slowly growing mass which called Holman-Miller sign shown in the lateral view of the skull. [9]

Computed tomography scan (CT-Scanning)

It is useful in determining the anatomy of the bone, especially when detecting bone fissures and foramina. It is also widely used for surgical planning and imaging guided-surgeruries.

Examination of patients using computed tomography in two projections (axial and coronary) makes it possible to judge the following data:

1. Tumor boundaries, and its size

2. Its relationship with surrounding tissues, especially bone

3. The degree of destruction of the base of the skull

4. Lobularity, density and the presence of cavities

With this examination in the projection of the nasal cavity, nasopharynx, paranasal sinuses, pterygoid and infratemporal fossa, and the maxillary space, a soft-tissue lobular formation with fairly clear contours, a relatively homogeneous structure is determined, with widespread growth causing displacement and bone destruction of the walls of the paranasal sinuses and nasal septum hard palate. JNA is located predominantly on one side of the skull base and is almost never strictly along the midline. With intracranial growth, the tumor component is determined, in the parasellar region with destruction of the bone structures of the skull base. [10]

Magnetic resonance imaging (MRI) is an alternative diagnostic method for JNA. Its advantages over CT are: no radiation exposure and the possibility of multiple examinations within a short period of time; examination is possible in a comfortable, gentle position for the patient; MRI allows you to well differentiate a recurrent tumor from cicatricial changes or concomitant sinusitis. Among the disadvantages of MRI, one should single out a longer study time, the impossibility of carrying it out in patients with magnetic or ferromagnetic prostheses, and less informativeness in relation to bone changes in the base of the skull compared to CT.

Morphological confirmation of the diagnosis of juvenile angiofibroma of the skull base was established earlier on the basis of tumor biopsy. But a biopsy of angiofibroma presents significant difficulties, since it inevitably leads to severe, difficult to stop bleeding. In this regard, at present, the indications for biopsy are significantly narrowed and remain as follows:

1. Ambiguity of the diagnosis after endoscopic and radiation diagnostic methods

2. Primary use of radiation therapy

3. In the long term after radiation therapy to exclude malignancy of the YUAO (rhabdomyosarcoma, esthesioneuroblastoma, etc.).

If it is necessary to conduct a biopsy of a lesion suspicious of SAD, it should be performed only in an otorhinolaryngological hospital, where there are all conditions for stopping bleeding, and, if necessary, complete surgical removal of the tumor. A piece of tissue during biopsy must be taken from the depth of the tumor, since its surface is usually devoid of the characteristic morphological signs of SAD. [9,10]

JNA blood supply:

The tumor is mainly supplied with branches of the external carotid artery (ECA) with access to the ICA in some patients. The main arterial blood supply comes from the IMA, which is branch of the ECA. Headsets are imported from many joins close to ECA and direct or indirect links of ICA. Supply is bilateral in almost 26.3–46.4%. Wu et al in recent meta-analysis found that 36% of JNA patients have dual supply. Interestingly, this study found that bilateral presentation is common in

well-defined tumors. [11]

Aim of Study

To study outcome and the role of Pre-operative Embolization of surgeries for Juvenile Nasopharyngeal Angiofibroma.

Patients and method:

A retrospective cross sectional clinical study conducted at Al-Sader Medical City during the period 2010 through 2020 included 15 male patients who were managed for JNA , in whom embolization was performed preoperatively

Patient sampling;

A total of 15 cases (all males) in age range 11-21 years with juvenile angiofibroma in variable stages.

Inclusion criteria:

Patient diagnosed with juvenile nasopharyngeal angiofibroma.

Exclusion criteria:

Recurrent cases.

Preoperative assessment and patient selection:

Before treatment, all patients were evaluated by full medical history and through clinical examination. Required investigations, imaging with CT scan and MRI were performed. Nasopharyngeal and nasal endoscopies were conducted for all patients.

Laboratory investigation:

In form of CBC, RBS, Virology screen ,RFT, blood group and Rh.

Blood preparation was done for all patient.

The diagnosis depend on clinical and radiological suspicion of JNA. According to Radkowski et al. staging system, tumors were staged .

Statistical Analysis:

Data of all patients were checked for any errors or inconsistency and then analyzed using the statistical package for social sciences (SPSS) version 24). Appropriate statistical tests and procedures were applied according the type of variables and requested outcome. Level of significance set at 0.05.

Results

There were 15 males enrolled in this study with a mean age of 16.4 ± 3.1 (SD) (range: 11 – 22) years, on the other hand, as it shown in (Fig. 1) more than half of the patients (8/15) aged between 15-17) years.

The distribution of the cases according to the clinical staging revealed that 6/15 (40%) had a staging of 2a, five patients with stage 1b, 3 with 2b stage and only one patient with 2c staging. Regarding the type of preoperative embolization, Polyvinyl alcohol PVA was the more frequently applied among the cases; 11/15, (73.3%), (Table 1).

Table 1. Distribution of cases according to the clinical staging and type of preoperative embolization

Staging	No. of patients	Percent
IIa	6	40.0
Ib	5	33.3
IIb	3	20.0
IIc	1	6.7
Total	15	100.0
Type		
Polyvinyl alcohol PVA	11	73.3
Embosphere (microsphere)	4	26.7
Total	15	100.0

Transpalatal approach was more frequently used than Endoscopic one, they were used in 10/15 (66.7%) and 5/15 (33.3%), of the cases, respectively, (Table 2)

Table 2. Types of clinical Approach

Approach	No. of patients	Percent
Transpalatal	10	66.7
Endoscopic	5	33.3
Total	15	100.0

The operative time ranged between 75 and 180 minutes with a mean (SD) of 126 (30) minutes (Table 3), furthermore, in 7 patients (46.7%) the operative time was 120 minutes , 3 patients had operative time 75-90 minutes and the remaining 5 patients need longer operative duration of 130-180 min, the amount of blood loss ranged 150-750 ml with an average of 300 ± 135 ml (Table 3). However, 7 patients had a blood loss of 300 ml, one patients with 350 ml and another one patient with 750 ml intra-operative blood loss while 5/15 patients had an intra-op blood loss of 150-250 ml

Table 3. Descriptive statistics of the operative time (minutes) and intraoperative blood loss

Measure	Mean±SD
Time (minutes) Range	126± 30 75± 180
Blood loss (ml) Range	300±135 150-750 ml

Furthermore, correlation analysis, cross-tabulation with Fisher's exact test were applied to assess the possible correlations between the studied parameters, using bivariate correlation analysis, Cross-tabulation with Fisher's exact test and curve estimation regression analysis, the correlation matrix is demonstrated in (Table 4). Among all possible statistical inter-correlation, only one significant direct (positive) correlation was reported between operative time and the amount of intraoperative blood loss, where the longer operative time was significantly associated with larger amount of blood loss, (R = 0.635, P = 0.011), (Table 4).

Table 4. Inter-correlation matrix between different studied parameters

		Age (year)	Type of approach	Staging	Operative time (min)	Intra-op blood loss (ml)	Recurrence
Type of approach	R*	0.190					
	P. value	0.499					
Staging	R	0.351	0.203				
	P. value	0.199	0.468				
Operative time (min)	R	0.302	0.213	0.189			
	P. value	0.274	0.446	0.501			

Cont... **Table 4. Inter-correlation matrix between different studied parameters**

Intra op blood loss (ml)	R	0.385	0.271	0.413	0.635		
	P. value	0.156	0.328	0.126	0.011		
Recurrence	R	0.118	0.277	0.239	0.127	0.000	
	P. value	0.675	0.317	0.390	0.652	1.000	
Hospitalization (day)	R	0.036	0.378	0.019	0.331	0.000	0.105
	P. value	0.899	0.165	0.946	0.228	1.000	0.710

As it shown in (Table 5), generally, longer operative time was associated with more advanced stage, patient with stage 2c had the longer operative time, compared to other patients, (P. value < 0.05)

Table 5. Comparison of mean operative time across staging

Staging	Number of patients	Operative time (min)	
		Mean	SD
2a (n=6)	6	135	25
1b (n=5)	5	99	20
2b (n=3)	3	133	15
2c (n=1)	1	180	0
Kruskal Wallis Test , H= 8.10, P. value 0.044, significant			

Discussion

Data analysis of our study revealed that mean patient's age was 16.4 ± 3.1 (range: 11 – 22) years, this finding is similar to the study done by *Martins MB et al.* mean patient age was 16 range from 10-29. [12]

In other study done by *Glad, H., et al.* found that patients were relatively younger with a median age of 15 years and range (of (10 – 24) years, due to wider range of ages and larger sample size of patients (51 cases were enrolled in his study). [13]

Blood Loss

The current study found that pre op. embolization was an effective method for bleeding control, as the mean amount of blood loss was 300 ± 135 ml, ranged between 150-750 ml. This result similar to *Subhash Kuma et, al* study in which the mean blood loss during surgery was 362 ml ± 176.2 ml (range 50 ml-1500 ml). [14] In *Martins, MB et al.* study they estimate that the mean blood loss was 300 mL which is comparable to our results. [12] A study carried by *Nicolai et al*, Intraoperative blood loss ranged from 80 to 600 mL (mean blood loss, 372 mL). This range are slightly lower because collected patients are operated with endoscopic approach and most of cases are early stage. [15] However, Mistry*, Rajesh C., et al.* The average blood loss was 828 ml (range 535-1570), which is higher than our study due to using different surgical approaches and selecting advanced stages. [16]

Operative Time

Regarding operative time: In our study the mean operative time was 126 minutes similar to the study done by *Martins, M B, et al* which was 120 minute. [12]

Another study by *Kasem, M, et al.* around 120 minutes. [16] A study done by *Gemmete, J. J., et al*. using

direct percutaneous embolization with onyx only. The result was slightly higher than our result due to use of Onyx only. Average total operative room time of 328.3 minutes (range, 39–503 minutes). [17]

Post Operative Hospitalization

Regarding the postoperative hospitalization: Most of our patient remain for two days this was comparable to *Roger, Gilles, et al where* most of his patients were able to be discharged within 48 hours after surgery. [18] And by *Ardehali M, et al. who found a mean hospital stay duration of 1.8 days for embolization patients.* [19]

However, result gained by *Peraza-McLiberty, Roberto A., et al.* Concerning hospitalization duration, postoperatively, a mean duration of 7.2 days was reported, with a minimum of 6 days minimum and maximum of 8; was longer than our result. He studied 49 patients, 21 had a conventional (Lefort I) approach and 28 had a combined approach with a conventional approach and an endoscopic procedure. [20] So, because of different surgical approaches and different stages, the hospital stay was longer.

Recurrence Rate

The recurrence rate was 13.3% among the studied group, where 2/15 patients had recurrence in comparable to Giavroglou*, C., et al* study recurrence rate was 14%. [21] A study done by *Ardehali, M, et al* which had recurrence of (19.1%), this slight difference due to larger number of samples. [19] Our results were better than Lutz*, J., et al.* study, where his recurrence rate was 25 percent, four of 13 patients. [22] This difference may be attributed to advance stage cases and different embolization techniques in that study.

Conclusion

The use of preoperative embolization in JNA patients has been very beneficial in shortening surgery time, improve bloodless of the operative site, with a significant reduction in intraoperative blood loss, reduction in postoperative hospitalization time, and reduction in recurrent rate.

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Role of Ginger Ethanolic Extract in Improving Renal Dysfunction –Induced by Gentamicin

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Abstract

Objectives: The present study was designed to evaluate the effect of ethanolic extract of *ginger rhizome* in improving renal dysfunction experimentally induced by gentamicin in male rats in order to give an experimental evidence for its established use. Duration: September 2020- December 2020. Design: In this study (25) Swiss albino male rats were used weighing (200-230) gm. which were divided randomly into five equal groups, (5) rats in each group as follows: G1 (control) which was received distilled water orally daily for 10 days. G2: received therapeutic dose of gentamicin sulphate (5mg/kg) intramuscularly once daily for 10 days. G3: received two fold dose of gentamicin (10mg/kg) intramuscularly once daily for 10 days. G4: received co-administration of gentamicin (5mg/kg) intramuscularly + ethanolic extract of *ginger rhizome* (250 mg/kg) orally daily for 10 days, while the G5: received gentamicin (10mg/kg) + ethanolic extract of *ginger rhizome* (250mg/kg) orally daily for 10 days. All the treatments were given in amounts levels of (0.1/100gm) B.W. Animals from each group were sacrificed at the end of 10 days and blood were collected and sera were separated for biochemical testes , while the kidneys removed for histopathological examinations . The findings: Significant increase ($P<0.05$) in the serum levels of uric acid, creatinine and blood urea nitrogen (BUN) in (G2&G3) as compared to (G1) group , while there were significant decrease ($P<0.05$) in serum levels of these testes in the groups (G4&G5) as compared to (G2&G3) groups beside normalizing the kidney histopathological architecture . Conclusion: It can be concluded that the co-administration of gentamicin along with *ginger rhizome* ethanolic extract has been exhibited more activity in improving renal dysfunctions .

Keywords: *Ginger extract , Rats , Renal dysfunctions*

Introduction

Gentamicin is an aminoglycoside antibiotic that is widely used for treating infections of gram negative bacteria [1] . However, complications that occur with gentamicin toxicity are considered the main reasons for prolonging hospital stays in the developed world which it has been estimated that more than 30% of patients treated with gentamicin for more than 7 days reveal signs of nephropathy [2, 3 & 4] . The specificity of gentamicin renal toxicity is related to its accumulation in the renal proximal tubules and in lysosomes 5 which lead to direct tubular necrosis and loss of tubular brush border integrity [6] . The mechanism of gentamicin-induced nephropathy is not completely known but [7] reported that gentamicin may inhibit protein synthesis in renal proximal tubular cells resulting in acute tubular necrosis followed by acute renal failure . Moreover gentamicin has been shown to increase the generation of reactive oxygen species (ROS) like superoxide anion (O_2^-), hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2) as well as reactive nitrogen species (RNS) in the renal cortex that lead to renal structural and functional deterioration [8 , 9 & 10]. The generation of ROS have the ability to cause cell death by necrosis or apoptosis [11] . It is notable that the using of many medications for renal diseases have side effects such as hepatitis , nephropathy , and allergic reactions [12] , therefore the need for less toxic alternatives is an important [13] . A potential source of such compounds can be obtained from medicinal plants which have more activity. Zingiber officinale Roscoe (Ginger) is the better component of curry belongs to the Zingiberaceae family

which has been used to treat disease including headache , cold , arthritis and renal functions [14] . The mechanisms of these renoprotective effects may involve both oxidative stress reduction and significant 6- gingerol antioxidant effects [15]. The major active ingredients in ginger are bisapolene , zingiberene , and zingiberol which have the pharmacological effects such as analgesics , sedative , antipyretic , and antibacterial effects in vitro and in animals [16] .

Materials and Methods

Animals:

This study was performed under the guidelines supervision of Ethical Committee for lab. Animals work in the College of Veterinary Medicine, University of Baghdad. Twenty five Swiss albino male rats about three months of age with body weight ranged between 200-230 gm were used to perform the experiment of the present study. Rats were housed in plastic cages of 20×50 ×75 cm dimensions, placed in a special housing room belongs to the Department of Physiology, Biochemistry and Pharmacology / College of Veterinary Medicine /University of Baghdad for two weeks for acclimatization. Standard rodent diet (Commercial feed pellets) and tap water were freely available.

Plant materials:

Freshly samples of *Zingiber officinale rhizome* were purchased from Iraqi local market in Baghdad. The samples were oven- dried for 96 h. at 40 ºC . Dried plant materials were grounded by using an electrical blender to fine powder which stored in a dry place in the dark for the experimental study [17] .

Extraction of *Zingiber officinale* rhizome:

The rhizome fine powder of 100 gram was homogenized in 500 ml of 70% ethanol and left in a conical flask at room temperature for 3 days. Then , the mixture was filtered through a fine muslin cloth and a filter paper (Whatman No. 1) . The filtered solution was transferred in hygienic and germ - free petri dish and evaporated at 40 ºC in an incubator for 3 days . The final concentrated extract was then lyophilized and yielded ethanolic extract of rhizomes of Zingiber officinale [18] .

Preparation the concentration of *Zingiber officinale* ethanolic extract:

Stock solution was prepared by mixing 5 gram from dried extract with distilled water and completed the volume to 10 ml to get a concentration of 500 mg/ ml , then the extract was given to rats orally at a dose of 250 mg/ kg according to [17] .

Preparation of Gentamicin Solution:

Gentamicin Sulphate injection (80mg/2ml) ampoule was used. The solution was prepared by taking 1ml (40mg) of gentamicin sulphate from the ampoule and completing to (8ml) of normal saline to get a concentration of (5mg/ml) and it was given to rats intramuscularly at a dose of 5mg/kg [19] .

Experimental design:

Total of (25) male rats were divided randomly into five equal groups, 5 rats in each group. Treatments began with schedule as follows:

G 1 (control) : was administered distilled water orally for daily for 10 days.

G2 : was administered therapeutic dose of gentamicin (5 mg/ kg) intramuscularly once daily for 10 days , then given distilled water orally for 14 and 28 days.

G3 : was administered two fold dose of gentamicin (10mg/kg) intramuscularly once daily for 10 days.

G4 : was administered co-administration of gentamicin (5mg/kg) intramuscularly + ethanolic extract of *ginger rhizome* (250 mg/kg) orally daily for 10 days.

G5: was administered gentamicin (10mg/kg) + ethanolic extract of *ginger rhizome* (250mg/kg) orally daily for 10 days . All the treatments were given in amounts levels of (0.1/100gm)

Parameters studied:

After the end of the study the blood samples of each group were collected via the heart puncture. Blood was kept in gel tubes and serum was isolated after centrifugation at a speed of 3000 (rpm) for 15 minutes , which were stored at (- 20 ºC) until analysis for :

1. Biochemical testes:

- Serum uric acid levels

- Serum creatinine levels

- Serum blood urea nitrogen (BUN) levels

2- Histopathological examinations of of kidneys sections

At the end of the experiment, all the animals were anesthetized and killed after blood collection, the kidneys were dissected, cleaned and preserved in (10 %) formalin till the preparation of histological sections. Several tissue sections were prepared according to [20].

Statistical Method:

The statistical analysis of the data of the experiment was measured by using the IBM SPSS Statistical (version 26.0), Using of one-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means of the groups. The results were expressed as mean ± standard error and ($P < 0.05$) was considered statistically significant [21].

Findings

The levels of serum uric acid are represented in table 1 which showed significant ($P<0.05$) increase in their values in serum of rats in (G2 & G3) groups that received gentamicin at the dose levels (5 & 10 mg/kg), respectively as compared to (G1) control group, while there were significant ($P<0.05$) decrease in the values of serum uric acid in (G4 & G5) groups that received gentamicin 5 mg/kg + 250 mg/kg ethanolic extract of ginger rhizome and gentamicin 10 mg/kg + 250 mg/kg ethanolic extract of ginger rhizome , respectively as compared to (G2&G3) groups .

Table 1: The effects of gentamicin and Zingiber officinale rhizome ethanolic extract on serum uric acid in rats (mg/ dl).

Groups	Mean ± SE
G1 : Control distilled water orally	1.47 ± 0.15 B
G2 : Gentamicin (5mg/kg) I/M	4.25 ± 0.18 A
G3 : Gentamicin (10 mg/kg) I/M	5.47 ± 0.36 A
G4 : Gentamicin (5mg/kg) I/M + (250 mg/kg) ginger rhizome ethanolic extract orally	1.52 ± 0.18 B
G5 : Gentamicin (10 mg/kg) I/M + (250 mg/kg) ginger rhizome ethanolic extract orally	1.67 ± 0.14 B

♦ Values are presented as mean ± SE

♦ Capital different letters denoted to ($P<0.05$) significant differences between groups.

The levels of serum creatinine are represented in table 2 which showed significant ($P<0.05$) increase in their values in serum of rats in (G2 & G3) groups that received gentamicin at the dose levels (5 & 10 mg/kg), respectively as compared to (G1) control group, while there were significant ($P<0.05$) decrease in the values of

serum creatinine in (G4 & G5) groups that received gentamicin 5 mg/kg + 250 mg/kg ethanolic extract of ginger rhizome and gentamicin 10 mg/kg + 250 mg/kg ethanolic extract of ginger rhizome , respectively as compared to (G2&G3) groups .

Table 2: The effects of gentamicin and Zingiber officinale rhizome ethanolic extract on serum creatinine levels in rats (mg/ dl).

Groups	Mean ± SE
G1 : Control distilled water orally	0. 35 ± 0.10 B
G2 : Gentamicin (5mg/kg) I/M	2. 08 ± 0.18 A
G3 : Gentamicin (10 mg/kg) I/M	2.15 ± 0.26 A
G4 : Gentamicin (5mg/kg) I/M + (250 mg/kg) ginger rhizome ethanolic extract orally	0.40 ± 0.08 B
G5 : Gentamicin (10 mg/kg) I/M + (250 mg/kg) ginger rhizome ethanolic extract orally	0.47 ± 0.04 B

♦ Values are presented as mean ± SE

♦ Capital different letters denoted to (P<0.05) significant differences between groups.

The levels of serum blood urea nitrogen (BUN) are represented in table 3 which showed significant (P<0.05) increase in their values in serum of rats in (G2 & G3) groups that received gentamicin at the dose levels (5 & 10 mg/kg), respectively as compared to (G1) group, while there were significant (P<0.05) decrease in the values of serum (BUN) in (G4 & G5) groups that received gentamicin 5 mg/kg + 250) ethanolic extract of ginger rhizome and gentamicin 10 + 250 mg/kg ethanolic extract of ginger rhizome , respectively as compared to (G2&G3) groups .

Table 3: The effects of gentamicin and Zingiber officinale rhizome ethanolic extract on serum blood urea nitrogen (BUN) levels in rats (mg/ dl).

Groups	Mean ± SE
G1 : Control distilled water orally	22.00 ± 0.10 B
G2 : Gentamicin (5mg/kg) I/M	32.20 ± 0.18 A
G3 : Gentamicin (10 mg/kg) I/M	34,14 ± 0.26 A
G4 : Gentamicin (5mg/kg) I/M + (250 mg/kg) ginger rhizome ethanolic extract orally	24.20 ± 0.08 B
G5 : Gentamicin (10 mg/kg) I/M + (250 mg/kg) ginger rhizome ethanolic extract orally	24.30 ± 0.04 B

♦ Values are presented as mean ± SE .

♦ Capital different letters denoted to (P<0.05) significant differences between groups.

The results of kidney histopathological sections, figures (1& 2) showed dilated of renal tubules with vacuolar degeneration of epithelial cells and atrophy of glomerular tufts with dilated of Boman space , acute cellular degeneration of renal epithelial cells and severe congested blood vessels in rats of (G2&G3) groups that received gentamicin at doses (5 &10) mg/kg , respectively while the sections of rats that treated with gentamicin (5 mg/kg) + (250 mg/kg) ethanolic extract of ginger rhizome and gentamicin (10 mg/kg) + (250 mg/kg) ethanolic extract of ginger rhizome in (G4&G5) , respectively showed no clear lesions and no lesions in glomerular tufts and return the structures of kidney to the normal .

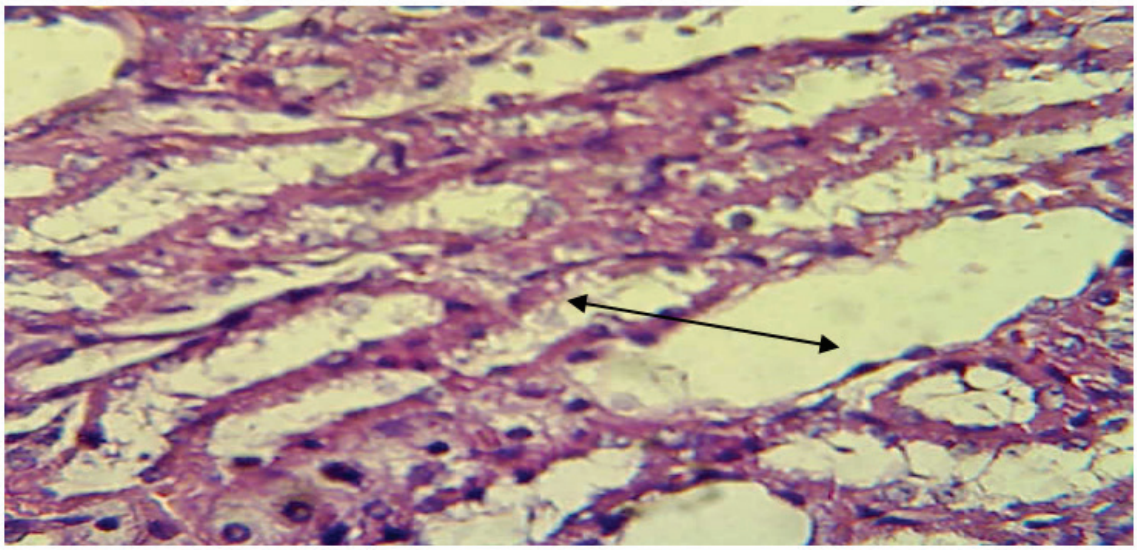

Fig.1 : Section in the kidney of rats in (G2) group shows dilated of renal tubules with vacuolar degeneration of epithelial cells (H&E stain 400X).

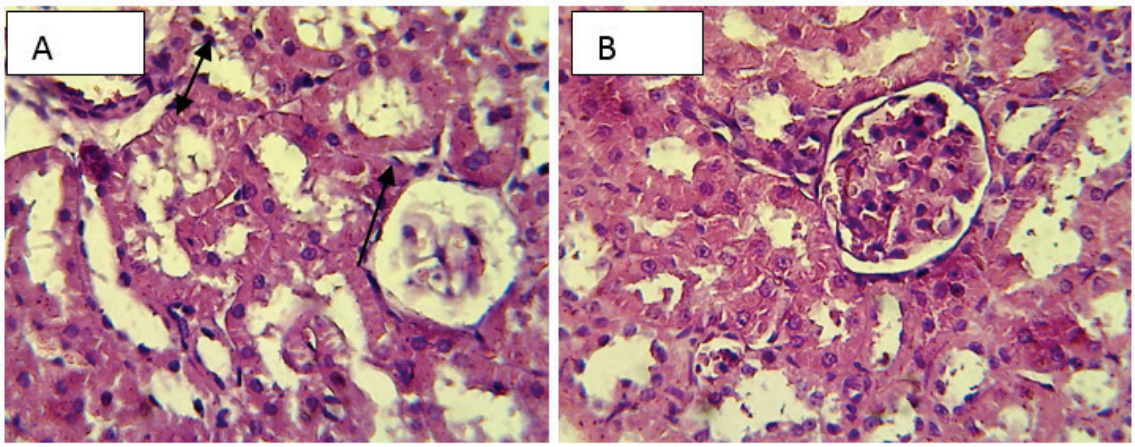

Fig.2 :Section (A) in the kidney of rats in (G3) shows atrophy of glomerular tufts with dilated of Boman space , acute cellular degeneration of renal epithelial cells and severe congested blood vessels , While Section (B) in the kidney of rats in (G4) shows no clear lesions (H&E stain 400X) .

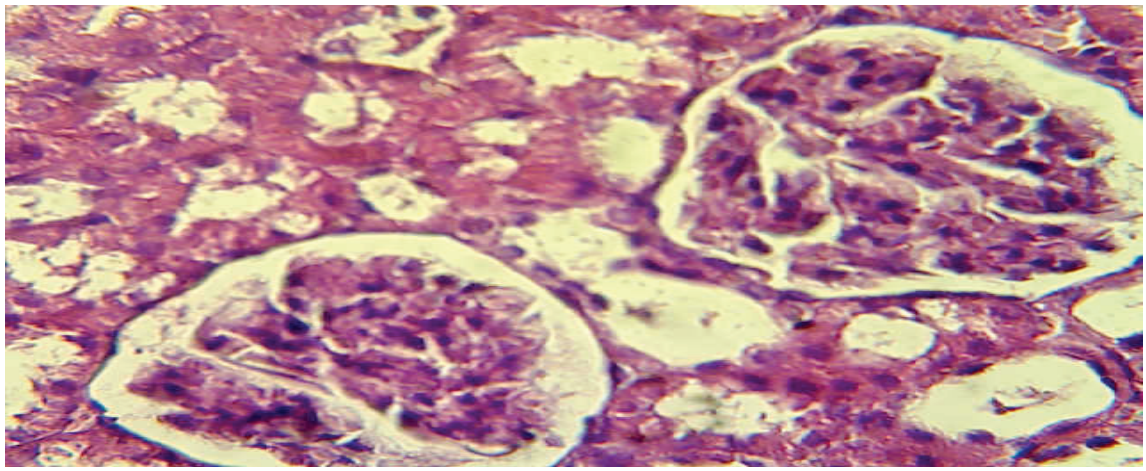

Fig. 3: Section in the kidney of ratsl in (G5) shows no clear lesions occurr in glomerular tufts and return the structures of kidney section to the normal (H&E stain 400X).

Discussions

The findings of the present study showed that gentamicin induces nephro deleterious effects by increasing the serum levels of uric acid , creatinine and blood urea nitrogen in rats that received gentamicin at doses of (5 & 10) mg/kg , while the rats treated with ginger rhizome ethanolic extract along with gentamicin showed decrease the serum levels of these values that return to the normal levels. The increase of these values may be related to the reduction in glomerular filtration rate as well as impairment of renal blood flow that occurred in rats treated with gentamicin [22 & 23] which also reported that uric acid is the primary catabolic product of protein and non protein nitrogen that excreted primarly by tubular excretion and increases in massive tissue destruction and renal diseases . Furthermore the increase in the levels of serum uric acid creatinine and BUN of rats that received gentamicin may be attributed to the result of membrane lipid peroxidation that occurred due to stimulation of reactive oxygen species (ROS) such as H_2O_2 and O_2 radicals which potentiate the contraction of mesangial cells that lead to change in the filtration surface area and ultrafiltration coefficient which causes retardation in glomerular filtration rate [24] . Also [25 & 26] reported that gentamicin-induced kidney injury is characterized by renal tubular epithelial cell necrosis, apoptosis, inflammatory responses and oxidative stress while in recent studies [27, 28 & 29] showed that higher values of plasma uric acid were found in rats on day (7) of treatment with gentamicin which associated with renal dysfunction and severe proximal renal tubular necrosis. The deleterious effect of gentamicin on kidney also involved severe proximal renal tubular necrosis that may followed by renal failure [30] . In the present study the elevation in serum values of uric acid creatinine and blood urea nitrogen in rats treated with gentamicin were brought to the normal levels in the rats treated with *ginger ethanolic* extracts along with gentamicin and this may be explained by the activity of the plant as antioxidant and its diuretic property [31 & 32] . The antioxidant activity of ginger rhizome ethanolic extract is due to its contain of polyphenolic compounds and phenols which have the potent antioxidant activity by scavenging the free radicals and serve as free radical inhibitors and inhibit lipid peroxidation [33 & 34] . In the previous study [35 & 36] demonstrated the presence of polyphenolic and flavonoid compounds in ginger rhizome extract which have the antioxidant activity . The presence of polyphenols and flavonoids in the *Z. officinale* extract might be responsible for the antioxidant and nephroprotective activities [37] . In this study we found that ginger could serve as a preventive agent against gentamicin tubular toxicity , protects the renal cells and reduces the severity of tubular damage caused by gentamicin and the co-administration of plant extract along with gentamicin in our study reduced the levels of raised serum uric acid creatinine and BUN suggesting that the contents of *ginger rhizome ethanolic* extract protected the integrity of kidney tissue

Conclusion

From the data of our study, we could conclud that the Zingiber officinale rhizome ethanolic extract has nephroprotective effects in rats that received gentamicin by decreasing the highly levels of serum uric acid creatinine and blood urea nitrogen. Our findings also suggest that ginger ethanolic extract could be used as a new potential drug in protecting against gentamicin which causes harmful nephropathy and renal dysfunction.

Source of Funding: Self

Conflict of Interest: Non

Ethical Clearance: The study was performed under the guide lines supervision of Ethical Committee for lab. animals work in the College of Veterinary Medicine , University of Baghdad.

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Hematological Alterations Induced by Zinc Oxide Nanoparticles and Preventive Role of *Moringa Oleifera*: *In vivo*

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Abstract

Increasing applications on nanomaterials have raised concerns about the toxicity of these materials toward human health. This study aims to illustrate the effects of Zinc Oxide Nanoparticles (ZnONPs) on hematological parameters in rats and to explore the protective action of *Moringa oleifera* (MO) against hematological changes induced by ZnONPs in rats. In our study, (24) albino rats were divided into four groups (6 for each group) and used. The studied rats included rats which received 100 mg/Kg ZnONPs for 60 days , while the control group were rats without treatment. In the ZnONPs+MO group, rats were co-administered with a combination of ZnONPs at a 100mg/kg dose with MO at a 300 mg/kg dose for 60 days. The MO group animals were treated with 300 mg/kg of MO for 60 days. The animals were sacrificed and blood samples were obtained by cardiac puncture in the end of the experiments. Various blood parameters were measured using HORIBA-Medical automatic hematology analyzer. Rats treated with ZnONPs showed a significant decrease ($p<0.05$) in red blood cell counts (RBCs), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), hematocrit value (Hct), and platelet count (Plt) count, except white blood cell count (WBCs) in comparison to the control group. Then, co-administration of M. Oleifera along with ZnONPs exerted a significant recovery effect in hematological disorders. Thus, these consequences established that *M. Oleifera* shown a defensive impact against ZnONPs induced hematological alterations in male rats.

Keywords*: Nanomaterials, hematological changes, anemal group, blood parameters ,M. Oleifera*

Introduction

It was expected that applying nanotechnology in medicine would have a revolutionary effect on healthcare[1]. Nano particles (NPs) are particles with a size of 1–100 nm[2]. Metal oxide nanomaterials (NMs) such as zinc oxide nanoparticles (ZnONPs) are used in cosmetics and skincare products and as drug delivery systems[3]. Nanoparticles were shown to penetrate through cell membranes and barriers of biological tissues resulting in toxic consequences because of their relatively small sizes. In addition, a high toxicicity levels are induced on the target cells as a result of nanoparticle release [4]. MNPs have the ability to enter the body in different ways, e.g through skin, inhalation or gastrointestinal tract , and to circulate through lymphatic system or blood , and to finally accumulate in different organs[5]. Following acute oral route exposure, previous studies have demonstrated more toxicicity of the nano-forms of various particles than their micro-counterparts[6].

More recently, a new science branch called nanotoxicology has appeared intending to clarify the possible impacts of nanoparticles and related parameters that affect the nanomaterial cytotoxicity[7]. In vivo and in vitro studies showed a toxic effect ZnONPs, thus exposure to ZnONPs should be considered and controlled precisely[8]. In biological media, the bioavailability of the chemical compounds at toxic levels

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can result in changing different hematological indices[9]. The evaluation of hematological parameters may have diagnostic adverse effects of foreign compounds on blood constituents[10]. In the diagnosis of actual physiological status of the organisms, blood parameters are considered as important indicators[11]. Hematological diseases have gained epidemic proportions in the world. Since professional care is not immediately available, costly, inconvenient and time consuming, several people look for medicinal herbs and plants to treat, enhance and boost their health[12]. Among these plants are vegetables, which contain considerable different nutrients and are good sources of Vitamins, proteins, essential amino acids, minerals and antioxidants [13].The leaves of *Moringa oleifera*, which are utilized as vegetables in different countries worldwide were shown to possess positive impacts on certain hematological parameters [14,15].

Therefore, this study aims to confirm the deleterious effect of ZnONPs on hema tological parameters and to assess the role of leaf extracts of *M. oleifera* inhibiting or preventing alterations induced by ZnONPs in the blood.

Materials and Methods

Chemicals

Zinc oxide nanoparticles were purchased from US Research Nano materials, Inc. Company (Houston, TX USA). CAS NO: 1314-13-2, Particle Average Size: 18 nm, nearly spherical, Specific Surface Area: 40–70 m2/g, Purity: 99.95 %). Powdered ZnONPs were dispersed in the fresh sterilized physio logical saline solution. *Moringa Oleifera* was obtained in the form of powder (Pure *Moringa Oleifera* Leaf Extract Veggie Capsules) from GreeNater PREMIUM, USA, and dissolved in normal saline.

Animals and experimental groups

The present study was conducted on (24) adult male albino rats of 5–6 months age and weighing 195-225 gms. Rats were housed in plastic cages under hygienic conditions at 25±2°C and 12 hour light-darkness cycles. Throughout the experiment, the animals had free access to feed and tap water. They were divided randomly into 4 groups each containing 6 rats:

- Control group: Healthy rats without treatments.

- ZnONPs-treated group : Rats received ZnONPs at 100 [16] mg / kg / day dose.

- ZnONPs + MO treated group: Co-treat ment group received *Moringa Oleifera* extract at 300 [17] mg/ kg body weight dose , plus ZnONPs at a dose of 100 mg /kg / day

- MO-treated group: Rats received *Moringa Oleifera* extract at 300 mg/body weight dose.

The rats received their respective daily doses for 60 consecutive days. Both drugs were given orally via gastric gavage. All animals in the 6 groups were anesthetized with diethyl ether and sacrificed at the end of the experiments.

Haematology

After blood sample collection by the cardiac puncture of rats, they were anticoagulated and blood parameters were measured by the automatic hematology analyzer (HORIBA - Medical / France). These parameters included red blood cell count (RBCs), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit value (Hct), platelet count (PLT), as well as white blood cell count (WBCs).

Statistical Analyses

All results were performed as mean± standard deviation of six animals in each group. All parameters for inter-group differences were analyzed by the one-way analysis of variance (ANOVA) followed by the Duncan analysis. In all analyses, the P-value of <0.05 was considered as statistically significant.

Results

Figure 1. shows the hematological para meters used in our study. In comparison with the control group rats, the rats treated with ZnONPs showed lower values of red blood cells count (RBCs), hemoglobin (Hb) concentration, mean corpuscular volume (MCV),mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit value (Hct) and platelet count (PLT), while on the contrary, the white blood cell count (WBC) showed higher values when compared with the control group animals. The results also demonstrated no effective alteration in all

hematological parameters with co-administration of *Moringa Oleifera* extract when compared with the ZnONPs -intoxicated rats ($p<0.05$).

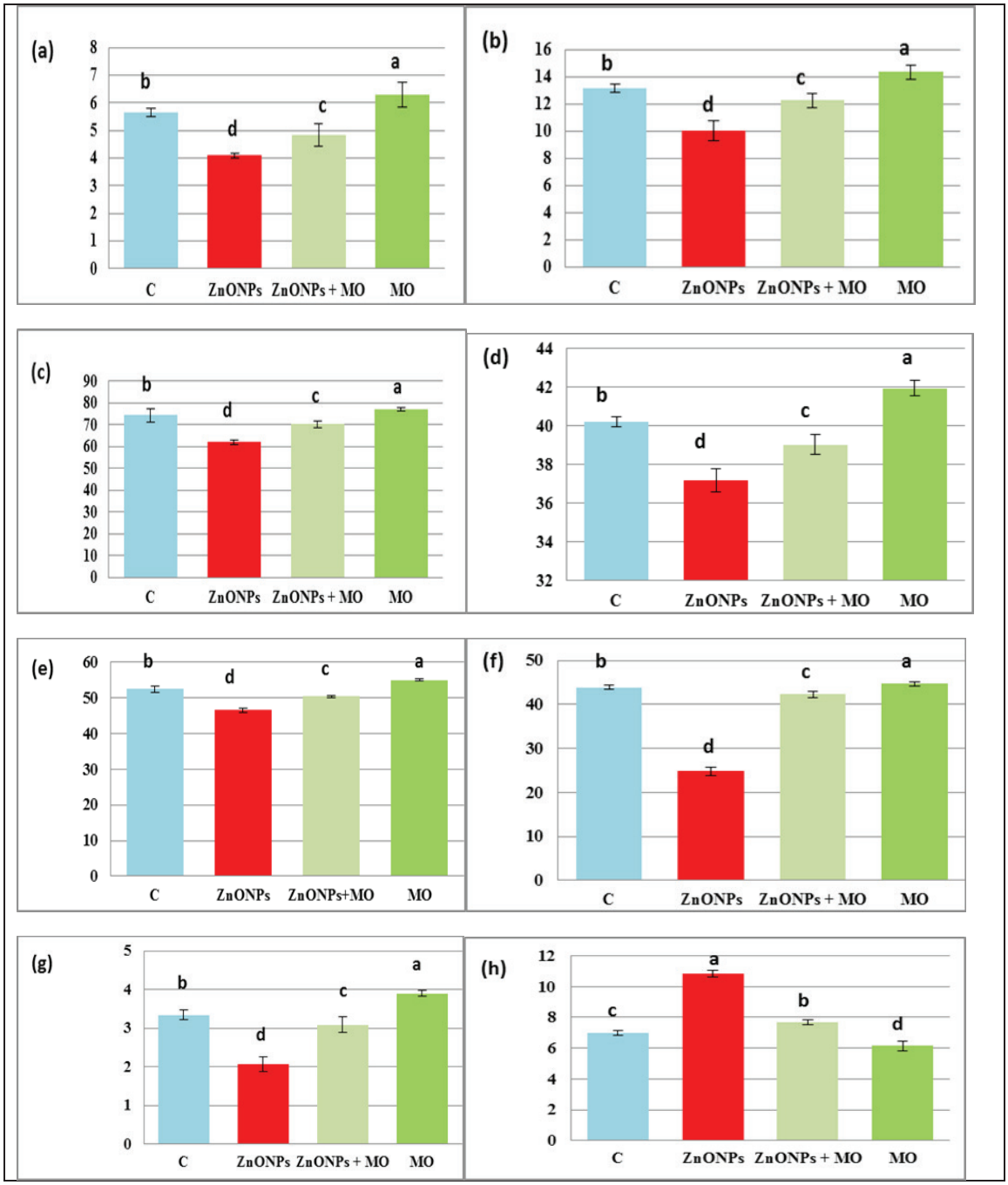

Figure 1: Levels of (a) red blood cells count RBCs (× 106 cell/µl), (b) hemoglobin Hb (gm/dl), (c) mean corpuscular volume MCV (fL),(d) mean corpuscular hemoglobin MCH (Pg), (e) mean corpuscular hemoglobin concentration MCHC (g/dl) ,(f) hematocrit value Hct (%),(g) platelets count Plts (× 105 cell/ µl) and (h) white blood cell count WBCs (× 103 cell/µl) in the blood of different treatment groups. Data are expressed as Mean± SD (n=6). a–d Different superscript letters denote significant differences within column ($p < 0.05$) Test = one-way ANOVA, post hoc = Duncan. .

Discussion

Because of their small size, nanoparticles, can travel to different parts and organs of the body after ingestion. These nanoparticles are able to cross the small intestines and then go to the blood circulation[18]. Blood is the most sensitive act as an indicator of countless metabolic disorders[19]. The analysis of blood parameters is associated with risk assess ments since the hematological system possesses a high predictive value for human body toxicity (91%)[20].

It was revealed in our study that there was a significant decrease in RBCs count after ZnONPs administration in comparison with the controls, which indicates hypoxia and anemia occurrence. This decrease in RBC count indicates that there is red blood cell destruction and a lowered rate of erythropoiesis[21]. The RBCs are normally produced from the haemo poietic stem cells in the bone marrow and undergo erythropoietin-aided maturation. Therefore, it can be concluded that the RBC count alteration could result from the haematopoietic system[22]. The high Hb degradation and catabolism can lead to low Hb levels . The decrease in Hb content can be associated with the low RBC count which, in turn, indicates induction of anemia[23]. Hematocrit value is a main blood viscosity indicator [24]. A haematocrit value lower than normal is an indication for anaemia occurrence[25]. This can be resulted from a low erythrocyte count, a low hemoglobin content in each RBC, or both. It is often acquired from the myeloid tissue damage by chemical compounds such as toxins or from using medications which inhibit enzymes required for haemopoiesis[26].

The red blood cell indices especially RBC count, PCV value, and Hb conce- ntration are correlated with the total red cell population; The MCV represents the red blood cell size, while MCH and MCHC are calculated mathematically for hemoglobin concentration determination [27- 31]. Platelets come after RBCs as the second most cell population found in the blood. The drop in platelet count resulting from either reduced platelet production or high platelet destruction is termed as thrombocytopenia[32]. The platelets are produced from megakaryocytes and play essential roles in haemostasis[33]. The white blood cells or (leukocytes) contribute to human body's immunity and defense against the invasion of antigens[34]. They are normally synthesized by the body in response to any foreign substance invasion[35]. These haematological parameters were shown to be significantly increased in the ZnONPs +*M.Oleifera* group when they were compared to the ZnONPs group. This could be attributed to the phytochemical components in the extract as well as the existence of Vitamins and minerals. These phytochemical components are well-known haemopoietic factors that directly affect the blood cell production in the bone marrow [36,37]. The results in our study seem to coincide with this claim and restoration of these functions could entail the existence of the extraxcts of *M. oleifera*.

Conclusion

The results of this study revealed that Zinc Oxide Nanoparticles possess a toxic effect on blood cells. *M. oleifer* extracts given to rats orally at the dose of 300mg/kg body weight improved full blood count in rats. It was effective to protect the hematological alterations induced by Zinc Oxide Nanoparticles. However, there is a need for future studies to prove *M. oleifer* as an effective protective agent against haematological disorders induced by metal nanoparticles.

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Ethical Clearance : No need

Source of Funding: None

Conflict of Interest: The authors have declared no conflict of interest

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The therapeutic Role of Medicinal Plants in Treating Corona Epidemic Disease (*Eucalyptus* as Applied Study)

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Abstract

The study was conducted to know the effect of treatment with the hot aqueous extract of the green leaves of *Eucalyptus camldulensis* on the initial symptoms associated with Coronavirus disease. Three concentrations of the hot aqueous extract were used, which are (0,25,50 $g.L^{-1}$) boiled distilled water, and the results in kind showed the infected volunteer patients the following: The results showed that there was an effective and significant increase in the use of the hot aqueous extract by the volunteer patients at a concentration of 50 g of green eucalyptus leaves per liter of water only. The percentage of those recovering from the symptoms of the disease was 63% when they inhaled the water vapour of the leaves for a period of 15-30 seconds, once a day for the age group (25-45) years, while the percentage of recovery for patients from the group (45-65) years was 55%. The results recorded a recovery percentage of 48.1% for infected patients and volunteers, and for the age group of 25-45 years, from the initial symptoms associated with the Coronavirus, such as fever, coughing and shortness of breath, and a recovery percentage of 29.6% and for the older age group from 45 to 65 years, when they inhale the steam of boiling water of the leaves of the extract for a period of 15-30 seconds and only once a day.

Key word: *Corona virus , Eucalyptus camldulensis, aqueous extract*

Introduction

Interest in medicinal and aromatic plants has increased in most countries of the world until it is traded this morning ,It is truly a call to the nature that God Almighty created and mocked for human beings, where there is a high response to traditional medicine (folk medicine) and this trend no longer includes only those wishing to receive treatment for various diseases who are hopeless to treat them with modern medical means, but, it also includes scientists, doctors, who began to move towards extracting medicinal herbs and medicinal plants free of chemical compounds, after it was proven that the negative symptoms of these combinations persist on the human body, Also, medicinal plants are a source of many biologically active compounds that have curative and scientifically proven effects for many diseases [(1)]. Research has gathered recently to treat many diseases by using medicines of plant origin, because most of the manufactured medicines, despite their high effectiveness, cheapness and large production, have serious side effects, and microbiology has developed resistance to their appearance. According to a study conducted by the World Health Organization, 91 countries use natural plants, which led many scientists to return to nature and experiment with alternative treatments and divide all plant extracts with no side effects to prove their effectiveness against microorganisms because they contain medically effective substances [(2)], Eucalyptus camldulensis belongs to the myrtacene family, and it is one of the evergreen perennial trees that is widely cultivated in Asia, Australia and most Arab countries. They may reach a height of several meters, It has a fragrant scent that repels insects, its narrow leaves are lanceolate-shaped and evergreen, considered as Ornamental plant and windbreaker plant [(3)].Eucalyptus is important in the treatment of respiratory infections and has been used to treat respiratory tract infection with

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excessive secretions and has inhibitory efficacy against microorganisms [4]Also, the medicinal part of the plant that is used the aromatic leaves that have many benefits, especially in the medical field, as well as oil is extracted from them. The effect of the aqueous and alcoholic extract of eucalyptus leaves in inhibiting the growth of Gram-positive bacteria isolated from the pharynx and tonsils were also studied by [5]. The aqueous extract had a higher inhibitory effect than the alcoholic extract on the growth of multiple antibiotic-resistant bacterial isolates. The best inhibitory effect appeared on the growth of streptococcus pyogenes with a diameter of 28.0 mm, as [6] emphasized the importance of the essential oils of eucalyptus leaves in the treatment of many respiratory diseases. Also, the results of the chemical detection of some components of eucalyptus leaves by [7] indicated that they contain terpenes, fluorides, and phenolic compounds represented by Tannins and terpenes, and these results are consistent with [8], The research aims to know the effect of the hot aqueous extract of fresh eucalyptus leaves on sick volunteers with symptoms of Corona and that is after it was proven and diagnosed that they had the disease by a hospital in Basra province or by making irradiated swabs to detect their infection with the disease.

Materials and Methods

The study was conducted to know the effect of the hot aqueous extract of green leaves of the eucalyptus plant on volunteer patients and those infected with Coronavirus disease in Basra province. and that is after it was proven and confirmed that they were infected in governmental hospitals of Basra province and by doctors, and after taking the required laboratory swabs, at ages ranging between 65_25 years, and the symptoms associated with the disease were at the beginning of their appearance, including fever, cough and shortness of breath.

Classification of eucalyptus leaves:

The green leaves of the green eucalyptus plant were classified by Dr. Afaq Ibrahim Juma, a teaching officer at the College of Agriculture, University of Baghdad, specializing in forest trees.

Preparing the aqueous extract:

The aqueous extract was prepared after weighing 25 gm and 50 gm of green eucalyptus leaves separately in (one liter) of boiled distilled water if the harbome method was adopted after its modification by not separating the filtrate from the sediment of green leaves and the treatments were as follows:

1- (zero) g / L control treatment boiled distilled water only

2-25 g / L distilled water

3_50 g / L distilled water

Processing method:

Treatment is done with the hot aqueous extract of the tender green eucalyptus leaves by exposing volunteer patients and those with initial symptoms of the Coronavirus to inhale the steam of boiling water with eucalyptus leaves for a period of 15-30 seconds, twice a day in the morning and before bedtime at night.

Chemical detection of eucalyptus leaves:

The chemical detection units for eucalyptus leaves were not completed due to the large number of studies that conducted the chemical detection of the active components of plant leaves, which were relied upon in this study, which confirmed that it contains soaps, alkalis, phenols and coumarins and also from [9], where he relied on the study of the active chemical components also in eucalyptus leaves, where a study proved that the aqueous extract has an acidic medium and contains phenolic compounds and flavonoids as well.

Statistical Analysis

A factorial experiment conducted in a The Randomized Complete Block Design (RCBD) with three replicates was used, and the averages were compared according to the least significant difference test (L.S.D),at a probability level of 0.05 [10].

Results

The results in Table (1), which represents the effect of exposure to water vapor of the hot aqueous extract of eucalyptus leaves for a period of 30-15 seconds, twice a day in the morning and before sleep at night, and for the two age groups (45-25) years and (65_45) years. The results were recorded after two days of treatment, where

eye observations of the patients showed clear efficacy in the response of the age group (45_25) years of volunteer patients to recover from the accompanying symptoms such as coughing, fever and shortness of breath from exposure to a high concentration of aqueous extract 50 g / l, by 63% of infected patients compared with zero% for the control treatment, While the percentage of recovery from symptoms associated with patients infected with Coronavirus for the age group (65_45) years when exposed to the hot aqueous extract and at a concentration of 50 g / L was 55.5% of the infected patients. As for when exposed to the hot aqueous extract at a concentration of 25 g / l, the percentage of cure of disease symptoms for the infected patients within the study and for the age group 45-25 years was about 48% and by 29.6% for the age group 65_45%,

Discussion:

The results used are explained in the two tables (1,2) of the study to the extraction with hot water for the leaves. Eucalyptus can 1-provide an amount of the active compounds that can be obtained from the leaves represented by Tannins, saponins and terpenes, which have been shown to have active effectiveness against many microorganisms associated with respiratory colds [(11)]These results are also achieved with the findings of (8) in that the results of the chemical detection of eucalyptus leaves showed that the leaves contain active compounds such as alkaloids and phenolic compounds represented by tannins and terpenes. He also confirmed [(12).] That eucalyptus, when inhaled in a hot water bath, acts as a nerve agent and causes muscle relaxation because it contains flavoidal compounds such as rutin . The reason for these results may also be due to the effectiveness of eucalyptus because it contains eucalyptol cineole. It is the main compound that contains the volatile oil, and it is a turbine compound responsible for giving effective properties to stimulate microorganisms. This explains the widespread uses of eucalyptus oil in medicines used to treat coughs, colds, influenza, and respiratory infections, and confirms its anti-microbiotic properties [(9)].

Table (1) Effect of the hot aqueous extract of green eucalyptus leaves for patients with primary symptoms of Coronavirus disease

Extract concentration(g/L)	Infected patients(age categories)		The average of Extract concentration
	Categories(25-45 years)	Categories(45-65 years)	
0	0.00	3.67	1.83
25	4.33	2.67	3.50
50	5.67	5.00	5.34
The average of Infected patients(age categories)	3.33	3.75	
L.S.D 0.05	Extract concentration :0.784 Infected patients:0.641 interaction :1.109		

Conflict of Interest: None

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Evaluation of Biofilm Formation in *Klebsiella Pneumoniae* and Antibiotic Resistance

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Abstract

Biofilm are densely packed communities of germ cell (bacterial cells), that grow either on living or inert surface, surround themselves with secreted polymers, the role of biofilm formation and development by bacteria has been suggested to be an important stage in the pathogenesis of numerous bacterial species. In this study *klebsiella pneumoniae* has been investigated to form biofilms and its relation to antibiotics resistant. The antibiotics which are used in this study are Ciprofloxacin (CIP) and Cefotaxime (CTX) because most of the isolates showed resistance to these antibiotics. The experiment was design to check the cause of UTI in the different types of patient by taking their urine as a specimen sample. Out of 160; 24 samples showed *Klebsiella pneumoniae,* which cause the urinary tract infection. The organisms were identified by the DNA isolation and checking with PCR using *16SrRNA*. Antibiotic susceptibility for the different groups of antibiotic were done by using disk diffusion method against all *Klebsiella pneumoniae* isolates. *Klebsiella pneumoniae* are important infectious bacterium and become more dangerous with their capacity of multi-drug resistant. Therefore, there is need to find out alternate therapy to eradicate the infection of this bacterium by reduce the formation of biofilm which may lead to decrease the antibiotics resistance.

Key words: *Klebsiella pneumoniae, UTI, antibiotic resistance, biofilm formation.*

Introduction

The *Enterobacteriaceae* family contains different microorganisms a Gram-negative *Klebsiella pneumoniae* is one of the recognized species in this family. This microorganism was easily found in each component of ecosystem which includes soil, air, water, animals and humans [(1)]. World wild Due to drug resistance this organism is resulting in high mortality and morbidity. This organism is a Gram-negative, rod-shaped, lactose-fermenting bacillus and encapsulated bacterium with polysaccharide capsule [(2)]. They produces large, smooth ,elevated and very mucoid colonies when grow on a solid medium such as Mac Conkey medium and pink in color because lactose fermention [(3)]. It is a non-motile commensal normal flora of the human gastrointestinal tract which widespread in the environment [(4)].

Klebsiella pneumoniae is opportunistic pathogen in humans which is facultative anaerobe and mainly found in skin, mouth, and intestines of humans and animals. Persons with compromised immune systems have great susceptible to become a major cause of community acquired and hospital- acquired infections in humans by this pathogen [(5)]. As this organism responsible for severe pneumonia, septicemia, respiratory infections, infections related surgery and UTIs [(6)]. A large degree of resistance was seen in *Klebsiella pneumoniae* against wide range of antibiotics such as fluoroquinolones, aminoglycosides and β-lactam antibiotics [(7)] becoming an global emerging problem for treatment with reference to the choice of antimicrobial agent forinfection which is acquired by hospital [(8)].

Virulence factor such as capsule synthesis, lipopolysaccharides formation, biofilm formation, iron uptake (siderophores) and fimbriae (types 1 and 3), play amajor role in pathogenesis of K. pneumonia. This virulence factors were encoded by many virulence genes which after their expression in bacteria permit its product for helping on bypass or attack the defense system of animals and responsible for various types of diseases [(9)].

The infection in urinary tract by *Klebsiella* depends on bacterial resistance to immune system and/or elimination by host defense mechanism. These types of infection caused by bacteria that can be specified as a primary attribute, which do not produce single virulence factor but successful colonization and growth in host depends on the various gene products where immune system is impaired or damaged and may not be fully functional [10]. The urinary tract infection caused by *Klebsiella pneumoniae* is depend on the virulence factor such as type 1 fimbriae (pili) which initiates fundamental adherence of bacteria in urinogenital system, but not so effective to initiate or cause an infection to intestine and lung . The important and recognizable virulence factor involved in higher degree of infection in *Klebsiella pneumonia* and other bacteria is Quorum Sensing (QS). QS is responsible for change in community of bacteria for their mobility alteration, Different enzymes production which may help them to invade and establish in tissues of host, toxic compound production, slime and capsule components production and formation of aggregates in the form of flocks and biofilm [11]. Biofilm formation with the help of QS can be recognized as intrinsic drug resistance factor in this types of aggregates makes impermeability in a structure and stop antibiotic to interact with a bacteria [12]. However other mechanism like transmission of resistance genes, increases the expression of efflux pumps, increases antibiotic metabolism, also favors persisted cells [13]. Therefore, it is essential to study the relationship between antibiotic resistance and biofilm formation in *Klebsiella pneumoniae* [14].

Materials and Methods

Collection of urine samples

A total of 160 urine sample were collected from patients suffering from Urinary Tract Infection from both sexes (males and females), during the study period from (September / 2019 –December / 2019) from patients attend in general Baladruz hospital and Al- Batool Teaching hospital.. To confirm these results were interpret on the basis Symptoms of UTI patients. From total 160 samples isolated microorganism were monitored for their identification based on their physicochemical properties including colony characters, Gram nature and biochemical using Analytical Profile Index system. (Forbes, 2007).

Antimicrobial Susceptibility Testing

It was isolated 24 samples of *K. pneumoniae* which were monitored to check antibiotics sensitivity testing using the Kirby- Bauer method formerly called as Disc diffusion method. The experiment was performed as per CLSI (Clinical and Laboratory Standard Institute guidelines) protocol, formerly recognized as NCCLS (National Committee for Clinical Laboratory Standards).

Molecular methods for *Klebsiella Pneumoniae* detection

After DNA extraction from urine samples, all isolates of *k. pneumoniae*, checking this with PCR using identified gene. This gene was amplified by using *16SrRNA* specific primer. (27 F AGAGTTTGATCCTGGCTCAG), (1492 R TACGGTTACCTTGTTACGACTT).

Effect of antibiotics on biofilm formation ability

To check the impact of antibiotics on biofilm formation ability of all isolates .Wells of sterile 96-well U shaped-bottomed polystyrene microplates were filled with 200 µl of nutrient broth (NB) with bacterial concentration equivalent to McFarland standard no. 0.5. This broths having bacterial suspension was then adjusted with the antibiotic concentration in it, as follows for Ciprofloxacin (CIP) and Cefotaxime (CTX) 0, 250, 500, and 1000 µg/ml concentration and incubated aerobically at 37°C for 24 h. Each bacterium with adjusted antibiotic concentration was tested in four experiments. Control wells were performed by adding the bacteria without antibiotic. To visualize biofilms, the contents of the wells were aspirated and the wells washed three times with 200 µl sterile Phosphate Buffer Saline (PBS) to remove loosely adherent cells. After drying in air, the wells were stained with 200 µl 0.1% crystal violet solution for 15 min at room temperature. Excess stain was rinsed off by placing the plate under running tap water. Thereafter, the plates were dried in a 37°C incubator for approximately 30 min to ensure they were completely dry.Stained biofilm in each well was dissolved in 200 µl ethanol: acetone (80:20) (v/v). The absorbance of dissolved biofilm was measured at 595 nm using a microplate reader whereas for control the absorbance was taken at 600 nm. The reading was taken

and on the basis of that average O.D and SD values for each concentration was checked. Optical density cut-off (ODc) was assigned as an average OD of negative controls + (3× standard deviation (SD) of negative controls).

Results

Sample description

For study the causative microorganism of illness with the symptoms of UTI amongst 160 patients their urine samples were collected and screened for the isolation. The samples were collected from Baladroz and Al-Batool hospitals in Diyala city. Using bacteriology culture test, bio chemical test and API 20 E system and the bacterial isolates from urine were showed in Table 1. In molecular test (16SrRNA gene) 24 patients gave positive.

Table 1: Frequency and percentage of microorganism isolated from urine samples of patients with UTI.

Name of isolated bacteria	No	%
Klebsiella pneumonia	24	15%
E. coli	45	28.12%
Citrobacer sp.	16	10%
Proteus sp.	27	16.87%
Pseudomonas sp.	23	14.37%
No growth	25	15.62%
Total	160	100%

Antibiotic susceptibility of *Klebsiella pneumoniae*

The results of this study showed that the highest percentage of inhibitory activity of antibiotic against *K. pneumoniae* was for impenem (79.2%), tobramycin (79.2%) and chloramphenicol (66.7%), while the lowest percentage was for piperacillin (12.5%).The highest percentage of antibiotic resistance by *K. pneumoniae* was for piperacillin (70.8%), cefotaxime (100%) and ciprofloxacin (100%), while the lowest percentage was for impenem (8.3%). The differences between antibiotic resistance was high significant ($p<0.05$).

Table 2: Sensitive, intermediate and resistance of *K. pneumoniae* isolates.

Antibiotics	Sensitive		Intermediate		Resistance		P value
	NO.	%	NO.	%	NO.	%	
Ampicilln-sulbactam (20 mug/ ml)	13	54.2%	4	16.7%	7	29.2%	0.07
Impenem (10 mug/ ml)	19	79.2%	3	12.5%	2	8.3%	0.001***

Cont... **Table 2: Sensitive, intermediate and resistance of *K. pneumoniae* isolates.**

Nalidixic acid (30 mug/ ml)	10	41.7%	2	8.3%	12	50.0%	0.03*
Piperacillin (100 mug/ ml)	3	12.5%	4	16.7%	17	70.8%	0.001***
Tobromycin (10 mug/ ml)	19	79.2%	1	4.2%	4	16.7%	0.001***
Nitrofurantion (300 mug/ ml)	12	50.0%	4	16.7%	8	33.3%	0.14
Chloramphenicol (30 mug/ ml)	16	66.7%	1	4.2%	7	29.2%	0.001***
Tetracycilln (30 mug/ ml)	14	58.3%	0	0.0%	10	41.7%	0.41
Ciprofloxacin (5 mug/ ml)	0	0%	0	0%	24	100%	0.0001****
Cefotaxime (30 mug/ ml)	0	0%	0	0%	24	100%	0.0001****
P value	0.007**		0.55		0.008**		

Detection of *16SrRNA* of *K. pneumoniae*

All the isolates (24) were exposed to molecular detection through PCR amplification of *16SrRNA* by 16S-R and 16S-F primers. Results of the amplification of 16srRNA gene of *K. Pneumoniae* are shown in Figure 1.

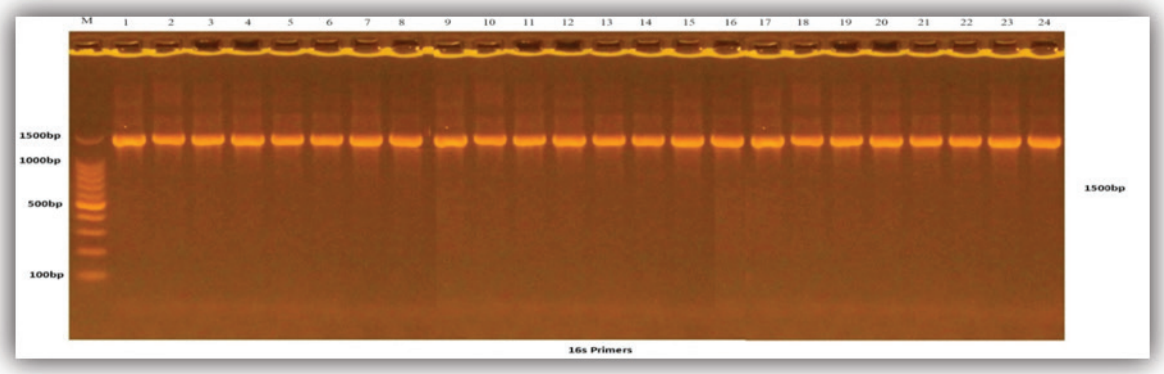

Figure 1: Agarose gel electrophoresis of DNA from urine samples directly showing PCR products for 1500 bp of 16SrRNA gene when compared to the molecular ladder (1500-100).

Biofilm and different concentrations of Ciprofloxacin antibiotic

Data of current study show the comparison between optical density (OD) value of biofilm at concentrations (0, 250, 500 and 1000 mg /ml) for Ciprofloxacin or cefotaxime antibiotic, where the study showed there is high significant differences between optical density (OD) value of biofilm at all concentrations (250, 500 and 1000 mg / ml) compared with OD value in zero order ($p<0.05$) (Figure 2).

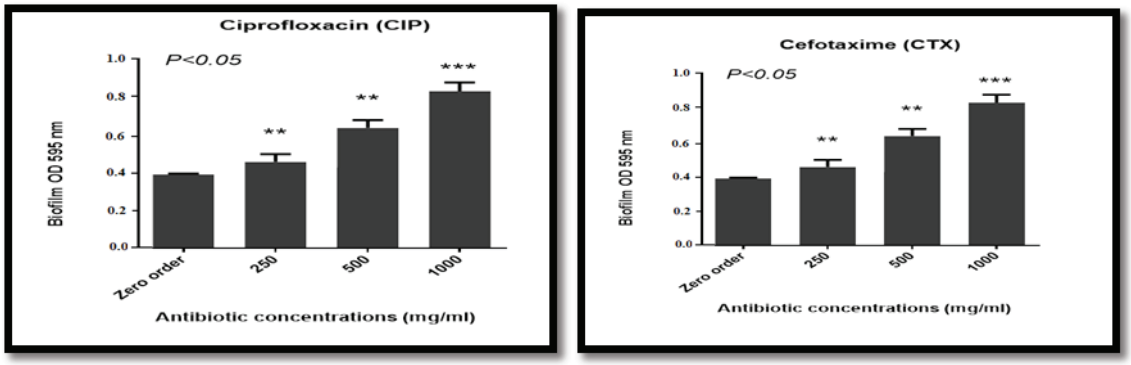

Figure 2: Comparison among biofilm formation at different concentrations of ciprofloxacin or cefotaxime.

Discussion

Klebsiella pneumoniae are the microorganism having potential to cause variety of illness and diseases which commonly includes urinary tract infections, community and nosocomial pneumonia and bacteraemia [15].This microorganism was also seen to be responsible for 'pyogenic liver abscess' (PLA), from last ten year. *K. pneumoniae* infection, was also responsible to cause endophthalmitis, meningitis and necrotizing meningitis in Asian countries including Hong Kong, Singapore, Korea and Taiwan [16].

Klebsiella pneumoniae antibiotic resistance could be recognised a dangerous problem as this organism is associated with different type of deadly diseases including UTIs [17]. The resistance against antibiotic was observed in two different ways extended spectrum β-lactamases (ESBLs) and Expression of carbapenemases. From this the ESBLs One mechanism involves the expression of in which bacteria resist some lactum containng antibiotics but especially monobactams and cephalosporins but carbapenemases could posseses ability to act on almost all reported β-lactams [18]. Similar type of finding were observed in this studies where least amount of resistance was observed in Impenem and Amphicillin after that amphicillin- sublactum was promising for the inhibition of pathogens and in terms of resistance and it is show least resistance against *Klebsiella*. Cefotaxime was shown to have less effect on *klebsiella* in terms of inhibition but they have higher degree of resistance with in all cell wall inhibitors [19]. In this studies it was observed that the cefotaxime and ciprofloxacin were have high resistance against this bacterium.

In these studies bacterial genomic samples were extracted and amplified by using PCR with some of the marker primer. All isolates (24) were subjected to molecular identification through PCR amplification of *16SrRNA* using K 16S-F and K 16S-R primers. The results showed that the amplified fragments were about 1500 bp. All (24) isolates gave positive results (1500 bp bands), and identified as *K pneumoniae*.

The formation of biofilm is somewhat a tedious process having no of steps involved in it firstly there is a adhesion of the microorganism to the substratum by using different adherence factors, microorganism then in second stage producing a microcolony after succeeding in adhesion, microcolony enlarges a become in the form of matured biofilm in the third stage, at the last and fourth stage some of these bacteria were released in the form of free planktonic cells from biofilm and hence if the success to bound another place initiate new cycle for the formation of biofilm. The biofilm formation is one the important reason for the drug resistance and they are also proposed to be less susceptible to killing by host-defense mechanisms. The drug resistance mechanism

by the biofilm is due to various factors includes immpermeability of antimicrobial agent through matrix produced by organism, and gene transfer. Investigations into bacterial-biofilm formation on solid surfaces has been a major focus of research into bacteria pathogens over the last decade [20].

Conclusion

The most bacterial isolates were considered as multidrug resistant, and the clinical isolates were resistant to most antibiotics (except imipenem and tobramycin) and the high ratio of resistance to cefotaxime and ciprofloxacin in clinical isolates. Amplification of *16SrRNA* of *K pneumoniae* confirmed the identification of this bacterium. Polymerase Chain Reaction (PCR) for *K. pneumoniae* detection considered relatively rapid, cost-effective, and easy application and repeatable. Biofilm formation in bacteria using microtiter plate and ELISA reader more frequent than other methods.

The research is going on for the elucidation of the gene involved in the production of biofilm and making their libraries to study different genes playing important role in biofilm formation and use of mutant to study their expression. On the basis of obtained from the experiment biofilm formation assay it was seen that all the isolates which are resistant to antibiotic such as Ciprofloxacin (CIP) and Cefotaxime but in presence of antibiotics also were increased their ability of biofilm formation.

Ethical Clearance: The project of this study was taken from the ethical committee of College of Medicine/ University of Diyala.

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Conflict of Interest: NIL

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Study the Effect of Poly Aromatic Hydrocharbons by Using Biochemical Tests in Chicken Embryos

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Abstract

The major risk of polycyclic aromatic hydrocarbons (PAHs) in all ecosystems originates from human race activity such as petroleum refining and other industrial processes. PAHs cause several effects at low doses in animals in different ecosystem. The aim of this study was to assess the treatment of AL-Dura refinery wastes before being discharged to the river by studying their effects on the liver and renal functions in chicken embryos which used as a model exposed *in vivo*. One hundred and twenty local eggs were incubated horizontally at 37.5±0.5˚C with a relative humidity of 65% in an egg incubator. Sixty eggs were used for hatchability study, were as other sixty eggs were used for biochemical analysis. On day 15th of incubation, the eggs were randomly divided into three different treatment groups and marked for identification. The first A and second group B were exposed by injections with the residues of AL-Dura refinery (after treatment) and at the concentration of (2, 4) ppm respectively and 0.2 ml / egg into the air cell with sterile siring, then sealed with melted paraffin. Control group was injected with Olive oil. On day 18th of incubation the samples of blood were collected from embryos. Liver function was measured using a biochemical assay with diagnostic kits of specific enzymes (ALT, AST, and ALP). Ceratinine and urea level were measured to asses renal function. The result showed a signenificant increase p≥ .05in the level of liver enzymes (ALT, AST), ceratinine and urea by increasing the exposure dose, were as there is a decrease in the level of ALP with the increasing of exposure dose. Hatchability rat were significantly ($p < 0.05$) decreased with dose exposure increase. Mortalities in the chicken embryos of all groups showed dose-dependent relationship. That shows there are a clear effect of oil waste on liver and renal function, and an indication of the inefficient treatment of these waste before being discharged to the river.

Keyword: *PAHs, chicken embryos, ALT, AST, ALP.*

Introduction

PAHs are well known to be toxic to avian [1] .Any toxic substances in nature can cause different changes in organisms include cellular, morphological, teratological and biochemical. When animals exposure to PAH at low doses that causes several effects related with immune and metabolic effects[2].

There is a comprehensive studies addressed the effects of PAHs on adult animals, other wise few literatures have describe the effects of PAHs on embryonic and first life stage in animals. These early developmental stages require less volume test and space that allowed using large numbers of test organisms and replicates and provide powerful statistical analysis of the results besides their sensitivity [3] .Toxicity tests with early live stage of organisms are often used to assess the toxic potential of substances and environmental samples.

For this reason, the chick embryo model is an excellent alternative to the much more expensive mammals for toxicological studies, and it is a useful tool when a study on the target species is difficult or inapplicable [4].

Chick embryos were used to detect the teratogenicity effects when exposed to BPA during sensitive periods in organogenesis induces oxidative damage and biological membranes and cellular structures damage of the brain,

and kidney, of chicken embryos [5].

Other study demonstrates the sensitivity of the chicken's reproductive system to adverse effects of contaminants commonly found in ground water [6]. Also Arleen [7] found mixed-function oxidase induction due to PAH_S in chick embryos relation with dose-response.

Liver is considered as a main target organ of environmental pollutants and any foreign substances or xenobiotics. [8] The highest capacity of metabolism is present in the liver. The level of liver enzymes in the blood was studied as an indication of dysfunction or damage in the body. The enzyme systems that metabolize PAHs are widely separated in the cells and tissues of humans and animals. For example, liver transaminases (ALT, AST) enzymes are indicators of cell necrosis in the liver tissue .Other researchers were mentioned that injection of BaP and other PAHs intraperitoneal or subcutaneous during the first 15 days of life, are caused liver and lung tumors within half a year [9].

Thus the current study was designed to evaluate the treatment of Al-Dura refinery wastes before they are discharge to the river by studying their effects in chick embryos liver and kidney which used in this study as a model exposed in vivo due to its low cost, easy accessibility and their sensitivity [10].

Material and Method

- Experimental Design

One hundred and twenty fertilized eggs with the average egg-weight of 54.5± 0.5g were purchased from the local market at March of 2019 and assigned randomly into three groups of 40 eggs each for PAHs administration on 15th day.

- Experimental group

Group A: PAHs (2 PPM/egg).

Group B: PAHs (4 PPM/egg).

Control group: (Olive oil)

Eggs were incubated horizontally at 37.5±0.5˚C in an egg incubator with a relative humidity of 65%. After72 hr. of incubation, all eggs was candled to confirm their viability. Dead eggs were removed. On day 15th of incubation, each egg was sterilized with 70% ethanol and egg shell was opened to obtain access to the air cell. Groups A and B were injected by the air sac method [11], administered as single dose of 20µg and 40µg/egg of PAHs respectively. Control group received no PAHs and served as healthy control. All eggs were covered by a wax to ensure the embryo's health until sampling takes place. At embryonic day 18, the egg shell was broken at the air chamber and blood was collected for biochemical tests. Blood were collected from each group by opening the eggs at the wider end. About 0.5 ml of blood was collected from heart of each chick embryo, was dispensed in tightly sterile tubes containing anticoagulant EDTA. Serum was separated from blood by centrifugation at 3,000 R.P.M for 10 minutes at room temperature, and stored at - 20 C until required for biochemical analysis to urea, ceratinine, (ALP), (AST) and (ALT) using commercially available kits. The rest of the injected eggs (20 eggs from each group) were placed in an incubator for hatching, at 19th day of incubation, tray rotation was stopped, and eggs were put in individual baskets and incubated at 37.5 °C and 70% humidity for hatching

Chemicals:

Two stock solutions contained mixed mixture from wastes of AL-Dura refinery dissolved in olive oil were prepared by modifying a procedure described by [12]. Solutions were stored at room temperature, protected from light, until needed for egg injections

Statistical Analysis

Data including embryonic mortalities, and hatchability were calculating by compared differences in ratios in each group. Tukey's test and One-way analysis of variance was applied to assess the significance variance in the biochemical analysis. Data were significantly different from each other considered at a p value ≥ .05.

Result and Desiccation

Hatchability and mortality:

Hatchability of each group, on the fertile eggs basis, was calculated at day 21 of incubation. Increasing in the embryonic mortalities was seen with increases in the dose of PAHs injected. Hatchability rat were significantly ($p < 0.05$) decreased with dose exposure increase table (1).

This decline in the hatch abilities was due to embryonic mortalities and teratogenic effects of PAHs which caused default in the hatching processes in fully developed chick embryos. The results are in agreement with [13] who observed that there are physiological abnormality development risks in the chicken embryo and hatched chicks that could be exposed to Ochratoxin A *in vitro*.

Table (1): Show hatchability% and Mortality% of test and control group

Groups	Total no. of eggs	No. of hatched eggs	Hatchability%	Mortality %
Con.	20	18	90	0
A	20	10	50	5
B	20	8	40	10

Also Albers (**2006**) [1] observed that exposure to PAHs in early life of birds cause's developmental abnormalities, decline in hatching success, and biochemical disturbance. Mortalities in the chicken embryos of all groups of this study showed dose-dependent relationship.

Effect of PAHs on liver function:

The results of control and treated chick embryo liver and kidney biochemical studies were presented in the fig 1and 2. In these results there is a significant increase ($p < 0.05$) in the level of liver enzyme (AST, ALT) and decrease in ALP levels with increasing of exposure dose as shown in fig 1.

The results are in agreement with [14] who mentioned considerable changes in chick embryo compared to the control ($p<0.01$) in some biochemical parameters such as ALT,AST, ALP, and amylase after treatment with MA (meglumine antimonite) .

In the present study, increased serum AST, (47.67±2.8,71.7±3.6,43.13±1.16) iu/l, ALT (9.75±2.4,19.50±4.6,7.33±1.8)iu/l concentration and decrease in ALP (12.1±03,10.0±0.2,14.9±1.1) iu/l levels in the chicks' embryo groups(A,B,and Con.) respectively clarify or explain liver damage [15].

The determination of serum enzymes was a useful quantitative indicator for estimating the extent and kind of liver damage in chicken as well as, many other species that were given toxic substances through feed[16], and air [17].

When liver cell plasma is injured, a variety of enzymes is released into the circulation, that cause increasing in the levels of enzyme in the serum [18].

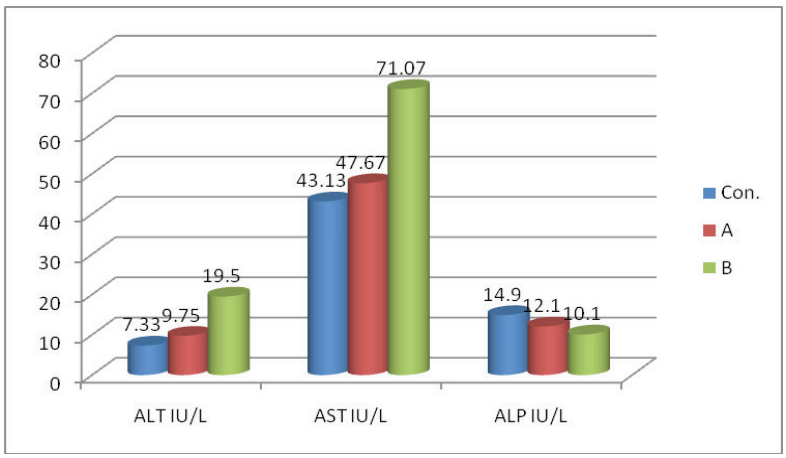

Fig (1): Show the level of liver enzymes (ALT, AST, and ALP) in the serum of chick embryos for groups A, B, and control.

The increase in ALT and AST generated more energy when stressful conditions increase, as a result of increased demand and precursors of carbohydrates to maintain the glycolytic pathway and tolerance in stable levels in the Krebs cycle (Tri Carboxylic Acid) [19].

ALT is found in the cells of various avian tissues and its level varies due to tissue damage. AST is useful for detecting hepatocellular malfunction or disorder and elevation is frequently associated with liver injury.

ALP is an enzyme found in some avian tissues(bone, kidneys, intestine and liver).The increased ALP activity was associated with the Hepatocytes performance due to industrialization increased with an increased biliary pressure , which lead to a blockage of the bile duct due to increase in the concentration of ALP [11].

Changes have been observed in the activity of ALP in tissues, organs and blood of fish when exposed to poisons [20]ALP is playing an important role for energy production during crips cycle (TCA), its help in transport of metabolites across the cell membrane and carboxylic acid cycle [20]ALP is considered as biomarkers for cell toxicity and genetic toxicity [21].

Significant reduction in ALP level in this study is agreement with other researcher which mention that after injection with Benz (k) fluoranthene the alteration in ALP enzyme activity and PCV values was apparent is indicative of the high sensitivity of chick fetuses [22]This result is consistent with studies dealing with the effect of chemical contamination , as the amount of this enzyme decreases compared to the control group .This increase can be adopted as evidence of chemical contamination after chronic exposure.

Effect of PAHs on renal function:

Kidney function in avian species is depended on concentration of urea, uric acid, and creatinine [23]. These three biochemical components and their concentration in peripheral blood have been shown reliable indicators for nitrogen metabolism and renal function [24].

Creatinine and urea values of groups A and B, show significant ($p < 0.05$) differences in their sera concentration (0.41±002mg/dl, 0.61±0.01mg/d for creatinine, 10.66±0.3 mg/d,14.04±0.5mg/dl for urea respectively compared with the values obtained from the chicks embryo belonging to control group (0.27±0.02mg/d for creatinine, 7.06±0.2 mg/dl for urea) as shown in (fig 2).

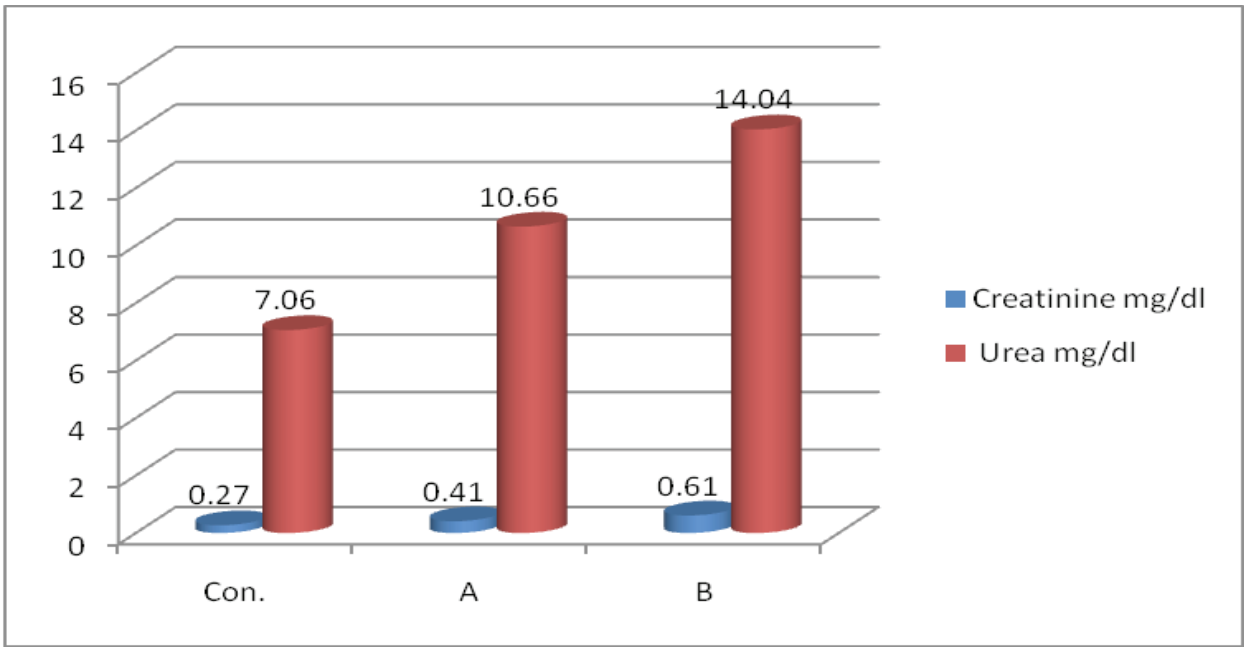

Fig (2): Show the level of Ceratinine and Urea in the Kidney of chick embryos for groups A, B, and control.

These results considered as pathological indicators confirmed the toxicity of PAHs with respect to liver function. Nephrotoxicity also observed by [25].who confirmed that significant increase in ceratinine and serum uric acid levels are indicative of nephrotoxicity in broiler chicken. Nephrotoxic and Hepatotoxic activity of PAHs in different animal species, as indicated by altered serum biochemical profile, is well established.

In the present study, increased serum ALT,AST,concentration and decrease in ALP levels are indicative of liver damage. In the same way, increased urea and ceratinine levels may be due to nephrotoxic effects of *in vivo* inoculation of PAH.

Conclusions

Results showed that exposure to PAHs increased the intensity of these changes such as increased in the level of hepatic enzyme (ALT, AST) and decrease in ALP which means that there is an effect on liver function, as well as there are an rise in ceratinine and urea level which effect on kidney function. That shows an indication of the inefficient treatment of refinery inputs of hydrocarbons into the river before being discharged.

These findings also suggested that there are teratogenic and toxicological risks in the developing chicken embryos that could be exposed to PAHs in *vivo*.

This study shows that the chickens' embryo is a suitable organism to perform early life stage tests. The high sensitivity of chickens' embryo to PAHs supports their use for ecological risk estimations in areas around the river under the influence of refinery activities or other inputs of hydrocarbons into the river.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of Ministry of science and technology in Iraq

Conflict of Interest: The authors have read the manuscript and declare that the work is ready for submission and there is no conflict of interest.

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Study of Epstein - Barr virus Infection in Relation to the Immunohistochemical Expression of Bcl-2 gene in Tissues of Patients with Adenocarcinoma of the Colon

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Abstract

Background: EBV infection in tissue micro-environment is challenged by the precisely regulated survival and apoptosis mechanisms. Abnormal bcl-2 proto-oncogene expression in colonic carcinomas allows accumulation and propagation of these genetically altered cells.

Objective: To analyze the relevant concordance of BCL-2 gene , EBNA1 s and LMP-1-EBV expression in issues from a group of Iraqi patients with colonic adenocarcinomas.

Patients and Methods: One hundred (100) tissue biopsies, belonged to (40) patients with colorectal cancers, (40) patients with benign colon tumors, and (20) apparently normal colorectal control tissues, were enrolled in this study. The detection of EBNA1 s and LMP-1-EBV as well as BCL-2 was done by immunohistochemistry (IHC).

Results: The positive-IHC results of EBNA1 detection in 40 tissues with malignant colonic tumors were 57.5%, while in 40 colonic benign tumors tissues were 32.5 %. In those apparently healthy colonic control tissues, EBNA1 detection was 20%. The positive results of LMP-1 –EBV -IHC detection where 45 % from colonic carcinoma group, while the percentage of benign colonic tumors was 30%(12 out of 40). Lastly, in AHC was15% showed positive signals. Malignant colonic tumors showed 47.5% positive results of Bcl-2-IHC detection while benign colonic tumor tissues showed 40% positive bcl-2-IHC reactions and in the colonic control tissues was 20%.

Conclusion: It concluded from the present high rates of EBV infection in colonic adenocarcinoma along with the concordance bcl-2 expression that they could played an important role in the development and progression of both malignant and benign colonic tumors in our group of Iraqi patients.

Keywords: *malignant and benign colonic tumors, Colonic carcinogenesis; EBNA1; LMP-1-EBV; Bcl-2; Immunohistochemistry.*

Introduction

Colonic cancers are one of the major cancer problems worldwide that ranking the third most common cancers and third leading cause of cancer-related deaths in both men and women. Worldwide, the annual newly diagnosed cases are approximately 1.36 million, constituting about 9.7% of all cancer cases. WHO stated that approximately 55% of colonic cancers were reported in more developed regions [(1)].

Previous studies found that environmental conditions, lifestyles, sequential genetic changes and viral agents are the major risk factors for colorectal cancers [(2)]. Among others, microbial-epithelial interactions have proposed as a potential oncogenic trigger for the development of colonic cancers [(3)].

(In contrast to gastric carcinoma and MALT-type lymphoma where both were strongly associated with *Helicobacter Pylori* infection , a direct causal

link between various microbial infections (as, *Helicobacter pylori, Streptococcus bovis, Escherichia Coli, Bacteroides*, JC virus, cytomegalovirus, human papillomavirus, Epstein-Barr virus (EBV)) and CRC has not been established yet [4].

Previous data regarding the role of EBV expression in colorectal cancer are sparse as well as contradictory and without a clear evidence of the active role of EBV in colorectal carcinogenesis (4). In addition, the EBV detection assays vary significantly across these studies, having a significant impact on the obtained results [4,5].

The expression patterns of the 6 EBV nuclear antigens (EBNA1, -2, -3A, -3B, -3C and -LP), 3 latent membrane proteins (LMP1, -2A, and -2B), and multiple non-coding RNAs (EBERs and miRNAs) define the distinct latency programs linked to the types of cancers associated with EBV infection

Earlier studies revealed that LMP1-mediated upregulation of Fascin was depending on the NF-κB, where NF-κB and Fascin have both contributed to an invasive migration of LMP1-expressing lymphocytes [6,7]. It is evident, and based on these studies, that EBV infection and Fascin expression have a strong correlation with malignancy progression in several human carcinomas especially moderately- and poorly-differentiated grades [7].

The deregulation of Bcl-2 family proteins can cause various pathological consequences including the development of cancer. The anti-apoptotic protein Bcl-2 (B-cell lymphoma-2) is an important member in that family that controls the release of pro-apoptotic factors which are responsible for the activation of caspases (by stabilizing the mitochondrial outer membrane) [8,9]. An aberrant expression of Bcl-2 has been implicated in several cancers, including CRC[5]. However, the data obtained from different researches were often in disagreement [9].

Neither the function nor the prognostic value of Bcl-2 expression in patients with CRC is clear. This was evidenced from the contradictories in data from many studies that have found expression of Bcl-2 to correlate with better prognosis [9,10], while many other investigators demonstrated that Bcl-2 was a poor prognostic marker for cancer patients [11] or Bcl-2 expression in CRC has no prognostic significance [12].

Under physiological conditions, the pro-apoptotic members (like Bax and Bak) are sequestered and inhibited by anti-apoptotic relatives (like Bcl-xL, Bcl-2 or Mcl-1). It was found that anti-apoptotic proteins are overexpressed in different cancers, supporting the avoidance of cell death. In CRC, high expression of these anti-apoptotic members has been shown to correlate with lower tumor differentiation [13].

Materials and Methods

This research was designed as a retrospective one. The study groups collectively comprised of (100) colonic tissue which have processed as formalin-fixed and paraffin embedded blocks and retrieved from the archives of the period from 2014 till 2020 from many hospitals and private histopathological laboratories in Baghdad, Babylon, Kerbela, and Al-Najaf provinces and enrolled in this study as both patients and control samples from patients whom their age ranged from 17 to 75 years.

These blocks included 40 biopsies from patients with colonic cancers, 40 biopsies from patients with benign colonic tumors and 20 biopsies from patients with apparently normal colonic tissues and used as an age- and sex- matched control group.

Trimming process and tissue sectioning of the tissue blocks were conducted in the histopathological department of Teaching laboratories at the Baghdad Medical City Complex where one paraffin embedded (4 mm) thick-tissue section was stained with hematoxyline and eosin, while other tissue sections were stuck on positive- charge slides for EBV-EBNA 1 and LMP 1 antigens as well as Bcl-2 detection by using Mouse and Rabbit Specific HRP/DAB immunohistochemistry detection kit (purchased from Abcam, UK). The details of techniques for performing IHC reaction as well as these antibodies conducted according the instructions of that manufacturing company and done in the Research Laboratories of the Clinical Communicable Diseases Research Unit, at College of Medicine, University of Baghdad as well as in the Advanced Microbiology Research Laboratory at College of Science, University of Babylon.

The specification of this IHC kit after proper use of the detection system gives an intense brown signals at the specific sites of the expressed proteins in tissues with a positive test. These signals evaluated under light microscopy using × 100 lens for counting the positive cells, where given intensity and percentage scores based on the counted in 10 different fields of 100 cells in each sample where their averaged percentage within 10 fields was determined and given a scale of 0-3 for relative intensity where 0 corresponding to no detectable IHC reaction, and 1, 2, 3 equivalents to low, moderate, and high intensity, respectively. Reactions assigned to one of the following percentage scores where 1%–25% = score 1, 26%–50% = score 2 and r > 50% = score 3.

Statistical Analysis

T test, ANOVA test, and Chi square applied by using the SPSS program (version-22) and Excel application.

Results

I. Study Group Characteristics

The archival specimens collected in this study were related to patients with colonic tumor whom ages were ranged from seven years to eighty five years. The mean age of patients in colonic carcinoma (CC) (56.7 years) was higher than the mean age of the benign colonic tumors (BCT) (52. 3 years), whereas the mean age of apparently healthy control(AHC) was (49.8 years). The distribution of gender frequency in this study where 52.5%, 55% & 60% were males in CC, BCT & AHC, respectively; while, 47.5% ,45%& 40% were females in CC, BCT & AHC, respectively. This study has revealed that well differentiated carcinomas were seen in 47.5% of CC group, while 37.5% and 15% of CC have moderately and poorly differentiated grade carcinomas, respectively. Anatomically, 6 cases of colonic carcinoma were in the cecum (15%), 11 cases in the transverse colon (27.5%), 2cases in the ascending colon (5%), 2 cases in the descending colon (5%), 11 cases in the sigmoid (27.5%), and 10 cases in the rectum (25%) (Table 1).

Table 1. The Study Group Characteristics

		Malignant colonic tissues (CC), N= 40 (%)	**Benign colonic tissues(BCT), N = 40(%)**	**Healthy colonic tissues (AHC) N=20(%)**
Age	Mean of Age (Years)	56.7	52.3	49.8
	Range of Age	17 – 85	24 -74	38 – 75
Gender	Male	21 (52.5)	22 (55)	12 (60)
	Female	19 (47.5)	18 (45)	8 (40)
Grade	Poorly	6 (15)		
	Moderately	14 (37.5)		
	Well	18(47.5)		

Cont... **Table 1. The Study Group Characteristics**

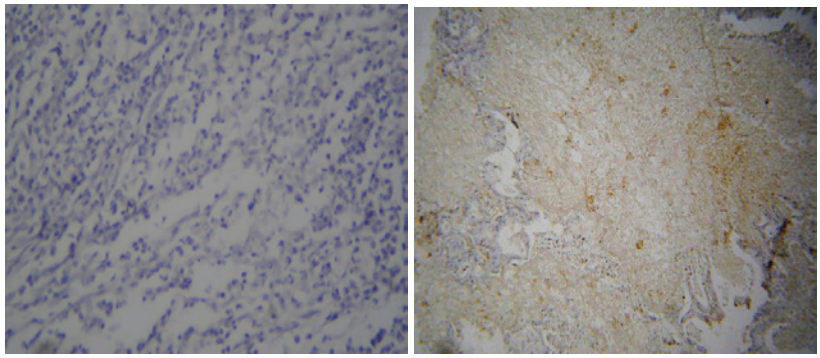
Site of tumors	Cecum	6 (15)		
	Transverse colon	11 (27.5)		
	Ascending colon	2 (5)		
	Descending colon	2 (5)		
	Sigmoid	11 (27,5)		
	Rectum	10 (25)		

II. EBV-EBNA1 - Associated Colonic Tumors:

Twenty- three out of forty (57.5%) tissues with **colonic** carcinoma showed positive immunohistochemical (IHC) reactions for EBV-EBNA1 in this study. The highly signal scores were found in moderate grade score (Score II) (25%; 10/40). While the highly intensity signals were found in weak (Intensity I) 22.5% (9/40) (Table 2 and Figure 1). The benign **colonic** tumors group revealed 32.5% positive signals which represented 13 out of 40 tissues in this group. The highly signal scores was found in low grade score (Score I) (17.5% ; 7/40).While the highly intensity signals was found in moderate (Intensity II) 17.5% (7/40) Lastly, 20% (4 out of 20 cases)of **colonic** control tissues group presented positive signals for EBV-EBNA1 –IHC test. However, in comparison to the percentage of EBNA1 –IHC in healthy **colonic** control group as well as in the group of benign **colonic** tumors, the differences between the percentages of EBNA1 –IHC in tissues of patients with **colonic** carcinoma and each of these groups are statistically significant (P value = < 0.004).

Table 2: The percentage of EBNA1 –IHC score and intensity signaling in colonic tumors

EBNA1 –IHC		**Colonic carcinoma (n=40)**		**Benign colonic tumors (n=40)**		**A.H control (n=20)**		**P-value (χ^2test)**
		No.	%	**No.**	%	**No.**	%	
Negative		17	42.5	27	67.5	16	80	0.004
Positive		23	57.5	13	32.5	4	20	
Signal Score	I	6	15	7	17.5	2	10	0.007
	II	10	25	4	10	1	5	
	III	7	17.5	2	5	1	5	
Signal Intensity	Weak	9	22.5	5	12.5	3	15	0.009
	Moderate	8	20	7	17.5	1	5	
	Strong	6	15	1	2.5	0	0.00	



A **B**

Figure (1): Colonic Carcinoma Showing The Results of Immunohistochemistry Staining Protein Over Expression Using Biotinylated Anti –EBNA1 Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxylin (Blue).A- Colonic cancers with negative EBNA1-IHC reactions (40X).

B- Positive EBNA1-IHC reaction with low score and high signal intensity (40X).

III. The Results of LMP-1 –EBV -IHC Signal score and intensity signaling in colonic tumors.

Table (3) shows the positive results of LMP-1 –EBV -IHC detection where 45 % (18 out of 40 tissues) from colonic carcinoma group showed positive signals including 27.5 % (11 out of 40 tissues) in the low score (score I), while the percentage of benign colonic tumors was 30%(12 out of 40) showed positive signals including 12.5% (5 out of 40 tissues) in the moderate score (score II) .Lastly, in AHC was15% (3 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the low score (score I) (Fig. 2).

The percentage of LMP-1 –EBV expression in cells that were evaluated for the intensity of LMP-1 –EBV -IHC reactions showed 30 % (12 out of 40 tissues) in the weak intensity(I), while the percentage of benign colonic tumors was (30%) showed positive signals intensity including 17.5 % (7 out of 40 tissues) in the moderate score (score II). Lastly, in AHC was15% (3 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the weak score (score I) Fig. (2). Statistically, significant differences were noticed between scoring and intensities of tissues at ($P<0.05$) in colonic tumors group.

Table 3: The percentage of LMP-1 –EBV-IHC score and intensity signaling in Colorectal Tumors

LMP-1 –EBV -IHC		Colonic carcinoma (n=40)		Benign colonic tumors (n=40)		A.H control (n=20)		P-value (χ2test)
		No.	%	No.	%	No.	%	
Negative		22	55	28	70	17	85	0.007
Positive		18	45	12	30	3	15	
Signal Score	I	11	27.5	4	10	2	10	0.04
	II	5	12.5	5	12.5	1	5	
	III	2	5	3	7.5	0	0.00	
Signal Intensity	Weak	12	30	4	10	2	10	0.03
	Moderate	5	12.5	7	17.5	1	5	
	Strong	1	2.5	1	2.5	0	0.00	

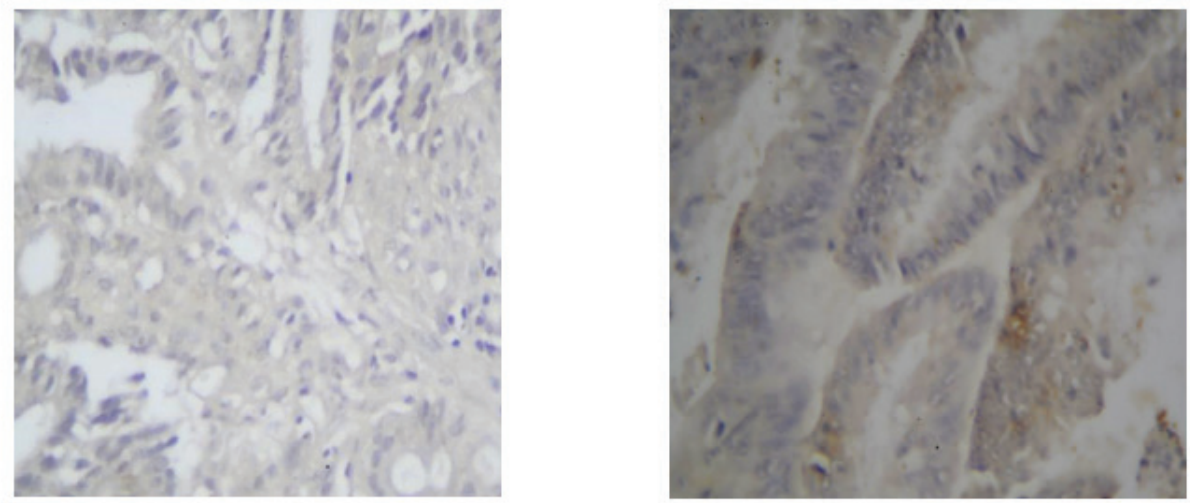


Figure (2) : Infiltrative Colonic Carcinoma Showing Results of Immunohistochemistry Staining Protein Over Expression Using Biotinylated Anti – LMP-1 –EBV Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Heamatoxylin (Blue).
A- Colonic Cancer with negative staining for LMP-1 –EBV (40x). .
B- LMP-1 –EBV -IHC-reaction with high signal score and strong signal intensity (40x).

IV. The Results of BCL2 -IHC Signal score and intensity signaling in colonic tumors.

Table (4) shows the positive results of bcl-2 -IHC detection where 47.5 % (19 out of 40 tissues) from colonic carcinoma group showed positive signals including 20 % (8 out of 40 tissues) in the low score (score I), while the percentage of benign colonic tumor was 40%(16 out of 40) showed positive signals including 17.5% (7 out of 40 tissues) in the moderate score (score II). Lastly, in AHC was 20% (4 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the low score (score I) (Fig. 2).

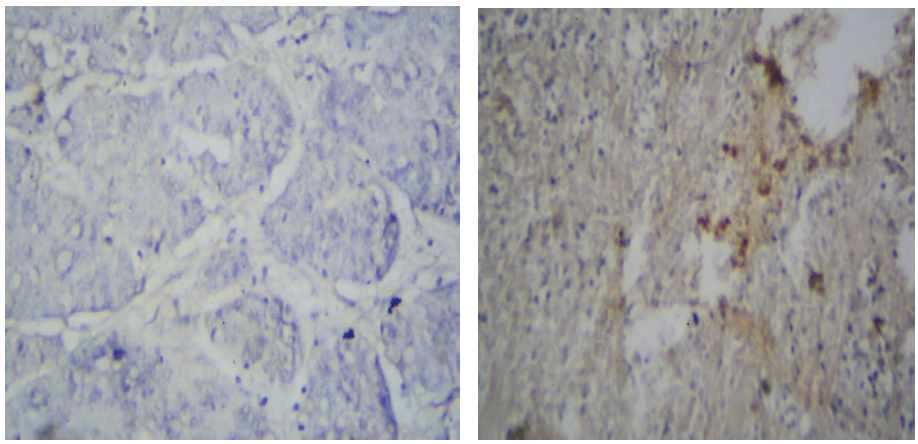
The percentage of bcl-2 expression in cells that were evaluated for the intensity of bcl-2 -IHC reactions showed 25 % (10 out of 40 tissues) in the weak intensity(I), while the percentage of benign colonic tumors was (40%) showed positive signals intensity including 20 % (8 out of 40 tissues) in the moderate score (score II). Lastly, in AHC was 20% (4 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the weak score (score I) (Figure 2). Statistically, significant differences were noticed between scoring & intensities of tissues at ($P<0.05$) in colonic tumors group.

Table 4: The percentage of BCL-2-IHC score and intensity signaling in colonic tumors

BCL-2 -IHC	Colonic carcinoma (n=40)		Benign colonic tumors (n=40)		A.H control (n=20)		P-value (χ2test)
	No.	%	No.	%	No.	%	
Negative	21	52.5	24	60	16	80	0.008
Positive	19	47.5	16	40	4	20	

Cont... **Table 4: The percentage of BCL-2-IHC score and intensity signaling in colonic tumors**

Signal Score	I	8	20	6	15	2	10	0.009
	II	7	17.5	7	17.5	1	5	
	III	4	10	3	7.5	1	5	
Signal Intensity	Weak	10	25	3	7.5	2	10	0.007
	Moderate	6	15	8	20	1	5	
	Strong	3	7.5	5	12.5	1	5	



A **B**

Figure 2: Infiltrative Colonic Carcinoma Showing The Results of Immunohistochemistry Staining Protein Over Expression Using Biotinylated Anti – bcl-2 Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxylin (Blue).
A- Colonic Cancer with negative staining for bcl-2 (40x).
B- bcl2 -IHC-reaction with high signal score and strong signal intensity (40x).

V. Spearman's Rho Statistical Testing of Age, Grade, EBNA1 –EBV; LMP1-EBV and BCL-2- IHC to Evaluate the Studied Markers in Colonic Tissues.

A strong positive relationship (with highly significant correlation) was found between **EBNA1 –EBV; LMP1-EBVand BCL-2-** markers in **colonic** tissues (r = 0.648, P = 0.008) and (r = 0.564, P = 0.006), respectively. Similarly, there is a strong positive relationship (with highly significant correlation) between **BCL2** and grade of **colonic** tissues (r = 0. 683; p= 0.009). However, there are no significant correlations among EBV and other markers (as illustrated in table 5).

Table 5: Spearman's Rho Statistical Testing of Age, Grade, EBNA1 EBV; LMP1--EBV and *BCL-2* -IHC to Evaluate the Studied Markers in Colonic Tissues.

Spearman's rho		Age groups (years)	Grade	EBV-EBNA1	EBV-LMP-1
Grade	r	-0.149			
	P	0.377			
EBV-EBNA-1	r	0.070	0.123		
	P	0.862	0.512		
EBV-LMP-1	r	0.080	0.153		
	P	0.794	0.449		
BCL-2	r	0.165	0.683	0.648	0.564
	P	0.371	0.009*	0.008*	0.006*

*Correlation is highly significant (P<0.01).

Discussion

CRC, like other human carcinomas, is a complex multistep process that involves early steps of tumor cell invasion within the microenvironment and then an eventual entering the bloodstream. Consequently, specific genetic and epigenetic changes are required to fulfill these metastasis steps [14]. Several studies had reported that the Fascin gene (produces an actin-binding protein that is upregulated in cancer cells specially during epithelial to mesenchymal transition) plays an important role in the progression of human carcinomas, including CRC and has correlated with poor prognosis in human CRC [6, 15,16].

In the present study, twenty- three out of forty (57.5%)and 45 % (18 out of 40 tissues) tissues with colonic carcinoma showed positive immunohistochemical (IHC) reactions for EBV-EBNA1and EBV-LMP-1, respectively.

Epstein-Barr virus by alternating its envelop proteins has exhibited a dual tropism to both B and epithelial cells. As such, this viral infection commonly associated with Burkitt and Hodgkin lymphoma (as B-cell lymphomas) as well as nasopharyngeal, gastric and probably rectal carcinomas (as an epithelial malignancy [17]). An activation of clearly blown colorectal cancers by Epstein-Barr virus can result by the viral wide- ranged effects that result in cell damage by various processes such as metabolism, cell cycle regulation, apoptosis, protein synthesis, angiogenesis and cellular connections [18].

Boguszakova and associates study on patients with colonic adenocarcinoma and adenoma failed to show EBV DNA in the investigated biopsies [19]. Similar no positive findings were reported by Yuen et al. and Cho et al. who tested the presence of EBV in colorectal carcinomas from Chinese patients using ISH for EBERs [20, 21].

In Karpinsky et al. study on 186 colorectal cancer specimens by PCR, 19% positive samples for EBV-DNA found. (16). In addition, Samaha et al. and Kon et al. (10, 12) concluded that rectal lymphoepithelioma-like carcinoma probably related to EBV. However, Yanai and co-workers by using ISH for EBER-1 found that

EBV infection may be related to 60% of IBD diseases such as Crohn's disease and ulcerative colitis cases [22].

During EBV latent infection enhances the oncogenic properties via compromising immune system and enhancing chronic inflammatory microenvironment. This virus encodes 6 viral nuclear antigens (EBNA-1, -2, -3A, -3B, -3C and LP), 3 viral latent membrane proteins (LMP-1, -2A and -2B), 2 small viral non-coding Ribonucleic acids (EBER-1 and -2) and rightward transcripts of BamHI-A, where the main function of these proteins is to evade immune surveillance and to play as "oncogenes" in EBV - infected cells, by silencing the anti-EBV effect of INF-γ and modulating anti-viral cytokines (TNF-α, IL-1β and IL-6) and mimicking the IL-10 characteristics [23, 24].

Bcl-2 is well documented as a key inhibitor of apoptosis that play a major role to maintain normal balance between both apoptosis and cellular survival. The defects in the apoptotic pathway in mitochondria are tightly related to carcinogenesis. In addition, abnormal activation of Bcl-2 has been implicated for the CRC evolution, yet, and to date, the exact role of Bcl-2 expression as well as its prognostic value in CRC patients has not been consistently established [25].

Positive results of bcl-2 -IHC detection were detected in 47.5 % of tissues from colonic carcinoma group, including 20 % with low score (score I), while the percentage of bcl-2 -IHC detection in benign colonic tumor was 40% including 17.5% with moderate score (score II) and in AHC was 20% including 10 % with low score (score I).

One eligible meta-analysis that investigated the prognostic value of Bcl-2 in CRC found that high expression of Bcl-2 has significantly increased survival of CRC patients and this is contradictory to the anti-apoptotic function of Bcl-2, and might be related to the interactions of other proteins enrolled in the apoptotic pathways such as p53(9). Highly expressed Bcl-2 was more frequently correlated to highly differentiated and A/B Ducks' staged in tumors. As such, down-regulation of bcl-2 has been associated with higher risk of malignant transformation of colorectal adenoma, and was proved that lacking of Bcl-2 expression correlated with invasion, metastasis and recurrence of CRC [26].

Many previous studies have tested the prognostic significance of the counteracting expression levels of Bcl-2 and Bax with clinicopathological parameters of colorectal cancers (27, 28), and found that Bax/Bcl- 2 ratio determines the cell susceptibility to apoptosis, where lower ratio lead to resistance of cancer cells to apoptosis result in poor prognosis and more infiltrative growth and tumor progression and aggressiveness [29].

Other study [30], and regarding the location of primary tumors from where resected, have demonstrated a critical role in determining tumor characteristics and prognosis of the cancer.

The finding of [31] study indicated that abnormal bcl-2 gene activation is an early event in neoplastic development or progression of colorectal carcinoma, since higher levels of bcl-2 expression in dysplastic and malignant than non-neoplastic cells were found.

However, several other studies [32,33] reported higher rates of bcl-2 expression in adenoma than carcinoma, indicating an important role of bcl-2 to play in the early stage of the adenoma-carcinoma sequence and being followed by other genetic changes, such as p53 accumulation.

Ethical Clearance: The study was ethically approved from the College of Medicine / University of Baghdad, College of Medicine / University of Babylon and Ministry of Health in Iraq. The patients were asked for their permission to give samples and information.

Source of Funding: Self-funded.

Conflict of Interest: Nil

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A Qualitative Study: Availability of Health Facilities as Part of Supporting the Implementation of the Health Referral System

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Abstract

The referral problem in Southeast Sulawesi is still quite complex. Based on preliminary studies conducted at several health centers, it was found that there were several problems related to human resources, time, facilities and infrastructure. The basic thing that was found was lack of availability of facilities and infrastructure. The results of observations at several Public Health Care found that several other complaints related to the BPJS (Social Security Administrator) referral system that were felt were the lack of facilities in primary health services and some damaged facilities so that cases that should have been handled at primary / secondary services were immediately referred to tertiary hospitals. This research is a qualitative research, The research was conducted at the Public Health Care of Kendari City and Bau-Bau City from February 2020 to completion. aspects of facilities and infrastructure as an important aspect in the implementation of The referral system is still inadequate in terms of the quantity of facilities and infrastructure as well as compliance with the compendium. The referral system is still inadequate in terms of both the number of drugs and the suitability of the formulary.

Keywords**:** *health facilities, referral systems, health services, policies*

Introduction

Realizing access to quality health services is one of the goals of the resolution for developing health financing that was ratified by the World Health Assembly in 20051. According to the World Health Organization (WHO) in 2010, the determining factor for its success is readiness and investment in health services, especially in infrastructure. and health workers who have good ability in primary health services, namely FKTP (First Level Health Facilities)[1]

In the National health insurance (NHI) Era, FKTPs are at the forefront of the health service system. So that the demand for excellent service facilities is important for FLHF. To support these services, the government supports financing through the capitation system. Capitation is a financing system that is calculated based on the number of NHI memberships in the FLHF. However, there are still many challenges to be faced. Funding support and membership are still not optimal. The high public demand for health services is not supported by a good financing system. As a result, every year, the financing for NHI experiences a deficit. The impact is that health services are disrupted. There are many cases where health facilities are unable to provide good services due to the lack of supporting infrastructure for services[2]

First Level Health Facilities (FLHF) as gatekeepers in the NHI era are required to provide comprehensive primary services with quality health services. The National Health Insurance Program has made access to health services more open and increased utilization. In the NHI era, health services used a tiered referral system with the division of tasks and functions of each health service provider[3]

The referral system is organized with the aim of providing quality health services, so that service objectives are achieved without having to use expensive costs. This is called effective and efficient. Efficient also means reduced waiting time in the referral process and reduced unnecessary referrals because it can actually

be handled at the original health service facility, either with the help of the latest technology or appropriate technology or low cost technology, which can still be accounted for[4]

On January 1, 2020, BPJS Health contributions officially increase, this is in accordance with Presidential article 34 of Presidential Decree No. 75/2019, mentions contributions for participants who are not wage workers and non-workers according to the old provisions of article 34 of Presidential Decree No. 82/2018 for Class 1 Rp. 80,000 / person / month, class II Rp. 51,000 / person / month and class III Rp. 25,500 / person / month, now based on article 34 of the Presidential Decree No. 75/2019 rose. Class 1 Rp. 160,000 / person / month, class II Rp. 110,000 / person / month and class III Rp. 42,000 / person / month. The cause of the increase in BPJS health output is due to a deficit. The health insurance fund finances run a deficit in which the amount of the obligation to pay health service claims is higher than the ability of BPJS health to collect revenue from participant contributions. The main source of the NHI program deficit is insufficient contributions to finance the program, in addition to the challenges of collecting contributions from informal sector participants and controlling health service costs. One of the policies taken was to improve the referral and return referral system[5]

The number of visits in 17 municipalities in Southeast Sulawesi in the last 3 years experienced an increase in the number of visits at both the Nursing and Non-Nursing public health center. In 2016, there were 590,372 visits, then increased in 2017 to 947,008 visits and increased again to 1,347,967 visits. The increase in the number of visits is also proportional to the increase in the number of non-specialist cases referred to in the last 3 years, namely in 2016 of 2,283 non-specialized referral cases, in 2017, which was 4,842 cases then in 2018 it increased to 6,137 cases[6]

Kendari City and Bau-Bau City are 2 cities in Southeast Sulawesi, consisting of 15 public health center for Kendari City and around 17 public health center for Bau-Bau City. From referral figures in Southeast Sulawesi 2 this area has a fairly high referral rate at the public health center Nursing for non-specialized cases compared to other districts, namely 278 referrals for Bau-Bau City and 130 referrals for kendari city[7]. The implementation of a system will not run well if its implementation is not in accordance with the provisions of the policy or guidelines. One of the problems in implementing the referral system is the limited resources and essential infrastructure in health institutions to provide minimal health services[8]

Based on the description above, it is necessary to make efforts in structuring an effective and structured referral system in this case the availability of health facilities as a support in implementing the health referral system, so it is necessary to conduct a study to analyze the implementation of the non-specialized case health referral system in Kendari City and City Health Centers.

Material and Methods

This research is a qualitative research is a research method based on the philosophy of post-positivism, used to examine the conditions of natural objects, where the researcher is the key instrument, the sampling of data sources is done purposively and snowball, the data collection technique is by triangulation, the data analysis is inductive, and qualitative research results emphasize meaning rather than generalization. The technique of taking informants used in this study was purposive sampling[9]. Sources of data and research information were taken from informants who were related to the evaluation of the implementation of the health referral system at the public health center. In qualitative research, the researcher is the key instrument (researcher as key instrument). The data analysis used by the author is the analysis of the interview data using the QSR NVIVO 12 application[10]. The validity test of the data in this study is an effort to check the accuracy of the research results by applying certain procedures, while the qualitative reliability indicates that their approach is consistent and reliable by always documenting all their research procedures and documenting as many steps in the procedure as possible[11].

Sampling Procedure

As for the information in this research, namely: Head of Primary Benefit Guarantee (PBG) in the Kendari branch work area and 4 referral patients at inpatient public health centerand the Bau-Bau branch of the Health BPJS Office (archipelago), which consists of the Head of the Bau-Bau City Inpatient Community Health

Center, Bau-Bau City inpatient Community Health Center Doctor, Head of Primary Benefit Guarantee (PMP) in the Bau-Bau branch work area and 4 referral patients at inpatient public health center. In collecting data through documentation, behavioral observation, or interviews with participants / informants.

Preprocessing

In its services, public health center always depend on the availability of facilities and infrastructure to support and facilitate consumers in utilizing services, even for diagnosis of non-specialized diseases. Therefore the referral system from the public health center is strongly influenced by the availability of facilities that can support the examination of these patients.

In the tiered referral system, the role of public health center is very vital. Public health center are the first health facilities to provide services, before they are referred to in stages. As a result, the number of patients has increased. This increase was not matched by an increase in services at the public health center, because the medicines were still limited.

Image Segmentation

Based on in-depth interviews conducted by researchers with informants, it was found that referrals to several health centers were often carried out when the diagnosis of a disease required X-rays because there were no facilities or radiology personnel, so patients had to be referred to other health facilities. The same condition applies to patients who need a pregnancy ultrasound. In addition, referrals are also often given to eye examination patients because they require specific equipment specifications that are not available at the public health center. General laboratory tests such as DHF, malaria and TB also often have to be referred because there are no adequate laboratory facilities.

Availability of drugs is drugs that are used for health services at the public health center in accordance with the needs that should be for medical services to the community in their working area. The level of drug availability is a drug supply both in the type and amount of medicine in a certain period required for community medical services. The unfulfilled availability of drugs at the public health center will lead to a drug void in the public health center which results in suboptimal treatment services for the community[12]

Area Research

This research will be carried out at the Kendari City Health Center and the Bau-Bau City Health Center and the time of the research is February 2020 to completion.

Findings

In its services, public health center always depend on the availability of facilities and infrastructure to support and facilitate consumers in utilizing services, even for diagnosis of non-specialized diseases. Therefore the referral system from the public health center is strongly influenced by the availability of facilities that can support the examination of these patients.

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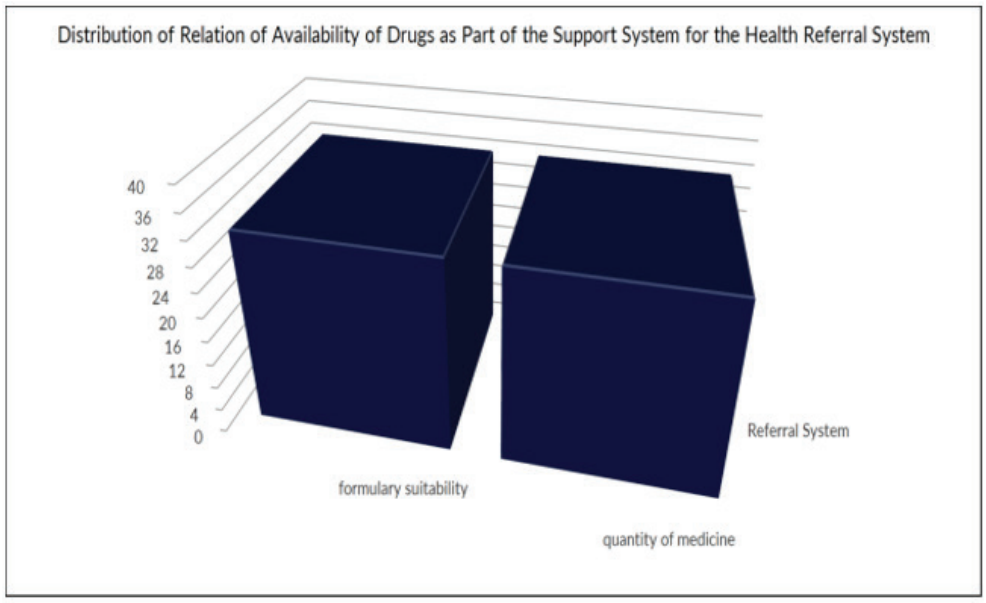

Fig. 1 Distribution of the Relationship of the Availability of Drugs as Part of the Support System for the Health Referral System

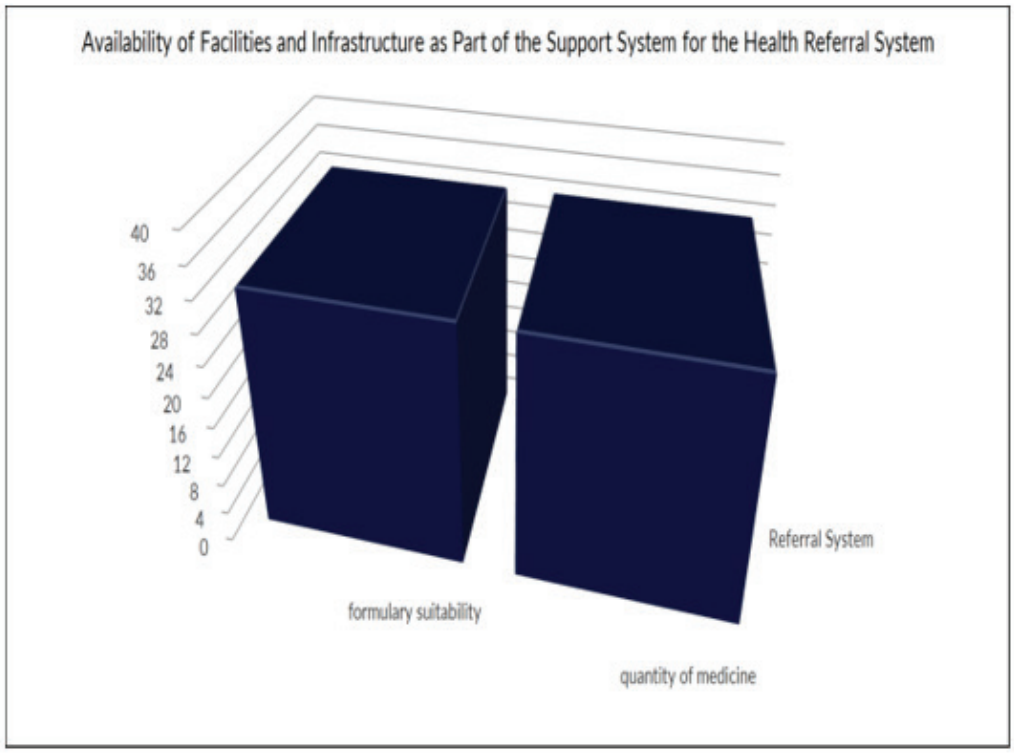

Fig.2 Distribution of the Relationship of the Availability of Facilities and Infrastructure as Part of the Support System for the Health Referral System

Based on in-depth interviews conducted by researchers with informants, it was found that referrals to several health centers were often carried out when the diagnosis of a disease required X-rays because there were no facilities or radiology personnel, so patients had to be referred to other health facilities. The same condition applies to patients who need a pregnancy ultrasound. In addition, referrals are also often given to eye examination patients because they require specific equipment specifications that are not available at the public health center. General laboratory tests such as DHF, malaria and TB also often have to be referred because there are no adequate laboratory facilities.

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Discussion

In more in-depth interviews, what public health center often do in conditions of examination that are constrained by facilities and infrastructure is to refer to other places, be it to other public health center with more complete equipment in this case, usually for patients who need laboratory examinations, or to health facilities. Others are managed by the private sector or the government for patients who need equipment with certain specifications that are not available at the public health center. Several informants also stated that the existing facilities are updated annually or equipped with a proposal scheme to be budgeted based on priority needs based on capitation funds received from BPJS. As for the urgent conditions.

The availability of adequate medical equipment facilities can improve the performance of the public health center in conducting examinations of patients and is a must for the referral process due to the limitations of these facilities, if the facilities and health support facilities are incomplete, the process of diagnosing what patients will be disturbed and this causes health workers must refer patients to the hospital so that it will have an impact on increasing the number of referrals in the hospital[13]

To fulfill the need for medicine, the public health center then proposes drug needs to the pharmacy warehouse at the health office which will be delivered directly to the public health center from the pharmacy warehouse if available at the pharmacy warehouse. In certain conditions, when drug needs are not available from the pharmacy warehouse, public health center carry out their own procurement using the capitation fund budget. Provision of drugs in health facilities is carried out by referring to Fornas and drug prices listed in the drug e-catalog. Drug procurement in e-catalogs uses an e-purchasing mechanism, or if there are operational problems it can be done manually. Several public health center also complained about several drugs that were difficult to obtain, even those that were procured independently with the public health center capitation funds.

The adequacy of medicines at the public health center is also greatly influenced by the responsiveness of the public health center pharmacy installations in managing drugs. Analysis of the drug management / management process must be carried out, because the inefficiency and inefficiency of drug management will have a negative impact on pharmaceutical service activities in the provision of health services as a whole, both medically, socially and economically[14]. Research states that the knowledge and training of drug managers is related to drug storage at the public health center in Jambi City, this shows that the high knowledge of drug administrators can maintain the circulation of drugs in and out of the drug so as to ensure the availability of drugs in the future[15].

Conclusion

The mode of facilities and infrastructure as an important aspect in the implementation of the referral system is still inadequate in terms of the quantity of facilities and infrastructure and conformity to the co-endium and the format of medicines as an aspect that plays an important role in the implementation of the referral system is still inadequate both in the quantity of medicines and the suitability of the formulary.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: None

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Formulating a Standardized Dental Record Format for Dental Surgeons in India - A Pilot Study on Dentists at Chennai and Ahmedabad

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Abstract

Background: Dental record management remains to be a much unexplored arena in forensic odontology. While some developed countries have pre-set guidelines for their licensed dental surgeons to follow, India lacks one such protocol. As a result, majority of dental surgeons in India do not maintain patients' records properly. This not only hampers the quality of dental practice, but also compromises its potential as a valuable forensic aid.

Materials and Methods: This study is a descriptive cross-sectional study. A comprehensive case history format was drafted for dental record management by optimizing the protocols followed by some developed countries. The protocols were supplied to 20 dental surgeons from Chennai and Ahmedabad, who were chosen by stratified simple random sampling. The dental surgeons were asked to follow the format for a month and give detailed feedbacks thereafter, which were collected in feedback forms.

Results: Out of 25 participants, only 15 of them completed the study. The feedback contained both positive and negative comments, along with some with effective suggestions. As a collective opinion, the dental surgeons felt that the case history was too exhaustive. Though the dental surgeons unreservedly welcomed most parts of it, some dentists had difficulties in adopting certain sections in their routine practice and rather suggested removal or modification of those divisions.

Conclusion: The feedbacks and suggestions given by the dentists clarified the feasibility of the prepared dental record format. However, further research on larger scales would be helpful in arriving at definite conclusions.

Keywords: *dental records, forensics, ethics, dentolegal, patient, dentist*

Introduction

Forensic dentistry is an unsung speciality that deals with dental aspects of legal science. Being considered either as a sub-speciality of forensic science or forensic medicine, forensic dentistry has failed to gain its identity as a distinct speciality. Though dental evidences are paramount and dental tissues are lauded as highly reliable tissues for age estimation, individual identification and sex determination purposes, very few understand the ambit of forensic dentistry as a separate subject[1]. Apart from aiding in criminal cases and mass disasters for establishing identity, there are some minor areas where the role of forensic dentists is inevitable. One of such domains includes dental record management.

Maintenance of records is essential in any professional practice. In dentistry, dental records play an important role for a successful dental practice. Dental records not only help the dentist to keep a track of the

treatments done on a patient, but also aid in standing as a legal proof of the treatment performed[2]. In the present days, it is not uncommon to see patients alleging dentists for not doing an ethical treatment and to hear them blame the dentist for any occurrence of unexpected events during the treatment, even if the patient fails to disclose proper medical history. This necessitates the need to record a proper case history for every patient and store all the records appropriately.

Apart from standing as a valid medico legal tool, dental records can also be used to identify a person in cases of crime and mass disaster with post-mortem dental profiling methodologies[3]. As every person is characterised by a unique dental arch with a distinct combination of dental traits, dental evidences are lauded as valid and reliable information to arrive at a conclusion. If proper dental records are maintained, they can be used as ante-mortem records and compared with the post-mortem findings for identification and exclusion purposes[3].

Forensic dentists are usually in demand in some countries because teeth do not decompose in a human and provide a wide range of information because of which identification becomes simple and at times, accurate[4]. In western countries, many civil and criminal cases have been solved with the opinions given by forensic dentist. An essential dental record should contain all the details of the patient which can be stored in the file or in electronic devices[5]. The awareness of the importance of forensic dentistry is very low in India. A few studies have proven that there needs to be more awareness on the importance and the knowledge on the applications of forensic dentistry in clinical practice as well as in the legal scenarios[6]. As forensic dentistry is often overlooked, the scope of sorting out of legal issues stay out of the ambit of the dentists, thereby keeping the interests of the dentists very low in forensics[7].

Forensic dentistry is a much-welcomed discipline in the occidental world. The National Health Service Scheme in the UK has provided various schemes and set up protocols for a hassle-free dental practice for all practitioners in the UK. The dental professional bodies such as the those found in British Columbia and Ontario in Canada, Australian Dental Association and the American Dental Association have also set up guidelines for their affiliated private dental practitioners to keep them at check. All private practitioners of these bodies are legally obliged to make dental records for every patient and store them sufficiently for a definite period of time[8-11]. In India, the understanding of forensic dentistry among dentists is very limited and there is a definite need to positively reinforce the vitality of forensic dentistry in their day to day practice[12]. In fact, very few dentists in India keep a record of the treatments done on their patients and only a small fraction stores them for a long period of time[13]. Also, there is no uniformity in the dental record pattern in India for the private clinical practitioners. A uniform dental case history record will help the dentists communicate among themselves, the patients' needs better and also to foster a standard system of private dental practice all over the country.

Although some knowledge and awareness studies have been conducted to stress on the importance of forensic dentistry in general, there are no studies in the literature which involved a field work to test a case history format among private dental practitioners in India[14-17]. Though there are some reviews and questionnaire-based studies published by Indian authors, there are no experimental studies on dental records from a forensic perspective. In order to check if such a thought would work in reality in India, a study was planned in which private dentists were asked to test the efficiency of a newly formulated case history pattern with guidelines to assist them in recording an ideal case history.

Study Outline

A total of twenty dentists were selected for the study by stratified random sampling, out of which ten dentists were from Chennai and ten dentists were from Ahmedabad. A comprehensive dental case history record was formulated, containing the patients' demographic data, medical history, dental history, family history, personal history, extra oral and intra oral examination including hard and soft tissue examination, provisional diagnosis, investigations (whenever needed), final diagnosis and treatment plan. To support the case history format and to aid in maintaining proper dental records effectively, guidelines were made for recording case history format and storing photographs, radiographs, dental models, patients' informed consent, referral correspondence, drug and laboratory prescriptions,

investigation reports, digital records and business records along with their case sheets. The formulated document was registered for copyright protection in accordance to Copyright Act of India, 1957 (Diary. No: 10848/2020-CO/L; Registration. No: L-96042/2020).

All the chosen dentists were personally met, and a written informed consent was obtained from all of them. They were provided with the case history pattern and all the guidelines and were given a period of five to seven days to read and understand the format. All the dentists were requested to stick to the given protocols as much as possible and give a feedback back at the end of the stipulated time. If they dentists could not follow any of the guidelines, they were requested to give the reason in the feedback. The dentists were assured that their feedbacks would not contain their identities and remain confidential.

After the fifteen-day time, the dentists were asked to return the filled feedback forms. Upon receipt of any incomplete feedback forms, the dentists were contacted and informed for requesting them to send again a completed feedback form. All the received feedbacks were segregated and tabulated under different headings in Microsoft Excel Spreadsheet for analysis. As the results obtained were purely qualitative, statistical analysis was not possible. So, the results have been explained descriptively.

Results

Out of the twenty dentists approached in the study, fifteen dentists responded and five of them failed to respond. The response rate was seventy-five per cent. All the feedbacks were consolidated and are presented below.

Dentists find the case history format lengthy, time-consuming and difficult to be followed in daily practice. They suggest that it can be shortened by retaining only significant details. Many dentists opine that it is difficult to enquire details such as income, religion, occupation, address and date of birth from the patients since certain patients hesitate to reveal their personal information. Few dentists feel uneasy to enquire female patients about their menstrual history and believe that its relation to dental treatment is insignificant. Vital and constitutional signs are not checked for any patients by almost all the dentists. Only those patients who are about to undergo any surgical procedures such as extraction and implant, all the vital signs are noted for. But almost all the dentists agree to record only BP and pulse for all patients routinely. Also, most dentists do not include BMI, height and weight in their clinical examination. Almost all the dentists the format can be concise, unlike the exhaustive one that was given to them. Overall, dentists appreciated these guidelines and found it useful and beneficial. Some stated they have started to follow these guidelines. A few felt that these guidelines are not feasible in their clinics. Except a very few, all dentists obtain informed consent from patients who are undergoing any surgical procedures such as implant, major surgeries, etc. and even for simple procedures in cases of medically compromised patients.

Dentists feel secured in obtaining consent from patients who refuse to undergo the advised treatment. Few dentists commented that most patients do not understand the nature of the treatment, hence they suggest that informed consent should also be made available in the clinic location's vernacular language. Only some feel that consent taking is difficult to practice, if the patients are apprehensive and non – compliant. Most dentists find that it is difficult to maintain extra-oral radiographs. This is because, mostly it would be referred to a radiology facility. Many dentists said prescription of OPGs for all patients is not possible. Others using digital radiography such as RVGs maintain them as records in a software. Manually developed radiographs are difficult to store because they tend to fade away after a period, hence not stored by many.

Photographs are maintained by all dentists for patients undergoing orthodontic treatment. Some state that photographs are also taken for patients with lesions, during surgical procedures or even implant placements. Others find that photographs were helpful in selection of shade for cosmetic restoration procedures. Photographs benefit a couple of dentists in educating their patients. All dentists retain dental models of orthodontic patients only. The models prepared for prosthetic and other purposes are generally given to the patients after delivery or even discarded due to space constraints in the clinics. In most of the clinics, prescriptions are given to the patient as per the guidelines, but not stored. Very few retain the prescriptions along with the patients' records.

Excepting some, all dentists follow the guidelines for laboratory prescriptions, but they do not store it. Some dentists find that it is not feasible to practice the guidelines in their clinics. Some dentists follow the guidelines for referrals, if at all need arises. Few dentists feel that it is not feasible in their clinics, hence they do not follow. Almost all dentists follow the guidelines for storing medical records, but do not store the reports. Quite a lot of dentists store digital records and manage them with the help of a software. A few dentists, who don't use digital records, do not follow the guidelines. Apart from a very few, all dentists store business records in their clinics. Many dentists think that it is difficult to maintain dental records for seven years. Some dentists agree to store the records for a period of three to four years.

Discussion

Dental records maintenance has not been practiced much effectively in India. There are a few studies that were done in forms of surveys in a few regions of the country. In all the studies recorded in the literature, it had been clearly demonstrated that more than half of the study population never practiced record maintenance. In fact, some dentists even lacked the knowledge of its significance and therefore, these dentists had no idea about its forensic implications. Astekar *et al*., Preethi *et al*., Gupta *et al*., and recently, Sarode *et al.,* had performed questionnaire-based studies in various regions of India and published results that lie in harmony with this fact[4,13-14,30].

There are quite a few international publications that resemble this study. Firstly, a baseline dental record was framed by Schleyer *et al*., from ten different sources was a novel idea of qualitatively analysing dental records in both paper-based and electronic versions[31]. Secondly, Acharya *et al*., came up with a new case history pattern for practising dentists and endorsed the idea of practicing a uniform case history pattern for effective patient care[32]. Both these studies framed a pattern for case history format but had no proper guidelines for handling the components of records, which includes photographs and radiographs and also for ancillary practices such as informed consent and business records, which are seemingly the unavoidable ingredients of today's private clinical practice. Delattre did a study among forensic dentists from a forensic perspective and understood that uniform methods of maintaining ante-mortem records would help forensic dentists perform better[33]. Waleed and co-workers compared the dental records of dental students and private dentists against an ideal template[34]. Though an ideal template was used in the study, there was no information about exposing it to the subjects involved in the study. This study absolutely contrasts the present study, where each and every dentist that participated in the study practiced the proposed guidelines for a period of time. This makes the present study as one of its kind in India.

The practice of dental record maintenance has also become digitally available since the past few years. Though such systems offer a lot of benefits to the dentists and make their practices easy, some dentists still prefer conventional method of paper-based record management system[35]. This is the foremost factor that also allowed the guidelines to support paper-based record maintenance as well. The set guidelines for this study is a mixture of optimal adoptions of the protocols commonly practiced in The United States of America, United Kingdom, Canada and Australia. They were framed in the intention of not failing to include any of even the slightest significant information, that could be used as a resourceful forensic aid. These formulated guidelines were then forwarded to experienced and iconic clinical practitioners of both the cities as they would stand as ideal sources of genuine and well-grounded feedback.

As per the obtained results, many dentists felt that the format was lengthy, because most of them did not even have the habit of maintaining records and tried it in their clinics for the first time. But even those who routinely make case history records felt it was time consuming and said that bringing this style of recording case history was not possible. Nevertheless, some of them felt that it was not necessary to document a patient's case history in their busy day-to-day practice. Astekar *et al*., Preethi *et al*., Gupta *et al*., and Sarode *et al*., have already brought to light the attitude of Indians towards case history[4,13-14,30]. This study experimentally strengthens their observations. A dentist who took part in the study suggested a separate case history format for paediatric patients. This seemed to be a very nice idea.

All the dentists in the study elicited proper medical history, including surgical history and allergic history. But they never asked for any pernicious habits unless any typical lesion was noted in their patients' mouths. If all the patients were asked for perilous habits as a question of routine, potentially malignant lesions can be prevented, well before they manifest[36]. With respect to the treatment plan, the dentists had different opinions regarding planning of the treatment. Though treatment plan should be advised by the dentists, the decision is left to the patients. The patients' decisions can be non-scientific, multifactorial and may not correspond to the phases[37]. Hence, the dentists suggested removal of phases from the treatment plan section, as they feel it is not suitable for a private clinical practice. Most of the dentists welcomed the idea of the guidelines that were made for writing a maintaining a case record. But the idea of obtaining informed consent from all the patients from every patient seemed to be a daunting task for most of the dentists. Though the idea was liked by the dentists, they wanted it to be implemented only for risky procedures. But delineating risky and riskless procedures is a tricky task as some minor procedures can also lead to unpleasant consequences.

Dentists who practised manual means of radiography had difficulties in storing the records. But clinics that were equipped with facilities for digital radiography had no difficulties in storing any kind of radiographs. Photographs were only made and retained of all patients who underwent orthodontic treatments as given in the guidelines. Some dentists also stored photographs of patients who had any rare and vulnerable lesions or underwent any major surgeries or implant surgeries. From a forensic odontologist's point of view, it can be very helpful for identification purposes[20]. At least, a photograph that contains the frontal profile of the patient, such a passport photograph could be stored for all patients, if not possible as mentioned in the given proposal.

All casts, especially the ones made for orthodontic purposes, are properly stored but the dentists preferred the idea of either taking photography of the casts before discarding or giving it to the patients for storage. It is better to take photographs and store them in the clinics than to give it to the patients or do both as it is a more responsible means of storing records. Concerning the prescriptions, all the dentists practise the guidelines for drug prescriptions, but some had difficulties in storing them. While most dentists were not interested in storing laboratory prescriptions, some dentists felt that the guidelines could not be practised. It is good to retain copies of prescriptions[2]. At least, dentists could write the prescribed drugs or prosthesis in the patients' case records to keep a record of them. On the other hand, most of the dentists had difficulties in practising referral correspondence. The need of referral correspondence has to be reinforced among the dentists and therefore be advised to send authenticated emails to the referred person, as a minimum.

Preserving medical records of patients with significant history is helpful. Most of the dentists did not store them in their records but they told that they made a detailed note of it in the case history records which was more than enough. Dentists who had software easily managed records through digital means. If all clinics adopt digital means of storing records, then record maintenance becomes much simpler in all aspects. This view is supported by a review paper by Devadiga, which was about dental record maintenance[24].

The only component of the proposal most dentists were extremely sceptical about is the prospective storage of records for a period of seven years, as mentioned in the guidelines. Alternatively, some dentists suggested a retention period of four years. At the outset, for a standardised format, beginning with a retention period of four years would be a prudent decision. A lesser period of retention of records is not recommendable, as chances for legal claims are higher within such a short timeframe[2].

Nonetheless, there are admittedly some limitations in the study. Some dentists could not use some of the guidelines completely, due to the short timeframe. Furthermore, this study had been done in only in two cities of the country. On the flipside, this study leaves behind a myriad of avenues for research for those who are interested to take this idea forward, with more dentists, longer testing period and by carrying out this study in many other locations within the country. This may not only add to the credibility of the study but may also derive contrasting conclusions. Thus, this recording format can be used as a standardised tool of dental record

maintenance for the whole of India.

Conclusion

The study was done with the intention of bringing uniformity in dental record maintenance patterns, throughout the country. A uniform scheme of record maintenance not only helps the dentist in keeping the patient records safely, but also helps the patient in a variety of ways. A forensic dentist has the responsibility of ameliorating the judicial standards of dental practice and render dentistry, free from pitfalls or loopholes that compromise justice to both dentists and the patients. Therefore, a standard system of dental practice is imperative to keep the morality and ethics of dentistry at check.

Ethical Clearance: Not applicable for this study

Source of Funding: Nil

Conflict of Interest: Nil

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Drug Interaction of *Hypericum Perforatum* with Routine Chemical Drugs

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Abstract

In traditional medicine, *Hypericum perforatum* L. is used as an antidepressant, diuretic, analgesic, antiseptic and wound healing, anticonvulsant, rheumatism and chronic gastrointestinal spasms, abortion, sciatica, and for the treatment of infectious diseases such as syphilis, tuberculosis, dysentery, pertussis, worm excretion, and malaria. *H. perforatum* interferes with cardiac drugs, anesthetics, narcotics and analgesics, antibiotics, hormonal compounds, antiviral compounds, anticonvulsants and antidepressants, organ transplantation compounds, and iron compounds. Despite the medicinal effects of the valuable plant *H. perforatum,* the single use of this plant is very effective and valuable in the treatment of diseases and disorders, but its use with other chemical drugs due to the occurrence of drug interactions requires caution in consumption.

Keywords: *Medicinal plant, Hypericum perforatum, Drug interaction, Chemical drug*

Introduction

Hypericum perforatum L. is a plant of the Hypericaceae family. This plant is a valuable herbal medicine for humans and a dangerous weed for livestock [1, 2]. This plant is an herbaceous and perennial plant and has many herbaceous and permanent stems of 50 to 100 cm, the cross section of the spherical stem and has two protrusions. Flowering stems that emerge from the crown every year and are usually red and have many branches at the end. The leaves of this plant are petiole less, opposite, elongated, with rounded ends and without cuts. The leaves have three types of broad leaves, narrow leaves and medium leaves [3, 4]. In traditional medicine, *H. perforatum* is used as an antidepressant, diuretic, analgesic, antiseptic and wound healing, anticonvulsant, rheumatism and chronic gastrointestinal spasms, abortion, sciatica, for the treatment of infectious diseases such as syphilis, tuberculosis, dysentery, pertussis, worm repellent, malaria [5, 6]. It grows mostly in Europe, from Siberia to northwest China, Asia Minor, the Mediterranean, North Africa, Canada and Australia. In Iran, it grows in the north, northwest, northeast and west, Fars, Kohkiluyeh and Alborz Mountains [7].

This plant contains compounds such as flavonoids such as epigallocatechin, rutin, hyperoside, isoquercetin, quercetin, quercetin, amentoflavone, biopogenin, astilbin, myricetin, myclinin, kampferrol and lutolin. Phenolic acids such as chlorogenic acid, caffeic acid, pico maric acid, folic acid, p-hydroxybenzoic acid. Fluoroglucinols such as hyperforin and adiporphorin. Tannin compounds, volatile oils such as 2-methyl octane, non-oxane, 2-methyldecane, non-sodium, α-pinene, β-pinene, α-terpinol, geraniol, myrcene, limonene, caryophylene and humolen. Saturated fatty acids such as isovaleric acid (3-methylbutanoic acid), palmitic acid, myuric acid and stearic acid. Alkanols such as 1-tetrasuzanol and 1-hexacosanol. Vitamins and their analogues are other constituents of the herb tea such as carotenoids, choline, nicotinamide, nicotinic acid [8-13].

Methodology For Review

In this study, relevant articles from ISI, PabMed, Scopus, google scholar, Magiran and ISC. databases were searched and related articles were used for review.

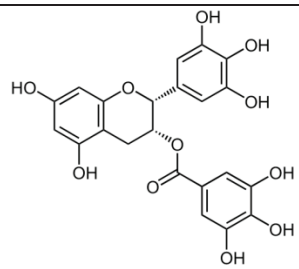
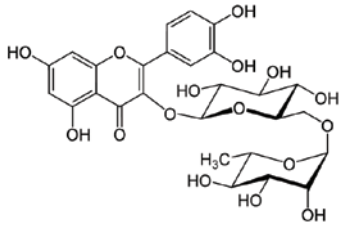
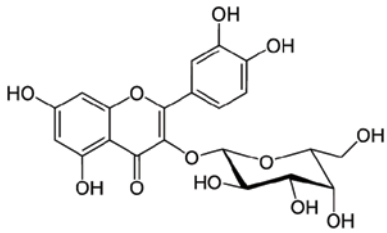
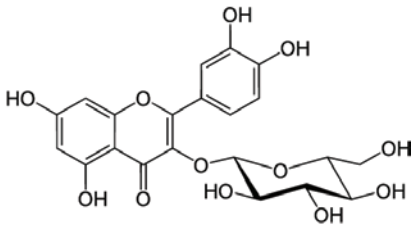
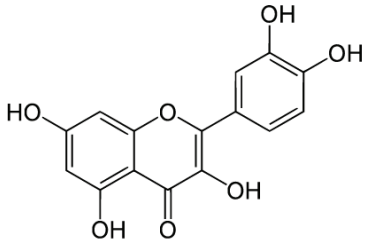
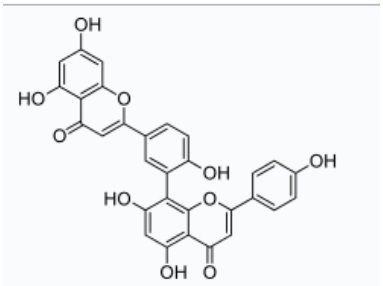
Results

As shown in Table 1, *Hypericum perforatum* has drug interactions with a number of chemical drugs.

Table 1. Drug Interaction of *Hypericum perforatum* with Chemical Drugs

Plant	Interfering drug	Interference type	*Ref.*
Plant name: Hypericum perforatum L. Known as: Perforate St John's-wort, common Saint John's wort and simply St John's wort Herbal family: Hypericaceae	Digoxin	Hypericum perforatum reduces the therapeutic effect of digoxin and reduces the sub-curved level of digoxin.	[14-17]
	Nitrates	Reduce its sublingual absorption	[18]
	Anesthetics	Delay and prolong the effect of the drug	[17]
	Narcotic	Increase sleep time and sedative effect of the drug	[16,17]
	Piroxicam, Tetracyclines, Sulfonamides, Chlorpromazine, Thiazides	Increased sensitivity to light	[14-16]
	Theophylline	Exacerbation of asthma symptoms and decreased serum levels of the drug through induction of the Cyp450 system	[14,19]
	Warfarin	Increase the dose of the drug by reducing the effectiveness of the drug	[17]
	Iron	Inhibition of iron absorption due to the presence of tannic acid in the plant	[17,19]
	Estrogen	Uterine bleeding and reduced fertility	[14,17]
	Cyclosporine	Decreased serum levels of the drug through induction of the Cyp450 system	[15,12]
	Pseudoephedrine	Intensify the effect of the drug	[9]
	Indinavir	Decreased serum levels of the drug through induction of the Cyp450 system	[17,20]
	Antidepressants	Interaction of Hypericum perforatum with antidepressants can increase serotonin levels and cause serotonin syndrome.	[21]
	Ranolazine	This medicine should not be taken with ranolazine heart medicine	[22]

Table2. Chemical Structure of bioactive compounds of *Hypericum perforatum*

Name	Chemical Structure
Epigallocatechin	
Rutin	
Hyperoside	
Iso quercetin	
Quercetin	
Amento flavone	

Discussion

Studies have shown that *Hypericum perforatum* interferes with cardiac drugs, anesthetics, narcotics and analgesics, antibiotics, hormonal compounds, antiviral compounds, anticonvulsants and antidepressants, effective compounds Organ transplants and iron compounds. Despite the medicinal effects of the valuable plant *Hypericum perforatum*, the single use of this plant is very effective and valuable in the treatment of diseases and disorders, but its use with other chemical drugs due to the occurrence of drug interactions requires caution in use. Studies have shown that medicinal plants due to their active ingredients and medicinal and antioxidant compounds have beneficial effects on human health and have a therapeutic effect on various organs of the body and various diseases [23-33].

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Pathological Evaluation of Canine Hepatic Cirrhosis and Necro-inflammatory Activity

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Abstract

Background: Hepatic fibrosis is a common pathological result of chronic liver diseases, which is a dynamic process of liver decomposition mediated by cellular mediators in response to injury.

Objective: The present study aims to evaluate the histopathological features of dog-liver fibrosis and / or cirrhosis.

Materials and Methods: 40 liver samples were collected; 12 liver fibrosis suspected animals were performed the histopathology to determine the main features of canine liver fibrosis.

Results: The histopathology revealed chronic hepatitis with liver islands separated by strips of fibroblasts and collagen, moderate numbers of macrophages, lymphocytes and plasma cells. Also in fibrosis between chronic hepatitis/cirrhosis had a wide range of severity and inflammation was observed primarily around fibrous tissue or was surrounded by fibrous tissue but extended to the liver parenchyma. The histochemical study revealed to a deposition of collagen fibers in the portal, subscapular and interlobular regions.

Conclusion: The histopathology revealed a range of lesions vary from mild hepatic degenerative changes to severe fibrotic features associated with canine hepatic fibrosis and early cirrhosis.

Key words: *Pathological, Hepatic, Fibrosis, Canine.*

Introduction

Cirrhosis/ liver fibrosis is a common pathological result of chronic liver disease, cirrhosis is a dynamic process of liver decomposition mediated by many cellular mediators in response to an inflammatory process [1]. The infected animals with liver chronic diseases can cause a harm affection in either health status and economic as a results to the inflammatory changes related to this disease [2].

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Hepatic star cells (HSCs) are a major component of cirrhosis, in the fibrous liver, HSCs quiet across different to reproductive, migratory and perxing myofibroblasts, show transcription properties and pro-fibrous secretions so-called cell activation and the secretion of ECM molecules that accumulate and form the deterrent tissues in space from the diss that leads to inflammation [3]. Liver diseases, especially chronic hepatitis and copper-related hepatitis, are common in dogs, with varying amounts of fibrosis, necrotizing, and inflammation among individual patients [4].

Fibrosis is an integral part of chronic liver disease, hepatitis caused by various detogenal sedation raises fibrous tissue deposition in parenchyma, which replaces normal functional liver cells, reshapes blood vessels in the system, waives liver functions[5]. Cirrhosis is characterized by the gradual accumulation of

extracellular matrix components (ECM) in the liver with persistent inflammation, the collagen profile of the liver changes, with increased relative amounts of first and third collagen types accompanied by modification and binding of ECM components [6]. Histologically cirrhosis is well estimated on the sections of the hematosis-eosin or with histochemical spots (titular mason or red sirius), which show collagen deposition with varying degrees of architectural distortion [7]. The current study concluded that the canine hepatic fibrosis was associated with a serious inflammatory events represented by multiple lesions vary in it severity in which the histopathology revealed a range of lesions vary from mild hepatic degenerative changes to severe fibrotic features associated with canine hepatic fibrosis and early cirrhosis.

Materials and Methods

The 12 dogs with clinical evidence of acute or chronic liver disease from 40 liver samples were collected, which were presented to liver biopsies to the faculty of veterinary medicine at the University of Basrah. The samples of dog fibrosis / cirrhosis were a medical procedure performed to obtain a small piece of liver tissue to diagnose liver fibrosis during the period from August 2019 to February 2020.

The livers were collected and thoroughly examined, after a clinical evidence of liver disease, the liver histopathology were detected from the diseased and control Dogs. All dog owners provided informed consent prior to enrolling in the study. Subsequently, the histological steps was conducted according to [8] in which the specimens were washed in water for removal of the blood then placed it in a plastic container, which contains formalin a concentration of 10%. These specimens were washed with tap water to remove excess fixation. The obtained specimens used for routine hematoxylin and eosin stains and Masson's trichrome staining, the stigma of these special stain used allows for the identification of more clinically important information than is available on the slicks of the hicnosis and eosin alone, and important for establishing a specific diagnosis. This method yields specimens for histological examination to study the microscopic changes in the tissue of the liver fibrosis.

Results

The histopathological results (H&E stain) revealed that the liver showed islands of liver cells separated by strips of fibrous and collagen cells, moderate numbers of macrophages, lymphocytes and plasma cells, besides, there was a dilation of center vein and filled with inflammatory cells infiltration mainly polymorphic inflammatory cells, in addition marked area of degenerative changes as form of vaculation of hepatocyte particular in the pericentral vein region (figure 1). The Fibrosis among the chronic hepatitis group/cirrhosis was a wide range of severity and inflammation was observed primarily around fibrous tissue or was surrounded by fibrous tissue but extended to the liver parenchyma (most inflammatory cells were lymphocytes, with slight infiltration by macrophages and justices) (figure 2).

The histochemical results showed a sever deposition of collagen fibers in the portal trait to demonstrate the portal fibrosis associated and liver cirrhosis which appeared surrounded the bile ducts, hepatic artery and portal vein (figure 3). Moreover, it showed a deposition of collagen fibers in the subscapular region and interlobular regions referred to periportal fibrosis (figure 4).

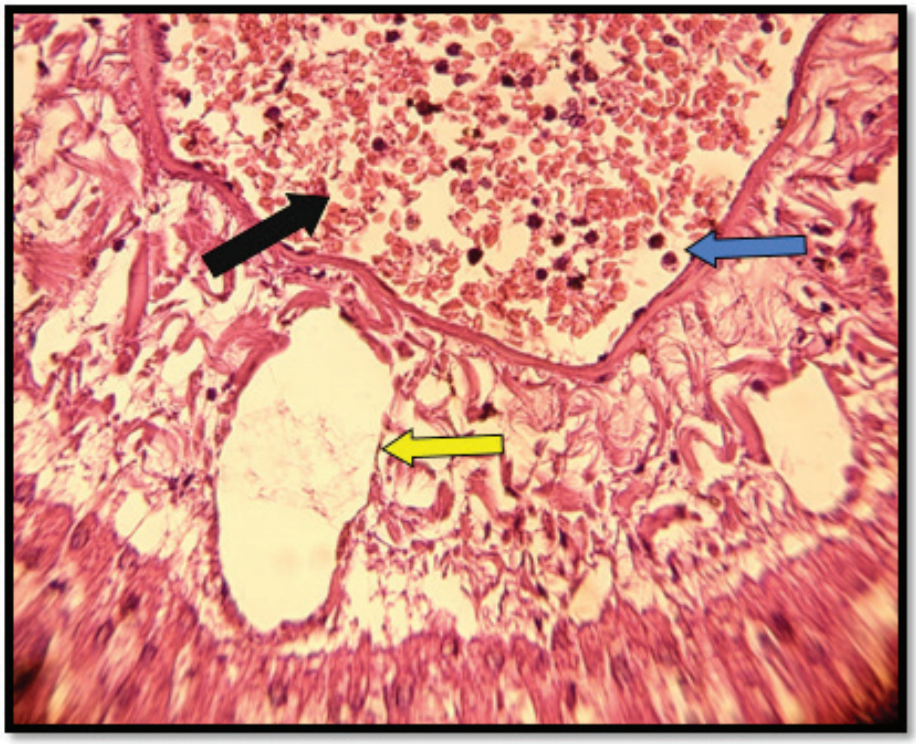

Figure (1) Histopathological section of liver fibrosed animals showed dilation of center vein (black arrow), filled in mononuclear inflammatory cells (blue arrow), in addition there are area of degenerative changes in the form of cystic dilation of hepatocyte particular in the pericentral vein region (yellow arrow) (H&E stain, 400X).

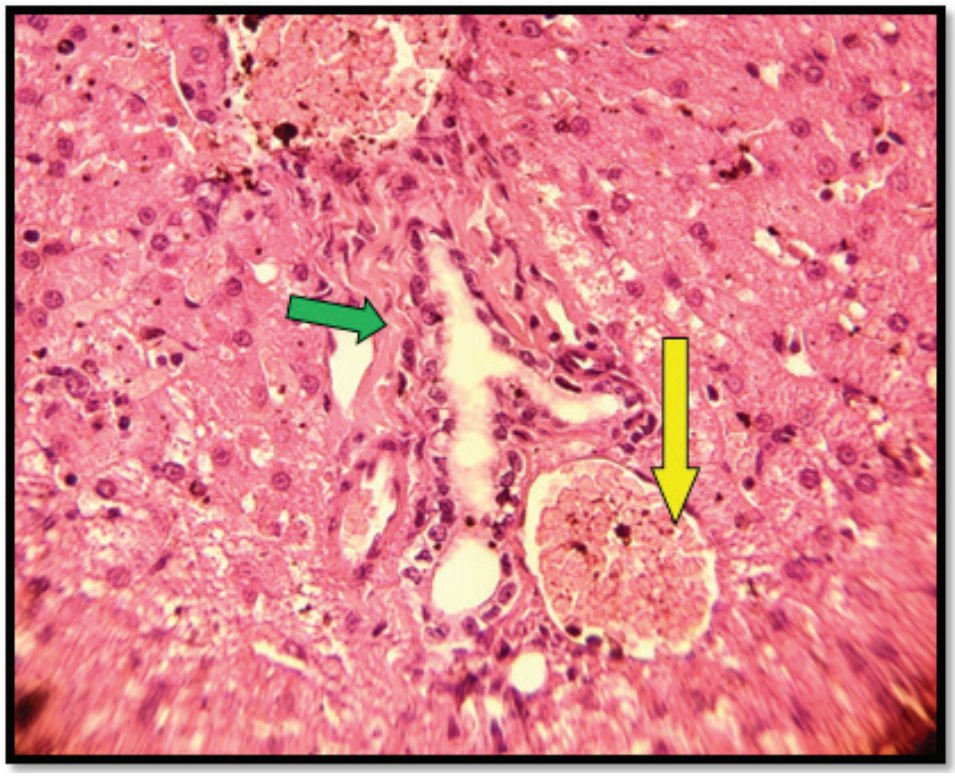

Figure (2) Histopathological section of liver fibrosed animals showed marked fibrosis present within portal area (green arrow) and the inflammation was observed primarily around the fibrous tissue, with portal vein congestion (yellow arrow). (H&E stain, 400X).

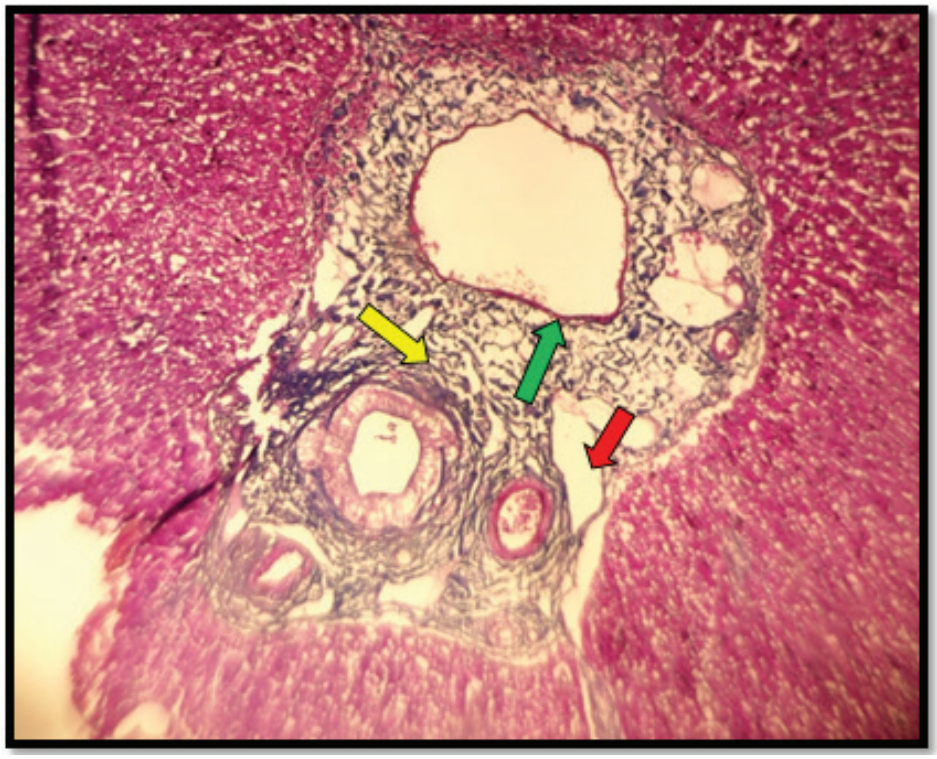

Figure (3): Histochemical section of liver of liver fibrosed animals show sever deposition of collagen fibers stained blue in the portal trait to demonstrate the portal fibrosis associated with liver cirrhosis appeared surrounded the bile ducts (yellow arrow), hepatic artery (red arrow) and portal vein (green arrow). (Masson's trichrome, 100X).

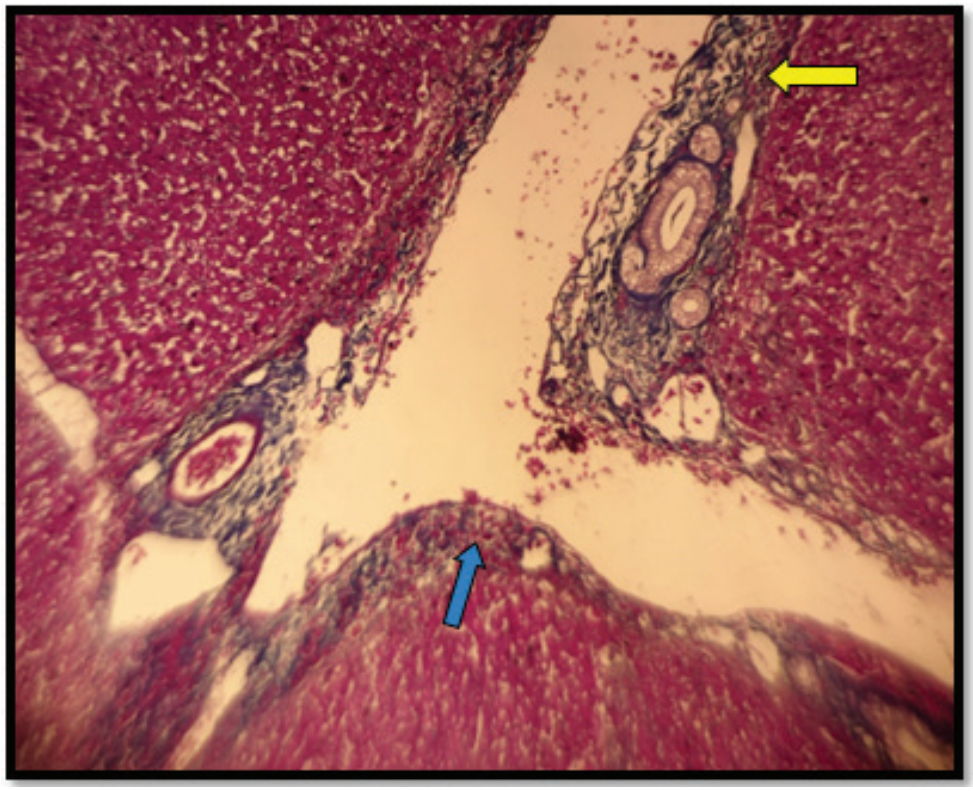

Figure (4): Histochemical section of liver of liver fibrosed animals show deposition of collagen fibers in the subscapular and interlobular regions (blue arrow) referring to periportal fibrosis (yellow arrow) (Masson's trichrome ,400X).

Discussion

Cirrhosis is the final stage of chronic hepatitis and is defined as a diffuse distribution characterized by cirrhosis of the liver and the conversion of the normal liver structure into structurally abnormal nodules, partial or total nodules, and is considered irreversible [9].

The microscopical results showed vary degrees of severity extend from inflammatory cells infiltration to the fibrous tissue lesions in the liver fibrosis animals, therefore, the histopathological result revealed dilation of center vein, also the central vein filled in inflammatory cells infiltration mainly mononuclear inflammatory cells, marked area of degenerative changes as form of vacuolation of hepatocyte particular in the pericentral vein region.

In other sections, there was Fibrosis between the chronic hepatitis group/cirrhosis was a wide range of severity and inflammation was observed primarily around fibrous tissues or was surrounded by fibroblast tissue but extended to the liver parenchyma (most of the inflammatory cells were lymphocytes, with a slight infiltration of macrophages and niatrovia), and in another section the livers deteriorated or necrotic, and the limit plates were damaged with some, Similar results were also recorded by [10] , which these findings in consistence with , who mentioned that liver fibrosed animals extent of hepatic fibrosis, inflammatory cells, cellular debris and fibrosis connective tissue.

Besides, there is fibrous connective tissue with the formation of two sublobules, fibrous connective tissue prominent in gate areas and thin bands tend to encircle small groups of liver cells. In addition, it noticed degradation with balloons and the presence of mild lymphatic inflammatory infiltration, associated with moderate disease, as well as the proliferation of kupffer cells.

The histochemical studies were good diagnostic tools for evaluation of the many hidden aspects of inflammation and repair [11] ; therefore, the current study used several special stains to estimate the role of the tissue against the invaders and the pathological changes.

The histochemical study of the liver fibrosed animals revealed to deposition of collagen fibers in the portal region of the liver, which stained blue to demonstrate the collagen deposition referring to moderate fibrosis in masson's trichrome, stain; also nuclei stained black and cytoplasm of hepatocytes and erythrocytes stained red. On other hands, the other histochemical section of the liver of liver fibrosed animals show severs deposition of collagen fibers stained blue in the portal trait to demonstrate the portal fibrosis associated with a liver abscess in masson's trichrome stain which appeared particularly in the surrounded area of bile ducts, hepatic artery, and portal vein. Also, the other histochemical section of the liver of the liver fibrosed animals showed pericentral vein deposition of collagen fibers stained blue to demonstrate the pericentral vein fibrosis in masson's trichrome stain.

Moreover, the histochemical section of the liver of the liver fibrosed animals which displayed the severe elastic collagen fibers deposition that stained blue around of portal vein revealed to perivascular fibrosis. Nearly similar results clarified by [12] who reported that the collagen fibers which stained blue moreover, the cytoplasm of hepatocytes are stained red with this histochemical stain. The above results were consistent with our microscopical results, which showed severe area of fibrosis particularly in the pericentral vein region as well to the portal area of the affected liver.

Conflict of Interest: None declared.

Source of Funding: Self, no other funding sources.

Ethical Clearance: The ethical committee, college of veterinary medicine, university of Basrah, Iraq, approved the study.

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Study of Electrical Injuries in Fatal Cases in Tertiary Care Hospital

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Abstract

Background and Objective: With wide spread use of electricity at home and work place the amount of electrical related injuries are on the rise. The present study attempts to know age, sex distribution pattern of electrical injuries, cause of death, manner of death, and Histopathological changes in skin with electrical contact mark and in internal organs.

Methods: Study was conducted on deaths due to electrocution brought to Osmania General Hospital mortuary during the period of one year. Specimen of skin with contact mark and internal organs like heart, kidney was sent for Histopathological examination. Data from police reports and picture evidence from the site of the accident were retrieved.

Result: Total number of 60 cases was studied in one-year period. Much of the casualties were men, belonging to the working age community. 58% of victims died instantaneously due to shock. 37% of the fatalities were due to complications of severe flash burns. High voltage electrocution constituted 56.66 % of cases. Low voltage electrocution constituted 43.33%. High voltage electrocution was identified as flash burns, low voltage electrocution was identified as contact mark. Typical Histopathological changes were seen in skin and heart specimen sent for examination. Histopathological changes in kidney were nonspecific.

Conclusion: The diagnostic parameters for electrocution are the electrical contact mark. Histopathological changes were used as supportive evidence in determining cause of death. Human negligence, electrical defective appliances and connexions and lack of safety precautions were the key factors for electrocution death.

Key Words: *Shock, High voltage, Low voltage, Flash burns, Electric contact mark, Septicaemia.*

Introduction

Electricity is essential in life that without it life is hard to conceive. But the benefits and convenience of electricity are often correlated with the dangers. Electricity can often result in morbidity or mortality, both of which are via accident.[1-5] Electricity has also been used for suicide reasons in extreme situations [6-11] and homicidal purposes.[2] Deaths attributed to electrocution around the world display a distinct trend. Accidental fatalities from electrocution are not prominent in the west because of strong protection and high awarenessMany suicide reports have been registered, however. Accidental deaths from electrocution are more frequent than suicide deaths, despite this in developed countries like India. The typical domestic supply of AC current in India ranges from 220-250 volts, compared to 110 volts in western countries and for industrial use in India its 440 volts.

There is as much risk as electricity is used. It is a threat. Accidents and injuries due to incompetence and

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inability to enforce effective protocols are unavoidable. Electricity has now become an integral aspect of our way of life, unavoidable, invaluable yet harmful. The lowest recorded electrical shock death voltage is 38 volts.[12] In residential, commercial, and industrial settings, electrical injuries can be prevented by following specific guidelines, incorporating electrical appliances and even by enforcing electrical protection regulations properly.

If electrical shocks trigger accidental death, the police can focus most or entirely on medical reports to decide causes of death. Doctors are the key source of proof for the decision-making process. The key purpose is to ascertain the facts, the cause, and the manner of death in the event of an electrocution, and to decide whether death was due to negligence or accident.

It is impossible to determine the absolute cumulative number of electric injuries because of the number of non-fatal incidents. This research is confined to fatal electrical events. In certain cases, the characteristics of electrical contact were lacking, which contribute to the hunt for diagnostic electrocution criteria.

It is also impossible to prove that a man has been electrocuted and there could be no marks from electrocution, but forensic pathologists ought to know about the fatal effects of electricity in order to help the investigation of those incidents.

Methodology

Place of Study: The present study involves study of electrical contact marks in fatal electrocution cases brought for autopsy to Osmania Medical College and General Hospital. **Study period:** One-year October 2019 to September 2020

Sample Size: 60 electrocution cases

Inclusion Criteria: Subjects included for the present study includes all age group, sexes, incidents occurring at home and work place.

Exclusion Criteria: Cases of electrocution as a cause of death indirectly. For instance , a person falls from height after being electrocuted and dies as a result of head injury.

Statistical Analysis: The statistical analysis was done using SPSS 20 software and data was presented in the form of tables

Methods: Skin with contact mark along with portion of normal skin as control is dissected and sent to Department of Pathology for Histopathological examination. Under the low power and the high-power microscope sections were observed. Observations were made, recorded, and photographed.

In cases where electric contact mark is absent or not evident, internal organs such as heart and kidney were sent for histopathological examination, sections from those organs were examined and observations were made, recorded, and photographed.

The retrograde analysis requires the gathering from inquest report, the deceased ‘s family and police about electrocution circumstances. Photographic evidence was obtained from the scene of the accident.

Results

Table 1: Distribution of cases according to gender, age group, season and voltage

SEX	NO OF CASES	PERCENTAGE
MALE	50	83.33%
FEMALE	10	16.66%
TOTAL	60	100.00%
AGE GROUP		
1 TO 10 yrs	2	3.33%
11 TO 20 yrs	13	21.66%
21 TO 30 yrs	25	41.66%

Cont... **Table 1: Distribution of cases according to gender, age group, season and voltage**

31 TO 40 yrs	10	16.66%
41 TO 50 yrs	6	11.66%
51 TO 60 yrs	2	3.33%
61 TO 70 yrs	1	1.66%
71 TO 80 yrs	1	1.66%
SEASON		
Summer	13	21.66%
Rainy	35	58.33%
Winter	12	20.00%
VOLTAGE		
High Voltage	34	56.66%
Low Voltage	26	43.33%

Table 1: Distribution of cases according to gender, age group, season and voltage

Majority of the cases were males accounting 83% and females were 17%. The majority of age group electrocuted was 21 to 30 years with 41.66% followed by 11 to 20 years with 21.66%. Most of the electrocution cases were seen mostly in rainy seasons with 58.33%. Most of the electrocution caused was due to high voltage around 56.66%.

Table 2: Distribution of Cause of Death In Fatal Electrocution Cases and Pattern Of Injuries In Electrocution Deaths

Cause Of Death	No of Cases	%
Shock	35	58.33%
Septicaemia	15	25.00%
Toxaemia	4	6.66%
Coma	4	6.66%
S & H	2	3.33%
INJURIES		
Contact Mark	34	45.94%
Flash Burns	27	36.48%
Contact Mark with Flash Burns	8	10.81%
No Contact Mark/ Flash Burns	5	6.75%

The shock was the cause of death in 56% of the cases and followed by septicaemia with 26%. Most injuries were due to contact mark with 45.94% and flash burns seen in 36.48%, contact mark with flash burns with 10.81% and only flash burns with no contact mark seen in 6.75% of the cases.

Table 3: Distribution of Cases with Histopathological Changes in Organs

Organs	Total Specimen	HPE Changes Seen	Percentage
Skin	35	35	100%
Heart	35	17	48.57%
Kidney	35	22	62.85%

The histopathological changes due to electrocution was seen in skin in 100% of the cases followed by kidney and heart with 62.85% and 48.57% respectively.

Cause of Electrocution	No of Cases	Percentage
Human Negligence	26	43.33%
Faulty Equipment's & Connections	18	30.00%
Lack of Protective Measures	16	26.66%

Table 4: Distribution via Cause of Electrocution

Majority of the cause of electrocution was mostly due to human negligence with 43.33% followed by faulty equipment's and connections and due to lack of protective measures in 30% of the cases

Table 5: Pattern of Electrical Injury with Respect to Voltage

Voltage	Total Cases	Flash Burns	%	Contact Mark	%
High Voltage	34	27	79.41%	7	20.58%
Low Voltage	26	4	15.38%	22	84.61%

Table 6: Voltage Distribution in Work Place and Home

Place	Voltage	Total Cases	Cases Found	Percentage
Work Place	High Voltage	34	20	58.8%
	Low Voltage	26	14	53.84%
Home	High Voltage	34	8	23.52%
	Low Voltage	26	10	38.46%

Majority of the electrocution cases occurred at the work place and mostly due to high voltage and low voltage. With 59% and 54% respectively.

Discussion

Number of cases being 60 in a span of 1 year is a significant number compared to earlier studies, where 220 cases were reported in 22 years[13] and 36 cases were reported in 3 years[14] in which all cases were males as compared to present study where 83.33% of victims were male. Age group most affected is the working class, which suggests that electrocution deaths are occupation related deaths.56.66% of deaths from high voltage occurred on job as compared to 86% in earlier studies.[13] 57% of deaths from low voltage were on job as compared to 45% in earlier studies.[13] 21% of deaths from high voltage occurred at home. 39% of deaths from low voltage occurred at home. Electrical accidents involving high voltage form 54 % and Low voltage form 46 % of total electrocution. Flash burns are present in 78%, while contact mark is present in 30% of cases of high voltage accidents. Contact marks are present in 89%, while flash burns are present in 11% of cases of low voltage accidents. The above statistic indicates that high voltage electrocution invariably present as flash burns and low voltage electrocution present with contact mark in correlation with findings of Wright and Davis,[13] Vincent D Maio.[15] 56 % died instantaneously due to shock as against 88.9%.[14] 35 % died due to complications of flash burns. 8 % died due to head injury consequent upon fall. 1% died due to shock and hemorrhage. All the cases were accidental electrocutions unlike observations made by Trubner and Puschel,[16] R.K.Wright and J.H.Davis.[13] Except for petechial hemorrhages over surface of heart, no specific gross internal findings of organs was observed at autopsy, which is in correlation with Bernard Knight's observation. [17] Maximum number of cases was seen in rainy season, which suggests that wet surface plays major role in electrocution as against findings of R.K.Wright and J.H.Davis[13] where there is predominance of cases in summer. Six cases of electrocution presented with neither contact mark nor flash burns. Death due to electrocution was confirmed by supportive Histopathological changes in skin, heart and kidney. Histopathological changes were seen in the, entire skin specimen, which had contact mark. Textbook description of vacuolation and honeycomb[18] appearance in the layers of skin was not observed. Instead, breach or break in epidermis along with separation of epithelial cells adjacent to breach is seen with vertical orientation of nuclei, which is in correlation with findings of Tedeschi,[19] Bernard Knight,[17] Ludwig,[20] Herbert Fischer.[21] In India voltage that is supplied for domestic purpose being 220v is higher when compared to that in western countries where it is 110v. This may be cause for separation of epithetical cells and absence of honeycomb appearance. Histopathological finding in heart, which includes wavy appearance of myofibrils, is in correlation with the literature available.[17] Sections showed stretching of myocardial fibres along with elongation and wavy appearance in 47% of specimen sent. Histopathological finding in kidney is non-specific and suggests disseminated intravascular coagulation, present in 61% of cases, correlates with findings of Bernard Knight.[17] In few cases, petechial haemorrhage was found along the margins of lungs, which was sent for Histopathological examination. Histopathological findings showed congested vessels and foci of haemorrhage. In cases where contact marks were evident, Histopathological findings were used as supportive evidence for cause of death. In cases where no contact marks were found, positive Histopathological findings were used to give the cause of death as electrocution. Causes of electrocution were investigated and found that human negligence was main cause of electrical accidents (41%), faulty electrical equipment's and connections contributed to 31 % of accidents and lack of protective measures caused 28 % of electrocution, which agrees with study done at Armed Forces Institute of Patology.[22]

Employees of electrical work even though provided with protective gloves, harness, belts were negligent of not using the same and they should ensure that main line should be devoid of current supply while they are working.

Domestic accidents, which consists 28% of total electrocution, are due to faulty electrical equipments or connections and human negligence. One fourth of domestic accidents occur in bathrooms involving boiler switches and immersion coils. Another important cause observed was contact with live wire while drying of wet cloths over metal wire (one fourth of total domestic cases) in correlation with Theodore Bernstein study.[23]

Houses constructed illegally with close vicinity to high-tension wire lead to electrical hazard with the dwellers. Short circuit with television cable wire has led to electrical accidents to people who tried to connect the cable wire.

Conclusion

The diagnostic parameters for electrocution are the electrical contact mark. Histopathological changes were used as supportive evidence in determining cause of death. Human negligence, electrical defective appliances and connexions and lack of safety precautions were the key factors for electrocution death.

Ethical Clearance: Ethical clearance was obtained from our College institutional ethics committee i.e Osmania Medical College and General Hospital prior to the commencement of the study.

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Conflict of Interest: Nil

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Forensic Estimation of Stature from Foot Length: An Autopsy based Study

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Abstract

The present study was conducted on 224 dead bodies of Keralites (167 males and 57 females) aged between 20 and 60 years. The correlation coefficient calculated in males for the stature and right foot length (0.779) and left foot length (0.778); and the right foot length (0.653) and left foot length (0.638) in females were significant at 0.01 level. Males had significantly higher mean values for both stature and foot length compared to females. Regression equations were derived from right and left foot length for estimating stature in males and females. Stature (Y) could be calculated from the right foot length (X_1) in males with the equation $Y = 53.050 + 4.802\ X_1$. For the left foot length (X_2) in males, the equation was $Y = 53.618 + 4.7762\ X_2$. In females, the equations were $Y = 78.538 + 3.597\ X_1$ & $Y = 78.724 + 3.601\ X_2$ respectively.

Key Words: *Foot Length, Forensic Stature Estimation, Human Identification, Physical Anthropology*

Introduction

Establishing the identity of a person is a major concern in Forensic medicine and Forensic Anthropology. Identity means the determination of individuality of a person[1]. The primary characteristics of identity are those of sex, age and stature and they may at once serve to disprove a supposed identity. Recovering of unknown bodies either in full or in part, as remnants or fragments or bodies in charred or putrefied state is a day to day affair in medico-legal practice.

Stature of an individual is an inherent characteristic, the estimate of which is considered to be an important assessment in the identification of unknown human remains. Most of the body parts bear a more or less constant relationship with stature.

Materials and Methods

The study was conducted on 224 dead bodies of Keralities aged between 20 and 60 years brought for autopsy in the Department of Forensic Medicine at Medical College, Thiruvananthapuram, Kerala, India. Of these, 167 were males and 57 were females. The period of study was from November 2010 to November 2011. Dead bodies having any significant congenital or acquired deformities including fractures of spinal column or long bones and segmented, charred, mutilated or decomposed bodies were excluded. Past history of the cases were recorded in detail to rule out nutritional deficiency, abnormal growth pattern and hormonal imbalance. Whenever such abnormalities were noted, those cases were omitted from the study. The inquest reports were scrutinised and relevant data were recorded in the Proforma.

Instruments Used for Measurement

A two meter long steel scale fixed to a straight wooden pole was used to measure stature in centimetres.

A Mitutoyo Digital Sliding Calliper (made in Japan) having an accuracy of 0.01 mm was used for measuring foot length. During the measurement, a wooden board

was used to keep the foot straight and perpendicular and a rectangular wooden block was used at the head end for measuring stature. (Fig 1)

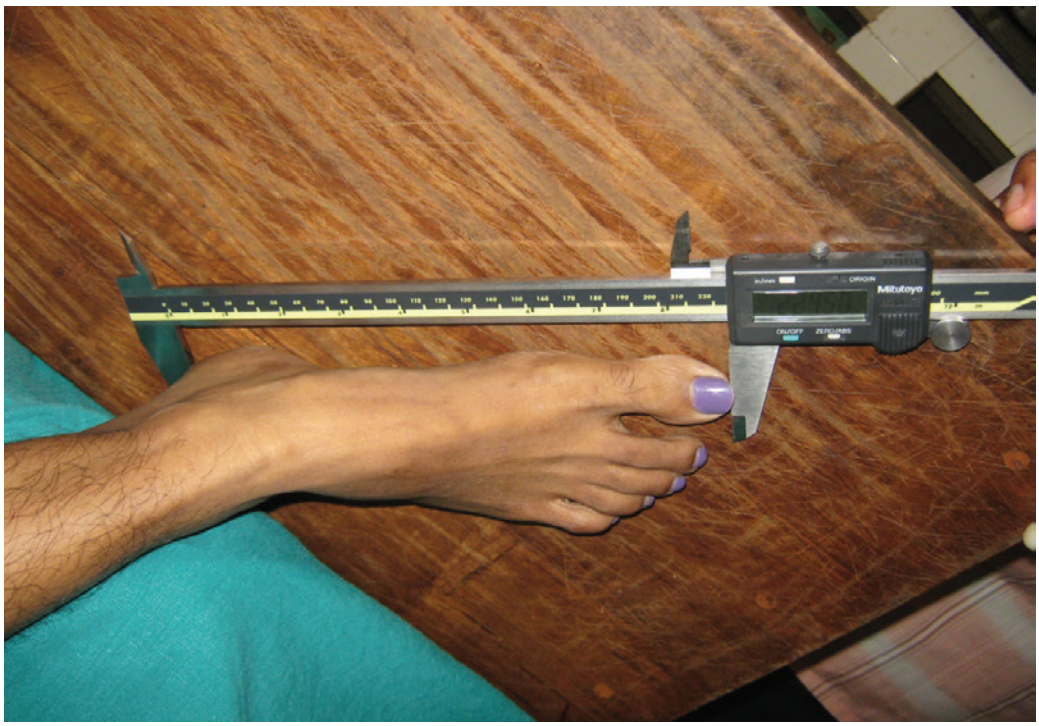

Fig 1: Measuring foot length using digital sliding calliper.

Statistical Analysis

The collected data were analysed using multiple regression analysis to derive formulae to estimate stature from foot length. As part of regression analysis, Pearson's product moment coefficient of correlation (r), Analysis of Variance (ANOVA) and Multiple Correlation Analysis (R) were done. In addition, paired sample 't' test and independent sample t-test (student t-test) was also applied in order to find out whether there is any significant difference between right and left foot length. Data were analysed separately for males and females.

Results

The data collected was statistically analysed and the results of the study were tabulated and are given in Table 1.

Table 1: Range, mean, standard deviation and correlation coefficient (r) values of anthropometric measurements in adult males and females.

Sl.No.	Gender	Parameters	Range (cm)	Mean	Standard Deviation	Correlation Coefficient
1.	Males	Stature	149-185	165.71	7.68	
2.		Right foot length	19.87-26.70	23.46	1.25	0.779*
3.		Left foot length	20.15-27.05	23.48	1.25	0.778*
4.	Females	Stature	145-175	155.39	5.71	
5.		Right foot length	19.32-23.44	21.36	1.04	0.653*
6.		Left foot length	19.02-23.36	21.29	1.01	0.638*

*Significant at 0.01 level

There was significant positive correlation between stature and the foot length at the 0.01 level in males and females.

Regression Equations

The regression equations derived for the present study are given in Table 2:

Table 2: Regression equations derived

	Gender	Regression model highly significant at the F value	The regression equation obtained
From rt. foot length	Male	F = 254.62; p < 0.01	Y* = 53.050 + 4.802 X1**
From lt. foot length		F = 252.48; p < 0.01	Y = 53.618 + 4.7762 X2***
From rt. foot length	Female	F = 40.81; p < 0.01	Y = 78.538 + 3.597 X1
From lt. foot length		F = 37.71; p < 0.01	Y = 78.724 + 3.601 X2

*Y = Stature**X_1 = Right foot length ***X_2 = Left foot length

Discussion

From the above findings, the feet were observed to be longer in males compared to females. This finding is in agreement with the findings of Giles and Vallandigham (1991), who used measurements obtained from young adult male and female recruits in the US army[2]; Baker and Scheuer (1998) who studied UK residents[3]; Ashizawa et al. (1997) who studied Javanese, Filipinas and Japanese[4]; Ilayperuma et al. (2009) who studied Sri Lankans[5] and Rani et al. (2011) who studied Indian subjects[6].

Prior studies as those done by Philip (1990), who studied Indian subjects[7]; Sanli et al. (2005) who conducted a study on Turkish subjects[8]; Patel et al. (2007) who studied the inhabitants of Gujarat region in India[9]; Ilayperumana et al. (2009)[5]; Chavan et al. (2009) who studied the inhabitants of Maharashtra region[10] and Rani et al. (2011)[6] had showed a high positive correlation between stature and foot length. The present study showed results in line with the published data.

The present study derived regression equations for estimating stature from right and left foot length which can be applied for forensic stature estimation to help law enforcement. Further study on the same population is recommended to validate the findings of the study.

Ethical Concerns: Human Ethics Committee, Medical College, Thiruvanathapuram, through Institutional Ethics Committee (IEC) and Institutional Review Board (IRB) approved the study on 12/11/2010. The data was collected during routine medico-legal autopsy examination and no information which could reveal the identity of the deceased are published.

Conflict of Interest Statement: The authors declare no conflict of interest. The research was self funded.

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Risk Analysis of Musculoskeletal Disorders (MSDs) Complaints on Batik Crafters Under The Assistance of Ademos Indonesia

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Abstract

Introduction: Batik is one of Indonesia's original works of art that is continuously preserved in several regions in Indonesia. Several people working as batik crafters in Bojonegoro, East Java, Indonesia are under the assistance of Ademo. Based on the preliminary study conducted, most batik crafters had started to complain of pain in several parts of their body, which was considered due to work postures and non-ergonomic work stations. Thus, this study was conducted to analyze the risk of Musculoskeletal Disorders using RULA and NBM.

Materials and Methods: It was an observational study with cross sectional design. Data collection was conducted by conducted observation using RULA (Rapid Upper Limb Assessment), NBM (Nordic Body Map) and interviews with batik crafters in home industry. The population in this study was 42 batik crafters in a batik industry under the assistance of Ademos Bojonegoro. This study utilized total sampling technique by which the whole population were taken as the sample.

Results: The results showed that 76.2% of batik crafters had a very high risk of MSDs on the right side of the body and on the left side of the body; 50% of batik crafters had a high risk of MSDs; and 50% had a very high risk of MSDs. The results of observations using NBM showed that 42.85% had a high risk of MSDs.

Conclusion: This study concludes that all the batik crafters are in an emergency circumstance experiencing musculoskeletal disorders and need an ergonomic work station as soon as possible.

***Key words**: batik crafters, ergonomic, RULA, NBM, MSDs*

Introduction

Batik is one of the works of art from Indonesia which has now penetrated the international market [(1)]. Along with the development of batik, every region in Indonesia has begun to produce batik with its respective regional characteristics, including Bojonegoro Regency, East Java, Indonesia.

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Several traditional batik crafters in Bojonegoro Regency are under the assistance of Ademos, an organization that focuses on the empowerment of rural communities by synergizing with government, private sector, and non-governmental organizations. High market demand for batik fabric makes an occupation as a batik crafter as one of the main sources of income for some people.

Batik fabric is usually produced in a wholesale system. It causes the batik crafters to work overtime in one time working on a batik making project for one client which is certainly full of pressure since there is a target that they must be able to complete a number

of fabrics agreed upon at the beginning within a certain period of time. Based on a preliminary study that has been done, the process of making batik is still done in the traditional way. The crafters can work more than 8 hours per day. During the batik making process, they can work in a sitting position and bend over for hours. This static work posture for a long time was evident during the initial interview that some batik crafters had started to feel some health impacts, one of which was frequent pain in the lower back. Lower back pain occurs since the load on the vertebrae is tripled in the sitting position compared to the standing position (2). Accordingly, batik crafters have musculoskeletal disorders.

Musculoskeletal Disorders (MSDs) are complaints of aches, pains, and so on in the muscular system such as tendons, blood vessels, joints, bones, nerves, and others caused by work activities ([3]). Musculoskeletal complaints are complaints of the skeletal muscles felt by a person ranging from very mild to very painful complaints ([4]). Muscles that receive static loads repeatedly and for a long time can cause complaints in the form of damage to joints, ligaments and tendons.

Furthermore, the results of the preliminary study also showed that most of the batik crafters under the assistance of Ademos Bojonegoro were female. Based on a study conducted by Wijnhoven, the prevalence rate of musculoskeletal pain was higher in women than men. For muscoloskeletal pain at any location, the prevalence ration of symptoms amon womern was 50% hinger than men [(5).] Another complaint that many batik crafters felt was cramps due to work posture which tended to be static for a long time and required a high enough concentration. Batik crafters were required to do a work posture that lead to awkward position since they did not have knowledge to make work stations more comfortable and ergonomic for their work. Additionally, they also did not understand that working posture with awkward position can have a negative impact on their health. If not handled properly, these complaints can affect the productivity of their work, in this case, the batik fabric target that they have to complete every day. Low back pain has a significant impact on the productivity of batik crafters. Referring to a study conducted in 2003, workers with low back pain will experience a decrease in work productivity by up to 60% [(6)].

This study conducted a risk analysis using RULA considering that RULA can assess the burden on posture that is not ergonomic more than some other risk assessment methods [(7)]. Thus, the researcher intended to conduct a risk analysis of Musculoskeletal Disorders on batik crafters working under the assistance of Ademos Bojonegoro using the RULA method.

Materials and Methods

This research is a descriptive study. Based on the type of research, it is an observational research. Besides, based on its location, it is a research conducted in the field using a cross-sectional design since the observations were conducted at a certain time or period. This study was conducted in Bojonegoro Regency with batik crafters in several districts under the assistance of Ademos as the target respondents with a total population of 42 batik crafters, all of which were used as research samples. The variables studied were the level of risk from ergonomics as seen from posture, type of work, age, working hours, and exercise habits as independent variables. Importantly, the dependent variable of this study included complaints of musculoskeletal disorders. The instrument used was a Nordic Body Map questionnaire to obtain data related to musculoskeletal complaints felt by respondents. The level of ergonomic risk was calculated using the RULA observation sheet.

Characteristics of Batik Crafters	Group	Distribution	
		N	%
Gender	Male	7	16.7
	Female	35	83.3
Age	12-16 years	9	21.4
	17-25 years	6	14.3
	26-35 years	13	31.0
	36-45 years	10	23.8
	46-55 years	3	7.1
	56-65 years	1	2.4
Period of Service (Years)	1-3 years	7	16.7
	> 3 years	35	83.3

Results

Characteristics of Respondents (Batik Crafters) Under the Assistance of Ademos Indonesia

Tabel 1. Distribution Table of Characteristics of Batik Crafter under The Assistance of Ademos Indonesia

The distribution of characteristics of respondents (Batik Crafters) under the assistance of Ademos Bojonegoro includes gender, age, period of service (years). For the gender category, most of the workers with a total of 35 batik crafters (83.3%) were females, while the rest with a total of 7 batik crafters (16.7%) were males. For the age category, most of them with a total of 13 batik crafters (31.0%) were aged 26-35 years. In addition, for period of service, most of them with a total of 35 batik crafters (83.3%) had been working for >3 years.

Complaint Levels of Musculoskeletal Disorders (MSDs) Based on Rapid Upper Limb Assessment (RULA)

Table 2. Frequency Distribution of RULA Final Score for the Right Side of the Body of the Batik Crafters Under the Assistance of Ademos Indonesia

Risk Categories	Frequency	Percentage (%)
Low	0	0
Moderate	0	0
High	10	23.80
Very High	32	76.2
Total	42	100.0

Based on the Rapid Upper Limb Assessment (RULA) method, 10 respondents (23.8%) of the 42 respondents (batik crafters) under the assistance of Ademos were at high risk of experiencing Musculoskeletal Disorders (MSDs), indicating immediate corrective action on work posture was needed in the near future. Moreover, 32 respondents (76.26%) are at very high risk so that corrective action on work posture was needed at the moment.

Table 3. Frequency Distribution of RULA Final Score for the Left Side of the Body of the Batik Crafters Under the Assistance of Ademos Indonesia

Risk Categories	Frequency	Percentage (%)
Low	0	0
Moderate	0	0
High	21	50
Very High	21	50
Total	42	100.0

Based on the Rapid Upper Limb Assessment (RULA) method, 21 respondents (50 %) of the 42 respondents (batik crafters) under the assistance of Ademos were at high risk of experiencing Musculoskeletal Disorders (MSDs), indicating immediate corrective action on work posture was needed in the near future. Moreover, 21 respondents (76.26%) were at very high risk so that corrective action on work posture was needed at the moment.

Complaint Levels of Musculoskeletal Disorders (MSDs) Based on Nordic Body Map (NBM) Method

Based on the results of observations and interviews with 42 respondents using the NBM checklist sheet regarding the severity of MSDs, the following data were obtained:

Table 4. Frequency Distribution of Nordic Body Map (NBM) Final Score of batik Crafters under The Assistance of Ademos Indonesia

Risk Categories	Frequency	Percentage (%)
Low	6	14.28
Moderate	16	38.10
High	18	42.85
Very High	2	4.28
Total	42	100

By using the Nordic Body Map (NBM) method to assess the complaint levels of Musculoskeletal Disorders

on 42 respondents, it was found that 6 respondents (14.28%) had a low risk, 16 respondents (38.1%) had moderate risk, 18 respondents (42.85%) had a high risk and, 2 respondents (4.28%) had a very high risk.

Discussion

Characteristics of Batik Crafters under the Assistance of Ademos

Most of the batik crafters under the assistance of Ademos were dominated by females or housewives who initially just filled their spare time by making batik which then continued to make batik activity their permanent job. All batik crafters, both males and females, experienced a high and very high risk of musculoskeletal disorders complaints. Based on the physiological aspect, males have stronger muscle abilities than females. Thus, it is natural for women to experience complaints or pain in their body more easily when doing risky work[8]. This is in line with a study conducted by researchers that there is a significant relationship between gender and complaints of Musculoskeletal Disorders[5]. In relation to this study, although both males and females were in the same risk category for MSDs, it was found that the number of women was higher. Theoretically, females' muscle capacity is not as strong as males', so immediate prevention efforts need to be done in order to prevent females from experiencing unwanted events that can affect their quality of life and productivity.

More importantly, results showed that most of the batik crafters under the assistance of Ademos were in the productive age range of workers (26-45 years). However, based on the results of RULA calculations and observations using NBM, all batik crafters were in the category of experiencing a high and very high risk of musculoskeletal disorders. This is because the older a person is, the less muscle strength he/she has, so the risk of these workers experiencing complaints of musculoskeletal disorders also increases[9]. Besides, at the age of 30 years and over, degeneration occurs in the form of scar tissue damage and reduced fluid in the bones and muscles which also affects their stability[8]. Thus, in that age range, batik crafters do have a high risk of experiencing musculoskeletal disorders.

Period of Service

The results also showed that most of the batik crafters had worked > 3 years and had experienced a high and very high risk of musculoskeletal disorders. According to a previous study conducted by some researchers, a longer period of service has a greater likelihood of experiencing MSDs[10]. The period of service is one of the factors that can affect complaints of lower back pain[4]. Longer period of service or work will affect muscular system caused by several factors, one of which is continuous static load and repetitive activities[11].

Complaint Levels of Musculoskeletal Disorders (MSDs) Based on Rapid Upper Limb Assessment (RULA)

Based on the calculation of RULA on 42 batik crafters under the assistance of Ademos Bojonegoro, most of them had a very high risk to the right side of the body. The left side of the body also had the same high and very high risk. It was because batik crafters used their right hand more so that the right hand was not in an ergonomic position. It was also not supported by an adequate work station since there was no hand support or armrest, while the left hand was in an awkward position because it did not have adequate armrests as well. The greater the angle formed by the shoulder, the higher the complaint of shoulder pain felt[12]. Fatigue and injury to muscles are two of the effects of an ergonomic work attitude.

During the making of batik tulis (hand-drawn batik), a crucial step that requires a long time is *mencanting* (the design phase using a traditional tool called a *canting*) since it requires high patience and accuracy. *Mencanting* in hand-drawn batik industry is carried out in a sitting position and often with a slightly bent back. sitting posture for too long causes weak abdominal muscles and curved spine so that workers get tired easily[4]. In addition, if the work position is carried out continuously and for a long time, it will cause complaints to the musculoskeletal system.

Furthermore, the placement of the fabric used by the respondents was too low, resulting in neck flexion. The neck that is bent down for a long time can cause pain, supporting finding of a study on workers working in front of computer[13]. They found that placing the monitor too low can increase the activity of the upper neck muscles and can cause pain in the upper neck and shoulders. Several studies conducted in the textile business which

were also carried out using the RULA method showed that many workers experienced complaints of pain in the neck area and waist area[14],[15].

Another factor causing complaints to the musculoskeletal system is that the worker's body position is not ergonomic in which there are demands for tasks, work tools, and workstation designs that are not in accordance with the worker's abilities[16]. Based on the identification results, most of the work positions were not ergonomic for example that there was no back support so that the back could not relax; the knees could not be bent 90 degrees (the seat was too low); and there was no armrest that caused the hands tired. During that time, batik crafters worked continuously in a non-ergonomic position (bent); sitting on a *dingklik* or a small stool without a backrest. This position is dangerous because it causes musculoskeletal disorders. Study of Musculoskeletal Disorders (MSDs) confirmed that 67.7% of cases were experienced by batik crafters who experienced MSDs complaints on the neck, right back, and waist[17].

Complaint Levels of Musculoskeletal Disorders (MSDs) Based on Nordic Body Map (NBM) Method

Based on research conducted on 42 batik crafters under the assistance of Ademos Bojonegoro using the Nordic Body Map (NBM) assessment sheet, more varied results were obtained. Based on the checklist, the most frequent complaints by workers were pain in the upper and lower neck, right shoulder, back, waist, right upper arm, right and left wrist, and knee. Musculoskeletal disorders (complaints in the parts of the skeletal muscles that a person feels from very mild to very painful) can be grouped into two: 1) reversible complaints or muscle complaints that occur when the muscles receive a static load, and 2) persistent complaints or muscle complaints that are persistent[4].

Based on the Nordic Body Map (NBM) checklist, batik crafters often complain of pain in several points, especially in the upper body, because they are in a constant position for a long time without doing light stretching movements. In fact, the duration that a person is in a non-ergonomic condition can reduce the body's ability which can eventually lead to injury[18]. Such monotonous conditions will make crafters experience neck pain or stiff, causing headaches[14,15,19] and it can spread to the shoulders, hands, and back. This is also in line with a study conducted in the textile industry using the Nordic Body Map method which found that a lot of workers complained of pain in torso, neck, shoulders and several other places[20].

Additionally, batik crafters do the entire work process in a sitting position. Working in a sitting position for more than 95% of the total working hour can increase the risk of neck pain[21]. Unnatural posture which is also caused by inadequate work station design can also increase complaints of pain in the upper body[22].

One of the complaints of musculoskeletal disorders felt by respondents could be due to the respondent's working period of more than 3 years. It supports a study which found that workers with longer period of service are more likely to experience MSDs[23]. The other two causes of MSDs complaints on batik crafters include the static work posture in a sitting position for a long time and minimal movement. This can lead to increased load on muscles and tendons, which causes blood flow to the muscles to be blocked and results in fatigue and pain[24].

Conclusion

Based on the results of the study conducted, all batik crafters have a high and very high risk of musculoskeletal disorders. This shows that this condition is dangerous and must be addressed immediately to prevent batik crafters from experiencing occupational diseases which can also have a fatal impact on their quality of life and also their productivity. To reduce complaints of Musculoskeletal Disorders (MSDs) experienced by batik crafters under the assistance of Ademos Bojonegoro in the moderate category, it is necessary to take action to improve work stations and work postures to reduce the complaints experienced.

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Ethical Clearance: This study is undergone ethical clearance through The Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Commision. Protocol number is: 292/HRECC.FODM/VI/2020

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Health Tourism Through *Panchakarma* with Special Reference of *Sharadiya Virechana*

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Abstract

Globalization is the process of international integration arising from interchange of the world's views, products, ideas and other aspects of the culture. *Ayurveda* has holistic approach towards health and diseases, lifestyle, using medications, diet and their fewer side effect .Globalization helps to propagate *Ayurveda* worldwide with the help of health tourism.

India is emerging as a great destination for health tourism because it has several factors like excellent medical treatment at low cost, easy accessibility, picturesque locations for excellent holidays etc. Health tourism is not only beneficial for the foreigners but our country can also be benefitted financially. It will also help to spread the importance of *Ayurveda* worldwide. The physician can extend a helping hand in globalization of *Ayurveda* by being in their own motherland. Patients from industrialized nations seek health tourism because of high quality treatment at low cost.

Panchakarma is one of the specialty of *Kayachikitsa* presents unique approach of *Ayurveda* with *shodhana* procedures of the body. *Panchakarma* provides promotive, preventive, curative and rehabilitative aspect. There has been phenomenal increase in the demand for *Panchakarma*. *Sharad ritu* is the time for *Pitta prakopa*. The *pitta* which was accumulated in *Varsha ritu* gets provoked by *ushna guna* during this season. If proper care is not taken during this time, it will cause *Pittaja vikara*. *Virechana* is the method of purification, conducted in *Sharad ritu* for preventing and controlling *pitta prakopa* as a part of *Ritukalina shodhana*.

In this current paper, we would like to discuss different aspects of health tourism with special reference to *Sharadiya Virechana,* which will contribute to propagate *Ayurveda* globally.

Keywords: *Globalization, Health tourism, Panchakarma, Sharad ritu, Virechana, Ritukalina shodhana, Pitta prakopa.*

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Introduction

The mutual exchange of technology and knowledge around the world is known as Globalization. It is a process of international integration arising from the interchange of world's views, products, ideas and other aspects. Internationalization holds great potential for *Ayurveda* in order to collaboration with other traditional

and modern medical systems around the world to find cures for global health care challenges, and to be part of modern lifestyle.

India, having one of the richest and most diverse heritages in the world is not an exception to this global trend. *Ayurveda* is the world's most ancient methodical medical knowledge system. Ayurveda due to its holistic approach using lifestyle medication, healthy diet, less side effects, has attracted a large population in different countries around the world. There is a growing demand for natural products in the international market. Global awareness on this traditional system has increased manifold over the past few decades, leading to the acceptance of such medicine systems.

Health tourism or medical tourism is a niche tourism developed by the competing tourism promoting countries in order to attract people travelling with the prime purpose of getting healthcare. Health tourism is not only beneficial for the foreigners, but our country can also be benefitted financially. It will also help to spread the importance of *Ayurveda* worldwide. The physician can extend a helping hand in globalization of *Ayurveda* by being in their own motherland. Patients from industrialized nations seek health tourism because of high quality treatment at low cost.

Panchakarma therapy is considered as a complete holistic approach to the elimination of the root cause of each and every chronic disease. The *Doshas* which are removed by *Langhana* and *Pachana Dravya* they can get aggravated sometimes, but the *doshas* which are removed by *Sanshodhana* procedures they cannot get vitiated again.

Amongst the 5 *shodhana* therapies, *Virechana* is the best therapy for *Pitta* elimination. When the *Tikshna* and *Sheeta* qualities of *Pitta* suddenly react with the *Ushna* quality of *Sharad Rutu*, aggravation of *Pitta Dosha* is seen. As a means of *Ritukalina Shodhana*, *Virechana* is specially recommended in Sharad *Rutu*. It should be done in healthy individuals for maintaining health and also in patients suffering from diseases in which *Virechana Karma* is indicated.

Virechana therapy can be proved beneficial for the foreign nationals who reside in a country which predominantly shows environmental changes seen during *Sharad Ritu*. This therapy will help to eliminate the possibility of formation of the *Pitta – Raktaj* diseases. It is safe to carry out for the delicate stature foreigners. In this way by being in our own country an *Ayurveda* physician can contribute towards globalisation of *Ayurveda* through Health tourism.

Aim: Globalisation of *Ayurveda* through Health tourism

Objective: To Review Health Tourism through *Panchakarma* with special reference of *Sharadiya Virechana*

Materials and Methods: Thorough review of literature related to topics was done through the *Ayurved* Compendia, various other *Ayurved* texts and textbooks of contemporary science. The references from internet and journals were also critically reviewed. The study had initiated for Affirmation of globalisation of *Ayurveda* through Health tourism with special reference of *Sharadiya Virechana*.

Review of Literature

Health Tourism

With the advent of globalization and culture of consumerism, people begin to travel to make use of wide variety of alternatives that bring satisfaction and healthy living. Now a day's people are more conscious in maintaining a healthy body, mind and soul. Health tourism comprises of two terms healthcare and tourism. Health tourism is becoming a preferred form of vacationing as its covers a broad spectrum of medical services and mixes leisure, fun and relaxation together with wellness and healthcare.

Health tourism is also known as medical tourism, wellness tourism, healthcare travel or medical value travel. Medical tourism is highly promoted by big corporate hospitals in developing countries by providing high quality medical services at cheaper prices to patients from industrialized nations. Health tourism is projected as a new segment in travel and healthcare business. Globally, medical tourism is said to be US $ 40 billion industry.

Benefits of Health Tourism

For the tourists:

· People from industrialized nations seek health tourism because of high costs of treatment in their home country.

· Health tourism destinations provide high quality treatment at low cost

· People get an opportunity to visit a new country and enjoy its tourism products during the recuperative period.

· Wealthy patients from third world countries also choose for health tourism as they get better service and care from the health tourism provider.

· Some health tourism destinations provide alternative medicines and traditional form of treatments. Patients wish to take alternative medicines like Ayurveda, Yoga through health tourism.

For the country:

· High quality treatment at a fraction of the cost, in comparison to western countries, makes India an ideal healthcare destination for highly specialized medical care.

· India is a reliable destination to get high quality of ancient treatment like authentic Ayurveda, Yoga etc, which are highly on demand.

· The use of non-toxic, harmless herbs resources, the waiting time for treatment in India is much lower compared to other countries.

· Country can be benefitted by increase in the number of foreign visitors

· This will also help to raise the finances, which will lead towards country's development.

For Ayurveda:

· Classical form of Ayurveda can be practiced by the physicians

· Through the tourists from various countries the knowledge of Ayurveda can be propagated globally

· The physician can work more efficiently in his mother land with easy availability of space, equipments, authentic drugs and trained staff, which is not possible if the doctors sets up the clinic out of India

Panchakarma:

These are the *Pradhana-karma* (main procedure) of Panchakarma as described in *Bhrihattrayee*. Besides *Pradhana-karma* there are *Purva-karma* (pre procedure) and *Pashchata karma* (post procedure). These three steps comprise *Panchakarma*. *Purva-karma* covers *Snehana* (oleation) and *Swedana*(fomentation). *Paschata-karma* includes the procedures indicated after *Pradhana karma* that mainly comprise of *Samsarjana Karma* (diet regimen after *Panchakarma*)

The *shodhana* procedures are, *Vamana*, *Virechana*, *Basti*, *Shirovirechana* and *Raktamokshana*

Speciality of *Panchakarma*

The *Doshas* which are removed by *Langhana* and *Pachana Dravya* they can be aggravates sometimes, but the doshas which are removed by *Sanshodhana* procedures they cannot occur again. Hence for eg : Likes roots of trees which are to be completely destroyed neither they occur again, in same way *doshas* should removed completely from its roots neither they will occur again. Hence *Panchakarma* treatment is best among other types of *chikitsa*[1].

PITTA DOSHA & VIRECHANA

Virechana is regarded as the most important amongst all the therapies for *Pitta* by *Ayurveda*. It extracts the entire pathogenic root of *Pitta Dosha* and when it is overcome, the *Paittika* manifestations in the body get pacified like the fire chamber which becomes cold when the fire is removed.

Virechana therapy is recommended in *Ayurveda* for treatment of *Pitta*. It not only eliminates *Pitta* but also acts on *Kapha* & *Vata Dosha*. Mild purgation in fact is recommended in *Vata Chikitsa Upkrama* in *Ashtang hruday sutrasthan*. Thus *Virechana* acts on *Tridosha*. But as it is most significant purification treatment for Pitta Dosha it is recommended in *Sharad Rutu* due to aggravation of *Pitta* during this period. It should be done in healthy individuals for maintaining health and also

in patients suffering from diseases in which *Virechana Karma* is indicated.

This evacuative therapy eliminates aggravated *Dosha*, excreta, alleviates diseases, improves strength and complexion and if administered properly, endows the person with a long life. *Virechana* sharpens memory, increases aquity of organs, energy, and stability of all *Dhatu*, *Agni* and delays ageing.

Pathogenesis of *Pitta prakopa* in *Sharad Ritu*

Accumulation of *Pitta* in *Varsha Rutu* occurs due to *Amla Vipaka* of food & medicines that occur naturally in this season. *Pitta* does not reach the level of aggravation because of external cold due to rain. *Pitta* accumulation occurs when internal qualities like *Tikshna* acts together with external qualities like *Sheeta*. When these *Tikshna* and *Sheeta* qualities suddenly act with the *Ushna* quality in *Sharad Rutu*, aggravation of *Pitta Dosha* is seen. Due to this aggravation there are higher chances of getting diseases related to *Pitta Dosha Prakopa*. Such as burning sensation,temperature, perspiration, suppuration, sloughing, fainting, gangrenes, moisture, intoxication, prostrations, sore or acrid eructation etc.

***Sadharana* kala for *Ritukalina Shodhana*[2]:**

***Chaitra* =** *KAPHA shodhana*

Falguna

Vaishakha Magha

Jyestha *Pausha*

Aashad Margashirsha

***Shravana* =** *VATA shodhana* *Bhadrapad* *Ashwin*
Kartik = *PITTA shodhana*

Sharad Ritu:

Sharad Ritu is considered to be breeding period for the disease.

Sharad ritu (autumn): *VisargaKaal*

Bharatiya month: *Ashwin* and *Kartik*

English month: October and November

ROLE OF *VIRECHANA* IN *SHARAD RUTU* FOR PREVENTION OF *PITTA*

Though *Swasthavritta* is not among *Ashtanga* of *Ayurveda*, it has its own importance in day to day life. *Ayurveda* guides society with the concept of daily-seasonal-social regimens as an integral part of maintenance of life. Critical equilibrium and homeostasis in *Tridosha* is responsible for health and ill-health. *Chaya* (increase in its own site), *Prakopa* (increase causing the spread from own site), *Prashama* (pacification) are the three natural stages those occur according to daily periodical and seasonal variations.

Sharad Ritu is observed to exist usually from mid September to mid November in India. In this *Ritu, Vata* undergoes stage of *Shamana* where as *Pitta* attains stage of *Prakopa*. *Acharyas* suggested various purificatory procedures, changes in diet and behavioural regimens in order to conquer the natural variation of *Dosha* in particular season. *Virechana* stands the best line of management for *Pitta-Raktha* and *Vathaja* conditions. It is a major therapy along with usage of *Tiktha Dravyas* indicated as a part of seasonal purificatory therapy in *Sharad ritu*

VIRECHANA

Nirukti: The word '*Virehchana'* is derived from '*Richa' Dhatu* (i.e. detachment of relation). The word "*Recana*" is commonly used as evacuation. Thus, *Virechana* means *Maladeh Nirharanam* i.e. removal of *Mala* from the body. It means that which eliminates the impurity from the lower part of the gut[3].

CLASSIFICATION OF VIRECHANA DRAVYA

Sukhma virechana, Mridu virechana, Teekshna virechana

Acc. to *Sharangadhara –*

Anulomana – hareetaki, Sramsana – kritamalaka, Bhedana – katuka, Rechana - trivritta

Speciality of *Virechana* over *vamana*:

· *Virechana* is regarded as the best one among all the therapeutic measures for *Pitta* elimination.

· *Virechana Karma* is easily accepted by the patients and foreigners because it includes *snehapana* and purgation which can be done at home

· *Vamana* is generally avoided by patients due to fear of complications and they are difficult to manage than *virechana*. Hence *virechana* is safer.

· *Virechana Karma* is the act of expelling *Doshas* through Adhobhaga i.e. *Guda*. The *Doshas* even from the *Amashaya* are taken to the *Pakwashaya* and they are removed through *Gudamarga*.

· It is the process of elimination of *Mala* either in *Pakwa* or in *Apakwa Avastha* but along with excess fluid portions.

· It is less stressful procedure

· . It is considered the best treatment for morbid and increased *Pitta Doshas*. *Virechana* is even a treatment for *Pitta Samsargaja Doshas*, *Kapha Samsrista Doshas* and also for *Pitta Sthanagata Kapha.*

· It is worth mentioning that *Virecana*, unlike the modern purgatives, is not merely an act to open the bowel, but is a complete therapeutic measure which has systemic as well as local effects.

Indications:

Virechana yogya rogi: *Pinasa, kustha* (soriasis), fever. *Rajayakshma*, cough, asthma, *galagraha, galaganda, slipada*, diabetes, *mandagni* (improper or delayed digestion), due to incompatible diet indigestion*, visuchika, alasaka, adhomarga raktapitta*, snakebites,mental disorders, diarrhea, swelling*, pandu, mukhapaka* etc. specially *pitta pradhana vyadhi*[4].

Contra-indications:

***Virechana ayogya rogi*:** delicated(weakness), any injury at anal region*, muktanala, adhogami raktapitta* (bleeding through anal region), after fasting, weak sense organs, improper and delayed digestion, immediately after giving *niruha basti, kama vasna chitta*, indigestion, *navajwara, madatya rogi*, alcoholic, distention of abdomen, injury due to any foreign body or *shalya*, those who is having any trauma or injury by accident, those who had given *snehana* in more amount, *atiruksha, krura kostha, kshata, garbhini* etc. are said to be *Avirechya[5]*.

Benefits:

The benefits of *virechana* are : It improves intelligence, gives strength to sense organs, stableness in *dhatu,* increases *utsaha* (feels powerful and energetic), increase hunger, also delayed ageing when the person takes virechana(accordingly to seasonal vairiations),it also destroyed *PittajanyaVyadhi*[6].

For example, When the water is removed from the Pond then aquatic plants and animals will not survive and died, like that *Virechana* will removes all *pittaja* diseases by eliminating pitta from the body.

Virechana Karma

The whole procedure has following steps:

I. *Purvakarma*: It includes following,

1. Collection of the drugs to manage the complications, & to handle the emergency.

2. Detail examination of the person, indications, contra-indications etc.

3. *Snehana* and *Swedana*: *abhyantara snehapana*, *bahya snehana* and *swedana*

5. Kala: early morning when previously taken food is digested, *abhyanga* & *Swedana* should be carried out during the gap of these 3 days.

6. Diet & regimen: During *Snehapana*, *drava*, *ushna, anabhishyandi ahara* should be consumed. *Ushonodaka* is mandatory, one should follow *brahmacharya* & avoid *vyayama*, *krodha*, *shoka*, *hima* & *atapa*, *pravata, yana, adhwa bhashya, atyasana sthiti, dhuma* & *raja* also should be avoided

7. Diet before *Virechana*: Diet containing *jangala mamsa rasa, yusha* & diet with *snigdha*, *laghu, ushna* quality & *kapha avriddhikara aharas* are necessary since *manda kapha* state is required during *Virechana*. If during *snehapana* itself, *dosha paka* occurs in *durbala* & *bahudosha* patients & *swayam pravritti* of *doshas* starts then one should give *bhedaneeya bhojana*[7,8]

8. *Matra vinischaya*:. The *matra*, should be decided according to *agni* & *kostha* of the person

II. *Pradhana Karma*: It starts from intake of medicines, up to completion of *Vegas*.

1. *Virechana yoga sevana*: Initially *snehana* & *swedana* should be completed successfully, followed by 3 days of gap wherein bahya *Snehana* & *Swedana* must be completed. On the previous day of the *karma, laghu, ushna* & *kapha avriddhikara* drugs should be taken

2. Assessment of *Virechana: Kaphanta Virechana* is desired.

Chakrapani has categorized *Shuddhi* under

1. *Antiki* (end product)

2. *Vegiki* (no. of vegas)

3. *Maniki* (quantity of vomited material)

4. *Laingiki* (Observations on the person[9]

III. *Paschat Karma*: Rules and regulations regarding diet and the regimen i.e. Sansarjana Krama which is decided depending upon the type of *shuddhi*[10]

Observations and Results

· Health tourism is a golden way to explore *Ayurveda*, and also contributes towards economic growth of the country

· *PanchaKarma* therapy is considered as a complete holistic approach to the elimination of the root cause of each and every chronic disease.

· *Virechana Karma* is safe and effective treatment modality for *pitta* disorders especially in *sharad ritu* and can be effectively used even for the delicate person.

· Thus, *Sharadiya virechana* should be practiced every year

Conclusion

· Health tourism destinations provide high quality treatment at low cost in India.

· By being in own country an *Ayurveda* physician can contribute towards globalisation of *Ayurveda* through Health tourism.

· In tourist, Practicing *virechan* therapy in *Sharad Rutu* to healthy person for maintainance of health and to diseased person to cure disease will help to expand the *ayurveda* globally.

Ethical Clearance- Taken from Institutional Ethical committee

Conflict of Interest: NIL

Source of Funding- Self

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Analysis of Platelet Volume Indices in Patients with Acute Coronary Syndrome in Gujarat, India

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Abstract

Background: Coronary artery disease (CAD) is growing in heaps and bounces with increasing incidences every year due to increase in cardiovascular risk factors like diabetes mellitus, obesity, smoking, hypertension, dyslipidemia and sedentary life style. Platelets have always been a cornerstone of thrombosis, aggravation of atherosclerosis and its complications, such as acute coronary syndromes which include acute myocardial infarction (AMI), Non ST Elevation MI (NSTEMI) and unstable angina (UA), and sudden cardiac death. Platelet aggregation and activation plays a pivotal role in thrombus generation in cardiovascular diseases. The aim of this study is to evaluate platelet indices in coronary artery disease and to reveal a clinical discussion based on them.

Methods: A total of 65 cases were studied; 21 patients had stable coronary artery disease for which they were admitted for a coronary angiogram. 27 patients had Troponin negative unstable angina (UA) or troponin positive NSTEMI or STEMI having corresponding ECG findings. The third group had a normal cardiogram.

Conclusion: Platelet Volume Indices studied were — mean platelet volume (MPV), platelet large cell ratio (PLCR), platelet distribution width (PDW) was increased in ACS as compared to stable CAD and the control group. Large platelets are functionally notorious and are a predisposing contributor for developing myocardial infarction. Larger platelets can be diagnosed routinely by CBC's, Platelet volume indices are common, cheap and easily available tool for screening future thrombotic cardiovascular diseases.

Keywords: *Myocardial infarction, platelet volume indices, coronary artery disease*

Introduction

In our new century, Coronary artery disease (CAD) has been growing with increasing incidence and prevalence across the world. Cardiovascular risk factors such as diabetes, old age, hypertension, dyslipidemia, smoking, anxiety and obesity increase the probability of developing coronary artery disease manifolds. Platelets play a major role in development of cardiovascular disease and progressive atherosclerosis, which can complicate in the form of acute coronary syndromes like unstable angina, NSTEMI and STEMI. Platelet activation, aggregation and platelet hemostatic plug formation is responsible for acute coronary events.[1] Platelet size determines platelet activity. Larger sized platelets are enzymatically and metabolically active as compared to small platelets and release large amount of Thromboxane A2.[2,3] Nowadays availability of automated cell counters has made the platelet volume available routinely in majority of clinical laboratories. The mean platelet volume shows changes in the rate of platelet production or platelet stimulation in the context of IHD and its clinic-pathological correlation.

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Materials and Methods

The study was conducted at Dhiraj Hospital, Smt B K Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth deemed university, Vadodara, Gujarat on 65 patients over a period of 12 months in 2019. Three groups were randomized. Group A: Acute coronary syndrome patients, non ST elevation ST elevation MI or Unstable angina in ICCU - 27 patients; Group B: patients with chronic stable angina admitted for coronary angiography - 21 patients; and Group C: normal healthy controls with normal ECG- 17 patients (Table 1).

4ml Blood was collected in di-potassium EDTA tubes from all the patients on day 1 and also on day 6 for patients by a sterile puncture. The platelet volume indices were studied in one hour of puncture using the ERBA H-560 five part automated cell counter and all platelet indices were calculated i.e. platelet distribution width and platelet large cell ratio, mean platelet volume and platelet count.

Inclusion criteria

1. Patients admitted with acute coronary syndromes, i.e. unstable angina, NSTEMI, STEMI.

2. Patients who have history of previous of antiplatelets therapy.

Exclusion criteria

1. Critically ill patients.

2. Patients with any bleeding or clotting disorder.

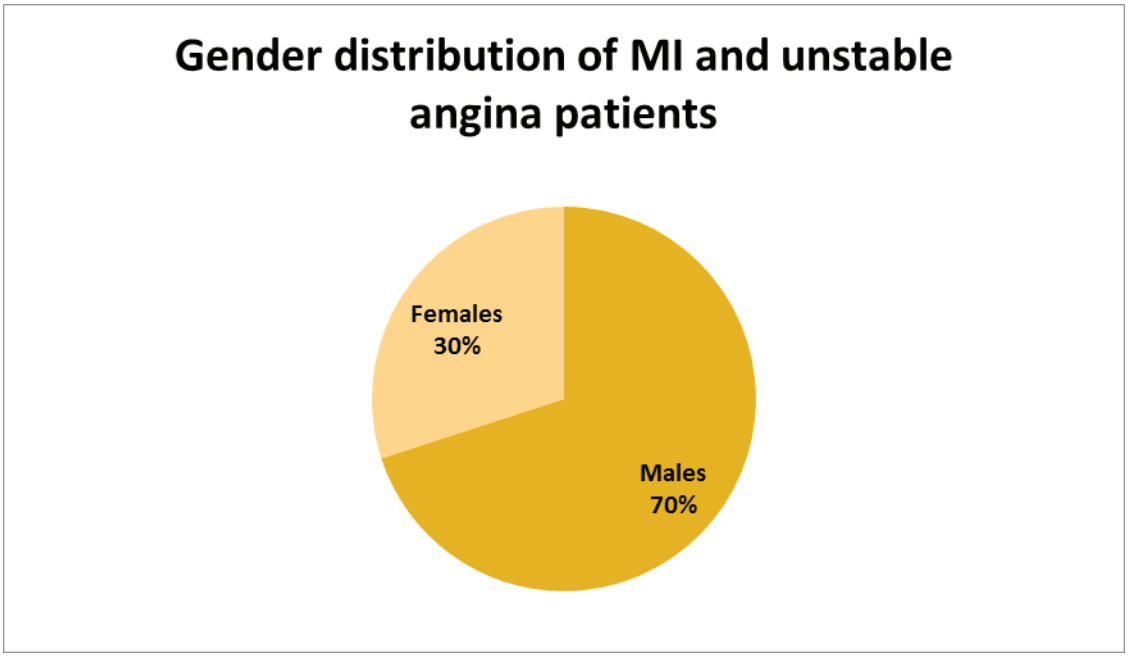
3. Patients having any platelet disorder such as immune thrombocytopenia.

Table 1: Group distribution of the patients

S.no.	Groups	Number of Patients
1	Group A: NSTEMI, STEMI, Unstable angina	27
2	Group B: Stable CAD	21
3	Group C: Normal healthy controls	17
		65

Results & Discussion

In this study, 70% of ACS patients (myocardial infarction and unstable angina) are males (graph 1). The most common age group in unstable angina group is 50-60 years and of patients in myocardial infarction group is 60-70 years. The table 2 shows that in patients of group A, the mean values of MPV and PDW are 8.2 fl and 17.9 % while in group B, the mean values of mean platelet volume and platelet distribution width are 7.6 fl and 16.5 % while in normal controls the mean values of mean platelet volume and platelet distribution width are 6.8 fl and 15.9 % respectively. This shows that these platelet volume indices are elevated in acute coronary syndrome patients.



Graph 1: Gender distribution of MI and Unstable angina patients.

Table 2: Mean value distribution of platelet indices in all groups of patients.

S.no.	Platelet Indices	Group A	Group B	Group C
1	Platelet Count	203 x 109/l	235 x 109/l	274 x 109/l
2	Platelet distribution width	16.9	15.5	14.9
3	Platelet large cell ratio	30.4 %	27.3 %	23.1 %
4	Mean platelet volume	8.2 fl	7.6 fl	6.8 fl

Automated blood cell counters are now readily available in most laboratories state-wise. The parameters for platelets which can be studied by these are, i.e., platelet distribution width, platelet count, platelet large cell ratio, platelet volume indices and mean platelet volume. In the 21st century, CAD and its related mortality and morbidity have increased in incidence and prevalence in all developed and developing countries. Atherosclerotic cardiovascular risk factors like; diabetes, old age, hypertension, smoking, dyslipidemia, metabolic syndrome and obesity are always fuel to this increasing non communicable pandemic. It is emphasized here that Platelet volume indices are now being researched as a significant parameter in ACS. Our study unveils platelet volume indices (PVI) in the spectrum of acute coronary syndromes and ischemic heart diseases. The results revealed that Mean Platelet Volume directly proportional to the thrombotic state in acute coronary syndromes and the larger platelets play significant role in infarction. Larger sized platelets can be found out by platelet studies and the patients with could benefit from early primary prophylaxis and intervention.[4]

The current study showed higher MPV mean value (8.2 fl) in unstable angina and myocardial infarction groups compared to group B and group C which had MPV of 7.6 fl and 6.8 fl respectively. Our study was cross sectional so follow up details could not be tracked down and the diagnostic benefit of these values cannot

be established. This finding is also supported by Ranjith M P et al [5] done in Jabalpur India, M M Khandekar et al [4] done on 210 patients, and also by Pervin S et al [6] among 142 patients.

There are plenty of evidences to support the hypothesis that generalised platelet activation occurs before an acute ischemic cerebrovascular and coronary event. The enhanced platelet consumption causes the bone marrow to release megakaryocytes at the site of the atherosclerotic plaque.

Platelets are proven to play an essential role in the progression of atherosclerosis and atherosclerotic cardiovascular diseases including myocardial infarction and cerebrovascular accidents. To provide a larger surface area for clotting factors to form hemostatic plug, platelets change in shape from discoid to spherical. Platelet indices such as PDW and MPV can be easily studied and they are elevated during platelet aggregation and activation. Platelet distribution width measures the difference in platelet sizes, and its large values suggest larger reticulated platelets.[7]

Conclusion

Our study revealed that high values of mean platelet volume leads to the widespread thrombosis in cardiovascular syndromes and the large platelet play an important role in thrombosis. As larger platelets are functionally more active, they appear to be one of many contributory factors for cardiovascular thrombosis. Patients having large platelets can be diagnosed by pathological blood tests as Platelet Volume Indices are reported as a side product of all blood count's study. In summary, Platelet volume indices provide a cheap, easily available and important investigation, which is useful for future acute cardiovascular events.

Ethical Clearance- Taken from sumandeep vidyapeeth deemed university ethical committee.

Source of Funding- Self funded

Conflict of Interest - Nil

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Cyclooxygenase-2 Level in Bilharzial and Non-Bilharzial Related Bladder Cancer among Iraqi Patients

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Abstract

Bladder cancer has become common cancer globally. It is the most common urological cancer; it comprises a significant part of urologists' work. 80 % of bladder cancers are superficial at diagnosis; they have not invaded into the muscle. The residual 20% are muscle-invasive, which carry a much worse prognosis. Emerging evidence mark that some parasites such as the blood fluke Schistosoma haematobium, Clonorchis Sinensis and small liver flukes Opisthorchis viverrini are causative agents of malignancies such as bladder cancer caused by schistosomes and cholangiocarcinoma by liver flukes. Infection with Schistosoma haematobium leads to urogenital schistosomiasis, which has been correlated with the occurrence of bladder cancer. The mechanisms responsible for this association have not yet been clearly identified. This study clarifies the association between cyclooxygenase-2 (COX2) and bladder lesions associated with Schistosoma. The result shows there is a high expression of COX2 in the Bilharzia related bladder cancer (BBC) while there is low expression of COX2 in non-bilharzia related bladder cancer (NBBC). In conclusion, conceder the positive expression of COX2 among Iraqi patients with Schistosomal-related bladder lesions is high. There may be a strong association between high rates of bladder cancer and urinary schistosomiasis in Iraq, as the vast majority of COX2 lesions were positive.

Keywords: *Schistosoma, Bladder cancer, COX2, TCC, SCC.*

Introduction

The bladder cancer refers to any of several types of malignant growths of the urinary bladder. Abnormal cells multiply without control in the bladder in a disease. Urinary bladder tumors contribute greatly to the global incidence of human cancer with about 550,000 new cases worldwide each year. Of those, around 425,000 occur in men, and about two-thirds occur in high-income countries [(1)].

There are two main types of bladder cancer specified: Transitional cell carcinoma (TCC). It one of the most recurrent tumors correlating with exposure to exogenous carcinogens such as smoking and most prevalent in Western and industrialized countries. The second type is squamous cell carcinomas (SCC), which are more recurrent seen in some country of Middle Eastern and African countries, where urinary schistosomiasis is an endemic disease [(2)]. Around 75% of squamous cell carcinoma in the Middle East and Africa was caused by Schistosoma haematobium infection [(3)].

One of the main risks factor is Schistosoma haematobium infection that causes urogenital schistosomiasis, which is linked to bladder cancer. According to the (WHO), schistosomiasis infect 200 million individual and is endemic more than 76 tropical developing countries. Schistosomes are parasitic blood flukes, which have an intermediate invertebrate host: freshwater snails and a mammalian host [(4)].

Cyclooxygenase-2 (COX-2) is another inflammatory mediator involved in tumour growth [(5)]. It is an inducible

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enzyme (also called prostaglandin synthase) that converts arachidonic acid to prostaglandins [6] . For most normal human tissues, cyclooxygenase-2 (COX-2) is not detectable but can be found in inflammation [7]. COX-2 overexpression may play a role in carcinogenesis, the underlying mechanisms of COX-2 tumorigenicity linked to several factors, including apoptosis inhibition, angiogenesis promotion, and activation of carcinogens such as aromatic amines, immune surveillance modulation, and increased invasiveness of tumour cells. It was associated with the development and progression of different forms of bladder cancer, including bilharzia bladder cancer [8].

COX2 is useful as a signal marker for bilharzia-associated bladder cancer, which may clear the way for preventive and therapeutic approaches using Cyclooxygenase 2 inhibitors to control bladder cancer in addition to chronic inflammation. [8] .

Materials and Methods

The current study was done during the period from October 2019 to June 2020. The study includes 50 patient of Iraqi people with bladder carcinoma. The cases collected from Al-Sadr Teaching Hospital in Al-Najaf province, Ghazi al-Hariri hospital for surgery specialist/ Baghdad, Al kadhimiya teaching hospital/Baghdad and Al-Diwaniyah Teaching Hospital.

Fifty cases divided into two groups of patients, patients with bladder cancer with history of schistosomiasis, patients with bladder cancer without a history of schistosomiasis. The cases include 39 male and 11 female. The age of patient range from 21 to 75 with mean 51.6 years. The samples included two types, paraffin-embedded block from hospital (45 samples) archive and fresh tumor biopsies (5 samples). The fresh biopsy transferred to the laboratory in formalin 10% to prepare tissues blocks embedded in paraffin to prepare for immunohistochemistry.

Preparation of fresh biopsy and paraffin-embedded block sample for immunohistochemistry it is proceed by placed the fresh sample in 10% neutral buffered formaldehyde immediately after removal. Then dehydration in various concentrations of ascending ethanol (70, 80, 90 and 100%). Clearing with xylene and then infiltration with paraffin wax at 60°C. Embedding in molten paraffin templates and they were left at laboratory temperature. The two type of sample were Sectioned by the rotary microtome (5µm thick sections) and then place the section on the water path and then placed on positive charge slide. The sections were de-paraffinized with xylene and then hydrated through 100%, 90%, 70% and 50% ethanol.

After dewaxing and hydrate the paraffin section add retrieval solution (Sodium citrate buffer) and incubate at 95°C for 15-20min then cooling the slide for 15min. After the slide cooled washed with phosphate buffer solution (Elabscience china) for two minx3 times. Add 3% H2O2 to the slide over tissue part for 10 min to eliminate endogenous peroxidase activity. Wash with phosphate buffer solution for two minx3 times. Then drain the PBS with absorbent paper, and then add Normal Goat Serum to the section Incubate for 30 min at 37°C. Dry the liquid around the section with absorbent paper, and draw a circle around the tissue with pap pen. Add primary antibody with proper dilution ratio, incubate at 37°C for 1~2h. Wash with phosphate buffer solution 2 min ×3 times. Then add polymer helper and incubate at 37°C for 20 min. Wash with phosphate buffer solution 2 min ×3 times. Add Polyperoxidase-anti-Mouse/Rabbit IgG and incubate at 37°C for 20~30 min. Then wash with phosphate buffer solution 2 min ×3 times. Add DAB working solution and take control of the DAB colouration period, the colour of tan or brownish-yellow is the positive signal. Wash the section with deionized water terminates the chromogenic reaction. Drain the slide with absorbent paper. The tissues' sections were stained with hematoxylin. This process also includes immersion the sections in xylene and different concentrations of ethanol. The prepared sections were mounted with DPX and covered with a glass coverslip. After that, the slides were cleaned and labelled to become ready for microscopic examination .

Statistical Analyses

Version 26 of the SPSS statistical program was used for all statistical analyses. The chi-square Pearson test was used and statistically relevant P. values of 0.01 or less were considered .

Result

In this study, pathologic evidence of urinary

schistosomiasis was shown in 21 (42%) patients. However, the true frequency of such infection among Iraqi patients may be higher than the estimated figure depending on certain factors such as the biopsy site, the number of biopsies taken, and the intensity of infection. Besides, there may be other patients in the Iraqi society with urinary schistosomiasis who did not have the opportunity for urinary bladder biopsy due to neglected lower urinary tract symptoms or inadequate referral to urology departments in major hospitals.

The age range of patients was between 21 and 75 years, with a median age of 51.6 years. The high frequency was recorded in age group (45-52) years and (69-76) years, which constituted 44% of the total number of patients. The present investigation showed that 78% of the patients were males and only 22% were females).

The histopathology examination of the sample microscopically shows 34- sample diagnostic as transitional cell carcinoma with their percentage 68% of the entire cases. In addition, 16-sample diagnostic as squamous cell carcinoma with the percentage 32%. The result shows transitional cell carcinoma is the predominant type of bladder cancer, whereas in schistosomiasis - endemic regions, squamous cell carcinoma is the most common type . The result shows there are 15 samples contain the schistosomiasis ova and 35 samples not contain. The 15 samples divided to the two types of cancer; transitional cell carcinoma 2(13.33%) and squamous cell carcinoma 13(86.66) .the sample nil from ova divided to the two types of cancer transitional cell carcinoma 32(91.42) and squamous cell carcinoma 3(8.57%).

The gene expression of the cyclooxygenase-2 in the whole cases displayed in (table 1). The expression estimate by staining intensity among the positive cells ranged from weak to dark brown reaction product. The staining intensity divided into the four types; weak staining, low staining, medium staining and high staining. The result shows high expression of the cyclooxygenase-2 in the squamous cell carcinoma cancer type (10 cases) while the transitional cell carcinoma (4 cases). In addition, low and weak expression of the COX2 appears in a high number of cases in the type transitional cell carcinoma. The COX2 expressed in medium rate in transitional cell carcinoma (4 cases) and squamous cell carcinoma (5 cases).

Table 1 : The gene expression of the cyclooxygenase-2 in the whole cases

No.	COX Expression	TCC	SCC
1	Weak	12	0
2	Low	14	1
3	Medium	4	5
4	High	4	10
Total = 50		34	16

TCC : Transitional Cell Carcinoma ; SCC : Squamous Cell Carcinoma

The gene expression of COX2 based on the pathological characteristic of bladder sections shown in table (2). In the table, descript the COX2 expression in two groups. The two groups divided according to the patients if have bilharzia history or not; the group split to bilharzia bladder cancer and non-bilharzia bladder cancer.

The result shows high expression of Cox2 in 14 samples; all of them is bilharzia related bladder cancer cases. In contrast, the medium expression appears in nine samples divided to 6 bilharzia related bladder cancer cases, and three samples are non-bilharzia bladder cancer cases. The low expression appears in 15 samples divided to 1 sample Bilharzia related bladder cancer cases, and 14 samples are non-bilharzia related bladder cancer. The weak expression appears in 12 samples; all of them are non-bilharzia related bladder cancer.

Table 2 : The gene expression of COX2 based on the pathological characteristic of bladder sections

Expression	Entire Cases	BBC	NBBC	P.Value
Weak	12	0	12	<0.005
Low	15	1	14	
Medium	9	6	3	
High	14	14	0	
Total	50	21	29	

BBC : Bilharzia bladder cancer ; NBBC : Non-bilharzia bladder cancer

Discussion

Usually, COX-2 does not exist in tissues but is caused by certain stimuli, including inflammatory cytokines, growth factors and oncogenes [9]. However, COX-2 is not expressed in the normal human urinary bladder epithelium [10] .

Cyclooxygenase-2 expression showed in transitional cell carcinoma cases was not significantly than in the early stages. This is consistent with the insufficiency of COX-2 as an indicator of tumour aggression [7]. Other studies were unable to connect COX-2 associated expression to tumour status, including level, histological gradation or remote metastasis [11]. Wülfing et al. (2004) Found that TNM and histologic grading were not associated, but there was a significant interaction between transitional carcinoma of cells and squamous carcinoma of the cells [12] .

Most bladder squamous cell carcinoma expressed COX-2 markedly. Conversely, transitional cell carcinoma was less COX-2 expressed. These variations in expressions can be due to etiological variations. The development of SCC is closely linked to chronic inflammation of the urinary tract, so COX-2 plays a leading role in inflammatory carcinogenesis. T. Shirahama and Sakakura (2001), Moussa et al. (2009) agree with these findings; they shown the COX-2 is high in urinary bladder squamous cell carcinomas [7,9] .

Genetic and clinical research found that COX-2 is a critical aspect of carcinogenesis. COX-2 expression plays a significant role in cancer growth and tumorigenesis [13] . Increased COX-2 protein can contribute to tumorigenesis by affecting many biological characteristics like cell adherence, angiogenesis, including apoptosis and invasiveness [14].

The findings strongly show that Cyclooxygenase-2 is involved in the early carcinogenesis of squamous bladder cell. Unlike squamous cell carcinoma, transitional urinary bladder cell carcinoma expressed COX-2, as previously reported, less heterogeneously. This result nearly similar to [6]. The gene expression there are statistically significant differences between the infection size NBBC& BBC measured by COX2. The result showed a high expression level of COX2 in the BBC with the P-value <0.005 reverse NBBC that show low expression of COX2. The high level of COX2 expression in BBC probably related to the Schistosoma worm placed the ovum in the bladder wall, and the ovum caused damage to the bladder lumen by travelling through the urothelium. These travel casings are damaged by chronic inflammation through their interaction with host immune cells and subsequent formation of granuloma [15] . These chronic inflammation lead to macrophages activated at inflammatory sites that lead to increase the pro-inflammatory cytokines IL-1, TNF-a and TGF-b, are COX-2 expression inducers. This expression can contribute to the mechanisms by which chronic inflammation initiates BBC cell transformation [16].

Conclusions

Take care of people with schistosomiasis in their lives who are at risk of developing bladder cancer and

do periodic check-ups for them and Monitoring cox 2 levels for people who have had a previous infection with Bilharzia, and use anti-cox 2 if levels elevated. COX-2 are essential markers, which differentiate between Schistosoma-related bladder cancers and non-Schistosoma.

Ethical Clearance: Taken from University of Al-Qadisiyah ethical committee

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Effect of Iron Deficiency Anemia on HbA1c Levels in Patients of Diabetes Mellitus with Controlled Plasma Glucose Levels

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Abstract

Background: Hemoglobin A1C (HbA1c) is an indicator of patient's glycemic status over the previous 3 months. HbA1C levels increase in presence of Iron deficiency anemia.

Aim: To analyze the effect of iron deficiency anemia on HbA1c levels in patients of diabetes mellitus with controlled plasma glucose levels.

Material and Methods: In the present study, total 50 patient of diabetes mellitus with iron deficiency anemia with controlled plasma glucose levels were taken as a cases and 50 iron-sufficient non-anemic healthy individuals were taken as a controls. Medical history was taken from all participant and data of HbA1c, ferritin, FBS, hemoglobin, peripheral smear examination, red cell indices were collected from laboratory. Statistical analysis was carried out by student's t-test, Chi-square test, and Pearson's coefficient of regression.

Results: HbA1C level (6.90 ± 0.81%) were increased in iron-deficient individuals as compared to controls. In females, HbA1C (7.08 ± 0.78%) were more elevated than males. When classified on the basis of FBS, HbA1C was elevated more in subjects having FBS between 100-126 mg/dl (7.31 ± 1.52%) compared to the those with normal FBS (<100 mg/dl).

Conclusion: A positive correlation found between iron deficiency anemia and increased HbA1C levels, especially in the controlled diabetic women and individuals having FBS between 100-126 mg/dl. Therefore, presence of iron deficiency anemia should be checked before altering the treatment regimen of diabetes mellitus patient.

Keywords: *Iron deficiency anemia, Hemoglobin A1C, Diabetes Mellitus*

Introduction

In the world, fifty percent of anemia is attributed to iron deficiency anemia. In India, Iron deficiency anemia is one of the most prevalent forms of malnutrition. The storage from of iron is ferritin which reflects the iron status.[1] Hemoglobin A1C constitutes 5% of the total hemoglobin in normal adults and up to 15% in patients with diabetes mellitus Hg b A to HbA1c conversion takes place during the entire life span of the red blood cell and the rate of this reaction is faster in diabetics because of the higher prevailing glucose concentration, resulting in a higher concentration of HbA1c.[2] Anemia may be associated with more rapid erythrocyte turnover, lowering concentrations of HbA1c, or with slower turn over or changes in the three-dimensional configuration of hemoglobin (Hb) in such a way as to increase the glycation of its N-terminal valine, leading to higher HbA1c.[3]

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HbA1c is a glycated hemoglobin which reflects the patient's glycemic status over the previous 3 months. Glycated hemoglobin, including HbA1c and other hemoglobins, constitute the HbA1 fraction of adult hemoglobin (HbA).[4] HbA1c provides an integrated measure of glycemia. HbA1C is less susceptible to short-term regulation than plasm blood glucose levels. Diagnosis of type 2 diabetes mellitus is done by HbA1c determination that has approval of WHO and ADA.[5]

Clinically HbA1C levels depend on following three major factors.

1. The mean age of RBCs in the circulation

2. HbA1c in reticulocytes when released from the bone marrow;

3. Hb glycation rate as red blood cells (RBCs) become older, a function of glucose concentration to which Hb is exposed.[5]

The prevalence of anemia is estimated about 10% to 30% in patients with diabetes. Iron deficiency anemia constitute about one-third of patients with anemia. Previous studies have shown that iron deficiency elevates HbA1c levels independent of glycemia.[6]

According to study conducted by Alap L. Christy et al., iron deficiency anemia has positive correlation with increased level of HbA1C concentration. These positive correlation was seen especially in patients of DM with controlled fasting glucose levels and in the subjects having FPS between 100-126 mg/dl. Therefore, presence of iron deficiency anemia should be considered before altering the treatment regimen for diabetic patient.[1] Study conducted by Absra Solomon et al demonstrated that diabetics with IDA had slightly lower compared to the diabetics with non-IDA patients.[2] Jari Intra et al concluded that mean level of HbA1c were significantly higher in anemic subjects (5.59%) compared to non anemic subjects (5.34%) ($P<0.0001$). These results suggest that clinicians should be cautious about diagnosing prediabetes and diabetes in individuals with anemia.[7]

HbA1c is most generally utilized as a significant marker of glycemic control, and that why it is important to exclude those factors which could falsely elevate its levels. On the basis of these, aim of our study is to estimate HbA1C levels in iron-deficient anemic persons with fasting blood glucose levels less than 126 mg/dl to see the impact of anemia on HbA1C concentrations.

Materials and Methods

The present study was conducted at Department of Biochemistry, SBKS MI & RC, Piparia, Gujarat in which 50 known case of iron deficiency anaemic individuals with diabetes and 50 iron sufficient non diabetic individuals were included from March 2019 to March 2020. We used the data of patients who attended the Dhiraj General Hospital (DGH) were included in the study.

Inclusion Criteria-

- Iron deficiency anemia should be consider when Individuals having low hemoglobin levels (less than 12 gm% in males and less than 11 gm% in female), predominantly microcytic indices (mean corpuscular volume-MCV less than 76 fL), and hypochromic indices (mean corpuscular hemoglobin –MCH less than 27 pg/cell).

Exclusion criteria-

- Subjects having FBS >126 mg/dl or RBG >200 mg/dl or PP2BS >200 mg/dl
- Hemoglobinopathies,
- Hemolytic anemia,
- Hypothyroidism,
- Pregnancy and
- Renal failure was excluded.

Laboratory Investigations

HbA1c, plasma glucose, peripheral smear, hemoglobin, MCH, MCV, mean corpuscular hemoglobin concentration (MCHC) and serum ferritin levels data were gathered from central clinical laboratory, DGH, Piparia.

Measurements

HbA1c was analysed by HPLC method using Hb Vario HbA1C analyzer. Plasma glucose was analysed by glucose oxidase/peroxidase method using fully

automated chemistry analyzer EM 200. Hemoglobin, MCV, MCH, and MCHC were analysed by Sysmex KX 21 automated counter. Serum ferritin was analysed by Nephlometry method on Mispa- i2 analyzer. For all the investigations, SOPs were used.

Statistical Analysis

Medcalc software was used for all statistical analysis. Data were presented as Mean and SD values. Comparison of two groups means were carried out by applying a student's t-test. Determination of correlation between two variables were carried out by applying Pearson's coefficient of correlation. Categorical data was analyzed by $\chi 2$ test. Logistic regression analysis was used for determined odds ratio and 95% confidence intervals. Interpretation was done according to p-value. A p-value less than 0.05 ($p< 0.05$) * - significant and **p < 0.001 – highly significant.

Results

The present study included 50 patients of iron deficient diabetic persons and 50 normal iron sufficient non diabetic healthy persons.

Subject characteristics

Mean ± SD of HbA1c among cases was 6.90±0.81 %. Mean ± SD of FBS among cases was 106.92±15.62. Mean ± SD of age among male and female cases was 50.91±9.87 and 45.86±9.66 respectively. Mean± SD of hemoglobin levels in male cases were 9.24 ± 0.97 and in female cases were 8.52 ± 0.89 gm/dl. Mean± SD of MCV and MCHC in cases was 64.43 ± 8.46 fL and 18.87 ± 2.32 pg/cell respectively.

Table 1. Baseline characteristic among iron deficient and non-iron deficient anemic individuals

	Male		Female		Total	
	IDA	**NA**	**IDA**	**NA**	**IDA**	**NA**
Number	22	21	28	29	50	50
Age	50.91 ± 9.87	52.95 ± 12.60	45.86 ± 9.66	49.45 ± 10.13	48.08 ± 9.98	50.92 ± 11.25
HbA1C	6.67 ± 0.81	5.08 ± 0.52	7.08 ± 0.78	5.23 ± 0.55	6.90 ± 0.81	5.17 ± 0.54

Tables 1 shows that HbA1c concentration was significantly higher in females compared to the males.

Table 2: Comparison of HbA1C levels in individuals based on their age

	Male		Female		Total	
Age (Years)	**IDA**	**NA**	**IDA**	**NA**	**IDA**	**NA**
≤ 50	6.73 ± 0.88	5.05 ± 0.45	7.08± 0.69	5.15 ± 0.55	6.94 ± 0.78	5.11 ± 0.51
>50	6.88 ± 0.74	5.12 ± 0.62	7.48 ± 0.98	5.35 ± 0.56	6.83 ± 0.88	5.25 ± 0.58

Tables 2 shows that HbA1c levels was significantly higher in subjects above 50 years of age compared to the subjects below 50 years of age.

Comparison of Fasting blood sugar and HbA1c levels.

Mean±SD fasting blood sugar (FBS) levels in iron deficient diabetic individuals was found to be 106.92 ± 15.62. In patients of well controlled diabetes mellitus with normal FBS (FBS levels <100 mg/dl) had HbA1c of 6.43 ± 1.07%. In subjects who had FBS between 100-126 mg/dl had a significantly higher mean A1C value of 7.33 ± 1.55% (Table 4, Fig. 3). Individuals with FBS between 100-126 had odds ratio for HbA1C >6.5 % was 5 fold higher in females and 4 fold higher in total population, but it was non-significant in males.

Table 3: Logistic regression analysis for effect of sex, FBS and age on HbA1c levels (6.5 %)

	Female		Male		Total	
	Odds ratio	**95% CI**	**Odds ratio**	**95% CI**	**Odds ratio**	**95% CI**
Female sex	0.702	0.367-1.284				
PG 100-126	5.592*	1.913- 16.305	2.336	0.827-6.583	4.072*	1.909-8.663
Age >50	0.727	0.336-1.658	0.750	0.323-7.129	0.867	0.448-1.601

*p < 0.05 – significant, CI- Confidence interval.

Table 3 shows, patients with FBS levels in pre-diabetes range have higher odds ratio for having HbA1c in diabetic range (>6.5%).

Table 4: HbA1c (%) distribution in male and female having FBS <110 and FBS 100-126

	Female		Male		Total	
	IDA	**NA**	**IDA**	**NA**	**IDA**	**NA**
HbA1c in FPG <100	6.91 ± 1.19*	5.10 ± 0.58	6.66 ± 0.88*	5.45 ± 0.59	6.83 ± 1.02*	5.09 ± 0.61
HbA1c in FPG 100-126	7.73 ± 1.65*	5.85 ± 0.48	6.98 ± 1.19*	5.79 ± 1.01	7.31 ± 1.52*	5.69 ± 0.71

IDA, iron deficiency anemia; NA, no anemia; P value (<0.05*). As shown in the Table, patients with FBS in pre-diabetes range have higher HbA1c levels when compared to those with FPG <100 mg/dl and non-anemic subjects.

Mean hemoglobin and ferritin levels for cases were found to be 8.44 ± 1.26 and 11.36 ± 7.59, respectively.

Discussion

HbA1C reflects long term fluctuation of blood glucose concentration instead of recent glucose control in blood. A diabetic patient may still have a high level of HbA1C who shows good glucose control recently.

The most frequently occurring fraction of hemoglobin A1 is glycated Hb(HbA1C) In RBCs, glucose reacts with N-terminal valine of both beta chains which form an aldimine linkage. Then it undergoes

rearrangement and form a more stable ketoamine link. According to ADA guidelines, HbA1C considered as the primary target for glycemic control. It is considered as a diagnostic criterion for DM. It is also elevated in other conditions such as hemoglobinopathies, chronic renal failure, pregnancy, and nutritional anaemias.[1]

Iron deficiency anemia is one the most common anemia amongst the nutritional anaemia in India. The study conducted by Sluiter et al. demonstrated that glycation of hemoglobin is an irreversible process and therefore HbA1C concentration will be increased with cell age. In iron deficiency anaemia, the RBC production is low which ultimately increase the average age of circulating RBCs results into increase in HbA1C concentration.[8]

In patients of diabetes mellitus and non-diabetes mellitus, number of studies were done but in well-controlled diabetes mellitus patients not much studies were conducted. The well controlled plasma glucose levels for three consecutive months correlates very well with controlled HbA1c.Therefore, it is expected that HbA1C level is less than 6.5 % in patients of DM with controlled plasma glucose levels.[9]

As shown in the results, HbA1C concentration was significantly higher in iron deficient anemic individuals who had less than 126 fasting plasma glucose level. In the present study, HbA1C levels were demonstrated according to the age, sex and plasma glucose level.

In the present study, there were higher mean hbA1C concentration in more than 50 years of age. But the probabilities of higher HbA1C in diabetics were statistically non-significant. It has been found that there is no significant correlation between HbA1C. Therefore, our findings nullify the role of elderly age with increasing the HbA1C level in iron-deficient individuals. HbA1C level were increased in males, but odds ratio was not significant.

The conditions such as iron deficiency anemia can spuriously increase HbA1C levels therefore iron deficiency should be considered before altering the treatment of patients. There were significantly increase HbA1C levels in iron deficient patients who had FPG between 100-126 mg/dl which suggest that iron deficiency anemia gives false picture of glycemic status. The mean HbA1C was around 6.4% who had FPG levels <100 mg/dl which were higher than those of controls. Thus iron deficiency anemia has significant role for increasing the HbA1C level in both the groups. There is no significant correlation was found between ferritin and HbA1C.

Conclusion

Presence of iron deficiency anemia increase the level of HbA1c in patients of diabetes mellitus with controlled plasma glucose levels. HbA1C is more elevated in diabetic patients who had plasma glucose levels between 100 to 126 mg/dl. Our observations can be attributable to the fact that iron deficiency anemia should be considered before altering the treatment of diabetic patients.

Conflict of Interest – Nil

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Ethical Clearance - Approval for the study was taken from Institutional Ethics Committee.

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Punishing the Suicide Survivors- The Justifiability

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Abstract

Suicide is a product of social abnormality, and originates from social incapability and mental distress; it cannot be appropriately addressed under the orders of law. In India, day by day, thousands of individuals irrespective of gender, age, social status and educational qualifications are ending their lives by committing suicide. The privilege to end one's own life has become much talked about subject all through the world. In India, Criminal Law students are very often asked a question that 'What is that act, which if completed is not made punishable but the failure to complete it or the mere attempt is made punishable?' The answer is "suicide" and 'attempt to commit suicide' is made punishable under Section 309 of the Indian Penal Code. An individual who is successful with regards to taking his own life gets away from all worldly miseries as well as legal action where as one who fails in suicidal attempt is brought into the trawl of criminal courts. The issue with regard to de-criminalization of attempted suicide has been questioned with controversies, besides moral and human point of view. The present article aims at discussing the various factors responsible as to why a person attempt to commit suicide and the attention towards the ongoing debate of retaining or deleting Section 309 of the Indian Penal Code, 1860 by testing its validity.

Key words- *Suicide, attempt, decriminalization, offence, life, death*

Introduction

Thomas Jefferson once remarked: *"the care of human life and happiness and not their destruction is the first and only legitimate object of good government"*

Suicide means deliberate termination of one's own physical existence or self murder. Suicide as a predicament is the combination of social, psychological, philosophical, moral, ethical and legal reasons behind it. A demonstration of suicide requires grave mindfulness in light of the fact that there is nothing a normal reasonable man fears more than his death, and that panic in the huge majority of cases, is as consistent as it is inescapable.[1] According to "Suicide Death rates in India are amongst the Highest in the World" *The Week* (December 10, 2018), Suicide came to be ranked first in India as the cause of death in this age group; whilst it is ranked as third globally. According to the recent report published in '*Crime in India 2018 Statistics* (National Crime Records Bureau, Ministry of Home Affairs) under the Chapter II (Suicide in India) recorded a total of 1,34,516 suicides were reported in the country during 2018 showing an increase of 3.6% in comparison to 2017 and the rate of suicides has increased by 0.3 during 2018 over 2017.'[2] Every year, more than 1,00,000 people commit suicide in our country. There are various causes of suicides like professional/career problems, sense of isolation, abuse, violence, family problems, mental disorders, addiction to alcohol, financial loss, chronic pain etc. NCRB collects data on suicides from police recorded suicide cases. From this it is clear that police recorded cases does not include the unreported cases and in India many suicide cases are not reported and mainly the attempted cases are reported minimal because attempt to commit suicide is still considered an offence. According to the WHO, around 8 lakhs people die of suicide every year and up to 25 times as many make a suicide attempt. And in 2019, *National Crime Records Bureau* provides that the

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average rate of suicide in India is 10.4. In India, attempt to commit suicide is made punishable under Section 309 of Indian Penal Code, 1860. There is always conflicts because of judgments given by our Courts about whether right to life includes right to die within the meaning of Article 21 of the Constitution of India.

Objectives:-

- To analyse the validity of 'attempt to commit suicide as an offence' from the societal point of view.

- To ascertain the Indian judicial trend relating to attempt to commit suicides.

Legislative Provisions:-

- **Section 309 of Indian Penal Code, 1860-** Attempt to commit suicide: Whoever attempts to commit suicide and does any act towards the commission of such offence, shall be punished with simple imprisonment for a term which may extend to one year or with fine, or with both.

- **Article 21 of the Constitution of India, 1950-** Right to Life and personal liberty: provides that, "No person shall be deprived of his life or personal liberty except according to procedure established by law."

- **Mental Healthcare Act** passed on 7 April 2017 and came into force from 29th May, 2018. **Section 115 of (MHCA), 2017-** Presumption of severe stress in case of attempt to commit suicide-

(1) "Notwithstanding anything contained in Section 309 of the Indian Penal Code any person who attempts to commit suicide shall be presumed, unless proved otherwise, to have severe stress and shall not be tried and punished under the said Code. (2) The appropriate Government shall have a duty to provide care, treatment and rehabilitation to a person, having severe stress and who attempted to commit suicide, to reduce the risk of recurrence of attempt to commit suicide."

Judicial Decisions

In ***Maruti Shripati Dubal v. State of Maharashtra*** [1987 (1) BomCR 499] the Bombay High Court was of the view that Sec. 309 of IPC is violative of Art. 14 of the Constitution for being discriminatory in nature and for violating arbitrarily the equality guaranteed to the persons. The provision was further held to be violative of Art. 21 as right to life include the right to terminate one's own life also. In ***Chenna Jagadeeshwar & Anr. v. State of Andhra Pradesh*** [1988 Crl.L.J.549] the Andhra Pradesh High Court, however, rejected the above contention made by the Bombay High Court and held that Sec. 309 IPC is not violative of Article 14 and Article 21 of the Constitution. In **P. *Rathinam/N. Patnaik v. Union of India*** [1994 AIR 1844] where the Supreme Court held that S, 309, I.P.C. is violative of Art. 21 of the Constitution, as there exists under A. 21 a 'right to die'. In ***Gian Kaur vs. State of Punjab*** [1996 AIR 946] the decision of Ratinam was overruled holding that Article 21 cannot be construed to include within it Right to Die as a part of the Fundamental Rights guaranteed therein, and therefore, it cannot be said that Section 309 is violative of Article 21.The Supreme Court in the case of ***Aruna Ramachandra Shanbaug v Union of India and Ors*** [(2011)4 SCC 454] had recognised passive euthanasia in 2011 and had even laid down elaborate procedures to execute the same. SC allowed "passive euthanasia" of withdrawing life support to patients in a permanently vegetative state but rejected out rightly 'active euthanasia' of ending life through the administration of lethal substances. In ***Common Cause (registered society) v Union of India & Anr*** (Common Cause) [W.P. (Civil) 215 of 2005] , Court recently in 2018, clarified that Article 21 covers within its ambit only passive euthanasia and not active euthanasia. Finally, the Court goes on to say that the right to live with dignity also includes the smoothening of the process of dying in case of a terminally ill patient or a person in permanent vegetative state with no hope of recovery and that is why it also recognises Advance Directives akin to a 'Living Will' through which persons of sound mind and in a position to communicate can indicate the decision relating to the circumstances in which withholding or withdrawal of medical treatment can be resorted to. The SC ruled that the interest of the patient shall override the interest of the State in protecting the life of its citizens and that right to live with dignity attaches throughout the life of the individual.

Concept of Attempt to Commit Suicide:-

The word 'Suicide' got its source from the Latin word 'Suicidium' which is derived from *Sui Caedere* meaning 'To Kill Oneself". More appropriately 'Suicide'

can be defined as a "self-intended lethal behaviour for extinguishing one's own life". The concept of decriminalization of attempted suicide is based on the philosophy of humanism and comparison. It recognizes the autonomy of the individual, freedom of choice to live or die with dignity.[3] It is a very sensitive issue that polarized the world; and one of the most perplexing issues which the world faces today. Few developed countries have given nod to decriminalize attempted to commit suicide. India too seems to have joined the club and is about to decriminalize attempted suicide; with a proposal to delete the relevant provision i.e. Section 309 of The Indian Penal Code relating to attempt to commit suicide. Differences arise in the question of what constitute an offence. Basically, a certain type of conduct is regarded as offence, because the conduct is supposed to cause harm to others.[4] Moreover, it has been argued that irrespective of harm caused to others by one's actions, there exists the element of "public morality", which holds the fabric of the society together. This means that individual autonomy should be coupled with public morality and the responsibility of an individual to the society. When criminal law chooses a particular conduct for punishment and distinguishes it from another conduct, it must take into account the magnitude of the harm likely to be caused.[5]

The move towards de-criminalization probably began with the feeling that for "certain types of conduct labelled as criminal in a particular legal system, the use of criminal sanctions is not really appropriate. This stems from a variety of considerations including the eternal debate as to the proper scope and limitations of criminal law in enforcing morality. Also, particular types of conduct against which criminal sanctions have been applied, may in due course of time, come to be regarded as not appropriate for the use of such sanctions for example conduct that is worth moral condemnations or disapprobation may not necessarily be made the subject matter of criminal sanction.[6]

Personal and social reasons, financial problems and poor health are considered as major reasons of suicide in India. The common modes of suicide are poisoning, drug overdose and hanging. Nowadays, it is found that the suicide rate is increasing in every age group of person especially in the teenagers and the elderly person (above the age of 60 years), which will pose additional challenges. Recently, I visited and enquired in the Private Hospital **SUM; Faculty of Medical Sciences of Siksha 'O' Anusandhan (SOA Deemed to be University), Bhubaneswar, Odisha** and got to know from the records of patients from Psychiatric ward that around 40% of Teenagers, 25% of Old age people, 20% of Married women and rest 15% people from other category have attempted suicide. For the Teenagers; love failures, failure in exams and pressure from parents and peer group are the main causes to take an attempt in such early age. For the elderly; social isolation, depression, functional disability, and the feeling of being a burden on their family have been cited as reasons for suicides. For the married women; adjustments with the husband and in laws and responsibilities in the matrimonial home, even dowry related issues are pertinent causes for attempted suicide by married women.

The act of attempted suicide is always given more importance than the act of committed suicide as because in case of the later the culprit is no more alive to get punishment, but in the former case the culprit is still alive to get some deterrent punishment so as not to repeat the same offence in future.[7] Section 309 of the Indian Penal Code categorically makes attempt to commit suicide punishable, but time and again there arose several debates on the necessity of retaining or deleting this section from the statute book. The impact of legislation is reflected in a study of **100 attempted suicides in a Government Hospital Psychiatric Department** in **Balangir, Odisha**. Where 19 of the 56 males i.e. 33.9% and 05 out of 44 females i.e. 11.3% were aware of the existing legal provisions relating to suicide attempts, however the majority did not know that 'attempt to commit suicide' is a punishable offence.

According to World Health Organization, *Suicide Prevention* (2012), attempted suicides are usually twenty times more common than the completed suicides. It is estimated that, of all those who engage in non-fatal suicidal behaviours, one-third repeat the tendency within a year and nearly 10% eventually commit suicide. Many of the countries worldwide no longer criminalize suicidal attempts; however India, continues to hold it as a punishable offence, despite the enactment of the Mental Health Act, 2017.[8]

Psychiatric illness constitutes a major cause for non-fatal suicidal behavior. Risk factors include depression and other mental disorders. Other risk factors are childhood adversities such as sexual/physical abuse, alcohol/drug abuse, stressful life events such as death of a loved one, loss of a job or relationship, financial bankruptcy, imminent criminal prosecution and suffering from, or having recently been diagnosed with, a terminal illness.[9] Essentially, people who attempt suicide are in need of help rather than punishment in view of association with a high psychiatric or psychological morbidity. The philosophical dilemmas surrounding the individual's right to life and death has been debated, *albeit* inconclusively, across several disciplines with differing outlook and perspectives.[10]

As per direction of World Health Organization, **September** is considered as **Suicide Prevention Month**. Because, over 800,000 people per year lose their lives by committing suicide. Suicide is considered the leading cause of death for men less than 50 years of age. It is the 10th biggest cause of death worldwide. Two of the leading factors that contribute to suicide are isolation and feeling like a burden. So, as a human being, it's our duty to check in on these kinds of people around you and listen without judgement.

Conclusion

The Indian law criminalizing attempt to commit suicide was in place from the time of colonial rule, and Britain itself had decriminalized suicide attempts in the year 1961 through Suicide Act, 1961. In view of these issues and the decriminalization of attempted suicide in many other countries worldwide, there was ongoing debate between lawmakers and various stake holders on removal of this antiquated law in India. This was lead by the judiciary and welcomed by health professionals and activists. As a matter of first importance the quick impact would be improvement in detailing of suicide attempts and getting immediate clinical consideration unafraid of prosecution. Despite of the fact that a significant percentage of the population knows that suicidal attempts are punishable, the number of persons who try to commit suicide is very high. This means that conditions sometimes become so pressurising that if they fail, people are not in a position to think about the repercussions. This means that while laws prosecute efforts to commit suicide, this rule has not been able to discourage and prohibit persons from seeking to commit suicide, however technically speaking. That is why the Law Commission of India and the Honourable Supreme Court are of the opinion that it should be decriminalised instead of prosecuting attempts to commit suicide, and that the State should make efforts to advise and rehabilitate the victims of such situations. This is explained by the countries that have decriminalised attempts to commit suicide on the basis that the person who takes such a drastic action is now a victim of conditions and suicide is not a common life function. Circumstances and conditions have already punished him. And if he fails, punishing him for his failure amounts to doubly punishing him, which ultimately magnifies his pain. Rather than punishment, he requires empathy, support and counselling.

Indian law makes 'Attempt to Commit Suicide' punishable under Section 309 of Indian Penal Code, 1860 and it is an offence against the human body under Chapter 16 of IPC. Even after passing the Mental Health Care Act, 2017 which impliedly decriminalize attempt to commit suicide, the problem is not solved. Complete repeal of the provision is the need of the hour; else the shadow of criminalization will choke the neck of suicide survivors.

Ethical Clearance: Not required, as the article is based on aspects which are doctrinally taken.

Source of Funding : Self

Conflict of Interest : Nil

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The Antibacterial Effect of Ethanol Extract of Garlic (*Allium sativum* L.) on Methicillin Resistant *Staphylococcus aureus* (MRSA) *In Vitro*

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Abstract

Objective: To determine the antibacterial activity of ethanol extract of garlic against Methicillin-Resistant *Staphylococcus aureus*. **Method:** Dilution test was performed on 9 different concentration of ethanol extract of garlic (1024, 512, 256, 128, 64, 32, 16, 8, 4 mg/mL) dissolved in DMSO 10% and MRSA suspension on Mueller Hinton broth medium to determine the Minimum Inhibitory Concentration. The Minimum Bactericidal Concentration was determined by the result of streaking of MRSA inoculation on the nutrient agar plate. **Results:** The observation through Mueller Hinton broth medium has shown turbidity both before and after incubation because of garlic ethanol extract color which is blackish-brown and thick, while in the nutrient agar medium, MRSA bacteria grew on the media with concentrations 4, 8, 16, 32, 64, & 128 mg/mL and unable to grow at concentrations of 256, 512, and 1024 mg/mL. **Conclusion:** The MIC of ethanol extract of garlic on MRSA could not be determined, and the MBC in this study was 256 mg/mL.

Keywords: *Allium sativum, Methicillin Resistant Staphylococcus aureus, antibacterial activity.*

Introduction

Antibiotic resistance is a major problem and threat to public health. CDC reports at least 2 million United States citizens are infected with antibiotic-resistant bacteria each year, and 23 thousand of them died[1]. One of the bacteria known for its resistance is Methicillin-Resistant *Staphylococcus aureus*. *Staphylococcus aureus* is a Gram-positive opportunistic bacteria that live as normal flora of the skin and nose but can be pathogenic in some people with weakened immune systems[2]. The main transmission of Staphylococcus infection occurs due to direct contact with human lesions, objects contaminated with human lesions, respiratory tract, and human skin[3]. Penicillin and β-lactam resistance in *Staphylococcus aureus* is caused by modification of the penicillin-binding protein[4]. A study on 643 patients conducted at Dr. Soetomo Hospital Surabaya proved that 8.1% of them were detected as carriers of MRSA[5]. The widespread and inappropriate use of antibiotics can significantly increase the risk of MRSA infection[6].

Vancomycin, as the drug of choice for MRSA infection, has been reported for its resistance status to MRSA[3]. The results of the nasal swab of the paramedics who served in the ICU room at Ratu Zalecha Martapura Hospital showed that there were 14 samples with the *Staphylococcus aureus* strain and 7 of them were Vancomycin Resistant *Staphylococcus aureus* strains[7]. Other research also found that most VRSA was found among MRSA strains[8]; therefore, there is a need for new and effective drugs against MRSA.

Allium sativum L. or garlic is one of the most consumed kitchen ingredients in the world. Besides being popular as a food spice, garlic is also used by many people as traditional medicine. This family of Amaryllidaceae

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is believed to have high potential as anti-microbial, anti-inflammatory, and antioxidant[9,10]. Crushed garlic has been shown to have broad-spectrum antibacterial activity against Gram-positive, Gram-negative bacteria and acid-fast bacteria, including *Escherichia*, *Staphylococcus*, *Streptococcus*, *Salmonella*, *Klebsiella*, *Proteus*, *Mycobacteria*, *Vibrio*, and *Clostridium* species[10,11]. The active compound possessed by garlic also acts as an antifungal, antiprotozoal, and antiviral agent[12]. Several studies have shown that garlic can be an alternative medicine for cardiovascular diseases, including hypertension and stroke. Garlic has also shown benefits in several treatments for hyperlipidemia, cancer, diabetes, and its use and safety if consumed by children. Besides smell breath, nausea, and vomiting, especially when consuming it raw, garlic has not shown any adverse side effects[9].

Various studies have confirmed that the pharmacological effects of *Allium sativum* have the potential to be a source of new drug development. This study is expected to be able to obtain data on the potential antibacterial activity of *Allium sativum* against MRSA, which in the future can be used as an alternative treatment of infections due to MRSA.

Materials and Methods

Bacterial used for study:

Methicillin-Resistant *Staphylococcus aureus* used in this study were procured from the Laboratory of the Clinical Microbiology Department, Faculty of Medicine, Universitas Airlangga, Indonesia.

Preparation of garlic ethanol extract:

Garlic was collected from a local market in Surabaya, Indonesia. The garlic bulbs were peeled, washed, cut as thin as possible to reduce water content, and dried at room temperature for 2 days. The dried onion bulbs were crushed into a fine powder. Afterward, the powder was extracted by maceration method using 96% ethanol. Ethanol was used as an extraction material because it can dissolve secondary metabolites in garlic, selective, was not easy to grow fungi, non-toxic, and has good absorption ability[13]. The content of antibacterial compounds in garlic is more stable with alcohol compared to water[14]. The result of this extraction is a thick blackish brown extract with a distinctive pungent odor.

***In vitro* study**:

Minimum Inhibitory Concentration (MIC)

About 2048 mg of garlic ethanol extract were diluted with 2 mL of 10% DMSO, yielding a concentration of 1024 mg/mL. Afterward, a serial dilution method was performed to obtain 9 concentrations of garlic ethanol extract, 1024, 512, 256, 128, 64, 32, 16, 8, and 4 mg/mL. Mueller Hinton broth media was inserted with a bacterial suspension equivalent to a standard of 0.5 McFarland for each test tube, then incubated at 37□C for approximately 24 hours. Minimum Inhibitory Concentration (MIC) was determined by observing turbidity in the test tube visually[15].

Minimum Bactericidal Concentration (MBC)

The dilution test results with various concentrations were then streaked on the nutrient agar plate's surface using the inoculation needle and incubated again for 24 hours at 37°C. Media that showed a visualization of clarity and was not overgrown with bacteria was defined as the Minimum Bactericidal Concentration (MBC)[16].

Result

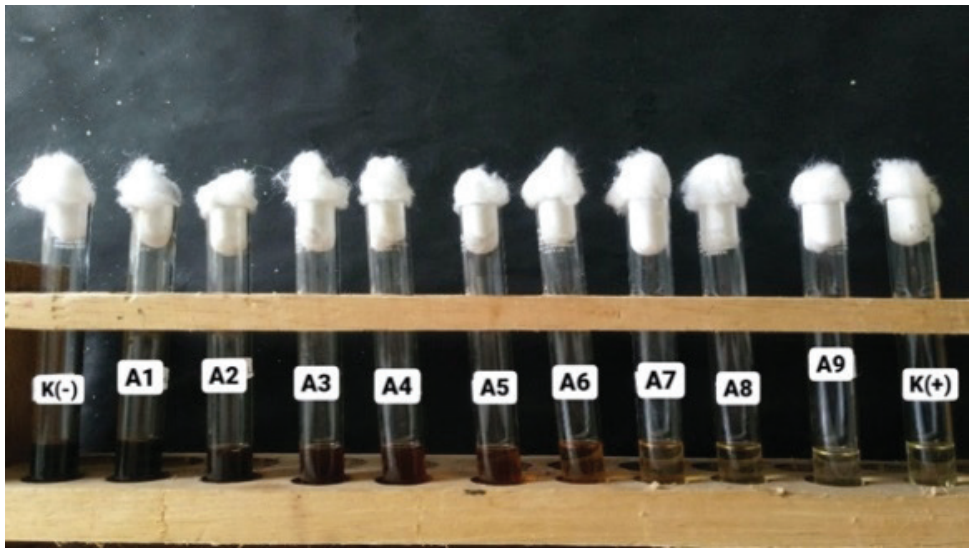

Figure 1. Dilution test tube before the incubation process

(K (-)) Negative control. Contains Mueller Hinton broth medium and garlic ethanol extract. (A1) Concentration of 1024 mg/mL. (A2) Concentration of 512 mg/mL. (A3) Concentration of 256 mg/mL. (A4) Concentration of 128 mg/mL. (A5) Concentration of 64 mg/mL. (A6) Concentration of 32 mg/mL. (A7) Concentration of 16 mg/mL. (A8) Concentration of 8 mg/mL. (A9) Concentration of 4 mg/mL. (K (+)) Positive control. Contains Mueller Hinton broth media and MRSA bacterial suspense.

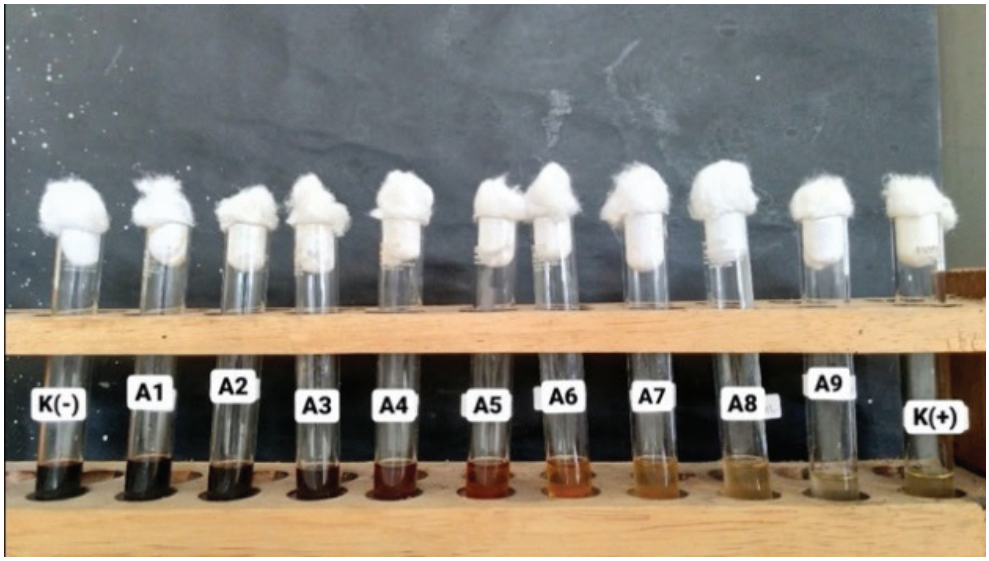

Figure 2. Dilution test tube after the incubation process

(K (-)) Negative control. Contains Mueller Hinton broth medium and garlic ethanol extract. (A1) Concentration of 1024 mg/mL. (A2) Concentration of 512 mg/mL. (A3) Concentration of 256 mg/mL. (A4) Concentration of 128 mg/mL. (A5) Concentration of 64 mg/mL. (A6) Concentration of 32 mg/mL. (A7) Concentration of 16 mg/mL. (A8) Concentration of 8 mg/mL. (A9) Concentration of 4 mg/mL. (K (+)) Positive control. Contains Mueller Hinton broth media and MRSA bacterial suspense.

All of the samples tested showed turbidity both before and after the incubation process. It is caused by the original color of the garlic ethanol extract, which is blackish-brown. Therefore, the MIC of garlic ethanol extract could not be determined.

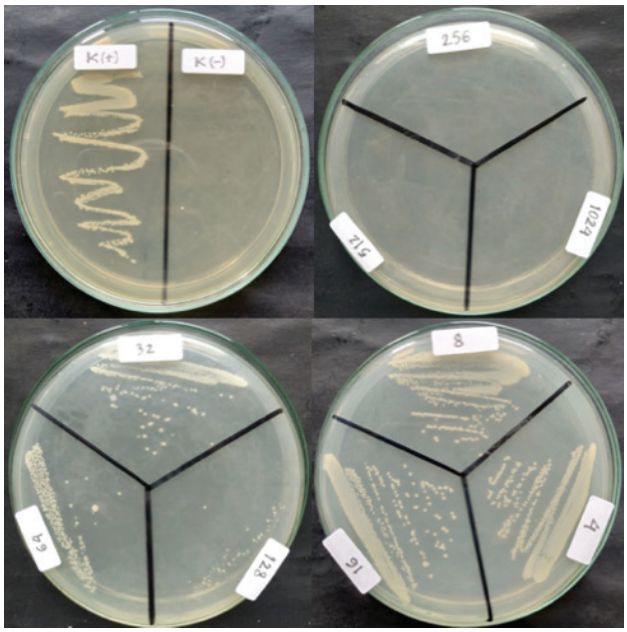

Figure 3. Results of streaking on nutrient agar plate media

After being streaked and incubated again for approximately 24 hours, K (-) or negative control showed no bacteria growing, indicating that the ethanol extract of garlic was not contaminated with bacteria, so the risk of bias could be avoided. Nutrient agar plates that have been streaked with concentrations of 4, 8, 16, 32, 64, and 128 mg/mL indicate growing bacteria's presence. Meanwhile, the results of streaking with concentrations of 256, 512, and 1024 mg/mL showed no bacteria grow. Therefore, it can be concluded that the MBC of garlic ethanol extract is 256 mg/mL.

Discussion

The garlic ethanol extract's blackish-brown color in this study might be caused by the Maillard reaction due to the lack of temperature and humidity control in the extraction process when drying at room temperature. The Maillard reaction occurs due to heating and drying, which causes a reaction between reducing sugars and amino acids in garlic. This reaction causes a change in the smell, original taste, and color of the extract[17].

The antibacterial effect of garlic extract itself is played by thiosulfinate compounds, including allin, allicin, and dialyl sulfonate[18]. Allicin has a strong antibacterial role. The crushed garlic will produce allicin compounds, which play a role in inhibiting the synthesis of DNA, RNA, and bacterial protein[19,20]. Allicin's precursor is a non-proteinogenic amino acid alliin ((S-allyl-L-cysteine sulfoxide)[21]. Crushed garlic activates the allinase enzyme, which then converts the alliin precursor to allyl sulfenic acid (2-propenesulfenic acid), which is unstable and highly reactive to room temperature. Then, two molecules of allyl sulfenic acid condense spontaneously with water elimination, which then forms allicin[22,23]. Allicin is very sensitive and unstable to heat. In ensuring the presence of allicin compounds in garlic extract, this study was conducted at room temperature[22]; therefore the maceration extraction method was used in this study.

The results of streaking on a nutrient agar plate in this study reveal no bacterial colonies that are growing at concentrations of 256, 512, and 1024 mg/mL. It indicates that the ethanol extract of garlic has bactericidal properties against MRSA. Later these results also demonstrate that its concentration is directly proportional to its antibacterial activity. The greater the concentration of the extract means, the greater the inhibition and bactericidal properties. It is marked by fewer of the colony of bacteria that grows on media with high extracts.

The bactericidal effect of garlic can be influenced by the characteristics of the bacteria itself. The antibacterial effect of the allicin compound in garlic has been shown to be three times more effective against Gram-positive bacteria than Gram-negative bacteria. Lipids in the membrane of Gram-positive bacteria such as *Staphylococcus aureus* play a role in helping allicin compounds penetrate into bacteria, so allicin can affect the activity of bacterial RNA synthesis and protein synthesis (one of which is by exchanging disulfide compounds) by inhibiting the activity of enzyme-free thiol groups then causes damage and even death of bacteria[23]. This proves that the main target of allicin compounds as antibacterials is RNA[14,24]. Although the molecule is unstable, allicin can easily pass through the bacterial cell membrane because of its hydrophobic nature, so it can reach the bacterial cellular compartment, which reacts quickly with the free thiol group[25].

However, Gram-negative bacteria such as *E. coli* which have lipid content on the membrane, are ten times higher than *Staphylococcus aureus*, making allicin compounds unable to reach their destination due to being trapped in the lipid content[14]. Allicin, which affects the enzyme thiol group's activity that acts on bacteria, makes these bacteria unable to change or modify the enzyme that plays a role, so the possibility of these bacteria being resistant to allicin and garlic is very small[11].

The results of this MBC study are slightly different from several studies, including research conducted by Johnson which showed MBC garlic was 104 mg/mL[26] and Venâncio which showed MBC of garlic against MRSA was 64 mg/mL[27]. The difference in MBC results in other studies influenced by several factors, including the location of plant growth, harvesting period, storage, and plant age. The extraction method used, the extraction tool used, the solvent used can also affect the differences in the results of each study[28]. In addition, different types of MRSA and different environments that MRSA develops also have different patterns of resistance and gene mutations against antibacterial compounds[29].

The ability of garlic to inhibit and kill resistant bacteria such as MRSA can also be seen from other studies whose results are in line with this study[26,27]. Further research is needed due to differences between MIC and MBC from several other studies, further research on individual isolates or active ingredients that act as antibacterial agents in garlic, and further research on garlic's antibacterial activity with other experimental methods.

Conclusion

The MIC of ethanol extract of garlic against MRSA bacteria in this study could not be determined because the extract was turbid and blackish-brown, so the difference in turbidity in each tube with different concentrations was difficult to observe visually. The MBC of garlic ethanol extract against MRSA bacteria in this study was 256 mg/mL. This indicates that the ethanol extract of garlic has antibacterial activity against MRSA bacteria.

Ethical Clearance: This experimental study protocol had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Manifestation of Face Asymmetry and Emotions in the Drawing Test Taken by Students from Different Ethnic Groups of the Small Siberian Region of Russia in the Republic of Altai

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Abstract

The study was conducted in the Republic of Altai, one of the Russian regions, with the participation of students of different ethnic and gender backgrounds. The main idea was to clarify the anthropoesthetic preferences when choosing a sexual partner; however, in this work, attention is paid to a characteristic feature of the drawn face – its asymmetry. One of the original projective psychodiagnostic methods was used – face drawing, which made it possible to determine the coefficient of asymmetry of its structural elements and the manifestation of emotions in relation to a potential sexual partner. The algorithm for obtaining a face pattern, drawing reference (cephalometric) points, and a module for calculating asymmetry was described in detail. In addition, the authors made an attempt to show the connection between asymmetry and the space occupied by facial structural elements with the emotional background.

Keywords: *anthropoesthetic preferences, face drawing test, projective techniques, sexual partner.*

Introduction

The impression people make on each other can be read on the face [1–4]. Even when looking at a stranger, an appropriate mimic reaction is recorded, and rather informative signals are recorded on the most impenetrable face, which is transmitted to the outside world [5–7]. The emotionality of a person is most clearly reflected in the face. In the process of direct communication, it shows the most complex "texts" of states, thoughts, interests, and intentions of communicants [8–10].

As soon as a person realizes that he or she is at the mercy of emotions, he or she begins to rethink and assess the situation. In this case, the assessment of emotions occurs automatically. This indicates that emotional experiences arise in a person; he or she does not choose them [11].

In addition, more and more information began to appear about emotional sex differences, i.e. differences between men and women in the demonstration of emotions and feelings [12].

This test allows a person to express him/herself through a drawing. The test results are more informative due to their projective nature and low susceptibility to control by consciousness. Besides, when interpreting the drawings, it comes to the problem of information quality.

Unfortunately, no research based on a detailed analysis of human face images based on drawings [13,14] and describing in detail the asymmetry of drawing details and psychological phenomena associated with asymmetry in the field of projective research methods has been found.

In view of the foregoing, the following goal was set – to determine the coefficient of asymmetry of facial structural elements and the manifestation of emotions in relation to a potential sexual partner, using the original projective psychodiagnostic method (drawing test).

Material and Methods

The participants in this study were residents of the Republic of Altai from among students of various ethnic and gender backgrounds. At the time of the study, all the participants reached adulthood, the estimated average age was 19.7 years, which corresponds to the adolescent stage of ontogenesis in terms of age periodization.

In total, 462 people were examined, including 22 young Kazakh women; 149 young Russian women; 130 young women and 47 men ethnographically related mainly to the southern group of Altaians (self-name "Altai-Kizhi", Telengits), hereinafter referred to as "Altai-Kizhi"; 55 young Russian men; 59 young mixed-race women. The uneven distribution of the respondents is explained by the low occupancy of student groups at educational institutions with young men. The minimum number of young Kazakh women and the lack of young men of this ethnic group in the analysis can be explained by the small composition of the Kazakh population, concentrated mainly in one of the remote regions of the Republic of Altai and, therefore, their frequency of occurrence in the student environment. The identification of ethnic groups in this study was carried out on the basis of a family survey map, in which the respondents noted their belonging to a particular ethnic group at the level of three generations.

Ten Phe-Martin head and face contours developed by European anthropologists, presented in the classic work on anthropology [15], on pre-prepared separate A4 sheets (Figure 1) were used as stimulus material.

The participants were given the task of drawing on the chosen contours the missing parts of the face of a potential (future sexual) partner as the subject wanted him or her to look like. In addition, the task was accompanied by the statement that in this part of the task, the subjects' artistic abilities were not evaluated.

Reference cephalometric points, well known and practically worked out by more than one generation of anthropologists, were applied on the test material obtained as a result of the first stage of the study (Figure 2), presented in the form of a face drawing [15–25].

In the study, only those cephalometric points were used that reflected the specificity of this work.

Structural elements of all received facial patterns were measured. As a result, it became possible to calculate their average size. In addition, it was possible to compare the structural elements of a face pattern, drawn with due account for the preferences when choosing one or another preferred general head contour in representatives of different sex and ethnic groups.

The resulting measurements, the sizes of interest in millimetres, accurate to hundredths, were used to calculate the coefficient of facial asymmetry, using the modulus of difference between the parameters on the left (L) and on the right (R), dividing by the sum of the same parameters:

(L-R)/(L+R), thereby calculating the asymmetry indicator for each feature.

Data processing was carried out by using the STATISTICA 6.0 software [26]. To analyze the differences in the structural elements of a face drawing between sex and ethnic groups, the authors used nonparametric statistics, the Wald-Wolfowitz test, which is used to compare data from independent samples.

Findings

When considering the results of this study as a process or some kind of technology for obtaining information from respondents, one should turn to the stages of its implementation. In this case, the first stage of the experiment will be the statement of a problem – stimulus. The second stage involves the processing of the stimulus that causes a number of objective changes in the subjects' bodies, up to and including changes in the activity of the nervous system. The third stage is the design of the stimulus in the form of a drawing, i.e. presenting subjective information on a sheet of paper. In this sequence of events, the only thing the experimenter obviously understands is the problem statement. He or she does not know a reflective degree of the brain.

In this regard, Simonov noted that "while studying the human brain, science deals with correlates (speech, electrophysiological, biochemical ones) of mental processes, but it does not have access to their subjective side. The methods of science cannot make a person feel pain, pleasure, joy, despair, and other emotions experienced by another person. This opportunity can only be given through empathy, the role of which has not yet been fully evaluated by either the theory or practice of education" [27].

First of all, the analysis starts with the factor that has recently found wide application in the section of natural and human sciences. This is asymmetry, and, as for the subject of this study, asymmetry of a face drawing,

which was obtained using the metric of the main facial structural elements in relation to the central axis of the mid-sagittal plane. As a result of the measurements and calculations of the asymmetry index, it turned out (Figure 3) that a larger amount of displacement fell on the left half of the drawn facial parts, which has negative values in the figure. In this case, the left side is viewed from the side of a face drawing, and not from the side of the person who draws or analyzes. In addition, one can see an increase in the large value of the asymmetry coefficient, starting from the upper face height to lower face height, respectively. Another thing to be clarified is that the face division into face heights is rather conditional and in literary sources, one can find a sufficient variety of options for its execution, this variety can be particularly found in the technique of drawing faces and in the literature on physiognomy.

In this work, the authors considered it necessary to use three face heights, the boundaries of which run through the eyebrows separating the upper face height and the wings of the nose, separating the lower face height from the middle one. In Figure 3, they are represented as gaps between data.

Moreover, in the details of the face, i.e. in its structures, the asymmetry coefficient is especially pronounced on the wings of the nose, lips (a point in the raised part of the intranasal depression above the contour of the red border), and the mouth as a whole. The least asymmetric were eyebrows, their medial and lateral borders. Thus, the effect of the dominance of facial structures is observed on the left side of the drawing, and this effect generally reflects the ethnic and gender components without any statistical differences between the groups.

On the one hand, this can be explained by the inequality of the functions of hands; the overwhelming majority of respondents drew with their right hand. In addition, based on modern ideas about the localization of functions, it should be understood that along with the dominance of the left brain in right-handers and the right brain in left-handers, there can be simultaneous participation of brain halves, as well as their alternate dominance in controlling movements [28].

In an article, the author cites Aristotle's reasoning about linking the concepts of "harmony" and "order". "He presents harmony as a mutual transition of order and disorder. But the main thing in Aristotle's philosophy was the concept of the "middle", the most moderate, equilibrium state, which he considered the idea of a sage who avoids extremes. The "middle" is between "excess" and "lack", it is completeness, which is no longer insufficient, but not yet excessive, and in this sense, it is perfect" [29].

When confronting symmetry with asymmetry [30–35], it must be borne in mind that the visual mass of a symmetrical figure will be greater than the mass of an asymmetric figure of a similar size and shape; symmetry creates balance in itself and is generally considered beautiful and harmonious. However, there is also a downside – it is often devoid of dynamics and may seem static and boring; asymmetry, as the antipode of static symmetry, usually brings dynamics to the composition [36–38].

An interesting review of facial symmetry is given in the article by Butovskaya *et al*., thereby emphasizing the factors of health, attractiveness, and reproductive success [39].

Excessive details of the drawing in its right part are perceived by the left brain. It is known that signals from the right halves of both eyes (left visual field) go to the right brain, and from the left halves (right visual field) – to the left brain [28,40].

Translating the results of this part of the study into the mainstream of emotions and the search for connections between the respondents' emotional response and the asymmetry revealed in the process of experimental material processing, it would be logical to provide some literary data that, in the authors' opinion, are very close to the issue under study in their content.

In the literature, there are proponents of two types of concepts. Proponents of the first type tend to believe that the right brain is emotionally negatively oriented, while the left brain is responsible for all positive emotions. However, proponents of the second type argue the same, while separating the ability to process from the ability to experience emotional information [41–43]. In experiments with differently emotionally coloured patterns, it was found that the more active area in the process of experiencing emotions was largely predetermined by

the experimental conditions. Besides, according to the author, the results of the study also depend on whether a person experiences emotions him/herself or they are induced by external stimuli [28].

In this regard, relying on the data from literature sources, it can be assumed that emotions experienced by the respondents when drawing the face of a potential sexual (future life) partner are positive.

In healthy people, the facial expressions of the left side of the face reflect the emotional state to a greater extent than the facial expressions of the right half, which also indicates the predominance of mechanisms of the right brain in the processes of expressing emotions. Similar data on the asymmetry of mimic expressiveness of the facial halves were obtained on macaques, and this indicates phylogenetic tendencies towards a more significant manifestation of the mechanisms for expressing emotions in the right brain. A more pronounced manifestation of emotions in the left half of the face is confirmed in special model experiments, in which it was shown that emotions are better recognized in photographs composed of two left halves of the face [43].

The space occupied by the drawing is associated with emotional manifestations, experiences of the past, present, and future. The left side is the subject's past, inactivity, passivity, negative emotions; tendency to reasoning, daydreaming, planning, but there is no real activity, actions. The higher the drawing is located on the left side of the sheet of paper, the more pronounced it is, the deeper the roots of these phenomena are. The right side is reality or the future, positive emotions, activity, actions, initiative. The higher the drawing is on the right side, the more pronounced it is. Normally, the drawing is located in the middle, without obvious displacement up, down, to the right or left [44]. Consequently, taking into account the above author's opinion, the position of the facial parts in the ethnic-gender groups of students represented in the space of the drawing indicates positive emotions, their activity in planning, daydreaming, reasoning, and real activity in relation to a sexual partner.

The facial expressions of the left side of the face represent the emotional condition of healthy individuals to a greater degree than the facial expressions of the right half, which also suggests the prevalence of right-brain mechanisms in the processes of communicating emotions. Severe asymmetry indicates that a person experiences a feeling of insecurity, a sense of imbalance, has poor control, and his or her behaviour may be characterized by hyperactivity and excessive spontaneity [44].

The above can be supported by the opinion of Shulgovsky: It is suggested that the psychosensory sphere is organized in two spaces (times) – past and present, while the psychomotor sphere – in the real space (present and future) of the external world. Omitting the details of the description of patients' mental states in this literary source, the author makes a thorough conclusion: all this may mean that normally two sides of the brain work simultaneously: the right brain is based on the past, while the left brain is based on the future [45].

However, the opinion of [46–49] is of interest in terms of the brightness of feelings of an event in the case of the right-hand position of the drawing. In this case, the drawing is accented on the lower right side of the face, which indicates current reality. In the authors' opinion, this is very interesting in terms of social psychology. It may be suggested that when choosing a sexual partner, the respondents do not see the future, it does not have a clear shape, i.e. the "here-and-now" paradigm is the main paradigm of being for them. Most likely, they have not developed the option of necessity, according to system administrators. The option of necessity is understood as the need to have a sexual partner, spouse, friend, authority, etc. They do not see the real future, including in matters of choosing a partner. What is the future for them in today's realities? How is it structured? How is it coloured? How is it formed ideologically? As strange as it may sound, there is a certain explanation for this phenomenon. The fact is that in the world, there has been a tendency associated with an increase in human life expectancy. This tendency apparently prolongs some stages of ontogenesis, in particular, the duration of childhood. As a result, "adolescent infantilism" began to manifest itself more and more. In addition, due to the tendency of life expectancy, traditional marriage with a lifelong partner is also undergoing a transformation.

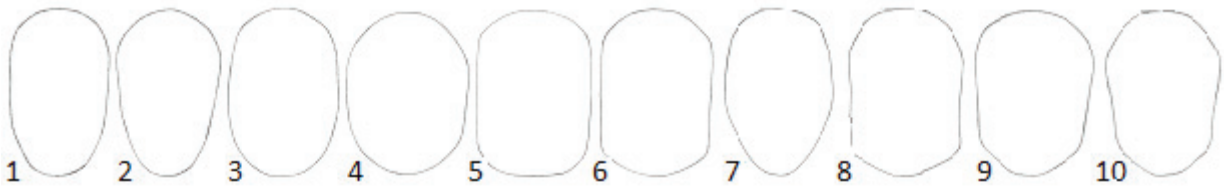


Figure 1. – Stimulus material. Corrected general outline of the head and face

(according to Phe-Martin)

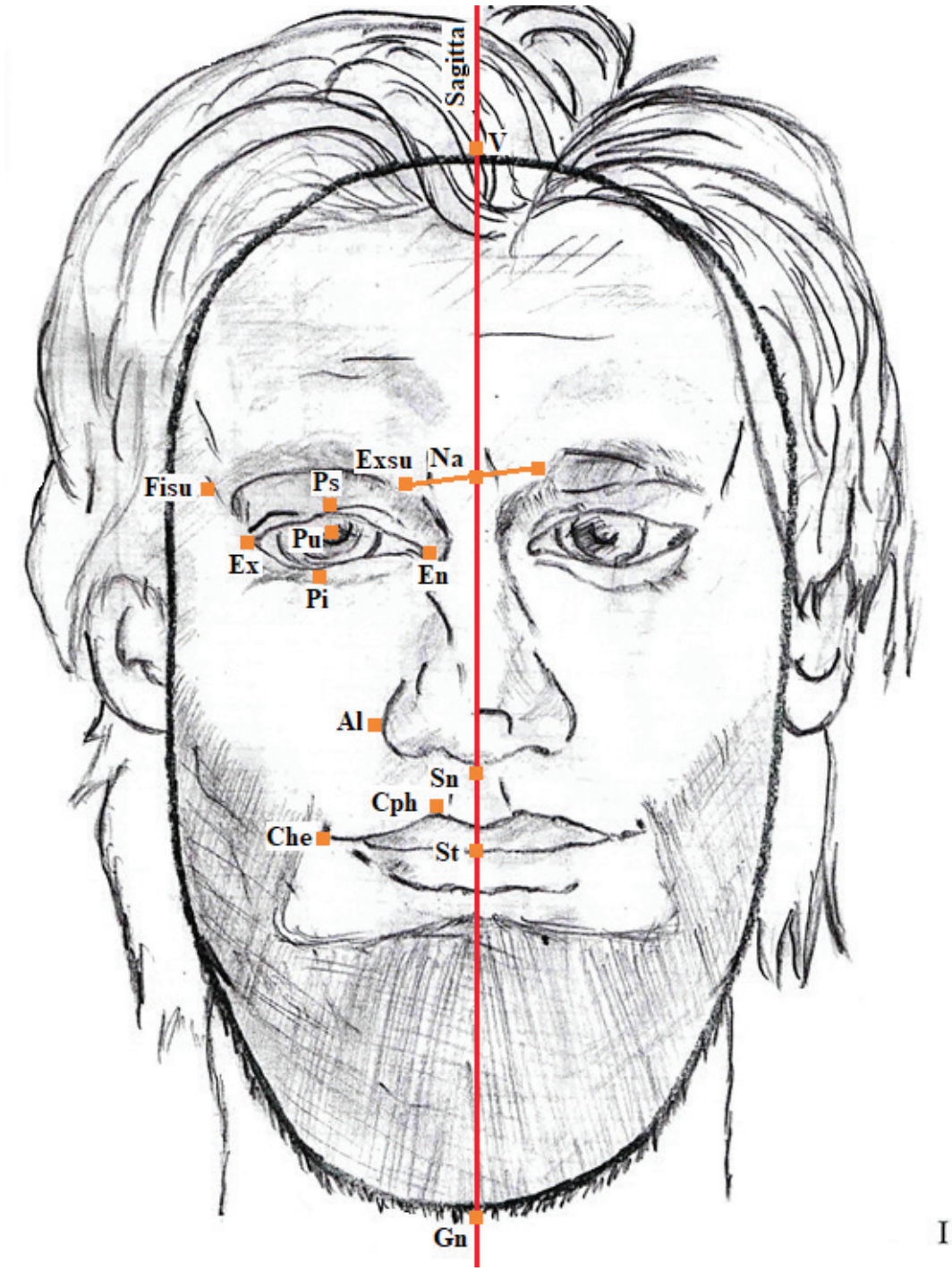
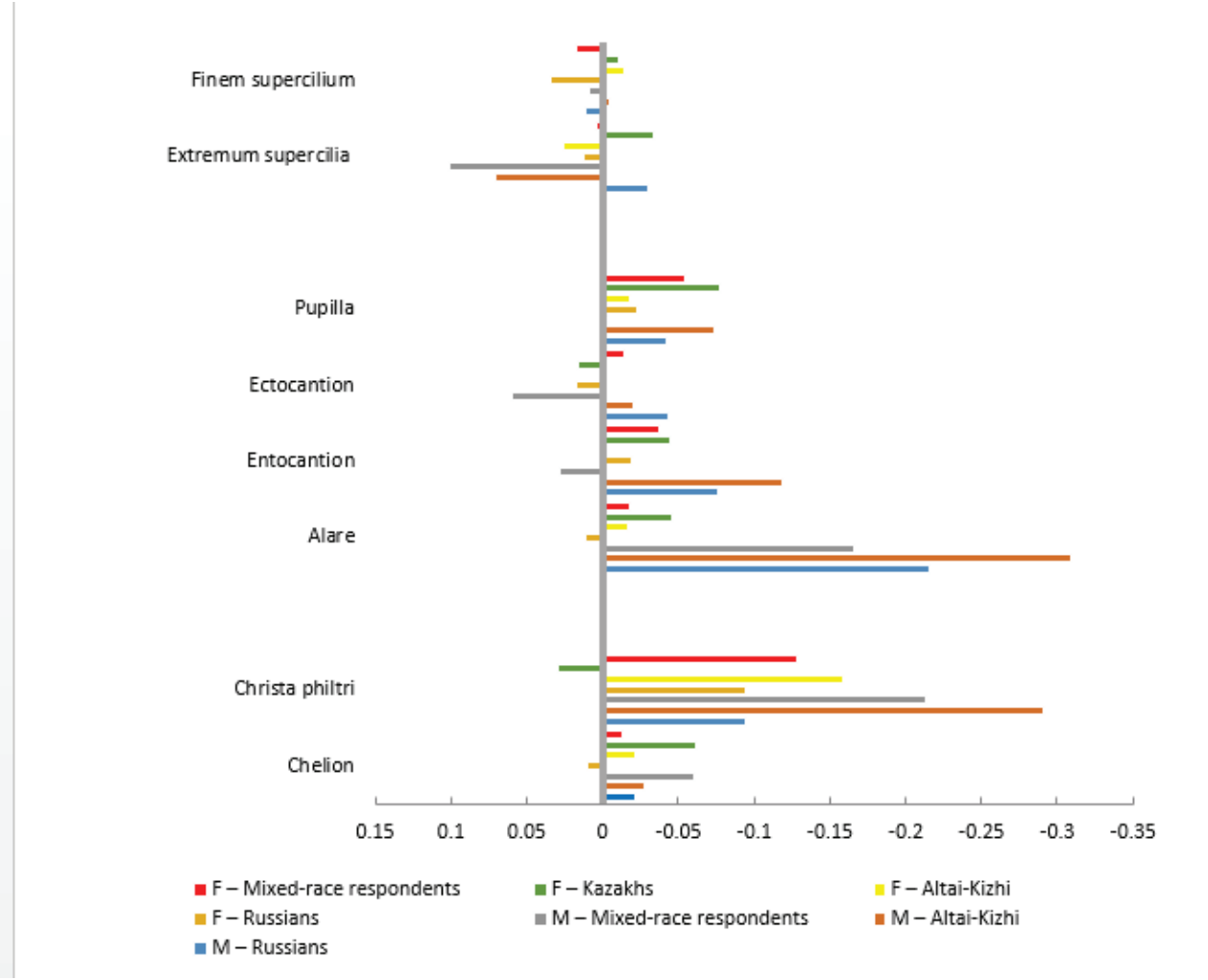


Figure 2. An example of test material obtained during the experiment



*F (female), M (male). The diagram covers adolescent individuals.

Figure 3. Asymmetry coefficient of facial structural elements in the drawings of respondents from different ethno-gender groups inhabiting the Republic of Altai

Conclusion

The results of the experimental part of this study made it possible to establish a pronounced directional asymmetry of the lower face height in the structural elements of the drawn face of an imaginary sexual partner. The right visual field, functionally involved in this process and perceived by the left brain, most likely reflects students' positive emotional reactions. The nature of the spatially presented asymmetry of the face drawing indicates the lack of any prolonged-in-time prospects for choosing a partner and does not depend on gender and ethnicity. In this regard, drawing the face of a sexual partner can be a reliable indicator of social ties among young students in terms of their attitude both towards marriage in general and towards the choice of a sexual partner.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Clearance: The ethics committee of the Gorno-Altaisk State University approved the research program of students. No contradictions with Declaration of Helsinki (1964 and with further amendments) appeared during the research.

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Family Meals Experiences Families with Adolescents: A Descriptive of Literature Review

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Abstract

Background Family meals play an important factor in family relationships and are considered as a symbol of family communication. Family meals were associated with increased dietary consumption and improved psychosocial health. However, few studies have focused on the experience of eating together as a family. The aim of the current literature review was to explore the benefits and challenges of eating together as a family, and how family members adopt and modify family meals from their childhood experiences.

Methods Studies were included if the researchers examined family meals or family dinners or family Meals experiences of families with adolescents; nine articles met the criteria. The studies published between 2010 and 2019 taken from Scopus, PubMed, and CINAHL databases.

Results This literature review of family meals provides an understanding of parents' and children's experience in the family meals. The results are divided into three themes including: 1) childhood family meal experience; 2) the role and responsibility of family members; and 3) family perception.

Conclusion Parents' childhood experiences influenced the types of food, norms, and cultures, that the family displayed at current meals. The family roles and responsibilities had changed from the past. Parents tried to preserve the family meals as an opportunity to connect the present with past experiences. Parents could spend this period interacting with their children and transmitting the norms and culture to the next generation.

Keywords: *family meals, adolescents, experience, perception*

Introduction

Family meals play an important factor in family relationships and family meals are essential for bonding and communication [1,2]. Family meals facilitate effective communication and engagement between family members [3,4]. Meals can provide an exclusive opportunity to participate with families, create food skills, and establish opportunities for healthy consumption in the family meal [5,6]. These can also provide the means to pass down cultural traditions from generation to generation [7,8]. However, due to the busy lives of contemporary families, family meals are happening less often. Several researchers in quantitative studies on adolescents from differing racial/ethnic cultures suggest that family meals are associated with increased dietary consumption [9-12] and improved psychosocial health [13-15].

According to the above findings, the deep understanding of parents' experience during childhood and parents' perception of the benefits and challenges related to family meals need to be examined. Moreover, in view of cultural evolution across generations, the researchers were also interested in how family meals

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have changed from the past. Therefore, the researchers examined this literature review to describe family meals in today's society. The purpose of this literature review was focused on the human experiences, behavior, and perception regarding family meals of eating together as a family.

Methods

The researcher performed a search for relevant articles in the databases of Scopus, PubMed, and Cumulative Index of Nursing and Allied Health Literature (CINAHL). The keywords used to search were consisted of (1) family; (2) meal; (3) adolescent; and (4) experience. The components of the PICO model (P = population; I = intervention; C = control; and O = outcome) were applied. We used the keywords and MeSH terms as noted in Table 1. Criteria for papers to be included in this review met the following criteria: 1) family has adult parents; 2) family has family meal experience together; 3) family includes at least one child 1-18 years old living at home; 4) accessibility of the full text of articles in detail; 5) published in peer-reviewed journals in English; and 6) published between January 2010 and December 2019. This review excluded unpublished master theses and doctoral dissertations without peer-reviewed process.

Table 1: Search terms and MeSH terms use for literatures search

Population	Intervention	Control	Outcome	Boolean operator
Family	Family meals	-	Experience	AND
Parent	Family Dinner		Perception	
Children	Family Activity		relationship	OR
kid	Family participation			

Summary of main findings

A total of 1,371 articles were identified in the initial databases. After duplicates were remove, 920 articles remained. Of these, 330 were excluded based on irrelevant studies, and 112 were excluded based on title and abstract screening a full-text review. Finally, nine papers met the criteria and were included in this review. The results of nine studies consisted of the family meal from family members' perspective, the benefits and challenges of eating together as a family, and how family members adopt and modify family meals from their childhood experiences.

Table 2. Theme and subthemes describing family meals experiences with adolescents

Themes	Subthemes describing family meals experiences with adolescents
themes 1. Childhood family meal experience	· Childhood family meal experiences · Mealtimes are generally a time for family members to shear the day's events
themes 2. The role and responsibility of family members	· The role and responsibility of family members in meal planning · The role and responsibility of family members
themes 4. Family perception.	· Family perception on eating out with children · Family perception on the benefits of family meals · Family perception on the challenges of family meals

Childhood family meal experience

The majority of the parents agreed having dinner with their family during their childhood was important and family meals could strengthen family bonds [1,16,17]. Parents also shared that the family meal was one of the memorable events in their childhood life because it was a good opportunity to exchange information among family members [16,18]. In addition, family meals provide an opportunity for parents to transfer values, goals, and culture to their children [1,17,19]. For instance, children could learn the traditions behind certain foods, especially during special events like Thanksgiving, Christmas, and New Year.

The role and responsibility of family member

Each family in the studies clearly identified the role of family members for the family meal which could be divided into two sub-themes including: 1) the role and responsibility of family members in meal planning [5,19]; 2) the role and responsibility of family members in dinner [5,19]. The majority of parents prepared the dinner menu for their children without asking their opinion because the parents knew their children's favorite dishes and food allergies [19]. Few families allowed the children to participate in weekly food planning by preparing a whiteboard for family members to write about their preferred meal [20,21]. Then, some families will go out to the grocery store together on the weekend to buy the food and store the refrigerator for the whole week [21].

Family perception

Family perception is the ways that parents perceived family meals and their attitudes toward these meals. It could be divided into three sub-themes in order to explain the perception of family, including: 1) family perception on eating out with children [18,22,23]; 2) family perception on the benefits of family meal [20,23]; and 3) family perception on the challenges of family meal [17,20]. Most of the families agreed that some parents still used the same rules as dinner in their childhood such as avoiding electronic devices and unruly behavior [23-25].

The vast majority of families agreed family meals were important as time the families could spend together, exchange the information during the day, and transfer the norms and cultures with their children [22,24]. Some challenges that parents reported for frequent family meals were that family members had difficulties planning a time to all be present [20,23]. Few parents also reported family members were hungry at different times [20,24].

Discussion

This literature review of family meals provides the understanding of parents and children's experience in the family meals. The results could explain the family meal into four themes including: 1) childhood family meal experience; 2) the role and responsibility of family members; and 3) family perception.

Family meals that parents remember as children influenced their current family meals with their own children [26,27]. For instance, the types of food, the norms and cultures could transmit from generation to generation. Family meals provide an opportunity for family members to stay and have social interaction together. Family meals provide opportunities for parents to talk, listen, attend, and teach their children. For instance, family meals offer parents a chance to be role models. Parents can set an example of healthy eating and polite table manners [28,29]. Parents with a good experience during childhood were likely to transmit the positive memory and perception of family meals to their children [7,27]. On the other hand, parents with a bad childhood experience felt that dinner was not necessary for family members. For instance, some parents had too little time for cooking, or were not good cooks. Their children found increased health risk behaviors [30]. Therefore, a good experience during childhood is very important for the children because childhood will become parents in the future [1].

This study found interesting results on the role and responsibility of family members in two phases including: 1) meal planning; and 2) cooking. Majority of parents did not plan for a daily dinner menu. Parents preferred to plan for the meal just before dinner time. Nowadays, parents are employed so time constraints were the main barrier to prepare the dinner [5]. Having less time had influenced the parents to plan the meals just before dinner time such as driving home from work, calling their spouse before the meal. Employment did not decrease the frequency of family meals but increased the likelihood of eating store bought or fast food instead

[31]. Hence, parents may not have enough time for family meals planning activities with their children such as discussing food choices, going out for shopping at the grocery store.

Then, the vast majority of parents reported females were responsible for the family meals. Parents were in-charge of the family meals including planning for the meals, cooking and cleaning. Cooking is considered to be an integral part of the domestic duties of a married woman. Parents perceive that feeding family members are deemed to be "womanly" operations thus and thus lead to the continued development of gender in families [32]. However, most families tried to encourage their children to participate in cooking such as preparing ingredients. Currently, most of the family members are fundamentally responsible for the running of the household particularly cleaning after the meal.

This study found parents usually have similar rules on the dinner table. Almost all families agreed that technology [25] such as television and telephones were not allowed during the dinner because the cellular technology may interrupt the conversation and interaction among family members [4,27,33]. However, family mealtimes provided opportunities for children to learn valuable life lessons [33]. Mealtime also provided a means of teaching manners and educating children to behave during dinner, particularly families with two or more small children [34]. For instance, parents were not allowed to argue at the table, or parents are not allowed to leave the table. Therefore, family meals are the perfect time for family members to spend the time together so family members should refrain from using the distracted technology during the dinner [25,33].

Lastly, this study also reported the perceptions of benefits and challenges of the family meals. Most parents agreed that family meals were important [27,35] and studies have shown that meals may have significant health effects over the lifespan as well as eating behavior in adulthood [36,37]. Parents mostly planned to eat together every day because which is a good opportunity for parents to connect their children [17,38]. Hence, socialization is crucial for children that childhood have to learn and practice from their family before getting to the real world. For challenges, parents reported some families had different schedules which were hard to gather family members in the specific time. Nowadays, most parents are employed in low-wage occupations with less flexible schedules, these factors may influence the family meals relationship [38,39]. Therefore, the future study should develop the strategy to maintain and support the family meals in employed parents.

Conclusion

This review was developed to understand human experiences, behavior, and perception regarding family meals. This current review found parents' childhood experiences influenced the current family meals including types of food, norms, and cultures. In some households, women were still responsible for planning and cooking the meals. However, the roles and responsibilities for family meals had changed from the past. Spouses and children were involved in preparing the ingredients and cleaning after the meals. As technology becomes more widely available, family meals can easily be interrupted by urgent matters that leave family members distracted and less likely to interact with each other. Most families agreed to develop rules for banning the use of technology during dinner, particularly mobile phones. Therefore, parents tried to preserve family meals as the time period that allowed family members to stay together. Parents could spend this period to interact with and transmit the norms and cultures to their children.

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Epidemiology of Hydatid Disease in Iraq: A Study of Hydatidosis Patients in Baghdad Province

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Abstract

Cystic echinococcosis (CE) is a zoonotic disease worldwide caused mainly by the metacestode *Echinococcus granulosus*. Hydatidosis disease is common in all countries of world, especially in the Middle East, in addition to Asia, Africa and Europe. A person becomes infected with this disease through accidental consumption of water, soil or food contaminated with the faeces of infected dogs, which is the most common type of method of infection. This parasite is transmitted to dogs when it ingests the infected organs of other animals that contain hydatid cysts. A study was conducted to study the infection rate between women and men in Baghdad governorate. The results showed a highly significant increase in the incidence of infection with hydatid cyst among females (70.00%) in comparison to males (30.00%). The oldest age of hydatidosis patients was between 31-40 years (30.00%) with a significant differences between age groups and gender, while the lowest percentage was at age 41-50, in addition 51-60 age reached (10.00%). In regard to the profession, the highest value of infection recorded in housewives (60.00%) and the lower percentages (6.67%) in the employees' category. However, the most infection rate was within under primary education level (40.00%), while the lowest percentage was at university education level (3.33%). The incidence of liver infection (100%) is higher than the other organs, Moreover, it is the only affected organ that contains a large number of hydatid cysts, Compared with lung, kidneys and spleen, which each recorded only two cyst for each of these affected organs in hydatid cyst patients, which was represented by significant differences at ($P<0.01$) and ($P<0.05$).

Key word: *Epidemiology, Echinococcus granulosus, hydatid cysts Cystic echinococcosis, Hydatid disease.*

Introduction

Hydatic disease, caused by the larvae of *Echinococcus granulosus*, is a serious disease, which can be fatal and, found anywhere in the world, but particularly in endemic areas such as the Mediterranean Basin, New Zealand, Australia, Eastern Europe, North Africa, the Balkans, South America and Middle East[1]. The intermediate host can be sheep/goat (pastoral hydatidosis) or moose /reindeer/caribou (sylvan hydatidosis). Humans are occasional intermediate hosts. The most common site of occurrence of hydatid cysts in humans is the liver (50%–93%)[2]. People become infected by devour (swallowing) the eggs[2]. Tapeworm larvae may be placed at different locations in the body where they form a fluid-filled sac known as a hydride cyst. The cysts contain immature forms of the tapeworm and can excess in size from 510 cm or more over a period of time, while some cysts may die, others can remain survive for many years. Cysts also contain 'daughter cysts' which, if released, may diffuse to other areas of the body[3] (Heymann, 2015). Many substances have recently been used to activate and modify the host's immune system in order to control the growth and development of the cyst[4]. The aim of the current study is to estimate the epidemiological of hydatid cyst disease in Baghdad governorate, which had a high incidence of infection.

Material and Methods

Patients group

The current study included sixty patients (42 females and 18 males) suffering from hydatidosis disease with ages between (10 - 75 years) by specialized medical surgeons after their conversation with the

surgery department in Private Nursing Home Hospital, The martyr Ghazi Al-Hariri Hospital, and Red Crescent Hospital, during the period from May 2017 to May 2018. All cases have hydatidosis disease according to identification of the parasite's structures by imaging techniques, including ultrasound, x-ray diagnosis and accentuated surgery operation of each patient. Then, the patient's demographic, epidemiologic and clinical data were recorded in a questionnaire sheet including age, sex, educational level, cysts number of infected members, patients job and status of the cyst fluid.

Cysts collection

Collected the specimens (hydatid cysts) after surgery and placed in sterile and refrigerated containers and transferred to the laboratory for analysis.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study percent. Chi-square test was used for significant comparison between percentage (0.05 and 0.01 probability) in this study.

Results and Discussion

Presented study included sixty personnel, suffering from hydrated cyst disease. According to the gender patients group consist of 42 (70%) females and 18 (30%) males (table 1).

Table (1): The percentage of study group (patients) according to the gender

Group	Gender	
	Female	Male
Patients	42 (70.00%)	18 (30.00%)
P-value	0.0023 **	
** (P<0.01).		

The results of the current study showed that the percentage of female infections (70%) was higher than that of males (30%) this is due to females responsible for cooking and preparing food, which makes them in direct contact with the pathogens.

Regarding patients age, the results of the present study showed that the infection rate of hydatid cysts increases in the age group (31-40) years in proportion 8 (13.33%), while the lowest were six patients at the age range (41-50) and (51-60) year. There were a significant difference (P<0.01) between age groups (table 2). From this table it is noticed that the age groups (31-40) years and (21-30) showed the highest number of infected patients. This result may due to the type of social life, because this group is able to work outside the home and thus be more susceptible for infection, with non-compliance with public health rules and indifference to eat foods, as well as spend a long time outside the home, making them more compatible with the causes of infection. These results were similar to the previous results conducted by Al-Qadhi[5], Al-Jobbory[6], Al-Ezzi[7] and Taher,[8] who showed that they age range between (21-40) years had the highest rate of disease incidence, but, in another study conducted by Al-Ubaidi[9] , Al-Shaimary *et al*.[10] and Saida and Nouradin[11] who concluded that the infection increases with age.

Actuality, hydatid cyst is affected youth individual, but occurs the symptoms delayed for many years which may reach decades, in which condition the cyst are formed in the affected organ.

Table (2): The percentage of infection according to the age

Age Groups (Year)	Percentage of infection (%)
10- 20	8 (13.33%)
21-30	14 (23.33%)
31- 40	18 (30.00%)
41- 50	6(10.00%)
51- 60	6(10.00%)
61- 70	8 (13.33%)
Total	60
P-value	0.00709 **
** (P<0.01).	

The nature of the profession practiced by people; a factor may increase the chances of infection rates. Present results recorded highest rate of infection among women (housewives) 36 (60%), while the lowest one 4 (6.67%) among employee

(table 3(. Increasing incidence of hydatid disease has been observed in housewives, this may be due to the daily work of women cleaning, preparing food, farming and washing vegetables, which may be contaminated with parasitic eggs, making contact with the infectious stage more easily.

Freelancers (who are only males) represents 10 percentage of the whole patients counts, due to their lifestyle, which make them spend most of their time in environment exposed to different disease factors, otherwise the most infected of both gender are from very poor environments.

Table (3): The percentage of infection according to the job

Patients with hydatid cysts	
Job	**Percentage (%)**
Housewives	36 (60.00%)
Students	8 (13.33%)
Freelancers	6 (10.00%)
Pensioners	6 (10.00%)
Employees	4 (6.67%)
Total	60(100%)
P-value	0.0001 **
** (P<0.01).	

Other studies indicated that a high incidence of hydatid disease in working classes of people. Therefore, focus on health information programs for all society is important to increase awareness of health, hygiene and avoid eating leafy vegetables, unless they are sure to be washed properly[6,8,12,13].

The role of education level indicates as active factor in the infection of hydatid cyst parasites, as shown in (table 4). The infection rate showed high level in people with uneducated individuals 24 (40.00%), followed by the primary certificate 18(30.00%) and middle school certificate16 (26.67%), while the less proportion of patients with university degree less frequent 2(3.33%).

The results of this study showed the importance of education, a large proportion of patients with low educational level which emphasizes the role of education as an important factor in avoiding infection of hydatidosis, leading to lack of health awareness and failure to follow preventive measures and neglect diagnosis and early treatment of the disease.

Table (4): Percentage distribution of hydatid cyst in patients according to educational level

Education level groups	Percentage of infections (%)
Uneducated individuals	24 (40.00)
Primary certificate	18 (30.00%)
Middle School certificate	16 (26.67%)
University degree	2 (3.33%)
Total	60
P-value	0.0001**
** (P<0.01).	

These results were consistent with Campos-Bueno *et al*.[14], Wang *et al*.[15] and Taher[8] who found the incidence of hydatid cyst infection increased significantly in patients with low education.

Infection can include more than one organ, the present study shows that the infected liver was in 60 patients followed by lung, kidney and spleen with 2 patients (table 5).

The results of the current study were agreed with most studies that proved the liver is the most common infected organ [16,17]. Further studies suggested that the liver is the generality affected organ by hydatidosis,, because liver is the first refinery for oncosphere, which hold these embryos in large numbers and attach by its hooks then begins to form of the cyst, and this is called the initial infection[18], these results correspond to Al-Jobbory,[6]; Al-Ezzi,[7] and Hussain,[13]. Followed by lung, kidney and spleen which were recorded the equal rate (it should be noted that three cases had the liver also infected with these cysts) this may be due to the escape of very few embryos entering the systemic cycle to reach different organs and tissues of the body such as the lung, kidney and spleen.

As well as that all organs of the body are targeted by parasite embryos except teeth, hair and nails[19]. The presence of cysts in more than one organ depends on the host's body resistance to infection, then parasite evasion to escape from the immune system and spread disease[20]. In contrast, Al-Ubaidi,[9] noted that the high rate of infection of the hydatid cysts in the lung compared to the proportion of the liver.

Table (5): The frequency number of infected organs according to the number of cysts.

Number of cysts	Number according to infected organ				
	Liver N=60	Lung N=1	Spleen N=1	Kidney N=1	P-value
One cyst	30	2	2	2	0.0001 **
Two cysts	6	0	0	0	0.0437 *
Three cyst and more	24	0	0	0	0.0001 **
P-value	0.0001**				
**: (P<0.01) , *: (P<0.05)					

The relationship between the number of cysts and the type of infected organ showed that the liver had the highest rate of infected organ in 30 cases, followed by lung, spleen and kidney only two and the liver was the only infected organ with two or three cysts or more. This relationship showed a significant difference at (P<0.01) and (P<0.05) (table 5). This may be due to, the liver is the more influenced organ by oncosphere that carried out via portal vein flow, a first stop on the way of the oncosphere of the small intestine, is the liver[17].

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Comparison the Effectiveness between Defective Cough and Yoga Breathing Exercises in Reducing Breathlessness on Tuberculosis Patients at Matahari Room, Dr. M. Yunus Hospital, Bengkulu

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Abstract

Introduction: The decrease of vital lung capacity on pulmonary tuberculosis (PTB) patients can reduce oxygen supply to the lungs. Lung capacity is very crucial within breathing process; decreased its capacity can cause the distruption functions in the body's cells, which leads to the breathlessness on tuberculosis patients. This research presents the study to examine the Effective Coughing (EC) and Yoga breathing Exercises (YBE) as a technique to reduce the breathlessness for patients who suffer from tuberculosis at Regional Public Hospital, DR.M.Yunus, Bengkulu.

Methods: This study was quantitative research with a quasy experiments design. The population was all patients (men and women) with pulmonary tuberculosis (PTB) at RSUD DR.M.Yunus, Bengkulu. The samples were 74 patients, which selected toward patients who have complaints of breathlessness; 34 patients used for intervention group, and 34 patients for control group. This, according to the research' aim, compared the treatment effect by using effective coughing intervention and Yoga Breathing Exercise as well.

Findings: The results showed a significant change after given effective cough (EC) (value=2.30), but there was a small change after given with Yoga Breathing Exercises (YBE) (value=2.84).

Conclusions: In short, our research finding there was a significant effect of using EC and YBE for reducing the breathlessness. The impications of this research can be implemented as respiratory management intervention on patients who suffer with pulmonary tuberculosis (PTB).

Keywords: *Effective Cough, YBE, Breathlessness, PTB, patients.*

Introduction

Tuberculosis is a contagious bacterial infection caused by bacteria Mycobacterium tuberculosis where its bacteria can attack on apex of lung. Some researches devoted its malignancy has infected nearly a third of the world's population, and one of the main causes of death, its incidence has been increasing since early 1980s. As cited from Word Health Organization (WHO) reports in 2009, the incidence of PTB is 9.4 million with a prevalence of 14 million, and a mortality rate reaches 1.3 million. Moreover, WHO also reported several cases of tuberculosis prevalences; Indonesia is at third ranks after India and China, which nearly 700 thousand cases with death rates is still 27/100 thousand population[1].

Regarding to the above cases, there are many incidences of PTB, which have been occurred in several cities of Indonesia, for instance Bengkulu province. In Bengkulu, particularly between 2018 to 2020, the number of tuberculosis's suffers has been an increase rapidly. This, a frightening disease, which requires

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immediate treatments. All sufferers experienced breathlessness are due to the accumulation of secretions in the airway[2]. In 2020, data obtained from Public Health Centers in Bengkulu desiphered the number of Pulmonary Tuberculosis cases, which classified based on sexes, districts, and cities; South Bengkulu was 2,110 people, Rejang Lebong 2,561, North Bengkulu 1,917, Kaur 2,062, Seluma 1,769, Mukomuko 2,625, Lebong 1,443, Kepahiang 1,142, Cental Bengkulu Tengah 981, and Bengkulu city 4,398[3].

Based on all above upon, this research aims to investigate a technique to reduce the breathlessnees on PBT patients underlying the motives to compare the effectiveness of effective cough and Yoga breathing exercises for reducing the breathlessness on tuberculosis patients at Regional Public Hospital, Dr. M. Yunus, Bengkulu.

Methods

This research was a type of quantitative research with a quasy-experimental design; it used a form of non-equivalent control group design (non-randomized control group pretest-posttest). The research population was men and women who suffered from tuberculosis or have complaint of breathlessness at Regional Public Hospital, Dr. M. Yunus, Bengkulu. The chosen samples were 74 patients; 34 for intervention group and 34 patients were used for control group.

Our main research target aims to compare the effect of treatments between the subject group who given with intervention I (effective cough), and subject group with intervention II by giving treatment with Yoga breathing exercises.

Findings

Based on our analysis results on univariate to determine the frequency of distribution between effective cough, Yoga breathing exercises, and reduction of breathlessness on tuberculosis patients, as follows

Table. 1. Comparison: Frequency of Distribution, with (n-74)

No	Action	Amount (n)	Percentage (%)
1.	Cough Effective (CE)	37	50.0%
2.	Yoga Breathing Exercise (YBE)	37	50.0%
Total		74	100.0

As shown from the Table.1 above described the comparison of distribution percentages; half of respondents 37 (50.0%) on tuberculosis patients at Regional Public Hospital, Dr. M. Yunus, performed an effective cough. Similarly, the result of Yoga breathing exercises, its distribution showed 37 (50.0%).

From the results of bivariate analysis, which conducted to determine the difference the decreased of breathlessness between effective cough and Yoga breathing exercises on tuberculosis patients at RSUD DR. M. Yunus Bengkulu, as follows

Effective Cough Frequency Distribution

This vividly describes the differences of breathlessness before and after giving treatments of effective cough and Yoga breathing exercises at Matahari room, Regional Public Hospital, DR.M. Yunus, which given within May-August 2020, with (n = 37.

Table 2. Comparison of Breathlessness, with (n-37)

Group	Mean (mg/dl)	St. Deviation	t	Sig (2-tailed)
Effective Cough				
Before	4.05	0.78	8.99	.00
After	2.30	0.93		
Yoga Breathing				
Before	4,14	0,71	9,72	.00
After	2,84	0,76		

The results of analysis as described from the table above, the average value of breathlessness of group before giving the effective cough intervention was 4.05. Meanwhile, after giving effective cough exercise, its value was 2.30. This vividly showed that there was a decrease or significant difference (Sig, 0.00) of breathlessness after giving effective cough intervention.

Furthermore, this is similar to the phases of invervention with Yoga breathing exercise, eventhough there was a small change the number of figure. Our analysis showed the average of breathlessness before giving Yoga practice was 4.14. Thus, there was a decreased after giving Yoga breathing exercise, as shown with 2.84. This result indicated that there was a significant different (Sig, 0.00) of breathlessness on PTB patients after giving the treatment by using Yoga breathing exercse (YBE).

Characteristics of Respondents

Based on the data obtained of univariate analysis that the results were much influenced by age factor as shown from the average age of respondents; the oldest patients with 50 years old, and youngest patients, 20 years old. These indicated that there was a significant age range for Tuberculosis patients. In India, 80% or mostly tuberculosis cases occurred on patients who were more than 45 years old[4], which happened due to the decrease of lung function and along with increasing of age with risky disease that triggered tuberculosis on patients. This is unsimilar to the finding[5], which stated that the age of more than 45 years is a risk of suffering from tuberculosis. In short, this research sums up with a relative age; on the age of 20 years there could be tuberculosis**.**

The gender who participated in this research were women who had a greater proportion, namely those who have given effective coughing 62.1% of 23 respondents, while Yoga Breathing Exercise was 67.6% of 25 respondents. Furthermore, men group who were effective with cough 37.9% of 14 respondents, and Yoga Breathing Exercises 32.4% of 12 respondents. The proportion of tuberculosis incidence is relatively higher amongst females than males. This result is different with Lubkin's findings (2009)[6], because mostly tuberculsosis attacks on men than women; his findings were caused by some reasons, for instance a lot of people smoke, and work outside of home, which were regardless of their health[7].

Effective Cough and Decreased of Breathlessness

The results showed that there were differences results between effective coughing (EC) and Yoga breathing exercises (YBE) on decreasing breathlessness on tuberculosis patients at Regional Public Hospital, DR. M. Yunus, Bengkulu. Cough is caused by acute respiratory tract infection (ARTI) improves within 3 weeks toward 90% of patients. Infection of pertussis is suspected by previous immunized adults with persistence or severe cough for 2 - 3 weeks. If there is no treatment with ACE inhibitors, acute respiratory tract infections and abnormal chest radiology, up to 90% of cases of persistent cough are caused by postnasal drip, ARI or gastroesophageal reflux disease (GERD). History of nasal or sinus congestion, wheezing or a burning sensation in the heart (heartburn) should be quickly evaluated and treated[8].

This condition often causes a persistent cough in a cough state with no other visible symptoms.

Bronchogenic carcinoma is suspected when coughing with unexplained weight loss, fever with night sweats, especially amongst people with a history of smoking and exposure.

Persistent cough accompanied by mucus secretions that are often suspected by chronic bronchitis amongst smokers or bronchiectasis on patients with a history of recurrent pneumonia or complications, chest radiology, those can help. Dyspneu at rest or activity is generally absent on patients with persistent cough. Thus, Dyspneu requires assessments of further evidences of chronic lung disease or congestive heart failure[9]. The purpose of Effective Cough is a cough technique that emphasizes the maximum inspiration starting from expiration, with the aim of stimulating the opening of collelateral system, increasing the distribution of ventilation, lung volume, and facilitating cleaning of respiratory tract[10].

Effective coughing (EC) and deep breathing exercises (DBE) are effective coughing techniques that emphasize maximal inspiration starting from expiration. These are aimed at stimulating the opening of collateral system, increasing ventilation distribution, and lung volume and facilitating airway clearance and lung expansion, and mobilizing secretions, and also preventing side effects. All these, from retention of secretions, such as pneumonia, ateletaksis and fever. This research is supported by some researches like Donesky-Cuenco D1, Nguyen HQ, Paul S, Carrieri-Kohlman V. Yoga therapy can reduces stress-related dyspnea and improve functional performance on people with chronic obstructive pulmonary disease. This finding is accordance with Purwanti's finding (2006)[11], her research analysis defines that cough is effective in reducing congestion in COPD patients. Moreover, effective cough is quite effective for reducing breathlessnees than Yoga breathing exercises on tuberculosis patients.

Based on the results of discussion above, our findings devote that effective coughing is more effective than Yoga breathing exercise for reducing breathlessness on tuberculosis patients.

Conclusion

Based on the theoretical description and research findings above. This outlines some important points of conclusion: (1) The characteristics of respondents in this research were mostly female, with the age range between 20-30 years. (2) There was the difference the decreasing breathlessness on tuberculosis patients before and after given effective cough and Yoga breathing exercises. (3) There was a difference on reducing the breathlessness with effective coughing and Yoga breathing exercises. (4) This study devotes the effective cough technique is more effective to reduce the breathlessness than Yoga breathing exercises on tuberculosis patients.

Suggestion

Regarding to the results of our findings in this research, it recommends in particularly at Regional Public Hospital, DR. M. Yunus of Bengkulu to implement the effective cough therapy and Yoga breathing exercises toward pulmonary tuberculosis patients who got breathlessness. For nurses or healthcares, both techniques can contribute to the development of comprehensive nursing care for tuberculosis patients; both outpatient and inpatient health services that focus on reducing symptoms and preventing recurrences. Therefore, this gives some important points in term of EC and YBE' practice, as follows

a. It is expected that the provision of effective cough therapy and Yoga breathing exercises toward tuberculosis patients can be carried out as early as possible after having free from the acute phase. Moreover, this can be carried out within daily activities.

b. Within its implementation should involve family participation to provide supports and care for patients, especially tuberculosis patients.

c. It is expected to be a counseling and facilitator for tuberculosis patients in increasing daily activities.

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Ethical Clearance: This research has already taken an ethical clearance, which is issued by Regional Public Hospital, Dr. M. Yunus, Bengkulu city, Indonesia.

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Conflict of Interest: The authors have declared that there is no conflict of interest in term of results of publication as well.

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Maternal and Child Health in Underdeveloped Areas and State Borders

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Abstract

Background: Mother and child are two vulnerable groups in the family. The health of families in underdeveloped and border areas is still slowly progress. The high degree of maternal and child health in each family in underdeveloped and border areas is a benchmark for a national's public health.

Purpose: The study aims to explore the various factors that correlate with the health of mothers and children in families who live in underveloped and state borders by using the perspective of supply and demand.

Methods: The research was conducted with observational analitic by survey in underdeveloped areas and state border in 3 border districts in 9 sub-districts with 18 villages in Nusa Tenggara Province, Indonesia. There were 396 families selected from the population by multistage random sampling.

Results: The results showed that pregnancy examinations or Antenatal Care services, delivery by health personnel in health facilities; Immunization factors and family planning participation are indicators of Maternal and Child Health, and home environmental factors, healthy lifestyle factors, infectious disease factors, non-communicable diseases, socio-economic factors, and accessibility factors are some of the factors related to MCH.

Conclussion: This study concluded that environmental health factors, healthy lifestyle and behavior, infectious and non-communicable diseases, socio-economic, and accessibility factors are related to the health of mothers and children in families living in underdeveloped areas and state borders and Antenatal Care services, delivery by health personnel in health facilities; Immunization factors and family planning participation were reflective indicators of Maternal and Child Health in families who live in underdeveloped areas and state borders.

Keywords: *Maternal and Child Health, underdeveloped and state border areas, family health.*

Introduction

The family as the smallest unit of society is an important part that affects the health status of a region's community. The mother and child group is one of the vulnerable groups in the family. Families who live in underdeveloped areas and state borders are a minor burden for the health development of a country. Several family-based studies provide results on the importance of family health in supporting public and state health in a broad sense.

In order to develop people who live in bucolic and underdeveloped areas, solid support is needed, not only in one aspect, but the support must be integrated with financial support, planning, evaluation, exchange of information and coordination, technology, promotion,

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and extension of liability in terms of healthcare[1]. The expenses incurred by the family because family members are sick can result in disruption of the family economy[2]. A study and the results stated that the degree of family health can be improved if you pay attention to the health elements of vulnerable family members, such as mothers and children, especially in terms of maternal ANC services, family socioeconomic status, as well as regional access disparities[3].

Another study on low and middle income underdeveloped areas in several Asian countries found that high child mortality rates with environmental health were a determinant factor[4]. The availability of health service facilities and infrastructure for Primaryc Health Center (PHC) and their networks in border areas had not been fulfilled properly, the pattern of health services at the PHC in the border area is not optimal[5]. A study in communities in the border areas of Indonesia and Malaysia, found that the obstacles experienced in health services for residents at the border were the need for referrals to hospitals which were often constrained by transportation, both cost and long distances with poor road conditions[6]. The purpose of this study was to explore the various factors that correlate with the health of mothers and children in families who live in underveloped and state borders.

Material and Method

This research was conducted by analytic observational through a survey in 3 districts (9 sub-districts and 18 villages) in underdeveloped and border areas in East Nusa Tenggara Province, Indonesia from August to November 2019. The sample size was 396 families selected by multistage random sampling. Family heads and housewives were selected as respondents. The independent variables of this study were nutritional factors, Healthy life style and behaviour, health of the home environment, infectious and non-communicable diseases, socioeconomic, accessibility, mental and spiritual factors, while the dependent variable was maternal and child health. All variables are in the form of an ordinal scale and analyzed using the Spearman correlation test at a 95% confidence level with 5% alpha..

Result

From the three districts, it is known that the average age of the household heads is 40.40 years (standard deviation 12.28) with the youngest 18 years and the oldest 83 years. The average number of family members in one house is 5 people (standard deviation of 2 (two) people), with the smallest number being 1 (one) person and the most being 11 people. More detailed information can be found in table 1 (Table 1).

Table 1. Distribution of Respondents Based on Information from The Head of The Family (n=396)

No	Variable (based on the family head)	Frequency	SD
1.	The average age of the head of the family	40.40 years	±12.28
2.	The average number of family members in one house	5 people	±2
3.	The head family by men	93.4%	
4.	Family marital status	96% married	
5.	Education based level	86% primary and secondary	
6.	Work in informal sector	69.2 %	
7.	The average of family in working age	2	±2.1

At Antenatal Care Service (ANC), the highest in the category of the examination was for all family members

who had babies (86.4%) with the highest proportion in Belu Regency (97%) and the lowest in Rote Ndao Regency (76.5%). For the variable of childbirth by health personnel, the highest was in the category of examinations by health workers (90.4%) with the highest proportion in Belu Regency (100%) and the lowest in Rote Ndao Regency (80.3%). The variable number of infant deaths and the variable number of maternal deaths for the three districts is homogeneous with no incidence in the last year. The highest immunization coverage variable was in the category of all family members who immunized with Southwest Sumba Regency having the highest proportion (94.7%) and the lowest in Rote Ndao Regency (89.4%). The highest stunting variable was in the no-incidence category (88.1%) with the highest proportion in Southwest Sumba (99.2%) and the lowest in Belu Regency (79.5%). The highest participation variable for family planning programs was in the category of family members who participated (43.9%) with the highest proportion in Belu Regency (86.4%) and the lowest in Southwest Sumba Regency (0.8%). The highest variable of exclusive breastfeeding in all categories was giving exclusive breastfeeding to babies (89.9%) with the highest proportion in Belu District (99.2%) and the lowest in Southwest Sumba (77.3%).

The results of the cross tabulation of each variable show that the health factor of the home environment has a significant correlation with the health of mothers and children with the spearman correlation coefficient (Rho) of 14.2% where alpha is less than 0.05. Clean and healthy lifestyle factors have a significant correlation with maternal and child health with a Spearman correlation coefficient (Rho) of 29.7%, infectious disease factors have a significant correlation with maternal and child health with a spearman correlation coefficient (Rho) of 34.5%. Non-communicable diseases have a significant correlation with maternal and child health with a spearman correlation coefficient (Rho) of 20.7%, socio-economic factors have a significant correlation with maternal and child health with the spearman correlation coefficient (Rho) 19.3%, accessibility factors are significantly correlated with maternal and child health with a spearman correlation coefficient (Rho) of 10.4%, while nutritional, mental and spiritual factors do not correlate with maternal and child health.

Table 2. Correlation between Maternal and Child Health with Other Variables (n=396)

No.	Correlation between Maternal and Child Health	Spearman Correlation (*rho*)	*P sign*
1.	Home environmental health	0,142	0,005
2.	Clean and Healthy Living Behavior	0,297	0,000
3.	Infectious diseases	0,345	0,000
4.	Non Communicable diseases	0,207	0,000
5.	Social Economy	0,193	0,000
6.	Accessibility	0,104	0,039

Discussion

The results showed that the MCH indicator with the Antenatal Care (ANC) service variable was dominated by the frequency of services in border areas, while those in underdeveloped areas showed high incompleteness of services, even though antenatal care was an important support for family health status[7].

Complete ANC services ensure better care, delivery and even post-delivery processes, controlled maternal and child health and of course become the basic capital for the development process in the region[3]. Belu Regency as a representation of the border region has a better number of ANC services compared to the other two areas which can be caused by other supporting factors such as sufficient awareness and knowledge of prospective mothers and mothers about antenatal care services in health services, awareness of family heads, accessibility, the activity of Posyandu's cadres (MCH integrated services in villages), and socio-economic factors.

A healthy delivery is that which takes place in an adequate health facility and is handled by a health worker. Indicator of coverage of maternal and child

health programs is the number of deliveries assisted by health personnel[8]. The study found that in Rote Ndao regency have the highest in terms of not utilizing childbirth with health personnel, due to access and socio-economic factors that make it difficult to reach services and availability of transportation at any time, although the ANC factor is quite good. The distance traveled and the availability of personnel are important factors in accessing adequate health services for each family[9].

The use of adequate childbirth services can reduce the high level of maternal and child health problems. The availability of health personnel at service facilities plays an important role in the healthy delivery rate in health facilities. The monitoring of the growth of children under five in border areas is relatively low due to a lack of understanding and awareness from the family and the sub-optimal activity of health service cadres. Toddler health determines the nutritional status and health of the mother itself and of course has a direct effect on family health, because toddlers are a vulnerable group[10]. The habit of consuming vegetables and fruit in the family correlates with the nutritional status of the family, including nutrition in high-risk age groups such as pregnant women and infants and toddlers. Humidity in the house is at high risk for the spread of respiratory diseases. Humidity is also triggered by the condition of the floors of the houses, earthen floors are still found in several families in the archipelago and underdeveloped areas. The family's socio-economic and knowledge factors play a role in this. Access to and availability of family latrines is one of the requirements for a healthy house, the availability of latrines is a serious concern of the government with the many triggers it is hoped that families seek to provide latrines independently.

Healthy Lifestyle, such as non-smoking, bathing/ tooth brushing habits, washing hands with soap, and regular and measurable physical activity. Smoking also interferes with the fulfilment of the main needs of family members who should have priorities such as health and education. Smoking is a trigger for lung cancer and tuberculosis.

The incidence of TB in the family is also triggered by the humidity of the house and the density of the occupancy. The facts obtained indicate that areas with relatively humid housing conditions indicate the number of TB sufferers that still exist. Humidity, poor lighting, and overcrowding influence the incidence of TB, therefore the risk of infection is higher. Sufficient knowledge followed by breaking the chain of transmission by modifying the shelter and early detection can reduce TB cases. Other infectious diseases such as diarrhea, which attacks high-risk groups, can interfere with the health status of the family, especially under five.

The facts in the families in the three regions show that in the border areas the number of families who do not suffer from hypertension is relatively high. This is due to the limited consumption and intake of marine-sourced foodstuffs supported by environmental conditions with more intake of plant-based food and livelihoods, most of which depend on agriculture. Communities whose livelihoods are fishermen and live around the coast tend to be more susceptible to hypertension than people who live and make a living in agriculture.

Another factor that is also described in this study is the incidence of DM and it is known that the highest cases are in underdeveloped areas, this can be caused by several triggering factors such as lack of physical activity and exercise, high carbohydrate food intake and an unbalanced diet and regular health check behavior has not become a habit as an early detection effort.

Costs incurred by the family when sick can cause socioeconomic status to be disrupted and if it is not supported by funding or insurance, the family's ability to be paralyzed. Per capita income is an important factor in improving family welfare including the health of vulnerable groups, namely children[11].

Providing adequate accessibility to the family means fulfilling the right to a healthy life for family members, even though the distance, time, and location of the house is a challenge[12,13,14]. The ability to access health facilities is important given the high mortality rate due to delays in finding health facilities due to the distance factor[15,16,17]. The travel time factor is a factor other than distance which is also related to the health status of the family, at some locations it is found that it is close but the travel time is long and vice versa, the distance is long but the travel time is short due to the available road and bridge network. In underdeveloped areas, family accessibility is lower than in border areas due to the more available road and bridge networks[18].

Families who are closer to access to health services have a much higher life expectancy than families who live far from access to health services[19].

The government's role in the field of health services is very important and requires more efforts, especially in the reach of services in remote areas in underdeveloped areas and state borders by scale up the budget the supporting infrastructure and the availability of health personnel[20,21]. The health of mothers and children of a family is very much determined and shaped, especially by awareness of childbirth in adequate health facilities with the helpers of which are health workers, with intensity of service and regular antenatal care [22]. Family planning follows a life cycle from early childhood to adulthood to old age. Family planning programs help regulate pregnancy spacing, meet the needs and concerns of all age groups, and immunization ensures the health and immunity of each family against attacks by various infectious diseases and contributes to family health, especially for children as the successor of the family generation.

Conclusion

This study concluded that environmental health factors, healthy life style, infectious and non-communicable diseases, socio-economic, and accessibility factors are related to the health of mothers and children in families living in underdeveloped areas and national or state border.

Ethical Clearance: This study had passed ethical clearance issued by Ethical Committee of the Faculty of Nurse, Universitas Airlangga, Surabaya, Indonesia.

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Conflict of Interest: Nil.

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A Comparative Study of Patients' Attitude Before and after Receiving Healthcare Services at Bam Pasteur Hospital

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Abstract

Background: The human attitude to events, including health and disease, and his interpretations determines the quality of his life. The purpose of this study was to evaluate the attitudes of patients before and after receiving the services of Pasteur Hospital in Bam.

Methods: This is a descriptive study. The research population included 110 patients referring to men's surgery and women's surgery who were studied by simple sampling method. The attitude of patients before and after receiving healthcare was measured by a researcher-made questionnaire. Data analysis was performed using spss24 software Through descriptive and analytical tests such as one-way ANOVA, two-population mean comparison and pair comparison statistical tests.

Results: The results of statistical tests showed that the average total attitude score of clients after receiving services (2.58) was more than the mean of attitude of clients before receiving services (2.41) (p=0.017). Paired t-test showed significant relationship between the attitude score of the patients towards medical staff visitors (p=0.017) and Patient discharge unit (p = 0.001) before and after receiving the service, but no significant relationship was found between the other units.

Conclusion: It can be concluded that the attitude of the clients after receiving the services has been positive. Therefore, to improve the delivery of services and to increase the satisfaction of clients in some sectors, managers can be more precise planning to improve quality.

***Keyword**: Attitude, Hospital, Customer, Quality, Bam*

Background

One of the important topics studied in the field of psychology is the attitude that is important in people's lives. In general, the attitudes of people about social issues in a society, are the determining determinants of people's intentions in dealing with social issues.[1,2] An attitude is a reflection of the way a person feels towards one thing or subject.[3] Some thinkers like Frankl and Aaron Beck believe that human attitudes to events, including health and disease, and his interpretations, determine his quality of life.[4]

In a general, each customer after receiving services or buying goods and using it, is satisfied or dissatisfied. Satisfaction is the existence of a positive feeling that ultimately results in the consumer. This feeling is due to supplier performance and fulfilment of customer expectations. According to equalization, higher or lower

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of expectations of the customers with the received goods and services, a feeling of satisfaction, tastes or dissatisfaction is created.[5] The word "satisfaction" means a lot of meanings. Most of these meanings reflect the concept that satisfaction is the emotional response of a customer that derives from a different understanding between customer expectations and product performance. Understanding the satisfaction leads us to the activities that achieve it has a tremendous effect.[6]

Many factors contribute to patient satisfaction. The comfort of patients in receiving services, the physical and hardware structure of hospitals, the medical personnel's exposure to patients and the professional skills of medical personnel among their colleagues and exposure to patients in difficult situations, and ultimately what the patients themselves have in their minds and expect, all have an effect on patients' satisfaction.[7,8-9] The direct relationship between expectations and satisfaction makes suitable factors for research and investigation. Expectations of patients are formed with various physical, psychological and social needs, and the agreement reached between them and receiving appropriate services leads to satisfaction.[10,11]

The results of Ismaili's Shahmirzadi study showed that improving the quality of services provided in public health facilities by implementing interventions to improve the physical environment, guaranteeing committed services, increasing employee empathy with referrals, and receiving feedback on issues and the results of the treatment are necessary from the referral.[12] To use customer perceptions and attitudes to evaluate the quality of services, tools must accurately measure these perceptions, because decisions based on inaccurate information will result in harmful effects for the organization.[13]

Assessing the views and opinions of patients has been booming in recent years due to the competition between hospitals in gaining trust, cooperation and participation as much as possible, reducing costs, and ultimately increasing income and productivity. Since consumer behavior and customer satisfaction are considered to be a sign of quality of service and a criterion for determining weaknesses and strengths in the process of production and supply of services, assessing the views of patients and users of the service as one of the methods Important and critical points in the evaluation of hospitals and medical institutions have been considered.[14,15]

Material & Methods

This study was a descriptive study. The research population includes patients admitted to hospital and Sample size done by obtaining the number of patient's admittance within two months from the admission and medical records section using the Cochran formula, taking into account 0.1 degree of freedom and assuming 0.05 degree of confidence. 110 people were estimated. In this research, a simple sampling method was used.

Data were gathered through a researcher-made questionnaire. Validity of the questionnaire was confirmed by experts in terms of content and structure. To assess the reliability of the questions, Cronbach's alpha coefficient was used which was 0.8.

The SPSS software version 24 was used for analysing. The statistical tests used to analyze the data were descriptive statistical tests and for statistical analysis of variables, one-way ANOVA, test the comparison of the mean of the two societies and the pair comparison test was used.

Table 1: Frequency and percentage of demographic variables

Variables		Frequency	Percentage	Cumulative frequency
Gender	Female	56	50.9	50.9
	Male	54	49.1	100
Education level	No Diploma	59	53.6	53.6
	Diploma	34	30.9	84.5
	Undergraduate	7	6.4	90.9
	Bachelors or higher	10	9.1	100
Hospital section	Men's surgery	81	73.6	73.6
	Women's Surgery	29	26.4	100
Age	Less than 20	26	23.6	23.6
	20 to 40	50	45.5	69.1
	More than 40	34	30.9	100
Residential area	City	62	56.4	56.4
	Village	48	43.6	100
Days of admission	1 to 3 days	66	60	60
	3 to 5 days	28	25	75
	More than 5 days	16	15	100
Number of visits	0	50	45	45
	1 time	14	13	58
	Two times	22	20	78
	More than 3 times	24	22	100

Findings

Based on the data collected from the questionnaire, the total number of respondents was 110 and demographic analysis results is shown in table1.

Regarding the normal distribution of the population, parametric tests have been used to analyze the data. it shows that there is no meaningful relation between the attitude of the clients and the demographic variables.

The relationship between the attitude score of clients before and after receiving health care was applied to the nursing staff, the admission unit, service unit, the guard unit and the welfare service unit from the t-test. The results showed that there is a significant difference between the clients' attitude's mean score before and after receiving health care from the medical staff and the discharge unit. In this sense, the attitude of the clients after the receiving health care has a significant effect on the medical staff and the discharge unit, which shows that the attitude of the clients towards the medical staff and the clearing unit has been positive (Table 2).

Table 2: Comparison of attitude score before and after getting services

Variables	Attitude Score Before Getting Services	Attitude Score After Getting Services	P Value
Total Attitude Score	2.4174	2.5883	0. 017
Attitude Score for the medical staff	2.7309	2.9309	0. 017
Attitude Score to the clearance unit	2.0424	2.4485	0. 001

Discussion

In this study the patient's attitude was positively changed after receiving the services compared to the hospital before hospitalization. So, the overall attitude of the patient before the admission was 2.41 and after receiving the service was 2.58. Expectations of customers are based on past experiences of the organization, business executives and advertisers. After receiving the service, the customer compares his imagined service with the expected service. If the service is thought to be less than expected, the customer will lose interest in the service provider. If the service is supposed to be more than or even equal to the expected service, the customer will still use the service.[16] According to Berkowitz, no part of the marketing mix is as visible as promotion in health services. Nasiripour et al in their research concluded that multiple marketing activities had an impact on other variables such as age, occupation, hospital near home, education level and marital status.[17] Hospitals can provide and perform their services in a way that is distinct from other hospitals to be selected by patients.[18]

Patients spend hours, alone and with the presence of maximum one family member as guest, sitting on bed or in the room, or sleeping. He concludes that the experiences of patients in the hospital are important as their temporary place of residence. Nurses should consider their patients' perceptions and, in relation to patients and families, consider options for improving patients' experiences by equipping a separate room in the hospital, the possibility of short visits to the home and etc. Therefore, nurses can personalize the care and improvement of patients' quality of life.[19]

The patient's attitude toward the medical staff of the hospital before and after receiving services was 2.73 and 2.93 after receipt of the service, which is statistically significant. Golda et al stated that there has been an assessment of patient experience, such as the assessment of health care users from providers and service delivery

systems since 2002, but today the assessment of the doctor's emphasis is stronger. Patient experience scores from physicians, today are increasingly required to maintain a certificate of practice and the ability to participate in specific health plans and online clinicians' credits.[20]

Clinical interactions between patients and physicians have led to significant improvements in the reduction of blood pressure, headache, glucose management, and general symptoms associated with multiple diagnosis, with the frequency of patient questions and explanatory remarks by physicians.[20] The patient empowerment approach includes dialogue and cooperation relationships as well as consumer participation in deciding on their services. A powerful predictor of improving mental health outcomes in patients with chronic illness.[21] The study by Ayoub et al showed that the physician-patient relationship is focused on providing high-quality medical care. The most urgent need for patients is to have a medicine that takes care of the patient and does not excite the patient.[22] One of the reasons for the positive attitude of the patients towards the medical staff is the continuous presence of the medical staff in the hospital for the development of health, as well as their appropriate treatment with patients, and the provision of advice and training to the patients.

The average patients' attitude score toward the clearance unit also had a positive change, so that the attitude of the clients before receiving the services was 2.04 and after receipt of services was 2.44. The reasons for the positive attitude of patients towards the clearance unit may be the cost-effectiveness of the unit, as well as the speed of clearance by the unit. Sarvandi and Shahroodi point out that due to the increase in the number of chronic patients or elderly patients hospitalized, the need for proper discharge of the patient from the day-to-day hospital is increasing.[23]

There is no significant difference between the attitude of patients with age, education, gender, place of residence before and after receiving health care. In the study of Sadr et al. and Dolatkhah et al, there was no significant difference between the attitude of the patients and their age, while Sajjadi et al and Hamedanizadeh et al in their study showed a significant relationship Between age and attitude.[24,25,26-27] There was no significant difference in the relationship between the attitude score of the clients and their education before and after receiving the health care. This finding is consistent with Sadr et al and Sajjadi et al.[24,26] This is despite the fact that Taba'i et al referred to a meaningful relationship between attitude and educational level.[28]

There was no significant difference between the attitude of clients and their gender before and after receiving health care. This finding is consistent with the study of Sajjadi et al, Dolatkhah et al, and Ganji et al.[25,26-29] While Hosseini Seresht et al point to a meaningful relationship between attitude and gender scores.[30] There was no significant difference between clients' attitude and their place of residence before and after receiving health care. This finding is consistent with the study of Sajjadi et al.[26]

Conclusion

One of the most important reasons for quality is meeting the needs and expectations of the customer. For this reason, it is suggested that hospitals, using marketing science, provide educational services to enhance communication and specialist skills for nursing staff, and, with more respect, the patient's rights seek to satisfy their needs. Organizing training courses for admission personnel to provide guidance to patients and adolescents at the time of admission, increasing the number of service forces and patients to provide further guidance to patients.

Ethical Clearance- This project has been approved by the Ethics Committee of Bam

University of Medical Sciences

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Conflict of Interest – no Conflict of Interest

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Preoperative Serum CA125 and Plasma Fibrinogen Levels as Predictors of Lymphovascular Space Invasion in Endometrial Cancer

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Abstract

Background: Lymphovascular space invasion has been shown to be a predictor of lymph node metastasis and decreased survival in patients with endometrial cancer. Unfortunately, LVSI status is most commonly not available until after hysterectomy at the time of preparation of the final Pathology report. Therefore; LVSI status represent additional information to be considered in decision making process regarding whether to perform lymphadenectomy or to deliver adjuvant therapy. Thus, it was important to find biomarkers to predict the risk of LVSI before surgery.

Objective: To evaluate the value of serum CA125 and plasma fibrinogen for prediction of lymphovascular space invasion in women with endometrial cancer.

Study Design: A prospective observational cross-sectional study.

Settings: was carried out in the Department of Obstetrics and Gynecology/ Baghdad Teaching Hospital, and Nursing Home Hospital in Medical City complex, Baghdad, Iraq, over a period of 1 year starting from the first of December 2017 to the end of November 2018.

Patients and Methods: Fifty-five women with endometrial cancer undergoing surgery were included in this study. We compared the preoperative level of serum CA125 and plasma fibrinogen with postoperative histopathological report regarding LVSI, the results were statistically analyzed.

Results: (16.4%) of the patients with positive lympho-vascular invasion. CA-125, Fibrinogen, were highly significantly increased in patients with positive LVSI. Sensitivity was (88.9%), specificity (95.7%), negative predictive value (95.0%), positive predictive value (91.0%),and accuracy of the test was (92.3%)when the cutoff value for CA125 was (28.75U/ml) while for plasma fibrinogen in cutoff value (317mg/dl)the sensitivity was (100.0%), specificity (95.7%),negative predictive value (100.0%), positive predictive value (95.9%) and the accuracy of the test was (97.8%)

Conclusion: The preoperative assessment of serum CA 125 and plasma fibrinogen could be used to predict the LVSI in endometrial cancer.

Keyword: *Lymphovascular space invasion, CA125, plasma fibrinogen, endometrial cancer*

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Introduction

Endometrial carcinoma (EC), or carcinoma of the corpus uteri, is the sixth most common cancer in women worldwide, with an estimated 287000 new cases per year. In developed countries, with 142 000 new cases

annually, endometrial carcinoma is the most common gynecologic malignancy, causing 33 000 deaths every year. The lifetime cumulative risk for a woman to develop an endometrial carcinoma is 1.0% worldwide (0–74 years). [1] The median age of patients at the time of diagnosis is 61 years, and ninety percent of them are older than 50 years. As endometrial carcinoma is partly associated with general socioeconomic status, the global incidence is expected to rise as the population in developing countries adopts a more westernized lifestyle along with its detrimental health effect, primarily due to dietary factors. [2]

Endometrial carcinoma by definition is a malignant tumor of the endometrium. It is divided into two types according to the histologic characteristics. Type I endometrial carcinoma is an endometrioid adenocarcinoma that is related to exogenous estrogen exposure. Type I endometrial carcinoma comprises three subtypes that are categorized by their histology into well (grade 1), moderately (grade 2) and poorly (grade 3) differentiated tumors. As obesity becomes more common, the incidence of type I endometrial carcinoma is expected to rise, because of the production of estrone by adipose tissue. Typically, type I endometrial carcinoma develops in perimenopausal, obese women with an endometrial hyperplasia as a precursor. Type II endometrial carcinoma is not related to estrogen exposure, occurs typically in older women and generally has a much poorer prognosis than type I disease. Type II carcinoma includes papillary serous or clear cell subtypes and may exhibit a mixed endometrioid component. [3]

Epidemiology

Endometrial cancer is the sixth most commonly occurring cancer in women and the 15th most commonly occurring cancer overall. There were over 380,000 new cases in 2018 while in 2012 it was around 320 000 new cases of endometrial cancer were diagnosed worldwide. [5] The highest incidences in 2012 are estimated in the USA and Canada (19.1/100 000) and northern (12.9/100 000) and Western Europe (15.6/100 000). Although endometrial cancer is conventionally thought to be a cancer of the postmenopausal period, 14% of cases are diagnosed in premenopausal women, 5% of whom are younger than 40 years. [6] The increased incidence of endometrial cancer in Europe and North America could be related to a greater overall prevalence of obesity and metabolic syndromes in these regions, in addition to the ageing of the population. Projections show that the number of cases will increase to 42.13 per 100 000 in 2030 in the USA. [7] In Iraq the prevalence of endometrial cancer was 1.87% [8]

Study design and setting

This is a prospective observational cross sectional study was carried out in the Department of Obstetrics and Gynecology/ Baghdad Teaching Hospital, and Nursing Home Hospital in Medical City complex, Baghdad, Iraq. This study was conducted over a period of 1 year starting from the first of December 2017 to the 30th of November 2018.

Patients

Fifty-five patients with endometrial cancer undergoing surgery were included in this study.

Exclusion criteria

- Patients who received chemotherapy or radiotherapy before surgery (for a reason other than endometrial cancer).
- Patients with chronic inflammatory disease.
- Patients with acute inflammatory condition.
- Patients with chronic liver disease.
- Patients with recent deep venous thrombosis.

Method

Pre-surgery: sample of 5 cc of blood were collected from the patients and then divided into :

1. 3 cc of blood was put it in a specialized EDTA tube and within few hours transfer it to the a specialized private laboratory (called Blue lab. In Al-Harthyia/ Baghdad) to investigate for fibrinogen

2. 2 cc of blood were centrifuged and send to the lab to investigate for CA-125.

Post-surgery: follow up the patient's histopathological reports. All patients underwent a total hysterectomy or radical hysterectomy with bilateral salpingo-oophorectomy (BSO) and pelvic lymph node

dissection. The specimens were reviewed by pathologists at the Department of Pathology in Teaching lab center. A tumor was considered lympho-vascular invasion-positive when tumor emboli were noted within a space that was clearly lined by endothelial cells.

Then patients divided into two groups (positive and negative lympho-vascular space invasion), then we compare the CA-125 and plasma fibrinogen between two groups.

The results were 9 patients with positive lympho-vascular space invasion, and 46 with negative lympho-vascular space invasion.

Statistical Analysis

All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 22 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Multiple contingency tables conducted and appropriate statistical tests performed, Chi-square used for categorical variables and t-test was used to compare between two means. One-way ANOVA analysis was used to compare between more than two means. ROC curve was used to clarify validity tests.

In all statistical analysis, level of significance (p value) set at ≤ 0.05 and the result presented as tables and/or graphs. Statistical analysis of the study was done by the community medicine specialist.

Results

CA-125 and Fibrinogen were highly significantly increasing in patients with positive lymphovascular invasion than those with negative lymphovascular invasion, while Platelet significantly increase and no significant association were found regarding PT and WBC with lymphovascular invasion (table 1).

Table 1: Distribution of the variables according to the relation with lymphovascular space invasion.

Parameter	Lympho vascular space invasion		P-Value
	Positive (N=9)	Negative (N=46)	
	Mean±SD	Mean±SD	
CA-125	37.1±11.51	23.46±7.47	<0.001*
fibrinogen	564±64.4	289.6±111.8	<0.001*
PT	11.76±0.83	11.63±0.74	0.665 NS
Platelet	331±48.21	292.87±47.62	0.033**
WBC	8.3±0.76	8.35±1.69	0.933 NS

* Highly significant ** significant NS=Not significant

Highly significant association were found between tumor stages and lymphovascular space invasion, while no significant association between age group and mean age of both groups with lymphovascular space invasion (table2)

Table 2: Relation between age group and stage with lymphovascular space invasion.

Parameter		Lympho vascular space invasion				P Value
		positive		negative		
		No.	%	No.	%	
Age group	<50	1	14.3	6	85.7	0.964
	51-60	3	17.6	14	82.4	
	61-70	4	18.2	18	81.8	
	>70	1	11.1	8	88.9	
	Mean age	60.7±6.36		61.7±9.12		0.5
Stage	1A	1	2.2	45	97.8	<0.001*
	1 B	1	50.0	1	50.0	
	2	3	100.0	-	0.0	
	3 A	2	100.0	-	0.0	
	3 C	2	100.0	-	0.0	

* Highly significant

CA-125 were increased in patients with positive lymphovascular space invasion more than those without invasion, this increment is not higher than normal level of the marker (≤35). Fibrinogen were increased in patients with positive lymphovascular space invasion more than those without invasion (table 3).

Table 3: CA-125 and fibrinogen means regarding to the lymphovascular space invasion.

	Lympho vascular space invasion	
	positive	negative
	Mean ±SD	Mean ±SD
CA-125	37.10±11.51	23.46±7.47
fibrinogen	564.0±64.4	289.6± 111.8

Regarding to the validity test of the CA 125 to predict the lymphovascular space invasion, it was found that Sensitivity were (88.9%), specificity (95.7%), negative predictive value were (95.0%), positive predictive value (91.0%) and the accuracy of the test was (92.3%) when the cutoff value of the CA 125 was (28.75U/ml) (table 4)

Table 4: Validity test of CA-125 in prediction of lymphovascular space invasion in endometrial cancer.

Validity parameter					
Cutoff value	**Sensitivity**	**Specificity**	**NPV**	**PPV**	**Accuracy**
28.75U/ml	88.9%	95.7%	95.0%	91.0%	92.3%

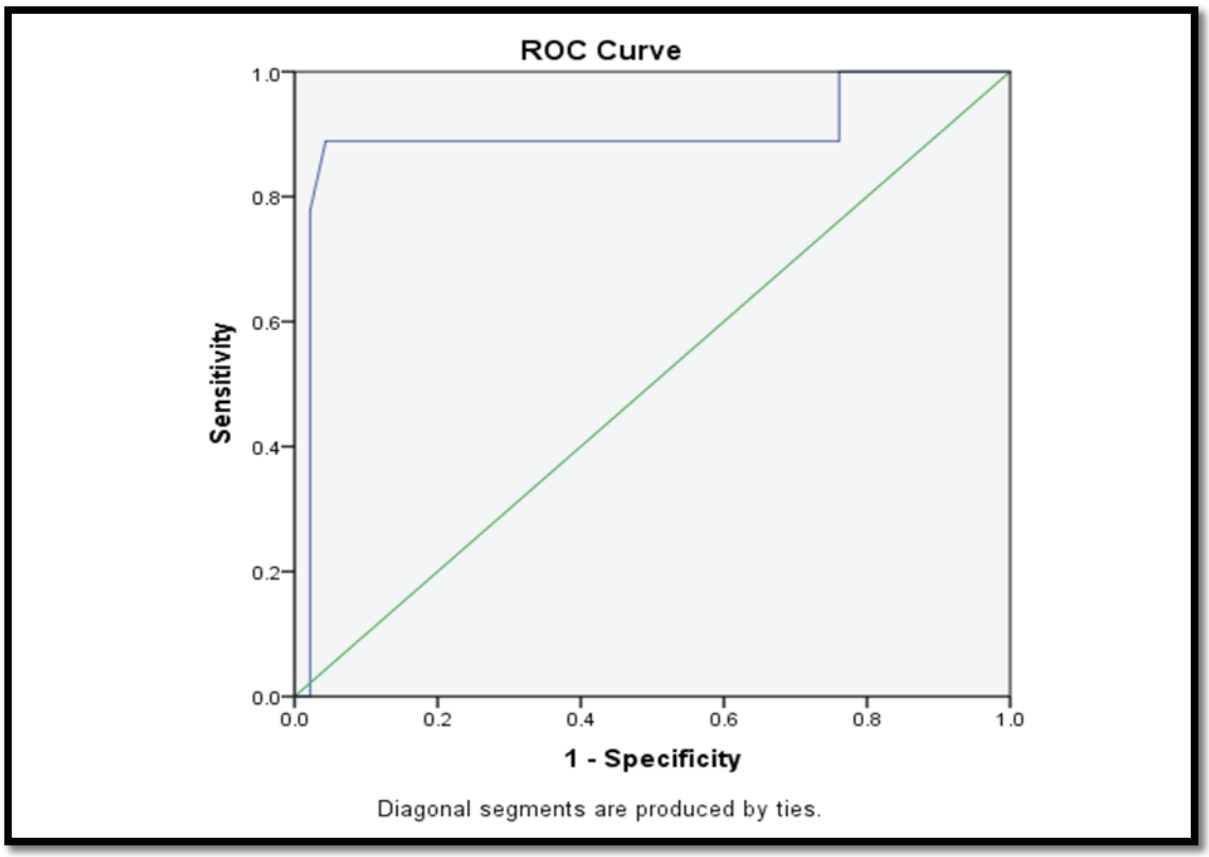

Figure 1: ROC curve for serum level of CA 125 for prediction of lymphovascular space invasion in endometrial cancer

As regarding to the validity test of the fibrinogen, it was found that Sensitivity were (100.0%), specificity (95.7%), negative predictive value was (100.0%), positive predictive value (95.9%) and the accuracy of the test was (97.8%) when the cutoff value of the fibrinogen was (317mg/dl) (table 5).

Table 5: Validity test of Fibrinogen in prediction of lymphovascular space invasion in endometrial cancer.

Validity parameter					
Cutoff value	**Sensitivity**	**Specificity**	**NPV**	**PPV**	**Accuracy**
317 mg/dl	100.0%	95.7%	100.0%	95.9%	97.8%

Discussion

Endometrial cancer is typically a disease of the peri-/postmenopausal period with a median age at diagnosis of 63 years, and more than 90% of cases occurring in women >50years. [9] This is in agreement with the current study, when the mean age of patients was (61.56±9.1) years. But it is more than (55.15±8.54) that mentioned by Zhou X et al. [10] this may be due to difference in sample size collection when the former study was with larger sample size (N=211). The majorities of the patients were in stage I (IA and IB) (89.2%) , this is in agreement with Yang B et al, [11] when both stage IA and IB is found in (75.4%) of the studied group. Data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program demonstrated that stage I was found in 73% of patients. [12] This is could be bias because we are in tertiary centre and receive many cases and also there could be missing in the reports of other cases whom been having surgery in other centres or private hospitals. In our study the positive rate of lymphovascular space invasion in endometrial cancer had no relationship with age. This is in agreement with Zhou X et al. [10] labeled that lymphovascular space invasion as a predictor for poor survival and nodal metastasis in patients with many types of cancer (ovarian, vulva, cervical, rectal and breast cancer). Mariani et al, [13] have published several studies with the objective of identifying new prognostic factors in EC. They and others have suggested that lymphovascular space involvement was an important prognostic factor for relapse of disease and poor survival, we didn't study the prognostic effects of lymphovascular space invasion on survival or recurrence due to short period of our study. Lymphovascular space invasion has also been shown to be an important prognostic factor for pelvic lymph node metastases in patients with endometrial cancer. [14] Therefore, they believed that expecting lymphovascular space invasion before surgery could help to better adapt surgical staging and adjuvant therapies. The current study found that (16.4%) of the patients with positive lymphovascular space invasion,this is in line with results of other studies evaluating patients with low risk endometrial cancer, which had found incidence rates of lymphovascular space invasion of 12% to 16.9%. (48,78) A study by Koskas M et al, [14] for predicting lymph node metastasis in endometrial cancer found that the metastatic LN rate with positive lymphovascular space invasion was 28% while metastatic LN rate with negative lymphovascular space invasion was 4%. Suh DH et al [15] with many other studies mentioned that serum levels of CA 125 were an independent risk factor for LN metastasis, this may be considered as a guide for weather patient need for lymphadenectomy or not. It has been reported that high CA125 values were associated with advanced surgical stage, lymph node metastasis, and poor prognosis. Hsieh CH, et al, [16] in multivariate analysis using a logistic regression model showed that lymph node metastases had the most significant effect on the elevation of CA125 levels. The ROC curve determined that the best cutoff value was 40 U/mL; the sensitivity and specificity for screening lymph node metastases were found to be 77.8% and 81.0%, respectively. Therefore, they thought a CA125 level greater than 40 U/mL could be considered a criterion for full pelvic lymphadenectomy in the surgical staging of EC. In our study the mean level of CA 125 was 37.10±11.51 in positive lymphovascular space invasion and 23.46±7.47 in negative lymphovascular space invasion patients and the ROC curves showed that the cutoff value of the CA 125 was 28.75 U/ml with a sensitivity (of 88.9%), specificity (95.7%), PPV (91.0%) , NPV (95.0%) and the accuracy was (92.3%) In Zhou X et al, [10] results section, the mean level of CA125 was 103.94 U/mL in lymphovascular space invasion positive patients and 31.199 U/mL in the lymphovascular space invasion negative group. The ROC curves showed that the cutoff value of CA125 was 21.1 U/mL with a sensitivity of (76.5% and a specificity of (69.4%) The results of the logistic regression analysis revealed that CA125 was significantly related to lymphovascular space invasion in endometrial cancer. Regarding to the fibrinogen, our study shows the relationship between the level of fibrinogen and lymphovascular space invasion in patients with endometrial cancer. Krenn-Pilko S et al, [17] revealed that the fibrinogen increment was related to tumor metastasis, prognosis and staging in patients with malignant type of tumor and fibrinogen had the uppermost concentrations level of plasma coagulation factors is a plasmin and thrombin substrate. The development of gynecological malignancies due to tumor cell invasion and metastasis will destroy a large amount of procoagulant materials in patient blood and also lead to secrete large amounts of tissue factors, and may start the clotting system. The formation of a large amount of thrombin fibrinogen was then converted

to fibrous protein, thereby increasing the content of fibrin. Most solid tumors contain considerable amounts of fibrinogenrelated products. Briët JM et al, [18] in a retrospective multicenter study included 436 endometrial cancer patients found that levels of plasma fibrinogen can be used as a prognostic independent parameter for disease-free survival and overall survival of endometrial cancer patients.

In the sub-cohort of women with endometrioid histology, an increased fibrinogen concentration at presentation was predictive of pelvic nodal involvement. The plasma fibrinogen level may be an independent risk factor in EC. [19]

In the present study the mean level of fibrinogen in positive lymphovascular space invasion patients were (564.0±64.4) mg/dL and the ROC curves revealed that at a cutoff value of fibrinogen was (317 mg/dl) with a sensitivity of (100.0%), specificity (95.7%), PPV was (95.9%), NPV (100%) and the accuracy was (97.8%). While in a study carried by Zhou X et al, [10] found that the mean level of Fibrinogen was 278 mg/dL in positive lymphovascular space invasion patients and 249 mg/dL in the negative group. The difference was statistically significant. The ROC curves showed a cutoff value of Fibrinogen of 258 mg/dL with a sensitivity of (71.4%) and a specificity of (64.4%).

Limitation of the study:

1. Small sample size with short period of the study

2. Single center study.

3. As in all cross-sectional study the temporal relationship between the cause and effect cannot be improved

Conclusion

The preoperative assessment of serum CA 125 and plasma fibrinogen could be used to predict the LVSI in endometrial cancer.

No **Conflicts of Interest**

Self-funding Source

Ethical Clearance: from the Ministry of health and Environment/ scientific committee

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Effect of Oxymetholone on The Sperm Quality and Sex Hormone Profile in Male Rats

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Abstract

The objective of present study was to evaluated the effect of Oxymetholone drug on sperm parameters and sex hormone in adult male rats. Twenty male rats sexually mature were randomly divided into two groups; Each group has 10 rats. The treated group administered orally with Oxymethololne drug 5 mg/g body weight/ day of for 60 days while the control group administered orally normal saline per day. SPSS analysis of data generated with P< 0.05 considered statistically significant. The results showed a significant decrease ($p < 0.05$) in the mean sperm count, the motility and viability in treated group compared to control. Also, LH (luteinizing hormone) FSH (follicle stimulating hormone) and T (testosterone) of the treated group were significantly lowered compared to the control. The administration of Oxymethylene induces low sperm quality with reduction in sex hormone.

Key words *: Oxymetholone, sperm parameters, hormonal profiles.*

Introduction

Anabolic androgenic steroids (AAS) are among the most important compounds used in the medical field as well as used illegally by young athletes and non-athletes who use this stimulant to increase muscle mass and improve their functional performance [(1)] and AAS are synthetic derivatives of male sexual hormone testosterone and are small molecules that can spread negatively in various body tissues [(2).] The first effect is the anabolic effect or muscle building, which is an increase in protein synthesis, which leads to muscle growth and an increase in its size, while the second effect is the effect of ender, genetic or masculinity stimulant, including sperm production, roughness of the voice and frequent hair appearance; Although his pharmaceutical industry mainly possesses low-end and genetic effects called anabolic steroids, both effects are inseparable [(3).] Oxymethlone is a synthetic anabolic steroid that is structurally associated with male testosterone [(4,5)] Uses Oxymethlone and other synthetic androgens to treat a variety of conditions, including hypogonadism and delayed puberty; androgens are also used to correct vascular edema, manage breast cancer, promote a positive balance of nitrogen after injury or surgery, and stimulate red blood cell production; athlete athletes consume large amounts of androgens in an effort to improve athletic performance [(5).] Hypothalamus -Pituitary Axis Hypothalamic- Gonodal (HPG) to regulate spermatogenesis; When the anterior pituitary gland's response to the stimulation of the hormones released to the canend (GnRH) decreases, the level of testosterone in the blood will rise, resulting in a decrease in FSH and LH secretion due to LH stimulation of testosterone production. If a specific cause, such as testicular damage, is caused by low levels of testosterone in the blood, resulting in an increased response of the anterior pituitary gland to stimulate the hormones releasing (GnRH), causing an increase in the secretion of LH and FSH hormones, thereby increasing the production of testosterone by the cells of Leydig Celle [(6)] and testosterone stimulates the maturation of spermatocyte [(7)]. Based on the above and for the purpose of knowing the effect of the sports stimulant Oxymetholone on

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reproductive hormones and sperm parameters, this study was designed to investigate the effect of the drug on the reproductive system of adult male rats.

Materials and Methods

1.Source of drug

Oxymetholone was obtained in a dose (50mg) from a gym in Baghdad governorate. This dose is for humans and it is in pill form. Adult white male rats were used in this study using the concentration (5mg / kg)

2. Animal:

Three-month-old mature and healthy adult male rats weights ranging from (190-220 g) were procured from animal house of Faculty of Education for Girls-University of Kufa . They were maintained under controlled light schedule (12h Light:12h Dark) at room temperature (28 ºC) and with constant humidity (40 -50%). The animal acclimatized for a period of 7 days before the start of treatment . During this period, they were fed with standard rat chows/pellets and water.

Experimental trial design procedure:

3. The treatment group which included (10) adult male rats, as these animals were administrated orally with oxymetholone at a dose (5mg/kg) and by (1ml) for (60) days. The control group, which included (10) adult male rats, as these animals were administrated with normal saline (1ml) for 60 day

4. Animal sacrifice

Weights were recorded for all animals and then the animals were drugged by injecting them with the mixture of Xyline and ketamine, by taking (0.5ml) of ketamine and (0.1ml) of Xyline per (250g) of body weight. A T-shaped hole was made in the abdominal cavity and then blood samples were drawn from the heart directly by heart stab to obtain a blood sample of (2-5ml), after which the genitals (testes and epididymis) were raised. Removal of fatty tissue from around the organs to record their weights using a sensitive balance, while the tails of barnacles are used to study sperm characteristics. After that, the organs were placed in plastic containers containing formalin solution at a concentration of (10%), and then subjected to microscopic examination to study the tissues of these organs.

5.Preparation of serum samples:

After the animals were sacrificed, blood samples were collected and placed in gel tubes and left to clot in order to separate the blood components from the serum. Then these tubes were centrifuged at a rate of (3000rmp) for a period of (10) minutes. After the separation process is completed, the serum is transferred into a serum test tube and kept at a temperature of (-20) degrees Celsius until the results are read [8]. Hormones were measured using several Enzyme Linked Immunosorbent Assay (ELISA) which are known ways to measure the concentration of hormones, including follicle stimulating hormone, luteinizing hormone, testosterone and progesterone, and that several kit to stack hormones of the reproductive system in humans can be used to measure the level of hormones in the animal. Except for FSH, the modus operandi was followed by the information attached with the kit measurement kit.

6- Sperm count:

The sperm was collected from the right tail of the spray after sacrificing the animals and dissecting them, where the sperm was placed in (10ml) of the solution in a petri dish, after which we calculate the sperm by placing a drop on the glass slide and examining it under the optical microscope [9].

Statistical analysis:

The statistics of the results were analyzed using the SPSS statistical system for the purpose of comparing control and transaction totals using the t test.

Results

1- Sperm quality analysis:

Results of sperm quality analysis are presented in the Table 1. The obtained data showed that the sperm concentration, motility and vitality in the treatment group that administered orally with Oxymethololne drug (5mg / kg) for to 60 days decreased in compared to the control group at each evaluated time ($P<0.05$).

Table 1. The obtained data showed that the sperm concentration

Sperm Parameters	Number of animal	Treatment group M ±SD	Control group M ±SD
Average sperm concentration per mg testis (million / mg)	10	24 ± 3.06	49 ± 2.67
Average testicular sperm concentration (million / ml)	10	33 ± 5.68	77 ± 7.29
Average sperm concentration in epididymis delicacy (million / ml)	10	25 ± 2.69	72 ± 6.2
The percentage of live sperm in the epididymis	10	30 ± 1.12	90 ± 7.49
The percentage of abnormal sperms in the epididymis delicacy	10	58 ± 9.42	17 ± 5.31
The percentage of motile sperm	10	20 ± 0.6	65 ± 8.22
The degree of sperm activity		1 ± 0.08	2.5 ± 0.62

Table No. (1) shows the decrease in sperm parameters for the comparison group

Five rats per group

Significant difference * $p < 0.05$

2- Hormones:

The results of the statistical analysis showed that there were significantly differences ($p<0.01$) in testosterone concentrations, follicle stimulating hormone, luteinizing hormone and progesterone, with a significantly decrease ($p<0.01$) observed for the control group.

Table No. (2) shows the decrease in hormones for the comparison group

Hormones (U/ml)	Number of animal	Treatment group M ±SD	Control group M ±SD
Testosterone	10	2.7± 1.1	6.6± 3.6
Progesterone	10	0.7± 0.7	2.4± 0.5
LH	10	2.5± 1.8	6.8±1.9
FSH	10	2.6± 1.4	7.8± 1.4

Table No. (2) shows the decrease in hormones for the comparison group

Five rats per group

Significant difference * p <0.05

Discussion

1-Changes in sperm standards:

The current study showed decrease that sperm concentration, motility and vitality in treatment group. The results of this study were in agreement with previous studies [10-12]. Naturally mature sperm production is the basis of male fertility [13] and the production of sperm and testosterone in the testicle is regulated mainly by FSH and LH, which have been released from the pituitary gland and are the main organizers of sperm formation [14], and the low level of reproductive club nutrients may have prevented the initiation of sperm formation in animals. Because the suppression of the nutrients of the canons prevents the signal responsible for the initiation and completion of the process of the formation of sperm during the natural maturation of the developing or developed sperm [15], the initiation of the process of the formation and maintenance of sperm naturally and qualitatively requires the presence of sufficient levels of hormone nutrients for the feeding and testosterone and usually the insufficient levels of these hormones are associated with severe abnormalities in the sperm and may lead to the state of sperm and weakness , In addition, the function of the ancillary gonads also depends on the presence of adequate levels of testosterone in the circulatory system [16,17]. It develops sperm cells, sperm cell differentiation and sperm protruding, and contributes to the generation of FSH receptors in sertoli cells [14]. Androgen also has an important role in the final stages of the formation of the sperm [18] It stimulates the transformation of the freshness of the round sperm to a long-range during the cycle of sperm formation, as well as the deficiency of androgens disrupts the process of freeing sperm [19] by changing the sperm contact points with sertoli cells, leading to the premature separation of the round sperm from the cells of sertoli [20].

2- Changes in hormones:

The current study showed a decrease in testosterone and hormonal FSH,LH this is consistent with previous studies [21,10]. It may be attributed to the simultaneous use of doses of anabolic androgens which leads to metabolism and change in the endocrine glands [22,23]. This may lead to a tendency to increase daily doses of external anabolic androgens which has caused a decrease in the secretion of internal androgens [24], as expected from the continued use of anabolic steroids. Testosterone reduction may be attributed to testicular size atrophy and lack of gonad hormones [10,11]. Low testosterone and FSH and LH hormone levels may be attributed to the fact that anabolic steroids administered from outside the body lead to adverse reactions to the secretion of the gonads, which may be due to the direct effect of hypothalamus to reduce gnRH secretion, which results in a decrease in the secretion of both FSH, LH and therefore the least biosynthesis of the release of testosterone hormones from testes [25]. In addition, androgenic steroids may produce oppressive effects on the testes and on the production of adrenal androgenic hormones [26,27]. It may be attributed to the fact that anabolic steroids increase the secretion of prolactin [28,29]. It may be because testosterone or its receptors may facilitate prolactin secretion and estrogen stimulates the release of prolactin from the anterior pituitary gland [30, 31].

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A Mini Review on Covid-19 Infection and Severe Outcome on Cancer Patient

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Abstract

Introduction December 2019, the world witness a new strand of pathogen known as coronavirus-2 (SARS-CoV-2), later recognized as the novel coronavirus disease (COVID-19). By April 1st 2020, the confirmed cases were over 800,000 around the world with over 40,000 fatalities. Individuals with cancer are more vulnerable to COVID-19 impact because of compromised health-status.

Methods A comprehensive search method was applied by searching the google scholar using the keywords to select eligible studies to be included in the review

Results The findings showed that patients with cancer, especially thoracic malignancies that were infected with COVID-19 infection during the hospital stay developed severe cardiorespiratory symptoms that warranted use of a mechanical ventilator. The cancer patients with COVID-19 had increased rate of hospitalization and a poor outcome as well as a higher risk of mortality when compared to healthy individuals. It is recommended that cancer patients taking anti-tumour drugs should undergo early screening for COVID-19 because the anti-tumour drugs also suppress the immune system. Hence, their dosage should be regulated appropriately in case of COVID-19 co-infection.

Conclusion. In risk-mitigation pandemic scenario in COVID-19 period, efforts should be made not to compromise the prognosis of cancer in these patients by adhering to treatment guideline.

Key words: *cancer patients, COVID-19 novel coronavirus, exercises.*

Introduction

December 2019, the world was exposed to an unknown pathogen known as coronavirus-2 (SARS-CoV-2) which was later recognized as the novel coronavirus disease (COVID-19) (1). By the 20th of March 2020, the number of infection has increased to 638,146 cases and 30,039 established deaths reported in more than 150 countries (2). By the 1st of April 2020, the confirmed cases were more than 800,000 patients around the world with more than 40,000 fatalities (3). Toward mid of 2020, COVID-19 has infected nearly 2.9 million individuals in over 200 countries with more than 200,000 deaths (4).

COVID-19 is linked with manifestations ranging from an asymptomatic state to severe conditions of pneumonia, severe respiratory distress syndrome and fatality (1). Individuals with cancer are more vulnerable to viral infections, especially if with concurrent history of chronic disease. Generally, poor health condition or immunocompromised status can mean poor treatment

outcome alongside with anticancer treatments [5]. Dai, Liu [6] indicated that patients with cancer and infected by COVID-19 would experience a more unsatisfactory outcome than healthy populations. To the best of the researcher's knowledge, there are insufficient data on the systematic assessment of COVID-19 on patients with cancer within a representative population. Another study conducted by Liang, Guan [7] showed in their study that cancer patients with superimposed COVID-19 infection had complex outcomes and complications, some of the patients requiring admission into ICU for mechanical respiratory assistance, this finding was also similar in the study by Zhang, Zhu [8]. On the 10th of April, the city of New-York in the USA had reported a whopping number as high as 180,458 COVID-19 cases with acute respiratory distress syndrome, a severe complication that shorts down the respiratory system and recording about 9,385 deceased cancer patients, amounting to approximately 8.4% mortality figure [9].

During this sudden outbreak, the most vulnerable were the elderly especially those with the immunocompromised conditions such cancer, the rising death number was majorly among these age-groups, more so that, the health officers in Italy and the US were very concerned about the rising fatality in the nursing home because more deaths figure were reported during this short duration [10, 11]. Although the exact cause of deaths could not be unravelled, a research gap was created concerning which particular marker could be used to trace or monitor which stage in the pathogenesis of cancer and its treatment confers the risk of severe COVID-19 to prompt quick medical response [12]. Robilotti, Babady [13] showed in their study that having a profound knowledge or information about COVID-19 and cancer association is critical to be able to provide a standard and quality treatment to symptomatic cancer patient with confirmed COVID-19 infection. In their study, 40% (500 out of 2000 cases) were admitted for COVID-19, while 20 % developed acute respiratory disease with demand for mechanical ventilation while 12 % passed away within 30 days of confirmation of COVID-19. Generally, cancer patients with COVID-19 infection present with a significant rate of hospitalization and an acute outcome. Presently, the increasing incidence of COVID-19 in the United States is still at large, and there are still limited and comprehensive investigations or analysis to show that patients with cancer are at higher risk of severe condition [12, 14].

More so, there are few studies on the lasting adverse effects of COVID-19 in hospitalized patients with cancers especially haematological cancers, and because many these cancer patients would be treated with anticancer medications which suppress bone marrow function thus targeting the immune system and exposing them to a higher risk of public and hospital-contacted nosocomial infections and COVID-19 [15].

The Objective of the Review

There are many studies on COVID-19 pandemics in several countries. However, research on COVID-19 association and outcome on patients with cancer are still limited, and the address question is to understand whether cancer patients infected by COVID-19 have a higher risk and severe outcomes than the healthy population or whether these patients experience a worse prognosis and higher mortality.

Methodology

Selected studies were chosen by typing the keywords in google search wherein searched up till 2020 was obtained. Observational studies with retrospective studies, and comparative studies from various search-results included in the mini review. A total of 3000 studies and 89 full-text articles were assessed. Six studies met the inclusion criteria (COVID-19 impact and outcome on patient with cancer in 2020). The main outcomes of this review were overall survival, cancer-specific outcome and complication following COVID-19 infection on patients with cancer

Table 1. Summary of selected studies on effect of COVID-19 infection and severe outcome on cancer

Author/ Year	Title	Study Population	N	Methods	Results	Outcomes
(16)	Thoracic Cancers International COVID-19 Collaboration (TERAVOLT): Impact of type of cancer therapy and COVID therapy on survival.	Cancer patients with COVID-19	295	Data from Global consortium from 59 different hospitals in 9 countries Total of 295 patients with thoracic-malignancies infected with COVID-19 To determine the effect of COVID-19 on the cancer patients, provide information, direction and treatment outcome and identify the risk factors for the COVID-19 disease and fatality.	The result showed that COPD and HTN are the most recorded comorbidities The average age was 68, 31% female,69% male and total of 79% present/past smokers 73% NSCLC, 14% SCLC, 4% meso and thymic 49% of patients with stage IV disease 73% of patients require admission for cardiorespiratory features despite been on chemo or chemo-IO or RT and COVID-19 infection was treated with antibiotics 67%, antivirals 33%, and steroids 30%.	The study reported that patients with thoracic malignancies infected with COVID – 19 during the admitted time developed severe symptoms, more so are at higher risk of mortality
(4)	Case fatality rate of cancer patients with COVID-19 in a New York Hospital system	Cancer patients with COVID-19	218	Data on patients who tested positive for COVID-19 were selected from an electronic medical record in a single medical centre in New-York from the 18th of March to the 8th of April, 2020 and correlated with an existing cancer database from a period of one month	127 (58%) males and 91 (42%) females. HTN, DM and Chronic Lungs Disease are the most recorded comorbidities 164 (75%) patients have solid tumours, and 54 (25%) has hematologic Malignancies. COVID-19 related death on the cancer patient: Total of 61 (28%) death with a case mortality rate for 37% (20/54): hematologic malignancies 25% (41/164) for solid malignancies The number of fatalities was significantly higher among older age.	The Age-adjusted case mortality rate in cancer patients with COVID-19 contrasted with patients without cancer in the institution indicated a significant increase in the mortality rate among COVID-19 patients with cancer. Rates of ICU admission and ventilator use was slightly higher for the COVID-19 infected cancer patient
(15)	COVID-19 in persons with haematological cancers	Cancer patients with COVID-19	354	A cohort study conducted in China involving 128 patients hospitalized for haematological cancer (HC) and 226 healthcare-provider HCP 13 of 128 (10%) hospitalized-HC patients developed COVID-19 while, 16 of 226 of HCP had developed COVID-19, and 11 of whom were hospitalized Co-variates were contrasted with the 115 HC patients without COVID-19 with 11 hospitalized HCP with COVID-19	Most patients were adults over the age of 60 years (234, 56%) The median age of subjects with and without COVID-19 was 35 years (range, 23–53 years) and 49 years (range, 33–59 years; P = 0.082) Cardio- and cerebrovascular diseases were the most common comorbidities The result showed that there were no significant differences in baseline co-variates among patients who have HC positive or not-positive for COVID-19. The cases rate for COVID-19 was higher in hospitalized patients with HC: 10% (95% [CI], 6, 17%) compared with 7% (4, 12%; P = 0.322 in HCP. Case mortality rates were HC: 62% (32, 85%) and HCP: 0 (0, 32%; P = 0.002).	Admitted patients with HC had more severe presentations and at higher risk and mortality rate. The 13 patients with HC experienced more severe COVID-19 and more fatalities compared with HCP with COVID-19. However, the author was not able to detect the precise risk factors for COVID-19 in admitted patients with HC.
(13)	Determinants of COVID-19 disease severity in patients with cancer	Cancer patients with COVID-19	423	Record from the 10th of March to the 7th of April 2020 of 423 cases of symptomatic COVID-19 from 2,035 patients with confirmed cancer was diagnosed at Memorial Sloan Kettering Cancer Centre	The average age was 60, (234, 56%) Diabetes, hypertension, chronic kidney disease and cardiac disease were the most reported comorbidities 40% were admitted for COVID-19, of these: 20 % developed critical respiratory disease 9% of them needed mechanical ventilation 12 % passed away within 30 days.	The cancer patients with COVID–19 had increased significant rate of hospitalization and a poor outcome

Cont... **Table 1. Summary of selected studies on effect of COVID-19 infection and severe outcome on cancer**

(6)	Patients with cancer appear more vulnerable to SARS –Cov-2: A multicentre study during the COVID – 19 outbreaks.	Cancer patients with COVID-19	641	A multicentre study on cancer patients with COVID-19 carried out in 14 hospitals over 2months in Hubei, China, among 105 cancer patients and 536 age-matched non-cancer patients tested positive with COVID-19. Four main outcomes analysed were death, admission into the ICU, development of severe/critical features, and requirement of mechanical ventilation.	Median ages of patients with cancer were 64.0, IRQ = 14.00) and patients without cancers were 63.5, IQR = 14.00) Comparing the outcomes in COVID-19 patients without cancer, the patients with cancer had 1.higher observed-death rates [OR, 2.34; 95% confidence interval (CI), (1.15–4.77); P = 0.03], 2.higher rates of ICU admission [OR, 2.84; 95% CI (1.59–5.08); P < 0.01] 3.higher rates of having at least one severe or critical symptom [OR, 2.79; 95% CI, (1.74–4.41); P < 0.01], and 4fold higher chances of mechanical ventilation requirement COVID-19 patients who did cancer surgery had a higher risk, severe outcomes and critical symptoms with higher mortality than those who have non-metastatic cancer who had only radiotherapy	The result indicated that cancer patients with COVID-19 had a higher risk than healthy individuals and at high risk of mortality and severe outcomes. More so, the result demonstrated that cancer patients appear more susceptible to COVID -19. Also, patients with COVID -19 who have non-metastatic cancer witnessed the same frequencies of severe health circumstances to those noticed in noncancerous patients.
(8)	Clinical characteristics of COVID-19-infected cancer patients: a retrospective case study in three hospitals within Wuhan, China	Cancer patients with COVID – 19	28	A retrospective cohort study on cancer patients with COVID-19 Data were collected through medical record over two months: Jan.13 to the 23rd of February, 20202 Univariate and multivariate analyses were employed to determine the factors linked with severe outcomes, length of hospital stay, intensive care unit and requirement of mechanical ventilation, and death fatality.	From a total of 28, 17 (60.7%) patients were male. Median (inter-quartile range) age was 65.0 (56.0–70.0) years. Lung cancer was the most common type of cancer reported A total of 15 (53.6%) patients had severe events, and the mortality rate was 28.6%. 53.6% of the patients had severe events 21.4% were admitted to ICU 35.7% had life-threatening complications, and 28.6% of the patients died. The cancer patients had worsening conditions and poor outcomes from the COVID-19 infection with several features of pulmonary complication.	The study outcome shows the vulnerability of cancer patients in the current pandemic. Poor treatment outcome and the possibility of mortality

Discussion

COVID-19 has dealt a massive setback on most of the countries around the world. The consequences of COVID-19 is fatal, and it created severe outcomes among people with an underlying health condition, especially cancers patients. COVID-19 has infected nearly 2.9 million individuals in over 200 countries, with the devastating number of deaths over 200,000, and the US has the highest fatality [(4)]. With the on-going risk of COVID-19 transmission and concurrent adverse outcomes, the long term psychological effect on cancer patients health will be heightened, and treatment safety on patients with these chronic diseases will be compromised. The six studies in the table demonstrated that patients with cancer especially thoracic malignancies that were infected with COVID-19 infection during the hospital stay developed severe cardiorespiratory symptoms that warranted admission into ICU and requirement of a mechanical ventilator and eventually higher mortality risk.

The age-adjusted case mortality rate in the cancer patients with COVID-19 in contrast with patients without cancer showed a significant increase in the rates of admission into intensive care unit ICU with mechanical ventilator use and more mortality among COVID-19 patients with cancer. Patients with haematological cancer HC was shown to have a devastating outcome, admitted patients with HC had increased severe presentations, higher risk and highest mortality. From the review analysis, it was shown that thoracic and haematological malignancies were the two with the most recorded severe COVID-19 infection and more fatalities

among the cancers. According to Mehta, Goel [4], the number of fatalities was significantly higher among the older age, and the cancer patients with COVID–19 had increased significant rate of hospitalization and a poor outcome. Also, patients with COVID-19 who have non-metastatic cancer showed the same frequencies of severe health circumstances to those noticed in noncancerous patients. The study outcome shows the vulnerability and poor outcome of cancer patients in the current pandemic, illustrating that patients with cancer demonstrated more susceptibility to COVID-19, poor treatment outcome and possibility of increase mortality rate. However, a contrary outcome was noted by only one study which indicated that there were no significant differences in baseline co-variates among patients with HC positive and no-HC patients infected with COVID-19.

Cancer is regarded as a chronic illness that gradually causes a significant decrease in the strength of the immune system, thereby reducing the individual capability to ward off infections [17]. A higher rate of severe outcomes and death were reported among the cancer patients with hematologic cancer (which included patients with lymphoma, myeloma, and leukaemia) thus recording the highest figure, followed by patients with lung cancer. [6]. These poor and worsen outcomes were associated with down-grading and weakening of the immune system because of the malicious or dysfunctional state of the lymphocytes, white blood cells or plasma cells. Generally, hematologic malignancy reduces the capability of the immune system to fence-off virus and other infections, which perhaps, is the main synergistic cause of rapid and deteriorating effect of COVID–19-associated high mortality rate and severe outcomes in these patients [6, 18]. Amongst solid tumours, patients with lung cancer have the utmost risk of contracting COVID–19 infections, because of the reduction in lungs function in lung cancer patients with superimposed acute COVID-19 infection, thus, contributing to the cardiorespiratory failure in this population [19].

It was indicated that the difference between the timeline in the hospitalization period and the severe events among cancer patient infected by COVID–19 and non-cancer patients infected by COVID–19 differs [6]. Cancer patients with COVID–19 had a mean length of hospitalization of 27 days (SD 9.52) while non-cancer patients infected by COVID-19 had a mean length of hospitalization of 17.75 days (SD 8.64); which varies significantly (Wilcoxon test, $P < 0.01$). The severe medical events (composite endpoint) clearly defined as a circumstance demanding admission into an intensive care unit (ICU), the procedure of using mechanical ventilation, or mortality was very feasible and significant among the cancer patients infected with COVID-19 when compared to non-cancer patients with COVID-19 infection. In fact, the need for ICU intervention was seen to be 3-fold high among cancer patient infected with COVID-19 than when compare to the non-cancer patient with COVID-19 infection[20].

Recommendations

This review provides vital information on the vulnerability and outcome of COVID-19 on patients with cancer. Cancer patients infected by COVID –19 are linked with a significantly poor severe outcome and mortality rate, suggesting that the need for a dynamic and stringent approach is needed to decrease the spread of COVID-19 and to develop early accurate screening and pathogen identification in this susceptible population [4]. It is recommended that healthcare provider in-charge should increase surveillance and increase medical equipment availability. Special attention on cancers patients, especially hematologic cancer on admission for COVID-19 infection should be monitored adequately and precisely if on bone marrow suppressing drugs as well as those with progressive cancer. Persistent preparedness is another vital approach needed for adequate cancer treatment, especially during the on-going COVID-19 infection along with daily testing and screening of individuals for COVID-19. Cancer patients on anti-tumour drugs should have dynamic screening for COVID-19 because the chemotherapy drug are also associated with stiff adverse effects. Lastly, patient's drugs dosages should be routinely reviewed in patients with immunocompromised status like cancer and other chronic medical comorbid diseases.

The Benefit of Exercise For Cancer Patients.

The treatment of cancer patients is a critical health matter that creates physical, emotional, mental health and discomfort. Cancer leads to a massive effect on patients with a high burden of disease. Cormie, Trevaskis [21] stated that exercise is harmless and effective adjunct therapy in cancer patients' care. Exercise is significantly

essential in all the health centre around the world because integrating exercise as part therapy represents a standard practice of keeping patient mentally and physically fit [22, 23].

Several studies have shown that introducing exercise into the management of cancer patient is significantly effective in improving patients' quality of life. If cancer patients perform moderate tolerable exercise frequently, they will have significantly immune boost, improve quality of life symptoms, possibly lengthening their survival, happiness, mental condition and physical status [24, 25]. Experts explained that cancer patients who perform exercise regularly would reduce their manifestations by stimulating changes in the immune system, metabolism, systemic inflammation, angiogenesis and redox status. Exercise outcome on cancer patient had a positive effect when patients performed it on a routine of six metabolic equivalent task 6-METs or more [26]. The American college of sports medicine (ACSM) admonishes the healthy person to perform twenty-sixty minutes of aerobic exercise at least 3-5 times/week and twenty repeated circle of resistance exercise with an intensity corresponding to 12-to-16 rate of exertion (20 is the maximum exertion rate) 2-to-3 times per week. Cancer patients can engage in 150 minutes of moderate-intensity aerobic exercise or aerobic combined to resistance exercise. The exercise-intensity is calculated using metabolic equivalent task (MET), 1-MET is equivalent to the expense-energy at rest. Moderate-intensity exercise is equivalent to 3-to-5.9 MET. High-intensity or vigorous exercise has a MET count of 6 and is referred to as an activity that produces perspiration and change in cardiac and breathing rates [26]. An adjunct therapy such a high-quality exercise would significantly reduce the neuropathic pain level and improve functional quality of life QOL [27]. High quality and regular aerobic exercise are highly advised for cancer patients or should be referred to an exercise physiotherapist: who has experience in cancer patients care [28, 29].

Conclusion

In a risk-mitigation pandemic scenario, efforts should be made not to compromise the prognosis of cancer in these patients by departing from guideline-recommended radiotherapy practice. If patients need for triage arise, important factors for triage such as potential for cure, relative benefit of radiation, life expectancy, and performance status should be employ in decision-making. The exposure and poor outcome of COVID-19 infection is increased in cancer patients as well as high mortality risk. Adequate and standard treatment procedures should be strictly followed. More so, exercise is a harmless and effective adjunct therapy in cancer patients' care. Exercise will be significantly essential in all the centre around the world to integrate exercise as a portion of standard practice in cancer care because high-quality, regular and tolerable aerobic exercise would sufficiently boost the immune system and improve functional QOL.

Conflict of Interest Declaration: The research team declares that there is no conflict of interest in the course of this study

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Exosomes and their Role in Immunity, Metabolic, Cardiovascular, Neurodegeneration, Reproduction and Development

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Abstract

Exosomes are produced by an endocytic cellular route which consists of three steps:first: Cellular membranes invagination forms the endocytic vesicles, then,The inward budding of the endosoma membrane starts at the second level, and multivesicular bodies emerge.. In third and last step the multivascular bodies combine with the cell membrane and Produces the substances of the vesicular**,** exosomes are used to transfer Set cell prostaglandins to specific cells exosomes Represent a new mode of contact between cells, which in many biological processes may play a significant role, for example, immune response,Transduction of signals,antigen presentation- Intercellular communication mediated by exosome Cells usually communicate with surrounding cells by direct cell-cell touch, involving interaction of the gap between the cell's surface proteins and protein c interactions Communicate with regional cells via induction soluble Factors like: hormones as well as cytokines Improve the transmission of the signals, Electrical and chemical signals**.** And also Exosomes may play a role in the process of metabolic conditions and cardiovascular disorders they were found to hold metabolites and allowing intra - cellular communication by exosomal miRNA exchange between skeletal muscles , adipose tissue, pancreatic b-cells, and the mouse liver and the human .

Key words: *Exosomes, APCs, miRNA, EVs, neurodegeneration*

Introduction

Exosomes are membrane vesicles of 30-150 nm size which are produced endogenously by nearly every type of cells. Rose Johnstone had first used the word (exosomes) , who discovered exosomes In Reticulocytes of sheep in1970 (1) These exosomes were known as Cell Homeostasis by-products until 1990 ,When it was discovered the revolution in exosomes Find that the β-cells produce functioning exosomes that transform antigen (2)

- Exosomes formation and its role in immunity

Exosomes are produced by an endocytic cellular route which consists of three steps:first: Cellular membranes invagination forms the endocytic vesicles, then,The inward budding of the endosoma membrane starts at the second level, and multivesicular bodies emerge.. In third and last step the multivascular bodies combine with the cell membrane and Produces the substances of the vesicular (exosome) (3)

Exosomes are special because of their protein and Lipid content, that presents an important hint for Recognizing them. Exosomes usually involve fusion Proteins and transport proteins As in phospholipases and other lipids releated proteins .(4)

The exosomes are also riched in Lipids such as cholesterol, sphingolipids, phosphoglycerides, ceramids and also are enriched With long saturated chains of fatty acid (5)

Study Researchs indicates that exosomes are used to transfer Set cell prostaglandins to specific cells (6) exosomes also have miRNA in large amounts (7) exosomes Represent a new mode of contact between cells, which in many biological processes may play a significant role, for example, immune response (8) Transduction of signals (9)antigen presentation (10) - Intercellular communication mediated by exosome Cells

usually communicate with surrounding cells by direct cell-cell touch, involving interaction of the gap between the cell's surface proteins and protein c interactions Communicate with regional cells via induction soluble Factors like: hormones as well as cytokines Improve the transmission of the signals[11] Electrical and chemical signals (e.g. nuclear elements, fatty acids and other signals) Also interested in communication [12] Exosomes are now accepted with a cell of protein , lipids and nucleic cell-specific cargo Acids can function as a new communication between cells Method. The theory of is based on evidence exosomes from parental cells may communicate directly with target cells , which leads to a future infusion of cell behavior and phenotype characteristics [13] the Exosomal biological applications are very dependent on effective supply of genetic materials, that can be accomplished through interactions between receptors, direct fusion of membranes or integration via endocytosis[14] When the exosomes are internalized fuse with the endosome compact membrane resulting in the transmission of its genetic material horizontally into Accurate cell cytoplasm.

The biologically active Molemolecules that impact were seen in exosomes the following pathways are used to target cells:1- direct attach occurs when surface ligands Stimulate Target cells 2- Move to recipient cells with active receptors;3- Recipient cell epigenetic reprogramming with function proteins , lipids and RNAs.[15] (Fig:1) exosomes play an important role in immunoregulation in the immune system, such as antigen presentation, immune activation, immune suppression and Immune tolerance by intercellular contact exosome-mediated. CD4 + T derivative exosomes Dendritic cells (DCs) can connect cells and cell CD8 + T cells via peptide / major complex histocompatibility Interactions between MHC / TCR and ICAM-1 / LFA-1 Conduct DC apoptosis and thus mediate antigen-specific DCmediated T cell silence[16] The outcomes that Exosomes produced by B-cell lines are co-stimulating MHC class II and Adhesion molecules indicated the stimulation of exosomes which stimulate CD4 T-cells Clones directly[17]

Exosomes produced by antigen presenting cells (APCs) Surface MHC Class-I and MHC Class-II molecules and therefore may be potentially stimulating. CD8 and CD4 T cells, respectively. APC-derived at high levels ,peptide-MHC-bearing exosomes (p-MHC) complexes serve as Ag-presenting vesicles for cells, lines and hybrids, and for ready T cells[18,19,20] The stimulating activity of T-cells by free exosomes appears to be less effective than for the parent APCs.[21,22] It could be. Explain the low ability to activate native T cells in vitro by free APC-derived Exosomes[23]The low stimulating potential of free exosomes on T-cells in Vitro also was likely due to its tiny size and Brownian vesicle diffusion Motion, In fact, when Exosomes derived from APC are coated at high levels of latex or when direct loading increases the amount of p-MHC complexes per exosome it is significantly increase their T-cell inducing capacity in the exosome with peptide in culture [24,25,2

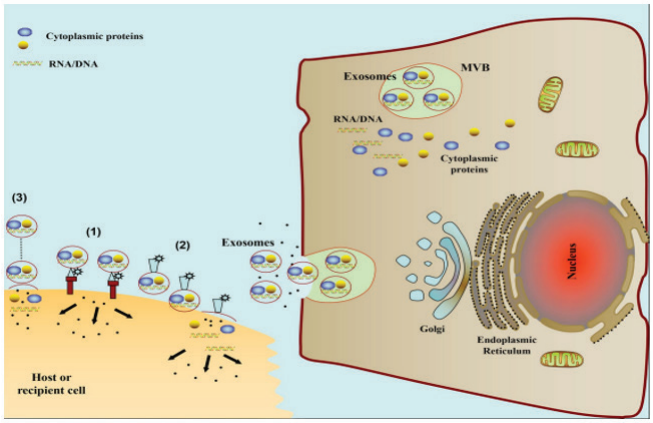


Fig:1: model signaling pathways in exosome mediated cell- (1) Cells receiving signals through direct exosomes to-cell communication Ligands that are surface-bound. (2) Transfer of active receptor exosomes to cells of the recipient. (3) Exosomes can reprogram recipient cells epigenetically by Protein, lipids and RNAs provide functional (13)

Even when it is no serious immune reaction should be observed in Mice had been multiple times administered with with a comparatively tiny(mouse dose or exosomes derived from human cells) for prolonged Time periods[27,28]Transfusions of Blood and /or plasma for decades do not seem to be related with exosomes-mediated immune response, even,no (human leukocyte antigen(HLA) , extracellular uptaking and intracellular exosomes producing In this way ,can induce immune response and this considerd as dose-dependent manner. Latest studies with modified exosomes have however shown a role of exosomes triggered both adaptive and innate Immunity, support their effectiveness for the advancement of treatment and its possible contribution in Organising the immune reactions to Infectious or cancerous agents(fig:2) The Purpose of exosomes in regulation of immunity seem to be probable if antigenic is transferred and presented of Peptides antigens, DNA-inducing cGASSTING distributions (Cyclic GMP-AMP synthase) Receiver signaling by Cells (an immunity process by which it senses Cytosolic DNA induces inflammatory response Genes and IFN reaction type I MiRNA, and specific signaling induction Surface ligand pathways exist on the exosomes.

Exosomes derivative from Antigen-presenting cells (APCs) Bring p-MHC-II [majo histocompatibility complex together with Antigenic peptide II (p)] and costimulative Signals, and display the antigenic peptide directly causes activation of different T cells. moreover, for purposes still to be Illustrated, exosomal T-cell stimulation is Less effective than that resulted by APCs[29,30] Tumor eradication and growth deficiency were measured in mice by Single intradermal infusion reported

of APC-derived exosomes loaded with MHC-II a peptide tumour[31] The strength and the effectiveness Of the CD8 + Cytotoxic T cell -antitumor immunity It also indicated controlled antitumor response

Indirect presentation of antigen due to various transfer of antigenic peptide on exosomes to APCs which really, effect, naïve T and/or Activation of B cells immature dendritic mouse cells triggered by immunogenic derivative of exosome Peptides activate APCs indirectly, and stimulate particular multiplication of the CD4 + T cells , exosomes produced by dendritic cells of humans enhance a T Helper response ,(production of IFNg) in culture[32] exosomes function in antigen presentation is also important in bacterial infections , such as (Mycobacterium tuberculosis and Helicobacter pylori),

In which exosomes may improve antibacterial properties of immune system response ,by inducing antigene presenting of bacteria from exosomed (derived from macrophages) This could have an influence later on Adaptive Immune Response[33] the Production of IFNa and IFNg, tumor necrosis factor

a (TNFa), and exosomal interleukin produced by macrophages which leads to to maturation of dendritic cell and activation of CD4 + and CD8 + T Cells [34]

The exosomal nucleic acid cargo, which is DNA and miRNA, was involved in the regulation of immune response both of innate and adaptive immunity. the Intracellular DNA of bacteria such as: (*listeria, Franciscella tularensis*) are located into exosomes that having the ability to enhance cGAS-STING signalling pathways in neighboring cells inducing innate immune efficiently

Responses. As for *Listeria,* however, which takeplace at the expense repressing of T cells and therefore reduces antibacterial defensive capabilities[35] Where as the significant role of exosomes in Fungal immune response cAnd parasitic infections is obure; afew other researches concerned exosomes derived from parasites have noted such an exosomes could be involved In pathogenesis of disease[36,37] human monocytes which take up exosomes Including parasitic DNA may cause STING-dependent DNA sensing which is considerd a decoy process to improve parasite survival[38]

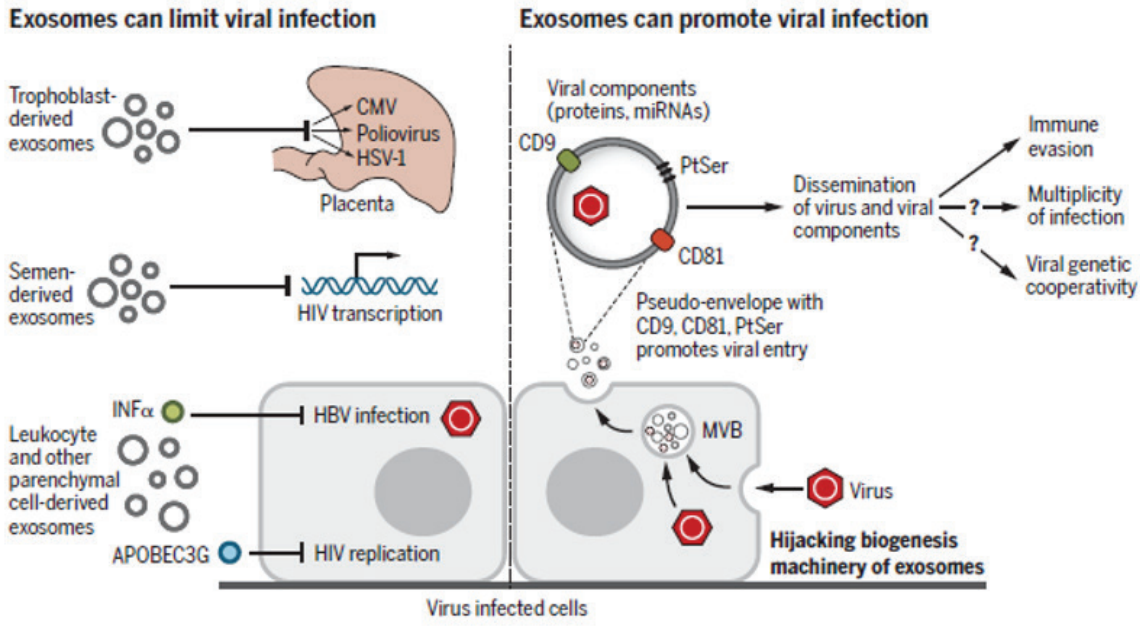


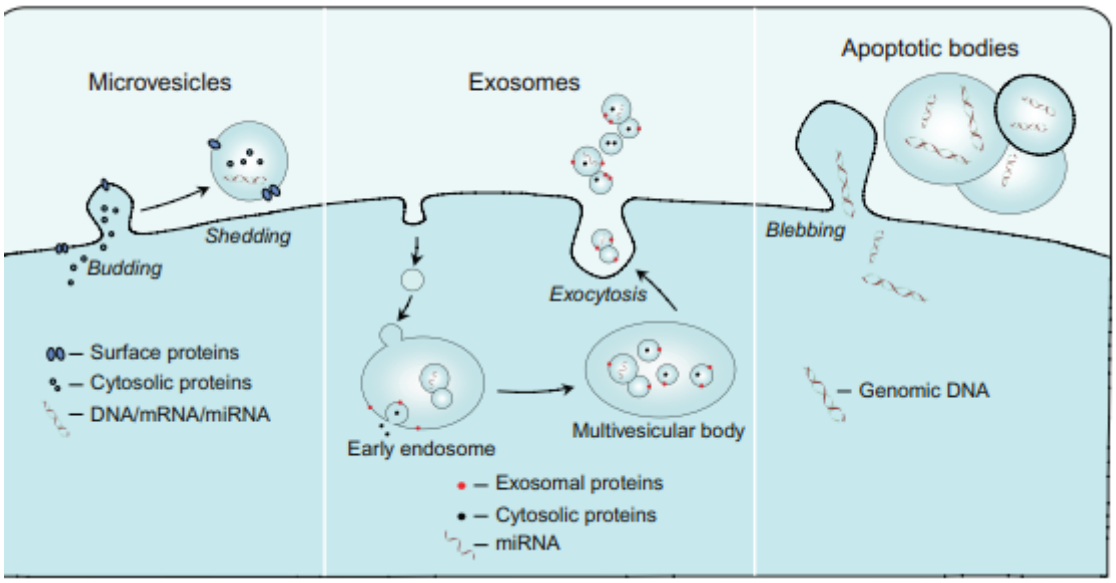
Fig. 2: Role of exosomes in viral infection Virus-infected exosomes could really reduce or enable viral infection

Exosomal cargo, including such cIFNa or APOBEC3 Gan supress infection by reducing or restricting the viral multiplication or improve antiviral immune response. Exosomes can assist as a pseudoenvelope improving Viral entry tetraspanins (CD81, CD9) and PtSer interaction and uptake to the host cell and to enable them evade immune antiviral. Co-transport of aviral Elemes (proteins and miRNA) can also improve pathogenicity exosome-mediated viral transmission could take an active part in genetic cooperative viruses and Infection multiplicity. CMV, and cytomegalovirus (39)

-exosomes role in metabolic / cardiovasicular diseases:

Patients with metabolic diseases have been well known notably resistance to insulin and diabetic mellitus type 2 (T2DM), or over nearly twice probability of developing increased CVD, which include atherosclerosis, stroke and coronary artery disease(40) coronary artery disease is largest cause of death around that leads to T2DM death and excess threat of women's mortality comparison to male(41) Relatively small extra cellular vescles(EVs) about (50 nm to 2 mm) are produced by the membrane of many cell types into different body fluids , including plasma, milk, sweat, saliva, blood, urine, tears, etc. There are many EV categories, exosomes, microvesicles (MVs),and apoptotic bodies formed by various

Processes.-Mechanisms. The most attention is given recenltly to exosomes which are extracted from cells when the plasma membrane fuses within multivesicular bodies (MVB; also known as multivosicular endosomes, MVE). in a carefully controlled mechanism and their contents released(42). One of the features of all EVs is the Phosphatidyl serine (PS) presence on the ground surface; loss of asymmetry of membrane when blebbing (apoptotic bodies) or budding (MV) and internal folding membrane during the formation of vesicles in MVBs exosomes .(fig:3)This could be determined by the label attachment ANNEXIN V, an frequently used flowcytometric reagent of Apoptotic cell examination. More significantly, a variety of groups find MVs without PS on the external membrane that does not seem required for MV preparation(43,44) which are 0.2-2.0 mm microvesicles coming from the cell membrane by budding and thus can contain common cell origin surface markers (45)



(Fig:3): Schematic representation of (microvesicles,exosos,and apoptotic bodies) formation processes

Asignificant percentage of the heart volume is formed by thecardiac muscle, while nonmyocyte is the most prevalent Cardiac fibroblast (CF) containing approximately 90 % of nonmuscular cells Endothelial cells (ECs) with important functions in cardiac homeostasis are in contact with those cells. In addition, several research indicate the presence of cardiac derived- progenitor cells (CPCs) which are involved in the response of post-injury , the existenc of such cells in the heart indicates the significance and the need to find the ways of heterocellular communication such communication occurs in which . EXOs are the primary mediation between the different populations of the cells. (46)

Exosomes may play a role in the process of metabolic conditions and cardiovascular disorders they were found to hold metabolites and allowing intra - cellular communication by exosomal miRNA exchange between skeletal muscles , adipose tissue, pancreatic b-cells, and the mouse liver and the human(47)

Reciprocal adipocyte signaling and macrophages via exosomes in the spontaneous leptin gene-knockout pathway of the RBP4 (retinol binding protein) suggests obesity (protein 4) in macrophage activation and insulin resistance(48) Obesemice had a fat diet show separate exosomal circulating

miRNAs that are sufficient to stimulate insulin lean mice resistance, probably via inhibition of proliferator-activated peroxisome receptor alpha presence in fatty tissues (49) fCachexia, a dangerous disease of lossing weight and muscle mass associated with chronic conditions such as cancer or paraneoplastic metabolisms disorders (such as: new-acquired diabetes in pancreatic cancer) Cancer cell-derived exosomal can be aggravated mouse and human adipocyte and muscle cell (50)

Adrenomenomedulline,a peptide hormone was detected that stimulates lipolysis. in human pancreas produced exosomes mouse and induced cancer cells and adipocytes in humans (51) Such results support for exosomes from cancer cells change non-cancer cell metabolism, including pancreatic islet cells, adipocytes, and thus supporting the production functionally in the paraneoplastic and cachexia syndrome. mouse and human cell culture xosomes Supernatant (, cardiac Fibroblast, cardiomyocytes and cardiac orginator cells seemed to be correlated with metabolic disorders such as atherosclerosis, diabetes-related disorder metabolic and cardiovascular disease (CVD) heart failure(52)

- **Exosomes role in neurodegeneration**

The development of exosomal biogenesis and the control of neuronal secretory vesicles new insights into

the putative were provided by cells communication and pathogenesis between exosomes of diseases that cause neurodegeneration. Exosomes may enable or reduce unfolded accumulation and abnormally folded brain proteins[53,54]

Exosomes may be involved in removing misfolded proteins ,Hence, practicing neuroprotective and detoxifying features,or assist in the proliferation and accumulation in misfolded proteins, supports effectively protein aggregates are "infective" and leading to the spread of diseases. GW4869 Pharmaceutical blockage(Inhibiting the flow of MVBs inward) or improvement of development of exosomes with monensin (with such an intracellular Ca2 + increase). adecrease in or generation of MVB increase of the transmission, respectively

PrPsc's contagious prion protein Creutzfeldt – Jakob disease in combination in vitro , Tau and Ab (generated with b-amyloid) by the amyloid precursor cleavage the Alzheimer's disease, protein [APP], exosomes, including patients, are considered exosomes derived from brain spinal fluid (Tau); supernatantderived mouse cell culture exosomes and supernatant exosomes of the mouse and human cell culture lines (Ab). Tau 's pathological spread in vitro ,exosome accumulation has been reported in vivo and in vtrio [55,56] The exosome biogenesis can be neuroprotective exosomes can trigger neurotoxic impairments the formation of oligomers, or even may move them out of cells, inhibition of exosome secretion in vivo but the suppression of exosome secretion in vivo utilize GW4869 in Transgenic TDP-43A325 T mice were detrimental, so it seemed to reduce pathogenic TDP-43 clearance of neurons the exosomal role in the pathophysiology of neurodegenerative disease and ASD needs more researchs , Such stimulation is empowered in large part by the intrinsic characteristics of exosomes to pass efficiently through blood – brain barrier, a working vascular network as a way to keep toxins , or drug far from brain [57]

-Exosomes role in reproduction and development

since small EVs are identified in the intercellular information exchange, numerous studies have indicated the impact of these vesicles in process of replication , tiny EVs shown in follicular fluid [58] and oviductal fluid [59] secreted by embryos in culture media [60] Furthermore, the information of molecular pathways of EVs promotes new technology progress of bio-roles, diagnostic and treatment potential(61) such significant structure is an ovarian follicle within the ovary, the microenvironment formed by theca cells , granulosa, cumulus and the

Oocyte , in the antral follicles which are formed by folliculogenesis having the follicular fluid , the microenvironment of follicle is managed via endocrine , paracrine and autocrine through its development , hence the contact between the cells is essential for the growth of oocytes and follicles.

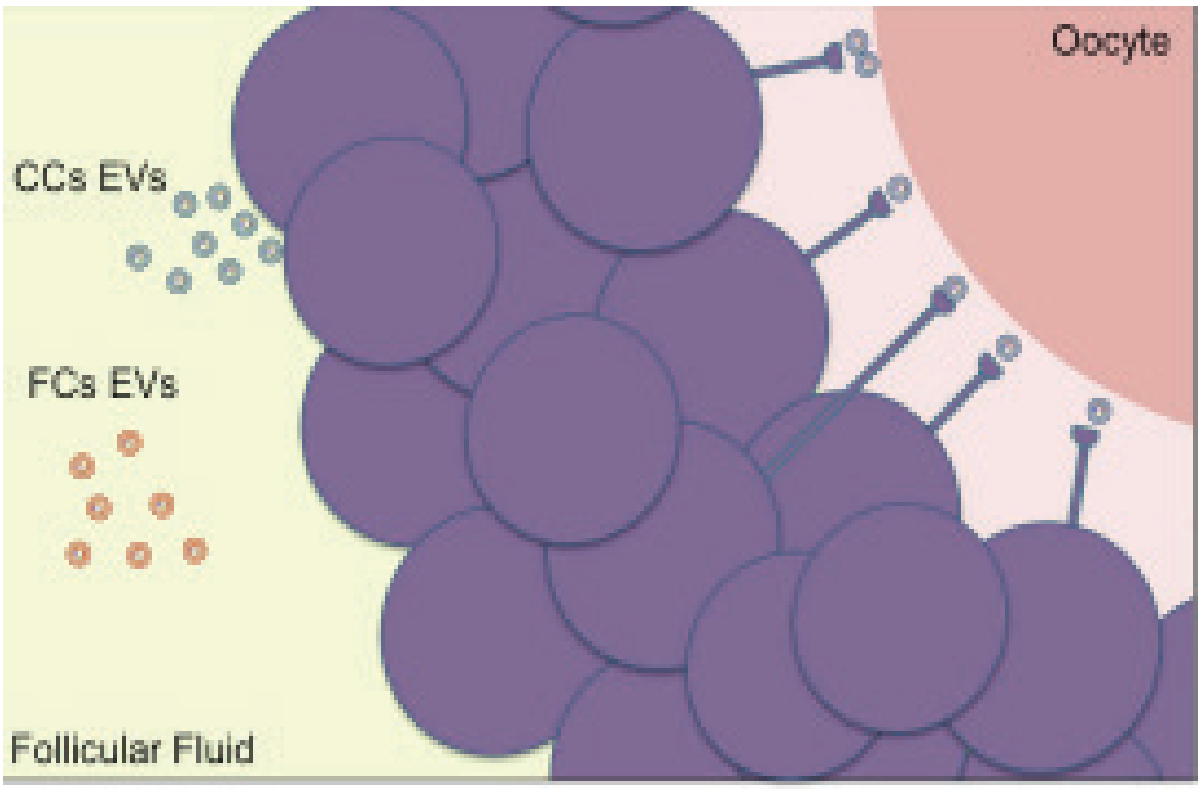
Folicular cells could also secrete EV in follicular fluid,that assist in transmitting of information between cells(fig: 4) [62]

EVs of follicular fluid has important biological content due to the development of follicle and oocytes for example, effects from these EVs can be detected in cumulus and cells of granulosa hormones and development able to effect theollicle environment that lead to alter biogenesis of EVs and contents, therefore, ovarian follicle physiology an essential to increase the application of EVs in the reproductive driven strategies.[63]

Researchers find alternative enhancement strategies for fertility levels and increase the probability of pregnancy in women by reproductive assistance and advanced materials for medication. ,inescapable correlation is between fertility and reproductive functions effective folliculogenesis, Oogensis, implantation, embryo development and pregnancy cell-to - cell contact is affected by exosomes, necessary cross-talk between mother and fetus and gene and protein expression rates during pregnancy from Reproductive system[64,65,66]

It has been shown that exosomes have an important role to play in transporting; the ability to modulate molecular cargos , normal follicular transcription and translations development, proliferation and distinguishing of granulous cells, gametogenesis, oocyte maturation , fertilization rate, embryo development, development of blastocysts, and appears result pregnancy [67,68] Identifying the exosomes in the reproductive system demonstrates their potential roles in pre- and post-conception intercellular communication during maternal aging advanced.

However, the efficiency and abundance of human exosomes is recently used as an indicator for pregnancy and pregnancy related diseases[69,70]



(Fig;4): Extracellular vesicles can hold and move bioactive molecules such as proteins in the follicular fluid RNAs, miRNAs, lipids and metabolites which contribute to oocyte maturation[71]

Ethical Clearance: Nil

Source of Funding- Self

Conflict of Interest :Nil

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Serum Heavy Metals Level and OS among e-ic Waste Exposed

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Abstract

The current study is performed in Thi-Qar Province which included a collection of whole blood samples from e-ic repair workers. The survey questionnaire was used which is a standardized tool for this research and provides for grouping and processing of data and used standardized research instruments such as questionnaires and interviews of a larger group of subjects to get a clearer and richer examination of the data necessary for further analysis. A total of 100 whole blood samples were taken with different ages ranged from 16 years up to 56 years. Other study groups were 30 subjects from non-e-waste workers. Daily work time approximately about 6-8 hours for 1 year up to 30 years. The results showed that the percentage of diabetic patients among the e-waste population is only (6.5%) all of the diabetes cases were arise for workers whose exposure exceeded 10 years. The heavy metals concentrations in the serum were measured by using atomic absorption spectrophotometry and the levels of (Pb) were unexpected, the mean serum Pb levels (24±54 µg/L) of non-e-waste workers was near to that of e-waste workers (24.6±34 µg/L) at ($p \leq 0.05$), Cd results reported high concentrations in the serum workers of e-waste (35.4±53 µg/l) compared with workers as non-e-waste (9.3±17.2µg/l) at ($p \leq 0.05$). The results of selenium showed an increase in serum Se levels in both groups, (209.4±195.5 mg/L) for the exposed workers compares with (178.2±29.5 mg/L) for unexposed workers. The other important aspect on which the study focused is the SOD1/catalase antioxidant system. As the study showed a decrease in SOD1 (15.2±2.8ng/ml) in workers of e-waste proportion in comparison to the comparison group (36.8±60ng/ml) at ($p =\leq 0.05$). Also, the catalase concentration was lower in e-waste workers (23.9±8ng/ml) more than (28.8±6ng/ml) at ($p \leq 0.05$) in non-e-waste workers.

Keywords: *Metals, OS, e-ic, Superoxide dismutase, Hypertension, Diabetic*

Introduction

Products of e-ic and electrical have become an integral current economy part and, with newer technologies development, the life spans of such products are becoming shorter. Consequently, the e-ic waste volume is drastically increasing around the world [(1)] .The hazards create from the e-waste from heavy metals presence (e.g. Cd, Pb, and Se, etc.). [(2)]

***Pb*:** Pb is commonly used in electrical products as the main soldier part (as a tin alloy) and as PbO_2 in cathode glass ray tube (monitors and televisions) along batteries of Pb-acid. Also, there compounds were utilized in cables of PVC and other materials as stabilizers.[(1)] Pb is utilized widely in goods as e-ic being a main solders component (as tin alloy). Pb considered as extremely toxic for humans, plants and animals. It able to body-build up via recurrent acquaintance with irreversible properties on system of nervous, predominantly in children where nervous system is developing.[(3)] Also, Pb has been utilized in solders in circuit printed boards and different components.[(4)(5)(1)]

Cd is utilized in batteries of (Ni-Cd), surface mount resistors as devices chip, detectors as infrared, and chips of semi-conductor.[(6)] Cd take place in specific components i.e., resistors of SMD chip, detectors of infra-red, and chips as semi-conductor. It is stabilizers for plastic where few older cathodes as ray tubes contain Cd. Compounds of Cd are bio-accumulative and toxic with irreversible health influences possible risk. Likwise, Pb and Cd might accumulate over time in the body, with exposure for a long-term leading to bone structure and kidneys damage. Compounds of Cd as well as Cd are

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well-known cause of carcinogens for human, principally via contaminated dust and fumes inhalation.[7] Cd prompts OS in animals and cultured cells and minimizes antioxidant humans levels.[8]

Superoxide dismutase/ catalase: Exposure to toxic chemicals increases free radical formation. [9] Free radicals (FR) are molecular fragments or molecules having a single unpaired $^{e-}$. Generally, FR are chemically reactive, some (e.g. HO•)[10]. Mostly important FR in several disease conditions are derivatives of O_2, principally radicals of hydroxyl and superoxide. Due to its ability to lose e-, primarily a metal is thought to be toxic via ROS generation. Therefore, high concentrations exposure to a single metal being heavy may cause buildup and possibly, oxidative damage.[11] (Limón-Pacheco & Gonsebatt, 2009). Although ROS, might of negative side influences at high intensities, also they are vital biological molecules being signaling which employ protective and therapeutic influences in contradiction of diseases.[12][13][14] Due to high ROS levels are implicated strongly in disease pathogenesis and progression, antioxidants (is in vivo molecule can prevente or slow FR oxidation) that exist in concentrations as low in comparison to the oxidizable substrate which able to scavenging such ROS following exerting useful signaling influences. For example, the mitochondrial-produced $O2^{-}$ is dismutation to H_2O_2 by superoxide dismutase, which is subsequently reduced to water via the glutathione peroxidase/reductases/NADPH system.[15]

An inequity between antioxidants and oxidants in oxidants favor, possibly causing damage, is termes of 'OS'. Oxidants are made as a normal aerobic metabolism product which might be yield at rates being elevated under conditions as pathophysiological.[16] Oxidation is reaction chemically which transforms e- from substance being specific into an agent of oxidizing which cause cells damage.[17] Antioxidants terminate such reactions chain via removing completely the principal stray media and other oxidation reactions prevention from themselves oxidizing. As a result, antioxidants usually remove O_2.

Antioxidants of enzymatic activity (3 proteins) are considered as 1st defense line versus OS body: (SOD), (CAT), and glutathione peroxidase.[18] (Ighodaro & Akinloye, 2018). In this study we focused on SOD1/ CAT;

1. SOD catalyze the dismutation of O_2^{-} to H_2O_2:

$$O^2- + O^2 - + 2H+ \rightarrow H_2\ O_2 + O^2$$

CuZn-SOD is detectd in all the virtually mammalian organelles cytoplasm. [19] with 2 subunits of protein, every one having a catalytically active Cu and Zn atoms. SOD1is highly present in the cytosol but is also partially localized In the mitochondrial matrix. ([14]

2. CAT was enzyme as 1st antioxidant that characterized and catalyzes the 2-stage conversion of $H_2\ O_2$ to H_2O and O_2:

CAT –Fe (III) + $H_2\ O_2$ → compound I

Compound I + $H_2\ O_2$ → CAT –Fe (III) + $2H_2O$ + O^2

CAT comprises 4 subunits of protein, everyone having group as haem and NADPH molecule. CAT is located principally within peroxisomes of cells that also have most of generating capable enzymes of $H_2\ O_2$. [20]

Methods

The study population was 100 E-ic repair workers who were exposed to e-ic waste asked them to take part in our study. Another group of evidently non-exposed e-waste is represented as the control group (50 individuals). After signature, the consent form, workers, and control groups followed the questionnaire include several clear questions; age, weight, length, working time, duration of exposure, diabetic or non-diabetic patients, and date of injury. Also, they were asked smoker or non-smoker, having daily drugs, endocrine diseased, if they have hypertension or other diseases. All metals being heavy were measured by Spectrophotometer of Atomic Absorption (AAS). Superoxide dismutase and catalase were measured by ELISA kits from MyBioSource / American.

The statistical case-control analysis of the current study was done with (SPSS) 24.0 and Microsoft Excel 2010. Data as numerical with normal distribution were designated as standard deviation and means, correlation used for finding the relationship between two groups.

Data as categorical were designated as % and count. Chi-square is utilized to compare the relationship between variables. To compare study groups used t-test.

Results

Table1: Demographic characteristics for workers as e- and non-e waste workers.

Characteristics	Workers of e-waste	Non-e-waste worker	p-value
Age mean±SD	33.83±9.5	34±9	0.9
BMI Means±SD	26±4.5	27.3±4.5	0.5

Table 2: Clinical Characteristics for workers as e- and non-e waste workers.

Characteristics	Workers of e-waste	Non-e-waste worker	P-value
Diabetic	6.5%	0%	0.127[c]
Diabetes family history	28.3%	35.3%	0.445[c]
Smoking	4.3%	0%	0.217[c]
Drugs	12.0%	17.6%	0.407[c]
Endocrine disease	2.2%	5.9%	0.292[c]
Hypertension	3.3%	11.8%	0.064[c]
Another disease	8.7%	0%	0.076[c]

c .chi-square, P < 0.05

Table 3: Human CAT quantitative comparison between exposed and non-exposed workers.

Groups Test	Workers of e-waste	Workers of non e-waste	P- value
Catalase ng/ml	23.9±7.9))	28.8±6))	0.6
SOD1 ng/ml	15.2(±2.8)	36.8 (±60)	0.05

*P < 0.05

Table 4 :Cd, Se, and Pb serum concentration for workers as e- and non-e waste workers.

study groups metal	Workers of e-waste Mean ($\pm SD$)	Workers of non e-waste Mean ($\pm SD$)	p-value
Cd(mg/l) Minimum maximum	35.4(±53) 0.508 175.7	9.3±17.2 0.51 72.3	0.000*
Se Minimum maximum	209.4±195.5 1.07 893.5	178.2±29.5 98.6 259.1	0.2
Pb Minimum maximum	24.6(±34) 1.1 145.8	24.1±53.9 1.8 231.4	0.95

*P < 0.05

Discussion

This study explores the effect of e-waste recycling on the health of e-waste repair workers and it's linkage to diabetes and other diseases. Diabetes has been recorded in e-waste workers (6.5%) less than (49%) which is reported by. [21] All of them have a diabetes family history. Also, the rate of thyroid disease was (2.2%) dispute with [21][22]. Hypertension rate was (3.3%), and other diseases were (8.7%).

The current study showed an increase in the levels of heavy in the e-waste population. Unexpectedly, the mean serum Pb non-e-waste workers levels were near to that of workers of e-waste. workers of e-waste will be unprotected to high levels of Pb by route of general followed by components being harmful which emerge from e-waste is either via ingestion, inhalation, or dermal contact .[23] This convergence of Pb concentration in both study groups indicates the presence of other sources of Pb exposure. I have suggested the first major source is car exhaust fumes (A samples were taken from the city center). Pb-exposed might be happened via in Pb breathing dust or fumes.[24] Pb exposure might be happened via Pb dust ingesting, dust of Pb able to settle on clothes, water, food, and other objects. If Individuals smoke, drink, or eat in places in which Pb is being stored or processed, they might ingest dust of Pb. Not cleaning hands prior to eating or touching mouths are ways also cmight ingest Pb. Few studies proved that Pb via skin can be absorbed.[25] The second possible reason for Pb poisoning was smoking and passive smoking .[26] Pbed gasoline and Pb paint and water was likely the primary source of Pb exposure for individuals living in regions with corrosive water supplies and Pb pipes .[27]

The results of serum Cd level reported high differences of significant in workers of e-waste compared with workers of non-e-waste. The present study results have an agreement with .[28][29] E-waste is an important source of Cd. Cd is used in galvanized metal due to its inability to corrode, and also in the manufacture of pigments and the manufacture of negatively charged materials so it is frequently used in the battery industry. [30] In the same way as Pb, (Cd) transports to humans. [23] So, it was suggested three key routes to intake Cd; (1) inhalation: As soon as in the lungs, inhaled dose 10%-50% is absorbed, based on size of particle, specific Cd solubility of compound inhaled, and exposure duration. [30] Leaves of tobacco accumulate as high Cd levels from soil, and smoke of cigarette is main exposure ources for tobacco smokers leaves accumulates high Cd levels from soil, and smoke of cigarette is the main exposure source

for smokers.[31]) (2) Ingestion: Mostly Cd ingested orally goes via GT unchanged being individuals normally absorb just almost 6% of Cd ingested, wherease up to 9% might be absorbed in those with deficiency of iron.[32] (ATSDR 1999). Cd also, in H_2O is easily absorbed compared to Cd in food (5% in H_2O compared to 2.5% in food).[33]The elevated Zn or Cr presence in diet declines Cd uptake.[34][35] (3) Dermal: skin absorption is not route of significant entry of Cd; just around Cd 0.5% is skin absorbed [35]

On the other hand, for non-exposed workers, the Cd concentration was still above the maximum permissible limit by the WHO. It was suggested the first route "food is generally the largest source of Cd exposure. Cd levels in some foods can be increased by the application of phosphate fertilizers". [36] The second route was passive smoking

The other important aspect in which the study focused on is the SOD1/catalase antioxidant system. As the study showed a decrease in SOD1 in workers of e-waste in comparison to the comparison group. Also, the concentration of catalase was lower in e-waste workers more than in non-e-waste workers. As it's known SOD1/CAT was increasing according to high ROS, but with high levels of ROS, the equation turns in favor of FR. On the other hand, Superoxide dismutases might be damaged only by few xenobiotics, such as cyanides,azide, HCl or diethyl-dithio-carbamate, and H_2O_2. Enzymatic activity increasing might be provoked via molecular activity enhancement vice versa. It is well-known that frequently SOD decrease or increase activity accompanies OS and an reactive O_2 increase formation of species.[37]

Conflict of Interest – Nil

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Association of Single-nucleotide Polymorphisms rs2243250 of IL4 gene in Diabetes Type 1 Patients with Acute Tonsillitis

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Abstract

Diabetes mellitus type 1 one of diseases which is a difficult metabolism condition, it is characterize high level of glucose in of the human blood stream and is a consider prolonged autoimmune disease alignment sever inflammatory factor. The newest studies reveal that innate immunity high role in diabetes type1. The samples in this study consist of (135) Iraqi male with diabetes type 1 with acute tonsillitis in three group (G1: 4-18 years, G2: 11-18 years and control) all samples diagnosed by biochemical methods added to the clinical method. Alleles were determined by amplification of the polymorphic site by real time polymerase chain reaction. The result of genotyping study of rs2243250 of IL4 revealed that the T allele frequency showed (0.42 %), (0.41%) and (0.69%) in groups G1,G2, control respectively, while the result revealed that the allele frequency C in groups G1,G2, control was (0.58%), (0.59%) and (0.31%) respectively. The study can be concluded that the genetic variation of rs2243250 of IL4 gene was associated with diabetes mellitus type1 comparison in control (healthy women) in Iraqi men.

Key words: *Interlukine4, Tonsillitis, Diabetes, Gene and Inflammation*

Introduction

Type 1 diabetes mellitus (T1D) is one of the autoimmune disease that occurrence damage in pancreatic beta cells lead to insulin deficiency which leads to hyperglycemia and a tendency to ketoacidosis [1]. T1D commonly presents in childhood or adolescence; however the disease can appear at any age. Patients with T1D constitute 5-10% of all people with diabetes [2]. The effect of complicating factors, such as aging, diet, immune cell metabolism, microbial pathogens, microbiomes, and epigenetic changes, which modulate the immune response that destroys β-cells, are still incompletely understood [3]. T1D has been linked with augmented frequency of infections [4]. Moderately it may be clarified through a decreasing immune response by T cell [5]. Also decreasing in neutrophil role and function through diabetes [6], American Diabetes Association (ADA) delivered guiding principle on flu, cold and pneumococcal infection in diabetes [7,8]. Diabetic patients have an augmented hazard of bacteriuria also infections in urinary tract [9], besides the infections in skin and mucous tissue such as candida infections in addition to foot infections [10], the association among infection in respiratory tract with diabetes residues undecided [11]. Almost altogether nucleated cells such as endo/epithelial also macrophages are strong manufacturers of IL-1, TNF-α and IL-6. IL-1 group is one of cytokines that performance an essential role of inflammatory replies and guideline of immune system [12], the aim of study association of single-nucleotide polymorphisms rs2243250 of IL4 gene in diabetes type 1 patients with acute tonsillitis.

Material and Methods

The samples in this study consist of (135) Iraqi male with diabetes type 1 with acute tonsillitis, the age of male about 4-18 years old, all samples diagnosed by biochemical methods added to the clinical method. The samples divided into three groups: group 1 (G1) included 50 sample age about 4-10 years with diabetes type 1 with acute tonsillitis, and group 2 (G2) included 50 sample age about 11-18 years with diabetes type 1 with acute tonsillitis, the group 3 included 35 control, the work was performed in University of Baghdad, Collage of Science. Venous blood of sample was

collected after fasting about (10-14) hours by syringes. It was put in two type of tube the first tube containing EDTA for purpose DNA extraction and second type of tube without (anti–coagulant) for biochemical tests. Serum was separated at room temperature after clot (15-25) minutes by centrifugation at (3000 rpm) 20 minutes. The measurement of biochemical tests as fasting blood glucose test determined by (biosystem kit, Spain), HbA1c was mesuered by kit (), while concentrations of insulin by ELISA kit (DRG Instruments Mbh, Germany) and interlukine 4 was assessed by ELISA kit ().Molecular study**:** Genomic DNA was extracted by using (Quick-gDNA™ Blood MiniPrep kit). Then DNA was stored at −70 ºC. The genotyping study was performed by TaqMan® SNP Genotyping Assays, 5′-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. The component of reaction (table 1) and the condition of reaction RT-PCR (Table 2).

Table 1: The component of reaction

Component	20 µL (Final volume)
2X TaqMan® Master	12.50 µL
20X Assay Working	1.25 µL
Nuclease-free	-
DNA Sample Volume	11.25 µL

Table 2: The conditions of reaction

Steps	Predesigned SNP		
	Temp.	Duration	Cycles
Enzyme activation	95°C	10 minutes	HOLD
Denaturation	95°C	15 seconds	40
Annealing/ Extension	60°C	1 minute (scanning)	

Statistical Analysis

To detect differences between categorical data was used statistical analysis System program (2012). Least significant difference (LSD) test (Analysis of Variation-ANOVA) was used to find out relate between means.

Results and Discussion

The results of this study to detect genetic variation of diabetic type 1 in patient with high frequency of tonsillitis aged from 4-18 years comparison with control group, the results revealed that the high significant differences of fasting blood sugar (P≤0.01) (7.811), the level of fasting blood sugar in G1 (212.09 ± 2.87), in G2 (217.16 ± 1.50), while in control (103.29 ± 4.10). The level of HbA1c in groups also showed high significant differences between groups of experiment, (P≤0.01) (0.687) in G1 (15.81 ± 0.26) and G2 (16.11 ± 0.21) while in control (7.24 ± 0.25). The insulin concentration in samples of experiment observed that high significant differences (P≤0.01) (4.576), the level of insulin in G1, G2 and control (0.694 ± 0.21), (1.529 ± 0.26) and (10.78 ± 1.71) respectively. The result showed high significant differences of IL-4 (P≤0.01)(153.31), the concentration of IL- 4 (pg/MI),(471.62 ± 67.23), (585.89 ± 62.58) and

(210.69 ± 26.52) in G1,G2 and control respectively **(Table 3)**.

Table 3: Comparison between difference groups in parameters study

Group	Mean ± SE			
	FBS	**HbA1c**	**Insulin**	**IL-4 (pg/MI)**
G1: Patients	212.09 ± 2.87 a	15.81 ± 0.26 a	0.694 ± 0.21 b	471.62 ± 67.23 a
G2: Patients	217.16 ± 1.50 a	16.11 ± 0.21 a	1.529 ± 0.26 b	585.89 ± 62.58 a
Control	103.29 ± 4.10 b	7.24 ± 0.25 b	10.78 ± 1.71 a	210.69 ± 26.52 b
LSD value	7.811 **	0.687 **	4.576 **	153.31 **
** (P≤0.01).				

The genotype variation of IL-4 (rs2243250) gene and allele frequency between groups of experiment (G1,G2, control), the results revealed that TT genotype appeared high significant (P≤0.01) differences (χ^2 = 9.361) between groups, the genotype TC also showed high significant differences (P≤0.01) (χ^2=8.026) between groups, similarity high significant differences (P≤0.01) (χ^2 =9.478) of genotype CC between groups. In addition to, T allele frequency showed (0.42 %), (0.41%) and (0.69%) in groups G1,G2, control respectively, while the result revealed that the allele frequency C in groups G1,G2, control was (0.58%), (0.59%) and (0.31%) respectively Table 4.

Table 4: Genotype of IL-4gene (rs2243250) and allele frequency in patients with diabetes type 1

Genotype	G1		G2		Control		Chi-Square-χ^2	P-value
	No	**%**	**No**	**%**	**No**	**%**		
TT	18	36.00	12	24.00	20	57.14	9.361 **	0.0017
TC	6	12.00	17	34.00	8	22.86	8.026 **	0.0056
CC	26	52.00	21	42.00	7	20.00	9.478 **	0.0001
Total	50	100%	50	100%	35	100%	---	---
	Allele Frequency %							
T	0.42		0.41		0.69		---	
C	0.58		0.59		0.31		---	
** (P≤0.01).								

This work exposed that the patients with diabetes type1 in groups G1 and G2 recorded high significant increasing in level of fasting blood sugar, HbA1C and IL-4 while high significant decreasing in level of insulin concentration versus in the control group. The result agree with this study that mentioned the rising character of inflammation for diabetic patients both type 1 and 2 pathophysiology and linked metabolic confusions, has causes increasing importance in aiming inflammation to develop avoidance this disease [13]. Other studies mentioned that object of inflammation in diabetic patients or hypoglycaemic achievement was connected with serine kinase (Ikappa B kinase-beta) inhibition, which associates with the post-receptor achievement of insulin hormone [14]. Advancement in understanding about the pathophysiology of diabetes type 1 has been make in matching with the progresses in the immunology. Anderson and Bluestone verified that collapse in immune tolerance (centrally and peripherall) instruments give to the appearance of T cells auto reactive in mice thinness with diabetes [15]. various studies have revealed that common and rare diseases are additional common in diabetic patients rather than in the universal population, while other studies have not detected connection [16,9]. Diabetic patients showed to have an more risk factor of bacteriuria and infections in urinary tract [17,18] also infections in skin and mucous membrane, such as Candida infections in foot [10]. Although there are many studies in this field remains the relationship unclear among infection respiratory tract and diabetes [11]. In addition, most studies did not report for difference in types of diabetes, ADA suggestion issue guidance about influenza or pneumococcal immunization in diabetic patients [8] but others specific information of infection treatment is deficient. the instructions of the Physicians in American College of infections in respiratory area do not refer to a particular approach for diabetic patients [19,20]. The result of genotyping study revealed that the T allele frequency showed (0.42 %), (0.41%) and (0.69%) in groups G1,G2, control respectively, while the result revealed that the allele frequency C in groups G1,G2, control was (0.58%), (0.59%) and (0.31%) respectively. There are practical studies are fundamental to dissect downstream influences in molecular variations on this locus, also the instruments of their involvement to relationship between increasing of risk for diabetes type 1 with autoimmune pathology [21]. In addition to, sequencing as well as genotyping of the IL2 region in T1D cohorts discovered the relations with rs3136534, rs6836189 with increased relationship of rs17388568 to widespread connection instability crossways the locus [22]. Other locus rs2243250 and rs2227284 are significantly related with asthma as well as allergic rhinitis, the environmental factors with genetic hazard factors participate an significant role in the progress of respiratory diseases [23]. IL-4, IL-6, also IL-12 plane in PB were connected with functions of lung and cellular immune role in children with middle to severe asthma [24]. The homozygous genotype TT of locus C-589T rs2243250 was extensively related with allergic rhinitis diseases [25]. Other studies have examined the relationship between IL4 -590C/T polymorphism and diabetes mellitus. Arababadi, 2010 revealed an important variation in the genotype also allele frequencies in -590C/T SNP between diabetes type 2 patients with DN and healthy cohorts in a Rafsanjan people from Iran southeast [26]. Neelofar *et al*., 2017 showed an connection between IL4 (590C/T) and IL6 (174G/C) in patients have to chronic kidney disease with T2DM from North Indian [27]. Further, Alsaid *et al*. [28] founded that the heterozygous genotypes of IL4 -590 CT also IL13 -1112 CT in Egyptian patients with DM may be considered at the same time as danger factors whereas the homozygous genotypes (CC) perhaps considered defensive for diabetes mellitus. While other study revealed no relationship between IL4 (590C/T) SNP with microvascular complications of diabetes mwllitus type 2 in Caucasians [29].

Conclusion

There are association of single-nucleotide polymorphisms rs2243250 of IL4 gene in groups of diabetic patient type 1 with acute tonsillitis contrary in control groups, the genotype and allele frequency of rs2243250 of IL4 gene, allele C was recorded a highly significance in patients compare with control, allele T frequency lower significant in patients than in control.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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Adaptability of Biofilm Formation in *Streptococcus Pneumoniae* to Various Growth Conditions

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Abstract

Streptococcus pneumoniae is one of the major general causes of important diseases world-wide such as pneumonia and meningitis with a high morbidity and mortality in adults especially in elderly people. Pneumococcal biofilms are essential for colonization and persistence, and it is important to continue to investigate the environmental conditions which affect their formation. This study came to find out the adaptability of biofilm formation in *S. pneumoniae* that causes pneumonia in Diyala city and diagnose them with PCR method by using *16SrRNA* for pneumococcal detection in addition to the roads bacteriology culture test. In this study, 192 sputum samples were collected from Bacteriology Unit of Baquba teaching hospital, Chest Diseases Consultation Clinic and Al-Khalis General Hospital. It appeared that 122 (64%) samples showed no growth while 70 (36%) showed positive growth. *Streptococcus pneumoniae* isolates were obtained from 32 isolates. The results of this study showed amplify 1500 bp domain of *16SrRNA* by PCR, which was positive in 32 (100%). In addition, the PCR product of the samples were sent for sequencing to identify the *Streptococcus pneumoniae* strains. The results appeared that the strains of *S. pneumoniae* that cause pneumonia in Iraq especially in Diyala city are K10 and D39 strains. Biofilm formation assay was measured by using microtiter plate in three different media and the membrane formation was measured at four times of bacterial growth phases to show the adaptability of biofilm formation in different environmental conditions in different media culture. Data of the current study showed that the ability of pneumococcal to form biofilm was better when using a poor medium such as nutrient broth, and the highest value was at the stationary phase.

Key words: *Streptococcus pneumoniae, pneumonia, biofilm formation, 16SrRNA*

Introduction

Streptococcus pneumoniae is a major human pathogen with high morbidity and worldwide mortality, primarily from community-acquired pneumonia, meningitis, bacteremia and medium otitis [(1)] The pneumococcus is, however, also a transient commensal that asymptomatically resides and proliferates in the human nasopharynx [(2)]. Accordingly, the ability to acquire and the metabolization of sugars is of great importance for the health of this microorganism *in vivo*. In addition, the pneumococcus lacks a complete set of respiratory protein genes and is for this reason unable to generate energy by respiration [(3)]. The genomic abundance of genes involved in sugar transport further supports the significant role of carbohydrates in the lifestyle of *S. pneumoniae* .Over 30% of the transporters in the *S. pneumoniae* genome were predicted to be involved in the uptake of carbohydrates [(4)], and those findings were confirmed by a recent functional genomic approach targeting carbohydrate transport. As well as, several putative carbohydrate degradation pathways have been

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annotated in the genome sequences of *S. pneumoniae* [5]. The growth *in vivo* needs to rely on alternative nutritional reservoirs. In the human nasopharynx, the glycol proteins lining the epithelial surfaces appear as good candidates to serve as carbon and energy sources for pneumococcal growth. *S. pneumoniae* is able to grow on mucin as a sole carbon source [6]. Mucins are major macromolecular components of the mucus that cover the epithelial surfaces. Each mucin has a unique and characteristic sequence of tandemly repeating amino acids rich in serine and/or threonine, these structures are heavily O-glycosylated glycoproteins [7]. The deglycosylation activity of both exo- and endo glucosidases has been previously demonstrated in *S. pneumoniae* [8]. Biofilms are highly-structured communities of cells which produce an extracellular matrix and adhere to abiotic or biological surfaces. Antibacterial resistance is an inherent feature of biofilms and the protective biofilm matrix enables evasion of host immune responses, facilitating persistence, and dissemination of bacteria [5]. Pneumococcal colonization precedes disease and has been found to be harder to eradicate than invasive disease inpatients as treatment with antimicrobial agents do not eliminate the major of bacteria carried in the nasopharynx [9]. More, environmental factors which affect the formation of biofilms, like the source of carbon, the pace of flux and the physical surface properties to which bacteria adhere [10]. The capacity of *S. pneumoniae* to formation biofilms have been tested on abiotic surfaces for a variety of materials include polyvinyl chloride, polystyrene and glass, the last one of which the strongest was linked formation of biofilm. The formation of pneumococcal biofilms was observed when using poor culture media and the difference in pH and temperature also affects the formation of biofilms [10]. Treatment of bacterial biofilm is complicated by the mechanisms for biofilm growth. Many clinically relevant biofilms are poly microbial and mixed species biofilms have complementary metabolic strategies for obtaining nutrients and degrading host immune molecules [11]. Thus, it is important to identify the adaptability to vary growth conditions of biofilm formation to introduce novel or re-emerging effective approaches to combat bacterial biofilms.

Material and Methods

Samples Culture

All the sputum samples were culturedon blood agar plates and then incubated to 37□ aerobically for 24 h., addition, cultured them on Chocolate agar in candle jar with 5% CO2 for 24 hours. After the incubation period the growth appears been moved pure colony on the Blood agar for the purpose of its purification bacteria were diagnosed by biochemical tests and other diagnostic methods. Diagnosis of bacteria isolates were diagnosed according to Forbes *at el. (*2007).

Molecular methods for *Streptococcus pneumoniae* detection

DNA Extraction of *S. pneumoniae*

Genomic DNA was is extracted from bacterial growth according to the protocol of ABIO pure Extraction.

Agarose Gel Electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria. The primers used to amplify *16SrRNA* are: 27F=5`AGAGTTTGATCCTGGCTCAG-3`and 1492R=5`TACGGTTACCTTGTTACGACTT3.

Standard Sequencing

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The results were received by email then analyzed using genious software.

Microtiter Plate Biofilm Assay

The microtiter biofilm assay was performed as described by Merrit and colleagues (2011), with some modifications. This assay allowed for qualitative and quantitative observation of the amount of biofilm formed [8].

Results

Molecular methods for *S. pneumoniae* detection

The genomic DNA was extracted directly from 32 samples and was used for PCR to determine *S. pneumoniae* infection. Whole DNA extracted were subjected to 1% agarose gel electrophoresis.

Detection of *16SrRNA* in *S. pneumoniae*

All 32 samples gave positive PCR results of the 1500 bp domain of 16SrRNA gene, as shown in Table (1). The PCR products were analyzed using 1% agarose gel electrophoresis which showed bands of the amplified DNA for 16SrRNA as shown in the Figure (1).

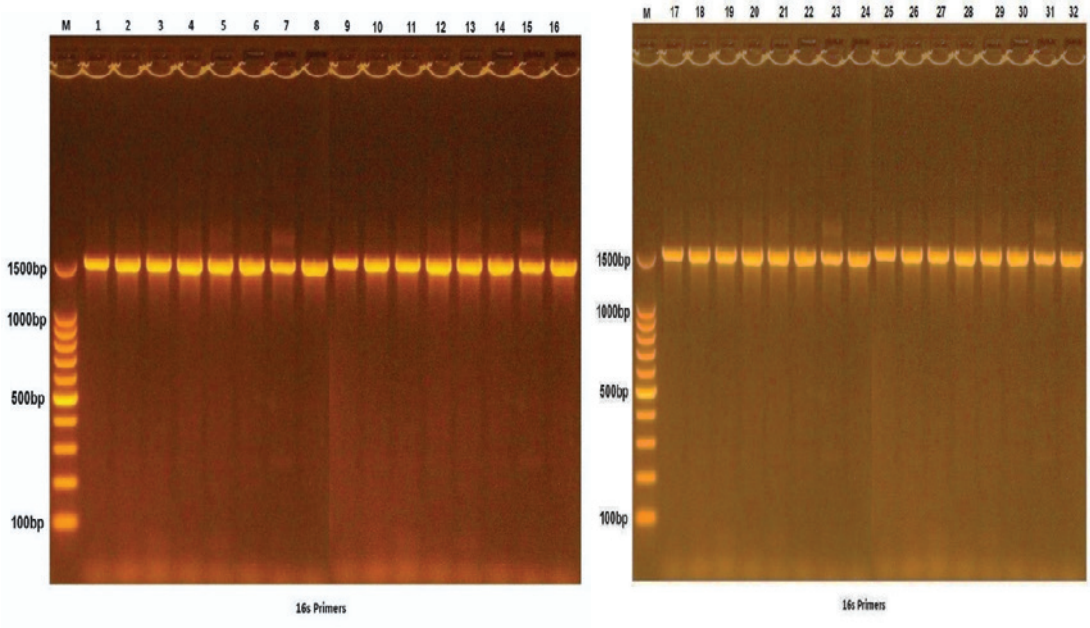

Figure (1): Agarose gel electrophoresis of DNA from PCR products were loaded directly showing PCR products for 1500 bp of 16SrRNA gene were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp DNA ladder.

DNA Sequencing

The DNA of *S. pneumonia* was extracted from the broth media and sent for sequencing in order to know the strain of *S. pneumoniae* which is most common cause's pneumonia in Iraq especially in Baqubah city. The results showed that all the DNA of *S. pneumoniae*, which was extracted related to *S. pneumoniae* strain x and the identity between them 100%.

Biofilm formation and different types of media at different times

Data of current study show the comparison between optical density (OD)595nm, value of biofilm at different phases (Lag) control, Log (3 hour), Stationary (6 hour) and death phase (24 hour) for each media (Nutrient Broth, Blood Broth and B.H.I Broth).

In nutrient broth, the highest concentration was at stationary phase (0.420±0.023), while the lowest concentration was at death phase (0.088±0.032). The differences between phases in nutrient broth were significant ($p<0.05$).

In blood broth, the highest concentration was at stationary phase (241±0.044), while the lowest concentration was at death phase (100±0.024). The differences between phases at blood broth were significant ($p<0.05$). In B.H.I broth, the highest concentration was at stationary phase (0.272±0.041), while the lowest concentration was at log phase (131±0.016). The differences between phases at B.H.I broth were significant ($p<0.05$). When comparison between different media for each phase, in lag phase the concentration is equal in all media (Figure 2). In log phase, the highest concentration was at nutrient broth media (0.333±0.028), while the lowest concentration was at blood broth (102±0.025). The differences among the media at stationary phase were significant ($p<0.05$).

In the stationary phase, the highest concentration was at nutrient broth media (0.420±0.023), while the

lowest concentration was at blood broth (0.241±0.044). The differences in biofilm formation among the media at stationary phase were significant ($p<0.05$). In the death phase, the highest concentration was at B.H.I. broth media (0.192±0.012), while the lowest concentration was at nutrient broth media (0.088±0.032). The differences between media at death phase were significant ($p<0.05$).

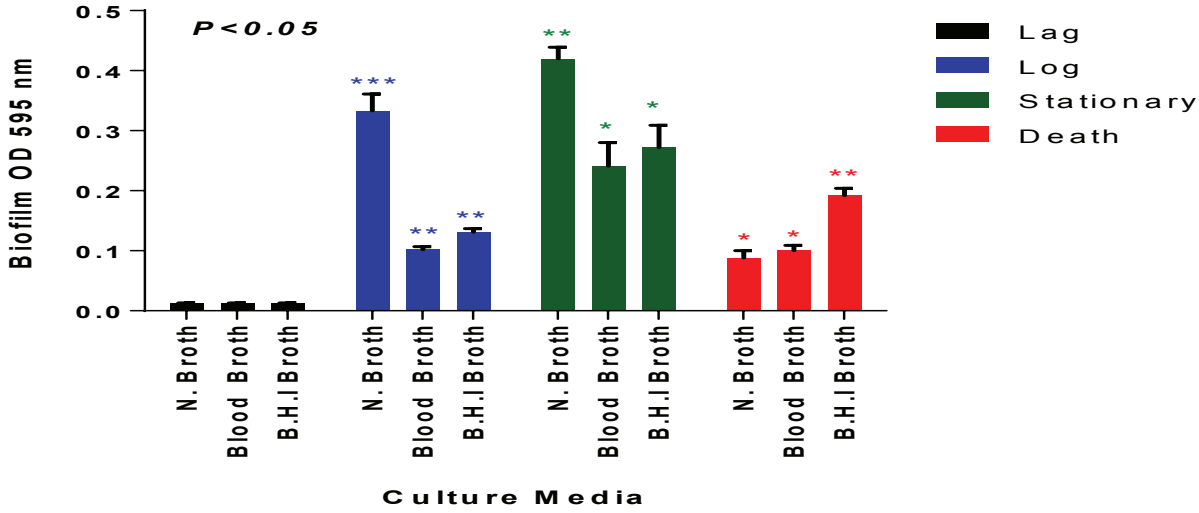

Figure (2): comparison between optical density (OD) values of biofilm at different times for each media.

Discussion

Streptococcus pneumoniae was classified the fourth-most common microbial cause of fatal-human infections, and one of the world's most common causes of severe diseases, like invasive-pneumococcal disease and community acquired., pneumonia. In addition, *S. Pneumoniae* was seen as a significant cause of pneumococcal disease-related morbidity and mortality worldwide [12]. Pneumococcal infections ware significant threats to public health, and these infections were aggravated by a growing increase in antibiotic tolerant and resistant pneumococcal strains [13]. In addition, about 3-5 million people die each year as a result of pneumonia, meningitis and bacteremia [14]. It was also documented that 67% of the approximate 14.5 million cases of pneumococcal disease in among children per year are documented in Africa and Asia, and about one million children under the age of 5 died in 2013 to pneumonia [3]. The environmental factors have been reported to play a role in raising the rate of CAP accidents, including the weather and gender [15]. Over winter months, more CAP was recorded than in other seasons during the year, and the prevalence of CAP among men is higher than among women. Also suggested as risk factors for contracting pneumococcal infections are becoming a tobacco smoker and alcohol misuse [16].

Two methods were used for diagnosing *S. Pneumoniae*, which are bacteriological methods including culture and antibiotic sensitivity, molecular methods using PCR to detect important genes including, *16SrRNA*. In addition, DNA sequencing was done to identify the strain of *S. pneumoniae* that causes pneumonia in Baqubah city [17].

In this study, the DNA of *Streptococcus pneumoniae* was extracted due to strains K10 and D39. Phylogenetic comparison of *16SrRNA* sequences became a powerful method for the systematic classification of microbial organisms [11]. Analysis of *16SrRNA* gene sequences became the primary method for determining prokaryotic phylogeny, which is the current basis for prokaryotic systematics. They were amplified to confirm the presence of the isolated *Streptococcus pneumoniae* strains. Sequence analysis confirmed the identification of *Streptococcus pneumoniae* isolates. *16SrRNA* sequence analysis has demonstrated considerable genomic diversity between isolates, and numerous sequences. It

was reported that *16SrRNA* was used to identify the strain of *Streptococcus pneumoniae* because it is housekeeping gene in this bacterium and find in most of *Streptococcus pneumonia* strains but they have different sequencing. However, the conventional methods for the detection of microorganisms are often unreliable. The use of the present PCR method is simpler, faster, and could solve some kinds of problems [15].

Most microorganisms take biofilm formation as a way of life in nature, and many unique properties of this multicellular community have been characterized. The experiment provided the influence of extracellular macromolecules in the development of *Streptococcus pneumoniae* biofilm. Under the experimental conditions described here, pneumococcal cells adhere to the surface and they form a biofilm [18].

In this study, the effect of the culture medium type, as well as the difference in incubation time, affected the formation of the biofilm *in vitro*. The results of this study were identical to other researches which found that biofilm formation in poor agricultural medium was better than the rich media [19]. In addition, the current study agreed with a study by Moscoso *et.al*, 2006, who found that pneumococci were grown in culture medium either supplemented with 0.08% yeast extract (C + Y medium) or not supplemented. In addition to medium C, the following media were used: Todd-Hewitt broth with 0.5% yeast extract, and CDM. As a result, biofilm was better in the poor agricultural medium like CDM [20].

The environmental variables, for example temperature and availability of nutrients, also influence biofilm-formation. The 34 ° C atmosphere results in biofilms that, by resisting antimicrobial agents, are dense and more functional than the biofilms produced at 37 ° C. At last, the availability of nutrients too affects the production of biofilms because nutrient rich-media did not support the developmental of biofilm [21,15]. The results of the current study showed that biofilm formation in poor media such as nutrient broth was better that in rich media and the cell density plays a role in biofilm formation in *Streptococcus pneumoniae* [22].

Ethical Clearance: In this study, the project was taken from the ethical committee Medicine College / Diyala University.

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Conflict of Interest: Nil

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Formal Reconciliation to Adjudge Psychological and Physical Abuses Committed During Civil War in Acehnese Society

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Abstract

The civil war caused many psychological and physical trauma for common people. As a way to end this such war, related parties usually take formal and social measures in peace agreement. In this case, the formation of the truth and reconciliation commission (TRC) in Aceh is one of a task to accomplish after the signing of memorandum of understanding in Helsinki (MoU Helsinki), and the implementation of law number 11, 2006 on the Acehnese government. On one side, the situation has urged TRC to be formed in Aceh, however local regulation has not specifically organized the forming of the commission. Reconciliation in Aceh comes with its own character by stressing more on the application of religious, custom, and local wisdom approaches without forgetting the effort of truth revealing on some cases of heavy human rights violation which occurred during conflict. The Acehnese society is a society who lives with their Islamic values and specific custom.

Keywords: *reconciliation, truth-seeking, local wisdom, forgiveness*

Introduction

The establishment of Truth and Reconciliation Commission (TRC) in Aceh is one of duty, which has to fulfil after signing memorandum of understanding in Helsinki (MoU-Helsinki) between Government of Indonesia and The Free Aceh Movement (GAM/ *Gerakan Aceh Merdeka*). The importance of TRC establishment is also mention in Law Number 11/2006 about The Government of Aceh (UUPA)[1]. In one side, the circumstance enforce the establishment of TRC in Aceh, however the regulation in Aceh was not fulfil yet. Those matters are difficult after The Decree of Constitution Court deciding The Law Number 27/2004 concerning Truth and Reconciliation Commission was against constitution[2]. It is mean that TRC Law in national level has not its supremacy and cannot be established anymore. So, it has to remake again in the new format. Therefore, the order in UUPA that TRC Aceh is the part of national TRC was not being established.

There are some alternative to fulfil law requirement for Aceh TRC. First alternative, propose *Peraturan Pemerintah Pengganti Undang-Undang* (Perpu/ Government Regulation In Lieu of Law). However, Perpu will be made in emergency situation. It is the difficult to explain that TRC Aceh is in emergency situation. The process of making Perpu will take too much time, due to the process between President and House of Representative (DPR RI) have a lot of step to fulfill. Another reason is that Perpu is only for temporary, so, if in one year it is not to be the law, it is mean that Perpu will not be valid anymore[3]. Second alternative, is to establish *Qanun* TRC. This alternative is really possible due to all of legislation process will be set up in Aceh. Therefore, the legislation process can be fasten, but the minus point is that *qanun* (a local bylaw in Aceh) has limit teritorial authority, it is mean that it cannot reach perpetrator staying outside Aceh.

Formal establishment of Truth and Reconciliation Commission

The establishment of TRC in Aceh has differential among TRC in other countries, such as South

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Africa, Rwanda, Yugoslavia, and other places. The reconciliation in South Africa after the apartheid regime as an example that people choose to forgive rather than to sue the perpetrator who did human rights violation in the past[4,5,6]. The reconciliation process in South Afrika is the resultant of negotiation between apartheid regime and Nelson Mandela who was still in prison. This reconciliation was meant as an allurement to forgive and to forget a past period, so the normal life can be started and the circle revenge can be avoid[7,8,9,10]. In others word, the function of social-politics to forget the past periode is really important. The primary purpose is to build civilized-life, to stop hostility between color skin and dark skin[7]. However, the reconciliation experience from South Africa is not completely appropriate with TRC in Aceh. Reconciliation in Aceh has their own specialties. It has a custom, religion, and local wisdom approach. Below will be discussed the specialties of Aceh TRC.

Reconciliation approach based on religion, custom, and local wisdom

Reconciliation in Aceh has its own character focusing on religion, custom, and local wisdom. However, those character will not forget the truth seeking process for gross human rights violation which was happened during conflict. People of Aceh are the society living with Islamic value and special custom. Both Islamic value and special customs are the part which cannot separate each others. For this reason, in Acehnese culture has proverb that *Adat ngon hukom lagee zat ngon sifeut* (custom and Islamic value are part that cannot separate each others). Brotherhood, understand each others, and forgiveness can build trust-feeling among parties who was conflict in the past. Those principle is known as *meusuloh* (peace) which is followed by custom ceremony such as *pesijuek*, *meumat jaroe* (*shakehand*), etc. Generally, each district in has their own specific approach *meusuloh*. *Peusijuk* is custom ceremony to make brotherhood between parties who had conflict before.

The word *Suloh* is taken from verses of Quran Sura al-Nisa (114)[11]:

"In most of their secret talks there is no good: But if one exhorts to a deed of charity or justice or conciliation between men, (Secrecy is permissible): To him who does this, seeking the good pleasure of Allah, We shall soon give a reward of the highest (value)."

Suloh is the part of reconciliation which is based on indigenous people. This approach is as alternative dispute resolution which is really effective to prevent conflict which is happened again. *Suloh* can be functioned as a tool to cut the circle of revenge which is possible happened again. Suloh is one of method to solve conflict with custom approach. It is unwritten law and uncodified. Soepomo give definition of custom law as a custom law, which is unwritten and uncodified, most of them is Islamic law[12,13]. That law is unwritten in state *gazzete* like most of state law. Although it is unwritten, it life in social culture as a living law.

Customary law in Islamic law is known as '*urf* (habit). It is one of resource in Islamic law. The function of this resource is to support others resources in Islamic law such as *mashalih al-mursalah, istihsan, istishab*, etc. These resources will be used when the special cases laws are not mention in Quran and Sunnah. In the dignity of Acehnese social life, they do not have revenge feeling, because it is against Islamic value. The resolving method using customary law can avoid revenge[12,13]. So, the power to get peace can be used in a positive way such as building bright future. However, Acehnese is still use self-defense to protect themselves from threat which is called *tuengbeela*. However, it will be used only in emergency.

Peace and reconciliation have a meaning to rebuild a brotherhood that was destroyed during the conflict, and to heal heart feeling in peaceful and brotherhood. In Aceh history was implemented the process of reconciliation using customary approach between The Government of Republic of Indonesia and DI/TII. This reconciliation was known as *Piagam Blangpadang* (Blangpadan Charter) which was held on December 18-21, 1962. This event was named as Musyawarah Kerukunan Rakyat Aceh (The Deliberation of Reconciliation People of Aceh). This event was known as reconciliation. The prominent people as delegation from The Government of Republic of Indonesia, Acehnese, and DI/TII attended this event. The main point of this event is to solve conflict during ten years between DI/TII and Indonesia, which was really disturbed the process of developing Aceh in multi-aspect. In the other sides, the customary law has a international power after United Nation on Declaration of Rights Indigenous People which is declared on September 13, 2007. The declaration consist

of the protection to indigenous people to develop their customary, to protect tradition, faith and believe, to adopt legal resources, etc.[14,15,16].

Since the Islam and local custom aspects are dependable one upon another, thus in Aceh there is one wise word saying *adat ngon hukom lagee zat ngon sifeut* (the custom and Islamic law cannot be separated). Brotherhood, maintaining social relationship, and forgiving are aspects that can develop mutual trust among people involving in conflict intentionally or unintentionally. For Acehnese people, there is principle known as *Meusuloh* (peace process) which is followed by local custom such as *peusijuek* (showering), *meumat jaroe* (shaking hands for forgiveness), and many other ways of *meusuloh* (peace process) with its specific character for each area in Aceh. In addition to the above matters, to fulfil the need for regulation at the state level, president can propose for Emergency Law or Perpu to fasten the process of truth revealing and reconciliation. The apparent problem is the forming of the Emergency Law is only temporarily, thus unless it is legalized within one year period, then the Law is considered as void. Another alternative is by immediately forming *Qanun* (Law) on the truth and reconciliation commission in Aceh[14,15,16]. This is as the most possible alternative to be implemented in the nearest moment as the whole arrangement process of the law can be done locally. Yet, this solution makes the scope of the truth and reconciliation commission becomes narrower since this commission is of the national scale level[17].

Specific Model for Aceh Reconciliation

Generally, there are two reconciliation models, which is, can be implemented in Aceh. Those reconciliation models will not forget the truth seeking. Those models have adopted Aceh specialties value, and the principals of justice. This resource come from Indonesia Human Right Law and International Human Right Law. Those models are specified into 2 models. First, Truth Seeking, Victim reparation, and Forgiveness. This model is to process truth seeking, to do victim reparation, and to forgive each other. It has to along with increasing victim welfare that can make religion, custom, and local wisdom approach. The reason of suggestion of this model is in Indonesian Constitution (UUD 1945) does not tolerate retroactive cases happening in the past. This is mention on Article 28I (1): Rights to life, rights to are not tortured, rights freedom of thinking and from the bottom of one's heart, rights to have a religious, rights to are not be slaved, rights to occupy as person in front of law, and rights to uncharged based on retroactive law, is the human rights which cannot added and cannot deducted in every circumstance. Because of the difficulties of retroactive punishment, Victim reparation and forgiveness are more essential to establish in the society. Second, truth seeking, tribunal process, and forgiveness. The models is to seek the truth, to make tribunal process, and forgiveness[14,15,16]. These models are almost same with the first model. It has to along with increasing victim welfare that can make religion, custom, and local wisdom approach. The differential between first model and second model is in the tribunal process. For gross human rights violation, it will be faced to Indonesian Human Right Court. For case which is not identified as gross human rights violation will be handle with reconciliation based on community approach and using Aceh local value.

Conclusion

Reconciliation process is the process to heal hurt feeling due to conflict in the past. It need special treatment in healing all of hurt feeling. Approaching Aceh specialties focus on religion value, custom, and local wisdom which is a part of reconciliation. Victim welfare, justice, and forgiveness are the part which cannot put on separate place. However, they are the unity connecting one each other. Ideally, all of mistake can be forgiven, but we cannot close the door for the victim who has hurt feeling and cannot forgive the mistakes. Forgiveness come from the bottom of one's heart, and cannot be forced from third parties.

Conflict of Interest

There is no conflict of interest.

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Ethical Clearance

Ethical clearance from the institutional ethical committee obtained for the study.

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Analysis of Health Behavior of Adolescents in Tuban, Indonesia

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Abstract

Adolescence is a period associated with increased risk behaviors such as smoking, sex, fighting, the use of sharp weapons and so on. These conditions coincide with changes in social and school environment. The purpose of this study is to explain how health risk behaviors are to adolescents. The research design used in this study was analytic with a cross sectional approach. The population was all high school/vocational school adolescents with a total of 29,963 boys and girls, with a sample size of 395 using cluster sampling technique. The independent variable of the study is risk behavior and the dependent variables of the study are smoking, bullying, use of sharp weapons, driving, drug consumption, sex, health consultation, nutritional fulfillment, suicide attempt, alcohol consumption, fighting and physical activity. Data collection was carried out using a questionnaire adopted from YRBSS (Youth Risk Behavior Surveillance System) which was translated into Indonesian. The collected data was tabulated and analyzed using Logistic Regression. The results showed that all health behavior including risk behaviors, although based on statistical test results the behavior of alcohol and drug consumption was not significant because (p value) was 0.000>0.005. Smoking behavior had a regression coefficient of 6,904 with a significance level (p value) of 0.000<0.005 and an OR (odds ratio) of 996,365, which means that adolescents have 996 times the chance to smoke. All variables are included in health risk behaviors; however, smoking is the riskiest health behavior carried out by adolescents.

Keywords: *Adolescence, Health Risk Behavior, Tuban, Indonesia.*

Introduction

Adolescence is a period marked by the transition from childhood to adulthood. In general, the adolescent age group ranges from 12-18 years of age, often associated with the onset of puberty. Puberty is a biological phenomenon that is experienced by every teenager and is triggered by the increase of adrenal and gonadal hormones, including the development of secondary sexual characteristics and changes in muscle and fat. In addition, adolescence is also a period associated with the increase of risk behavior and emotional reactivity. These conditions coincide with changes in social and school environment[1]. However, until today there is no explanation on the highest risk behavior in adolescents and the factors that influence adolescents to do so.

Youth is generally considered a period of good health. However, risk behaviors such as the use of hazardous substances, poor diet or premature sexual activity are topics that often arise. Risk behavior is understood as behavior with unexpected consequences that go hand in hand with damage and loss. These risk behaviors are understood to be directly or indirectly related to health and well-being. The fact that is often found is that risk behaviors generally appear or begin during adolescence[2]. Risk behaviors in adolescents include smoking, anti-social behavior, alcohol consumption and early sexual intercourse[2][3].

Previous research showed that out of 1087 students in the UK, 50% of the students had risk behavior and 18% had high-risk behavior[4]. Meanwhile, a research in Indonesia that involved 368 students in Sidrap Regency

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revealed that 2.4% have had sex, 27.4% have smoked, 11.7% have consumed alcoholic beverages, 2.7% have used drugs, 6.5% have experienced acts of violence and all respondents behave in a high manner[5].

WHO set Sustain Development Goals (SDG's) which must be achieved by 2030. This achievement would not be possible without investing in the health and well-being of adolescents[6]. On the other hand, risk behavior in adolescents has a direct or indirect impact on the health and well-being of adolescents, including poor educational attainment, future morbidity and premature death[3].

Understanding the impact of risk behaviors on health in adolescents and studying the importance of adolescent health are important investments to achieve the SDG 2030 target. Achieving this target requires efforts to prevent or reduce the effects that may occur. Nurses have a crucial role in realizing this effort. Health promotion through Health Promotion Model (HPM) theoretical approach can be carried out by nurses to overcome risk behaviors in adolescents. Health promotion encourages a lifestyle and behavior that allows a person to maximize their potential through individual, organizational and community change. HPM combines the perspectives of nursing and behavioral science with the factors that influence health behavior. This model offers guidance to observe complex biopsychosocial processes that motivate a person to engage in behaviors that lead to improved health[6,7].

Method

The research design used in this study was analytical with a cross sectional approach. The population in this study were all high school/vocational school students in the Tuban district with a total of 29,963 teenagers. The participants in this study were teenagers who attended SMA/SMK in Tuban district selected from the population.

The inclusion criteria in this study were: Adolescents registered as high school/vocational school students in all areas of Tuban district. The sample size in this study was 395 respondents and the sampling technique in this study was carried out using cluster sampling.

The independent variable in this study is health risk behavior and the dependent variables in this study are smoking, physical activity, bullying, sex, use of sharp weapons, fighting, drug consumption, alcohol, health consultation, nutritional fulfillment, suicide attempt and driving. The instrument used in data collection was a questionnaire adopted from the YRBSS (Youth Risk Behavior Surveillance System) which was translated into Indonesian and tested for its validity and reliability. The statistical test used logistic regression to analyze the dominant behavior.

Results and Discussion

The characteristics of the respondents were mostly female (65.1%), half were 16 years old (50.1%), more than half had a normal body mass index (54.2%), and all have experienced puberty.

Table 1. Distribution of Respondent Characteristics

Characteristic	Total n = 395	%
Gender		
Male	138	34,9
Female	257	65,1
Age		
15 years old	22	5,6
16 years old	198	50,1
17 years old	133	33,7
18 years old	27	6,8
19 years old	15	3,8
Body Mass Index		
Thin	137	34,7
Normal	214	54,2
Fat	44	11,1
Puberty		
Have Experienced	395	100
Have Not Experienced	0	0

The health behavior of adolescents in Tuban Regency shows that driving has the highest risk percentage, namely 80.5%. This is followed by the second highest risk behavior, namely nutritional fulfillment 45.8% and bullying 26.3%. Meanwhile, other behaviors have a

lower risk percentage.

Table 2. Percentage of Adolescent Risk Health Behaviors.

Behavior	Total n = 395	%
Smoking		
Risk	90	22.8
Non-Risk	305	77.2
Physical Activity		
Risk	75	19
Non-Risk	320	81
Use of Sharp Weapons		
Risk	45	11.4
Non-Risk	350	88.6
Driving		
Risk	318	80.5
Non-Risk	77	19.5
Alcohol Consumption		
Risk	23	5.8
Non-Risk	372	94.2
Fighting		
Risk	90	22.8
Non-Risk	305	77.2
Sex		
Risk	36	9.1
Non-Risk	359	90.9
Nutrition Fulfillment		
Risk	181	45.8
Non-Risk	214	54.2
Bullying		
Risk	104	26.3
Non-Risk	291	73.7
Consultation with a Doctor Regarding Disease/ Medication		
Risk	59	14.9
Non-Risk	336	85.1
Attempted Suicide		
Risk	25	6.3
Non-Risk	370	93.7
Use of Drugs		
Risk	5	1.3
Non-Risk	390	98.7

Based on the data presented in Table 3, it can be concluded that smoking, physical activity, use of sharp weapons, driving, fighting, sexual behavior, nutritional fulfillment, bullying, health behavior and suicide attempt have significance of <0.05, which means that these behaviors are included in health risk behaviors in adolescents. Meanwhile, although drug consumption statistical test results showed no significance (>0.05), there were adolescents who consumed drugs, hence this behavior is considered as a risk behavior. Smoking held the largest odds ratio, namely 996,365, which is the most influential variable in the occurrence of health risk behaviors in adolescents.

Table 3. Analysis of Health Risk Behavior in Adolescents in Tuban Regency

Variable	B	S.E.	Wald	df	Sig.	Exp(B)	95,0% C.I. for EXP(B)	
							Lower	Upper
Smoking	6,904	1,285	28,883	1	,000	996,365	80,337	12357,166
Physical Activity	5,356	,882	36,837	1	,000	211,862	37,575	1194,551
Use of Sharp Weapon	6,834	1,564	19,102	1	,000	928,530	43,342	19892,195
Driving	3,673	,837	19,241	1	,000	39,385	7,629	203,313
Alcohol Consumption	,121	1,913	,004	1	,949	1,129	,027	47,973
Fighting	6,544	1,189	30,286	1	,000	695,064	67,585	7148,223
Sex	5,927	1,552	14,584	1	,000	374,881	17,902	7850,399
Nutrition Fulfillment	5,214	,819	40,570	1	,000	183,851	36,954	914,684
Bullying	6,388	1,035	38,107	1	,000	594,441	78,221	4517,476
Health	5,382	,997	29,142	1	,000	217,561	30,823	1535,616
Attempted Suicide	5,058	1,566	10,432	1	,001	157,311	7,306	3386,999
Drug Consumption	15,610	12650,371	,000	1	,999	6017306,980	,000	.
Constant	-133,391	25300,746	,000	1	,996	,000		

The majority of adolescents have started smoking since the age of 13 and even some have started since the age of 8 and under. The types of cigarettes consumed include electrical cigarettes, tobacco and cigars, with the number of cigarettes consumed ranging from one to over ten a day. Adolescents obtain cigarettes from friends or by being in debt in order to 'buy' cigarettes. The frequency of smoking starts from a few times a month until almost every day. Some teenagers have tried to quit smoking, but have not succeeded.

In Indonesian adolescents, the tendency to smoke in junior high school is increasing. On the other hand, it is relatively stable in high school and there was a decline from 2009 to 2016 in college [8]. Based on the results of Riskesdas (2018), the proportion of smokers over 10 years of age in Indonesia is still relatively high. In East Java and Tuban Regency itself, the percentage almost

reaches 30%, where this is considered high as it is close to the national figure[9].

In Indramayu Fisherman Village, the highest health risk behaviors that appear among adolescents are smoking, physical fighting and alcohol consumption[10]. Early initiation of smoking is associated with the desire to be called a 'real man' or 'manly' and this behavior is supported by the family[11]. Several factors that influence risk behaviors among adolescents in mining areas include family, peers and school transition[12]. According to Sadzaglishvili (2017), there are several factors that have the greatest influence on adolescents in doing various risk behaviors, including: the intention of adolescents to carry out these behaviors and this is influenced by attitude, social norms, adolescent beliefs and expectations, self-esteem, affect and emotion and self-efficacy (individual feelings about the adequacy, efficiency and ability of individuals in coping with life). Apart from intention, gender and environment also greatly influence adolescents in engaging in risk behaviors[13].

The high number of adolescents who smoke and the fact that smoking is the highest-risk behavior among other risk behaviors is not solely the fault of the adolescents, but also many parties who should be responsible and take part in minimizing or even stopping adolescents who smoke. Among all the factors that influence adolescents to smoke, intention is the biggest factor, as any action or behavior with weak intention will not occur or be carried out. Thereby increasing the understanding of adolescents about the dangers of smoking, as well as taking a spiritual approach to convey a message that smoking causes more harm than good should be done. By doing this, adolescents will realize the negative aspects of smoking and will want to change for the better. In addition, an adolescent starts to develop social responsibility necessary to enter adulthood, so they should be responsible for the positive and negative impacts that come along with whatever action they do.

Researches state that experience affects smoking behavior in adolescents[13][14]. This experience can be obtained from friends and peers. The experience of being forced to smoke or not being considered a friend if not smoking affects the smoking behavior of adolescents. Adolescents have the need to be accepted by their peers, which causes many teenagers to follow their friends and start smoking.

This is consistent with the adolescent development task which states that in adolescence, teenagers want to be accepted by their peer groups and begin to escape emotional dependence on adults, and learn to make their own decisions.

The serious smoking behavior among adolescents corresponds to Pender's Health Promotion Model, health promotion behavior is influenced by interpersonal and situational influences. Research shows that there are 85.4% of adolescent passive smokers in Indonesia[15]. Parents who smoke strongly influence the behavior of teenagers who smoke.

Conclusion

Adolescent health risk behaviors in Tuban Regency include smoking, alcohol consumption, use of sharp weapons, bullying, sex, driving, health consultation, suicide attempt, drugs, nutritional fulfillment, physical activity and fighting. Smoking is the highest health risk behavior for adolescents and it is 996 times more likely for a teenager to engage in this behavior. Further research on the factors that influence adolescent smoking behavior should be conducted, support from family, environment and school, so that the right solution can be given to reduce or even stop smoking among adolescents.

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The Genetic Aspect of Non-Syndromic Cleft Lip and Palate towards Candidate Genes in the Etiology : A literature Review

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Abstract

Cleft lip and cleft palate (CL/P) is a cleft lip deformity indicated by an opening or an uncommon cleft in the lip or roof of the mouth (palate). The differences in ethnicity, gender, and the correlation with genetic factors influence the prevalence of Non-syndromic CL/P. This study was conducted through a literature review on genes that were allegedly associated with Non-syndromic CL/P. Genetics play a role, to a greater or lesser extent, in all diseases. Besides, palatogenesis involves many diverse genes in a complex process. In this case, oral cleft phenotypes develop when this process is disrupted in some manner because of gene dysfunction. Various genetic approaches, including genome-wide and candidate gene association studies as well as linkage analysis, have been undertaken to identify etiologic factors, but results have often been inconclusive or contradictory. Therefore, it concludes that the genetic basis of CL/P is still controversial because of the genetic complexity of clefting.

Keywords: *Cleft Lip, Cleft Palate, Genetic*

Introduction

Cleft lip and cleft palate or orofacial cleft is a cleft lip deformity indicated by an opening or an uncommon cleft in the lip or the palate (roof of the mouth). There are three primary types of orofacial cleft, namely cleft lip (CL) that is a cleft appeared only in the lip, cleft palate (CP) that is a cleft in the palate area, and cleft lip palate (CLP) that is a cleft appeared from the palate through the lip.[1] Non syndromic cleft lip with or without a cleft palate or abbreviated as *NSCL/P* is a type of deformity that can happen separately or simultaneously and it strongly influences a child's growth and development as a series of cause and effect, such as speech problems, hearing problems, dental and oral problems, feeding problems and malnutrition, respiratory disorders, psychological disorders, and facial aesthetics.[2,3] The proportion of genetic and non-genetic contributions for some types of disease or abnormality is various. However, the proportion of variants of each observed disease in a population caused by variants in genetic factors is known as heritability and it can be calculated through several methods, for example, by knowing the concordance rate.[4] The genetic aspects are known for playing a vital role in *NSCL/P*. However, up until now, the specific genes have not been fully identified due to a high number of gene interactions that are involved.

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Method

The method of this study used a literature review with an electronic database. This study used secondary data, namely, articles collected from a search engine in

some publication sources. During the search, the author used several criteria for keywords as follows: "cleft palate", "facial clefts", "genetic", "cleft lip", dan "non syndromic". In addition, a combination of the following terms was used: "Genetic cleft lip palate", "Genetic cleft lip palate" and "non-syndrome".

Etiology of Cleft lip and Palate

The incidence of *NSCL/P* is not surely known due to its multifactorial causes and it is influenced by several factors besides the genetic and non-genetic factors. In genetic aspects, there is a gene playing a role in the occurrence of *NSCL/P*. Non-genetic factors that play a role in cleft lip are nutritional deficiency (folic acid), chemical substances/drugs, rubella virus, radiation, infection of infectious disease, endocrine disorders, smoking, drinking alcohol, and trauma (mental trauma and physical trauma).[5]

Epidemiology of Cleft lip and Palate

The epidemiology of Cleft lip and Palate occurs two times higher in boys, while *Cleft palate* occurs two times higher in girls. In the Czech Republic, it has been found 2,147 babies with *Cleft lip* and or *Cleft palate*. The overall incidence is 1 out of 600 live births.[6] Twelve studies conducted in several locations in North America reported that the level of prevalence for white people, black people, Hispanic, and American Indians was around 0.6 to 3.92 per 1,000 births. Six studies conducted in several locations in Europe reported that the level of prevalence for white people and Arabs was around 1.02 – 1.94 per 1,000 births. There is a difference in the prevalence of births with orofacial clefts *(OFC)* among ethnicities. American Indians had the highest level of prevalence followed by Japan, Chinese, and white people, while black people had the lowest level of prevalence.[7] The data of global prevalence for orofacial clefts are not fully completed and, up until now, the international level of prevalence has not been established yet.

Genes Contributed to Cleft

1. Transforming growth factor – alpha (TGFa)

The TGFa gene is located at the 2p13 chromosome. TGFα plays a role in the regulation process of palate development. The previous genetic studies showed a significant correlation between the transforming growth factor-alpha (TGFα) and CL/P.[8,9]

2. Transforming growth factor - beta 3 (TGFβ3)

Transforming Growth Factor-Beta 3 (TGF-β3) is one of the strongest candidate genes for cleft lip and palate in humans.[10,11] TGF-β3 (located at the 14q24 chromosome) has a wide spectrum of biological activities and they are known to be able to induce palatal fusion[12]; over the last few years, several studies had been conducted to explain the correlation between TGF-β3 and cleft lip and palate.[13,14,15]

3. Muscle Segment Homobox Gene 1 (MSX1)

MSX1 is at the 14p16.1 position to code protein from 297 amino acids that are functioned as the transcriptional repressor during embryogenesis. MSX1 plays a role as a strong candidate causing NSCL/P, based on cleft palate and the complete failure of the development of incisor teeth.[16]

4. Interferon regulatory factor 6 (IRF6)

The human interferon regulatory factor 6 gene (*IRF6*) located at the 1q32.2 chromosome is responsible for the majority of patients with Van der Woude syndrome. The risk of NSCL/P relapse has been reported higher to infect an individual with the C allele, namely single nucleotide polymorphism (SNP) rs2235371, that encodes the Val274 amino acid from the product of IRF6 gene.[17]

5. T-box-containing transcription factor 22 (TBX22)

Mutation in the TBX22 gene is correlated with the causes of a syndrome related to the X chromosome with cleft palate and ankyloglossia. The T-box TBX22 transcription factor plays an important role in the normal craniofacial development as what has been reported in a finding related to non-sense mutation, frameshift, splice-site, or missense in patients with CPX. The TBX22 mutation is reported to occur in around 4%-8% of patients with non syndromic cleft palate.[18]

6. Retinoic Acid Receptor α (RARA)

The retinoic acid receptor α (*RARA*) is one of the candidate genes for causing NSCLP.[19] RARA gene is located in the 17q12-21 chromosome region. The RARA

gene plays an important role in the development stage that its functions are mediated by retinoic acid receptor alpha (RAR-α), including the regulation of development, differentiation, apoptosis, granulopoiesis. A study in a transgenic rat that has been knocked out showed the role of the RARA gene in facial development.[20]

7. Poliovirus receptor-related 1 (PVRL1)

The poliovirus receptor related-1 (PVRL1) gene is located in the 11q23.3 chromosome region that encodes the nectin-1 and cell adhesion molecules (CAMs) (OMIM #600644). An experiment in a rat reveals that mRNA PVRL1 is expressed in the medial edge epithelium (MEE) of the palate that is developing, it showed that there is an involvement of normal PVRL1 gene in the fusion of palatal shelves during palatogenesis.[21,22,23]

8. Methylenetetrahydrofolate reductase (MTHFR)

MTHFR gene is a gene playing a role in coding for making *methylenetetrahydrofolate reductase* enzyme. The enzyme functions to folic metabolism and it has been learned in an abnormality, such as NSCL/P. This reaction is needed for an advanced process in changing amino acid types, namely homocysteine into methionine that functions to produce protein and other important compounds for the metabolism. MTHFR gene has a sequential order of complete DNA of 20.373 bp in length consisting of 11 exons, located in the 1p36.22 chromosome (at the chromosome's long arm of 1, area 3, *band* 6, *sub-band* 2).[24,25 ,26]

9. Bone Morphogenetic Protein 2 (BMP2)

BMP2 gene encodes the ligand secreted from the TGF-β superfamily. The ligand from this superfamily binds several TGF-β receptors that play a role in the activation of a transcription factor, especially SMAD protein, in the regulation of gene expression. BMP2 gene is located in the 20p12.3 chromosome (the chromosome's short arm of 20, area 1, band 2, sub-band 3). It is recorded that a significant association between the polymorphism of *BMP2* rs235768 A>T with the incidence of CB/L as a risk factor causing CB/L in the Iranian population.[27]

10. Bone Morphogenetic Protein 4 (BMP4)

BMP4 gene with a sequential order of complete DNA and 11.2 kb in length consists of four exons and is located at 14q22.2 chromosome (at the chromosome's long arm of 14 and area 2, *band* 2, *sub-band* 2). BMP4 gene encodes the ligand secreted from the TGF-β superfamily. Mutation in this gene is correlated with orofacial disorder and microphthalmia.[28,29]

Conclusion

A genetic study is needed to provide an understanding of the pathophysiology of NSCLP. It has been an effort to identify the genetic disease related to NSCL/P to allow disease prevention and provide suggestions in the clinical management for controlling the risk factors.

Ethical Clearance: This study was approved by the ethical committee of the Faculty of Dentistry, Universitas Airlangga, number 606/HRECC.FODM/IX/ 2019.

Conflict of Interest: There was no conflict of interests regarding the publication of this study.

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Evaluation of Routine hematological parameter and Red Cell Distribution Width (RDW) in Myocardial Infarction patients admitted at Dhiraj Hospital

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Abstract

Background: - An increased systemic and local inflammation in vascular rupture plays a key role in the pathophysiology mechanism of acute coronary syndrome (ACS). Routine complete blood count (CBC) is one of the most common and conventional blood test that physicians test cycle.

Objective: To study the level of routine hematological parameters and red cell distribution width in Myocardial Infarction.

Methodology: - A cross sectional type of study carried out in the department of biochemistry, Smt. B.K. Shah Medical Institute & Research center, Sumandeep Vidyapeeth Vadodara Gujarat. A total 100 myocardial infarction patients with age group of 25-70 of either gender were collected from Dhiraj General Hospital. Pathological parameter were analyzed by BACKMAN COULTER SYSMEX Kx-21N.

Results: - In the age wise distribution of MI patients, we found more number in 58 to 68 age group. The mean levels of pathological parameter were observed with in reference range. In the observation of age wise pathological parameter we found that there increases mean levels of leucocyte count and the mean level of RDW (18.50±1.80) is increased in 58-68 age group.

Conclusion: - RDW is one of the predictive biomarkers for diagnosis of myocardial infarction with cardiac markers especially cardiac troponin I (cTn-I). Platelet levels can be monitored in MI patients in early phase to find out the status of clot.

Keywords: *Myocardial Infarction, Red cell width distribution, Total Leucocyte count (TLC)*

Introduction

Myocardial infarction (MI) is one of the major cause of morbidity and mortality in all over the world. Acute myocardial infarction is a clinical condition which can be characterized by pathological changes, electrocardiographic changes, elevated levels of biochemical indicators for myocardial necrosis.(1) Consequently, identification of novel risk factors is of great potential value to improve risk stratification and facilitate targeted prevention of MI. Red Blood Cell distribution width (RDW) is a simple measure of the broadness of erythrocyte size distribution, conventionally called anisocytosis (2).

Leukocytes play a key role in the pathophysiology of ACS, given their effect on the instability of atherosclerotic plaques. In the initial stage, leukocytes permeate endothelial cells and become activated when reaching the tunica intima. They induce the formation of micro vascularity there and, as a result, make plaques more susceptible to rupture. (3) RDW which is a part of

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a standard complete blood count (CBC) is a measure of variations in the volume of red blood cells. An elevation in RDW is known as anisocytosis.

Elevated red cell distribution width (RDW) values are independent predictors of prognosis in patients with different Ml types; it has also been noted that elevated levels of RDW are associated with the presence and severity. Platelets play a key role in the pathophysiology of ACS. Compounded with fibrin, platelets form coronary thrombus. (2) Platelets participate in the creation of blood clots and deliver mediators which develop and sustain a local inflammatory response Platelet distribution width (PDW) indicates a varied size of platelets. The number of large immature platelets in patients with ACS is caused by an increased bone marrow activity during the process known as thrombocytopoiesis. (4)

The red cell distribution width (RDW) has also been proposed as an independent predictor. It has been reported that the diagnostic accuracy of troponins within 2-4 h of symptom onset is limited due to the specific kinetics of this protein in the injured myocardium (5).The identification of patients with elevated troponin levels is especially useful for selecting the appropriate treatment in patients with of AMI (6).The RDW is a measure of the heterogeneity of red blood cell size obtained from red blood cell size distribution curves. This parameter has been shown to be predictive of morbidity and mortality in various cardiovascular diseases, such as heart failure, stable coronary artery disease and acute myocardial infarction (AMI) (7, 8). So looking into above aspect the present study was planned to study the levels of routine hematological parameters and red cell distribution width in Myocardial Infarction patients admitted at Dhiraj Hospital.

Materials & Methods:

The present study is the type of cross sectional type of study carried out in the department of biochemistry, Smt. B.K. Shah Medical Institute & Research center, Sumandeep Vidyapeeth Vadodara Gujarat after getting ethical approval Institutional ethical committee (Approval No- SVIEC/ON/MED1/Ph.D./18015*)*. A total 100 myocardial infarction patients with age group of 25-70 of either gender were collected from Dhiraj General Hospital, Sumandeep Vidyapeeth Vadodara Gujarat within the one year of duration. The age group were further categorized into 4 different categories with ten intervals. The selection of Myocardial infarction patients was made after review by cardiologist on the basis patient details, ECG and cardiac biomarker (above 99th percentile)

Patients with diabetes mellitus, chronic muscle disease, renal disease, autoimmune disease, arthritis, any inflammatory disease, recent surgery and any other disease except AMI were excluded from the study.

Approximately 3 ml blood sample was taken in EDTA vials from each and every Individuals having ischemic type of chest i.e. MI under all aseptic precautions and mix well and wait for 30 minutes. The parameter of complete blood count (CBC) were analyzed by BACKMAN COULTER SYSMEX Kx-21N. The patient confidentiality was mentioned at each and every levels.

Statistical Analysis

Data were analyzed with the help of software program Statistical Package for Social Sciences (SPSS version 21, Chicago). The statistical differences between cases and control were determined by student independent sample t-test. The p-value is less than 0.005 ($P \leq 0.005$) were considered as statistically highly significant.

Results and Observation

Table- 1 observation of pathological parameter in MI patients

Parameter	Mean ±SD
Age	52.56 ± 11.56
Hemoglobin (gm %)	13.70 ± 1.71
Red Blood Cells (mc/µl)	5.36± 4.90
Total Leukocyte count (cells/ cumm)	12370.12 ± 10702.42
Platelet (lakh/ µl)	276480.00 ± 108331.19
Red cell Distribution Width (%)	17.60± 1.89*

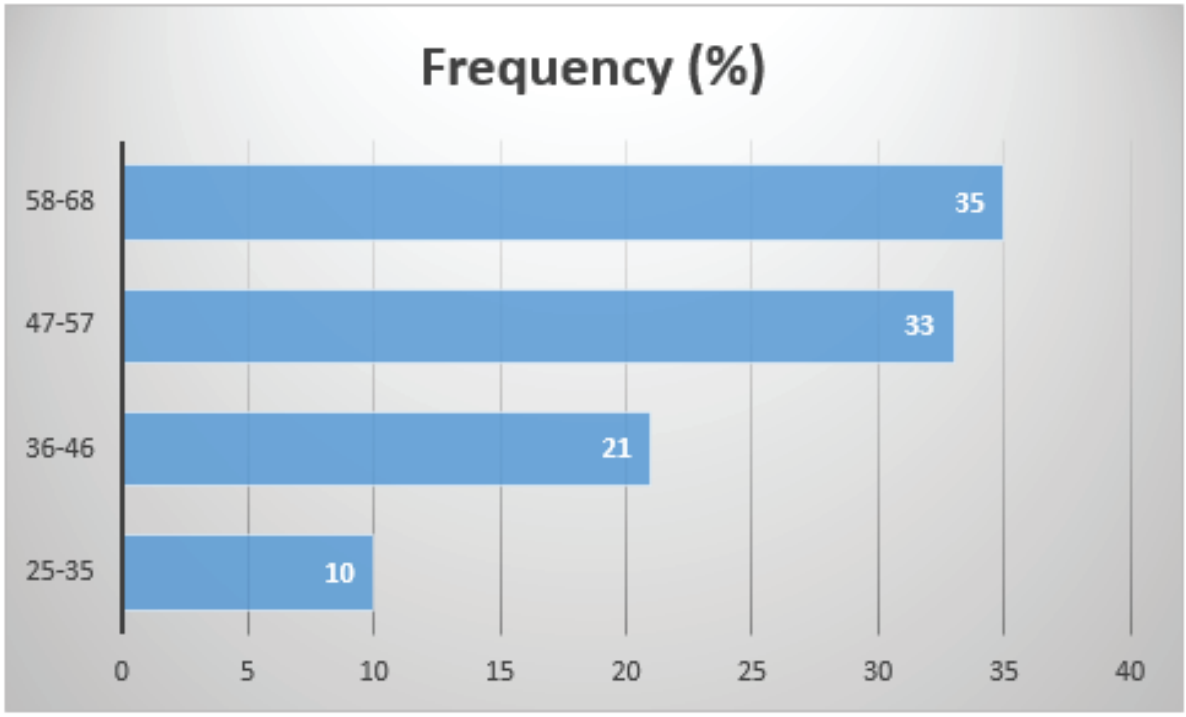

Figure-1 Cluster bar showing the percentage of MI patients in different age categories

Table – 2. Observation of pathological parameter in various age group categorization.

Age Group	Hemoglobin (gm %)	Red Blood Cells (mc/µl)	Total Leukocyte count (cells/cumm)	Platelet (lakh/ µl)	Red cell Distribution Width (%)
25-35	13.81 ± 1.86	5.32 ±1.11	10166.67±2454.08	316777.78 ±84241.88	18.29 ±2.38**
36-46	13.85 ±1.44	4.98 ±0.98	11914.29 ±5273.98	253666.67 ±126141.72	15.53 ±1.82
47-57	13.48 ±1.63	6.27± 8.58	11312.59± 4220.13	274906.25 ±102639.16	14.67±1.92
58-68	13.63± 1.88	4.82±0.84	10601.69 ± 5051.11	284137.93 ±116591.39	18.50±1.80**

Discussion

In clinical laboratories, the cycles of each test starts with the preparation of patient, and continues with biological sample collection, preparation and incubation the sample in suitable conditions and finally finishes by reporting the result. It is sometimes necessary to redo a test to obtain a reliable result. Sometimes there is a period of time between sample collection and doing or redoing a test.

Complete blood count (CBC) is one of the most common and routine laboratory tests that is the first step to diagnose an illness and since this test is become easy and quick, it can give valuable information to the physicians. [9]

In the current study, we have observe various pathological parameter in myocardial infarction patients and fond average age of MI patients is 52.56 ± 11.56. That means the middle group ages people getting affected more with myocardial infarction. This might be their sedentary life style and dietary pattern. The mean hemoglobin is 13.70 ± 1.71 which is within normal ranges indicates that the levels of hemoglobin is within the normal ranges. The mean levels of RBC count is 5.36± 4.90, the mean levels of leucocyte count is 12370.12 ± 10702.42, the mean levels of platelet count is 276480.00 ± 108331.19. The level of red blood cells and platelet were observed within the normal ranges. This may be due to the supplementation of thrombolytic agent. The mean levels of Total Leucocyte count were observed increases or found above the normal ranges, which may be due to rupture of the vasculature or its involvement in the pathophysiology of the myocardial infarction or ischemia.

We have also observed the number of Myocardial infarction patients in different age group and found there is more number of MI in 58-68 age group i.e. 35, which means the middle age group of having more prone towards myocardial infarction.

We have also observed the levels these pathological parameter in various age group and fond all the pathological parameter were lying in normal ranges.

Red blood cell distribution width (RDW) is a parameter routinely measured by most modern hematology analyzers. RDW is defined as the quotient of standard deviation of red blood cell volume and its mean volume and is expressed as a percentage according to the following formula: RDW = (standard deviation of red blood cell volume/mean cell volume) × 100. Higher RDW values reflect greater variations in red blood cell volume. [10]

RDW is an important risk factor for both mortality and cardiovascular events in patients with SCAD or acute coronary syndrome. It has not yet been determined whether anisocytosis is the cause of the poorer prognosis observed among these patients or if it is simply a marker of multiple pathological states connected with said prognosis. In contrast to the markers of inflammation and oxidative stress, which are not routinely analyzed, RDW provides valuable information concerning prognosis in patients with CAD [11]. It is believed as per the various reports that the Red cell distribution width (RDW) is one of the predictor biomarker for the myocardial infarction. In this study we have also observed the RDW status in myocardial infarction patients and found significantly higher (17.60± 1.89). We have also found the level of RDW in various age group and observe increased levels in 18.29 ±2.38 in 25-35 and 18.50±1.80 in 58-68 age group respectively.

Conclusion

RDW is one of the predictive biomarkers for diagnosis of myocardial infarction with cardiac markers especially cardiac troponin I (cTn-I). Platelet levels can be monitored in MI patients in early phase to find out the status of clot and other pathological parameter can be done as a part of routine laboratory investigation.

Source of Funding: - Self

Conflict of Interest: - None

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Original Study

Knowledge and Awareness about Identification of Domestic Violence, Abuse and Neglect of Victim among Dentists

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Abstract

Domestic violence is an age-old social evil which remains hidden from the eyes of the society for long. The incidence of domestic violence (DV) is at epidemic proportions of violence, abuse and neglect of any individual have a significant impact on the social system. Majority of the studies report violent and abusive injuries involve the head and neck region but many times dentists fail to report abuse. The various reasons could include loss of patients, economic loss and fear of dealing caregivers or of getting involved. Above all these, the majorly lack of sufficient knowledge about identification uncertainty of diagnosis and agencies where to report. The purpose of this paper is to assess the knowledge regarding identification of domestic violence, abuse and neglect and reporting agencies of maltreatment.

Aim & Objective: The aim of the present study is to assess knowledge and create awareness regarding identification of domestic violence, abuse and neglect and reporting agencies of maltreatment among dentists of Mysuru city of India.

Method: A self-administered, structured questionnaire written in English given to the dentists who give their consent.

Results: 91.7% (n=100) of the participants had heard about Domestic Violence, neglect and abuse and 73%(n=67) had come across victims of Domestic violence, neglect and abuse. Among them, 9.2% (n=10) had come across them frequently and 32 (29.8%) occasionally.

51 respondents said that the domestic violence victims are adult females, elderly female patients (16.6%) followed by children between 12 to 15 years of age (13.7%)

Conclusion: Many dentists will come across and identify the victims in their practice, but only few dentists will make an attempt to report. The main reason being lack of knowledge and proper training. Thus, there is a need for all of us to get trained in this aspect. Recognizing reporting the violence is everyone's responsibility.

***Key Words**: Domestic Violence, forensic dentist, Dentist, Knowledge & Awareness, Abuse And Neglect.*

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Introduction

Violence, abuse and neglect of any individual has a momentous impact on the social organization. Majority of the studies report violent and abusive injuries occupy both head and neck section, which places dentists at front of abuse discovery.

Domestic violence (DV) is an age-old social evil which remains hidden from the eyes of the society from long time. The incidence of domestic violence it is at epidemic proportions.[1,2,3]

Abusers establish and maintain power over victims through the use of a variety of coercive tactics, which may include physical, emotional, sexual and financial abuse. Domestic violence also otherwise called intimate partner violence is a problem of epidemic proportion. In recent years it has emerged as a significant risk factor for many chronic health problems including obesity, substance abuse, mental disorder and gastro intestinal problems.[3]

With large amount of domestic voidance occurring in both region, dentists have the unique opportunity to play a role in conducting routine examination of victims and identify the victims.[2]

The dental experts are the first peoples to watch noticeable injuries on the patient's head and neck together with ligature marks, scratches, abrasions, scrapes, and bruises.[4-7] Dentists have a chance to play a proactive job in helping these casualties. Reporting is the beginning of protection.

In spite of the dental specialist's remarkable opportunity, a national review appeared; just 41/% dental specialists conceded that they consistently screened for abusive behavior at home just when they see persistent with injury of head and neck.

Many times, dentists fail to report abuse. The various reasons could include loss of patients, economic loss and fear of dealing caregivers or of getting involved. And majorly lack of sufficient knowledge about identification uncertainty of diagnosis and agencies where to report.[7]

When victims are identified and referred to appropriate resources, they can receive necessary care and counseling therefore affording protection from further violence, disease, and also death and helping to break the cycle of violence from harming future generation.[10]

Most patients have positive views of their dentists and trust them that the dentists also have social responsibilities.[11]

A Countrywide survey of dentists discovered that those who had acquired any training about domestic violence were more likely to screen for domestic violence and to intervene than those who had obtained none.[12] it seems that Little has been achieved to discover positive approaches to instructing or education dentists to verify or deal with domestic violence. While a preceding learns confirmed our tutorial to be effective for dental students,[13] domestic violence training for dentists has no longer been addressed in any posted Report.[14]

Recognizing reporting the violence is everyone's responsibility. In India, the commonness of abusive behavior at home ranges from 6-60%,, with considerable variation across the states[3]

India is a sub continent with a wide variation in its population distribution, socio financial differences and cultural beliefs and taboos, these beliefs have induced social domestic violence is a displayed as social supremacy especially concerning intimate partner violence.

The intention of this paper is to assess the knowledge concerning identification of domestic violence, abuse and neglect and reporting agencies of maltreatment.

Aim & Objective

The aspire of the present study is to evaluate the knowledge and create consciousness regarding identification of domestic violence, abuse plus neglect in addition to reporting agencies of maltreatment among dentists of Mysuru city of India.

Methodlogy

A self-administered, structured questionnaire written in English was given to dentists who were eager to take an interest in the examination and give their consent. A self-administers, prepared questionnaire written in English will be given to the dentists who give their consent. The investigator visited the dental clinics and hospitals on a scheduled day and issued the questionnaire to the dentist in their respective work places and once the questionnaire was filled collected it personally. Members were approached to react to everything in the poll by picking the most proper choice. Privacy and Secrecy of the respondents was assured.

Ethical clearance was obtained from Institutional ethical committee of JSS Dental College and Hospital, affiliated to JSS Academy of Higher Education and Research, Mysuru.

Questionnaire included 15 close ended questions related to domestic violence identification and reporting. the self-administered questionnaire was in english language. The questionnaire be validated by few experts in the field of dentistry in Mysore and those experts were not considered for the main study. The data thus collected will be retrieved and will be amiable to statistical analysis using SPSS software version 22. Descriptive statistics such as Mean, Standard Deviation, percentages, in addition to frequencies was applied for data analysis.

Results

The total no of participants were 109 practicing dentists of the city. Though there are about 216 no of dentists only those who consented to participate were given the questionnaire. The results of the study with the response from participants is depicted as follows. 91.7% (n=100) of the participants had heard about Domestic Violence, neglect and abuse and 73%(n=67) had come across victims of Domestic violence, neglect and abuse. Among them, 9.2% (n=10) had come across them frequently and 32 (29.8%) occasionally.

51 respondents said that the domestic violence victims are adult females, elderly female patients (16.6%) followed by children between 12 to 15 years of age (13.7%). Of the 87 respondents for finding difficulty in recognizing the victims, 42 said they had found some difficulty and 46 of them did not have any difficulty.

Among 86, 47(43%) elicited reason for violence and 38(34%) of them could not elicit the reason from the victims. The major reason quoted by the dentists for not eliciting the reason for domestic violence in because of lack of proper training in how to elicit the reasons and other reason being most commonly the victim is accompanied by the abuses themselves. Few others were worried of offending the victims by asking about the violence and loosing practice time and also loosing patients because of being inquisitive, thus the patients may not follow up the appointments.

Not many (74%) dentists knew that 50% of domestic violence injuries are mainly in head and neck region. Only 19 participants attempted to report the victims to the concerned authorities. Others did not know which are the agencies to report the victims (n=79). though 86 of the respondents felt that the dentists be able to definitely play a part in reporting domestic violence but they required adequate training to do so(n=97) among them, 75% preferred to attend a workshop to enhance their knowledge regarding ways to identify the domestic violence victims and how to elicit reasons and where to report them.

Discussion

Abuse is any act which endangers physical or emotional health of the individual. The abusers are generally victims own family members, their spouses, adult children or caretakers. Victimizers originate from varying backgrounds, paying little heed to financial status and instructive, family, strict or social foundation.

The various types of abuse could be Physical abuse, Psychological, abandon, financial exploitation and Social mistreatment.

Violence, abuse and neglect of any individual have a major collision on the social system. Majority of the studies[15-20] report violent and abusive injuries involve the head and neck region, as these areas are exposed and accessible and because the head often is considered representative of the whole being, The dental experts are among the primary people to watch obvious wounds and checks on the patient's head and neck, which places dental specialists at the front line of misuse discovery.

In the article, Domestic Violence and its relation to Dentistry: A Call for Change in Canadian Dental Practice,[15] The authors discuss about highest victims of domestic violence and abuse are women as is found similar in our study also, worldwide women are considered as the weaker section of the society thus victimized in domestic violence. Violence against women is measured to be an significant public health problem for women and their children.

The next most affected by domestic violence are children, similar to our study few other studies which claim that the abused children have shown lowered

intellectual abilities (difficulty in reading, writing, scoring) when compared with the non abused children, these children have displayed overall deterioration in their psychological development. Very often these children also are witnesses of other forms of domestic violence in their families like their mother or grandparents being the victims of domestic violence making even more impact on their psycho social behavioral changes. [21-25]

Apart from women and children the other population most abused is the elders as is found even in our study. A study done by Raju and Prakash, in 2010 found that the elder individuals distress from depression, sickness or physical impairments were more at risk of being abused than those of similar age and normal health status. This implies that an elderly with physical or mental impairments may be perceived as a burden by the care-givers. Such stressful and bitterness spills out in form of abuse and neglect of elderly people. [21]

The authors in an article titled "Changing Dentists'' facts, attitude, and behaviors regarding Domestic Violence through an Interactive Multimedia Tutorial" mentioned regarding a tool called "AVDR," an acronym for "asking, validating, documenting, and referring." AVDR means: 1) asking the patient about the injury; 2) providing validating messages that battering is wrong and it is not the victim's fault in any circumstance; 3) documenting signs, symptoms, or any verbal disclosures that the patient has shown or given in the patient's records in writing and with any pictures; and 4) referring victims to a DV specialist, which may include counselors, authorities, or shelters[3,26]. This was developed to explain and simplify the dentist's role in addressing DV. This intervention can be used when abuse is suspected but not disclosed, and it allows dentists to help their patients. Hence, AVDR tutorial is a quick & simple way to educate oral health professionals about the importance of recognizing DV among their patients and learning how to help them by providing strategies for assessment and intervention.[26,27]

Few studies [28-33] have shown brief domestic violence intervention training can be effective in improving knowledge and attitudes among dentists, but could lead to false confidence in staff and should be followed by in-depth practical training and the advancement of proper procedures for managing misuse casualties.

Because dentists routinely assess a patient's head, neck and mouth, they have a unique and excellent opportunity to recognize whether or not a patient is being abused.[31,33]

Conclusion and Recommendation

Though many dentists in the study did come across and identify the victims, very few made an attempt to report. The main reason being lack of knowledge and proper training. Thus, there is a need for all of us to get trained in this aspect. Recognizing reporting the violence is everyone's responsibility.

Dental specialists like forensic dentists have a chance to play a proactive job in helping these casualties. Owing to the fact victims are mostly adult females, extra caution to screen for maltreatment should be an integral part to be taken to while examining the patients of this age group.

Since most of them were not aware of the reporting agencies, Dental offices can aid community awareness of abuse in different ways. Pamphlets on abuse, resource materials from agencies that help the victims can be displayed.

This study was a sincere effort put forward to understand the awareness of dentists regarding domestic violence which is a social crime. In this attempt we found out the dentists are interested to learn and know more about the domestic violence identification, counseling and reporting agencies by forensic dentists. These are the areas which are not thought explicitly in the course curriculum of dentistry in India. In this juncture, Workshops and inclusion of topics in curriculum may be planned and arranged.

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Original Research Article

Histopathological Study of Urinary Bladder Neoplasms

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Abstract

Inroduction:- Urinary bladder tumor is the 7th most common tumor worldwide**.** Urinary bladder carcinoma has varying type of histological variants and complexity with an aggressive biological behaviour.

Methods:- A total of 37 cases of urinary bladder neoplasia [histological proven] of patients admitted in a tertiary care teaching hospital were studied retrospectively over the period from June 2019 - Oct 2020 in the Department of Pathology. On the basis of histology, these cases have grading by the means of WHO(2016)/ ISUP classification.

Result:- Out of 37 cases, male were more commonly affected (28 cases) than female (9 cases). A peak age incidence of cases was in the 6th decade.

Conclusion:- In our study, males (75.67%) are more frequently affected compared to females. The most frequent neoplastic findings in urinary bladder is invasive papillary urothelial carcinoma (75.66%).

Keywords: *Invasive urothelial carcinoma, Urothelial carcinoma with glandular differentiation, Squamous cell carcinoma of urinary bladder.*

Introduction

Neoplastic lesions of urinary bladder present with higher rate of morbidity and mortality. On global scale with tumor burden it ranks 7th. Amongst all urinary bladder carcinoma, the most common lesion is urothelial carcinoma which stands for ninty percent of all cases of urinary bladder carcinoma.[1] According to Indian Cancer Registry data for male, it ranks 9th most common cancer, which stands for 3.9% of all cancers.

Urinary bladder carcinoma has varying type of histological variants and complexity with an aggressive biological behaviour. Inspite of advances in surgical techniques along with intravesical and systemic therapies, rate of disease progression, recurrence and eventual death with non-muscle invasive urothelial carcinoma and muscle invasive carcinoma are up to 30% and 50% respectively.[2] Risk factors from external environment like smoking, schistosoma hematobium infection forms the basis for etiology of bladder cancer and points out it's biological plausibility as well.[3]

Thus, it is important to be familiar with physical examination, cystoscopic evaluation, and histopathologic analysis as far as contemporary bladder cancer diagnosis and treatment are concerned.

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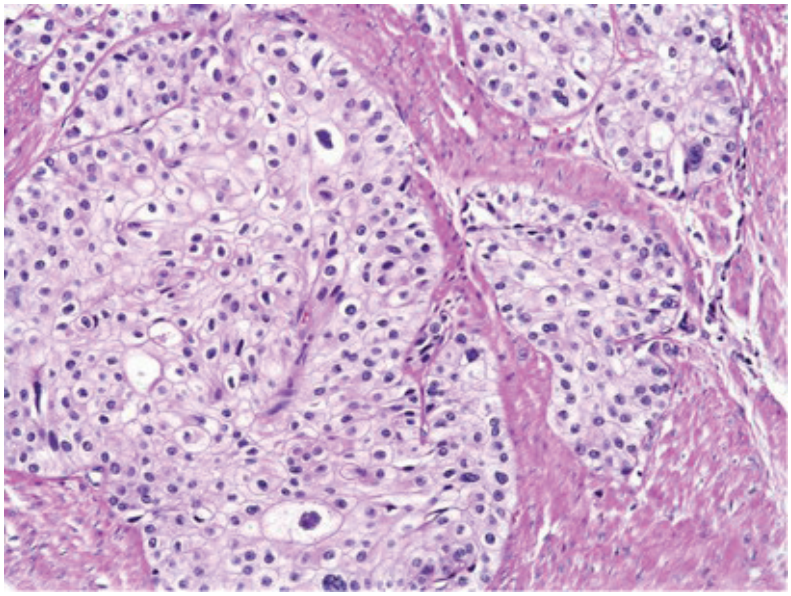

Figure-1: Invasive urothelial carcinoma.

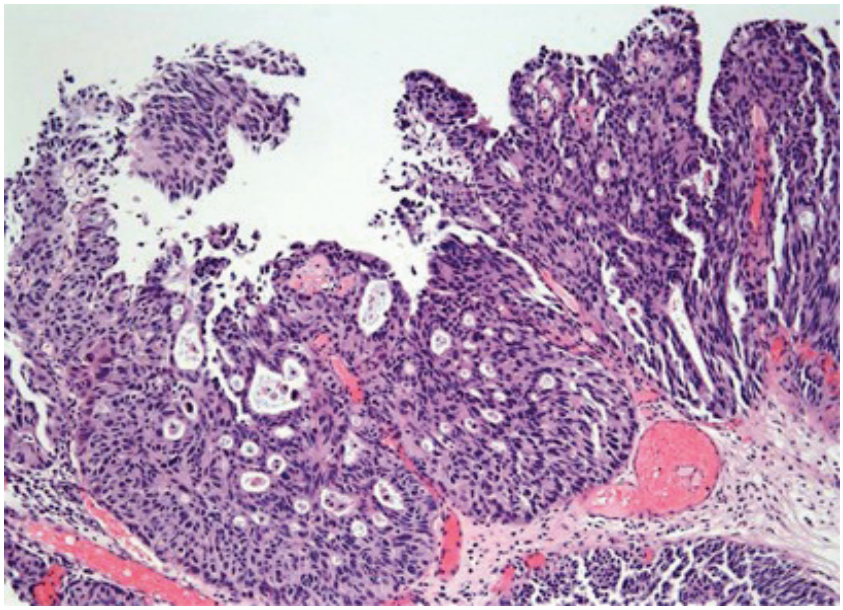

Figure-2: Urothelial carcinoma with glandular differentiation.

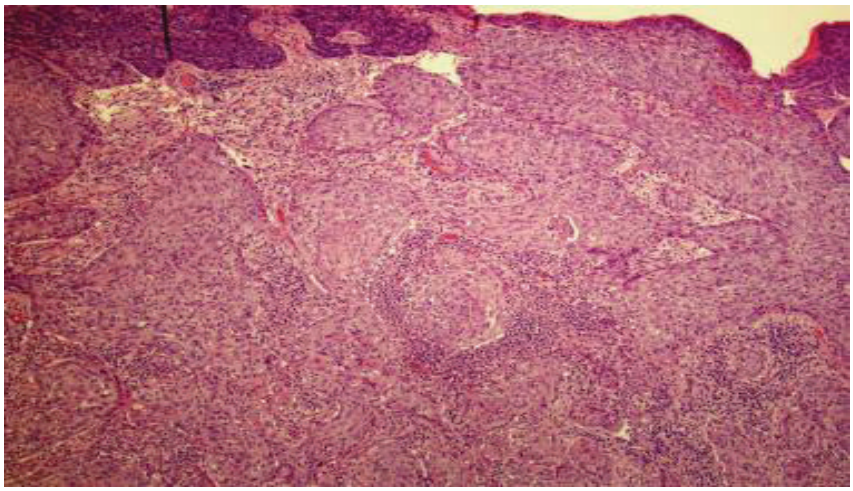

Figure-3: Urinary bladder squamous cell carcinoma.

Aims and Objectives

The aims and objectives of present study are:

1.To show the histopathological features of varying type of neoplasms in the urinary bladder lesions.

2.To quantify urinary bladder neoplasms with varying types in reference to age and sex distribution.

Material and Methods

A total of 37 cases of urinary bladder neoplasia [histological proven] of patients admitted in a tertiary care teaching hospital were studied retrospectively over the period from June 2019- Oct 2020 in the Department of Pathology.

Cystoscopic biopsies received were submitted as whole after measuring the size.

Radical cystectomy specimen were cut opened, fixed in formalin and size of bladder, both ureters, urethra, both seminal vesical (in male), prostate (male) was noted.

Entire external surface of bladder along with prostate was painted.

All surgical margins (both ureter, seminal vesical, urethra, vas deferens) were submitted along with at least 4 sections of tumor.

Additionally, random section from the uninvolved wall were taken and submitted.

Tissue were fixed and preserved in formalin, then passed through ascending grade of alcohol and xylene and finally embedded in melted paraffin wax.

Then blocks were prepared, single block was made for each section, thin sections of 4 to 5 microns thickness were cut, slides were prepared and stained by H & E stain.

On basis of histology these cases have grading by the means of WHO(2016)/ISUP classification.

Inclusion Criteria:

*It includes cystoscopic biopsies and radical cystectomy biopsy for the present study.

Exclusion Criteria:

Autolysed Specimen, Inadequate Biopsies

Observations and Results

A total of 37 cases, 28 urinary bladder biopsies (TURBT) and 9 cystectomy specimen were studied over the period from June 2019 to Oct- 2020 in the Department of pathology.

TABLE 1: INCIDENCE OF URINARY BLADDER NEOPLASMS WITH RESPECT TO GENDER

Histological type	Male	Female	Total	Percentage(%)
Leiomyoma	**0**	**1**	**1**	**2.70**
PUNLMP	**1**	**1**	**2**	**5.40**
Non-invasive Urothelial Carcinoma,Low Grade	**2**	**2**	**4**	**10.81**
Invasive Urothelial Carcinoma, Low Grade	**15**	**2**	**17**	**45.94**
Invasive Urothelial Carcinoma,High Grade	**8**	**3**	**11**	**29.72**

Squamous cell Carcinoma	**2**	**0**	**2**	**5.40**
Embryonal Rhabdomyosarcoma, Botryoid type	**0**	**0**	**0**	**0**
Total	**28**	**9**	**37**	**100**

Males were more commonly affected than females. In the present study there were 28 male patients (75.67%) and 9 female (24.32%) with M:F ratio of 3.11:1

TABLE 2: CLINICAL PRESENTATION OF URINARY BLADDER NEOPLASMS

Complain	Male	Female	Total	Percentage(%)
Incidental USG finding	1	0	1	2.70
Abdominal pain	1	1	2	5.40
Burning Micturition	1	1	2	5.40
Hematuria	26	6	32	86.48

In symptoms hematuria stands at top with 86.48% cases followed by burning micturition (5.4%), abdominal pain (5.4%) and incidental USG finding (2.7%).

TABLE 3: AGE – WISE DISTRIBUTION OF URINARY BLADDER NEOPLASMS

Age group (Years)	Total no. of patients	Percentage(%)
<30	1	2.70
30-39	3	8.11
40-49	7	18.92
50-59	8	21.62
60-69	**10**	**27.03**
70-79	7	18.92
80-89	1	2.70
Total	**37**	**100**

Age of the patients in our study ranged from 1 year to 80 years with highest number of cases (10cases) in the 7th decade, followed by 8 cases in the 6th decade.

Discussion

The present study has laid emphasize on need of biopsy examination in the diagnosis of bladder lesions. Now a days, a synergetic approach of cystoscopy, histopathological biopsy, and urine cytology are in mainstay for diagnosis in urinary bladder lesion and its monitoring.[4]

In our study, the focus is on the histopathological diagnosis along with histological grading and staging of bladder tumours. For histological grading we used, WHO/ISUP (2016) classification in our study.

TABLE 4 : Comparison of Age-wise distribution of urinary bladder neoplasms with different series.

Age group (years)	Present study (%)	Vaidya et al5 2013 (%)	Shreshtha et al6 2016(%)
<30	2.70	2	2
30-39	8.11	4	8
40-49	18.92	7	21
50-59	21.62	20	29
60-69	27.03	33	30
70-79	18.92	20	7
80-89	2.70	7	3

In our study highest number of cases are in 60-69 year age group comprising 10 cases followed by the 50-59 year age group comprising 8 cases . The above observation has well similarity with Vaidya et al[5] and Shreshtha et al.[6]

TABLE: 5 Comparison of Male: Female Ratio with other series

Study	Male:Female Ratio
Present study	3.11:1
Shah et al7 (2016)	2.29:1
Vaidya et al5 (2013)	4.5:1
Lim et al8 (2009)	5.0:1

As shows in above table , the male to female ratio from various series highlights the association of bladder neoplasms and increased male susceptibility with wide variation in ratio. Male preponderance can be subjected to higher rate of cigarette smoking in male and more industrial exposure in male than female.

Conclusions and Summary

Total 37 TURBT as well as Radical Cystectomy specimens submitted to Department of Pathology at our institute during June 2019 to October 2020 were studied clinically and Histopathologically.

In our study, most of the bladder tumors seen in cystoscopic biopsies as well as cystectomy specimen were of urothelial origin.

Maximum numbers of patients are in age group 60-69 years (27%)

Males (75.67%) are more frequently affected as compared to female.

The most frequent Neoplastic findings in Urinary bladder is Invasive papillary Urothelial carcinoma (75.66%) .

Squamous differentiation was seen in 7.4% of urothelial carcinoma.

Most of these tumors were low grade Invasive urothelial carcinoma.

A biopsy or TURBT may show different grade from the one that will be found in the surgical specimen because it may happen that biopsy was taken from different area.

Leiomyoma (~2%) is rare tumors arising in bladder while pure squamous cell carcinoma comprising of ~5%.

It is important to recognize that grading is highly subjective and that in future various molecular and immunohistochemical studies will provide better reproducibility.

Ethical Clearance: Taken from Institutional ethical committee.

Sources of Funding: Self-funding.

Conflict of Interest: None

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Melatonin Receptor A Gene, Melatonin Receptor C Gene and Ovocalyxin-32 gene polymorphisms associated with some Egg Production in Local Iraqi Chicken

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Abstract

The aim of this research is to detect genetic polymorphisms of some candidate genes associated with egg production trait and to find the relationship between some of these genetic sites and the productive traits of Local Iraqi chickens that will be used for the national poultry breeding programs to increase quality egg production in endogenous chicken. Genomic DNA were extracted and then amplified three genes (melatonin receptor a gene MTNRa, melatonin receptor c gene MTNRc and ovocalyxin-32 gene (OCX-32). (MTNRa) gene amplified by PCR-SSCP method. The distribution of percentages in female Iraqi Local chicken studied for (MTNRa) were 73, 20, 5 and 2% for TN, TNA, NN and TT SSCP-genotype respectively. It turned out that phenotypic polymorphisms of MTNRa gene was significant ($p<0.05$) with Serum Melatonin and Magnesium concentrations, in addition to First egg weight FEW and Body weight at maturity. (MTNRc) gene amplified by PCR-RFLP method, the distribution of percentages of MTNRc genetic location in female Iraqi Local chicken Flock studied were 23, 61 and 16 % for MM, Mm, mm RFLP-genotype respectively, it turned out that phenotypic diversity of MTNRc gene was significant ($p<0.05$) in mean of Serum Melatonin and Magnesium concentration, in addition to First egg weight FEW and Body weight at maturity. (OCX-32) gene amplified by PCR-SSCP method. The distribution of percentages in female Iraqi Local chicken studied were 98 and 2% for WS and SS (SSCP-genotype) respectively. It turned out that phenotypic polymorphisms of OCX-32 genetic site were significant ($p<0.05$) with serum Melatonin and Magnesium concentration and Body weight at maturity. The present research suggests that the genes MTNRa, MTNRc and OCX-32 could be used as a possible marker in Marker Assisted Selection (MAS) and genomic selection programs for local Iraqi chickens.

Key words: *Local Iraqi chicken, genetic polymorphism, Melatonin receptor c, egg production traits, Local chicken, crossbreeding, breeding poultry and improvement, Melatonin receptor a gene, ovocalyxin -32 gene, OCX-32, MTNRa*

Introduction

Local Iraqi chickens are valuable genetic resources due to their adaptability to harsh conditions when raised in rural area or when reared in outer system as free-range chickens, these chickens responded well to improve their environment conditions, especially, nutrition and exhibited improvement in egg weight and egg mass, in addition, it was found that they classified as a good performance for egg production[1], because of breeding program for local chickens in developing countries are still out of competition with commercial breeding company that has access to technology advantages and economics of scale. It was strongly needed to establish breeding programs that allows improving performance of local chickens[2]. Iraqi chicken characterized by high viability to adapt to the prevailing environmental conditions such as their heat resistance and resistance to some of the endemic diseases. Also characterized by their efficiency to moderate productivity and their needed a simple diet, especially in the free-range system and rural breeding but the egg production chain

has to be short and variable range from one series to another for the same individual, and because of modern trends in the use of molecular genetics technologies for genetic improving in the selection programs[3]. Egg production is a polygenic inheritance trait with low to moderate heritability, which depends on the period-involved[4]. Multi traits selection to improve fitness and simultaneously increase egg yield is therefore difficult to accomplish by traditional, direct phenotypic selection. Thus, selecting individuals with additional information on their genotype for markers associated with QTLs for fitness and reproduction (marker-assisted selection, MAS) is preferred[5] Molecular markers were used to map QTLs related to chicken growth and reproduction such as body weight (BW), Egg number (EN), and Egg Mass (EM) in the past decade[6]. So, the using of molecular marker in breeding has a huge advantage, for the time being, molecular markers are widely used in poultry breeding[7], thus, the aim of the present paper was to detect genotype polymorphism for egg production traits genes of local Iraqi chickens.

Materials and Methods

The experiment was conducted in poultry farm in the Ministry of Science and Technology/ Agricultural Research Directorate / Animal resources and fisher's center/ poultry department and DNA laboratory of Biology department college of Science/ Babylon University during the period from 20/2/2019 until 15/8/2020, it has conducted on 100 Local Iraqi chickens raised at an experimental farm, where all hens were individually kept in cages. The hens in the period of laying eggs (28-47 weeks old) were mated with male Iraqi chicken at ratio of 1 cock: 10 hens.

Genotypes of candidate genes were determined by PCR-RFLP and PCR-SSCP techniques. extraction and purification of DNA from hen's blood[8], amplification of DNA by using appropriate primers corresponding to each gene and determination of the genotype by electrophoresis band on 2% agarose gel. Details of the primer sequences, annealing temperature, and other details are presented in Table1.

Table 1 Sequences of PCR primers for genomic DNA

Genetic position	accession number	Primer sequence (5` – 3`)	Chr. number	Length (bp)	Annealing temperature (C°)	Reference
MTNRc SNP	NC_006091	(F): GGTGTATCCGTATCCTCTAA (R): GACAGTGGGACAATGAAGT	4	372	52	Li, 2013[6]
MTNRa mRNA	NM_205362.1	(F): CCTGGCAATTGCAGACTTGG (R): TTCTGGGTCAACAGCCACAG	4	595	63.1	Designing genes, using NCBI BLAST method
OCX-32	AM076826.1	(F): TGCAGTCAAGTAAATCAGGGTAGA (R): ACTAGCAAAGTTTTTCAGTAGGTCC	9	589	60	

PCR-RFLP and PCR-SSCP Analysis

An *MboI* recognition site was created by the MTNRc SNP. The PCR products of this locus were digested with the restriction enzyme *MboI* at 37°C for 20 minuets, ran on a 2% agarose gel, and stained with red safe to detect the SNP by cleavage of the MTNRc amplicon. PCR-SSCP analysis of the MTNRa and OCX-32 SNP was performed according to Sivaraman[9]

Traits and statistical analysis

Associations between genotype and reproductive traits were assessed using one–way ANOVA, were used to find the association between the categorical variables,

P value (P≤ 0.05) was considered statistically significant. SPSS, Statistical Analysis System, Version24, 2019. Significance of the least squares means was tested with the Duncan's Multiple Range test.

Results

The present study includes (100) samples as local Iraqi chicken's flock. The genetic positions of this study were detected genetically in order to investigate if there are any genetic relationship for these genetic loci with Egg production trait.

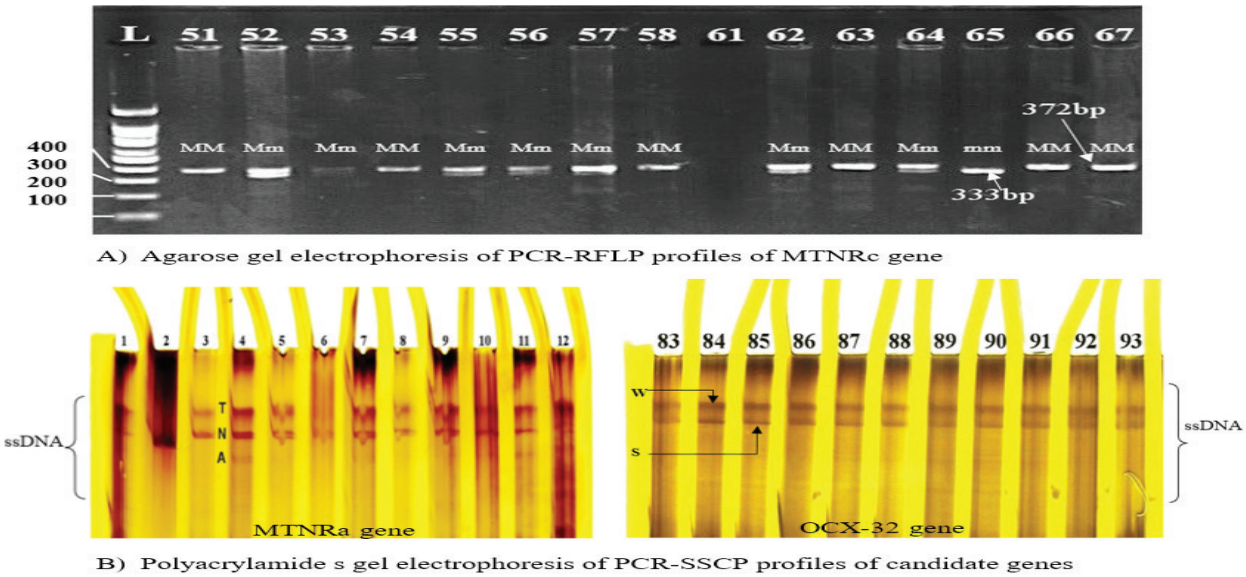

Figure 1: Gel electrophoresis of (A) PCR-RFLP and (B) PCR-SSCP profiles of candidate genes.

Figure 1 (A) shows the electrophoresis results of PCR products that were digested with *MboI* restriction enzyme for MTNRc gene and Figure 1 (B) shows the electrophoresis results of amplification of the target site of conformational polymorphism of the Melatonin receptor a and OCX-32 genes using PCR-SSCP method.

Table 2 Allele and genotype frequencies of genes in Local Iraqi chicken.

Genes	Genotype Percentage (%)				Allele			*HWE
MTNRa	TT	NN	TNA	TN	T	N	A	P≤0.01
	2%	5%	20%	73%	95%	97%	20%	
MTNRc	MM	Mm	mm		M	m		P≤0.01
	23%	61%	16%		0.54	0.47		
OCX-32	WS	SS			W	S		P≤0.01
	98%	2%			0.49	0.51		

*HWE: Hardy–Weinberg Equilibrium; significant differences (P≤ 0.01)

Table 3 polymorphism relationship of local chickens for some Physiological and egg production parameters.

Genes	Traits genotype (M± SE)	Serum Melatonin Hormone (pg/ml)	Serum Magnesium (mg/dl)	First egg weight (FEW)(gm)	body weight at maturity (BWM) (gm)
MTNRc	MM	132.62±19.378 a	2.96±0.067 a	34.14±0.93 a	1471±41.20 a
	Mm	92.77±5.046 b	3.23±0.102 a	32.68±0.513 ab	1324±20.32 b
	mm	100.28±8.241 b	2.5±0.242 b	31.23±0.902 b	1382±62.63 ab
MTNRa	TT	90.83±0.02 b	3.6±0.01 a	29.7±0.03 b	1108±0.04 b
	NN	120.46±12.865 a	2.1±0.4 b	35.34±0.968 a	1283±70.68 ab
	TN	106.61±7.392 ab	3.13±0.081 a	32.64±0.509 ab	1390±24.361 a
	TNA	92.47±7.78 ab	2.97±0.216 ab	33±0.792 ab	1332±24.279 ab
OCX-32	WS	103.97±5.82	3.043±0.08	32.81±2.419	1332.69±25.651
	SS	62.3±0.01	3.7±0.01	31.35±0.35	1201.25±23.67
	Sig.	0.000	0.000	NS	0.000

M: Mean; SE: Standard error; different letters per class indicates significant differences (P≤ 0.05); NS: non-significant

The detection of allelic and genotypic frequency plays an important role in animal breed selection. Based on these frequencies, assumption and their association with phenotype traits would support to create new individuals with desirable genotype and phenotype. The frequencies of MTNRa, MTNRc and OCX-32 alleles and genotypes are shown in Table 2 and the Genetic and Phenotypic correlations of candidate genes showed in Table3. At the MTNRa polymorphic locus, N allele showed higher frequency than T and A alleles and accounted for 97% in the population. Moreover, the appearance of T allele was higher than A resulting in highest percentage of TN genotype in Local Iraqi chickens (73%). The analysis of MTNRc which restricted by *MboI* restriction enzyme, amounted to 23%, 61% and 16% of the genotypes are MM, Mm and mm respectively, meaning that spread pronounced the heterozygous Mm genotype, followed by found the pure homozygous MM and mm genotype and when followed Allele frequency for MTNRc gene in Table 2 showed 0.54 for M allele and 0.47 for m allele. the percentages of phenotypic occurrences of the exon OCX32 gene in local Iraqi chicken showed high significant differences ($p<0.05$) for the different genotypes, which amounted to 98% and 2% of the genotypes are WS and SS respectively, meaning that there is a clear prevalence of

the heterozygous formations carrying the WS genotype, followed by rarely found the pure homozygous SS genotype and the frequency ratios for alleles were (98%) W haplotype allele and (100%) for S haplotype allele In addition to the disappearance of the genotype WW. When corelated relationship of the polymorphism of MTNRa gene on some of the physiological parameters of important PCR-SSCP genotypes, it was found a significant difference ($p \leq 0.05$) with (melatonin and magnesium) and from the significant value of Serum concentrations of melatonin 120.46 (pg / ml) for the NN genotype, and were set a significant at 3.6 and 3.1 (mg/dl) magnesium concentration for TT and TN, respectively, there was a significant difference among genotypes with FEW and BWM, significant value for FEW (35.34 gm.) at NN genotype and BWM (1390.02gm.) at TN genotype. Correlation polymorphism of MTNRc gene on Melatonin (pg/ml) and magnesium (mg /dl) serum concentrations record a significant difference ($p \leq 0.05$) and the highest value recorded by MM genotype, and the results found that there is a significant difference ($p \leq 0.05$) by the mean weight of the first egg (FEW) 34.14 gm. and body weight at sexual maturity (BWM)1471.04 gm. for the genotype (MM). The corelated relationship of the polymorphism of OCX-32 gene on some of the physiological parameters of important PCR-SSCP genotypes, it was found that there was a significant difference for physiological parameters (melatonin, and magnesium) and from the value of Average serum concentrations of melatonin for the WS and SS genotypes were set at 103.97 pg/ml and 62.3 pg/ml respectively, serum concentrations of magnesium for the WS and SS genotypes were set at 3.043 mg/dl and 3.7 mg/dl respectively, egg production trait polymorphism relationship results found that the mean weight of the first egg (FEW) for the genotypes (WS, SS) was 32.81and 31.35 grams, respectively, and since no significant differences between the genotypes were identified but for body weight at sexual maturity (BWM), it became apparent that they recorded significantly the highest value ($P<0.05$) (1332.69) gm. For WS genotype, while the lowest value of 1201.25 gm was recorded by the genotype (SS)

Discussion

Egg production is an important economic trait in the poultry industry[5]. Our findings confirm significant impact of melatonin receptor a (MTNRa) on female reproductive traits (FEW and BWM) in addition to significant difference ($p \leq 0.05$) with serum Melatonin hormone (pg/ml) and serum Mg(mg/dl) concentration in local Iraqi chicken, some of these results similar to Li[10] which studied association of three melatonin receptor genes with reproductive traits in Erlang Mountain chicken and reported that chickens at MTNRa studied by sequencing produced their first eggs earlier with genotype EE (but, perhaps consequentially, eggs of lower weight). In poultry breeding programs, egg number at 300 days of age (EN) is used as the most valuable indicator of total egg production potential. Recently, many researchers have sought correlations between markers of candidate genes and reproductive traits in chickens [11; 12; 13]. The MTNRa gene studied in this study are located on chromosome 4 to which highly significant QTL effects on production traits have been mapped in previous studies[14;15;10]. Interestingly, the expression of the MTNRa mRNA has been found lowest level in small yellow follicle and increased in the granulosa layer of the chickens' ovarian follicles[16] but relationship between MTNRa gene polymorphisms and seasonal reproduction activity associated with melatonin concentration is what influences the ovarian activity leading to its effect on the resulting eggs and egg production in general[17]. The search for molecular markers that influence reproductive traits of chickens has been well reported by[18;15; 19; 20]. Ommeh[21] identified polymorphisms in the GnRHR (gonadotropin-releasing hormone receptor) and NPY (neuropeptide Y) genes but discovered no association of the two polymorphisms with total egg production. Li[10] was found specified polymorphisms a statistically significant association of the MTNRa with EN, demonstrating that animals with the FF genotype produce their first egg at a lower age (AM) and produce more eggs at 100 days of age after maturity (EN) than those with the EE, EF ($P < 0.05$). AL-Rekabi[2] concluded that, there is a clear significant difference for phenotypic polymorphism of the genotypes of the growth hormone (GH) gene and its receptor (GHR) on quality and egg production traits in local Iraqi chickens. in present study a statistically significant association of the N allele in both studied flocks, suggesting strong balancing selection (overdominance). Previous studies have found that the GnRH-I gene, vasoactive intestinal polypeptide receptor-1 (VIPR-1) gene[22],

follicle-stimulating hormone receptor (FSHR) gene[18], significantly affects age maturity or broodiness. In the current study we have shown that allele N in Local Iraqi chicken for MTNRa gene are associated with (BWM, Melatonin Hormone, Magnesium and FEW). The Hardy-Weinberg equilibrium (HWE) is the primary starting point for all genetic investigations of populations, whether the goal is to discover or estimate the effects of all forces disrupting HWE[23]. Determining the presence or absence of a deviation from it is important, because it allows inferences regarding the effects of selection or other factors that shape the population composition (i.e., the general trend of animal husbandry can be traced by Hardy Weinberg equilibrium) and then form the study of the relationship between different allelic variants of the target genes Relevant economic features of different strains or productivity trends are the next step to explore the success of its purpose[24]. In our current study, it is important to continue the resulting flock subjecting to Hardy Weinberg equilibrium because the purpose of this research maintaining the traits of local Iraqi chickens that Distinguishing, (like a high viability to adapt to the prevailing environmental conditions such as their heat resistance and their needed a simple diet, especially in the free-range system and rural breeding) subject to Hardy Weinberg equilibrium[1] Egg production is a complex process that not only involves the reproductive system but also depends on the availability of specific nutrients and the efficiency of their utilization. For example, melatonin, enhance the egg laying productivity of hens. Higher serum melatonin levels in hens associated with more egg production, increasing promoted the maturation and development of oocytes[25]. There are few reports that are related to the effects of melatonin application on the yield of egg production in hens, the egg-laying rate was positively associated with blood melatonin levels. Furthermore, melatonin implantation at a dose of 10 mg significantly improved their egg-laying rate. In addition to the increase in the egg-laying rate, the total egg weight, and also improved the quality of the eggs[26]. We found there was a significant increase in the concentration of melatonin hormone with the genotype MM for MTNRc gene Table (3), which means MM genotype of the MTNRc gene is associated with the significant increase serum melatonin hormone. our results similarly recent studies which found Birds with the AG (Mm at our study) genotype for the MTNRc SNP had shorter AM than those of AA (MM) genotype for the MTNRc SNP, lacking the MTNRc *MboI* restriction site, exhibited statistically significantly higher WFE ($P < 0.05$), they exhibited statistically significantly lower EN values ($P < 0.01$) than those with both the GG (mm) and AG (Mm) genotypes that were homozygous and heterozygous for the restriction site, respectively [6; 22]. The organic matrix proteins involved in eggshell formation and the important role of *OCX–32* during the termination phase of eggshell formation. Moreover, *OCX–32* expression levels were related with Quality and Egg Production Traits[27]. There are several reports in the literature of variation within this gene and subsequent examination of its influence on various shell quality traits[27;28;29]. The results of our study similar to [28]Takahashi,2010 which identified haplotypes of OCX32 utilizing three SNPs within exons 2, 3, and 4 in an F2 generation of a line cross. In view of the extreme filtering applied to the consistency traits of the eggs in local Iraqi chicken, this tends to be a very large number. Maybe the heterozygosity of the OCX32 protein confers a selective benefit, thus leading to the preservation of genetic diversity in the OCX32 gene[29]. From the results Ovogalyxin-32 gene Table (3) in this gene we found that there is a highly significant difference $p \leq 0.05$ with serum Melatonin hormone, serum Magnesium and Body weight at maturity that mean this gene closely related with these phenotypes (PHTs). As the ovocalyxin–32 SNPs are located in gene coding regions with nonsynonymous substitutions of the protein sequences as deposited in the DDBJ database, the association between haplotypes and these parameters suggests that these substitutions may be directly responsible for these phenotypic alterations[28]. Fulton[29] conclude that the presence of multiple PHTs has consequences for the application of whole genome selection and high-density SNP analysis in which SNPs are assumed to be in high LD with all surrounding variants. This is correct if only two haplotypes occur within a line but erroneous if multiple haplotypes occur. The haplotype analysis of OCX32 presented here clearly showed that just two haplotypes occur are common in local Iraqi hens, it is plausible that the statistically significant linear trends manifested by PHTs in local Iraqi chickens which reflected ongoing selection targeted to the chromosomal region surrounding the OCX32 gene in the studied flock. The wide variation in some physiological parameters

between species of birds may be due to the circadian rhythms, the effects of diet, gender, and age. Like we assume that the increase in the serum magnesium level was due to increased biosynthesis and accumulation in the egg yolk[30]

Conclusions

1- Local Iraqi chicken responsive to the Hardy Weinberg equilibrium law for MTNRc genetic location by PCR-RFLP.

2- Phenotypic diversity used by PCR/RFLP of MTNRc gene was significant ($p<0.05$) in mean of melatonin concentration, body weight at sextual maturity, serum Mg conc., FEW.

3-Phenotypic polymorphisms of MTNRa gene were significant ($p<0.05$) with serum Magnesium concentration, First Egg Weight, body weight at sextual maturity, and serum melatonin hormone.

4- phenotypic polymorphisms of OCX-32 gene was significant ($p<0.05$) with serum melatonin hormone, Mg concentration and BWM.

5- the genes MTNRa, MTNRc and OCX-32 could be used as a possible marker in Marker Assisted Selection (MAS) and genomic selection programs.

Conflict of Interests: The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests**.**

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Ethical Approve: We declare that the study does not need ethical approval.

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Short-term Evaluation of Water Sorption of Glass Ionomer Cements

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Abstract

Background: The bonding of cement materials to enamel and dentin may be affected by dimensional changes which may be happened after water absorption of the cement when it is in water when the filling used by the person. The aim of our research was to see how much water may be noticed inside prepared cements compared with those with other traditional cements.

Method and materials: tow light-cured (1 and 2) and another two chemically-cured (4 and 5) were prepared. Two types of cements used by the Iraqi dentists also used, first one light-cured (No.3) and the other was chemically-cured (No.6) . Thirty samples were produced having 4mm height and 9mm diameter. Five samples from each type of cement were prepared. Then we record the weight of each sample was recorded after setting of the material before putting them in water using very. To remove water from the specimen material a desiccator was used for one day. Samples were kept in water. After different immersing times of 5,10,15,30, and 60 minutes, one, three, seven, ten day, and fourteen the weight of each sample was recorded. The value recorded for water absorbed was rounded to the nearest 0.1mg/cm^2. **Results:** light-cured cements absorb more water than chemical-cured cements. The highest value was noticed for cement (5) then for cement number (2). The least value was noticed for cement number (3).**Conclusion:** cements cured by light absorbed more water than chemically-cured cements. Modifying cement´s powder components showed no effect on water sorption, while liquid chemical composition showed noticable effect on water absorbed bythe tested cements.

Key words:*, light-cured, chemical-cured, Water sorption, glass ionomer cement.*

Introduction

Water absorbed by any material represents the amount of water that adsorbed on the surface and that amount absorbed within the body of a material during the manipulation or when the filling used by the person in. Serious dimensional changes and warpage in the material may be happened after high amounts of water absorbed. That water is measured gravimetrically in µg/mm^2 after few days of immersion in water. [1] A modification of glass cements by replacement the polyacid with one grafted with unsaturated polymerizable hydrophilic resins facilitated the production of resin-modified (light-cured) cements. HEMA which is a hydrophilic resin is introduced in the composition as a cosolvent. It will react or copolymerizes with the modified polyacid. Due to this chemical structure the cements are expected to absorb water easily. [1]

Materials and Methods

Preparation of glass powders:

The solid part or powder of glass cement consisted mainly of silica (SiO_2), alumina (Al_2O_3), and calcium fluoride (CaF_3). Other minor constituents were added such as cryolite (Na_3AlF_6) to lower the fusion temperature of the powder mixture. Two powders were prepared containing the following compositions:

Powder I: The materials, their amounts, and weight percent are described in Table (1).The alumino-silicat ratio for powder I formula was 0.68 and fluoride contents was 42.2%.

Powder II: This powder was modified to produce new formula (table 2). The alumino-silicat ratio for this powder formula was 0.57, while total fluoride content

was 29.2%.

Table (1) Components of powder I

Material	Weight(gram)	Weight%
SiO2	57.7	27.0
Al2O3	39.0	18.3
CaF3	37.4	17.5
Na3AlF6	52.8	24.7
AlPO4	26.6	12.4

Table (2) Components of powder II

Material	Weight(gram)	Weight%
SiO2	70.4	37.0
Al2O3	40.2	21.2
CaF3	40.2	21.2
Na3AlF6	15.2	8.0
AlPo4	24.0	12.6

Preparation of the liquid part:

The liquid part for the tested glass ionomer cements was prepared from the aqueous solution of polyacrylic acid (PAA) in a concentration of 50% which is a yellowish viscous liquid. The Maleic acid is also added, as a white powder, to form a copolymer with polyacrylic acid, and to prevent increased viscosity of the acid over the time. Tartaric acid was also added. These materials were mixed until the powder acid particles were dissolved and water was finally added. Hydroxyethylmethacrylate (HEMA) material as a liquid was also used. Two types of light-cured glass ionomer cements were produced, cement (1) composed of (powder I) and (light-cured liquid), cement (2) composed of (powder II) and (light-cured liquid).

Two types of chemical-cured cements were produced, cement (4) composed of (powder I) and (chemical-cured liquid), and cement (5) composed of (powder II) and (chemical-cured liquid). Two types of commercial cements were used for comparison purposes as control cements. Cement number (3) which was cured by light and cement (6) which was a chemical-cured.

Preparation of specimens:

Specimens of the cements were made by preparing the moulds from pink modeling wax to create a mould of 9 mm diameter and a height of 4 mm. A celluloid strip was fixed below the mould. The cement powder and liquid were mixed to 3:1 ratio for 30 seconds and packed into the mould to one side to avoid entrapping of air into the mixed material. Another celluloid strip was fixed on the top of the mould and the material was pressed between two celluloid strips for five minutes and it was left to set for one hour. For light-cured materials, the mixed cement material were packed inside the mould and then light-cured for 60 seconds from both sides. Five samples were prepared from each type of cement. Specimens with 4 mm height and 9mm diameter were produced after setting of cement material, the specimens were removed from the moulds and finished by stone burs and then by sandpaper.

Water sorption measurement:

After one day of setting and before immersing in distilled water the weight of each specimen was recorded using weight measuring balance. To absorb water from the specimen material a desiccator containing silica gel material with moisture indicator was used for 24 hours. After that the weight of specimens were recorded to obtain the net weight of the specimen material. Then specimens were kept inside closed containers with 20 milliliters distilled water at room temperature (25ºC ± 2). After that the weight of the specimen recorded after removing them from water after different immersing times of 5,10,15,30,60 minutes, one, three, seven, ten and fourteen days until the weight stay unchanged in the last three readings means no more water absorbed.

Table (3) Water sorption values within 24 hours.

Type of cement	Weight of absorbed water (gm)	Coefficient of water sorption (mg/cm2)	Final value of water sorption coefficient (mg/cm2)
1	0.0753	10.88	10.9
2	0.1146	16.56	16.6
3	0.0314	4.54	4.5
4	0.1016	14.68	14.7
5	0.1171	16.92	16.9
6	0.0575	8.31	8.3

After each immersing time, the specimens were removed with tweezers and passed it on dry, clean paper until their surfaces became without superficial moisture and exposed to stream of air for fifteen seconds. After one minute from the removal from water the specimen was weighed. The difference in weight of each specimen will show the amount of water absorbed by each specimen during the different times of water immersion. The final value of the water absorbed was calculated to the nearest 0.1mg/cm^2 as follows:-

Results

The mean value of amount of water absorbed by the specimens during ten days period of time, and coefficient of water absorption (mg/cm^2) for each type of tested cements were presented in Table (4). Generally, the results of water sorption of the tested cements revealed that, chemical -cured cements showed lower values than light-cured types. The greatest water sorption value was noticed for cement (1). While the lowest water sorption value was recorded for cement (4). Figure 1 showed the distribution of water sorption values of all tested cements within ten days. The mean values of water absorbed by specimens of tested cements within 24 hours were presented in table (3). The high value recorded in our work of water sorption test was for chemical-cured with powder II cement (5) followed by light-cured with powder II cement (2). Both of these cements contained powder (II) in their consistency.

Table (4): Water sorption values within ten days

Type of cement	Weight of absorbed water (gm)	Coefficient of water sorption (mg/cm2)	Final value of water sorption coefficient (mg/cm2)
1	0.0271	3.92	3.9
2	0.0250	3.61	3.6
3	0.0250	3.61	3.6
4	0.0208	3.01	3.0
5	0.0259	3.74	3.7
6	0.0222	3.21	3.2

The low value recorded was for light-cured commercial cement (3). No clear difference was observed between the resin-modified and conventional glass ionomer cements, but there was an increased amount of water absorbed by the conventional types of cements than their counterparts containing the same type of powder. Figure (2) showed the distribution of different values of the amount of water taken up by the cement specimens of the different tested groups within 24 hours of water immersion.

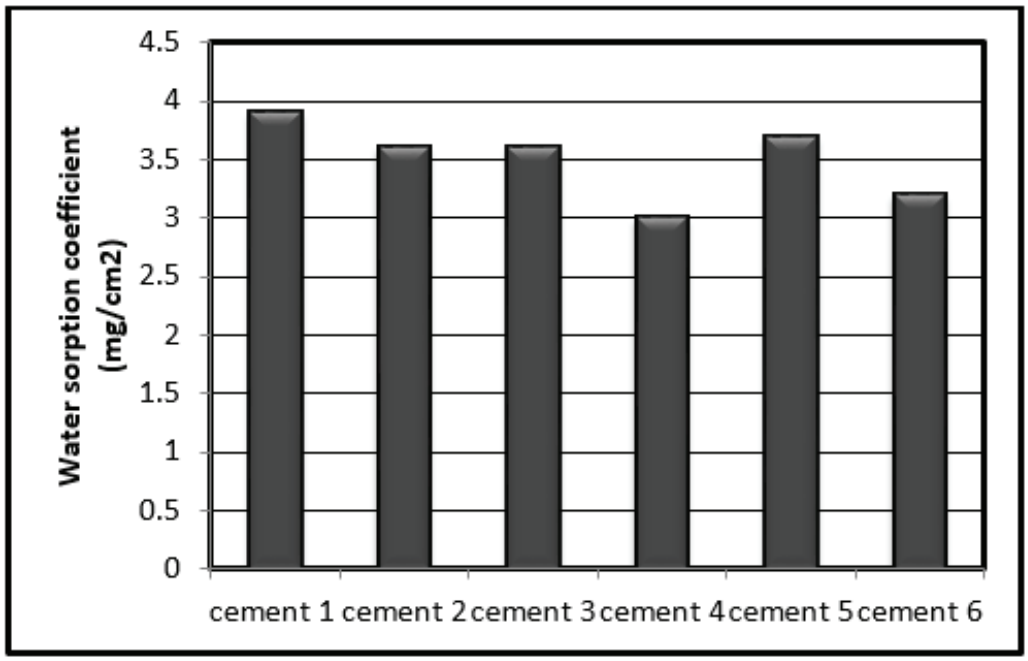

Figure (1) Water sorption values after ten days.

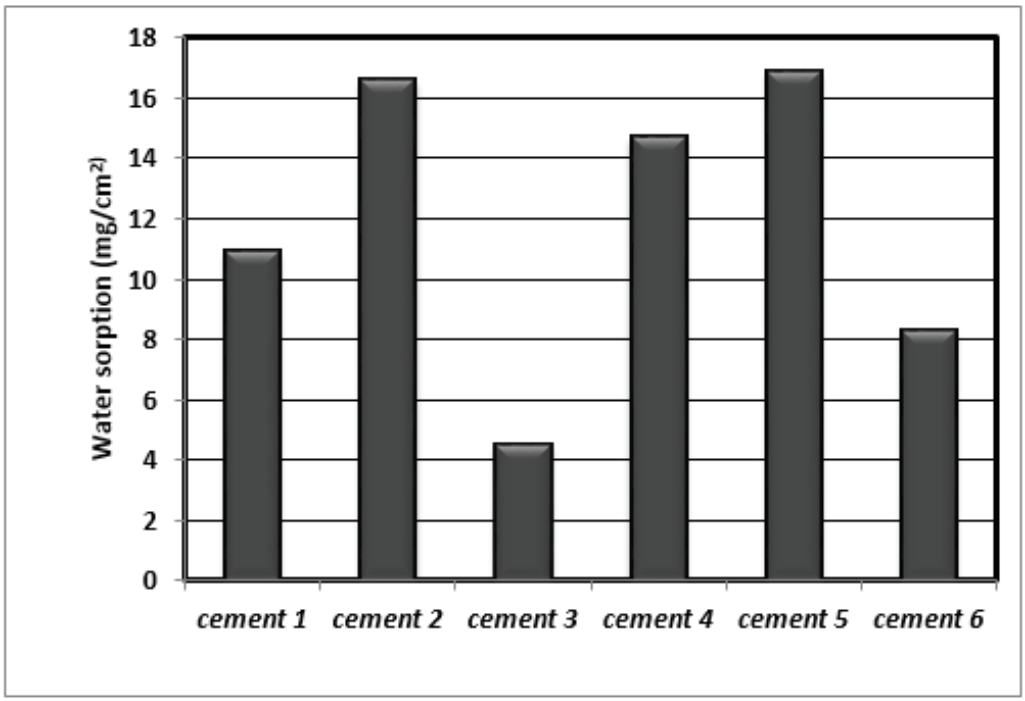

Figure (2) Water sorption after 24 hours

Discussion

Conventional glass ionomer cements absorb (2%) water when placed in an aqueous medium reaching to equilibrium in amount depend on the ratio of powder to liquid employed in cement formation and the liquid formation. It has been found that cements formed from high powder/ liquid ratio contains less amount of water within the ionic polymer structure, which may be attributed to low water absorption capacity.[2] HEMA has been used as major component in cement cured by light because it acts as both cosolvent and comonomer. To increase their solubility and make a workable solution, a polymerizable monomer like HEMA is incorporated. [3] The incorporation of this polymerizable hydrophilic resin in resin- modified cements expected to absorb water easily.[1] From the results of Cattani et al (1999) it was found all types of tested specimens absorb great amount of water during the first 24 hours. The chemical-cured types of glass ionomer cements absorbed more water than the light-cured types of glass ionomer cements.[1] In this study a similar results were found. After 24 hours of immersion in distilled water, chemically-cured glass ionomer cements absorbed greater amounts of water than those light-cured. The amount of water absorbed from cements in this study differs from those in other studies due to: -

1- Different cements have been used with their different compositions.

2- Different methods of water absorption measurement were used.

From the results found by Akashi the polymeric matrix of light-cured glass ionomer cements is considered hydrophilic in its nature and for this reason it may absorb water with time. While with chemical-cured glass cements, the water absorption is less.[4,5] Yap (1997) and Ellakuria et al (2003) found similar results. The results of our study in pointing out that the greater amount of water absorbed by the cement material may be due to the presence of high proportion of functional hydrophilic groups contained within the matrix of these materials.[6,7]

Comparing the amount of water within the set cement immersed in water, for a period of seven days (in the work of Wilson)[8] and for ten days (in this study), there was the same picture of water absorption where the resin-modified type of cements absorbed water more than conventional cements except cements (2 and 5) were both of them contained powder (II) which was the modified powder containing high alumino silicate ratio which probably has some effect on this property of cements. The chemical composition of the cement may be the reason for greater amount of water absorbed. The composition of glass ionomer cement which contains ion leachable glass and polycarboxylic acid may also bind water molecules. This may also explain the effect of changed powder formula on the acid-base reaction of cements. Cross linked matrix of polymer formed by photo-polymerization of HEMA which contains polar hydroxyl group. However, this type of polymer is sensitive to water uptake.[1]

Conclusions

Within the limitation of this work the following conclusions were drawn:Within the first 24 hours conventional cements absorb water more than resin-modified cements. Chemical-cured cements absorbed less water than light-cured cements after 24 hours. Changed cement formula did shown effect on water absorbed.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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Profile Quantitative Hepatitis B Surface Antigen (qHBsAg) of Chronic Naïve Hepatitis B Patients in Dr. Soetomo Hospital, Surabaya, Indonesia

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Abstract

This study aimed to evaluate the profile of qHBs Ag profile, and also to investigate the correlation between qHBs Ag and HBV DNA. Seventy samples of chronic-naïve hepatitis B patients in Dr. Soetomo Hospital were analyzed in a cross-sectional study. Patients were categorized according to the HBe Ag positive (n=30) and HBe Ag negative (n=18), also based on qHBs Ag 1000 IU/mL and qHBs Ag >1000 IU/mL. qHBs Ag was correlated with HBV DNA. qHBs Ag by CLEIA method from Sysmex, KOBE HISCL, HBV DNA was measured by real-time Polymerase Chain method from Gene Xpert, Cepheid. 70 patients naïve CHB treatment showed a median of ALT level 60.21±70.76 U/L. 30 patients showed a positive-HBeAg, 18 patients showed negative-HBeAg, 22 patients were not evaluated (N/A). Positive-HBeAg patients had 70% qHBsAg >2500 mg/dL and median HBV DNA 7.49×10^7 IU/mL. Negative-HBeAg patients had 55.6% HBsAg ≤1000 mg/dL and median HBV DNA 9.66×10^2 IU/mL. qHBsAg correlated with HBV DNA ($p < 0.001$). This data demonstrated that quantitative HBsAg was associated with a phase of HBV-infection, quantitative HBsAg showed a moderate correlation with DNA HBV, quantitative HBsAg levels might be a predictor of initiation therapy for CHB patients.

Keywords: *Chronic-Naïve Hepatitis B Patients, Hepatitis B, Quantitative Hepatitis B Surface Antigen, Surabaya.*

Introduction

Hepatitis B virus (HBV), a small DNA virus, is Estimated globally as much as 400 million people are infected with chronic HBV infection worldwide. A High prevalence can be found in developing countries such as Indonesia. HBV patients are approximately 4.0-20.3% of the healthy population of Indonesia. However, The java island proportion is lower than the other islands[1,2]. The risk of developing chronic HBV infection based on the age of infection was as much as 90% if infection occurred from perinatal to 6 months and 20%-60% if infection occurred between the age 6 of the month-5 years[3].

HBs Ag is a seromarker used routinely to diagnose acute or chronic viral hepatitis B, screening blood donors or organ donors, surveillance persons at risk of acquiring or transmitting (HBV)[1]. Quantitative HBs Ag has been proposed to be used as monitoring the course of chronic hepatitis B infection including the immune tolerance, immune clearance, immune control/inactive carrier phase as well as reactivated negative HBe phase[4]. The decline of HBs Ag levels could be an early predictor to know viral efficacy for HBV therapy[5].

Appearance and disappearance of HBs Ag in infected HBV infection persons generally adhere to characteristic patterns. The highest level of HBs Ag is in

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the immune tolerance phase, declines during the immune clearance phase, and decreases slowly but progressively in seroconversion HBe antigen. The lowest HBs Ag level is in the inactive carrier phase, but increases in HBeAg-negative patients[6].

HBsAg, HBV quantification, and HBe Ag may be useful biomarkers to monitor therapy for HBV infection[5]. The novelty of this research so far was that there were no studies for clinical implication research of HBs Ag quantitative in Indonesia up to now.

The present study aimed to assess the clinical implication of quantitative HBs Ag in chronic HBV infection. We sought here to demonstrate the potential utility of quantitative HBs Ag in reactive HBe-Ag and non-reactive HBe-Ag patients. We also investigated the correlation between quantitative HBs Ag and the level of HBV DNA.

Methods

This was a case-controlled study. As many as 70 treatment-naïve chronic hepatitis B patients were enrolled in this study. Inclusion criteria were as follows age between 18-70 years old, positive HBs Ag at least 6 months. Exclusion criteria were no other chronic hepatitis causes including autoimmune hepatitis, or other viral hepatitis such as hepatitis C virus, hepatitis D virus, or human immunodeficiency virus and no history of anti-HBV treatment. In addition, qHbs Ag was quantified by Sysmex, KOBE HISCL-5000 with detectability range of 0.05-2,500 mg/dL, HBV DNA quantification by Gene Expert, with a detectability range 10-1,000,000,000 IU/mL.

The frequency and median values were determined for categorical and continuous variables. The differences between groups were analysed using the Mann-Whitney U test for ordinate data, while Fisher Exact test with continuity correction for continuous dichotomous variables, and The Spearman Rank test for correlation analysis. Statistical analyses were performed by SPSS 17 version.

Results and Discussion

A total of 70 patients, including 39 (55.7%) male and 31 (44.3%) females, with a median of age 36.86±12.73 years. The baseline median of Aspartate Transaminase (ALT) level was 60.21±70.76 U/L. Of these patients, 22.9% had ALT >2 ULN mean 137.6±118.7 U/L, 77.1% had ALT >2 ULN mean 37.4±14.82 (U/L). Among this prevalence, 30 patients were positive HBe Ag, 18 patients were negative HBe Ag, and 22 patients were not evaluated (N/A). Characteristics of 70 chronic hepatitis B patients are shown in Table 1.

Table 1. Characteristics of patients.

Characteristics	Value (percentage)	Mean
Total sample (n)	70	
Gender Male/Female n (%)	39 (55.7%)/31 (44.3%)	
Age (n)	70	36.86±12.732
Total HBe Ag Reactive	30 (62.5%)	
Total HBe Ag-negative	18 (37.5%)	
ALT (U/L)		60.21±70.765 (U/L)
ALT >2 ULN (U/L)	16 (22.9%)	137.6±118.7 (U/L)
ALT £2 ULN (U/L	54 (77.1%)	37.4±14.82 (U/L)

Based on this result, 30 patients were classified as positive HBe Ag and 18 patients were classified as negative HBe Ag. Patients with positive HBe Ag showed higher AST and ALT than negative HBe Ag patients (p <0.05). Of these positive HBe Ag showed a median of AST 40 U/L (range 14-236) and median ALT 65 U/L (range 20-559). Besides, positive HBe Ag patients had ALT 2 ULN (60%) higher than ALT >2 ULN 40%. Negative HBe Ag patients who had ALT £2 ULN 77.1% were higher than patients who had ALT >2 ULN 22.2% (p=0.343). positive HBe Ag patients had a median HBV DNA lower than negative HBe Ag patients (p <0.001).

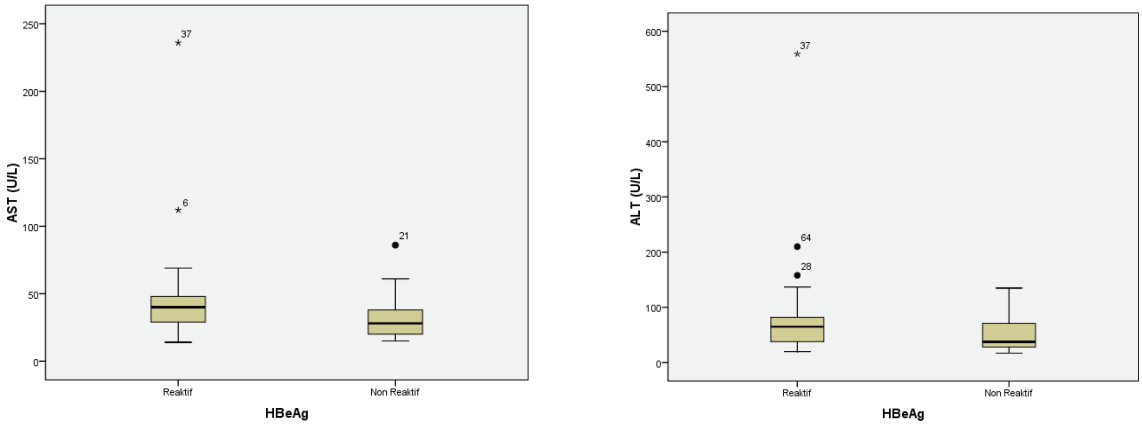

Figure 1. The differences AST and ALT levels between HBe Ag positive and HBe Ag negative.

Table 2. Baseline demographic data comparing HBe Ag positive and HBe Ag negative patients.

	HBe Ag Positive (30)	HBe Ag Negative (18)	p
AST	40 (14-236)	28 (15-86)	0.044[a]
ALT	65 (20-559)	37.5 (17-135)	0.041[a]
ALT >2 ULN	12 (40%)	4 (22.2%)	0.343[b]
ALT £2 ULN	18 (60%)	14 (77.8%)	
HBs Ag £1000 (mg/dL))	5 (16,7%)	10 (55.6%)	0.001[a]
HBs Ag >1000-2500 (mg/dL)	4 (13,3%)	4 (22.2%)	
HBs Ag >2500 (mg/dL)	21 (70%)	4 (22.2%)	
DNA HBV (IU/mL)	7.49×10^7 (26-7.22×10^8)	9.66×10^2 (10-2.71×10^8)	<0.001[a]

[a]Mann-Whitney test

[b]Chi square with continuity correction

However, between positive HBe Ag patients who had HBs Ag £1,000 mg/dL 16.7%, HBs Ag >1,000 mg/dL, 13.3%, HBs Ag >2,500 mg/dL 70%. negative HBe Ag patients who had HBs Ag £1,000 mg/dL 55% and HBs Ag >1,000 22.2%, HBs Ag >2,500 mg/dL 22.2% (p=0.001).

Table 3. HBV DNA levels in patient with HBs Ag >1,000 IU/mL and HBs Ag £ 1,000 IU/mL.

	DNA HBV (n)	Median	p
HBs Ag £1,000 IU/mL	27	7.70×10^3 (10-4.19×10^6)	< 0.001[a]
HBs Ag >1,000 IU/mL	41	5.00×10^6 (10-7.22×10^8)	

[a] Mann Whitney

Patients with HBs Ag £1,000 mg/dL had a median HBV DNA 7.70×10^3, patients HBs Ag >1,000 IU/mL had median HBV DNA 5.00×10^6 ($p < 0.001$).

Table 4. Correlation between HBs Ag and HBV DNA.

	HBs Ag vs HBV DNA	
Coefficient correlation HBs Ag vs HBV DNA	$r_s = 0.599$	p <0.001[a]

[a] Spearman correlation

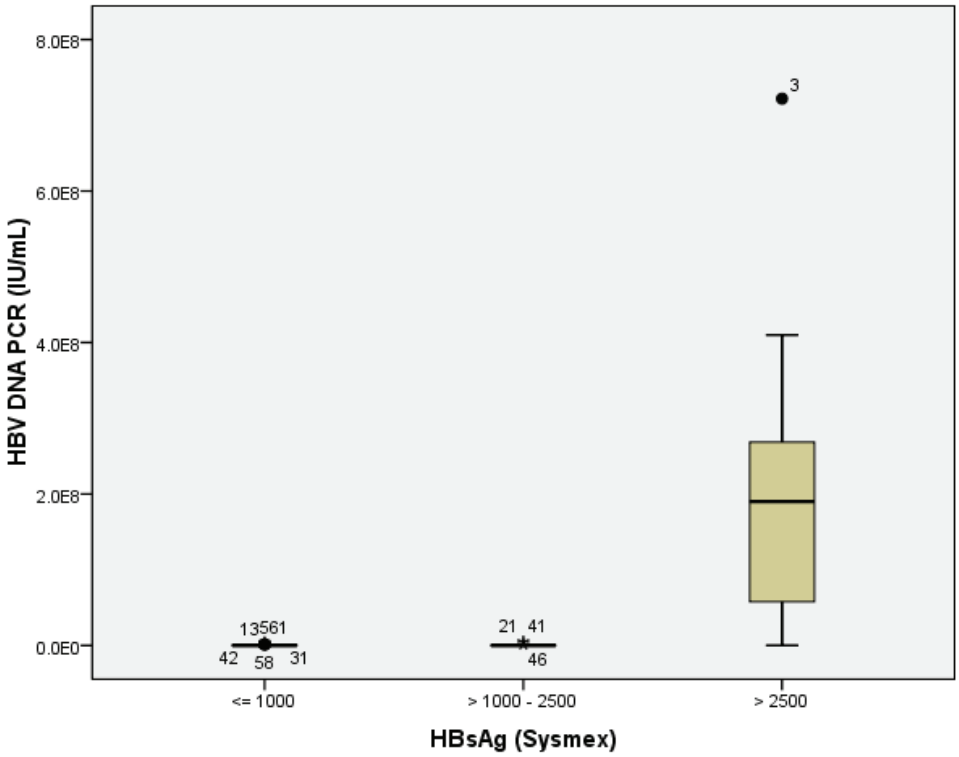

Figure 4. Correlation between quantitative HBs Ag and HBV DNA.

Indonesia is one of the major countries with a high prevalence of hepatitis B virus (HBV) infection. The endemicity of HBV in Indonesia is intermediate to high with a geographical difference. The most common HBV subgenotype in Indonesia is B3, followed by C1[7].

Chronic hepatitis B patients run through four phases, an initial HBe Ag positive-chronic infection Hepatitis B is characterized by high HBs Ag, positive HBe Ag, normal ALT, and null or minimal histologically damages. The second phase is HBe Ag positive chronic Hepatitis B, whereby the immune system recognizes HBV as a foreign-invader which can cause extensive liver cell inflammation. The third phase is HBe Ag-negative chronic infection or inactive phase showing persistently normal alanine aminotransferase (ALT) and lowers HBV DNA levels (<2,000 IU/mL). The fourth phase is characterized by late reactivation of infection with persistently or intermittently increased HBV DNA and ALT levels accompanied by progressive liver damage, then so-called HBe Ag- negative chronic hepatitis[8,9].

Based on the four phases of chronic hepatitis B, this study showed that among naïve Hepatitis B chronic patients, positive HBe Ag had higher AST and ALT than negative HBe Ag patients. Between positive HBe Ag patients, most of them had HBs Ag >2,500 IU/mL. Among HBe Ag negative patients, most of them had HBs Ag £1,000 IU/mL. Therefore 18 patients were immune tolerance, 12 patients were in immune clearance phase, 14 patients were in low replicative phase, and 8 patients were inactive carriers, and 10 patients were HBe-negative hepatitis B (data were not shown).

Positive HBe Ag patients had a higher HBV DNA than HBe Ag negative patients (median 7.49 x 10^7 IU/mL VS 9.6×10^2IU/mL) ($p<0.001$). Between quantitative HBsAg and HBV DNA showed a moderate correlation with a coefficient correlation $r_s= 0.599$ ($p<0.001$) especially in patients with quantitative HBs Ag >2,500 IU/mL. This finding was similar to a study by Gupta *et al* who stated that serum HBsAg correlates with HBV DNA in CHB patients, especially in high serum HBV DNA, HBe antigen-positive, and treatment-naïve group. Furthermore in a study from Pratiwi *et al.* who reported that quantitative HBs Ag had a significant correlation with HBV DNA in CHB patients[10]. These findings were similar to some previous studies, that HBs Ag titer and HBV DNA were higher in HBe Ag-positive patients than HBe Ag negative patients[4,11,12]. The kinetics of serum HBs Ag during the natural history of chronic HBV, infection have been studied, but the factors affecting them remain unclear.

HBs Ag titers varied significantly in different phases, with the highest in patients with HBe Ag-positive chronic HBV infection, while that among HBe-Ag positive patients, HBs Ag titers were correlated with HBV DNA [13]. These mechanisms are probably caused by the two different but cross-regulated synthesis pathways of HBs Ag and HBV DNA. HBs Ag is secreted in the serum as Dane particles and noninfectious filamentous or spherical subviral particles which are derived from different open reading frames of cccDNA[14], and can also be produced from HBV DNA integrated into the host genome[15,16]

In Another study from Thomas *et al*, HBe-positive patients showed a mean quantitative HBs Ag 5,410.17 IU/ml and mean HBV DNA 1.86×109 IU/ml with a correlation coefficient of -0.184 (p=0.256), while a mean quantitative HBs Ag 5,229.24 IU/mL and mean HBV DNA $1.8X10^7$ IU/mL with correlation coefficient -0.84 (p=0.256) while in HBe-negative patient. HBV DNA levels were significantly higher in positive HBe Ag patients, but there was no significant correlation between quantitative HBs Ag levels and HBV DNA[17]. HBe Ag-negative patients had higher levels of HBs Ag and HBV DNA, there was no significant correlation between HBs Ag and HBV DNA levels in CHB with predominant genotype D in Iran[18]. There are 2 forms HBs Ag, the first intact virion which includes small, medium, and large protein in the envelope, that is related to viral infectivity, the second are subviral particles in serum that are produced abundantly. These are predominantly S proteins and to a lesser extent are M and L proteins (these are not infectious but are strongly immunogenic, stimulating antibody production). This study suggested that examination should be used for two forms of HBs Ag and quantitate them, so the relationship between HBs Ag and HBV DNA could determined[18].

Monitoring quantitative HBs Ag has been suggested as a predictor of treatment response, especially for IFN- based therapy in chronic HBV-infection[4–6]. Since Chronic HBV infection had a highly dynamic based on

four chronological phases, this study aimed to determine HBs Ag levels in Reactive HBe Ag and non-reactive HBe Ag of HBV. HBs Ag levels were higher in reactive HBe Ag compared to nonreactive HBe Ag. We found a moderate correlation between HBs Ag and DNA HBV in chronic hepatitis B patients. The weak association between DNA HBV and HBs Ag in a narrow window of time for each HBV-phase may reflect a disconnection between HBV replication and HBs Ag production during persistent HBV infection. They are most likely due to various reasons such as the integration of HBV into the host genome that potentially provides a separate template for the production of HBs Ag or the cytokine dependant modification of viral replication pathways leading to disruption in the stability of cytoplasmic viral capsids[4]. Furthermore, the dissimilarity of quantitative HBs Ag as a predictor and discrepancies in studies about the association of HBs Ag and DNA HBV may be due to the highly dynamic nature of HBV infection as well as the influence of HBV genotype on HBs Ag-levels[4]. This study did not correlate the association between HBs Ag and HBV genotype.

Conclusion

In summary, these data demonstrated that (i) quantitative HBs Ag were associated with the phase of HBV-infection, (ii) while HBs Ag titers had higher in HBe Ag-positive CHB than HBs Ag-negative CHB, quantitative HBs Ag showed a moderate correlation with DNA HBV, and (iii) quantitative HBs Ag levels might be a predictor of initiation therapy for chronic hepatitis B patients. Further study with larger samples, examining genotype, monitoring therapy and liver biopsy as a need for future study.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical Approval: This study had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Role of Sonoelastography in Predicting Positive Sperm Retrieving in Patients with Non-Obstructive Azoospermia

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Background: Infertility is an important public health problem , almost 10-30 % is due to azoospermia. Elastography is a new ultrasound modality which simply could evaluate the internal elasticity of the testis & finally give an idea about the presence or absence of sperms.

Objective: To evaluate the validity of sonoelastography in predicting positive sperm retrieving in patient with azoospermia.

Patients and Methods: A cross sectional study performed in Al-Sader Teaching Hospital / fertility center & radiology department during the period from 1st September 2017 to 1st September 2018, included 98 patients /196 testes) with azoospermia by B-mode U/S, Doppler study (RI) & Elastography (strain ratio) , then biopsies obtained for all patients

Results: Only (136) testes were subjected to biopsy. Testicular volume & strain ratio were the most important factors which suggest that the testis will give a positive or negative sperm retrieving. Low volume + high strain NSR (bad prognosis) while high volume + low strain PSR (good prognosis).

These findings of SR were 75.7% sensitive, 93% specific, with 78% and 92% positive and negative predictive values, respectively and an accuracy of 89%.

Conclusion: Strain Elastography results have been validated and were differed significantly between patients with abnormal sperms count than those without. This Technique provides a promising way to get more accurate diagnostic results.

Keywords: *Azoospermia , Definition, causes, Types, Non-obstructive Sonoelastography*

Introduction

World Health Organization (WHO) and American Society Committee of Reproductive Medicine Practice (ASRM) defines infertility as "Inability of sexually active couples who do not use contraception to have a spontaneous pregnancy after a year of unprotected sex" Azoospermia, both obstructive and non-obstructive, affects almost 20% of cases with male infertility [(1)].

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Azaospermia mean "no sperm in the ejaculate in at least two semen analyzes and two semen samples obtained in two weeks interval" [(2, 3)] . Many causes of azoospermia are divided into pre-, post-, and testicular. Azoospermia can be as a result of inappropriate hormonal stimulation, impaired spermatogenesis or obstruction. In most cases, obtaining physical and laboratory data, including semen volume, testicular volume, presence of bilateral vas deferens, and serum FSH levels, helps to distinguish between the three categories [(4)].

Elastography is a method that has evolved and gained momentum over the past two decades. The

detected tissue stiffness can be made using the Young's modulus or elasticity (E), which is obtained as the ratio between stress (uniform compression) and the resulting deformations (Strains). The tissue stiffness can then be changed and presented in the form of an elastogram, which is usually color encoded [5]. There are two types of elastography; strain and shear wave elastography.

Patients and Methods

A cross sectional study was conducted from 1st September 2017 to 1st September2018, at Al-Sadder teaching hospital / the fertility center & radiology unit included 98 patients with azoospermia proved by seminal fluid analysis (SFA). In all patients, a thorough clinical examination was performed after a full medical history obtained from all patients. ultrasound study was done by specialist expert radiologist using B-mode, Doppler study & elastography (SR). ES always done before a testicular biopsy to avoid altering the elasticity of the testis with invasive tests with bleeding or scarring. Biopsy results were obtained by two embryologists with at least 5 years of experience in this field. The results of the biopsy was presented as "no sperms, 300K, 500K, 1M& 4M , etc. ."Where K & M represent number of sperms in thousands& millions respectively. Sonoelastography also done and the SR was calculated. Based on static image, average of at least three times assessment was reported for each testis.

Patient preparation after patients consent, standard examination and operation protocols were followed strictly. Statistical analysis and data management were applied with the aid of SPSS software version 20. Statistical analysis and tests were applied according to the type of variables at a significance level (P. value) of 0.05

Results

A total of 98 patients were enrolled in this study, of them (36) had a bilateral biopsy and the remaining (62) had a unilateral biopsy, the total number of testes was 136. The mean age of the patients was 31.7 ± 6.9 (Range : 20-60) years , the other features are shown in (Table 1)

According to the biopsy, the patients were divided into two groups ; group 1, those with positive sperm retrieving(PSR) included 33 testes and group 2 (103 testes) with negative sperm retrieving (NSR). Mean strain ratio is significantly higher in those with NSR (0.89±0.04) than with PSR 0.44±0.1,(P<0.05). Mean volume is significantly lower in those with NSR than PSR (8.25±0.4) vs. (10.4±0.5), respectively, (P<0.05), (Table 2).

No significant statistically correlation was found between volume and SR as (R= 0.100 and P. value= 0.300), (Figure 1).

Using Receiver Operating Characteristics (ROC) Curve analysis revealed an area under curve was 0.871 which is an indicator of an excellent test as shown in (Figure 2). Furthermore, when strain ratio at cutoff point of 0.385 cross-tabulated against Biopsy result (PSR and NSR), it showed a Sensitivity of 75.5%, Specificity 93.2%, positive predictive value of 78% , negative predictive value of 92.3 and accuracy of 89%, (P. value < 0.001), (Table 3).

Table1. Baseline characteristics of the patients

Variable			Range
Age (year) mean ± SD		31.70 ± 6.90	20 – 60
Weight (kg) mean ± SD		79.95 ± 7.92	61 – 96
Height (cm) mean ± SD		169.0 ± 6.0	155 – 186
BMI (kg/m²)mean ± SD		28.17 ± 2.65	18.8 – 34.8
Biopsy n (%)	Unilateral	62 (63.3%)	-
	bilateral	36 (36.7%)	-
Total		98 (100.0%)	-
SD: standard deviation			

Table 2. comparison of SR and testicular volume between NSR and PSR groups

Variable	NSR (n = 33)		PSR (n = 133)		P. value
	Mean	SD	Mean	SD	
SR	0.89	0.04	0.44	0.10	0.0001*
Volume	8.25	0.40	10.4	0.50	0.002*

SD: standard deviation
* Significant

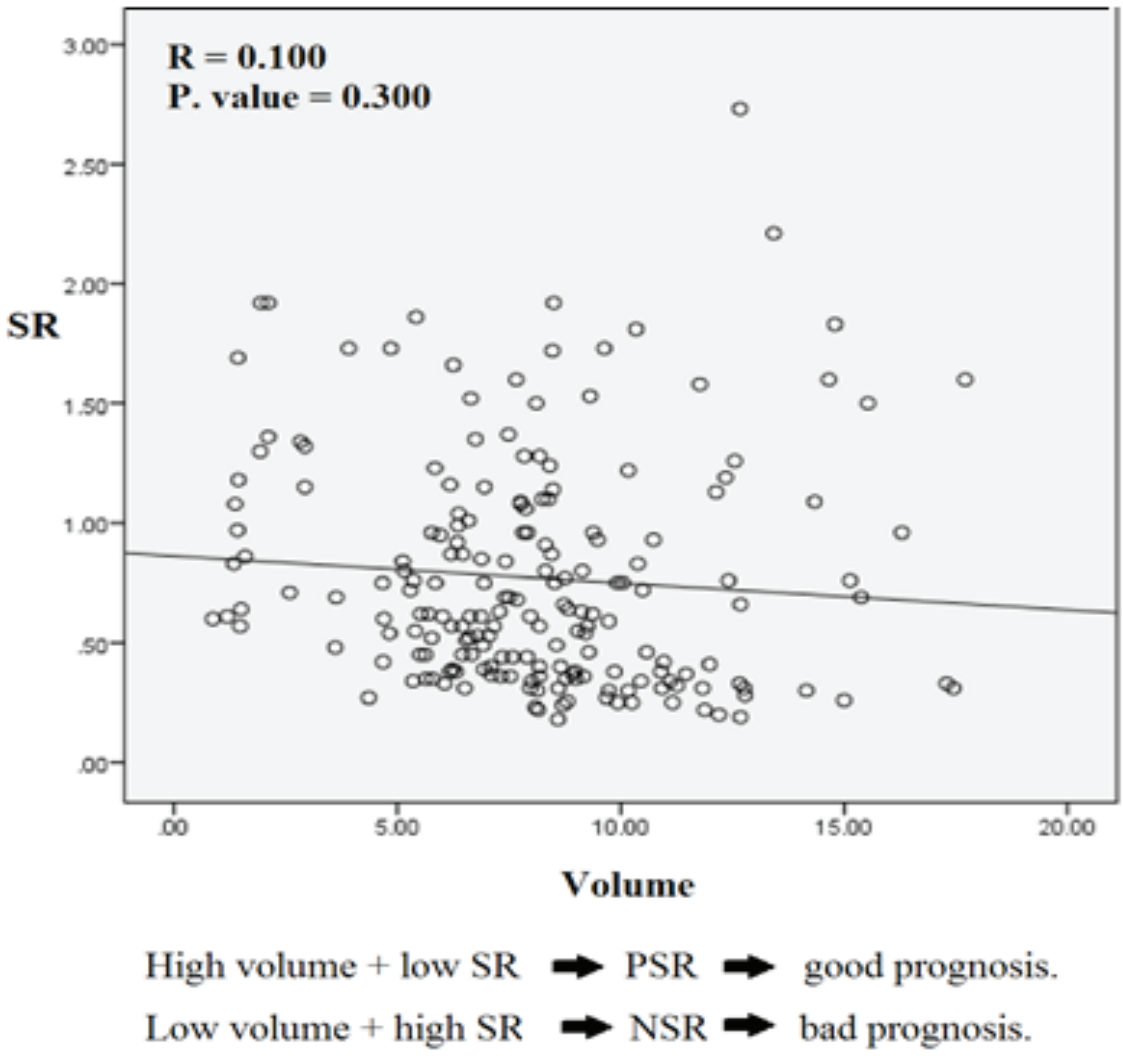

Figure 1. Correlation between testicular volume and SR .

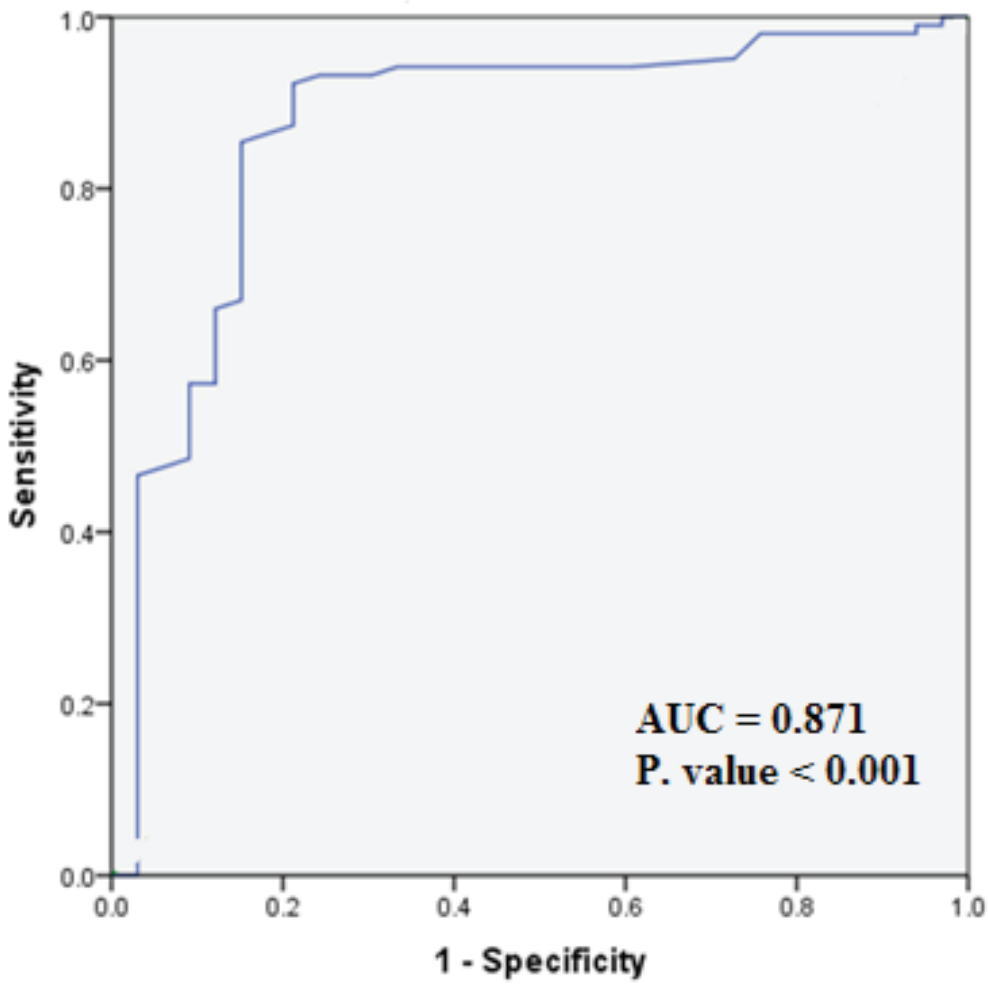

Figure 2. ROC curve for validity of strain ratio in differentiation between PSR and NSR.

Table 3. validity of strain ratio in differentiation between PSR and NSR (Cutoff value = 0.385)

PSR		Biopsy		Total
		NSR		
SR	< 0.385	25 (75.8%)	7 (6.8%)	32 (23.5%)
	≥ 0.385	8 (24.2%)	96(93.2%)	104(76.5%)
Total		33(100%)	103(100.0%)	136 (100.0%)
P. value < 0.001				

Discussion

Nowadays, imaging techniques are more often used to assess the causes of infertility, traditional U / S methods (B-mode and color Doppler) using the latest technologies (SR and SWE elastography) were the first investigation line in male infertility and assessment of testicular tissue and spermatogenesis[6,7]. As the strain elastography is dependent on the criteria of compressions, it produces tissue strain, however, the deformation degree in soft tissues is larger than that in stiff one [8]. Few testicular focal lesions including the small diameter lesions of less than 10 mm, azoospermias and microlithiasis , especially if not palpable, have been investigated by ES [9,10]. Biopsy results from male patients with azoospermia showed that as the histological criteria increased in grade, seminiferous tubules adianeter and spermatogenic epithelium height are gradually decreased, but there is a gradual increase in thickness of lamina propria [9]. Previous studies, proved an increased stiffness of tissues in azoospermic cases and that SR is a useful azoospermia diagnostic tool .

Other several studies have documented that SR can objectively provide data with good accuracy in diagnosis compared to elastography [10-12]. Our study have shown that in patients with abnormal parmaeters of sperms , the SR was much higher. On the other hand, testicular volume was suggested to be a good indicator of spermatogenesis, and a variation was proved between infertile and fertile cases [13]. Moreover, Seminal volume sperm motility and count appeared to be significantly correlated as documented in previous studies [14,15]. Condorelli and his colleagues [16] found that the testicular volumes sperm parameters of bio-function were significantly associated and that testicular volume decrease exacerbated by these parameters. Elastography was approved to be useful in structural analysis of tissues since any pathological changes in tissues can be detected [17]. Our findings also show that the mean volume is much lower in patients with non-obstructive azoospermia than those with obstructive one. As the seminiferous tubules contribute to almost 80% of testicular volume , they are responsible for spermatogenesis, hence the sperms count associated with testicular volume. Using ES we can discriminate soft from hard testicular tissue regions, this based on that a backscattered sonographic signals suffer from displacement if the tissue is slightly compressed &decompressed. Lesser displacement seen when tissue get stiffer than normal tissue. So, tissue elasticity may be correlated with pathological conditions. Unfortunately, we couldn't include the hormonal analyses of our patients because most of them didn't perform a hormonal assays before biopsy.

Conclusion

Strain Elastography results have been validated and were differed significantly between patients with abnormal sperms count than those without. This Technique provides a promising way to get more accurate diagnostic results. However, extensive research can guide the road to approve the role of imaging methods in male infertility assessment . Hence, we recommend further studies with larger sample size, to discover the beneficial applications of this modality with inclusion of hormonal status of the patients.

Ethical Clearance: All ethical issues approved by the authors and patients data were collected and Managed in accordance with the World Medical Association, declaration of Helsinki, 2013

Conflict of Interest: None

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Supervised Classification Approaches for Brain Tumour Classification Using Fused Wavelet Features

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Abstract

In this study, an efficient pattern recognition technique is developed for Brain Image Classification (BIC) into normal or abnormal. Wavelet transform features with supervised classification have a potential role to play in bringing Magnetic resonance Images (MRI) of the brain into practical clinical use.The developed pattern recognition technique uses Discrete Wavelet Transform (DWT), Dual tree M-band Wavelet Transform (DMWT), and Stationary Wavelet Transform (SWT) for feature extraction, *k*-Nearest Neighbour (*k*NN) and Naive Bayes (NB) for classification and is considered as an effective and accurate tool for brain image analysis for cancer classification. Also, the predominant coefficients are chosen from the combined feature space by rank features of statistical feature selection approach. Results show that the proposed system acts as a pre-treatment predictor for BIC with an accuracy of 88.5% for *k*NN and 95.5% for NB classification.

Keyword: *Brain tumour, wavelet transforms, supervised classification, k-nearest neighbourand naive Bayes.*

Introduction

MRI is emerging as one of the most powerful tools for the study of the human brain. Recent advances in MRI technology have enabled the acquisition of brain images in high resolution and thus providing a more clinically relevant utility to study brain tumour in the context of pattern recognition. The potential of MRI images to diagnose and classify the brain tumour images has been reviewed.

DWT with three families of filters; 'bio3.7', 'db8', and 'sym8' are well analyzed for BIC[1]. It uses energy features of sub-bands extracted from each level of decomposition up to 5th level. Then, the features are analyzed independently by Support Vector Machine (SVM) classifier. A hybrid approach is described in for MRI-BIC system. The DWT sub-bands are fed to Principal Component Analysis (PCA) algorithm to reduce the dimension of feature space. Then, kNN classifier and Neural Network (NN) are designed to classify the MRI images.

Texture features from the run-length matrix and intensity features based BIC system is discussed[2]. It extracts features from the clustered tumour region after preprocessing. Then the features are reduced by PCA and singular value decomposition techniques, and a simple NN is used for classification. Feature extraction by DWT and feature reduction by PCA is employed in for BIC[3]. It uses extreme learning machine and back propagation NN for classification.

Topographic sparse coding based BIC system is implemented[4]. The gray matter of the brain is obtained after a series of preprocessing stages such as segmentation and smoothing. Then, unsupervised feature learning is employed for classification and comparison is made with PCA and self-learning NN also. A deep learning NN for BIC is described[5]. It classifies the abnormal brain image classes only. The main disadvantage of NN is high computational cost due to the increased number of layers, and also fine-tuning of NN is a time-consuming process.

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Stockwell transform-based BIC system is described[6]. After taking the Stockwell transform, Sammon mapping is employed to reduce the feature space. NB classifier is applied to classify the MRI images. Wavelet and statistical-based features are used for BIC system[7]. It uses wavelet coefficients along with the first or second-order statistical features as features and multi-layer perceptron, random forest and NB as classifiers for brain image analysis.

Automatic BIC system using salp swarm algorithm is described[8]. It uses decision tree, kNN and NB as learning algorithms for BIC with the help of three levels vortex wavelet transform. Feature reduction is also employed by PCA. Features from the Fourier transform spectrum are employed for BIC[9]. It uses the SVM classification technique for classification after feature reduction by PCA.

In this paper, MRI-BIC system using fused wavelet features by two supervised classification approaches; *k*NN and NB is developed. For feature reduction, the system uses statistical *t*-test instead of PCA, and thus the wavelet coefficients are directly used for the classification. The organization of the paper is as follows: Section 2 discusses the methods used for MRI-BIC are described briefly. The performances of MRI-BIC system using 200 images of MRI brain images using *k*NN and NB are discussed, and the conclusion is made in the final section.

Methods and Materials

The workflow of MRI-BIC system consists of three stages; feature extraction, selection and then classification. To represent the input MRI images in multi-resolution, which provides different sub-band coefficients, they are given to DWT, DMWT, and SWT. After representation, the coefficients are selected using the ranking approach by a statistical t-test. A predefined number of predominant rank features are selected from the sub-bands of each transform coefficients according to their rank. Then the selected features are classified by KNN and NB classifier.

Feature Extraction

In this section, the three transformations; DWT[10], SWT[11] and DMWT[12] used for the extraction of features for MRI-BIC system are discussed.The wavelet analysis leads to a signal representation in time and frequency domains simultaneously. The basic idea is to use a cutting window that is shifted along with the signal and calculate the spectrum for each of these shifted positions (translation). This process is then iterated, changing the window size (dilation). The result is a collection of time-frequency representations with different resolutions, hence the definition of multi-resolution analysis. The goal of the multi-resolution analysis is to apply the divide and conquer strategy: separate the signal in multiple components that can be independently analyzed and processed by different algorithms. Let‘s define the function $\psi(t)$ called mother wavelet as follow:

$$\psi_{\tau,\sigma}(t) = \frac{1}{\sigma}\psi\left(\frac{t-\tau}{\sigma}\right) \quad (1)$$

where σ is the scale factor (dilation), τ the translation factor and ψ represents a basis function that can be designed to taste as long as it fulfils general wavelet properties such as admissibility, regularity and vanishing moments.

The main drawback of conventional wavelet is that it has shift-invariant against the input samples. Thus the size of the sub-bands obtained from the conventional wavelet is not equal to the size of the input data. This is due to the downsampler in the decomposition filter. To remove the shift-invariant of conventional wavelet, SWT[11] is designed by just removing the downsampler. Thus, the resolution of the sub-bands does not change. Though the wavelets are multi-resolution in nature, it provides information in three directions; vertical, horizontal and diagonal only. To get more information from other directions, DMWT is used. The decomposition is obtained by using *the M*-band filter bank and dual *M*-band filter bank, and then the linear combinations of the sub-bands are obtained with more information[12]. After decomposition by DWT, DMWT and SWT, the coefficients in each sub-band are considered as features. In the later stage, dominant coefficients are selected for the further classification process.

Feature Selection

After representation, the feature space of each transform becomes high (except DWT) as they provide more information in different sub-bands. It is well known that not all the wavelet coefficients carry

significant information, and thus a feature selection approach is needed. Without feature selection, the use of all coefficients reduces system performance. After representation, the coefficients are selected using statistical *t*-test. The *t*-test is based on the average of the features extracted from normal and abnormal MRI images which is given by Eqn. (2)

$$t(x) = \left(\bar{y}_1(x) - \bar{y}_2(x)\right) / \sqrt{\left(s_1^2(x)/n_1 + s_2^2(x)/n_2\right)} \quad (2)$$

where $\bar{y}_1(x)$ is the means of normal, $\bar{y}_2(x)$ is the means of abnormal, $s_1^2(x)$ is the standard deviations of normal and $s_2^2(x)$ is standard deviations of abnormal features.

A predefined number (N) of predominant rank features are selected from the sub-bands of each transform coefficients according to their rank. In this study, three different sets of features are computed and named as FV-I-25, FV-II-50, and FV-III-75. The first variable FV indicates the feature vector and the second variable denotes the feature set number, and the last variable indicates the number of features selected from each transform. These set of features are the inputs to the classification system for BIC.

Classification

Machine learning is often applied to problems where it is difficult to construct a physical model of the processes generating the data but where a reasonable number of examples are available. In this study, two classifiers are such as *k*NN[13] and NB[14] are used for classification. *k*NN is a similarity-based approach based on the minimum distance measure between the testing samples and the training samples. For *k*NN, *k* numbers of training samples (i.e. nearest neighbours) are used to label unknown samples according to the distance measure. The distance metric can be any similarity measure statistics. This study uses Euclidean distance in Eqn. 3 as a distance measure with *k*=1. The common advantages of KNN are that it is computationally simple, less prone to noise and bias. The Euclidean distance between two points $u = (x_1, y_1)$ and $v = (x_2, y_2)$ is given by

Euclidean distance

$$(u, v) = \sqrt{(x_1 - y_1)^2 + (x_2 - y_2)^2} \quad (3)$$

An NB classifier is a statistical-based approach based on the elementary Bayes Theorem with strong independence assumptions, i.e. all samples in the data set are unrelated to each other. Given a *N*umber of classes in the data set and *f* is an observation (features) in the data set with a known probability density of the class $p(f_N)$. If the class prior probability $p(N)$ is known, then the posterior probability $p(N|f_N)$ of the observation can be expressed as:

$$p(N|f_N) = \frac{p(f_N)}{p(N)} \cdot \frac{L}{p(f_N)} \quad (4)$$

Where, *L* is the predefined number of labeled observations used to measure the likelihood of the observation, irrespective of their class labels.

Results and Discussions

To simulate the proposed BIC system discussed in section 2 and analyzes the performance of the system, REpository of Molecular BRAin Neoplasia DaTa (REMBRANDT) database[15] images are used. MRI images are acquired from 130 subjects with a size of 256x256 pixels[16]. From the database, 100 images per category (normal and abnormal) are selected. Figure2 shows sample normal images (top row) and abnormal images (bottom row) in the REMBRANDT database[17].

To predict the abnormality, a training set composed of both normal and abnormal MRI images (the inputs) and the corresponding label to the images (the outputs) is required. These are prepared with the help of *k*-fold cross-validation with *k* equal to 10. This approach validates the classifier performance with different training, and testing samples and also all the samples undergo into training and testing phase as well. At first, the images in each category (class) are divided into 10-folds with an equal number of images in each group. Each iteration images in one fold are used to test the classifiers, and the images in remaining folds are used to train the classifiers[18]. This procedure is repeated for 10-iterations so that all the images are used to test the classifiers.

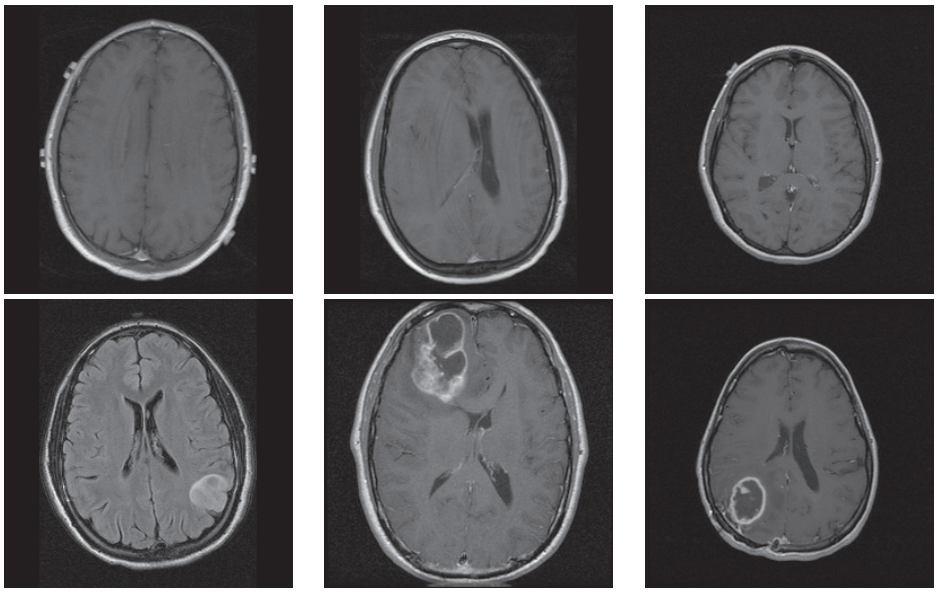

Figure 2 REMBRANDT database images

In each iteration, the correctly and misclassified number of images are noted and finally, the total number of correctly and misclassified images at each category are calculated, and the following performance metrics such as sensitivity, specificity and accuracy are computed to analyze the performance of the MRI-BIC system. The definitions are as follows:

- **Sensitivity:** It gives the correct classification rate of abnormal images and is given below:

$$Sensitivity = \frac{P}{P + N} \quad (4.1)$$

Where, True Positive (TP) and False Negative (FN) are the correct and misclassified abnormal images respectively.

- **Specificity:** It gives the correct classification rate of normal images and is given below:

$$Specificity = \frac{N}{N + P} \quad (4.2)$$

Where, True Negative (TN) and False Positive (FP) are the correct and misclassified normal images respectively.

- **Accuracy:** It gives the overall classification rate of the system and is given below:

$$Accuracy = \frac{P + N}{P + N + N + P} \quad (4.3)$$

Tables 1 to 3 display the evaluations of BIC system in terms of accuracy, sensitivity and specificity which are computed using the above formula.

Table 1 MRI-BIC system performance in terms of accuracy

Different wavelet transform (DWT, SWT and DMWT) Levels	Accuracy (%)					
	FV-I-25		FV-II-50		FV-III-75	
	kNN	NB	kNN	NB	kNN	NB
1	73.5	82.5	71	78.5	76	84.5
2	80	88	88.5	95.5	83.5	90
3	76	85	68	75	77	83
4	65.5	73.5	66.5	74	74.5	82.5
5	78	85	72.5	80.5	80.5	89

Table 2 MRI-BIC system performance in terms of sensitivity

Different wavelet transform (DWT, SWT and DMWT) Levels	Sensitivity (%)					
	FV-I-25		FV-II-50		FV-III-75	
	kNN	NB	kNN	NB	kNN	NB
1	80	90	75	82	76	84
2	80	89	89	96	84	90
3	80	88	84	92	85	90
4	79	87	78	86	80	88
5	82	90	82	90	80	90

Table 3 MRI-BIC system performance in terms of specificity

Different wavelet transform (DWT, SWT and DMWT) Levels	Specificity (%)					
	FV-I-25		FV-II-50		FV-III-75	
	kNN	NB	kNN	NB	kNN	NB
1	67	75	67	75	76	85
2	80	87	88	95	83	90
3	72	82	52	58	69	76
4	52	60	55	62	69	77
5	74	80	63	71	81	88

It can be seen from Table 1 that the FV-II-50 set produces the highest accuracy of 95.5% while using the features of different wavelets from 2ndlevel of decomposition by NB classifier when compared with FV-I-25 and FV-III-75 set. For the same set of features, kNN gives an accuracy of 88.5%. It is noted that the performance of MRI-BIC system is improved while increasing the predominant coefficients from each wavelet transform from 25 to 50 features. Further increase in feature dimension reduces the performance as it adds redundant features in the feature space.

Conclusion

A pattern recognition technique for BIC system is presented using three different wavelet transforms; DWT, DMWT and SWT. It also highlights the potential role of *k*NN and NB in developing pattern recognition applications for the classification. The automatic feature

selection can infer relationships between normal and abnormal MRI images. The following feature sets such as FV-I-25, FV-II-50 and FV-III-75 are employed for performance evaluation. These sets are extracted for each level of decomposition and each wavelet transform as well. Then they are given to the *k*NN and NB for classification independently. Results show that the FV-II-50 set produces the highest accuracy of 88.5% (*k*NN) and 95.5% (NB) at 2nd level combined wavelet features.

Ethical Clearance- NA

Source of Funding- Self

Conflict of Interest –Nil

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Calorie Restriction and Moderate-Intensity Continuous Exercise Decrease Free Fatty Acid Levels and Visceral Fat Weight on High Calorie Diet Female Mice

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Abstract

Objective: This study aimed to analyze the effect of a combination of calorie restriction and moderate-intensity continuous exercise on FFA levels and visceral fat weight in female mice exposed to a high-calorie diet.

Material and Method: This research was randomized posttest used only control group design with female mice as the sampl Balb/c mice (Mus musculus), 8 weeks old, 20-30 grams. It was randomly divided into four groups, namely C (control group), G_1 (moderate-intensity continuous exercise group), G_2 (calorie restriction group), and G_3 (combination group calorie restriction and moderate-intensity continuous exercise) where in every group consisted six female mice. Providing a high-calorie diet using ad libitum plus D40 as much as 3-5% of calories (0.0325 mL/gBB) for 4 weeks. The provision of a high-calorie diet was done using the sonde technique. Calorie restriction was done 3x/week in 4 weeks by limiting calorie intake to 50% of standard foods. The moderate-intensity continuous exercise was carried out by swimming for 15 minutes plus 6% gBB loading, it was done 3 times/week for 4 weeks. The measurement of cholesterol, visceral fat weight, and FFA level was done at the end of treatments.

Results: The results found that the mean of cholesterol of C C (171.00±47.74 mg/dL), G_1 (126.00±15.92 mg/dL), G_2 (131.50±22.37 mg/dL), G_3 (121.17±21.23 mg/dL) dan (p=0.032). The mean weight of visceral fat at C (0.68±0.23 grams), G_1 (0.25±0.08 grams), G_2 (0.28±0.06 grams), G_3 (0.20±0.03 grams) and (p=0.000). The mean FFA levels were at C (1511.34±6.19 µmol/L), G_1 (1419.31±53.61 µmol/L), G_2 (1458.20±28.89 µmol/L), G_3 (1451.55±43.77 µmol/L) and (p=0.004).

Conclusion: Based on the results of the study, it could be concluded that the provision of calorie restriction and moderate-intensity continuous exercise significantly reduced cholesterol levels, visceral fat weight, FFA levels and in female mice exposed to a high-calorie diet.

Keywords: *Calorie restriction, moderate-intensity continuous exercise, free fatty acids, visceral fat, cholesterol*

Introduction

The increasing prevalence of overweight and obesity is a global problem, both in developed and developing countries [1]. The prevalence of overweight and obesity in women is greater than in men [2,3]. In 2016, 39% of the population aged over 18 years or more than 1.9 billion people were overweight and 13% of the population over 18 years of age or 650 million people were obese [4]. In Indonesia, based on the results of Basic Health Research[5] the prevalence rate of overweight increases every year with a prevalence rate of 8.6% in 2007, 11.5% in 2013, and 13.6% in 2018, while the obesity prevalence rate is 10.5% in 2007, 14.8% in 2013, and in 2018 to 21.8%.

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This is a serious problem that will threaten the quality of human resources [6].

Overweight and obesity are conditions of abnormal or excessive fat accumulation in adipose tissue and become one of the main public health problems [7,8,9]. Moreover, overweight and obese can increase the risk of chronic diseases, such as type 2 diabetes mellitus[7,10,11], coronary heart disease[7,12], several types of cancer[13,14,15,16,17], osteoarthritis and musculoskeletal disorders [18]. Overweight and obesity are often associated with multifactorial diseases resulting from lifestyle, unhealthy eating patterns, and lack of physical activity [19]. Poor diet and an inactive lifestyle are contributors to increased fat accumulation [20]. Poor diet such as high-calorie consumption in normal subjects is considered to be a major contributor to the increase in body fat accumulation, especially visceral fat, and an impact on weight gain [21]. Body fat is stored in the form of triglycerides (TG) in adipose and liver tissue which are stimulated by blood glucose [22]. When glucose as the main energy source is unable to meet energy needs, body fat deposits, especially TG, will be hydrolyzed into free fatty acids (FFA) and glycerol as an energy source through fat metabolism [23]. FFA is released from adipocytes into the circulation through lipolysis and circulating FFA concentrations can increase in obese subjects due to an increase in adipose tissue mass [24]. The level of FFA concentrations can affect the increase and decrease in insulin resistance [25]. Lifestyle modification is recommended to be one of the foundations in the management of overweight and obesity [26]. Controlling energy intake and energy expenditure is one of the main mechanisms in regulating energy balance [26].

Calorie restriction occurs when the intake of food as an energy source is reduced and it causes the utilization of energy sources in the body to increase, especially body fat deposits. When the calorie restriction is in the form of fasting, there is a decrease in blood glucose levels which causes a decrease in insulin secretion and an increase in lipolysis [27]. The high lipolysis due to calorie restriction can increase the hydrolysis of TG to FFA and glycerol [28]. Increasing FFA as the main energy source can result in a decrease in body fat accumulation. According to Maughan et al. [29] weight loss can come from oxidation of the substrate in the body, oxidation of water and loss of water due to the release of glycogen. Besides, physical exercise also has a positive effect in reducing the increased risk of overweight, and obesity by reducing body fat deposits. During physical exercise, there is a decrease in blood glucose which results in a decrease in insulin and stimulates an increase in the hydrolysis of TG to glycerol and FFA as an energy source [30]. Exercise will stimulate an increase in growth hormone (GH) which has a role in metabolism to stimulate fat to become a more dominant source of energy inactivity than glucose [31]. 30 minutes of moderate-intensity exercise can increase the production of FFA to be used as an energy source [32] and have more potential to reduce visceral fat accumulation [33].

The combination of calorie restriction and moderate-intensity continuous exercise can be used as a non-pharmacological approach to reducing levels of FFA and body fat deposits. However, the effects of calorie restriction combined with moderate-intensity continuous exercise exposed to a high-calorie diet are not well known. Therefore, this study aimed to analyze the effect of a combination of calorie restriction with moderate-intensity continuous exercise on FFA levels and visceral fat weight in female mice exposed to a high-calorie diet. The hypothesis in this study was that there is an effect of a combination of calorie restriction with moderate-intensity continuous exercise on reducing FFA levels and visceral fat weight in female mice exposed to a high-calorie diet.

Materials and Methods

This research was a true experimental research design with the randomized control group posttest-only design using 24 Balb/c female mice (Mus musculus), 8 weeks, 20-30 grams and randomly divided into four groups, namely C (n=6, control group) G_1, (n=6, moderate-intensity continuous exercise group), G_2 (n=6, calorie restriction group), and G_3 (n=6, combination group calorie restriction and moderate-intensity continuous exercise). The research was conducted at the Embryology Laboratory, Faculty of Veterinary Medicine, Airlangga University. The experimental animals were placed in a room with a temperature of 26±2 °C and humidity of 50-60%, moreover the lighting was set to a light-dark cycle with a regulation of 12 hours of light and 12 hours of darkness. Before the treatment begins, the sample was given a swab test to determine the ovulation period and

the estrous phase as a starting point for providing calorie restriction and moderate-intensity continuous exercise. All of these research procedures had been approved by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University, Surabaya Number 11/EC/KEPK/FKUA/2020.

Diet conditioning with ed libitium plus sonde D40. Sonde D40 was offered at a dose of 3-5% of calories (0.013 gBB) or 0.0325 ml/gBB [34]. The D40 solution was given at 10.00 a.m. Calorie restriction was done by limiting calorie intake by 50% of standard food or by 5 grams of feed/day. Calorie restriction is done 3x/week at night and a high-calorie ed libitium diet and Sonde D40 at noon 7 times/week for 4 weeks. The moderate-intensity continuous exercise was carried out by immersing the mice with a load of 6% of the bodyweight of the mice tied to the tail rank [35] for 15 minutes [36]. Swimming training interventions were given 3 times/week for 4 weeks. The intervention was given at 15.00 p.m. Swimming intervention was carried out in a tub with a diameter of 55 cm and a height of 60 cm with a water temperature of 25±1°C and a water depth of 50±1 cm. In groups C and G_2, they were still given time to play with water as far as their feet on the same day and time to minimize the influence of water. Diet restrictions and physical exercises were given on different days.

One ml of blood was taken from the left ventricle of the mice. The visceral part of the mice was collected 24 hours after the last intervention. Blood was centrifuged for 10 minutes at 3000 rpm. The serum was separated, and stored at -80 °C for analysis of FFA levels the following day. Measurement of serum FFA levels using Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalog No: MBS026749; MyBioSource; San Diego, California, USA) with a concentration unit of µmol/L. Measurement of body weight, and visceral fat weight using a digital scale with a scale of 1 x 10-3 mg.

Cholesterol levels were measured using tail blood which was dripped on the Easy Touch GCU system with Easy Touch II Blood Test Strips by Bioptik Technology, Inc. based on [37].

Visceral fat weight is fat tissue taken in the visceral area of mice and measured using a digital scale with a scale of 1 x 10-3 m.

Statistical analysis used the Statistical Package for Social Science (SPSS) software version 21 (Chicago, IL, USA). The normality test used the Shapiro-Wilk test, while the homogeneity test used the Levene test. The difference test used One way-ANOVA, and continued with the Least Significant Difference (LSD) post hoc test. The linear correlation was used Pearson product-moment model. The data were presented as mean±SD and, the statistical analyzes used a significant level (p<0.05).

Results

The results of the statistical analysis of the mean body weight in the first week to the final week after giving a combination of calorie restriction, and moderate-intensity continuous exercise could be seen in Figure 1.

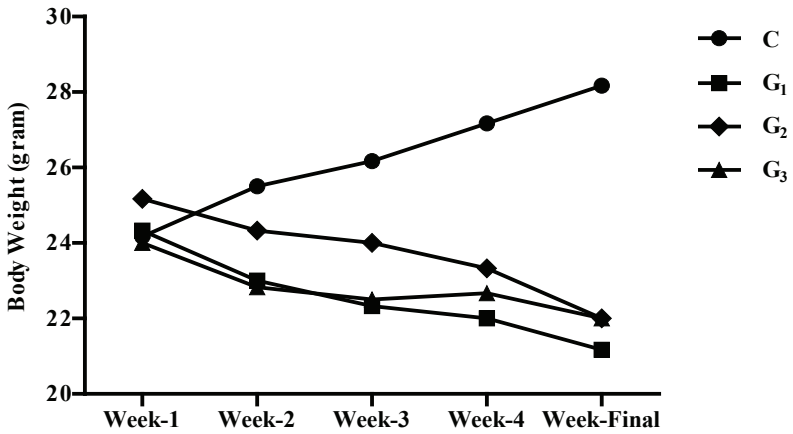

Figure 1. The Mean Body Weight of Mice in Each Group.

Note: C (control group), G_1 (moderate-intensity continuous exercise group), G_2 (calorie restriction group), and G_3 (combination calorie restriction and moderate-intensity continuous exercise).

Figure 1 showed that there was an increase in mean body weight at the initial week (week-1), and body weight. For the end of the week (week-final) in C, while in G_1, G_2, and G_3 group, there was a decrease in mean body weight at the end of the week (week-final) compared to body weight in the initial week (week-1). The results of the analysis of the mean visceral fat weight could be seen in Table 1.

Table 1. Result of Statistical Analysis of Mean Visceral Fat Weight

Group	n	Mean±SD (gram)	ANOVA (p-values)
C	6	0.68±0.23	0.000
G1	6	0.25±0.08*	
G2	6	0.28±0.06*	
G3	6	0.20±0.03*	

Note: One way-ANOVA, followed by LSD post hoc test, was used to compare the differences among groups. The data were presented as mean ± SD. * Significant vs control group (C) ($p<0.05$). C (control group), G_1 (moderate-intensity continuous exercise group), G_2 (calorie restriction group), and G_3 (combination calorie restriction and moderate-intensity continuous exercise group).

Based on Table 1, it proved that the mean visceral fat weight at C is higher than G_1, G_2, and G_3. The results of the One way-ANOVA analysis exhibited that there was a significant difference in the mean weight of visceral fat in each group (p=0.000). The results of the LSD post hoc test provided the data that there was a significant difference in the mean weight of visceral fat between G_1, and C (p=0.000), G_2 with C (p=0.000), G_3, and C (p=0.000), while G_1, and G_2 (p=0.671), G_1 with G_3 (p=0.539), and G_2 with G_3 (p=0.303) did not show a significant difference in mean visceral fat weight ($p>0.05$). The results of the analysis of the mean cholesterol levels were apparent in Table 2.

Table 2. Results of Statistical Analysis of Mean Cholesterol Levels.

Group	n	Mean±SD (mg/dL)	ANOVA (p-values)
C	6	171.00±47.74	0.032
G1	6	126.00±15.92*	
G2	6	131.50±22.37*	
G3	6	121.17±21.23*	

Note: One way-ANOVA, followed by LSD post hoc test, was used to compare the differences among groups. Data were presented as mean ± SD. * Significant vs control group (C) (p<0.05). C (control group), G_1 (moderate-intensity continuous exercise group), G_2 (calorie restriction group), and G_3 (combination calorie restriction and moderate-intensity continuous exercise group).

Based on Table 2, it showed that the mean cholesterol levels in C are higher than those of G_1, G_2, and G_3. The results of the One way-ANOVA analysis proved that there were significant differences in the mean cholesterol levels in each group (p=0.032). The results of the LSD post hoc test offered the data that there was a significant difference in the mean cholesterol levels between G_1 and C (p=0.016), G_2 and C (p=0.031), G_3 and C (p = 0.008), while G_1 and G_2 (p=0.750), G_1 with G_3 (p=0.780) and G_2 with G_3 (p=0.551) did not show a significant difference in the mean of FFA levels (p>0.05). The results of the analysis of the mean FFA levels were observable in Table 3.

Table 3. Statistical Analysis Results of Mean FFA Levels.

Group	n	Mean±SD (µmol/L)	ANOVA (p-values)
C	6	1511.34±6.19	0.004
G1	6	1419.31±53.61*	
G2	6	1458.20±28.89*	
G3	6	1451.55±43.77*	

Note: One way-ANOVA, followed by LSD post hoc test, was used to compare the differences among groups. Data were presented as mean ± SD. * Significant vs control group (C) (p<0.05). C (control group), G_1 (moderate-intensity continuous exercise group), G_2 (calorie restriction group), and G_3 (combination calorie restriction and moderate-intensity continuous exercise group).

Based on Table 3 showed that the mean FFA levels in C were higher than those of G_1, G_2 and G_3. The results of the One way-ANOVA analysis showed that there were significant differences in the mean FFA levels in each group (p=0.004). The results of the LSD post hoc test exhibited that there was a significant difference in the mean FFA levels between G_1 and C (p=0.000), G_2 and C (p=0.024), G_3 and C (p=0.012), while G_1 and G_2 (p=0.089)), G_1 with G_3 (p=0.153) and G_2 with G_3 (p=0.763) did not show a significant difference in the mean of FFA levels (p>0.05). The results of the correlation analysis of FFA levels with final body weight and visceral fat weight were shown in Figure 2.

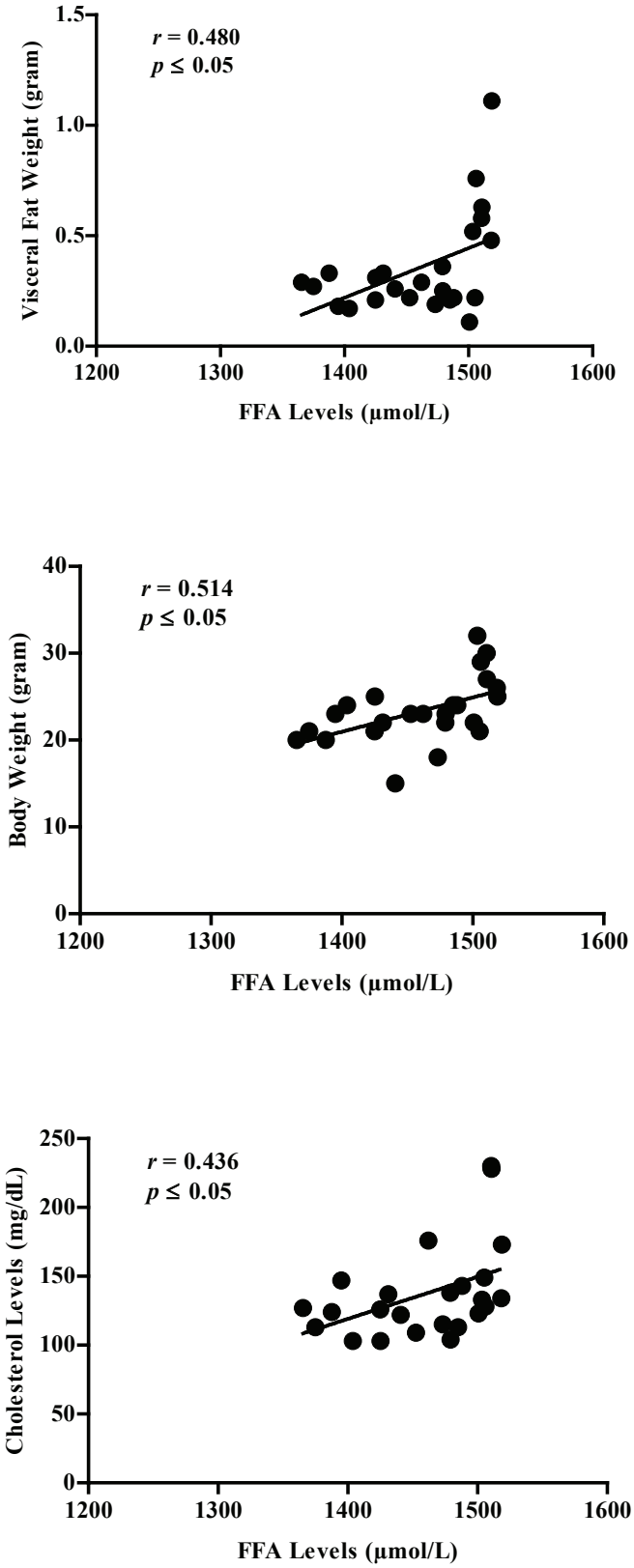

Figure 2. The correlations between FFA levels with visceral fat weight, body weight and cholesterol levels. The significant linear correlation among parameters was visualized in the plot model ($p<0.05$). * Significant with $p<0.05$ by Pearson's product-moment correlation test.

Based on the results of the study found a significant relationship between FFA levels and final body weight, visceral fat weight and cholesterol levels. The results of the Pearson product-moment linear correlation analysis found that FFA levels were positively correlated with final body weight (r=0.514, p<0.05), visceral fat weight (r=0.480, p<0.05) and cholesterol levels (r=0.436, p<0.05).

Discussion

Based on the results of the analysis, it had been found that the mean body weight of mice had a difference between before and after treatment (4 weeks) and there was a tendency to lose weight in G_1, G_2 and G_3, while in C the mean body weight increases (Figure 1). Weight loss in G_1, G_2 and G_3 is probably due to metabolic factors and the effects of calorie restriction treatment, exercise as well [38]. Calorie restriction and exercise had a positive effect in reducing body fat stores. During calorie restriction and exercise, there was a reduction and decrease in food intake as an energy source which caused the utilization of energy sources in the body to increase, especially body fat deposits. When calorie restriction was in the form of fasting and exercise, there was a decrease in blood glucose levels which caused a decrease in insulin secretion and an increase in lipolysis [27]. Increased lipolysis due to calorie restriction could increase the hydrolysis of triglycerides to FFA and glycerol [28]. The increase in FFA as the main source of energy could result in a decrease in body fat stores, thus caused lower body weight of mice in G_1, G_2 and G_3 compared to C.

Another factor that determined the difference in weight gain and loss was due to appetite stimulation marked by the hormone Ghrelin [39]. Ghrelin is divided into 2 types, namely Acylated Ghrelin (AG) which stimulates appetite and Desacyl Ghrelin (DG) which functions as a trigger for adipogenesis (Mackelvie, 2007). According to King [40], calorie restriction and exercise lead to appetite suppression that is mediated by AG which is inhibited by DG [41]. Emphasis on AG causes no binding on the Growth Hormone Secreting Receptor (GHSR) type 1a with AG, so that appetite stimuli cannot be sent to the hypothalamus and pituitary [42]. Inhibition of appetite stimulation and continuous use of energy during calorie restriction and exercise results in reduced calorie intake and more calorie expenditure, resulting in disturbed energy balance, which causes weight gain in G_1, G_2 and G_3 to be lower than in C. Conversely, the increase in body weight in C is higher than that of G_1, G_2 and G_3 due to an imbalance between calories intake and expenditure. According to Restuastuti [43] in his research, it is concluded that an imbalance between calories included and calories expended causes weight gain [44]. Likewise, according to Surabhi [45], the imbalance between calories in and calories out causes excessive accumulation of fat tissue in the body, leading to weight gain [38]. Excess calories are converted into glucose and stored in the liver in the form of glycogen [46]. Glycogen levels in the liver had a certain optimal limit so that if blood glucose levels are sufficient, while glucose intake continues, a mechanism for converting glycogen into fat will be stored in adipose tissue in the form of a fat pad [47]. This was why the weight gain in C is higher than that of G_1, G_2 and G_3 (Figure 1).

The mean visceral fat weight in G_3 was lower than that of C, G_2 and G_3 then there was a significant difference in the mean visceral fat weight in each group. These results are in line with the results of previous studies conducted by Verheggen et al. [48] shows that exercise combined with calorie restriction is the right recommendation in reducing visceral fat weight. This is probably because the combination of calorie restriction and moderate-intensity continuous exercise is effective in reducing visceral fat weight. Exercise causes an increase in lean body mass and a decrease in fat mass [48]. Therefore, the combination of calorie restriction and moderate-intensity continuous exercise was an appropriate method to use in reducing visceral fat weight. Calorie restriction could reduce the weight of visceral fat. Likewise, moderate-intensity continuous exercise could also cause a decrease in visceral fat weight, so that the combination of calorie restriction and moderate-intensity continuous exercise would be greater in reducing the weight of visceral fat. It was because of the combination of calorie restriction and moderate-intensity continuous exercise which could increase lipolysis of larger fat stores as an energy source. A greater increase in lipolysis could lead to a greater decrease in visceral fat weight.

Moreover, the mean of FFA levels in G1 were lower than those in C. These results are in line with the results of research conducted by Yu et al. [49] concluded

that swimming exercises performed for 8 weeks on Sprague-Dawley Rats significantly reduced FFA levels compared to controls. The decrease in FFA levels in G_1 is likely due to the influence of the intervention. During physical exercise, there is a decrease in blood glucose which results in a decrease in insulin and stimulates an increase in the hydrolysis of TG to glycerol and FFA as an energy source [30]. Exercise increases growth hormone (GH), which has a role in metabolism to stimulate fat to become a source of energy used for muscle contraction during exercise [31], causing the FFA concentration in blood circulation to decrease [49].

Exercise is an optimal way that can be used to lose weight [49], improve metabolic health and increase insulin sensitivity [50]. During exercise, triacylglycerol will be hydrolyzed into FFA, then FFA is released into the blood circulation to provide a source of muscle energy during exercise [51]. During intense and intermittent exercise, muscle glycogen particles are broken down, releasing glucose molecules which are then oxidized by muscle cells through anaerobic and aerobic processes to produce adenosine triphosphate (ATP) which is required for muscle contraction during exercise [52]. Exercise performed acutely can increase non-esterified fatty acids in the circulation by increasing sympathetic nerve activity through the release of adrenaline by activating lipase sensitive hormone (HSL) and adipose triglyceride lipase (ATGL) [30]. This lowers blood glucose which will stimulate a decrease in insulin and is associated with greater oxidation of fatty acids and reduced muscle glycogen degradation which can decrease circulating FFA in muscles [32].

Long-duration exercise can increase fat oxidation as energy production [53]. This is indicated by an increase in blood ketone concentrations, a decrease in insulin concentrations and an increase in lipolysis [54,55,56]. The enhanced lipolysis state with reduced lipogenesis theoretically contributes to a decrease in body fat storage. Physical exercise helps reduce waist circumference and visceral fat, lowers blood pressure, increases high-density lipoprotein (HDL) cholesterol, lowers plasma triglyceride concentrations, and especially promotes an increase in glucose transporter type 4 (GLUT-4) in muscle fiber membranes, improves glucose transport from blood to muscles, which lowers blood glucose [57]. Muscle as a whole is a major contributor to total body glucose and FFA metabolism [58], individual muscles can contribute differently depending on their fiber composition. Type 1 red muscle fibers are thought to be more sensitive to insulin, with a greater oxidative capacity for glucose and FFA [53], whereas type II white muscle fibers contain less mitochondria, are considered less sensitive to insulin and contribute less on FFA oxidation [59].

While the mean of FFA levels in G_2 were lower than those in C. These results are in line with the results of research conducted by Kostogrys et al.[60] concluded that there was a significant difference in FFA levels after caloric restriction (CR) administration compared to controls in mice. The decrease in FFA levels in G2 is likely due to the influence of the intervention. Throughout calorie restriction, the body will respond to energy use by taking fat deposits using the lipolysis method [27]. The increased lipolysis state with reduced lipogenesis theoretically contributes to the decrease in body fat stores [51]. High lipolysis causes TG to be hydrolyzed into glycerol and FFA [28]. FFA is mobilized into the blood circulation as a source of energy during calorie restriction, causing FFA levels to decrease [51].

Calorie restriction is a calorie restriction that is carried out within a certain time which is useful for reducing body fat deposits [61] and losing weight [50]. According to Chaouachi et al. [62], calorie restriction is avoiding eating, drinking and cravings for 11-13 hours. Calorie restriction in Islam is known as fasting which is divided into 2 types, namely compulsory fasting (Ramadan fasting) and sunnah fasting (eg fasting Monday and Thursday. Fasting does not eat or drink for 12-19 hours every day depending on geographic location [63]. Calorie restriction can reduce body weight, cholesterol, low-density lipoprotein (LDL) and triglycerides and increase insulin sensitivity [64]. Physiologically calorie restriction can reduce the function of the digestive organs for 12 hours [65], Calorie restriction can reduce blood pressure, glucose [27], leptin and c-reactive protein (CRP) [66]. During caloric restriction, mitochondrial protein increases. In active tissue [67]. Calorie restriction after a high-calorie diet can trigger an increase in mitochondrial sirtuin 3 (SIRT3) transcription, then stimulate antioxidant productivity (SOD and GPX1) and suppress oxidative stress [68]. When in a calorie restriction condition, the body responds to energy use by taking fat deposits using the lipolysis

method [27]. The increased lipolysis state with reduced lipogenesis theoretically contributes to a decrease in body fat stores[51].

High lipolysis causes TG to be hydrolyzed into glycerol and FFA [28]. FFA becomes a source of energy and is mobilized to the blood circulation, causing a decrease in FFA [51]. The provision of calorie restriction by fasting can improve the standard of life than the untreated restriction treatment in obese rats and an increase in FFA levels [69]. According to Alvarez-Curto and Milligan[24] explained that the concentration of FFA circulating in the bloodstream increases in obese subjects due to an increase in the amount of adipose tissue. This is in line with the results of this study which showed that the FFA levels in the control group were higher than the FFA levels in the calorie restriction group.

Based on the results of the study, it showed that the mean FFA levels in G_3 were lower than those in C. These results differ from the results of research conducted by Bruss et al.[70] concluded that giving calorie restriction increased FFA synthesis in mice. The difference in the results of this study may be due to differences in the form of intervention given. In this study, a combination intervention between calorie restriction and exercise was provided, whereas previous studies only provided calorie restriction without exercise. The combination of calorie restriction and exercise causes an increase in energy demand, increasing in the lipolysis process to increase the hydrolysis of TG to FFA and glycerol to meet primary energy needs [28]. Based on the research results of Gopalan et al.[50] concluded that the combination of calorie restriction and exercise reduces body weight, depots adipose tissue and reduces TG in muscle. A decrease in TG can decrease FFA production. Decreased FFA production can reduce insulin resistance [25] and lose weight [50].

The combination of calorie restriction with exercise has been recommended to reduce body weight, improve metabolic health and increase insulin sensitivity and can be used as a non-pharmacological treatment method [50,61]. This is because exercise triacylglycerol will be hydrolyzed into FFA, then FFA is released into the blood circulation to provide a source of muscle energy during exercise [51]. During the provision of calorie restriction with exercise, there is an increase in energy needs, which causes a decrease in blood glucose levels, which causes a decrease in insulin secretion and an increase in lipolysis [27]. Increased lipolysis as a result of calorie restriction intervention with exercise can increase the hydrolysis of TG to FFA and glycerol to meet primary energy needs [28]. When the provision of calorie restriction with exercise is carried out continuously, it can reduce body fat deposits, especially visceral fat, thus causing FFA production to decrease [32,33].

The combination of calorie restriction with exercise could reduce food intake which affected the production of fatty acids in the body. During exercise, hypoglycemia occurred, which could increase glucagon. When providing calorie restriction with exercise, blood glucose levels decrease which caused a decrease in insulin secretion and an increase in lipolysis [27]. The increased lipolysis state with reduced lipogenesis theoretically contributed to a decrease in body fat stores. High lipolysis causes TG to be hydrolyzed into glycerol and FFA [28]. FFA was used as a source of energy and mobilized to the blood circulation, caused FFA levels to decrease.

Conclusion

Based on the results of the study, it can be concluded that the provision of calorie restriction and moderate-intensity continuous exercise 3x /week for 4 weeks was effective in reducing serum FFA levels and visceral fat weight in female mice exposed to a high-calorie diet. Further research is needed to reveal further about the mechanism that might be involved such as hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL).

Conflict of Interest: The authors declared that there is no conflict of interest.

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The Effect of Imbalanced Levels of Some Serum Biochemical Elements on Senile Cataract Formation

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Abstract

Background: Cataract is the clouding of lens which subsequently may impair vision. The main subtypes of senile cataract are cortical, posterior sub-capsular and nuclear sclerosis. Electrolyte disturbance has been linked with the development of age related cataract.

Aim of the Study: To assess the correlations between the levels of the blood biochemical elements (Ca^{++}, Na^{+}, K^{+}, Cl^{-}) and the formation of senile cataract.

Patients & Method: Blood samples of 35 patients of cataract and 35 of non-cataract/ healthy people have been analyzed for calcium and electrolytes levels, all cases were female whose age ranged between (50 – 70 years). (5 mL) of blood was taken from the patients' veins for the study. The serum was separated of each sample by a centrifuge at (4000) rpm for (4 min) and used for the mentioned biochemical exams. (FUJIFILM/ DRI-CHEM NX500i) biochemical analyzer was utilized in the study. The results were obtained directly from the analyzer.

Results: In cataract patients group, there was a significant variations in calcium level (high, normal, and low) as well as sodium level (low and normal) compared to the control. There was no significant differences in the level of (K^{+} and Cl^{-}) between two groups.

Conclusion: In the current study, there was a significant differences in calcium and sodium level in cataract patients group compared to the control. These variations might be one of many factors that contribute in the formation of cataract.

Keywords: *Cataract, Electrolytes, Calcium, Sodium, Potassium, Chloride.*

Introduction

Cataract in an ophthalmological disease is characterized by the clouding of lens which subsequently may impair vision. In 2010, cataract was responsible for more than 50% of blindness worldwide. (1) Age related cataract or senile cataract develops due to age related changes with the body's ability to detoxify the reactive oxygen species or even to repair the damages induced by these substances. (2,3)

The main subtypes of senile cataract are cortical, posterior sub-capsular and nuclear sclerosis. Cortical cataract arise from the opacity of the lens cortex and posterior sub capsular cataract affect the back of the lens next to the capsule. Nuclear cataract arises from the lens center and induces hardening or sclerosis in the nuclear brown pigment deposition. (4)

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Electrolyte disturbance has been associated with multiple manifestations, and many of the studies had linked serum electrolyte disturbance with the development of age related cataract. [5,6]

The aim of the study is to assess the correlations between the levels of the blood biochemical elements (Ca++, Na+, K+, Cl-) and the formation of senile cataract.

Patients & Method

The study was conducted in (Al-Nahrain Eye Specialty Center) in Ramadi – Iraq. Inclusion criteria included, female with and without cataract. Some cases had diabetic, osteoporosis, which were placed into consideration. The ages' range, that the study focused on, was between (50-70) years Numerous clinical diagnostics, such as pupillary reactions to light, near visual acuity and distance, intraocular pressure measurement, and Slit lamp Biomicroscope were conducted to determine cataractous patients. Two cases were nuclear cataract type while the rest was mixed between Cortical &Posterior sub-capsular. Blood samples of 35 cataract patients and 35 of non-cataract/ healthy people have been analyzed for calcium and electrolytes levels. (5 mL) blood was taken from the patients' veins for the study. The serum was separated of each sample by a centrifuge at (4000) rpm for (4 min) and used for the mentioned biochemical exams. (FUJIFILM/ DRI-CHEM NX500i) biochemical analyzer was utilized in the study. The results were obtained directly from the analyzer.

The data was analyzed by Statistical Package for the Social Sciences (SPSS), version 24.0.

Result

In this study, 35 senile cataract patients and 35 healthy people were selected. All the cases were female whose age ranged between (50 – 70 years). Two of the selected senile cataract cases were Nuclear, and 33 cases were mixed cortical posterior sub-capsular. The cases were conducted by an ophthalmologist, cortical posterior sub-capsular cataract was predominant as it is illustrated in (Figure 1)

Cases	Calcium (8.62-10.23 mg/dl)			Potassium (3.4-5.1 mmol/L)			Sodium (134 - 148 mmol/L)			chloride (95 - 110 mg/dl)		
	Normal	Low	High	Normal	Low	High	Normal	Low	High	Normal	Low	High
Senile cataract	19	11	5	33	2	0	29	6	0	34	1	0
Healthy	35	0	0	35	0	0	35	0	0	35	0	

Table (1) Levels of biochemical elements in cataract patients and healthy people

Senile cataract	N.O. Patients	Calcium (8.62-10.23 mg/dl)		Potassium (3.4-5.1 mmol/L)		Sodium (134 - 148 mmol/L)		chloride (95 - 110 mg/dl)	
		High	Low	High	Low	High	Low	High	Low
Diabetic	7	3	2	0	0	0	3	0	1
Osteoporosis	23	14	11	-	-	-	-	-	-

Table (2) Cases of senile cataract with diabetic and osteoporosis

Biochemical elements (Na^+, K^+, Cl^-) and Ca^{++} levels in patients blood's serum were measured using (FUJIFILM/ DRI-CHEM NX500i) biochemical analyzer) Those patients were questioned to determine whether they have had diabetic, high blood pressure, or osteoporosis. This further and profound investigation was aimed to see if the mentioned diseases are correlated with the obtained results or not. Table (1) demonstrates the conducted study on the normal and up-normal levels of the electrolytes and calcium in cataract patients.

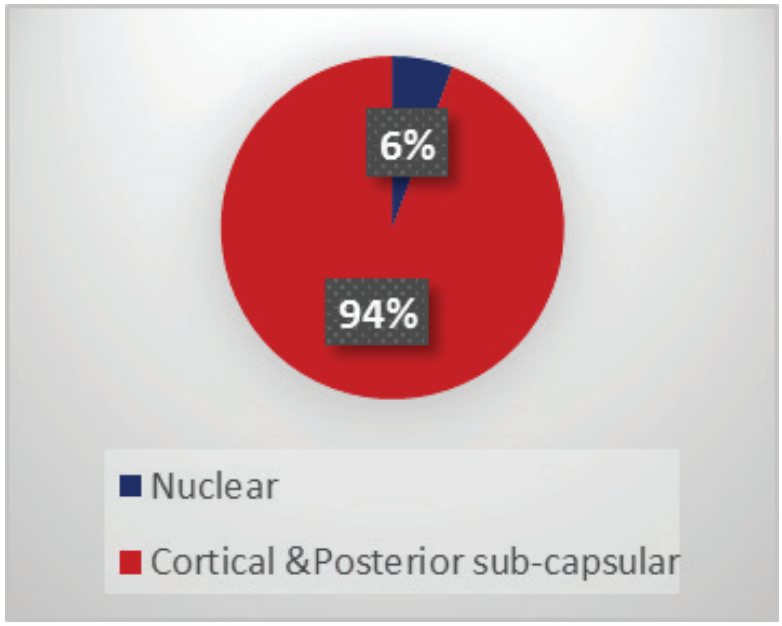

Figure (1): percentage of selected patients with different types of cataract

Surprisingly, significant differences were obtained when the result of the levels of calcium and the electrolytes in cataract patients were compared with the healthy people. Some cases were found to be normal, however, massive differences were acquired in calcium and sodium levels. Figure (2 and 3) show the percentage of high and low levels of biochemical elements in cataract patients

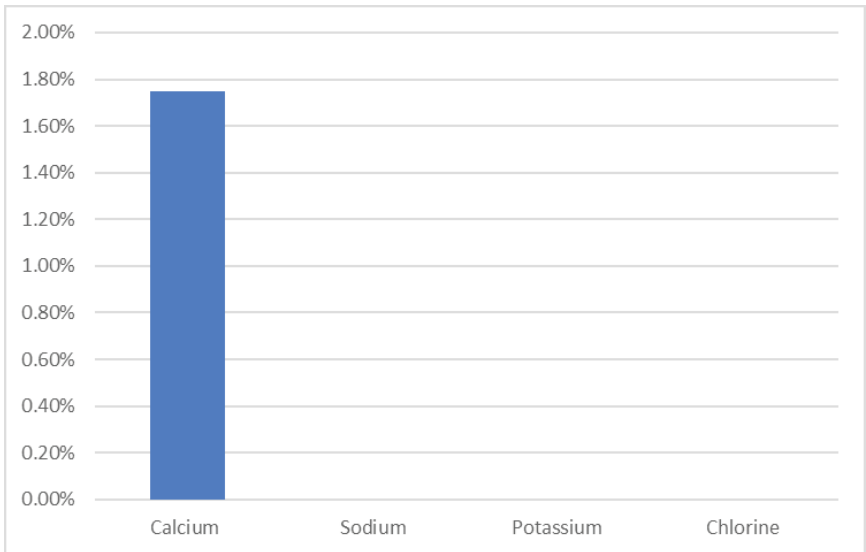

Figure (2) The Percentage of high levels of Biochemical Elements in Cataract Patients

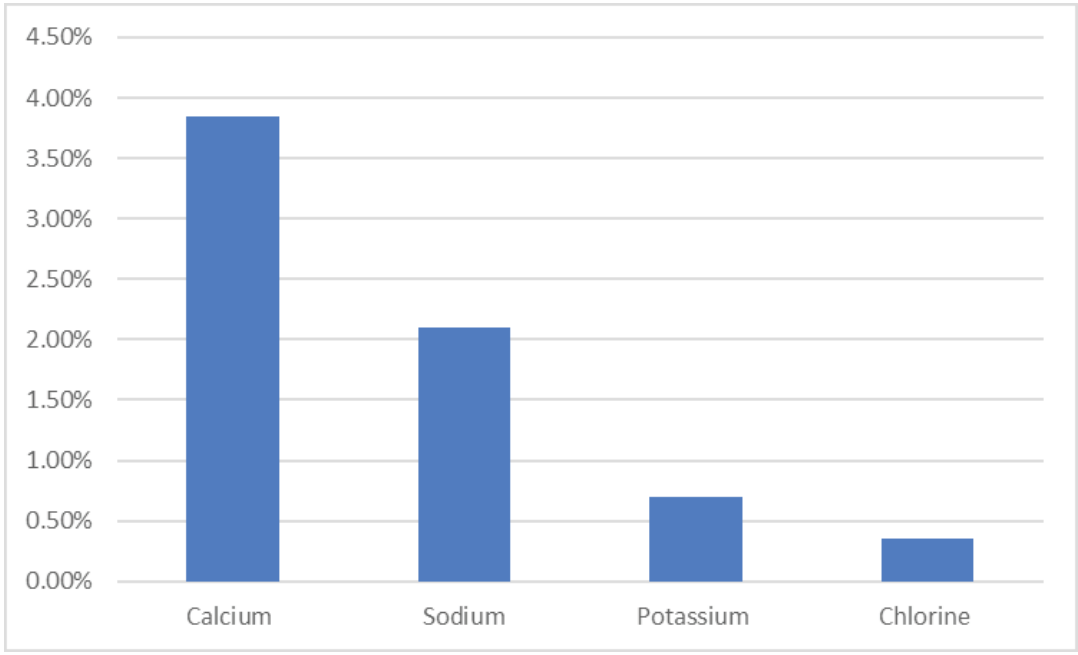

Figure (3): The Percentage of Low levels of Biochemical Elements in Cataract Patients

Discussion

Numerous scientific studies have been conducted all over the world aiming to find out whether there is a correlation between the levels of calcium and biochemical elements/electrolytes (Na^+, K^+, and Cl^-) in human serum and cataract formation. Based on the results, that those studied were come up with, it was suggested that nutritional factors play a significant role in raising or lowering the levels of calcium and electrolytes in cataractous patients. Likewise, protein accumulation, which is one of the major causes of blindness, and oxidative stress are considered and depended on to find out any correlation between those factors and cataract formation. [7] Our study was concentrated on only women whose ages are between 50-70 years. The study suggests that the levels of those

Elements in the serum of senile cataract patients were lower in most cases, as it is shown in table (1), compared to the control. Many studies have been shown significantly different results where the levels of (Ca^{++}, Na^+, K^+, and Cl^-) were high, normal, and low. These variable results could be attributed to the nutrition system and dietary supplements in different spots all over the world. Our study is coordinated with the study that was conducted in Iran by Ipchi Sheshgolaei P, and co-workers. Iranian study was shown that there was not a remarkable difference in K^+ level in both cataract and healthy patients. However, lower values ($P<0.05$) were shown in the level of Na^+ in cataract patients compared to the control (44.0±2.13 vs. 49.7±2.5 µg/dl). [8] In contrast, an Italian-American and Pakistan published studies have been stated that no difference was obtained when comparing the blood biochemical elements levels in cataract patients and the control. [9,10] . In 2016, a study, that was published by Mathur and Pai, was illustrated a high serum Na+ in cataract patients compared to the control, while there was an insignificant change in the level of K in both cataract and control cases. [11]

In the current study, it was found that (3.85%) of senile cataract patients was diagnosed with low serum calcium level. Those individuals were diagnosed previously with osteoporosis disease. Osteoporosis is a bone disease that is formed when the production of bones decreases and the losing of bones increases. The major factors that inducing the formation of osteoporosis are deficiency of vitamin D3 and estrogen hormone, as well as, calcium insufficiency**.** [12] Eleven patients (with osteoporosis), as they are indicated in table (2), have been obviously shown to have low calcium level.

When the level of calcium in blood decreases, the body alters its path and absorbs calcium in blood quicker

from the bones. As a result of that, the bone's mass decreases, and the formation of osteoporosis occurs. [13] The selected cases, that matched the control, was shown that cataract is linked along with a 43% elevated risk of osteoporosis. [14] In 2013, a study by Nemet, Arie Y. and co-workers suggested that there is an association between the minerals' levels, calcium for instance, and the formation of cataract and osteoporosis. [15] Low level of calcium in the blood leads to a deficiency in vitamin D3, and the formation of osteoporosis takes place. When an individual run into an osteoporosis issue, there is a big chance to get infected by cataract. Five out of 35 selected cases have shown to have a high calcium level which could be as a result of cataract formation. Level of calcium in the lens elevates because of cataract formation. Further investigation and (ion-sensitive microelectrodes) measurement for instance, need to be conducted to figure out the reason behind the elevation of calcium in the blood, and how it correlates with the level of it in the lens. [13,16] There is a matter that must be put into consideration, which is the selected gender and the aging factor. Postmenopausal women tend to have a low serum calcium level. Thus, parathyroid hormone levels tend to elevate and a significantly transformation of the body's bones takes place. [17] As it is mentioned above that low calcium level is one of the causes of osteoporosis, and osteoporosis is a factor that triggers cataract's formation. Serum sodium level should be normal to keep the water electrolyte level equilibrated in the lens membrane, which is in charge of the permeability of the lens. **[18]** Low serum calcium levels in senile cataract patients were obtained in many previous studies, which match our results. [12]

(2.1%) of the senile cataract patients were diagnosed with low serum sodium level in compression with the control. Low serum sodium level (>134 mmol/L) is due to numerous factors such as liver disease, kidney disease, medication uses, and so on. In addition to that, elevating the glucose level, which is considered as an osmotically active material, in the blood plays an important role in falling down the level of sodium in blood. An investigation was conducted, and it was found that most of these patients have had diabetic mulitas. When glucose level increases, the osmosis of the serum increases. Therefore, the water gets out of the cells, and a reduction of sodium level occurs. [19,20]

Chlorine and potassium levels in compression with the control were insignificant. Only two patients have had slightly low potassium levels, while one patient was diagnosed with a low chlorine level. Those patients may have had other diseases that have been reduced the levels of (Cl^- and K^+). In conclusion, Dietary system in different places around the world impacts the levels of electrolytes and calcium in blood by elevating and lowering it. High level of calcium leads to cataract formation by changing the molecular structure of the lens and the lens heightens the light scattering. In contrast, low serum calcium level causes osteoporosis which leads to cataract formation. Decreasing the level of Na+ in the blood may due to diabetic. High glucose level in the blood makes the water gets out and the Na content decreases. The levels of K+ and Cl- in the current study were insignificant.

Ethical Consent: Ethical approval for this study was obtained from every patient before participation in the study.

Conflict of Interest: there is no conflicts of interest.

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Original article

Effect of Information, Education and Communication (IEC) on Knowledge and Attitude regarding Premarital HIV and Haemoglobin Genotype Testing among Adolescents

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Abstract

Background: Premarital screening is a test done for younger adults and couples considering marriage for prevention of genetic, infectious and blood transmitted diseases. Adolescent and college young adults must be educated about potentially life-threatening infectious and genetic disorders for the promotion of their health, well-being as well their offspring too. For providing accurate and unbiased education and information, the IEC is considered to be an effective intervention strategy. **Materials and Methods:** The study was done to assess the effect of IEC on knowledge and attitude regarding premarital HIV and Haemoglobin genotype testing among adolescents in a selected Pre-University Colleges, Bengaluru. An evaluative approach with quasi experimental pre-test and post-test control group design was used. A study sample comprised of 60 adolescents in experimental group and 60 adolescents in control group. The sample was selected using lottery method of the simple random sampling technique. The Structured knowledge questionnaire and Three point Likert scale was used to assess the knowledge and attitude level of adolescents respectively. **Results:** Study results revealed that the experimental group which received IEC showed significant difference in the improvement of knowledge ($p < 0.001$) and the attitude score ($p < 0.001$). **Conclusion:** Based on the study findings, it can be concluded that IEC is a best strategy to spread awareness through proper use of materials to young people to achieve a desired positive outcomes in terms of improving knowledge and positive attitude on prevention of life threatening infectious diseases and genetic disorders.

Keywords: *Information, Education, Communication, Knowledge and Attitude,*

Introduction

Adolescent is one of the most important phases in human development characterised by physical, psychological, sexual and social maturation[1]. When adolescent become sexually matured they may have high risk behaviour[2] like having early sexual initiation before marriage, unprotected sexual intercourse, sex with multiple partners, and unprotected sex with partners result in sexually transmitted infections, genetic disorders which make them more susceptible to get STD, unwanted pregnancy, and unsafe abortion and contributes to different adverse effects[3]. In worldwide hemoglobinopathies such as the sickle cell disorders and the thalassemia syndromes are the most common genetic disorders, typically inherited as autosomal recessive disorders from healthy-carrier parents to children through genes. Among the blood-borne viruses HIV, HBV and HCV are important and have

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several implications[4,5]. Information education and communication (IEC) is the process of learning that empowers people to make decision, modify behaviours and change social conditions in relation with health. An IEC approach are used to reach target groups very easily and provides self-belief concerning premarital testing of HIV and Haemoglobin genotype, so as to protect and sustain their own health and that of their partners including the children[6,7]. Hence, IEC intervention is deemed to be the best method to provide information to the adolescents regarding premarital screening and get to know about health challenges and prevention of any risk of transmitting disease to spouse and children because they are specially targeted audience for the same as they are the most vulnerable group towards getting sexually transmitted infections. The World Health Organization (WHO) and the World Bank recognises the important role of IEC in the achievement of health, nutrition, and population goals and recommended its inclusion in health programmes for sustainable and better health outcomes[8,9,10]. The study was done to evaluate the impact of IEC on knowledge and attitude regarding premarital HIV and Haemoglobin genotype testing among adolescents. It was hypothesized that there was a significant improvement of knowledge level and changes in attitude among adolescents received IEC intervention.

Material and Methods: The study adopted a quantitative research approach with a quasi-experimental pre-test and post-test control group design. The study was carried out at selected Pre-University Colleges, Bengaluru. Before conducting the study, an administrative permission was obtained from selected Pre-University Colleges. Both male and female adolescent, aged 15-21 years and who are available at the time of data collection were the focus population for this study. They were 120 adolescent that is 60 in experimental group and 60 in control group were selected from two different schools to eliminate possible contamination of the study participants. A lottery method of simple random sampling technique was used for sample selection. Ethical clearance was approved by the ethical committee of the institution. Data was collected after the details of the study were explained to them. Informed consent was obtained from each participant. Assurance was given to participants that anonymity of each individual would be maintained and were informed of their right to withdraw anytime during the course of the study. The tool used for data collection were socio demographic proforma, structured knowledge questionnaire to assess the knowledge level and Three point Likert scale to assess the attitude level of participants. The reliability, content validity was established for all tools. After the selection of participants, the pre-test assessment of knowledge and attitude were done. The experimental group received information, education and communication on premarital HIV and Haemoglobin genotype testing by using various methods and materials include a lecture, PPT slides, leaflet, poster, educational video and booklet. The post-test assessment of knowledge and attitude was carried out after seven days of intervention. At the end of the research study, the control group was also provided with the same content of all the information which is provided to the experimental group. The data analysis has been done using SPSS 19 version. Data was analyzed using descriptive and inferential statistics.

Results

Subject demographic characteristics: The frequency and percentage distribution of baseline characteristics are described in Table-1.

Data presented in Figure- 1 show that in experimental group 46 out of 60 (77%) of them had inadequate pre-test knowledge score. After the IEC intervention, majority of them that is 27 out of 60 (45%) of them had adequate post-test knowledge score. Whereas in the control group majority of them that is 43 (72%) had inadequate post-test knowledge score.

The data presented in Figure- 2 show that in the experimental group 31 out of 60 (52%) had positive pre-test attitude score before IEC intervention given. After the IEC intervention in the experimental group, majority of them 50 out of 60 (83%) had positive post-test attitude score. Whereas, in the control group, majority of them 51 out of 60 (85%) and 36 out of (60%) had neutral attitude score in pre-test and post-test respectively.

Effectiveness of IEC on knowledge: The experimental group mean post-test knowledge score (M=12.9, SD=2.60) was higher than the control group mean post-test knowledge score (M=7.63, SD=2.54). There was a significant gain in knowledge level ($t(118) = 11.21$, $p<0.001$) among the experimental group (Table 2).

Effectiveness of IEC on Attitude: The experimental group mean post-test attitude score (M=36.38, SD=4.10) was higher than the control group mean post-test attitude score (M=30.60, SD=4.18). There was a significant change in attitude level (t (118) =7.63, p<0.001) among the experimental group (Table3).

The chi-square test show no significant association was found between knowledge score and selected socio-demographic variables, but there was a significant association found between attitude score and selected socio-demographic variables like age and life style practice, at 0.05 level of significance.

Discussion

College and University students as a population are particularly vulnerable to getting infectious diseases. Young adults are expected to gain sexual experience by starting sexual relationships at an earlier age or having multiple sexual partners[11]. Inadequate knowledge of HIV and AIDS regarding prevention measures can produce the false perception of the disease among youth population. The youth are much more prone to HIV infection as well as other sexually transmitted infections as a result of a lack of correct health information[12]. Now a day, the youths are indulging in unhealthy practices at relatively early age. Easy accessibility to internet facilities is one of the reasons. According to the World Health Organization (WHO), approximately 240 million people are carriers of genetic disorders and at least 200,000 affected individuals are born annually which is approximately equally divided between sickle cell anaemia and thalassemia[13]. Many studies have been conducted to evaluate outcome of educational intervention on knowledge and practice regarding HIV and its transmission. But very fewer studies have been done to determine the effectiveness of IEC on pre-marital HIV and genotype testing among adolescents. The World Health Organization (WHO) and the World Bank recognises the important role of IEC in the achievement of health, nutrition, and population for better health outcomes[14]. Pre-marital HIV testing contributes to the prevention of HIV infection by diminishing heterosexual transmission between partners and indirectly also protects any potential child from contracting the virus. Worldwide, youth are often viewed as immature individuals who are not ready to make the right decisions regarding their sexual lives, and parents are expected to monitor and inform their decision about sexual and reproductive health. Genetics educational programs that target youths may improve knowledge of genetics and create a public perception that further supports genetic testing[15]. Providing sexual communication and education on sexual and reproductive health is important to prevent sexual risk among adolescent[16]. IEC is a method that empower individual to make decision and promotes positive behavioural changes. It helps to improve the understanding and attitudes towards prevention of genetic blood disorders. The findings of the present study reveal that the adolescent's knowledge about HIV and genetic disorder and its treatment was relatively low and the poorest attitudes were observed towards before intervention. Adolescent's limited knowledge about HIV could be attributed to their lack of access to sexual health information. After IEC intervention there was a significantly gain in knowledge and attitude on premarital HIV and Haemoglobin genotype testing. There was no treatment and apparently no way to stop the spread of HIV infection, except by engaging in dubious efforts to change people's behaviour. In a similar study, it was observed that IEC has significantly increased the knowledge and positive attitude of the study population[17].

Conclusion

Adolescents period is an important part of the development process. Due to engagement in high risk behaviour there is an increasing rate of prevalence of spread of infectious and genetic disease. It may cause short term and long term health effects. IEC has role to increase awareness, knowledge and attitude about Premarital HIV and Haemoglobin Genotype Testing. The similar intervention can be conducted in the curriculum of secondary education is recommended.

Table-1: Frequency and percentage distribution of adolescents according to their socio demographic variables N=120

S.No	Socio demographic variables	Experimental group (n=60)		Control group (n=60)	
		f	%	f	%
1	Age in years 16-17 18-19	 43 17	 72 28	 40 20	 67 33
2	Gender Male Female	 31 29	 52 48	 34 26	 57 43
3	Class PUC I PUC II	 23 37	 38 62	 32 28	 53 47
4	Religion Hindu Christian Muslim	 49 6 5	 82 10 8	 55 2 3	 92 3 5
5	Types of parent marriage Marriage between relatives Marriage between non-relatives	 7 53	 12 88	 11 49	 18 82
6	History of HIV in the family Yes No	 1 59	 2 98	 - 100	 - 100
7	History of SCD/ thalassemia in the family Yes No	 - 60	 - 100	 - 60	 - 100
8	Awareness about premarital HIV and Haemoglobin genotype testing Yes No	 - 60	 - 100	 - 60	 - 100

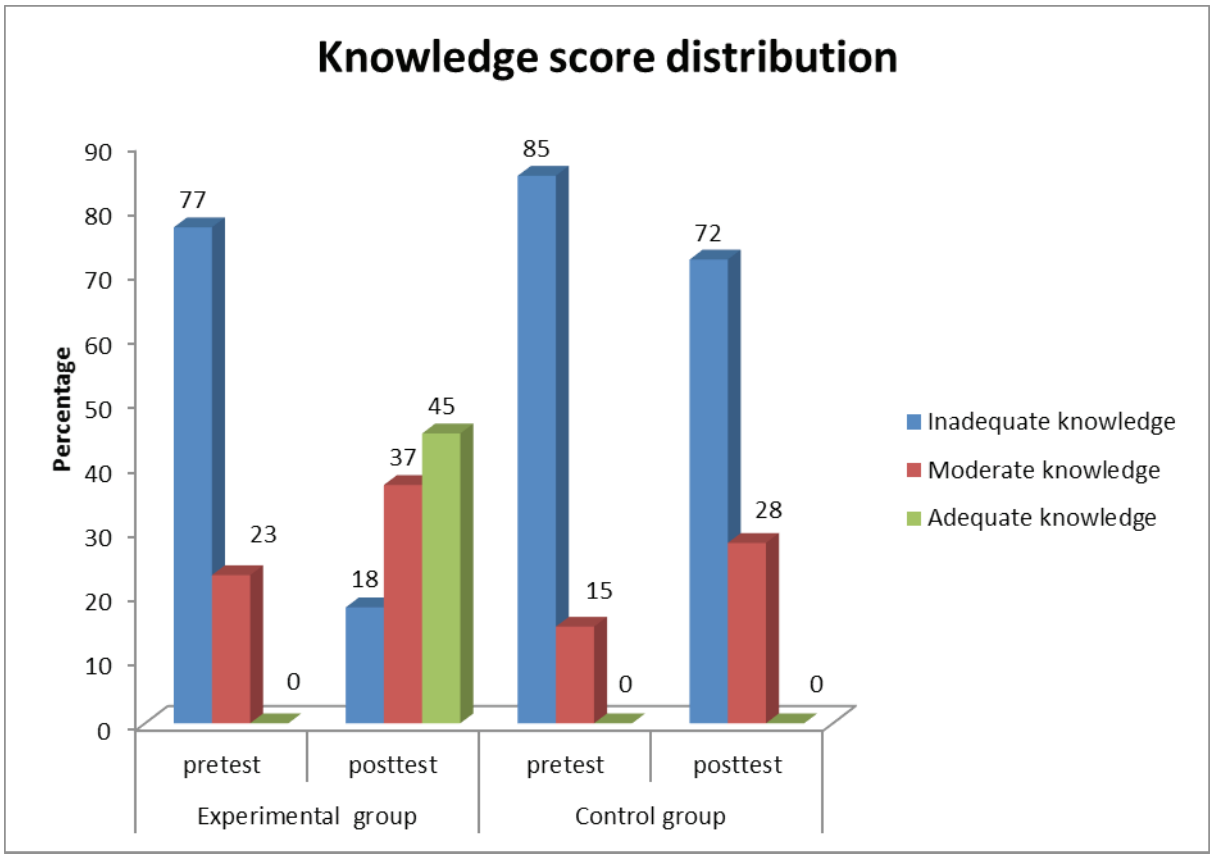

Fig 1: Frequency and percentage distribution of adolescents according to pre-test and post-test knowledge score N=120

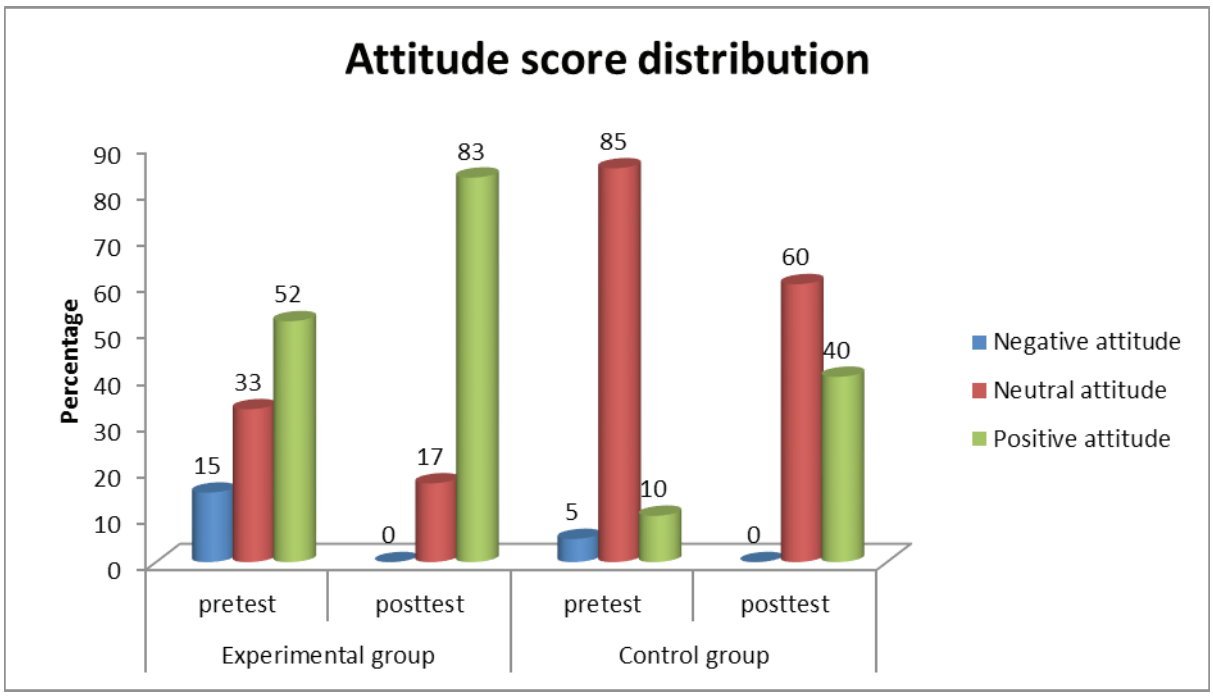

Fig 2: Frequency and percentage distribution of adolescents according to pre-test and post-test attitude score N=120

Table- 2: Overall knowledge score of adolescents

Group	n	Mean	SD	MD	t-value	p value
Experimental group	60	12.900	2.602	5.267	11.210	(p<0.001)
Control group	60	7.633	2.544			

* significant at 0.05 level of significance; df=118

Table- 3: Overall attitude score of adolescents

Group	n	Mean	SD	MD	t-value	p value
Experimental group	60	36.383	4.109	5.783	7.636	(p < 0.001)
Control group	60	30.600	4.187			

*significant at 0.05 level of significance; df=118

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Psychological Impacts, Hand Hygeine Practices & and Its Correlates in View of Covid-19 among Health Care Professionals in Northern States of India

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Abstract

Background: The novel coronavirus disease 2019 presents an important and urgent threat to global health. 190 countries with more than 300 000 confirmed cases and 14 510 deaths[1]. This critical situation, health care workers on the front line who are directly involved in the diagnosis, treatment, and care of patients with COVID-19 are at risk of developing psychological distress and other mental health symptoms and transmission between people through close contact and droplets[2-3].

Method: Descriptive correlation survey design was to assess psychological impacts, hand hygiene practices & and its correlates in view of covid-19. Probability Stratified cluster staging sampling techniques were used to select 320 health care professionals.

Result: The findings revealed that the effect of noval coronavirus covid-19 among health care professionals 43.8% had mild fear, 45.9% moderate fear, and 10.3% a severe fear, and their mean value (14.58), S.D (6.96) and the variance was 48.54. Level of mild perceived stress was 46.87%, moderate perceived stress was 44.06% and 9.06% had severe perceived stress their mean value (14.417), S.D (7.09) and variance was (50.38).Researcher is also assessing the level of practice was 94.37% had good practice, 5.62% had weak practice their mean value (18.22), S.D (3.15) and variance was (9.92) regarding the prevention of novel coronavirus covid -19 among Health care professionals. The correlation between fear and perceived stress was intermediate correlation, fear and practice had weak positive correlation and in between perceived stress and practice was showing weak positive but nearly close to no correlation. Fear was significantly associated with workplace was, perceived stress was significantly associated with Gender, No. of family members was statistically significant at $P<0.05$.

Conclusion: Novel coronavirus covid-19 was affecting their psychological factors among health care professionals those are working in hospitals.

Key word: *Fear, Health care professionals, Novel coronavirus covid-19, Perceived stress, Practice*

Introduction

Covid-19 was indicate human-to-human transmission and thus the risk of much wider spread of the disease[4]. The coronavirus is contagious during the incubation period (2-10 days). There's no specific treatment for the coronavirus, and you'll be prescribed medicine that would treat your symptoms[5-6]. A vaccine is in the works, and that will stop the infection, but the vaccine won't be done for a few months, as it has to undergo the same steps as any new treatment, including clinical trials and approvals from oversight bodies[7].

This pandemic situation is developing the challenges for healthcare staffs are currently facing. This critical

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situation, health care workers on the front line who are directly involved in the diagnosis, treatment, and care of patients with COVID-19 are at risk of developing psychological distress and other mental health symptoms[8-9]. The ever-increasing number of confirmed and suspected cases, overwhelming workload, depletion of personal protection equipment, widespread media coverage, lack of specific drugs, and feelings of being inadequately supported may all contribute to the mental burden of these health care workers[10]. The various research studies have reported that the health care professionals affecting from adverse psychological reactions due to the (SARS-Cov) outbreak[11]. Studies showed that those health care workers feared contagion and infection of their family, friends, and colleagues, felt uncertainty and stigmatization, reported reluctance to work or contemplating resignation, and reported experiencing high levels of stress, anxiety, and depression symptoms, which could have develop the long-term psychological effects. Similar concerns about the mental health, psychological adjustment, and recovery of health care workers treating and caring for patients with COVID-19 are now arising[12-13].

Irrespective of the measure implemented, health care workers must have the required IPC education and training about the correct use of PPE and other IPC precautions, including demonstration of competency in appropriate procedures for putting on and removing PPE required for direct care of patients with COVID-19[14-15].

This study aims to observe various points. First, it aims to find out how it's producing changes in the affective state of the health care professionals. Next, it is expected, that we assess the level of fear perceived stress and the practices of health professionals due to the overwhelming situation they are living in hospitals around the world. This survey is the first attempt to measure the psychological consequences this pandemic is having. The results of the study will help determine the mental health care that might be needed.

Material and Methods

Research Approach: Quantitative research approach.

Research Design: Descriptive correlational survey

Source of Data Collection: Data was collected from the Health care professionals those are working in selected hospital of northern states of India.

Population: population consist all the Health care professionals. The accessible population of study consists of health care professionals in selected hospitals. The target population was the selected hospitals of northern India.

Sample: Health care professionals working in selected hospitals of northern states of India.

Sample size and Sampling Technique:

The sample size for the final study consists of 320 health care professionals working in selected hospitals of northern India. Probability cluster sampling technique was used to select the sample. The rational was the numbers of Healthcare workers are having a busy schedule due to coronavirus Covid-19.

Ethical Consideration:

Research proposal will be approved by ethical / DRC committee of university. Prior permission will be taken by concerned authority of SGT University. Informed consent will be taken from each selected sample.

Result and Findings

Findings related to frequency and percentage distribution of selected demographic Variables:

According to the Age group the maximum participant was in age group of 23-31years (91.56%). Gender was 82 (25.62%) was male and 238(74.37%) was females. Work tittle 31(9.6%) was doctors, 170 (53.12%) was Nurses, 12(3.75%) teaching faculties, 82(25.62%) was Intern students, 4(1.25%) Lab technicians, 21(6.5%) was other health care professionals. Marital status 63(19.68%) was married and 257(80.31%) was unmarried health care professionals. Number of people in family members was 1-5 members (72.18%),6to 10 (24.68%),11 and above members (3.12%).Working experience of health care professionals was less than 1year was (54.37%),1-2 years(21.25%),3-4(13.75%) and 5years and above was 10.62%. Work place those are working in Government hospital 39(12.18%), private hospitals 229(71.56%) and 52(16.25%) was working in semi government hospitals. Awareness regarding the

prevention of coronavirus covid-19 the 315(98.43%) samples are aware and 5(1.56%) was not aware. Working in covid-19 designated wards was 61(19.06%) and 259(80.93%) was designated in non covid-19 wards. Presence of any vulnerable /suspected people are presently residing along with your family is 31(9.68%) and 289(90.31%) was not living along with vulnerable or suspected peoples. Any personal medical insurance 103(32.18%) are having or 217(67.18%) are not having any personal medical insurance.

Findings related to the Frequency and Percentage distribution of Fear, perceived stress and level of practice among health care professionals working in selected hospitals

Table 1: Depicts The Level Of Fear Regarding Corona Virus Covid-19 Among Health Care Professionals. n=320

V V variable	LEVELS OF FEAR		
	Mild Fear (0-12) F (%)	**Moderate Fear (13-24) F (%)**	**Severe Fear (25-36) F (%)**
Fear a Level of fear regarding coronavirus Covid-19 among health care professionals	140(43.8%)	147(45.9%)	33(10.3%)

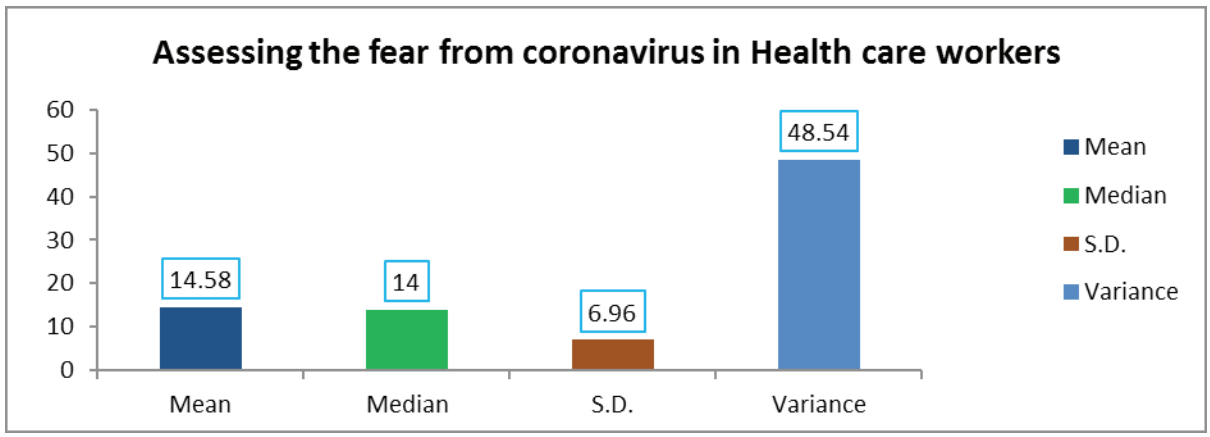

Figure-1: bar diagram showing the mean, median, SD and variance of fear among health care professionals

The Level of perceived stress was 150(46.87%) had mild perceived stress, 141(44.06%) had moderate and 29(9.06%) among health care professionals.

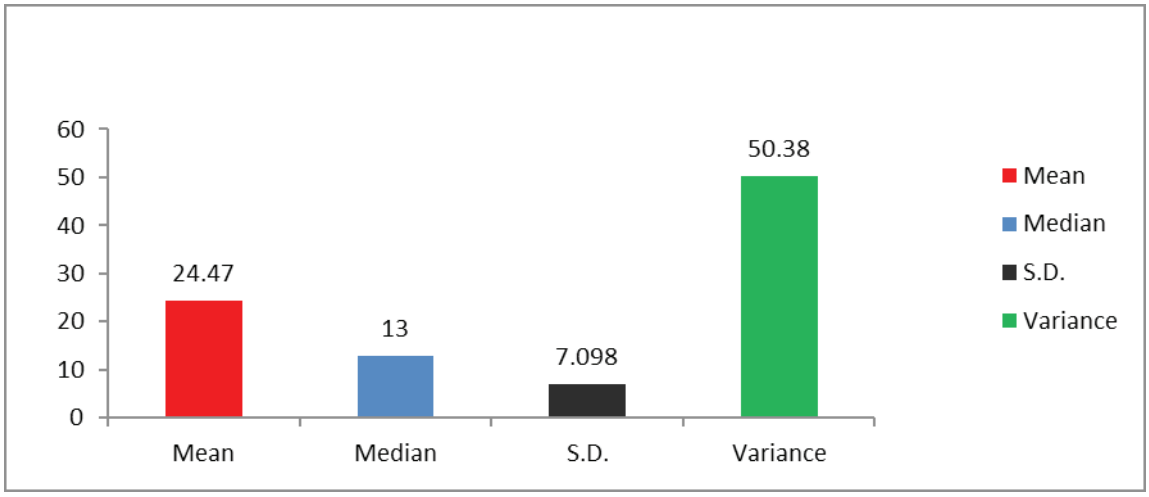

Figure- 2: bar diagram showing the mean, median, SD and variance of perceive stress among health care providers working

· The level of practice was 302(94.37%) had good practice and 18(5.62%) had weak practice their mean value (18.22), median (19) Standard deviation (3.15) and variance was (9.92) regarding the prevention of coronavirus covid -19among Health care professionals.

Findings related to the correlation in between the variables Fear, perceived stress and level of practice among health care professionals working in selected hospitals

· The karl pearson coefficient of correlation method was used to calculate the correlation in between fear and perceived stress was (r= 0.64) intermediate positive correlation was found.

· In between fear and practice the correlation was (r = 0.042) interpretation was a weak positive correlation in these two variables.

· Correlation in between perceived stress and practice was (r=0.029) showing weak positive correlation but very nearly close to the no correlation in these two variables respectively. Hence, research hypothesis H_1 was accepted and null hypothesis H_{01} was rejected.

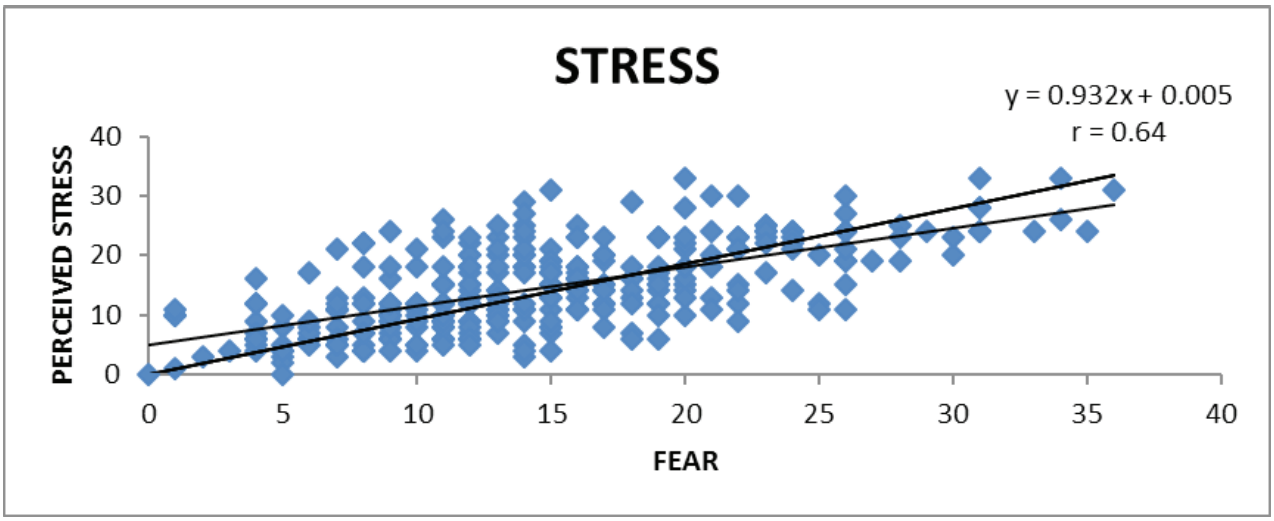

Figure- 3: Scattered dotted diagram shows that the correlation in between fear and perceived stress

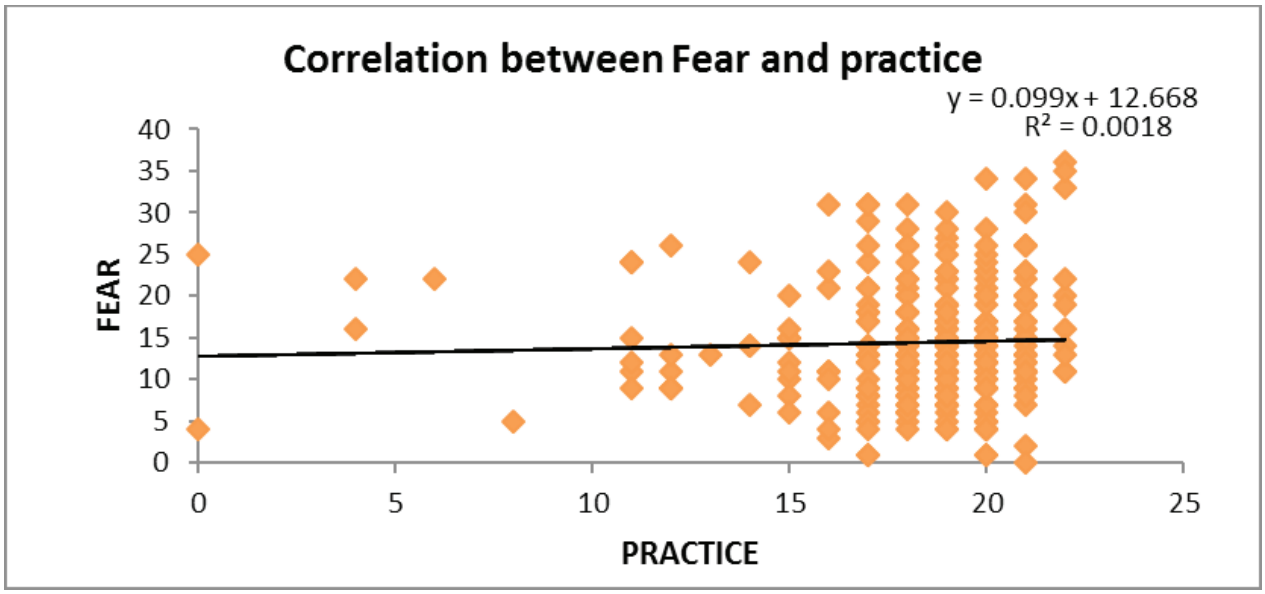

Figure-4: Scattered dotted diagram shows that the correlation in between fear and practice

Findings related to association of level of fear, perceived stress and practice score among health care professionals with selected demographic variables

· Chi-square was applied to survey group with respect to fear scoring in that workplace (χ^2=10.68) was significant at 0.05 level of significance and other demographic variables are found to be non-significant at 0.05 level of significance.

· Level of perceived stress was significantly associated with Gender (χ^2=8.36), Number of family members (χ^2=10.515) value was significant at the level of significance P<0.05 and There is no association was found in between perceived stress other selected demographic variables.

· Level of Practice was not found significant at the level of significance was P<0.05 with any selected demographic variables.

· Hence, research hypothesis H_1 was accepted and null hypothesis H_{01} was rejected.

Findings related to single factor measure of ANOVA showing the variance in between variables fear, perceived stress and practice.

· The ANOVA test was applied to assess the variance in between the level of fear with selected demographic variables in survey group, there was significance of variance in Workplace (F=7.650, P=0.0005) that found to be statistically significant at 0.05 level of significance.

· Workplace (F=3.284, P=0.038) was statistically sowing the significance of variance at 0.05 level of significance of health care professionals with their level of perceived stress score except the workplace other demographic variables are found to be statistically non significant at 0.05 level.

· Thus, the level of practice score was found to be non significant with the all selected demographic variables at the 0.05 level of significance.

Findings related to single factor measure of ANOVA showing the variance in between variables fear, perceived stress and practice.

· There was a significant difference in between the three variable means that was statistically significant as evaluated by single factor measure of ANOVA (f value=40.164, p value=1.77). Hence, research hypothesis H_2 was accepted and null hypothesis H_{02} was rejected.

Discussion

To assess the Level of fear, perceived stress regarding novel coronavirus Covid-19 among

the health care professionals working in selected hospitals.

In the present study the Level of fear was 140(43.8%) had mild fear, 147(45.9%) had moderate fear, and 33(10.3%) had a severe fear, and the Level of perceived stress was 150(46.87%) had mild perceived stress, 141(44.06%) had moderate and 29(9.06%) had severe perceived stress regarding coronavirus covid-19 among health care professionals. In congruence with these findings some studies are conducted by **Shuai Liu, Lulu Yang, Chenxi Zhang et.al (2020)** was conducted a study on Online mental health services in China during the COVID-19 outbreak. The study result shows that the rapidly increasing numbers of confirmed cases and deaths, both medical staff and the public have been experiencing psychological problems, including anxiety, depression, and stress.

Zhou Zhu, Shabei Xu et.al (2020) was conducted a study on COVID-19 in Wuhan: Immediate Psychological Impact on5062 Health Workers. The outbreak of COVID-19 has laid unprecedented psychological stress on health workers (HWs). The study shows the result as we received 5062 completed questionnaires (response rate, 77.1 percent). 1509 (29.8 percent), 681 (13.5 percent) and 1218 (24.1 percent) HWs reported stress, depression and anxiety symptoms. This study concluded that Women and those who have more than 10 years of working, concomitant chronic diseases, history of mental disorders, and family members or relatives confirmed or suspected are susceptible to stress, depression and anxiety among Health workers during the COVID-19 pandemic. [24]

To assess the Level of practice regarding Novel coronavirus Covid-19 among the health care professionals working in hospitals.

The level of practice was 302(94.37%) had good practice and 18(5.62%) had weak practice regarding the prevention of novel coronavirus covid -19 among Health care professionals. In congruence with these findings some studies are conducted by **Ali Alfahan Samia Alhabib (2020)** 237 participants were included in the analysis. The study Result shows that Participant who received hand hygiene training within the last 3 years (2012-2014) scored higher on a knowledge scale. In the result found that there was an overall positive attitude from participants toward hand hygiene practice. In total, 87.54% acknowledged that they routinely used alcohol-based hand rub, 87.4% had sufficiently decontaminated hands even under high work pressure, and 78.6% addressed that this practice was not affected by less compliant colleagues. **Haozheng Cai, Baoren Tu (2020)** was conducted a study on Psychological Impact and Coping Strategies of Frontline Medical Staff in Hunan Between January and March 2020 During the Outbreak of Corona virus Disease 2019 (COVID-19) in Hubei, China. Study questionnaires were completed by 534 frontline medical staff. The responses showed that they believed they had a social and professional obligation to continue working long hours. Medical staff was anxious regarding their safety and the safety of their families and reported psychological effects from reports of mortality from COVID-19 infection. This study concluded that the COVID-19 outbreak in Hubei resulted in increased stress for medical staff in adjacent Hunan province.

Conclusion

Noval coronavirus was affecting the psychological health like fear perceived stress of health care professionals those are working in hospitals but it will not affecting their practices in hospitals regarding prevention of covid-19.

Conflict of Interest: NIL

Source of Funding- Self

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Natural Honey- as an Effective Adjunct in the Management of Radiation Induced Oral Mucositis

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Abstract

Background - Malignancies are second most common cause of death in the world. Betel quid chewing, smoking, alcohol and low socioeconomic status contribute to its high prevalence. Its treatment protocol includes surgery, radiotherapy, chemotherapy or a combination. Oral mucositis is an unavoidable most frequent, painful, distressing clinical side effect of radiotherapy.

Methods – The study group consisted of 102 subjects diagnosed with carcinoma of head and neck and planned for radiotherapy and had developed oral mucositis, among which 51 were in control group and 51 in interventional group. The control group received 0.9% normal saline while interventional group received natural honey as topical application. Both the groups were evaluated by World Health Organization oral mucositis assessment scale and symptoms were assessed on verbal basis at baseline (I visit) and along with four follow-up visits at an interval of 10 days, for total period of 40 days by 2 investigators. The study duration was of 2 years.

Conclusion- This study concludes that the topical application of honey can be used as an adjunct in alleviating the symptoms and in effective management of radiation mucositis as it is an easily available, simple, potent and inexpensive agent.

Key words*: Apitherapy, Honey, Normal saline, Radiation induced mucositis, Radiotherapy.*

Introduction

Malignancies are second most common cause of death in the world.[1] In South and Southeast Asia, prevalence of head and neck cancer is high. It is ranked one of the eighth most common malignancies in world.[2,3]Cultural habits like betel quid chewing, alcohol consumption, reverse smoking and low socioeconomic status including low intake of fruits and vegetables contribute to its high prevalence.[1] Around 90% of oral carcinomas occur in middle aged men with majority of them being squamous cell carcinomas.[5] Incidence of oral and pharyngeal cancer is more in developing countries. Most involved sites of oral carcinomas are lateral border, dorsal surface and ventral surface of tongue, floor of mouth, lips, gingiva, palate and least being salivary glands. [6,7]

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Its treatment protocol includes surgery, radiotherapy, chemotherapy or combination of all.[8] Generally patient with head and neck carcinoma receives an accumulative dose of 60-70 Gy of radiation for primary treatment depending on size and location of tumour. Its early side effects include mucositis, oral ulcerations, nausea and alopecia. Late side effects include xerostomia, dysphagia, trismus, lymphadenopathy, increased prevalence of dental caries. [9]

Oral mucositis is unavoidable most frequent, distressing, painful, clinical side effect of radiotherapy. [10] The term "mucositis" was introduced in late 1980s to describe inflammation of oral mucosa induced by radiotherapy (in over 80% of the patients), chemotherapy (in 40-80% of patients) and bone marrow transplantation (in over 75% of patients).[11]At present, oral mucositis is considered to be the most serious condition resulting in epithelial and sub epithelial injury.[12]

Exposure to ionising radiation gives rise to radiation epithelitis of oral, pharyngeal and laryngeal mucosa towards 2nd and 3rd weeks of conventional fractionated radiotherapy.[13] Effective management of oral mucositis is of prime concern as there are no drugs for avoidance/ mitigation of radiation-induced mucositis. Palliative procedures consist of symptomatic management and adherence to basic oral care to prevent infections and for proper maintenance.[14]

Medicine in this 21st century is an era of artificial intelligence, laser etc but the traditional ancient ways of treatment are still accepted and practiced by people around the world with lots of faith, one among which is apitherapy in which bee products such as honey, pollen, propolis, royal jelly and bee venom are used to treat illness and promote healing. Clinical implications of apitherapy includes colds, surgical and pressure wounds, burns, sore throat, oral infections and as a general prophylactic agent.

Honey is an age-old remedy from times of Egyptian civilization, but very recently it has found a place in symptomatic management protocols.[1,9] Honey has high viscosity, osmolarity, hygroscopic nature, rich nutritional properties and acidic ph.[15,16,17] In mucositis, mucosal ulcers, inflammation and the impaired mucosal barrier often permits the development of superadded bacterial and fungal infections. Honey is known to have antibacterial properties and enhances epithelisation, thereby improving wound healing.[9] Therefore, honey is considered as an agent that holds promise in reduction of ulceration and inflammation of mucosa.[1]

Material and Methods

The aim and objective of the study was to evaluate the efficacy of natural honey in the management of radiation induced oral mucositis.

To recommend natural honey as an effective adjunct in the management of radiation induced oral mucositis if found effective.

It was a double-blinded, interventional controlled study conducted on patients reported to Bharath Hospital and Institute of Oncology for receiving radiotherapy for head and neck carcinomas and resulted in the development of mucositis. The study population was selected from Oncology centre by randomised sampling and was carried out in the Department of Oral Medicine and Radiology, protocol was approved by the Institutional Human Ethics Committee of HCG-Bharath Hospital & Institute of Oncology ethical committee.

(a) Inclusion criteria:

Patients willing to be a part of the study and sign the written informed consent.

Patients with histologically confirmed head and neck carcinoma and planned for external beam radiotherapy with total radiation dose ranging between 60-70 Gy and presented with oral mucositis.

(b) Exclusion criteria:

Patients with systemic diseases like diabetes.

(c) Withdrawal criteria:

Patients not willing to be a part of study after signing the written informed consent.

All patients were strictly advised to avoid alcohol, smoking, spicy and acidic food and to maintain good oral hygiene by following the instructions of brushing their teeth with soft toothbrush and fluoridated toothpaste after each meal and before going to bed, during course of radiotherapy.[7] Study group consisted of 102 subjects divided by randomized sampling into

equal number among both interventional and control groups by assigning every 2nd patient to interventional group, satisfying inclusion criteria. Measured amount of commercially available Dabur natural honey (manufactured by Dabur India Limited, one of the India's largest Ayurvedic medicine and natural consumer products manufacturer) was labelled as A and given to interventional group subjects and instructed to apply 4gms at once using cotton pallet topically 4 times per day (total of 16 grams/day) after which patients were instructed not to consume any food for half an hour. Similarly, 20ml 0.9% normal saline was labelled as B and given to control group and instructed to rinse it for 3 minutes, 4 times per day. The same protocol was followed by all 102 subjects for total period of 40days, including 4 follow-ups at an interval of 10 days each. The study was conducted for a duration of 2 years. At baseline visit followed by each visit after 10 days two investigators who were blinded regarding natural honey and 0.9% normal saline evaluated the severity of lesions clinically using the WHO oral mucositis assessment scale, and symptoms were assessed on verbal basis at baseline and at each follow-up visit thereafter. None of the patients reported with adverse effects of using natural honey or 0.9% normal saline and no drop outs in the study. WHO oral mucositis assessment scale by WHO handbook 1979 (Adapted from Athar and Gentile 2009)

Grade 1 – Soreness with or without erythema

Grade 2 – Erythema, ulcers, patients can swallow solid food

Grade 3 – Ulcers with extensive erythema, patient cannot swallow

Grade 4 – Mucositis to the extent that alimentation is not possible

The data obtained were tabulated and analysed using SPSS software for windows. Descriptive statistics included Mean, standard deviation, Frequency and percent. (Table 1) Pearson Chi-Square test and General Linear Model Repeated Measure ANOVA was used in the present study for tests of within subject's effects.

Table 1: Showing number of subjects in both Control group and Interventional group from baseline visit followed by four follow up visits.

Descriptive Statistics				
	Group	**Mean**	**Std. Deviation**	**N**
Base	CONTROL	1.7255	.56845	51
	INTERVENTIONAL	1.8824	.55307	51
	Total	1.8039	.56357	102
V1	CONTROL	1.9216	.56011	51
	INTERVENTIONAL	1.7451	.59475	51
	Total	1.8333	.58162	102
V2	CONTROL	2.1961	.60065	51
	INTERVENTIONAL	1.2549	.65858	51
	Total	1.7255	.78547	102
V3	CONTROL	2.4118	.57189	51
	INTERVENTIONAL	1.0392	.63121	51
	Total	1.7255	.91367	102
V4	CONTROL	2.6275	.63121	51
	INTERVENTIONAL	.7255	.56845	51
	Total	1.6765	1.12718	102

Results and Discussion

Application of natural honey for oral mucositis in the patients for 40 days showed diverse results pertaining to each group.

No progression in the grade of oral mucositis was observed among patients from interventional group with grade I and grade II mucositis when natural honey was topically applied from first visit (Figure 1a, 1b, 1c, 1d, 1e) till the fourth visit (Figure 2a, 2b, 2c, 2d, 2e).

Baseline visit:

Figure 1a Figure 1b Figure 1c Figure 1d Figure 1e

Fourth visit:

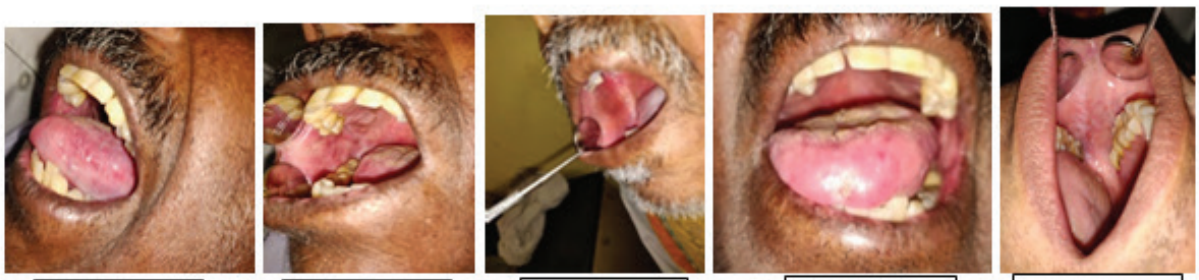
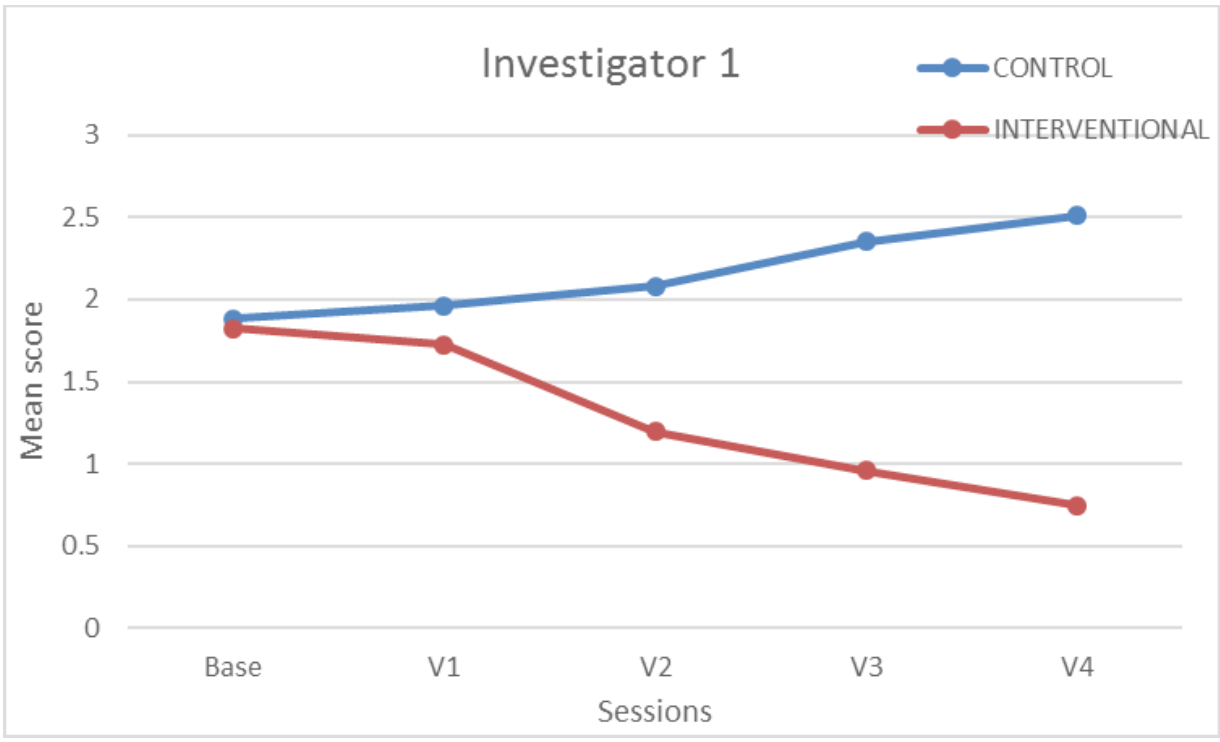


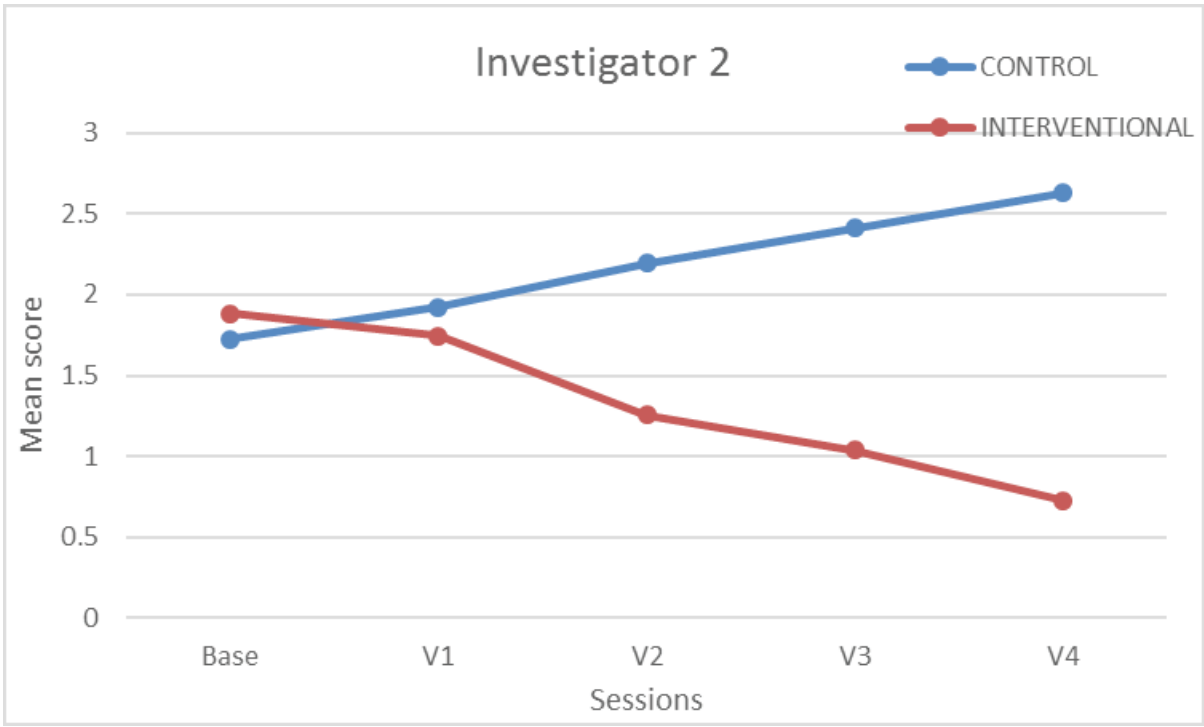
Figure 2a Figure 2b Figure 2c Figure 2d Figure 2e

According to investigator 1 few patients among grade I and II mucositis showed gradual reduction of erythema and symptoms. Patients with Grade III mucositis on first visit did not show significant results, on following visits they showed arrest of progression and reported with symptomatic relief in subsequent follow ups on application of natural honey. There was no healing of ulcers noted among grade III mucositis patients. According to investigator 2 majority of patients among grade I and grade II mucositis showed symptomatic relief in first 10 days and gradual reduction in erythema. Patients with Grade III mucositis on their first visit did not show significant results, in follow up visits they gradually showed arrest of progression of lesion and then reported with symptomatic relief in their succeeding follow ups on application of natural honey.

In control group those who were treated with 0.9% normal saline, showed neither symptomatic relief nor arrest of the progression of condition according to both the investigators 1 and 2, In turn the condition deteriorated to increasing grades of mucositis in each follow up visit. (Graph 1, Graph 2)



Graph 1: Line graph showing variations in mucositis grading in interventional and control group from base line data up to completion of 4th visit – According to investigator 1.



The present study proved that topical application of natural honey provides symptomatic relief to patients and arrests further progression of lesion to increasing grades of mucositis. (Table 2, Table 3)

Table 2: Tests of Within-Subjects Effects according to investigator 1 showing P Values of both Control and Interventional groups.

Measure: Investigator 1						
Source		**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Control group		1.675	4	.419	2.872	0.023
Interventional group		72.812	4	18.203	124.862	0.0001
Error		58.314	400	.146		

Table 3: Tests of Within-Subjects Effects according to investigator 2 showing P Values of both Control and Interventional groups.

Measure: Investigator 2						
Source		**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Control group		5.322	4	1.330	11.405	0.0001
Interventional group		54.420	4	13.605	116.633	0.0001
Error		46.659	400	.117		

Radiation therapy results in radiation injury not only to tumour cells but also to normal oral mucosa, tongue, salivary glands and alveolar bone.[11] The oral mucosa has a relatively high mitotic rate, thus absorb radiotherapy rays which leads to development of a debilitating condition called oral mucositis.[18]

Basement membrane of oral mucosa contains a basal layer composed of rapidly dividing, radiosensitive stem cells. As radiation cycle progresses, mucous membrane begins to show areas of redness and inflammation. Nearing second week of therapy some of these cells might die.[19] As a result of radiotherapy mucous membrane becomes atrophic, emerging from fibrosis of the underlying connective tissue and relatively being avascular. At dose of 10-20 Gy erythema and hyperkeratosis is seen as initial signs, which further results in severe mucositis at dose of 30Gy.[9] The early symptoms of mucositis include erythema, diffuse ulcerations leading to white-to-yellow pseudo membrane formation as a result of separation of irradiated mucous membrane from underlying connective tissue. Mucositis causes severe discomfort at the end stage of therapy leading to difficulty in drinking, eating, swallowing and speech further leading to nutritional problems, dehydration and weight loss. [20,21]

Mucositis is described in five overlapping stages: Initiation, Primary damage response, Signal amplification, Ulceration and Healing.[22] Development of mucositis is related to radiation dose, fraction size, volume of irradiated tissue, fractionation scheme and type of ionizing irradiation.[23]

The baseline management for oral mucositis is targeted at symptomatic relief, and at its four defined pathogenesis: firstly most important is to check basal

cell layer growth by modifying transforming growth factor beta3,[24] second mechanism is stimulation of epithelisation by encouraging rapid recovery of cell loss, [25,26] third is chemical protection of mucosa using Amino-Thiol group of compounds, lastly the physical protection by shield use, conformation therapy or intensity modulated radiotherapy.[27] The common controlling pathway includes use of saline mouth rinses, opioid analgesics, ice chips, topical application of anti-inflammatory like lidocaine and benzydamine hydrochloride mouth washes, antimicrobials, subcutaneous or topical granulocyte macrophage colony stimulating factor and cytoprotective agents. [28,29,30,31,32,33,34] Local antibiotics are given in the form of lozenges, that helps in preventing bacterial colonisation and reduces inflammation of damaged mucosa. Low-energy He/Ni laser treatment may promote proliferation of mucosal cells and wound healing, it is tried for treatment of chemotherapy/radiotherapy induced mucositis.[35]

Honey is formed from transformation and concentration of nectars from flowers by two processes, interaction with the upper digestive tract secretion of honeybees and concentration is by water loss in beehives. It contains moisture, fructose, glucose, sucrose, maltase and other compounds along with trace elements. [17,36] There are various types of honey like avocado honey, basswood honey, beechwood honey, blueberry honey etc. In present study commercially available Dabur honey by Dabur corporate office, Kaushambi, Sahibabad, Ghaziabad, Uttar Pradesh, India was used as it is easily available and standardized by FSSAI (Food Safety and Standards Authority of India). Pure honey is ubiquitous, cheap, natural and exhibits antibacterial, analgesic and tissue nutritive factors to stimulate re-epithelisation in damaged mucosa, thereby it can be considered as a justified agent to use in radiation mucositis.[9] It has powerful impact on B lymphocytes and T lymphocytes proliferation and also in activation of macrophages,[37] by inhibiting cyclooxygenase pathway it also inhibits inflammatory pathway. It stimulates process of granulation tissue, angiogenesis, rapid epithelisation and proliferation of fibroblasts. Honey exhibits bactericidal activity because of phytochemical agents (methylglyoxal and methyl syringate) low pH, defensin, peptide, and high osmolarity.[38]

The important factors like its hygroscopic nature, acidic pH prevents bacteria growth; inhibin converted from glucose oxidase and gluconic acid, enzymes and tissue-nutritive minerals and vitamins help to repair tissue directly.[9] Honey aids in arresting inflammatory lesion involved in radiation mucositis by stimulating formation of granulation tissue, promote angiogenesis, rapid epithelisation, and proliferation of fibroblasts and stimulate re-epithelisation of damaged mucosa which plays important role in healing process, as a result of enhanced epithelisation of mucosa. [39,40] Natural resin obtained from honey acts as potent inhibitor of human colon adenocarcinoma cell growth and carcinogenic induction. Tissue oxygenation takes place by sealing damaged mucosa from air when wound is coated with honey.[41]

The results of our study were consistent with those of Biswal et.al[35] where study group undergoing radiotherapy was treated with topical application of natural honey. There was significant reduction in the symptomatic grade III and grade IV mucositis among honey treated patients when compared to controls. Similar results were obtained by Rashad U M et al[41] where patients were provided with honey for topical application and no patients in study group developed grade 4 mucositis.

The observation was consistent with M Motallebnejad et.al[36] study in which patients were instructed to take 20ml of natural honey 15mins before and after radiotherapy, but in their study the patients were instructed to consume honey instead of topical application. Their study also showed same results, in which mucositis score at the end of each week was significantly lower compared to control group.

Conclusion

The present study concludes that topical application of honey can be used as an adjunct in effective management of symptomatic radiation induced mucositis as it helps in alleviating symptoms while the limitations of the study include inability to predict contact time of honey, unreliable verbal standardization of symptoms by patients and limited accessibility to posterior regions of oral cavity. It is necessary to validate these limitations to obtain better results and for authentication of the study.

Ethical Clearance – Taken from HCG-BHIO ETHICS COMMITTE – IECREF Number: ECM/06/19

Source of Funding – Self

Conflicts of Interest – Nil

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Cheilitis Granulomatosis - A Case Discussion

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Abstract

Introduction: Cheilitis granulomatosa is an idiopathic, cosmetically unpleasant, non-specific, painless, persistent granulomatous inflammation which affects one or both the lips. It is considered under the entity orofacial granulomatosis with very high rate of recurrence inspite of good treatment.

Presentation of case: A 45 years old female patient reported with the chief complaint of swelling in the upper and lower lips for 2 years which had gradually increased in size since the onset. The swelling was asymptomatic, firm in consistency. On histopathological examination the condition was diagnosed as cheilitis granulomatosa

Discussion: Cheilitis granulomatosa is considered as an entity of orofacial granulomatosis. It is asymptomatic, persistent swelling of the lips. The primary goal of management should be elimination of the predisposing factor. Intralesional corticosteroids are considered as gold standard in the management of conditions. In severe cases surgical management is helpful.

Key words: *Cheilitis, Corticosteroids, Granulomatosis, Intralesional injection, Lip swelling*

Introduction

In 1985, the concept of orofacial granulomatosis was introduced by Wiesenfield and colleagues to include lesions that resemble Crohn's disease, clinically and histologically, but occur in patients who do not exhibit the gastrointestinal manifestations of Crohn's[1,2].

The exact etiology of cheilitis granulomatosa is undefined but the list of predisposing and aggrevating factors include exposure to sun, allergens, food allergies, and systemic illnesses like Crohn's disease, Sarcoidosis and Tuberculosis. On proper investigations we couldn't rule out the exact etiology, but we considered sunlight to be a predisposing factor as the patient was a farmer working for long durations in the sun[3].

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Case history:

A 45 year old female patient reported with a chief complaint of asymptomatic swellings of upper and lower lips. Patient gave a history of the swelling persisting for more than 2 years and with mild degree of regression since the onset (Figure 1). Patient was otherwise healthy and there was no history of similar complaints in the family.

On clinical examination, the lips were diffusely swollen, smooth, non-ulcerated with no evidence of any discharge on or around the swelling and a mild degree of surrounding erythema. The lips were chapped, normal in color and non-tender. The lips were firm in consistency and non-tender, non-reducible, non-compressible, non-fluctuant and non-pulsatile, with neither local rise in temperature nor positive transillumination.

On investigating the case, the vital signs were normal. Lab investigations for hematology showed normal values for CT,BT, ESR, DLC, RBS, HbsAg, HIV, serum folate, Iron, Vitamin B12 . Haemoglobin which was 9.5 grams/dl. The tuberculin skin test to rule out tuberculosis was negative.

There were no clinical or radiographic evidences of any odontogenic cause.

The patient was referred to an oral maxillofacial surgeon for biopsy of the upper lip that revealed stratified squamous parakeratinized epithelium, overlying edematous connective tissue stoma and delicate collagen fibres and chronic perivascular inflammatory cells infiltrating with some focal noncaseating granulomas in the submucosal connective tissue consisting of lymphocytes, histocytes, epitheloid cells and Langerhans type of multinucleated giant cells .The histopathological study was suggestive of a granulomatous lesion of the lip.

Based on the clinical and histopathological findings the case was diagnosed as Cheilitis granulomatosa. Thereafter treatment was planned with intralesional injection of triamcinolone 40mg -1ml mixed with 0.3 ml of normal saline which was deposited in both upper and lower lips at three different sites in between the junction of vermillion border and lining mucosa. After 2 intralesional injections there was around 40% reduction in swelling of the upper and lower lips (Figure 2).

A total of 6 intralesional injections were given to the patient every alternate week. After completion an 80% reduction in the swelling was acknowledged. Two more intra lesional injections were planned following which total remission was observed (Figure 3). The patient was followed-up for 6 months without any recurrence after which patient did not report.

Figure 1: The appearance of upper and lower lips on the first visit to the hospital.

Figure 2 : The appearance of the lips after 2 injections , showing about 40% reduction in the swelling.

Figure 3: The appearance of lips after intralesional

Discussion

Cheilitis Granulomatosa (CG) presents as a persistent asymptomatic and recurrent lip swelling not associated with any particular age, sex or race. It is a presentation of orofacial granulomatosis which is a nonspecific granulomatous inflammation presenting as facial or lip swelling, cheilitis, ulcerations, gingival enlargement, mucosal tags and sometimes lymphadenopathy. The other differential diagnosis includes angioedema, Cheilitis glandularis, actinic cheilitis Melkersson-Rosenthal syndrome, Chron's disease, Sarcoidosis and tuberculosis[4].

Few of the differentiating features helped us to eliminate differential diagnosis and to arrive at final diagnosis. Presence of mucopurulent discharge as a result of pressure build- up most commonly in the lower lip due to the blockage of the salivary duct, surfacing the dilated salivary gland orifices as in cheilitis glandularis was missing in our patient. In this scenario patient had asymptomatic swelling of the lips for more than 2 years which helps in eliminating the condition angioedema where the symptoms regress with in 4 days. Actinic cheilitis usually affects lower lip as the upper lip is relative less exposed to sunlight. This condition usually results In loss of normal lip architecture which was not

seen in our patient. Even though patient presented with cheilitis the other components like facial paralysis and fissured tongue were missing to consider the condition as Melkersson-Rosenthal syndrome[5]. Endoscopy was performed to rule out gastrointestinal disturbances which helped in wiping off Chron's disease from our list. Oral manifestation of sarcoidosis are the presence of lesions on tongue, gingiva, palate and cheeks which were absolutely not found in our case thus excluding sarcoidosis. Tuberculosis was ruled out by the negative result of tuberculin skin test[6].

Non-specific granulomatous inflammation was seen on histopathology which confirmed cheilitis granulomatosa.

For the management a synthetic glucocorticoid triamcinolone which acts as anti-inflammatory and immunomodulator was chosen in our case because of its delayed release and high concentration[7]. It works by blocking the release of arachidonic acid from membrane phospholipids there by preventing the synthesis of prostaglandins and leukotriens, the mediators of inflammation. Similarly the condition was treated by Coskun et al. with a combination of intralesional steroid and metronidazole[8]. Likewise Stein and Mancini have used oral prednisolone and minocycline for treating cheilitis granulomatosis in children[9]. A combination of intralesional triamcinolone, metronidazole and minocycline was used by Dar et al. and they observed improvement in one month. Clofazimine was used by Gibson et al. for treating the condition. Miralles used 1000mg metronidazole to treat such cases[10].

The elimination of the differential diagnosis help in the proper final diagnosis. Elimination of all possible odontogenic causes is important in further management of this condition.

Conclusion

The complete resolution of the condition and prognosis may vary from patients to patients due to their immunological responses and adaptation to treatment. The primary treatment should be the elimination of the etiology of the condition. If the condition does not subside with a single drug usage, combination of drugs will be helpful. There is a need for extensive studies to find the etiology and the cure.

Conflicts of Interest - Nil

Financial support - Nil

Ethical Clearance – Patient's consent was obtained for publication of images and/or information about them.**injections showing complete remission of the swellings.**

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Bio Functional Evaluation of EGFR Expression as a Prognostic Indicator in Oral Squamous Cell Carcinoma and a Recommendation for Personalized Targeted Therapy with Anti EGFR Inhibitors

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Abstract

Background: Carcinoma is a complex, multifactorial and a devastating disease. Understanding the molecular changes that leads to disease enables one to develop strategies to prevent, provide an early diagnosis and serve as targets for therapy. Epidermal Growth Factor Receptor (EGFR), a 170-kDa cell-surface protein is a tyrosine kinase receptor from the ErbB family and is involved in many biological processes that affect tumour development, growth progression, differentiation, inhibition of apoptosis and development of metastasis. Methods: Study included 54 cases of oral squamous cell carcinomas. EGFR expression by immunohistochemistry has been studied, correlated with clinical parameters, known prognostic factors and patient survival to note its significance.

Conclusion: Head and Neck Squamous Cell Carcinoma (HNSCC) with or without regional lymph node metastases, are challenging to treat effectively. The prognosis for the patients with advanced stage of the disease still remains poor. The correlation of EGFR overexpression with tumour size, grade, stage and survival suggests that EGFR expression can be considered as having prognostic significance and is a potential target for therapy with EGFR inhibitors in HNSCC. Further studies are required to assure the viability of EGFR levels in saliva as non-invasive prognostic marker and also as an indicator of recurrence.

Keywords*: Epidermal Growth Factor Receptor (EGFR), EGFR inhibitors Immunohistochemistry, Squamous Cell Carcinoma.*

Introduction

Carcinoma is complex, multifactorial and devastating disease.[1] HNSCC is ninth most common carcinoma in the world.[2]Oral cancers accounts for one third of the global burden of cancers which depends on the exposure to major risk factors like tobacco smoking, chewing areca nut and alcohol consumption.[3]Understanding the molecular changes that leads to the disease enables one to develop strategies to prevent, provide an early diagnosis and serve as targets for therapy.[1] HNSCC generally develop through accumulation of multiple genetic and epigenetic alterations in multistep process which could include deletion or methylation of tumour suppressor regions, mutations, amplification or overexpression of oncogenes. Some of these alterations occur in early tumorigenesis and are identified in tissues that are chronically exposed to common carcinogens and in premalignant lesions of head and neck.[4] In recent years mutation of p53, over-expression of EGFR, cyclin

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D1, Bcl-2 and transforming growth factor (TGF-alpha) are found to be of prognostic importance.[5] EGFR, 170-kDa cell-surface protein is a tyrosine kinase receptor from ErbB family and is involved in many biological processes that affect tumour development, growth progression, differentiation, inhibition of apoptosis and development of metastasis.[4,] Verma *et al*[6]., have observed overexpression of EGFR in 40%–80% of head and neck cancers.[6,7] Its overexpression is associated with malignant phenotype and results in poor prognosis.[8] Studies have prompted development of clinical trials testing EGFR inhibitors as single-agent therapy or in combination with conventional cytotoxic therapy.[9]The present study aims to establish the prognostic value of EGFR expression in oral cancers which could likely benefit patients with the use of EGFR inhibitors and to study the expression of EGFR in oral squamous cell carcinomas and to correlate it with prognostic factors that could guide initiation of anti-EGFR therapy.

Materials and Methods

It was a retrospective and a prospective in-vivo, observational study of EGFR expression in 54 oral squamous cell carcinomas undertaken at JSS Dental College and Hospital in Department of Oral Medicine and Radiology, Department of General Pathology, JSS Medical College, JSSAHER, Mysuru from year 2018 till 2019 with the approval of institutional ethical committee. The formalin fixed paraffin embedded sections were studied for various morphological parameters and subjected to Immunohistochemistry (IHC) staining with EGFR and the expression was correlated with clinical parameters, known prognostic factors and patient survival.

Immunohistochemical staining: 3 - 4 µm thick sections were fixed on Poly-L-Lysine coated slides, deparaffinised and rehydrated. The sections were incubated with primary antibody EGFR (DAKO, FLEX Monoclonal Mouse Anti-Human EGFR), for 30 minutes, washed with wash buffer and further incubated with labelled polymer – HRP (DakoEn Vision + Dual Link System – HRP, DAB+, Code K4065). The bound antibodies were visualized using a DAB-chromogen, counterstained with haematoxylin and mounted with DPX. The staining pattern, the percentage of stained cells and the intensity of the stain was evaluated by two independent observers.

Evaluation of EGFR staining pattern: To quantify EGFR expression a total immunostaining score was calculated, using the product of a proportion score and an intensity score. The proportion score described the estimated fraction of positive stained tumour cells (0 = none; 1 = <10%; 2 = 10–50%; 3 = 50–80%; 4 = >80%). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). The total score obtained by multiplying the two scores and it ranged from 0 to 12. Tumours were categorized into samples with high EGFR, low EGFR and no EGFR expression using a cut-off value of: 0 = none, 1–3 = low, 4-12 = high according to Laimer*et al*.[5]

Statistical methods: The data was represented in terms of frequency distribution tables. The proportion of subjects revealing the expression according to the various subgroups such as pathological staging, age, location and lymph node status was estimated along with 95% confidence interval. The possible associations between EGFR overexpression, treatment outcome, and other categorical variables were evaluated with Fisher's exact test. Association of continuous variables was evaluated with t-test. The log-rank test was done to compare survival among groups of patients. Survival curves were generated by the univariate and multivariate analysis to get Kaplan-Meier survival curves. A significance level of .05 was used for all statistical tests.

Results and Discussion

In the present study, majority (56%) of the patients were males with a male to female ratio of 1.85:1 and the male patients showed higher EGFR expression. The mean age of presentation was 52 years and age range were between 30-75 years. EGRF expression was predominantly of high grade in patients above 40years. The tumours were seen predominantly involving the tongue (63%) (Figure 1), while the remaining cases were seen to involve the floor of the mouth (15%) (Figure 2), retromolar area (11%) and the posterior palate (11%).

Figure 1: Carcinoma of tongue

Figure 2: Carcinoma of floor of the mouth

Correlation of EGFR expression with sex and age distribution and site of involvement showed no significant correlation (0.109). There was no significant family history among the subjects included in the study. 74% of patients were exposed to some form of tobacco and they showed increased EGFR levels compared to subjects not exposed to either forms of tobacco. 37% of cases were exposed to alcohol while the remaining 63% were not exposed to alcohol, the EGFR expression in both the groups showed no significant difference. On gross examination 14 cases were of <2cms and 40% of these cases showed low EGFR staining. The 28 cases which were of 2-4 cms and 12 cases which were >4cm showed high EGFR positivity. The intensity of EGFR staining of the tumour cells became stronger with increase in tumour size, with the 'p' value showing statistical significance (0.0001). On grading 56% of cases were moderately differentiated, of which majority (96%) showed high EGFR scores (Figure 3). 30% of cases were well differentiated of which few (17%) showed low EGFR score (Figure 4) and remaining 14% were poorly differentiated and all of which showed high EGFR score.

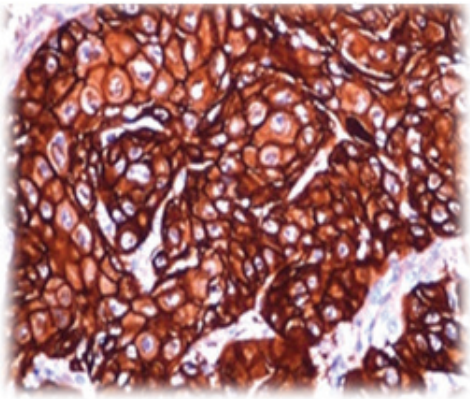

Figure 3: High grade

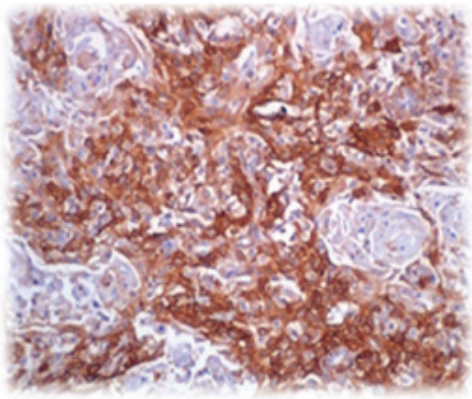

Figure 4: Low grade

Majority of tumours were of stage II and III and there were equal number of stage I and IV tumours. Stage III tumours showed nodal involvement in only 40% of cases. In stage IV tumours 56% of cases had metastasis in single ipsilateral lymph node which was >3cms but <6cms in its greatest dimension, 33% of cases showed multiple ipsilateral lymph node involvement, while only one case showed a single node involvement of >6cms. 30% of tumours of stage I showed low EGFR score. 95% of stage II, and 100% of stage III and IV tumours showed high EGFR levels and this was statistically significant. Survival was better among tongue tumours, with no history of exposure to tobacco, better differentiation, lower TNM staging and low EGFR levels. (Table 1)

Table: 1 Correlation of EGFR expression levels with prognostic factors and survival.

Variables		EGFR Low	EGFR High	Survival (%)
Site	Tongue	03	31	88%
	Floor of mouth	00	08	60%
	Retromolar region	00	06	
	Posterior palate	01	05	
History of Tobacco	Negative	00	14	100%
	Positive	04	36	76%
Grade	Well Diff	03	14	100%
	Moderately Diff	01	29	80%
	Poorly Diff	00	07	25%
TNM Staging	Stage I	03	07	100%
	Stage II	01	21	97%
	Stage III	00	12	93%
	Stage IV	00	10	11%
EGFR levels	Low	04	00	100%
	High	00	50	66%

HNSCC are one of the most leading cancers in India and the incidence is high compared to the rest of the world.[10] EGFR is a tyrosine kinase transmembrane cell surface receptor with an extracellular ligand binding domain and an intracellular tyrosine kinase domain and mediates its mitogenic effect by binding to the cell-surface receptor.[8,11] It is highly expressed in several human tumours and contributes to the pathogenesis of disease. Increased levels of EGFR expression, as detected by IHC have been shown in up to 90% of HNSCC tumours.[12] The high expression of EGFR correlates with a more aggressive clinical course and has been reported to be a useful diagnostic and prognostic marker. In recent years, it has also been considered a promising target for monoclonal antibody therapy.[13]

Age has been recognized as a prognostic factor for tumour recurrence, the risk of head and neck cancers (HNC) increases with age and most oral cancers occur in people older than 40years as seen in the present study. The age distribution is consistent with other studies.[14, 15] and shows significant correlation with EGFR expression (0.004). Men in most countries have a two to five-fold greater risk of HNC than women, this finding is substantiated by the male predominance seen in the present study with high EGFR expression. This difference is likely related to higher rates of substance abuse, particularly tobacco use, among men.[16]The tumours were seen predominantly to involve the tongue with lateral border being most common site. There was no significant difference in EGFR expression with site of involvement and this was consistent with other studies.[14, 15]

Approximately 90% of patients with HNC have a history of tobacco use, tobacco smoke contains 5,000 chemicals including polycyclic aromatic hydrocarbons, N-nitrosamine's and Azarenes, which have shown to possess antigenic, cytotoxic, mutagenic, carcinogenic and genotoxic properties. These chemicals induce DNA adducts, mainly 6-methyl-guanine which interfere with DNA replication throughout the genome and can damage proliferating cells.[17]In the present study there was a predominance of tobacco users which was similar to that seen in previous study by Maiti GP *et a.l*[18] and there was a statistically significant correlation of smoking with EGFR over expression. Zhou J *et al*[19], in his study stated that long-term use of smokeless tobacco increases the risk of HNSCC.[19] Rettig *et al*[17]. in their study have concluded that tobacco in both smoked and smokeless forms is most important risk factor for both development and prognosis of HNSCCs.[17] Studies have also shown a dose-response effect between duration of smoking and increased risk of cancer and risk reduction after cessation of smoking.[20, 21]In the United States 7% of oral cavity cancers are attributable to chewing tobacco including betel quidor and areca nut, while it is as high as 53% in India and 68% in Sudan.[17]Alcohol is second to smoking as etiological factor in development of HNSCC and it is difficult to distinguish the separate effects of alcohol and smoking.[22]The risk of developing cancer in patients who both smoke and consume alcohol has a multiplicative effect rather than an additive risk.[23]Alcohol is not carcinogen, but acetaldehyde, the first ethanol metabolite produced in gastrointestinal tract by alcohol dehydrogenase (ADH) enzymes forms DNA adducts that interfere with DNA synthesis and repair, this has been shown to be toxic, mutagenic and carcinogenic.[24]In the present study the EGFR expression in alcoholics and non-alcoholics showed no significant difference.

As seen in the present study, Chen *et al* [25]and Santini *et al* [26]have demonstrated a positive correlation between the tumour size and EGFR expression. Majority of tumours of 2-4cms and all cases with size >4cm, showed high EGFR expression. The intensity of EGFR staining of tumour cells became stronger with increase in tumour size, with 'p' value showing statistical significance (0.012).

HNSCC is graded according to Broder's classification histologically into well, moderately, or poorly differentiated tumors.[4] Moderately differentiated tumours were more predominant in the present study and this observation was consistent with Laimer *et al*[8], Rodrigo *et al*[27]and Scambia *et al*[28], while Verma et al,[6] Vats *et al*[14] and Zafar *et al*[15] have found predominantly well differentiated tumours. There was a statistically significant association of EGFR expression with tumour grade (0.02) in the present study and most studies have confirmed similar findings.

The most frequently used WHO system for staging cancer is the TNM staging systems, the importance of which includes an indication of patient prognosis, a

guide for treatment choice, a comparison of treatments in same stage patients and a facility for the exchange of information between clinicians.[29] In the present study all tumours of stage I and 47% of stage II showed low EGFR score, while 57% of Stage II, and all tumours of Stage III and IV showed high EGFR scores and it was noted that EGFR scores increased with increasing TNM staging and it was found to be statistically significant (0.02).

There was significant difference in survival in favor of patients who had lower proportional EGFR score of 0 to 2 compared to higher scores of 3 and 4 (0.002) which indicates that patients with higher EGFR expression have poorer prognosis compared to patients with lower EGFR expression. Similar results were seen in the study by Laimer *et al*[8]., who has found high EGFR expression correlating significantly (0.05) with poor survival.[8] Survival analysis has shown better survival in patients <40years, with tumours <4cms, with no history of exposure to tobacco, better differentiation, lower TNM stage and low EGFR levels.

Conclusion

The correlation of EGFR overexpression with tumour size, grade, stage and survival suggests that EGFR expression can be considered as having prognostic significance and is a potential target for therapy with EGFR inhibitors in HNSCC. These findings need to be verified on larger cohort for further consideration and further studies are required to assure the viability of EGFR levels in saliva as non-invasive prognostic marker and also as an indicator of recurrence.

Ethical Clearance – Taken from Institutional Ethical Committee – Number: 5813

Source of Funding – Self

Conflict of Interest – Nil

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Lipoid Proteinosis - A Rarity in An Adult

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Abstract

Background: Lipoid proteinosis is a rare genodermatosis with autosomal recessive inheritance. It is a multisystem disorder characterized by progressive deposition of hyaline material in the skin, mucous membrane and different organs of the body. The disease is presented by dermatological, laryngeal, ophthalmological, and neurological manifestations. Involvement of oral mucosa may lead to xerostomia and dysphagia; involvement of larynx and eyes results in hoarseness of voice at early infancy and moniliform blepharosis. Extracutaneous involvement includes neurological manifestations like psychosis, partial complex seizures, anger, panic attacks, and progressive amnesia. Till date around 300 cases have been reported in literature.

Case Report: A 63 years old male patient reported with chief complaints of pain in the right and left cheek region, difficulty in swallowing food and dryness of mouth.

Conclusion: Lipoid Proteinosis requires multidisciplinary opinion and evaluation by dermatologists, ophthalmologists, otolaryngologists, neurologists and psychiatrist. Clinical features and histopathological evaluation aids in the confirmation of the disease. Lipoid proteinosis should be considered one among the differential diagnosis when patient reports with difficulty in mouth opening, enlarged tongue, difficult in chewing and swallowing.

Key words *– Amyloidosis, Autosomal recessive, Genodermatosis, Lipoid Proteinosis, Moniliform blepharosis.*

Introduction

Lipoid proteinosis (LP) known as Urbach and Wiethe disease and Hyalinosis cutis et mucosae, first reported by Urbach and Wiethe in 1929. [1,2]

LP is an autosomal recessive inheritance causing deposition of hyaline material in skin, oral cavity and various organs. Involvement of larynx results in hoarseness of voice.[3] Affects mucosal membranes of the upper respiratory tract, digestive tract etc. [4] Infiltrations of oral mucosa leads to xerostomia and dysphagia.[5] Restricted tongue movements, limited mouth opening and speech difficulties. Cutaneous and extracutaneous involvement include waxy papules and moniliform blepharosis and neurological manifestations. [6,7] Histopathologically hyaline-like material is Periodic-Acid Schiff (PAS) positive.[5]

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Case Report

A 63 years old male patient reported with multiple complaints of pain in right and left cheek region, difficulty in swallowing food and mouth dryness.

History of moderate, radiating pain, initiates on chewing food and subsides on stopping, history of hearing impairment and speech difficulty, decreased appetite in the past 8 months leading to weight loss of

5 kgs. Medical anamnesis divulged no other relevant history.

On examination, there were multiple waxy aggregates measuring between 4-6mm below nostrils (Figure 1) and multiple yellowish waxy papules measuring 4-7mm on ventral surface of tongue (Figure 2).

Figure 1: Waxy beaded papules below the nostrils

Figure 2: Yellowish waxy papules noted on the ventral surface of the tongue.

The tongue appeared enlarged, firm, loss of papillae on dorsal surface and crenated lateral borders bilaterally (Figure 3).

Figure 3: Macroglossia with crenation's along the lateral borders and loss of papillae on dorsum surface of the tongue.

Yellowish waxy papules measuring 4-6mm observed on lower labial mucosa, non-tender on palpation (Figure 4). Restricted mouth opening and blanched buccal mucosa with non-tender soft to firm mucosal corrugations.

Figure 4: Yellowish waxy papules noted on the labial mucosa.

Provisional diagnosis of amyloidosis and differential diagnosis of LP, erythropoietic protoporphyria (EPP), xanthomatosis, and papular mucinosis were considered.

Laboratory investigations:

1. Complete hemogram.
2. Serum protein electrophoresis.
3. Serum albumin, globulin and A/G ratio.
4. Urine analysis.
5. Electrocardiography.

Complete hemogram did not disclose any abnormal values.

Radiographic investigations:

6. Orthopantomograph, true lateral cephalometric projection and Posteroanterior view of skull.

Generalised horizontal and vertical alveolar bone loss suggestive of chronic generalised periodontitis on panoramic radiograph. No evidence of intracranial temporal calcifications on true lateral cephalometric skull view and posteroanterior view.

Histopathological investigations:

7. Incisional biopsy for light microscopy.

Light microscopic evaluation of histopathological specimen showed stratified squamous para-keratinized epithelium with underlying connective tissue hyalinised and fibrous stroma with fibres, fibroblasts, blood vessels, proliferating endothelial cells with numerous eosinophilic amorphous aggregates in connective tissue. Similar aggregate found around blood vessels and proliferating endothelial cells. The sections were congo red negative thus eliminated amyloidosis. PAS positive, showing hyaline deposition around blood vessels and connective tissue (Figure 5). The features were consistent with Histopathologic diagnosis of LP. Patient was referred to dermatologist, ENT, gastroenterologist and psychiatrist for evaluation.

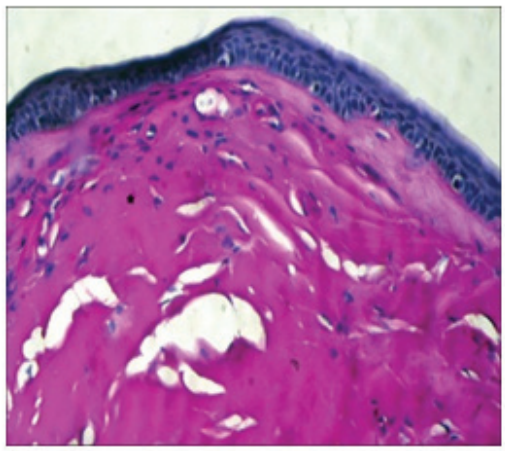

Figure 5: Subepithelial amorphous material shows PAS positive hyaline material

Discussion

LP is life threatening condition. Its protean clinical manifestations can be ruled out and confirmed by characteristic clinical findings and histopathology.[8] "Lipoid proteinosis cutis et mucosae" derived from belief of Urbach's that condition is because of abnormal deposition of lipids and proteins within tissues.[1] LP generally seen in infants, but this case report presents case of LP in 63 year old male with psychological disturbances without parental consanguinity, family history and hoarseness of voice.

Literature mentions that disorder is by pathogenic mutations in extracellular protein 1 gene (ECM1) present on chromosome 1q21.[9] A study in 2002, performing chromosome analysis mapped that disorder is due to chromosome 1q21 locus, which encodes secretory glycoprotein, and plays role in structural and functional biology of skin. Reduced expression of this protein results in aberrant hyaline deposition in tissues.[1,10] Kowalewski et al. postulated that ECM1 glycoprotein plays role in regulating blood physiology and anatomy of skin, as substantiated by alteration in microvasculature of dermis.[11] Total of 52 pathogenic mutations have been reported; among 10 exons of gene, nearly 50% of mutations occur in exons 6 and 7, mutations in exon 6 results in severe clinical manifestations and 7 displays mild clinical manifestations.[12] Mutations can be heterozygous and homozygous type.[13] The unstable

extracellular matrix might cause neighbouring cells to overproduce proteins and other materials resulting in excess substance production which accumulates in tissues and deposits, the hallmark feature of LP.[14] It is characterized by generalized thickening, scarring, infiltration of mucosae and deposition of hyaline like material in skin, oral cavity, larynx and internal organs. [15] Infiltration of oral mucosa may lead to xerostomia and dysphagia, which were also apparent in our case. Infiltration of larynx leads to hoarseness of voice since infancy, a typical sign missing in our patient.

Cutaneous findings are of two stages, First stage in early childhood, usually self- limiting with formation of haemorrhagic bullae and pustules with prolonged healing time and delayed resolution with scarring. In severe cases, acneiform scarring with verrucous cutaneous lesions and dental anomalies. In second stage waxy yellow papules with increased thickening in face, trunk, flexure and extremities are appreciated. [16,17]

Neurological signs and symptoms are extracutaneous and extra mucosal features, detected by brain CT. [8] Depression and impairment of emotion noted in present case, which were also reported in earlier reports along with mental retardation and tension-type headache.[18]

Common oral symptoms include thickening of tongue, lingual frenum, difficulty in opening mouth, speech and tongue movements which were prominent features of our case. Many studies have reported association of diabetes mellitus with LP, as it causes deposition of amorphous material in capillary vessels or in pancreas.[17] Clinical differential diagnosis can be differentiated by histopathology.

The treatment options are limited with symptomatic relief including CO2 laser ablation, dissection of vocal cords and blepharoplasty. Skin lesions treated by cryotherapy, dermabrasion and chemical peeling. Intralesional heparin and etretinate are used.[1]

The prognosis of LP is good and patients with this disorder usually have normal life expectancy even after upper respiratory tract is involved and breathing is compromised.[13]

Conclusion

All though LP is rare chronic cutaneous disease with multisystemic manifestations in early infancy, it can be seen in elders without exhibiting symptoms at early childhood. A multidisciplinary approach comprising of dermatologists, ophthalmologists, otolaryngologists, neurologists and psychiatrist is of prime importance in the diagnosis and management. The significant role of oral diagnostician in overall approach towards this condition cannot be overemphasized.

Conflicts of Interest - Nil

Financial support – Nil

Ethical Clearance – Patients consent was obtained for publication of images and/or information about them.

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Efficiency of Chamomile Essential Oils on Sleeping Quality of First-year University Students

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Abstract

Objective: This is the quasi-experimental research proposed to study the efficiency of chamomile essential oils on sleeping quality of the first-year university student.

Methods: The sample was selected on 20 students with poor sleeping quality. The study was done weekly in 2 weeks, intervened with chamomile essential oil. Research tools were the 3-parts questionnaires including personal information, quality of sleep and environmentally influencing sleep. Data were analyzed in statistically parameters.

Results: The sleeping quality of the 1st year university students in chamomile group, compare mean and standard deviation of experimental group found that the mean pre-test sleep quality was M = 80.20, *SD* = 6.65, with poor sleep quality overall and after 2 weeks of trial participation M = 55.65, *SD* = 10.32, which had moderate sleep quality. In experiment with the experimental group, it was found that after 2 weeks of using chamomile essential oil, the sleep quality of the experimental group improved. According to statistical comparison, t = 2.494, *p*-value = .022.

Conclusion: This study uses of chamomile essential oil showed an improvement in the sleep effect of the experimental group. To be concluded that chamomile essential oils improved the sleeping quality significantly as the well-known lavender essential oils. However further study in larger group is recommended.

***Key words**; chamomile oils, sleeping quality, mental health, university students.*

Introduction

Sleeping is necessary for life. Inadequate sleep is one of the most common health problems. It can be found in all age group. The cause may be illness, stress or environmental problem. Some patient needs medication for sleep.[1] Inadequate sleep may affect to physical and mental of the patient and increase the risk of cardiovascular diseases, diabetes, cancer, depression and obesity.[2] The leading factors related to health care behaviors were health care knowledge, attitudes, towards health care and perception of the benefits of health care sleep and rest refreshed body, joyful mind.[3,4]

There are many herbals reported as sleep assisting effect. McKenna et al [5] reported Chamomile (*Chamaemelum nobile*) was use as herbal for thousands of years since Egypt, Greek and Roman empire. It is popular in Europe to reduce stress, gastric irritation, dysmenorrhea, muscle spasm, inflammation and promote wound healing.[6] Chamomile in essential oil is used to relieve stress and improve sleep. It composed if many compounds as flavonoid, coumarin, phenolic acids, gamma aminobutyric acid and volatile oils. The component of each plant is different depend on the place and climate but remaining their properties.[7] Some

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reported the allergic reaction of Chamomile as skin irritation or allergy. However, it was minimal.

Chamomile is a medicinal plant widely used as tisane or oil thanks to its anti-infammatory and calming effects. Hence, two researcher teams have tried to evaluate the inhibition action of chamomile (Matricaria recutita) aqueous extract on mild steel corrosion in HCl solution.[8,9] On the other hand,[9] confirmed that the extract was a mixed-type inhibitor with predominantly anodic behavior.

Figure 1: Chamomile

Essential oil needs diffuser as volatile inducers, it can stimulate the human's sensory as smell to the olfactory bulb in the nose and reflex as emotional calm down.[10]

It is not yet known exactly why or what mechanisms we need to spend a third of our lives asleep. [11] About 8 hours and 15 minutes per night [12] shows the importance of a good night's sleep. While sleeping although the body has reduced movement reduce energy metabolism, but the control system of important organs such as the hormonal system and the central nervous system work increases. [11] The sleep cycle is formed by two main mechanisms that work together:

1. Circadian rhythm is like the life clock of the human body. This system is under the control of the hypothalamus that regulates the secretion of the hormone melatonin. External factors such as light and darkness determine or alter the functioning of the body's clock,[13] observed when traveling across countries where time differences may cause the body to adjust to it, there may be insomnia.

2. Homeostasis is an equilibrium system of the body such as blood pressure, body temperature. By this system will cause drowsiness and the number of hours we sleep each night will depend on this system. Studies have shown that the neurotransmitter adenosine is a stimulant to this equilibrium. For example, if the body is fatigued, tired from all day activities this system will drive drowsiness faster than usual, [14,15] these mechanisms that the body needs rest. No one man can sleep for more than a few days.[15]

Sleep time the body releases important hormones that affect the circulatory system, cardiovascular system, immune system such as prolactin, insulin, and others especially during periods of deep sleep. The pituitary gland produces and secretes the Growth Hormone (GH) many times more. Therefore, having an important effect on growth body metabolic processes and stimulate the production of proteins to repair cells to function normally.[16,17]

The preliminary research was interviewed 20 first year university student and found most of them had sleep problem including environmental disturbance and anxiety of the class. It effects to the study as they were sleeping in the class and cannot get the knowledge, reflex to the examination score. So, we interested in

the efficiency of Chamomile essential in relaxing and promoting sleep quality with less side effect. It can be applied to other preparation such as spray, balm or vapor oil for any kind of usage.

Objective

To study the efficiency of Chamomile essential oils on sleeping quality.

Methods

This is the quasi-experimental design on two groups with pre-test and post-test evaluation. The demographic data were gender, age underlying disease, religious, department, and monthly income. The population was 159 first-year university students in College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkram Education Center. The study was performed in January to September 2020. The sample was purposively selected of 20 students with insomnia and without underlying disease or allergy to the essential oil. Data were collected by 3-part questionnaire; demographic data, sleeping quality, and factors disturbing sleep.

The population had 28 items questionnaire to evaluate sleeping quality scoring 1-4 in each item. Pre-test evaluation was performed. The sleeping quality has 20 items with 4 levels evaluation as minimal, mild, moderate and strongly agree. The factor disturbing sleep has 8 items with the same evaluation choices. The criteria for measuring the quality of sleep were in which is divided according to the following criteria 1 - 28 points are considered good sleep quality, 29 - 56 points were considered moderate sleep quality, 57 - 84 points were considered poor sleep quality and 85 - 112 points were considered severe poor sleep quality.

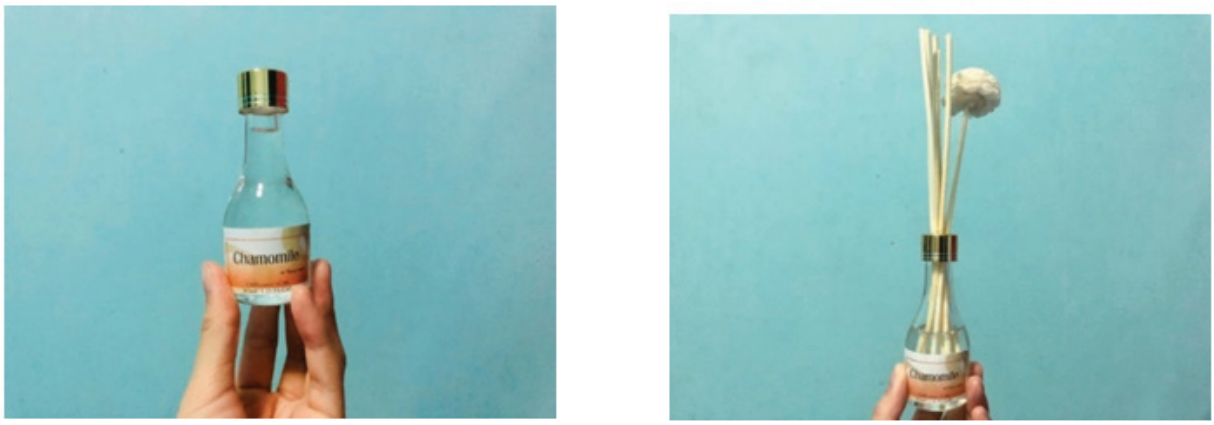

Figure 2: Chamomile in essential oil

Chamomile in essential oil 30 ml is the product of Panita Prouct Co., Ltd. It composed of 95% ethyl alcohol and pure essential oil. Five pieces of wood stick were put in for five minutes then reverse them up. The bottle was placed within one meter from the bed in room temperature for two weeks in the test group. The control group had Lavender in essential oil in the same manner. Post-test was performed at the end of first and second week.

The questionnaire had content validated by three experts with Index of item-objective congruence (IOC) over 0.5. Data were collected and analyzed by paired sample t-test and independent t-test with 95% confident, $p< 0.05$.

Results

Part 1 Demographic data

The sample of 20 university students, all are female, mostly Buddhism in the age group of 18-22 years. Medical secretary and Thai Traditional Medicine are the majority of 22.5% each and over the half has monthly income less than 5,000 THB as showed in Table 1.

Table 1 Demographic data

Demographic data	Experimental group (n = 20)	
	N	%
Female	20	100.0
18–22 (yeas old)	20	100.0
Religious		
Buddhism	19	95.0
Christian	1	5.0
Monthly income (THB)		
< 5,000	11	55.0
5,001-10,000	9	45.0
Major of study		
Medical secretary	4	20.0
Thai Traditional Medicine	5	25.0
Public health	2	10.0
Child care	2	10.0
Elderly care	0	0.00
Beauty care	5	25.0
Health promotion	0	0.00
Chinese medicine	2	10.0

Part 2 Pre and post-test of Chamomile essential oil

The comparison of pre-test, a week and 2 weeks later. The average score of sleeping quality in pretest was 80.2 + 6.65 (mean + *S.D.*). After 2 weeks of using Chamomile essential oil, the average score of sleeping quality decreased to 55.65 + 10.32 (mean + *S.D.*). The comparing showed statistically significant decreasing of the sleeping quality score with $p < 0.05$. From the experiment, the experimental group found that after 2 weeks of using chamomile oil, the sample group had improved sleep quality, 15 people accounted for 75% and 5 were the same sleep quality accounted for 25%.

Table 2 Sleeping quality score pre-test, a week and 2 weeks later of Chamomile essential oil (*N*=20)

No.	Pre-test		1 week later		2 weeks later		Summary Sleep quality
	score	Sleep quality	score	Sleep quality	score	Sleep quality	
1	92.00	severe poor	75.00	poor	58.00	poor	better
2	93.00	severe poor	73.00	poor	56.00	moderate	better
3	75.00	poor	78.00	poor	62.00	poor	same
4	78.00	poor	74.00	poor	55.00	moderate	better
5	88.00	severe poor	72.00	poor	44.00	moderate	better
6	75.00	poor	80.00	poor	55.00	moderate	better
7	74.00	poor	79.00	poor	82.00	poor	same
8	80.00	poor	77.00	poor	71.00	poor	same
9	91.00	severe poor	73.00	poor	61.00	poor	better
10	76.00	poor	69.00	poor	66.00	poor	same
11	76.00	poor	70.00	poor	47.00	moderate	better
12	73.00	poor	80.00	poor	55.00	moderate	better
13	79.00	poor	75.00	poor	56.00	moderate	better
14	84.00	poor	80.00	poor	63.00	poor	same
15	71.00	poor	84.00	poor	56.00	moderate	better
16	85.00	severe poor	76.00	low	41.00	moderate	better
17	74.00	poor	81.00	low	43.00	moderate	better
18	77.00	poor	72.00	low	44.00	moderate	better
19	82.00	poor	71.00	low	44.00	moderate	better
20	81.00	poor	65.00	low	54.00	moderate	better

Compare mean and standard deviation of experimental group found that the mean pre-test sleep quality was M = 80.20, SD = 6.65, with poor sleep quality overall and after 2 weeks of trial participation M = 55.65, SD = 10.32, which had moderate sleep quality as shown in Table 3.

Table 3 Mean (*M*) and standard deviation *(SD)* sleeping quality score pre-test, and 2 weeks later of Chamomile essential oil. (n = 20)

Experimental group	M	S.D.
pre-test	80.20	6.65
2 weeks later	45.78	10.32

In experiment with the experimental group, it was found that after 2 weeks of using chamomile essential oil, the sleep quality of the experimental group improved. According to statistical comparison, t = 2.494, p-value = .022. This study used of chamomile essential oil showed an improvement in the sleep effect of the experimental group as shown in Table 4.

Table 4 Sleeping quality score pre-test, and 2 weeks later of Chamomile essential oil (*N* = 20)

Experimental group	M	S.D.	t	p-value
			2.49	.022*
pre-test	80.20	6.65		
2 weeks later	45.78	10.32		

Discussion

This is a quasi-experimental research with quasi-equivalent control group design to study the efficiency of Chamomile essential oil on sleeping quality of the first-year university student. The study was performed during January to November 2020. The sleeping quality was scored and evaluated as bad, low, moderate and good quality.

The population were screened for their sleeping quality and 20 sample with poor sleeping quality were selected. Pre-test evaluation by questionnaire were performed. The questionnaire was evaluated and approved by three experts. A weeks and two weeks post-test were followed. Data were analyzed by descriptive statistic, paired sample t-test.

This research found that experimental group found that the mean pre-test sleep quality was poor sleep quality overall and after **2** weeks of trial participation which had moderate sleep quality. And chamomile essential oil showed an improvement in the sleep effect of the experimental group. Chamomile belongs to the daisy family (Asteraceae) and is characterized by white petals and yellow stamens in the center. It will bloom and smell good in early summer from March to April. And will bloom until the end of June.[18] With this fragrance, chamomile flowers are dried and used to make tea, essential oil and the extract from chamomile flower is widely used. A research study in a group of female volunteers found that the scent of chamomile oil has a beneficial effect on calming comfort. In addition, clinical studies have been performed in groups with anxiety disorders by consuming chamomile extract for 8 weeks, there was a significant reduction in anxiety. Chamomile has the potential to promote better sleep. Chamomile flowers contain the active ingredient apigenin, which helps calm the central nervous system and reduce anxiety and helps to fall asleep easily at night, helps to relax like a sleeping pill reduce the compression of the muscles, reduces nervousness, refreshes the body and makes it more comfortable to sleep. So, it can be said that chamomile essential oil it certainly contributes to sleep.

Conclusion

Lack of sleep or poor quality can lead to feeling tired. Not refreshed, in a bad mood, easily irritated, feeling drowsy in the afternoon of the day if this accumulated sleep deprivation continues until it can affect other health problems that follow. Quality sleep will depend on the number of hours and the right time to go to bed. Deep sleep is when the brain and body work in a systematic relationship to restore the body and maintain good health. Eastern medicine has long been using herbs for relaxation and sleep. Chamomile has a long history of use dating back to the ancient Greco-Roman period. The apigenine in chamomile has a calming effect. Relieve anxiety and helps to sleep soundly a good night's sleep takes care of your physical and mental health from within. Will help you wake up with a cheerful mind and helps keep the body healthy.

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Comparison of Casein Phosphopeptide Amorphous Calcium Phosphate Fluoride and Fluoride Varnish on Remineralization of Early Caries Lesions Around Orthodontic Brackets

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Abstract

Objectives: Orthodontic treatment increases the risk of caries and enamel white spot lesions incidence on the buccal surface of teeth. This study aims to compare the effect of Casein PhosphoPeptide Amorphous Calcium Phosphate Fluoride and fluoride varnish on remineralization of white spot lesions around orthodontic brackets.

Materials and Methods: In this in-vitro study, 60 premolars teeth were etched for 15 seconds. Then brackets were bonded with Relicane bond. All teeth were immersed in a demineralization solution for 4 days.

The lower part of the bracket was painted with nail polish to prevent the effect of a remineralizing agent to serve as a control group. The teeth were divided into 4 groups, Group 1: treated with fluoride varnish for 3 weeks (one-time application), Group2: treated with fluoride varnish for 6 weeks (two-time application), Group 3: treated with casein phosphopeptide and amorphous calcium phosphate with fluoride (CPP-ACPF) for 3 weeks (twice a day), Group 4: treated with CPP-ACPF for 6 weeks (twice a day). Remineralizing agent was applied on the occlusal of bracket for every tooth Area.

then the depth of specimens in treatment and control groups was measured by AutoCAD 2007. Pair-T test and repeated measured ANOVA were used for data analysis by SPSS 23.

Result: There was no statistically significant difference between each group of treatment. However, mean remineralization depth in group fourth and mean remineralization area in group three were higher than other groups.

Conclusion: Fluoride varnish and CPP-ACPF have the potential to treat the white spot lesion which is created during orthodontic treatment. According to less fluoride varnish use, it seems this substance is a better option in case of patient's cooperation is not good.

Keywords : *Tooth remineralization , Amorphous calcium phosphate, Fluorides, orthodontics*

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Introduction

A fixed orthodontic device can lead to the accumulation of plaque in the tooth surface which is the major cause of spot lesions after orthodontic treatment (WSL).[1-4]

Fluoride is one of the most common materials used topically for the treatment of WSL.[5]

Fluoride is available in various forms including mouthwash, toothpaste, tooth powder, gel and varnish that all of them are effective in WSL treatment. The advantage of fluoride varnish is not needing the patient's cooperation and could be used by the clinician.[4-8]

Fluoride causes hypermineralization in the enamel surface layer which prevents the Ca and phosphate penetration to the lesion.[2-8] The other concern is undesired effects like fluorosis and toxicity in high doses.[9]

Another material with anti-caries property is casein phosphopeptide and amorphous calcium phosphate (CPP-ACP) with dairy used.[9] This product derived from milk and suggested to prevent caries and enamel demineralization by releasing elements during the extension of caries and increase enamel remineralization. As a result, if it is used in the early stages of disease, it can stop caries and improve the affected areas.[6]

MI paste (GC America, Alsip III) is a new material with nanoparticle structure which is prescribed in recent years for WSL remineralization. The active constituent of this product are CPP that fixe and replace Ca, phosphate and fluoride in tooth surface and cause WSL deep remineralization.[2] In addition to the potential of all anti-caries material like fluoride, CPP-ACP is safe, tasty and tolerable by humans.[9]

Sampath et all shows that CPP-ACP increases remineralization on 40 orthodontic patients.[6] While Huang showed that varnish and Mi Paste have no special effect on WSL.[2]

Some studies showed that the simultaneous use of CPP-ACP and fluoride have a synergic effect on remineralization.[2] MI Paste Plus is a new product with this compound, 900ppm fluoride and CPP-ACP. Regarding the difference in fluoride products and CPP-ACP effects on WSL, this study is designed to compare the effect of fluoride varnish and Mi Paste Plus in WSL remineralization around the orthodontic brackets.

Materails and Methods

60 healthy premolars that were extracted due to orthodontics selected and kept in normal saline. All surface plaques were wiped by rubber cap and Pamis Powder. The place of the bracket on the tooth buccal surface was marked. Etching acid was placed for 15 seconds on the tooth surface and washed for 30 seconds with water. All brackets were bonded with Relicane bond (made in India, manufacturing RelX composites) and cured with Kerr light cure device (made in USA, Detroit Dental Manufacturing Company). It is necessary to mention that the bracket of all teeth were the same (Roth, Dentarum, Germany) and bonded by one operator. Then, all teeth were placed on demineralization solution (containing 2mmol/L calcium chloride+ 2mmol/L tri-sodium phosphate+75 mmol/L buffer acetate) with pH=4.6 for 4 days. Then, they were exited from the demineralized solution and washed with deionized water and divided randomly into four groups (15 per each group). The lower part of the bracket was covered with nail polish to prevent remineralization solution effect and remained on tooth until all teeth were cut. It is worth noting that the ethical aspect of the present study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (ID: IR.UMSHA.REC.1396.668).

Then fluoride varnish and MI Paste Plus (casein phosphopeptide amorphous calcium phosphate fluoride) were rubbed to the teeth according to the following protocol:

Method I: fluoride varnish was used on the buccal surface in occlusal of bracket once in three weeks according to producer instruction.

Method II: fluoride varnish was used on the buccal surface in occlusal of bracket twice in 6 weeks.

Method III: CCP-ACPF was used twice a day with a micro-brush according to the producer instruction on the buccal surface in the occlusal bracket for three weeks.

Method IV: CCP-ACPF was used twice a day with a micro-brush on the buccal surface of the occlusal bracket for 6 weeks according to the producer instruction.

All four groups were kept in separate containers containing synthetic salvia with the given formula (NaCl 0.4, KCl 0.4, CaCl2.2H2O 0.795, NaHPO4.2H2O 0.78, Na2S.2H2O 0.005, Urea 1.0, H2O 1.0) g/L in order to

simulate mouth condition.

After three weeks, teeth of the first and third groups were removed from synthetic salvia and their bracket was removed also. Then, they were cut vertically[5] by using a uniaxial cutting machine, made in Mashhad, Iran. Each cut was rubbed with sandpaper to reach 50-micron thickness. Each cut was evaluated under a polarized microscope (Dewinter technologies, Italy) with 40X magnification. The photo of each sample was captured and the mineralization depth and area in each tooth in both treatment and control sides were measured by AutoCAD 2007 software. Teeth of second and fourth groups were removed from the solution after 6 weeks, with the same cut and were observed under a polarized microscope. The enamel in one specimen of the third group broke during removing brackets and excluded from the study. Two specimens of the second group were broke with cutting machines and destroyed. Two specimens of the first group, three specimens of the second group, one specimen of the third group, and two specimens of the fourth group were destroyed during enamel sandpapering and excluded the study. Finally, 13 specimens remain in each group except group 2 with 10 specimens.

Statistical Analysis

A paired T-test was used for comparison of means of two outcomes (area, depth) in each method separately, and repeated measures ANOVA test (RM-ANOVA) was used for comparison of the mean of treatment and control between the four methods. All the analysis was performed by SPSS 23.

Results

In this study, two treatment methods were used for surface remineralization. Then teeth were cut and observed to measure the depth and extent of lesion remineralization under a polarized microscope. A figure of specimen of each treatment and control group is shown in Figure 1A to E.

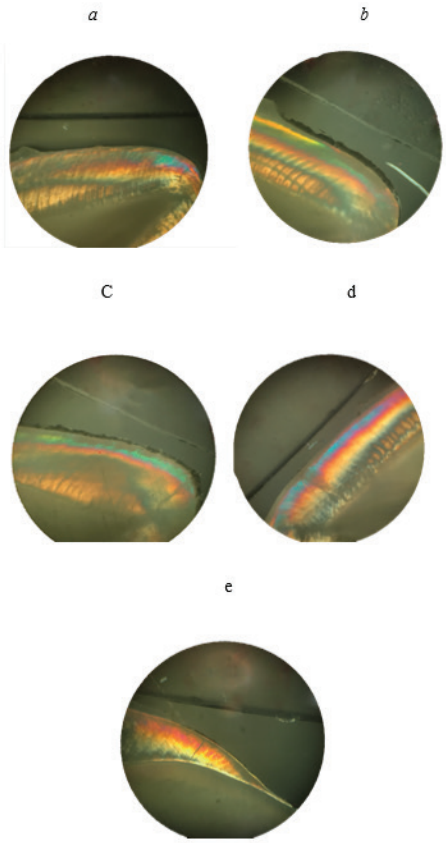

Fig. 1 a,tooth enamel 3 weeks after using fluoride varnish .b, tooth enamel 6 weeks after using fluoride varnish.c, tooth enamel 3 weeks after using CPP-ACPF.d, tooth enamel 6 weeks after using CPP-ACPF.e, An image of gingival part of bracket as control

Our result showed that without considering the four treatment methods, area and depth are significantly different in the two groups of control and treatment. (p<0.001) (Table1).

Table 1 The result of Paired t-test to compare area and depth of remineralization regardless of group

Group		N	Mean(SD)	T	p-value
Area	Treatment	49	0.252±0.29	5.873	<0.001
	Control	49	0.004±0.02		
Depth	Treatment	49	0.317±0.28	7.921	<0.001
	Control	49	0.007±0.03		

The result also shows that in each of the treatment method, area and depth were significantly different between the two groups of control and treatment (Table2). For example, the depth response in the first method for the treatment group is 0.298±0.312 And for the control group, it was 0.015±0.034 (Table2).

Table 2 The results of paired T-test and repeated measures ANOVA test.

Variable	Method	Treatment (SD)	Control (SD)	T-value	P-value*	P-value**
Depth(mm)	I	0.298(0.312)	0.015(0.034)	3.373	0.006	F=1.362, p=0.267
	II	0.276(0.295)	0.004(0.013)	2.875	0.018	
	III	0.307(0.217)	0.009(0.033)	5.371	<0.001	
	IV	0.376(0.306)	0.000(0.000)	4.436	0.001	
Area(mm2)	I	0.143(0.157)	0.013(0.030)	2.872	0.014	F=0.354, p=0.787
	II	0.262(0.322)	0.006(0.019)	2.464	0.036	
	III	0.362(0.402)	0.001(0.005)	3.252	0.007	
	IV	0.243(0.224)	0.000(0.000)	3.911	0.002	

***: Paired T-test statistic**

****: RM- ANOVA test statistic**

SD: Standard Deviation

I: fluoride varnish was used on buccal surface in occlusal of bracket once in three weeks according to producer instruction, ***II***: fluoride varnish was used on buccal surface in occlusal of bracket twice in 6 weeks, ***III***: CCP-ACPF was used twice a day with micro-brush according to the producer instruction on the buccal surface in occlusal bracket for three weeks, ***IV***: CCP-ACPF was used twice a day with micro-brush on the buccal surface of occlusal bracket for 6 weeks according to the producer instruction.

This result was also the same for other treatment methods. But the findings showed no significant difference between the depths of different treatment methods (p=0.267) and also between the area of different treatment methods. (p=0.787)

Discussion

Based on results, it is clear that four treatment methods have no statistical significant different in tooth surface remineralization and depth. However, mean remineralization depth in group four (treated with CPP-ACPF for 6 weeks (twice a day)) and mean remineralization area in group three (treated with CPP-ACPF for 3 weeks (twice a day)) were higher than other groups.

White spot lesions (WSL) which are visible clinically are seen in about 50% of orthodontic patients.[11] Different methods have been introduced to prevent WSL during orthodontic treatment. Among them laser, bonding material and fluoride-releasing composites, self-etch bonding, and fluoride releasing material and calcium depositing materials like fluoride varnish and CCP-ACPF can be referred to Miresmaeili et al.[12]

Advantage of fluoride varnish was adhesiveness to tooth surface, long-term fluoride release and ease of use (4-8). Todd et.al reported that fluoride varnish caused 50% reduction in demineralization compared to control group.[13]

Fluoride reduces demineralization which imposes this effect through antibacterial effect and producing hydroxyl apatite fluoride which is more resistant to acid.[14]

CPP-ACP is another remineralization method that in addition to anti-caries characteristics of fluoride varnish, its desired taste and odor can be optimal for patients.

The study of Srinivasan etal. on the remineralization potential of CPP-ACP toothpaste and CPP-ACP and fluoride toothpaste indicates higher remineralization in simultaneous use of both materials.[15]

Different direct and indirect methods like abrasion, scratching and indentation or atomic force microscopy and micro-hardness were used to evaluate demineralization and remineralization of enamel.[16] In this study, enamel lesion remineralization was measured by using polarized microscope. In method like indentation and scratching, only the instance surface is evaluated and the accurate amount of caries, remineralization and demineralization extension is not possible. In micro-hardness, a diamond blade entered into the sample and based on this, surface hardness was measured.[17] The amount of micro-hardness depends highly on experimental conditions and in addition of demineralization and remineralization, other factors are effective on it. Micro-hardness is a physical criterion which can be used to determine the tooth mineralization degree. This criterion is directly related to the arrangement of enamel crystals and its mineral content. [18] We can say that polarized microscope is the most objective method for measuring demineralization and remineralization.[15]

It can be said that the reason of significant statistical difference in N.Srinivasan study, compared with this study that results were not statistically different, was the difference in enamel remineralization measurement method that used micro-hardness.[15]

Jialing Li et.al concluded in a review study that CPP-ACP in the long-term has considerable effect on WSL but it has no significant difference compared to long- term effect of fluoride.[19]

In a study by Jayanth Jayarajan et.al study, the effect of CPP-ACPF on tooth remineralization was compared with CPP-ACP. Based on the results of this study, both materials had higher remineralization compared to synthetic salvia but CPP-ACPF had higher remineralization compared to CPP-ACP which is likely due to fluoride release.[20]

In this study, the mean remineralization area and depth difference in all treatment groups was significant compared with control group. In the current study, by increasing the use of fluoride varnish, the remineralization area increases which can be due two times of using fluoride varnish but mineralization area of CPP-ACPF, it has reduced which is likely due to the higher penetration of this material to the depth and increase in depth remineralization which is opposite to fluoride varnish feature that increases remineralization in the surface layer. The mineralized surface prevents the penetration of remineralization materials to depth.[2,8] Low mean remineralization depth in group 2 compared

to group 1 indicates this issue. It is worthy to mention that none of these differences were significant statistically.

Conclusion

Using fluoride varnish and CPP-ACPF can be effective in WSL remineralization. Regarding the lower number of fluoride varnish, compared to CPP-ACPF, it seems that varnish is a better option to prevent WSL.

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Conflicts Factor and Stress Related to Health Problems of Official Employees

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Abstract

Objective: The objectives of this research were to 1) measure frequency of conflicts in the work detail, behavior, work relationship, and work stress, 2) study the relationship between conflicts in the organization factors of work detail, behavior, work relationship, and work stress, 3) analyze the work stress related to health problems, and 4) analyze the ability to explain the variation of conflicts in the organization on work detail, behavior, work relationship, work stress toward health problems.

Methods: The sample was 132 official employees collected by simple random sampling. The research referred to a quantitative technique that analyzed data using descriptive statistics, inferential statistics, Pearson's product-moment correlation coefficient, chi-square, and multiple regression analysis.

Results: The research proved that the majority often conflicted in work detail, behavior, work relationship, which their work relationship was an aspect that the respondents conflicted the most. The results revealed that conflicts in work detail, behavior, and work relationship were related to work stress, which also affected health problems. The variable of work stress can predict health problems accounted for 53%. Work relationships can explain the variation of health problems estimated at 52% at the significance level of 0.05.

Conclusion: This research can effectively implement conflict management and develop a good relationship in the organization.

Key words: *Stress, health broblems, work relationship, official employees*

Introduction

Conflict is a normal phenomenon that arises from different opinions or perspectives, or interests in a situation. There are many root causes of conflict, such as taking interdependence, incompatible goals, personality differences, and communication problems. Conflict can sometimes trigger quarrels, estrangement, and internal war. However, conflict can initial both positive and negative outcomes. On the positive side, conflict can issue a greater decision. For instance, according to disagreement over an organization's direction, a leader may learn from a follower who proposed a newer idea. It may be a better choice for the current situation.

On the contrary, conflict can increase stress and anxiety among individuals, which may decrease productivity and satisfaction.[1,2] Types of conflict consist of intrapersonal conflict, interpersonal conflict, intragroup conflict, intergroup conflict, Intra-organizational conflict, and inter-organizational conflict.[3] When conflicts arise, effective site-based management depends on collaboration and integrative problem solving as collaboration will help individuals share their better comments and seek the most excellent decision.[4]

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Presently, organizational management must be aware of conflicts that arise in the organization. The leader must find causes of conflict and seek for best ways to deal with the conflicts to restore a peaceful life to the organization.[5] However, in a conflict situation, the leader must deal with a conflict by separating the person from the problem, listening to them actively and carefully, talking with neutral language, compromising, seeking the collaborative conclusion, reducing their stress that may affect their health, and building better workplace atmosphere. Besides, effective conflict management must focus on communication because good communication helps everyone understand why the conflict arises, the causes of conflict, and the best solution for the situation. The way to find out solutions is based on how much people appease and respect each other; the more they respect each other, the easier solve the problems as results of their self-verification and self-enhancement, personality, emotional intelligence, interaction patterns, and partner support.[6,7] Conflicts in a workplace will impact individual behavior because pressure, anxiety, and stress will make unhappiness to the people. It will transform into a severe atmosphere. However, the way to reduce conflicts in a workplace refers to integrating, obliging, and compromising.[8]

When conflicts arise, health changes will result from anxiety, dissatisfaction, and bad mood.[9] People in conflicts always express their negative behavior, which directly triggers their physical and emotional health.[10] Thus, if there is a conflict in the organization, the leader should restore a great atmosphere and deal with the conflict suddenly. The leader should communicate and harmonize dispute sides to reduce stress in the workplace[11] because practical corporate communication will resolve conflicts and reduce stress problems.[12] Moreover, the integrative experience will reduce task conflict and stress.[13] However, the leader should understand the management role, the organization's relationship, coordination among different cultures in the organization, and how to create satisfaction.[14]

The authors are interested in conflicts and stress in a workplace-related to the official employees' health problems. The authors expect that this study's findings will be an advantage for organization management in all aspects.

Objectives

1. To measure the frequency of conflicts in the work detail, behavior, work relationship, and work stress.

2. To study the relationship between conflicts in the organization factors of work detail, behavior, work relationship, and work stress.

3. To analyze the work stress related to health problems.

4. To analyze the ability to explain the variation of conflicts in the organization on work detail, behavior, work relationship, work stress toward health problems.

Hypothesis

1. Work detail, behavior, and work relationship factors are moderately related to work stress.

2. Work stress affects health problems.

3. Conflicts in work detail, behavior, work relationship, and work stress can explain the variation of health problems.

Methodology

This research employed the quantitative technique to measure the frequency of conflicts in the work detail, behaviour, work relationship, and work stress. The sample was 132 official employees collected by simple random sampling. A questionnaire used in this research was delivered to the respondents from January to September 2020.

Data Analysis

The research analyzed demographic characteristics by the descriptive statistics such as percentage, mean, standard deviation, then comprehended the degree of conflicts in work detail, behavior, work relationship by the mean and standard deviation. The researchers also analyzed the relationship between conflict factors in work detail, behavior, work relationship, and work stress by Pearson's product-moment correlation coefficient. Multiple regression analysis was also used to analyze work stress towards health problems.

Results

The results showed that most of the research respondents were female (75%) aged between 26 and 35 years old (63%). Most of them had an educational background in bachelor's degree (84%) with 3 – 5 years work experience (69%). They earned income from 15,001 – 20,000 Baht a month (53%). The majority often agreed that conflicts often arose in the organization because of conflict factors in work detail, behavior, and work relationship. By list, the respondents also thought that conflicts often arose in the organization because of work relationship (M = 4.11), followed by work detail (M = 4.10), and behavior (M = 4.08), respectively (see Table 1).

Table 1 Descriptive statistics of conflict factors in work detail, behaviour, work relationship (N=132)

List	M	SD	Frequency
Conflicts in work detail			
1. Haggle about work responsibility (i.e., who must do, who should do)	4.17	0.81	often
2. Conflicts in the sequence of step among working groups	4.05	0.86	often
3. Other work groups disagree with your group	4.07	0.90	often
Conflicts in behaviour			
4. Coworkers have different opinions about the work	4.14	0.90	often
5. Coworkers disagree with you on the job that you have done	4.09	0.95	often
6. Coworkers disagree with you on the job that you are working	4.12	0.95	often
7. Your works conflict to work detail	4.01	0.95	often
8. You recognize that there are work conflicts in your working group	4.06	0.95	often
Work relationship			
9. How often does a conflict happen in your organisation?	4.07	0.93	often
10. How often does a conflict happen because of emotional aspect?	4.16	0.80	often
11. How often do you express indignation in the organisation?	4.05	0.74	often
12. How often do you feel that conflicts in the organisation come from personality?	4.06	0.90	often
13. How often do you feel that some coworkers have negative behaviour that impacts other coworkers?	4.20	0.90	often
Total	**4.13**	**0.76**	**often**

Table 2 showed that most agreed that conflicts often arose in the organization because of work stress factors that consisted of changes in the organization and work goals (M = 4.15). Due to changes in the organization, most respondents often felt that their jobs put a lot of pressure or stress and worried them. By this factor list, the question addressed how often they feel they can not prioritize their job, which the average score was lowest; it implied that the organization's changes had less affect their work stress. According to the list of work goals, most respondents were often angered because their works were out of control which the results showed the highest score. However, fewer respondents felt that they could not solve many problems according to the lowest average score.

Table 2 Descriptive statistics of conflict factors in work stress (N=132)

List	M	SD	Frequency
Changes in the organisation			
1. How often do you feel worried about your jobs or concerned about something out of control?	4.13	0.81	often
2. How often do you feel you cannot prioritize your job?	4.02	0.73	often
3. How often do you feel that your jobs make you are stressed and worried?	4.17	0.86	often
4. How often do you feel that you cannot handle any problems?	4.09	0.87	often
5. How often do you feel you can handle any work problems or obstacles?	4.13	0.83	often
Work goals			
6. How often do you feel anythings related to your works is being as you expected?	4.12	0.91	often
7. How often do you feel you cannot implement your works?	4.13	0.87	often
8. How often do you feel you can control anything as planned?	4.13	0.87	often
9. How often do you feel anger because your works are out of control?	4.17	0.90	often
10. How often do you feel you cannot solve plenty of problems?	4.11	0.92	often
Total	**4.15**	**0.78**	**often**

Table 3 proved that the organization's conflict factors in the behavior and work relationship were related to work stress with a statistical significance level of 0.05. The results supported Hypothesis 2.

Table 3 Correlation matrix (Pearson Product Moment Correlation Coefficient) of conflict factors in the work detail, behaviour, work relationship and work stress (*N*=132)

Measure	1	2	3	4	5	6	7	8	9
Conflicts in the organisation	1.66	0.47	1						
1. Work detail	1.86	0.48	0.00*	1					
2. Behaviour	1.65	0.58	0.00*	0.00*	1				
3. Work relationship	1.48	0.53	0.00*	0.00*	0.00*	1			
4. Changes in the organisation	1.85	0.79	0.00*	0.17*	0.00*	0.00*	1		
5. Work goals	2.00	0.81	0.02*	0.73	0.00*	0.02*	0.00*	1	
6. Work stress	1.92	0.71	0.00*	0.34	0.00*	0.00*	0.00*	0.00*	1

Note: 1 = Mean

2 = Standard Diviation

3 = Conflicts in the organisation

4 = Work detail

5 = Behaviour

6 = Work relationship

7 = Changes in the organisation

8 = Work goals

9 = Work stress

Table 4 showed multiple correlations equated .83, that variable of work stress from working in managing problems or obstacles related to work can predict health problems, accounted for 53% statistically. The result was involved in Hypothesis 3.

Table 4 Regression coefficients and statistics of work stress towards health problems (*N*=132)

Measure	Unstandardized Coefficients		Standardized Coefficients	t	p-value
	B	Std.Error	Beta		
Constant	0.45	0.21		2.11	0.04
Changes in the organisation 1. How often do you feel worried about your jobs or concerned about something out of control?	-0.27	0.22	-0.57	-1.19	0.24
2. How often do you feel you cannot prioritize your job?	0.27	0.31	0.46	0.87	0.39
3. How often do you feel that your jobs make you are stressed and worried?	-0.03	0.16	-0.05	-0.19	0.85

Cont... **Table 4 Regression coefficients and statistics of work stress towards health problems (*N*=132)**

Measure	Unstandardized Coefficients		Standardized Coefficients	t	p-value
	B	Std.Error	Beta		
4. How often do you feel that you cannot handle any problems?	-0.01	0.22	-0.03	-0.08	0.93
5. How often do you feel you can handle any work problems or obstacles?	0.132	0.14	0.78	2.20	0.03*
Work goals 6. How often do you feel anythings related to your works is being as you expected?	0.02	0.09	0.05	0.22	0.82
7. How often do you feel you cannot implement your works?	0.06	0.11	0.10	0.54	0.59
8.How often do you feel you can control anything as planned?	-0.06	0.09	-0.19	-0.64	0.52
9. How often do you feel anger because your works are out of control?	0.13	0.10	0.21	1.132	0.20
10. How often do you feel you cannot solve plenty of problems?	0.12	0.09	0.25	1.24	0.22

Note: p-value <0.05, R=.83, R2=.53, F= 4.57

Table 5 proved that multiple correlations equated to .77 so that work relationship can explain the health problems (52%) at a significance level of 0.05. The results supported Hypothesis 3, not Hypothesis 4.

Table 5 Regression coefficients and statistics of conflict factors in the work detail, behaviour, work relationship, work stress towards health problems (*N*=132)

Measure	Unstandardized Coefficients		Standardized Coefficients	t	p-value
	B	Std. Error	Beta		
Constant	0.31	0.29		1.06	0.29
Work detail	-0.06	0.24	-0.06	-0.26	0.79
Behaviour	-0.00	0.15	-0.00	-0.03	0.97
Work relationship	0.44	0.22	0.51	1.97	0.05*
Work stress	0.13	0.10	0.22	1.29	0.20

Note: p-value <0.05, R=.77, R2=.52, F= 7.77

Conclusion and Discussion

The research found that most of the research respondents were female (75%) aged between 26 – 35 years old (63%). Most of them had an educational background in bachelor's degree (84%) with 3 – 5 years work experience (69%). They earned income from 15,001 – 20,000 Baht a month (53%). The majority often agreed that conflicts often arose in the organization because of conflict factors in work detail, behavior, and work relationship. By list, the respondents also thought that conflicts often arose in the organization because of work relationship (M = 4.11), followed by work detail (M = 4.10), and behavior (M = 4.08), respectively. The research also proved that the organization's conflicts in work detail, behavior, and work relationship were related to the accumulated work stress at a significance level of 0.05. The study analyzed work stress and investigated that work stress affected the employees' health problems according to multiple correlation at .83, which implied work stress factors from working in managing obstacles can statistically predict health problems (53%). This paper disclosed the multiple correlations for analyzing how much conflicts in work detail, behavior, work relationship, and work stress impact health equated to .77. This result also proved that work relationships could explain the health problems (52%) at the significance level of 0.05, which supported Hypothesis 3.

Hypothesis testing

Hypothesis 1 expected that conflicts in work detail, behavior, and work relationship were moderately related to work stress at a significance level of 0.05. The results were involved in Fransman [6] who proved that human behavior in the organization depended on self-verification, self-enhancement, personality, emotional intelligence, interaction patterns, and partner support. Besides, according to conflicts in the different work environment, the employees should understand why conflicts arise, where sources of conflict be, what conditions leading to the organization's conflict situation are, conflict resolution behavior, and how to deal with a conflict situation. Thus, they will be able to handle conflict management.

Hypothesis 2 expected that work stress affected health problems. The research investigated that work stress had affected the employees' health problems according to multiple correlations at .83, which implied work stress factors from working in managing obstacles can statistically predict health problems (53%) at a significance level of 0.05. The results were related to McClure [10] who proved that serious behavior expression impacted health.

Hypothesis 3 expected that conflicts in work detail, behavior, work relationship, and work stress could explain health problems. This research disclosed the multiple correlations for analyzing how much conflicts in work detail, behavior, work relationship, and work stress impact health equated to .77. This result also proved that work relationships could explain the health problems (52%) at the significance level of 0.05, which supported Hypothesis 3, not Hypothesis 4. The results were consistent with Wood [15] who performed that positive work efficiency depended on how much a leader can make the employee satisfied with their job. However, the leader who can manage the conflict should understand the management role, the organization's relationship, coordination among different cultures in the organization, and how to create satisfaction.[14] The results were also related to Thomas [12] and McClure [10] who proved that effective corporate communication would resolve conflicts and reduce stress problems.

Recommendations

As this research found the official employees often conflicted and had much work stress that affected their health problems, to reduce its frequency of conflicts in any aspects such as the work detail, behavior, and work relationship, the employees should change their personality and behavior for the better. The employees should not procrastinate for work stress because it will cause the work to pile up and not clear it out, affecting the employees' health. After finishing their work every day, the employees should exercise and relax to reduce their stress.

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Effectiveness of Behavior Modification Program for Diabetic Patients in Suan Kluai Health Promoting Hospital, Ban Pong District in Ratchaburi Province, Thailand

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Abstract

Background- This study was an experiment for changing behavior in patients with diabetes mellitus. Because behavior modification and promoting knowledge of patients will make the quality of life of patients with diabetes with good quality and reduce the cost of treatment, reduce the complication of patients.

Objective - To measure blood sugar level with pre-post joining to change the behavior and comparison of knowledge before and after participation in health behavior modification program of patients with diabetes.

Material & Methods – Our study was a quasi-experimental design. The samples were diabetic patients with similar qualifications. Simple random sampling consisted of 60 participants, divided into 30 experimental and 30 control groups. The experimental group was modified by the researcher-developed self-care behavior. The data was collected by using questionnaires. Spending time to 12 weeks for behavior modification. Analyze data from experimental and control groups. Compare the mean of self-care behavior scores before and after joining the program by using the pair *t*-test statistics. We also compare the mean of the self-care behavior score of the control group and the experimental group using independent *t*-test statistics.

Results - The results of this study showed that the blood sugar levels of patients with diabetes after participation in activities the experimental group lower sugar levels 24 people, 80.00 %. By comparing the knowledge scores before and after the participation in the health behavior modification model of diabetics, it was found that the experimental group had a mean of 2.78 and the control group had a mean value of 2.50. The experimental group's mean knowledge score was higher than the control group. This activity pattern is appropriate for the patient.

Conclusion – The successful behavior changes in patients with diabetes. The patient's needs must be taken into account by a patient-centered patient to decide and choose a course of treatment to raise awareness of the patient. However, medical personnel should educate and as a consultant to give patients confidence in taking care of themselves to have a good quality of life forever.

Keywords: *Diabetics Mellitus, Behavior Modification Model, DM patients, Health Promoting Hospitals*

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Introduction

Diabetes mellitus (DM) is caused by a disorder in the pancreas' functioning that is used to make the hormone insulin.[1] DM is a general term for heterogeneous disturbances of metabolism, for which the main finding is chronic hyperglycemia.[2] The cause is either

impaired insulin secretion or impaired insulin action, or both. Usually, the hormone insulin is responsible for the metabolism of sugar used for energy in the body.[3] However, when the body has insufficient insulin hormones to use sugar, it cannot be used as energy. Causing the accumulation of blood sugar levels.[4] DM is a chronic metabolic noncommunicable diseases (NCDs), has attained epidemic proportions worldwide. [5,6] As of 2015, > 415 million adults have diabetes mellitus, and this number is estimated to increase to 642 million by 2040.[7] Lin et al[8] reported that DM is mostly women and is in the reproductive. In Thailand, the number of DM patients increased to 9.80%, and each day, 200 patients died from DM or about eight people/ hour.[5] The cost of treating DM is level 2 for the chronic disease group. The cost of measuring blood sugar levels will be 30.10 - 133.78 USD per person, and the cost of drugs used for treatment will start at 13.38 USD, and if complications arise, the cost will increase according to the complications. The government sector has lost about 602.68 USD per person for DM treatment.[9] In 2015, Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong district Ratchaburi Province, there were 685 patients with DM, 710 patients in 2016, and 737 patients 2017. Furthermore, in 2018 there are 738 patients with DM. Which tends to have more cases every year; 251 patients had complications from DM, most of them had an eye and kidney complications representing 4.87 % of all people with DM. Suttithum et al[10] found that providing information and knowledge, teaching the necessary skills, creating an environment that is conducive to learning by providing brochure-type, media exercise video, creating a collaborative learning environment among patients build confidence and encourage. Setting common goals for self-care behavior change will enable patients to take better care of themselves and adjust behaviors that are appropriate to their lifestyle, resulting in lower blood sugar levels.

Therefore, the researcher sees that the behavioral modification of patients with DM is diabetics should be aware and accept their health first to set common goals for behavior change in various fields, including diet, exercise, drug use and doctor visits, foot care, and complication prevention behaviors.[11] DM patients can control the blood sugar level to be in a normal range and reduce the occurrence of complications. The results of this research, the researcher hopes that the hospital director of Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province, and relevant personnel will be able to use the findings of this research to promote correct health behaviors. Furthermore, it is suitable for DM patients to reduce the incidence of complications, mortality, lower government costs, and give DM patients a better quality of life.

Objective

1. To measure the blood sugar level of patients with diabetes before and after participating in the program of changing health behavior of patients with diabetes.

2. To compare knowledge before and after participating in the diabetes health behavior modification program.

3. To analyze the effectiveness of the health behavior modification program of patients with diabetes.

Methods

Research model

This research was a quasi-experimental research design in patients with diabetes of Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province. Use the self-care ability development model, namely group relations activities, problem analysis review, joint decisions to solve problems, and providing self-care knowledge for five subjects is to control food, exercise, drug use, and doctor visits, foot care, and complication prevention behaviors. After educating, the researcher distributed the diabetic self-care manual to the experimental group to review their self-care at home and explore the results of the next trial.

Population and Sample

2.1 The population used in this study was diabetic patients admitted to a chronic disease clinic of Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province, 738 patients.

2.2 The sample consisted of diabetic patients admitted to chronic disease clinics of Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province. All 60 patients were divided into two groups by selecting patients with

similar qualifications in terms of age, blood sugar level. Simple random sampling by drawing lots choose to be a control group of 30 patients and an experimental group of 30 patients.

Instruments

We created the questionnaire were divided into two categories.

1. Questionnaires were developed from the literature review is divided into two parts:

Part 1 is a questionnaire for personal information consisting of age, gender, nationality, occupation, income per month, marital status, weight, height, waist circumference, education level, number of family members, the duration of diabetes, before-after blood sugar level, family history of diabetes, and about self-care while having diabetes 16 questions.

Part 2 was a questionnaire about self-care for five subjects to control food, exercise, drug use, doctor visits, foot care, and complication prevention behaviors. The question is a numerical rating scale of three levels 29 questions.

2. Experiment equipment there is a comprehensive content of awareness and acceptance of their health behavior modification in various fields such as control food, exercise, drug use and doctor visits, foot care, and complication prevention behaviors. It consists of 3 sets of tools:

2.1 Set 1: Model for developing self-care to the researchers, there are also public health experts at Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province become a research assistant in organizing activities according to the experimental plan. Including preparing the patient review, analyze problems assessment, decision setting realistic goals, educating, and practicing self-management and monitoring and evaluation.

2.2 Set 2: Presentation slides presenting five topics of self-care knowledge of patients such as control food, exercise, drug use and doctor visits, foot care, and complication prevention behaviors.

2.3 Set 3: Diabetes Home self-care guide with content about diabetes, causes, symptoms, interpretation of hypoglycemia, unconsciousness from hyperglycemia, complications, and exercise characteristics of the researcher applied an exercise model from the Parhira [12] research to enable the experimental group to review home care.

Content validity

Conducted content correlation from 3 experts to check for content validity. The questionnaires were used with 30 people, which looked like a sample group (Try out), and analyzed the questionnaire's confidence. By using the Cronbach alpha coefficient, receive the confidence value of the questionnaire total equal to 0.79.

Data collection

We collected the data at the Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province. Throughout the research period, the experimental group was collected on Tuesday, and data collection is divided into two groups as follows:

1. The control group collects the following information:

1.1 First time collecting general information on October 1, 2019, to collect data on self-care behavior and the blood sugar level and hand out self-care manuals to control groups for use in home self-care. Then, set up a control group at the second meeting for the next four weeks.

1.2 Second time on November 1, 2019, the control group received regular care following a general treatment procedure, namely blood glucose measurement. Weighing Measure blood pressure, measure waist circumference, take a history, see a doctor and take-home medication, and the third meeting in 4 weeks.

1.3 Third time on December 1, 2019, the researcher collected the self-care behavior data. Take blood to measure blood sugar levels and give information to the control group like the experimental group.

2. The experimental group participated in the behavior change program as follows:

2.1 First time: General data was collected on October 1, 2019, to collect self-care behavior data. Blood sugar level, then make an appointment to attend the workshop

for the 2nd time for another four weeks.

2.2 The second time, on November 1, 2019, the experimental group participated. Behavior modification programs that applied the operating procedures followed the Orem theory and the literature review.

2.2.1) Step 1: Build relationships, prepare patients with analytical activities, problem situations, and set goals for action according to their circumstances by organizing group relations activities to dissolve behaviors and promote good relationships. Facilitate better learning in this activity. The experimental group was paired to discuss and exchange blood sugar levels behavioral health problems that result in an inability to control blood sugar levels, health problems, or complications that follow and guidelines for managing self-care problems.

2.2.2 Step 2: It is a period of evaluation and decision. Five topics of self-care knowledge of patients include controlling food, exercise, drug use and doctor visits, foot care, and complication prevention behaviors. It creates a collaborative learning atmosphere among the patients with media for lectures and group activities.

2.2.3 Step 3: The phase of the action and the evaluation of the action in this phase uses a participatory learning process. To consider past self-care behaviors setting goals practical. Building confidence in self-care and complimenting a decision encourage and motivate participants to take care of themself when they return home.

2.2.4 Describe the self-care manual for DM patients, give them back to review, and then make an appointment for the third trial group for another four weeks.

2.3 The third time, on December 1, 2019, after participating in the 12-week program, the researcher collected the self-care behavior data. Furthermore, sent to the public health officials Suan Kluai Subdistrict Health Promoting Hospital, Ban Pong District Ratchaburi Province, performed a blood test to check blood sugar levels, and we analyzed the findings.

Data Analysis

Socio-demographics data were analyzed for the samples by distributing frequency, percentage, mean, standard deviation. Compare the mean of self-care behavior scores before and after joining the program by using the pair *t*-test statistics. Furthermore, compare the mean of the self-care behavior score of the control group and the experimental group using independent *t*-test statistics.

Ethical Consideration

The present study was approved by the Ethical Committee from Suan Sunandha Rajabhat University Ethics Committee certificate number: COA. 1-013/2019 and the directors of five faculties. Each participant received explanations about the study and had their rights protected throughout, including confidentiality and the right to refuse or withdraw from the study. The participants also received information 60 sheets and signed a consent form.

Results

In total, 60 patients with DM, divided into experimental groups of 30 patients, a control group of 30 patients. From the data analysis, it was found that most diabetics have an average age of 51-60 years, the average income is approximately 334.11 - 501.17 USD/ per month, most of them are single, qualifications are in the first to 6 years of education. There are 4-6 family members. The duration of diabetes is 5-10 years, and most have no family history of diabetes. The average patient's weight was 49 kilograms, height 165 and 160 centimeters, waist circumference 30 inches, and most of the patients received knowledge from doctors, nurses, and patients, by obtaining knowledge of drug use, meeting a doctor, eating and exercise.

From Table 1, the blood sugar of a total of 30participants had a decreased post-program participation level of 24 participants (80.00%), The same sugar level of 5 participants (16.67%) and the sugar level increased by one participant (3.33%), with the mean sugar level of about 110.16 mg /dL.

Table 1 Mean and standard deviation blood sugar levels in the experimental group diabetics patients (*N*=30)

Blood sugar level	Number	Percent (%)	M	S.D.
			110.16	33.35
Same	5	16.67		
Decrease	24	80.00		
Increase	1	3.33		

From Table 2, it was found that knowledge of self-care of the experimental group before joining a model of behavior modification of patients the average was 2.40 and after joining with an average of 2.78. Prior to joining the behavior modification model of the patients, the control group had a mean of 2.41, after the participants had a mean of 2.50. Comparing the pre-post cohort to the patient behavior modification model, it was found that this model of behavior modification was significantly suitable for the patients at a significance level of 0.05.

Table 2 Mean and standard deviation knowledge score self-care experimental and control groups diabetics patients (*N*=60)

Sample group	Before experiment		After experimental		t	p-value
	M	SD	M	SD		
Experimental group	2.40	.03	2.78	0.02	2.96	0.00*
Control group	2.41	.02	2.50	0.01	1.20	0.11

Note: Significance Level $p < .05$

From Table 3, it was found that knowledge of self-care the mean of the experimental group was 2.78, and the control group had a mean of 2.50. Statistical t-test equal to 3.27, showed that the behavior modification model of patients with DM was suitable for people with diabetes this time.

Table 3 Compare scores of knowledge self-care of experimental and control groups diabetics patients (*N*=60)

Sample group	M	SD	t	p-value
			3.27	0.00*
Experimental group	2.78	0.02		
Control group	2.50	0.01		

Note, Significance Level 0.05

Discussion

Our results on the effectiveness of the behavior modification model in patients with DM. From analysis and the summary of the research results can be discussed as follows: The blood sugar level of patients with diabetes before and after participating in a model of behavior modification of patients in the experimental group have a lower sugar level this is consisted with the study of Parhira [12] found that the effect of exercise by Muay Si Kong on physical fitness, glucose and blood fat levels of type 2 diabetics, Maharat Nakhon Ratchasima hospital, It was found that the patients with type 2 diabetes who exercised by Muay Si Kong fitness for 12 weeks had a statistically significant lower blood sugar level than before, at 0.05. Boehme et al[13] found that the trend of people with diabetes tended to increase particularly the elderly population aged between 51-60 years, which in this research found that the sample was between 51-60 years old the most and in line with research by Lipska et al[14] found that the occurrence of diabetes in the elderly is caused by an inability to control blood sugar levels.

Behavior modification patterns in patients with DM consist of relationship group activities education. This was because the knowledge score of self-care of diabetics in the whole experimental group increased the score level. The study of Ulittaphon [15] studied the use of a program to modify the health behavior of DM patients. Using the conceptual framework of beliefs in health and self-efficacy theory of type 2 diabetes patients, Khao Phanom hospital, Khao Phanom District, Krabi province was found that the self-care behavior of diabetic patients receiving the health behavior modification program was significantly higher than that of the previous trial at 0.05.[15] Average of the score cognitive behavior of diabetes according to the conceptual framework of the beliefs in the health of the patient; Diabetes after the program this was significantly higher than the control group at .05 level, indicating that the health behavior modification program was by using the conceptual framework, beliefs in health and self-efficacy theory positively affects the health behaviors of type 2 diabetes patients.[15] Phongprapapan et al[16], who found that empowering behavioral modification of diabetic patients in the community of Purnawat temple found that the total score of health behavior of the sample after the empowerment process activity for health behavior modification. Have more points before doing the strengthening process activities. Statistically significant at the level of 0.01.[16] In line with the study of Pennim [17] found that the behavior modification program of type 2 diabetic patients with HbA1C value were greater than 7%, was found that before joining the health behavior change program, patients had little awareness about diabetes. Most members are unaware of the importance of blood sugar levels after joining the program. It was found that the patients understood more about diabetes in terms of complications. Understanding their own blood sugar values self-care behaviors in promoting blood sugar control of diabetic patients [17]. Inconsistent with the research of Suttithum et al[10] who conducted the effects of self-care ability program on self-care behavior and HbA1C levels of type 2 diabetes patients at Lan Saka hospital results of comparison of self-care behavior was found that the control group and the experimental group had no different mean scores for self-care behavior.

Conclusion

This model of behavior changes in diabetes it consists of a planning process about relationship building, establishing common health care goals, doing group relations activities, important education in patient self-care was appropriate for the patient. Because of the opportunity for patients to set goals in their own health care. Make patients aware of their health care and to cooperate in doing this activity, the blood sugar level of the experimental group decreased. This has a positive effect on the control of blood sugar levels of patients improving patient quality of life.

Limitations

Our study has some limitations. Some patients do not have time to participate in a diabetes behavior modification program because most of them are employed. The questionnaire for the Suan Kluai Subdistrict Health Promoting Hospital workers, Ban Pong District in Ratchaburi Province, commented that most patients were trained several times. However, the patients were unable to implement recommendations. Thus, making the patient unable to control the blood sugar level. This may be due to the inadequate awareness of the severity of the disease, and some patients think that taking medication or injections regularly can lower blood sugar levels as well. Therefore, not paying attention to

taking care of themselves. Therefore, raising awareness of self-care is important, and allowing patients to set goals for their health care is an important consideration for healthcare professionals. So that patients have the best quality of life with their diseases.

Implications for future research

Our study indicated knowledge for patients with care. The education about self-care for five subjects is to control food, exercise, drug use and doctor visits, foot care, and complication prevention behaviors as a preliminary knowledge for the patient to modify the behavior to be correct. Focus on involving patients in planning and setting goals for self-care to make patients aware and take responsibility for health care. This study provides guidelines for promoting appropriate patient care for patients in each area due to differences in personality factors and cultures; those designing behavioral modification should take this into account.

Declaration of Conflicting Interest

The authors declare no conflict of interest.

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Stress and Happiness in Life of Students at Rajabhat University in Thailand

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Abstact

Background: Stress is related to happiness in student life, it is a condition in the body and stimulated to respond from inside and outside the body. The body or mind is out of balance, with each person showing different stress affects the happiness in life in each period of that person.

Objective: To examine the relationship between stress and the happiness of Rajabhat University students in Thailand.

Material & Methods: This research was contextual research by using a cross-sectional survey. The samples were students selected the sample group by stratified proportion random simple and not overridden using a random number table. The sample consisted of 190 students. The research instruments were self-reported questionnaires. The statistics used for data analysis were descriptive using inferential statistics to analyze relationships with static chi-square.

Results: The results of the research, most students have a high level of stress and rare happiness in life. The study of the relationship between demographic factors, stress, and happiness in students' lives found that level education and family income have a relationship to the stress and gender associated with happiness. Stress was associated with happiness at a statistically significant level of 0.05.

Conclusion: In this research, there are suggestions to study the cause of stress and ways to stress management and the factors that contribute to the happiness of students to enable students to continue living in quality teaching and learning

Keywords: *Stress, Happiness, University students, Students' life*

Introduction

Happiness is one of the essential outcomes of human life, and it is an important concept in social sciences.[1] Feelings of happiness enhance an individual's intellect, strength, and productivity.[2] Happiness is something that every human being desires. Since happiness is a good feeling, having a life is something that a person wants to keep their feelings long-lasting.[3] The contemplative education center, Mahidol university provided meaning to happiness, meaning comfort, joy, happiness perception of the positive emotions in mind due to the satisfaction with the expected living conditions.[4] Causing a feeling of self-worth, positively causing the creation of things. There are many elements that cause happiness. It assesses a person's happiness based on their perception of emotional feelings. The assessment of thought happiness results in a person's satisfaction in various areas of life and creating positive or negative emotions.[5] Identifying the factors deriving happiness has attracted many researchers across the globe. From

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exploring the national statistical office's happiness situation in the first half of 2020 using the mental health questionnaire among those aged for more than 15 years, it was found that the male had happiness.70.76% and female 69.47%.[6] In addition, there are survey results in students was found that 68.70% of the students had a high level of happiness score.[7]

Nowadays, stress is found in people of all ages. The slightest stress can affect human life in the first place, and unable to use daily life not causing trouble in society.[8] Good stress management affects happiness in life today because of happiness and enables that person to live happily in society.[9] They can also provide advice and care for those with stress to manage their stress and have a higher level of happiness. Eysenck and Eysenck [10] said that a group of people who have changed once in a lifetime have a stressful one. A group of secondary school students in the 6th year of study at the tertiary level.[10] After students choose to study in different institutions, there will be many changes, such as transfer of schools, adaptation to university life, adaptation to friends, teaching adaptation, and doing activities.[11] A living life that requires a different life may result in students having stress.[12] In addition, teaching and learning make students take on greater responsibility. It could affect students' adjustment in tertiary education. In an educational environment, these stressors are likely to be of a long-term nature, inducing physical and spiritual tiredness that affects one's health and affecting one's perception of completing an assignment/project, finally deteriorating their learnability.[13] Additionally, educational environments may change with the impact of new educational technologies that affect the students' stress level and learning abilities.[14] These negative emotions are caused by the existence of sources of stress, or "stressors", in students' lives.[15] In addition, many of students are facing challenges due to the pressure from their homes and parents to succeed.[16] For example, the facilities for learning and teaching activities are inadequate.[17]

Stress refers to the condition in which the body and mind respond to stimuli from both inside and outside the body.[18] As a result, there are changes within the body in both structural and chemical reactions to counteract that threat. Causing the physical and mental imbalance by dividing stress levels into four levels.[19] Mild Stress refers to a small amount of stress and disappears in a short time. Occur in everyday life, this level of stress is not threatening to lifestyle. Individuals have automatic sample adjustments. It's a kind of adaptation, and the adjustment requires little energy. It is a condition in which the body is relaxed. Moderate Stress refers to the stresses that occur in daily life due to threats or encountering important events in society; people will react in the form of anxiety, fear, etc., considered normal in general, not severe until causing harm to the body.[20] It is a stress level that makes a person more active. High Stress is the degree to which a person has been exposed to an event that causes high stress. Can not adapt to reduce stress in a short time considered in the danger zone. If not mitigated, it can lead to chronic stress and various diseases later. Severe Stress is a high level of stress that continues continuously that causes the person to fail to adapt to being bored, discouraged, exhausted, and unable to control themself. The physical symptoms or diseases easily follow.

Happiness is a part of undergraduate students' university life in the transition from late adolescence to early adulthood. Therefore, the age that has been adjusted, for example, with changes in areas such as being in the environment and study atmosphere, differs from secondary school education, which affects life, activity, and work socialize. Living with other foreign origin students, there is parenting diverse thoughts and behaviors different to affect the way of black life and quality of learning.[21] During at least four years of university life, the students are important and necessary at the university or manufacturing institution graduates must give important to the design of the learning system and facilities that facilitate life for graduates.[22] In line with the association of higher education institutions, Thailand said that "higher education will focus on developing quality students to be both a good person and a good person life on campus is the happiest time of life, Because all students are in a bright age with brain power mind and body power for development.[23] Therefore encouraging students to be happy is most important because it is one of the factors of quality a good life and will result in the power to study and promote to continue to do creative activities in life for both oneself and society.

From the above information, it can be concluded that the level of happiness affects students' living. Student stress and proper stress management inevitably result in students being happy in teaching, resulting in students being interested in studying and participating in various activities. That students are happily interested and continue to be quality people of the country. Therefore, the researcher is interested in studying the relationship between stress and these Rajabhat University students' happiness. The researcher sincerely hopes that the results of this study will benefit senior management or those involved in using the study as a guideline for improving students' quality of life. To have happiness and good quality of life in teaching until the end of the next course.

Objective

This study aimed to examine the stress and happiness in life levels, and to examine the relationship between demographic factors and happiness in life of university students.

Methods

Study Design

This research was explanatory research using a cross-sectional survey. To examine the stress and happiness in life levels, and to examine the relationship between those factors and happiness in life of university students.

Setting and Sample

The population was students at a Suan Sunandha Rajabhat University in Thailand. Which consists of Applied Thai Traditional Medicine Health Sciences, Traditional Chinese medicine, Secretary of Medicine and Public Health and Public Health enrolled in semester 1, the academic year 2020. There were 827 students and a sample number of 190 students.[24] The samples were students select the sample group by stratified proportion random simple and not overridden using a random number table for each subject. The questionnaire was distributed to students with a randomly selected list of students who had readiness and convenience in answering the questionnaire. Inclusion criteria were students in all five classes, years 1-4 registered enrolled in semester 1, the academic year 2020. Exclusion criteria included students who are not comfortable participating in the research; they can cancel participation in this research at any time.

Instruments

The research tool was a questionnaire, divided into three parts:

Part 1: Questionnaire about happiness. Researcher, translation from Hills and Argyle. [25] There were used to measure the level of happiness. The questionnaire was a 6-Likert scale measure; there were 29 items. These are 17 positive questions, 12 negative questions. The respondents were the respondents themselves. Content validity determination, the researcher brought the tools to the three advisor experts to check the content validity, correctness of language, scoring criteria, and various recommendations have been made to correct them and used to experiment with students with similar characteristics was not a sample of 30 subjects Cronbach's alpha coefficient 0.87.

Part 2: Questionaire about stree from Department of Mental Health Stress Questionnaire (SPST - 20) contains 20 questions was a 5-Likert scale. Which has been revealed to the public developed by Suan Prung hospital, department of mental health. It has been revealed to be able to be used without breaking ethics and experimenting with students with similar characteristics was not a sample of 30 subjects Cronbach's alpha coefficient 0.91. Stress is divided into four levels: a score ranged of 0 - 23 refers to the low-stress level, 24 - 41 refers to the moderate stress level, 42 - 61 refers to the high-stress level and score 62 or more points refers to the extremely high stress level.

Part 3: Personal factors were gender, ages, level of education, GPA, living, marital status, family income, income sufficiency of the student, source of income, and source of income contains ten questions. The respondents were the respondents themselves by checklist.

Data Collection

Data were collected by using questionnaires. Data were collected during 1 May – 1 August 2020. We used a convenience sample of 190 students who were willing to participate in the study. The participants then signed a consent form, and each student spent around

10-15 minutes completing the self-report questionnaires. Checked all questionnaires, and if an incomplete questionnaire was found, the participant was asked to complete the questionnaire. However, respondents who were not willing to participate could withdraw anytime.

Data Analysis

Analyze general data happy stress levels by descriptive statistics using frequency, percentage, mean and standard deviation, and inferential statistics. Analyze the relationships between personal factors with happiness in life, stress, and happiness with Chi-square. Statistical significance was set at <.05.

Ethical Consideration

The present study was approved by the Ethical Committee from Suan Sunandha Rajabhat University Ethics Committee certificate number: COA. 2-169/2020 and the directors of five faculties. Each participant received explanations about the study and had their rights protected throughout, including confidentiality and the right to refuse or withdraw from the study. The participants also received information 160 sheets and signed a consent form.

Result

In total, 190 students, most of the students were female, aged 22 years, most studied in the third year with a GPA of 3.01-3.50, living at the most dormitory, thier parrents marriage is together, and family income enough but not left to collect. The income sufficiency of student has enough but not left to collect. Most of the income source comes from parents, and most students do not take extra work.

The students had a mean score of 52.23 stress. Overall, most students have a high level of stress. The students had the highest stress level in the moderate of 85 students accounting for 44.70 percent. Then 53 students had a high level of stress, 27.90 percent of which was a low level of stress, 46 students accounting for 24.20 percent, and six students with the lowest stress level accounting for 3.20 percent (see Table 2).

Table 1: Number, Percentage, Mean and Standard Deviation of Student Stress (*N* = 190)

Stress level	Number	Percentage	Mean	SD
			3.10	0.80
Stress is low	46	24.20		
Moderate stress	85	44.70		
High levels of stress	53	27.90		
Severe stress	6	3.20		

From Table 2, students had the mean of happiness in life of 3.09 refer to rare happy, with the sometime unhappy in life 123 students representing 64.70%, followed by had happy 38 students of them representing 20.00%; some were not happy 25 students accounted for 13.20% and were unhappy at all, the least was one student accounting for 0.50%.

Table 2: Number, Percentage, Mean and Standard Deviation of Student Life Happiness (*N* = 190)

Happiness in life	Number	Percentage	Mean	SD
			3.09	0 0.64
Not happy at all	1	0.50		
Unhappy	25	13.20		
Sometimes unhappy	123	64.70		
Happy	38	20.00		
Sometime happy	3	1.60		
Aways happy	0	0.00		
Very happy	0	0.00		

From Table 3, analyze the relationship between personal factors as follows: sex, age, year, GPA, shelter, their parent's marital status, family income, the sufficiency of the money received each month itself, the main source of income, and extra work with happiness in life found that the age and marital status of their parents has a relationship to the happiness in the life of the students. Their parents' age and marital status affect the happiness in student life. However, sex, year, GPA, shelter, family income, the sufficiency of the money received each month itself, the main source of income, and extra work is no relationship with the happiness in student life.

Table 3: Relationship between personal factors and happiness in student life (*N* = 190)

Personal factor	p-value
Sex	0.69
Age	0.00*
Year level	0.98
GPA	1.00
Shelter	0.16
Father-mother's marital status	0.04*
Family income	0.28
The sufficiency of the money received each month itself.	0.21
Main source of income	0.08
Extra work	0.08

* Statistical significance at level 0.05.

From Table 4 analyzed the relationship between stress and happiness in students' lives. It was found that stress was related to the happiness in the student's life, meaning that the students were having different levels of stress resulting in their happiness in life. There were also statistically significant differences at the 0.05 level.

Table 4: The relationship between stress and happiness in student life (*N* = 190)

Variable	p-value
Stress	0.03*

* Statistical significance at level 0.05.

Discussion

This research found that stress was associated with happiness and gender was related to happiness statistically significant at a level of 0.05. Overall, students have a high level of stress and sometimes unhappy.

Students had the most moderate stress levels. The students were stressed at a high level, and then stress is low and had the least severe stress levels, respectively. The teaching and learning system with a teacher advisor help give advice and counseling to students. Through various technology, channels are making it easier to reach students. These allow students to manage stress quickly and appropriately. Abiola et al[26] who found psychological distress due to academic stress among clinical students in a Nigerian tertiary institution. The authors identified academic stress as the highest in medical students, followed by allied-health students, and the least in those who are not in any of these two disciplines.

Most students were sometimes unhappy. Followed by happy, unhappy, and not happy at all, respectively. Happiness in life at the happiest and happiest level. Students were not happy in life at this level. Education level and family income had a relationship to the stress of the students not consistent with the research of there was no statistically significant difference in the perceptions of male and female students on the causes of academic stress among the students in higher education.[27]

Gender has a relationship to the happiness in the life of the students not consistent with the research of Ouiprasert [28] found that the age difference the happiness in life was not significantly different at 0.05 level. Aungkana [29] found that marital status does not correlate with happiness. Sawangphol [30] found that age difference had a no different effect on happiness at work. Consistent with the research of Ouiprasert [28] found that sex, marital status of parents, education level different there was a statistically significant difference in working happiness at a level of 0.05. Consisted study of Aungkana [29] found that age, work experience there is no relationship with happiness. The earlier empirical research on causes of happiness can be traced back to the work of Easterlin [31] Scitovsky [32] and Hirsch [33] These studies considered economic factors to explain happiness. The studies such as Wadsworth [34] emphasized socio-demographic factors to explain happiness. These studies explored the U-shaped relationship between age and happiness. Some other studies considered marital status,[35]success in education,[36] and physical health as causes of happiness.[37] Frey and Stutzer [38] argued that institutional factor such as individual's participation in political matters plays a vital role in the life satisfaction.

Analyze the relationship between stress and happiness. The research results were found that stress is related to happiness in life. Because stress is a condition in which the body and the mind are stimulated in response to stimuli coming from the inside and outside the body. As a result of anxiety unbalanced body or mind, with different expressions of stress, affect the happiness in life of each moment of that person. In line with research by Silva and Figueiredo-Braga [39] found that first and second-year students had lower stress levels than third and fourth years. Research by Norman and Goldberg [40] found that male and female students had different study stress. The female students had higher stress than the male students.

Conclusion

Most of the students are female, aged 22 years, mostly studying in the third year, with a GPA of 3.01-3.50, living at the most dormitory, father-mother's marriage Is together, family income is fair but not accumulated, the adequacy of the money the students received each month was sufficient but not the rest most of the source of income comes from parents. Most students do not take part-time work. Students had the most moderate

stress levels. Students are happiest in life at the level of unhappiness in life. The age and marital status of their parents had a relationship to the happiness in the life of the students, and stress is related to happiness in student life. Executive and educational administrators, the research results could be used as a guideline for stress management activities and increasing students' level of happiness to have a better quality of life. This study's results could be combined with the assignment of duties and responsibilities to senior students to organize a mentor system to help new students create a good image for the university in the future. In addition, the causes of stress should be studied thoroughly to prevent the occurrence of severe stress if it is caused by teaching and learning to suit the students further.

Research limitations

This research only studied students at one university should have studied at other universities to compare research results and apply the information to promote stress management happy creation to suit the students. In addition, the data was collected by using questionnaires alone. There should be more qualitative data collection, such as in-depth interviews, grouping, to confirm more quality quantitative research data.

Implications for Future Research

This research study examines the relationship between stress and happiness of life students. The results can be used as information to make students happy to reduce the stress that arises during university studies. The current teaching and learning system that focuses on student center and the application of technology to be used in teaching and learning reducing stress for students is therefore important for the university administrators to focus on. To make students happy in university studies and graduate with high quality. However, other factors that should be studied are the cause that promotes the students' stress, such as the environment, teaching, quality of teachers, and so on to know the cause of stress. These study findings will also help the academic staff and university management/administrators design and implement policies toward refining the teaching procedures in higher education. Moreover, this study would be of great value to academic staff and university administrators' academic stress and coping strategies.

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Knowledge, Attitude and Practice Regarding Covid-19 Pandemic among Dental Practitioners of India: A Questionnaire-based Survey

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Abstract

Background- Owing to high viral load in saliva, dental practitioners are not only susceptible for exposure during Covid-19 outbreak but also post pandemic era. During the period of evolving facts and recommendations of WHO for maintaining precautions this study is an effort to understand the preparedness of dental practitioners to resume their practice.

Objectives- The objective of this study is to evaluate the knowledge, attitude and practice of dental practitioners regarding the Covid-19 pandemic.

Methods- This cross-sectional study was conducted among dental practitioners of India through an online questionnaire-based survey to collect data. The questionnaire was divided into sections containing structured multiple-choice questions about the knowledge, attitude and practice of dental practitioners.

Result- Upon analyzing 311 responses it has been observed that majority of dental practitioners were aware of common symptoms of Covid-19 and about the modes of transmission of the disease. They possess adequate knowledge about use of Personal Protective Equipment (PPE) with 88.4 % suggesting to use it while performing aerosol generating procedure. Their awareness about a Covid-19 patient becoming noninfectious is inadequate with only 10% reporting 30 days. Attitude and practice of dental practitioners regarding Covid-19 were stratified on the basis of years of practice**.**

Conclusions -Dental practitioners possess adequate knowledge about standard precaution protocol although they are less aware about transmission-based precaution specific to Covid-19 situation. This study attempts to highlight some facts about Covid-19 which will enlighten the dental practitioners before resuming practice.

Key-words: *SARS-CoV-2, Covid-19, Dental Practitioners, awareness*

Introduction

Recent outbreak of coronavirus disease 2019 (Covid-19) in the Wuhan city of China, has rapidly advanced into a public health crisis and has a cascading effect in other parts of the world. On 11th March 2020 ,World Health Organization (WHO) had declared Covid-19 to be a pandemic.[1] Covid-19 caused by novel corona virus officially known as severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) belonging to a family of single-stranded RNA viruses known as Coronaviridae seems to be more contagious than SARS-CoV and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) virus.[2] This family of viruses

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are known to be zoonotic or transmitted from animals to humans. In Covid-19 case the source of spread is the Chinese horse shoe bats and the intermediate host is Pangolin.[3] The SARS CoV-2 has an incubation period of 5 to 14 days which is usually followed as quarantine period for potentially exposed persons in many countries. [4] Several patients remain asymptomatic during this period but are potential carrier of the virus. Common symptoms of the Covid-19 infection are fever with dry cough, sore throat, shortness of breath, fatigue, and some atypical symptoms include muscular pain, diarrhea, vomiting, headache and confusion. [2]

Dental clinics pose increased risk for spread of infection due to close contact with patients. Salivary viral load is of utmost concern for dental practice. The positive rate of Covid-19 in patients' saliva can reach 91.7%. Even after patient recovery, recurrence during the convalescence period was reported. SARS CoV-2 enters the cell in the same path as SARS coronavirus, that is, through the angiotensin converting enzyme2 (ACE2) cell receptor, which may promote human-to-human transmission. [5,6]

Dental treatment includes many aerosol generating procedures like ultrasonic scaler and high-speed handpiece thus increasing the chances of cross infection between dental practitioners and patients. Dental clinics and hospitals in countries affected with Covid-19, should follow strict protocol and guidelines for infection control. Standard Operating Procedures issued by several government organization should be followed. This article is an attempt to assess the knowledge, attitude and practice (KAP) of dental practitioners regarding the Covid-19 pandemic through questionnaire-based survey.

Methods

This cross-sectional study was conducted among dental practitioners who worked in private dental clinics, hospitals or dental institutions in different cities of India. Ethical approval for the study was obtained from the Institutional Review Board of the Institute of Dental Sciences (IDS) Bhubaneswar, India (No. SOADU/IDS/IRB/18/24). The online questionnaire was prepared to collect data using Google forms. The questionnaire was distributed personally to dental practitioners via a quick response (QR) code as well as posted on various social media platforms like Facebook and WhatsApp. It was also e-mailed to few dental practitioners. The study was done for a period of 1 week starting from 23rdApril 2020 to 30th April 2020. The participants were selected among members of different state branches of Indian dental association. A pilot study using the web-based questionnaire was conducted among 25 randomly chosen dental practitioners. The responses received from the pilot study was used to modify and validate the questionnaire with the help of expert in the field of Public health dentistry. With power set at 80%, sample size was determined to a minimum of 250 responses using G power 2.0 software based on the findings of pilot study.

The questionnaire consisted of structured multiple-choice questions which was divided into sections. First section had 4 questions regarding knowledge of dental practitioner's about common symptoms, incubation period, mode of transmission and screening methods for Covid-19 and next section consisted of 3 questions regarding their attitude and last section had 7 questions pertaining to practice during Covid-19 pandemic. Total 311 responses were received and results were analyzed using the statistical package for the social sciences (SPSS, version 21.0, Chicago: SPSS Inc).

Results

The study consisted of 311 responses forming a response rate of 62% from 500 questionnaires being sent. Majority of the respondents (46.6%) were having 0-5 years of clinical experience. Total number of responses received were divided on the basis of regions like east, west, north and south regions of India, with the largest number of responses (48.8%) coming from the dental practitioners of eastern part of India. (Table-1)

Because this pandemic affects the general dental practitioners, we have stratified our data according to years of clinical experience rather than stratification on the basis of qualification. The stratification of results showing KAP about Covid-19 related to years of practice were analyzed and summarized in Table-2

As depicted in Table-2 most of the dental practitioners were aware of the common symptoms of Covid-19 infection with 88% suggesting fever with dry cough and 37.6% suggesting sore throat. The dental procedure which in their opinion can transmit Covid-19

virus most aggressively was responded by 74.3% suggesting ultrasonic scaling and 22.5% suggesting tooth preparation. Their awareness about a Covid-19 patient becoming non-infectious is inadequate with only 10% reporting 30 days while 45% reported 15 days. (Figure-1)

Which preprocedural mouth rinse do dental practitioners think is most effective was responded correctly as 0.2%povidone iodine by less than 51% respondents while 43% suggested 0.2%chlorhexidine which is ineffective for SARS-CoV-2. (Figure-2) Choice of mask during diagnostic work-up had correct response by only 43% suggesting N95 mask and disposable gown. In present Covid-19 situation the choice of radiograph for diagnostic purpose is extraoral radiograph which was suggested by 92% as compared to only 8% suggesting intraoral radiograph and readiness to use rubber dam has been shown by 65% of dental practitioners.

Table-3 depicts stratification of results showing KAP about Covid-19 related to region of practice. It shows that response trends are nearly comparable in all different regions of India. KAP related to Covid-19 is not showing marked difference among different regions of practice.

Table -1 Sociodemographic characteristic of participating dental practitioners

	Characteristic	**n (%)**
Region of India	East West North South	152(48.8) 60(19.2) 48 (15.4) 51(16.3)
Years of practice	0–5 5-10 10-20 >20	145(46.6) 86(27.6) 66(21.2) 14(4.5)

Table-2 Stratification of results showing KAP about Covid-19 related to years of practice

		Overall N=311	**0-5years N=145**	**5-10years N=86**	**10-20years N=66**	**>20years N=14**
Knowledge	Common symptom in patients with Covid-19 infection Fever with dry cough- Sore Throat	274 (88.1) 117 (37.6)	138 (95.1) 67(46.2)	75 (87.2) 30 (34.8)	64 (96.9) 19(28.6)	13 (92.8) 1 (7.1)
	Which should NOT be given in suspected Covid-19 infected patient Paracetamol Ibuprofen Aceclofenac Ketorolac	50(16.1) 207 (66.6) 34 (10.9) 71 (22.8)	21(14.4) 83 (57.2) 12 (8.2) 26 (17.9)	19(22) 52 (60.4) 05 (5.8) 10(11.6)	7(10.6) 55 (83.3) 0(0) 4 (6)	01(7.1) 11 (78.5) 01(7.1) 01(7.1)
	How many days after recovery a confirmed patient of Covid-19 may become non-infectious? 7 days 15 days 21 days 30 days	20 (6.4) 141 (45.3) 119 (38.3) 31 (10)	9 (6.2) 70 (48.2) 57 (39.3) 9 (6.2)	7 (8.1) 37(43) 31 (36) 11 (12.7)	2 (3) 30 (45.4) 25 (37.8) 9(13.6)	2(14.2) 4 (28.5) 6 (42.8) 2 (14.2)
	Dental procedure can transmit Covid-19 virus most aggressively Ultrasonic scaling Tooth Preparation Extraction with forceps	231 (74.3) 70 (22.5) 10 (3.2)	102(70.3) 35(24.1) 08(5.5)	70 (81.3) 14 (16.2) 2(2.3)	48 (72.7) 18 (27.3) 0 (0)	11 (78.5) 3 (21.5) 0 (0)

Cont... **Table-2 Stratification of results showing KAP about Covid-19 related to years of practice**

Attitude	Category of patients they are attending No procedures at all Emergency procedure Routine procedures	123 (39.5) 180 (57.8) 08 (3.6)	64(44.1) 78(53.7) 3(2)	33(38.3) 51(59.3) 2(2.3)	20(30.3) 43(65.1) 3(4.5)	6(42.8) 8(57.1) 0
	Screening measures employed Travel and Contact History with Temperature Recording Rapid Screening kit with above	145(46.6) 166 (53.3)	64(44.1) 81(55.8)	42(48.8) 44(51.1)	32(48.4) 34(51.5)	7(50) 7(50)
	How far agree for using Tele-consult? Strongly agree-162 (52.1%) Agree-129 (41.5%) Disagree- 18 (5.8%) Strongly disagree-2 (0.6%)	162 (52.1) 129 (41.5) 18 (5.8) 2 (0.6)	71(48.9) 64(44.1) 8(5.5) 2(1.5)	47(54.6) 36(41.8) 3(3.4) 0	35(53) 25(37.8) 6(9)	9(64.2) 4(28.5) 1(7.1) 0
Practice	Intend to use for infection control and prevention during diagnostic work up? Regular Surgical mask and autoclavable gown with face shield Use of N95 masks and autoclavable gown	163(52.4) 148(47.5)	75(51.7) 70(48.2)	48(55.8) 38(44.1)	32(48.4) 34(51.5)	8(57.1) 6(42.8)
	Infection control and prevention during Aerosol Generating Procedure? Regular Surgical mask, gloves and autoclavable gown only N95 masks and autoclavable gown Personal Protective Equipment (PPE) with N95 mask and face shield-	19(6.1) 41(13.2) 275(88.4)	12(8.2) 19(13.1) 124(85.5)	4(4.6) 10(11.6) 72(83.7)	3(4.5) 5(7.5) 58(87.8)	1(7.1) 13(92.8)
	Radiograph preferred for diagnostic purpose Extraoral Radiograph Intraoral Periapical Radiograph	288 (92.6) 23 (7.4%)	137(94.4) 8(5.5)	79(91.8) 7(8.1)	60(90.9) 6(9)	12(85.7) 2(14.2)
	Agreement for the use of Rubber dam Strongly agree Agree Disagree Strongly disagree	202 (65) 85 (27.3) 13 (4.2) 11 (3.5)	90(62) 43(29.6) 4(2.7) 7(4.8)	60(69.7) 22(25.5) 4(4.6)	41(68.1) 16(24.2) 5(7.5) 4(6)	10(71.4) 4(28.5)
	Aerosol generating procedures, how virus load can be reduced Preprocedural mouth rinse High Volume Evacuator Aerosol reducing Air Purifier	148 (47.6) 123 (39.5) 189 (60.8)	77(53.1) 36(24.8) 32(22)	40(46.5) 20(23.2) 26(30.2)	32(48.4) 23(34.8) 5(7.5)	5(35.7) 7(50) 5(35.7)
	Preprocedural mouth rinse do they think is most effective? 0.2% povidone-iodine mouthwash 0.2% Chlorhexidine mouthwash Cetylpyridinium chloride (CPC)	158 (50.8) 134 (43.1) 58 (18.6)	49(33.7) 68(46.8) 38(26.2)	34(39.5) 40(46.5) 11(12.7)	40(60.6) 23(34.8) 3(4.5)	11(78.5) 3(21.5) 0
	Equipment to reduce bio aerosol in your clinic post-COVID to infection? Air Purifier with HEPA filter High volume evacuator (Nu Bird) Ultraviolet germicidal irradiation (UVGI)	175 (56.3) 145 (46.6) 113 (36.3)	70(48.2) 79(54.4) 73(50.3)	56(65.1) 40(46.5) 22(25.5)	40(60.6) 20(30.3) 18(27.2)	9(64.2) 5(35.7) 0

Table-3 Stratification of results showing KAP about Covid-19 related to region of practice

		Overall N=311	East N=137	West N=53	North N=63	South N=58
Knowledge	Common symptom in patients with Covid-19 infection Fever with dry cough- Sore Throat	274 (88.1) 117 (37.6)	127 (92.7) 10(7.2)	51 (96.2) 02 (3.7)	61(96.8)) 2 (3.1)	50 (96.2) 8 (7.1)
	Which should NOT be given in suspected Covid-19 infected patient Paracetamol Ibuprofen Aceclofenac Ketorolac	50(16.1) 207 (66.6) 34 (10.9) 71 (22.8)	19(14.4) 92 (67.1) 12 (8.7) 14 (10.2)	11(20.7) 34(64.1) 01 (1.8) 07(13.2)	13(20.6) 42 (66.7) 2(3.1) 6 (9.5)	06(10.3) 34(78.5) 03(5.1) 15(23.8)
	How many days after recovery a confirmed patient of Covid-19 may become non-infectious? 7 days 15 days 21 days 30 days	20 (6.4) 141 (45.3) 119 (38.3) 31 (10)	11 (6.4) 54(45.3) 56 (38.3) 16 (10)	4 (8.1) 28(52.8) 16 (30.1) 5(9.4)	4 (6.3) 28(45.4) 25 (37.8) 6(9.5)	1(14.2) 31 (53.4) 22 (37.9) 4 (6.8)
	Dental procedure can transmit Covid-19 virus most aggressively Extraction with forceps Ultrasonic scaling Tooth Preparation	10 (3.2) 231 (74.3) 70 (22.5)	06(5.5) 106(70.3) 25(24.1)	1(2.3) 34 (64.1) 18 (33.9)	2 (3.1) 48 (76.1) 13 (20.6)	1 (0) 42 (72.4) 15(25.8)
Attitude	Category of patients they are attending No procedures at all Emergency procedure Routine procedures	123 (39.5) 180 (57.8) 08 (2.6)	62(45.2) 72(52.5) 3 (2.1)	21(39.6) 32(60.3) 0	21(39.6) 40(63.4) 2(3.1)	19(32.7) 36(62) 3(5.1)
	Screening measures employed Travel and Contact History with Temperature Recording-134 (43.1%) Rapid Screening kit with above	145(46.6) 166 (53.3)	57(41.6) 80(58.3)	21(39.6) 32(60.3)	34(53.9) 29(54.7)	33(56.8) 25(43.1)
	How far agree for using Tele-consult? Strongly agree-162 (52.1%) Agree-129 (41.5%) Disagree- 18 (5.8%) Strongly disagree-2 (0.6%)	162 (52.1) 129 (41.5) 18 (5.8) 2 (0.6)	79(57.6) 51(37.2) 6(5.5) 1(0.7)	24(45.2) 24(45.2) 4(7.5) 1(1.9)	33(62.2) 26(49) 4(7.5)	26(44.8) 28(48.2) 4(6.8)

Cont... **Table-3 Stratification of results showing KAP about Covid-19 related to region of practice**

Practice	Intend to use for infection control and prevention during diagnostic work up? Regular Surgical mask and autoclavable gown with face shield Use of N95 masks and autoclavable gown	163(52.4) 148(47.5)	69(50.3) 68(49.6)	27(50.9) 26(49)	35(55.5) 28(44.4)	35(60.3) 23(39.6)
	Infection control and prevention during Aerosol Generating Procedure? Regular Surgical mask, gloves and autoclavable gown only N95 masks and autoclavable gown Personal Protective Equipment (PPE) with N95 mask and face shield	19(6.1) 41(13.2) 275(88.4)	9(6.5) 15(10.9) 113(82.4)	2(3.7) 4(7.5) 47(88.6)	4(6.3) 7(11.1) 52(82.5)	4(6.8) 9(15) 45(77.5)
	Radiograph preferred for diagnostic purpose Extraoral Radiograph Intraoral Periapical Radiograph	288 (92.6) 23 (7.4)	127(92.7) 10(7)	51(96.2) 2(3.7)	59(93.6) 4(6.3)	56(96.5) 2(3.4)
	Agreement for the use of Rubber dam Strongly agree Agree Disagree Strongly disagree	202 (65) 85 (27.3) 13 (4.2) 11 (3.5)	89(64.8) 38(27.7) 6(5.5) 4(2.9)	35(73.5) 13(24.5) 1(1.8) 4(7.5)	46(73) 13(20.6) 4(6.3) 1(1.50	24(41.3) 21(36.2) 2(3.4) 2(3.4)
	Aerosol generating procedures, how virus load can be reduced Preprocedural mouth rinse High Volume Evacuator Aerosol reducing Air Purifier	148 (47.6) 123 (39.5) 189 (60.8)	76(55.4) 61(44.5) 60(43.7)	20(37.7) 16(30.1) 30(56.6)	23(41.2) 28(44.4) 20(31.7)	31(53.4) 13(22.4) 10(17.2)
	Preprocedural mouth rinse do they think is most effective? 0.2% povidone-iodine mouthwash 0.2% Chlorhexidine mouthwash Cetylpyridinium chloride (CPC)	158 (50.8) 134 (43.1) 58 (18.6)	60(43.7) 61(44.5) 16(11.6)	15(28.3) 28(52.8) 10(18.8)	32(50.7) 21(33.3) 9(14.2)	27(46.5) 24(41.3) 7(12)
	Equipment to reduce bio aerosol in your clinic post-COVID to infection? Air Purifier with HEPA filter High volume evacuator (Nu Bird) Ultraviolet germicidal irradiation (UVGI)	175 (56.3) 145 (46.6) 113 (36.3)	76(55.4) 63(45.9) 50(36.4)	29(54.7) 30(56.6) 18(33.9)	35(55.5) 26(41.2) 26(41.2)	35(60.3) 26(44.8) 19(32.7)

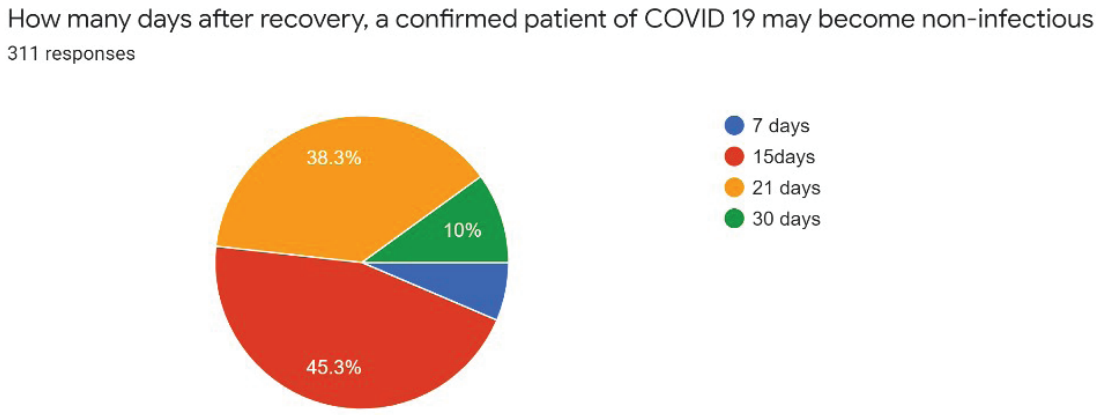

Figure-1 Awareness regarding confirmed patient of Covid-19 becoming non-infectious

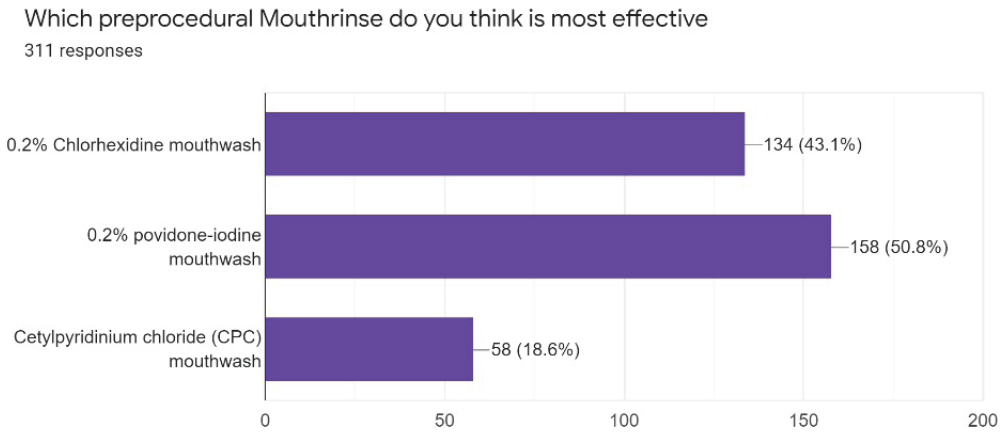

Figure-2 Knowledge about effectiveness of preprocedural mouth rins

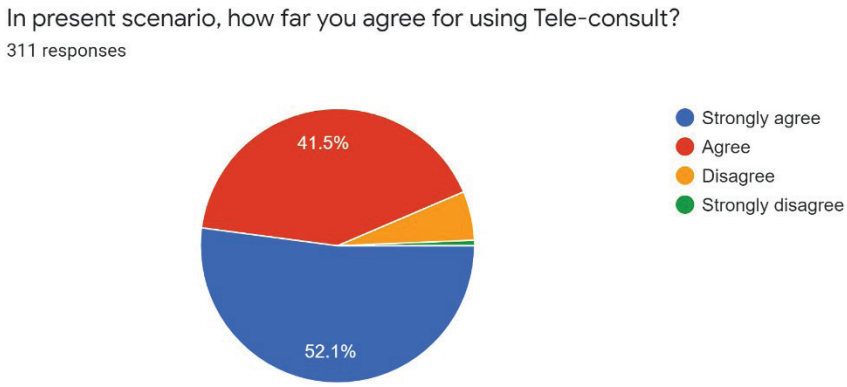

Figure-3 Awareness about Teleconsultation during Covid-19

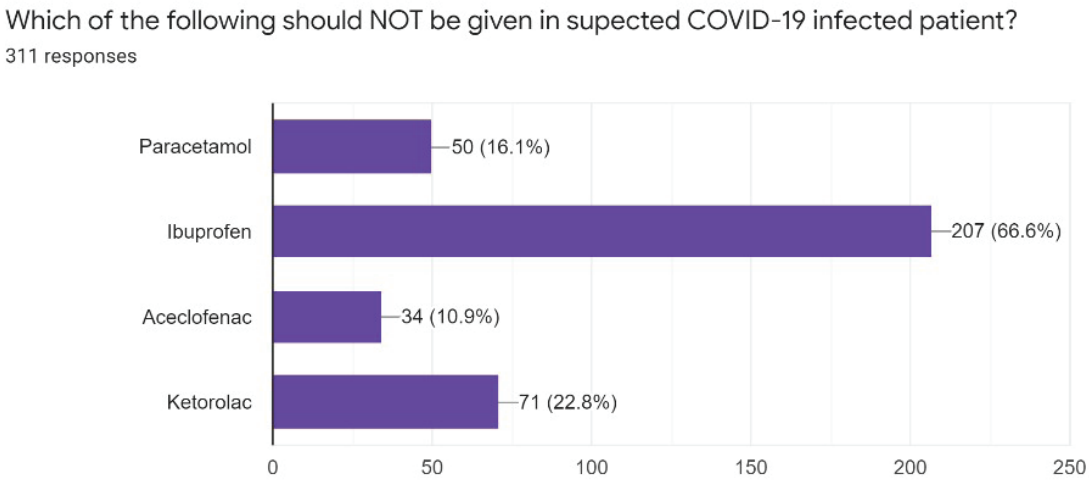

Figure-4 Knowledge about choice of analgesic in suspected patient of Covid-19

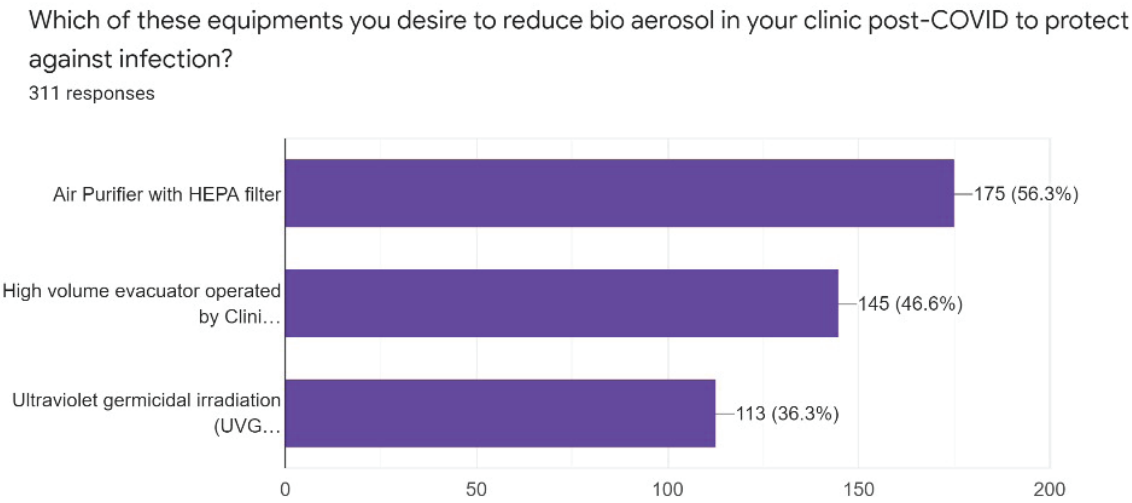

Figure-5 Awareness about ways to reduce aerosol in the dental clinic

Discussion

With the objective of assessing the knowledge, attitude and practice of Indian dental practitioners regarding the Covid-19 pandemic, this study was done through a questionnaire-based survey. After evaluating the responses, it has been observed that dental practitioners need to update themselves regarding both standard and transmission specific precautions for Covid-19. Dental practitioners should follow the guidelines issued by WHO and Indian Dental Association to categorize the patient and assess the risk and benefit while treating the patient.[7] Although WHO guidelines are still changing as this is a new disease.

Teleconsultation should be used via App based, Web based or Telephonic to assess patients' urgency for need of treatment. Questions related to recent travel history to affected countries, coming in contact with infected person at home or workplace, and/or presenting with common symptoms of Covid-19 should be asked. Patient consent form should be filled and signed by

patient before starting any treatment. A strict protocol for teleconsultation should be followed for scheduling appointment. However only 51% have strongly agreed to it. Therefore, spreading the awareness of teleconsultation among dental practitioners is the need of the hour. (Figure-3)

In response to the question about the screening measures for Covid-19 almost 92 % of dental practitioners were well aware and have reported contact and travel history with temperature recording alone or with rapid screening test as primary screening measure during the Covid-19 pandemic situation. Incorporation of the contact and travel history can significantly reduce the transmission as well as the burden of the disease. A non-contact forehead thermometer using infra-red rays is recommended for the screening. If a patient had a positive history of contact and/or symptoms, such a patient should not be given appointment through teleconsultation. As of now, there is no universally accepted rapid screening test available for chair side use. Each kit has their own sensitivity and specificity. However, dental practitioners should constantly update themselves about availability of such kits and start using whenever available.

Presently, in India, dental care facilities are either completely closed or are working only for emergency patients. In present survey 41 % of dental practitioners had reported that they are doing no procedure at all and 35% were dealing with only emergency patients. The reason could be that most of the dental clinics are one or two room operatories with little or no scope to maintain PPE protocol. Patients who were in urgent need of dental care might have suffered, although this step taken by maximum dental practitioners, will certainly reduce the spread of infection.

On being questioned which analgesic should not be given in suspected Covid-19 infected patient 67% suggested Ibuprofen while Ketorolac was suggested by 22%. Day [8] in his paper had suggested against the use of ibuprofen as it aggravates the respiratory and cardiovascular complications. He also suggested that ibuprofen's anti-inflammatory properties could diminish the immune system and slow down recovery. Nevertheless, the evidences are not enough for concluding remark. Further research in this aspect will enlighten dental practitioners. (Figure4)

To et al.[5] have reported that salivary glands remain the potential reservoir for the asymptomatic Covid-19 infection. In response to the question about how many days after recovery, a confirmed patient of Covid-19 may become non-infectious. 45.3% respondents have suggested 15 days, 38.3% have opined 21 days while only 10% have suggested 30 days which is actually the period up to which recurrence is observed in a patient as reported by Chen et al. [9]. They suggested the role of active surveillance of SARS-CoV-2 RNA for its infectious potential.

National Institute for Occupational Safety and Health (NIOSH) classification of filtering respirators describe efficiency of N95 respirators to be 95 % and of N99 respirators to be 99%. Because of tight fit these respirators minimize facial seal leakage and avoid inhalation of small airborne particles. In contrast, surgical masks are loose fitting, provide barrier protection against large droplets and prevent contact of hand with face.[10] In response to the question regarding diagnostic work-up, 33% of dental practitioners intend to use regular surgical mask and autoclavable gown with face shield while 46% have preferred use of N95 masks and autoclavable gown. However, during aerosol generating procedure 88.4% intend to use Personal Protective Equipment (PPE) with N95 mask and face shield and only 12% intended to use N95 masks and autoclavable gown. These results suggest that dental practitioners are aware about precautions to be taken, but its affordability and practicality remains obscure.

The most important concern in the dental clinics and hospital is to avoid droplet and aerosol-based transmission of SARS CoV-2. Many dental procedures generate large amount of aerosol mixed with patient's saliva and blood during dental treatment.[11] In response to the question on how viral load could be reduced during aerosol generating procedure, 60% of respondents have suggested the use of high volume evacuators and 47% have suggested to use preprocedural mouth rinse and 39% were of the view to use aerosol reducing air purifier. The use of high volume evacuators can effectively reduce the production of droplets and aerosols.[12]

In response to the question which preprocedural mouth rinse they think to be the most effective; 50%

have selected 0.2% povidone-iodine mouthwash while 0.2% Chlorhexidine mouthwash was selected by 43%. Chlorhexidine, which is commonly used as mouth rinse in dental practice, may not be as effective to kill SARS CoV-2. Preprocedural mouth rinse containing oxidizing agents such as 0.2% povidone iodine or 1% hydrogen peroxide is suggested as SARS CoV-2 is susceptible to oxidation.[13,14] The dentist should be sensitized about this aspect of usage of mouthwash to reduce the viral load significantly.

It has been reported that the use of rubber dam could significantly reduce airborne particles in approximately 3-foot diameter of the operational field by 70%. Rubber dam with high-volume evacuator would reduce splatter and aerosol. In such case execution of four-handed dental practice is also needed. A preprocedural mouth rinse would be strongly recommended in cases where rubber dam cannot be employed. [12]

In response to their choice of equipment to reduce bio aerosol in their clinic post Covid-19, 56% have opined in favor of Air Purifier with HEPA filter (High Efficiency Particulate Air) filter, 46.5% have opined for High volume evacuator operated by Clinician (NuBird), while 36.5% have positive opinion towards Ultraviolet germicidal irradiation (UVGI) device. The protocol for use of airborne contaminant removal suggest a minimum of 6 Air Changes per Hour (ACH). HEPA air filters provide 12 ACH with 20 minutes of use whereas UVGI provides 6 ACH with 15 minutes of use.[15] (Figure-5) These steps taken to combat the Covid-19 pandemic will certainly reduce the burden of disease.

Dental practitioners possess adequate knowledge and awareness about certain aspects of prevention and control of infection. Their response related to standard operating protocol are appropriate. Dental practitioners and their assistant should be sensitized about transmission-based precaution specific to Covid-19 situation which will help defeat this pandemic. The different modes of transmission include direct transmission (through cough, sneeze and droplet inhalation), contact transmission (through eye, oral and nasal mucosa) and air-borne transmission (aerosol and droplets mixed with the patient's saliva and blood).[11] This reinforces the importance of good hand hygiene. Dental practitioners should be more cautious to avoid touching their own mouth, nose and eyes. [16] Other than the questionnaire there are additional aspects for dental practitioners to pay attention for a safe practice. First, thorough disinfection of all surfaces with 0.1% sodium hypochlorite or 70% isopropyl alcohol at dental clinic is of great importance because the virus remains viable on surfaces for 2 hours up to 9 days. [17] Second, disinfection and sterilization after every patient which includes flushing of spittoon and suction drainage with 1% sodium hypochlorite (NaOCl). Third, hand hygiene two-before and- three-after guideline, proposed by the infection control department of the West China Hospital of Stomatology, Sichuan University should be followed.[11] Fourth, The medical waste generated after the treatment of patients with suspected or confirmed Covid-19 infection is regarded as infectious medical waste and should be disposed according to guidelines.

Conclusion

Amongst precautions for several infectious diseases, Covid-19 is a major challenge to the dental practitioners. Because of the novelty, the guidelines and recommendations to combat this disease are still evolving. This study is highlighting the lesser known facts about Covid-19 which may enlighten dental practitioners before resuming practice.

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Ethical Permission: Approved

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Pit and Fissure Sealants as a Viable Preventive Tool for Dental Caries

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Abstract

Dental caries remains to be a global disease burden and the current ways of preventing it are through maintenance of good oral hygiene, watching over the diet intake and pit and fissure sealant application. Pit and fissure sealants are still underused in dental practice. With the availability of the various type of sealants ranging from glass ionomer sealants to resin sealants, we need to improvise our preventive protocols for dental caries. This article provides crisp and compact information on the current status and usage of dental sealants to encourage the fraternity to use it more often.

Keywords: *Pit and fissure, sealant, resin, glass ionomer, fissurotomy, enameloplasty*

Introduction

Pit and fissure sealants, introduced in the 1960s prevented dental caries by inhibiting bacterial growth which can lead to a full-blown carious lesion. Evidence shows the application of sealants aided in better prevention of caries than with no sealants.[1] For nearly 5 decades, controlled caries in primary and permanent teeth and are still underutilized despite the evidence of their efficiency and the availability of clinical practice guidelines.[2,3]

Pit and fissure sealants are a part of comprehensive caries management under primary prevention due to its action of reducing the risk of caries by penetrating and sealing the anatomical grooves and fissures.[4,5] On the note of secondary prevention, sealants can also inhibit the progression of non-cavitated carious lesions.[6] The success of sealants for caries prevention depends on its long-term retention. The visual and tactile examination can help determine this aspect. [7,8]

Newer modifications of sealants continue to emerge which puts the dentist in dilemma which makes a continuous review of the available options necessary to aid the clinicians in decision making.[2,9]

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DEVELOPMENT OF PIT AND FISSURE SEALANTS:

Previously enamel fissure eradication or fissurotomy was used which involved the widening of the fissures to make them self-cleaning.[10] Treatment of pits and fissures with ammoniacal silver nitrate was also used.[11] But none of these methods showed much reliability. Hyatt in 1923 introduced an even more invasive procedure that required class I cavity preparation including all deep pits and fissures followed by a prophylactic restoration which remained as a choice of treatment till the 1970s.[12]

Buonocore documented the method of resin bonding to enamel that was etched using 85% phosphoric acid for 30 seconds to increase the adhesion of self-curing methyl methacrylate resin material to dental enamel.[13] Cueto came up with the first sealant in the mid-1960s which consisted of methyl cyanoacrylate which wasn't marketed and it was also vulnerable to bacterial disintegration in the oral cavity.[14] Bowen invented a viscous resin, called bisphenol-a-glycidyl dimethacrylate, and this has become known as BIS-GMA. It was resistant to disintegration in the oral cavity.[15] This BIS-GMA was used along with ultraviolet light later by Buonocore successfully.[16]

TYPES OF SEALANT MATERIALS AND PLACEMENT TECHNIQUES:

Broadly two types of pit-and- fissure sealants are available which are resin-based and glass ionomer cement (GICs) based sealants. Resin-based sealants can be self-cured or light-cured using visible light or a combination of the two.[7]

GICs have a fluoride-releasing property and are of 2 types conventional and resin-modified(RMGIC).[17] GI based sealants are not moisture sensitive and are easier to bond on tooth surface without acid etching of enamel. GI sealants ability to release fluoride acts as an added adjuvant for caries prevention.[18] Resin when added to glass ionomer, it is called a resin-modified glass ionomer (RMGI) which was also used as a sealant. Setting reaction of RMGI sealants is initiated by photoactivation of the resin component and acid-base reaction of the GI component. It offers added advantages of physical characteristics and lesser sensitivity to water and longer working time compared to conventional ionomer sealants.[18,19]

Compomer which is the polyacid modified resin-based composite material was also used as a fissure sealant. It combines the advantageous properties of visible light polymerized resin-based sealant with the fluoride-releasing property of the GI sealant. It offers several advantages such as better adhesion, lesser water solubility than GI sealants and better workability than resin sealants.[20]

HOW TO IMPROVE RETENTION OF A SEALANT?

Complete cleaning of the occlusal surface with hydrogen peroxide, pumice, as well as air abrasion and pre-treatment with acid has been shown to improve retention of the sealant.[21] Evidence shows the reduced occurrence of caries with improved retention.[1] Unco-operative child or patients with special health care needs or cases of the partially erupted tooth may pose a problem for isolation of teeth for sealant placement. Such cases are best dealt with GI based sealants which are not moisture sensitive.[22] For successful retention, strong penetration of the sealing material into the pits and fissures is a pre-requirement. The morphology of the fissures seemed to significantly influence the depth of penetration of the sealing material. "Y"-shaped fissures showed a shallower penetration than "U" or "V" shaped fissures.[23] Long term success rate is influenced by the usage of a bonding system in resin-based sealants and pre-treatment methods such as laser irradiation, air abrasion and pre-heating of the sealant.[24-29]

TECHNIQUE FOR RESIN SEALANT PLACEMENT:

Enamel preparation: Careful cleaning of the pits and fissures before acid etching is recommended by most manufacturers.[30] Fluoride before sealant placement is contraindicated usually as it inhibits enamel etching and decreases enamel solubility. Although some studies proved sealant retention may not be impaired by fluoride application before sealant placement.[31-33] Researchers have studied various methods of mechanical preparation such as air abrasion, sandblasting, fissurotomy before sealant placement but it was found that enameloplasty is not necessary. Rather it would make the tooth more susceptible to caries if the sealant disintegrates.[34, 35]

Isolation: Isolation is the most critical step for resin sealant application. Even exposure of 0.5 seconds to saliva can contaminate the etched enamel which may require re-etching.[36] Four-handed dentistry was found to increase sealant retention by 9% when the method of surface cleaning was controlled.[37]

Acid etching: 35% and 37% concentrations of phosphoric acid for 20 seconds is commonly used for this purpose.[38] Earlier for primary teeth enamel etching time was twice that of the accepted time for permanent enamel, namely i.e. 120 seconds for primary enamel and 60 seconds for permanent enamel to ensure the elimination of identified prismless enamel. Although this finding was found not to be clinically significant for sealant retention. Another added advantage of shorter etching time is decreased chances of salivary contamination.[22] Post etching, rinsing time of 30 seconds and drying the tooth for 15 seconds should be enough to remove all acid etchant residues and achieve the characteristic enamel frosty appearance.[22]

Bonding agents: The retention of sealants was found to be higher when the etch-and-rinse adhesive system was used than self-etch adhesive system.[39] Studies have also found that the adhesive system enhances

the retention of the fissure sealant by increasing the penetration into enamel porosities and thus increasing the bond strength.[40]An evidence-based 2008 report from the American Dental Association and the American Academy of Pediatric Dentistry supports the use of adhesive systems before sealant application for better sealant retention.[21,41]

Evaluation of sealant post-placement: Post curing the sealant the operator should check for voids, bubbles or any deficient material. Sealant retention should be checked using the explorer in an attempt to remove the sealant. In the case of sealant dislodgement, the fissures should be re-checked for any remaining food debris that may have caused the debonding of the sealant material. Redoing of the sealant application is done. Attention should be paid to excess sealant material which may cause high points and hamper normal occlusion.[42]

SEALANTS IN PRIMARY TEETH:

Pit and fissure sealants are indicated for primary teeth that have deep retentive or stained pits and fissures.[42] Sealants are found to be beneficial for children and young people with medical, physical, or intellectual impairment in preventing caries. [43]

To quicken the sealant application procedure in children, a shorter etching time may be considered to decrease the chances of salivary contamination.[44] Shorter appointments can also be achieved by the usage of self-etch bonding agents.[45,46] Evidence also indicates usage of GI sealant as an alternative when salivary contamination is expected.[47]. However, evidence on the use of GI sealants in primary teeth are very limited and calls for further research in this area.[48]

CARIES PREVENTION BY SEALANTS:

A Cochrane review concluded that resin-based sealants application on permanent molars is effective for prevention of caries in children and adolescents and reduced caries by 11-15% when compared with no sealant application. Evidence was not sufficient to comment on the effectiveness of GI sealants or another different type of sealants on caries prevention. While this review threw light on the effectiveness of sealants in preventing caries, the efficacy related to caries remains unclear.[1]

ESTEROGENICITY:

Bisphenol-A (BPA) precursor of bisphenol-a dimethacrylate (Bis-DMA) and bisphenol-a glycidyl dimethacrylate (Bis-GMA) are the most commonly used monomers in resin-based sealants. They are known to have an estrogenic property which may result in potential reproductive and developmental human toxicity.[49,50] BPA is not present in monomers as a raw material but as BPA derivatives that can sometimes be hydrolyzed and found in saliva.[1] High level of BPA was found in salivary samples post 1 hour of sealant treatment and urine samples.[51] But the American Dental Association(ADA) and American Academy of Pediatric Dentistry report dismissed the occurrence of the adverse effect after sealant placement.[5,19]

Immediate cleaning of the sealed surface or the removal of the oxygen inhibition layer of the unreacted monomer, which is present on the outer layer of the sealant surface to reduce the amount of unreacted monomer with pumice or rotating rubber cup was suggested to reduce the potential BPA exposure.[51]

Conclusion

Pit and fissure sealants are one of the measures of primary prevention of dental caries. It has a history of being efficient in caries prevention. It is also important to select the type of sealant to be used according to the patient's age group, medical conditions, tooth eruption time and co-operative nature. This simple procedure can change the dynamics of caries disease in high caries risk patients. Therefore, dentists should be encouraged to take up this preventive tool in their regular dental practice.

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Etiologic Factors Influencing the Pattern of Mandibular Fractures – A Retrospective Study

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Abstract

Objectives: Fractures involving the face, especially the mandible causes aesthetic disfigurement and hamper masticatory function. Ever changing economic and population trends have caused a shift in the etiologic factors responsible for mandibular fracture. This study records the epidemiology of the mandibular fractures in Bhubaneshwar region of Odisha and to appreciate the importance of demographics and contribution of individual etiological factors towards such injuries.

Methods: A retrospective study was conducted during between September 2013 to March 2017, in the department of Oral and Maxillofacial Surgery, Institute of Dental Science. Patients admitted in the facility for the treatment of mandibular fractures were included in the study. The data was collected from patient records.

Results: Out of a total of 197 patients, 90% affected were males, with 42.6% aged between 20-29 years. The most common cause was Road Traffic Accidents (RTAs)with 83% and para-symphysis fractures was seen in 42.7%. Etiology was found to be influenced by gender using Chi-square ($p < 0.05$) and site of injury was found to highly significant with respect to age.

Conclusion: Mandibular fractures were found to be associated with younger aged males and road traffic accidents. This could be prevented with stringent and safer traffic regulations.

Key words: *Mandibular fractures, etiology, retrospective study*

Introduction

The face is a significant part of the human body, necessary for many vital functions such as speech, sight, smell and most importantly in establishing the identity of an individual. The shape of face is determined by the bone structure of the skull, and each individual is unique through the anatomical variations present in the bones that make up the viscero-cranium. The shape of the facial structure can be attributed mainly to the maxilla, mandible, nasal bone and zygomatic bone.

Of these, the mandible is the only movable bone of the skull. It houses the lower teeth and is formed from the fusion of the right and left processes. One fifth of all facial injuries involve the mandible. Mandible has been found to be the second most affected site in facial injuries[1–4]. The injury to the mandible is given special consideration as, apart from the aesthetic disfigurement, the presence of teeth adds a concern regarding functionality of mastication. The mandible includes areas of weakness such as the areas adjacent to the mental protuberance, mental foramen, mandibular

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angle and the condylar neck. The bone tends to fracture at these areas of tensile strain.[5] But the condylar neck because of the thickening on its inner surface transmits pressure to the TMJ and base of skull, increasing the fatality of mandibular fractures[1].

The etiology and pattern of mandibular fractures vary from geographic regions based on its economic and population growth trends. In India, an alarming increase in vehicular ownership combined with expansion of road network has caused as change in the trends of facial fractures[6]. There is also a change in the age groups involved in the road traffic accidents with an increase in the younger adults being more affected[4]. Hence, even though there is no lack of literature elucidating the factors associated with mandibular factures, there is a need to re-evaluate these factors given the change in the economic, cultural and social environment of the recent times.

This study intends to record the epidemiology of the mandibular fractures in Bhubaneshwar region of Odisha and also to appreciate the importance of demographics and contribution of individual aetiological factors towards such injuries.

Material and Methods

A retrospective study was conducted in the department of Oral and Maxillofacial Surgery, Institute of Dental Science. Patients admitted in the facility for the treatment of mandibular fractures during the time period of September 2013 to March 2017 were included in the study. The data was collected from patient records. The search criteria included all mandibular fractures including condylar fracture, para-symphysis fracture, symphysis fracture, mandibular body fracture.

Eligibility Criteria:

1. Patients with fractures in the mandible.
2. Patients reporting to the OPD during the time period of the study.
3. Patients who consented to be a part of the study.

The patient records with incomplete information were not included in the study.The data was entered into a digital proforma designed to record the necessary variables required for the study. A tabular form was prepared for all the cases in Microsoft excel including the name of the patient, age, sex, site of fracture and mode of injury. The site of fracture classified into symphysis, parasymphysis, mandibular body, mandibular angle, condyle, coronoid and ramus fractures. Mode of injury for all the cases fell under three categories - Road traffic accidents, Fall and Assault. Ethical Clearance was obtained from the Institutional Review Board for conducting the study. The data was analysed for descriptive statistics and Chi-square was used to associate sex and age with type and region of fracture. The p value was set at >0.05.

Results

A total of 197 patients with mandibular fractures were included in the study. Of these 19 (10%) were females and 178 (90%) were males. (Graph 1). The M/F ratio was 9.3. Majority of the study sample (42.6%) belonged to 20-29 years, with the Mean ± SD found to be 30.9 years ±11.1.(Graph 2). It was observed that the most common etiologic was road traffic accidents contributing to 83% of the mandibular fractures (Graph 3). Aetiology was found to be influenced by gender using Chi-square at p value of 0.004. But however, it was evident that age of the study sample did not play a role. (p = 0.660) (Table 1 and 2). With 42.7% subjects reporting with para-symphysal fractures, making it the most frequently involved site in mandibular fractures. (Graph 4) The next common site of injury was condyle (n=80). This was found to highly significant with respect to age but not gender. (Table 3 and 4)

Table 1: Influence of gender on etiology of fractures:

Gender	Etiology			Chi-square value	p-value
	Fall	Accidental fall	Road traffic accidents		
Males	18 (10.1%)	8 (4.5%)	152 (85.4%)	11.257	0.004*
Females	7 (36.8%)	1 (5.3%)	11 (57.9%)		

Table 2: Influence of age on etiology of fractures:

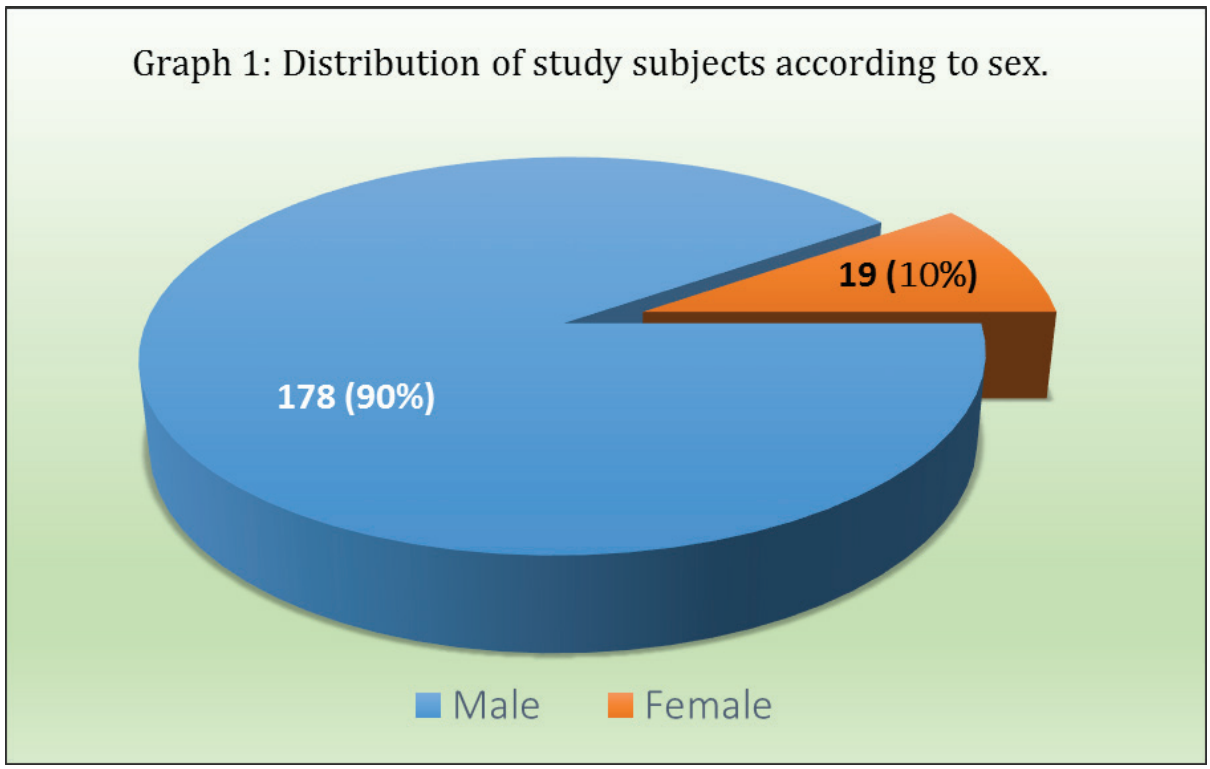
Age group	Etiology			Chi-square value	p-value
	Fall	Accidental fall	Road traffic accidents		
Under 9 years	0 (0%)	0 (0%)	1 (100%)	9.498	0.660
10-19 years	3 (16.7%)	1 (5.6%)	14 (77.7%)		
20-29 years	5 (6%)	5 (6%)	74 (88%)		
30-39 years	11 (19.3%)	2 (3.5%)	44 (77.2%)		
40-49 years	4 (20%)	1 (5%)	15 (75%)		
50-59 years	2 (18.2%)	0 (0%)	9 (81.8%)		
60 years and above	0 (0%)	0 (0%)	6 (100%)		

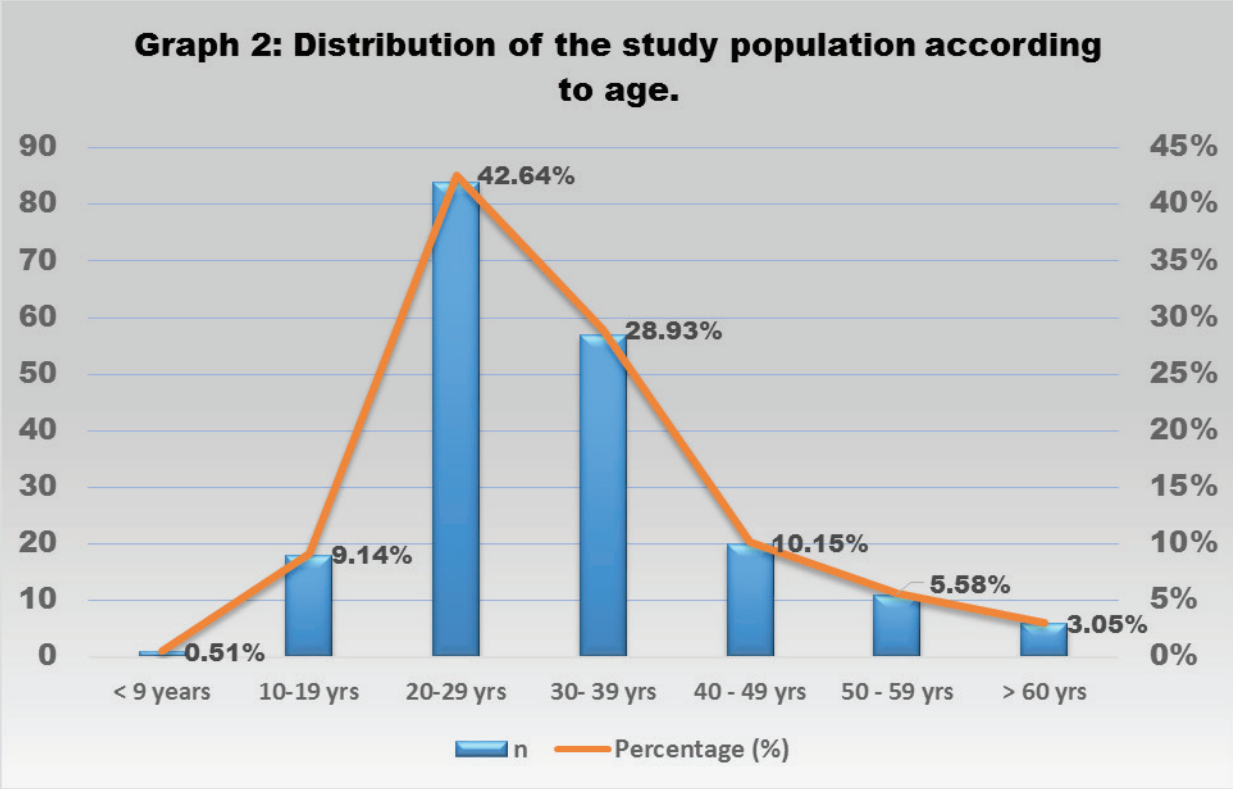
Table 3: Influence of gender on site of fractures:

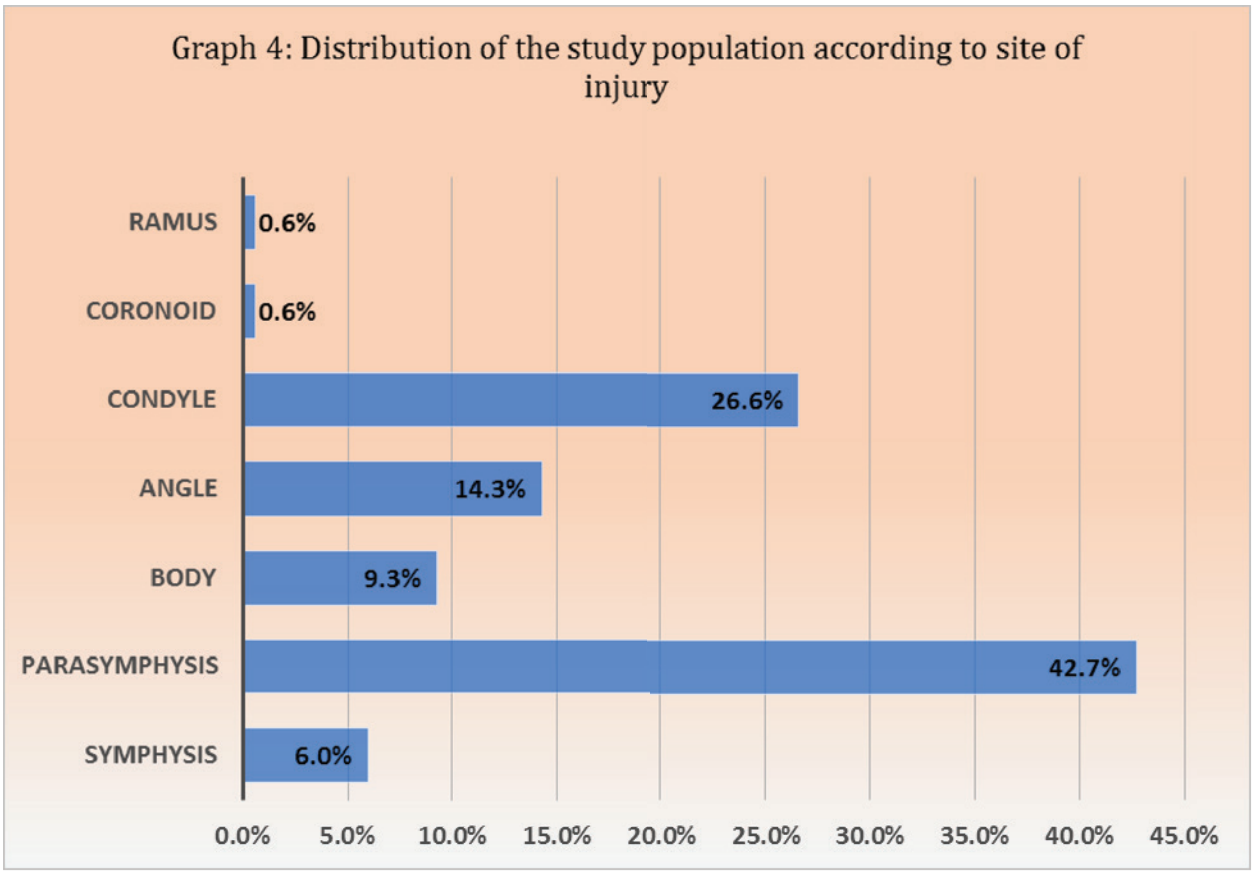
Gender	Site of fracture							Chi-square value	p-value
	Symphysis	Parasymphysis	Body	Angle	Condyle	Ramus	Multiple fractures		
Males	8 (4.5%)	56 (31.5%)	11 (6.2%)	22 (12.4%)	9 (5.1%)	1 (0.6%)	71 (39.7%)	4.933	0.552
Females	0 (0%)	5 (26.4%)	2 (10.5%)	2 (10.5%)	3 (15.8%)	0 (0%)	7 (36.8%)		

Table 4: Influence of age on site of fractures:

Age group	Site of fracture							Chi-square value	p-value
	Symphysis	Parasymphysis	Body	Angle	Condyle	Ramus	Multiple fractures		
Under 9 years	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	80.194	0.000*
10-19 years	0 (0%)	5 (27.8%)	2 (11.1%)	3 (16.7%)	4 (22.2%)	0 (0%)	4 (22.2%)		
20-29 years	2 (2.4%)	27 (32.1%)	4 (4.8%)	11 (13.1%)	3 (3.6%)	0 (0%)	37 (44%)		
30-39 years	2 (3.5%)	17 (29.8%)	5 (8.8%)	5 (8.8%)	5 (8.8%)	0 (0%)	23 (40.4%)		
40-49 years	1 (5%)	7 (35%)	2 (10%)	4 (20%)	0 (0%)	0 (0%)	6 (30%)		
50-59 years	1 (9.1%)	3 (27.3%)	0 (0%)	1 (9.1%)	0 (0%)	0 (0%)	6 (54.5%)		
60 years and above	1 (16.7%)	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	2 (33.3%)		




Graph 2: Distribution of the study population according to age.
90
80
70
60
50
40
30
20
10
0
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
42.64%
28.93%
10.15%
9.14%
5.58%
3.05%
0.51%
< 9 years
10-19 yrs
20-29 yrs
30- 39 yrs
40 - 49 yrs
50 - 59 yrs
> 60 yrs
n
Percentage (%)


Graph 4: Distribution of the study population according to site of injury
RAMUS
0.6%
CORONOID
0.6%
CONDYLE
26.6%
ANGLE
14.3%
BODY
9.3%
PARASYMPHYSIS
42.7%
SYMPHYSIS
6.0%
0.0%
5.0%
10.0%
15.0%
20.0%
25.0%
30.0%
35.0%
40.0%
45.0%

Discussion

Maxillofacial trauma involving fractures of the mandible have been on a steady rise. In the present study young adults (20-29 yrs) were found to be more prone to mandibular injuries. The changing socio-economic scenario makes possession of vehicles more easy and affordable but the younger drivers are usually found over-speeding and violating traffic rules, thus predisposing them to facial traumas. This is accordance with the findings of various other studies [1–3,5,7–14]. But studies by Ogundare et al(2003)[15] and E.T Adebayo et al (2003)[16] found higher prevalence of mandibular fractures in patients aged >30yrs, in Columbia (USA) and Nigeria respectively.

Mandibular fractures had a strong gender predilection with 178 males being affected out of 197 subjects. This is supported by outcomes of most of the studies[2,5,8,11,12,16–19]. Presently in this region, ownership of vehicles is largely skewed with males predominating, thus explaining the observation. Also lady drivers could be deemed as being more cautious and adhering to vehicular rules and regulations in general, thereby reducing the chances of Road Traffic Accidents. Chi square confirmed this difference and it's association with Road Traffic Accidents. But the attention should be drawn to the fact that only few of these studies[1,10,13,15,20] had a difference between the sexes by such a large margin as is seen in our study.

Road traffic accidents were found to be the leading cause of mandibular fractures followed by fall and sports injuries. This was similar to other studies[1–3,5,9,12,21] conducted in the India. These conclusions also matched with studies in Nigeria by E.T. Adebayo et al[16], in Saudi Arabia by Mahmood Samman et al[18], in Bangladesh by F Sultana et al[8], H. E. Al Ahmed et al[10] in UAE, in Iran by Mohammad Hoesin Kalantar Motamedi[13] and Fu-Yu Lin et al[7] in Taiwan probably because these countries also reflect similar socio-economic circumstances. However findings of the studies conducted by Jung Hoon Lee et al[14] in Korea, Ogundare et al[15] in United States, Ricardas K and Tadas Keizeris[20] in Lithuania, Cihan Bereket et al[19] in Turkey, Petrus Pereira et al[11] in Brazil, and Edwards et al[17] in United Kingdom were in clear contrast where, assaults or inter-personal trauma was the leading cause.

The most common site for the mandibular fracture was para-symphysis, followed by condyle. The least involved sites were coronoid and ramus. Studies by Fu-Yu Lin et al[7], Sultana et al[8], Sobodh Natu et al[5], Barade et al[2], Kamath et al[1], Kar et al[3], Vyas et al[21], Subhashraj et al[9] were also in concurrence with this finding. The para-symphysis is anatomically weak due to comparatively longer roots of canines, making them an easy site fractures in RTAs. However, Garkoti et al[12], Mahmood Samman et al[18], Cihan Bereket et al[19], Lee et al[14], Ahmed et al[10] and Motamedi[13] found condyle to be most frequently involved. Ricardas Kubillus and Tadas Keizeris[20] and Ogundare et al[15] found the angle of the mandible to involved while Adebayo et al[16] concluded that commonest site to be body of the mandible.

Conclusion

Road traffic accidents involving the highly impressionable younger age groups were found to be the leading cause of mandibular fractures. Most of which are preventable with stringent traffic regulations and safer traffic measures. A slight oversight in our study was that we did not differentiate if the traffic accidents involved four wheelers or two wheelers and driving while under the influence of alcohol. This, if done would have guided a clearer path in targeting the susceptible group.

Conflicts of Interests: The authors declare, they do not have any conflicts of interests

Funding: None

Ethical Issues: None

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Aggressive Adenomatoid Odontogenic Tumor of Maxilla involving Impacted Third Molar: A Rare Case Report

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Abstract

Adenomatoid odontogenic tumor (AOT) is a rare tumor comprising about 2.2% to 7.1% of all odontogenic tumors. It is a neoplasm of odontogenic origin having slow-growth rate, noninvasive and benign in nature. Here, we report a case of a young female patient who presented with an aggressive AOT in maxilla involving the impacted third molar. Usually, the treatment for AOT comprises of enucleation and curettage, whereas due to the fast growth rate and aggressiveness of the lesion, subtotal maxillectomy was performed in this case. The authors have described about the clinical, radiographic, histopathological and surgical aspect of the case.

Keywords*: Adenomatoid odontogenic tumor, Maxilla, subtotal maxillectomy*

Introduction

Adenomatoid odontogenic tumor (AOT) is a neoplasm of odontogenic origin having slow-growth rate, is noninvasive and benign in nature[1]. AOT usually presents with an impacted tooth and contributes about 2.2% to 7.1% of all odontogenic tumors[2]. It has high female predilection in the age range of 20–30 years. The most common site of occurrence is anterior maxilla, usually associated with the impacted canine.

Stafne was the first to report AOT as a separate entity but the term AOT was not coined by him[3]. Nomenclature of AOT was not possible due to its similar histologic features with other odontogenic tumors until 1971, when WHO adopted the term "adenomatoid odontogenic tumor" proposed by Philipsen and Birn[2,3]. There is a lack of consensus on pathogenesis of the tumor, but according to the developmental theory, some authors believe it to develop from the epithelium of a dentigerous cyst[4].

Here we report a case of AOT of the maxilla. The case presents certain atypical features such as enormous size and aggressive behavior contrary to its smaller size and slow growing nature. The involvement of maxillary third molar is also not so frequently seen associated with AOT, maxillary canine being the favored site. Considering the aggressive nature of the lesion, treatment was done with subtotal maxillectomy under general anesthesia.

Case Report

A 22-year-old female patient reported to the Department of Oral and Maxillofacial Surgery complaining of painless swelling in the left side mid-facial region and upper left posterior teeth region with surface ulceration since last two months. The swelling was insidious in origin, but its rate of growth was very fast. There was deviation of regional teeth and ulceration

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over the swelling due to occlusal trauma from mandibular teeth. Due to difficulty in chewing food and marked extra-oral swelling, she consulted a local physician and was subsequently referred to our institute.

On clinical examination, there was marked mid facial diffuse swelling on the left side of the face with normal-appearing overlying skin. (Fig.1) Intraorally the swelling was evident both on the buccal and palatal aspect with obliteration of vestibular sulcus from maxillary canine to the third molar region. Palatally, the swelling extended up to the mid palatal raphe. There was marked displacement of maxillary molars and premolars with missing maxillary third molar. The swelling on the maxillary tuberosity region was impinging on the mandibular molars resulting in ulceration of the overlying mucosa. (Fig.2) The swelling was soft in consistency indicating cortical bone resorption both buccally and palatally. On aspiration, scant mucoid fluid was noted.

CT scan demonstrated a large cystic expansile radiolucent lesion with corticated borders on the left maxillary region with marked expansion of both buccal and palatal cortices. Left maxillary sinus was completely obliterated by the SOL (Space Occupying Lesion) and approximating to the orbital floor. Orbital floor was slightly elevated anteriorly by the SOL. Displaced maxillary third molar was seen intra-lesionally near to the orbital floor. There was no area of calcification inside the SOL. (Fig.3) Based on these clinical and radiological findings, a provisional diagnosis of malignant neoplasm of connective tissue origin of the left maxilla was made, and an incisional biopsy was done. Histological findings revealed the presence of solid nodules of epithelial cells forming sheets, rosettes, and whorled masses in a scanty connective tissue stroma with a fibrous capsule surrounding the tumor. (Fig.4) Histopathological diagnosis was adenomatoid odontogenic tumor (AOT).

Considering the extensive size and rate of growth of the lesion, a subtotal maxillectomy was planned. With standard Weber–Ferguson incision, sufficient access to the tumor mass was obtained, and a subtotal maxillectomy preserving the infraorbital rim and floor of the orbit was done. (Fig.5) Complete resected specimen measuring 8cmX7cm was sent for histopathological examination. (Fig.6) Biopsy report reconfirmed it to be AOT. The surgical defect was packed with a whitehead varnish-soaked gauge pack. A surgical obturator was given to hold the pack in place. Postoperative course of healing was satisfactory and uneventful. (Fig.7) Patient is under regular follow up with interim obturator prosthesis.

Fig.1 Preoperative View (A) Frontal (B) Profile

Fig.2 Preoperative Intraoral View

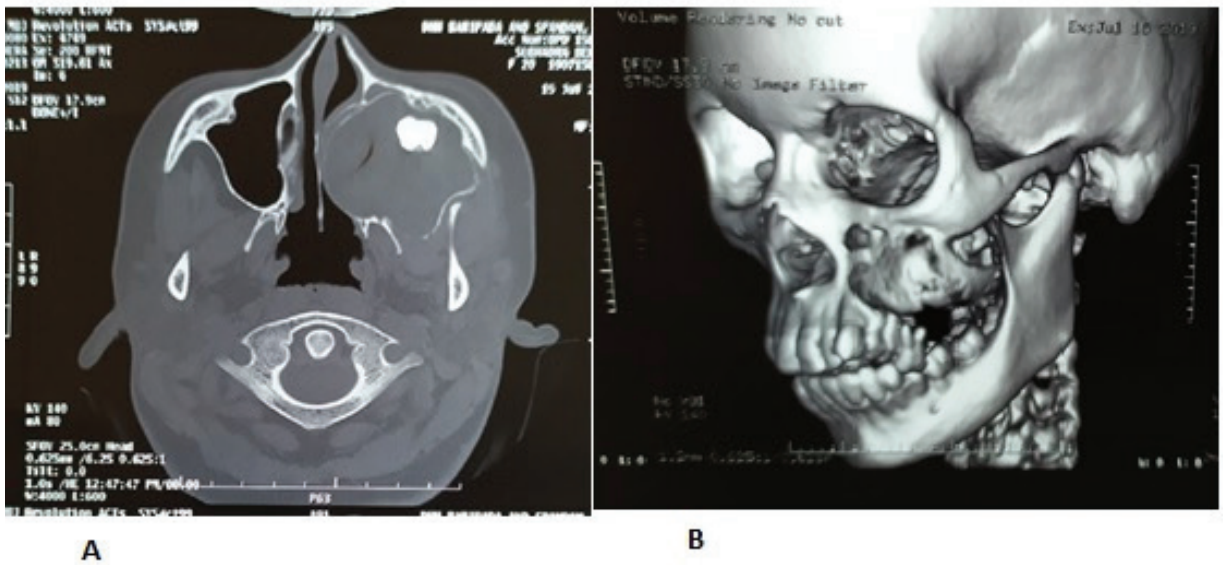

Fig.3 CT Scan showing the enormous size of the tumor. (A) Axial View (B) 3D View

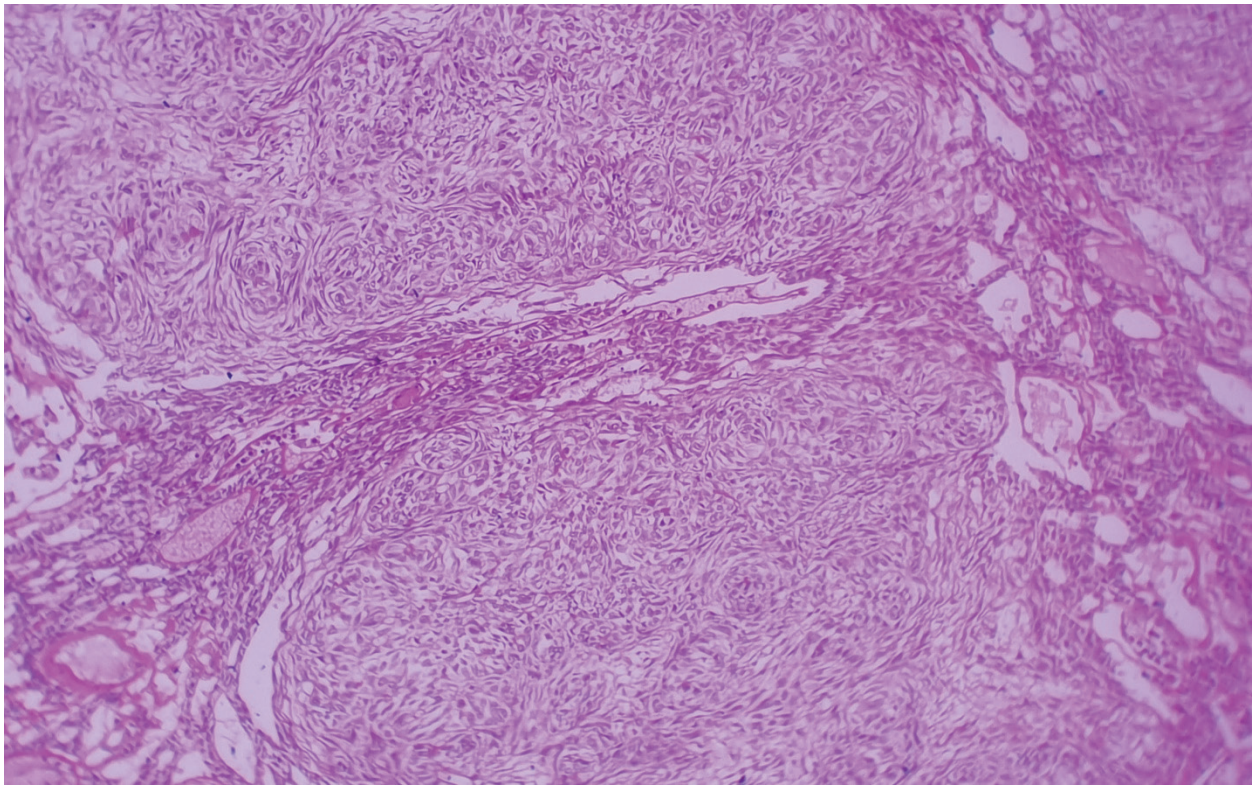

Fig.4 Histopathological photomicrograph.

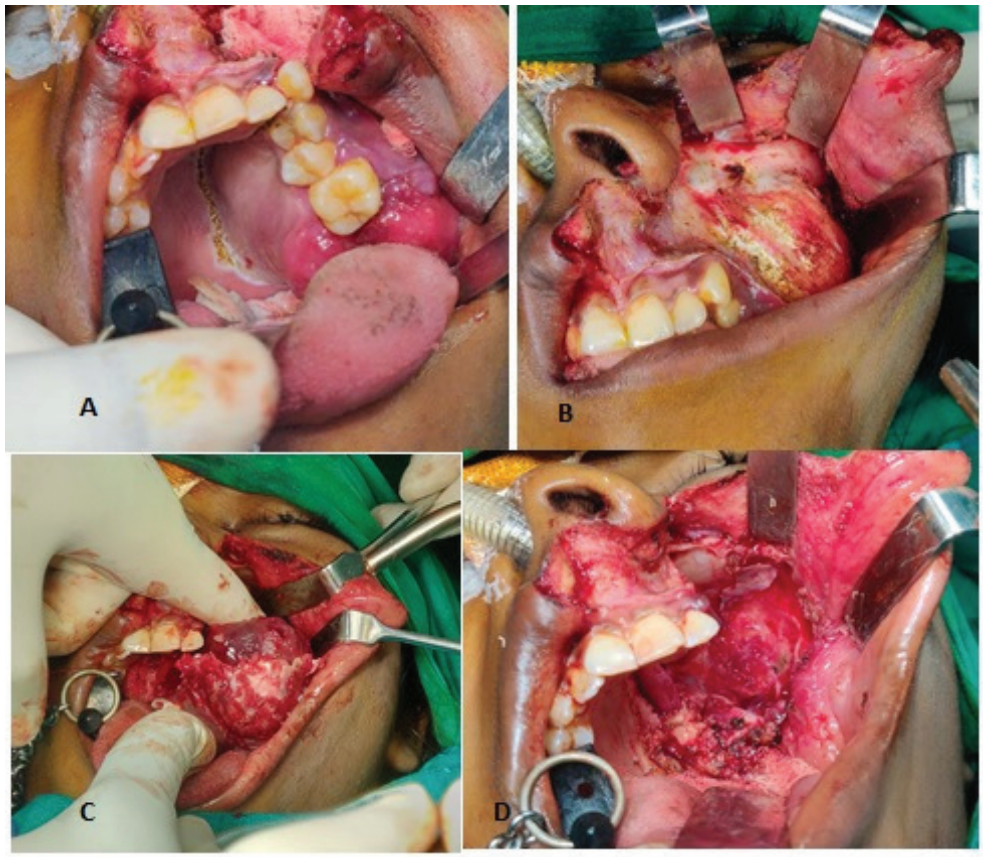

Fig.5 (A-D) Intraoperative photographs

Fig.6 Surgical Specimen after resection.

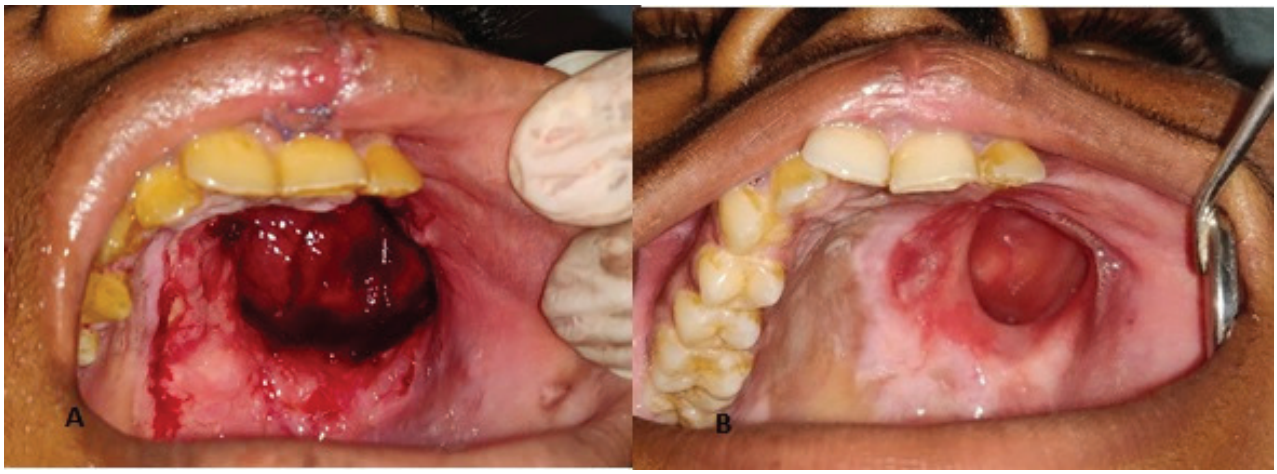

Fig.7 Postoperative intraoral view (A) 7 days postoperative photograph (B) 6-months postoperative photograph showing satisfactory healing.

Discussion

Histologically three different variants of AOT are described. The follicular variant accounts for 73% of AOT cases and is associated with an unerupted tooth. The extrafollicular variant is not associated with an impacted tooth and accounts for approximately 24% of AOT cases. The peripheral variant accounts for 3% of the cases and affects the gingival tissues[5]. In the present case, the lesion was a follicular variant as associated with impacted maxillary 3rd molar. The lesion was very fast growing contrary to its nature and attained a huge size within two months of history as reported by the patient. Due to its fast growth rate and ulceration of the overlying mucosa our first provisional diagnosis was a malignant tumor of connective tissue origin.

Unequivocally AOT is also misdiagnosed as dentigerous cyst on clinical and radiologic examination. Roughly 77% of the follicular variant of AOT is reported to be misdiagnosed as dentigerous cyst[6]. Both AOT and dentigerous cyst present as pericoronal radiolucent area around the impacted tooth. Unlike Dentigerous cyst, AOT is most commonly seen enclosing the entire crown and root of the impacted tooth as it grows from the Hertwig's epithelial root sheath[7].The dentigerous cyst is frequently seen associated with the impacted third molar, enclosing mostly the coronal part of the tooth[8,9]. Due to this difference in presentation, the treatment options in both AOT and dentigerous cyst may vary. In AOT, a marsupialization procedure with the intention to allow the involved tooth to grow to the occlusion is less likely to be successful as there is little or no peri radicular bone available around the root of the involved tooth. In the present case, although the site of the lesion was favorable for dentigerous cyst, an aggressive course of the swelling deferred our provisional diagnosis more towards a malignant growth.

The size of AOT is usually within 1–3 cm in diameter. Rarely in literature, do we get cases of large AOTs[10,11]. The large size of this lesion has been credited to a higher growth potential in younger patients and usually delay in seeking treatment. In the present case the patient was a young female but there was no delay in seeking treatment. This suggests more aggressive nature of the tumor. The treatment of AOT usually consists of enucleation and curettage. However, in our case, the enormous size of this lesion achieved within a short period with cortical perforation and ulceration in overlying oral mucosa suggested the aggressiveness of the neoplasm. Therefore, subtotal maxillectomy was planned and carried out.

Conclusion

The clinical behavior of AOT has been widely studied in the past by many authors and it has been tagged as slow growing benign neoplasm of odontogenic origin, with favored anatomical site, age and gender of the patient. But due to some unknown factors, this seemingly docile tumor may behave aggressively masquerading as a malignant neoplasm. The present literature has very few case reports on aggressive AOTs. The cause of aggressive behavior in some cases of AOT is still to be found out to help the surgeon in formulating an adequate treatment protocol for these cases.

Conflicts of Interests: The authors declare, they do not have any conflicts of interests

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Ethical Issues: None

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Original Research

Myrrh Oil Reduces Gingival Inflammation and Inhibits Gram Negative Dental Plaque Bacteria at Early Stages – A Randomized Control Trial

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Abstract

Aim: To assess the anti-plaque, anti-inflammatory and antimicrobial efficacy of Myrrh oil as an adjunct to scaling and root planing in the treatment of gingival inflammation. **Materials and Methods**: Subjects with moderate to severe gingivitis were recruited for this parallel arm, double blind randomized controlled trial. All subjects underwent SRP at baseline and were randomly divided into Group A (placebo control) and Group B (Myrrh oil). Plaque index (PI), gingival index (GI) and microbiological analysis was carried out at baseline, 48 hrs and 1week interval **Results**: Thirty subjects completed the study. Myrrh oil showed anti-inflammatory and antibacterial efficacy. There were no statistically significant differences between the groups with respect to plaque score at baseline *(p=0.25)*, after 48 hours *(p=0.16)* and after 1 week *(p=0.37)*. There was a significantly lower gingival inflammation *(p=0.02)* recorded in the Myrrh oil group after 48 hrs. A greater reduction in inflammation from baseline at 48hrs was observed, although there was no statistically significant difference in gingival inflammation between the groups after1 week *(p=0.39)*. A significantly greater number of fields were observed with score 1 and lesser number of fields with 2 for gram +ve *(p<0.001)* and gram –ve *(p=0.002)* bacteria in Myrrh Oil group indicating overall lesser gram +ve and Gram –ve bacterial count in comparison to commercially available Myrrh oil. No adverse effects were reported by any subject. **Conclusion**: Myrrh oil when used as an adjunct with scaling and root planning significantly reduced the gingival inflammation in 48 hrs and gram-ve bacteria after 1 week.

***Key words**: myrrh oil; essential oil; anti-inflammatory; antibacterial; gram staining*

Introduction

Gingival and periodontal diseases are caused by bacteria and bacterial products in the dental plaque. [1,2] Dental plaque exists as a biofilm on the surfaces of tooth and mucosal surfaces. [3,4] In addition to mechanical plaque removal by scaling and root planing (SRP), chemical plaque control is often advocated as an adjunct to reduce plaque formation and plaque-associated gingivitis. [5] Although chlorhexidine gluconate has been considered as the gold standard among anti-plaque agents, altered taste sensations, staining of teeth, and development of resistant microorganisms have been observed as its undesirable side effects when used on a long term basis. [6] Similarly, several chemical astringents have been used to reduce gingival inflammation and gingival bleeding. It necessitates the development of alternate anti-plaque and anti-inflammatory agents for prevention of dental plaque and gingivitis. In this endeavour, several plant and animal-based natural products have found use in the treatment of oral ailments[7]; these include Triphala *(Emblica officinalis[8])*, Turmeric *(Curcuma longa)* [9], Basil (*Ocimum tenuiflorum*)[10], Neem (*Azadirachta indica[11]), Miswak (Salvadora persica)[12]* and Honey.

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[13,14]

Myrrh essential oil is an extract of Myrrh gum which is harvested from the species *Commiphora myrrha.* It is a plant extract with medicinal properties with a history that dates back to the birth of Jesus in 6th BC where the three kings who travelled to visit Jesus after his birth, bearing gifts of gold, Frankincense, and myrrh. Myrrh is said to have therapeutic properties since ancient times. Myrrh is has a stimulating action for blood circulation, soothes pain and provides relief from swelling. It helps in the regeneration of skin and accelerates the healing of skin ailments.

Historically, Myrrh tincture has also been used as a mouthrinse in the treatment of oral ulcers and gingival inflammation.[15] It has also been used topically in the treatment of inflammation of the oro-pharynx.[16] There is no recent reported literature or product where Myrrh oil has been clinically used in the treatment of periodontal disease. Therefore, the present study was carried out to assess the anti-plaque, anti-inflammatory and antimicrobial efficacy of Myrrh oil as an adjunct to scaling and root planing in the treatment of gingival inflammation.

Materials and Methods

Study Population and Study design

Patients reporting to the Outpatient Department of Periodontics and Oral Implantology were screened for recruitment in the study. This study was a parallel arm, double-blind, randomized controlled clinical trial. The institutional review board approved the study and written informed consent was taken from the patients before inclusion in the study. Patients fulfilling the following eligibility criteria were selected:

Inclusion criteria

- Aged 18–35 years
- No history of any systemic diseases
- Possessing a minimum of twenty permanent natural teeth with no visible signs of untreated caries
- Moderate to Severe gingivitis (Gingival Index score ≥ 2)[17]

Exclusion criteria

- Patients with a history of use of antibiotics within the last three months
- Pregnant women and lactating mothers
- Medically compromised patients
- Smoking or smokeless tobacco users
- Patients with removable or fixed appliances
- Participants with a known history of allergy to any chemical or herbal products

Randomization, blinding and allocation

Randomization of test and control products was done by a simple coin test method by a blinded examiner (RM) to Group A, and Group B. Group A subjects received Glycerol (Kazima Perfumers, New Delhi, India) as a placebo control while the subjects in Group B received Myrrh oil (Kazima Perfumers, New Delhi, India) respectively.

Procedure

Microbial dental plaque and gingival inflammation were assessed at baseline using plaque index (PI) [18] and gingival index (GI). [17] Supragingival plaque samples were also collected at baseline from the buccal surface of the upper first permanent molar using a curette. Each participant underwent scaling and polishing following the assessment of baseline plaque and gingival inflammation status and collection of plaque samples. Subsequently, the participants were supplied with the allocated placebo/test product dispensed in amber-coloured droppers to mask the colour hue. They were instructed to apply the two drops of the dispensed product twice daily with their finger on the gums for 1 minute and rinse with water after tooth brushing. The technique of application and tooth brushing (Modified Bass [19]) was demonstrated by a trained investigator to all participants. The post-intervention assessment and sample collection was done by the same investigators after 48 hours and after 1 week.

Microbiological Analysis

For microbial analysis, gram staining was used. [20] Collected microbial plaque samples were spread on

sterile microscopic slides and were stained with Gram's stain. Slides were then examined at 100X magnification with a light microscope. Quantitative assessment of the bacteria was done by visualizing five random non-overlapping fields. Scoring was done as per the number of bacteria visible in each field. Fewer than five visible bacteria were assigned a score of 1, 5-10 visible bacteria were scored as 2, 10-20 visible bacteria were scored as three and more than 20 visible bacteria were scored as 4. To compute the number of bacteria, the number of observed fields was multiplied with the score. All clinical recordings and microbial sampling was done by a single investigator (BL) who was blinded to the allocation.

Statistical Analysis

A sample size analysis was estimated using G*Power software version 3.0.10 (Universitat Kiel, Germany) with 5% significance level, 80 % power and effect size of 0.5 estimated the total sample size to be 27. Since there were two groups and it was a parallel arm study 20 subjects were enrolled in each group taking in account the possible 10% drop-outs during follow up. Data was analyzed using Statistical Package for Social Sciences Version 20.0 (SPSS Inc, Chicago Illinois, USA). Collected data were tested for significant differences within the groups and between the groups with the Student's paired and unpaired t-tests. A value of $P \leq 0.05$ was considered statistically significant for all analyses.

Results

A total of 30 (males =14; females 16) subjects aged 24.19 ± 2.63 years completed the study successfully. 5 subjects dropped out in each group owing to non-compliance (Fig 1). None of the subjects reported any adverse effects or any minor side effects due to any of the products during the study period. Table 1 presents the comparison of clinical parameters between Group A and Group B at baseline, 48 hours and after 1 week. There were no statistically significant differences between the groups with respect to plaque score (PI) at baseline *(p=0.25)*, after 48 hours *(p=0.16)* and after one week *(p=0.37)*. Although there was a significant decrease in the plaque scores from baseline to that at 48 hrs, it increased significantly after an initial decrease (Figure 3) as observed after one week. Also, there were no statistically significant differences between the groups with respect to gingival inflammation (GI) at baseline *(p=0.51)*. However, there was a significantly lower gingival inflammation *(p=0.02)* recorded in the Myrrh oil group after 48 hrs.

Further, a greater reduction in inflammation was seen from baseline to that at 48hrs (Figure 4), although there was no statistically significant difference in gingival inflammation between the groups after1 week *(p=0.39)*. The intra-group comparisons (Table 2) revealed that there was a significant reduction in the plaque score and gingival inflammation at 48hrs and 1 week from baseline in both groups. However, there was no statistically significant further reduction in plaque score (*p=0.107*) and gingival inflammation *(p=0.112)* from 48hrs to 1 week.

Comparison of microbial count between Group A and Group B at baseline and 48 hours (Table 3) and one week (Table 4) reveals that there was no statistically significant difference in the number of gram +ve and Gram –ve bacteria between the groups at baseline. However, after 48 hrs, a significantly greater number of fields were observed with score one and lesser number of fields with score 2 for Gram +ve *(p<0.001)* and Gram –ve *(p=0.002)* bacteria in Myrrh Oil group indicating overall lesser gram +ve and Gram –ve bacterial count.

After one week, there was no significant difference between the groups in the Gram +ve bacterial count but showed a significant reduction in the microbial count of Myrrh oil group in the fields with score 4 (more than 20 visible bacteria) *(p=0.042)* and overall count *(p=0.018)* indicating a gradual decrease in the total number of a gram-negative microorganism after one week in the Myrrh oil group.

Table 1. Comparison of plaque score and gingival inflammation scores between Group A (Placebo) and Group B (Test) at baseline, 48hrs and after 1 week.

	Group	N	Mean	SD	95% CI		p value*
					Lower	Upper	
Plaque Score							
Baseline	Group A	15	3.27	0.40	-0.13	0.47	0.25
	Group B	15	3.10	0.39			
after 48 Hrs	Group A	15	1.86	0.15	-0.47	0.08	0.16
	Group B	15	2.05	0.49			
After 1 week	Group A	15	2.41	0.25	-0.09	0.24	0.37
	Group B	15	2.34	0.18			
Gingival Inflammation							
Baseline	Group A	15	1.86	0.31	-0.12	0.16	0.51
	Group B	15	1.94	0.34			
after 48 Hrs	Group A	15	1.48	0.36	0.04	0.56	0.02
	Group B	15	1.18	0.33			
After 1 week	Group A	15	1.03	0.44	-0.71	-0.19	0.39
	Group B	15	0.93	0.33			

*N= Number of subjects; *Unpaired t test; p < 0.05; SD= Standard Deviation; CI=Confidence Interval*

Table 2. Intra-group Comparisons of Plaque Score and Gingival Inflammation for Group A and Group B

		N	Mean	SD	SEM	95% CI		P Value#
						Lower	Upper	
Group A	**Plaque Score**							
	At Baseline	15	3.27	0.40	0.10	3.05	3.49	<0.001
	After 48 Hrs	15	1.86	0.15	0.04	1.77	1.95	
	After 1 Week	15	2.41	0.25	0.07	2.27	2.55	
	F=92.28; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (<0.001)*; 48 hrs-1 week (<0.001)*						
	Gingival Inflammation							
	At Baseline	15	1.86	0.31	0.08	1.69	2.03	<0.001
	After 48 Hrs	15	1.48	0.36	0.09	1.28	1.68	
	After 1 Week	15	1.03	0.44	0.11	0.79	1.27	
	F=18.89; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (=0.021)*; 48 hrs-1 week (=0.005)*						

Cont... **Table 2. Intra-group Comparisons of Plaque Score and Gingival Inflammation for Group A and Group B**

Group B	Plaque Score							
	At Baseline	15	3.10	0.39	0.10	2.89	3.32	<0.001
	After 48 Hrs	15	2.05	0.49	0.13	1.78	2.33	
	After 1 Week	15	2.34	0.18	0.05	2.24	2.44	
	F=92.28; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (<0.001)*; 48 hrs-1 week (=0.107)*						
	Gingival Inflammation							
	At Baseline	15	1.18	0.33	0.09	1.00	1.36	<0.001
	After 48 Hrs	15	0.93	0.33	0.08	0.75	1.11	
	After 1 Week	15	1.35	0.54	0.08	1.19	1.51	
	F=18.89; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (<0.001)*; 48 hrs-1 week (=0.112)*						

*N= Number of subjects; SD = Standard Deviation; SEM=Standard Error of Mean; # One-Way ANOVA Test; * Tukey's Test for multiple comparison; p < 0.05; SD= Standard Deviation; CI=Confidence Interval*

Table 3. Comparison of bacterial count between Group A (Placebo) and Group B (Test) at Baseline and after 48 hrs.

			At Baseline			After 48 Hrs		
Parameter	**Group**	**N**	**Mean**	**SD**	**p value***	**Mean**	**SD**	**p value**
Gram+ve (<5)	Group A	15	0.53	0.64	0.793	1.73	1.33	<0.001
	Group B	15	0.6	0.74		3.27	0.59	
Gram +ve (5-10)	Group A	15	1.73	1.67	0.432	4.67	2.69	<0.001
	Group B	15	2.27	1.98		1.73	1.03	
Gram +ve (10 - 20)	Group A	15	5.6	2.97	0.866	1.6	2.5	0.793
	Group B	15	5.4	3.44		1.8	1.52	
Gram +ve (>20)	Group A	15	7.2	4.59	0.473	1.6	2.95	0.556
	Group B	15	5.87	5.42		1.07	1.83	
Gram +ve Total	Group A	15	15.07	2.91	0.407	9.6	2.97	0.046
	Group B	15	14.13	3.16		7.87	1.25	
Gram –ve (<5)	Group A	15	0.67	0.72	0.63	1.53	1.25	0.01
	Group B	15	0.8	0.77		2.67	0.98	
Gram -ve (5-10)	Group A	15	2.13	1.6	0.201	4.67	3.18	0.002
	Group B	15	3.07	2.25		1.47	1.92	
Gram -ve (10-20)	Group A	15	4	2.45	0.457	1.4	1.92	0.148
	Group B	15	3.2	3.3		2.8	3.1	
Gram -ve (>20)	Group A	15	7.73	6.32	0.564	2.93	2.81	0.765
	Group B	15	6.4	6.2		2.67	1.95	
Gram -ve Total	Group A	15	14.53	3.11	0.386	10.53	1.92	0.135
	Group B	15	13.47	3.5		9.6	1.35	

*N= Number of subjects; *Unpaired t test; p < 0.05; SD= Standard Deviation; CI=Confidence Interval*

Table 4. Comparison of bacterial count between Group A (Placebo) and Group B (Test) 1 Week

Parameter	Group	N	Mean	Std. Deviation	95% CI		p value*
					Lower	Upper	
Gram+ve(<5)	Group A	15	0.73	0.88	-1.41	-0.19	0.012
	Group B	15	1.53	0.74			
Gram +ve (5-10)	Group A	15	2.40	1.88	-0.94	2.01	0.466
	Group B	15	1.87	2.07			
Gram +ve (10 - 20)	Group A	15	2.80	2.40	-1.63	2.03	0.825
	Group B	15	2.60	2.50			
Gram +ve (>20)	Group A	15	8.53	3.96	-1.93	5.66	0.322
	Group B	15	6.67	5.98			
Gram +ve Total	Group A	15	14.47	3.02	-0.65	4.25	0.144
	Group B	15	12.67	3.52			
Gram -ve(<5)	Group A	15	0.93	1.03	-1.25	0.31	0.231
	Group B	15	1.40	1.06			
Gram -ve (5-10)	Group A	15	1.60	1.72	-2.64	0.24	0.099
	Group B	15	2.80	2.11			
Gram -ve (10-20)	Group A	15	5.20	3.67	-0.99	3.79	0.241
	Group B	15	3.80	2.65			
Gram -ve (>20)	Group A	15	6.13	3.34	0.09	4.71	0.042
	Group B	15	3.73	2.81			
Gram -ve Total	Group A	15	13.87	2.59	0.39	3.88	0.018
	Group B	15	11.73	2.05			

*N= Number of subjects; *Unpaired t test; $p < 0.05$; SD= Standard Deviation; CI=Confidence Interval*

Figure 1. CONSORT Workflow Diagram

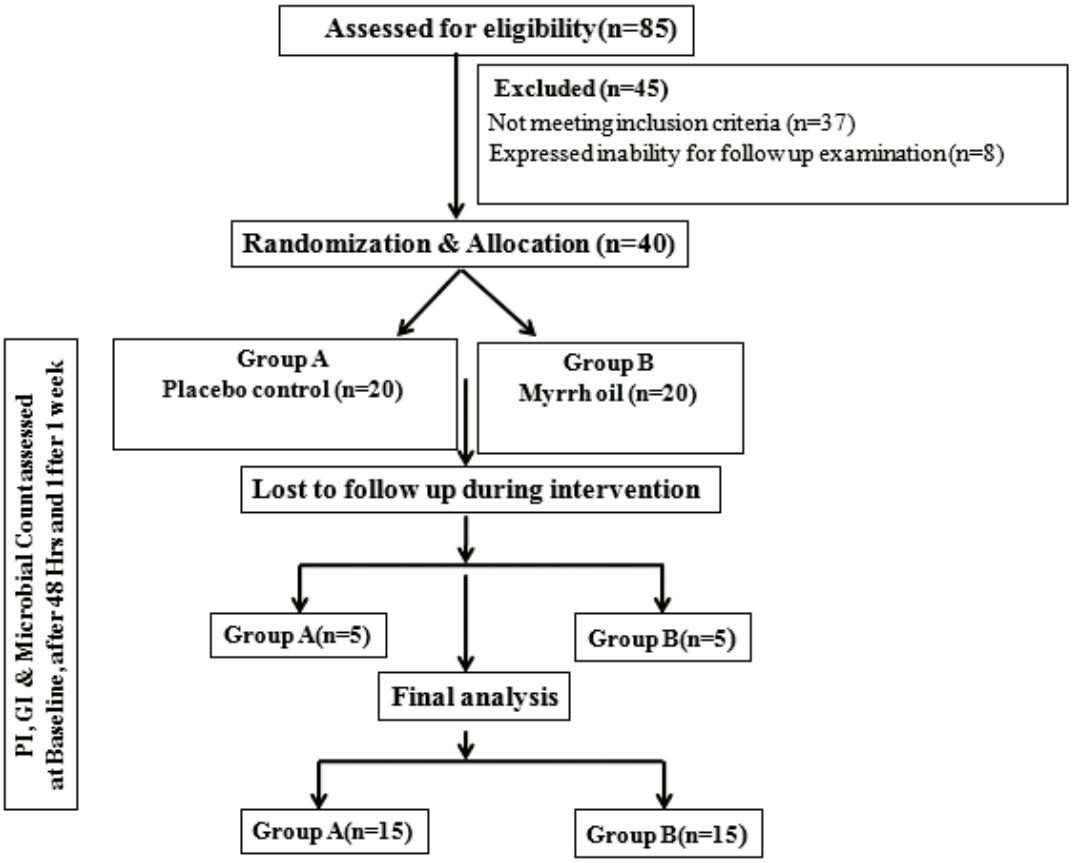
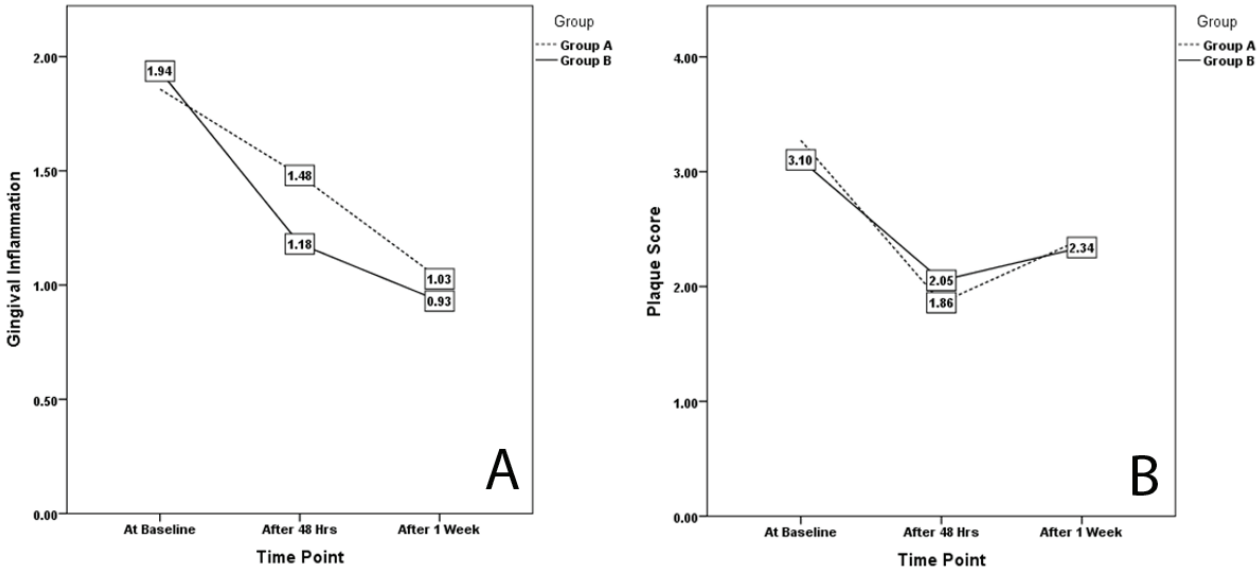


Figure 2. Gingival Inflammation Score (A) and Plaque score (B) of Group A (Placebo) and Group B (Test) at baseline, 48hrs and after 1 week.



Discussion

The microbial dental plaque remains to be the primary agent in causing inflammatory periodontal disease. [21,22] Although mechanical plaque inhibition continues to be the mainstay in the prevention of periodontal disease, several plaque inhibiting products have been regularly used as an adjunct.

The present study assessed the anti-plaque, anti-inflammatory and antimicrobial efficacy of Myrrh oil for seven days. While there was no significant difference in their anti-plaque efficacy over a week, there was a significant reduction in the gingival inflammation after one week in Myrrh oil group. There, however, was an increase in the plaque level after 1 week after it initially decreased after 48 hours.

We found a significant antibacterial effect of Myrrh oil, a plant extract leading to reduction of bacterial count (Gram –ve microorganisms) 7 days after the intervention compared to baseline levels. This finding was in accordance with a study by Sukhabogi et al. [23] who compared three different herbal products with a chlorhexidine gel and found to be effective in reducing plaque and gingival scores. Al-Mobeeriek et al. [24] in an animal study reported that myrrh suspension promotes healing and repair of damaged tissue in comparison to Chlorhexidine and Tetracycline. Similarly, Gupta et al. [25] in their *in vitro* study also demonstrated the antimicrobial efficacy of aqueous and ethanolic extracts of Triphala on primary plaque colonizers. Chandrashekar *et al.* [26] in their *in vitro* studies also demonstrated the antibacterial efficacy of eucalyptus plant extracts on plaque microorganisms. Plant-based medicines are adjunct to mechanical plaque control methods and are being established as an alternative to chlorhexidine. Absence of adverse effects by plant products when used over a long period of indicates their safety when used as an anti-plaque, anti-inflammatory or antimicrobial oral formulation. Prakash and Shelke et al. [8] have shown that Triphala is efficacious in reducing microbial plaque and controlling gingivitis. Herbal dentifrice formulations containing *Cinnamomum camphora* and menthol have been found to reduce plaque in patients with gingival inflammation. [27]

Myrrh (*Commiphora myrrha*) oil contains a-pinene, cadinene, limonene, cumin aldehyde, eugenol, m-cresol, heerabolene, acetic acid, formic acid and other sesquiterpenes and acids.[28] The antibacterial and anti-gingivitis effect is mainly attributed to these ingredients.[29] Myrrh has been reported to be highly effective in the treatment of inflammatory diseases such as Rheumatoid arthritis. In an in-vivo study by Su et al. [30] revealed a reduction in elevated expression levels of TNFα, PGE_2, IL-2 and Nitric Oxide in serum after treatment with Myrrh and Frankincense. The previous study by Ljaljević Grbić et al. [31] reported antibacterial, antifungal properties of Myrrh oil. Mohamed et al. [32] demonstrated the susceptibility of Gram-positive and Gram-negative bacteria Myrrh oil (MIC 2–5 µL /mL to 100 –1000 µL /mL).

Conclusions

This was first of its kind study to assess the anti-plaque, anti-inflammatory and the antibacterial inflammation of Myrrh oil on its topical intraoral application. Although gram staining tells us about the presence of gram-positive and the Gram negative bacteria, it is a weak indication of antibacterial activity as compared to culture studies and polymerized chain reaction. Also, in such kind of studies, Hawthorne effect may also act as a bias as it influences the performance of the participating subjects. Within the limitations of the study, it can be concluded that Myrrh oil is an efficacious anti-inflammatory and antibacterial oral formulation when used as an adjunct to scaling and root planning in the treatment of gingivitis.

Ethical Permission: Approved

Conflicts of Interests: None

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Ulcer in the Disguise-A Rare Case Report

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Abstract

Tuberculosis (Tb) is an infectious disease which usually affects the pulmonary system and sometimes occurs in other body organs including the oral cavity. The present article is on a case study of a 25-year female diagnosed with TB osteomyelitis which presented a non-healing and non-painful ulcer both intra and extra orally. The diagnosis was confirmed based on histopathology. The patient underwent anti-TB therapy and her oral and systemic conditions improved rapidly. Although oral manifestations of tuberculosis are rare, clinicians should include them in the differential diagnosis of various types of oral ulcers. An early diagnosis with prompt treatment can prevent complications and potential contaminations. Tuberculosis of the oral cavity is rare and have become a forgotten diagnosis of oral lesions. Dental practitioners need to be aware that TB may occur in the oral cavity and should be considered in the differential diagnosis of any ulcerated, indurated non-healing lesion of the oral cavity, especially in lower socioeconomic groups. Besides, efforts should be made to control oral TB by early detection and referral of the patient to a physician for proper management. Clinicians are to be careful about ulcers in disguise.

Keywords: *Tuberculosis, ulcer, Langhans cells, epithelioid cells*

Introduction

Tuberculosis (TB) is a transmissible chronic granulomatous disease caused by Mycobacterium tuberculosis.[1] India alone accounts for nearly one-fifth of the global burden of tuberculosis.[2]The incidence of TB in underdeveloped countries is increasing. Depending on the organ system involved, tuberculosis is classified clinically as pulmonary and extra-pulmonary. Pulmonary tuberculosis remains the most common form of the disease. Extra-pulmonary involvement in tuberculosis is uncommon, accounting for approximately 10% to 15% of all the patients.[7] TB mainly affects the lungs but also affects intestine, meninges, bones, joints, lymph glands, skin and other tissues of the body.[3] Oral tuberculosis lesions are rare and it is estimated that only 0.05- 5% of total tuberculosis cases may be presented with oral manifestations.[4] This article aims to report a case of primary tuberculosis and to emphasize the importance of early diagnosis with various diagnostic tests to lessen the risk of exposure to an infected patient's contact.

Case Report

A 25-year-old female reported to the department of oral medicine with a chief complaint of ulcer in the right cheek as well as in right buccal mucosa for two months. On examination, there was an ulcer extra orally in the right cheek (Fig 1a&b) measuring about 4x3 cm. Intraorally there was a non-healing ulcer concerning carious 46 teeth in the buccal mucosa of size 2x1.5 cm (Fig 2). It was painless mild tender on palpation. A single lymph node was palpable and enlarged. An intraoral radiograph revealed radiolucency concerning 46. OPG revealed severe bone loss. (Fig 3a&3b). Her detailed medical history revealed that she was diagnosed for TB and had weight loss and was under medication 6 months back. There was no other abnormality elsewhere in the oral cavity. Based upon the clinical examination, a differential diagnosis included aphthous ulcer, traumatic ulcer, infections (bacterial, fungal and viral), drug reaction and malignancy, including primary squamous cell carcinoma and lymphoma. Since there was no history of any kind of trauma and the ulcers were

chronic, painless and non-recurrent, the possibility of traumatic or aphthous ulcers was ruled out. Moreover, the patient was not on any systemic medication; thus the possibility of an ulcer due to drug reaction was also ruled out. An incisional biopsy is done. Histopathologic examination revealed an ulcerated stratified squamous surface epithelium in association with fibrovascular connective tissue. The connective tissue exhibited granulomatous inflammation containing epithelioid cells, Langhans giant cells and lymphocytic infiltrate with areas of necrosis. (Fig 4a&4b).

This raised the possibility of granulomatous infection, including tuberculosis. Manteaux test was positive. The blood test was within normal limits.ESR was raised. Based on the clinical, radiological and Histopathological findings the case was diagnosed as TB osteomyelitis.

Figure 1a. Extraoral Examination

Fig1b Extraoral Examination

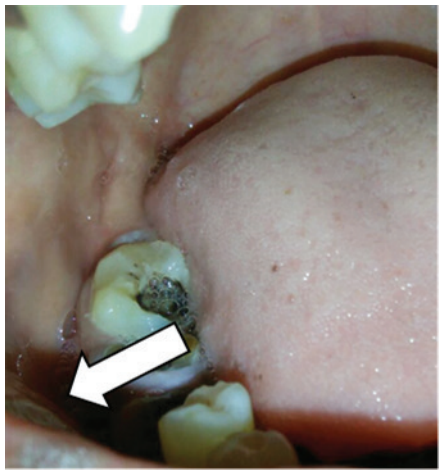

Fig 2. Intraoral examination

Fig 3a OPG examination

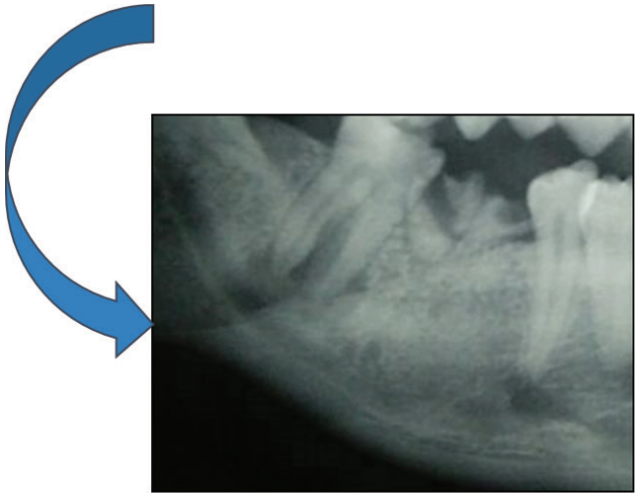

Fig 3b OPG revealed a severe bone loss

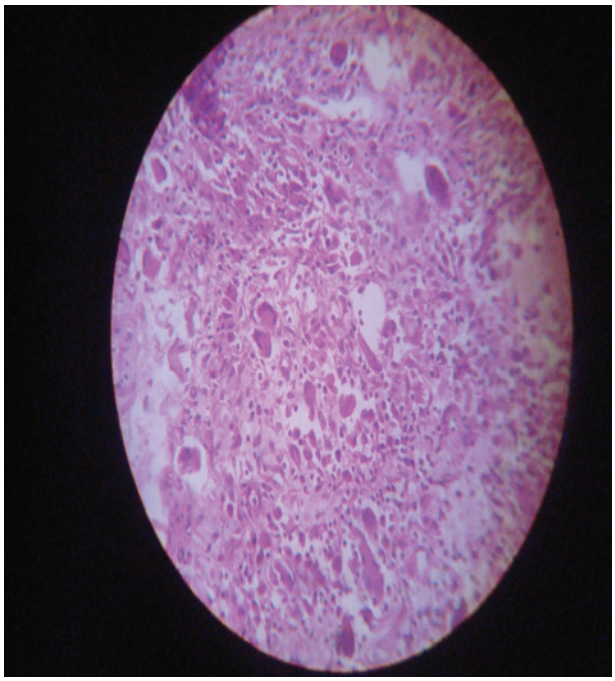

Fig 4a (Langhans cells)

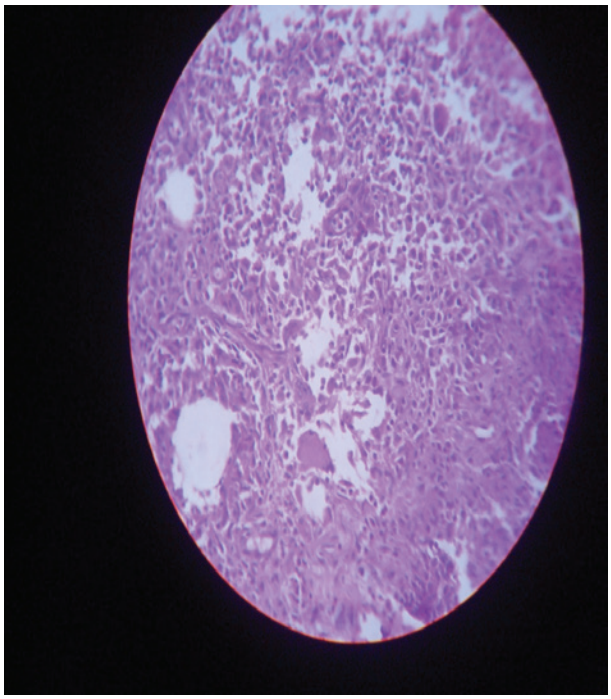

Fig 4b(Epitheloid cells)

Discussion

Although the manifestation of TB has a rare occurrence nowadays it seems to be more chronic as a consequence of the outbreak and emergence of drug resistance TB(MDR &XDR).Global Burden: WHO(2013):TB incidence :8.6million rel death:1.3million.

Oral TB lesions Are of two types primary and secondary. Primary ulcers are uncommon, seen more in young patients, presents itself as a single painless ulcer with an enlarged lymph node. A secondary ulcer is more commonly seen in middle-aged and elderly persons, presents as a single painful ulcer with an irregular border, indurated and associated with pulmonary diseases. The various forms of TB ulcer are ulcer, nodule, Tuberculoma, TB Granuloma and TB osteomyelitis. Clinically difficult to differentiate oral TB from Primary Syphilis, Deep fungal disease and Squamous cell Carcinoma so Biopsy is confirmatory.[5]

Figure 5. Diagnosis of TB

Conclusion

Tuberculosis of the oral cavity is rare and have become a forgotten diagnosis of oral lesions. Dental practitioners need to be aware that TB may occur in the oral cavity and should be considered in the differential diagnosis of any ulcerated, indurated non-healing lesion of the oral cavity, especially in lower socioeconomic groups. Also, efforts should be made to control oral TB by early detection and referral of the patient to a physician for proper management. Clinicians are to be

careful about ulcers in disguise.

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Oral Pyogenic granuloma: Retrospective Review of 49 Cases in a Tertiary Care Dental Institution of Odisha

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Abstract

Pyogenic granuloma (PG) also known as "Lobular capillary hemangioma," appears as an overgrowth of tissue due to irritation, physical trauma, or hormonal changes in the oral cavity. this present study is on a series of cases comprising of 49 cases of pyogenic granuloma is the first of its kind reported from Eastern India. Even though patients usually do not report the hospital for pyogenic granuloma unless the lesion is large and incapacitating, such number in four years is not very less. Therefore it may be deduced that pyogenic granuloma is not rare in this part of India.

Keywords: *Pyogenic Granuloma, hemangioma, gingival, Buccal mucosa, palate*

Introduction

Pyogenic granuloma (PG) also known as "Lobular capillary hemangioma," [1] appears as an overgrowth of tissue due to irritation, physical trauma, or hormonal changes in the oral cavity.[2]It was first reported in the English literature by Hullihen in 1844.[3]The term Pyogenic granuloma was termed by Hartzell in 1904. [4] The name is ambiguous because it is neither a granuloma nor its origin is infectious, it is a capillary hemangioma of the lobular subtype, are often prone to bleeding. It is not truly "pyogenic". Frequently seen in young females usually in the second decade of life, due to various to hormonal changes. Clinically appears to be reddish pink to purple depending on the vascularity, with smooth or lobulated surface, pedunculated or sessile mass with a broad base. Painful when associated with continuous physical trauma. These lesions often bleed profusely with little trauma. The most common site is the gingiva (approximately 75%) followed by lips, tongue, buccal mucosa, palate, and floor of the mouth. [5] Histopathologically, PG is classified into lobular capillary hemangioma (LCH) &and non-lobular capillary hemangioma (non-LCH) [6]. Confirmatory diagnosis is done only after the study of histopathological sections.

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Methodology

Patient records of Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Siksha 'O' Anusandhan Deemed to be University were retrospectively reviewed from December 2015 to December 2019 for identification of patients diagnosed with Pyogenic granuloma. Clinical and histopathological features of those cases were identified and tabulated. The frequency of each feature was observed. Clinical features like age, gender, site, and clinical size were recorded. Histological types of pyogenic granuloma were categorized as lobular and non-lobular capillary hemangioma.

Result

Upon reviewing the patient record, 49 cases were

identified. The mean age of the study population was found to be 34 with male to female ratio as 11:38. Gingiva was found to be the predominant location (n=39/49) followed by buccal mucosa (n=4/49), tongue(n=3/49) and palate(n=3/49). The median size of the lesion was found to be 1.5cm with a minimum of 0.5cm to a maximum of 8cm. histological two types were identified, lobular (n=4) and non-lobular (45) capillary hemangioma. Results have been tabulated in table number 1.

Table 1.

Characteristics	Number of cases (%)
MEAN AGE	34
GENDER	
Male	11
Female	38
SITE (n=851)	
Gingiva	39
Buccal mucosa	4
Tongue	3
palate	3
TUMOUR SIZE (in cm)	
Median	1.5
Range	0.5-8
HISTOPATHOLOGY	
Lobular	4
Non-lobular	45

Figure 1. Pyogenic granuloma in buccal mucosa

Figure 2. Pyogenic granuloma in gingival

Figure 3. Pyogenic Granuloma in the palate

Discussion

The present series comprising of 49 cases of pyogenic granuloma is the first of its kind reported from Eastern India. Even though patients usually do not report the hospital for pyogenic granuloma unless the lesion is large and incapacitating, such number in four years is not very less. Therefore it may be deduced that pyogenic granuloma is not rare in this part of India.

Pyogenic granuloma has been considered as a reactive lesion arising as a response to various stimuli such as chronic low-grade irritation [7,8], trauma [7,8], hormones [9], and few drugs[10]. Although the lesion does not manifest pus formation and does not suffice granuloma definition

[7,8] still the misnomer terminology 'Pyogenic granuloma' is prevailing. Trauma plays a role, especially in extra gingival PG. poor oral hygiene is also reported to be an etiologic agent. [7,8]

Mean as well as the median age of the study population was found to be 34 years with a female predominance. Female prevalence has also been observed by [11-13]. Unusually high female prevalence in

this study may be due to the age of presentation of PG during puberty or pregnancy. The principal oral site to be affected was found to be gingiva. This is invariably reported by every author. [11, 12, 14-17]. Mandibular gingiva was found to be a more frequent location compared to maxillary gingiva {21/37}. This finding is contradicted by with few authors. [11-12]Uncommon locations are palate, lip, tongue, and buccal mucosa.[7,8,18]Cases in our study cohort also demonstrated an unusual location like buccal mucosa (n=4), tongue (n=4) and palate (n=4). Clinically it is manifested as a lobulated exophytic erythematous lesion. It may be pedunculated or sessile. Size usually does not exceed 2.5 cm [8] whereas the maximum size in our study cohort was 8cm. eight cases were above 3 cm. The larger size may be attributed to late presentation. The development is usually slow and painless. The surface of the lesion is characteristically ulcerated and friable covered by a fibrinous membrane.

Microscopically PGs can demonstrate two types of arrangement, lobular and non-lobular capillary hemangioma. 45 out of 49 in this case series were non-lobular capillary hemangioma. Numerous small and large blood vessels are seen in lobular or diffuse arrangements in lobular and non- lobular capillary hemangioma respectively. Those blood vessels are lined by mitotically active plump endothelial cells or flat endothelial cells. [19] Attributed to a few structural and immunohistochemically differences Toida et.al [13] have suggested different pathogenic factors for lobular and non-lobular capillary hemangioma.

Differential diagnosis of PG includes benign lesions like peripheral ossifying fibroma, peripheral giant cell granuloma. [8]Malignant neoplasm like a metastatic tumour, Non-Hodgkin's lymphoma, angiosarcoma and Kaposi's sarcoma [11, 20] also needs to be differentially diagnosed.

Adequate follow-up data is not available in this study population. Adequate excision is the usual treatment of choice. Recurrent lesions may be the result of inadequate excision or employing non-surgical techniques for treating PG. a further well-planned retrospective study on immunohistochemical features of this lesion would throw light upon many unanswered questions regarding Etiopathogenesis and nature of vascular proliferations.

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Conflict of Interest: The author declares that there was no conflict of interest.

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"Antifungal Stewardship" A Pertinent Exigency in Mycobial Treatment

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Abstract

"Stewardship" is an ethic that exemplifies the conscientious, and sustainable forethought with the management of resources. *Antifungal stewardship"* is evidence-based interventions for judicious antifungal usage in healthcare setup, to get the best clinical outcomes and minimize adverse events and selective exhaustive pressures. This review briefly catalogues the seven core elements and supplementary recommendations by Mycoses Study Group Education and Research Consortium. Appraisal of AFS requires a set of metrics which have been categorized into quantitative metrics, quality metrics, patient outcome and microbiological metrics and performance metrics or outcome measures. The four domains about barriers concerning antimicrobial stewardship programs which include "outer setting", "inner setting", "individuals involved" and "the intervention", have also been elucidated. At the present scenario, fungal infections are increasing especially in immunocompromised and cancer patients thus financial strain imposed by these on health care is expected to escalate over time as the populace of individuals at risk augment. AFS comes to the rescue to promote a wholesome utilization of currently available antifungal drugs to stem the tide of the emergence of resistance and morbidity of invasive disease.

Keywords: *Antifungal Stewardship; Mycobial Treatment; Antifungal Resistance.*

Introduction

"Stewardship" is an ethic that exemplifies the conscientious, righteous and sustainable forethought with the management of resources.[1] *"Antimicrobial stewardship"* refers to a rational set of measures with coordinated interventions which sponsor the accountable use of antimicrobials (means against bacteria, fungal, virals, and parasitic infections) which encompasses actions at the individual, national and global level, and across human health, animal health and the environment.[2,3] "*Antifungal stewardship"* is evidence-based interventions for monitoring and directing judicious antifungal usage in healthcare setup, to achieve the best clinical outcomes and minimize adverse events and selective exhaustive pressures.[4] The ideologies of antifungal stewardship are comparable to those of established antimicrobial stewardship programs under which resource (antifungal) optimization is executed based on the spectrum of activity, drug pharmacokinetics-pharmacodynamics, duration, route of administration, and multidisciplinary partnership, judicious planning, leadership support, and financial sustenance.[5] This review is a précis on the underlying principles and motivations supporting the development of antifungal stewardship, their contrasts against

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antibacterial stewardship agendas, core elements and recommendations governing the implementation of antifungal stewardship programs, evaluation metrics and barriers and pitfalls in their achievement.

The rationale behind the realization and performance of antifungal stewardship stratagem

At the outset, principles governing the modus operandi of antimicrobial pharmaceuticals designed to combat noninfectious diseases is highly contrasting to the mechanisms and challenges met by drugs administered for infectious diseases: the implicated agent in the later possesses an intrinsic or subsequently acquired aptitude to acclimatize to the hostile milieu, which includes the host's defence or the administered antimicrobial themselves. Like all microorganisms, mycobes (fungi) can develop resistance on exposure to antimycobials (antifungals), which is retained in a given population by swift multiplication cycles or is transferred to different microorganisms through conjugation or by plasmid sharing or through chromosomes itself.[6] The materialization of antifungal resistance has sweeping and grave penalty on the safety, quality, and economic effects in healthcare setup.[7] Consequently, unsuitable antifungal usage has led to an increase in antifungal resistance all over the world.[8] Moreover, overuse of antifungals precipitates higher toxicity owing to overexposure to concerning drugs.[9] Sustainable and equitable use of antifungals is another justification as roughly 481 million of the world population has no access to amphotericin B and 5 fluocitocyne.[10] The diversity of fungal infections, invasive infections, multidrug resistance and breakthrough fungal infections continue to rise paralleling the increased use of immunosuppressives and invasive devices, with un-reprimanded antifungal consumption, posing exhausting demand on the antifungal reservoir. These are associated with high mortality, morbidity, direct costs and poor outcome.[11] Under-recognition of invasiveness of a fungal disease, diagnostic delays owing to the time-consuming work with traditional fungal culture, add to lengthened empiric and prophylactic therapy, contributing to 42–66% of antifungal use.[12] Furthermore, when prescribing the drug is very sensitive part as numerous fungi targeting drugs have intricate pharmacokinetics requiring customized dosing regimens in the perspective of drug-drug interactions and patient comorbidities.[13,14]

Antibacterial versus antifungal stewardship- a juxtaposition

Although antifungal stewardship takes after the principles and norms of better and earlier established antibacterial stewardship, nevertheless there occur contrasts between the two schools. While antibacterial stewardship relies upon CRP, procalcitonin and culture (earlier) for diagnosis whereas antifungal stewardship is reliant on culture, galactomannan, beta-D-glucan and computerized tomography for the same. For antibacterial stewardship, resistance reports are set, prophylactic measures are defined, tailored treatment is sufficiently developed, while for antifungal stewardship these parameters, in order, are improving, not clearly defined and not as well developed. Also, as regards consensus in treatment, antibacterial stewardship programs stand out under being better while antifungal stewardship needs to be improved.[15] Additional differences include the incorporation of antifungal stewardship higher up in the hierarchy of health care system (secondary or tertiary care hospitals), and fewer medical specialities but antibacterial stewardship is more global in implementation. Furthermore, antifungal treatments and prophylaxis are protracted with intricate pharmacokinetics against antibacterial treatments.[16]

Core Recommendations for Antifungal Stewardship

Core elements are standard principles of stewardship programs whose realization and execution is reliant on capital, proficiency and subsistence at the national and global level. The following tabular description briefly catalogues the core elements and supplementary recommendations by Mycoses Study Group Education and Research Consortium[17], to combat respective challenges.

Table 1. Core Recommendations for Antifungal Stewardship

CORE ELEMENT	RECOMMENDATION
1. ENGAGEMENT OF SENIOR MANAGEMENT LEADERSHIP TOWARD ANTIMICROBIAL STEWARDSHIP	Antifungal stewardship aspirations should be incorporated into tactical hospital plans and guidelines with senior leadership engagement, answerability, and committed resources.
2. ACCOUNTABILITY AND RESPONSIBILITIES	Facilitation of ongoing collaborative strategies with core members of the stewardship team having profound comprehension and clinical skill in the management of invasive fungal disease approaches to revamp antifungal dosing and duration and managing drug interactions, antifungal toxicities, formulation of surveillance strategies.
3. AVAILABLE EXPERTISE ON INFECTION MANAGEMENT	Centres that habitually manage invasive fungal disease should have access to timely conventional and non-culture-based diagnostic testing for Candida and Aspergillus species.
4. EDUCATION AND PRACTICAL TRAINING	Development of targeted educational programs to take in hand information gaps in the analysis of laboratory results, discrimination of colonization and infection, indications for prophylaxis or empiric therapy, and antifungal therapy dosing and monitoring.
5. OTHER ACTIONS AIMING AT RESPONSIBLE ANTIMICROBIAL USE	Whenever achievable, infectious diseases consultation should be performed for patients with invasive fungal diseases. Treatment bundles should be launched to improve the prospects of timely delivery of interventions, introduced sequentially, guided by data-driven strategies to capitalize on patient outcomes and provider education. Ongoing interventions strategies such as “handshake stewardship rounds” or post prescription review and feedback are an indispensable element.
6. MONITORING AND SURVEILLANCE	Establishment of local surveillance systems with programs having access to antifungal susceptibility testing to develop snowballing antifungal susceptibility information and therapeutic drug monitoring for triazoles; Facilitation of “real-time” interventions by steady communication of results of rational diagnostic testing (culture and non-culture-based); patient record screening by a clinical pharmacist to assess drug-related parameters and potential interactions.
7. REPORTING AND FEEDBACK	All facilities should have the machinery to track antifungal usage, promote benchmarking, and assess patient-level outcomes and straight data feedback to prescribers.

Metrics for appraisal of antifungal stewardship programs

The periodic assessment, modification and implementation of antifungal stewardship programs require a set of measurement tools for comprehensive appraisal and to present a conduct audit. These set of measurements or metrics, for simplicity and organization, have been categorized as quantitative metrics, quality metrics, patient outcome and microbiological metrics and performance metrics.

(a) ***Antifungal quantitative metrics:*** The multitude of measurements included under this category of metrics include DDD (defined daily doses) adjusted for bed occupancy[18], prescribed daily doses adjusted for bed occupancy[19], DOT (days of therapy) total[20], DOT adjusted for bed occupancy[21], duration of therapy[22], antifungal acquisition costs (commonly used) [21], antifungal cost expressed as a proportion of the pharmacy budget and potential cost savings[23]. Average maintenance dose per day for a drug is referred to as DDD.[24] This metric used mainly in adults, is not appropriate for use in pediatric patients, can be incorporated for international benchmarking, does not require administration data and facilitates cost scrutiny.[25] DOT is the aggregate sum of calendar days during which a patient received any amount of an antifungal as documented in the eMAR (electronic medication administration record) and or BCMA (bar code medication administration) data.[20] This is appropriate for use in the pediatric patient but is not as useful for international benchmarking and is reliant on administration data, which may not be accessible in all institutions. The sensitivity of monitoring of costs is dependent on prescription changes of high-cost drugs. Consumption data accuracy is of little use outside electronic prescribing and none are indicative of the quality of prescribing.[25]

(b) ***Antifungal quality metrics:*** This metric mainly targets assessment of appropriateness and compliance of antifungal therapy with institutional guidelines.[12] Included under this blanket term are quality prescribing indicators[26], the proportion of patients treated with the drug of choice for the indication (Choice of the drug)[12], Proportion of patients prescribed appropriate dosing for the indication (Drug dose)[12], documentation of these indications and treatment plans[27], Therapeutic drug monitoring[12], intravenous to oral switch[12], dose optimization[12], de-escalation of empirical antifungal therapy[28], usage of first-line drugs for invasive disease[29], timeliness of antifungal regimens[30], and use of diagnostic tests and expert assessment[12]. These parameters serve as a complement to metrics determining quantity. These, however, are labour-intensive processes which demand manpower and resource.

(c) ***Patient outcome and microbiological metrics:*** Various parameters include several episodes of candidemia, the incidence of invasive fungal disease adjusted for days of hospitalization, mortality, readmission, length of stay, relapses, implicated Candidal species, azole-resistant candidemia and adverse drug reactions precipitated by the use of antifungals. These strategies are increasing labour and capital intensive and there exists a difficulty to assess data and implicate outcomes to a particular intervention.[31]

(d) ***Performance metrics or outcome measures:*** These include invasive fungal disease in targeted groups (requires prospective surveillance), antifungal drug expenditure (revolutionizes in response to changing practices and depends on the cost of prescribed drugs) and structural measures.[32]

Quality measure components as per the typology of implemented intervention

Antifungal prescribing can be modelled after antibiotic prescribing strategies, distinguished into three categories, namely restrictive interventions, persuasive interventions and structural interventions as tabulated hereunder[33]. Figure 1 depicts a logical sequence of steps towards implementation of antifungal stewardship.

Table 2: Different types of intervention and its components

Type Of Intervention	Components
Persuasive	-education -audit and feedback - guidelines and clinical pathways
Restrictive	-formulary restriction, -preauthorization - antifungal cycling or scheduled switch
Structural	-computerized records and decision support, exemplar: computer physician order entry (CPOE)

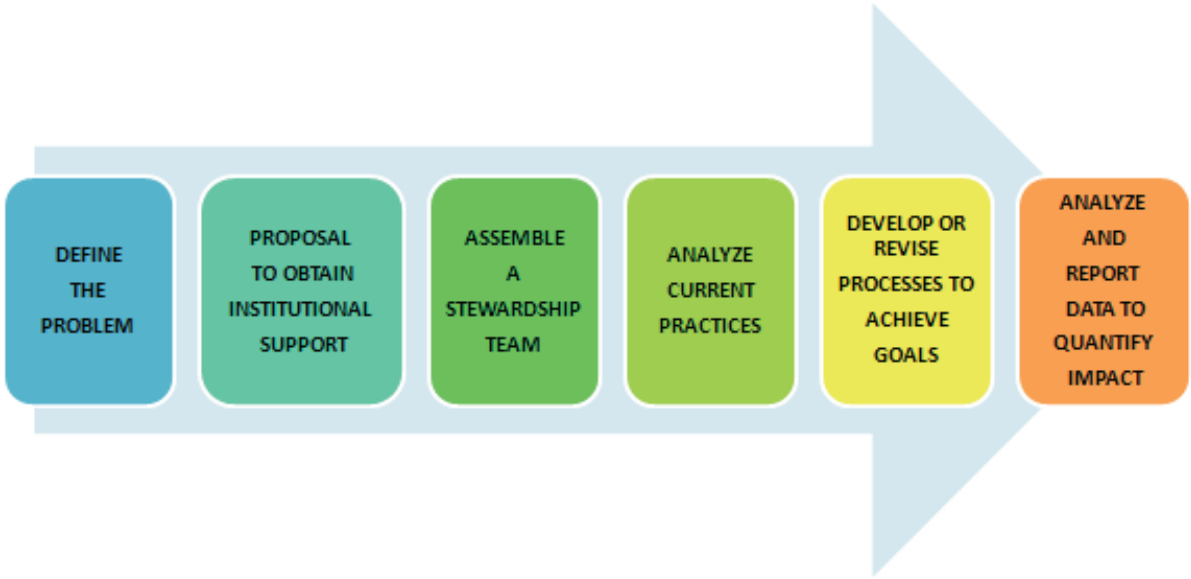

Figure 1: Flow-diagram depicting sequential steps towards implementation of antifungal stewardship program.

Barriers and pitfalls in the implementation of antifungal stewardship programs

Kaljee et al. presented four domains about physical barriers for physicians concerning antimicrobial stewardship programs which included *"outer setting", "inner setting", "individuals involved"* and *"the intervention"*.[34] The "outer setting" domain encompasses contending burden on resources, the dearth of supportive approaches, scarcity of physicians to assume supervisory and managerial roles, lack of infectious disease professional and microbiology amenities, inadequately established networking webs operating within hospitals, health systems and epidemiologically paralleling countries and selective strain on drugs due to prescription demands from pharmaceutical companies. Hierarchical constitution and their effect on interpersonal relationships are the primaries coerce under "inner setting" barriers. The workforce including the prescribers, administrators, and health staff may be deficient in information and acquaintance necessary for sustainable use of antifungals resulting from

inadequate educational opportunities, under-scrutinized prescribing practices and over-reliance on learning from experience rather than evidence-based practice, makes the "individuals involved" a significant barrier in the implementation of AFS. These incorporate highlighting stewardship virtues like forming collaborative alliances and supervisory boards, enhancing and strengthening communiqué proficiency, popularizing local guidelines de-escalation strategies and restraining prophylactic drug application. Supplementary barriers include dissolution of stewardship teams, understaffed stewardship workforce, lack of health information technology (IT), Physicians' fears and concerns concerning accountabilities and legal responsibilities. Furthermore, developing countries have poor access to speedy, sensitive, and specific fungal diagnostics to assist precise and well-timed diagnosis, leading to unwarranted empiric recommendations. Reports suggest that a 9-12 hours postponement in the therapy of invasive candidiasis, precipitates a 20% increase in mortality. Additionally, these barriers have also been grouped into domains like *"Environmental context and resources", "Social Influences", "Behavioral Regulation", "Knowledge", "Beliefs About Consequences", "Social/ Professional Role and Identity", "Goals", "Intentions", "Reinforcement"* and *"Skills"*.[35]

Conclusion

Fungal infections cause financial strain on health care, is expected to escalate over time as the populace of individuals at risk augment. Increasing fungal infections and empirical use of antifungals, possessing intrinsic and acquired resistance, are an intensifying communal health concern in susceptible patient populations across the globe, particularly in developing countries. Research indicates that prescriptions tailored for antifungal treatment are inappropriate in 40% of prophylaxis prescriptions, 50% of preemptive prescriptions, 78.6% of empirical prescriptions, and 25% of tailored therapy prescriptions.[15] Hence, principles of antifungal stewardship come to the rescue to promote a wholesome utilization of currently available antifungal drugs. 50% in intensive care units dwindled by antifungal usage after realization of antifungal stewardship programs. Providentially, innovative proteomic and molecular diagnostic paraphernalia are improving the standards of AFS. The various key elements guiding stewardship ethics like prescription authorization, clinical guidelines for treatment, education, pharmacodynamic dose optimization, post prescription feedback, computer-assisted decision support programs and pharmacy-based dosing programs strengthen the impact of stewardship agenda, promoting viable utilization of resources, to stem the tide of various fungal disease mortality and drug resistance.

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Inspecting the Cause & Thereby Managing Implant Failure in Dentistry

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Abstract

Dental implants are becoming the most popular mode of treatment nowadays, both partially and fully edentulous patients. Although several meta-analyses show 93% survival rates of dental implants, failures in dental implants are also seen. This review article aims to identify the failing implants at its earliest, making awareness among the dentists to identify the causes of the failure and thereafter the treatment modalities to manage such cases.

Keywords*: Dental implants; implant failure management; dentistry; treatment modalities*

Introduction

Implant dentistry has progressed much in the twentieth century. Though an implant-supported restoration offers a predictable treatment for tooth replacement, failures may do occur. Implant failure occurs due to a variety of causes like improper treatment planning, improper maintenance by the patients and surgical and prosthetic reasons etc. Researches show that implants are more successful in mandible then that of the maxilla. It has also been seen that implants are more successful in host bone then grafted bone. In 1990 in North American literature a team ailing implant was introduced. Ailing implant refers to a clinically stable implant affected by bone loss and pocketing. A failing implant refers to a feature similar to the ailing implant showing refractory to the therapy and continues to become worse. Finally, a failed implant is the one that must be removed immediately, as it shows mobility and the progressive destruction of the surrounding tissues.[1-3]

Criteria of evaluation of implant success: In the year 2000, the American Academy Of Periodontology gave the criteria for implant success. They are

a) Absence of persistent signs/ symptoms, such as pain, infections, neuropathies, and violation of vital structures.

b) Implant immobility.

c) No continuous peri-implant radiolucency

d) Negligible progressive bone loss (less than 0.2 mm annually)offer physiologic remodelling during 1st year of function.

e) Patient / Dentist satisfaction with an implant-supported restoration.

Causes of implant failure: According to 2003 American Academy of periodontology, it concluded that "To have a successful dental implant, the patient should be evaluated at regular intervals to monitor their peri-implant status, the condition of implant-supported prosthesis and plaque control"

The cause of implant failure can be divided into four categories :

a) Patient-related factors: It is related to the general health status of the patient(eg . patient having a disease like diabetic Mellitus, osteoporosis, on

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irradiation therapy etc), patient have a smoking habit or not, quantity and quality of bone at implant site of the patient and most importantly oral hygiene maintained of the patient.[4]

b) Important characteristics: It involves the material, dimension, shape, coating, and loading of the implant. Research shows that a diameter of 5 mm is three times and 6mm is six times stronger than 3.75 mm diameter implant. Studies show that most fracture implants are of molars and pre-molars area and that too more in the maxilla than mandible. The Implant surface coating can be of two types titanium oxide(Tio2) coating, ceramic coating, or diamond coating. Of these ceramic coating have the best prospect. [5]

Biochemical failures of the implant range from the loosening of the implant to the breakage of implant components. This type of failure is best avoided by a good understanding of screw joint mechanics and knowledge of the implant system used. In Implant-restoration connections the torque is applied to the screw causes the thread to engage and thereafter continued torque causes screw to elongate. The stress developed in the screw clamps the implant components together. This is known as pre-load. This pre-load gets reduced after cyclic loading. So proper torque should be applied to give maximum pre-load possible. Nowadays manufactures are making the implant components to gain high pre-loads, allowing them to control torque force and avoid screw loosening.[4,5]

Intraoral screw joint separating forces include:-

(i) Off-axis occlusal contacts

(ii) Lateral excursive contacts

(iii) Interproximal contact between natural teeth and implant restoration

(iv) Protrusive contacts

(v) Parafunctional forces

(vi) The non-passive framework that attaches the implants

Settling of Implant occurs when the rough surface interfaces get blunt under load since they are the only contact surface when initial torque is applied. Studies show that 2 – 10% of the initial pre-load gets loose as a result of settling. This reduction is termed as embedment relaxation. This process of screw loosening occurs in two stages. In the first stage, the preload reduction occurs due to masticatory forces and in the second stage, the continuous prolonged reduction occurs below a critical level allowing full loosening of the screw. This screw loosening or screw fracture occurs mostly in single implant crowns and mostly in the posterior segment then the anterior segment.[6]

(c) **Implant location**: The most common type of failure occurs due to poor treatment planning of implant location and poor surgical execution. Ideally, restoration should guide the placement site of the implant. So surgical guide should be used for a good understanding of the restorative aspect of the implant by the surgeon. Malpositioned implant leads to biomechanical problems of the implant. Ideally, two-stage implants should be placed with the platform of implant 3-5 mm apical to the gingival margin of the like tooth of the contralateral side. The implant should be placed with at least 1mm bone circumferentially which can allow some amount of crestal bone loss. The positional error occurs more frequently where multiple teeth are to be replaced by implants, as the surgeon gets more freedom of space to place implants. Hence the skilful technicians use pink porcelain to camouflage and give a more aesthetic result. Even well-placed implants will devoid good aesthetics if the soft tissues around the implant are not managed well by giving interim restorations.[7]

(d) **Clinicians experience**: The clinician should be experienced enough to select the patient properly, have a proper treatment planning, do the surgical procedure ideally(threshold level for bone survival during implant site preparation is 44°C to 47°C with drilling time less than 1 minute.) and do the post-implant recall visits effectively The clinician must diagnose the peri-implant pathology effectively, then go for treatment strategy whether non-surgical or surgical.

Classification of Implant failure:- It can be classified as (1) Early failure (2) Late failure.

(1) Early failure: These failure occurs shortly after surgery and is characterized by lack of osseointegration due to overheating during surgery, contamination and trauma etc.

(2) Late failure:- They occur in those implants which are thought to be successful for some time but eventually fails after prosthetic restoration is made. It occurs due to metal fatigue of implant, overloading, and continuous loss of supporting tissue due to the presence of peri-implantitis.

In 1986, Albrektsson suggested the success criteria of implant based on the rate of MBL (**M**arginal **B**one **L**oss). He said during the first year of implant 1mm of MBL is allowed followed by 0.2mm annually in the subsequent years. This is still referred to as the "Gold standard for implant success. [6-9]

But recently due to the emergence of a wide verity of implant systems, this criterion shows to be inaccurate. Though in the 1st year 1mm MBL is allowed, there are four hypothetical patterns of MBL in the conjugative years –

(I) Low rate of MBL over years(Albrektsson's pattern)

(II) Low rate of MBL in the first few years followed by rapid loss of bone support.

(III) The high rate of MBL in first few years followed by almost no bone loss and

(IV) The continuous high rate of MBL

This MBL rate should be assessed regularly in the subsequent visits to avoid implant failure through

(i) Peri-implant probing – the presence of bleeding on gentle probing (0.25N) is useful for observing the condition of mucosal infection.

(ii) Mobility

(iii) radiographs

(iv) Occlusal analysis

(v) Prosthetic evaluation

(vi) Bilateral culturing use of inflammatory markers in peri-implant crevicular fluid and genetic diagnosis are few secondary tools.[6-9]

Treatment For Implant Failure :

When planning implant rehabilitation or when facing implant failure a dentist must always consider how many teeth are necessary for adequate function. In 1992, the WHO(World Health Organisation) stated that throughout life, the retention of a functional esthetic, natural dentition of 20 teeth without requiring prosthesis, should be the treatment goal for oral health.[10]

For ailing implants, non-surgical treatment can be tried like

(I) Occlusal interferences are to be checked and removed

(II) The fit of implant-supported prosthesis and abutment is to be checked and evaluated.

(III) Debridement of granulation tissue locally should be done.

(IV) Antimicrobial mouth rinses to be used chemical disinfectants like H2O2, chlorhexidine gluconate to be used to improve clinical outcomes.

(V) Antibiotics are to be administered both systemically as well as locally.

But if all these fail, in case of a failed implant, having pain on function, mobility, radiographic bone loss greater then half of the length of the implant, uncontrolled exudate, must go for surgical intervention. Surgical intervention includes

(i) Evaluation of mucoperiosteal flap and removal of peri-implant inflammatory granulation tissue.

(ii) Regenerative surgeries using bone grafts and barrier membrane to fill the osseous defects and improve soft tissue conditions around the implant.

(iii) In the case of failed implants, there should be meticulous removal of granulation tissue & wider implants with improved surface properties are implanted in the same sucket. But this also leads to about 71% success as per studies.

(iv) In case of fractured abutment screw, the retrieval of the fractured abutment is done by artery forceps, ultrasonic scaler or repair kit.[11]

Besides all these other restoratives options are considered to replace the missing area eg. Fixed partial denture, removable partial denture, implant-supported

removable partial denture etc.[10-12]

Conclusion

A failed dental implant is now considered as an ever-growing problem in the coming years as the number of implant patients are increasing day by day. The dentists should identify and simplify the process of selecting appropriate alternative occur failure occurs in implant patients. In the coming days, the patient should be informed regarding all the possible treatment modalities after implant failure and give their consent to the most appropriate treatment for them.

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Case Report

Ocular Prosthesis Fabrication with Visible Light-Cured Resin Custom Tray: A Case Report

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Abstract

A well fitting custom ocular prosthesis warrants an accurate impression of the anopthalmic socket. Problem arises when neither the stock ocular tray nor conformer is available or ill fitting in nature. This case report elaborates the fabrication of an ocular prosthesis with emphasis on chair side method of custom ocular tray fabrication. Visible light cured resin denture base material has been used for preparation of ocular tray. This method is simple, quick and precise.

Keywords*: Ocular Prosthesis, Ocular tray, Visible light cured resin denture base material*

Introduction

Loss of eye is an unfortunate incident and often allied with trauma, glaucoma or cancer. [1] Early rehabilitation of the anopthalmic socket is essential for psychosocial well-being of patient. [2] A well-adapted custom ocular prosthesis (COP) not only improves aesthetic but also the ease of eye movement. [3] This warrants an accurate ocular impression. Routinely ocular impression is made with stock ocular tray. [4] A custom ocular tray is preferred in absentia of stock tray and in highly irregular anopthalmic socket. [5] This case report enumerates the fabrication of COP with an emphasis on chair side method of custom ocular tray preparation.

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Case Report

A five-year-old boy was referred to the department of prosthodontics, for rehabilitation of the lost eye. History of the patient suggested, enucleation of right eye one year back due to trauma. A shrunken right eye with healthy intraocular tissue bed was revealed upon clinical examination (Fig. 1A, B). Depth of fornices was adequate for retention of ocular prosthesis. So, a COP was planned for the patient and consent taken for the same.

The procedure was initiated, with fabrication of custom ocular tray, using visible light cured resin denture base material (VLCRDBM) (Dental Avenue DMG supertech: India). [6] Outline (indelible colour pencil) of the tray was made on right eyelids, followed by adaptation of a sheet of VLCRDBM over it (Fig. 2A). The marked portion of the adopted sheet was cut and a sectioned needle cap (2ml dispovan syringe) was attached to its centre for passage of impression material (Fig. 2C). The whole unit was readapted over the right eye and then polymerized with a handheld UV light source for initial stabilization. A protective eye glass

(3M: India) was used for left eye during the procedure. Definitive polymerization of the ocular tray was done in a light polymerization unit (sibari Sr 620; Sirio Dental S.R.L: Italy) as per manufacturer's instructions (Fig. 2D). The polymerized tray was perforated and verified for fit, after necessary corrections (Fig. 3A).

Ocular impression was made with this custom tray and light bodied poly vinyl siloxane elastomeric impression material (Aquasil; Dentsply Caulk:USA), when the ocular muscles were at neutral gauze. [7] The impression material was slowly injected through the stalk of ocular tray to avoid air entrapment. A functional ocular impression was made by asking the patient to move his eyes in all direction (Fig. 3B). The obtained ocular impression was analysed for any defect and then invested with irreversible hydrocolloid impression material to create the mould of defected right eye. Wax pattern (Inlay wax, Maarc; Shiva Products) of the ocular prosthesis was prepared from this mould.

Try in of ocular wax pattern was done for verification of contour, comfort and ease of eye movement (Fig. 4A). [8] Shade and position of the right iris was synchronized with that of normal left eye (Fig. 4B). The selected shade of iris button was then cut from the stock ocular prosthesis and secured in desired position of the ocular wax pattern, with the help of a disposable needle cap.

Processing of the ocular wax pattern was done, with heat polymerized acrylic resin (DPI; India). Characterization of the polymerized blank sclera was done with red fibres obtained from high strength heat polymerize acrylic material (Lucitone; Dentsply: USA), to simulate the blood vessels of eye. The characterized prosthesis was covered with a layer of heat polymerize clear acrylic resin (Acralyn-H; Asian Acrylates: India) again, to avoid irritation of eye.

The definitive ocular prosthesis was delivered to the patient, with proper education for its insertion, removal, and maintenance (Fig. 4C). No ophthalmic lubricant was advised for this patient, as the lacrimal flow was adequate. He was further educated about the change of prosthesis with age.

FIGURES

Fig. 1A. Preoperative extra ocular view

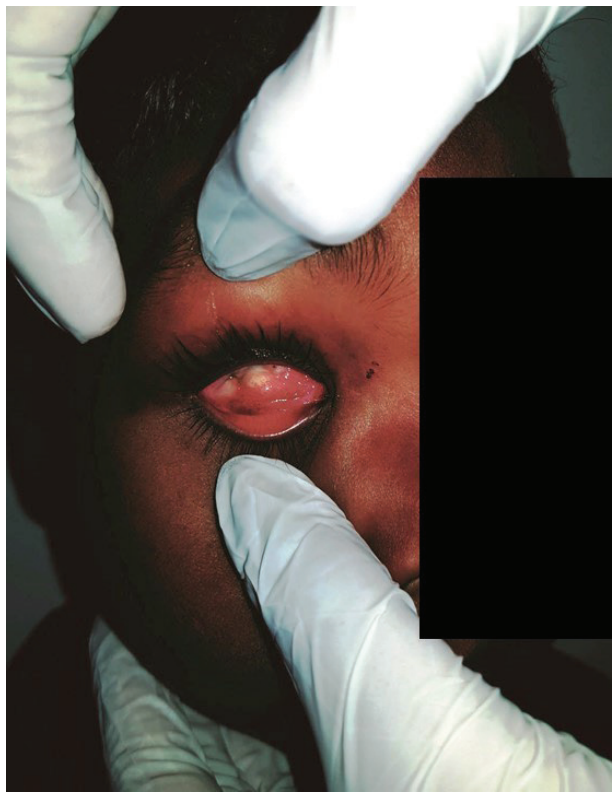

Fig. 1B. Preoperative intra ocular view

Fig. 2A. Out lined upper and lower eye lids

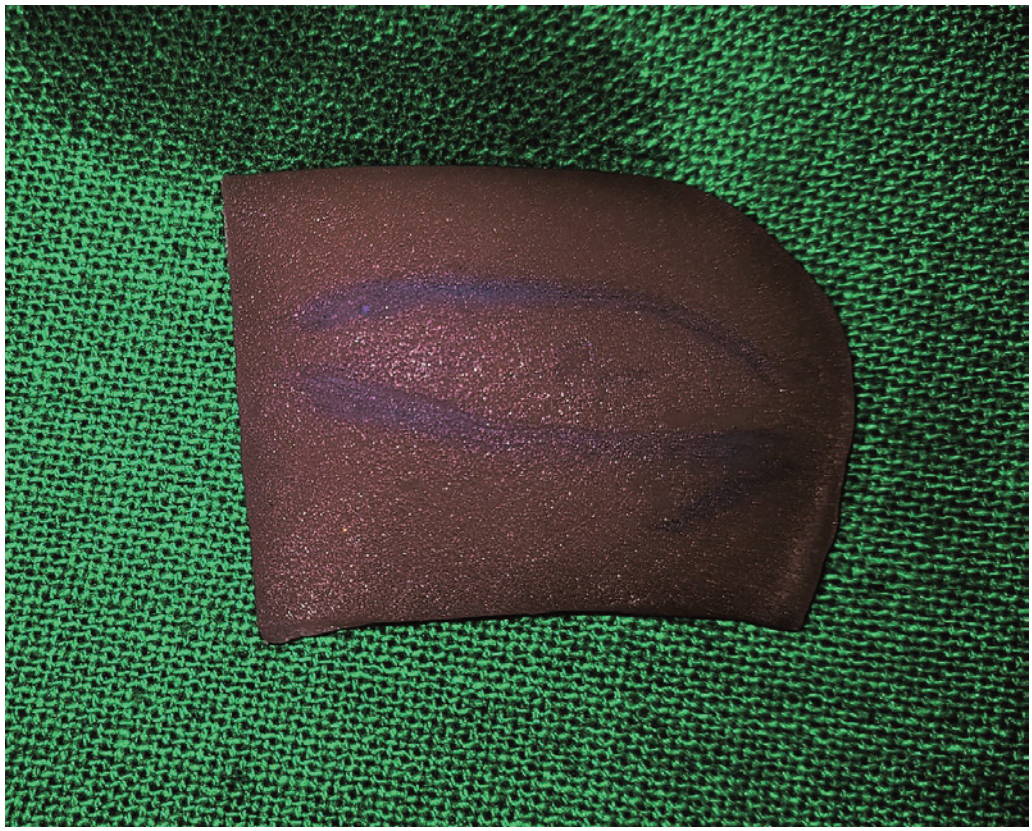

Fig. 2B. Transfer of markings of out lined upper and lower eye lids to sheet of VLCR denture base material

Fig. 2C. Adopted sheet of VLCR denture base material with attached needle cap

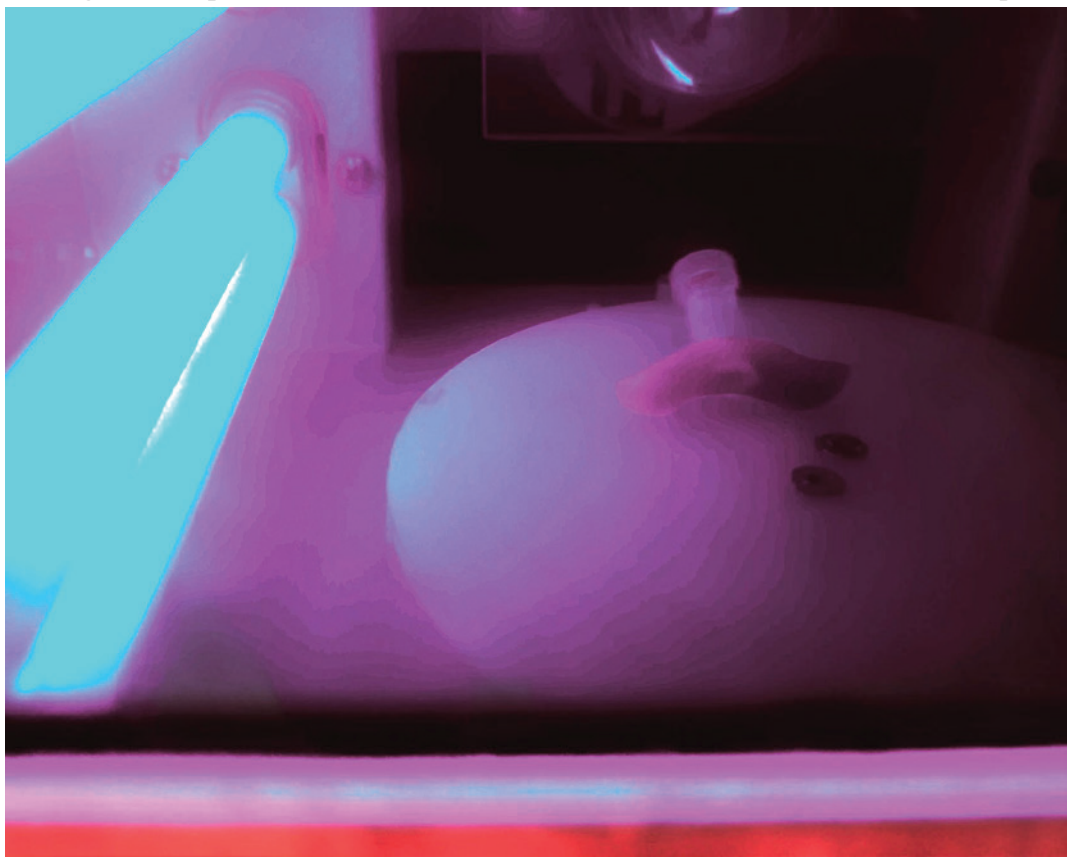

Fig. 2D. Light polymerizing machine polymerizing the adopted ocular tray

Fig. 3A. Verification of ocular tray

Fig. 3B. Ocular impression

Fig. 4A. Ocular wax pattern try-in

Fig. 4B: Positioning of iris in the ocular wax pattern

Fig. 4C: Post-operative view of patient with definitive ocular prosthesis

Discussion

A well-adapted COP to ocular bed not only improves mobility but also eliminates voids, pooling of stale tears and ulceration. [3] It is achieved by an accurate functional impression of the anophthalmic socket. Literature suggests, direct use of stock ocular prosthesis as an ocular tray in favourable situation. [9] But this technique requires many stock ocular prostheses, for size and shade matching.[9]

Custom ocular tray has been fabricated in the past, with an array of methods like, duplication of old ocular prosthesis of patient, conformer or wax shell. [5, 10] However, most of them are time consuming and not so accurate.

In this clinical report, custom ocular tray has been prepared chair side with VLCR denture base material. This material is routinely used for fabrication of denture base and custom tray by chair side.[6] Being malleable in nature, it gets closely adopted to the master cast.

The malleable property of VLCR denture base material has been used in a similar manner, to closely adapt to the contour of affected eye lid of the patient. Custom ocular tray prepared by this method requires fewer adjustments, as its shape is in harmony with shape of affected eye. The only disadvantage of this technique is that, a light polymerization unit is mandatory for this procedure. A chair side fabrication of ocular tray with VLCR denture base material is precise and swift. It makes an accurate impression in the absence of stock ocular tray or conformer.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Ethical Permission: Approved

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COVID-19: What the World Should Learn from India?

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Abstract

The pandemic, COVID-19 is not the first but savagely has become a global attraction. First detected in China, it has spread to India including other countries. Various intervention strategies like restriction on travel, lockdown, screening and extensive research has contained the widespread infection in India. While intensifying preparedness for the unprecedented threat posed by COVID-19, the Government of India (GoI) has constituted an inter-ministerial committee represented by the ministers of the Ministry of Health and Family Welfare (MoHFW), External Affairs, Home, Civil Aviation as well as the National Disaster Management Authority. India has constantly been the centre of discussion because of the way it has handled the pandemic so far. This article reflects the preparedness and potential plan by India to scale-up COVID-19 management.

Keywords**:** *COVID-19, Pandemic, Preparedness, Management; India*

Introduction

The novel coronavirus infection recently labelled coronavirus disease 2019 (COVID-19) by the WHO (World Health Organization) Director-General at the media briefing on February 11, 2020; was diagnosed and identified by 15 laboratories across the world.[1] In December 2019, increasing clusters of people were affected with novel severe acute respiratory syndrome coronavirus (SARS-CoV-2)-infected pneumonia (COVID-19) first reported from Wuhan, a metropolitan in People's Republic of China. By March 11, 2020 COVID-19 was declared as a Pandemic by WHO.[2] CoVs represent a major family of viruses, which have been implicated in several multi-country outbreaks of severe respiratory illnesses such as the Middle East Respiratory Syndrome CoV (MERS-CoV) and SARS. The SARS-CoV-2 was defined by WHO as "a new strain of coronavirus that has not been previously identified in humans".[2]

In India, the first covid case was diagnosed in 30th January 2020 at Kerala.[3] As of 24th April 2020, total confirmed cases are 23,077 with a recovery rate of 20.57 per cent.[4] India has had its own share of various outbreaks/epidemics like the H1N1, H5N1, avian influenza, Ebola, SARS, Zika and Nipah which were effectively handled with suitable research. India has been at a continuous risk for any emerging outbreaks causing rapid spread of infection, with its 1.3 billion population living in densely populated areas in resource-constrained environments. The published studies from China reported that most cases were among the patients aged above 50years, predominantly men and had chronic medical conditions.[5-8] The common symptoms included fever, fatigue, dry cough, myalgia, dyspnoea, expectoration and diarrhoea. There have been substantial investments in readiness for quick responses and to generate a favourable environment to commence therapeutic or disease prevention research.

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Ethics Preparedness

India despite its huge population showed some amazing preparedness skills during the time of this pandemic. The term ethic preparedness refers to "the capability of the public health system, to protect and have the ability to quickly respond to by having in place an ethical framework that would build trust and guide measures to recover from health emergencies". The Indian Council of Medical Research (ICMR) has worked with the Department of Health Research (DHR), MoHFW and GoI in enhancing the national capacity for early diagnosis of infections that have an epidemic potential by setting up a network of well-equipped Virus Research and Diagnostic Laboratories (VRDLs) for managing epidemics that can lead to public health crisis. The network is led by the ICMR-NIV (Indian Council Of Medical research-National Institute of Virology), Pune, which is well equipped with capability to handle highly contagious pathogens having a Biosafety Level 4 (BSL4) laboratory to set the stage for prompt action.[9] For the confirmation of coronavirus disease 2019 (COVID-19) diagnosis, reverse transcription polymerase chain reaction (RT-PCR) has been recommended by WHO. India developed an in vitro transcribed (IVT) RNA as positive control for laboratory diagnosis of SARS-CoV-2 targeting E gene.[10] Due to limited supply of positive controls available from the WHO, India generated in-house positive controls for the screening and confirmatory assays. This gene-specific IVT RNA was synthesized for all the gene targets used in real time PCR. India's successful establishment of diagnostic system including in-house positive control was beneficial in providing timely diagnosis and accelerated clinical management and isolation of SARS-CoV-2 patients and controlled further spread.[10]

The ICMR Bioethics Unit at the ICMR National Centre for Disease Informatics and Research, Bengaluru, have also developed a web portal with all important information and resources. The ethical committee of India in accordance with the National Ethical Guidelines, are generously reviewing all the protocols including the informed consent form, protection of confidentiality, and prevention from any stigmatization, rapid post-trial access, local roll out and surveillance, benefit sharing with the community, publication of results in a timely manner. It also incorporates provisions made for isolation, quarantine, travel restrictions, availability of appropriate training to work in such challenging situations, etc.[11,12] India has constantly maintained its ethical values during public health emergencies.[13]

Testing Process and Tracing by Integrated disease surveillance programme (IDSP)

The 1.3 billion person country, due to lack of enough test kits is using a gigantic surveillance network to trace and quarantine infected people.[14] A potential plan to scale up COVID-19 testing in the public sector was prepared in India. A large number of laboratories have been included in the ICMR network of COVID-19 testing and private lab chains have registered with ICMR to increase testing capacity. On April 8, the Supreme court ordered that all COVID-19 tests must be provided for free. On April 5th, the government placed restrictions on the export of most diagnostic testing kits. The national guidelines on surveillance, case investigation and laboratory detection of COVID-19 cases have been developed by the National Centre for Disease Control (NCDC) and made available to all States.[15] The scope has been expanded and now includes all symptomatic individuals who have undertaken international travel in the last 14 days; all symptomatic contacts of laboratory confirmed cases; all symptomatic healthcare workers; all hospitalised patients with Severe Acute Respiratory Illness (fever and cough and/or shortness of breath); asymptomatic direct and high-risk contacts of a confirmed case, who should be tested once between day 5 and day 14 of coming in his/her contact. Since mid-February, India has included testing for patients (with no travel or contact history) having severe acute respiratory illness (SARI) from the surveillance system.[16]

The MoHFW, GoI started the IDSP in 2004, for strengthening the disease surveillance systems for epidemics / outbreak detection and response. In addition of maintaining a rapid response team across several locations of the country, it also aims in strengthening laboratory systems for the same. Using a network of IT-enabled systems, it started watching out for COVID-19 soon after the first case arrived in India in late January, and is now monitoring hundreds of thousands of people.[17] A containment zone is set up and everyone inside is ordered to stay at home, in areas

where workers identify clusters of disease. Door-to-door inspection is done by Social workers for finding people with suspected infections. Symptomatic persons are then tested for the virus, including members of their household and close contacts. Persons found positive are taken to isolation units or hospitals.[14] In states like Kerala, for people found ambiguous, phone records are obtained for investigating the contact histories of people with COVID-19.[14] The Odisha state government took the initiative to appeal people returned from foreign countries to register themselves through "toll free number-104 or online portal *https://covid19.odisha.gov.in"* in consideration of the coronavirus outbreak. An incentive of Rs 15,000 was assured for registration and home isolation as a preventive step towards spread of coronavirus.[18]

According to Epidemiologists, the strategy to "trace and quarantine contacts", including lockdown of 21 days from late March, of roughly 1.3 billion inhabitants, taken by the government; have helped in containment of the virus in most places.[14]

Isolation, Quarantine, Lockdown, containment to mitigation

In India, airport entry screening has been initiated including thermal screening at the international airports, major and minor seaports and land crossings. Existing visas from China have been cancelled, and travellers returning from COVID-19 affected areas have been quarantined.[19] So far, more than half million incoming airline passengers and thousands coming through seaports have undergone the screening procedure.[19] All international flights (from March 26th) till April 15th were banned, excluding approved all-cargo operations and flights. All visas (from mid-March) were also suspended, except for a few categories. Passenger flights of domestic routes on were banned from March 25th. The country's rail network suspended most passenger services. All public transport services for roadways were also suspended till April 15th.

On 24th March, a nation-wide lockdown was declared from March 25th and on April 14 it was further extended till 3rd May.[20] A conditional withdrawal of the lockdown is allowed from April 20th where the virus spread has either been contained or prevented. Some states have announced that they will not relax the lockdown including Delhi for at least one week and Telangana till May 7th. All activities are suspended since March 25th except shops dealing with essential commodities like perishables, groceries and dairy. The government has announced a gradual exit strategy to mitigate the economic and social costs of the lockdown. Since 20 Aprilth, some parts of the economy, including agriculture, logistics, infrastructure, e-commerce and factories located in rural areas, plantation activities such as tea with maximum of 50% workers, have been allowed to return to work in areas where no COVID-19 cases have been detected (so-called green zones). Construction activities for those roads, irrigation projects, buildings and industrial projects where workers are available on site have been allowed. Services provided by self-employed people such as electricians, electronics repairers, plumbers, motor mechanics and carpenters, have been allowed to operate. Exemptions given from April 20 are not applicable in COVID-19 hotspots or containment zones. Since March 25th, following nation-wide lockdown, no religious congregation is permitted and all places of worship are closed for the public, all social / political / sports / entertainment / conferences / workshops / cultural / religious function / gathering have been prohibited across the country. In case of funerals, congregation of not more than twenty persons is being allowed. Educational institutions and universities are also closed following second lockdown.

The government has also allowed people to take up jobs under the National Rural Employment Guarantee Act (MGNREGA), a large rural public job programme targeting the poor in rural areas.

According to an analysis by Dr VK Paul, chairman of one of the 11 Empowered Groups sets up by the Centre, "lockdown has been effective in slowing the doubling rate of COVID-19 and saved lives. The lockdown decision was timely as, the around 23,000 cases in India today could have been 73,000. The lockdown has helped increase the doubling rate from 3.3 days to 10 days. From the last three days, the doubling rate has been 10 days, which is a good sign".[21]

The India Epidemic Intelligence Service or India EIS programme (applied field epidemiology training programme) run by the NCDC, in collaboration with the US Centers for Disease Control and Prevention (CDC),

has been actively supporting the COVID-19 response.[22] At present, 45 EIS officers are supporting various States in this pandemic containment exercise, including Kerala, Maharashtra, Nagaland and Uttar Pradesh for an early detection and rapid response and generating evidence that can be used for policymaking and implementation. To prevent clusters of cases and community transmission, India has included active surveillance and expanded testing, contact tracing, isolation and management of cases after triage.

Drugs

India is the biggest manufacturer of a drug Hydroxychloroquine (HCQ). An ICMR advisory said HCQ was found to be "effective against coronavirus" as a preventative medicine in laboratory studies and in-vivo studies. However, only a few clinical trials have shown limited success of the drug. Yet, after the statement of US President Donald Trump, of HCQ being a "game-changer" in the fight against COVID-19, shipment orders from countries around the world hasn't stopped.[23]

India is also working on getting access to the patented "Remdesivir" which is now being touted as the best shot against COVID-19. "Initial data based on an observational study shows that the drug (Remdesivir) is effective. We will wait for the results from the WHO solidarity trial and also see if some other companies can work on this to proceed further," said Raman Gangakhedkar, head scientist at ICMR.[24]

Building public trust and improving communication

To engage the community, Gram Sabhas are organizing awareness drives regarding clinical presentation of the novel CoV diseases, preventive measures, and the need for reporting especially in the villages in border districts. A 24×7 call centre has been operational at the NCDC from an early stage of COVID-19 outbreak which provides information and guidance about testing facilities and their location, sample collection facilities, as well as other aspects of the pandemic.[25] Recently, the MoHFW has prepared some frequently asked questions (FAQs) and fact sheets and ICMR has been preparing press statements and posting updates on COVID-19 on its website on a daily basis.[26]

The ICMR has partnered with the Global Health Strategies to receive guidance on communication and networking activities.[27] Nodal communication officers have been appointed in each of the institutes who coordinate with the Communication Unit at ICMR Headquarters office and public relations officers for media related activities. A social media policy has been released and workshops have been held to train scientists on effective communication skills.[28] These small initiatives can go a long way in improving communication capabilities and build strong public support.

On March 26th, the central government issued INR 1.7 trillion rupees (USD 23 billion) aimed at ensuring that families belonging to poor and lower to middle income groups are able to carry out their daily lives smoothly under the lockdown. In the fiscal package announced on March 26th, the government has committed to pay employee provident fund contributions on behalf of employees and employers (12% each) for the next three months along with amendment of pension regulations which is going to help 48 million workers.

Government has also received a lot of funding for management of COVID from private and public sector as well as many eminent businessmen, celebrities and citizens of India to the PM-CARES Fund.

The government has created an application software, "arogya setu" and urged public to follow it for correct information and prevention procedures to combat COVID and also has asked public to rely on government sources only and not fall a prey of fake news.[29] Government has also asked social media platforms to curb on COVID-19 related fake news and misinformation.

Amidst lockdown, Prime minister, Narendra Modi united all the citizens of India on March 22nd by asking to clap, ring bells and conches, for showing gratitude to doctors, paramedics, police and other personnel associated with essential services.[30] On April 5th, he also influenced people to switch off the lights of their homes and light up lamps, candles or mobile phone torches for nine minutes at 9 pm to display the country's collective resolve to defeat coronavirus.[31] Both these events were hugely successful with active participation from all

sections of the society, bringing back the nation together.

Health workforce

The union home ministry has also set up four additional Inter-Ministerial Central Teams (IMCT) rounding upto 10, for examining lockdown violations, social distancing norms and attacks on medical professionals.[32] The teams consists of public health specialists and officers of the National Disaster Management Authority (NDMA), whose expertise can be leveraged by the state government for management of COVID-19 pandemic. These teams have been sent to the states like Maharashtra, Madhya Pradesh, Rajasthan and West Bengal for making ground assessment of the situation prevailing in select districts and reviewing the implementation of the lockdown measures.

On March 19th, hospitals were asked to defer elective surgeries and the government capped the price of sanitizer bottles and banned exports of masks, ventilator machines.

On March 24th, the government announced an emergency health fund of INR 150 billion (USD 2 billion) for treating COVID-19 patients and strengthening the medical infrastructure of the country including for rapidly ramping up the number of testing facilities, Personal Protective Equipment (PPE), Isolation Beds, ICU beds, Ventilators and other essential equipment announced. Training of medical and paramedical manpower is also being undertaken and widespread news regarding prevention from corona is being circulated [4]. Some states (including Gujarat, Assam, Odisha, Jharkhand, Rajasthan, Goa, Karnataka, MP and J&K) have set up hospitals dedicated for the management of COVID-19. The March 26th central government package includes insurance cover of INR 5 million per health worker fighting COVID-19 to be provided under Insurance Scheme.

Health ministry confirmed that for containing the spread of the novel coronavirus in the country, social distancing and lockdown have been very crucial. A study by experts at ICMR using mathematical modeling estimated that if distancing and quarantines are strictly followed, India may be able to reduce its COVID-19 cases by up to 62 per cent.[33] Accessing about the current situation, they revealed that 15 districts have had no new case in last 28 days. Besides these heartening numbers, now there are 80 districts in the country that have reported no new cases in last 14 days.[32]

WHO views on India

"India's response to COVID-19 has been pre-emptive, pro-active and graded with high-level political commitment. India has shown 'whole of government' approach and is adopting 'whole of society approach'," said WHO regional emergencies director of South-East Asia, Roderico Ofrin.[16] He further added that "ICMR has isolated the virus. India is only the fifth country to do so, and the second in the region after Thailand. We are aware that India is engaging in research on vaccines and better diagnostics which includes serological testing. This will be important to better understand the outbreak".

Conclusions

India being a densely populated country has highest risk of COVID transmission. With the current measures taken, the spread of COVID-19 has been able to show a recovery rate of 20.57%. The public health interventions along with the lockdown has made the world recognize India's strong health and political domain. By a strong national will and determination, India can surely overcome the impact of pandemic COVID-19.

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A Questionnaire based Cross-Sectional Survey on PRF

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Abstract

Progress in regenerative endodontic procedures needs approval by clinicians, but there is little or no evidence. This study aimed to examine the awareness, mindset and experience of regenerative endodontics among Indian dental residents. For the survey they used a self-administered questionnaire which consisted of three parts; part A focused on the background and experience of dentists, part B pursued the viewpoint, views and decision of the dentist about the utilize of PRF and Regenerative techniques, Whereas part C contained questions focused on their clinical experience. Findings indicate that maximum respondents knew about PRF during postgraduate training but 90% are willing to attend PRF and REP training. However, few claimed to have used any form of regenerative therapy in their work. Finally, it can be concluded that findings are an indication of optimistic dental residents concerning the use of regenerative endodontic methods; however, further analysis and routine training is needed.

Keywords: *PRF, Regenerative Procedures, Dentist, Survey*

Introduction

Platelet Rich Fibrin (PRF), a patient blood-derived and autogenous living biomaterial, is rapidly being studied and utilized by clinicians worldwide as an autologous adjunctive biomaterial to facilitate bone and soft tissue healing and regeneration.[1] The gold standard for in vivo tissue repair and regeneration includes the interplay of platelets, scaffold (fibrin matrix), growth factors, leukocytes and stem cells.[2]

Such main components are both active components of PRF which, when combined and equipped, are implicated in the core processes of tissue healing and regeneration, that act naturally and in synergy to stimulate, improve, and accelerate tissue healing and to regenerate soft or bone tissue including extracellular matrix synthesis, cell differentiation and proliferation, angiogenesis and chemotaxis.[3] In terms of structure, the three-dimensional fibrin membrane is proficient of imitating the extracellular matrix and provides the framework or scaffold for cells to act optimally during healing and regeneration.

Restoration of dentin pulp in endodontics and restorative dentistry is a longer-term goal. There was increasing concern about the application of the tissue engineering theory to endodontics. Theoretical implementation for regenerative and tissue engineering methods to dentistry has an enormous opportunity to address a range of medical needs. Nevertheless, there is a need for implementation of this novel therapy-detailed awareness of PRF and an appropriate capacity to conduct it is of primary importance.

The dental professionals are the next wave who will make up the majority of future researchers whose work associates over the next decade. The aim of this

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study is therefore to consider their views, their degree of understanding and their future recognition of this progress in endodontics. It would also help to determine that further focus has to be paid to PRF curriculum and training programmes. There is little research in the empirical literature that provides details on endodontists' views, values and behaviours concerning the implementation of PRF. It is necessary to consider the reaction of the endodontic society to this modern age of care. The endodontic practitioners would be the foremost professionals to advise as well as educate patients about new treatments regarding the use of REPs. This study aimed to gather endodontist opinions on the employ of PRF and REPs. Different perspectives are required to help establish ethical standards and determine the need for endodontic professionals to accept PRF and REPs.

Materials and Methods

110 copies of the questionnaire on the issues of PRF were distributed among dental professionals throughout the region. The survey consisted of three parts: The first section included questions about respondent profile including a year of study, age and demographics. The second section included 13 questions about information and thoughts on using PRF. The third component covered their participation in clinical experience. The response feedback was measured by the figure of answers as a proportion to all answers to provide an insight into the participants' prevailing views.

Results

110 Copies of the questionnaire circulated and the surveys completed were received, resulting in a cumulative response rate of 100%. The findings of the questionnaires are listed in Table 1.

Profile of Participants:

Most of the participants were in the age group of fewer than 35 years (86%). 62.5% of dental professionals who participated were from Odisha, 18.2% were from West Bengal, the rest of the participants belonged to Bihar, Jharkhand, Chhattisgarh and other areas. The majority (55%) of dental professionals devoted more than 20hrs/ week in clinics. Majority of dental professionals who responded to the questionnaire were endodontists(39.1 %) and general practitioners(20.9%). According to the survey, the majority of respondents have practised less than 10yrs(74.3 %), 10-20yrs (21.1%) and more than 20yrs (4.6%).

Knowledge, attitude and opinion towards regenerative endodontics and PRF :

Around 88% of participants were aware of the potential application of PRF. Most of them knew about its centrifugation protocol, different forms and properties. From Figure 1, it can be seen 49% of participants noted that the biggest obstacle accepting PRF as regenerative dental treatment was a need for specialized equipment and 26.9% noted about their lack of experience. 40% of participants had ethical concerns regarding the use of PRF. Around 70 of participants agreed that PRF as a regenerative therapy will be a superior management alternative than implant procedures and 28 of them were not sure of it. A majority of participants (90%) agreed that dental professional association would encourage PRF for their benefit in regenerative dentistry(Figure 2) and 60% advocated the introduction of PRF as regenerative therapy into the undergraduate course of dentistry. More than half of the participants (58.9 per cent) completed the Continuing Dental Education Program (CDE) on regenerative endodontics and PRF, and 90 per cent of the participants are interested to engage in further instruction and/or continuing education on the use and use of PRF to enhance their understanding of it.

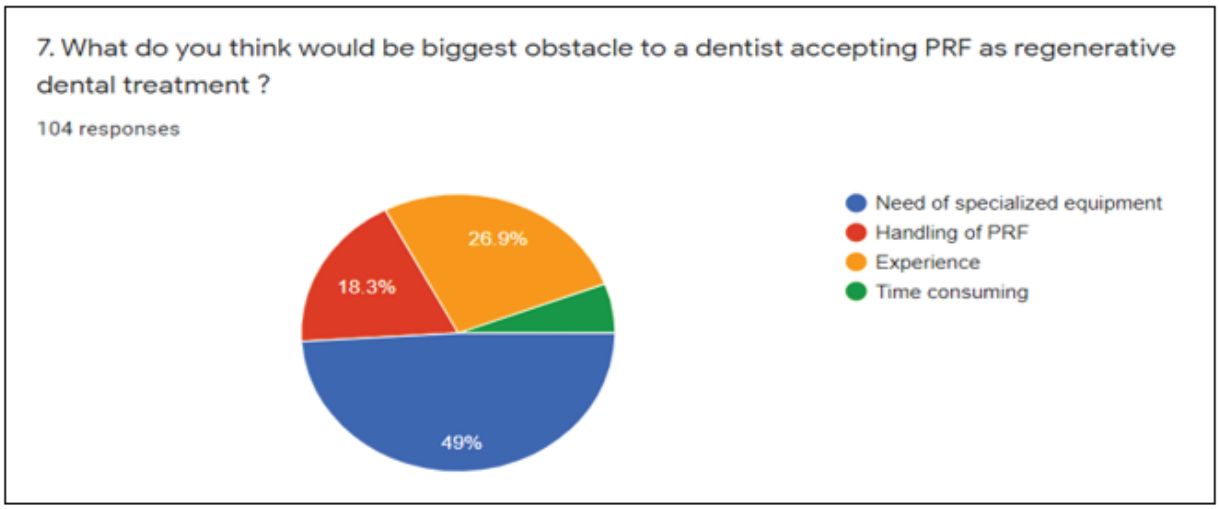

Figure 1. Responses for the biggest obstacle to a dentist for accepting PRF as regenerative treatment.

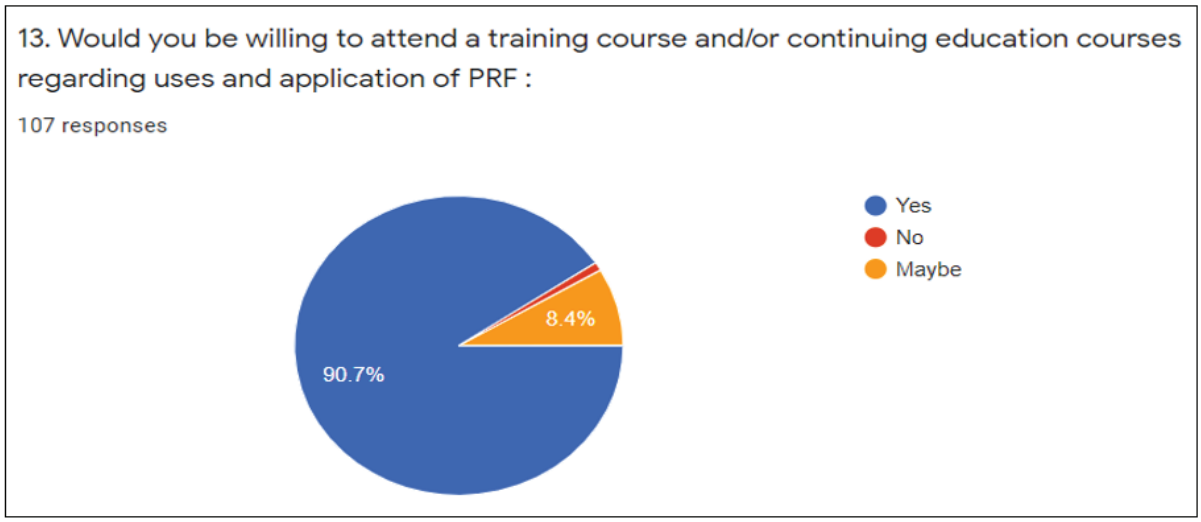

Figure 2. Responses for participants willing to attend training courses regarding the use and application of PRF

Clinical Practice:

85.5% of participants considered that PRF can be applied to continued root formation, healing of periradicular bone and revitalization of pulp tissue within the root canal. 78 dentists agreed on the periapical tissues healing be can be improved by PRF as a tissue engineering process following non-surgical endodontic treatment and 27 of them are unsure regarding it.

Some participants indicated that necrotic premature teeth in their work accounted for 10-25 per cent (57 per cent) of cases. Periradicular lesions were stated to account for the majority of patients (45.7 per cent) between 26% - 50% of all cases observed. The majority of participants (36.8 per cent) found the application of calcium hydroxide accompanied by a mineral trioxide aggregate apical plug and obturation content backfilling to be the best treatment for premature necrotic teeth. About a quarter of participants (27.4 per cent) found tribiotic paste application and pulpal regeneration to be the best therapy for immature necrotic teeth.

71% of practitioners would like to recommend a patient to store dental stem cells and explain its prospects and 22.4% are not sure about it. 50% of respondents agree that it is the most successful treatment choice

and will suggest PRF and regenerative dental therapies to their patients, while 31.8% think it is the safest and most efficient treatment process. 55.7% of dentists considered the greatest limitation for use of PRF are increased risk of transmitting infectious agents, can't be used as allogenic material and faster resorption rate. Finally, 63.2% of participants regard successful for PRF as treatment outcome and 36.8% are not sure about it.

Discussion

The biologically based procedures designed to repair damaged components, including dentine and root components and pulp-dentine cells, are demarcated in the regenerative endodontic processes.[4] The harmonized biological spurs and mechanical regulators encouraging cellular development have greatly expanded the recognition of regenerative therapy for dental tissues. PRF technology aims to extract from a patient's blood sample the essential elements that could be used to enhance healing and promote tissue regeneration, and to prepare it in a clinically functional form such as membrane (A-PRF, L-PRF, or GCF) or injectable liquid (i-PRF).[5] Improved understanding of the growth, biological and physiological properties and characteristics of PRF in tissue healing and regeneration over the past two decades has resulted in more effective therapeutic applications, especially in the areas of periodontology, implantology and oral surgery.

PRF gives off a variety of growth factors. Between them, platelet-derived growth factor, the transforming growth factor-beta, matrix glycoproteins and vascular endothelial growth factor which are slowly released for at least 1 week.[6] The enhanced accumulation of such growth factors indicates an improvement in the healing of soft and hard tissues.[7] Moreover, several studies have indicated that PRF can promote regeneration of the osseous and soft tissue while reducing inflammation, pain and adverse effects.[8] Due to these physiological properties, the use of platelet concentrates has become increasingly popular over the last 15 years to improve wound healing and advance the clinical benefits of bone replacement grafts.[6,8]

Adequate research interest and expertise among service providers are paramount to achieve clinical relevance to human beings. This research was undertaken to collect data from the dentists in our country about the level of understanding, expertise and clinical status of PRF and other REPs. Throughout this survey, residents reacted quite strongly, where nine of 10 felt that PRF should be used in dentistry. In the survey conducted by *Epelman et al.2009* concluded that "due to the increased interests and activities that are ongoing in this field, dental practitioners' opinion towards REPs reported that almost 89% of practitioners are willing to save teeth and dental tissues for stem cell banking".[9]

The benefits of "PRF as a scaffold is that it has an architectural flexible trimolecular or equilateral fibrin intersection that facilitates cytokine interaction and cell migration".[1] Many of the participants suggested that regenerative procedures should be integrated into dentistry and were prepared for saving teeth and dental tissues with the application of PRF and not favouring implants over as a treatment choice. Half of the participants used regenerative methods, like bones, membranes, or bioactive materials, in their research already, signifying that numerous regenerative techniques are already in use. Yet only one-fourth respondents discovered useful regenerative techniques in the treatment of necrotic immature teeth in 20% of their patients. More than half of the participants also find the best management for necrotic immature teeth to be the application of $Ca(OH)_2$, followed by MTA apical plugs and backfilling with the sealing content. This offers an overview of the idea that people are not being trained about advanced regenerative endodontic approaches. Continuing education and training services are required about all procedures involving dentin-pulp regeneration from the easiest type of blood clot revascularization (BCR) to more complex technique, including the development of tissue-engineered pulpal structures in the laboratory and their implantation in clean along with shaped root canals.

A large number of participants thought that PRF can be used in different applications such as continued root regeneration of developing teeth, periradicular bone recovery and pulp tissue revitalization inside a root canal. The positive outcome of the regenerative procedures can be assessed as the capability to achieve periapical lesion recovery response, apical tooth root closure, root lengthening and dentinal wall response.[10,11,12] According to *Pinto et al.2017, "*The periapical reaction to lesion healing was 88.9 per cent for BCR and 100 per

cent for both PRP and PRF. To dentists, these results are positive news. It indicates that REPs are very effective in disinfecting periapical bacteria, which helps treat isolated lesions and avoids the need for complex apical surgery".[13]

Clinicians may have an aversion to PRF as they need venous blood drawn from the patient's arm at the time of treatment, more time and effort in the procedures required to administer the dental therapy and more cost for the PRF kits.[14] Some of the respondents knew about other REP procedures but were unsure about the outcomes. A survey found that 55% per cent of dentists were unsure as to whether REPs should work.[15] The inability to use REPs to treat immature traumatized due to less experience or trust in the result, might lead millions of children with lost natural teeth that can be rescued. To modify professional attitudes and achieve greater approval of RETs for use in helping to preserve traumatized teeth for children, it was important to decide whether the PRF techniques could make regenerative methods more effective or not.

It was estimated that in the United States 2 million dental implants are inserted per year.[16] Implant placement may surpass a few endodontic therapies and also REPs.[16] Around 50% of respondents felt regenerative procedures will be a preferable management choice than tooth implant procedure. Epidemiological research showed 97% of 1,4 million endodontically treated tooth remained functional following 8 years[17], with efficacy or survival levels achieved by more than 90% of endodontic procedures and implants. .[18,19] The high level of performance poses a threat for researchers and practitioners of tissue engineering. Most attendees would consider REPs only if they were the most successful care choice. For researchers developing REPs, the message from this analysis is that they would need to build a base of proof to convince patients and endodontic practitioners that latest regenerative-endodontic therapies are successful and also as safe as the existing endodontic therapies that these seek to substitute.

Endodontist curriculum, instruction, and ongoing education have evolved to persist with the availability of newer treatments.[20] Dentists who participate in regenerative therapy have a social and ethical imperative to be active and qualified, but there are few training programmes in REPs. In addition to existing curriculum standards, additional educational or qualification systems will be needed, and these concerns may impose a greater academic strain on clinicians. This seems that the participants expect new training activities to take place, as almost all were able to undergo training courses in REP.

Conclusion

The participants in the study were overwhelmingly positive about the implementation and effectiveness of PRF and regenerative treatments. The groundbreaking character of this sample avoided differentiation with endodontists and other health care practitioners 'views, values and attitudes. It is not clear if the same passion intended for stem cell adoption and regenerative therapy occurs among doctors, dentist, and other health care professionals. Further study work by health professionals is required for evaluating ethical recommendations along with examining the possible acceptability and drawbacks of providing patients with stem cell and regenerative therapies.

Conflict of Interests: The authors declare that they have no competing interests.

Ethical Issues: None

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Ridge Split Techniques: A Literature Review

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Abstract

Extraction of teeth leads to loss of alveolar bone in several patterns. The bone loss can occur in a horizontal or vertical direction or a combination of both. Horizontal ridge width deficiency possesses a challenge for the clinician to obtain primary stability during loading and there is risk of fracture of bone segment. Therefore different techniques have been described to gain the ridge width. One of the techniques used is ridge split technique which was first described by Simion et al in 1992. It is a technique of bone manipulation in such a way that it becomes a site for implant placement without removing any bone. Various types of techniques like 3 staged approach, one staged approach etc are described for various sites and situation that help in obtaining an adequate width for loading. This article specifically deals with the different types of ridge split techniques and its description in a simplified manner.

Keywords*: Alveolar bone, Horizontal ridge width, and Ridge split technique*

Introduction

The architecture of the alveolar bone is completely dependent on the tooth architecture. Dependency is so much so that the shape and volume of bone revolves around inclination, axis of eruption and the form of teeth. Therefore, atrophy of the alveolar bone is bound to occur on tooth removal.(1) Pietrokovski and Massler et al in 1967 studied the amount of bone tissue loss following unilateral tooth extraction and concluded that buccal bone resorbed more than the corresponding lingual or palatal side. Therefore, shifting the center of the ridge more palatally or lingually.(2) Studies have also proved that maximum bone resorption occurs in the initial three months following extraction.(3) Schropp et al in 2003 observed that 2/3rd of the bone width reduction occurred in the first three months.(4) Araujo and Lindhe et al in 2005 in an animal study observed alterations in bone width at different levels and stated that the buccal bone wall was thinner than the palatal or lingual bone wall and the width of bone decreased towards the base.(5) These studies proved that there is maximum reduction in bone width in the first three months making it difficult for the placement of implants in those regions.

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As per the classification provided by Les Tolstunov in 2014[6],

Ridge width	>10mm	8-10mm	6-8mm	4-6mm	2-4mm	<2mm	6-10/2-4mm	2-4/ 6-10mm
Ridge Deficiency	No deficiency	Minimal	Mild	Moderate	Severe	Extreme	"Hourglass"	"Bottleneck
Classification	0	I	II	III	IV	V	VI	VII
Indication for surgery	Hard tissue surgery not indicated.	Hard tissue surgery is rarely indicated	GBR or ridge split is often indicated	Ideal for ridge split. GBR or block bone graft can also be done.	Ridge split and block bone graft.	Large extra oral block bone graft	GBR can be done at mid ridge level	Ridge reshaping or GBR at the top of the ridge can be done.

Simion et al in 1992 was the first to introduce the ridge split technique in horizontally deficient ridges.[7] In the maxilla there is more cancellous or elastic bone, therefore the staged approach of expansion of ridge followed by loading after 2-4 months is preferred and for mandible that has dense cortical plates is preferred for crestal split or the greenstick fracture.[8]

CONCEPT OF BONE COLLAPSE AND BONE RESORPTION

Before going ahead with the ridge split procedure (RSP), it is important to understand the difference between bone resorption and bone collapse that defines bone deficiency in a particular region. On extraction of single or multiple teeth, the cancellous or spongy bone undergoes bone resorption. But the cortical bone undergoes collapse resulting in loss of function or stability as shown in **figure 1A**. This leads to buccal and lingual cortical plate lying proximal to each other with minimal amount of medullary bone within it. In case of extraction that includes trauma, both cortical and medullary bone loss occurs together resulting in a true corticomedullary bone loss. Thus , the ideal cases for a ridge split procedure includes a mild medullary bone resorption with mild to moderate loss of cortical bone and an alveolar width ranging from 3 to 5mm having a good alveolar height.

It is very difficult to separate two proximal cortical plates with very little interpositional medullary bone. The medullary bone is the area where the actual split occurs. Therefore, more the amount of interpositional medullary bone, easier it is to split the cortical plates and more predictable results can be expected. On flattening the coronal bone a good amount of cortical plate can be obtained.

SURGICAL PRINCIPLES OF RIDGE SPLIT PROCEDURE (RSP)

1. Development of a vascular bone flap.

2. Healing mostly occurs by the secondary intention.

3. Osteocondensation is one of the processes by which healing occurs.

4. Osteomobilization of cortical plates.

5. Healing occurs differently in maxilla and mandible.

MANDIBULAR TWO-STAGE ALVEOLAR RIDGE-SPLIT PROCEDURE[9]

STAGE 1: CORTICOTOMY

In the process of RSP, corticotomy is of prime importance because of the dense nature of the bone in the mandible. Three types of corticotomies are involved i.e. the crestal corticotomy, apical corticotomy and the vertical corticotomies (two in number) as shown in **figure 1B**. Corticotomy is usually done as a window in

the form of a rectangle as shown in **figure 1C**.

SURGICAL PROCEDURE

It starts with a crestal incision more towards the lingual side for the reflection of a full thickness mucoperiosteal flap. Two full thickness vertical incisions are given at 90 degrees from the crestal incisions in the adjacent teeth in the mesio or disto line angle to release the flap. The reflected flap should expose the bone atleast 1 to 2mm beyond the planned corticotomy site. After reflection of the flap, the corticotomy is performed with the help of thin fissure bur or piezoelectric tips. The crestal and the apical corticotomy are performed parallel to each other with a distance of 10-12mm between them. Corticotomies form a rectangular shape.

Of the four corticotomies, the most important corticotomy is the crestal one since it will be reopened to start the split. The depth of the cut should be the depth of the cortical bone i.e. 1.5 -2.5mm. The corticotomies are extended to the medullary layer of bone. This results in end of the first stage and the site is sutured with a resorbable 5-0 suture and the patient is recalled after 4-5 weeks for the commencement of the second stage.

STAGE 2: SPLITTING, EXPANSION, AND GRAFTING

In this stage, Osteomobilization is done by splitting the bone along the medullary bone leading to the movement of the bone buccally by forward movement. If in the stage 1, the buccal bone has been evenly weakened throughout then the Osteomobilization becomes easy. Therefore, they cause movement with the help of special osteotomes and spreaders.

SURGICAL PROCEDURE

The second stage of the surgery should be started only with a crestal incision as shown in **figure 1D**. Overzealous exposure of the bone is not required. Buccal periosteal vascularization is maintained by prevention of reflection of the flap.[10]

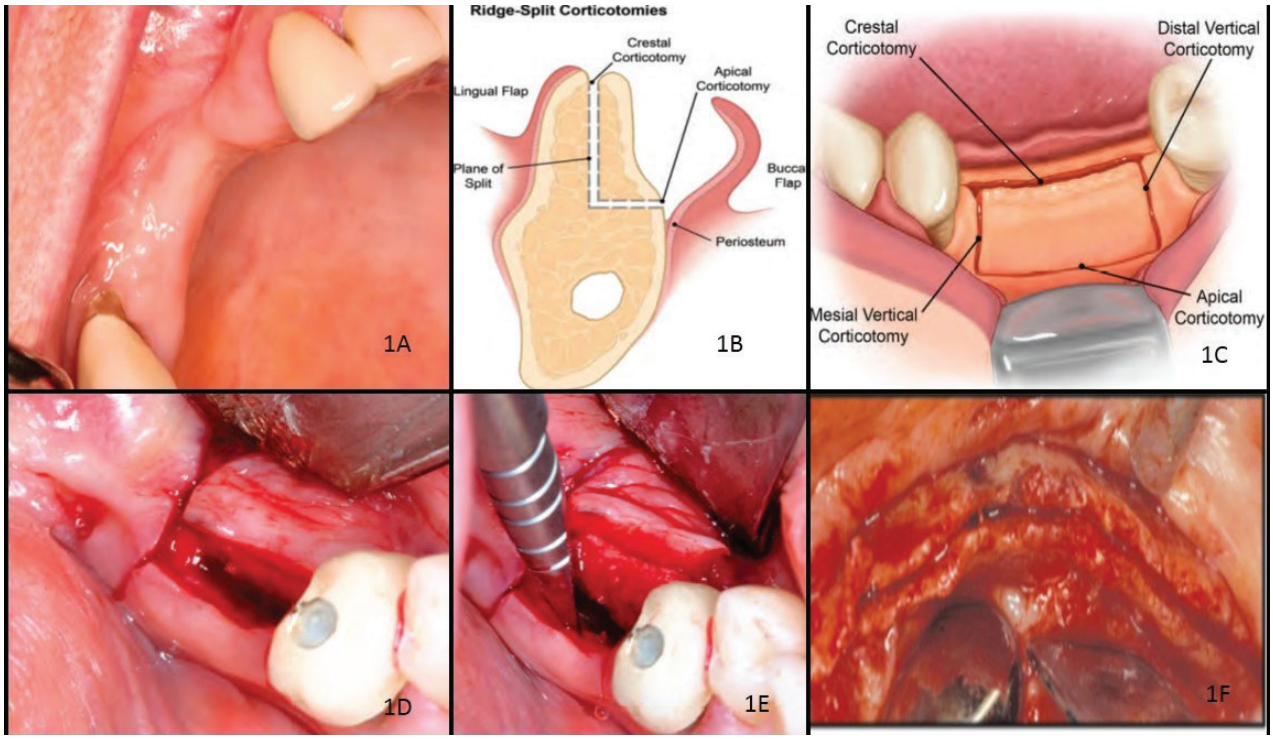

Figure: 1A (Volumetric bone loss), B (Buccal bone split), C (The four corticotomies for RSP), D (Exposure of crestal site), E (Buccal bone fractured using chisel), F (Combination of flap)

Partial thickness flap could be used to allow visibility as well as to maintain the vascularization of the bone that is to be splitted in **figure 1E**.[11]

Elian et al in 2008 gave a technique where he used a full thickness flap for the crestal area and a partial thickness flap for the rest of the elevated flap as shown in **figure 1F**.[12]

According to Tolstunov, the best way to identify the crestal groove is to vertically drop a number 15 blade and create a mark or indentation on the spongy bone where the next set of instruments (osteotomes) can be used. Variety of instruments along with osteotomes is shown in **figure 2A**.

SPLITTING

For this process to take place osteotomes and chisels are a prerequisite. The force required is a gentle tap with light to medium force to produce a greenstick fracture of the buccal segment of the bone. There are two types of buccal segment of bone i.e. "Island flap" and the "Book flap". Jensen et al in 2010 stated that once the I flap is obtained a movement of around 3mm is possible.[13] Maximum thickness or the most available thickness that provides a good result through an I flap is 2mm. **Jensen et al in 2009** conducted a study to check the marginal bone stability using 3 different flap approaches and concluded that alveolar widening by crest splitting by all three approaches resulted in alveolar width increase after 1 year but the full thickness flap resulted in some amount of gingival recession and facial bone loss.[14]

GRAFTING

The most ideal grafting material would be the one that maintains the split during the time of healing and provide osteoconduction. Xenografts like Puros, Allo-Oss, Bio-Oss and Nu-Oss can be used. The packing needs to be done by applying light pressure as shown in **figure 2B**. Once the graft has been assimilated, the graft needs to be isolated from the oral environment by the usage of barrier membranes. Non resorbable membrane would require a surgical reentry for its removal while resorbable doesn't. However, a resorbable membrane interferes with the tissue healing and predictable outcome. After desired placement of membrane, suture is given as shown in **figure 2C.**

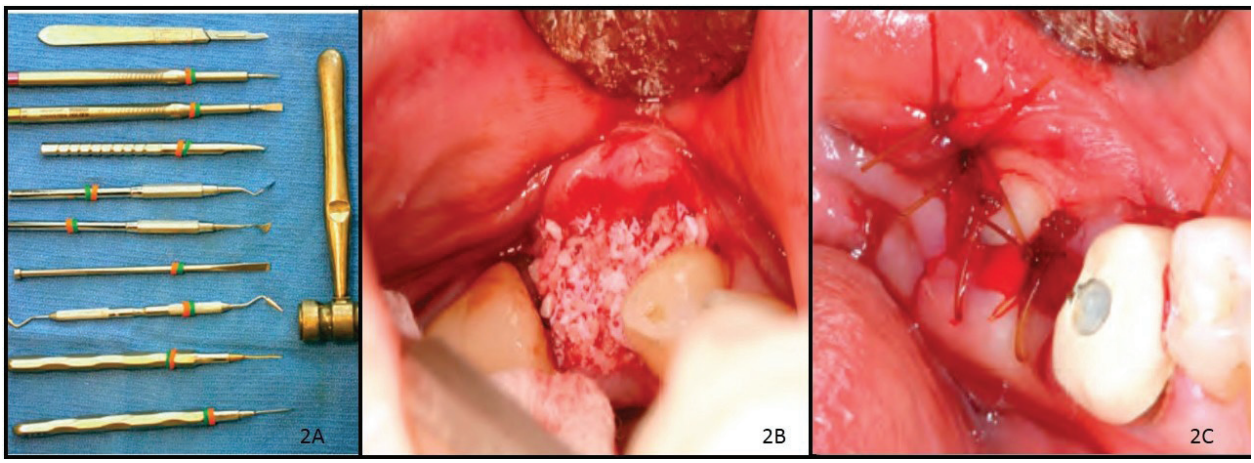

Figure: 2 A (Instruments required for RSP), B (Placement of bone graft of choice), C (Suture placed)

THREE STAGE SPLIT CREST TECHNIQUE (HU ET AL,2008)[15]

Hu et al in 2008 came up with a three stage procedure for the split crest technique. In the **first stage**, only corticotomy is done following mid crestal incision and reflection. It ends with primary flap closure and the patient is recalled after **2-3 months**.

In the **second stage**, a split crest knife is used to give an incision at the crest to only view the ridge crest or a limited full thickness flap. Then particulate bone graft is placed in the interpositional gap and then suture is placed with primary closure. Then patient is recalled

after 4-6 months for the implant placement.

In the **third stage**, patient is recalled after 4-6 months for implant placement.

ALVEOLAR RIDGE SPLIT TECHNIQUE USING PIEZOSURGERY(Moro et al 2017)[16]

Vercelotti et al 2000 introduced the use of piezoelectric surgery in the treatment of atrophic jaw. The advantage of using piezosurgery is that it is safer, easier and decreased the complications that are encountered when done with osteotomes.

In this study the bone regeneration was evaluated and found both vertical and lateral augmentation occuring with values of 3.2±0.4 mm and 5.2±0.7 mm. Use of piezosurgery reported no cases of infection and post operative complication. This study has used various kinds of piezo tips for the comfortable cutting of the bone.

The first set of tips that were used in this study was the square shaped tips which had the advantage of performing the osteotomies faster and efficiently as shown in **figure 3A, 3B**. The second set that were used in this study were with blunt edges that had the advantage of being less aggressive to avoid damage to vital structures. After splitting of alveolar bone, desired bone graft is placed along with suitable membrane as shown in **figure 3C**.

MAXILLARY SINGLE-STAGE ALVEOLAR RIDGE-SPLIT PROCEDURE[18]

Maxilla is composed mostly of spongy or the cancellous bone which is soft in nature. Here in maxilla, a crestal corticotomy along with limited vertical corticotomies are required with a split thickness flap which comprise the single stage approach.

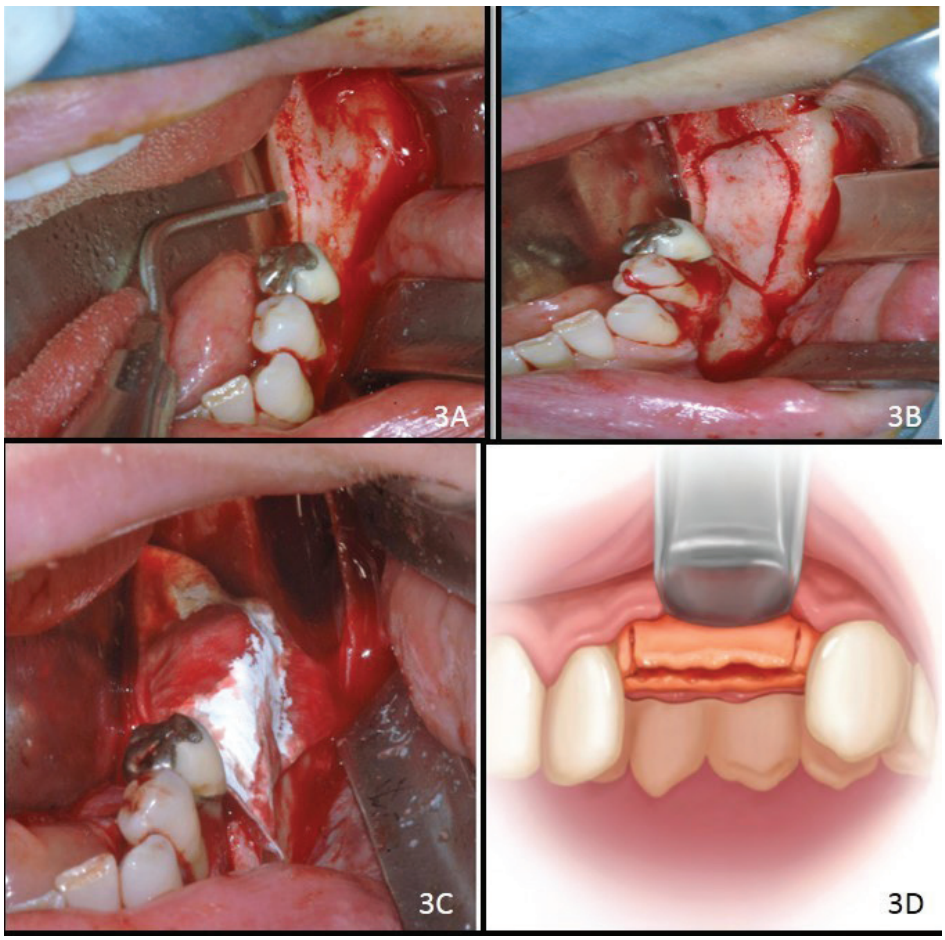

Figure: 3 A (grooving of the center using low power of Piezo), B (Prepared osteotomy site), C (Resorbable membrane covering the grafted materials), D (Maxillary single stage ridge split procedure)

The buccal flap is reflected only to the extent where proper visualization of the buccal bone (2-3 mm) can be done. Crestal incision must be given slightly to the palatal side to visualize the crestal aspect of the bone. A combination flap is the most preferred type used. The next step is to go ahead with the crestal corticotomy. The corticotomy involves a crestal corticotomy with the two small vertical corticotomies to assist in limiting the fracture of vertical component and propagating a horizontal one.

The crestal corticotomy can be initiated using a 15c blade, piezo tips or 701 bur. But the best way to initiate is the use of 15C blade to make a small initial indentation to gain access to the medullary bone. Further chisel osteotomes are used to widen the split and go apically till the vestibular depth and then the pressure is transferred laterally for the green stick fracture to take place. The best way to go about is to stay parallel and towards the palatal plate which transfers the pressure superiorly and prevents buccal thinning or fracture. The small vertical osteotomies guide the fracture in a vertical direction as shown in **figure 3D**. This controls the amount of greenstick fracture and also preserves the integrity of the close by teeth or roots.

APICAL U-SHAPE SPLITTING TECHNIQUE(WU ET AL IN 2019)[19]

In the anterior maxilla, ridge splitting technique with immediate loading is preferable.[20,21] There are many advantages to this technique that includes less use of bone substitute, immediate loading and usage of the cancellous bone for fracture less bone spreading. More the cancellous bone, better outcome can be predicted. During RST, splitting of the bone segment might result in greenstick fracture of the segment due to the concavity owing to its pattern of resorption. The principle for U shape splitting concentrates on expanding the thinnest area or the most concave area leaving alveolar crest alone.

SURGICAL PROCEDURE

Mucoperiosteal flap is reflected for the bone able to be seen. In this procedure, bone is scored in a U shape till the cancellous bone. The osteotomy is done just above the lower portion of the concavity to the depth of the cancellous bone. The vertical osteotomy was done 1 mm of the adjacent roots and the cuts are extended beyond the undercuts. Then, the released bone end was gently leveled out using a periosteotome through a greenstick fracture so as to create enough space for implant placement. Following which an immediate implant placement can be done as shown in **figure 4**.

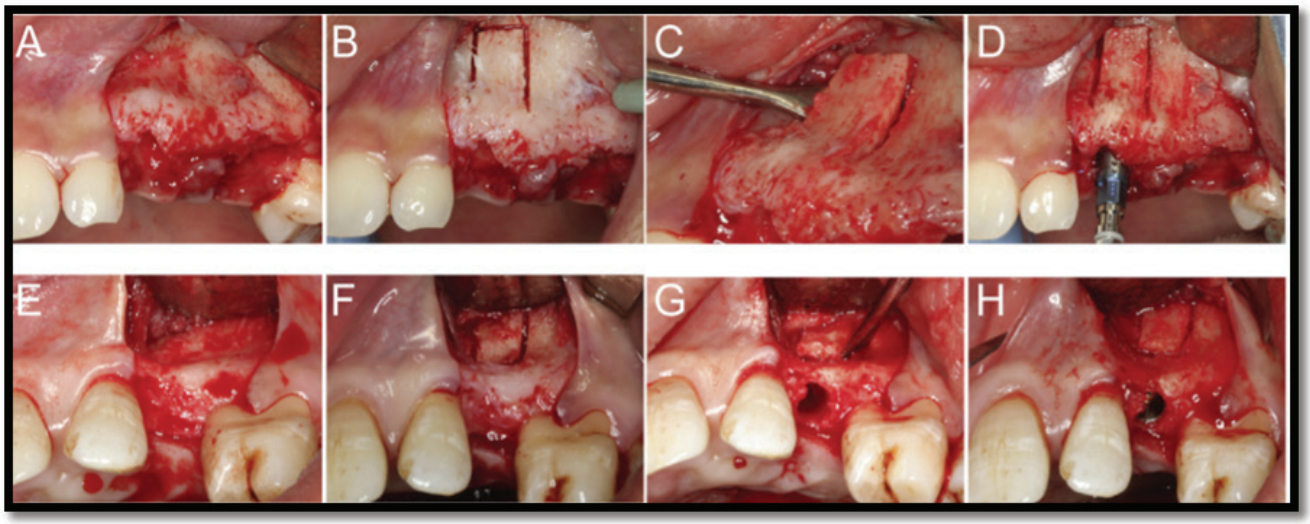

Figure 4 –Apical u shape split procedure

Wu et al in 2018 reported that the use apical u shape split procedure resulted in greater ridge width gain in comparison to GBR technique.[19] The advantages of this technique over GBR include its ability to maintain the space. This technique is however not free of limitations. Deeper defects resulted in jeopardizing the bone flap and the elevation is not possible in that case. Deeper defect resulted in thinner bone flap which resorbs faster due to lack of blood supply. Therefore, this technique holds well for the less deep defects.

CONCEPT OF BONE EXPANSION

One of the most important complications of implant dentistry is the atrophic ridge in the maxilla. Narrow crests make it difficult for the implantologists for the placement of implants which led to discovery of ridge expansion procedures. Summers in 1994 first introduced the expansion osteotomes which were cylindrical and conical in shape. The shape of the instrument explains a lot about the mechanism by which bone expansion in maxilla occurs. The first thing that needs to be understood is that the maxilla is composed of less dense bone and more of spongy bone and trabecular bone. Summers expansion osteotomes are cylindrical-conical in shape with steady escalation of the instrument diameter wherein the base of each instrument corresponds to the active part of the next one which can be clearly seen in the **figure 5A**.[22]

BONE SPREADING TECHNIQUE (BST) (Nishioka et al 2010)(23)

This technique is basically an alternative Summers osteotomy technique which involves less trauma to the bone while expanding it followed by implant placement. Screw spreaders are used with increasing diameters to expand the bone laterally as shown in **figure 5 B, C.**

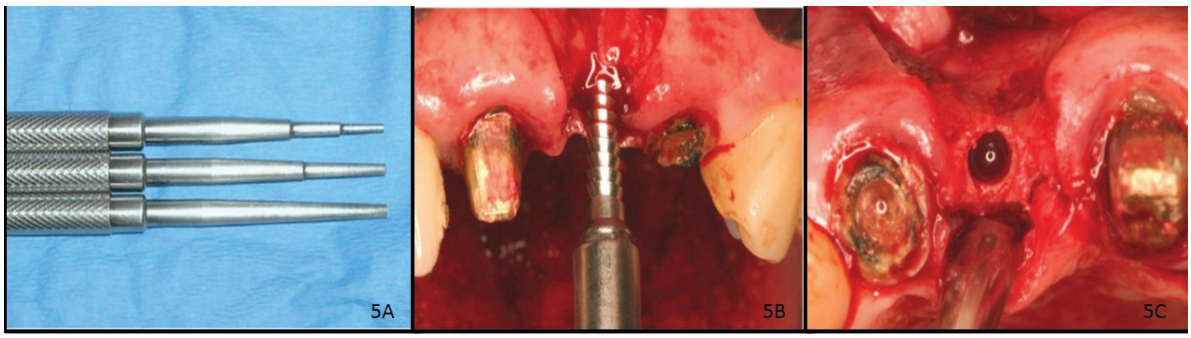

Figure: 5 A (Summers expansion osteotomes), B (Placement of thread former), C (Broadening of the cavity)

Nishioka et al suggested that this technique was feasible for the patients with a spongy bone width of 2.5mm between the cortical walls.[24] This technique allows the clinicians for implant placement in areas where the bone thickness is very less. It has a control system that controls the horizontal dimension to a certain width and also prevents excessive expansion of the medullary bone.

SPLIT-CREST TECHNIQUE

This technique uses the piezo surgery device and the ultrasonic bone surgery device to make precise cuts and osteotomies for splitting the alveolar crest giving more predictable outcome. Piezoelectric transducers have more energy in terms of bone cutting. Piezo-electric materials when subjected to intense electric field, typically in the 500–750 V/mm range.[25]The rationale of the treatment is the trauma that is caused to the bone due to the use of chisels. In the mandible, the stresses are more due to the presence of dense bone. Therefore the use of oscillating instruments is effectual and less strenuous on the patient.

EXTENSION CREST TECHNIQUE

Chiapasco et al suggested the use of a new device in 2006 called as the extension crest device as shown in **figure 6 A, B**. It was anticipated to be used for the sagittal osteotomy in case of atrophied ridge. The extension crest device provides a slow and steady expansion of the sagittal osteotomy and also reduces the risk of the buccal plate fracture.[26]

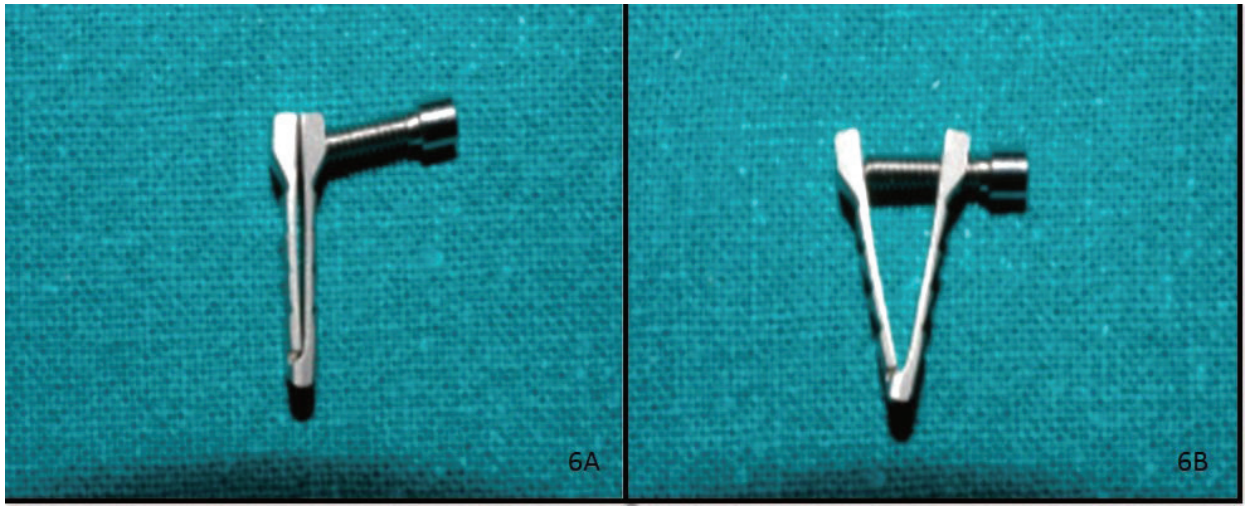

Figure: 6 A (Non activated extension crest device), B (Activated Extension crest device)

COMPLICATION

- Fracture of the buccal plate
- Infection and uncontrolled bleeding
- Neurosensory complication in case of nerve damage(Rare)

Conclusion

The literature has demonstrated different techniques of the ridge split procedure which can be used in case of resorbed ridges. The staged approach is always preferred over single stage ridge split procedure as they have higher implant success rate. Although it is an invasive procedure, it has successfully helped in rehabilitating highly resorbed ridges and should be used as and when necessary. The disadvantages however are the technique sensitivity that comes with the practitioner's experience and confidence.

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Ethical Issues: Approved

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Rehabilitating an Ocular Defect with a Custom Made Ocular Prosthesis: A Clinical Report

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Abstract

An ocular prosthesis is a replacement of the ocular part of the eye in patients due to trauma, benign or malignant lesion or congenital anomaly, there is the loss of the eye. Prosthetic rehabilitation with a customized ocular prosthesis that is proportioned and similar in appearance to that of the contralateral natural eye is needed. This maintains the volume of the orbital socket and also helps create an illusion of the presence of a healthy eye. It provides the individual confidence to adapt to a normal lifestyle and prevent them from going into psychological distress. Various techniques have been advocated for the fabrication of the ocular prosthesis comprising the use of the stock prosthesis, custom made prosthesis and micro-implants for the rehabilitation of the ocular part of the eye. A cosmetically approvable and pleasing ocular prosthesis are those which reproduces the colour, appearance and orientation of the iris to that of the contralateral natural eye. This clinical report describes a simple, economical and time-saving method for the fabrication of the customised ocular prosthesis in a patient with the ocular defect.

Keywords: *ocular defect, ocular prosthesis, eye, ocular impression, iris*

Introduction

Loss of any maxillofacial tissue or organ per se causes a substantial impact physically, physiologically, socially as well as on the psychosomatic aspect of the affected person and which can be a result of any congenital anomalies, trauma, or because of any surgical intervention. [(1)] Eyes form a major part of the human facial feature and appearance. Loss of eye not only compromises the appearance but also hampers the self-esteem of the individual. The surgical interventions associated with the removal of the eye are evisceration, enucleation, and exenteration. Based upon the surgical technique undertaken, rehabilitation of the ocular or orbital defect is done either using the available stock eye prosthesis or customised eye prosthesis or sometimes with the use of micro-implants for better retention. [(2,3)]

The Stock eye prosthesis is available in various shapes, size and colours which are used for short-term rehabilitation of the ocular defects. [(3,4,5)] . A customised ocular prosthesis is advantageous than the stock prosthesis as they help in better eyelid movements with equal distribution of pressure thereby reducing the occurrence of any ulceration, proper fit and natural appearance obtained from the size and shape of the iris, customising the sclera part of the prosthesis according to the patients need[(6,7,8)]. This clinical report describes a simplified technique used in the fabrication of customised ocular prosthesis to achieve optimal fit and giving the patient natural appearance.

Case Report

A 37-year-old male visited the Department of Prosthodontics, Institute of Dental Science, Bhubaneshwar with the complaint of ill-fitting eye prosthesis. The patient history revealed that he suffered from anterior staphyloma after which the ocular part was surgically enucleated 12 years back, and he was

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using implant-supported ocular prosthesis for the past 5 years. The patient complained of watering and itching of the eye with the use of the ocular prosthesis. Upon examination, the right eye bed was able to coordinate and synchronous movements like that of the left eye. The right eye socket appeared to shrunken as compared to the left eye (Figure 1). The patient was explained regarding the treatment protocol and fabrication of the semi customised ocular prosthesis was planned.

Irreversible hydrocolloid along with the Plaster Of Paris was used to make a primary impression of the extraoral eye impression. To make, the impression stable small pieces of gauges were incorporated into the Plaster Of Paris. Upon the retrieval of the impression, a cast is poured using Type -III Dental Stone (Figure 2). On this primary cast, a custom tray is fabricated using the pink self-cure resin (DPI-RR Cold Cure). In this custom- tray, a large hole was made in the centre to which a 10ml syringe was attached that served as a passage for the light body polyvinylsiloxane impression material (Affinis) (Figure 3A). Five to Six escape holes were made for the mechanical locking of the impression material to the tray. First, the custom tray was checked for the fit inside the eye socket and further adjustments were done according to the need. The patient was made to sit in an upright position, the custom tray along with the syringe containing the light body polyvinylsiloxane impression material was placed into the socket. Once the position is confirmed, then the light body material was injected through the inlet and the patient was asked to do different eye movements i.e. lateral, upward and downward movements so that a proper impression can be recorded (Figure 3B).

Once the second impression is obtained, a mold was obtained by immersing the secondary impression into the alginate mixture in a bowl until the level of the syringe hub tip (Figure 3C). As the alginate sets, a cut is made as shown in the figure to retrieve the impression.

The wax pattern was obtained by pouring the molten wax (baseplate wax and inlay wax combined in the ratio of 1:2) inside the alginate mold. As the wax hardens, the alginate mold was cut and the pattern was retrieved which was further shaped and carved to simulate as the scleral part of the missing eye. The wax pattern was then checked inside the patient's right eye socket for the shape, size, fullness and retention on doing various eye movements i.e. the lateral movements, circular motion and up and down movements (Figure 4A). Following this, the shade of the iris button was matched with the contralateral natural eye, then it was trimmed from the stock prosthesis and iris button was positioned in the centre of wax pattern by taking the contralateral iris as a reference by marking the distance between the inner canthus and outer canthus of the eye (Figure 4B).

Following the wax trial, the flasking , dewaxing and packing of the mold was done with heat cure tooth coloured acrylic resin mixed with part of heat cure clear acrylic resin (COLTENE, Heat cure acrylic, India) (figure-5A, B). The shade of the scleral part was initially matched with the contralateral natural eye. After the curing has been done, the ocular prosthesis is trimmed, finished and polished with pumice powder. Fine red embroidery threads are painted on to the scleral part of the eye this mimics the blood vessels that is present in the natural eye giving the prosthesis a life-like appearance. To keep the blood vessel fibres in place, the scleral portion is painted with a thin layer of monomer polymer liquid which is then allowed to set (figure 5C). The ocular prosthesis was polished, checked for any sharp edges, later was inserted into a patient's eye (figure 6). The patient was given certain post insertion instructions which he had to follow for the maintenance of the prosthesis. He was recalled for a follow after 7days, 15days and 1 month.

Post Insertion Instruction[9]:

1. Never use rubbing alcohol or any other astringent to clean the prosthesis as it can break the prosthesis.

2. Remove the ocular prosthesis at night while sleeping. Frequent removal and insertion of the prosthesis are not advised as it can irritate the socket.

3. Always store the prosthesis in cold water when removed.

4. Always clean the ocular prosthesis with antibacterial soap between your fingertips.

5. If there is any feeling of dryness, the patient is instructed to use eye drops along with the artificial eye.

7. The patient is advised for follow-up visits once a year at least.

Figure 1 : Patient with right ocular defect

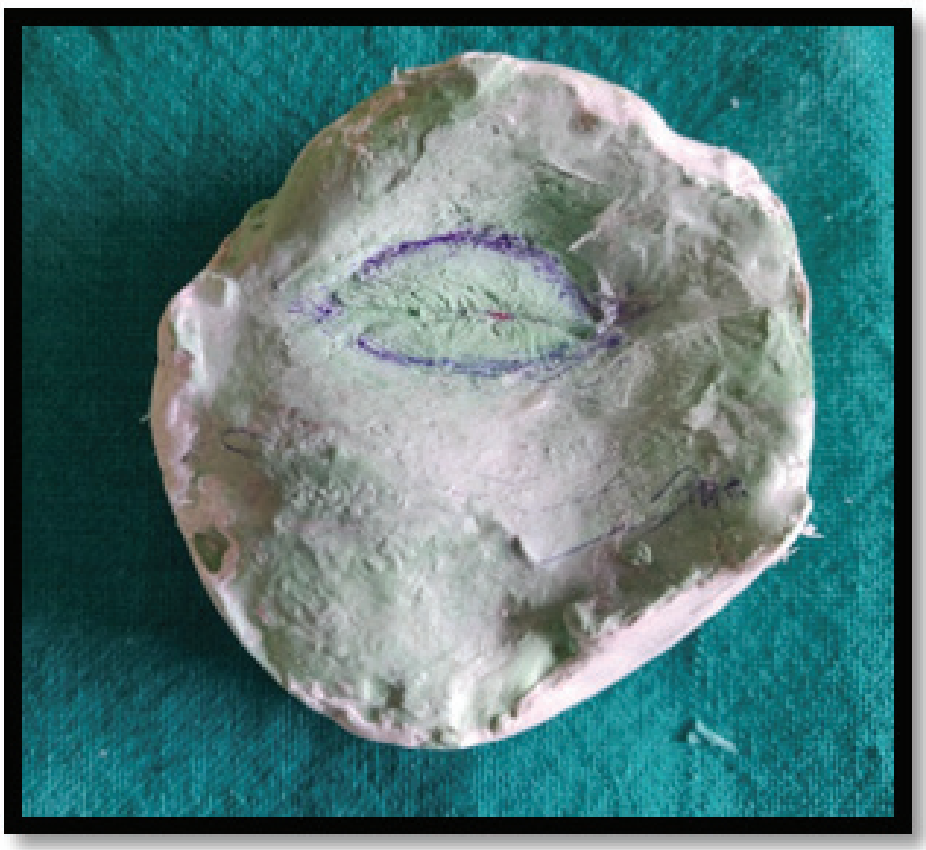

Figure 2 : Primary Cast

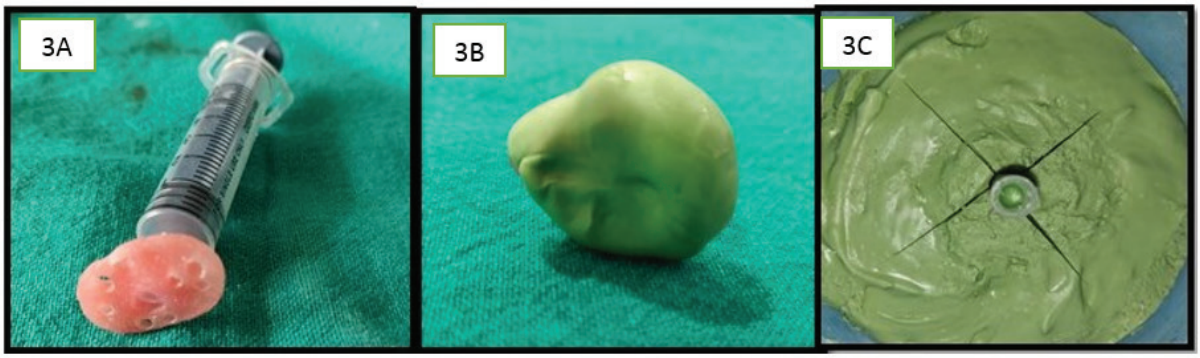

Figure 3 : A: Customised impression tray with injector, B: secondary impression using light body polyvinylsiloxane impression material, C: secondary impression immersed into alginate mixture to obtain a mold.

Figure 4: A: Wax Trial, B: Positioning of the iris button

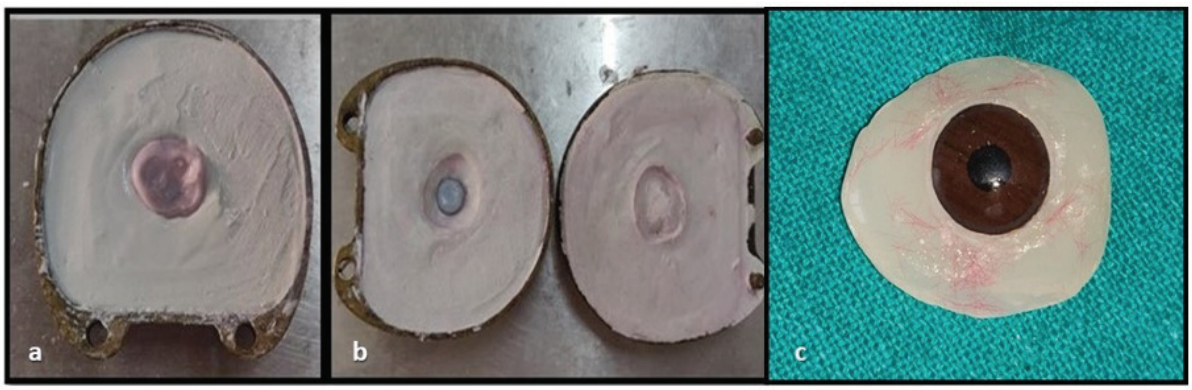

Figure 5 : A: Investment of the Wax Pattern B : Dewaxing and Packing of the Mold

Figure 6 : Patient with Definitive Ocular Prosthesis

Discussion

Ocular defects are a major maxillofacial defect that requires prosthetic rehabilitation to improve the quality of life of the patient and also boost their confidence. [10, 11] After the surgical removal of an eye, the prosthodontists play a major role in prosthetic rehabilitation procedure by providing the patient with an ocular prosthesis changing his appearance after the loss of the eye.

The customized ocular prosthesis is one of the most acceptable treatment options that satisfy the patient's need and also helps the clinician to achieve the desired goals through the meticulous work duplication of the sclera and matching of the iris. This not only requires the skill and efficiency of the clinician but also is a time-consuming procedure.

A stock ocular prosthesis is available in different shapes, sizes and colour according to the need of the patient. [12] With the advancement in techniques and availability of the newer materials, enucleated eye socket can be rehabilitated with a modified stock ocular prosthesis according to the precise fit of the patient eye. But the aesthetic of the customised ocular prosthesis is better than that of a stock ocular prosthesis. Furthermore, in the customised prosthesis, the scleral convexity can be reduced according to the patient's various eye movements which is difficult in case of stock eye prosthesis. The custom ocular prosthesis also prevents the shrinking of the eye socket thus retaining the shape of the eye socket, improves the muscular functions of the eyelids thus gradually providing a natural appearance to the rehabilitated prosthesis[13].

However Subjective errors may take place while the centralisation and placement of the iris button in the wax pattern, which is the limitation of the technique described.

Conclusion

The aesthetics of a custom made ocular prosthesis was a boon to the patient as compared to

that of a stock ocular prosthesis. The technique described in the clinical report is a simple, cost-effective procedure that provides the patient with an aesthetically appealing ocular prosthesis thus increasing the quality of life of the patient that has a deep psychological as well as the physiological impact on his personality and appearance.

Consent for publication: The patient has given valid and informed consent for publication of this case and related images

Conflict of Interests: The authors declare that they have no competing interests.

Ethical Issues: None

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Hypersensitivity- An Update

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Abstract

Dentinal hypersensitivity (DHS) can be defines as a short sharp pain that occurs as a response to various stimuli on exposure of dentinal tubules to the external environment and which cannot be described as any other disease. The most important part of the treatment is to identify the etiology behind the sensitivity and treating the condition accordingly. Differential diagnosis may include pulpitis, carious teeth, chipped tooth, cracked tooth syndrome etc. The treatment modalities depend upon whether the sensitivity is due to gingival recession, tooth wear lesion or periodontitis or related causes. This review highlights various strategies to counter this disease as well discusses various desensitizing agents recently in use like Novamin, Biomin, Calcium phosphate dentifrices, Nanoparticles and herbal desensitizing agents.

Keywords: *Dentinal hypersensitivity, Desensitizing agent, Pain*

Introduction

Dentine hypersensitivity (DHS) is a very common but an exigent task to manage among the dentists. Although many agents and techniques have been studied and applied, it still remains a testing job to manage and treat this condition. It is a circumstance that affects the overall quality and standard of living of people.(1) As per Gillam et al, there is still a lack of buoyancy among the dentists in managing this condition.(2) The objective of this review is to provide an update on the modus operandi to treat dentinal hypersensitivity and to also provide an update on the latest desensitizing agents and procedures.

DHS can be clinically described as hyper responsiveness in the form of pain of the exposed or disclosed dentinal tubules to different types of stimulus. (3) Further, it has a characteristic short and sharp pain which occurs as a result of exposure to various types of stimuli like evaporative, osmotic, tactile, chemical or thermal and also it cannot be attributed to any other form of defect or pathology.(4) However, the Canadian Advisory Board proposed for modification of this description slightly by replacing the term pathology with disease.(5) Rationale for this is to challenge the clinicians to consider other possibilities for the discomfort or pain like cracked tooth syndrome, carious tooth, uncontoured restorations, fractured cusps etc.

Synonyms used for this condition are root sensitivity, cervical sensitivity, cementum sensitivity or hypersensitivity and dentine sensitivity.

Prevalence

The prevalence of hypersensitivity varies from 4 to 57% making it a very regular finding among the general population with a female predilection.(6) People with periodontitis are mostly affected. Around 60 to 90 % people with periodontitis have hypersensitivity. (7) Teeth affected include cuspids and bicuspids of both the arches, mostly the buccal site.(8) 90% of the dentinal hypersensitivity have been observed to be at the cervical region of the tooth.(9) However, clinically it has been observed that the occlusal sites have also been affected due to the excessive occlusal wear and various abusive habits. In a systematic review by Zeola et al in 2019, the prevalence ranges from 11 to 33 percent.(10) Dentinal hypersensitivity although being the most common pathology, has been treated with less success according to some studies.(11) Thus validating the purpose of this

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review. DHS has also been associated with the gingival recession among 23% individuals.[12] Hypersensitivity following or during periodontal therapy have also been observed.[13] Therefore, for the DHS to occur exposure of the dentinal tubules to the external environment is mandatory. However, the liaison between the wearing of tooth, gingival recession and abrasion has not been explored.

ETIOPATHOGENESIS

Three mechanisms have been proposed-

- Direct Innervation
- Odontoblast Receptor
- Hydrodynamic/ Fluid movement

Of the three theories that have been proposed, the fluid movement theory of Brannstrom and Astrom is the most widely acknowledged. [14] The reason being it stresses on the dynamics of fluid movement in the dentinal tubules that activates the nerve endings resulting in pain sensation. Although, the other two theories (Direct innervation and Odontoblastic receptor) provide evidence for the causation of sensitivity but are undermined by their respective drawbacks. Direct innervation theory fails to prove the synapse between the nerve fibres and the odontoblast and Odontoblast receptor theory fails to explain the extension of the odontoblastic processes to the DEJ. Hydrodynamic theory on the other hand states that when exposed dentinal tubules are subject to external stimuli, it results in alteration of the rate of fluid flow in the tubule causing a change in pressure that activates the nerve endings causing DHS.

The rate of flow of the fluid in the dentinal tubules is dependent on the diameter of the tubule according to Poiseuille's law. Thus, as the diameter of the dentinal tubule increases towards the pulp, the rate of flow thus increases and sensitizing the nerves near the junction of pulp and dentin to cause pain or sensitivity.[15] At a molecular level, the etiology of DHS has been based on 3 hypotheses, none of which can be discarded. The first hypothesis is based on the expression of various receptors (thermal, mechanical, chemical) by the afferent fibres. The second hypothesis is based on the passage of fluid within the tubules on stimulation. The third is based on the presence of ion channels on the odontoblastic processes that extends to the pulp-dentin complex.[16]

Clinically, it occurs due to the exposure of the dentin either due to removal of enamel (faulty tooth brushing, attrition, parafunctional habits, trauma from occlusion) or due to denudation of cementum due to loss of periodontium(recession, following periodontal surgery, periodontitis).[17] The pathogenesis can be described based on two phases namely Localization of lesion and Instigation of lesion. Localization is basically exposing the dentine to the external environment and initiation or instigation of lesion is through the erosive agents that eliminate cementum and the smear film.[18]

SCREENING OF DENTINAL HYPERSENSITIVITY

Screening of the patients on their first or subsequent dental visits is an age old but effective method to identify the sensitivity issue. Questions like whether the patient is having any discomfort in their daily activities like drinking, eating or while breathing through the mouth can help screen patients suffering from this condition. Frequently the initial symptoms is tend to be ignored by the patients leading to difficulty in managing DHS in later stages and more invasive procedures need to be used to manage the condition. Therefore, proper screening of the patients needs to be done to efficiently manage and treat in a cost effective process.

HISTORY

Proper history regarding the types of food or drinks that the patient consumes on a daily basis needs to be taken after proper screening of the condition. History regarding the type of pain also needs to be taken. Whether the pain lingers on consumption of cold foods and drinks and the localization of the pain also needs to be asked. The systemic health of the patient is an also an important factor that needs to be considered and hence proper history regarding medical and dental problems needs to be enquired. History regarding extreme intake of certain foods like citrus fruits, carbonated drinks and wines needs to be taken. A proper history would decide the road of management that the clinician needs to consider to manage the condition.

OBJECTIVE METHODS TO DETECT DENTINAL HYPERSENSITIVITY

The methods could be divided into two parts i.e. stimulus based assessments or response based assessments.

The stimulus based assessments include various types of stimuli like-

- Tactile
- Thermal
- Osmotic
- Electrical

ü Tactile stimuli – The simplest method is to use a sharp explorer and run the pointed end over the area of complaint.

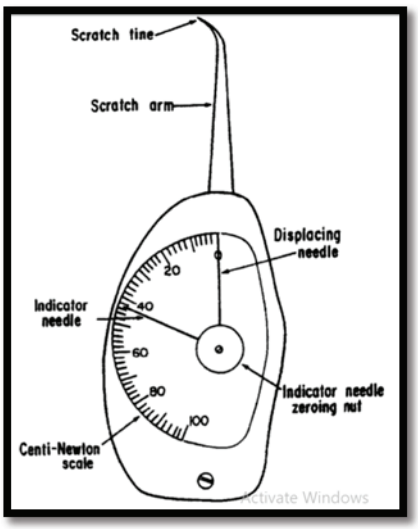

Figure 1 – Hand held scratch device

Kleinberg et al in 1990 devised an instrument to measure the sensitivity calculated by the force of displacement when pressure is applied as shown in Figure 1. A tooth is declared to have no sensitivity issues if it does not respond till 80 centi-newtons.[19]

Figure 2- Yeaple's probe

Another method was used by Schiff et al in 1994 using Yeaples probe (Figure 2) which is sensitive to pressure and uses electromagnetic force to control the amount of force applied.[20]

ü Thermal stimuli – Simplest method is to use a three way syringe at the sensitive site to record the findings. However, Smith and Ash et al in 1964 developed a device that uses a thermister probe to allow flow of current to that area and record the finding as shown in Figure 3.[21]

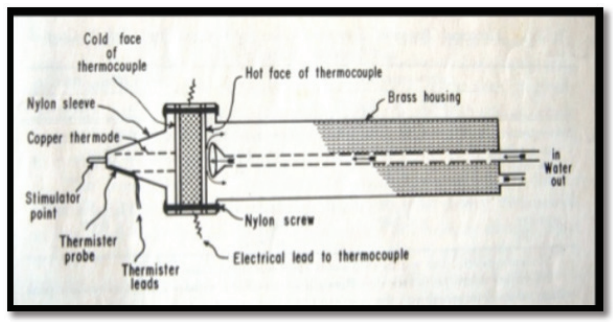

Figure 3- Smith and Ash device

ü Osmotic stimuli – Mcfall and Hamrick et al in 1987 devised an osmotic method to identify hypersensitive teeth by the use of sweet stimuli using the principle of osmosis.[22]

ü Evaporative stimuli – Brannstrom and Astrom et al first demonstrated that air blast can cause movement of fluid due to the evaporation of the fluid on that area.[14]

These assessments have some limitations that needs to be considered which is repeated stimulus can result in wrong outcomes as the threshold will increase and also the number of teeth that would be tested in a particular appointment would be less.[23]

However the response based assessments like Visual analog scale(VAS), Verbal response scale(VRS) would provide a more consistent outcome since a steady stimulus would be applied.[24]

DIFFERENTIAL DIAGNOSIS OF DHS

It is very important to eliminate the other possibilities that could cause similar kind of pain as that of sensitivity. Some of the defects can be eliminated by taking proper history like whether the pain lingers following removal of stimuli or how long the pain does last or is the pain occuring at night time only. Some defects that could be in the differential include broken tooth (bite test), cracked tooth syndrome, carious tooth with periapical pathology(tooth tender on percussion), gingival infection or inflammation(dull pain of pain deep into bone).[25] Symptoms like dull pain, lingering pain, referred pain from other sites removes the possibility of DHS. On exclusion of these defects it can be concluded that the pain is due to hypersensitivity.

MANAGEMENT OF DENTINAL HYPERSENSITIVITY

Management strategies should be based on the etiology of the DHS. The main objective should be to remove the etiological factor to prevent relapse of the condition and therefore the management should be strategized accordingly.

A. If the condition is mechanical trauma causing gingival recession, then it can be managed by various ways such as History of good plaque control, Diagnosis of mechanical trauma being the underlying cause, Patient should be made aware of the involved sites, Patient should be educated about brushing technique (Modified Stillman's technique), Patient should be advised to refrain from consumption of carbonated drinks and sweet foods, At office desensitizing treatment for pain relief, At home desensitizing agent and mouthwash should be advised with appropriate brushing technique, Follow up should be done for the patient regarding severity of DHS and brushing technique should be assessed, In case the DHS persists, surgical intervention to be done for root coverage.

B. If the condition is tooth wear lesions, then it can be managed by various ways such as History of excessive enamel loss and etiology to be identified (traumatic brushing, parafunctional habits, endogenous acids or exogenous dietary habits), Severity of tooth wear to be recorded with an apt index, Patient advocated an oral hygiene agenda to prevent tooth wear and diet dairy needs to be maintained, High fluoride remineralization is advised, Professional desensitizing treatment to be done, Systemic condition to be checked by medical practitioner for GERD, At home desensitizing toothpaste to be advised with appropriate brushing technique to avoid trauma by extreme brushing, Regular follow up to be done for tooth wear and potential etiology to be discussed, along with assessment of diet diary, Endodontic followed by prosthodontic intervention to be done if DHS persists.

C. If the condition is periodontal disease, then it can be managed by the following ways such as Previous history of periodontal problems and poor oral hygiene, Patient shown involved site and probable cause for exposure to be explained to the patient, Risk factors of periodontal disease to be discussed with patient with proper medical and personal history regarding deleterious food habits, Patient advised appropriate at home oral hygiene maintenance protocol, Management to be done non surgically followed by treatment for DHS, Periodontal parameter to be recorded for specific sites, Follow up to be done, If it persists, surgical treatment to be done alone with at home desensitizing procedures and maintenance of oral hygiene, Follow up with proper recording of periodontal parameters with desensitizing treatment to be done.

DESENSITIZING AGENTS

Scherman and Jacobsen et al in 1992 classified based on the basis of –[26]

- Method of application
- Treatment at home
- Treatment at dental office

v Mechanism of action

v DESENSITIZING NERVE- KNO_3

v PRECIPITATION OF PROTEIN COMPOUNDS- $ZnCl_2$, $AgNO_3$, Gluteraldehyde

v DENTINAL TUBULE OCCLUSION- NaF, SnF, KHC_2O_4, $Ca_3(PO_4)_2$, $CaCO_3$, Arginine

v ADHESIVE SEALERS- Flouride varnish, GIC, Composite, Resins

v LASERS

v HERBAL DESENSITIZING AGENT- Propolis, Triphala, Lavanga, Palakya, Cinnamon

There are various desensitizing agents available in market such as

A. Potassium nitrate(SENSODYNE PRONAMEL): It's mechanism of action is due to its oxidizing nature and/or due its crystallization process. Zawaideh et al in 2017 stated that ProNamel reduced the change in microhardness occuring due to acids.[27]

B. Oxalates (Oxagel): It's mechanism action is as a precipitation of oxalates and occlusion of tubules thus preventing dentinal fluid flow.[28] Pereira et al in 2005 stated that the oxalates tend to be resistant to dissolution by acids.[29] However Cunha Cruz et al in 2011 stated that there is no added benefit from the use of oxalates.[30]

C. Calcium Phosphate dentrifices(G.C TOOTH MOUSSE): It's mechanism action is as combining ACP and Casein Phosphophopeptide to localize at the plaque area and results in remineralization of enamel at a very fast rate.[31] Tung et al in 2003 stated that they imitate the natural process of sclerosing dentin and is very biologically compatible.[31]

D. Amorphous calcium sodium phosphosilicate (NOVAMIN): It's mechanism action is as effectively delivering silica, calcium, phosphorus and forms an occlusive layer over dentin. Kakodkar G et al in 2013 stated that it has the dual advantage of bleaching and densensitization.[32] Millemann J et al in 2012 stated that it has the potential clear white spot lesions.[33]

E. Fluoride containing Bioglass (BIOMIN, NUPRO): It's mechanism action is as high flouride release and the substantivity for 8-12 hours and its microparticles contents that help penetrate the tubules deeper providing better effect. Cruz et al in 2018 stated that incorporation of bioactive glass helps accentuate the process of treating DHS.[34] Reddy et al in 2019 concluded that Biomin proved to be better than other dentrifices like strontium chloride and potassium nitrate at the end of 4 weeks.[35]

F. Arginine (PRO ARGIN TECHNOLOGY): It's mechanism action is as using the physiological pH of saliva for deposition of a calcium rich layer in tubules and its arginine, calcium carbonate and bicarbonate contents as a buffer. Hamlin et al in 2009 concluded that dentrifice containing 8% Arginine and calcium carbonate significantly reduced hypersensitivity when as a pre procedural toothpaste. Bekes et al in 2017 conducted a study to evaluate the efficacy of *% Arginine and calcium carbonate and concluded that it reduced DHS over a period of 8 weeks.[36]

G. Nanoparticles Hydroxyapatite (DESENSIBILIZE NANO-P PASTE): It's mechanism action is as a Nano hydroxyapatitie particles have the same structure, morphology as the HA in enamel and dentin, thus replaces it better. Wang et al 2016 concluded that Desensibilize Nano-P paste effectively treated DHS significantly comparable with Pro argin toothpaste and potassium nitrate.[37]

H. TRIPHALA: It's mechanism action is as obtunding action on the dentinal nerves for its eugenol content. Kumari et al in 2013 stated that a herbal desensitizing dentrifice containing Triphala, spinach, suryakshara helps in reducing DHS over a period of 12 weeks.[38]

I. PROPOLIS: It's mechanism action is as occlusion of the dentinal tubules for its resins, wax calcium and aluminium contents and thus preventing fluid movement. Kripal et al 2019 in an invitro study showed that Propolis varnish significantly reduced the open tubules, thus has a desensitizing effect.[39]

Conclusion

Dentinal hypersensitivity is a pathology that needs to be managed carefully and in meticulous manner. In this review several strategies and desensitizing agents

have been discussed which if used as and when required could possibly treat hypersensitivity issues. However, there are many desensitizing agents that are undergoing research which could provide faster relief and thus give an effective treatment to the patients.

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Ethical Issues: Approved

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In Vitro Evaluation of Antimicrobial Efficacy of the Different Concentrations of Chitosan against *Staphylococcus epidermidis* in Disinfection of the Root Canal System

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Abstract

During endodontic procedures, along with mechanical instrumentation, irrigants are imperative for pathogen eradication. As the strains of pathogens are becoming antibiotic resistant at a constant ascending pace and also the potentially harmful effect is associated with synthetic drugs, so studies on natural alternatives for irrigation are being done. Chitosan, a natural alternative, has been evaluated as an antimicrobial medicine. Recently, in cases presenting with persistent infections after endodontic treatment, it was noted that of all the microbial species identified, the prevalence of *s.epidermidis* was the most. In this current study, an analysis of the antimicrobial effectiveness of chitosan as a potent root canal irrigant, for disinfecting the root canal was conducted. **Methodology:** By the serial dilution method, the minimum inhibitory concentration was determined. Dilution of the test solution was done using 10% DMSO solution. After which, incubation in a rotary incubator, at 180 rotations per minute, the temperature of 37℃, for 24 hours was done. For obtaining the zones of inhibition of different concentration (0.2%, 0.5%, 0.75, 1%) of chitosan irrigant, the agar diffusion method was applied and the resultant clear zone was measured with a ruler. **Result:** Chitosan (liquid) had promising antibacterial activity against *Staphylococcus epidermidis*, with a 16 mm zone of inhibition at 100 µg/ml. **Conclusion:** Within the limitations of the present study, it was observed that, even at lower concentrations, chitosan (liquid form) effectively hindered the further growth of *S.epidermidis*.

Keywords*: Chitosan, Antimicrobial efficacy, S. epidermidis, root canal system*

Introduction

It has been established that the preeminent cause behind pulp infection and periapical disease is microbes.[1,2] Polymicrobial character is specific to endodontic infection, which is of intraarticular or extraarticular origin. In primary intraarticular infection, apart from fungi(*candida spp*), viruses, the commonly found pathogens are gram-negative anaerobic rods, *Fusobacterium, dialister*, spirochetes, *Tannerella forsythia,* gram-positive anaerobic rods, gram-positive cocci. Endodontic pathogens associated with extraarticular infection are usually anaerobic bacteria, *Actinomyces spp, Prevotella spp., Fusobacterium nucleatum* to name a few.

Enterococcus faecalis (E. faecalis) and *Candida albicans (C. Albicans*) are commonly detected in root canals that are already endodontically treated but still showing signs of infection.[3-5] But in some recent studies, the presence of microbes other than commonly found ones are mentioned. *Murad et al* stated that, when investigated by checkerboard DNA-DNA hybridization, *S.epidermidis* along with *E.faecium* were the most prevalent microbes in cases of persistent

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endodontic infection.[6] With the total occurrence rate of staphylococcal species in the oral cavity (saliva) being 83.9 %, s.epidermidis is the second most frequently found species in the oral cavity (41.4%). Bacterium *s.epidermidis* is a facultative anaerobe, i.e., it can survive in both the aerobic and anaerobic situation.[6]

During endodontic procedures, in conjugation with mechanical instrumentation, irrigants are imperative for thorough pathogen eradication from the root canals. The antimicrobial efficacy as shown by irrigants is of extensive levels. The features supposed to be present in quintessential endodontic irrigants are superior antimicrobial efficacy, non-cytotoxic, prudent towards the tissues of the periapical region, endotoxin inactivation, lubricating nature and breakdown of tissue.[7-9]

Of all the irrigants used in endodontics, Sodium Hypochlorite(NaOCl) is considered as the gold standard.[7] The major disadvantage of NaOCl is that when near vital tissues, it irritates leading to severe inflammatory reactions.[10-12]

As the strains of pathogens are becoming antibiotic resistant at a constant ascending pace and also the potentially harmful effect is associated with synthetic drugs, so studies on natural alternatives for irrigation are being done.

Chitosan, a natural alternative, has been evaluated as an antimicrobial medicine. It is a Polysaccharide and cationic. The chemical structure of chitosan (Figure1) comprises of "copolymers of glucosamine and N-acetylglucosamine". The main characteristic feature of chitosan being biocompatible, biodegradable, bioadhesive, non-cytotoxic along with its ability to chelate for varying metal ions, even in acidic condition, makes chitosan an enticing potent irrigant in endodontics.[7]

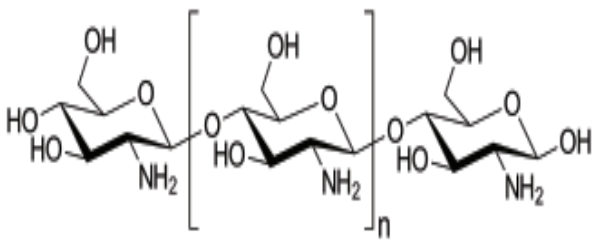

Fig 01: chemical structure of chitosan

So, the current study mainly aims at the analysis of the effectiveness of chitosan, in two forms, the powder and the liquid form, against *S.epidermidis* as a potent irritant in the root canal system.

Material and Methodology

The Agar-well diffusion method was used for antibacterial assays of chitosan.

Bacterial strain: Biofilm-positive strain of *s. epidermidis* was incorporated in this study. [Strain: RP62A / ATCC 35984]

Bacterial Culture*:* Agar was taken as the culture media for the culture of the *s.epidermidis*. For testing the efficacy, three Petri dish were taken, one for the reference control group ampicillin, and remaining two for the two forms of chitosan viz., the powdered form and the liquid form. Into all three of the pre-sterilised Petri dish of 6 mm depth, the culture media was dispensed and the bacterial lawn was prepared that was fully punched. After 30 minutes of the preparation of the lawn, 5 wells were prepared on the lawn. 50 µl of molten nutrient agar was then put as a base in all the 5 wells. Following which, 100 µl of the test compound, chitosan was poured in the well, containing the molten nutrient agar. Each of the 5 wells in the Petri dishes, had a different concentration of chitosan(0.25%,0.5%,0.75%,1%). Incubation of the plates was done at a temperature of 37°C, duration of 18-24 hours and was then refrigerated until further use.

The powder form of chitosan was from Bangalore Fine Chemicals (BFC), India. (Item model number: BFC260274). To prepare the stock solution of powder form of chitosan, the addition of 1gram of chitosan powder to 100 ml of 0.1M acetic acid under mild heat (40-50 °C) with continuous agitation for almost 5 hours was done. The insoluble chitosan of higher molecular mass was removed by filtration.

The chitosan in the liquid form came in a glass bottle of 2% concentration. The different concentrations of both the forms of chitosan were achieved by diluting the stock solution with 10% DMSO solution(dimethyl sulfoxide)

The Minimum Inhibitory Concentration (MIC): The lowest concentration at which no visibly appreciable turbidity can note is known as Minimum Inhibitory

Concentration. The preparation of the bacterial culture was done as such so that the McFarland standard turbidity of 0.5 could be obtained. The determination of MIC was gained by serial dilution of 0.25%, 0.5%,0.75% and 1% of chitosan. At 180 rotations per minute, the incubation of the well plates was done for 24 hours, at a temperature of 37°C

Measuring The Zone Of Inhibition: For determining the zone of inhibition, agar well diffusion method was chosen for this study. Onto a trypticase soy agar plate, *S. epidermidis* was sub-cultured, followed by incubation for a period of 24hours, at a temperature of 37°C. As a reference for adjusting the turbidity, 0.5 McFarland's standard was considered. On completion of 24hours, suspension of bacterial colonies in the culture medium was done. The turbidity of the bacterial suspension was adjusted according to the standard. (Figure 2).

On the superficial layer of the trypticase soy agar plate, an even layer of bacterial suspension was laid out. It was allowed to dry for some time of 5minutes. Already sterilized Whatman disc, having a radius of 3mm was saturated in 20 µL of chitosan, in both powdered and liquid form, at different concentrations. While maintaining an adequate distance between the discs, the placement of the trypticase soy agar plate was carefully executed. Following which, incubation for 24 hours and at a temperature of 37°C was done. From the zone of inhibition, the evaluation of the antibacterial activities was done by measurement of the diameter of the zone, using an electronic digital calliper (6 inches).

Reference Control: As a reference control, ampicillin having a concentration of 30µg/ml was taken.100µl aliquot was analysed. The zone of inhibition of ampicillin had a diameter of 21mm (average).10% DMSO solution having null cytotoxic and antibacterial activity was taken.

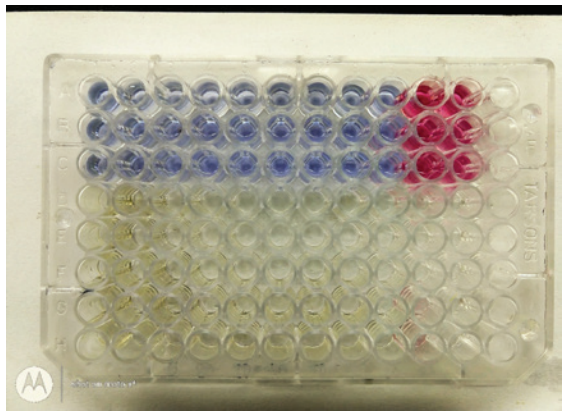

Figure 02:Minimum Inhibitory Concentration of chitosan against Staphylococcus epidermidis

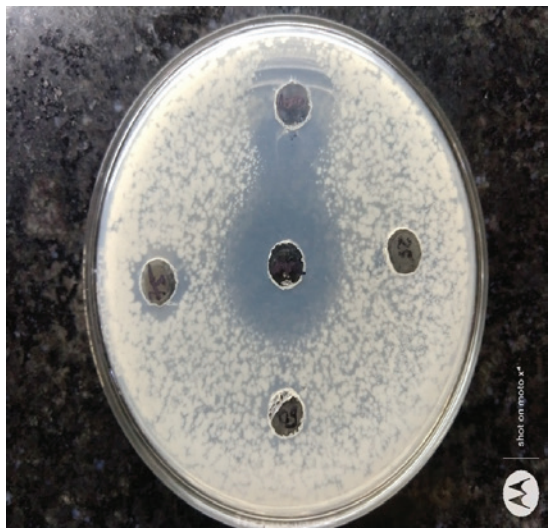

Figure 03: Antibacterial effectivity of chitosan (powdered form) against *Staphylococcus epidermidis*

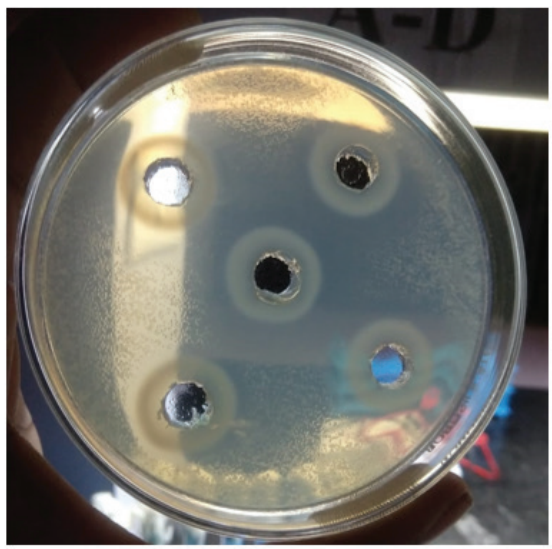

Figure 04: Antibacterial effectivity of chitosan (Liquid form) against *Staphylococcus epidermidis*

Results

The powdered form of chitosan had negligible antibacterial activity against *Staphylococcus epidermidis* (fig.03). Only 6 mm zone of inhibition was observed, which was very low when compared to the positive control group of standard antibiotic Ampicillin (30 µg /ml). (table:01). On the other hand, Chitosan in the liquid form had promising antibacterial activity against *Staphylococcus epidermidis* (fig.04). A 16 mm zone of inhibition at 100 µg/ml was observed, which was comparatively slightly higher than the positive control group. Even at lower concentrations of 0.25, 0.5 & 0.75 chitosan in the liquid form exhibited moderate antibacterial activity. (table:01) The Minimum inhibitory concentration (MIC) of chitosan (liquid) was recorded at 30 µg/ml.

Table 1: Zone of inhibition and minimum inhibitory concentration of the different concentrations of chitosan in both the powder and the liquid form

Sample	Concentration	Zone of Inhibition	MIC
Chitosan (powdered form)	25µg/ml	-	- - - -
	50µg/ml	-	
	75µg/ml	-	
	100µg/ml	6 mm	
	Ampicillin 30 µg/ml Positive Control	15 mm	
Chitosan (Liquid form)	25µg/ml	10 mm	30 µg/ml
	50µg/ml	12 mm	
	75µg/ml	15 mm	
	100µg/ml	16 mm	
	Ampicillin 30 µg/ml Positive Control	15 mm	

Discussion

Bacterium *S. epidermidis* is a facultative anaerobe, i.e., it can survive in both the aerobic and anaerobic situations. Character specific to S.epidermidis is biofilm mode of growth and its attachment to the uncoated abiotic surfaces. As endodontic infection is primarily biofilm-oriented, so for successful treatment and prognosis, disruption of the pathogens through thorough irrigation and ultimately leading to its eradication is of prime concern. [11]

For a good prognosis and for the outcome of endodontic treatment to be successful, along with proper instrumentation of the root canal, irrigation is also of prime importance. Chitosan, a potent irrigant, is isolated from the crustaceans (Phylum: Arthropoda). The shells of the organisms are then treated with a substance, alkaline in nature, preferably sodium hydroxide. When the chitin shells are deacetylated partially, chitosan as an end-product is acquired. It demonstrates antimicrobial activity against a wide range of microbes, including bacterial species, fungal species and also viruses as it interferes with the cellular walls and membranes of microbes, leading to lysis of the cell. Chitosan is biocompatible, biodegradable, bioadhesive and is non-cytotoxic by nature. [12]

The production cost being comparatively low, the serviceableness of chitosan has increased manifold in the field of medicine and pharmaceuticals. Not only in the medical field, but also in dentistry, in the branch of periodontology, the successful usage as a "barrier membrane" is evidenced. Another commendable property of chitosan is its ability to chelate for varying metal ions, even in acidic condition. The antimicrobial efficacy of different forms and concentration of chitosan was evaluated in this study. The current study revealed that 1% of chitosan had an antimicrobial effect compared to the control group,i.e., ampicillin 3%. The agar diffusion method and MIC was used to determine the concentration of chitosan against *S.epidermidis.*[13-15]

Previous studies advocated that despite the root canal system is complex, the antimicrobial property of chitosan was adequate. The outcome of this current study also complies with the already published literature. The cation active structure of chitosan ascribed to the superlative antimicrobial property of chitosan.[15] As the bacterial cell wall is negatively charged, so when the oppositely charged chitosan binds easily to it, the imperative functions of the bacterial cell wall gets disrupted, ultimately leading to apoptosis.

The effectiveness of the antibacterial property of a test material/compound is directly related to the size of the zone of inhibition. The larger the clear area on the disc, the more is the antibacterial efficacy of the compound against the microbe. Standard antibiotic, Ampicillin, which is a broad-spectrum antibiotic was taken as the reference control group. This group exhibited a zone of inhibition of 15mm.

Chitosan-liquid showed a zone of inhibition of 16mm at a concentration of 1%, which was slightly higher, by 1mm, than the reference control group, ampicillin. At a concentration of 0.25%, 0.5% and 0.75%, the zone of inhibition was found to be 10mm,12mm and 15mm respectively. So even at a lower concentration, Chitosan-liquid resulted in moderate antibacterial activity. At a concentration of 1%, Chitosan-powder showed a zone of inhibition of just 6mm, which is very low, as compared to the reference control group. At lower concentration of 0.25%, 0.5%, 0.75%, no zone of inhibition could be appreciated. When compared between the different forms of chitosan, i.e, the powder and the liquid form, it was seen that even when the concentration was kept the same, the two forms of chitosan showed a stark difference in the result. At a concentration of 1%, while chitosan-liquid showed result superior to that of the control group, chitosan-powder showed the almost negligible result.[16]

Also at lower concentrations (0.25%,0.5%,0.75%), the moderate antibacterial activity could be seen in case of the liquid form of chitosan. But no activity could be seen in the powdered form. So, on comparing the antibacterial activity between the two forms of chitosan, the liquid form showed a promising result. The poor result of the chitosan in the powdered form may be related to the particle size and particle solubility. The size of the particle influences the bacterial cell wall penetration. Also higher the degree of deacetylation, more is the solubility of chitosan in 0.1M acetic acid. It has been reported that lowering the particle size has a positive effect on its antibacterial property. Of all the methods of investigating the antibacterial properties, the

agar diffusion test is one of the entrenched ones. The major benefit of the agar diffusion method lies in the fact that the chemical properties of the test medicament don't undergo any change in the whole process. Just by asserting the specific microbial isolates present in the antimicrobial disc, the detection of the antimicrobial efficacy of the medicaments can be accomplished. In the current study, the largest zone of inhibition was exhibited by the liquid form of 1% chitosan against *S. epidermidis*. It showed a zone of inhibition which was more than the control group. After taking into account the disparity in the data of minimum inhibitory concentration and the zones of inhibition evaluated in the current study, the speculation that can be contrived is, even at a low concentration, liquid form of chitosan can be an irrigant of choice, a potent irrigant against *S. epidermidis*.[17]

Further researches including the antimicrobial effect of chitosan in the presence of other microbes, either a single species or multiple species, that are found in the biofilm is obligatory.

Conclusion

The antimicrobial efficacy of chitosan, a natural alternative to the conventional irrigant was evaluated in this study. Within the limitations of the present study, it was observed that, at a concentration of 1%, chitosan-liquid constrained the microbial growth of *S. epidermidis*, showing result superior to that of the control group(ampicillin), while chitosan-powder showed almost negligible result. At a concentration of 0.3 %, chitosan in the liquid form showed promising antimicrobial efficacy against *s. epidermidis.* So considering the antimicrobial properties that were evaluated in the study, chitosan can be possibly used as an endodontic irrigant.

Ethical Permission: Not Required

Conflict of Interests: None

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Intraosseous Injection: A Review

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Abstract

The purpose of this article is to detailedly study the intraosseous (IO) injection in dentistry. Successful treatment of patients relies on the effective manner of delivering local anaesthesia. The conventional aspirating syringe is the widely used method of administration of local anaesthesia. Problems with administration include delays between injection and effect, the long duration and post-operation numbness of patients. But newer methods have been developed that can aid in alleviating pain among which intraosseous injection is one. This article also focuses on uses, techniques, advantages, disadvantages, and site of administration of injection and duration.

Keywords*: Intraosseous anaesthesia; Techniques; Advantages and Disadvantages*

Introduction

Most of the dental treatments rely on effective management of pain. An inevitable side of dentistry is unsuccessful local anaesthesia. Several elements can contribute, which may be related to either the patient or the dentist. Factors related to the patient may be anatomical, pathological, or psychological. Whenever the conventional method of aspirating syringe fails, the advanced method of intraosseous injection can be applied.[1]

The intraosseous injection is a specialized method that enables the deposition of a local anaesthetic solution exactly into the cancellous bone close to the tooth which has to be anaesthetized. Due to the thickness of the cortical plate, the infiltration injections with lidocaine solution are not convincing for anaesthesia of the molar teeth of the mandible and hence clinicians do not endeavour infiltration in the posterior mandible. Overcoming this problem, the intraosseous injection allows direct access to the bone which is cancellous.[2] Hussein et al compared these two injection techniques, using 1.8mL of 2%lidocaine with 1:100,000 epinephrine, in the lateral incisor of the maxilla. The two techniques were indistinguishable except the intraosseous technique which had a shorter duration of anaesthesia as well as a quick onset. Many studies have been issued on intraosseous technique along with an effective rate of anaesthesia of pulp of 41-96% which has been based on the pathology, the tooth of concern, treatment, and method of assessment. Three devices are currently within easy reach. They are the Stabident system, X-tip system, and the Intraflow hydroxytryptophan anaesthesia. The intraosseous injection can be operated by utilizing the conventional or specialized instrument. When a vasoconstrictor-containing solution is used, intraosseous injection becomes more efficient.[3,4]

The specialized instrument comprises a suitable perforator and needle. The best intraarticular area is recognized by the help of a radiograph for anaesthetic injection. If it has not anaesthetized the gingiva then in the area of perforator a small volume (0.1ml) of anaesthetic solution is infiltrated. Within the attached gingiva the area to be perforated is present 2mm below the gingival margin of nearby teeth in a vertical plane intersecting interdental papilla. Well-fixed to a dental handpiece, the perforator is pushed on through the buccal cortex till a distinctive drop is experienced into the cancellous area of bone. The perforator is pulled out and the small 6mm 30 gauze needle is pushed out and the small spot in the bone and slowly about 0.2-0.5ml is injected.[5]

Reasons For Failure Of Anaesthesia

There can be different reasons for which local

anaesthesia may fail to work. Variations in the positions of nerves, as well as foramina, can lead to failure of anaesthesia in the conventional method. In dentistry, the important foramina do not have a constant location for regional block anaesthesia among patients. Furthermore, other causes for failing of anaesthesia can be the thickness of the bone, inflammation of the pulp, and pathological changes relayed to soft or hard tissues.[6]

Nerve Supply

There can be innervations from more than one nerve trunk which teeth may encounter. Failure of anaesthesia following infiltration injection and block techniques can be due to accessory nerve. Upper molar teeth are supplied by greater palatine nerves therefore an infiltration is not likely to attain transference buccally at upper first molar teeth. In the very same manner, maxillary anterior teeth are supplied by nasopalatine teeth. The long buccal nerve supply rarely supplies lower molar pulp and a long buccal block or mandibular infiltration may be needful for absolute anaesthesia in these cases. Pulpal supply of mandibular teeth may be contributed by the lingual nerve but it can be usually restrained by the lingual nerve block along with the inferior alveolar nerve block.[7]

Pathological Causes For Unsuccessful Anaesthesia

There can be numerous factors which may include anatomical alterations due to surgery or any trauma. Trismus can be another crucial factor which is the most common factor in dentistry. The solution is such a case can be buccal infiltration in the upper jaw with closed month. The palatal tissues inpatient with trismus can be anaesthetized by the use of intraosseous anaesthesia with help of advanced needle towards the palate via mesial and distal gingival papillae buccally.[8]

Bone As Barrier For Diffusion

The significant factor for the diffusion of anaesthesia is the thick cortical plate of the mandibular alveolar process that prevents infiltration in grown-up patients with the exclusion of the midline of the mandible.

Inflammation Of Pulp

A very commonly noticed fact is that tooth with inflamed pulp may be difficult to numb. The reason behind this is the low pH in an inflammatory area that influences the working of the local anaesthetic solution by reducing the concentration of unionized fraction that spreads via nerve sheaths. Likewise, inflammatory areas have an enhanced supply of blood as of vasodilatation which might rise anaesthetic 'washout'.Local anaesthesia in high anaesthetic is not practicable.[9]

Techniques

The intraosseous system which has been purposeful clinically are first, the Stabident system(Fairfax Dental Inc., Miami, FL) and secondly, the X-tip system (Dentsply, York, PA-the X-tip system newly has been procured by Dentsply International). Other systems have been advanced-the Intraflow (Pro-Dex Inc., CA) as well as the Comfort Control Syringe (Dentsply International).[10]

Stabident System

This system consists of a slow-speed handpiece driven perforator, a solid 27-gauze wire with a bevelled finish which when operated, through the cortical plate produces a small hole. The delivery of the anaesthetic solution to the bone, which is cancellous is via the 27-gauze ultra-short injector needle put into the hole produced by the perforator. Worthwhile benefits are given by the Stabident method. This permits a reduction in time amidst the anaesthetic injection and accomplishment of anaesthesia. Local anaesthesia requirement is reduced as one cartridge is generally needed.[11]

X-Tip System

A newly discovered system namely the X-tip was set in motion in the year 1999.Dr. Arthur(Kit) Weathers from Griffin innovated the technique of intraosseous injection with the X-tip. Dr Weathers invented a simple and beneficial approach to penetrate bone while simultaneously, giving dentists opportunity and proficiency to effortlessly re-enter the penetrated site after which the clinician can inject anaesthesia straight into the cancellous bone.

The system of X-tip is packed in a sterile packet. In the packet, there is a vial that has the X-tip material. The X-tip is made up of 9mm 27-gauze hollow perforator that is positioned inside a 7mm23-gauze cannula which is called a guide sleeve. So, the perforator overhangs 2mm

beyond the guide sleeve. This part has a 15mm extended universal latch-type fitting for a slow-speed handpiece. The perforator as well as a cannula, both are enveloped with a protective red cap. Also, an ultrashort needle of 27-gauze(8mm long) is present in this system.[12]

For use of X-tip, according to this technique, the first step is to take a radiograph of the interdental area which is to be perforated for the dentist to visualize and to verify sufficient interproximal bone is present between teeth thereby not rupturing the periodontal ligament. Various risk areas are also present which has to be taken care such as the mental foramen region, an impacted third molar area present horizontally, or a low-lying maxillary sinus area. Again due to the presence of mainly cortical bone in the anterior region i.e. in between the central incisors, IO anaesthesia will not be functioning properly. In such a case, clinicians have to give topical anaesthesia, and then after 0.3-0.4 ml of local anaesthesia has to be injected at the site selected to perforate in the buccal vestibule.[13]

The soft tissue site where perforation has to be done should lie in the attached gingiva,2mm coronally towards the mucogingival line between teeth. In the mandible, it is suggested to go distal to the tooth which has to be worked on for perforation because of the course of nerves present. At the same time, in the maxilla because of greater porosity in the bone it is of less importance. As there is a lack of attached gingiva in cases of periodontal diseases, this method of X-tip is unfit to be in use.

After fitting of perforator along with the guide sleeve to the slow-speed handpiece, one can start up the perforation process. It is achieved by a striking motion and this motion should be conducted no longer than 2s to prevent heating of the bone which may induce the necrosis of that region. During the perforation of the cortical bone, the operator can feel the piercing into the bone. The guide sleeve has to be gripped against gingiva with the help of a pair of cotton pliers while the perforator and slow speed are withdrawn. After this, an ultrashort needle (27-gauze) can be instituted via the guide sleeve. One-third of the cartridge should be put into the region. Slowly but surely the injection has to be given for 30 seconds. In the motive to attain hard tissue anaesthesia of duration for nearly 20 minutes, local anaesthesia with vasoconstrictor has to be used(suggested vasoconstrictor recommendation of epinephrine 1:200,000). The dentist employs a cotton plier to the pull-out guide sleeve and after which anaesthesia will work instantly.[14]

IntraFlow Anaesthesia

This is a technique that primarily focuses on the proper placement of the injection. For Intraflow, choice of the site, preparation of the site, and proper perforation are much-required considerations. For its successful execution, the answer is proper placement so that the anaesthetic solution won't flow within medullary areas. Generally, it is advisable to insert the solution distally to the tooth needed to be anaesthetized. For the determination of the desired site in this, the operator is needed to take a pre-operative radiograph.

The required area of perforation is cancellous bone, which is present adjoining the middle-third of the root to alveolar crest and also apically. The site of choice can be outlined along a line that has to bisect the papillae interdentally, present coronal to the mucogingival junction of the desired tooth distally.[8]

Comfort Control Syringe

The Comfort Control syringe is an electronically operated system for the delivery of local anaesthesia. It has 5 contrasting injection rates that are pre-set in the system for its utility. Using a two-stage delivery rate, the injection begins at an extremely slow rate to counter the initial pain usually associated with an injection. A digital panel shows the rate, elapsed time, and amount of anaesthetic inserted. This approach can make injections easier for dental professionals and make them comfortable for the patients.[8-11]

Advantages Of Intraosseous Injection

The IO injection benefits and eases the work of dental professionals. The Stabident system lessens time in achieving the anaesthesia after injecting the solution. Hardly,3-3 ½ minutes are needed for accomplishing anaesthesia when the solution is injected for 30 seconds even when topical anaesthesia and infiltration of the membrane are given for 60 seconds. In simple terms, we can conclude it minimizes the interval between administration of solution and tooth preparation.[11]

In cases where the patient has to undergo restoration, the IO injection benefits the patient for bilateral work of restoration in the mandibular arch. Moreover, lips and tongue remain unanaesthetized making the technique appreciable. The Stabident method uses local anaesthetic lesser when compared to traditional methods of delivery. The method is admired due to its painless technique of injection. Fastness is an important advantage of the procedure done.

Another advantage is without general injection in all the sites, anterior maxillary anaesthesia is feasible. In such cases, palatally no anaesthesia is required. To attain easy anaesthesia, the X-tip is an effortless and fruitful technique. It can be a piece of vital equipment to keep in reserve in clinics, which can be utilized particularly in the instance where there is no effect of block or infiltration anaesthesia when the tooth is treated.[12]

The plus point of interflow anaesthesia is its use when one has to select a different site for perforation and there is the proximity of roots. The fine target areas are the retromolar pad areas and edentulous areas. In places where the bone is thick or dense like in the buccal shelf area near the molars of the mandible, the approach of the technique can be done lingually too.

The Interflow system enables the introduction inside the penetration site, the injection site as well as removal in one step. There is no requirement for relocating the site of perforation. It is efficient for areas that are hard to imagine as such in the case of the first molar and at times the second molar region. It can be helpful when attached gingiva has finite bands or there is horizontal less loss of bone in the area of penetration. While the use of IO injection there is the immediate onset of anaesthesia so there is no time required to wait for its onset which is needed in instances of conventional methods.[1,13]

Disadvantages Of Intraosseous Injection

There are some disadvantages to the IO method. The perforator which is metallic segregates from the shank which is made up of plastic. The chances of such kind of segregation of metal perforator from the plastic shank often arise when the perforation is intricated and probably because of the extreme heating of the metal leading to the softening of the plastic hub. The Stabident system can't be used in the apical region of alveolar mucosa as it is infeasible to discover the hole to distribute the solution evenly. Another drawback can be leakage of the solution via the guide sleeve within the mouth. It happens particularly when the needle is not placed properly in the guide sleeve up to the hub. Post-operative pain can be also one of the disadvantages. The Intraflow anaesthesia equipment needs a high-price establishment and maintenance cost. Moreover, sometimes the device may cause leakage of anaesthetic solution.[14,15]

Selection of The Site

The Stabident as well as the X-tip system allows the clinician to look out the site of selection for perforation in the attached gingiva. This permits the penetration to be done via the thick cortical bone and has to be halfway amidst the adjacent roots of teeth. As the guide sleeve stays in the same place, the X-tip method can be favourably placed in apically in alveolar mucosa. When the interproximal area is absent, X-tip can help obtain pulpal anaesthesia. When a site has to be anaesthetized for the best results, one has to go to the distal side of the tooth. But an exception to this, it can be one of the cases such as in mandibular and maxillary 2nd molar cases in which one has to go to the mesial side of the tooth.[15]

Conclusion

The dental professionals encounter a failure of local anaesthesia at times. The causes behind the failure of the anaesthesia explained here can aid to master the skills and control the situation while facing any tough or demanding case. The equipment used in the intraosseous systems forms the basis for a route towards the experimentation and research work in dentistry. The present-day technique of the IO system is proficient and the duration of its action is lesser when compared with the conventional methods. Patients with less fear and pain can receive better treatment as lips and tongue numbness doesn't occur. Patient satisfaction and preference are more for intraosseous injection.

Ethical permission: Not Required

Conflict of Interests: None

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Role of PRGF (Plasma Rich in Growth Factors) in Periodontal Regeneration

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Abstract

Platelet-rich-growth-factors (PRGF), a modification of fibrin glue made from autologous blood, is being used to deliver growth factors in high concentration to sites requiring osseous grafting. Growth factors released from the platelets include platelet-derived growth factor(PDGF), transforming growth factor-beta (TGF-ß), platelet-derived epidermal growth factor (PDGF), platelet-derived angiogenesis factor, insulin-like growth factor 1(IGF1), and platelet factor 4. These factors signal the local mesenchymal and epithelial cells to migrate, divide, and increase collagen and matrix synthesis. Anitua et al in 2001 introduced the concept of PRGF and since then it has been used for a variety of purposes in the field of dentistry and oral implantology for proper wound healing and regeneration of periodontal tissues. This review aims to give a brief idea about the various uses of PRGF and the possibility of utilization in the future taking into consideration many studies that have been done. The main advantage of using PRGF is that it lacks the leukocyte content thus preventing damage to the tissues during healing due to the acid hydrolases and proteases released from the neutrophils. It has been previously used for treating intrabony defects, to increase the implant-bone contact area, for recession coverage etc. In other fields of medicine, it has been used to cure osteoarthritis, to treat chronic ulcers as well. Therefore, PRGF is a very effective blood concentrate with a high concentration of growth factors and its use should be increased in the clinical practice.

Keywords: *Healing, leucocytes, periodontal regeneration, plasma rich in growth factors*

Introduction

The field of dentistry is evolving to provide techniques that are minimally invasive and biologically accelerated which reduces patient morbidity and results in improved healing. Healing in dentistry is broadly related to repair and regeneration, both of which are two different terminologies. Regeneration refers to the complete restitution of the injured tissues with their proper architecture and normal function whereas repair is the type of healing that occurs in response to injury for normal functioning. The requirement of regeneration has given rise to many new biomaterials that have been used in dentistry. However, the disadvantage of the cost-effectiveness is persistent with these biomaterials. Recently the use of platelet-rich treatments has revolutionized the field of regenerative dentistry, specifically in the field of periodontics and oral implantology.[1] It has been able to do so due to the repair potency of the growth factors and the proteins released by the platelets.[2]

Megakaryocytes derived cell platelets have an intense tubular structure which helps in the eviction of the growth factors and the proteins. Thus they are a very good source for different types of growth factors and proteins that are required for regeneration. Platelets when activated, release various growth factors from the α granules. The justification for the utilization of

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platelets was to reinstate the clot with platelets which on stimulation or activation releases various growth factors like Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-β(TGF- β), Vascular endothelial Growth Factor(VEGF), Insulin-like Growth Factor(IGF), Thrombospondin, etc which help in the process of regeneration.[2] One advantage of using Plasma rich in growth factors is the exclusion of the RBC's and the WBC's resulting in accelerated wound healing with minimal post-operative complications. Furthermore, it can be easily be prepared from the patient's blood followed by its centrifugation thus it is cost-effective as well as saves a lot of time clinically.

PREPARATION OF PRGF

Anitua et al in 1999 affirmed the concept of PRGF.[1] It is completely autologous and biologically compatible and obtained by a single centrifugation process utilizing sodium citrate (anticoagulant) and calcium chloride (platelet activator). The beneficial effects of PRGF are dependent on the amount of growth factors, rate of release of the peptides and the centrifugation procedural considerations like the speed.[3]

PRGF Endoret technology for the collection of PRGF gel is the most effective techniques to obtain PRGF with maximum beneficial attributes and has provided clinical development in the field of Periodontics and Oral Implantology.[4]

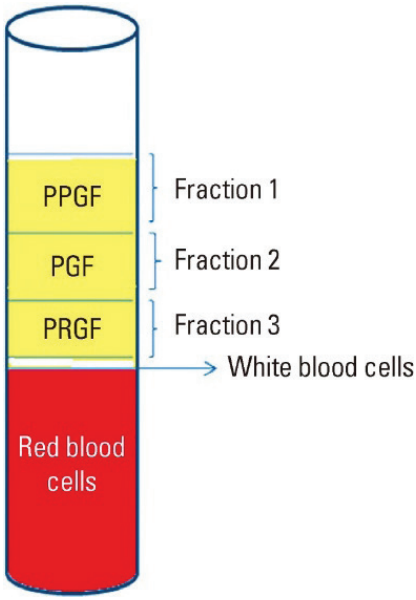

Figure 1 – Fractions obtained following centrifugation

Protocol includes –

· Venous blood should be collected from a healthy donor into 10 ml tubes that contains 3.8% sodium citrate

· Single centrifugation process should be performed in the PRGF Endoret system at a speed of 580g for around 8 minutes

· Fraction(F3) just above the buffy coat (collection of white blood cells) as shown in **Figure 1** should be pipetted out carefully and transferred to a separate disinfected tube or a petridish

· Addition of 10% $CaCl_2$ (Calcium Chloride) should be done for the stimulation of platelets to get activated (Around Fifty microlitre per ml of PRGF)

· Incubation for 60 minutes in the PRGF Endoret activator needs to be done at a temperature of 37ºC to obtain the PRGF in a gel consistency.

There are many techniques by which the platelets can be separated from the blood which includes gravitational platelet sequestration process, standard cell separators and plateletpheresis. However, the gravitational technique which utilizes the gravitational force to separate contents of blood is the most useful since it has a yield of 78% platelets with minimum RBC's and WBC's.[5]

ROLE OF GROWTH FACTORS IN TISSUE REPAIR & REGENERATION

The growth factor is a generalized term used for a group of proteins that function together in the body to promote the migration of the cells and carry out specific metabolic activities.[6] Following tissue injury, there are a series of events that occur at the cellular and molecular level which includes several stages of inflammation.[7–9] The events include proliferation and migration of various cells, the appearance of new blood vessels, formation of granulation tissue, etc. These events are initiated by the virtue of certain growth factors and cytokines that are released due to the process of platelet activation which occurs as a part of the healing process to injury. The primary clot is the most important since it acts as a scaffold to hold all types of growth factors and cells that would help repair the wound later on in the healing process.[10]

· PLATELET DERIVED GROWTH FACTOR (PDGF) –

PDGF is the most important growth factor responsible for the regeneration of periodontal ligament tissues. It has three types of isomers PDGF-AA, PDGF-BB and PDGF-AB. The concentration of PDGF in blood serum is around 15-50 milligrams per milliliter. Lynch et al discovered the effectiveness of PDGF in the regeneration of components of periodontium that includes cementum, bone and PDL.[11] The sources of PDGF include degranulating platelets, macrophages, fibroblasts, endothelial cells and smooth muscles.

PDGF is a major factor in the healing of wounds since it promotes the division of cells and is responsible for the cell to enter into the division cycle from the G_0 quiescent phase. It also has a chemotactic function and also prevents apoptosis of cells.

Major functions include: Angiogenesis, neutrophil chemotaxis, improves the function of osteoblasts and fibroblasts, helps in secretion of other growth factors and mitogenesis.[12]

Platelet-derived growth factor also helps in the regeneration of periodontium as per many recently conducted studies. **Nevins et al in 2012** conducted a study to check the effectiveness of using PDGF-BB along with tricalcium phosphate over 36 months in correcting a localized osseous defect and concluded that hr-PDGF-BB provides a long term results with stable clinical and radiographic parameters.[13] In an in-vitro study **Manoranjan et al in 2012** studied the effectiveness of PDGF-AB on PDL fibroblasts and concluded that PDGF-AB had maximum mitogenic response on PDL fibroblasts at 48hrs.[14] In an animal study, Cho Moon et al in 1995 compared the efficacy of GTR therapy with PDGF and GTR alone and concluded that GTR with PDGF provided better results than GTR alone.[15]

INSULIN LIKE GROWTH FACTOR(IGF)-

A.K.A **erythropoietic factor**, they are a family of proteins with one chain and consists of two isomers IGF I and IGF II which are functionally similar but regulated differently. IGF is usually secreted from the liver due to the action of growth hormone. Therefore its release and action are growth hormone-regulated which is secreted from the pituitary gland. It helps in the promotion of cell division.[8,9,16]

Not only does it encourage cell division but also prevents cell apoptosis. In an in vitro study, **Xie et al in 2017** studied the effects of insulin-like growth factor-1 on the periodontal ligament stem cells and concluded that IGF-1 promotes the proliferation of PDLSC by binding to its receptor and activating the AKT pathway and it also elevates the differentiation of osteoblasts and thus can be crucial in periodontal regeneration.[17] Platelets have a high content of insulin-like growth factors and are released on their activation. It is also released by fibroblasts on activation by PDGF. In an animal study, **Lynch et al in 1989** studied the combined effect of IGF and PDGF when applied after periodontal surgery and concluded that the combination accentuates the migration of fibroblasts and osteoblasts and also stimulates the activity of these cells to lay down collagen and new bone thus helping in regeneration of PDL cells.

TRANSFORMING GROWTH FACTOR(TGF)-

Transforming growth factor constitutes isomers i.e. **TGF-α** and **TGF-β** that are both configurationally and functionally different. They compete for the **epidermal growth factor** receptor and functionally activate epithelial and endothelial cells. TGF- β also has three isomers that are TGF β1, TGF β2 and TGF β3. When TGF α helps in the proliferation of different types of cells, TGF β has a dual-action where it can accentuate or minimize the proliferative action of cells.

TGF β has the property of altering the secretion of other growth factors like PDGF, EGF, TGF α etc.[18] It has the property for the proliferation of fibroblasts and collagen production and also downgrade the production of matrix metalloproteinases. In the process of wound healing, TGF β may produce fibrosis due to the excessive production of collagen.

In an animal study with transgenic mice, when TGF β was introduced, they were declared dead due to an inflammatory syndrome. Thus proving that it regulates the immune system. TGF β also augment bone formation when injected near periosteal cells.[19]

EFFECT OF PRGF ON THE ALVEOLAR BONE

Bone regeneration has to be one of the challenging things to achieve as it involves a cascade of events that includes the release of various cytokines, chemokines, and growth factors that have to occur. **Anitua et al in 2013** conducted a study in which the primary osteoblasts were isolated and tested with PRGF-Endoret and concluded that it promotes osteoblast proliferation thus can be used to regenerate bone.[20] Some of the factors that are essential for regeneration of bone include VEGF, TGFβ, PDGF-B, Insulin-like growth factor, etc. which are known to be secreted by the platelets on activation.[21] Another growth factor known as Hepatocyte growth factor(HGF) has been proved to be involved in the coupling process and regulates both osteoclasts and osteoblasts in an in vitro study by **Grano et al in 1996**.[22] The HGF released by the PRGF has also been shown to lower the expression of the pro-inflammatory Nuclear Factor of Kappa B thus allowing for a smooth coupling process.[23] The cytokines involved in bone resorption include TNFα, IL-6 IL beta are secreted by lymphocytes and macrophages when they are activated. However, due to the lack of leucocytes in PRGF, there is very little chance of release of these growth factors.[24] It was also observed that there was increased expression of osteocalcin and alkaline phosphatases which increased bone formation process as per the study done by **Lin et al in 2006**.[25] In an animal study done by **Eda et al in 2014** on rat cavalria proved that the use of PRGF in new bone formation was better than the use of PRP.[26] In another animal study done by **Rivera et al in 2013** proved that the combination of PRGF and Simvastatin produced better results in regenerating bone than the combination of PRP and Simvastatin although the results were not statistically significant.[27]

PRGF has also been used for the correction of intrabony defects as well. As per a study done by **Khalifehzadeh et al in 2019** Metformin when used in combination with PRGF provided better radiographic and clinical changes than other groups in patients with chronic periodontitis and a localized intrabony defect. However, in a split-mouth study done by **Ravi et al in 2017,** evaluated the efficacy of the use of PRGF along with GTR and concluded that there was no additive effect of PRGF when used along with GTR membrane compared to GTR alone in terms of clinical and radiographic outcomes.[28] Also in another study done by **Gamal et al in 2016** for treating intrabony defects, it was proved that although the level of growth factors was higher in the PRGF group but it was not statistically significant and the use of PRGF though showed improvement in clinical parameters in treating intrabony defect but the results were not statistically significant.[29] According to a systematic review done by **Panda et al 2016**, it was concluded that platelet-rich plasma when used along with bone grafts had an additive effect in treating intrabony defects but was not found to be effective when used along with GTR membranes.[30] Hence it can be established that PRGF definitely would have an additive effect in the treatment of bony defects and possesses an advantage due to the presence and release of various growth factors.

EFFECT OF PRGF ON SOFT TISSUE REGENERATION

PRGF, as already mentioned before is devoid of white blood cells and other pro-inflammatory products.[31] They also have a rich source of platelet-derived growth factors that are usually released during the healing of a wound on the activation of platelets. In an in vitro study done by **Anitua et al in 2009** stated that the proliferation rate of fibroblasts was increased in the presence of PRGF. **Vahabi et al in 2015** compared the efficacy of PRF (platelet-rich fibrin) and PRGF and concluded that PRGF had a strong effect in terms of stimulation and proliferation of fibroblasts and epithelial cells.[32] In a recently conducted human study done by **Jenabian et al in 2018** it was stated that when PRGF along with connective tissue graft for recession coverage using coronally advanced flap, the results showed significant differences in terms of Recession coverage, recession height and width and keratinized tissue width (KTW).[33] Following soft tissue injury, there is an increased expression of smooth muscle actin (α-SMA) in the fibroblasts resulting in increased production of myofibroblasts resulting in fibrosis, therefore weakening tissue characteristics.[34] Use of PRGF has shown to downregulate transforming growth factor-induced α-SMA expression thus reducing the possibility of healing by fibrosis and promoting regeneration.[35]

EFFECT OF PRGF ON IMPLANTS AND PERI IMPLANT MUCOSA

The platelet-rich PRGF has shown remarkable results in soft tissue regeneration and bone regeneration in preparing sites for the placement of implants.[1] The protocol of implant placement has seemed to evolve with time with the use of platelet concentrates in it. **Anitua et al in 2006** stated that the platelet concentrate PRGF could be used to create new bone formation at the border of bone and implant, thus enhancing osseointegration by placing the implant covering its surface with PRGF.[36] In an animal study conducted **by Birang et al in 2012** stated that PRGF improved not only the healing process when placed along with an implant but also improved the percentage of Bone implant contact.[37] PRGF has also been used following extraction at the immediate implant placement site and has proved to be beneficial in post-extraction healing and prevent bone loss.[38]

Conclusion

The main advantage of the use of PRGF is its healing property and the fact that it not only heals but allows regeneration. In this review, it has summarized the various usages of PRGF and their advantages in an abridged manner. However many studies are yet needed to be done to prove the exact mechanism of how it brings about regeneration. As mentioned in this review, PRGF can be used in various aspects of Periodontics and should be used in clinical practice daily.

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A Study to Evaluate the Analgesic Efficacy of Transdermal Diclofenac Sodium Patch in Minor Oral and Maxillofacial Procedure

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Abstract

Aims and Objectives: The aim and objectives of this study are to evaluate and determine the efficacy of transdermal diclofenac patch in control of pain following Routine Extractions, Transalveolar extractions, Soft tissue biopsies, to evaluate any adverse reactions following the usage of the transdermal patch and to relate its efficacy to the gender, pre-surgical condition of the patient and time taken for the procedure.

Materials and methods: A clinical study was done in the patients coming to the Oral and Maxillofacial surgery department, Institute of Dental Sciences, SOA University. A total of 537 patients were selected from our patients in Oral and Maxillofacial Surgery department who underwent routine extractions, Transalveolar extractions, Soft tissue biopsies etc.

Results: 500 patients who underwent minor surgical procedures under local anaesthesia with adrenaline. The patient has then prescribed a diclofenac patch for analgesic purpose and the efficacy and tolerability of the patch was evaluated. Using SPSS software, statistical analysis was done. All the patients were evaluated with VAS scale and Wong baker scale on 1st day, 2nd day and 3rd day. The mean age of the patient is 28.2 years and the mean value for the time taken for the procedure is 14.4 min.

Conclusion: Transdermal patch can be effectively used for analgesic effect following minor oral surgical procedures of lesser duration. It is effective for the management of mild to moderate pain relief as it has proved to be a good option as an analgesic especially in patients with gastric problems and for better compliance.

Keywords: *Analgesic Efficacy; Transdermal Diclofenac Sodium Patch; Minor Oral and Maxillofacial Procedure; Transalveolar extractions; VAS scale; Wong baker scale; Pain management; Drug administration*

Introduction

Managing pain is the most substantial problem for any oral and maxillofacial surgeon especially in the post-operative phase. Hence every surgeon should be well prepared to manage pre, intra and post-operative pain for which it is imperative to have in-depth knowledge about the biology of pain and recent theories and its management.[1] Pain can be managed both by pharmacological and non-pharmacological methods out of which post-operative pain management relies heavily on pharmacological methods. There are both opioid and non-opioid drugs to choose for the management of pain. Although opioids are potent analgesics, its drug dependency makes non-opioid a better option for general pain management.[2] Diclofenac sodium is a frequently used NSAID's, as it has antipyretic, anti-inflammatory and analgesic properties. Diclofenac sodium is a phenylacetic acid derivative. Its chemical nomenclature is:

2-[2-(2,6-dichloroanilino) phenyl] acetic acid monosodium salt.

Diclofenac can be administered through various routes such as through oral, intramuscular, intravenous, transdermal and rectal. Oral administration of diclofenac is quite disadvantageous due to the serious side-effects of the drug, which is chiefly due to the poor agent specificity, which results from the drug binding to specific receptors (e.g., prostaglandin). The adverse reaction of the drug mainly affects the gastrointestinal tract. Diclofenac when administered orally is not well tolerated as it can result in severe stomach ulcerations. Hence new methods are being ventured for the administration of the drug to reach the specific therapeutic drug concentration in the tissue without causing any systemic problems. Transdermal delivery is a befitting alternative for the administration where the drug concentration reaches a required therapeutic level in the tissue without reaching the gastrointestinal tract.[3]

Methodology

A clinical study was done in the patients reported to the Oral and Maxillofacial surgery department, Institute of Dental Sciences, Siksha O Anusandhan (Deemed to be University), after getting clearance from the ethical committee. The purpose of the study was explained to all the patients included in the study and written consent was obtained. A total of 537 patients were selected from our patients in the Oral and Maxillofacial Surgery department who underwent following treatment 1. Routine extractions, 2. Transalveolar extractions, 3. Soft tissue biopsies

Materials Used: Diclofenac sodium patch

Inclusion Criteria: ASA-1 category patients, Age – 15 to 50 years, Routine extractions, Transalveolar extractions, Soft tissue biopsies,

Exclusion Criteria: Those patients not fulfilling the inclusion criteria are excluded in the study; patients allergic to diclofenac or any other NSAIDs; those patients who were not willing for the study.

500 patients were selected for the study after excluding some of the non-complaint patients. Preoperative radiographs and routine blood investigations were performed before procedures wherever required. The Diclofenac Patch (Dicloplast) 100mg was administered after 30 minutes of the procedure for all the patients. The scheduled regimen for administration of diclofenac patch is Day 1 1st patch after 30 minutes of surgery, Day 2 and Day 3 new patch was given. The patch can be applied to any part of the body, preferably an area devoid of hair (Figure 1). Wong-baker faces pain rating scale and the Visual Analog Rating Scale was used to record the result. The patients will be assigned scores for every parameter at intervals of 6hrs, 12hrs and 24 hrs and 48hrs postoperatively. Furthermore, the subjects will have to set values for a total of 72hrs in the postoperative period.[4]

A special questionnaire was prepared, to record the relevant history. The parameters such as age, gender, pre-surgical condition and the pain rating scales were noted. Each patient received a patch, half an hour post-operatively which was applied over the bare skin of the patient. The patient was explained about the dosage of the transdermal patch and probable side effects of the application. Patients were instructed not to use any other analgesic or anti-inflammatory medications. In the unavoidable situation where they have to take these medications, they are asked to report it on call in which case they were excluded from the study. The amount of pain was recorded with visual analogue pain scale ranging from 0 (least pain) to 10 (maximum pain) and FACES pain rating scale (Wong baker). The patient and their attendant were explained about the ratings. The first rating was recorded by the operator and other ratings were enquired by the attendant over the phone. In case of any incidence where the patient had to take other medications like if the patient complained of fever, the patients were prescribed with other antipyretics. These patients were excluded from the study. Thus, 37 patients were excluded, and statistical analysis was done on the 500 patients. SPSS software was used for purpose of analysis

Ethical Permission and Patient Consent: Approval from the Institutional Ethical Committee of IMS and Sum Hospital, Siksha 'O' Anusandhan (Deemed to be University) was taken before conducting the study. Moreover, prior consent was taken from patients before the study and signature of the patients were taken in the consent form.

Results

In the descriptive study performed on 500 patients who underwent minor surgical procedures under local anaesthesia with adrenaline. The patient has then prescribed a diclofenac patch for analgesic purpose and the efficacy and tolerability of the patch was evaluated using the following parameters

Ø Pre-surgical condition (Symptomatic/ Asymptomatic)

Ø Gender predilection

Ø Duration of the procedure

Ø Visual analogue scale

Ø Wong-baker scale

Using SPSS software, statistical analysis was done. All the patients were evaluated with VAS scale and Wong baker scale on 1st day, 2nd day and 3rd day. The mean rating of Wong baker pain scale for day 1 is 2.6 (Standard Deviation or SD=1.07), day 2 is 0.9 (SD=1.09) and day 3 is 0.07 (SD=0.34). The visual analogue pain scale rating for day 1 is 3.1 (SD=1.35), day 2 is 0.9 (SD=1.07) and day 3 is 0.08 (SD=0.3). The mean age of the patient is 28.2 years and the mean value for the time taken for the procedure is 14.4 min.

As per the study by VAS scale and Wong-Baker scale, there is no statistical significance in comparison of pain rating according to the gender on day 1, 2 & 3 (Table 1 and 2) but as per pre-surgical condition, there is the statistical significance ($p>0.05$) among symptomatic and asymptomatic patients on day 1, 2 & 3 (Table 3 and 4). In contrast, there is no statistical significance on day 1&2 for the time taken during the procedure but significance in day 3 in both scales. There is no correlation between the time taken in minutes and Wong-Baker Pain Rating on Day 1. ($r = -0.01$) There was no statistical significance ($p>0.05$). There is no correlation between the time taken in minutes and Wong-Baker Pain Rating on Day 2. ($r = -0.02$) There was no statistical significance ($p>0.05$). There is a mild positive correlation between the time taken in minutes and Wong-Baker Pain Rating on Day 3. ($r = .133$) There was statistical significance ($p<0.05$). There is no correlation between the time taken in minutes and Visual Analog Scale Pain Rating on Day 1. ($r = -0.02$) There was no statistical significance ($p>0.05$) There is no correlation between the time taken in minutes and Visual Analog Scale Pain Rating on Day 2. ($r = 0.05$) There was no statistical significance ($p>0.05$). There is a mild positive correlation between the time taken in minutes and Visual Analog Scale Pain Rating on Day 3. ($r = 0.167$) There was statistical significance ($p<0.05$).

Table 1: Comparison of the Mean Wong-Baker Pain rating according to the gender from day 1 to 3.

Wong-Baker Pain Rating	n	Day 1		Day 2		Day 3	
		Mean	SD	Mean	SD	Mean	SD
Male	184	2.67	1.03	0.98	1.07	0.05	0.27
Female	316	2.65	1.11	0.98	1.11	0.09	0.39
Total	500	2.66	1.08	0.98	1.09	0.07	0.35
T-test	T test – p > 0.05, Not Significant			T test – p > 0.05, Not Significant		T test – p > 0.05, Not Significant	

The Mean Wong-Baker Pain rating on Day 1 was 2.67 ± 1.03 among males and 2.65 ± 1.11 among females; Day 2 was 0.98 ± 1.07 among males and 0.98 ± 1.11 among females; Day 3 was 0.05 ± 0.27among males and 0.09 ± 0.39 among females

Table 2. Comparison of the mean visual analogue pain rating according to the gender from day 1 to 3.

Visual Analog Pain Rating	n	Day 1		Day 2		Day 3	
		Mean	SD	Mean	SD	Mean	SD
Male	184	3.14	1.41	0.97	1.06	0.07	0.25
Female	316	3.14	1.33	1.00	1.08	0.09	0.35
Total	500	3.14	1.36	0.99	1.07	0.08	0.32
T-test	T test – p > 0.05, Not Significant			T test – p > 0.05, Not Significant		T test – p > 0.05, Not Significant	

The Mean Visual Analog Pain rating on Day 1 was 3.14 ± 1.14 among males and 3.14 ± 1.33 among females; Day 2 was 0.97 ± 1.06 among males and 1.00 ± 1.08 among females; Day 3 was 0.07 ± 0.25 among males and 0.09 ± 0.35 among females

Table 3. Comparison of the mean Wong-Baker pain rating according to the pre-surgical condition from day 1 to 3.

Wong-Baker Pain Rating	n	Day 1		Day 2		Day 3	
		Mean	SD	Mean	SD	Mean	SD
Asymptomatic	269	2.39	1.01	0.7	1.06	0.03	0.24
Symptomatic	231	2.97	1.08	1.32	1.04	0.13	0.44
Total	500	2.66	1.08	0.98	1.09	0.07	0.35
T-Test	T test – p < 0.05, Significant			T test – p < 0.05, Significant		T test – p < 0.05, Significant	

The Mean Wong-Baker Pain rating on Day 1 was 2.39 ± 1.01 among asymptomatic patients and 2.97 ± 1.08 among symptomatic patients; Day 2 was 0.7 ± 1.06 among asymptomatic patients and 1.32 ± 1.04 among symptomatic patients; Day 3 was 0.03 ± 0.24 among asymptomatic patients and 0.13 ± 0.44 among symptomatic patients.

Table 4. Comparison of the mean Visual Analog scale pain rating according to the pre-surgical condition from day 1 to 3.

Visual Analog scale Pain Rating	n	Day 1		Day 2		Day 3	
		Mean	SD	Mean	SD	Mean	SD
Asymptomatic	269	2.87	1.36	0.74	0.98	0.03	0.23
Symptomatic	231	3.47	1.28	1.29	1.1	0.13	0.39
Total	500	3.14	1.36	0.99	1.07	0.08	0.32
T-Test	T test – $p < 0.05$, Significant			T test – $p < 0.05$, Significant		T test – $p < 0.05$, Significant	

The Mean Visual Analog scale Pain Rating on Day 1 was 2.87 ± 1.36 among asymptomatic patients and 3.47 ± 1.28 among symptomatic patients; Day 2 was 0.74 ± 0.98 among asymptomatic patients and 1.29 ± 1.1 among symptomatic patients; Day 3 was 0.03 ± 0.23 among asymptomatic patients and 0.13 ± 0.39 among symptomatic patients.

Figure 1: Applied Diclofenac Transdermal patch

Figure 2. Comparison of pain rating according to the time taken for the surgery

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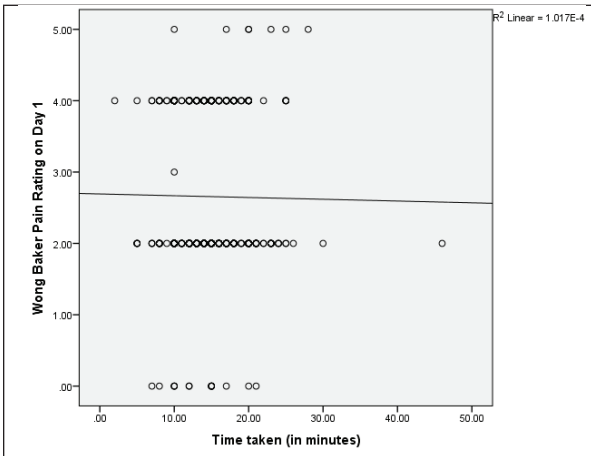
<td> 
</td>
<td>There is no correlation between the time taken in minutes and Wong-Baker Pain Rating on Day 1. (r = -0.01) There was no statistical significance (p>0.05).</td>
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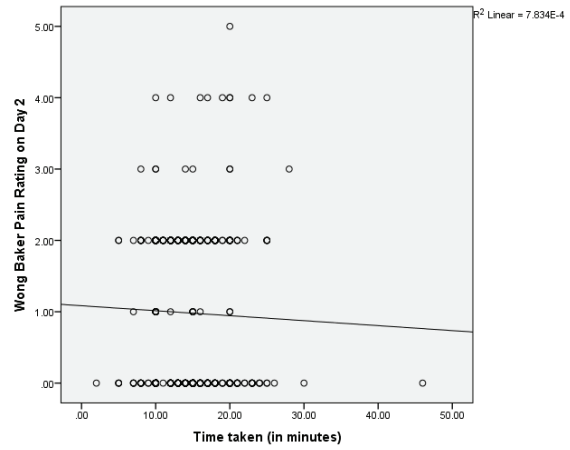
<td> 
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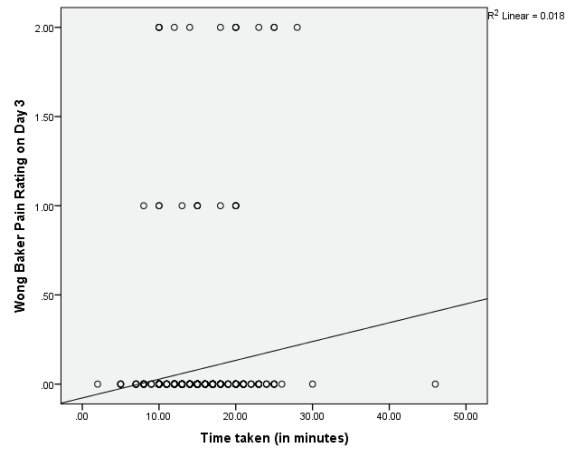
<td> 
</td>
<td>There is a mild positive correlation between the time taken in minutes and Wong-Baker Pain Rating on Day 3. (r = .133) There was statistical significance (p<0.05).</td>
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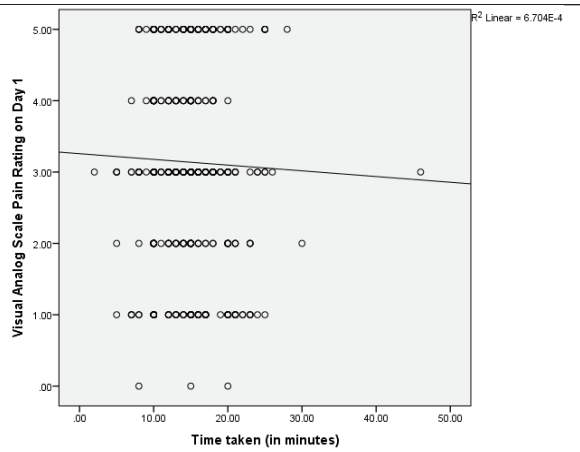
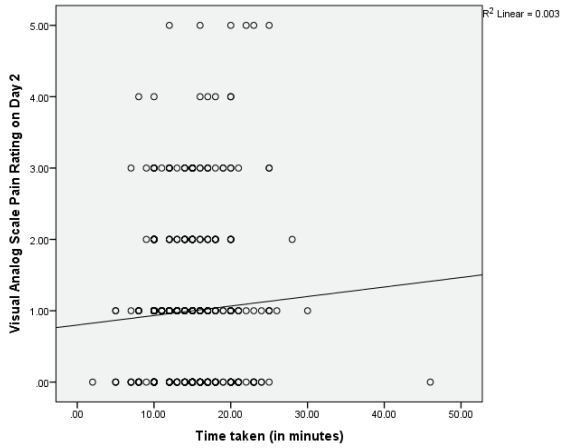
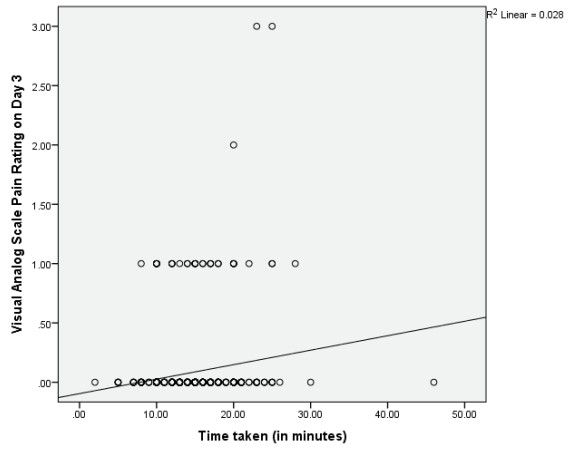
	There is no correlation between the time taken in minutes and Visual Analog Scale Pain Rating on Day 1. (r = -0.02) There was no statistical significance (p>0.05).
	There is no correlation between the time taken in minutes and Visual Analog Scale Pain Rating on Day 2. (r = 0.05) There was no statistical significance (p>0.05).
	There is a mild positive correlation between the time taken in minutes and Visual Analog Scale Pain Rating on Day 3. (r = 0.167) There was statistical significance (p<0.05).

Discussion

Management of pain is one of the frequently found complications associated with dental practices, mainly by oral and maxillofacial surgeons. Patients are sometimes coming with painful problems, and most of the times the treatment rendered causes pain postoperatively. Every surgeon must be ready to deal with pre-operative, intra-operative and postoperative pain and its treatment. To do this successfully it is necessary to know the biology of pain, recent theories and practices for its management.[5] Treatments for pain can be classified as pharmacologic and non-pharmacologic. Non-pharmacological approaches for the treatment of pain are psychological approaches, physical rehabilitation and surgical approaches. Pharmacological management is the mainstay of pain therapy. Drugs available for

management of pain belong to two major groups: The Non-Narcotic analgesics (NSAIDs and acetaminophen) and The Opioids (or Narcotics). Various formulations of Diclofenac are present for topical administration; Diclofenac sodium, Diclofenac diethylamine (DEA), and Diclofenac Epolamine (EPA).[6] The potassium salt of diclofenac (DFK) which was also administered orally was also checked for skin penetration capabilities. In comparison to the free acid than these salts, they have higher aqueous solubility as the previous is to have a solubility of 60 mm in the water at 25 °C and an intrinsic solubility value of 1.03 _ 0.7 mg/mL, as determined by using acid-base titration.[7,8] Diclofenac exercises analgesic, antipyretic and anti-inflammatory activities by COX 1& COX-II inhibitor with 4-fold advanced discrimination for the latter.

Transdermal patches are nowadays used in various treatment modalities including cosmetic purposes. The patch emerged as a product of various developments in the technology of skin science. Transdermal delivery at the earliest was studied and recognized by Dale Wurster. Most of his studies regarding proper dose, area of application, vehicle and device of the transdermal delivery system are significant.[9] Presently, it has been recommended that the vasculature of the dermis provides the main course to the deeper tissues to transfer anti-inflammatory drugs which are tightly bound. A rate-controlling membrane was used to regulate the rate of delivery of transdermal medications from a bandage to the skin for systemic circulation.

The major disadvantage of transdermal patches is the adverse skin reactions it causes, especially when it is used for a long period which results in extended contact with the skin. Two types of skin reaction have been observed by using patches, that is irritant contact dermatitis and allergic contact dermatitis out of which the former is more commonly seen. Most of the time it is the drug which causes the reaction rather than the other components of the patch. This kind of reaction causes poor patient compliance leading to the discontinuity of the treatment.[10,11]

There are various advantages of transdermal delivery over the oral route. First and foremost, it is used when there is a first-pass metabolism effect of the liver which impulsively metabolize the medications. Over hypodermic injections they have the advantage of being painless, not generating any kind of dangerous hospital waste and avoidance of any chances of transmission of diseases cross-contamination rendered from needle reuse.[12]Additionally, transdermal patches are non-invasive and can be self-applicable increasing the patient compliance. They provide extended-release of the drug for a longer period.

A disadvantage of the transdermal delivery system is that only a few numbers of drugs which can be administered by this route. Recent advances have enabled transport of molecules of molecular mass up to few hundred Dalton, which exhibit octanol-water partition coefficients that highly favour lipids and need doses of milligrams /day or less.[13] Transdermal delivery of peptides macromolecules, including recent genetic method employing DNA or siRNA (small-interfering RNA), has also been studied. The other usage of transdermal delivery includes delivery of vaccines, patient-regulated drug delivery system of fentanyl moderated by electricity to reduce pain (iontophoresis), extracting molecules (analytes) via skin e.g. Extraction of interstitial fluid for glucose monitoring.

Conclusion

This study was done to evaluate the efficacy of transdermal patch of diclofenac sodium in any minor oral surgery. Out of 537 patients who have prescribed the patch, 37 patients were excluded in the study as they took other medications due to either fever or swelling or if the patient were incompliant. The statistical analysis was made on the other 500 patients. The conclusion achieved after the complete study is that, there is no difference in pain relief between male and female patients. Further, there is a significant difference in pain scores between symptomatic and asymptomatic patients. From the graphs, it was evident that on day 1 and day 2 pain relief was better in asymptomatic patients when compared to symptomatic patients whereas on day 3 there is not much difference in scores, but this is significant - due to big sample size. The time is taken for the procedure also did not have much significance on the 1st and 2nd day whereas on 3rd day it had a mild effect. Hence, the transdermal patch can be effectively used for analgesic effect following minor oral surgical procedures of lesser duration. It is effective for the management of mild

to moderate pain relief as it has proved to be a good option as an analgesic especially in patients with gastric problems and for better compliance. The only drawbacks of this patch are that it is not waterproof and any contact of the patch with water will decrease its effectiveness moreover this patch has not shown promising results on symptomatic patients i.e. patients having pain before the surgical procedure. There are only a few clinical trials with larger samples out of which this is one of them, hence there is a need to have more trials in various fields of medicine and dentistry and the third-generation patches should be explored and developed for better transport of the drug transdermally.

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Biosensors Used in Dentistry: A Review

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Abstract

A bio-sensor is composed of six elements which include bio-receptor, transfusion element, chemically active interface, signal amplifier, signal processor and display. The analyze binds to the immobilized biological material and forms a product. The product linked change is then converted by the transducer into electric signals which can be amplified and measured. Oral fluid bio-sensors have the advantages of easy accessibility and noninvasive sample collection, making them a novel method in disease diagnostics. However, its limitations such as less sensitivity and specificity are overcome by the advent of new technologies such as microfluidics and nanofluidics. This review article discusses the concepts and applications of biosensors in the field of dentistry.

Keywords*: Biosensors, Types, Applications in Dentistry*

Introduction

Humans have been performing bio-analysis seeing as the sunrise of the time, utilizing the sensory nerve cells of the nose to detect scents or to detect the enzymatic reactions of the tongue to taste the food. Since biological organisms are the most efficient machines that are ever created, many scientists have an eagerness to apply as well as copy their efficiency to utilize it in man-made creations.[1] Scientists have developed a new means of chemical analysis by using bio-receptors from biological organisms that often have the highest selectivity of the biological recognition system. These bio-recognition elements when combined with various transfusion methods, created a rapidly expanding fields of bio-analysis as well as associated technologies that are known as biosensors.

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A bio-sensor is composed of six elements which include bio-receptor, transfusion element, chemically active interface, signal amplifier, signal processor and display.[2] The analyzer binds to the immobilized biological material and forms a product. The product linked modifications are then converted by the transducer into electric signals which can be amplified and measured.[3] This review article discusses the concepts and applications of biosensors in the field of dentistry.

Basic concepts of biosensors

A biosensor contains a sensor-element along with a bio-element. The bio-element may be an enzyme living cells, antibody or tissue. The sensing constituent may be an electric current, electric potential etc. This bio-element, as well as sensor-element, can be attached in one of the four possible customs like a membrane entrapment custom which is a semi-permeable membrane that segregates the bio-element where it is attached to the sensor.[4] The second one is a physical adsorption custom that is reliant on a combination of the van der walls forces, hydrophobic forces and hydrogen bonds as well as iconic forces to connect the bio-material to the exterior of the sensor. The third one is the porous

entrapment custom which is based on an encapsulation matrix formation around the biological material that helps in binding it to the sensor. And the fourth one is the covalent bonding custom has a sensor surface that is treated as a reactive group to which the biological materials can be attached.

Types of Bio-Sensors:

(A) Blood glucose bio-sensor - It is the biggest bio-sensor success story in commercial use for diabetes control. Rapid analysis forms a single drop of blood which is the analyzer that monitors glucose level in the blood.[5]

(B) A holographic bio-sensor - It uses holograms as sensing elements and it is used to screen pancreatic disorder by detecting trypsin in the duodenal fluid.[5]

(C) *Nanoshell bio-sensor* - It uses gold as a transducer, because of its high scattering level, so that sub nanograms/mm quantities of Ig in saline, serum and blood can be measured. It enhances chemical sensing by 10 billion times and is being urbanized for applications together with various cancer therapies as well as testing of proteins that associated with Alzheimer's disease.[5]

(D) *Bio-detector* - It is used for DNA sequences and mutation detection. Bio-detectors are faster than conventional PCR and also more efficient in producing the number of DNA copies.[5]

(E) *Immuno bio-sensor* - Used to detect Ag-Ab reaction for analysis of complex fluids to analyze micro-organisms, viruses, pesticides and industrial pollutants. They are designed for easy use by the untrained personnel.[5]

Components of a Bio-Sensor

There are 2 basic components of a bio-sensor:

(1) Biological component (a) Analyst (blood, serum saliva, urine and stools etc. (b) Sensitive bio-element created by biological engineering (enzyme, antibody, nucleic acids, cells and tissues etc).[6]

(2) *Physical component* (a) Detector transforms the signals that are being ensued from the interaction of the analyst with the biological element into other signals which are easily measured and quantified. (b)Amplifier/ display unit is the signal processors or the electronic component accountable for the display of the results in a user-friendly way.[6]

Role of oral fluid-based bio-sensors in dentistry:

Dental caries: Since dental caries is an irreversible multifactorial disease affecting the tooth, which often leads to cavitation, caries activity tests are performed to measure the caries activity which helps in motivating patients in caries prevention. However, caries activity tests consume a lot of time, and so to save the time of clinicians, a fiberoptic bio-sensor was developed to monitor tooth loss concerning *S. mutans* in human saliva. The finding of this bio-sensor confirmed that there is a decrease in absorbance of saliva with increased bacterial activity.[7] Hence this test can be used to monitor *S. mutans* activity in saliva as its levels correlate with dental caries.

Periodontitis: This is a type of chronic inflammation of the periodontal tissues caused by a continual bacterial infection that result in parameters such as probing pocket depth, bleeding on probing and loss of clinical attachment and radiographic assessment of bone loss provide information on the severity of periodontitis but not on disease activity. This need resulted in the development of biomarkers which can measure periodontal disease at the molecular, cellular, tissue and clinical levels. Several biomarkers associated with inflammation, soft tissue and bone destruction have been identified in GCF and saliva. IL-1B, MMP-8, TN F-A, IL-6 and C-reactive protein (CRP) are the biomarkers associated with periodontitis, for which saliva-based bio-sensors have been developed by a group. Another bio-sensors called the Integrated Micro-fluid Platform for oral diagnostics was developed which can detect biomarkers such as MMP-8 TN F-A, CRP and IL-6.[8]

Oral cancer: Oral cancer is the eighth most widespread cancer in men while it is fourteenth most common cancer in women worldwide. It is the most common cause of mortality and morbidity among the developing countries. Hence, for early detection and assessment of risk, various biological markers have been developed. The use of saliva-based bio-sensors for exfoliated cells in the oral cavity allows screening and identification of potential biomarkers for oral cancer. Besides, it can reduce anxiety and discomfort in the

patient compared routine biopsy techniques. Mi-RNA, are short non-coding RNAs encoded throughout the genome where some of these genomic regions are prone to alterations. An electrochemical bio-sensor method for the detection of oral cancer-related mi-RNAs at levels was developed which detects mi-RNA using a magnetic controllable gold electrode.[9] The advantage of this bio-sensor is magnetic beads-based enzymatic catalysis amplification which improves the sensitivity of bio-sensor.

Principles of detection

Bio-sensors work mainly via the following principles:[10]

(1) Photo-metric - Many of these optical bio-sensors are theorized on the phenomenon of surface plasma on resonance (SPR) which is an evanescent wave technique. This utilizes gold as an electrode. A photometric detection system is used to detect the optical signal.

(2) Electrochemical - Bio-sensors based on enzymatic catalysis of a reaction that produces or consumes electrons (the enzymes used for the catalysis are called redox enzymes).

(3) Piezoelectric - These acoustic devices utilize crystals undergo an elastic deformation when an electric potential is applied to them. The alternating current produces a wave in the crystal whose frequency is dependent on the elastic properties of the crystal.

(4) Thermal detection bio-sensor - Commonly used in the detection of pesticides and pathogenic bacteria, its high sensitivity to thermal changes without frequent calibration and intensity to optical and electro-chemical properties in the sample are some of its advantages.

Role of oral-fluid based bio-sensors in systemic diseases:

(1) DIABETES - Diabetes mellitus is a systemic disorder characterized by hyperglycemia, in which the patient should be regularly monitored to prevent further complications such as neuropathy, vascular diseases and predisposition to infection. For continuous monitoring of glucose levels, researchers have investigated the possibility of using GCF and saliva. The glucose level in saliva can be measured by a salivary nano bio-sensor which is an on-chip electrochemical sensing device. The charge transfer complex formed on the working electrode allows the direct oxidation of the glucose oxidase enzymes and thus determines salivary glucose levels.[11]

(2) *HUMAN IMMUNODEFICIENCY VIRUS* - It can also be used widely as a diagnostic tool for HIV and hepatitis C virus infection as it undergoes blood testing for Ab screening, particularly home testing. Electrochemical peptide sensors are fabricated for anti-HIV Ab detection which uses X-ray photoelectron spectroscopy to analyze salivary DNA on the sensor surface.[12]

(3) *PSYCHIATRIC DISORDERS* - Increased stress levels are observed due to change in lifestyle. This can lead to physical disorders such as cardiovascular, diabetes, obesity, and psychiatric disorders such as depression, schizophrenia, attention deficit and bipolar disorders. In saliva, cortisol and sAA are recognized as biomarkers for stress. A flow filtered ported, surface plasma on resonance (SPR) biosensor was developed to detect cortisol levels in saliva. In SPR bio-sensors, receptor molecules are immobilized on the gold sensor for detecting cortisol. For determining sAA levels, sAA bio-sensor is used which can act as a biomarker for autonomic dysregulation.[13]

(4) *DRUG ABUSE* - A serious problem with long term consequences. Illicit drugs can be detected in oral fluids, and in contrast to urine, they contain parent drugs rather than metabolites. Hence, they can be used as screening and confirmatory tests for illicit drug abuse. Lee et al developed giant magneto-resistive bio-sensors integrated with a portable reading system using competitive assays to trace tetrahydrocannabinol levels in saliva.[13]

Bio-sensors with saliva as an analyst:

(1) Bio-sensors using saliva help detect HIV-1, HIV-2, viral hepatitis, oral cancer, breast cancer, type-2 diabetes, periodontal disease and caries.[5]

(2) The oral fluid nanosensor test (OFNASET) is an automated, easy to use an integrated system that enables simultaneous as well as rapid detection of nucleic acid target and multiple salivary proteins. OFNASET

detector can also be used in the office of a dentist or another health care provider for various point-of-care disease screening and detection [14].

Applications of bio-sensors - cancer detection

a) Helps to spot cancer cells circulating in the blood either from a cancer patient, or treated tumour that has returned to the cancerous phase.[15,16]

b) Detects altered expression levels of micro RNAs, the new targets for cancer therapy, that are correlated with cancer type, tumour stage and response treatments.

c) Helps in detecting health-related problems associated with soft tissues including tendon and ligament, hard tissue as well as bone-related diseases such as osteoporosis and Paget's disease. [15,16]

Other uses:

A) Biosensors are used not only as diagnostic and prognostic tools in medicine but have various other uses[13]

B) Glucose monitoring in diabetic patients which is a historical market driver[2]

C) Pregnancy tests which detect the h CG protein in urine[1]

D) Estimates fracture risk and determine the response of bone to the treatment of bone disease[15]

E) Remote sensing of airborne bacteria example in counter-bio terrorist activities.

F) Determining levels of toxic substances before and after bio-remediation.[2]

G) Detection and determining of organophosphate.[17]

Conclusions

As bio-sensor technology advances a various range of applications broadens. Bio-sensors are now being made for the detection of microbial pathogens and their toxins, blood analysis, monitoring of glucose and other metabolites and other physiological monitoring and cancer detection. Oral fluid bio-sensors have the advantages of easy accessibility and noninvasive sample collection, making them a novel method in disease diagnostics. However, its limitations such as less sensitivity and specificity are overcome by the advent of new technologies such as microfluidics and nanofluidics. It is almost certain that in the forthcoming years home testing kits incorporating oral fluid bio-sensors will begin to appear, outperforming the routine laboratory tests in the diagnosis of disease.

Ethical permission: Not Required

Conflict of Interests: None

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Control Strategies and Behavioural Changes in the Elimination of COVID-19 from India

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Abstract

The massive spread of the novel coronavirus and its associated disease COVID 19 as a pandemic around the world has gripped the international community, crippled healthcare systems and raised enormous public health concerns. The universe attempts to restrict the virus until now have yielded little fruit because of the zoonotic transmission ability of the virus. Countless healthcare professionals have already become infected with the disease and dentists are considered to be especially vulnerable. This article reviews the origins of the virus, its modes of transmission, symptoms and the recommendation provided to the dentists to diagnose and manage a patient confirmed/ suspected of COVID 19 in the dental office.

Keywords**:** *COVID 19, Dentists, Coronavirus, Zoonotic, Healthcare*

Introduction

The rapid outbreak of COVID 19 in Wuhan, China, followed by its subsequent spread to the entire world, has led to a massive public health crisis. The disease is caused by a single-stranded RNA virus belonging to the family Coronaviridae.[1] These viruses are zoonotic and the world has witnessed previous outbreaks of the severe acute respiratory syndrome (2002) and the Middle East respiratory syndrome (2012), which are diseases caused by viruses of the same family.[2-4]

The widespread transmission of the novel coronavirus has seen even the health care professionals getting infected. Dentists are considered among the professionals at high risk for the disease given the particular kind of the interventions they render, which include aerosol generation, proximity to the patient's oropharyngeal region. Thus, through this article, we summarize the recommendations that dentists should follow in terms of diagnosing and managing patients who are confirmed/suspected of COVID 19.

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Backdrop[5]

- In December 2019, Wuhan city of China reported several pneumonia-like cases that rapidly spread to other provinces.
- On 7th January 2020, it was officially stated that the Novel Corona Virus was the pathogen responsible for the epidemic.
- On 30th January 2020, the WHO declared this outbreak as a matter of global health emergency.
- On 11th March 2020,this outbreak was declared a pandemic by the WHO.
- As of 28th April 2020, 210 countries are affected by this pandemic, with over 3 million positive cases and 211,658 deaths of Covid-19 worldwide.

About the novel coronavirus

According to research, the virus, which is a type of SARS virus (also named as SARS CoV-2), most probably originated from a specific species of bat, Rhinolopussinicus with pangolins as its intermediate

host. Taxonomy of viruses has been scientifically named as SARS CoV-2, although it is widely known as the COVID-19 Virus. Coronavirus (Family-Coronaviridae, Order-Nidovirales) consists of ssRNA as their genetic material with 'spike proteins' in the membrane along with some other membrane proteins and polyproteins. There are 4 genera of coronaviruses, α,β,γ and δ, with α and β affecting humans. It has been observed that 2019-nCoV uses ACE2 as a receptor to enter cells, similar to the SARS coronavirus, which is responsible for human-to-human transmissions.

The typical clinical features of this viral disease are fever, headache with dry cough sometimes accompanied by fatigue, sputum, myalgia and shortness of breath in severe cases. Majority of the patients detected are mild cases. It has an incubation period of 14 days on an average with upto 4% fatality rate. The symptomatic patients are main sources of transmission but it has also been observed that asymptomatic patients and patients in their incubation period can also be carriers of this virus.[6]

Routes of Transmission of Novel Coronavirus in Dental settings-

Most of the dental procedures produce aerosols which can remain suspended in the air for long periods, thus having the risk of inhalation by dental professionals. This becomes the most important concern in dental setups because the production of air droplets and aerosols is inevitable (Figure 1).

Dentists also have frequent contact with body fluids such as blood, saliva and contaminated dental instruments which can also act as a route of transmission for this virus. This virus can remain on the contaminated surfaces from 2 hours up to 9 days at room temperature, better at 50% as compared with 30% relative moisture.

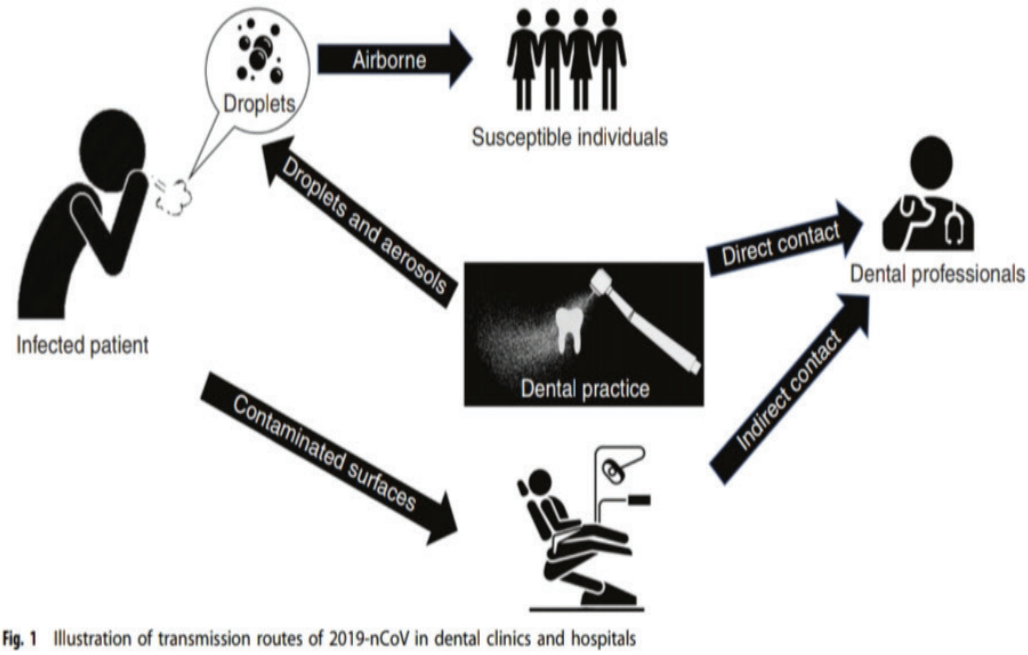

Fig. 1 Illustration of transmission routes of 2019-nCoV in dental clinics and hospitals

Figure 1: Diagram of transmission routes of the novel coronavirus in hospitals and dental clinics

The above-mentioned routes of transmission of this virus have posed a great risk for dentists to prevent its spread, especially in dental clinics as there is an increased chance of cross-infection between the dental professionals and patients.

The dentists should thus be aware of the possible transmission routes as well as ought to be able to recognize a suspected COVID-19 case. On 15th March 2020, the New York Times published an article titled as: The Workers Who Face. The Greatest Coronavirus Riskwhich stated that the dental professionals are at the greater risk of being affected by Covid-19.[7]

Scope of Green Recovery in Dental Practice-

It is very difficult to predict the future regarding dentistry amidst this outbreak. Instead of being pessimistic about the future, we must ensure to follow the guidelines being issued by dental authorities.[5] Now, the dental procedures have been classified into various groups-

a) Crisis Management of Life-Threatening Conditions.

b) Urgent circumstances that can be managed with the least invasive procedures and without aerosol production.

c) Urgent circumstances that need to be managed with invasive and/or aerosol-generating procedures.

d) Non-urgent procedures.

e) Elective procedures.

All the elective procedures, non-urgent and regular dental visits should be deferred. (Adult Elective Surgery and Procedures Recommendation). Currently, the main focus should be on Primary Care Dental Triage which comprises of 3As- Advice, Analgesia, Antimicrobial (where needed)[8]

Patients should be explained how to manage their symptoms without coming to clinics as the treatment options are severely limited. Whenever there is a need to prescribe any analgesic/antibiotic, it should be done after taking into account the complete medical history (including consumption of any over the counter drugs).

Table 1: Recommended analgesic doses for adults.

For moderate dental pain in adults, an appropriate 5-day regimen is either:
· paracetamol, 2 x 500 mg tablets up to four times daily (i.e. every 4-6 hours) or
· ibuprofen, 2 x 200 mg tablets up to four times daily (i.e.every 4-6 hours), preferably after food.
For severe dental pain in adults, an appropriate 5-day regimen is either:
· increase the dose of ibuprofen to 3 x 200mg tablets up to four times daily, preferably after food or
· ibuprofen and paracetamol together, preferably after food,without exceeding the daily dose or frequency for either drug, as above or
· diclofenac (1 x 50 mg tablet three times daily) and paracetamol together, preferably after food,without exceeding the recommended daily dose or frequency for either drug.

N.B. Maximum drug doses in 24-hour period: 4 g paracetamo l;2.4 g ibuprofen;150 mg diclofenac.

Do not prescribe diclofenac or high doses of ibuprofen (i.e. more than 1.6 g daily dose) for patients with moderate or severe asthma, those with hypersensitivity to aspirin or any other NSAID or those with renal impairment.

Table 2:Recommended analgesic doses for children

For dental pain in children, an appropriate 5-day regimen is either:			
· Paracetamol (500 mg tablets, or 120 mg/5 ml or 250 mg/5 ml oral suspension*), dose depending on age (see below): up to four times daily (max 4 doses in 24 hours):			
6-12 months	120mg	8-9 years	360-375mg
2-3 years	180mg	10-11 years	480-500mg
4-5 years	240mg	12-15 years	480-750mg
6-7 years	240-250mg	16-17 years	500mg-1g
or			
· Ibuprofen (200 mg tablets, or 100 mg/5 ml oral suspension*), dose depending on age (see below): preferably after food, upto three times daily unless indicated otherwise below:			
6-11 months	50 mg (4 x daily)	7-9 years	200 mg
1-3 years	100mg	10-11 years	300mg
4-6 years	150 mg	12-17 years	300-400 mg(4 x daily)

*Sugar-free preparation is available.

N.B. Combining paracetamol and ibuprofen is not recommended for children without consulting a pharmacist or medical practitioner.

Table 3:Recommended first-line antibiotic doses for adults (see Table 1 for duration)

For dental infection in adults,either:
· amoxicillin, 1 x 500 mg capsule 3 times daily, or
· phenoxymethylpenicillin, 2 x 250 mg tablets 4 times daily, or · metronidazole, 1 x 400 mg tablet 3 times daily.

N.B. For severe infections (e.g. extra-oral swelling, eye closing or trismus), the dose of amoxicillin and phenoxymethylpenicillin can be doubled.

Table 4: Recommended first-line antibiotic doses for children.

For dental infection in children, either:			
· Amoxicillin (250 mg capsules, or Oral Suspension* 125 mg/5 ml or 250 mg/5 ml) dose depending on age (see below); three times daily.			
6-11 months	125 mg	5-11 years	500 mg
1-4 years	250 mg	12-17 years	500 mg
For severe infection in children aged 6 months to 11 years, increase the dose of amoxicillin up to 30 mg/kg (max 1 g) three times daily. For severe infection in children aged 12-17 years, double the dose of amoxicillin. Or			
· Phenoxymethypencillin (250 mg tablets, or Oral Solution* 125 mg/5 ml or 250 mg/5 ml) dose depending on age (see below); four times daily.			
6-11 months	62.5 mg	6-11 years	250 mg
1-5 years	125 mg	12-17 years	500 mg
Or			
· Metronidazole (200 mg tablets, or Oral Solution* 200 mg/5 ml) dose depending on age (see below); threetimes daily unless indicated below.			
1-2 years	50 mg	7-9 years	100 mg
3-6 years	100 mg(2 x daily)	10-17 years	200 mg

*Sugar-free preparation is available.

There has been an issue regarding the use of Ibuprofen as analgesics in infected patients saying that it could worsen the condition although it hasn't been proved yet. Thus, it is recommended to prescribe paracetamol instead of Ibuprofen in confirmed and suspected cases. But Ibuprofen can still be used for dental pain management in patients not having COVID-19 infection.

✓The patients should be contacted before emergency dental treatment to assess his/her condition and decide whether to visit a clinic or not.

✓The pre-check staff should be instructed to measure the body temperature of the patient visiting the clinic using a contact-free forehead thermometer.

✓A questionnaire including questions regarding any symptoms shown by the patient, their travel history or contact history with any suspected or positive case of COVID-19 in the past 14 days should be provided to the patients to fill in.

✓All of the above procedures should be done at the reception area itself. If the reply to any of the above screening questions is in affirmation, the treatment should be postponed till 14 days and the dentist should immediately report to the nearby hospital. The dentist should always avoid treatment in such cases unless it is an emergency.

✓ Hand -hygiene should be maintained at all times. Dental professionals should wash their hands before and after any dental procedure and should also avoid touching the surroundings including their own eyes, mouth and nose.

✓ Dentists should always prefer using PPE (Personal Protective Equipment) which includes head caps, mouth mask, protective eye wears, face shield, gloves, shoe covers etc while performing any dental procedure that produces aerosol.

There are three levels of protection in the dental setup -

1. Primary protection -for staff to wear which includes head cap, mask, gloves, eyewear, face shield.

2. Secondary protection- Advanced protection for dental professionals.

3. Tertiary protection- strengthened protection where dental professionals come in contact with confirmed/suspected case of infection.

✓N95 respirators should be preferably used in procedures that produce aerosol. These disposable respirators should be discarded as soon as the procedure has ended. A combination of a surgical mask and full-face shield is advisable in cases where such respirators are not available. One must ensure that such surgical masks have cleared the FDA guidelines. Surgical masks should be changed even during the procedure if it becomes wet.[9,10]

✓ Pre-operative mouth rinses are advisable as it helps in reducing the oral microbial load. Chlorhexidine mouth rinses may not be as effective as oxidative agents such as 1% H_2O_2 or 0.2% Povidone as it is believed that nCoV is vulnerable to oxidation.

✓ Rubber Dam Isolation is by far the best way to reduce the aerosol and spatter especially in cases where aerator or ultrasonic devices are to be used. In such cases of rubber dam application, four-handed dentistry is advisable. If rubber dam placement is unachievable, manual devices such as hand scaler and materials such as cariosolv are recommended to reduce the aerosol production. Prioritize minimally invasive / Atraumatic Restorative Technique (hand instruments only).

✓ Anti-Retraction handpieces should be used to decrease the backflow of oral pathogens into dental units as an extra preventive measure for cross-infection.

✓Intraoral radiography should be avoided as it stimulates saliva secretion and coughing. Therefore, extraoral radiography such as CBCT, a panoramic radiograph should be considered as appropriate alternatives.

✓The dental waste such as soiled cotton, extracted tooth, needles, disposable PPE after use should be regarded as infectious medical waste and be disposed of in double yellow colour medical waste package bags with 'gooseneck' knot.

✓The instruments should be pre-treated, sterilized & properly stored for further use. Clean and disinfect the surfaces and equipment present within 6 feet of symptomatic patients. Dental clinics should be disinfected and cleaned properly and frequently including the doorknobs, desks, handles, dental chair etc.

Conclusions

The emerging threat of COVID 19 as a pandemic and its fast transmission rates make the healthcare professionals and especially the dentists vulnerable for contracting the disease and the dentists must maintain high standards of infection control in the clinical setup. Thus, it becomes important for the dentists to be well aware of the recommendations put forward to render dental treatment in these trying times without putting themselves, the staff and patients at risk of the disease.

Ethical Permission: Not Required

Conflict of Interests: None

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A Comparative Study of the Efficacy of Methylprednisolone Administration Route on Postoperative Sequelae Following Impacted Third Molar Extraction

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Abstract

Background: Surgery on the lower-impacted third molar typically includes injury to the heavily vascularized loose connective tissue due to its anatomical location, leading to inflammatory sequelae during the immediate post-operative stage, including postoperative pain, oedema and trismus.

Aims: This prospective, randomized, comparative clinical study was conducted in 30 patients to assess pain, oedema and trismus in patients undergoing surgical removal of impacted third molars.

Method: The study sample involves 30 patients, randomly categorized into 3 groups: group 1 (control; no steroids), group 2 (Intra-Muscular injection in Masseter muscle), group 3 (oral tablets). Patient aged between 18 and 30, with a similar anatomical position, and similar surgical difficulty, no allergies, without any systemic diseases are included. Syndromic patients, patients with periapical pathologies were excluded from the study. A single surgeon was working on all patients using a standard technique. On the 2nd and 7th postoperative day, linear oedema and maximal mouth opening were evaluated for both routes of methylprednisolone administration and compared.

Results: We observed a higher range of mouth opening found in masseter injection group relative to the oral tablet group on Day 2 & 7, for the steroid treatment groups. On postoperative days 2 and 7, the overall increase in the tragus-commissure, canthus-gonion and tragus-pogonion lines, for control & oral tablet groups were identical and higher than the masseter injection group.

Conclusion: Comparison to the control group following lower third molar surgery, intramassetric injection provided improved outcomes than tablet form in limiting oedema and trismus.

Keywords*: Methylprednisolone Administration; Postoperative Sequelae; Impacted Third Molar Extraction*

Introduction

Inflammation & analgesia are biological reactions of the body to trauma of several origins. Following any surgical procedure, inflammatory mediators are emitted into the tissues in reaction to analgesia, resulting in increased vascular dilatation and permeation, resulting in oedema.[1] In oral & maxillofacial surgery, surgical extraction of mandibular 3rd molars has been the most frequent surgical technique. Owing to the anatomical location of the affected 3rd molars, both soft and hard tissue lesions occur during surgery, leading to postoperative oedema & trismus.[2,3] Furthermore, management of postoperative reactions, can improve patient outcomes by all way. Methylprednisolone is a drug used as a corticosteroid to suppress the immune system and reduce inflammation.

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Mechanism of Action: Unbound glucocorticoids invade cell membranes and attach with certain cytoplasmic receptors, altering transcription and protein synthesis. Glucocorticoids can prevent leukocyte infiltration at the inflammation site through this mechanism, interact with inflammatory mediator production, such as prostaglandins and leukotrienes, and suppress immune function.[4]

Routes Of Administration- Oral; Intramuscular; Intravenous

Indications

1.Endocrine diseases- Primary & secondary adrenal insufficiency & Congenital adrenal hypoplasia

2.Rheumatic diseases-Rheumatoid arthritis & Ankylosing spondylitis

3.Allergic states

4.Dermatological diseases- Pemphigus Vulgaris

5.Ophthalmic diseases

6.Respiratory diseases

7.Neoplastic diseases- Leukaemia

8.Gastrointestinal diseases- Ulcerative colitis

Contraindications: Methylprednisolone is contraindicated when there is known hypersensitivity to components and systemic infection. Its use cautiously in chronic treatment (will lead to adrenal suppression), paediatric patients, obstetrics and neonates

Aim: Purpose of the study "to compare the efficacy of two different routes of administration of methylprednisolone on oedema and trismus after impacted lower 3rd molar surgery".

Inclusion Criteria-

- Age-18 -35
- Asa category-1
- Impacted mandibular third molar –

Ø Winter's classification- Mesioangular,

Ø Pell and Gregory classification-Class 11, Position B

Exclusion Criteria

Ø Smoking

Ø Pregnancy or lactation

Ø Medications interfering healing process

MATERIALS & METHODS

This randomised, prospective and controlled trial involved 30 patients requiring the extraction of a lower 3rd molar that had been impacted. In the control category (group-1), the first 10 patients did not receive preoperative or postoperative steroids. Two further categories were allocated to the remaining 20 patients, with 10 patients in each. Group-2 patients were administered methyl-prednisolone (single dose of 20 mg/ml) into the masseter muscle by parenteral injection; it was achieved in the immediate postoperative time by the intra-buccal method. Group-3 patients were administered methyl-prednisolone(single 20 mg dose) 1 h before treatment as oral medication.

Parameters To Be Evaluated

Trismus and oedema were examined after the surgical intervention and compared to preoperative measurements on day 2 and 7.

Using 3 facial lines Edema was evaluated (Fig. 1):

(1) lateral canthus of eye to gonion angle distance;

(2) tragus to mouth commissure distance on treatment side;

(3) tragus to the soft pogonion distance.

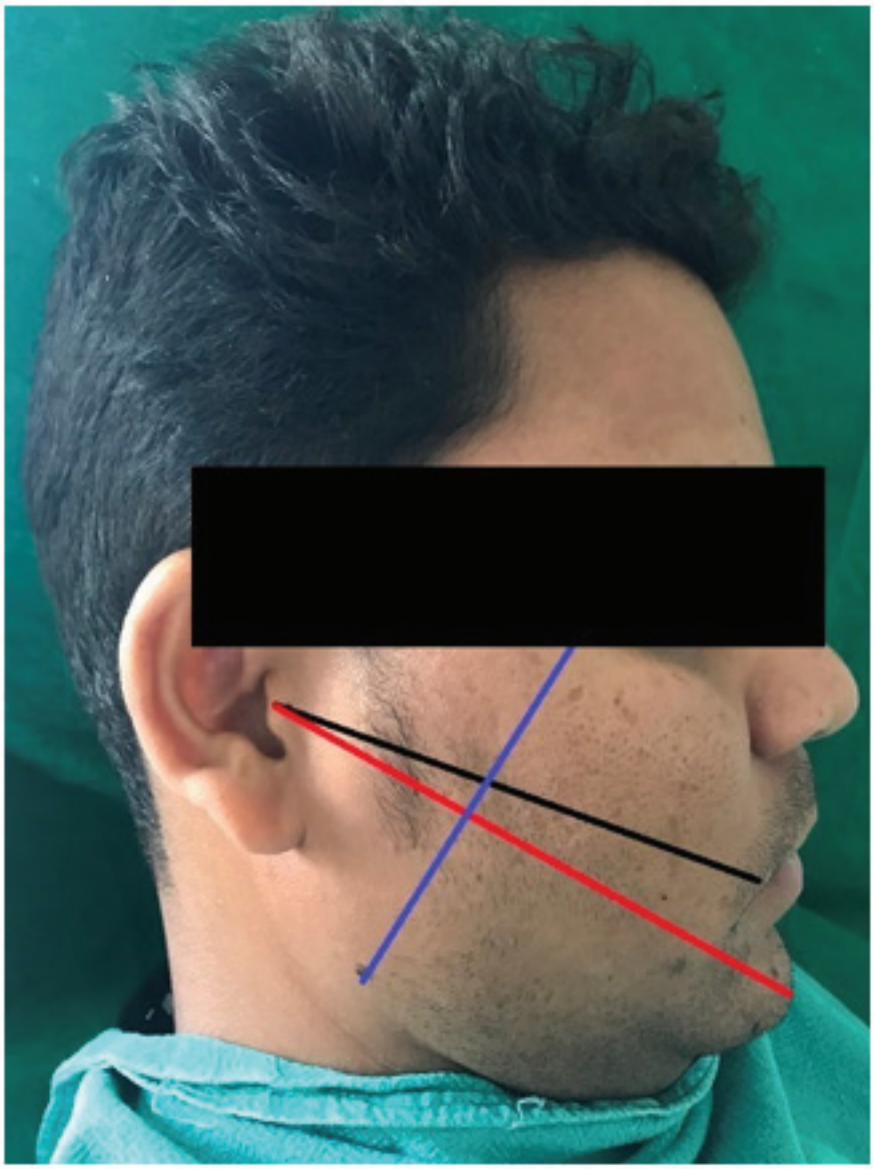

Figure 1. Linear demarcation measures for swelling: canthus–gonion line(blue line); tragus–commissure line(black line); tragus–pogonion line(red line).

The range between upper & lower incisional edges of central incisors was assessed for the evaluation of trismus.[5] For all patients, initial preoperative assessment has been performed. A single surgeon was working on all patients. Patients were prepared for the surgical operation, following standardized surgical and aseptic guidelines.[6] Inferior alveolar, lingual and long buccal nerve blocks were administered. To obtain access, a regular Ward's incision was used, and the tooth was extracted after sufficient bone cutting and tooth splits as were required. To ensure minimum trauma to the tissues, precaution was taken. The socket was furiously irrigated after extraction, using betadine solution combined with equivalent parts of regular saline. With 3-0 mersilk the flap was sutured. All patients were given routine postoperative instructions & were prescribed a 5 days course of antibiotic and analgesic. On a postoperative day 7, intra-oral sutures were removed.[7]

Results

Trismus: The opening of the mouth after the injection of the masseter & oral tablet of methylprednisolone on postoperative days 2 and 7 was observed to be better than in the control group. The higher range of mouth

opening found in masseter injection group relative to the oral tablet group on Day 2 & 7, for the steroid treatment groups.[8] This implies that methylprednisolone is more effective in regional injection form than the oral tablet group in reducing postoperative trismus.

Postoperative Swelling: On postoperative days 2 and 7, the overall levels of the rise in the tragus-commissure, canthus-gonion and tragus-pogonion lines, for control & oral tablet groups were identical and higher than the masseter injection group. [9] For masseter injection group, relevant change was not observed between pre-operative & post-operative measures in canthus-gonion distance and tragus-pogonion line on day 7. In the present study, masseteric methylprednisolone injection is most efficient in lowering swelling on a postoperative day 2 and day 7.[10]

Table 1. Randomised, prospective and controlled trial values

	Time	Control Group	Local Infiltration Group	Oral Tablet Group	Average
Interincisal Distance(cm)	Preop Postop day 2 Post-op day 7 Average	4.6 3.1 3.7 3.8	4.3 3.6 4.2 4.0	4.5 3.2 4.0 3.9	4.4 3.3 3.9
Tragus-commissure line(cm)	Preop Postop day 2 Post-op day 7 Average	11.2 12.4 11.8 11.8	11.6 11.9 11.7 11.7	11.5 12.4 11.8 11.9	11.4 12.2 11.7
Tragus-commissure line(cm)	Preop Postop day 2 Post-op day 7 Average	9.3 9.8 9.5 9.5	9.5 9.7 9.5 9.6	9.6 9.9 9.7 9.7	9.4 9.8 9.6
Tragus-pogonion line(cm)	Preop Postop day 2 Post-op day 7 Average	14.2 15.7 15.2 15.0	14.5 14.7 14.5 14.6	14.3 15.3 14.7 14.7	14.3 15.2 14.7

Disscussion

Inflammatory responses are a protective system in the tissues of the body. Oedema, trismus, pain and prolonged recovery, however, can decrease the patient's quality of life and are difficult to tolerate. With a large degree of possibility, these post-operative conditions are shown.[11]The effectiveness of corticosteroids has been researched in several trials. Since it has minimal mineralocorticoid production and maintains a level of therapeutic plasma in the early postoperative stage, methylprednisolone was chosen for this analysis.[12]

For both the surgeon & patient, this procedure is simple, quick and the injection site is near to the surgical field that is already anaesthetized. Pain is a subjective phenomenon and is caused by variables such as age, sex, levels of anxiety and surgical difficulty about which it is hard to achieve an objective conclusion and was not assessed in the present analysis.[13] The possible adverse

reactions with glucocorticoids depend on the therapy duration and intensity.[14] Chronic steroid usage can induce adrenal suppression, but it has been shown by Novak et al that no complications resulted from a single large dose / short term of methylprednisolone. In this analysis, side effects were not observed. In this present study, the predicted points (the tragus, lateral canthus, oral commissure, gonion, and pogonion) were used to assess oedema, as described by Antunes et al, for validity, convenience, low cost & procedure repeatability purposes, to compare oedema.[15, 16]

Conclusions

Our findings confirm the observation that methylprednisolone injection into masseter is successful in minimising postoperative oedema & trismus. To achieve the most accurate results and to mitigate adverse effects in patients, more trials with larger samples are required.

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Ethical Statement: This study was approved by the institutional ethical committee.

Conflict of Interest: The author declares that there was no conflict of interest.

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Acrocephalosyndactyly Syndrome: A Rare Case Report

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Abstract

Acrocephalosyndactyly or Apert syndrome is a rare disorder in which the fingers and toes are fused, giving the appearance of webbed hand and feet. Also, there is craniosynostosis, leading to severe mid-face hypoplasia and characteristic facial deformities. Here there is a genetic mutation in the fibroblast growth factor receptor 2 (FGFR2) gene with autosomal dominant inheritance. A case of an 8-year-old male child is reported here with frontal bossing, acrocephaly, prominent ocular hypertelorism with divergent strabismus, saddle-shaped nose, mid-face hypoplasia, crowded anterior teeth, sparse hair and severe bilateral syndactyly of the feet and hands.

Keywords*: Craniosynostosis, Facial Deformity, Malocclusion, Syndactyly.*

Introduction

Eugene Apert, a French doctor first described Apert syndrome which has acrocephalosyndactyly. [1] This is a rare autosomal dominant developmental disorder in which the individual is affected from birth with craniosynostosis, midface hypoplasia, webbed, fused hands and feet.[2] Deformities like a cone-shaped skull, frontal bossing, proptosis, prominent hypertelorism with divergent strabismus, saddle-shaped nose and a hypoplastic maxilla are observed. Dental malocclusion, high arched palate, ectopic, malaligned crowded teeth are seen. The mandible may be normal but due to a hypoplastic maxilla, the mandible may appear prognathic. Other systemic diseases have been associated with the syndrome.[3]A case of an eight-year-old male child affected by acrosyndactyl syndrome with all the classic features is reported for documentation.

Case Report

An 8-year-old male child reported to the dental hospital for enhancement of facial aesthetic appearance. History revealed that the child was born with facial deformities and fused hands and feet. He was otherwise normal, and the medical and family history was non-contributory.

On clinical examination, the face was deformed, (figure 1 and 2) and the fingers and toes appeared web-like and fused. (figure 3 and 4) He had malocclusion, high arched palate, pseudo-class III malocclusion, cross-bite and delayed eruption of permanent teeth. The skull was cone-shaped or acrocephalous, hypoplastic maxilla, bilateral divergent strabismus, hypertelorism with propoptosis, scanty hairline, saddle-shaped nose and frontal bossing. Features were suggestive of Acrocephalosyndactyly syndrome.

The patient was referred to a paediatrician for further evaluation to exclude other systemic diseases. The patient's mental function was evaluated and found normal. He was found not to be suffering from any cardiac, gastrointestinal, renal or respiratory diseases. Full skeletal radiographs were taken and revealed syndactyly, acrocephaly and dental malocclusion.

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13.

Figure 1: Frontal view showing divergent strabismus, hypertelorism, saddle shaped nose.

Figure 2: Profile picture showing ear tags and sparse hair.

Figure 3:Syndactyly in hands appearing like web.

Figure 4: Syndactyly of the toes appearing like webbed feet.

Discussion

Acrocephalosyndactyly syndrome (AS) or Apert syndrome is one of the craniosynostosis syndromes and is associated with syndactyly. which is a form of craniosynostosis. [4] It has been reported in the literature that parenting at an advanced age leads to AS. [5] Though it is of autosomal dominant inheritance, sporadic mutations occur like in the present case without any family history of AS. There is no gender or racial predilection and the disorder is quite rare occurring about ten to fifteen in a million live births. [6]

The pathology is in the mutation of FGFR2 gene which codes for a protein known as fibroblast growth factor receptor and is responsible for forming vascular channels, healing of wounds, the evolution of an embryo, regulates the division of cells, cell growth and cell maturation. This mutated gene causes defective signalling in pathways that initiate the skull bone fusion, hence there is premature craniosynostosis.[7] There is also a defect in the epithelial-mesenchymal interaction leading to defective membranous and endochondral ossification. [8]

The facial appearance results due to premature fusion of the coronal sutures so there is a short anteroposterior diameter and enlarged forehead giving rise to the acrocephalic skull. Depressed nasal bridge and hypoplasia of maxilla, as well as dental malocclusion, are common findings. [7,8] Ocular anomalies like hypertelorism, strabismus, propoptosis are also frequent manifestations.[9] Syndactyly of the fingers and toes are the classic features and most important differentiating feature between other craniosynostosis syndromes. [8-10]

Extremely rare systemic manifestations like vertebral mal-segmentation, hypogenesis/ agenesis of the corpus callosum, defects of the cerebrum and cerebellum, enlarged ventricles have been reported. [11] Obstructive sleep apnoea, respiratory infections, mal-nourishment, etc have been rarely mentioned in the English literature. Mental retardation may also be rarely seen. [12]

Oral manifestations like malocclusion and crowded teeth are due to the decrease in the anteroposterior maxillary growth. [11]

Hand and foot deformities are characteristic and syndactyly may be associated with polydactyly also which was not seen in our patient. Since the quality of life of these patients gets severely affected due fused hands and toes, surgical correction of hands and feet is a priority. The patient's feet are unable to bear the weight like normal feet, and they cannot wear normal shoes also. Many a times patients are most uncomfortable wearing shoes. Other craniosynostosis syndromes like Crouzon syndrome, Carpenter syndrome, Chotzen syndrome and Pfeiffer syndrome must be considered in the differential diagnosis.[12]

Conclusion

The present reported case had the characteristic facial features and syndactyly, all suggestive of AS besides sparse scanty hair and short stature. It may be emphasized that when there is a family history of AS then foetal DNA analysis must be performed to diagnose any DNA mutations like the FNGR gene mutation Moreover pre-natal diagnosis can establish craniosynostosis as well as syndactyly on prenatal ultrasonographic images. AS is a rare disorder without a complete cure and the aesthetic issues associated with disease leads to depression and low confidence in the patients due to their facial appearance. Hence the aim is to diagnose such developmental anomalies early in the foetus and consider medical termination of pregnancy.

Conflicts of Interest: None

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Ethical Permission: Approved

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Skeletal Bimaxillary Protrusion: Combined Surgical and Orthodontic Approach – A Case Report

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Abstract

Bimaxillary protrusion is one of the commonly seen skeletal deformity in Indian population. This skeletal condition is typically characterized by proclined and protruding anterior teeth with everted lips. If this condition is associated with macroglossia or any parafunctional habits like tongue thrusting or thumb sucking, an anterior open bite is often found. Minor discrepancies can be managed by orthodontic treatment alone by extracting all first premolars and retracting the anterior segment. But in severe skeletal cases, orthognathic surgery along with pre-and post-surgical orthodontics is the best option to get the ideal result. This article describes the case of a female patient with bimaxillary protrusion. The patient had a convex profile, anterior open bite, and incompetent lips. Chin had a relative deficiency with hyperactive mentalis muscle. On patients' consent and willingness for a shorter course of the treatment period, surgery first approach was planned. Within seven months of the total treatment period, a remarkable change in facial profile and a pleasing anterior occlusion was achieved.

Keywords: *bimaxillary protrusion, anterior segmental osteotomy*

Introduction

Bimaxillary protrusion is one of the commonly seen skeletal deformity in Indian population. This skeletal condition is typically characterized by proclined and protruding anterior teeth with everted lips. Lip incompetency, excessive gingival show and hyperactive mentalis muscle may also be found in this condition. Aesthetic correction in this group of patients is of primary concern. Minor discrepancies can be managed by orthodontic treatment alone by extracting all first premolars and retracting the anterior segment back. But in severe skeletal cases, orthognathic surgery is the only option to get the ideal result. Moreover, the conventional orthodontic treatment is more complicated in adult patients due to difficulty in achieving desired tooth movement, longer treatment time, compliance of the patient, different societal requirements from the patients' side and risk of worsening of periodontal status [(1)]. Cohn-Stock has been credited for reporting first anterior segmental osteotomy in the maxilla in the year 1921 [(2)]. After him, various modifications to the procedure depending upon the type of incision, degree of osseous movement and rotation of the osteotomized segment was reported. Until 1942 only anterior maxillary segmental osteotomies were performed. In 1959, Kole popularized this technique for both maxillary anterior and posterior segments [(3)]. Single-stage set-back osteotomy through a vestibular approach was first described by Wassmund [(4)]. Wunderer presented an important improvement of Wassmund's technique by recommending a predominantly palatal approach, which simplified the procedure [(5)]. In his technique, the palatal flap was

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raised and bony cut through the palatal aspect is made. Buccal soft tissue pedicle is kept intact for vascularity of the osteotomized segment. Down-fracturing concept was given by Bell in which the anterior segment was approached through a horizontal vestibular incision [6]. Despite aforesaid modifications, risk of necrosis of the osteotomized segment and devitalization of teeth persist. To overcome these shortcomings, the European craniomaxillofacial surgeon's Congress modified the technique for anterior maxillary osteotomy in the year 1996 in Zurich [7]. According to this modification, a horizontal incision from canine to the canine is given. Maintaining the soft tissue pedicle over the extraction socket, the vertical bone cut is given bicortical and joined by a horizontal cut 5mm above the canine root tip. The osteotomized anterior segment is then pushed back to the extraction space posteriorly. Further, a clockwise rotation of the segment is advised to counter the unaesthetic senile appearance. Choi et al. reiterated a clockwise pivotal rotation along with setback for the maxillary anterior segment to prevent the aged appearance [8].

Hullihen is credited to first describe the anterior mandibular subapical osteotomy in the year 1849[9]. Hofer made this procedure popular by recommending its use for dentoalveolar set-back as well as advancement. Kole advocated this osteotomy to treat anterior skeletal open bite by the interposition of a bone graft taken from the lower border of the mandibular symphysis [3]. McIntosh in the year 1974 described the total mandibular alveolar osteotomy for correction of anterior open bite. [10] In the present case, a bijaw anterior subapical osteotomy with the clockwise pivotal rotation of the maxillary segment was performed and a satisfactory improvement in facial aesthetics post-surgery has been achieved.

Case Report

A 22-year old adult female reported with the chief complaint of forwardly placed upper and lower front teeth. On extraoral examination, the profile was convex with an acute nasolabial angle, lip incompetence and a deficient chin point. On intraoral examination, maxillomandibular protrusion with anterior open bite was seen. There was Class I molar and canine relationship bilaterally. The excessive display of gingiva was evident in smiling. (Fig.1 and 2) Treatment objectives were alignment and levelling of both arches and surgical retraction of the maxilla and mandible with anterior sub-apical osteotomy. The patient was advised for orthodontic correction of the maxillomandibular protrusion with the extraction of all four first premolars followed by retraction of anteriors in both the arches. Due to social obligations, the patient wanted early results, so the patient was advised for a surgical correction first and brief settlement later.

Before surgery, the patient's radiograph and articulated casts were evaluated. As the patient had class I molar and canine relationship without vertical maxillary excess, with anterior open bite, so the problem area to be addressed was anterior segment only. The occlusal plane was divergent from the canine region leading to anterior open bite. Despite having a potentially competent lip patient was unable to close her lip. Anterior sub-apical osteotomy (ASO) was planned with the extraction of all four first premolars with set back and clockwise rotation of the maxillary and counterclockwise rotation of the mandibular anterior segment to correct open bite and protrusion.

The above treatment plan was discussed with the patient and her relatives highlighting a probable course of postoperative events like swelling, temporary paresthesia due to nerve injury, and a period of intermaxillary fixation. Psychological counselling of the patient was done with Visual Treatment Objective (VTO) for better acceptance of postoperative results.

The maxillary vestibular incision from canine to canine region was given, the mucoperiosteal flap was being raised superiorly. Buccal and palatal tunnelling was done on both the sides as in Wassmund's technique. Osteotomy cut was given 5 mm above the canine root apex up to the first premolar region. Buccal and palatal cuts were given through the mucoperiosteal tunnels. With fine osteotome, all the cuts were completed, and down fracture of the maxillary anterior segment was done. Around 6-7 mm of bone was removed from both the posterior aspect of the osteotomized maxilla, and it was repositioned posteriorly with a slight amount of clockwise rotation. In the mandibular arch, the above procedure was repeated with counter-clockwise rotation to correct the open bite (Fig.3)

After completion of the treatment, there was no anterior open bite with nearly ideal overjet and overbite. There was a reduced amount of gingival show on rest and smiling. Preoperatively patient was unable to seal her lip due to the skeletal defect, which improved drastically thus improving the facial esthetics. (Fig. 4 and 5)

Figure 1 Preoperative view. (A) Frontal (B) Profile.

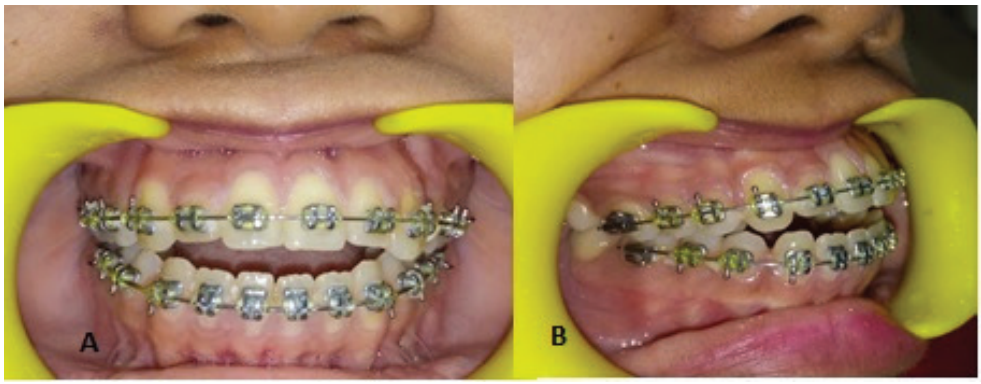

Figure 2 Preoperative intraoral view (A) Frontal (B) Profile

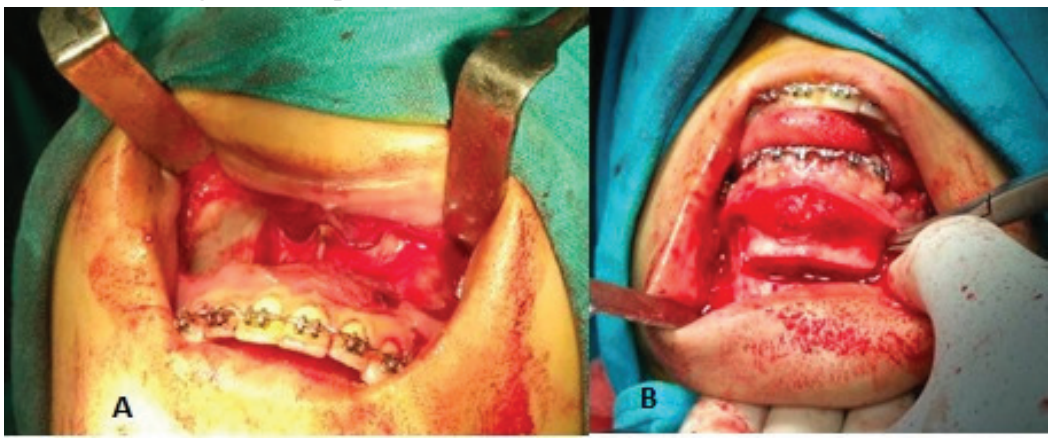

Figure 3 Intraoperative View

Figure 4 Postoperative view. (A)Frontal (B) Profile

Figure 5 Postoperative intraoral view (A) Frontal (B) Profile

Discussion

Anterior segmental osteotomy is prescribed in cases where the considerable movement of the anterior tooth segment is required, but tooth repositioning by orthodontic treatment alone is not possible. Reason for the difficulty in orthodontic treatment alone being the age of the patient making the physiologic movement of teeth considerably difficult, the extent of tooth movement required, periodontal status and duration of treatment [6,11]. In our case although all parameters were favourable for the orthodontic treatment, the patient insisted on fast treatment results, so she was advised for surgical correction. As the patient has anterior open bite along with bimaxillary protrusion, the osteotomized segments were counterclockwise rotated as suggested by Choi et al [8].

Any orthognathic surgical procedure deals with the facial harmony between soft tissue and hard tissue, thus the basic understanding of the facial musculature and effect of change in bony architecture should be considered in treatment planning. The changes anticipated after the surgery should be analyzed on macro as well as the micro aesthetic level to obtain pleasant and harmonious anatomy. The effect of age change on the soft tissue drape on the face is of vital importance. As we grow older, the lip coverage on the anterior teeth increases and teeth exposure decreases. Due to loss in tissue turgidity, the lips become saggy and muscles become relatively inelastic. These tissue changes result in the lengthening of the philtrum and deepening of the nasolabial fold. In any maxillofacial surgery, if the teeth exposure is severely decreased, then the face will look older [12,13]. In anterior segmental osteotomies, these soft tissue changes pose a significant challenge and thorough planning of the case before the surgery is mandatory.

Substantial increase in nasolabial angle and prominence of mentolabial fold was evident after correction of maxillary protrusion and open bite. A relative deficiency of chin was not addressed during surgery, as a setback and counter-rotation of the mandibular anterior segment made the lower lip fall back from its original position thus accentuated the chin prominence. With the correction of bimaxillary protrusion, there was a change in facial profile from convex to straight. The patient was satisfied with the change in esthetics and the result of treatment. The patient's psychological and social wellbeing was improved.

Conclusion

Anterior subapical osteotomy is a versatile orthognathic procedure which can correct the severe dentoalveolar discrepancy in a shorter period. Thorough preoperative planning between the surgeon and the orthodontist is crucial in formulating an effective, patient-specific treatment plan. Adult patients with severe malocclusion, poor periodontal health and desire to have a shorter course of treatment can be good subjects for this surgical procedure. The surgery is simple, with minimal complications and relapse rate. Soft tissue changes in response to the surgery are also very predictable and remarkable. In our case, the result obtained was highly favourable concerning interarch relationship, aesthetic outcome and patient satisfaction.

Conflicts of Interest: NIL

Source(S) Of Support: NIL

Ethical Issues: NIL

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Biomimetic Materials in Restorative Dentistry and Endodontics – A Review

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Abstract

In 1950, while researching the nerves in a squid, Otto Schmitt coined the word "Biomimetics" It means imitating life, literally. To seek to mimic or reproduce it artificially to preserve the same esthetics or work is the study of natural structural processes. Since tooth structure has no natural means for restoration, biomimetic methods can be implied to restore the teeth mechanically to its normal function and esthetics. There are two things of biomimetic dentistry. First, the damaged or absent dental tissue is replaced, leading to complete restoration to the tooth of function and aesthetics. And the substance used will regenerate the damaged dental tissue, replicate and imitate it. The bio-compliant and Physico-chemical products of biomimetic material have the benefits of improved biocompatibility, sealing ability, high strength, antibacterial properties and are used to preserve long term esthetic and restoring techniques. Recent biomimetic technologies have established the potential to transcend various considerable drawbacks of technologies of a previous age. This study would seek to provide a clearer explanation for biomimetic materials 'relative role in the sense of past and current dental materials.

Keywords*: Biomimetic Materials, Restorative Dentistry, Endodontics*

Introduction

"Biomimetics is characterized as the study of the structure, creation and function of substances and materials, as well as biological processes and mechanisms, particularly for the synthesis of similar products with artificial mechanisms that mimic them".[1] Biomimetic material is a substance created by biomimetic processes based on natural processes occurring in biological systems. The main concept of Biomimetics is to restore all ready tissues to completion via a strong fabric attachment, which can help the crown achieve its ultimate biological and esthetic functional outcome by providing for functional stresses.[2]

There is no biomaterial in dentistry that has the same mechanical, electrical and optical properties as the structure of the tooth and has the functional features of preserved teeth in operation. Employing biomimetic therapeutic methods, dental practitioners could develop and get closer to normal biological systems and their operation. There are two main perspectives to which the phrase "*Biomimetic* is applied: a purist viewpoint that focuses on recreating biological tissues and a descriptive perception that focuses on using materials that effect in a mimicked biological effect".

A biomimetic material can suit the portion of the tooth that it substitutes in a variety of respects, including the modulus of elasticity and the structure of the corresponding areas (e.g., enamel, dentin, dentoenamel, pulp). So this analysis would aim to present a clearer explanation of the biomimetic materials 'relative role in the sense of past and present dental material. Figure 1

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below shows the generation of biomaterials.

GENERATION OF BIOMATERIALS

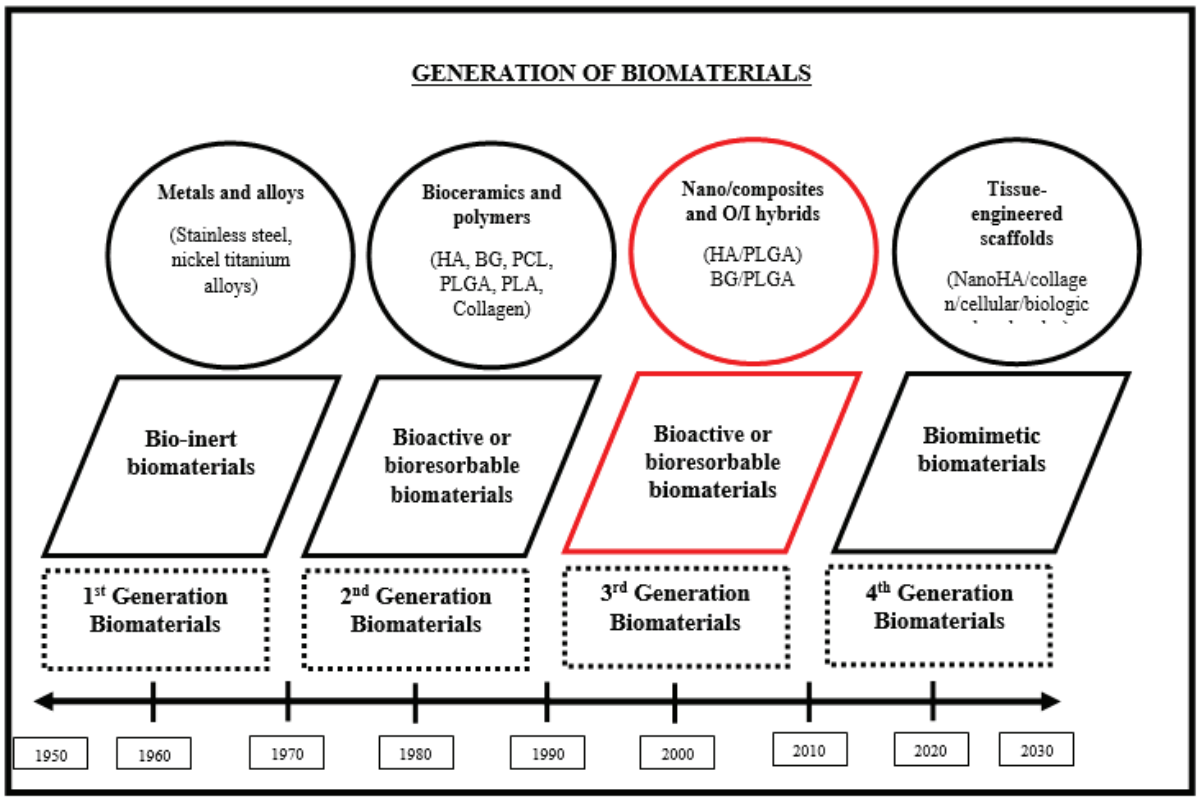

Figure 1. Generation of biomaterials

BIOMIMETICS IN RESTORATIVE DENTISTRY:

A biomimetic strategy in restorative dentistry should signify similar aesthetic and practical restorative materials to the natural tooth and its enamel and dentine layers. In 2006 Magne stated "The objective of Biomimetics in restorative dentistry is to reestablish all of the prepared dental tissues to full function by the creation of a hard tissue bond that permits functional stresses to pass through the tooth, making the entire crown into the final functional biologic and esthetic unit. The intact tooth in its ideal hues and shades, and more importantly in its intracoronal anatomy, location and mechanics in the arch, is the guide to reconstruction that governs success".[3]

Table.1 Features the resemblance of natural tooth substance to existing artificial materials.

	Elastic modulus (Gpa)	Thermal expansion coefficient (x 10-6 o/C)	Ultimate tensile strength (Mpa)		Corresponding material	Elastic modulus (Gpa)	Thermal expansion coefficient (x 10-6 o/C)	Ultimate tensile strength (Mpa)
ENAMEL	82	17	10		Feldspathic ceramics	60-70	13-16	25-40

Cont... **Table.1 Features the resemblance of natural tooth substance to existing artificial materials.**

DENTIN	14	11	40-105		Hybrid composites	10-20	20-40	40-60
					Glass-ionomer cements	4-10	35	4-5

GLASS IONOMER CEMENT:

Invented by Wilson and Kent in 1969, consisting of glass powder of fluoroaluminosilicate along with water-soluble polymers (acids). Once liquid and powder are combined, this results in a hardening reaction involving neutralization of the acidic group along with considerable fluoride release. It is labelled as biomimetic substance because it is synthetic, chemically adheres to the surface of the tooth, minimum shrinkage, less microleakage, high humidity structural stability, release fluoride, bactericidal nature, stimulate sclerotic dentin and also has similar properties to dentin. Nonetheless, due to their low tensile strength, they are not recommended in the use of high occlusal force and stress.

Modifications of Glass-Ionomer were Water settable GIC, Metal modified GIC and Resin modified GIC. Metal-based GIC has been improved by adding filler particles to maximize fracture resilience & wear resistance but esthetic properties have been compromised. Resin modified GIC was auto-cured, Light-cured, dual-cured depending on activator initiator system resulting in improvement of Optical properties as better translucency was achieved, diametral tensile strength was much higher but compressive strength and hardness are lesser, adhesion with dentin was higher than that of conventional GIC but microleakage was increased due to polymerization shrinkage of resin. Fibre-reinforced GIC involves the incorporation of a continuous network/scaffold of alumina and SiO2 ceramic fibres. Flexural strength is increased(15.6 Mpa) and Compressive strength is increased (200Mpa) and Fracture Toughness— 0.22 Mpam0.5. This proficiency is called the "Polymeric Rigid Inorganic Matrix Material" or PRIMM Alumina and SiO2 ceramic fibres

A recent advance in GIC modification is nano-hydroxyapatite/yttria-stabilized ZIRCONIA (HA/YSZ) containing GIC which defines a new class of restorative glass ionomer that promises the strength and durability ideal for permanent posterior restoration maintaining aesthetics.

GIC as Biomimetic Material in Endodontics – Glass-ionomer based sealer: used in obturation because of their dentin bonding property, enables adhesion between the material and canal wall but has minimum anti-bacterial property and removal is difficult in case of retreatment. "KT-308 (GC Corporation Company, Tokyo, Japan)" a GIC based sealer, offers improved zinc oxide-eugenol-based sealer which is resistant to coronal penetration of bacterial agents into the root canals.[4] "ZUT (University of Toronto, Ontario, Canada)" is an amalgamation of GIC and an antimicrobial silver-containing zeolite that is effective against E.faecalis and can therefore treat teeth with chronic apical periodontitis more effectively.[5] "Active Gutta-Percha (Brasseler USA, Savannah, GA, USA)" are Glass ionomer infused Gutta-Percha cones that bonds to GIC sealer and remarks to present adhesive bonding of the active Gutta-percha to intra-radicular dentin.[6,7]

Composite:

Bowen introduced composite in 1962 which offers colour stability, wear resistance, radioopacity and enhanced physical properties.[8]

Composition: Resin matrix–BIS-GMA or Urethane Dimethacrylate (UDMA) + Dimethacrylate monomers (TEGDMA), Inorganic Fillers: Quartz/Glass Particles (0.1 to 100μm) Colloidal Silica (0.02 to 0.04

μm), Coupling Agents: organosilanes, Pigments: oxides of titanium, Activator: Tertiary amines, Photo-initiator: Camphorquinoneand Chemical-initiator: Benzoyl Peroxide

Table 2. **Modifications of different biomimetic materials**

MODIFICATIONS	MATERIALS USED
REINFORCED FILLERS	· Electrospun nylon nanofibres containing highly aligned silicate single crystals · Nanofibres and e-glass fibres with SiO2 in the semi-interpenetrating polymer network(IBN) matrix · TiO2 Nanocomposites modified with Allytriethoxysilane(ATES)
CARIES PREVENTION FILLERS	· Nano-DCPA whiskers · TTCO-Whiskers · CaF2 nanoparticles with reinforcing whisker fillers · Polymer-kaolinite Nanocomposite
RESINS	· Epoxy-polyol matrix · Epoxy functionalized cyclic siloxane · Bioactive poly i.e methyl methacrylate/SiO2-CaO nanocomposite · Epoxy resin ERL-4221(3,4-Epoxycyclohexylmethyl-(3,4-epoxy cyclohexane carboxylate · Silsesquioxane(SSQ)
NANOPARTICLES SURFACE MODIFICATION WITH DIFFERENT SILANES	· 3-methacryloxypropyltrimehoxysilane(MPTS) · N-octyltrimethoxysilane(OTMS) · Equal masses of MPTS ad MPTS · Y glcidoxypropyl trimethoxysilane (GPS) · Allytriethoxysilane(ATES)

"Smart Dentin Replacement" (SDR) - is the first kind of flowable composite resin material with properties modified with lower polymerisation stress and decreased polymerisation; higher depth of cure and bulk-filling content that can be used in Class I and Class II cavities up to 4 mm in increments [9,10,11]

CERAMICS:

By firing at a high temperature, ceramic is classified as a substance made from non-metallic material. Ceramic applications are promising because they are strongly esthetic, have lengthy-term consistency in colour, tough compressive strength and hardness, outstanding biocompatibility and chemical inertness. Dental ceramics includes a phase of crystal and a phase of silica-based glass. Leucite crystal and lithium disilicate crystals are similar to porcelain dental prism rods. The enamel rod is 4-8um in diameter. It is a compact, systematized agglomerate of hydroxyapatite crystals. The cross-section has keyhole form characteristics.

"Ceramics are studied for the manufacturing of bone tissue and other dental applications too. Hydroxyapatite (HA), an essential inorganic part of the bone, is a ceramic dependent on calcium phosphate. New bone formation happens first on the scaffold surface in Novel ceramic bone replacement material called Ceraball. Ceraballs can act as a transporter for pluripotent mesenchymal stem cells, stromal cells and bone marrow".[12]

BIOMIMETICS IN ENDODONTICS

Calcium hydroxide:

Hermann introduced calcium hydroxide to dentistry at the turn of the 20th century and has been widely used in endodontics. It is a solid alkaline material with a pH of about 12.5 and has numerous biological properties that have impelled it to be used in many clinical contexts. Its dental use primarily relates to its antibacterial properties and the ability to promote repair and encourage the regeneration of hard tissues. The greatest advantage of calcium hydroxide is the bacterial effects deliberated by its elevated pH because certain endodontic micro-organisms are incompetent to live in the extremely alkaline atmosphere created by calcium hydroxide, as an intracanal treatment.

It was frequently used as a mineralising and antimicrobial agent. Based on whether the threat is succumbing to or subsisting with current odontoblasts, two forms of dentin may be produced. Reactionary dentinogenesis in which tertiary dentin matrix is covered by oriented controlled pre-existing odontoblasts and reparative dentinogenesis in which the secretion of tertiary dentin matrix occurs after the death of primary odontoblasts by an entirely new generation of secreting cells. Calcium hydroxide enhances the production of biomolecules (such as FGP, BMP, etc.), induces the release of extracellular matrix molecules (such as dentin sialoproteins, dentin phosphoproteins), has anti-inflammatory and antimicrobial activity.[13] "Controlled-release Calcium hydroxide charged microcapsules" based on ethyl cellulose (EC) and polylactic acid (PLA) has been developed to advance its biological efficiency.[14] Those formulas delayed the release of ions. Disadvantages of $Ca(OH)_2$: inconsistent treatment time, apical closing unpredictability, patient follow-up challenge, prolonged treatment, canal susceptibility to fracture during treatment, $Ca(OH)_2$ does not conform to dentine and lacks sealing capacity. Tunnel defects can act as microleakage pathways in dentine bridges.

Calcium Sulfate

It has been shown that calcium sulfate (CS) is fully bioabsorbable, osteoconductive, doesn't induce an inflammatory response, makes fibroblast migration and doesn't raise serum calcium levels either. It had been seen recently that Calcium Sulfate can be turned into a granular blend with poly-lactic acid to reduce the degradation rate.[15]

"Calcium Phosphate"

"Calcium phosphates" play a significant function in the mineralisation of biological and pathological compounds. Also commonly found in paste, cement, ceramics, and scaffolding.[16] The various calcium phosphate products described below in Table 2.

Table 3. Calcium phosphate materials

CALCIUM PHOSPHATE MATERIALS	EXAMPLES
Calcium phosphate ceramics	· Calcium hydroxyapatite [calcitite (calcite,Inc)] · Beta-tricalcium phosphate [synthograf, augment (miter,Inc.] · Biphasic calcium phosphates [triosit(zimmer)]
Calcium materials material from natural products	· Coralline HA[inerpore 200(interpore)] · Bio-oss(from sintered bovine bone)
Glass-ceramics	· Bioglass (American biomaterial corporation)

"Calcium Enriched Mixture (CEM)"

"It consists of calcium oxide, phosphorous pentoxide, sulfur trioxide, and silicon dioxide. It may promote stem cell differentiation and cementogenesis".

Mineral Trioxide Aggregate

MTA is a novel advanced technology for endodontics and appears to be important over other technologies. It has been used in both surgical and nonchirurgical applications since its invention by Torabinejad and colleagues in 1993. This is the first restorative material that makes cementum overgrowth gradually and may help the periodontal ligament to be regenerated. This should also be called 'Biomimetic Material.' MTA's capacity to cause dentine bridge formation may be attributed to its excellent sealing efficiency or biocompatibility. MTA can stimulate cytokine release from bone cells and enable osteoblasts to be attached in monolayer form.[17]

MTA can activate cementoblasts to generate a matrix for the formation of cementum. Studies indicate that MTA enables cementoblast attachment and growth, and building a mineralized matrix gene and protein expression. Irrespective of its origin, cementoblasts are the cells accountable for cementum production and regeneration, concluding that MTA is semiconductive materials.

Advantages: Resistance to marginal leakage reduces bacterial migration, Least toxicity of all the filling materials, Excellent biocompatibility, Hydrophilic sets in the presence of moisture. Moisture contamination is not an issue, Negligible Solubility**,** Super Sealing ability**,** Sufficient compressive strength to allow condensation of amalgam when it is used as a pulp capping agent, Reasonably radio-opaque and Non-resorbable.

Disadvantages: Difficult to manipulate, Prolonged setting time and Dissolves in acidic pH.

Calcium-Aluminate Cement (Binderware, São Carlos, Brazil),

This had been built by São Carlos Federal University. This consists of aluminium oxides (Al_2O_3), silicon (SiO_2), calcium (CaO), iron (Fe_2O_3) and magnesium. It enables the regulation of impurities like Fe_2O_3 (which promotes tooth darkening) and is free of CaO and MgO which prevents unnecessary expansion of the substance after contact with moisture.[18,19]

Biodentine™ (Septodont, France):

"In 2011, it was introduced which composed of tricalcium silicate, calcium carbonate and zirconium oxide and a water-based liquid containing calcium chloride as the setting accelerator.[20,21] Once biodentine makes contact with dentine, it contributes to the formation of tag- structures next to an interface layer which is called "Mineral Infiltration Area" and may contribute to adhesive properties".[22] It has shortened setting time (12 min), enhanced physical properties and induces mineralization and cell differentiation.[20]

"Bioaggregate (Innovative Bioceramix Inc., Vancouver, Canada)"

"Introduced in 2006, it is available as powder consisting of nanoparticles comprising dicalcium silicate, tricalcium silicate, amorphous silicon dioxide, calcium phosphate monobasic, tantalum pentoxide (radio pacifier) and liquid form has deionized water. As it is an aluminium free formulation, so it aids in periodontal regeneration and human PDL fibroblasts proliferation" .[21-25]

Bioactive glass:

The bioactive glass was first introduced by Hench et al, are surface-active glasses that bond chemically to bone materials. They are non-bone graft materials. These bioactive glasses contain different ratios of Sodium dioxide (24.5%), Calcium oxide (24.5%), silicon oxide and phosphorus (45%). "Bonding of BAGs to the living bones is accomplished by a bone-like apatite coating formed on their surface in the body environment due to their close bond with living bone, BAGs have been used as bone replacement products in different clinical conditions". Currently, there are two widely available glasses for use at bone locations, Bioglass with a partial size of 300-355μm. These are 100% organic bone restorative or regenerative materials. "Mohn et al' combined BAG particles with 50% bismuth oxide and used as the root canal filling substitute. BAG has an antibacterial influence, directly and indirectly, linked to pH".[26]

Conclusion

It is a biomaterial scheme that mimics the mechanical and physical properties of the affected tissue and thus provides a framework for the disease to adapt and alter therapeutic methods. Biomimetic dentistry is a

collaborative approach that can change everyday dental practice. This uses the strength of advanced molecular, environmental, and physical research to solve real clinical challenges. Biomimetic products serve as cement, filling materials, root canal sealer, and root-crown repair material and possess features such as root-reinforcing after obturation, strong sealing capacity, improved antibacterial properties and biocompatibility. Nevertheless, in terms of requirements for classifying them as ideal products, there are drawbacks. Multiple in vitro and in vivo experiments have shown good results, but long-term success requires more randomised and double-blind trials with biomimetic materials.

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Dental Caries Vaccines –An Emerging Viable Treatment for Modern Dentists

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Abstract

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth. It is a bacterial driven, generally chronic, site-specific, the multifunctional dynamic disease process that results when the PH drop results in net mineral loss over time. It is one of the most prevalent diseases affecting mankind. Cariogenic bacteria enter the dental biofilm early in life and subsequently invade the host tissue during favourable environment condition, to cause the disease. Many studies and researches show that this disease process can be interrupted. This article will give a clear idea of the method by which the host defence system can be aroused to combat dental caries caused by the crucial antigen related to *Streptococcus mutans*. Analyzing the fact that more effective public dental health care measures are needed to combat this dental caries, this article critically evaluates the recent signs of progress in the development of mucosal adjuvants, delivery systems for the dental vaccine, and also limitations of caries immunization.

Keywords: *Dental caries; Immunization; Vaccines; Streptococcus mutans*

Introduction

The fact that a specific bacteria cause dental caries and the function of salivary glands as mucosa-associated lymphoid tissue(MALT) as an effector site mucosal immune system, has provided a strong ground for the development of caries vaccine. Research efforts towards developing an effective and safe caries vaccine are facilitated by progress in molecular biology and mucosal immunology. Advancement in molecular biology led the cloning and functional characteristics of virulence factor from *Streptococcus mutans*, DNA technology and advancement in mucosal immunology led to the development of sophisticated antigen delivery system and adjuvants which stimulate salivary immunoglobulins. The virulent components of Streptococcus mutans are (1) Adhesins or surface fibrillar proteins which help in adherence to the salivary pellicle. (2)Glucosyltransferases ie. GTF enzymes (3) Cell wall-associated glucan binding proteins i.e GBP. These are primary target sites of the caries vaccine.[1]

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The Microbial characteristics of Dental Caries:

Streptococcus mutans is the primary etiological factor of dental caries, though S.sobrinus and Lactobacillus are also involved in the progression of the disease. The initial attachment of S. mutans to the hard surface of the tooth is through the interaction of bacterial proteins with lecithin in the dental pellicle. [2]The streptococcal adhesions which are referred to as an Antigen I/II or PAC are responsible to bind to tooth pellicle (Tooth pellicle is a thin protein film that is formed on the tooth surface of teeth enamel by selective binding of glycoproteins from the saliva, that is formed seconds after a tooth is cleaned. After binding to the tooth pellicle *S.mutan* secretes Glucosyltransferase(GTF) which synthesizes several forms of glucans. GTFs that synthesize the insoluble form of glucan (in *S.mutans* GTF-B and GTF-C) are closely associated with pathogenicity. This glucose

polymer provides scaffolding for the collection of more and more of S.mutans with other oral streptococci like *S.sobrinus*. *S.mutans* are huge producers of lactic acid which then leads to the demineralization of enamel and ensuing dental caries.[3]

Potent Molecular Targets for Dental Caries Vaccine :

Immune interventions can be undertaken at various stages of caries pathogenesis. They may be clearing S.mutans in the salivary phase before colonization or blocking the receptors for colonization or localized accumulation of S.mutans or inactivating GTF enzyme functions. Besides the antimicrobial property of salivary of IgA antibody can be enhanced or redirected by synergism with innate components of immunity eg. mucin or lactoferrin.

(a) Secretory Immunity And Synthesis of IgA :

IgA is the second most (first being IgG) abundant immunoglobulin class which constitutes 10-13% of serum immunoglobulins with a half-life of 6-8 days. It occurs in two forms -monomeric and dimeric forms (IgA1 & IgA2). The dimeric IgA is most prevalent & known as secretory IgA found majorly in saliva, tears, sweat, colostrum, GIT, respiratory tract etc. Initial oral immunity trials involved whole mutans streptococcal cells to induce salivary IgA response, which interfered in the colonization of S.mutans. The need for developing a subunit vaccine becomes obvious as the whole-cell approach was unlikely to be approached for the clinical purpose for safety reasons.[4]

Subunit vaccines, which contain structural elements of AgI/II adhesion family or encapsulated GTF and aluminium phosphate adjuvants, shows a modest & a short-lived salivary IgA response, following a dental prophylaxis. Since there is only a limited number of caries immunity trials are done with subunit vaccines or only adult volunteers, the full protective potential of the vaccines is under investigation.[4]

Studies show that in children an appropriate early immunization could set the S.mutans in a severe colonization disadvantage compared to other bacterias. This timing of immunization should precede the "window of infectivity" ie. the period in which children are more susceptible to mutans streptococci infection. Generally, it extends one and a half years to two and a half years. It is seen that immunisation of mother can lead to preventing transmission of bacteria to the infants during this critical window of infectivity. Some longitudinal studies show that children who became infected with S.mutans at 2 years developed significantly more carious lesion then the children who became infected with S.mutans at 4 years of age. Thus if the vaccine is given on time, it gives more time for maturation(i.e enhanced mineralization) in the oral environment. Since it is still unknown whether the child who escaped acquisition of S.mutans in their primary teeth will also remain free of infection during permanent teeth eruption, a booster immunisation dose is needed to inhibit a second attempt for colonisation by *S. mutans* on newly emerged permanent teeth. This booster immunisation period is before the additional window of infectivity i.e at 5th year of life.[4,5]

(b) **ADHESINS:** Adhesion from two principal human pathogens *Streptococcus mutans* (Antigen I/II, PAc or P1) and Streptococcus sobrinus (SpaA or PAg) have been purified. Studies show that vaccination with an intact antigen I/II can protect rodents, primates or humans from dental caries. Immunisation of mice with synthetic peptide residues from the alanine-rich region of antigen I/II suppressed tooth colonisation with S.mutans. This probably occurs by antibody blockade of initial colonisation events or antibody-mediated agglutination and thus eliminating the adhesin bearing bacterias from the saliva. S.mutans Ag I/II and S.sobrinus Sp A display structural similarities of alanine-rich tandem repeats in the N-terminal, one-third of the molecules predicted to be α-helical and proline-rich tandem repeats within the central portion to form an extended structure with many β-turns.[6]

(c) **GLUCOSYLTRANSFERASES:** S.mutans and S.sobrinus produce several GTFs. The GTF is important for its glucan synthesizing and binding property. The genes responsible for glucan synthesis are GTF-B, GTF-C and GTF-D in S.mutans. Studies show that vaccination can be achieved with GTFs of S.mutans or *S. sobrinus* since it induced a protective immune response in experimental dental caries rodent models after infection with several mutans streptococcal species.[6]

(d) **GLUCAN BINDING PROTEINS:** S.mutans have cell wall-associated Gbp mainly of three distinct types Gbp A (Russel,1979), Gbp B(Smith et all,1994) and Gbp C (Sato et all, 1979) Out of these Gbp B protein's N- terminal contains several immunodominant regions which are found to induce a protective immune response in humans and animals. In the experimental studies, protection can be achieved by subcutaneous injection of Gbp B in the salivary gland region or by mucosal application through intranasal route. Preliminary studies also show Gbp A to be less immunogenic then Gbp B and also the ability to induce protective immunity by Gbp A is problematic. Till now Gbps of S.sobrinus is not evaluated for its probable vaccination potential.[6]

DIFFERENT APPROACHES FOR CARIES VACCINE :

(I) **SUBUNIT VACCINE:** It contains the structural elements of AgI/II adhesin family, GTF or Gbp B. Furthermore multivalent subunit vaccines could be constructed which target different functions of the same component (eg. GTF catalytic & Glucan binding activities) Such vaccines would facilitate the variable mucosal response in target vaccine population.[7]

(II) **SYNTHETIC PEPTIDE VACCINES:** It utilises the fact that immunogenic reaction can be achieved in the body by vaccination with only alanine-rich repeat region of Ag I/II. Studies show that synthetic peptides derived from the alanine-rich region of Ag I/II induced high levels of serum IgG antibody. Fusion proteins containing PAcA also inhibited adhesive property of S.mutans to the tooth surface. Researches show that application of synthetic peptides containing immunodominant sequences of 3.8 – kDa antigen of S. mutans to the gingival mucosa elicit both salivary IgA and gingival IgG antibody reaction.[8,9]

(III) **RECOMBINANT VACCINES:** Recombinant vaccine approaches are based on the fact that salivary IgA response can be elicited by stimulation of either local lymphoid tissue or central inductive sites of the mucosal immune system like GALT. Local lymphoid tissue is the organised lymphoid tissue associated with major salivary gland (eg. the parotid) and minor salivary gland (eg. Glands present on lower lip). These are called duct associated lymphoid tissue(DALT). Central inductive lymphoid tissues are gut-associated lymphoid tissue(GALT) and nasal associated lymphoid tissue. These both comprise as an integrated system known as the common mucosal immune system. Studies show that intranasal route is a more effective route over the oral route for inducing salivary IgA responses, probably due to efficacy of antigen uptake, less antigenic competition and proteolytic activity than for oral vaccination. Nowadays mucosal vaccination strategies are being used for delivery of S.mutans antigens. In this context Cholera toxin(CT) is an exceptionally strong mucosal immunogen & potent mucosal adjuvant for human use because of enteric toxicity, though CT finding subunit conjugate with Ag I/II through intranasal vaccination-induced high levels of IgA in saliva. So exploration of other immunogens led to find the avirulent derivatives of Salmonella typhimurium to be another effective antigen delivery system. This is based on the fact that antigen of S.typhi has the tropism for the GALT. The fact that intranasal route requires lower dose then intra gastric route for inducing a response of comparable magnitude, intranasal route for caries vaccination will be a preferred route.[10]

Conclusion

As dental caries fulfils the criteria of infectious disease, the possibility of vaccination is thought of. Since WHO(World Health Organisation) vaccine effort is to reduce, rather than increase the number of different immunisations that a child receives, the approach that combines recombinant vaccines (i.e recombinant salmonella) felt to be most desirable for global application. Till date with more animal trials, less human trials, more cross-sectional studies and less longitudinal studies we are still in quest of an ideal dental caries vaccine that would give the broadest coverage to intercept the disease would work in both low and high-risk population, would be given in easy route like nasal/oral, would be inexpensive and would be delivered by individuals with little training. More paediatric clinical trials are needed to provide insights to best caries vaccine strategy for broadest protection. This may help greatly in improving the oral health of the developing countries.

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Platelet Rich Fibrin(PRF)-A Novel Generation of Regeneration in Endodontics: A Review

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Abstract

In the field of regenerative medicine, PRP/PRF whichwas will be is used as a surgical adjuvant became the new glorified approach. For the Regenerative endodontic treatment based on the tissue engineering concept, creating a favourable environment free of pathogens, for preservation and migration of stem cells, pathogen eradication, stem cell preservation, presence of scaffold facilitating spatial orientation and signal molecules released by cells is of utmost importance. In tooth revascularisation and revitalization, successful application of platelet concentrates as scaffolds have been reported. Being autologous, comprising of high growth factor concentrates and relatively hassle-free preparation in dental setup, platelet concentrates are preferred. The advancement in the regenerative endodontics field, with the introduction of the different generation of platelet concentrates, is a breakthrough.

Keywords*: Regenerative endodontics, PRF, Platelet concentrates, Tissue engineering.*

Introduction

Blood coagulation, that leads to fibrin/platelet clot and matrix formation acts as the initiator of the natural healing process. Platelet concentrate that was introduced, reinforced the process of wound healing, that ultimately progressed to tissue engineering, explicit in its way. All the essential and primary components involved in the process of wound healing, namely platelets, altruistic in growth factors, leukocytes, fibrin as the supporting matrix, growth factors and some other cells when combined, form a kind of engineered tissue.[1]

In the field of regenerative medicine, PRP/PRF which was used as a surgical adjuvant became the new glorified approach. For the regenerative endodontic treatment based on the tissue engineering concept, creating a favourable environment free of pathogens, for preservation and migration of stem cells, pathogen eradication, stem cell preservation, presence of scaffold facilitating spatial orientation and signal molecules released by cells is of utmost importance.[2]

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In tooth revascularisation and revitalization, successful application of platelet concentrates as scaffolds have been reported. Being autologous, comprising of high growth factor concentrates and relatively hassle-free preparation in dental setup, platelet concentrates are preferred. The second generation of platelet concentrate (PRF) is preferred over the first generation (PRP) as the addition of exogenous agents not required, formation of organized fibrin network, circulating immune cells that act against infection. All these make PRF an optimal bioscaffold for tooth revascularization and revitalization.[3]

Platelets And Platelets Concentrates:

Thrombocytes, also known as platelets, derived from bone marrow megakeratocytes, are discoidal anucleate cytoplasmic fragments with an average diameter of 2-3 μm primarily causes the hemostasis at the site of injury. The average lifespan of platelet being approximately 8-10days, 1.5-4 thousand per μL in peripheral blood is the normal platelet count for an adult human.

During tissue repair, two different types of dense granules, having serotonin, adenosine triphosphate and calcium, are released from activated platelets. Platelets also contain α-granules, rich in growth factors that exhibit chemotactic and mitogenic properties. These growth factors assist in promoting and modulating cell proliferation and function that leads to tissue healing and regeneration

Table 1: Compilation of some major research work done in the evolution of platelet concentrates.

Year	Research work
1954	While conducting experiments pertinent to coagulation of blood, Kingsley contrived the term PRP attributed to thrombocyte concentrate.5
1970	Fibrin glue, introduced by Matras.6 The polymerisation of fibrinogen with thrombin and calcium leads to the development of fibrin glue. But, the low concentration of fibrinogen in donor plasma, resulted in suboptimal quality and stability of fibrin glue.
1975	platelet-fibrinogen-thrombin mixtures.7
1979	A newer proposition, "gelatin platelet - gel foam", that was mainly used for the quality of gluey-effect, ensured platelet performance and showed superlative results in disciplines of general surgery, ophthalmology and neurosurgery
1986	Knighton et al originally coined the term "platelet-derived wound healing factors (PDWHF)", where the platelet concentrates promoted the successful healing of the wound.8
1988 1990	The term platelet-derived wound healing formula (PDWHF)", was coined by Kingsley et al5 and Knighton et al9
1997	Initially, during preparation, Whitman et al used the term PRP to name their product.10 But then the final product had more of a fibrin gel consistency and so it got labelled under the name of "platelet gel"
1998	The craze for the newer techniques started after the publication of an article by Marx et al.11 Before that, these techniques developed at a slow pace.
1993	Plasma rich in growth factors (PRGF), by Endoret(Victoria, Biotechnology Institute BTI, Spain) was commercialized and advertised on a large scale. But there was some major disadvantage associated with this technique, due to lack of ergonomics and specific pipetting techniques.4Apart from this, another product named Vivostat PRF (Alleroed, Denmark) was commercialized, even though it was nothing but one PRP product.
2000	A form of PC, based on a strong fibrin gel polymerisation, was developed by Choukroun et al in France and was marked as PRF. As PRF was different from other PRPs, its development was an important milestone and so it was imprinted as "second-generation" platelet concentrate.11
2006	Bielecki et al and Cieslik-Bielecka et al proposed that when compared with PRG (platelet-rich gel), PRP was not an active substance. On the other hand, PRG was biologically activated, with a fibrin matrix rich in active molecules.12-14 A newer notion of GCF (concentrated growth factors) was established by Sacco. The resultant fibrin rich blocks were larger, richer and denser too.15
2008	The focus of Everts et al was on the leucocyte component of PC and its forms; inactivated/non-activated form["platelet-leukocyte rich plasma (P-LRP)] and activated gel form platelet-leukocyte-gel".16,17
2009	Pioneer classification of platelet concentrate by Dohan Ehrenfest et al[4].
2010	Sohn in 2010 unveiled the concept of autologous fibrin glue mixed with bone graft, the sticky bone concept.18
2014	A-PRF, an advanced PRF claiming to contain a comparatively greater number of monocytes was introduced by Choukroun. A newer type, Titanium-prepared PRF(T-PRF) was introduced by Tunalı et al.19,20
2015	A technical note, with elaborate detail on the preparation of i-PRF, was given by Mourão et al.21

PRF- Platelet Rich Fibrin

Platelet-rich fibrin, a platelet concentrate of the second generation, can stimulate the proliferation of osteoblasts, gingival fibroblast and PDL cells. Also, it contains VEGF, which is a potent stimulator of angiogenesis.

This generation of platelet concentrate was developed in France in the year 2001 by Choukroun et. Al. After the name of its inventor, PRF is also called as Choukroun's PRF. The procedure of PRF preparation involves instantaneous centrifugation of the blood (at 3000rpm for 10mins) that is collected into test tubes, without an anticoagulant. After centrifugation, three layers are seen in the resultant product, viz, at the base level the Red portion of blood, PRF clot at the intermediate level and straw-coloured acellular platelet-poor plasma at the topmost level.[22]

Use of PRF in dentistry:

· In the procedures involving lateral sinus elevation, PRF when used along with bone grafts, hastens the healing process.

· In augmentation procedures, PRF in addition to the graft material protects and stabilizes the graft material.

· PRF used after tooth extraction preserves the socket.

· Regenerative procedures in the treatment of 3wall defect.

· Treatment of periodontic endodontic lesion

· Treatment involving the furcation defect.

· PRF enhances palatal wound healing graft.

· For filling of the cystic cavity

PRF act as an immune regulator. The α-granules, present in the PRF, release cytokines interleukin (IL)-1β, IL-6, TNF-α and growth factors TGF β1, PDGF, VEGF, EGF, on activation and degranulation, that initiates the healing process through stimulation of migration of cells and proliferation of cells. The function of the growth factors found in PRF is listed in table-2.

Table 2: List of cytokine/growth factors present in PRF

IL-1	Stimulates T-helper cells, inflammatory mediator
IL-6	B cell differentiation, T cell activator, antibody secretion stimulation, inflammatory and remodelling mediator
IL-4	Proliferation and differentiation of activated B cells, moderates inflammation, increases fibroblast synthesis of fibrillary collagen
TNF-α	Monocyte activator, stimulate fibroblast remodelling, increase phagocytosis and neutrophil toxicity modulates IL-1 and IL 6 expression
VEGF	Initiates angiogenesis
TGF β1	Fibronectin and collagen synthesis
PDGF	Helps in the regulation of cell migration and further cell proliferation. Also renders help for the mesenchymal cell survival and cicatrisation
IGFs 1 and 2	Acts as a mediator in cellular apoptosis and has chemotactic effects towards human osteoblasts.

Prevailing Prominence of PRF in endodontics (current status):

For the revascularization of pulpal tissue, PRF serves as an ideal medium due to the fibrin mesh. This fibrin mesh entraps the cytokines, causes a slow and progressive release of cytokines over time around the surrounding tissues, as the degradation of fibrin happens. an additional function of PRF is that it accelerates the healing of wound edges by offering a function and also structural support mimicking fibrin bandage.

Even though when a tooth is classified as necrotic, there might be the presence of some pulpal tissue that survived in the apical region. Regenerative endodontics id based on the concept, that in the presence of the favourable condition, proliferation and regeneration of these pulpal tissues can be achieved. Due to the sustained key growth factor release, flexible trimolecular fibrin scaffold, the enmeshment of cytokine, for apexification and pulpal regeneration, PRF acts as a valuable adjunct and an ideal pulp capping agent.

PRF application: Use in avulsion and tooth transplantation

PRF is known to show a reduction in inflammation. In traumatized teeth, the inflammatory process may also cause ankylosis. In a study by Johns et al, when a 24month reevaluation was done following PRF placement (prepared via choukroun's method), reimplantation and splinting, it was observed that the tooth was vital to both percussion and thermal testing. Also, no radiographic signs of inflammation or replacement resorption were observed. As the pulp tissue in the canal assists in stimulating revascularization, so after a traumatic avulsion of dentition, PRF is quite a valuable adjunct for pulpal revascularization.[23]

PRF application: For the treatment of the chronic periapical lesion

Local application of growth factors maximizes the healing potential and ultimately promotes tissue regeneration and healing in the periapical region. As the stem cells that are present in the apical tissues attract growth factors, the PRF rich in growth factor is used to stimulate bone regeneration.[24] In a study by Wilting et al, reported that when PRP was added to tricalcium phosphate, there was an 8%-10% increase in bone formation. When compared to the first generation of platelet concentrate (PRP), the second generation (PRF) shows a stronger and more organized fibrin network. Apart from initiating bone growth, healing, maturation, β-tricalcium phosphate bone graft when mixed with PRF, causes an enhancement in the desired treatment outcome.[25] In a study by K.B.Jayalakshmi et. al, it was stated that when PRF was added to β-Tricalcium Phosphate, there was an acceleration in the capacity of bone regeneration and there was both clinical and radiographic evidence of bone formation.[26]

PRF application: For endodontic management of the open apex

The major difficulty faced in the case of an open apex is limiting the extrusion of the material into the periodontal tissue. This can be overcome by using a matrix, that avoids extrusion and causes a reduction in the leakage of the sealing substance. Out of the various materials used for the barrier formation at the apical end, PRF membrane is one of the preferable ones. In cases of single visit apexification, PRF membrane when combined with MTA (mineral trioxide aggregate) creates an artificial root-end barrier and results in faster healing in the periapical region.[27]

Conclusion

The advancement in the regenerative endodontics field, with the introduction of the different generation of platelet concentrates, is a breakthrough. The second generation of the platelet concentrates, the PRF was developed when the first generation (PRP) wasn't meeting all the desired qualities. The major drawback of PRP was wide variation in platelet concentration and also variation in the different centrifugation guidelines. The PRF that was developed became a boon. PRF with autologous growth factors and cytokines makes it a desirable treatment option in the field of regenerative endodontics. Further studies should be conducted for evaluating its effect and treatment outcome in a wide variety of clinical cases and setup.

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Recent Advances of Endodontic Retherapy- A Review

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Abstract

Endodontic plays a major role in uplifting the quality of oral health. Retherapy offers the patient a subsequent prospect to save a tooth that would otherwise be certain for extraction. The prime objective of retherapy is to gain entry to the apical foramen by absolute confiscation of the root canal packing substance thus easing sufficient disinfecting and shaping of the absolute root canal system and terminal obturation. It can be concluded that with the emergence of modern instruments and techniques it is bygone just to extract teeth solely due to periapical disease and endodontic failures. Advances in scientific proficiency and technical ability have assisted us to improve the prognosis of treatment, but it does not always end up in the desired healing response while doing clinical practice.

Keywords: *Endodontic Retherapy; Recent Advances; Clinical Practices*

Introduction

Endodontic plays a major role in uplifting the quality of oral health. Retherapy offers the patient a subsequent prospect to save a tooth that would otherwise be certain for extraction[1]. The prime objective of retherapy is to gain entry to the apical foramen by absolute confiscation of the root canal packing substance thus easing sufficient disinfecting and shaping of the absolute root canal system and terminal obturation. Published works support a fortunate endodontic retherapy ranging from 80% to 88%.[2]

Role of Magnification:-

In endodontic retherapys, the microscopes are considerate in recognising and eliminating residual packing substances, such as sealer residue, pastes or gutta-percha, silver points and carrier-based substances, posts or separated instruments. It also encourages nonsurgical perforation repair, permitting clinician in cleaning the perforation site and to place perforation restore substances accurately.

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According to P. Sequeira et al, Curved canals are more prone to instrument separation than straight canals[3]. In spiral canals, rotary instruments (as well as lentulo spirals) separate quite frequently than other instruments. Ideally, 45–60 min is required to remove separated instruments from root canals, after this interval of time momentous thought is to be given to other treatment options. The use of an operating microscope is an imperative approach to remove the separated instruments. Good illumination and magnification, ideally with a microscope, will help to remove hard cement and this will help to understand the connection between packing substance and the root canal wall to minimize the threat of perforation.[1,2]

Marketable microscopes provide adaptable magnification cover from around 4x-25x, whereas loupes come with fixed amplification covers between 2.5x-6x.

Magnification can be divided into:-

- SHALLOW MAGNIFICATION (2x-8x),
- MEDIUM MAGNIFICATION (8x-16x), and
- HIGH MAGNIFICATION (16x-25x).

Shallow magnification is suitable for a silhouette of the working field. Medium magnification is the primary

proceeding around root canal therapy and endodontic surgery. The peak of magnification is to take hold for recognition of fine anatomy and also for documenting the exceptional details. With time modern microscopes have advanced noticeably while typical microscopes come with elementary optic and illuminating alternatives. Particular supplementary characteristics are the hallmark for endodontic purposes[4]. At the same time, the surgical method needs significant angular orientation to observe incised root surfaces and other sectioning details. As a result, the microscope should be provided with a 180°-tilt-able binocular orientation and an eyepiece with a reticle (A reticle is a set of fine lines, commonly in the shape of concentric rings that yields proper centring on the object in focus and allows for easier individual calibration (parfocaling) of the microscope).[1,2]

Additional refurbish for in-depth microscopic functions can be seen, rather than firm focal distance that restricts the microscope to an object distance of 200 mm, 250 mm or 300 mm[5]. Trouble free change between clinicians and uncomplicated adjustment to patients can be done with variable focal distance adaptors nowadays that are provided with apex notch microscopes, often following computerized zoom. Hassle-free and steeples acclimatization of both magnification and focus is allowed by the fine focus options.[1,2]

Role of Lasers

More resistant microorganisms lead to low conjecturing of the endodontic retherapy than the conventional endodontic treatment. Thus the use of high-power diode laser has paved the way towards disinfection of the earlier inaccessible areas. These lasers achieve higher exorbitant levels in disinfecting dental tissues than regular rinsing solutions. Alfredo et al proposed that utilisation of 980 nm diode laser (1.5 W) is secure in all modes nevertheless the presence/absence of dampness in endodontics.[1]

Due to comparatively substantial side effects of analgesics or NSAIDs, low-level laser therapy may be utilized to regulate the inflammatory process. In cases of acute and chronic pain lasers leads to tissue repair by the improvement of local microcirculation.[2]

ROLE OF ULTRASONICS IN REVIVAL OF POSTS AND HARD CEMENT

The emergence of ultrasonic and its implication into endodontics has provided with a handy companion for post confiscation. Ultrasonic energy has been proven to be a valuable companion in the confiscation of silver points, separated instruments, and cemented posts. These are appreciably more efficient than sonic instrumentation in reducing the amount of time required to dislodge a cemented 4mm-long prefabricated post under a constant tensile force.

Yoshida et al. found that contemporaneous application of two ultrasonic tips on a cemented post from either side further decreases the amount of lateral vibration required to remove cemented dowel-retained cast cores under intermittent tension. While performing access refinement procedures dominant advantages can be seen when piezoelectric technology is used in coordination with ultrasonic instruments. In constricted areas such as between a post and an axial walls, thinner and more parallel-sided ultrasonic instruments are easy to work with. Especially in an irregularly shaped canal, a parallel-sided ultrasonic instrument may be safely used below the orifice and lateral to a post. Light brush cutting motion employed with Ultrasonic instruments are best on the peripheral edge of a sectioned core to chip, break up and sand away substances such as cement, composite or amalgam. Removing these substances from the pulp chamber serves to diminish post retention. All of the nonsurgical ultrasonic procedures are performed dry. An ultrasonic tip with medium to high power in conjunction with irrigating spray is used to carefully remove core substance from the pulp floor**.** The same instrument, materials can be removed very precisely from the coronal part of the root canal without exposing the danger of perforation. The tips are coated with an abrasive substance, assisting in the confiscation of restorative substances[1].

A new bur **GYROTIP** has been designed especially for removing fibre posts. Drills consist of a heat-producing tip that softens the matrix that holds together the fibre of the fibre post. Fluted zones allow for safe confiscation of the fibre. Above the fluted zone, plasma bonded silica carbide reduces the heat generation. Piezon-type ultrasonic machine made up of diamond-

coated tips is specifically helpful in the confiscation of hard cement.

CONFISCATION OF GUTTA PERCHA:-

Confiscation of Gutta Percha using rotary system aims to remove GP by dividing the canal into thirds. The rotary instrument should turn at a speed ranging from 1200-1500 RPM to soften and engage Gp. Instruments such as

· Protaper universal retherapy files (by Dentsply), Mtwo R (size/taper-R15/0.05, R25/0.05) Recioroc (VDW) - this was created by Dr GassenYard, offers greater flexibility and resistance to cyclic fatigue than traditional niti files.

· The **Mani** GPR system is a 4-**file** system that is composed of a mixed pack of 4 instruments (1S, 2S, 3N, 4N) with 4 individual sizes. The 1 and 2 "S" instruments are stainless steel, and the 3 and 4 "N" instruments are nickel-titanium.

· **HyFlex® NT™** endodontic rotary NiTi file is designed to work in specific clinical situations, such as calcified canals, straight canals, retherapys and removing Gutta-percha. HyFlex® NT™ files are available in .06 and .04 tapers in 21mm, 25mm and 31mm lengths.

· **Neoendo Retherapy Files** comes as N1 - For Coronal one-third,

N2 - For Middle one-third and

N3 - For Apical one-third.

N1 and N2 come in 16mm and 18mm and N3 comes in 22mm and 25mm.

· **Endostar** RE Re Endo Rotary System is used for the confiscation of old gutta-percha packing from the root canal system. **Endostar** RE Endo Rotary System contains 4 instruments of different sizes and tapers marked from 1 to 4.

Tender touch with retherapy files is used. The files are never forced and always the recommended speed and torque setting is used. Along with files lasers are also effective in removing the substances. Studies conducted on the effectiveness of Nd; YAG laser for the confiscation of gutta-percha have shown that it is capable of softening gutta-percha.[3-5]

Ø **Lower setting: (100MJ, 15Hz, 1.5w):** Fairly clean root canals but in absolute elimination of gutta-percha from dentinal walls.

Ø **Increased power levels: (100MJ 20Hz, 2w):** More effective on the canal walls for disinfection.

RECENT ADVANCES IN IRRIGATION PROTOCOL: - The efficacy of endodontic treatment may be compromised due to the anatomical convolution and uncleansed portions of the canal for utilising only sodium hypochlorite in countering the production of microbial biofilm. Thus to tame the hindrance of normally available root canal irrigating solutions and medications, Thus to decontaminate the canal use of nanoparticles have been put forward.[6]

· ***Antibacterial nanoparticles* -** The electrostatic interlink betwixt negatively charged bacterial cells and positively charged nanoparticles accelerate the growth of the maximum number of NPs on the bacterial cell membrane. Loss of membrane permeability and unsuitable membrane functions associated with NPS. Antibacterial nanoparticles lead to extensive antimicrobial activity[7].

Zhang et al. evaluated that potency of Chlorhexidine with silver nanoparticles that significantly produced barrier effect on the Enterococcus faecalis biofilm[8].

· ***Antimicrobial photodynamic therapy (APDT)* -** APDT is a two pace plan of action that requires administration of a photosensitizing agent, preceded by the light radiance of the sensitive tissues leading to noxious photochemistry that finally culminates into the destruction of microorganisms[9-11].

· **Photon-induced photoacoustic streaming (PIPS) -** Sub ablative energies of 20 mJ at 15 Hz for a mean force of 0.3W at 50 μs impulses, PIPS has a radial firing stripped nib with laser stimulus. These stimulus prompt interactivity of water molecules with pinnacle force of 400W. Thus sequential shock waves are created leading to robust streaming of the germicidal fluid that is present inside the canal, without temperature rise[12, 13]. Unlike everyday laser applications, unique tapered PIPS nib is not much compulsory to be placed inside the canal but can be placed in the pulp chamber solely. Larger

instrument needs can thus be diminished to fabricate larger canals for penetration of irrigating solutions during treatment of the apical end and ramifications of the canal. Effective removal of both vital and non-vital tissues, bacterias, and disinfection of dentin tubules can be performed easily by this method [14, 15].

· **Gentle wave procedure-** It is a multi-ultrasonic disinfection procedure, a minimally invasive alternative to standard root canal treatment. This enables the fluids to extend through the entire root canal system providing efficient disinfection of the complex canal anatomies. In multi ultrasonic disinfection, multiple frequencies are generated at the same time and this technology is paired with optimized procedure.

1. The Gentle Wave technique performs dissipation of tissues eight to ten degree expeditiously than ultrasonic tools and needle irrigation[16].

2. The Gentle Wave leads to zero extrusion at the apex as it is a negative pressure system.

3. Teeth with minimum instrumentation (e.g.: size 15/04) are ideal for using the Gentle Wave device. The resulting fluid dynamics, multi-sonic sound waves, and sonochemistry permit medicated fluids to penetrate and outstretch complex areas as apical-thirds, isthmus, lateral fins, dentinal tubules, and other anastomoses. This disinfection unit is composed of a moveable treatment system with a disposable sterilized handpiece. Flushing suspension of sodium hypochlorite, distilled water and Ethylene diamine tetraacetic acid are present in disinfection unit.

4. Latest clinical survey reveals that only 3% of the patients experienced modest after-therapy discomfort. At 12 months, 97% of fortunate healing of the teeth treated with the Gentle Wave System was found [6].

CONCLUSION

It can be concluded that with the emergence of modern instruments and techniques it is bygone just to extract teeth solely due to periapical disease and endodontic failures. Advances in scientific proficiency and technical ability have assisted us to improve the prognosis of treatment, but it does not always end up in the desired healing response while doing clinical practice. So even if the preliminary mode of treatment is futile, clinicians should increasingly be expected to hold adequate knowledge and competency to conduct a more technically challenging strategy to retain natural denticulation.

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Frenectomy with Lateral Pedicle Flap: A Novel Surgical Approach for Maintaining The width of Attached Gingiva: A Case Report

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Abstract

The aberrant frenum is usually managed by a common periodontal plastic surgical procedure called frenectomy to create a zone of attached gingiva. This surgical approach may often lead to loss of interdental papilla creating unacceptable esthetic results in the maxillary incisor region. Thus this case reported frenectomy combined with a laterally positioned flap which was a better approach to achieve primary closure. And the lateral pedicle sutured in midline provided better and faster healing with excellent esthetic results. The esthetic results were far more superior then the classical frenectomy technique with a considerable gain in attached gingiva.

Keywords*: Frenectomy, Attached Gingiva, Lateral Pedicle Flap*

Introduction

Periodontal therapy aims to restore the gingival health, comfort and function of a patient with acceptable aesthetics. The presence of the adequate amount of keratinized gingiva protects the marginal gingiva from bacterial invasion, thus preventing further gingival recession aiding in plaque control and maintaining gingival health. [1] A frenum is a mucous membrane fold that consists of oral muscle and connective tissue fibres that attach the lip and the cheek to the alveolar mucosa, the gingiva and the underlying periosteum of alveolar bone. [2] There are four types of labial frenum attachment, mucosal, gingival, papillary and papillary penetrating. The frenulum mostly consisted of dense collagen fibres and elastic fibres. There are four types of labial frenum attachment, mucosal, gingival, papillary and papillary penetrating. The frenulum mostly consists of dense collagen fibres and elastic fibres.

The frenum is usually classified as path gnomic when it is usually wide or there is add inadequate amount of attached gingival or there is a shift of interdental papilla due to frenal extension[3]. Frenectomy is performed in such cases to restore function and aesthetics. In the classical frenectomy by Archer, interdental tissue and palatine papilla are completely excised leading to exposure of underlying alveolar bone resulting in scar formation in the esthetic zone[4]. The free gingival graft was advocated to prevent scar formation but led to a colour mismatch.[4] Miller advocated a technique of Frenectomy combined with lateral pedicle flap which increases the zone of width attached gingiva by increasing the collagenous band of the gingiva.[3] In the present case report modified surgical technique was designed for the management of maxillary aberrant frenum using lateral pedicle flap which led to the gain of attached gingiva, excellent aesthetic results as there was healing by primary intention with minimal scar formation and prevention of further progression of gingival recession.

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Case Report

A 22 year old female reported to the department of periodontics with a chief complaint receding gums in maxillary anterior teeth. In the extraoral examination, no abnormality was detected. However, she looks thin and emaciated. Intraoral examination revealed healthy but reduced supporting tissue surrounding the remaining teeth with good oral hygiene. And Tension test was positive indicative of labial frenum was inserted high on attached gingiva (fig1). All the teeth with adequate vestibular depth were present apart from the frenulum area. The patient was well aware and concerned about the frenum. And she is very cosmetically concerned. The surgical procedure was throughly explained to the patient and signed consent form was taken. All blood investigation were performed and were found to to be withing normal limits.

Under local anaesthesia on the buccal aspects of maxillary anterior teeth using 1:200,000 lidocaine hydrochloride with adrenaline. A horizontal incision was given first to separate the frenum from the interdental papilla (fig 2). The incision was extended apically towards the vestibule to separate the tissue along with periosteum was separated from the underlying alveolar bone. Further, the frenulum was excised. A second vertical parallel incision was given 3mm apical to marginal gingiva extending to the vestibule. A partial-thickness flap was raised and displaced laterally (fig3). With resorbable 4-0, vicryl sutures interrupted sutures were placed (fig4). Thus, ensuring to the attachment of the graft with the adjacent gingiva and alveolar mucosa. Transeptal fibres in between central incisors were remained undisturbed. Following suturing Coe-Pak was placed and it was removed after a week. Mild Analgesic was prescribed with oral hygiene instructions were postoperatively a greater width of attached gingiva was obtained in the midline with no loss of interdental papilla (fig5,6).

Figure 1. Pre-operative photograph

Figure 2. Horizontal incision.

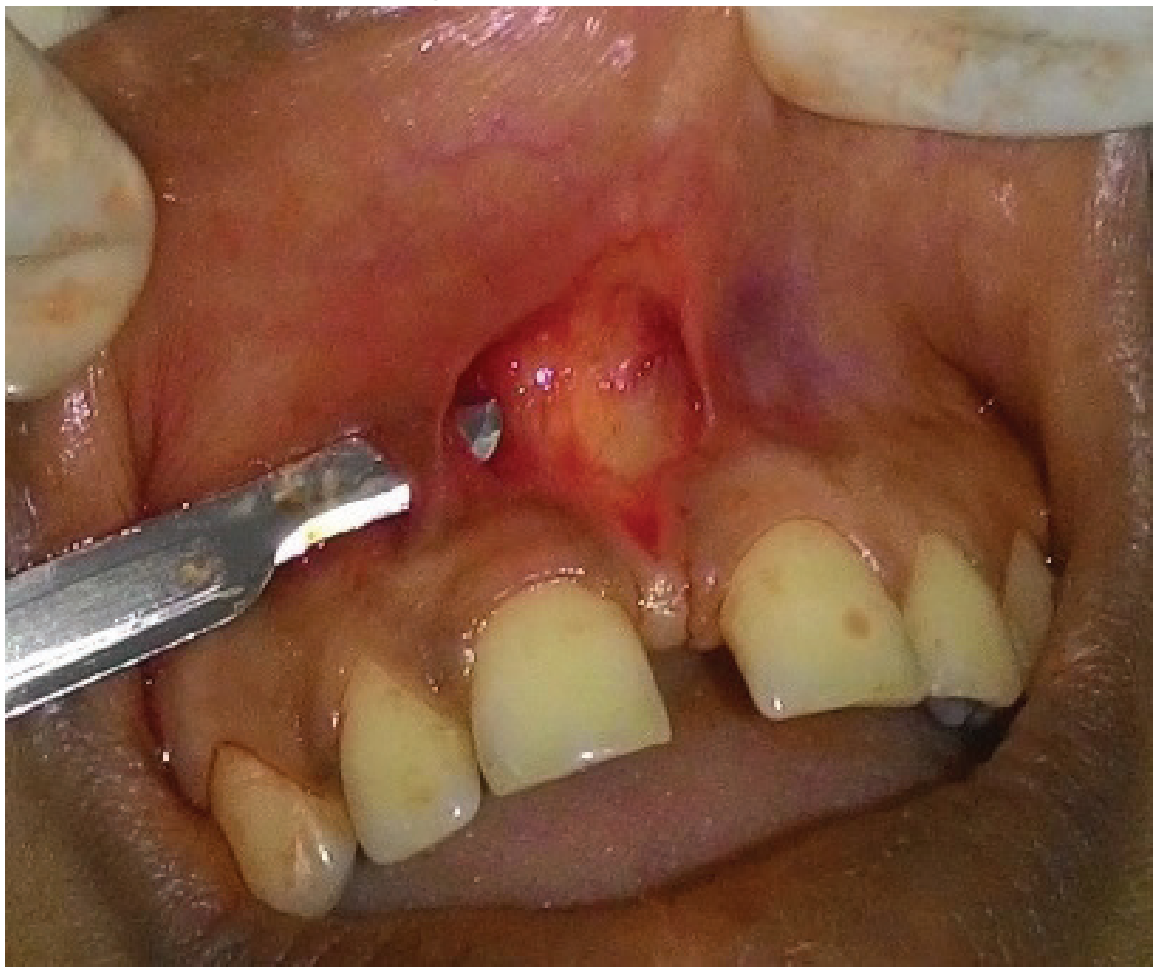

Figure 3. A second vertical parallel incision with a partial-thickness flap was raised and displaced laterally.

Figure 4. With resorbable 4-0, vicryl sutures interrupted sutures were placed.

Figure 5. One week post-operatively.

Figure 6. Six month postoperative photograph

Discussion

Over the years it has been documented that the amount of attached gingiva which is composed of dense collagenous connective tissue with keratinized epithelium is relatively avascular compared to alveolar mucosa and is considered important for the maintenance of gingival health[3]. Therefore to achieve these adequate anatomical dimensions, various surgical techniques were developed[5].

The frenectomy has travelled a long distance from classical techniques[3,6] to the more conservative and precise approaches [7]. Frenal relocation by Z- plasty[8] procedure along with soft tissue graft[9,10] and lasers application[10] have been added for better esthetic results and to facilitate proper healing [11, 12]. All the modifications of these surgical procedures have their own importance.

Frenectomy involves complete removal of aberrant frenum along with its attachment to the underlying bone[3] but this may result in loss of interdental papilla with exposure of underlying alveolar bone leading to marginal tissue recession[13,14.] Therefore frenectomy with lateral pedicle flap was planned in this case which is a modified method for increasing keratinized tissue causing increased resistance to plaque bacteria which is conducive to periodontal health[2]. Further, this provides a collagenous band in the midline maintaining the width of attached gingival which also closely matches the colour of the surrounding tissues. Pedicle when sutured back medially act as tissue dressing over the V-shaped defect which facilitates faster and better healing. Furthermore highly esthetics results were obtained due to undisturbed transeptal fibres and no scar formation.

Conclusion

The surgical technique presented in this article is a reliable and an effective way of maintaining the width of attached gingiva, easier to perform in an outpatient setting with excellent esthetic results.

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Ethical Issues: None

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Appraisal of Dimensional Accuracy in Five Brands of Extended Pour Irreversible Hydrocolloid Materials for FDP Impression

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Abstract

Aim. The purpose of this study was to assess and compare the dimensional accuracies of five brands of extended pour irreversible hydrocolloid materials for fixed dental prosthesis impression.

Materials and methods. Impressions of a master stainless steel model were made with five extended pour irreversible hydrocolloids and one addition silicone impression material. A total of ninety impressions (n=90) were made, with fifteen for each group of impression material. The stone dies retrieved from these impressions were analysed with Baty-Vision Systems - Venture 3D CNC machine, for dimensional accuracies. Comparison of continuous data between two groups was done with the Wilcoxon rank-sum test (Mann Whitney U test) and for more than two groups with the Kruskal Wallis test ($\alpha = .05$)

Results. All six impression materials showed some amount of error. On comparison of the median total absolute deviation of the various dimension of stone dies, the accuracies of impression materials in decreasing order appeared to be Neocolloid >> Affinis >> Coltoprint >> Zelgan >> 3M ESPE >> Plastalgin. On the contrary, a comparison of the impression materials, in terms of several dimensions of stone dies differing significantly from the stainless-steel model, the sequence in terms of accuracy was, Coltoprint > Affinis >3M ESPE > Neocolloid > Plastalgin > Zelgan.

Conclusions. Based on the dimensional accuracies of the stone dies retrieved from the impressions, Neocolloid was found to be the best impression material with the least possible deviation, followed by Coltoprint and Affinis.

Keywords*: Extended pour irreversible hydrocolloid; Dimensional accuracy; FDP*

Introduction

Dimensional accuracy of impression is as critical as the working cast, for precise fit and success of fixed dental prosthesis.[1-3] This warrants the use of the right choice of impression material.[4,5] Addition silicone has been the material of choice for a fixed dental prosthesis (FDP) until now.[6] Along with dimensional accuracy, it has good detail reproducibility, ease of handling, and superb elastic recovery. Even multiple casts can be poured from a single impression.[7,8] Nevertheless, the material is quite expensive, and the technique sensitive in nature.[9] Being intrinsically hydrophobic, it requires an extrinsic surfactant to avoid voids formation, at the margin of tooth preparation.[10] This necessitated the development of cheaper impression material, with dimensional accuracy similar to addition silicone.[11]

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A substitute to addition silicone can be irreversible hydrocolloid, owing to its ease of manipulation and cost-effectiveness.[12] Nevertheless, the irreversible hydrocolloid impression has low dimensional stability and needs to be poured immediately.[13, 15] Again, it has a low tear strength, tendency to stick to teeth with poor adhesion to the tray. So, the use of this material was initially restricted for the diagnostic cast.[15] But, the new generation of extended-pour irreversible hydrocolloids has extended pour time, colour-changing nature, high viscosity, and dust-free properties with better adherence to a tray.[16-18] So, the dimensional accuracy of these new generation irreversible hydrocolloids, need to be assessed and compared with addition silicone impression material for FDP impression.[19]

Dimensional accuracy of the impression is either assessed directly or indirectly on the stone cast, retrieved from the impression.[19] Literature shows the use of image analyser, coordinate measuring machine (CMM) Carl Zeiss Contura G 2, travelling microscope (ELFO, India Pvt. Ltd), micrometres, and digital modelling, for the dimensional measurement.[15, 20,21] Baty Vision Systems - Venture 3D CNC is a new machine in this category. The upgraded fusion software of this machine, inspects the model completely automatically, thereby making the scanning and best fitting process quick. Once the parts are measured, a full CNC program is generated automatically. This saves the time of skilled operators. Moreover, the procedure of CNC programming is easy to learn and repeat.

The present study was designed to assess and compare the dimensional accuracies of five brands of extended-pour irreversible hydrocolloid materials for fixed dental prosthesis impression. The null hypothesis stated that all the five brands of extended pour irreversible hydrocolloids and addition silicone impression material were similar, in terms of dimensional accuracy of impressions and therefore the generated casts.

Material and Methods

Six impression materials, with five brands of extended-pour irreversible hydrocolloids and one addition silicone impression material, was assessed in this study, for dimensional accuracy (Table 1). All the measurements were done indirectly on the stone dies retrieved from the impressions.[22]

The clinical situation for the fabrication of FDP was simulated with a master stainless steel model (Fig 1A). This model was having two prepared abutments (A, B) with six-degree occlusal convergence, the gingival finish line with 1mm width, and a ninety-degree shoulder.[23] Cross grooves were inscribed on the occlusal and proximal surfaces of these abutments for reference measurement. For proper orientation of the impression tray, a step was present on the base of the stainless-steel model.[23] The entire master model was designed with SOLID WORKS® software and was milled in high precision AGNI+ BMV 45+ TC24 milling machine, in accordance to the ANSI/ADA stipulations (8.015mm in height, 6.330mm diameter at the apex and 8.450mm diameter at the base of the abutment, with a 28.270 mm inter-abutment distance).[24, 25] Descriptions of all the nine locations of the stainless-steel model used for measurements are given in the schematic form (Fig 1B). This model provided a good baseline condition for comparisons of impression materials.

A single customized stainless-steel perforated tray, with metallic handle, was used throughout the study, to standardize impression making. The perforations were of 2mm diameter for the mechanical retention and were designed to hold the 4mm thickness of impression material (Fig 1A).[26]

All the six impression materials were manipulated in standard proportions as per the manufacturer's instruction, at room temperature (25±2℃). The irreversible hydrocolloids were mixed manually, with a clean rubber bowl and spatula, whereas a one-step double mix impression technique was used for the addition of silicone impression material. Care was taken to apply a thin layer of tray adhesive (VPS Tray Adhesive; 3M ESPE) to the intaglio surface of the tray, before loading of the addition silicone. To make the impression the loaded impression tray was pressed over the master stainless steel model. At that juncture, the whole assembly was submerged in a water bath having a temperature of 35°C±1°C, till the material set.[27] This mimicked the oral environment. The temperature of the water bath was regulated with a mercury thermometer.

A total of ninety impressions (n=90) of the master stainless steel model were made for six groups, with fifteen for each group of impression material. Following

the set, impressions were detached from the stainless model in a snap movement, parallel to the long axis of prepared abutments. The unsatisfactory impressions with voids and inaccuracies were discarded from the study. To simulate the clinical procedure, the approved impressions were air-dried and preserved in sealed plastic bags (100% relative humidity) at room temperature for half an hour.[28]

Type IV dental stones (Kalrock: Kalabhai Karson Pvt., Ltd, and Pearlstone: Asian chemicals) were used to pour the impressions. They were vacuum mixed (Multivac 4; Degussa), under standardized conditions, as per manufacturer's instructions, and vibrated (EWL 5403; KaVo EWL) into the impressions. After setting, stone dies were separated from impressions and ready for measurement.

Measurements of the stone dies were done with Baty Vision Systems - Venture 3D CNC machine supported by fusion software. This machine has a high-resolution 0.5μm scale for increased accuracy. For measurement, stone dies (n=90) were mounted on the jig of Baty Vision systems, to orient the occlusal surfaces of the abutments in the horizontal plane. Before this master stainless steel model was calibrated in the Baty Vision Systems - Venture 3D CNC machine. Measurements were made for abutment diameters (A- m1, m2, m3 and B- m5, m6, m7), height (A-m4, B-m8), and the inter-abutment distance between abutment A and B (m9). The obtained results were tabulated for statistical analysis.

Statistical Analysis

Measurements of all the nine locations, of the stainless-steel master model, as well as stone, dies (n=90 @ 15 per group) for six impression groups were tabulated and statistically analyzed using the software STATA 12.1. Descriptive statistics in the form of mean and standard errors were computed. Since the sample size was small, non-parametric tests were used for testing the hypothesis of a significant difference. A comparison of continuous data between two groups was done with the Wilcoxon rank-sum test (Mann Whitney U test) and for more than two groups with the Kruskal Wallis test. Cut of significant probability was tested at $\alpha = .05$, whereas highly significant values were tested at $\alpha = .01$ levels.

Results

The comparison of the nine dimensions of master stainless steel model with that of stone dies, retrieved from six categories of impression materials, are displayed in tabular form (Table 2). They are presented in the form of the absolute mean values, corresponding standard deviation, and P-value, of the nine dimensions of stone casts belonging to six groups.

According to the Table, all the six groups of impression materials were still found to have an error, for the abutment diameter (A- m1, m2, m3 and B- m5, m6, m7) and height (A-m4, B-m8). However, the inter-abutment distance between abutment A and B (m9) was not found to be significantly different, for any of the six impression groups.

On comparison of the median total absolute deviation of the various dimension of stone dies (m1, m2, m3, m4, m5, m6, m7, m8, m9) using Kruskal Wallis Test, a significant difference ($p = .0041$) was found between the groups (Table 3, Fig. 2). Groups having significant differences were further compared with each other using the Man-Whitney Test. It was seen that Neocolloid was having a significant difference with Plastalgin, 3M ESPE, and Zelgan, while Plastalgin was having a significant difference with Zelgan, Affinis, and Coltoprint ($\alpha = .05$). No other groups were found to have a significant difference. It appeared that the accuracy of impression materials in decreasing order are Neocolloid >> Affinis >> Coltoprint >> Zelgan >> 3M ESPE >> Plastalgin.

On the contrary, a comparison of the impression materials, in terms of numbers of dimensions of stone dies differing significantly from the stainless-steel model, a different outcome was found (Fig.3). As per the Figure, Coltoprint was found to be the best material, with the least number of dimensions differing from the stainless-steel model. Affinis followed next in order. So, the sequence in terms of accuracy were, Coltoprint > Affinis >3M ESPE > Neocolloid > Plastalgin > Zelgan

Table 1 Description of the impression materials

Type of impression materials	Trade name	Manufacturer
Irreversible hydrocolloid	Neocolloid	Zhermack
	Plastalgin	Septodont
	3M ESPE	3M
	Zelgan	Dentsply
	Coltoprint	Coltene
Addition silicon	Affinis	Coltene

Table 2 Comparisons of Dimensional accuracy of stone dies obtained from six impression material with master stainless steel model using Wilcoxon Signed rank test (n=15 for each group)

Dimension	Master stainless steel model	IRREVERSIBLE HYDROCOLLOID										ADDITIONAL SILICONE	
		Neocolloid		Plastalgin		3M ESPE		Zelgan		Coltoprint		Affinis	
		Mean ± SE	P VAL	Mean ± SE	P VAL	Mean ± SE	P VAL	Mean ± SE	P VAL	Mean ± SE	P VAL	Mean ± SE	P VAL
M1	6.366	6.35±0.01	0.087	6.44±0.03	0.125	6.41±0.03	0.255	6.41±0.03	0.255	6.38±0.02	0.820	6.372±0.007	0.4225
M2	9.842	9.54±0.04**	0.001**	9.41±0.05**	0.001**	9.52±0.03**	0.001**	9.42±0.05**	0.001**	9.56±0.03**	0.001**	9.496±0.019**	0.0006**
M3	7.100	7.15±0.01**	0.002**	7.05±0.05	0.443	7.13±0.03*	0.028	7.10±0.04	0.306	7.15±0.03	0.053	7.153±0.024*	0.0466
M4	6.990	7.07±0.02**	0.004**	7.18±0.08**	0.006**	7.24±0.1**	0.001**	7.08±0.03**	0.002**	7.00±0.02	0.741	7.015±0.015	0.1671
M5	6.369	6.37±0.01	0.955	6.47±0.05*	0.014	6.37±0.01	0.459	6.42±0.02*	0.047	6.41±0.03	0.211	6.373±0.029	0.4257
M6	7.109	7.12±0.01	0.733	7.03±0.06	0.233	7.13±0.01	0.155	7.05±0.05	0.733	7.15±0.03	0.255	7.183±0.042*	0.0404
M7	9.502	9.49±0.01	0.394	9.35±0.08*	0.026	9.49±0.01	0.532	9.36±0.06**	0.003**	9.52±0.02	0.363	9.477±0.021	0.3626
M8	6.990	7.04±0.02*	0.020	7.1±0.06*	0.027	7.15±0.07*	0.029	7.06±0.02**	0.001**	7.02±0.02*	0.088	7.077±0.033*	0.0306
M9	20.273	28.26±0.01	0.334	28.24±0.05	0.495	28.26±0.02	0.649	28.21±0.04	0.191	28.32±0.03	0.231	28.283±0.033	0.5699

N.B: * Significant at 0.05 level, ** Significant at 0.01 level of significance.

Table 3: Median total absolute deviation of the stone dies using six impression materials (n=15 for each group)

Group	Median total absolute deviation#	25th percentile Q1	75th percentile Q3	Rank sum	'p' value Kruskal Wallis test
Neocolloid	7.339	5.943	10.506	391.5	0.0041
Plastalgin	14.771	9.634	24.992	971.0	
3M ESPE	10.506	8.281	15.257	755.0	
Zelgan	9.301	7.615	14.004	690.5	
Coltoprint	9.882	7.409	13.389	644.5	
Affinis	9.529	7.301	13.897	642.5	

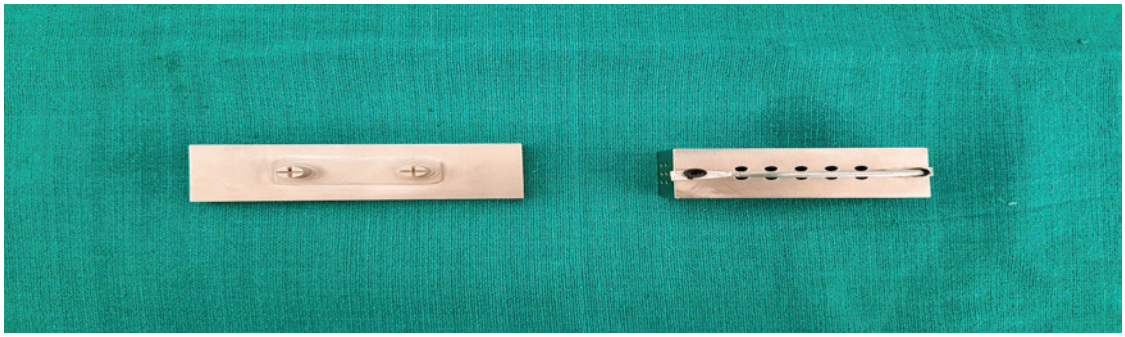
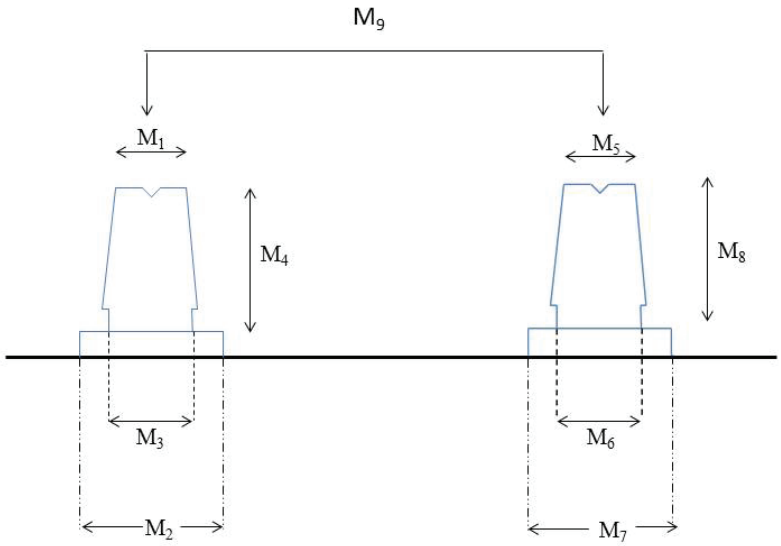


Figure 1A: Master stainless steel model with a custom tray



M_1 - Occlusal diameter of Abutment A(6.366). M_2 - Cervical diameter of Abutment A(9.842). M_3 - Diameter of undercut of Abutment A(7.100). M_4 - Occlusal-Gingival diameter of Abutment A(6.999). M_5 - Occlusal diameter of Abutment B(6.396). M_6 - Cervical diameter of Abutment B(7.109). M_7 - Diameter of undercut of Abutment B(9.502). M_8 - Occlusal-Gingival diameter of Abutment B(6.990). M_9 - Distance between Abutment A and Abutment B(28.273).

Figure 1B: Schematic diagram of nine dimensions of the master stainless model

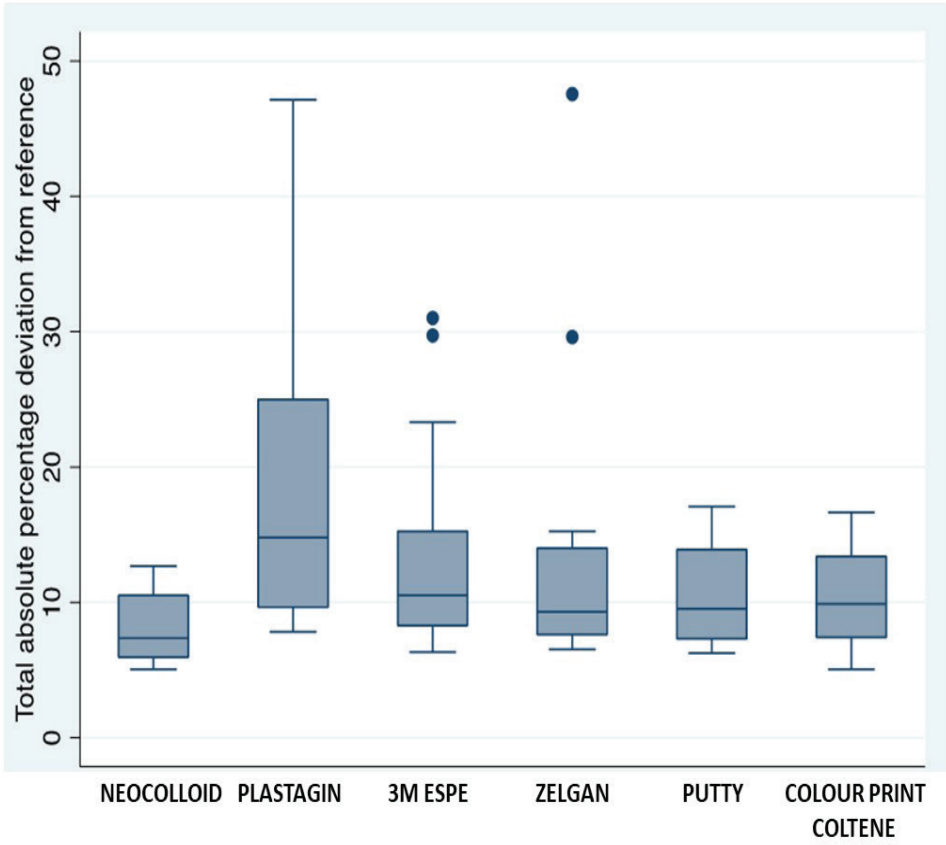


Figure 2: Median total absolute deviation of the stone dies using six impression materials (n=15 for each group)

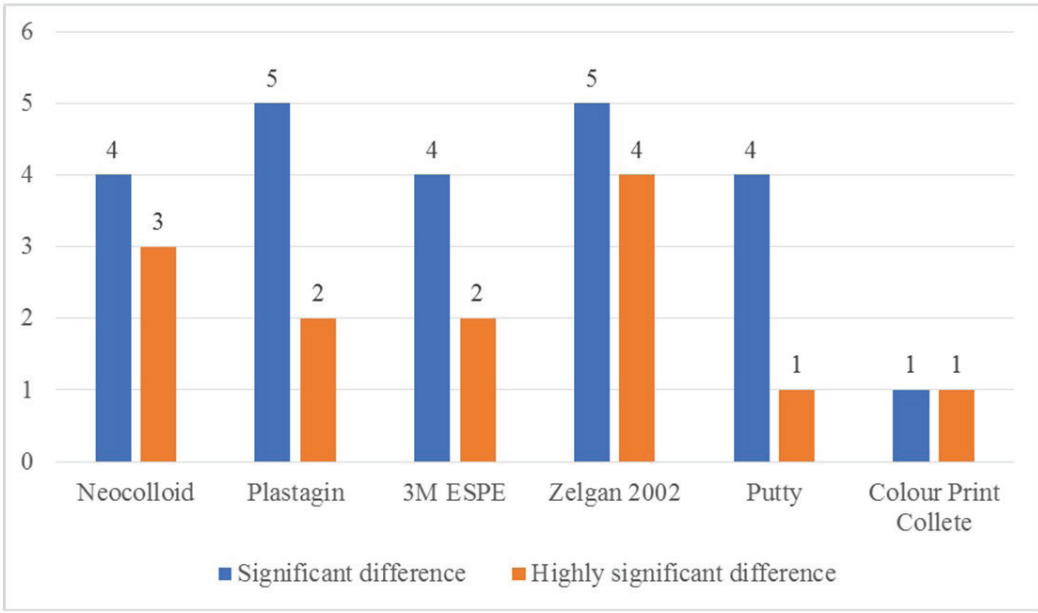


Figure 3: Comparison between the six impression materials in terms of the number of dimensions of the stone dies differing significantly from the stainless steel model (n=15 for each group).

Discussion

The results of the present study support the null hypothesis, as dimensional accuracy of most of newer generation of irreversible hydrocolloid were close to addition silicone (p = .0041). A significant difference among the six impression materials was noted only for dimension m2, m4 and m7, when they were compared with the standard dimensions of master stainless steel model individually (Table-1). This might be due to discrepancies in removals of impression from the master stainless steel model, or polymerization shrinkage of the impression materials. The presence of air bubbles, voids, or tears along the margin of impression, might have led to data related error.[29]

The result showed discrepancies of the stone dies and master stainless model, with positive and negative values, at different abutment dimensions (m1-m9). This would have led to incorrect outcomes, due to positive and negative values annulling each other out. To evade that, the data were transformed to absolute values for comparisons.[15]

In comparison, Neocolloid, Coltoprint, 3M ESPE, and Zelgan resembled closely with Affinis (not being significantly different), with Neocolloid having the minimum median total absolute deviation from the standard reference. However, Plastalgin was having the highest deviation, with a significant difference from Affinis as well as Zelgan and Coltoprint (Table 4). Since the absolute deviations were summed up here, the accuracy of impression materials might not have been significantly different. But as per the rank sum of the deviations from the stainless steel model, it might have seemed that the accuracy of impression materials in decreasing order are Neocolloid >> Affinis >> Coltoprint >> Zelgan >> 3M ESPE >> Plastalgin.

On the contrary, the sequence of dimensional accuracy of impression materials was found to be Coltoprint > Affinis > 3M ESPE > Neocolloid > Plastalgin > Zelgan when compared, in terms of the number of dimensions of stone models differing significantly from stainless steel model. Coltoprint and Neocolloid were having a minimum possible deviation from the master model, similar to addition silicone (not being significantly different). Other groups also had a median total absolute deviation similar to addition silicone were 3M ESPE and Zelgan. However, Plastalgin was found to have the highest deviation with a significant difference from addition to silicone (Table 4). Among the irreversible hydrocolloids, Coltoprint was found to be the best material followed by the Neo-colloid.

This outcome was found to agree with the results of some of the previous studies.[30] Peutzfeldt and Asmussen had found the dimensional inaccuracy of the irreversible hydrocolloids to be between 44 and 188 microns. Even dimensional accuracy of one of the irreversible hydrocolloids (Blueprint regular), was comparable with elastomeric impression materials.[30]

Similar results were echoed by Cohen and co-workers. They studied the dimensional stability of conventional brands of irreversible hydrocolloid impression materials under different storage conditions, at different time intervals of 10 min, 30 min, 1 h, and 24 h, before pouring.[31] The most accurate cast was obtained from immediate pouring. In another study, Chen et al had also suggested similar dimensional accuracy of irreversible hydrocolloid to those of elastomeric impression materials, provided it is poured within 24 h.[15] After 24 h, irreversible hydrocolloid impressions are relatively unstable. Faria et al had found the dimensional accuracy of irreversible hydrocolloid to be acceptable, comparable to that of elastomeric impression materials other than polyether, as long as they are poured in time.[11] In contrast, the new generation extended pour irreversible hydrocolloid has been found to maintain dimensional stability up to five days when stored adequately.[16-18] According to Frederic and Caputo, though the precisions of irreversible hydrocolloids are almost those of the elastomeric impression materials, they are inferior, when properties like water loss and the formation of surface roughness are taken into considerations.[25]

There are certain limitations to this study. Being an in vitro study, it does not resemble the oral cavity situation and only provides baseline data. Though the study focuses on the dimensional accuracy, it does not emphasize on the surface detail of the impressions. Moreover, sample sizes taken are small. Further clinical studies with bigger sample studies are required for the successful implementation of the inference of the present study.

Conclusions

Within the limitation of the study, it can be concluded that Neocolloid is the best material with the least possible deviation, with Coltoprint and Affinis closely following it when compared to each other. Plastalgin is the poorest material in terms of accuracy, having a highly significant difference with the best materials. Accuracy is only in terms of diameter and height of abutments and no significant difference between any of these impression materials when inter-abutment distances are concerned. Coltoprint can be regarded as the best material in terms of the least number of dimensions differing from the master stainless steel model.

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Dentist as an Expert Witness under Section. 45 of Indian Evidence Act 1872

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Abstract

Dentists of different specialties are sought for their expert testimony in person identification, injury related to oral and dental structures. Person identification may include live or dead, injured or healthy, skeletonized, or mummified. Dentists also opine in crime scene investigation, mass disaster victim identification, and professional negligence or injury. The dental surgeon evaluates the available dental evidence and drafts a report to testify in a deposition as a professional opinion in the medico-legal cases. This article discusses the demeanor of the dental surgeon as an expert in the court of law.

Keywords: *dental law, forensic odontology, medico-legal case, person identification, crime investigation*

Introduction

In Ancient India there existed traditional legal proceedings among social groups mainly based on the *Kautilya Arthashastra* written by Chanakya, which lays the foundation for law and order. This type of communal law existed until the establishment of the Indian Penal Code (IPC) in 1860 and the Criminal Procedure Code (CrPC) of 1861 which was later amended in 1969 and replaced with the Indian Evidence Act of 1872 by Sir James Fitzjames Stephen of British Raj. This law still exists with few additional amendments.[1,2]

Expert opinion is covered under Section 45 of the Indian Evidence Act which guides the judicial system to permit skilled professionals to share their knowledge in a particular trial, to impart proper judgment. The competency of the witness is always based on experience in a particular field.[3]

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Law is created to maintain peace and to settle a dispute among people in the society and when the process involves courtroom, it is referred to as an adversary process.[4] In such an event, a person who is not involved in the dispute - a witness, usually supports the legal proceedings and if this procedure involves scientific expert opinion to prove or disprove the evidence, these doyens are denoted as an expert witness.[5,6] The Judge is not expected to be an expert in all the fields, especially where the subject matters involve technical knowledge.[3] The expert opinions are utilized for ascertaining disputed questions in a judicial inquiry.[7]

Ethically an expert witness must be appointed by the court and should not back any particular party.[8] In the courtroom an expert witness also undergoes a cross examination, as they are advisory characters who help the Judge with scientific data. However, they cannot influence the judgment, as it is solely a decision made by the Judge. The presence of expert in the trial room is not mandatory, his/her opinion can presented as an affidavit, telephonic conversation or through video conferencing.[9]

Dentist as an expert witness - Link between Dentistry and Judicial system

A dentist by profession, treat dental ailments however, they can be considered as an expert witness in the court of law under certain circumstances such as; (a) Person identification (b) crime investigation cases, (c) Mass disaster victim identification (MDVI), (d) Professional liability cases and work-related injuries. Figure 1 summarizes the role of dental expert witness in the trial room. *Forensic Odontologists* are considered as the legal expert involving person identification and crime scene investigation while issues on professional negligence can be attended by any experienced dental surgeon. Keiser-Neilsen describes forensic dentistry as a subject that deals with proper handling and evaluation of dental evidence and presentation of these dental findings to the court of law. The dental expert must be able to correlate and apply clinical dentistry with dental law and legal medicine.[10]

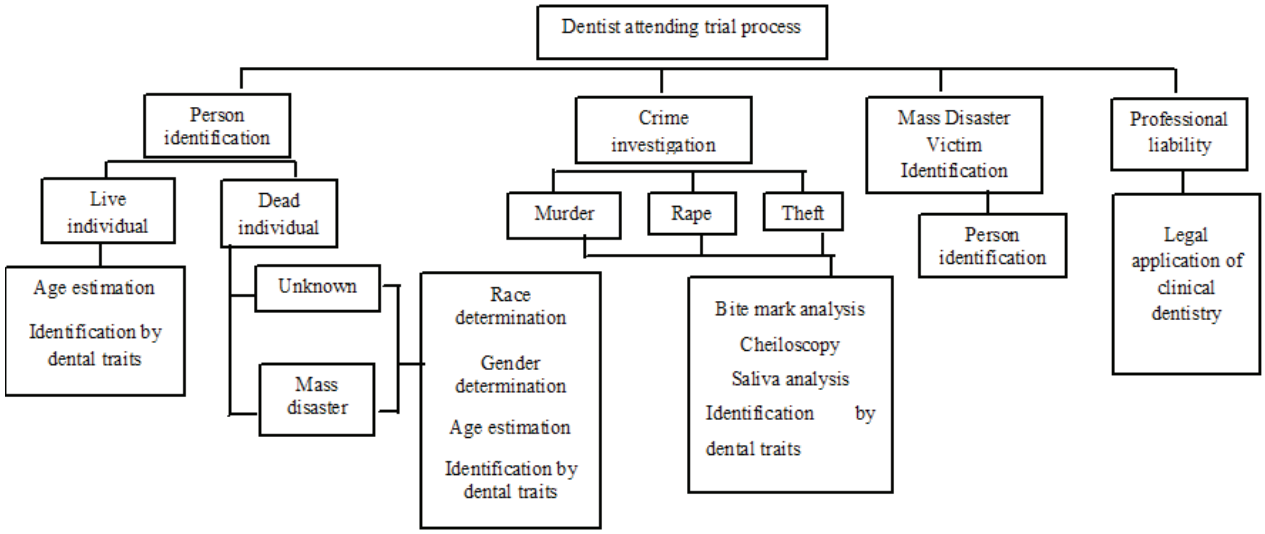

Figure 1. Schematic representation featuring dental opinion in court trial

Dental expert opinion on person identification:

The identification of a person involves dead as well as live. Live individuals are called for identification in case of age assessment specifically involving child labor or pornography. Whereas in living adult the dental traits can be used to recognize the criminal, which include bitemark analysis. Dental evidence have effectively contributed to the gang rape case of Nirbhaya in 2012, where the forensic dentist positively matched the accused dental alignment with the bitemark on the victim which proved to be a strong evidence for prosecution.[11]

Identification of a well preserved corpse is very easy, but the same becomes complicated with putrefaction. Highly skeletonized corpse pose a great challenge for forensic expert, dental hard tissues being stable in all thermal conditions can narrow down the positive identification. It is not only the teeth and their arrangement, the dental restorations, artificial denture, skull and its association with the facial bones, individual features of these bones may also clue about the identity of the deceased.[12] Age of an embryo can be estimated from the developing dental organ,[13] knowledge on eruption and shedding of teeth can clue on ageing in children and adolescence. In adults above the age of twenty five years, microscopic and molecular analysis such as racemization can guide in age assessment.[14,15]

Gender identification of a decayed corpse can be done by careful examination of bones, when the bone is highly fragmented, pulp within the tooth may offer a reliable source of gender prediction by expressing the amel-x gene.[16]

Dental expert opinion in crime investigation:

Any illegal activity is considered as crime[17] which includes murder, rape, theft, etc. Ancient Indian literature bridges law and medicine by investigation of death which can be caused by three ways (a) stop breathing, (b) physical injury, (c) poisoning. In India death cases demanding autopsy ranges from 10000 to 35000 per state in a year among which less than 10% account for homicide. Any medical/dental graduate is permitted to perform medical/oral autopsy. Although there is specialization in the relevant field, the availability of experts is less in India unlike countries such as USA, UK, but the objective of an autopsy is to aid the investigating team and to guide over the facts about death.[2]

In situations where the dead is skeletonized and only the skull is left out, facial reconstruction helps to scientifically re-establish the identity. On 10th of April, 2018 a decomposed corpse with a completely burnt face but with recognizable attire was recovered from the mangroves at Mumbai. When all other modes to trace the identity of that person proved futile, forensic odontologist at KEM hospital Mumbai created a face model over the available skull, with appropriate face wrinkles and other features using Plaster of Paris. Fortunately, the model was identified and the victim was prosecuted.[18]

Photographic superimposition is another method to identify the skeletonized skull where the clue on the identity of the victim is available. In April 2012 Sheena Bora murder case was busted and her skeletonized skull was successfully identified by photographic superimposition in November 2015[19] however dental DNA from the molar tooth was not sufficient to match the maternity in this particular case.[20] In cases where dental DNA from the teeth are not available, it could be obtained from long bones such as humerus.[21,22]

The 2007 New Year eve in Nithari – Uttarpradesh fumed with the flash news about the heap of skull and skeleton in the gutter. In this serial killings, the number of skulls, and not any other bone at the crime scene accounted for the number of the victims claimed to be killed. It was totally 39 skulls recovered which also aided in prediction of the sex of an individual with 90% precision. The age was assessed from the sutural closure of the skull as well as x-ray examination of the long bones.[23]

Dental expert witness in Mass Disaster Victim Identification (MDVI):

Mass disaster (MD) causes interference in the normal livelihood of human causing widespread damage to properties and raising the number of casualties. MD could be accidental eg. bombings, fire accidents, flight crash, drowning of marine vehicles, or due to natural calamities like earthquakes, hurricanes, typhoons, tsunami. Whatever is the cause, the outcome is similar exhibiting fragmented, co-mingled, or burned remains; which makes the identification of the corpse more difficult. In such an event forensic expert unit comprising of a forensic pathologist, forensic anthropologist, forensic odontologist, forensic photographer, police crime scene investigator, evidence technician/gatherer, fingerprint expert, DNA analyst, radiologist and radiographic technician, toxicologist, dental hygienist/ assistant extend an indispensable contribution. The identification of the dead in MDVI is usually established by comparing antemortem and postmortem records.[24]

Dental tissues being hard to resist the highest temperatures and are less vulnerable to these incidents, hence provide a better source for person identification. A complete intra-oral examination and radiographs of intra/extra-oral structures are recommended since the dental features may clue on race, socio-economic status, dietary habits, habitual behaviours, dental or systemic diseases.[25] Gender determination and age estimation is also possible with microscopic and dental DNA analysis. Usually, a healthy second premolar is extracted for DNA analysis from the pulp.[26]

The 1991 disastrous bomb attack, which was targeted to the former Indian Prime Minister Mr. Rajiv Gandhi ended up causing many fatalities, his dead body was shattered and co-mingled. His identity was established by the forensic odontologists from his dentition.[27,28]

In December 2004, tsunami took away the lives of many in Asia but the dental expert was hardly sought in the MDVI in Chennai when compared to Thailand.[29,30] Similarly in May 2010 Mangaluru, Karnataka Air India Express flight IX-812 crash barely utilized the forensic dentist. In some instances, the kin of the deceased was unaware of the dental status and the antemortem dental

records were not available. The state administration also failed to recognize the value of dental expert opinion at these instances and the importance of dentist/dental records must be emphasized.[30] Apart from the identification of the dead, a dentist can also contribute to recovery team by delivering emergency health measures to the injured in need.[31,32] Since there is no emergency at postmortem human identification, one must analyze the available evidence with utmost care to draft a report.[24]

Expert opinion on fellow professional for legal protection and litigation:

The health care professionals including dentist and general physicians are looked upon as noble professionals since they strive to keep the patients at comfort by relieving them of disease and saving their lives.[33,34] Hence, when the physicians or the surgeons are questioned on their quality of treatment, expert opinion is sought to prove or disprove the litigation which further decodes the medical scenario to the understanding of the jury and the common man.[8]

In India, the Consumer Protection Act (CPA) of 1986 was ordained for quick addresses of the consumer grievances and the consumer redressal forum (CRF) governs these issues. Experts need not a part of CRF but they must be available when called upon by the jury.[33,35] Section 2(1)(o) 1995 of the CPA denotes if the service at hospital or institution is provided free of cost then the patient cannot be considered as a consumer and the issue cannot be redressed in consumer forum, while the chargeable services are accepted for grievances, the liability covers negligence in diagnosis and treatment.[34] The litigation can be either dismissed under IPC section 88 citing that the harm was caused to the patient for their goodness or the surgeon/physician can be held behind the bars or let free with a fine amount under various sections of IPC (304A, Sec-336, Sec-337, Sec-338).[33,35] Any decision on the involved doctor cannot be solely concluded by the court, an opinion from a competent and experienced professional of that particular specialty is mandatory.[8,34,36] Globally there are several reports on dental negligence and malpractice mainly associated with dental extraction and oral surgical procedures. Most of these cases failed to prove the negligence and the judgement favoured the dentists based on the expert testimony.[35]

The legal workers including the lawyers and the judge should always overview the reliability of medical negligence offense, since no healthcare provider would intend to harm the diseased. However, that particular dental professional has to obtain written consent from the patient after explaining the inherent risks associated with the treatment procedure.[33,35] Also it is mandatory to maintain updated computerized records of all the patient's treatment protocol with appropriate consent, to prove the moralities at the court.[35,38]

Conclusion

Forensic identification deems multidisciplinary co-ordination including law enforcement officials, medical experts, laboratory specialists, etc. A dentist can provide accountable information to benefit the legal proceedings, just from the radiographs even when the corpse is not presented.[39] However, there exists an ambiguity over the character – witness and when a dentist is called upon at various circumstances of court events fear stings over. Still one must remember the fact that *No One Can Replace Others Role*, so every dentist must volunteer a testimony when required. Sometimes such an act may benefit a fellow dentist in litigation issues.[40]

Expert evidence may sometimes be the foundation of the case or at times it may not be essential. He serves the proceedings by providing missing links, strengthening a weak link, in a chain of evidence. An expert must be bold in presentation with sound knowledge.[7]

Forensic dental identification had become a routine protocol many countries but it is still evolving in India and the authorities concerned lack to value their importance.[30] Though any registered dentist can be offer an expert witness in India, special training on forensic is gaining popularity and it is mandatory in cases of medico-legal mortalities.

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Case Report

Misinterpretation of Section 304A IPC vis-a-vis 304IPC in Medical Negligence Cases – Deliberate or Inadvertent?

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Abstract

Negligence is specific tort and in any given circumstances is the failure to exercise that care which the circumstances demand. What amounts to negligence depends on the facts of each particular case. The degree of care required in a particular case depends upon the surrounding circumstances, and may vary according to the amount of risk to be encountered and to the magnitude of the prospective injury. A basic knowledge of how judicial forums deal with the cases relating to medical negligence is of absolute necessity for doctors. The need for such knowledge is more now than before. In light of higher premium being placed by the Indian Forums on the value of human life and suffering, but sometimes misinterpretations are done deliberately or inadvertently when the doctors are charged for culpable homicide not amounting to murder (304 IPC) instead of medical negligence (304 A IPC). A case of medical negligence is being discussed in which a gynaecologist has been charged for 304IPC instead of 304A IPC. The scenario was that, the patient died after readmission in private hospital, who had delivered a healthy baby, managed properly and discharged by the Gynaecologist and suffered undiagnosed complications after discharge at home. The charge was framed that the doctor was absent from the hospital and the case was handled by nurses on telephonic conversation with doctor. Earlier also the doctors had been prosecuted under 304IPC instead of 304A IPC in lower courts and even high courts (e.g. Jacob Mathews V. State of Punjab, Dr. Suresh Gupta V. Govt. of NCT Delhi). But in many cases, finally Supreme Court set aside the judgement of high court and held that the doctors could not be criminally prosecuted.

***Key Words*:** Medical negligence, Culpable Homicide, Gynaecologist, Delivery, High Court, Supreme Court

Introduction

Negligence is specific tort and in any given circumstances is the failure to exercise that care which the circumstances demand. What amounts to negligence depends on the facts of each particular case. The degree of care required in a particular case depends upon the surrounding circumstances, and may vary according to the amount of risk to be encountered and to the magnitude of the prospective injury. A basic knowledge of how judicial forums deal with the cases relating to medical negligence is of absolute necessity for doctors. The need for such knowledge is more now than before. In light of higher premium being placed by the Indian Forums on the value of human life and suffering, but sometimes misinterpretations are done deliberately or inadvertently when the doctors are charged for culpable homicide not amounting to murder (304 IPC) instead of medical negligence (304 A IPC).[(1)]These misinterpretations of 304A IPC and 304 IPC by law enforcement agencies are not uncommon which further complicates the medical practice. Sometimes it happens

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due to pleading by defence counsel in unprofessional manner also. The present case is also a classical example of the misinterpretation of law by law enforcement agencies.

This case report is based on the judgement of honourable high court of judicature at Bombay in Criminal anticipatory bail application no. 513 of 2018 dated 25.07.2018, in Dr. Deepa Sanjeev Pawaskar and Dr. Sanjeev Anant Pawaskar (The doctor couple) vs. the State of Maharashtra. The applicants had been filed for anticipatory bail apprehending their arrest in crime no. 71 of 2018 registered with Ratanagiri City police Station for offences punishable under section 304 IPC read with section 34 IPC. The main allegation under consideration was, "prescription without diagnosis and hence resulting into death of the patient amounts to criminal negligence on the part of the doctors". The whole thrust of the bail petition was the act of the applicant would fall under section 304A of the Indian Penal Code not under section 304 of the Indian Penal Code and therefore the court was considering the issue. After considerations, the Honb'le High Court rejected the plea thus upheld the FIR. This case was widely circulated and published recently on social media and traditional media also claiming applicability of section 304 IPC in medical negligence cases.

Case Report

The scenario was that patient died after re admission in a private hospital,who had delivered a healthy baby, managed properly and discharged by the Gynaecologist and suffered undiagnosed complications after discharge at home. The patient was readmitted and died in the hospital. The charge was framed against doctor that prescription was done without diagnosis resulting into death of the patient amounts to criminal negligence on the part of the doctors. Pranav Pramod Polekar and his wife Dnyanada used to visit the hospital of Dr. Pawaskar for antenatal check up. Dnyanada was registered with Dr. Pawaskar hospital and visited the hospital regularly. She had taken the medicine prescribed by Dr. Pawaskar regularly and she was informed that the expected date of delivery was approx. 18/02/2018. On 05/02/2018 she started having labour pains and therefore she rushed to Dr. Pawaskar hospital. The doctor and his wife were present. She was admitted in the hospital. Initially, the family members were informed that she would have normal delivery. On 06/02/18, she was advised to undergo sonography test at GuruKrupa Sonography Centre. The sonologist diagnosed "umbilical artery showed reduced diastolic flow with increased S/D Ratio s/o fetoplacental insufficiency". Upon seeing the Sonography report, doctors were of the opinion that she should undergo Caesarean Operation. On 06.02.2018, she underwent Caesarean Section. Dr. Ketkar was the anaesthetist. Dnyanada had given birth to a female child. The baby was admitted for routine child care.

On 08-02-2018 the baby was discharged and on 09-02-2018 at 5:00 PM Dnyanada was also discharged from Dr. Pawaskar hospital. Meanwhile, the doctor couple (applicant) had to go to Pune for some academic events therefore, they went to Pune on 08-02-2018 requesting Dr. Karmakar to attend the patient, if necessary in their absence. Applicants also informed the staff of their hospital, to call upon Dr. Girish Karmarkar in their Absence.

On 10-02-2018 patient started vomiting at home and her relative called upon Dr. Deepa Pawaskar (applicant) on telephone. The applicant asked them to call from any medicine shop and then she instructed to medicine shop owner to give some medicine which was said to be followed. On the same day, in the evening Dnyanada developed fever and continued vomiting and therefore, she was taken to the hospital of Dr. Deepa Pawaskar at 08:30 PM. The staffs have informed that the doctors are not available in the hospital. The staff nurse has called upon Dr. Deepa Pawaskar, who advised to admit the patient. The first informant has asked as to whether she should be taken to another hospital. However, he was informed that it was not necessary and the patient would be admitted for one day and on the next day, she would be discharged.

Dnyanada was being treated by two nurses, who were administering medicine on telephonic instructions of Dr. Deepa Pawaskar. The condition of the patient was deteriorating and the relatives, out of anxiety were informing the staff nurse. The relatives were insisting on shifting the patient to another hospital. However, the staff nurse informed the relative that they need not to panic and they are in touch with Dr. Deepa Pawaskar and she has guided them telephonically. When patient

didnot respond to the treatment, Dr. Karmakar visited the hospital at 10:30PM and patiently heard about the complaints of the patient and thereafter prescribed the medicine. On 11-02-2018 at about 03:45AM patient relatives realised that the tip of the nose and lips of Dnyanada had turned black and health of the patient deteriorated to large extent. The relatives had quarrel with the staff and thereafter at 04:00AM the staff has called upon Dr. Pawaskar (Applicant) and upon the instruction of the applicant, Dr. Ketkar visited the hospital at 04:30AM. By then patient was getting fits. Doctor Diagnosed poor prognosis and therefore, the patient needs to be shifted to Parker Hospital. The doctor was not able to state the current situation of the patient. No ambulance was available for transportation so she was transported by Dr. Ketkar's car. She was admitted in ICU of Parker's Hospital. She was kept on ventilator and at 07:00AM the doctor informed that Dnyanada expired.

The investigating officer submitted the investigation report to the District civil surgeon for the opinion. The relevant part of the opinion of the civil surgeon was as follows:

On post-mortem examination following relevant findings have been noted:-

· Pulmonary thromboembolism and bone marrow embolism in medium sized blood vessels.

· Intra-alveolar Haemorrhages and focal pulmonary edema.

· LSCS Suture site: acute non-specific inflammation

· Other organ congested

· Cause of death- pulmonary thromboembolism.

On perusal of other records the District civil surgeon opined as follows :

· Since the patient has undergone Caesarean operation and was readmitted on the very next day, it was incumbent upon the hospital to have examined the patient by the gynaecologist.

· The patient was admitted on telephone construction of Dr. Deepa Pawaskar.

· The Dr Deepa Pawaskar is responsible for the health condition of the patient.

· Dr. Deepa Pawaskar should have referred the patient to a specialist immediately.

· The absence of Dr Deepa Pawaskar was preplanned and therefore the patient not to have been admitted in her absence except checking pulse and blood pressure and no other test were performed on the patient.

OBSERVATION BY HON'BLE HIGH COURT

The whole thrust of the applicants in the present case is that the act of the applicant would fall under section 304A of the Indian Penal Code and not under section 304 of Indian penal code and therefore this court is considering the issue. The Honourable Court considering the issue as "Prescription without diagnosis and hence resulting into death of the patient amounts to the criminal negligence". This would be a case of culpable neglect which is defined as considerable on censurable or blameworthy neglect that is less than gross carelessness, but more than failure to use ordinary care (reference is given as Black law dictionary). An error in diagnosis could be negligence and covered under section 304Aof IPC. But this is case of prescription without diagnosis and therefore culpable negligence. The word "gross" has not been used in section 304A of theIndian Penal Code, yet it is settled that in criminal law negligence or recklessness, to be soheld, must be of such a high degree as to be "gross". On this basis thehonourable High Court justified applicability of 304 IPC.

OBSERVATION BY HON'BLE HIGH COURT NOT RELEVANT IN DECIDING THE CASE ON LAW POINTS

Indian Medical Association, Ratnagiri branch wrote a letter to protest the DC and SP, threatening to go on strikefor prosecuting doctor Pawaskar couple for offences punishable under section 304 of IPC.It is unfortunate that all private hospitals in Ratnagiri actually remain closed for 2 days and patients were forced to rush to Civil Hospitals.In whole judgement the matter of Strike by IMA is discussed at three places.

PLEADING OF DEFENCE COUNCIL

Applicant at the most beprosecuted under section 304A IPC not 304 IPC. Section 304A read thus,:-

· **Section 304 Acausing death by negligence:** Whoever causes the death of any person by doing any rash or negligentact not amounting to culpable homicide, shall be punished with imprisonment of either description for a term which may extendto 2 years or with fine or with both.

It is submitted that in no way, it can be said that this was a criminal negligence.The literature in respect of Pulmonary embolism may read as follows

· The patho-physiology of pulmonary embolism. Although the pulmonary embolism can arise from anywhere in the body most commonly it arises from calf veins.

· Unfortunately the diagnosis is often missed because the patient with pulmonary embolism presents with none specific signs and symptoms.

It is the case of civil law and the doctors could be made to pay compensation.At the most it is a negligence under section 304A and not criminal negligence.Besides these the defence counsel placed judgments of different case by the Supreme Court of India.[2]

EXPLANATION OF RELEVANT LAWS RELATED TO THIS CASE

As this case has been registered under section 304 of IPC and main contention of the Counsel of the applicant (accused) was it could be a case of civil law or at the most it is a negligence under section 304 A IPC, it would be proper to have a look on following legal facts.

INDIAN PENAL CODE

· It deals with substantive criminal laws of India.

· It defines offences and prescribes punishment.

SECTION 40 IPC Definition of Offence

· The word "offence" denotes a thing made punishable by this code.

ETHICAL NEGLIGENCE

· It is a violation of code of medical ethics.

· If the complaint is made and the fact is proved, the name of the doctor may be erased from the medical register.

ORDINARY NEGLIGENCE (black's law dictionary)

· Lack of ordinary diligence; the failure to use ordinary care (black's law dictionary)

· Absence of reasonable skill,or, wilful negligence, of a medical practitioner in the treatment of the patient which causes bodily injury or, death of the patient.

CRIMINAL NEGLIGENCE(black's law dictionary)

· Gross negligence so extreme that it is punishable as a crime.

GROSS NEGLIGENCE (black's law dictionary)

· A lack of even slightdiligence or care.

· Gross negligence is traditionally said to be the omission of even such diligence as habitually careless and inattentive people do actually exercise in avoiding danger to their own person or property.

· Failure to exercise even that care which a careless person would use.

· It is synonymous with reckless negligence, wantonnegligence, wilful negligence, wilful and wanton negligence, wilful or wanton misconduct, hazardous negligence, magna negligence.[3]

MEDICAL NEGLIGENCE

· The term "medical negligence" is an omnibus one, which has come in vogue to refer to wrongful action or omissions of professionals in the field of medicine, in pursuit of their profession while dealing with the patients.

· There are four essential components of negligence

1. The existence of the **duty to care**, which is owed by the doctor to the complainant;

2. The failure to attain that standard of care prescribed by the law, thereby committing the **breach of such Duty**;

3. **Direct causation**;

4. **Damage,** which is both casually connected with such breach and recognised by the law has been suffered by the complainant.(4)

This is the ordinary Legal meaning of negligence. But for professionals such as Medical practitioners and additional prospective is added through atest known as **BOLAM TEST** which is the accepted test in India. In case of **Bolam vs. Friern Hospital Management Committee,**(5) the Queen's bench division of the British Court held: "a doctor is not guilty of negligence if he has acted in accordance with the practice accepted as proper by a responsible body of medical men skilled in that particular art.It says that the standard of the ordinary skilled man exercising and professing to have that special skill" and not of the "highest expert skill".This is applicable to both "diagnosis" and "treatment".However, Supreme Court has now observed the need to reconsider the parameters set down in Bolam'stest that the errors of judgement do not necessary implynegligence.

ERROR OF JUDGEMENT(4)

· A doctor is not liable for an error of judgement or of diagnosis, if he has secured all necessary data on which to base a sound judgement.

SECTION 299 IPC- Definition of culpable homicide

· Whoever causes death by doing an act with the**intention** of causing death, or**with intention of causes such bodily injury** as it is likely to cause death, or with the **knowledge** that he is likely by such an act to cause death, commits the offenceof culpable homicide.

SECTION 300 IPC - Definition of murder

· Except in case hereinafterexcepted, culpable homicide is murder.

· Distinction between culpable homicide and murder,"culpable homicide" is a genus and "murder" is its species and all "murders" are culpable homicide but all "culpable homicide" are not "murder".(6)

· **Section 300 IPC definition of culpable homicide not amounting to murder (exception to the murder)**

1. Exception I - Culpable homicide is not murder if the offender, Whilst deprived of the power of self control by **Grave and sudden provocation causes** the death of the person who gave the sudden provocation or causes the death of any other person by mistake or accident.

2. Exception II - Culpable homicide is not murder if the offender, in the exercise in good faith of **the right of private defence of a person or property**exceeds the power given to him by law and causes death of the person

3. Exception III - Culpable homicide is not murder if the offender,being a public servant or adding a **public servant acting for the advancement of the public justice** exceeds the power given to him by law and cause death by doing an act.

4. Exception IV - Culpable homicide is not murder if it is committed without premeditation in a **sudden fight in the heat of passion upon a sudden quarrel.**

5. Exception V - Culpable homicide is not murder when the person whose death is caused, being above the age of 18 years, **suffers death or taken risk of deathwith his own consent**.

SECTION 304 IPC - punishment for culpable homicide not amounting to murder

o punishment for culpable homicide not amounting to murder.

o It has two parts: section 304 **I.P.C part I, section 304 IPC part II .**

o section 304 IPC Part I : The first part applies where the accused causes bodily injury to the victim with intension to cause death; or with intention to cause bodily injury as is likely to cause death.

o Section 304 IPC part II: comes into play when death is caused by doing an act with **knowledge** that it is likely to cause death, but without any intention to cause death or to cause such bodily injury as it is likely to cause death.

SCOPE-Before an accused is held guilty and punished under first part or second part of 304,a death must have been caused by the assailant under **any of the circumstances mentioned in the five exceptions to Section 300 IPC.**(7)

Therefore it is very clear that instant case cannot be placed under any of the five exceptions of section 300 IPC hence the doctors cannot be prosecuted under 304 IPC.

SECTION 304 A IPC CAUSING DEATH BY NEGLIGENCE

Whoever causes the death of any person by doing any **rash and negligent act not amounting to culpable homicide**, shall be punished with imprisonment of either description for a term which may extend to 2years or with fine or with both.

RELEVANT JUDGEMENTS OF DIFFERENT COURTS INCLUDING APEX COURT

Honourable Supreme Court in Jacob Mathew versus State of Punjab and others reported in (2005) 6 SCC

Indiscriminate prosecution of medical professionals for criminal negligence is counterproductive and does no service or good to the society.To prosecute a medical professional for negligence under criminal law, the hazards taken by the accused doctor should be of such the nature that the injury which resulted was most likely imminent.(1)

Hon'ble Supreme Court in Emperor versus Omkar Ram Pratap (1902) 4 Bombay LR679

An error of judgement on the part of a professional is not negligence Per Se. Higher the acuteness in emergency, the higher the complications more are the chances of error of the judgement.(8)

Hon'ble Supreme Court in ASV Narayan Rao versus Ratnamala and anr. reported (2013)10SCC 741

For an act to amount to criminal negligence, the degree of negligence should be much higher that is gross or of very high degree.(9)

Hon'ble Supreme Court in Dr Suresh Gupta vs government of NCT of Delhi and another AIR 2004 SC 4091

The prosecution will have to prove a case of high degree of negligence on the part of the doctor forsustaining of prosecution under section 304A IPC.(8)

Hon'ble Supreme Court in Mahadev Prasad Kaushik vs State of Uttar Pradesh and another (2008) 14 SCC 479

Honourable Supreme Court found that the offensive reveal is one punishable under section 304-A IPC and summons out to have be issued under section 304-A IPC.(10)

Hon'ble Supreme Court in Crl.R.P number 296 of 2014 has cautioned many times that of prosecution under section 304 IPC is not possible in any circumstances against doctor.Even prosecution under section 304A IPC can be sustained only if definite material showing high degree of negligence is there with the prosecution. (11)

Deficiency in prosecution arguments, defence arguments, judicial observations and judgements

1. Hon'ble High Court defined the culpable negligence as less than gross negligence and more than failure to use ordinary care and quoted reference of black's law dictionary. If it is a fact then culpable negligence shall be of lesser degree than criminal negligence,as according to black law dictionary,Criminalnegligence is synonym to gross negligence.

2. The observation that the error in diagnosis is covered under section 304 A IPC is wrong.In fact error in diagnosis is not negligence at all (usually).

3. Hon'ble Court has mentioned three times regarding strike of IMA and on reading judgement it appears that the presiding judge was unhappy with the strike by IMA.Such things should not be considered in discussing law points. Even the protest of the members of IMA against the lodging of FIR under section 304 was genuine.

4. The defence lawyer has argued wrongly that it cannot be considered under section 304 IPC and atmost the case can be considered under section 304A IPC and

not in criminal negligence. Actually section 304 IPC can never beimposed against any doctor for professional negligence. Section 304 A and criminal negligence is the sameand the defence lawyer should have argued for Civil negligence.

5. However the duty of Defence counsel is to protect any way to his client therefore it would have been proper that the Counselwould have place the scope of 304 IPC and would have pleaded that the complaint is not tenable under section 304 IPC and should be quashed. But defence counsel not pleaded like defence counsel but as judge.

Discussion

The term medical negligence is not defined or referred to anywhere in any of the enacted Indian law. Therefore it is interpreted by the Indian judiciary by referring general laws tending to give sufficient leeway to the doctors and expressly recognize the complexity of the human body, inexactness of the medical sciences, the inherent subjectivity of the process,genuine scope of the error of judgement and the importance of autonomy of the doctors.On persual of entire facts it is very clear the different types of negligence can be understood as follows:-

· Error of judgement- Not negligence usually.

· Ethical Negligence – it is the violation of code of medical ethics which is tried in the court of State Medical Council.

· Ordinary negligence orCivil Negligence - Failure to use ordinary care,or absence of reasonable degree of skill and care. It has to be tried in Civil Court like consumer forum.

· Criminal negligence - Gross negligenceso extreme that is punishable as a crime. Gross negligence as a lack of even slight diligenceor care.The omissions of even such diligence as habitually careless and inattentive people actually do exercise inavoiding danger to their own person or property.[3]In such cases the offender can be prosecuted under 304A IPC and the case can be tried in Criminal Court.

Section 304 IPC is meant to deal with general offences and includes act of commission only that to in regards to general crime. The scope of culpable homicide not amounting to murder is limited to thefive exceptions of definition of Murder. Professional negligence especially related to medical profession cannot be the example of these five exceptions thus cannot be brought under ambit of section 304 IPC. No judgement of Apex Courtjustified the prosecution of doctor under section 304 IPC.The apex court even cautioned all courts of India regarding prosecution of doctor even under section 304A IPC except in case of extreme recklessness.

Therefore it is very clear from the above discussion that in the judgement of instant case –

· That in incorrect manner the criminal negligence has been considered under section 304 IPC. The Civil negligence, error of judgement, ethical negligence etc. in 304A IPC.There is no discussion on therapeutic misadventure

· Some unwanted facts like strike by IMA which is likely to affect human psyche in judging a case was included in the judgement.

· Pleading by Defence Council was not proper.

Conclusion

Although the legislature have not guide the Judiciary clearly and finer details encompassing all the types of situation relating to medical negligence but **Hon'ble Supreme Court** of India through its various judgements have clarified many things to some extent.But vagueness still prevails and need to be addressed.It cannot be done by single agency but in policy making, various stakeholder like legislatures, Judiciary, medical jurist, Healthcare providers, law enforcement Agencies,medical and legal fraternity to avoid such situations. A comprehensive manual of the medical jurisprudence has to be framed and legalised by parliament of India for the guidance of law enforcement agencies, medical and legal fraternity in dealing with such cases.

Conflict of Interest: - None

Source of Funding: - Not required

Ethical Clearance: - Not required

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Occupational Stress and Its Impact on Work and Work-Life Balance among Nurses in a Large Private Hospital in South India

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Abstract

The job stress is common among all the professions. The nursing profession is combined with stressful work environments and heavy workload that are leading to work life imbalance. Stress has been a routine and accepted as part of the nursing profession and there is lack of research on occupational stress in south India. The aim of this study is to find out the factors of occupational stress and its impact on work and work life balance of nursing professionals in a large private hospital in south India. There are many reasons that affect work life balance of the nursing professionals. The study is focused on more on factors that leads to occupational stress. The universe of the study was nursing professionals in a large private hospital in South India. For this study purpose the researcher is used quantitative study method and descriptive research design. The hospital is having more than one thousand bed capacity and consists of 600 nursing professional including permanent and contractual nature of employment. The study sample consists of 60 respondents (permanent nurses) including both male and female nursing professional are selected. Simple random sampling method is used and collected primary data. The data is collected by using self-administered questionnaire. The questionnaires were distributed among in all the wards/units of the hospital. The data analysis was done through Microsoft Excel and the inferences were drawn and presented. The findings of the study reveal that the heavy workload, long working hours and insufficient staff are the main factors that lead to occupational stress. The stress really affected on their work life balance and nursing professionals were not able to concentrate on their work which will also lead to other health related problem.

Key words: *occupational stress, nurse, factors of stress, work life balance*

Introduction

The stressful working condition of an individual can indirectly impact the health by decreasing our capability or incentive to take part in further fitness encouraging behaviours and fitness practice such as healthy food consumption and workout. There are several societal demands that the human race feels obligated to fulfil. To achieve self-actualization according to Maslow, human race tends to fulfil all the basic needs in life right from physical needs to safety needs to emotional needs to our self-esteem. However, it is not easy for someone to fulfil all these needs at once and sometimes there are repeated failures in experiencing this satisfaction. This can result in increasing frustration and further compel a person to exhaust its limit in order to prove his/her value in the society. Status is again a very important aspect of our societal needs which drives the human race towards struggle and competition against each other.

According to [1]NIOSH (National Institute for Occupational Safety and Health, 1999) "Stress at work" has defined occupation pressure as all the risky mental

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and sensitive reactions that happen while the necessities of the work doesn't tie up with resources, experiences, or desires of the employee". It is observed in general that the world we live in privileges certain groups of people over certain others. Those who belong to this privileged group enjoy all the luxuries and comfort that come with it. Previously, the boundaries of these privileged groups were very rigid, but now with the changing norms in the contemporary society, the boundaries of these privileged groups are becoming more and more permeable. Due to this people tend to climb up the status ladder by working day and night to earn more profits. Economic status hence determines one's worth in the society and the pressure to achieve this is what makes people weaker to tension. Stress in nurses is a regional issue. [2]Apeksha Gulavani, Mahadeo Shinde, (2014) found that reasons for work pressure, heavy work task as well as bosses are two main considerations to fall worry among nurses This ends up in health related issues and there will be decrease in efficiency in working of nurses. The nursing occupation is well known to be difficult work which is present all over the globe and have attain on well-being of an individual's physical and psychological health. Job related issues are a cue attraction to manager since a well-known unfavourable outcome on workers' productivity, execution, work gratification etc.

Every individual's life is hanging upon the career. Their gratification in life is unconventional or incidentally determined by the vital influence on work. Due to globalization and financial justification, the roles are currently becoming burdened, job controlled, and unsecured. The following influences result in job-related pressure which contains a direct effect on work fulfilment, output, psychological and bodily wellbeing, absenteeism, and effect on the individual life and it affects the capability of the labours**.** [3]Viveka Marie. G, Dr. Umesh Maiya, (2015) identified that huge numbers female medical attendants working in multispecialty emergency clinic make the most of their work while offering administration to the individuals they are confronting wellbeing related issues because of work pressure. Work-life balance is the foremost cause aimed at the superiority of work-life and quality of individual life. The health career strains dedicated job and the excessive burden of attention to handle characteristic circumstances. So this requirement a lot of tolerance, promise, attention, fortitude, and self-assurance. This job demands extraordinary attentiveness and capability to character on diverse hours additional in difficulties. The worker during their career has the concern on particular life. A moment of oblivion could result in cost of individual life. So this vocation comprises more anxiety and stress and also needs to add unusual hours.

The study Occupational Stress, its Causes and Consequences for Job Performance, "the authors claim that stress can be particular in nature and that this particular stress can lead to anxiety, depression and other psychological problems",[4]Motowidlo, S. J., Packard, J. S., & Manning, M. R., (1986). They have also mentioned that stress is usually caused by certain instances in the workplace and that the frequency and intensity of such instances can lead to difference in subjective stress. According to [11]WHO (World Health Organization, 2019), "there are several threat issues for intellectual state which will be existing inside the working atmosphere. Utmost danger relay to interconnection among kind of labour, the administrative and supervisory atmosphere, the capacities of employees, and therefore the support obtainable for workers to hold out their work".

According to [3]Viveka Marie.G and Dr. Umesh Maiya, (2015) hospitals have got one among India's biggest and significant parts as far as income and business. Work Life Balance alludes to the viable administration of various jobs both at work and family has characterized Work Life Balance as "the degree to which an individual is similarly self-drawn in and similarly happy with-their work job and family job." this proposes one must find some kind of harmony among work and family life by organizing both at proficient and at individual level. Finding some kind of harmony among work and family is significant to have physical and mental state. Appropriate working conditions, working timetables and backing from individuals from the family will help accomplish great physical and mental state.

According to [1]NIOSH, (National Institute for Occupational Safety and Health, 1999) Job stress is more now than ever before and this is mostly because of the change in the work culture and the lifestyles of the people. To cope with scarcity of time and work load in the office people often end up having unhealthy food and the body does not get the required nutrients. As a result of lack of healthy body there is a lack of healthy mind-

set. People nowadays stay more frustrated and disturbed. In today's fastest growing business world, the ability to deal with occupational stress and to achieve proper work life balance is becoming more and more difficult. Employers or the supervisors of the employer expect more from the staff, and we are putting lot of additional pressure on ourselves to achieve greater result.

Methodology

The study was conducted in large hospital in south India. The study is focused on more on factors that leads to occupational stress. The universe of the study was nursing professionals in a large private hospital in South India. For this study purpose the researcher is used quantitative study method and descriptive research design. The hospital is having more than one thousand bed capacity and consists of 600 nursing professional including permanent and contractual nature of employment. The study sample consists of 60 respondents (permanent nurses) including both male and female nursing professional are selected. Simple random sampling method is used and collected primary data. The data is collected by using self-administered questionnaire. The structured questionnaires used consisted of demographic and occupational, work life balance variables with option of never, sometimes, and often were distributed among in all the wards/units of the hospital. The data analysis was done through Microsoft Excel and the inferences were drawn and presented.

Result

The majority of the study participants were female (93%) with 83% aged 21-30 years and 10% of them were belongs to the age group 31-40 years. [5]Arash Najimi, Ali Moazemi Goudarzi, Golamreza Sharifirad, (2012) conducted a study using Occupational Stress Inventory- Revised tool and the results showed that the level of stress in most of the nurses was in medium level. Job factors were more involved in job stress than demographic and other factors.

Table No.1 Demographic details of sample

Gender	**Female**	56 (93%)
	Male	04(07%)
Age	21-30	50(83%)
	31-40	06(10%)
	41-50	04(07%)
Marital status	**Single**	22 (36.67%)
	Married	38 (63.33%)
Experience in years	1-2	32(53.33%)
	3-4	05 (8.33%)
	5-6	09 (8.33%)
	7 & above	14 (23.33%)

Table No.2 Working relationships

Responses	Poor relation with supervisor	Poor relation with co-worker	Absence of communication from management	Working with the civic
Never	65.00%	70.00%	38.33%	45.00%
Sometimes	35.00%	26.67%	58.33%	46.67%
Often	-	3.33%	3.33%	8.33%

The distribution of sample based on the working relationship which may cause to occupational stress shows poor relation with supervisor, poor relations with co-worker, absence of communication from management and working with the public. Majority of study participants (65%) responded that they do not face problem of poor relation with supervisor. But 35% of them said that they sometimes face poor relation with supervisor. Study participants said that poor relation with co-worker (70%) never caused problem but 26.67% of them agreed that sometimes the poor relation with co-worker do cause problem in working relationship. Absence of communication from management (58.33%) sometimes leads to the problem and 38% never felt such problem. About 46% opined that sometimes the cause of their problem is work with civic and 45% respondents never felt any problem working with public. It also shows that nursing professional have good relationship with their supervisor and with their colleagues.

Table No.3 Causes for job stress

Causes for job stress	Never	Sometimes	Often
Nursing difficulties	17%	70%	13%
Heavy workload	2%	70%	28%
Tight work shifts	13%	47%	40%
Harassment at workplace	62%	23%	15%
Discrimination at workplace	70%	23%	7%
Poor relationships with colleagues or boss	75%	20%	5%
Lack of staff support	50%	38%	12%
Changes within the organization	33%	60%	7%
Exposure to infection	18%	62%	20%
Job insecurity	57%	38%	5%
Lack of opportunity for growth/promotion	45%	38%	17%
Needle-stick injuries	28%	50%	22%
Lack of break during the shift	18%	48%	33%
Exposure to work related violence or threat	67%	33%	0%

The distribution based on causes for stress in which majority of (70%) of respondents stated that nursing difficulties, followed by 70% of respondents stated heavy workload, 62% of them opined that of exposure to infection and a 60% of them are mentioned about changes within the organization will sometimes leads to occupational stress. [6]Funmilola Adenike Faremi, Matthew Idowu Olatubi, Kehinde Grace Adeniyi, Omowumi Romoke Salau, (2019) found that that workload stands as the first aspect of nurse's job that leads to in highest frequency of stress.

Table No.4 Occupational stress experienced by the study participant

Occupational stress experienced by the study participant	Never	Sometimes	often
Handling alone a large number of patients	20%	47%	33%
Inadequate staffs	12%	33%	55%
Long working hours	7%	27%	66%
No break during the shift	22%	32%	47%
Frequent night duty	32%	47%	22%
Nursing the patients without bystander	60%	30%	10%
Lack of opportunity for growth and promotion	50%	28%	22%
Nursing difficult especially chronic ill patients	53%	38%	8%

The distribution of occupational stress experienced by the study participant's clearly shows that the majority of them (66%) expressed that the most common job stress which is often faced is due to long working hours, followed by (55%) of respondents mentioned that inadequate staffing and 47% participant opined that lack of break period during the shift is also leads to occupational stress. The distribution of factors that hinder in balancing the work and family commitments shows that long working hours (49%) hinder them in balancing work and family commitment,23% of respondents' state that shift work,15% of them respondents were expressed that due to overtime and 3% of respondents stated that meetings/training after office hours that hinder them in balancing work and family commitments. Majority (65%) of the study participant sometimes do miss out quality time and 35% respondents had frequently miss out quality time with their family and friends with family due to work pressure**.** [7]Satpathy I, Chandra MP, Sasmita J (2014) found that because of imbalance nurse are unable to look after themselves which also leads to health problems and in professional life, impact of work life imbalance ends up in dissatisfaction in work.

The distribution of impact of the job stress on work-life balance reveals that the majority (53%) of the study participant agreed that job stress really impacted their work life balance and respondents also stated that in the workplace any form of stress/trouble will directly have the impact on their daily routine and not able to give quality time for the family. They also stated that they cannot concentrate on work and due to over workload in department that will lead to physical and mental health problems**.**[8] G.Suguna, C. Eugine France, (2017) found that vast majority of the nursing staff was disappointed with work life balance. Household linked matters and person's very own issues caused anxiety for nursing staff.

Discussion

The expectations from the management by the study

participants was to make a stress-free workplace. It is clearly reveals the expectation of nursing professionals from the management that sufficient support, need adequate opportunity for career development, open communication from the management, need training and development activities and proper recognition for their work from the management to make a stress- free workplace. From this study it is found that the main causes for occupational stress is nursing difficulties and heavy workload. It is also found that the most common job stresses faced by nurses are exposure to infection and lack of break during the shift work. The respondents are mostly happy with their workplace. Most of the respondents are dissatisfied with the facilities provided by the management. It is found that due to tight work shifts the respondents are not able to give quality time to their family and this hinders their work life balance. [9]Onur Balkan, (2014) examined the impact of work life balance on job stress and individual performance and found that, there were powerful relations among job related stress performances and work life balance components.

It is also found that the majority of respondents expect sufficient support from the management and an adequate opportunity for career growth and recognition and expect an open communication from the management which will reduce the occupational stress among the nurses and can be able to maintain work-life balance. The investigation on whether the job stress really impact work life balance, the majority nurses agreed that job stress really impact their work life balance, due to stress they cannot concentrate on work, over workload in the department will lead to mental stress and it will again show a significant donor on hypertension. The findings clearly reveal that nurses are not able to handle work life balance due to heavy workload and long working hours. Majority of nurses stated that job stress impacts their work life balance because they cannot concentrate on the work and it impacts their health and mental wellbeing. The research finding shows that it is very important to conduct mentoring session for the nurses where they can put forward the concerns and problems faced by them and release their stress. In order to reduce stress level and good work life balance, the hospital management need to encourage a positive and flexible working environment and that will help towards the improvement of the work force. [10]Dr. Bindiya Goyal, (2014) studied nurses and female doctors of treatment centre and private hospitals. It found that the facilities of work life balance strategies application through the healthcare Centre service for female doctors and nurses for good work-life balance in clinics and private hospitals will make nurses and female doctors more devoted and extremely fruitful thereby giving them absolute job satisfaction.

According to [11]World Health Organisation, (2019) a well-functioning atmosphere is a unique and important aspect is not only the reason for absence in dangerous conditions or stressful circumstance however wealth of promoting one's health. The management can have some policies to provide stress relief session and stress management programs, proper training, workshop on work-life balance for a stress-free workplace and managing work-life balance among nursing professionals.

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Suitability Tests for Bacterial Identification and Antibiotic Sensitivity Tests using Microscan Walkaway on Vitek 2

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Abstract

Culture or bacterial culture is the gold standard for diagnostic examination to detect the presence of microorganisms in the patient's body. The development of culture technology and culture-based automatic devices for diagnostic examination are widely researched. At present, there are several types of automatic blood culture instruments in Indonesia, namely Vitek 2 and Microscan Walkaway. This research aims to analyze the suitability of bacterial identification and test antibiotic sensitivity of Microscan Walkaway device from Beckman Coulter and Vitek 2 device from Biomerieux as a gold standard. The research conducted was an observational cross-sectional study. Sampling was done consecutively. The study sample consisted of 202 samples obtained from the results of positive isolates, during January-July 2019 at Dr. Soetomo hospital. Positive isolates were examined for bacterial identification and antibiotic sensitivity using Vitek 2 and Microscan Walkaway. The results were statistically analyzed using SPSS. Examination of bacterial identification using the Microscan device showed 34.2% of Gram-positive bacteria and 65.8% of Gram-negative bacteria, whereas with Vitek 2, results showed 34.7% of Gram-positive bacteria and 65.3% of Gram-negative bacteria. Both of these instruments showed identification accuracy of 98.56% for Gram positive bacteria and 100% for Gram negative bacteria with Kappa value: 0.814 and p <0.0001. The results of the accuracy test for antibiotic sensitivity of multidrug resistance bacteria showed compatibility with *p* value <0,0001. There is very good agreement between Vitek 2 and Microscan Walkaway in the identification of bacteria and antibiotic sensitivity.

Keywords: *Identification of bacteria, antibiotic sensitivity, Microscan WalkAway, Vitek 2*

Introduction

Bacterial infection is one of the ten most common causes of death in the world[1]. Microbal floral that is found in clinical specimens obtained from different parts of the human body consists of a variety of different organisms, both pathogenic and non-pathogenic. Traditionally, the diagnosis of bacterial or fungal infection relies solely on culture-based techniques and culture is considered as the gold standard for pathogen diagnosis and detection. However, some organisms may not be easily detected by conventional culture methods used in most laboratories due to various factors. In a conventional clinical microbiology laboratory setting, the majority of microbial cultures of specimens are carried out under aerobic conditions. Standard culture techniques heavily rely on morphological and biochemical characterization for identification, which can lead to decreased specificity. In addition, only a small portion of organisms can successfully be cultured in multi-pathogenic samples. This is mostly caused by various factors, such as rapid growth requirements, non-viable organisms or inhibition of pathogenic organisms due to the production of bacteriocins by other microbes present in clinical specimens[2,3]. These factors make accurate diagnosis and treatment of infections a challenge.

For proper initial empiric therapy, it is important to ensure correct identification of microorganisms for

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accurate diagnosis[4]. Proper antimicrobial therapy has shown to save costs and to help prevent the spread of antimicrobial resistance[5,6].

Automatic culture technique uses a large number of biochemical reactions for the identification of bacteria and broth microdilution for antibiotic sensitivity. In Indonesia, there are currently two automatic culture devices, namely Vitek 2 and Microscan WalkAway. Vitek 2 uses colorimetric technology coupled with the use of three longitudinal waves to provide a general profile of organisms which is important for clinical needs. Antibiotic sensitivity test uses a test card containing standard dilutions of different antibiotics according to the cutoff points established by the Clinical and Laboratory Standards Institute (CLSI). On the other hand, the MicroScan WalkAway utilizes fluorescent technology. MicroScan panels are conventional, consisting of 40 microdilution plate wells[7]. In this study, we aim to determine the suitability of the automated systems from two different manufacturers, BioMérieux (VITEK 2) and Beckman Coulter (MicroScan WalkAway).

Method

The study design used in this research is observational cross-sectional. Research samples in this study consist of the results of positive isolates from January-July 2019 at Dr. Soetomo Hospital. The samples were obtained from the results of each patient's culture, swabs, tissue, pus and peritoneal fluid that met the inclusion criteria. The sampling technique carried out in this research was consecutive sampling. Bacterial identification and sensitivity test were performed using the Vitek 2 and Microscan WalkAway devices. The data obtained was analyzed using SPSS version 24.0.

Result

The number of research isolates was 202 samples. In this study, there were 91 men (45%) and 106 women (52.5%) with ages ranging from 15 days (youngest) until 93 years old (oldest) with overall mean age of 52.72 and median of 58 years old. Research sample data is shown in Table 1.

Table 1. Research sample data with positive culture results (isolates).

Specimen	N	%
Peritoneum Fluid	1	0.5
Blood	22	10.8
Tissue	3	15
Pus	26	12.9
Sputum	56	27.7
Gaster Swab	1	0.5
Nose Swab	2	1
Underarm Swab	1	0.5
Wound Swab	4	2
Pharynx Swab	1	0.5
Between Finger Swab	1	0.5
Throat Swab	5	2.5
Swab urethra	1	0.5
Swab vagina	1	0.5
Urine	76	37.6
Total	202	100

Each sample was examined for bacterial identification and antibiotic sensitivity using the Vitek and the Microscan WalkAway devices. Results of the bacterial identification test were obtained automatically using the GP-67 and GN-93 cards with the Vitek 2 device and the PC-34 and NUC-73 cards with the Microsscan WalkAway device. Data of bacterial identification can be seen in Table 2.

Table 2. Data of bacterial identification on Vitek 2 and Microscan WalkAway devices.

Bakteri Vitek	N	%	Bakteri Microscan	N	%
Achromobacter dentrificans	1	0.5	Achromobacter xylosoxidan	2	1
Achromobacter xylosidan	1	0.5	Acinetobacter baumannii	11	5.4
Acinetobacter baumannii	15	7.4	Aeromonas hydrophila	1	0.5
Aerococcus urinae	1	0.5	Burkholderia cepacian	3	1.5
Burkholderia cepacia	2	1	Cedecea davisae	1	0.5
Citrobacter freundii	1	0.5	Chromobacterium violaceum	1	0.5
Citrobacter koseri	5	2.5	Chryseobacterium indologe	1	0.5
Elizabeth meningoseptica	1	0.5	Citrobacter koseri (ESBL)	1	0.5
Enterobacter aerogenes	1	0.5	Citrobacter koseri	4	2
Enterobacter gallinarum	1	0.5	Elizabethkingia meningose	1	0.5
Enterobacter cloacae	1	0.5	Enterobacter aerogenes (ESBL)	1	0.5
Enterococcus faecalis	14	6.9	Enterobacter cloacae (ESBL)	2	1
Eschericia coli (ESBL)	24	11.9	Enterobacter cloacae	3	1.5
Eschericia coli	29	14.4	Enterococcus durans/hirae	1	0.5
Ewingella americana	1	0.5	Enterococcus faecalis	8	4
Klebsiella oxytoca	1	0.5	Enterecoccus gallinarum	2	1
Klebsiella pneumoniae	20	9.9	Eschericia coli (ESBL)	22	10.9
Klebsiella pneumoniae (ESBL)	10	5	Eschericia coli	22	10.9
Kocuria kristinae	3	1.5	Klebsiella oxytoca (ESBL)	1	0.5
Proteus mirabilis	1	0.5	Klebsiella oxytoca	1	0.5
Providencia rettgeri	1	0.5	Klebsiella ozaenae	1	0.5
Pseudomonas puttida	2	1	Klebsiella pneumoniae (ESBL)	8	4
Pseudomonas aeruginosa	7	3.5	Klebsiella pneumoniae	17	8.4
Salmonella spp	1	0.5	Klebsiella rhinoscleromatis	1	0.5
Serratia fonticola	1	0.5	Proteus mirabilis (ESBL)	1	0.5
Serratia marcescens	1	0.5	Ochrobactrum anthropi	1	0.5
Sphingomonas paucimobilis	1	0.5	Providencia rettgeri	1	0.5
Staphylococcus aureus (MRSA)	5	2.5	Pseudomonas aeruginosa	6	3
Staphylococcus gallinarum	1	0.5	Raoultella ornithinolytic	1	0.5
Staphylococcus hominis	2	1.0	Salmonella enterica	1	0.5
Staphylococcus aureus	18	8.9	Salmonella enterica serot	2	1
Staphylococcus epidermidis	6	3	Serratia fonticola	1	0.5
Staphylococcus haemolyticus	12	5.9	Serratia marcescens	1	0.5
Stenophomonas maltophilia	1	0.5	Shigella sonnei	1	0.5
Streptococcus agalactiae	2	1	Sphingobacterium spiritivorum	2	1
Streptococcus alactolyticus	1	0.5	Staphylococcus aureus (MRSA)	6	3
Streptococcus mitis/oralis	2	1	Staphylococcus aureus	14	6.9
Streptococcus pneumoniae	1	0.5	Staphylococcus epidermidis	5	2.5
Streptococcus pyogenes	1	0.5	Staphycoccus haemolyticus	7	3.5
Streptococcus salivarius	2	1	Staphylococcus hominis	3	1.5
Streptococcus sanguinis	2	1	Staphylococcus hyicus	1	0.5
TOTAL	202	100	Staphylococcus capitis	1	0.5
			Stenophomonas maltophilia	1	0.5

Cont... **Table 2. Data of bacterial identification on Vitek 2 and Microscan WalkAway devices.**

			Staphylococcus sciuri	5	2.5
			Streptococcus mitis/oralis	2	1
			Streptococcus pneumoniae	1	0.5
			Streptococcus pyogenes	1	0.5
			Streptococcus salivarius	2	1
			Streptococcus sanguinis	2	1
			Vibrio species group	1	0.5
			Wautersiella falsenii	1	0.5
			TOTAL	202	100

Results revealed that the identification of bacteria using the Microscan WalkAway device showed 34.2% of Gram-positive bacteria and 65.8% of Gram-negative bacteria, whereas with Vitek 2, results showed 34.7% of Gram-positive bacteria and 65.3% of Gram-negative bacteria.

The top three Gram-positive bacteria identified by the Vitek 2 device were *Staphylococcus aureus* (18), *Staphylococcus haemolyticus* (12) and *Staphylococcus epidermidis* (6), while on the Microscan WalkAway the top three bacteria were *Staphylococcus aureus* (14), *Enterococcus faecalis* (8) and *Staphylococcus haemolyticus* (7).

The top three Gram-negative bacteria identified by the Vitek 2 device were *Eschericia coli* (29), *Eschericia coli* (ESBL) (24), *Klebsiella pneumoniSa* (20), while on the Microscan WalkAway the top three bacteria were *Eschericia coli* and *Eschericia coli* (ESBL) (22), *Klebsiella pneumoniae* (17), and *Acinetobacter baumannii* (11).

Antibiotic sensitivity analysis in this study was done to detect the presence of bacteria that cause multidrug resistance (MDR), namely: *Extended-spectrum beta-lactamase* (ESBL), *methicillin-resistant Staphylococcus aureus* (MRSA), and *methicillin-resistant Staphylococcus spp* (MRSS). Results are shown in Table 3, Table 4, Table 5 and Table 6. The p value <0.05 indicates a significant similarity. The suitability of antibiotic sensitivity between the Vitek 2 and Microscan WalkAway devices was analyzed according to the card type, namely AST GP-67 and AST GN-93 cards for the Vitek 2 device and ID/AST PC-34 and ID/AST NUC-73 cards for the Microscan WalkAway device.

Table 3. ESBL phenotype detection.

Device/Antibiotic	CTX	ATM	CAZ	CRO	CTX/CLA and CAZ/CLA
Vitek 2	34	34	34	34	34
Microscan WalkAway	36	36	36	36	36

According to the data in Table 3, it can be seen that the Microscan WalkAway device has the ability to detect more ESBL bacteria with value of 36, while the Vitek 2 device only detected a value of 34.

Table 3. MRSA detection.

Device	MRSA	Interpretation of MIC Oxacillin (>4 μg/l)	Cefoxitin screen result (Resistant)
Vitek 2	5	5	5
Microscan WalkAway	6	6	6

According to the data in Table 4, it can be seen that the Microscan WalkAway device has the ability to detect more MRSA bacteria with value of 6, while the Vitek 2 device only detected a value of 5.

Table 5. MRSS detection.

Device	MRSS	Interpretation of MIC Oxacillin (>4 μg/l)	Cefoxitin screen result (Resistant)
Vitek 2	20	20	20
Microscan WalkAway	22	22	22

According to the data in Table 5, it can be seen that the Microscan WalkAway device has the ability to detect more MRSS bacteria with value of 22, while the Vitek 2 device only detected a value of 20.

Table 6. Antibiotic sensitivity test of ESBL, MRSA and MRSS bacteria between Microscan WalkAway and Vitek 2.

Bacterium	Kappa	p-value
ESBL	0.732	0.0001
MRSA	0.720	0.0001
MRSS	0.688	0.0001

Statistical analysis results showed moderate suitability of ESBL, MRSA and MRSS between Microscan WalkAway and Vitek 2 devices, where *p* value was <0.05.

Discussion

As clinical microbiology laboratories have become increasingly dependent on automated systems, accuracy can be evaluated clinically as well as with reference samples. In this study, there were discrepancies in genus and species. Sensitivity test of various strains between the two systems that are most often used in hospital settings was performed. Other studies have evaluated bacterial identification and antimicrobial sensitivity performance of specific isolates of these systems. However, there are only a few studies with various clinical samples[8,9,10]. The result of bacterial identification using the Microscan device was 34.2% of Gram-positive bacteria and 65.8% of Gram-negative bacteria, while Vitek 2 device identified 34.7% of Gram-positive bacteria and 65.3% of Gram-negative bacteria. Both of these devices showed identification suitability of 98.56% for Gram-positive bacteria and 100% for Gram negative bacteria. It can be concluded that results were suitable and there was no significant difference, with Kappa value: 0.814 and *p* <0.0001. Results obtained in this study were align with a research conducted by Jin *et al*. (2011), where out of the 142 isolates identified up to the species level, Vitek 2, Microscan and Phoenix devices had accuracy of

93.7%, 82.4% and 93%. Microscan-Phoenix devices had p value of $p < 0.05$ and Vitek 2-Phoenix devices had p value of $p < 0.05$[10]. According to Hernandez et al (2017), the identification accuracy of Vitek 2 and Microscan devices can be influenced by differences in the number and distribution of organisms tested, software versions, including the number of bacteria tested by the device and the bacteria listed in the device database[7]. This could also be due to the lack of incubation time. Because of this, in a research conducted by Jossart and Courcol (1999), 24 hours of incubation time for non-fermenter bacteria was suggested[11].

Difference of bacteria identification was found between the two devices. *Chromobacterium violaceum* was identified with the Microscan WalkAway device, whereas *Staphylococcus haemolyticus* was detected with the Vitek 2 device. This could be due to the misidentification of bacteria during Gram staining, differences in the number of biochemical tests used and type of bacteria that can be identified by the two devices. *Chromobacterium violaceum* is a Gram-negative bacterium in the *Neisseriaceae* family. Meanwhile, *Staphylococcus haemolyticus* is a Gram-positive bacterium in the *Staphylococcaceae* family.

Phenotypic confirmation of bacteria that produces ESBL, namely positive isolates on the ESBL screen results, must be confirmed with cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), aztreonam (ATM) and a combination of CTX and CAZ with clavulanate (CLA) antibiotics. Results showed value of 34 for the Vitek 2 device and 36 for the Microscan WalkAway device. These results are similar to a study by Linscott et al (2004), where 49 isolates were tested using 4 commercial methods to confirm ESBL. The four devices had different sensitivity values, namely 100% for Microscan, 99% for Vitek, 97% for ESBL Etest and 96% for BD BBL Sensi-Disk ESBL[12].

Antibiotic sensitivity test results between the Microscan WalkAway device and the Vitek 2 device in detecting ESBL showed kappa value of 0.732 and p value of 0.0001. Kappa value of 0.720 and p value of 0 0.001 was obtained for MRSA, and kappa value of 0.688 and p value of 0.0001 was obtained for MRSS. According to these results, it can be said that there is an intermediate suitability between the two devices. Results obtained in this study are parallel to a research conducted by Gherardi et al (2012) where the categorical agreement for antibiotic resistance of Gram-negative bacteria was 97.5% and 98.1% between Vitek and Phoenix devices, whereas the categorical agreement for *methicillin resistant staphylococci* was 94.6% and 100% between the two devices[13].

Conclusion

The results of this study reveal that the Microscan WalkAway device shows equally good ability in bacterial identification and antibiotic sensitivity test with the Vitek 2 device. Further research using PCR to detect genes that cause resistance as a reference method is highly suggested.

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Effectiveness of Self Instructional Module (SIM) on Knowledge and Attitude regarding Care of Attempted Suicide Patients among Staff Nurses – A Pre Experimental Study

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Abstract

Suicide is a major public health problem and one of the leading causes of deaths worldwide. The effects of suicide go beyond the person who acts to take his/her life; it can have lasting effect on family, friends and communities. The role of a nurse specific to suicide prevention includes both system and patient level interventions.

Objectives of the study:

- To assess the existing knowledge and attitude regarding care of attempted suicide patients among staff nurses.
- To evaluate the effectiveness of self –instructional module regarding care of attempted suicide patients among staff nurses.
- To find out the association of post -test knowledge and attitude regarding care of attempted suicide patients among staff nurses with selected demographic variables.

Materials and Methods: In this pre- experimental research study non probability convenient sampling technique was used to select 60 staff nurses from selected Hospitals. A self- structured questionnaire and attitude scale was used to assess the knowledge and attitude of the staff nurses regarding the care of attempted suicide patients.

Result: The finding of the study revealed that majority 95% (57)of staff nurses had moderate knowledge and only 5% (3) had inadequate knowledge regarding care of attempted suicide patients in pre-test. After going through Self Instructional Module, 83.37 % (50) of the staff nurses had adequate knowledge and 16.66% (10) had moderate knowledge regarding care of attempted suicide patients. The attitude scale findings revealed that 100% (60)of staff nurses had neutral attitude regarding care of attempted suicide patients in pre-test and in post-test 100%(60) had positive attitude regarding care of attempted suicide patients.

Conclusion: Study concludes that going through Self-Instructional module helps method in improving knowledge and attitude of the staff nurses regarding care of attempted suicide patients.

Key words: *Self-instructional Module, Suicide, Staff nurses, Knowledge, Attitude.*

Introduction

Suicide is the act of human being intentionally causing harm to one. Suicide is often committed out of despair, or attributed to some underlying mental disorder which includes depression, bipolar disorder, schizophrenia, substance abuse etc. Financial difficulties, interpersonal relationships and other undesirable situations also play a significant role[1].

The Government of India classifies a death as suicide if it meets the following three criteria: - It is an

unnatural death; the intent to die originated within the person and there is a reason for the person to end his or her life. The reason may have been specified in the suicide note or unspecified[2].

Nursing encompasses autonomous and collaborative care of individuals of all ages, families, groups and communities, sick or well and in all the settings. Care of attempted suicide patients depends on the ability of staff nurse to understand the situation and work. Evidence suggest that the lack of knowledge and unfavourable attitude of the nursing staff working in psychiatric unit have direct impact on the patient care and health care facility[3].

Amongst youth, the strongest risk factors are depression, alcohol, or other drug use disorder, and aggressive or disruptive behaviours. Children who are perfectionists and over achievers may also be at risk if there are other problems, such as depression or family problems[4].

Suicidal tendencies occur in many medical conditions as their sequel or as concomitant symptoms. Symptoms and conditions behind these emergencies may include panic attacks and then eventually lead to suicide attempt[5].

About 800000 people commit suicide worldwide every year, of these 135000 (17%) are residents of India, a nation with 17.5 % of world population[6]. It is perceived that the implementation of special supervision is the most effective preventive method in the care of suicidal patients. Studies show that it is possible to provide nursing care in the prevention of further harm to the patient but in doing so the nurse may not necessarily be proficient in demonstrating the interpersonal caring skills essential for the development of a therapeutic relationship and hence patient recovery.

According to WHO data, the age standardized suicide rate in India is 16.4 per 100,000 for women (6th highest in the world) and 25.8 for men (ranking 2nd)[7].

Nebhinani M, Tamphasana L, etal 2013 conducted a cross sectional study among three hundred and eight students of two nursing colleges of north India to assess the attitude of nursing students towards suicide attempters. Suicide opinion questionnaire was administered to assess their attitude towards suicide attempters. Nearly half of the students believed that suicidal attempters were impulsive, self-punitive and non-believers in after life. One third of students considered them as rigid, weak personality, mentally ill and interested to get public attention. Overall attitude of students towards suicide attempters remained favourable for a half of the attitudinal statements and uncertain for rest half of the items[8].

Joseph C. 2005 conducted an evaluative study to assess the effectiveness of structured teaching program on knowledge of suicidal behaviour in adolescents among nurses in Bengaluru. One group pre- test, post-test design was used on a sample size of 60 nurses and the data were collected by using a structured interview schedule. Pre- test revealed the fact that nurses have a low level of knowledge with a mean score of 21.43 out of 47. After administering a structured teaching program, post test score rose to 40.43 which showed the effectiveness of structured teaching program[9].

Materials and Method

The selection of design depends upon the purpose of the study, research approach and variables to be studied. Keeping all that in mind, research design used for the present study is pre-test and post-test research design.

Research Setting:

The criteria of selecting the setting was availability of subjects, feasibility of conducting the study, economy of time, easy accesses, expected cooperation and administrative approval for conducting the study. Present study was conducted at ICU, Psychiatric units and emergency departments of SGT Hospital, Gurugram Hospital, Sanjeevani Hospital, Jaipur Golden Hospital (Delhi NCR).

Study Population:

Study population is the total number of people who meet the criteria that the researcher has established for the study from whom subjects were and on whom the study findings will be generalized.

Target population for the study comprised of staff nurse working in ICU, Psychiatric units and emergency departments of SGT Hospital, Gurugram Hospital,

Sanjeevani Hospital, Jaipur Golden Hospital of Delhi NCR.

Eligibility Criteria:

The researcher specified some inclusion and exclusion characteristics for the population to be considered as a sample. Accordingly the sample was selected. Population elements who met the eligibility criteria were selected as sample and other elements were excluded.

Inclusion Criteria for the present study:

- Staff nurses working at selected departments of selected hospitals of Delhi NCR.
- Staff nurses who were available at the time of data collection
- Staff nurses who were willing to participate.

Exclusion Criteria for the present study:

- Staff nurses who were not willing to participate.
- Staff nurses who have been already exposed to such kind of study.

Sample and Sampling Technique:

The sample were 60 staff nurses working in ICU, Psychiatric units and emergency departments of SGT Hospital, Gurugram Hospital, Sanjeevani Hospital, Jaipur Golden Hospital of Delhi NCR.

Non probability convenient sampling technique was used for the selection of staff nurses.

Variables

Three categories of variables are discussed in the present study.

Dependent Variable: Level of knowledge and attitude of staff nurses.

Independent Variable: Self Instructional Module.

Demographic Variables: Age, Sex, Qualification, Income, Total years of professional experience, Religion and Source of information.

Data Collection Tool and Technique:

The tool comprised of three sections:

Section I: Socio Demographic Data

It consisted of items like Age, Sex, Qualification, Income, Total years of professional experience, Religion and Source of information.

Section II: Structured Knowledge Questionnaire regarding care of attempted suicide patients.

It comprised of 30 questions each with a score of one.

Table1. Interpretation of Score of Structured Knowledge Questionnaire

S.No	Level of Knowledge	Score
1	Inadequate	0-10
2	Moderate	11-20
3	Adequate	21-30

Section III: Likert Scale to assess the attitude of staff nurses regarding care of attempted suicide patients. It comprised of 40 items each with a score of one.

Table2. Interpretation of Score of Likert Scale

S.No	Attitude	Score
1	Negative	1-13
2	Neutral	14-26
3	Positive	27-40

Pilot Study:

A try out of the tool was done on 06 staff nurses and all the items were clear to the subjects.

Data Collection Procedure:

After taking formal permission from the involved three hospitals, staff nurses were explained about the purpose and procedure of data collection. A verbal and written consent was taken from all the 60 subjects. Average time taken by the respondents to complete the pre -test was 15 -20 minutes. A Self Instructional Module was given and after 7 days a post test was taken.

Major Findings

Majority of the staff nurses 23.33%, belong to age group of 18 to 26yrs. According to monthly income, most of the nurses i.e., 76.66% had the income ranging from Rs 15000 -20000/month. As per the Gender, majority of the staff nurses i.e., 86.66% were females. With regard to Educational qualifications, most of the nurses were GNM, i.e., 53.33%. According to Religion, 60% of staff nurses belonged to the Hindu religion, i.e., 60%. According to the Family Type majority of staff nurses belonged to nuclear families, i.e., 66.66%. With regard to clinical experience, 56.66% of staff nurses had minimum of four years of experience. As per the position in job, most of the nurses were nursing officers, i.e., 70%. Regarding attending to the patients with attempted suicide, majority of them had not attended such patients i.e., 73.37%. Regarding attending any workshop on suicide prevention, majority of staff nurses had not attended any such workshops, i.e., 93.37%. Regarding professional experience of managing suicidal patients, out of all 30% had such professional experience.

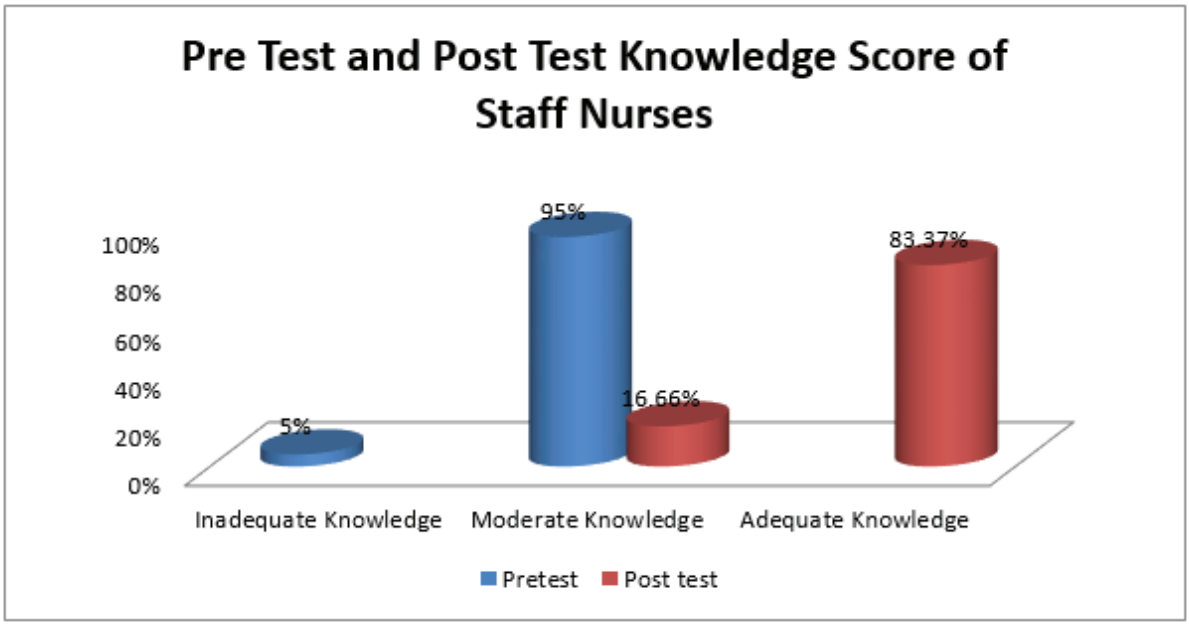

Fig 1 Pre-test and post- test knowledge score of Staff Nurses

As depicted in Figure 1, after data analysis and interpretation, it was found that majority of staff nurses that is 95% had moderate knowledge and 5% had inadequate knowledge regarding care of attempted suicide patients in pre-teat and in post- test 83.37% had adequate knowledge and 16.66% had moderate knowledge regarding care of attempted suicide patients.

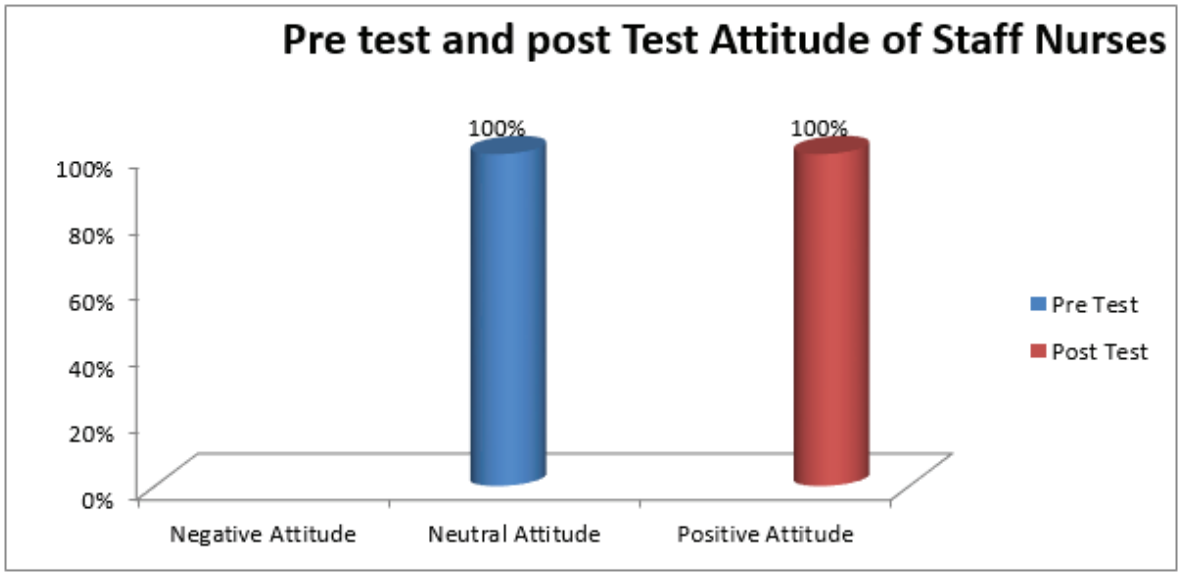

Fig 2. Pre- test and Post- Test Attitude of Staff Nurses

As shown in Fig 2, after data analysis and interpretation, all of the staff nurses i.e. 100% had neutral attitude regarding care of attempted suicide patients in pre-test and in post- test 100% had positive attitude regarding care of attempted suicide patients.

Data analysis and interpretation explains that there is significant difference in mean scores on knowledge as the 't' value obtained was higher than the tabulated value at 0.05 level of significance. So, it can be concluded that self-instructional module on knowledge regarding care of attempted suicide patients among staff nurses is effective.

Discussion

In the present study majority of staff nurses that is 95% had moderate knowledge and 55 had inadequate knowledge regarding care of attempted suicide patients in pre-teat and in post- test 83.37% had adequate knowledge and 16.66% had moderate knowledge regarding care of attempted suicide patients.

Chiaki Kawanishi (2006) did a study to assess the knowledge and attitude towards suicide among medical students by administering a brief knowledge and attitude assessment questionnaire concerning suicide to students in their first, third, and fifth years at a Japanese medical school. Participants numbered 160 (94 men with a mean age of 21.8 years, SD = 3.01, and 66 women with a mean age of 21.2 years, SD = 2.64); 59 first year, 52 third year, and 49 in their fifth year. The questionnaire consists of eight multiple-choice questions asking knowledge of suicide and one open-ended question asking attitude. In the knowledge part, only about half of the items (out of total 8 items) were answered correctly (mean score was 4.21, SD = 1.28). A significant difference was observed in prevalence of attitudes as categorical variables between student years (P = 0.001). Sympathetic comments increased along with student years, while critical comments decreased. Given the frequent and interventional opportunities of primary-care medical contacts, poor understanding of suicide from the medical viewpoint was of concern. Moreover, judgmental attitudes were common, especially in earlier school years. Better informed, more understanding physicians and other health professionals could contribute greatly to prevention.

Present study reports that all of the staff nurses i.e. 100% had neutral attitude regarding care of attempted suicide patients in pre-test and in post- test 100% had positive attitude regarding care of attempted suicide patients[10].

Vajona tapola (2014) conducted a study with an aim to describe the attitudes towards self□injurious patients among psychiatric personnel of 50 psychiatric personnel (N = 50) using Understanding Suicidal

Patients Questionnaire (USP) before and after the training. Psychiatric personnel attended a four-day training program, presenting evidence-based knowledge regarding self-injury assessment and treatment, using group exercises and reflective learning principles. The training program had statistically significant impact ($P < 0{\cdot}01$) on the following individual items of the USP scale: Patients who have tried to commit suicide are usually treated well in my work unit ($d = 1{\cdot}02$); A person who has made several suicide attempt is at greater risk of committing suicide ($d = 0{\cdot}64$); Because the patients who have tried to commit suicide have emotional problems, they need the best possible treatment ($d = 0{\cdot}57$). The results also suggested that the frequency of patient contact had impact on attitudes towards self-injurious patients.

In present study there is significant difference in mean scores on knowledge as the't' value obtained was higher than the tabulated value at 0.05 level of significance. So, it can be concluded that self-instructional module on knowledge regarding care of attempted suicide patients among staff nurses is effective[11].

Alin Thomas Cherian (2017) conducted a study to assess effectiveness of structured teaching program for staff nurses regarding care of attempted suicide in selected hospital of Indore. The study involved single group pre-test and post-test design. Simple random sampling was used to select 40 staff nurses working in Bombay Hospital, Indore. Structured knowledge questionnaire was given followed by structured Teaching Program. Post test was conducted after 8 days using same tool. Findings revealed that the mean post-test knowledge score (51.2) was higher as compared to pre -test knowledge score (14.10).

According to this study there is significant association between the post test scores of knowledge and attitude with that of income , education and years of professional experience to manage suicidal patients as computed by analysis of variance <0.5[12].

Inga-Lill Ramberg, Maria Anna Di Lucca, and Gergö Hadlaczky (2016) did this study aimed at investigating what impact other factors than knowledge might have on attitudes towards work with suicidal patients and suicide prevention. In 2007, 500 health-care staff working in a psychiatric clinic in Stockholm received a questionnaire with items concerning work with suicidal patients to which 358 (71.6%) responded. A set of attitude items were tested using structural equation modelling (LISREL). Three models were found to be satisfactory valid and reliable*: Job clarity, Job confidence* and *Attitudes towards prevention*. These were then used in regression analyses as dependent variables with predictors such as *experience of work with suicidal patients, perceived sufficient training, age* and *gender. Perceived sufficient training* was consistently the most important predictor for all three attitude concepts ($p < 0.01$, $\beta = 0.559$ for *Job clarity;* $p < 0.01$, $\beta = 0.53$ *for Job confidence;* $p < 0.01$, $\beta = 0.191$ *for Attitudes towards prevention). Age* was another significant predictor for *Job clarity* ($p < 0.05$, $\beta = 0.134$), as was *experience of patient suicide for Job confidence* ($p < 0.05$, $\beta = 0.137$). It is concluded that providing suicide preventive education is likely to improve attitudes towards the prevention of suicide, clarity and confidence regarding their role in the care for suicidal patients. These improvements may contribute to the prevention of suicide in health care settings[13]

Conclusion

Data Analysis and Interpretation explains that there is a significant difference between the pre and post- test mean score of knowledge and the 't' value obtained is higher than the tabulated value at 0.05 level of significance for 59 degrees of freedom. Hence, it can be inferred that obtained difference is not by chance but is true difference. So, it can be concluded that Self Instructional Module (SIM) on knowledge and attitude regarding care of attempted suicide patients among staff nurses is effective.

Conflict of Interest: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

It is to specifically state that "No Competing interests are at stake and there is No Conflict of Interest" with other people or organizations that could inappropriately influence or bias the content of the paper.

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Ethical Clearance: Ethical approval for the study taken from Institutional Ethical Committee, SGT University, Gurugram, Haryana.

Alignment with SDG Goals: This research is in alignment with 3rd SDG Goal i.e., Good Health and Well Being.

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Publication Ethics: Awareness & Knowledge among Medical Faculties

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Abstract

Publication ethics is related with ethical aspects of publication of scientific data to ensure high quality scientific publication in the current era of evidence-based medicine. Violation of publication ethics also known as scientific misconduct may be committed by researchers knowingly and unknowingly due to various reasons. Present study inducted 130 medical faculties from a medical college providing self-administered questionnaire regarding awareness about publication ethics using Google forms. Among those 94 faculties responded and 75% of the faculties were in favor of gift authorship and did not perceive it as a misconduct. 83% of the faculties were aware that violation of publication ethics would affect career of all the involved authors. Almost 56% believed that falsification or fabrication of data is acceptable and 58% faculties felt it acceptable to split the results of a study and publish it separately. Considering the lack of awareness among medical faculties scientific misconduct, it is recommended that a formal educational session of publication ethics is necessary.

Keywords: *publication ethics, awareness, medical faculties, scientific misconduct*

Introduction

In the present era of publish or perish, many of the medical faculties may fall prey for the different avenues of misconduct in publication of the research works. Routine emphasis on research ethics which secure and provide rights to the research participants in clinical trials are well established over period of years. But unethical practices in the research publication does also lead to numerous devastating consequences.

One of the most important and crucial issue is plagiarism. It can be in the form of copying literature, paraphrasing and copying ideas and concepts without proper acknowledgement to original sources. [1,2]

Apart from this even the issues like authorship, redundant or multiple publications, copyright and permissions, salami publications, data manipulation, favorable reporting, conflict of interest confidentiality and data retention are different area which may be misused for unethical publication behavior by the researchers. [3]

A recent example of such manipulation of data was done by the Indian wing of the multinational company Johnson & Johnson about more than 3600 faulty hip implants and it was reported by Union Ministry of Health & Family Welfare. J & J concealed the fact that the incidence of revision surgery was as high as 35% and instead reported it to be 12%. [4]

Many studies have been done so far in relation to knowledge and attitude towards only plagiarism among medical students and medical teaching faculties. Also standardized ATP (attitude towards plagiarism) questionnaire are available for assessing plagiarism.[5] But there are few researches which encompass all the above-mentioned criteria of publication ethics and hence the present study is undertaken with objective to assess the knowledge and attitude of medical faculties towards publication ethics.

Methodology

A cross-sectional study was conducted recruiting medical faculties as study participants by convenience sampling and a set of pretested and validated anonymous questionnaire were administered; created using *Google* forms and sent through a web link by *Whats app* message to 130 medical faculties working in a medical college. Out of those 94 medical faculties responded back consenting for participation in the study. The questionnaire consisted of questions pertaining to personal data, level of studies, department and designation at workplace of the respondents, their publication background and their exposure to relevant instruction on topics concerning publication ethics. It also addressed evaluation of the overall knowledge about scientific publishing, ethical matters including honorary authorship, duplicate publication, salami-publication, data falsification and the later effects of misconduct on one's carer and co-author liability.[3,6]

Results & Discussion

Questionnaire were duly filled and submitted by 94 medical faculties out of 130. As shown in the Fig. 1, Among those 74.46% had their previous research papers published in various journals. Out of them 90.42% had published research papers as corresponding author and 55.31% had taken permission from all the co-authors before adding the names as co-authors. Only 22.34% faculties had knowledge about COPE (Committee on Publication Ethics) or ICMJE (International Committee of Medical Journal Editors) guidelines. Among 94 faculties, 82.92% had never undergone any formal teaching in publication ethics though they had previously attended research methodology workshops.

Study participants were asked various questions pertaining to attitude towards publication misconduct. The responses were collected on Likert scale varying from strongly agree to strongly disagree. For statistical presentation of data as shown in Table 1, *Strongly agree* and *agree* responses were combined together while *Strongly disagree* and *disagree* were also combined as disagree. Majority (75%) of the faculties were in favor of gift authorship and did not perceive it as a misconduct. Most of the faculties (83%) were aware that violation of publication ethics would affect career of all the involved authors. Almost 56% believed that falsification or fabrication of data is acceptable and 58% faculties felt it acceptable to split the results of a study and publish it separately; not being aware of it as a type of scientific misconduct.

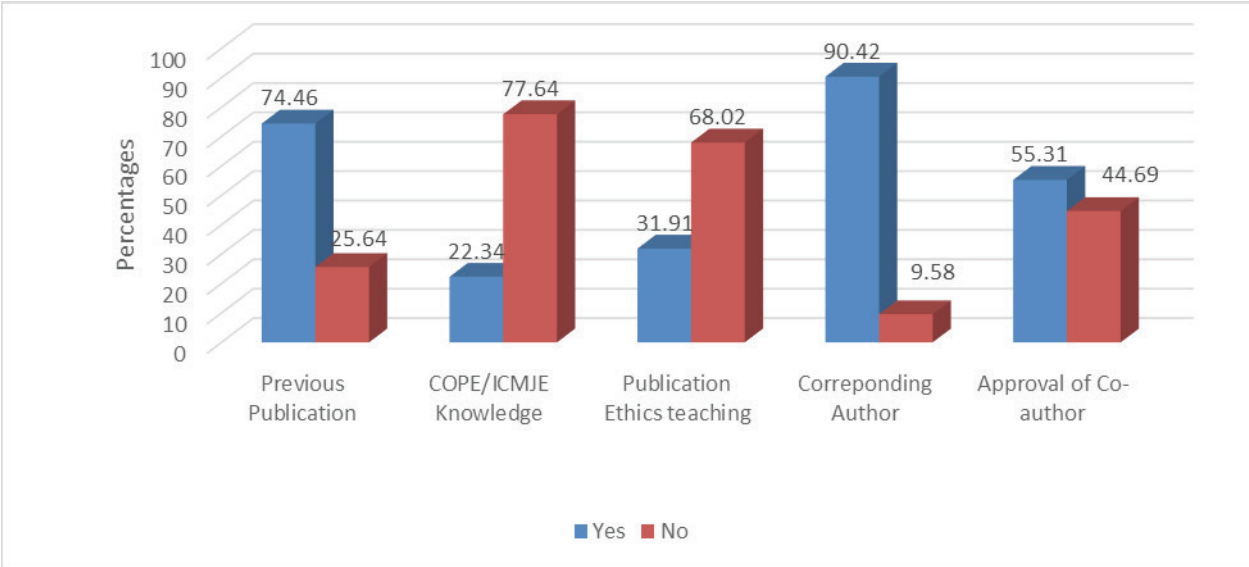

Fig.1: Knowledge of study participants about Publication Ethics

Table 1: Attitude of study participants towards Publication Ethics

SN	Questions pertaining to Attitude towards Publication Ethics	Agree (%)	Neutral (%)	Disagree (%)
1	Is it necessary to include the Head of the department as covering author, without him having substantially contributed to the study? (Gift authorship)	75	17	8
2	A violation of publications' rules will affect the career of the involved author(s)?	83	0	17
3	Are all coauthors equally responsible for Misconduct?	75	0	25
4	Suppose you observed that a colleague selectively deleted data to confirm a hypothesis ("massaging data," "cooking data"), this is accepted to increase validity of the research. (Falsification or fabrication of data)	14	30	56
5	Is it acceptable to split the results of a study and publish them separately? (Salami publication)	58	9	33
6	Mr. X, sends a manuscript to a journal and after waiting for 6 months without any response from the journal editors, fed up of waiting he sends the manuscript to other journal without informing first journal. Is it necessary to intimate the first journal authority? (Duplicate publications)	57	18	25

Conclusions

Considering their knowledge and attitude, majority of the medical faculties are lacking basic knowledge and also not aware of different practices which are deemed to be violation of publication ethics. Educating the medical faculties about the publication guidelines and ethics is the need of hour with sensitization programs. Changing the criteria from quantity to quality of research publications can be implemented when research papers are used for assessment of posts or grants.

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Considering Role of Probiotic on Respiratory Disease: Is Probiotic Possible to Treat COVID-19?

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Abstract

Background: COVID-19 is a new variant of the corona virus known as a pandemic disease. The number of cases has increased every day around the world. Unfortunately, treatment in management has not been satisfactory.

Purpose: This study aims to examine the role of probiotics in respiratory disease and the possibility of managing COVID-19 through an analysis of its function.

Method: This study is a review. Quality journals until 2020 were searched in the Pubmed database for the keywords 'respiratory' or 'asthma' or 'pneumonia' or 'lung' or 'influenza' and 'COVID-19'. Compiled data includesthe author, type of study, type of probiotic, duration of intervention, target population, results, conclusion and side effects that occurred

Results: We analyzed 9 experimental studies. Some studies related to respiratory disorders used the Lactobacillus Sp as probiotic. One research used Fructooligosacharide as additional ingredient. The research use different doses and timing of interventions 14 days - 6 months. The diseases covered in this report are asthma, pneumonia, and influenza. Probiotics can reduce symptomps, duration in hospitality risk, and quality of life.

Conclusion: The ability of probiotics in the management of respiratory diseases provides hope for the management of COVID-19, of course, it is balanced with further research that is able to analyze clear dosages, types and roles.

Keywords*: Respiratory disease, Role, Probiotic, COVID-19,*

Introduction

Covid-19 is a health problem in the world. Globally, there have been 88,383,771 confirmed cases of COVID-19, including 1,919,126 deaths, reported to WHO. There were 818,386 cases with a total of 23,947 deaths in Indonesia. The number of these cases continues to increase and changes every day[1]. Cases in East Java reached 92,613 cases [2]. Outbreaks of viruses and pathogens originating from zoonoses in the future are likely to continue[3].

Handling COVID-19 is currently at an unsatisfactory stage. There is no standard treatment for this disease and supportive treatment is the only strategy. Further, expanded clinical trials with better designs are still

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needed to evaluate the efficacy of this treatment although such treatments will be quite challenging to undertake in an epidemic era. One of the alternative treatments available is Probiotics. Probiotics are believed in previous studies to have benefits in overcoming health problems. Probiotics are live microorganisms that can be found in fermented foods and cultured milk, and are known as "health friendly bacteria," which exhibit a variety of beneficial health properties such as prevention of intestinal disease, boosting the immune system, for lactose intolerance and gut balance. microbes, exhibiting anti-hypercholesterolemic and antihypertensive effects, reducing postmenopausal disorders, and reducing diarrhea[4]This evidence appears to be adequate regarding the prevention and treatment of certain conditions while being only promising or even controversial when it comes to others. However, the development of probiotics for human consumption is still in its early stages.

Further research, in the form of controlled human studies, is needed to determine which probiotics and doses are associated with greatest efficacy and for which patients, and to demonstrate their safety and limitations [5]. The main focus of Probiotics is on three things, health improvement, infection control and disease management, through the use of various types of direct use of Probiotics or with the use of foods containing Probiotics[6].

At this time, there is not enough evidence to recommend specific anti-COVID-19 treatments. The decision to use this drug during the COVID-19 pandemic must be based on careful consideration of the potential benefits and risks for the patient. This study aims to examine the role of probiotics in respiratory diseases and the possibility of managing COVID-19 through an analysis of their functions.

Method

Participants in this study were all ages ranging from pediatric patients to adult patients. The experimental research included in this review. The data compiled are the first author, year of publication, method, types of probiotics, target population, and conclusion. A systematic search on the PubMed database, carried out for studies from the start of the database to 2020. We searched article using language in English with a searching strategy of applying 'asthma' or 'lung' or 'pulmonary' or 'pneumonia' or 'respiratory disease' or 'probiotic' and 'COVID-19'. Duplicate articles were eliminated. We screened potentially eligible trials by article titles and abstracts obtained from broad search, and then, the full text of these screened trials was assessed for eligibility according to inclusion and exclusion criteria.

Results

A study from Ahanchian et al (2016), used a randomized, double-blind, placebo-controlled, randomized clinical trial in 72 children aged 6-12 years for 60 days. The probiotics used were Lactocare ® containing ®, synbiotics containing 1 billion CFU / capsule, Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacteriuminfantis, Lactobacillus bulgaricus, and Fructooligosacharide. Synbiotics (a mixture of seven strains of probiotics plus fructooligosacharide can have an effect on reducing episodes of viral infection in children with asthma. [7]

Del Giudice et al (2017) used placebo-controlled, double-blinded, and randomized for 40 children for 4 weeks using 1 sachets/day. The probiotic used were Bifidobacteria, B longum BB536, (3x109 CFU), B infantis M-63 (1x109 CFU),and B. breve M-16 V (1x109 CFU) as a powder in 3 mg sachets. Bifidobacteria mixture can be increased significantly AR symptoms and quality of life in children with pollen-induced AR and intermittent asthma.[8]

Moura et al (2019) used a pilot longitudinal experimental and nonrandomized study for 30 patients from six to 17 years at least 60 days. All patients received beclomethasone at the initial visit, and one group also received a probiotic containing Lactobacillus reuteri (n = 14). Giving probiotics as an adjunct therapy for the treatment of children and adolescents with asthma improves the patient's clinical condition.[9]

Zeng et al, 2016 [10] used open-label, randomized, controlled multicenter trial for 235 respondents critically ill adult patients expected to receive mechanical ventilation for ≥48 hours. Probiotics used contain Bacillus subtilis and Enterococcus faecalis (Medilac-S). Patients were randomized to receive a probiotic capsule of 0.5 g three times daily via a nasogastric feeding

tube plus a standard preventive strategy or a standard prevention strategy alone, for a maximum outcome of 14 days. VAP progress is evaluated daily, and throat swabs and gastric aspirates are cultured at baseline and once or twice a week there after. Therapy with the probiotic bacteria B. Subtilis and E. faecalis is an effective and safe way to prevent VAP and acquisition of PPMO colonization in the stomach.

Mahmoodpoor et al, 2019 [11] used clinical trial for 100 critically ill adult patients who underwent mechanical ventilation for> 48 hours. The patients in the probiotic group received 2 capsules probiotic preparations and the control group received placebo daily for 14 days. Probiotic used *Lactobacillus* species (*casei*, *acidophilus*, *rhamnosus*, *bulgaricus*), *Bifidobacterium*species (*breve*, *longum*), and *Streptococcus thermophilus*. Probioticsfor the prevention of VAP is inconclusive but such an approach can reduce its length ICU and inpatient hospital.

Leyer et al, 2009 [12] used double-blind, placebo-controlled study for 326 children (ages 3–5)were treated twice a day for 6 month. Probiotic used Lactobacillus acidophilus NCFM (N 110), or L acidophilus NCFM withBifidobacteriumanimalissubsplactis Bi-07Respondentswere randomly assigned a placebo(N 104), Lactobacillus acidophilus NCFM (N 110), or L acidophilus NCFM in combination with Bifidobacteriumanimalissubsplactis Bi-07(N 112). Daily dietary probioticsupplementation for 6 months is a safe and effective way to reduce fever, rhinorrhea, and coughand duration and incidence of antibiotic prescription, as wellnumber of days absent from school due to illness, for children 3up to 5 years of age.

Bianchini et al, 2020 [13] used a prospective, randomised, and single-blind study for 87 patients. Probiotic used LGG Lactobacillus rhamnosus GG (Dicoflor 60 Immuno D3, Dicofarm, Italy), containing 1 billion LGG / drop, QIV is associated with adequate immunogenicity in children and adolescents with T1D in the presence of a good safety profile. Although systematic administration of LGG does not result in improved humoral response to influenza vaccine, probiotics have important anti-inflammatory effects.

Wang et al, 2018 [14] used randomized, double-blind, placebo-controlled pilot trial for 196 nursing home residents aged 65 and older. They are randomized received probiotic studies - 2 capsules or placebo (calcium carbonate) every day for 6 months. Probiotic used Lactobacillus rhamnosus GG (estimated 10 billion colony forming units of L. rhamnosus GG per capsule). Feasibility of a trial assessing the effects of probiotics on reduce influenza and other respiratory infections in treatment houses for residents aged 65 years and over.

Discussion

Most of them use lactobacillus Sp as a probiotic in research. Previous research supports the potential of lactobacilli to regulate the immune system, increase intestinal metabolic capacity and maintain the balance of the gut microbiota. However, the species and specific characteristics of the lactobacilli strains that provide probiotic benefits are still not well understood [15]. There are recommendations about probiotics containing Lactobacillus rhamnosus GG (LGG) against acute gastroenteritis and against antibiotic-related diarrhea[16]. One research used Fructooligosacharide as additional ingredient. Fructo-oligosaccharides (FOS) are medium-chain carbohydrate compounds known as prebiotics with natural functional ingredients. Several studies have shown the functional properties of FOS, such as decreased cholesterol and blood glucose levels, decreased blood pressure, better absorption of calcium and magnesium, as well as inhibiting the production of reductase enzymes which can contribute to cancer, stimulating the growth of non-pathogenic intestinal microflora (bifidobacteria). and boosts the immune system[17].

The dosage used also varies. Various dosages for Lactobacillus sp. and other probiotics have been studied in clinical trials. In general, higher probiotic doses (ie, more than 5 billion CFU per day in children and more than 10 billion CFU per day in adults) were associated with more significant study results. There is no evidence that higher doses are unsafe; however, they may be more expensive and unnecessary. The dosage of S. boulardii in most studies has ranged between 250 mg and 500 mg per day. Probiotics are generally sold as capsules, powders, tablets, liquids, or in foods. The specific amount of CFU contained in a particular dosage or serving of food may vary between brands. Patients should be advised to read product labels carefully to make sure they are getting the

right dose [18]

Probiotics have been explored in a number of clinical trials which are increasing exponentially for their health effects. Underpowered clinical trials are another problem in the probiotic field[16]. Most research used to study children and adults with almost similar effectiveness though respiratory diseases often attack children. Infants and young children have higher resting metabolic rates and oxygen consumption per unit of body weight than adults because they have a larger surface per unit due to body weight and because they are growing rapidly [19].

Most of the studies found a reduction in symptoms, risk of hospitalization, risk of ICU and nasal symptoms including the respiratory symptoms present in Covid-19, namely fever, rhinorrhea, and cough. Probiotics exert their beneficial effects by modulating the host's immune response, maintaining gut homeostasis and producing interferon thereby suppressing viral cytokine-induced storms. In fact, immune stimulation and cytokine expression are strain specific, and can vary according to the probiotic bacterial consortium[20]. Probiotics are live microorganisms that provide health benefits when consumed in sufficient quantities, including subtle immune activity and clearing of respiratory tract infections [21]. The main points of the role of probiotics are health promotion, infection control and management. disease [6].

All studies reviewed in this article did not analyze and report side effects of probiotic administration. There are studies that report side effects of probiotics such as minor gastrointestinal symptoms, such as stomach cramps, nausea, soft stools, flatulence, and taste disturbances, which occurred in subjects receiving probiotics[22]. Most probiotics are safe. There are rare occurrences of sepsis, endocarditis, and liver abscess during Lactobacillus use; In addition, fungemia has been reported with S boulardii, especially in patients with severe comorbidities. The most common side effects of probiotics are constipation, flatulence, hiccups, nausea, infections, and rashes [23].

Most of the studies agree and support probiotics in respiratory disease in general. This shows possible applications in the management of COVID-19. Probiotic research in the form of a randomized control trial needs to be carried out in the management of COVID-19. Several systematic reviews underscore the need for studies that address the role of clear, recommended doses and the broader affordable population. Better designed randomized controlled trials with larger sample sizes need to be done to improve the quality of research[24].

Much remains to be learned about the determinants of the various immune responses induced by different strains of bacteria. Deeper knowledge of the interactions between specific probiotics and existing microbiota, along with an understanding of how the dialogue between microbes and innate management systems becomes a beneficial / protective response is needed before we can achieve clinically effective bacteria-based strategies which maintains and improves respiratory health[25]. Physiology and pathology of the respiratory tract and digestive tract are closely related. The similarity of these two organs may be the reason why dysfunction in one organ can cause disease in other organs. In this sense, probiotics can act as immunomodulatory agents and activators of the body's defense pathways suggesting that probiotics can affect the severity and incidence of disease at the location of the distal intestine. There is more evidence that probiotics given orally are able to provide an immune response in the respiratory system [26].

Conclusion

Probiotics have many great benefits related to the respiratory system. This is likely to be able to support health services during the Covid-19 period. Recommendations for further research are randomized control trials that are able to analyze the ability of probiotics, dosages and their processes in the management of respiratory diseases. This research is expected to be able to be an alternative and supportive therapy during a pandemic.

Ethical Clearance: This manuscript has passed the ethical test process in Faculty of Medicine Universitas Airlangga.

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The Mediating Effect of Wisdom in the Relationship between Self-leadership and Quality of Service of Caregivers caring for Patients with Impaired Mobility and Elderly with Dementia

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Abstract

Background/Objectives: The purpose of this study is to analyze the mediating effect of wisdom in the relationship between self-leadership and quality of service in caregivers who provide nursing services to patients and elderly.

Methods/Statistical analysis: The subjects of this study were 96 caregivers from January to February 2021. Data analysis is performed using SPSS Win 25.0 program, and were analyzed by correlation between variables by Pearson correlational coefficients, and factors affecting service quality and mediating effects by multiple linear regression and Sobel test.

Findings: As a result of regression analysis to test the mediating effect of wisdom, in step 1, self-leadership had a significant effect on wisdom (β=.73, p<.001), in step 2, self-leadership affected the quality of service (β=.59, p<.001), in step 3, self-leadership had a positive effect on the quality of service (β=.26, p=.022), and wisdom also had a positive effect on the quality of service (β=.46, p<.001) and showed 44.9% explanatory power. It was found that wisdom has a partial mediating effect.

Improvements/ Applications: Education and training are needed to increase self-leadership and wisdom in caregivers in charge of visiting care. In particular, caregivers need to make efforts to acquire wisdom in their daily lives.-

Keywords: *Self-leadership; Quality of service; Wisdom; Dementia; Elderly; Caregivers; Mediating effect*

Introduction

Due to the rapid aging of Korea, the population aged 65 or older in 2020 is 15.7%, and it will continue to increase in the future, and by 2025, it is expected to reach 20.3%, entering a super-aged society. In 2019, the proportion of people over 65 who are satisfied with their current life was 25.0%, a decrease of 4.9% compared to the previous year, and the elderly showed lower life satisfaction compared to all age groups[1]. To analyze the cause of this situation, in the 2017 survey on the elderly, more than 75% of the elderly have two or more chronic diseases[2] and complex chronic diseases require continuous treatment and management, increasing the burden of public expenditure and personal out-of-pocket medical expenses. Moreover, it is difficult for the elderly of the low-income class to use sufficient medical care even though their health status is worse[3]. These physical disorders lead to depression and helplessness, leading to poor quality of life.

Therefore, systematic and continuous provision of care in daily life can help maintain and promote the health of patients or the elderly. The Health Insurance Corporation determines the level of the person who will be eligible for nursing care, and provides assistance to patients who have difficulty in maintaining their daily life because of

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their mobility, and in the case of elderly with dementia, it is judged as 4th and 5th grade and institutionalized so that they can receive care by caregivers at home.

In Korean culture, the elderly prefer to live in their own home rather than move to a care institution to receive care, and their families also want caregivers to visit their homes to provide services, unless they have to rely on high-quality hospital-centered care. The caregivers, who will perform these tasks, are the primary care workers providing direct services to long-term care recipients. It is contributing to the improvement of the quality of life of the people by supporting physical and household activities, reducing the burden on the family, and helping those who have difficulty living alone due to the elderly or senile diseases[4]. Since the nursing services they provide are very important to the health of the subject, education and research must be accumulated so that high quality services can be provided. Quality of service refers to the performance of a service with the attributes of reliability, responsiveness, ability, accessibility, courtesy, communication, honesty, stability, understanding and tangibility[5].

As a result of analyzing previous studies on factors necessary for caregivers to increase the quality of service, job commitment and self-leadership are highly correlated with job competency, and self-leadership has an important mediating effect in the relationship between job commitment and job competency[6]. In addition, caregivers' job competency and wisdom were highly correlated and were a major influence factor[7,8]. Therefore, it can be inferred that the roles of self-leadership and wisdom will be very important for caregivers to increase quality of service with job competency. And as a result of a study on caregivers' self-leadership affected the quality of service, and constructive thinking, action-oriented strategies, and natural reward strategies, which are sub-domains of self-leadership, were found to affect quality of service, and leadership explained quality of service by 39.1%[9].

Moreover, as most caregivers are middle age and old adults, the wisdom of middle-aged adults and interpersonal relationship harmony and productivity showed a high correlation, and wisdom appeared to have a mediating effect on the sense of crisis and productivity in the middle-aged[10], it can be expected that the wisdom attitude of caregivers will smooth relationships with the subject and provide their own services productively. Also, wisdom was an important factor in the health preservation of the elderly[11], and as it is thought that wise people will maintain good health and provide services well, so wisdom was included. Wisdom is an element that caregivers must have when performing their duties as they increase their ability to accept life and establish relationships with others with problem-solving and insight[7] . In case of wise caregivers, it is thought that they can provide care to the elderly with a positive attitude, sympathy, and balance.

Therefore, the researcher investigated the relationship between self-leadership and wisdom targeting caregivers in charge of visiting care, and by analyzing the mediating effect of wisdom in the relationship between self-leadership and quality of service, this study aimed to improve the quality of service of caregivers.

Materials & Methods

Subjects

The subjects of this study were 96 caregivers who belong to 3 nursing home care centers for the elderly located in D City. The number of study subjects was calculated using the G-power 3.1.9.4 program. The number of samples required to maintain 2 predictors, effect size of .15, significance level of .05, and power of .90 was 88, and 96 people were surveyed considering the dropout rate of 10%.

Instruments

Self-leadership

The self-leadership tool of Prussia, *et al.* modified and supplemented by Koh [12] was used. With a total of 20 questions, the higher the score, the higher the degree of self-leadership. Cronbach's α was .94 in the study of Kim [6], and .92 in this study.

Wisdom

The Korean wisdom scale (KMWS) developed by Kim[13] was used. A total of 43 questions consisted. The higher the score, the higher the degree of wisdom. At the time of development, Cronbach's α=.93, and in this study was .98.

Quality of service

The 'SERVQUAL' scale developed by Parasuraman *et al.*[5] used for caregivers by Jung[14] was used. The tool was composed of five factors including, reliability, responsiveness, assurance, empathy, and tangibility, with a total of 20 questions. In the study of Jung[14], Cronbach's α was .97, and in this study, it was .95.

Data collection

The researcher and research assistant directly visited the 3 elderly home visiting care centers located in D city, explained the purpose and method of the research to the center director, and obtained permission, and after meeting with the center director, the research purpose, method, and especially the data collection method were explained, and the research purpose was explained to the caregivers through the center director, and the questionnaire was completed after receiving a written consent. It took about 10-15 minutes to complete each questionnaire.

Ethical Consideration

Approval was acquired by the ethics committee of K University on the objective, methodology and protection of rights of participants (KNU_IRB_2020-99). During the study period the guidelines on ethical studies were observed. The consent form included details on anonymity and confidentiality, and it was explained that participation in the study can be stopped at any time if he/she wants to discontinue the study even after consenting to participate in the study according to his or her voluntary will, and that there is no disadvantage.

Data analysis

Using the SPSS/WIN 25.0 program, the frequency, percentage, mean and standard deviation of each variable were calculated, and the correlation between each variable was analyzed through Pearson's correlation coefficients, and the mediating effect of wisdom was analyzed using multiple linear regression, and the significance test for mediating effect size was analyzed by the Sobel test.

Result and Discussion

General characteristics of subjects

Subjects of this study were female care workers (100%) who are in charge of visiting care, ranging in age from 38 to 80 years old, with an average of 59.23±8.16 years old and 55.2% (53 persons) upper 60 years old. Most (95.8%) were married, and as for the educational background, 67.8% (65 people) had a high school diploma or higher, and the average working experience as a care worker was 57.31±45.99 months, ranging from 6 months to 12 years. 69.8%(67 people) had religion. It was found that more than half (69.8%, 67 people) had no other certifications than the care worker certification. 73 (76.0%) care workers had received maintenance training or job training more than once in the past year.

Degree of self-leadership, wisdom, and quality of service in subjects

The care workers' self-leadership scored 3.94±0.47 points out of 5 points, wisdom scored 3.82±0.57 points out of 5 points, and quality of service scored 4.16±0.50 points out of 5 points. [Table 1]

Table 1: Degree of communication ability, Empathy and Quality of service in Subjects (N=96)

Variables	M±SD	Range
Self-leadership	3.94±0.47	1~5
Wisdom	3.82±0.57	1~5
Quality of service	4.16±0.50	1~5

Correlation between self-leadership, wisdom, and quality of service in subjects

Quality of service and self-leadership (r=.59, p<.001), quality of service and wisdom (r=.65, p<.001), and wisdom and self-leadership (r=.73, p<.001) all showed high positive correlation [Table 2].

Table 2: Correlation between Self-leadership. Wisdom and Quality of Service in subjects

Variables	Self-leadership r(p)	Wisdom r(p)	Quality of service r(p)
Self-leadership	1		
Wisdom	.73 (<.001)	1	
Quality of service	.59 (<.001)	.65 (<.001)	1

Mediating effects of wisdom in the relation between self-leadership and quality of service in subjects

As a result of examining the autocorrelation of the dependent variable and the multicollinearity between the independent variable before testing the mediating effect, the Durbin-Watson index for autocorrelation was 1.86, which was close to 2, which was independent. The multicollinearity between the independent variables was less than 10 with the VIF index 2.11, and tolerance was 0.47, which is above the standard value of 0.1, and there was no multicollinearity, which meant the data was suitable for regression analysis.

As a result, in step 1 regression analysis, self-leadership, an independent variable, had a statistically significant affect on wisdom, a mediating variable (β=.73), and the explanatory power for empathy was 52.6%. In the second-stage regression analysis, self-leadership, an independent variable, had a significant effect on the quality of service, a dependent variable (β=.59), and the explanatory power for quality of service was 35.0%. In step 3, in order to test the effect of wisdom, a mediating variable, on quality of service, which is a dependent variable, as a result of regression analysis with self-leadership and wisdom as predictive factors and quality of service as dependent variable, self-leadership was found to have a positive effect on quality of service (β=.26), and wisdom also had a positive effect on quality of service (β=.46), and it showed 44.9% of explanatory power.

As a result of comparing the β values, it was confirmed that wisdom showed a partial mediating effect as much as 0.33. The β value of .26 in step 3 was lower than the β value of .59 in step 2, and because the effect of self-leadership was reduced by the parameter wisdom, the partial mediating effect of wisdom was confirmed. As a result of confirming the significance of the mediating effect coefficient, it was statistically significant (Z=3.78, p<.001) [Table 3].

Table 3: Mediating effects of wisdom in the relation between self-leadership and quality of service

Variables	B	β	t	p	R2	Adj. R2	F	p
Step1: Self-leadership→ Wisdom	.88	.73	10.21	<.001	.526	.521	104.19	<.001
Step2: Self-leadership →Quality of service	.63	.59	7.12	<.001	.350	.344	50.72	<.001

***Cont....*Table 3: Mediating effects of wisdom in the relation between self-leadership and quality of service**

Step3: Self-leadership, Wisdom → Quality of service					.449	.437	37.94	<.001
1. Self-leadership → Quality of service	.28	.26	2.33	.022				
2. Wisdom → Quality of service	.40	.46	4.09	<.001				
Z= 3.78, p<.001								

Discussion

By analyzing the mediating effect of wisdom in the relationship between self-leadership and quality of service, targeting caregivers who are in charge of visiting care for elderly with impaired mobility with conditions such as dementia and stroke, research was attempted to provide basic data to improve the quality of service of caregivers.

Self-leadership and wisdom in caregivers showed a positive correlation with quality of service. Caregivers' self-leadership had a significant effect on the quality of service [9]. Self-leadership is a skill for self-efficacy, the basis of behavioral control, and the learning process of self-completion. It is also the process of exerting influence on oneself to perform tasks and duties, motivating oneself to achieve goals and developing one's own framework. Therefore, it was confirmed that the self-leadership of caregivers can have an important influence in providing nursing services to the elderly.

Moreover, wisdom is the mental ability to quickly realize the reasoning of things and process things accurately [15]. It is also facilitated through life's internal and external experiences [16] and it can be said that caregivers after middle age are more likely to be wise because they have lived for a long time and have had a lot of experience. Generally, those who perform the duties of caregivers are middle-aged and elderly women, and these are subjects who are familiar with the act of care, and since they mainly perform duties related to nursing care, it is judged that wisdom and quality of service could derive a positive correlation. Wise middle-aged and elderly adults had higher self-esteem and higher ability to adapt in life than the lower-age group[17], and they were expected to be able to lead their work well, and this supported the results of this study. Also, in a study of 116 and 123 caregivers in charge of visiting care [7,8], wisdom and job competency showed a statistically significant positive correlation, and wisdom was found to be an important factor influencing the job competency of caregivers where, as the wisdom increased, the job competency was higher, and therefore, it is believed that the caregivers can provide high quality services by exerting high competence. Therefore, it is necessary to provide an opportunity to acquire wisdom in life while educating and training to demonstrate self-leadership of caregivers based on the results of this study so that excellent nursing services for the elderly can be provided.

Conclusion

According to the results of this study, wisdom was found to have a partial mediating effect in the relationship between self-leadership and quality of service in caregivers. In order for caregivers to increase the quality of care service for patients with impaired mobility and elderly with dementia, they need an opportunity to participate in leadership improvement programs and enrich their life experiences through wisdom. A follow-up study targeting or expanding the target to caregivers belonging to nursing hospitals is suggested.

Ethical Clearance: Not required

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Determination Levels of Tumor Necrosis Factor Receptor and Tolk like Receptor -4 and Bacterial Vaginosis in Women with Recurrent Spontaneous Abortion

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Abstract

The presents study included Determination levels of Tumor necrosis factor and Tolk like receptor -4 in women with recurrent spontaneous abortion in province of Babylon, 100 samples were collected, whereas 50 samples with recurrent abortion women and 50 healthy women as control groups **,**the results of presents study shown that the concentration of tumor necrosis factor receptor in serum of women with recurrent abortion and control was showed significantly differences between control and cases (P≤ 0.044), the Mean of tumor necrosis factor receptor (TNFR) in serum of patients women with recurrent abortion was 52.08 pg/ml,Whereas in control, the concentration of tumor necrosis factor receptor in serum of healthy women was 42.77 pg/ml.

Tolk like receptor 4 concentration in serum of women with recurrent abortion and control was significantly differences between cases and control (P ≤ 0.001), the mean of Tolk like receptor 4 concentration in serum of women with recurrent abortion was 3.95 pg/ml While In serum of women without abortion (control), the mean of Tolk like receptors 4 was 2.84 pg/ml.

The results of statistical analysis shown that the tolk like receptor-4 marker that is significantly differences at (p ≤ 0.05) positive correlation with age (r = .698**) , and its positively correlation with abortion (r= .505*) , the tumor necrosis factor receptor was significantly differences at (p ≤ 0.05) and positively correlation with age and the value of r was reached to .539* and its positive correlation with abortion (r =.854**)

Keyword: *Recurrent abortion, Tumor necrosis factor receptor and Tolk-like receptor 4*

Introduction

Recurrent spontaneous abortion is defined according to the American society for reproductive medicine " two or more consecutive pregnancy losses before 24 weeks of gestation''[1, 2] the gross risk of abortion is 15% after one abortion , 17-31%after two successive abortion , and 25-46% after three or more repeated abortion [3, 4].

Many epidemiological that are causes recurrent spontaneous abortion such as infection (0.5-5%), genetic (2-4%), anatomic (10-15%), thrombotic (≥15%), endocrine (17-20%), immunologic (20%) and environmental (40-50%), in women with recurrent abortion that causes rising NK cell count and autoantibody levels that lead to decreased blood flow in uterine during early pregnancy, Hence, autoimmunity play pathological role in causing recurrent abortion in pregnant women [5, 6].

Cytokines influence all steps of reproduction, including the risk of miscarriage, specifically, Th2 cytokines such as interleukin (IL)-4, IL-10 and IL-13] are associated with pregnancy success, whereas Th1 cytokine responses such as interferon (IFN)-γ, and tumour necrosis factor (TNF)-α predominate in spontaneous miscarriage. Although the complexity of the cytokine network at the feto-maternal interface has increased with the discovery of newer cytokines and improved understanding of the role of specific cellular subtypes (e.g. natural killer cells, dendritic cells and regulatory T cells), the evidence that a Th1 response in

the decidua may lead to miscarriage remains substantial.

Toll-like receptors (TLRs) are a recently identified group of vertebrate receptors that play a central role in determining the Th1/Th2 balance of immune responses. The human TLR family consists of 10 receptors that orchestrate the innate immune response by linking pathogen recognition with immune cell activation. Individual TLRs recognize a distinct, but limited, repertoire of conserved microbial products, and the best-characterized receptor-ligand pair is TLR4 and lipopolysaccharide (LPS or endotoxin). In most situations, TLR activation promotes the generation of a Th1-dominated immune response and inhibits Th2 cytokine production [7].

Material and Methods

Patients and control groups

The study was done on women that are attended to departments of obstetrics and gynecology at maternity and child teaching hospital, and some private clinics in Babylon governorate that are suffering from recurrent spontaneous abortion from 1/12/2019 to 1/6/2020. This included 50 patients and 50 pregnant women that are apparent healthy that are selected as a control groups

Blood Samples

Five milliliter of venous blood that are collected from women with recurrent spontaneous abortion and healthy women , the samples were placed in tubes containing a gel and then the blood was separated by centrifuge at 3000 rpm for 15 minutes, the serum was putted in Eppendrof tubes and then stored in a deep freeze at -20C˚ until used [8].

Immunological Markers

Tumor Necrosis Factor Receptors

Tumor necrosis factor receptor of human is measured by Boster's human TNFsR I ELISA kit was depend on standard sandwich enzyme-linked immune-sorbent assay, A monoclonal from mouse specific for TNFsR I has been precoated onto 96 well plates , the standard and sample are added to the wells, a biotinylated goat TNFsR lis added subsequently and then followed by washing with PBS buffer , Avidin-Biotin-peroxidase complex was added and unbounded conjugates were washed away with PBS buffer , HRP substrate TMB was used to visualize HRP enzyme reaction, TMB was catalyzed by HRP to produce a blue color product that change into yellow after adding acidic stop solution , the density of yellow color is a proportion to the human TNFsR l amount of sample captured in plate and absorbance is measured at 450 nm. .

· Tolk Like Receptors-4

Tolk like receptor 4 is detection by an enzyme-linked immunosorbent assay kit, the plate has been precoated with human TLR4 antibody,40µl from sample is added and binds to antibodies coated on the well, and then 10 µl biotinylated human TLR4 antibody is added and binds to TLR4 in the sample , then 50 µl streptavidin-HRP is added and binds to the biotinylated TLR4 antibody, after incubation unbound streptavidin-HRP is washed away during a washing step, 50 µl from substrate solution is then added an color develops in in proportion to the amount of human TLR4, the reaction is determined by addition 50 µl from acidic stop solution and absorbance is measured at 450 nm.

Statistical Analysis

Statistical analysis was carried out using statistical package for social science SPSS statistical software for windows version 24 to found means, Standard deviation, Least Significant differences by Duncan and Correlation by ANOVA

Results

Tumor necrosis factor receptors TNFR

The concentration of tumor necrosis factor receptor in serum of women with recurrent abortion and control was showed significant differences between control and cases ($P \leq 0.044$), the Mean of tumor necrosis factor receptor (TNFR) in serum of patients women with recurrent abortion was 61.91 pg/ml While the highest concentration in serum of women with recurrent abortion was 94.57 pg/ml and the lowest concentration of in serum of women with recurrent abortion was 15.55 pg/ml. Whereas in control, the concentration of tumor necrosis factor receptor in serum of women was 38.50 pg/ml, While the highest concentration of tumor necrosis factor receptor (TNFR) was 76.19 pg/ml and the lowest concentration of tumor necrosis factor receptor (TNFR)

was 14.43 pg/ml Table (1) and figure (1-1).

Tolk like receptors 4 TLR4

Tolk like receptor 4 concentration in serum of women with recurrent abortion and control was significant differences between cases and control ($P \leq 0.001$), the mean of Tolk like receptor 4 concentration in serum of women with recurrent abortion was 4.42 pg/ml While the highest concentration of Tolk like receptors 4 was 7.30 pg/ml and the lowest concentration of Tolk like receptors 4 was 2.42 pg/ml ,

In serum of women without abortion (control), the mean of Tolk like receptors 4 was 2.33 pg/ml ,Whereas the highest concentration of Tolk like receptors 4 was 4.59 pg/ml and lowest concentration of Tolk like receptor 4 was 1.29 pg/ml Table (1) and figure (1-2).

Table. (1). the variation in TNF-α level expressed as mean ± SD in patients and control groups.

Marker	Group	N	Mean	Lower-upper value	Std. Deviation	Std. Error Mean	t.test	P.value
TNFR	Control	45	38.5080	42.77-76.19	14.28940	2.13014	-6.402	0.000
	Patient	49	61.9129	15.55-94.57	20.79679	2.97097		
TLR4	Control	44	2.3317	1.29-4.59	0.96700	0.14600	-8.265	0.000
	Patient	48	4.4250	2.42-7.30	1.43500	0.21000		

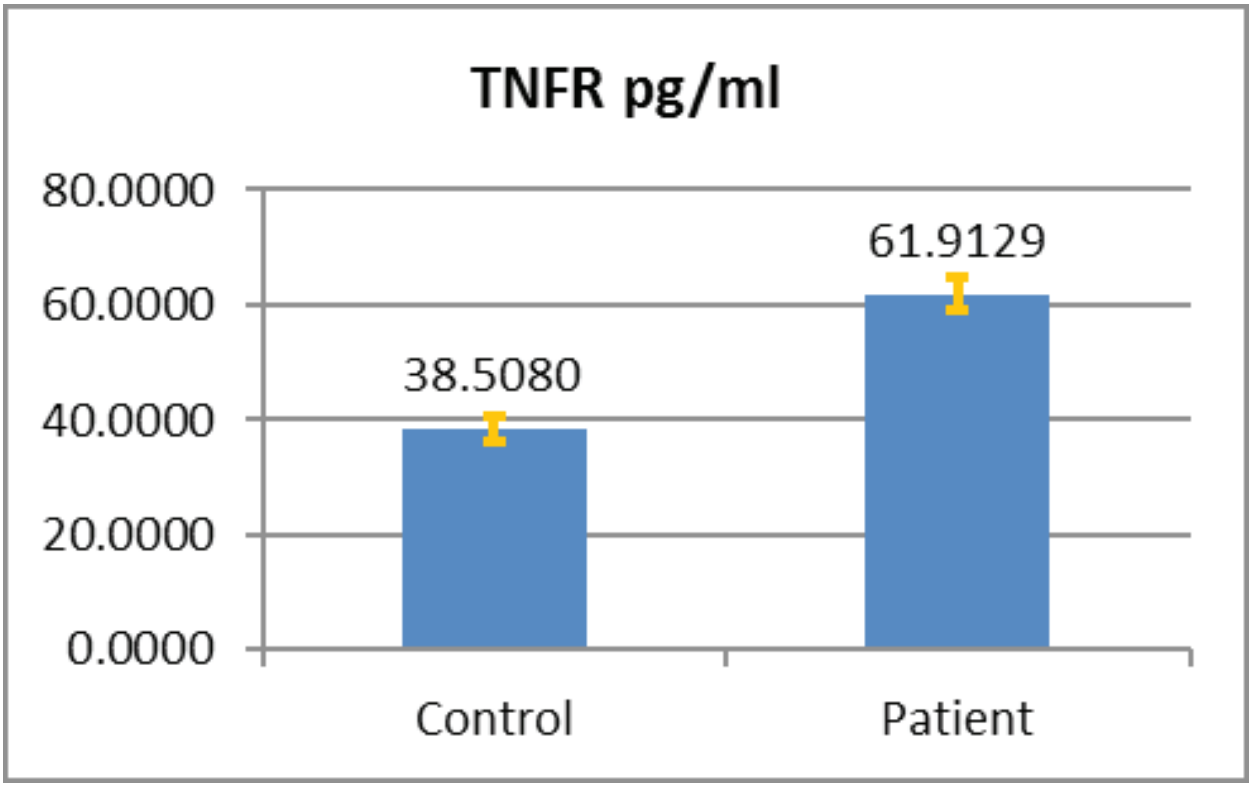

Figure (1-1): The concentration of tumor necrosis factor receptor in serum of control and women with recurrent abortion

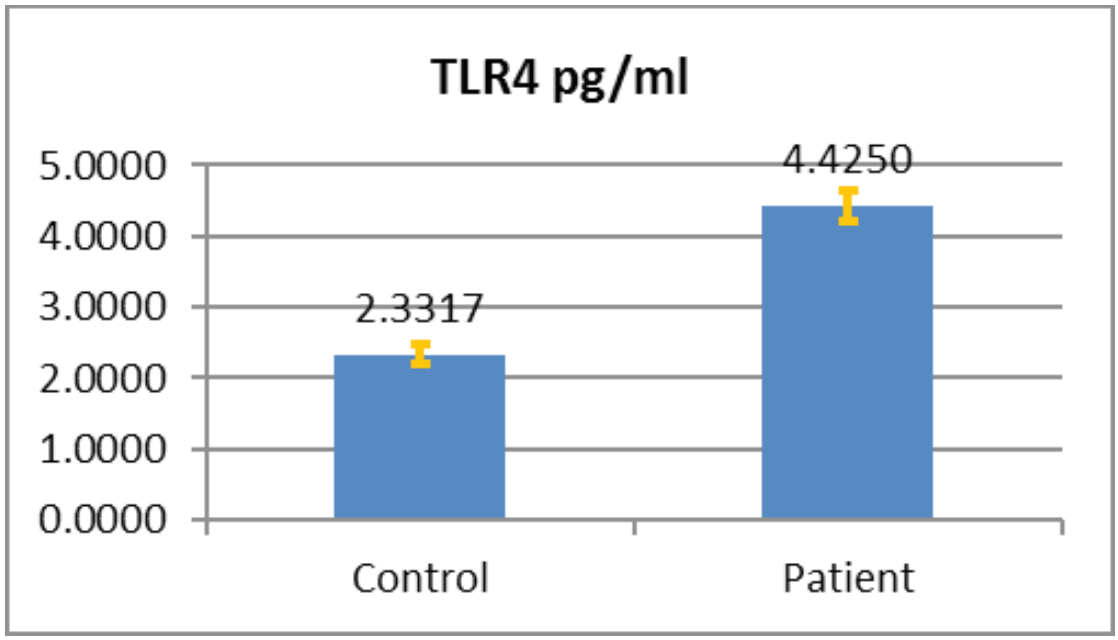

Figure (1-2) The concentration of Tolk like receptor 4 in serum of control and women with recurrent abortion

Correlations among TLR-4, TNFR, Age and Abortion

Table (2) correlation among TLR-4, TNFR, Age and abortion , the results of statistical analysis shown that the tolk like receptor-4 marker that is significantly differences at ($p \leq 0.05$) positive correlation with age ($r = .698^{**}$) , and its positively correlation with abortion ($r= .505^{*}$) , the tumor necrosis factor receptor was significantly differences at ($p \leq 0.05$) and positively correlation with age and the value of r was reached to $.539^{*}$ and its positive correlation with abortion ($r =.854^{**}$)

Table (2) correlation among TLR-4, TNFR, Age and abortion

Correlations					
		TLR-4	**TNF**	**Age**	**Abortion**
TLR-4	Pearson Correlation	1	-.019	. 698**	.505*
	Sig. (2-tailed)		.892	.005	.030
	N	56	56	48	56
TNFR	Pearson Correlation	-.019	1	.539*	.854**
	Sig. (2-tailed)	.892		.040	.021
	N	56	57	49	57
Age	Pearson Correlation	.698**	.539*	1	.702**
	Sig. (2-tailed)	.005	.040		.000
	N	48	49	50	50
Abortions	Pearson Correlation	-.205	.854**	.702**	1
	Sig. (2-tailed)	.130	.021	.000	
	N	56	57	50	58
**. Correlation is significant at the 0.01 level (2-tailed).					

Discussion

One of a pro-inflammatory cytokine is a tumor necrosis factor receptor (TNFR) that excreted by Th1 cells and placenta , it links with inflammatory mechanisms that regarding with an implantation , placentation and pregnancy loss.

The results of present study shown significantly differences increased in the level of tumor necrosis factor receptor in women with recurrent abortion which reached to 61.91±20.79pg/ml, While it was 38.50±14.28 in healthy without bacterial vaginosis, the current results was agreement with most other studies about tumor necrosis factor receptor concentration like the study of Hamad (2018) that shown significant rise in the level of tumor necrosis factor in women with recurrent abortion (78.5±6.61) While in healthy women, its concentration (59.5 ±6.35) Whereas Azizieh and Raghupathy, (2015) that had been shown the level of TNFR was significantly increased at 1st trimester (4159.8 pg/ml); 2nd trimester (3489.5 pg/ml) and the 3rd trimester (4149.2 pg/ml) in women with recurrent abortion as compared with 1st trimester (1176.4 pg/ml); 2nd trimester (4320.9 pg/ml) ; and the 3rd trimester (7307.4 pg/ml) respectively in the normal healthy women, these increased in level of tumor necrosis factor receptor in women with recurrent as compared with the healthy women may be related to the presence of the auto antigen that induces human maternal peripheral blood mononuclear cells ,and thus lead to the production of a large amount of TNFR in sera of women with recurrent abortion [9].

The results of present study shown significantly differences at ($P \leq 0.001$),increasing in the level of Tolk like receptor-4 in women with recurrent abortion which reached to 4.42 ± 1.43 pg/ml, While it was 2.33±0.96 in healthy without bacterial vaginitis.

Toll-like receptor 4 (TLR4) belongs to the pattern recognition receptor family, which plays a key role in the human defense mechanism and responds to invading pathogens with high selectivity and sensitivity (Gay et al.,2014 and Chen et al.,2018). TLR4 is sensitive to pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) and lipo-oligosaccharide. Moreover, TLR4 recognizes PAMPs from fungi, viruses, mycoplasmas, and bacterial vaginosis, in addition to PAMPs, TLR4 can be activated by certain endogenous ligands produced due to tissue injury and/or inflammation [10, 11]. This receptor–ligand interaction initiates an intracellular signaling cascade that leads to the subsequent proinflammatory response, thus, due to the involvement of TLR4 in various pathological conditions [12, 13].

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Ethical Approve: We declare that the study does not need ethical approval.

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Probiotics Intake as Adjunct Therapy for Infected Health-Care with SARS COV-2

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Abstract

Objectives: We performed this case-control observational study to evaluate the comparison of the length of duration of SARS COV-2 infection and the cycle threshold (Ct) value of reverse-transcriptase polymerase chain reaction (RT-PCR) nasopharynx swab between the probiotics intake (case) group and the non-probiotics intake (control) group.

Materials and Methods: Our study was a case-control study involving 15 cases and 15 controls match for RT-PCR positive results. The participants were healthcare consisted of registrars, consultants, and nurses. Each participant was interviewed by google forms using a structured questionnaire to collect socio-demographic characteristics, diet, therapy from a pulmonologist, and adjunct therapy.

Results: The total participants consisted of 15 males and 15 females. 4 participants in the case group had febrile, 1 participant with anosmia, 1 participant with febrile, nausea, and vomit before they consumed probiotics, and 9 participants without clinical complaints. One participant in the control group had fevered and cough, 14 participants without clinical complaints—1 participant with co-morbidities in the control group. The data of age, duration of infection, and cycle threshold (Ct) value were in the normal distribution. Analysis results using SPSS 21.00 show no significant differences in the course of disease between the case group and the control group. We found 2 participants in the control group had re-infection, while there was no re-infection in the case group.

Conclusion: The present study's finding may imply future care for the viral infection through the immunomodulation mechanism by probiotics consumption.

Keywords*: Probiotics; Immunomodulator; COVID-19*

Introduction

Immediate solutions are needed to deal with the new SARS COV-2 virus that has shocked the world. We must realize immediately about the strategies in the form of prevention and treatment of COVID-19. For example, prevention from the social side, social and physical distancing, wearing masks, avoiding crowds, and washing hands. Meanwhile, vaccines may not give protection in a short time [(1)].

The clinical appearance of COVID-19 varies widely, ranging from asymptomatic, mild symptoms, moderate to critical conditions, mostly needs the ICU treatment. The asymptomatic patient is dangerous for himself and hazardous for others because it causes others to become

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infected. Critical patients mostly died in the ICU. Transmission of SARS COV-2 is said to be through droplets, but changes in gut stability also contribute to the occurrence of COVID-19 [2]. The previous study detected SARS-CoV-2 ribonukleat acid (RNA) in the gastrointestinal tract and stool samples from patients [3] [4][5], and sewage systems [6]. Coronaviruses, including SARS-Cov-2, can invade enterocytes, thereby acting as a reservoir for the virus [5]. Indeed, extensive clinical studies from China indicate that gastrointestinal symptoms are common in COVID-19 and are associated with disease severity [4][5].

As a result of the SARS COV-2 virus attack, the world seems to have had a monster attack, and we haven't found a weapon to fight back. The group of scientists with various theoretical bases is trying to find a way out of prevention and treatment. The previous literature discussed probiotics' role promotes us to study probiotics' effectiveness against the SARS COV-2 virus. The hypothesis of the inhibition mechanism by probiotic against virus may come from the inhibition of viral entry and/or replication or by suppressing the immunologic response because of the infection (known as cytokine storm).

SARS-CoV-2 can infect the digestive tract and cause inflammation of the absorbing mucosa and sometimes diarrhea. The first US case of COVID-19 showing atypical symptoms, diarrhea, and other gastrointestinal (GI) [7]. Dysbiosis can exacerbate the immune response and the production of systemic inflammatory mediators. Oral probiotics can play a role in the gut and systemic effects of COVID-19. Also, inhaled microorganisms can directly activate the respiratory epithelium and immune system cells that fill them [8].

A Probiotic is a living microorganism that confers a health benefit on the host when administered in adequate amounts. Evidence is scarce about the relationship between COVID-19 and gut microbiota. Our case-control study about the advantages of using probiotics as one of the therapy modalities in healthcare workers with positive SARS COV-2.

Materials and Methods

Primary outcome

To analyze and compare the duration of infection in healthcare in the two groups of intake (case) and non-intake probiotic (control) during the infection.

Selection of subjects and study design

After passing ethical clearance (Ref.No: 0115/LOE/301.4.2/IX/2020), we collected the data from June to October 2020. We assessed 97 health care (registrars, consultants, and nurses) in Dr. Soetomo General Academic Hospital for eligibility. 67 participants were excluded because they did not fit the inclusion criteria (n =0), participants refused to participate (n = 0) or the data were not complete (n = 67).

We divided 30 eligible participants into two groups (15 cases and 15 controls). Fifteen participants consumed probiotics during the infection phase, while 15 participants never consumed probiotics in the control group. We interviewed and collected the medical records data to detect the clinical complaint and cycle threshold value of Rt-PCR from the nasopharynx swab specimen. This study compared the duration of infection, cycle threshold value, and clinical complaints of the two groups of participants.

Sample collection

The hospital has a schedule to examine healthcare routinely after working in high risk of infection transmission places. After the participant underwent an examination, the research team interviewed each participant with a structured questionnaire. We got the value of laboratory examination results and duration of infection from the medical records.

Viral Load Analysis

We obtain the viral load data from the cycle threshold value of the RT-PCR nasopharynx swab.

Statistical Analysis

Classical descriptive indicators describe the characteristics of the studied samples. Data shows as mean ± Standard Deviation (SD) for continuous variables and as number and percentage for categorical variables. The student's T-test (normally distributed data) compares the duration of infection of the case and control groups. All reported p values are 2-sided, and significantly when $p \leq 0.05$. We performed statistical

analysis with commercially available software (SPSS for Windows version 21.0; SPSS Inc., Chicago, IL, USA).

Results

From June 2020 to October 2020, 30 participants were enrolled (15 consumed probiotics and 15 which no probiotics consumed), and their nasopharynx swab specimens were collected to be analyzed. Table 1 reports participants' characteristics. We found that there were no significant differences in the duration of infection and Ct value in the two groups. From the gender aspect, there was not an important message between the two groups. An essential piece of news came from the information of re-infection events that happened in the control group. But we still can not explain the mechanism of the re-infection event.

Table 1. Characteristics of participants enrolled in this study

Variables	Cases, n (%) n = 15 (50.0)	Controls, n (%) n = 15 (50.0)	p value
Mean age ± SD (years)	34.73 ± 5.612	33.47 ± 3.871	0.21#
Male/female, n (%)	9/6 (60 %/ 40 %)	6/9 (40% / 60%)	0.52~
Re-infection	0	2 (0.13 %)	
Duration of infection	15.20 ±13.078	21.40±14.401	0.22*
Mean Ct value	29.74±5.45	25.32±7.93	0.08*

#Kolmogorov Smirnov

*T-test Unpaired

~Chi-square

Discussion

In much literature, probiotics in the incidence of gastrointestinal disorders are beneficial. According to Xu K et al. (2020), the use of probiotics can reduce secondary infections due to microbial translocation in severe cases of COVID-19. In mild and moderate cases, it can inhibit the progression of COVID-19 [(9)]. But giving probiotics in severe and critical cases needs to be evaluated more deeply [(10)]. We moved to investigate the effects of probiotics in mild to moderate patients of COVID-19, whether symptomatic or not. At this initial stage, we only observed data that was easy to obtain in the field. In the future, we hope that the detailed research comes to strengthen our results in this study.

In this case-control study, we focus on the Lactobacillus strain that is the main compound in the prebiotics. The zero conversion time in the RT-PCR result for SARS COV-2 was the main target after consuming the probiotic. The results show in table 1. There were no significant differences in the duration of infection. An explanation for this result is the sample size of this study was small. The starting of the Ct value between the two groups were not significantly different. It means that the participants have a similar Ct value before consumed probiotics. The excellent information about this study was the re-infection did not happen in the case group. The explanation about this phenomenon was unexplainable. Many works of literature said that probiotics could modify the immune response.

Although our study did not show the analysis results of the response time to clear the infection faster than the control group, several previous theories still accepted to be proven by a better number of cases than we have done. The study we conducted showed that there was no re-infection in the case group. This phenomenon may

support the article published by Baud et al. (2020) that probiotics can flatten the case curve of COVID-19 [1].

Effects on Virus Titres Reduction

The results of our observation show that the average day of rt-PCR zero conversion in the case group was 15.20 ± 13.078 days. We could not show the real result of the length of duration infection because the hospital examined the healthcare on a fixed schedule (checked 7 days after the first positive result, then multiplies 7 days).

Study of Gender

Our study shows that 50 % positive for SARS COV-2 are male, while 50 % are female. This data did not support the previous data that mention males bigger than female patients.

Study of Age

The mean age of the subject in the case group was 34.73 ± 5.612 years old (table 1). The young generation of participants may cause an improvement of clinical appearance in our study. Much previous literature said that older age gives a lousy prognosis.

Study of RT-PCR Results

A Ct value < 40 cycles denotes a positive result for SARS-CoV-2, whether a lower value indicating a larger amount of viral RNA was a basic policy to adjust someone positive COVID-19.

Limitation of The Study

The sample size in this study was small, and the dose of probiotics did not record. We did not examine the RT-PCR every day, so we can not obtain the infection's actual duration.

Conclusion

The study considered consuming probiotics for the prevention and treatment of COVID-19—further research based on the data we have obtained highly expected to solve the COVID-19 problem worldwide.

Ethical Clearance: Ethical clearance was taken from Health Research Ethics Committee Dr Soetomo General Academic Hospital

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Dentigerous Cyst Mimicking Odontogenic Keratocyst: A Rare Case Report with 12 Month Follow up

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Abstract

Dentigerous cyst is one of the most commonly seen odontogenic cyst in children. It is characterized by a unilocular radiolucent lesion that encloses permanent tooth buds. Buccal bony expansion and a missing tooth is the most common clinical feature. Various treatment modalities have been mentioned in the literature for management of dentigerous cysts. This case report presents the management of a left mandibular dentigerous cyst in a 10-year-old boy by surgical enucleation and its follow up.

***Key Words**: Dentigerous Cyst, Mandible, Molar.*

Introduction

A dentigerous cyst is a well-defined, odontogenic lesion that surrounds the crown of an unerupted tooth arising from the epithelial remnants of the tooth forming organ.[1] It can be developmental or inflammatory in origin and is usually asymptomatic unless infected. [2] In children and adolescent, increased incidence of dentigerous cysts and Keratocystic Odontogenic Tumour (KCOT) are found when compared to other pathologies. [3] Dentigerous cyst is managed by enucleation and marsupialization.[4] In this case report we present a case of unilateral dentigerous cyst resembling a KCOT in a 10 year old boy with its management and a 12 month follow up.

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Case Report

A 10-year-old male boy had visited the Department of Pediatric dentistry with a swelling since 3 days in relation to lower left back region of face. No history of pain, fever or other systemic symptoms were present. On extra oral examination (Fig 1A), there was a hard localized unilateral swelling of size 1.5x1.5cm on the lower left lateral aspect of cheeks inferior & distal to the lip commissure with no surface redness or increase in surface temperature. Intra oral examination (Fig 1B) showed restored lower left second primary molar with grade II mobility but no tenderness on percussion. There was a localized bony hard non tender swelling of the buccal and lingual gingival region extending from distal aspect of 73 to the mesial aspect of 36 with obliteration of buccal vestibule and expansion of the buccal and lingual cortical plates.

A panoramic radiograph (Fig 1C) showed endodontically treated 75 with complete root resorption and a unilocular well defined oval radiolucency measuring 2x1.7cm in relation to the tooth bud of 35 extending from the mesial aspect of 34 to the mesial aspect of 36. Based on the clinical and radiographic

findings, a provisional diagnosis of dentigerous cyst was made.

To evaluate the lesion three dimensionally a CBCT scan was advised which (Fig 1D,1E, 1F) showed a well-defined radiolucency measuring 17.85x20.63x18.25 extending from the periapical region of 34 to 36 and surrounding the crown of developing 35. There was expansion of buccal and lingual cortical plates with thinning of the plates and partial loss of buccal cortical plate along with the root resorption of 75. Based on the above findings from CBCT a diagnosis of Keratocystic odontogenic tumour (KCOT) was given.

A confirmed diagnosis of the pathology can be obtained only after histological examination. Management of KCOT requires a more aggressive approach with marginal bone resection [5] along with complete removal of the lesion when compared to that of dentigerous cyst wherein enucleation [2] is the standard approach. Considering these factors and the age of the child, surgical enucleation of the cyst under general anesthesia (GA) was planned after consulting with the Department of Oral and Maxillofacial Surgery and after obtaining informed consent from the parent.

Extraction of 75 was done following which buccal and lingual flaps were raised to visualize the lesion under General anesthesia. Part of the thin and friable buccal cortical plate was removed following which the entire cystic lesion was enucleated (Fig 1G,1H,1I). As the cyst was firmly attached to the neck of the developing 35 it was decided to extract it. Following saline irrigation the flaps were closed with non resorbable sutures. The biopsy specimen was sent for histopathologic examination.

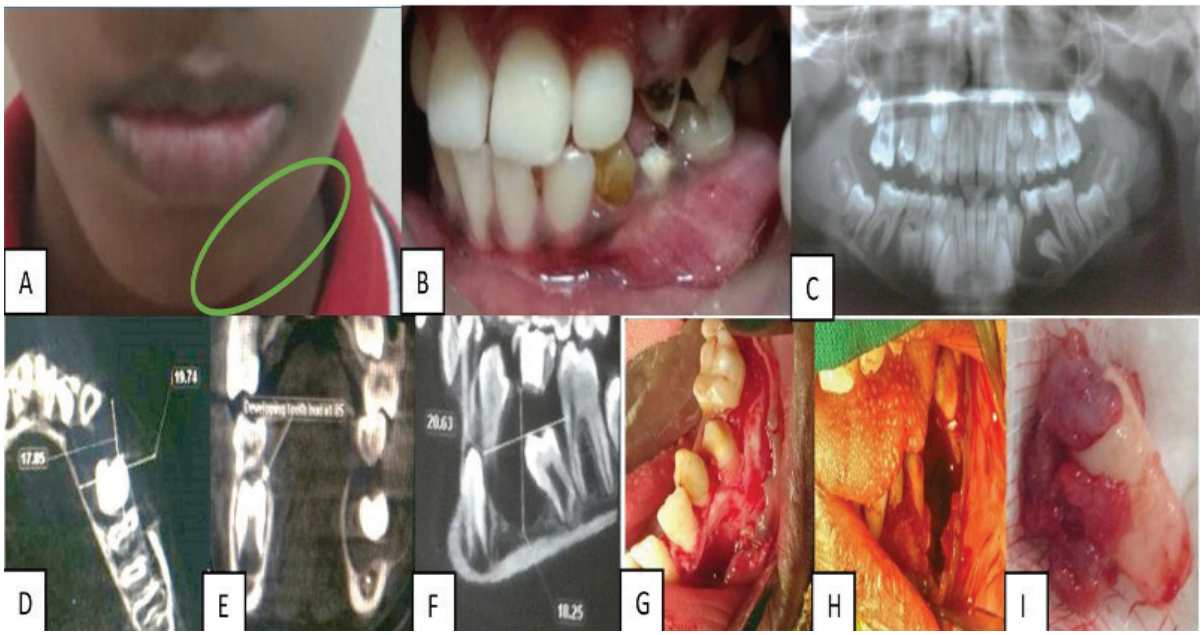

Fig 1: (A) Extra oral swelling on the left side. (B) Left buccal vestibule. (C) Orthopantomogram (D, E and F) CBCT (G) Intraoral vestibular incision (H and I) Removal of second deciduous molar with cyst.

Histopathologic evaluation revealed cystic lining of 2-3 cell layers of non-keratinized stratified squamous epithelium with fibro cellular connective tissue capsule which was attached to the cemento enamel junction of the tooth giving a confirmed diagnosis of dentigerous cyst (Fig 2A,2B).

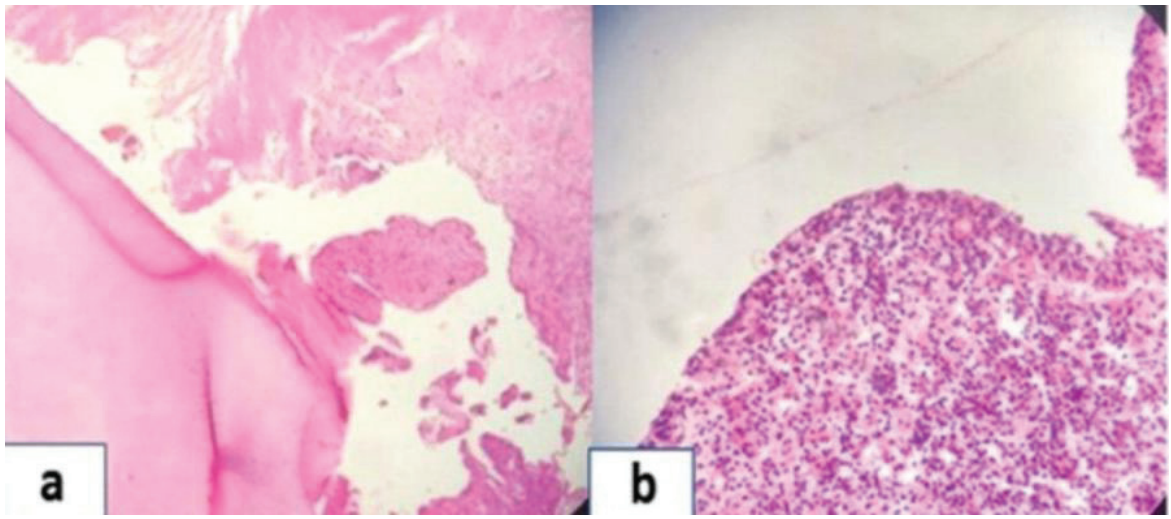

Fig 2: (A) Cystic lining attached to the CEJ (B) Stratified squamous epithelium lining the wall.

Patient was recalled after 1 week for suture removal and follow up visits at 1, 3, 6 & 12 months. During follow up visits, (Fig 3A,3B) uneventful healing of the lesion was noted.

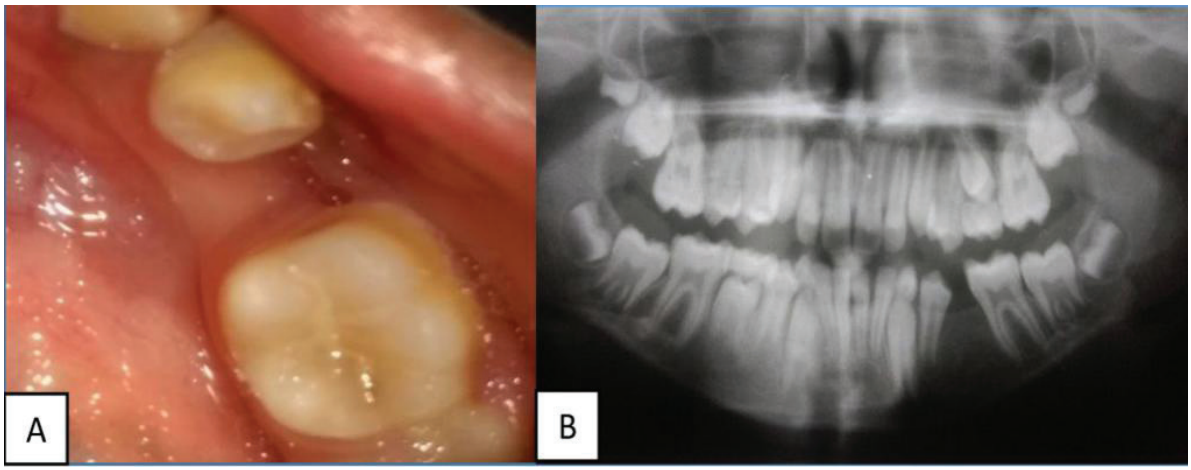

Fig 3: (A and B) Post op photograph and OPG at 12 months.

Discussion

Odontogenic cysts are among the commonest lesions encountered in children.[5,6] According to Manor et al[7] among the cystic lesions seen in children, developmental cysts were found at higher rate. A prevalence study by Li et al[3] on the occurrence of developmental cysts in children showed that 97.8% of the encountered lesions were dentigerous cyst and Keratocystic Odontogenic Tumour (KCOT). They also found that an increased risk of occurrence of KCOT as compared to dentigerous cyst in adolescent. This is in accordance with Tkaczuk et al[8] wherein out of 57 patients with true cystic lesions 33% were keratocystic odontogenic tumors and 30% were dentigerous cysts.

In children a dentigerous cyst may arise from an infected carious predecessor tooth or due to the continuous irritation of the follicle of the permanent tooth bud by obturating material of the treated primary tooth.[2] In the present case dentigerous cyst was of inflammatory origin developed from the pulpectomy treated deciduous second molar. Management of dentigerous cysts is commonly done by surgical enucleation and curettage with removal of the impacted tooth or teeth. However, if the lesion has larger dimensions and is causing tooth displacement and involves multiple teeth with extensive loss of bone marsupialization or decompression,

followed by enucleation later on can be attempted.[2]

In the present case, there was mixed opinion regarding diagnosis of the cyst suggested from OPG and CBCT, but considering age of the patient, enucleation of cyst was done with extraction of the associated tooth. On surgical exposure the cystic lining was fibrous and thick as opposed to the thin friable lining usually seen associated with KCOT. The lesion was removed as a whole which is difficult in case of KCOT. Extraction of developing second premolar was done as the lesion was found to be firmly attached surrounding the crown. The biopsy report confirmed the lesion as a dentigerous cyst due to presence of fibrous capsule that was attached at the CEJ which is characteristic of dentigerous cyst.[1]

Conclusion

Management of odontogenic lesions like dentigerous cyst in children can be challenging. Thus it is important to understand the nature of such lesions thoroughly backed by good clinical history and radiographic aids which help the clinicians to arrive at proper treatment protocol.

Conflict of Interest: No conflict of interest present.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient's parents have given their consent for images and other clinical information of the patient to be reported in the journal. The patient's parents understand that the name and initials of the patient will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

Source of Funding: Self

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The Effect of Using an Exercise-Nutrition Program on Some Physiological Variables on Diabetes Patients

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Abstract

The tremendous and astonishing technological progress in the modern era in all different fields has led to a lack of human mobility and thus a lack of physical and physiological competence, which made him vulnerable to many diseases called hypokinetic diseases such as cardiovascular disease, obesity, lower back pain, diabetes, and high blood pressure. **The Study Problem:** Diabetes is one of the most common and widespread diseases today, and failure to control this disease will lead to many complications, including cardiovascular disease, high-fat content, in addition to limb muscle atrophy, high blood pressure and peripheral nerve inflammation, which sometimes leads to leg amputation. The cause of death and this is the source of the problem. **The Study Objectives:** Knowing the blood sugar level of non-athletic patients aged 40-55 years before the program. Designing an athletic program for diabetics aged 40-55 years who are non-athletes for the research group. To know the effect of the program on blood sugar, HDL cholesterol, and PCV blood viscosity for the research group. Identify the effect of the program on the nerve signals received from the upper and lower extremities of the research group. **The study community** the human field: people with diabetes who are 40-55 years old who are not athletes. from 1/3/2020 to 1/7/2020. Hospitals and specialized centers for treating this disease and the Physiotherapy Center for Sports Medicine in Egypt. **Results:** the values of the blood sugar test indicate the significance of the differences between the pre-and post-tests and in favor of the post-test, considering that the drop in blood sugar level is a positive case. blood HDL ratio test indicates the significance of the differences between the pre-and post-tests and in favor of the post-test, considering that the drop in blood sugar level is a positive case. blood HDL ratio test considering the high level of HDL cholesterol is a positive case. **Conclusions:** 1-The diet and exercise program has a positive effect on low blood sugar levels. 2-The sports and nutritional program have a positive effect on the level of high-density lipoprotein (HDL) cholesterol in the blood. 3-The sports and nutritional program have a positive effect on blood viscosity (PCV). 4-The sports and nutritional program have a positive effect on the nerve signals coming from the upper and lower extremities.

Keywords: *Diabetes, Exercise-Nutrition Program, sugar level, cholesterol, non-athletic, blood viscosity*

Introduction

The tremendous and astonishing technological progress in the modern era in all different fields has led to a lack of human mobility and thus a lack of physical and physiological competence, which made him vulnerable to many diseases called hypokinetic diseases such as cardiovascular disease, obesity, lower back pain, diabetes, and high blood pressure Diabetes is a chronic disease that occurs due to genetic or environmental factors, which is greatly influenced by exercise, diet and lack of excessive eating, which means a lack of insulin secretion, which increases blood sugar, diarrhea, and

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diabetes has several types, the most common of which is What is known as the first insulin-dependent type (IDDM) and the second non-insulin-dependent type (NIDDM). Diabetes causes complications and symptoms in the arteries as there is a confirmed association between diabetes and atherosclerosis and diabetes may lead to atherosclerosis and the emergence of serious symptoms at an early age. Unusual angina or a clot in a coronary artery, or hardening of the arteries of the brain or kidneys occurs, and it also leads to pain in the extremities. It is known that peripheral nerve infections are complications and symptoms of diabetes, and the feeling of them differs from one person to another, and among the most famous of these symptoms are skin such as boils, abscesses, and fungal infections between the fingers and between the thighs [1,2].

And as we learned that diabetes causes peripheral neuropathy, which in turn will have three main effects on the lower extremities: sensory, motor, and subjective, especially if we know that the main function of the sensory nerves in the lower extremity is to act as a warning system to perceive the sense that is protective of harm and that perception The immediate sense of protection from harm is very important for healing, so the importance of research lies in identifying the effect of exercise and regular food programs on some physiological changes in diabetics that must be recognized early and protected with appropriate care, and despite all these different and different symptoms of diabetes, Among the simple diseases, if the patient, in cooperation with his specialist doctor, improves on following the diet, treatment and physical activity regimen, and in return, diabetes is the most dangerous disease if he neglects his treatment. [3–8]

Research Problem:

Diabetes is one of the most common and widespread diseases today, and failure to control this disease will lead to many complications, including cardiovascular disease, high-fat content, addition to limb muscle atrophy, high blood pressure, and peripheral nerve inflammation, which sometimes leads to leg amputation. The cause of death and this is the source of the problem. Therefore, it is imperative to use all methods and means that help to avoid complications of this disease, as sports and movement play an important and great role in the lives of sick individuals in addition to the correct diet as studies indicate that physical and movement activity With both insulin and diet, it is considered the main source for curbing diabetes, and among those methods that help to avoid complications of this disease is to use a regular exercise and diet program for people with diabetes and to know the effect of the program on some of the physiological changes associated with the disease through daily monitoring of blood sugar level or diarrhea. And examining the flow of blood through the blood vessels using modern devices, as well as recording the nerve signals coming from the brain to the limbs, so that we can know the progress of the patient.

Research Objectives:

1- Knowing the blood sugar level of non-athletic patients aged 40-55 years before the program.

2- Designing an athletic program for diabetics aged 40-55 years who are non-athletes for the research group.

3- To know the effect of the program on blood sugar, HDL cholesterol, and PCV blood viscosity for the research group.

4- Identify the effect of the program on the nerve signals received from the upper and lower extremities of the research group.

Research Hypotheses:

1- The program has a positive effect on blood sugar level, an increase in HDL cholesterol, and blood viscosity (PCV).

2- The program has a positive effect on the nerve signals received from the extremities.

Research Areas:

1- The human field: people with diabetes who are 40-55 years old who are not athletes.

2- Time-domain: from 1/3/2020 to 1/7/2020.

3- Spatial domain: Hospitals and specialized centers for treating this disease and the Physiotherapy Center for Sports Medicine in Egypt.

Diet for Diabetics:

Diet is an essential pillar for controlling diabetes, whether the patient is treated with diet only, tablets, or insulin injections. The doctor or dietitian often organizes a specific diet program that is determined according to the patient's age, weight, height, degree of movement activity, and the type of sugar, so that the nutritional program is varied, taking into account the ease of choosing different food items that suit the patient's local and personal dietary pattern [17–20]. The patient should take into account the following things to reduce blood sugar levels:

1- The food should be varied and complete, the proportion of carbohydrates in it 50-60% of the daily calories needed, proteins 15-20%, and the rest of fats

2- Regularly eating meals on time, so that they are three main meals.

3- Avoid eating large amounts of food at the same meal

4- That the food is eaten is approximately equal in quantity every day

5- Avoid eating sugar-sweetened foods such as juice, chocolate, Halawa, jam, molasses, candy, and Arabic sweets.

6- It is advisable to eat more foods that contain a high percentage of fiber, as it is absorbed slowly from the digestive system and helps to avoid a sudden rise in blood sugar. These foods include vegetables, legumes, grains with their skin (brown bread), and most fresh fruits

7- It is recommended to replace animal fats with vegetable oils to avoid high blood fats to prevent heart disease and atherosclerosis [20–23].

Research methodology and field procedures

1-Research Methodology:

The researcher used the experimental method as it is the most suitable method for solving the research problem

(One group method).

2-Research Sample:

The research sample was selected randomly for diabetics who were not completely insulin-dependent and whose ages ranged between 40-55 years and from males with this disease. The sample is 20 patients, and it is finally ready to implement the required training and food program. The researcher found the average ages, lengths, and weights of the samples as shown in Table (1).

Table (1): It shows the mean and standard deviations of height, weight, and age

Variables	Mean	SD
Age	48.11	5.55
Length	172.1	6.26
the weight	81.8	8.75

Search tools, devices, and utilities:

1-Research Tools:

1- Testing and measurement.

2- The interview.

2-Assistive devices and tools:

1- Doppler, a French-made device.

2- A German-made EMG, EEG (to measure the speed of nerve impulses).

3- Chemicals.

4- Pressure test device.

5- Syringes to draw blood 5 cm.

6- Bottles to hold blood samples with a volume of 10 cm.

7- A German-made micropipette.

8- An English-made photometer.

9- Sports equipment.

10- Electronic scale to measure height and weight.

11- Registration forms.

Field research procedures:

Filtering of sports exercises and nutrients for the sports and food program:

After reviewing many sources and references and conducting interviews with experts regarding exercise and foodstuffs that benefit diabetics, several exercises that help reduce blood sugar levels, in addition to appropriate nutrients for diabetics, have been selected.

Preparing the sports and nutritional program:

After the sports and nutritional program was designed and after presenting it to some of the specialists who were mentioned on the previous page in the sports and medical side to evaluate it in terms of the vocabulary it contains and the extent of its suitability for the research sample and the stresses contained in the program and the extent of the ability to apply exercises, especially since they are diabetic and so that we can achieve The desired goal was the specifications of the sports program as follows ...

· The period of the program lasted about (12 weeks) of training, at the rate of (3 training units) for each week and the days of Saturday - Monday - Wednesday.

· Total training units (36 training units).

· The time of one training unit (45 minutes).

· As for the method of administering the program, the researcher relied on the method of gradation in intensity.

While the nutritional program came parallel to the athletic program, see Supplement (2) the nutritional program.

Exploratory Experience:

The researchers conducted the exploratory experiment before starting the original experiment to control the measurement variables and identify the obstacles facing the researcher during the research period, starting from taking blood samples and measuring some physiological variables represented (high-density HDL cholesterol, PCV blood viscosity, neurological instructions coming from the limbs, and the percentage Blood sugar) and the extent of patients' acceptance of exercise, the availability of devices, their validity, and the ability of the assisting team to implement the program, so the experiment was conducted on 1/3/2020 at 10 am on 3 NIDDM types II diabetics. And to ensure the accuracy of the laboratory results obtained, the test was repeated after 7 days under the same conditions, and indeed the results were identical accurately.

Pre-Exams:

The tests are among the most important methods used in scientific research, using which the necessary information for scientific progress is collected [(9)]. The following tests were carried out on the research sample in the laboratories of Hospital in Egypt, namely (fasting blood sugar, HDL cholesterol, PCV, blood viscosity, the nerve signals received from the parties) on 12/3/2020 at ten in the morning, without eating breakfast.

Post Tests:

After completing the application of the sports and nutritional program, the post-tests were conducted in the same place and time and under the same conditions in which the pre-tests were conducted on 17/6/2020, and these results were taken to conduct the appropriate statistical treatments.

Statistical methods:

- Mediator
- Spring deviation
- Wilcoxen's test

Presentation, analysis, and discussion of the results:

Presentation, analysis, and discussion of the results of sugar, HDL, and PCV of the research sample:

Table (2): It shows the pre-and post-tests of the median, the spring deviation, and the Wilcoxen test of the research sample for the ratio of sugar, HDL, and blood viscosity ratio (PCV) in diabetics

Variables	Pretest		Post-test		Wilcoxen value		p-value	significance level
	Median	Interquartile Range	Median	Interquartile Range	Calculated	Tabular		
Blood sugar level	177	1	103	1	0	8	0.05	significance
HDL blood level	22	2	28	1	1	8	0.05	significance
Blood viscosity ratio	50	1.5	45	0.75	0	8	0.05	significance

Measuring unit: mg / 100 ml

From Table (2) it can be seen:

In the blood sugar test: the median of the research sample for the pretest test reached (175) with a spring deviation of (1), while the median in the post-test reached (105) and a spring deviation of (1) and the calculated Wilcoxen value was (zero), which is less than the tabular value The amount (8) is at the level of significance (0.05) and in front of the sample size (20), which indicates the significance of the differences between the pre and post-tests and in favor of the post-test, considering that the drop in blood sugar level is a positive case.

In the blood HDL ratio test: the mean of the research sample in the pretest was (21) with a spring deviation of (2), while the mean in the post-test reached (27) and a spring deviation of (1) and the calculated Wilcoxen value was (1), which is less than the value The tables and amounts (8) at the level of significance (0.05) and in front of the sample size (20), which indicates the significance of the differences between the pre and post-tests and in favor of the post-test, considering the high level of HDL cholesterol is a positive case.

In the PCV test: the median of the research sample for the pretest test reached (48) with a spring deviation of (1.5), while the mean in the dimensional test was (46) and a spring deviation of (0.75) and the calculated Wilcoxen value was (0) It is less than the tabular value of (8) at the level of significance (0.05) and in front of a sample size (20), which indicates the significance of the differences between the pre and post-tests and in favor of the post-test because the decrease in blood viscosity means a positive condition. The tests indicated that there is a reciprocal effect between what has been achieved of the quantities of development in the level of sugar, HDL, and PCV in the blood, which were caused by the sports and nutritional program prepared by the researcher based on age and type of diabetes, which included a clear decrease in the concentration of these two substances in the blood, as most studies and specialized research indicated that I studied the effect of exercise and nutritional programs on diabetics and their effect on reducing blood sugar levels. Participation in regular exercise leads to a reduction in insulin needs and an increase in the sensitivity of cells to insulin in diabetics and normal people by increasing receptors. Insulin

"studies also indicated that the diet has a clear link with diabetes, as" avoiding excessive food and adhering to specific food quantities by a nutritionist helps to regulate blood sugar and without it, diabetes cannot be controlled and its possible complications avoided "[16], as he sees Some researchers believe that exercise and nutritional programs greatly help to regulate blood sugar, reduce harmful fats, and increase High-density fats that protect against heart disease, atherosclerosis, reduce blood pressure, and most importantly of all, increase the effectiveness of insulin. When exercising, the effect of insulin on muscle tissue improves, thus increasing the consumption of blood sugar, as well as the effect of insulin on the liver increases and the rate of sugar release from it decreases [4,12] Some scientists also confirmed that practicing sports activity and following an organized diet leads to reducing the risk of developing atherosclerotic diseases caused by high blood sugar to an abnormal limit [11,13] and that increasing the level of HDL in the blood of the research sample works on the reverse transfer of cholesterol in the tissues to the liver to be Disposal, and that this increase means the positive relationship to the practice of sports activity [17,18] , and the lack of sources for the variable PCV blood viscosity, especially in the sports field, the researcher called for an explanation of the reason for the low blood viscosity in diabetics, which is the result of a decrease in the proportion of low-density harmful fats and an increase in high fats The density that protects against heart disease, atherosclerosis, lowers blood pressure, and most importantly of all, increases the effectiveness of insulin and smooth blood flow in blood vessels without p Fit.

Presentation, analysis, and discussion of the results of the speed of nerve impulsion in the middle sensory and motor nerve of the upper limb:

Table (3): It shows the pre-and post-tests of the median, the spring deviation, and the Wilcoxen test of the research sample for the velocity of nerve impulse in the sensory and average motor nerve of the upper extremity in diabetics

Variables	Pretest		Post-test		Wilcoxen value		p-value	significance level
	Median	Interquartile Range	Median	Interquartile Range	Calculated	Tabular		
The median sensory nerve (latency)	3.85	0.25	2.5	0.25	2	8	0.05	significance
The median sensory nerve (velocity)	28	2.5	45.5	2.5	0	8	0.05	significance
The median motor nerve (Latency)	4.5	2.5	3	0.25	1	8	0.05	significance
The median motor nerve (Velocity)	22	3	49	1.5	0	8	0.05	significance

Measuring unit: the mm \ s sensory nerve - the mm motor nerve.

From Table (3) it can be seen:

- In the test of the signal velocity of the median sensory nerve of the upper limb: the mean for the pre-test was (3,75) with a spring deviation of (0.25), while the mean for the post-test was (2.5) and with a spring deviation of (0.25), while the calculated Wilcoxen value was (2) and it is less than the tabular value of (8) at the level of significance (0.05) and in front of the sample size (20). This indicates the significance of the differences between the pre-and post-tests and in favor of the post-test, considering that the decrease in values means increasing the speed of instruction Nervous.

In the velocity test of the median sensory nerve signaling: Velocity

The mean in the pre-test reached (27.5) with a spring deviation of (2.5), while the mean in the post-test reached (45) and a spring deviation of (2.5). As for the calculated Wilcoxen value, it was (zero) which is less than the value Table (8) at the level of significance (0.05) and in front of sample size (20). This indicates the significance of the differences between the pre-and post-tests and in favor of the post-test.

Latency Test:

The mean in the pre-test reached (4) with a spring deviation of (0.25), while the mean in the post-test reached (2.5) and a spring deviation of (0.25), while the calculated Wilcoxen value was (1), which is less than the tabular value The amount of (8) is at the level of significance (0.05) and in front of the sample size (20) and since the calculated value is less than the tabular, this indicates the significance of the differences between the pre and post-tests and in favor of the post -Test because the decrease in the values indicates an increase in the speed of the nerve impulse in the average motor nerve.

Velocity Test:

The mean in the pre-test reached (20) with a spring deviation of (3), while the median in the post-test reached (47.5) and a spring deviation of (1.5) and the calculated Wilcoxen value was (zero), which is less than the tabular value of (8) At the level of significance (0.05) and in front of the sample size (20), and when the calculated value is less than the tabular, this indicates the significance of the differences between the pre and post-tests and in favor of the post-test because increasing the values means increasing the speed of the nerve impulse.

Presentation, analysis, and discussion of the results of the speed of nerve impulsion in the sensory and motor nerve of the lower extremity:

Table (4): It shows the pre-and post-tests of the median, the spring deviation, and the Wilcoxen test of the research sample for the speed of nerve stimulation in the sensory and motor nerves of the lower extremity in diabetics

Variables	Pretest		Post-test		Wilcoxen value		p-value	significance level
	Median	Interquartile Range	Median	Interquartile Range	Calculated	Tabular		
Sensory nerve (Sural \ Latency)	5	0.5	3	0.25	1.5	8	0.05	significance
Sensory nerve (Velocity \ Sural)	26	2	47	2	0	8	0.05	significance
Posterior tibial motor nerve (Latency)	5	1	2.5	0.5	1	8	0.05	significance
Posterior tibial motor nerve (velocity)	3.5	1.5	2.5	0.25	0	8	0.05	significance
Common fibular motor nerve (Latency)	5	1	3	0.25	0	8	0.05	significance
Common fibular motor nerve (Velocity)	4.75	0.75	2.75	0.5	1	8	0.05	significance

From Table (4) it is found:

In the Sural Latency test for the lower extremity:

The mean for the pre-test for the research sample was (4) with a spring deviation of (0.5), while the mean for the post-test was (2.75) and a spring deviation of (0.25), while the calculated Wilcoxen value was (1.5), which is less than the value. Table (8) is at the level of significance (0.05) and in front of the sample size (20). This indicates the significance of the differences between the pre-and post-tests and in favor of the post-test because the decrease in the values means an increase in the speed of the nerve impulse.

Sural / Velocity nerve stimulation test:

The median in the pre-test of the research sample reached (25) with a spring deviation of (2), while the median in the post-test reached (46) and a spring deviation of (2) and the calculated Wilcoxen value was (zero) which is less than the tabular value of (8) at the level of The significance of (0.05) and in front of the sample size (20), which indicates the significance of the differences between the pre and post-tests in favor of the post-test, given that the increase in the values indicates an increase in the speed of the nerve impulse in the sensory nerve.

Latency Test:

The mean in the pretest was (3,5) with a spring deviation of (1.5), while the mean in the post-test was (2.25) and a spring deviation of (0.25). As for the calculated Wilcoxen value, it was (1), which is less than the tabular value of (8) at the level of significance (0.05) and in front of the sample size (20), which indicates the significance of the differences between the pre-and post-tests and in favor of the post-test.

Velocity testing of the nerve signaling of the posterior bronchial motor nerve:

The mean in the pre-test reached (3.5) with a spring deviation of (1.5), while the mean in the post-test reached (2.25) and a spring deviation of (0.25). As for the calculated Wilcoxen value, it was (zero), which is less than the tabular value of (8) at the level of significance (0.05) and in front of the sample size (20), which indicates the significance of the differences between the pre-and post-tests and in favor of the post-test.

Latency Test:

The mean in the pre-test reached (4.5) with a spring deviation of (1), while the mean in the post-test reached (2.75) and a spring deviation of (0.25). The calculated Wilcoxen value was (zero), which is less than the tabular value of (8) At the level of significance (0.05) and in front of the sample size (20), this indicates the significance of the differences between the pre-and post-tests in favor of the post-test.

Velocity Test:

The mean in the pre-test reached (4.25) with a spring deviation of (0.75), while the mean in the post-test reached (2.5) and a spring deviation of (0.5). As for the calculated Wilcoxen value, it was (1), which is Less than the tabular value of (8) at the level of significance (0.05) and in front of the sample size (20), which indicates the significance of the differences between the pre-and post-tests and in favor of the post-test. Through the previous two tables, these tests represent the patient's special abilities in limb mobility the upper and lower extremities, which were affected greatly by the aerobic exercises and the nutritional program that the research sample followed and placed by the researcher, had a clear effect on increasing the speed of nerve signals to the lower and upper extremities, which was confirmed by many studies that "the reason behind nerve damage is due to the increase in blood sugar. Which damages the wall of the blood vessels that supply the nerves, and that the symptoms that occur depend on the type of nerve that the injury occurred to, and in most cases the injury occurs in the sensory nerve in the legs and sometimes the arm, as a result of diabetes "[7,27,28], and the high blood sugar for a long period leads to morbidity Sensory and motor neurosis as a result of the formation of harmful substances to these nerves, as well as due to the impact of the small blood vessels that feed these nerves, and this causes a lack of perfusion to these nerves and thus to their damage [7,26,27]. Also, the development occurring in the speed of nerve impulses Sensory and kinesthetic in the dimensional tests came in line with the statistically significant development of blood sugar that was previously discussed, and it is known that neuropathy is a difficult problem in the treatment of diabetes and

it is difficult to describe exercise. This is because the physiological changes associated with exercises such as high blood pressure and acceleration of the heartbeat depend on the integrity of the nerves, and therefore it may disappear in patients with diabetes. Baths or similar places, where the average person depends on these symptoms to feel tired during exercise and thus stop the exercise. The absence of it in diabetics removes this beneficial feeling [14,15,31].

Conclusions and Recommendations

Conclusion:

the researchers came up with the following conclusion:

1- The diet and exercise program has a positive effect on low blood sugar levels.

2- The sports and nutritional program have a positive effect on the level of high-density lipoprotein (HDL) cholesterol in the blood.

3- The sports and nutritional program have a positive effect on blood viscosity (PCV).

4- The sports and nutritional program have a positive effect on the nerve signals coming from the upper and lower extremities.

Recommendations:

In light of the results reached by the researchers regarding his research topic, the researchers recommend the following:

1- The necessity of education and education on the importance of regular exercise for diabetics.

2- Emphasis on following a suitable diet, especially for overweight people, to reduce weight and thus reduce blood sugar levels.

3- The necessity of self-monitoring of sugar at home and laboratory tests of the patient to avoid an increase in the level of sugar and its risks.

4- Emphasis on the use of aerobic exercises in the treatment of diabetics.

5- Seeking to conduct similar research in which it deals with the effect of exercise on the vision for diabetics.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from the departmental committee

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Comparison between the PaxView TB/NTM MPCR-ULFA Kit and Xpert MTB/RIF for *Mycobacterium Tuberculosis* Detection in Indonesian Clinical Isolates

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Abstract

The PaxView TB/NTM MPCR-ULFA Kit, which targets the IS6110 and mtp40 genes for Mycobacterium tuberculosis (MTB) detection, is a novel tool that substitutes gel electrophoresis for universal lateral flow assays. The sensitivity and specificity of this method were compared with those of established methodologies using Indonesian clinical isolates. In this study, 317 sputum specimens isolated from tuberculosis (TB) suspects were examined to evaluate the performance of the PaxView TB/NTM MPCR-ULFA Kit compared to that of smear microscopy and the Xpert MTB/RIF assay. Out of 317 cases, the rate of TB-positive samples evaluated by different methods was 33.4% (106/317; 95% CI 28.2-38.6) for smear microscopy, 37.9% (120/317; 95% CI 32.5-43.2) for the Xpert MTB/RIF, and 40.7% (129/317; 95% CI 35.3-46.1) for the PaxView TB/NTM MPCR-ULFA Kit. Compared to the Xpert MTB/RIF as a standard reference, the PaxView TB/NTM MPCR-ULFA Kit was found to possess a 92.5% sensitivity (111/120; 95% CI 87.8-97.2), a 90.8% specificity (179/197; 95% CI 86.8-94.8), 86.0% PPV (111/129; 95% CI 80.0-92.0), and a NPV 95.2% (179/188; 95% CI 92.2-98.3). The PaxView TB/NTM MPCR-ULFA Kit could be a useful molecular diagnostic tool to identify MTB in clinical samples in resource-limited countries, as this procedure is more cost-effective and sensitive than the Xpert MTB/RIF, and more convenient than conventional PCR gel electrophoresis approaches.

Keywords: *Mycobacterium tuberculosis, multi-plex PCR, universal flow lateral assay, Xpert MTB/RIF, smear microscopy*

Introduction

To this day, tuberculosis (TB) continues to be a major chronic infectious disease worldwide. According to the 2019 Global TB Report, the WHO estimated that 1.4 million people died due to TB and 10.0 million people developed TB worldwide in 2019. The current estimated tuberculosis incidence in Indonesia is 845,000, which ranks second globally, only surpassed by India. In Indonesia, notifications of people newly diagnosed with TB rose from 331.703 in 2015 to 562.049 in 2019. However, there is still a large global gap between the estimated number of incident cases and the number of people newly diagnosed (7.1 million in 2019), due to underreporting of detected cases and underdiagnosis, and Indonesia accounted for 10% of the global gap. It has been estimated that 96,700 people died from tuberculosis in 2019 in Indonesia[1]. Most deaths from TB could be prevented with early diagnosis and appropriate treatment[2,3], and therefore early TB diagnosis is essential to reduce its worldwide lethality.

There are many kinds of TB diagnostic tools, from the smear microscopy (SM) approach developed by Robert Koch in 1882 to modern sophisticated approaches including Xpert MTB/RIF (Cepheid, CA, USA). Smear microscopy has become the main method for diagnosing

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pulmonary TB in low- and middle-income countries. The procedure is simple, fast and inexpensive and very specific in areas with a very high prevalence of TB. The main drawback of SM is its limited sensitivity when the bacterial load is less than 10,000 organisms/mL[4,5].

Among the various existing TB diagnostic tools, molecular diagnostic tools are the fastest methods for the detection of *Mycobacterium tuberculosis* (MTB) in clinical specimens, and also possess satisfactory sensitivity and specificity[6]. The development of the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA), which was endorsed by WHO in 2010, was a major step forward in improving the diagnosis of TB and rifampicin-resistant TB (RR-TB) globally. Compared with the reference standard of culture, however, it still had suboptimal sensitivity (particularly among people with smear-negative TB and people living with HIV) and specificity[1]. And due to the expensive equipment, cartridges, and installation costs required by the Xpert MTB/RIF approach, this testing solution is not suitable for implementation in developing countries or in private sector unless international monetary aid is provided[7,8].

In house-PCR is the cheapest molecular diagnostic method, but requires cumbersome gel electrophoresis procedures. Therefore, PaxGenBio (Korea) developed the PaxView TB/NTM MPCR-ULFA Kit, which rapidly detects MTB without the need for gel electrophoresis after polymerase chain reactions (PCRs). Moreover, this procedure can be used with a conventional PCR instrument without the need for a specific expensive PCR device. Therefore, this study aims to evaluate the potential clinical applicability of the PaxView TB/NTM MPCR-ULFA Kit for the detection of MTB in clinical samples by comparing its performance with that of the Xpert MTB/RIF and SM, both of which have been extensively implemented worldwide.

Methods

Specimens

In this study, we utilized sputum specimens from TB suspects, which were acquired from Karang Tembok Hospital and Ibnu Sina Gresik Hospital from July 2019 to March of 2020 in the Surabaya City, East Java, Indonesia.

Ziehl-Neelsen Smear Microscopy

Ziehl-Neelsen direct AFB smear and grading was performed by the technicians from each institute. Briefly, smears are fixed on heated surface (60 °C for at least 10 minutes) and then flooded with carbolfuchsin (primary stain). Smears then are heated to almost boiling and are after the smears were allowed to sit for 5 min, then the slides are washed in distilled water. The slides were then decolorized with 3% HCI in 95% ethanol for approximately 1 minute and washed with water. The methylene blue (counterstain) was then flooded to the slide and allowed to sit for 1 min before the slides being washed with distilled water and let dry upright. The slides were then examined microscopically according to the International Union Against Tuberculosis and Lung Disease (IUATLD) method[9].

DNA extraction from specimens

After SM, the remaining sputum specimen was treated with Xpert MTB/RIF buffer. After proceeding Xpert MTB/RIF test, the remaining buffer treated sputum were stored in the refrigerator until maximum 20 days before DNA extraction from the TB bacilli for PaxView TB/NTM MPCR-ULFA test. DNA were extracted by the PaxView DNA Extraction Kit (PaxGenBio, Korea). Briefly, 1000 µl of the specimen pretreated with the Xpert MTB/RIF buffer was transferred into 1.5 ml screw capped tubes and then centrifuged at 13,000 rpm for 3 min. After the supernatant was discarded, 1000 µl of washing buffer were added (provided by the PaxView DNA Extraction Kit). This mixture was then centrifuged again at 13,000 rpm for 3 min, after that the supernatant was discarded. After washing the specimen once more, 100 µl of elution buffer were added into the tube, which was then transferred to a 95 ℃ heating block for 15 min. After centrifugation, 20 µl of supernatant were transferred into a new tube and used as a template.

Xpert MTB/RIF

Pretreated specimen was amplified with the GeneXpert kit following the manufacturer's instructions.

PaxView TB/NTM MPCR-ULFA Kit PCR and interpretation of the results

The PaxView TB/NTM MPCR-ULFA Kit includes multiple primer pairs, including two for MTB-specific

genes (IS6110 and mpt40), as well as for the mycobacteria rpoB gene. After multiplex PCR, the product identities were confirmed by universal lateral flow assay (ULFA), which is based on DNA-DNA hybridization with previously immobilized complementary DNA fragments on a nitrocellulose membrane. PCRs were performed with the following protocol: 50 °C for 4 min; 95 °C for 10 min; 25 cycles of denaturation (95 °C for 15 sec), annealing and extension (71 °C for 60 sec); 20 cycles of denaturation (95 °C for 15 sec), annealing (60 °C for 30 sec), and extension (72 °C for 30 sec).

After PCR amplification, 5 µl of PCR solution were added to the ULFA device inlet, after which 50 µl of running buffer (provided by the manufacturer) were added immediately. After 5 minutes, 50 µl of washing buffer were added into the inlet and the results were then read within 15 minutes.

Statistical Analysis

Demographic data were presented in mean±SD and percentages. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PaxView TB/NTM MPCR-ULFA Kit were compared to Xpert MTB/Rif as a reference standard using contingency tables 2×2.

Results

A total of 317 clinical specimens were compared the performances of the PaxView TB/NTM MPCR-ULFA Kit, SM, and the Xpert MTB/RIF. Demographic data was shown in Table 1. Out of 317 cases, the rate of TB-positive samples evaluated by different methods was 33.4% (106/317; 95% CI 28.2-38.6) for smear microscopy, 37.9% (120/317; 95% CI 32.5-43.2) for the Xpert MTB/RIF, and 40.7% (129/317; 95% CI 35.3-46.1) for the PaxView TB/NTM MPCR-ULFA Kit.

Table 1. Demographic Data of Study Participant.

Characteristic	Total (n=317)	%
Male Female	227 90	71.6% 28.39%
Age (year)	Mean 48.19±14.59	

Compared to the Xpert MTB/RIF as a standard reference, the PaxView TB/NTM MPCR-ULFA Kit was found to possess a 92.5% sensitivity (111/120; 95% CI 87.8-97.2), a 90.8% specificity (179/197; 95% CI 86.8-94.8), 86.0% PPV (111/129; 95% CI 80.0-92.0), and a NPV 95.2% (179/188; 95% CI 92.2-98.3) (Table 2)

Table 2. Performance of Pax View MPCR ULFA Compared with Xpert MTB/Rif as Reference Standard.

	Xpert MTB/Rif Positive		Xpert MTB/Rif Negative		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
SM	MPCR Positive	MPCR Negative	MPCR Positive	MPCR Negative				
Positive	96	3	7	0	92.5% (87.8 – 97.2)	90.8% (86.8 – 94.8)	86% (80.0 – 92.0)	95.2% (92.2 – 98.3)
Negative	15	6	11	179				
Total	111	9	18	179				

Nine samples (7.5%) of 120 Xpert MTB/RIF positive were PaxView TB/NTM MPCR-ULFA negative, and 3 (33.3%) of them were even SM positive. On the contrary, 18 samples (13.9%) of 129 PaxView MPCR-ULFA positive cases were Xpert MTB/RIF negative, and 7 (38.9%) of them were even SM positive. Compared to the Xpert MTB/RIF, the PaxView TB/NTM MPCR-ULFA Kit was found to possess a 92.5% sensitivity (111/120; 95% CI 88.4-97.4), a 94.2% specificity (179/197; 95% CI 90.9-97.5), a 91.5% PPV (111/129; 95% CI 86.6-96.3), and an NPV 95.2% (179/188; 95% CI 92.2-98.3) (Table 2).

Discussion

In this study, the TB detection rate of the Xpert MTB.RIF was 1.13 times (37.9%/33.4%) higher than that of SM, which is only a slight difference compared the 1.75-fold (84%/48%) in a study in Thailand[10], and 1.8-fold (65.5%/36.2%) in Ethiopia[11]. Generally, SM positive rates may vary depending on specimen quality, technician skill, and the number of samples treated in a day.

The PaxView TB/NTM MPCR-ULFA Kit showed a higher detection rate of MTB (40.7 percent) than that of the Xpert MTB/RIF (37.9 percent). In the study of another group on the detection of meningitis TB in India, the MPCR sensitivity was 1.7 times higher (87.2 percent /50.5 percent) than that of the Xpert MTB/RIF and 1.2 times higher (100 percent /82.9 percent) for pulmonary detection[12,13]. This may have been due to target gene differences, as the former is IS6110-specific while the latter is rpoB-specific. Generally, IS6110 has higher copy numbers than the rpoB gene, which only has one copy.

The challenge of using PCR based method for mycobacteria detection in clinical samples (especially sputum) is the extraction of nucleic acids from the samples, since they contain a unique lipid rich cell wall and rather difficult to lyse[14]. Three samples (2,5%) of 120 Xpert MTB/RIF positive were PaxView TB/NTM MPCR-ULFA negative and smear microscopy positive. This may have been caused by DNA loss during the DNA extraction step, as several manual washing steps are required for DNA purification.

The PaxView TB/NTM MPCR-ULFA Kit incorporates multiplex PCR and universal lateral flow assay (ULFA) and uses the IS6110 and mtp40 genes for TB detection. The IS6110 gene was widely known for its better sensitivity in diagnosing both pulmonary and extra-pulmonary TB, due to its multiple copies in the genome of the MTB complex[16]. MTB was detected more often by IS6110 PCR method than by smear microscopy and culture techniques among clinically diagnosed child TB patients[17]. The MTP40 gene is exclusively present in MTB, i.e., not in *M. bovis*, *M. bovis* BCG, or NTM[18]. The addition of the use of the MTP40 gene together with IS6110 has been shown to increase sensitivity[19]. But we did not analyze the sensitivity of each gene in this study. There were 11 samples out of 197 Xpert MTB/Rif negative were PaxView TB/NTM MPCR-ULFA positive and smear microscopy negative. This indicated that the use of the IS6110 and MTP40 genes in PaxView results in better sensitivity than the use of single rpoB gene.

The newly-developed PaxView TB/NTM MPCR ULFA Kit incorporates multiple polymerase chain reactions and simplifies the result-reading process by implementing ULFA instead of a cumbersome electrophoresis procedure. Another advantage of the PaxView MPCR-ULFA Kit is that it only requires a standard PCR device. In contrast, the implementation of Xpert MTB/RIF requires specialized and expensive equipment.

Although in-house PCR or MPCR targeting IS6110 has satisfactory sensitivity and cost-effectiveness compared to the Xpert MTB/RIF targeting the rpoB gene, in-house PCR has the disadvantages of being time-consuming and requiring a cumbersome gel electrophoresis step. Therefore, the PaxView TB/NTM MPCR-ULFA Kit substitutes the gel electrophoresis step with ULFA for rapid PCR result detection.

Conclusion

In conclusion, the PaxView MPCR ULFA Kit has an excellent capacity for the detection of MTB in clinical specimens, and this kit could be implemented in laboratories with standard PCR equipment, bypassing the need to purchase expensive equipment. Moreover, the results of this kit can be obtained easily and quickly, without the need for unpractical electrophoresis

procedures. Therefore, this approach could be very useful to detect MTB in clinical samples in conventional molecular laboratories in resource-limited countries or private sector.

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Ethical Approval: This study was approved by the Institutional Review Board of Airlangga University, under the project title "The evaluation of PaxView TB/NTM MPCR-ULFA compare to Xpert MTB/RIF for diagnosis of pulmonary tuberculosis" (Approval number: 164/EC/KEPK/FKUA/2019).

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Systemic Effects of Occlusal Disharmony on Corticosterone Serum Levels in Rattus Norvegicus

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Abstract

Background: Mastication process occurs over time and it can lead to occlusal reduction of the teeth. This causes reducing occlusal contact of teeth and it will lead to disharmony occlusion disorders. The occlusion disharmony is recognized by the body as a stressor that systemically affects the homeostasis in the body, especially the production of hormones.

Objective: The aim of this study was to determine the systemic effect of occlusal disharmony on corticosterone serum levels in Rattus norvegicus.

Method: The research method of this study used experimental design and conducted among 7 male Rattus Norvegicus in each groups. In intervention group, an occlusal reduction was ± 1 mm in all molars and for control group did not given any intervention. Moreover, at 1st day, 7th day, 14th day and 21st day was taken 2 cc of blood via infraorbital vein. The measurement of Corticosterone levels used Enzyme Linked Immunosorbent Assay (ELISA).

Result: Anova test results showed that corticosterone levels was significant difference between each groups ($p < 0.05$). Tukey HSD test showed there was a significant difference between intervention group and control group on 1st day, in intervention group on 7th day ($p < 0.05$).

Conclusion. The results of this study showed that there were differences of corticosterone serum levels among mice who experienced occlusion disharmony so it concluded that the dental occlusion disharmony had a systemic impact to corticosterone serum levels in Rattus norvegicus.

Keywords: *Occlusal disharmony, Corticosterone, Stress, Trauma occlusion, Temporomandibular disorders*

Introduction

The stomatognathic system is a unitary organ that has related functions each other. The organs are include mandible, maxilla, temporo mandibular joint (TMJ), tooth structures and other supporting structures such as masticatory muscles, facial muscles also head and neck. The stomatognathy system plays an active role in mastication or chewing process. In normal conditions, there is relationship and integrity of masticatory components system such as the teeth, muscles, TMJ, lips, cheeks, palate, tongue and salivary secretions[(1)].

If there is a problem in one of component system, it will impacts on occlusion [(1)]. Occlusion is a connection in jaw, where teeth of each jaw will connect on occlusion line curve. This occlusion is important for mastication process, speaking and swallowing processes. Occlusion is

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influenced by physiological and pathological conditions. The pathological condition of occlusion known as occlusal disharmony and it can be caused by several factors such as tooth loss, caries, attrition, periodontal tissue disorders, bruxism, and one-sided chewing habit [2]. Moreover, occlusal disharmony problems also can caused by systemic disorders such as cardiovascular disease, respiratory disorders, nutritional problems, diabetes, posture abnormalities and osteoporosis [3]. However, that mechanism of conditions are need further research.

Previous studies showed that occlusal disharmony affected a series of homeostatic processes in body, especially the regulation of Corticosterone hormone and bone metabolism [4]. This condition guests that occlusal disharmony as a stressor and affects physiopsychology of human, and stimulate the activity of hypopituitary system axis [5]. This situation is possible to increasing adrenocorticorticotropic hormone (ACTH) secretion and it influencing corticosterone secretion, so it will suppressing the immune system [6]. The immunology system can increase the inflammatory cytokines, such as interleukin (IL) -1, IL-6, tumor necrosis factor-alpha (TNF-α), and it activates the osteoclasts role. Osteoclasts are cells who scrapes the bone, dissolve bone and inhibit bone remodeling [7]. This condition causes reduction of mineral levels in jaw. Therefore, if the occlusion disharmony problems are not immediately prevented or treated, within a certain time period it will possible cause systemic problems and reduce quality life of the patient.

The aim of this study was to determine the systemic effect of occlusal disharmony on corticosterone serum levels in Rattus norvegicus. The measurement of corticosterone levels conducted at different time frames, it was to determining the trend if there was an increase in each variables. It is important to early detect and identify the abnormalities of bone and teeth caused by occlusal disharmony.

Materials and Method

This study used 7 male white rats of Rattus norvegicus in control group and intervention group, 8 weeks of old and 200-250 grams of weigh, and in good health status. Before conducted this research, all procedures obtained ethical research from ethics commission of Dental Faculty, Gajah Mada University, Yogyakarta. In intervention group, the researcher reduced occusal ± 1mm on molar teeth and prevented pulp perforation. The reduction procedure performed under anesthesia. While, the researcher didn't given any intervention in control group.

2 cc of blood serum was taken via infraorbital vein on 1st days, 7th day, 14th day and 21st day. Then the blood was put into a centrifuge tube and centrifuged at 1500 rpm for 20 minutes to get serum. The serum stored in deep freezer at -70 °C until all serum samples have been collected and are ready for corticosterone levels to be checked. The identification of corticosterone levels in serum used sandwich enzyme linked immunosorbent assay (ELISA) technique. The data analyzed by used One Way Anova and continued with Tukey HSD test with a significance p value <0.05.

Results

The results of this study showed that Corticosterone levels in serum become increased in intervention group on 7th day and become decreasing in intervention groups in 14th and 21st day (Table 1).

Table 1. Results of Corticosterone levels in serum (nmol/l)

Group	Mean ± Standard Deviation			
	Day-1	Day-7	Day-14	Day-21
Intervention	71.50±11,76	91.60±4.95	87.46±7.44	85.95±8.37
Control	70.38±12.65	71.56±16.52	74.01±8.17	74.64±3.84

The results of this study showed that the Corticosterone levels serum increased in treatment group on 7th day and it decreased in treatment groups on 14th and 21st day.

The Anova statistical test and continued with the Tukey HSD test to determine the average difference in each intervention groups. Anova test results showed that there was a significant difference in mean corticosterone levels of serum in experimental animals with p value = 0.003 ($p < 0.05$). The Tukey HSD test used to determine the differences of corticosterone levels between groups. The Tukey HSD test results showed showed that there was a significant difference of corticosterone levels between intervention group on 1st day and 7th day. The significant differences also showed between control group on 1st day and 7th day, and showed in intervention group on 7th day and control group on 7th day ($p < 0.005$).

The occlusal disharmony is a malocclusion which will reduce mastication function and lead to psychological stress [8]. Stress is psychophysiological response of an organism toward a disturbance or challenges [9]. Occlusal disharmony is recognized by a stressor body that affects a person's physiopsychology and stimulates the activities of axis hypopituitary system [6]. This condition can increasing the secretion of adrenocorticotropic hormone (ACTH) which affects the corticosterone secretion [6].

The results of this study showed that there was differences of corticosterone levels between control group and intervention group who experienced occlusal reduction as a form of occlusal disharmony. The increasing of corticosterone levels occurred on 7th day and decreasing on 14th and 21st day. In occlusal disharmony process, systemically the part of body (central nervous of brain system) received stressor. In the brain the stressor translated into a physiological process as a defense response from host. The part of brain stimulated hypothalamus, and play an important role to responding of stress. The hormonal system in hypothalamus start activate in HPA (hypothalamic-pituitary-adrenal) axis. The activated HPA axis way can stimulate the hypothalamus to secreting corticotropic releasing hormone (CRH) into the hypothalamic-pituitary portal bloodstream [10]. CRH stimulates the anterior pituitary to secreting ACTH. ACTH will circulate in blood and throughout to body until to adrenal glands. ACTH will influence the adrenal cortex to secreting glucocorticoid hormones. One of the secretion of glucocorticoid hormones during stress is corticosterone. Corticosterone secretion as a stress hormone and it will increase as a cellular physiological response [11].

The occlusal disharmony among experimental animals caused an increasing of corticosterone levels as an indicator of psychological stress [8]. The body's responses stress in 3 stages, first stage is warning reaction, second stage is resistance stage and third stage fatigue stage [12]. The highest level of corticosterone occurred on 7th day after occurred disharmony disorder. This assume that the resistance phase in intervention mice, where the pituitary secretes ACTH at their peak so the corticosterone secretion also reaches in highest level. Furthermore, the body's response will decline as a form of resistance to stress. This ndicated that the decreasion of corticosterone levels start at 14th and 21st day.

The increasing of corticosterone levels in intervention mice due to stress and lead to occlusal disharmony than affected host immune system. The effect on immune system can increase inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) which they activates the role of osteoclasts. The glucocorticoid stimulation secretes corticosterone, inhibits osteoblast activity and increases osteoclast activity [14]. Stimulated osteoclasts caused bone resorption, so it caused the extrication of calcium and phosphorus. The extrication of bone mineral will increases serum calcium and phosphorus levels [15]. In addition, glucocorticoids secretes corticosterone and inhibit calcium absorption in digestive tract [16]. This will reduce the bone remodeling process.

Based on the results of this study, it concluded that there were differences in corticosterone levels serum among mice who experienced occlusal disharmony. This suggests that the occlusal disharmony caused by occlusal reduction of teeth and it can causes stress and affects the corticosterone secretion.

Ethical Clearance: This study was obtained ethical clearance from the ethics commission of Scince Faculty of Gajah Mada University, Yogyakarta.

Conflict of Interest Satatment: The authors declare that they have no conflict of interests regarding the publication of this paper.

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Demographic Pattern of Covid 19 cases in and around Vijayawada, Andhra Pradesh

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Abstract

COVID-19 Pandemic has brought a threatening challenge to the world and as well as for Indian society and economy. In India, it has become a public health disaster and its' intensity increasing continuously. For the disaster risk reduction, and capacity building against the COVID-19 pandemic understanding of the relationship between socio-environmental conditions with the pandemic is very necessary. India is the 2nd largest country in terms of population and 6th leading economy in the world. This study represents the hospitalized patients with COVID-19. In this cohort, significantly more patients admitted to hospital were found to be in lower socioeconomic groups. patients with comorbidities should take all necessary precautions to avoid getting infected with SARS CoV-2, as they usually have the worst prognosis. These precautions include regular handwashing with soap and water or use of alcohol-based hand sanitizer, limiting person-to-person contact and practicing social distancing, wearing a face mask in public places, and overall limiting going to public areas at this time unless it is necessary.

Key words: *covid 19, challenge, disaster, environmental, precautions*

Introduction

World Health Organization (WHO) had declared Coronavirus disease 2019 (COVID-19) as global pandemic on 11th March 2020. Initially identified as novel beta-coronavirus (2019 nCoV) by Centre for Disease Control (CDC), International Committee on Taxonomy of Viruses officially renamed it as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS COV-2)[1] Since the onset of 1st cluster of unusual pneumonia cases in Wuhan in late December 2019, the rapid human to human transmission of virus via droplets and close contacts resulted in unprecedented, swift and frantic global spread affecting 215 countries and territories with confirmed cases globally more than 45 lacs and in India more than 90000 (as on 17th May 2020)[2]

WHO recommends following infection prevention and control (IPC) measures to mitigate COVID-19 spread: 1. Hand washing; 2. Social distancing of 6 feet; 3. Avoid touching mucus membrane of mouth, eyes and nose; 4. Respiratory protection and hygiene by using personal protective equipment (PPE), masks; 5. Identification of infected personnel and contact tracings; 6. Travel restrictions.[3] In India, the first case of COVID-19 was reported on 30th Jan 2020 in Thrissur, Kerala. With outbreak of cases within various states and union territories, the largest COVID-19 national lockdown was enforced from 24th March 2020 onwards, with exception to those associated with essential commodities.[4] Parallel establishment of isolation and quarantine centres, lab services, research and development, deployment of medical staff and procurement of PPEs were streamlined. These stringent drastic IPC measures were intended to flatten epidemic

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curve as well as prepare for possible surge.. With the population of 1.3 billion, implementation of social distancing faces a generic challenge in industrial zones accommodating gig-economy workers and slums that have unequal access to necessities including food, water, medicine, and sanitation supplies. Often, socio-political factors, economic interests, feasibility for implementation, and panic emotion influence decision-making process for any proposed measure.

Widely believed to have originated in the Hubei province in China in November, 2019, COVID-19, the infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is spreading fast across the world and was declared a pandemic by WHO on March 11, 2020.

As of July 5, 2020, the number of confirmed cases of COVID-19 worldwide stood at more than 11·2 million, with more than 529 000 deaths. 213 countries and territories have reported confirmed cases of coronavirus. In India, the first case was reported on Jan 30, 2020, among a group of students who arrived in the southern state of Kerala from Wuhan, the epicentre of the outbreak at that time. As of July 5, 2020, in India, more than 648 300 confirmed cases have been reported, of which around 235 433 (36%) were active, 394 227 (61%) recovered, and 18 655 (3%) died. These cases were spread over 35 (97%) of 36 states and union territories and 627 (98%) of 640 districts of the country.

Materials and Methods

This study has been carried out, in the year 2020, after approval from the ethical committee of NIMRA Medical College/General Hospital, 150 covid patients were conducted in the NIMRA medical college, Ibrahimpatnam

All the cases were examined in the NIMRA Medical Hospital, Vijayawada. The victims were said to have identified of covid 19 during the period from 1st April 2020 to 30th September 2020 The factors taken to enumerate the study are age, sex, occupation, socio economic status and comorbidity conditions

Observations and Results

The total admitted cases from 01st April 2020 to 30th September 2020 in NIMRA Medical College and General Hospital, Vijayawada were 150 . These cases were taken into study and the following observations were obtained.

In terms of age group of positive cases, this is how the numbers add up:

- 47 percent of cases are less than 40 years of age
- 34 percent are in the age group of 40 to 60 years
- 19 percent are above 60

It is important to read this data in light of the demographic of those who were tested. That data is currently not available. It is also important to read this data and analysis in light of India's massive young population and life expectancy of 69 years.

When it comes to gender distribution, India's number match up to the international trend. 76 percent of all cases in India are male and 24 percent are female. This reflects in fatalities as well. 73 percent fatalities are recorded among men and 27 percent among women. Now, while nearly 81 percent of those who have contracted COVID-19, the disease caused by the coronavirus, are under the age group of 60, when it comes to mortality, India again reflects the international trend - 63 percent of our mortalities are among those about 60

While many workers can work from home and carry out their jobs from a safe place, essential workers like those in retail and healthcare are forced to risk their health and that of their families. With only 29% of public able to work from home, social distancing is a luxury not everyone can afford. Many individuals have been practicing social distancing by working from home in recent weeks. While this arrangement can be a great way to reduce one's exposure to COVID-19, it's a luxury that's available to just 29% of Americans. The situation for the remaining 71% is uncertain, to say the least. A significant portion of the population has lost their jobs due to business shutdowns and mandated lockdown orders. Others employed in "essential services" have continued working as usual but may face a higher risk of potential exposure to the virus. We identified 103 possible work-related cases (14.9%) among a total of 150 local transmissions. The five occupation groups with the most cases were healthcare workers (HCWs) (22%), drivers and transport workers (18%), services and sales workers (18%), cleaning and domestic workers (9%)

and public safety workers (7%). Possible work-related transmission played a substantial role in early outbreak (47.7% of early cases). Occupations at risk varied from early outbreak (predominantly services and sales workers, drivers, construction laborers, and religious professionals) to late outbreak (predominantly HCWs, drivers, cleaning and domestic workers, police officers, and religious professionals).

Socioeconomic status is calculated based on several factors including education, social class and income.[5] which in turn determine a person's lifestyle. Low socioeconomic status has been shown to have a considerable impact upon health in various ways, particularly comorbidity status.[5] Poorer housing conditions and smaller spaces for living have been shown to be associated with increased exposure to infections and other communicable diseases.[6] Socioeconomic status has been a concern for many infectious diseases as it has been shown to be a risk factor for worse outcomes or more resistant organisms,[7] although this may be attributed to more specific factors, such as lower level of education or monthly income.[8] More specifically with regard to respiratory infections, people from impoverished backgrounds have been noted to have both a higher incidence of disease as well as a more severe infection.[9]. This study represents the hospitalized patients with COVID-19. In this cohort, significantly more patients admitted to hospital were found to be in lower socioeconomic groups.

In terms of fatalities linked to co morbidities, 86% of deaths recorded in India had significant co-morbidities like hypertension, diabetes, heart disease, chronic kidney disease. India has a massive crisis of lifestyle linked diseases and unfortunately has tag of diabetes and heart disease capital of the world. Add to that, the number of young people with these co-morbidities, it is reflected in the numbers - 37% of these deaths were among people below 60 years of age.

Due to COVID-19 being a relatively new and understudied disease, the data available is limited. However, from the cases that emerged, it was observed that comorbidities increase the chances of infection [7]. Based on current information and clinical expertise, the elderly, especially those in long-term care facilities, and people of any age with serious underlying medical conditions are at a greater risk of getting COVID-19[7]. The elderly, a vulnerable population, with chronic health conditions such as diabetes and cardiovascular or lung disease are not only at a higher risk of developing severe illness but are also at an increased risk of death if they become ill[15]. People with underlying uncontrolled medical conditions such as diabetes; hypertension; lung, liver, and kidney disease; cancer patients on chemotherapy; smokers; transplant recipients; and patients taking steroids chronically are at increased risk of COVID-19 infection[7]. The most common comorbidities identified in these patients were hypertension (15.8%), cardiovascular and cerebrovascular conditions (11.7%), and diabetes (9.4%)[10]. The fewer common comorbidities were coexisting infection with HIV and hepatitis B (1.5%), malignancy (1.5%), respiratory illnesses (1.4%), renal disorders (0.8%), and immunodeficiencies (0.01%)[10].

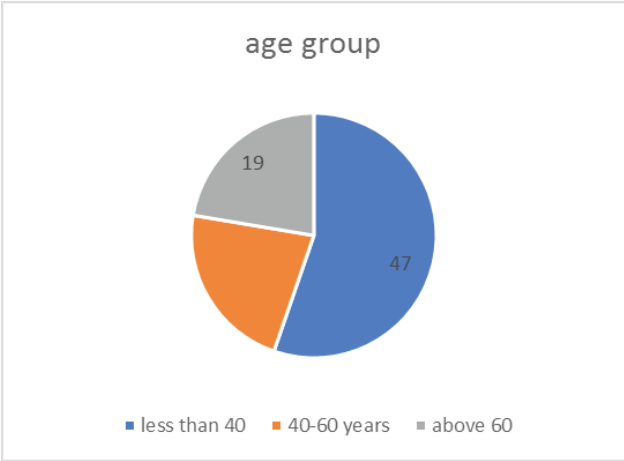

Figure 1 Age Group

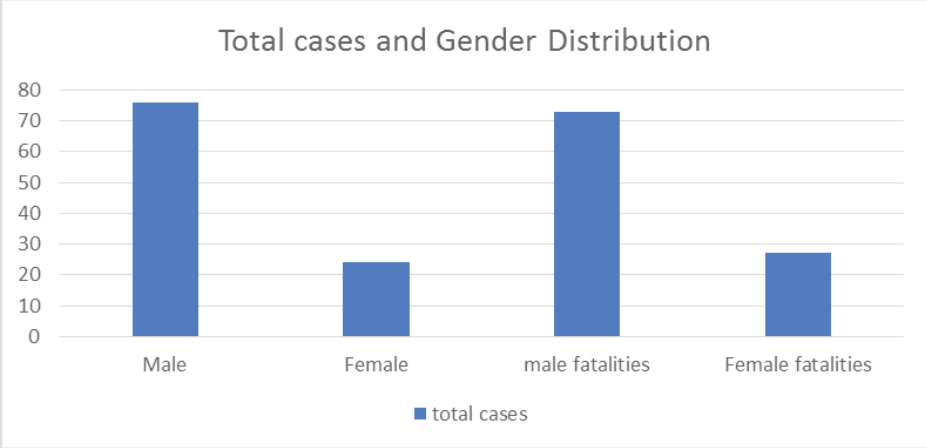

Figure 2 Gender Distribution

Figure 3 Occupation

Occupation	No.of affected
Health care professionals	22
Drivers and domestic workers	18
Service and sales workers	18
Domestic and cleaning workers	9
Public safety workers	7
Construction workers	5
Religious professionals	6

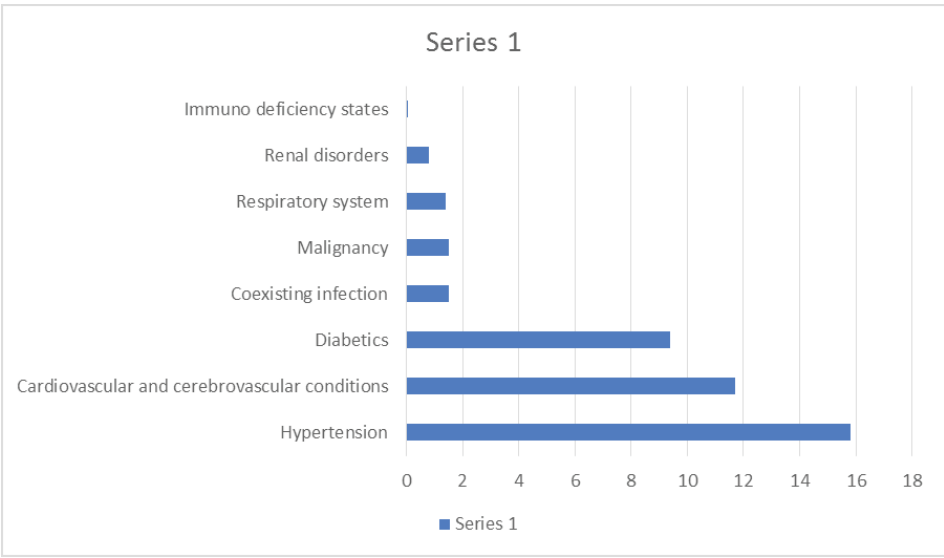

Figure 4 Comorbidity

Discussion

Age:

47 percent of cases are less than 40 years of age, 34 percent are in the age group of 40 to 60 years and 19 percent are above 60. Due to the substantially smaller sample of fatalities, as compared to cases of infection, the age distribution was divided into three categories: ages 0–40, 41–61, and 61 and above. For Indian women, these divisions correspond to (integer parts of) the median ages of puberty and menopause. The female to male ratio for COVID-19 infections and fatalities in these three age bins is given in Diagram I.

Gender:

Sex differences are also observed in the fatalities. 73 percent fatalities are recorded among men and 27 percent among women. Now, while nearly 81 percent of those who have contracted COVID-19, the disease caused by the coronavirus, are under the age group of 60, when it comes to mortality, India again reflects the international trend - 63 percent of our mortalities are among those about 60. The details are mentioned in Diagram 2.

Occupation:

we removed most occupations held by fewer than 150 people. From the remaining pool, we selected **5** well-known occupations. individuals on the front lines, whether they are taking care of patients or stocking grocery shelves, are placing themselves at risk to ensure our communities can continue to run smoothly. The five occupation groups with the most cases were healthcare workers (HCWs) (22%), drivers and transport workers (18%), services and sales workers (18%), cleaning and domestic workers (9%) and public safety workers (7%). Possible work-related transmission played a substantial role in early outbreak (47.7% of early cases). These details are furnished in Diagram 3.

Multiple comorbidities are associated with the severity of COVID-19 disease progression. Many of the poorer outcomes for COVID-19 have been related to cardiovascular comorbid conditions. However, this may be a direct result of the cardiovascular condition itself or attributed to other comorbidities along with a cardiovascular condition. Patients with type 2 diabetes were also more likely to have increased severity of COVID-19 death. Among other comorbidities, chronic obstructive pulmonary disease (COPD) has also been associated with poor disease progression. In this analysis, the smoking status of patients and severity of COVID-19 were also studied. Only one study was found to have an association with smoking and severe course of COVID-19. After this study was removed, there was no significant association with smoking and severe COVID-19 disease.

Conclusion

COVID-19 Pandemic has brought a threatening challenge to the world and as well as for Indian society and economy. In India, it has become a public health disaster and its' intensity increasing continuously. For the disaster risk reduction, and capacity building against the COVID-19 pandemic understanding of the relationship between socio-environmental conditions with the pandemic is very necessary. India is the 2nd largest country in terms of population and 6th leading economy in the world.[1]But COVID-19 has brought a threatening challenge to the Indian society and economy. The major challenge is to provide basics medical facilities to the huge numbers of incoming infected people if, the community spread takes place in the vast territory. Initially, the introduction of lockdown in the early stage of spreading has controlled the number of cases and restricts the community spread[2 , 3] eventually, but the question is how long it would be possible for India to stay at the home closing door of economic. The economic shutdown is not the permanent solution from this pandemic this emergency demand more constructive socio-economic and public health reforms form a political and apolitical organization. Over 180 countries have been affected by COVID-19, resulting in mass death worldwide. As cases evolve globally, it has been noted that persons with underlying chronic illnesses are more likely to contract the virus and become severely ill.

Due to SARS CoV-2 being a relatively new virus, the data available is limited. However, patients with comorbidities have more deteriorating outcomes compared with patients without. COVID-19 patients with history of hypertension, obesity, chronic lung disease, diabetes, and cardiovascular disease have the worst prognosis and most often end up with deteriorating outcomes such as ARDS and pneumonia. Also, elderly

patients in long-term care facilities, chronic kidney disease patients, and cancer patients are not only at risk for contracting the virus, but there is a significantly increased risk of death among these groups of patients.

Therefore, patients with comorbidities should take all necessary precautions to avoid getting infected with SARS CoV-2, as they usually have the worst prognosis. These precautions include regular handwashing with soap and water or use of alcohol-based hand sanitizer, limiting person-to-person contact and practicing social distancing, wearing a face mask in public places, and overall limiting going to public areas at this time unless it is necessary. Hence, there is a need for a global public health campaign to raise awareness, on reducing the burden of these comorbidity illnesses causing deaths in COVID-19-infected patients.

Ethical Clearance: This study has been carried out in the year of 2020, from the ethical committee of NIMRA Institute of Medical Sciences and Hospital, Vijayawada, Andhra Pradesh

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A Descriptive Study to Assess the Knowledge and Attitude Towards Care of Dying Patients among Nursing Interns

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Abstract

Background of the Study: Death is inevitable for all living beings. Nurses play a vital role in care of dying patients and their family. Regardless whether the death occurs, in the community or hospital nurses spends largest proportion of time during the care of dying patients. The nurses concern and support are valued by patients as well as the relatives

Aim: To examine the nursing intern's knowledge and attitude towards care of dying patient.

Method and Materials: A quantitative research approach with non-experimental descriptive study was adopted in this study. The stratified random sampling technique was used to select the subject for the study. Data collected from 100 subjects were analyzed by descriptive and inferential statistics using SPSS version 22.

Result: Among the selected subjects 83% had average knowledge, 69% had neutral attitude towards care of dying patients.

Conclusion: In the present study by assessing the level of knowledge and attitude of Nursing interns towards care of dying patients, it was found that 83% of Nursing interns had average knowledge and 69% of Nursing interns had neutral attitude towards care of dying patients. This chapter enlightens the need of the research study. The research reveals that there is no association between level of knowledge, level of attitude with selected demographic variables and there is no significant relationship between level of knowledge and attitude of Nursing interns towards care of dying patients.

Key Words: *Knowledge, attitude, nursing interns, care of dying patients.*

Introduction

Every person has the right to die with dignity. Life begins with birth and ends with death. Client's death is often viewed as personal failure on the part of health personal. Dying is the final portion of the life cycle for all of us. Death is inevitable for all living beings. And as health care providers, nurses play a principle role in the care of dying individuals and their families. Consequently, the care that nurses provide to terminal or dying patients may be affected by their own attitude towards death. [1]

When a patient is dying, treatment moves away from active efforts to cure the disease and concentrates instead on minimizing distress and controlling symptoms. The nurse's aim in caring for a terminally ill patient is to provide personal support in maintaining an acceptable life style and in enabling a peaceful death having regard to the patient's culture and beliefs, values, preferences

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and outlook of life. For relatives and friends of the dying patients, the emotions connected with losing a loved one can be intense. The response to a loss may reflect the intensity of the previous relationship and the prospect of impending loss can engender acute anxiety, distress and guilt at real or imagined past falling. An emotional response on the part of relatives to the impending loss and to the death when it occurs is a normal reaction and to be encouraged. [2]

Regardless of whether a death occurs in hospital ward or community settings among the health care team members, nurses will spend the largest proportion of time with the dying patients. So, nurses have an important role in caring for this group of patients. This part of nursing care is difficult and may be an emotionally painful, distressing and sometimes threatening. [3]

Looking after patients who are dying makes many of us feel a little uncomfortable. What we should be doing or saying is often unclear. Although we feel we should be doing something "to help" we can often be uncertain what that "something" is. Some of this uncertainty arises because being in contact with people who are dying is neither a common experience nor is it easy to talk about or seek advice on how we should be responding and helping.[3]

Objectives

v To assess the knowledge of nursing interns towards care of dying patient.

v To assess the attitude of nursing interns towards care of dying patient.

v To find out the relation between knowledge and attitude of nursing interns towards care of dying patients.

v To associate knowledge score towards care of dying patient with selected demographic variables.

v To associate attitude score towards care of dying patient with selected demographic variables

Methodology

Study setting

The study was conducted in YMCH, Mangaluru.

Study population

In this study, population comprised of the nursing interns in YMCH, Mangaluru

Research design

A non- experimental research design was used in this study.

Sample

The study was conducted among the nursing interns in YMCH, Mangaluru

Sample size

100 nursing interns in YMCH, Mangaluru

Sampling technique

Stratified random sampling technique was used in this study

Result

Table 1: Description of demographic characteristics

Sl.No	Demographic variable	Frequency	Percentage (%)
1.	Age		
	a) 18 – 21 years	90	90%
	b) 21 – 23 years	10	10%
2.	Gender		
	a) Male	08	08%
	b) Female	92	92%

Cont... **Table 1: Description of demographic characteristics**

3.	Education a) PUC	100	100%
4.	Religion a) Hindu b) Muslim c) Christian	29 09 62	29% 09% 62%
5.	Previous Knowledge a) Yes b) No	55 45	55% 45%

Table 2: Distribution of subject according to their level of knowledge

n =100

Level of knowledge	Scores	Frequency	Percentage (%)
Good	21 – 30	15	15%
Average	11 – 20	83	83%
Poor	0 – 10	02	02%

The table 2 shows that 83% of Nursing interns had average knowledge, 15% of them had good knowledge and remaining 2% had poor knowledge regarding care of dying patients.

Table 3: Distribution of subject according to their level of attitude

n = 100

Category	Score	Frequency	Percentage (%)
Positive attitude	101 – 150	29	29%
Neutral	51 – 100	69	69%
Negative attitude	0 – 50	02	02%

Table 3 reveals that 69% Nursing interns had neutral attitude, 29% Nursing interns had positive attitude and 2 % of them had negative attitude towards care of dying patients.

Table 4: Association between level of knowledge and selected demographic variables.

n=100

	Demographic variables	Median ≤ 16	Median >16	P value	Chi-square
1.	Age			0.920	.010
	a) 18-21 years	58	31		
	b) 21-23 years	07	04		
2.	Gender			0.164	1.935
	a) Males	07	01		
	b) Females	58	34		
3.	Religion			0.693	.735
	a) Hindu	17	12		
	b) Muslim	06	03		
	c) Christian	42	20		
4.	Previous Knowledge			0.916	.011
	a) Yes	36	19		
	b) No	29	16		

P < 0.05 * significant

The data presented in table 4 shows that, the calculated value (age= 0.920, gender= 0.164, religion= 0.693, previous knowledge= 0.916) is more than the P value (P>0.05) hence the null hypothesis **$H_0$2** is accepted for all these variables. Thus concluded that there was no association between knowledge score and selected demographic variables.

Table5: Association between attitude and selected demographic variables

n = 100

	Demographic variables	Median ≤ 89	Median >89	P value	Chi-square
1.	Age			0.697	.152
	a) 18-21 years	46	43		
	b) 21-23 years	05	06		
2.	Gender			0.426	.634
	a) Males	03	05		
	b) Females	48	44		
3.	Religion			0.826	.382
	a) Hindu	16	13		
	b) Muslim	04	05		
	c) Christian	31	31		
4.	Previous Knowledge			0.410	.679
	a) Yes	26	29		
	b) No	25	20		

P <0.05 * significant

The data presented in table 5 shows that, the calculated value (age= 0.697, gender= 0.426, religion= 0.826, previous knowledge= 0.410) is more than the P value (P>0.05) hence the null hypothesis $\mathbf{H_0}1$is accepted for all these variables. Thus concluded that there was no association between attitude score and selected demographic variables.

TABLE 6: Correlation between level of knowledge and attitude score of Nursing interns towards care of dying patients

	Mean	Median	S.D	R
Knowledge	16.11	16	3.323	-.078
Attitude	90.69	89	18.242	

The data presented in table 6 shows that there is no relationship between level of knowledge and attitude score of nursing interns towards care of dying patients, Hence $\mathbf{H_0}2$ is accepted. However there was strong negative correlation between level of knowledge and attitude towards care of dying patients.

Conclusion

In the present study by assessing the level of knowledge and attitude of Nursing interns towards care of dying patients, it was found that 83% of Nursing interns had average knowledge and 69% of Nursing interns had neutral attitude towards care of dying patients. This chapter enlightens the need of the research study. The research reveals that there is no association between level of knowledge, level of attitude with selected demographic variables and there is no significant relationship between level of knowledge and attitude of Nursing interns towards care of dying patients.

Recommendations

Similar study can be conducted with a large sample in order to enhance generalization of the findings. Similar study can be conducted among doctors. A comparative study can be conducted to assess the knowledge and attitude regarding care of dying patients among community and hospital nurses. A comparative study can be conducted to assess the knowledge regarding care of dying patients among doctors and nurses.

Ethical Clearance: Ethical clearance obtained from the ethics committee of the Yenepoya University prior to the conduction of the study. Administrative permission obtained from the concerned authorities. Written consent obtained from the samples and confidentiality will be maintained.

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Conflict of Interest-Nil

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Prudential Principles Notary in Making Deed Agreement Between Transplant Donor and Recipient

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Abstract

Introduction: Transplantation of organs in humans is one of the fastest-growing alternative treatments. The use of donors from non-family members is one of the considerations. Is a notarized agreement between the organ giver and the organ recipient necessary so that problems do not occur in the future?

Objective: This study's objective was to analyze the principle precautionary in a notarized deed agreement between the donor and recipient transplant.

Method Research: This research method uses a normative and empirical juridical approach with descriptive-analytical and normative research specifications.

Conclusion: This study found that the alleged buying and selling of kidneys leaves underhand agreements' ongoing problem. The paradigm that applies in the work of notaries is the principle of prudence in doing notarial deeds. Prudence comes from the word prudence, which is analogically closely related to the supervisory function management as it applies to banking. A notary caution is required to draft a transplant notary agreement between donors and recipients so that disputes do not occur in the future.

Keywords: *Transplantation Agreement; Notary; The Precautionary Principle.*

Introduction

Organ transplantation is a medical technology for replacing organs; medical patients were not functioning organs from another individual[1]. In implementing Kidney, transplants are directly related to parties such as the recipient (receiver kidney donor), tendons, and doctors. Measures such as renal transplant medicine begin with between the parties, giving birth to an agreement for an agreement transplant[2]. That means an agreement between the donor and the recipient to implement the kidney transplant voluntarily. The agreement is an act with which one or more binds himself to one or more people binds himself to something right against someone or another person[3].

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The status of kidney transplant agreements is often declaring contrary to Law number 36 of 2009 concerning health, which prohibits being traded commercial organs for kidney transplantation. However, there are still many internal problems with a medical transplant that requires the parties' agreement, even if it is not deep in the context of buying and selling organs[4].

Notaries in carrying out their duties and positions are required to implement the principle of prudence in the process of doing authentic deeds because there are parties who violate the terms valid of the agreement, such as providing false letters or false information into deeds made a notary[5]. It can prevent crimes that might plunge notaries into legal issues, as stated in the Law on Notary Position regarding the guidelines and guidance for notaries to act more carefully, thoroughly, and carefully in the process of doing authentic deeds.

Problems Medical agreements in kidney transplantation are still not a significant issue among the public. Many organ transplants have, even though donors and recipients have not used the agreement.

Buying and selling body organs has been rife among the public due to the difficulty of finding donors to improve the body's health and prolong its span of life[6]. Blood transplant donors and recipients are only required to show their identity. These identities are National Identity Card, family card, marriage certificate. However, this document is straightforward to forget. Because the proof of identity is often problematic, it is necessary to have an under-hand agreement made by donors and recipients whose contents are entirely in blood. There is no buying and selling of kidney organs, which are then legalized or legalized by a notary public. If the donor and the recipient are not in blood, they are required to do a notarial deed that states that there is no buying and selling of kidney organs[7].

Every authority given to a position must have a legal rule. As a limitation, the position can run well and does not conflict with other positions' authority. Thus, if an official (Notary) commits an action outside the predetermined authority, it can be categorized as an act of violating authority. A notary's authority in Article 15 paragraphs 1, 2, and 3 of Law Number 2 of 2014 concerning a Notary Position.

Research Methodology

This research's type of research is normative legal research, namely legal research that places the law as a norm system building. The norm system in question is regarding the principles, norms, rules of the statutory regulation. The juridical approach method used is by examining legal rules or regulations as a system building associated with a legal event[8]. The data used in this study are secondary data, which consists of primary and secondary legal materials, which from books, literature, papers, laws and regulations, and other data sources. Secondary data collection uses the method literature approach, namely a library research using s legal library materials that support this research. Secondary data in the form of primary legal materials in the form of legal materials are collected and processed, analyzed by normative methods, and then presented descriptively.

Discussion

Transplantation can only if its safety and proven benefits in the article on organ transplantation aim to provide protection and legal certainty for donors, recipients, organ transplant hospitals, and health workers providing organ transplants services. Article 65 of Law Number 36 of 2009 concerning Health explains the competency requirements for health workers and health service facilities in carrying out organ transplants and the need for *informed consent* in the implementation of organ transplants. According to Regulation of the Minister of Health Number 290 of 2008 concerning Approval of Medical Actions (*Informed Consent),* Article 1 states 65 Law Number 36 of 2009 concerning health states that organ transplantation can occur only by health workers who have the authority to carry out in individual health care facilities. Harvesting of organs or tissue from a donor must pay attention to the donor's health and obtain the donor and heir's approval or family[9].

The agreement letter has an essential function when agreeing in a letter because black and white evidence applies between the two parties. In the patient-doctor relationship, known informed consent is. Meanwhile, the relationship between donors and recipients is considered in the informed consent clause, whereas informed consent only involves medical activities[10]. The relationship between donors and recipients in organ transplants has a specific relationship regulated in law, where the main point is that there is no buying and selling of organs[11].

An agreement between the donor and recipient of the transplant needs to be where the agreement has several rules and functions, namely: As authentic evidence that certain parties have entered into an agreement or agreement; Become a basis for doing things that have been mutually agreed upon by the parties making them; The agreement letter can also be used as a guide when used to sue parties who violate the agreement specified in the agreement letter; The parties who have agreed will feel calm because there is certainty in the agreement letter; Can be used to know the rights and obligations between the parties who enter into the agreement or agreement; Can avoid disputes between the parties to the agreement, which is likely to arise in the future; and Used as a guide in resolving a problem or dispute that may arise as a result of the agreement.

Notaries must comply with applicable legal provisions regarding how far they can act and what they can and cannot do. If the prohibited provisions have, the deed concerned will lose its authenticity[12]. The Notary has moral integrity, honest, and aware of the limits of their authority. Must also have an intrinsic sense of justice, not be influenced by the amount of money, and not merely create formal evidence to pursue legal

certainty, but ignore the sense of justice. The code of ethics also plays an essential role in social control and prevents supervision or interference by the government or by the community. Besides, the code of ethics also plays a significant role in developing the notary profession to prevent misunderstanding and conflict as much as possible.

The paradigm that applies in the work of a Notary is prudence in making Notary deeds. The principle of prudence because in Article 16 paragraph (1) letter a of the notary position law, the article has not explained the obligations and examples of careful actions of Notaries in doing authentic deeds, so that the Notary's obligation to act carefully is not clear and creates multiple interpretations. Therefore, in carrying out its duties, the Notary must act more carefully and thoroughly in examining the documents and statements of the parties who wish to do an authentic deed so as not to cause legal problems with the deed he makes at a later date.

Conclusion

The agreement deed can also be used as a guide when used to sue parties who violate the agreement specified in the agreement letter. Therefore, the forms of the prudential *principle* that a notary should carry out in the deed-making process identify the complainant's identity, verifying the data of the subject and object of the observer carefully, giving a grace period in working on the deed, acting carefully, being careful and thorough in the deed work process, fulfilling all the technical requirements for making deeds are forms of precautionary principles that a notary public should not carry out. The Notary can prevent legal problems arising from the authentic deed he makes in the future.

Ethical Clearance: Yes.

Conflict of Interest: No

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Study of TP53 Gene-Codon 72 Polymorphism in Epithelial Ovarian Cancer (EOC)

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Abstract

Introduction: The tumor-suppressor gene (TP53) encodes p53 protein, the vital protein in the apoptotic cascade pathway which has been presented to be of crucial potential in the progression of cancers. Genetic variation SNP that were revealed to affect the regulatory biochemical functions of p53 are the exon 4 codon 72, (G)Arg/(C)pro polymorphisms. Aim: the present study aims to analyses genetic variation (SNP) of Tp53 gene, codon 72 and p53 protein level in Epithelial Ovarian Cancer (EOC). Methods: the study achieved on 26 EOC cell line specimens and 26 specimens as control group, using PCR-RFLP genetic analysis method and cell lysate p53 level determined by ELISA technique. Results: the percentage of allele polymorphism come as homologous GG variant 85% and 23% in EOC specimens and control respectively and (CC) (8% and 46%) in EOC case and the control in order. Heterologous pattern CG was (34% and 23%) for experimental and control group respectively.

Conclusion: our study proposes that 72 codon polymorphism (GG) is closely related to EOC progressions.

Keywords *: P53 Gene, p53 PCR-RFLP, Polymorphism, Ovarian cancer*

Introduction

Epithelial ovarian cancer (EOC) is one of the highest harmful inherited malignancy disorders and the fifth source of malignance-associated death of women. The global incidence of this disease has been estimated, about 239,000 case per year with 152,000 deaths record, through the year 2012[1]. The possibility of epithelial ovarian cancer development appropriates with age. Women with over 50 year age are more visible to develop ECO condition, even, other age can affected with this malignant cancer. Substantial attempt aims to improve the prognoses guidelines of EOC and detailed the early staging, along with invasive and chemo-therapeutic administrations, have enhanced the short-term progression of epithelial ovarian carcinoma. Modern records for incidence and death rates caused by cancer, indicate that the EOC related death cases have dropped about 14% percentage. The survival proportion for EOC during the last 5 years has been climbed from (36%) to (44%) of total cases of cancer. Among colorectal, women breast cancer conditions, and men prostate cancers conditions, epithelial ovarian cancer is the only cancer companied with significantly dropping of death curve [2]. Epithelial ovarian cancer subdivided into tow type according on the histological transformations and genetic variations, first type with slow-grading tumors combined with mutate form of PTEN , K-RAS and BRAF cell cycle controlling genes. The second type of EOC characterized by mutated TP53 genes and harmful high-grading clinically [3].

Tumor suppressor protein p53 have dominant role in genomic stability and integrity with a conventional defensive role against malignant transformations [4]. RNA Transcription regulatory protein (P53) contributes in control many cellular activates, including the gene

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expression of several genes included in cell cycle and genomic disturbance. When DNA damage is detected p53 protein blocks cell cycle by suspends cell division at point of transition from G1phse to S phase (G1/S checkpoint) and promoting DNA repairing, if genomic damage verified to be irreversible, p53 triggers programed cell death (apoptosis) [5]. P53 protein consists of four structural domains: N,-terminal transcription activating domain, a domain that binding to specific DNA sequence, a domain of tetramerization and transcription regulatory C-terminal region. [6-7]. functional disturbance of the p53 protein is a distinctive hallmark for different types of malignant tumor conditions [8]. P53 switching The pathways-traffic of several activates that determine the future of the cells dividing under extreme endogenous or exogenous conditions and inactivation of the p53 protein signaling cascade pathway is approved in most human cancers [9]. Several recent Studies have verified that different genetic changes and many abnormal environment aspects can be triggering of cancer development, so TP53 gene polymorphism has been consider a study matter due to it is importance as a key tumor suppressor gene [10]. Genetic alterations in the sequence that encoded for p53 which leads to synthesis of inactive or truncated protein, the abnormal production of proteins that interfere TP53 gene expression although, gene amplification that cause increasing in MDM2 level and other proteins contribute in normal metabolism of p53 in the cell, both of it cause a functional disturbance in p53 function and influence malignant tumors development [11].

There are well established links of possibility of cancer progression, onset of cancers and inclusive surviving of tumors with TP53 gene allelic C/G variant at codon 72 (12). Single nucleotide polymorphism in TP53 gene tightly connected with Epithelial ovarian Cancers (EOCs) case, as 97% of High Grad Serous Ovarian Cancer (HGSOCs) case show an polymorphism in TP35 gene sequence [13].

P53 gene codon 72 (Arg/Pro) polymorphism, in fourth exon, were inspected in many forms of human cancer, a guanine base replacement with cytosine base in this site results synthesis a contain an arginine amino acid instead of praline with impaired anticancer activity [14-15]. The pointed genetic variations which including exchange of (CGC) by (CCC), affects the tertiary structure assembly of the trans-activating domain of this protein and its critical activity. both allelic variants (Pro72 and Arg72) of the p53 protein [16]. This introduction com to confirm the prospective concern of this substitution on transcriptional level, triggering of apoptosis events and malignant transformation of affected cells [17]. We carried out a cell line based research to verify the frequency of (rs-1042522- G/C) SNP of goal sequence among woman undergoes EOC.

Material and Methods

Study Subject

The present experiments were achieved on EOC and control tissue culture provided by cell culture unit, collage of medicine, university of Babylon as a gift. A total 26 cell lines of EOC and control specimens was included in the study.

Determination of p53 level

Cell lysate was prepared by frozen at (4˚C) and resolving at (45˚C) for two times, cell lysate level of p53 protein was determined by ELISA according to manufacturing procedure of ELISA kit (Elabsit/ China).

TP35 codon 72 polymorphism (SNP) analysis

Genomic DNA from cell line culture for both EOC and control group were extracted using DNA extraction kit manufacturing procedure of (favorgen/korea). The segment of the TP53 gene covering the codon 72 (G/C) polymorphism (199 bp) was amplified by (PCR) using the following primers pairs : Forward primer , 5'-TTG CCG TCC CAA GCA ATG GAT GA-3' and reverse primer , 5'-TCT GGG AAG GGA CAG AAG ATG AC-3'. PCR achieved by using premix (promega/ Spain) separated in finally volume (20 µl). PCR reaction program carried denaturation at (94˚C/30 sec) and annealing at (55˚C/1 min), followed by extension at (72˚C/1 min),the thre steps repeated for35 cycle [18]. PCR products about (199bp) were processed with restriction enzyme (BstUI) concentration 0.1 µl (10 U/µl), RFLP reaction applied at 60˚C for 18 hour, then electrophoresed for analyzing. C Allele is not cleaved by BstUI producing a single band with size about (199bp). The G allele is cleaved by BstUI producing of double restriction fragments of (113 and 86 bp), heterozygous (CG) produce three bands of (199, 113 and 86 bp) [19].

Statistical Analysis

Statistical analysis out carried by SPSS version 23. as a mean ± SD for P53 level. allele frequency tested by Hardy–Weinberg equilibrium.

Results and Discussions

P53 protein level, cell lysate p53 level was determined by ELISA (figure 1), present study show that (38.4%) of EOC and (11.5%) have an detectable level of p53protein, there are non-significant increasing of p53 protein level mean in ECO lysate in contrasting with control (0.216pg/ml and 0.166pg/ml), respectively.

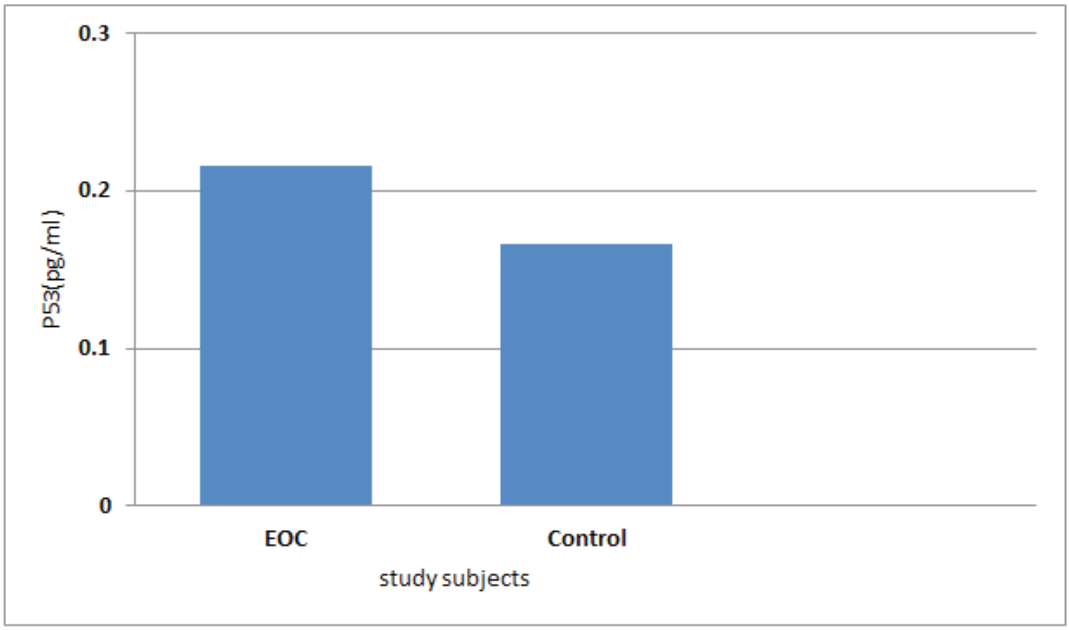

Figure (1) show means of P53 protein concentration of EOC and control specimens

Significant increase of mutant p53, levels in colorectal carcinoma patients compared to the control group [20]. The mean levels of p53 protein of carcinoma of colon were (0.12 ng/ml) [21]. Many experiments show an clear relationships between cytoplasmic p53 concentration and both of triggering of programed cells death and transformation of cancer cells, so this increasing in level considered an powerful clinical of cancers [22]. Extreme rising of cytoplasmic p53 protein ESCC tissue culture about (1.89) fold above that of the normal tissue [23]. Study observations com similar to fondness of Shim et al represented by significantly increasing of p53 in cancer patient specimens [24]. Several excremental analysis based recommendations, suggested that p53 is an important indicator of cancer cells behavior through treatment course [25].

TP53 codon 72 polymorphism, PCR-RFLP genotyping for codon 72 SNP (CG) showed in (figure 2)

The results presented in (table 1), demonstrate an non-significant increasing in Arg alleles patterns (GG) 85% in EOC case in contrast with 23% in control (p-value= 0.09), combined with non-significant decreasing in Pro alleles patterns (CC) in EOC case among the control (8% and 46%) respectively (P-value= 0.069).

Table (1) show genotype patterns of TP35 gene codon 72 polymorphism in EOC and control cases

Genotype	No. (%)		P-value	Phenotype	Mean ± SD of p53 level
	case	control			
GG	15(58%)	6(23%)	0.09	Arg / Arg	0.272 ± 0.072
GC	9(34%)	6(23%)	1	Arg/pro	0.315 ±.096
CC	2(8%)	14(46%)	0.069	Pro/ Pro	0.164 ± 0.042
Total	26	26	------	------	------
LSD value	------	------	------	------	0.899

In aspect of the several researches applied on EOC conditions but there is not recorded instructions about participation of mutated p53 in risk and pathological aspect of EOC[26]. This may be owed to small sizes sampling or mixed population included in study subjects. present study reviles high Arg allele (GG) at TP53 codon 72 in patient of EOC than in control, this result was similar to several studies cured out on TP53 gene codon 72. The percentage of three patterns was homozygote for the Arg allele (33%), homozygote for the Pro allele (17%) and heterozygote Pro/Arg patterns (50%)among women population screened for ovarian cancer [27]. meta-analysis proposes that the TP53 codon 72 CC / CG SNP are not related with an increased risk of breast cancer progression [28]. The percentage of homozygous (Arg=33%), homozygous (Pro=17%), and heterozygous (Arg/Pro=50%) among patients diagnosed for sever ovarian cancer; 62%, 6%, and 32% for health group [29].

These observations administrate that p53 -codon 72 SNPS may attend as peril element for tumors progression and this battle due to distinctiveness of malignant tumor nature, study subjects bulk and ethnic distinction. Our results propose that Arg/Arg pattern is associated with epithelial ovarian cancer progression. The polymorphism in codon 72 of p53 coding sequence is an instance of an risk variation which may have a critical implication in Epithelial ovarian carcinogenesis and stimulus DNA repair in cells and apoptotic cascade events. These outcomes may have a prolific consequence for gene-mediated therapeutic strategy in the treatment of EOC. To certify the link between p53 (codon 72) mutation and EOC, we suggest to achieving an additional cohorts with high volume of international subjects.

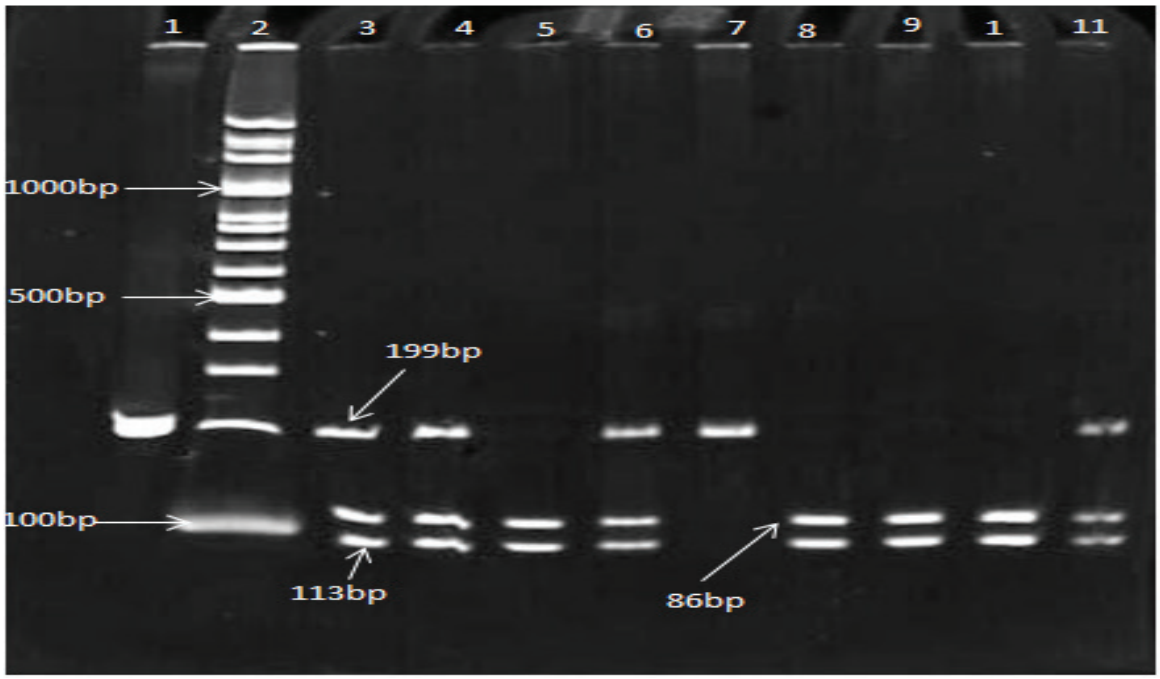

Figure (2) shows gel electrophoresis band pattern of p53 gene allels vesiolized on polyacrylamid gel under UV transumilation, Lin1 undigested product, Lin2 100bp marker, lin(3,4,6,11) CG, lin(5,8.9,10) GG, lin (7) CC pattern.

Conclusions

Epithelial Ovarian Cancer (EOC) is a serious disease that causes mortality in Iraq and worldwide. p53 protein is encoded by the tumor-suppressor gene (TP53) and plays a vital role in progression of EOC. The current study aims to investigate the genetic variation (SNP) of Tp53 gene, codon 72 and p53 protein level in Epithelial Ovarian Cancer (EOC). The obtained results show that the percentage of the homologous allele polymorphism GG variant is 85% and 23% in EOC specimens and control respectively, while (CC) variant gave 8% and 46% in EOC case and the control in order. On the other hand, heterologous pattern CG was 34% and 23% for EOC specimens and control groups, respectively. The findings of this study suggest that the polymorphism in 72 codons (GG) is directly related to EOC progression.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Incidence and Prevalence of Periodontitis among 90 Patients Diagnosed with Salivery Gland Tumor in Duhok City

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Abstract

Background and Objectives: Globally, there was (20-50) % periodontal disease's prevalence. This disease is one of the tooth loss causes that might be compromising the life quality, self confidence, esthetics, and mastication. The major problem that salivary gland tumors do is decreasing the amount of saliva in the oral cavity that led to change in oral flora and dehydration of the tissue, especially periodontal tissue. This study was held to found the direct relationship between saliva amount and periodontitis. **Methods:** This epidemiological study was held out by a collection of data from 90 patients that seeking out clinics in Duhok and Zakho and seeking Azadi general hospital complaining from periodontal problems. **Results:** 90 patients involved in this study 47(%52.22) of theme with history of parotid gland tumor, 24(%26.66) with submandibular salivary gland tumor, and 19(%21.11 with sublingual salivary gland tumor.68 (75.55%) are benign category while 22(24.44%) malignant patients having severe xerostomia are 28(31.11%) Moderate types of xerostomia are 14(%15.55) patients, while the mild type is 22(24.44%). And those with no xerostomia are 26(28.88%) patients.

Keywords: *periodontitis, xerostomia, salivary gland tumors, gum disease, parotid neoplasia.*

Introduction

Periodontitis is gum disease—a type of chronic inflammatory condition that is triggered by bacterial, viral, and autoimmune infections. Periodontitis causes destruction of the tooth-supporting structures; gums might be pulled away from the teeth, a loss of bones might happen; also, the teeth could be falling out or loosen. Majorly, periodontal disease occurs in adults. In dental health, the two major threats are tooth decay and periodontal disease[1]. Xerostomia can be specified as one of the subjective complaints related to the dry mouth caused by a reduction in the production of saliva[2]. The acuteness of dry mouth or xerostomia symptoms is ranging from mild oral discomforts to considerable oral diseases compromising the patient's health, life quality, and the intake of dietary. Dry mouth causes might involve autoimmune diseases, negative medication's effects, neck, and head radiotherapies, toxicity from chemotherapies, or other conditions (hormonal changes, infections, and uncontrolled diabetes). Malignant type salivary gland tumors: adenoid cystic carcinoma, polymorphous adenocarcinoma, basal cell adenocarcinoma, and mucoepidermoid carcinoma[4]. Infected, swollen, or obstructed salivary gland due to tumors of both types will cause a severe decrease in saliva productions [5]. Saliva is very important in oral lubrication, whereas producing suitable amounts related to stimulated and un-stimulated normal salivary secretions was vital to prevent esophageal and pharyngeal conditions, dental caries, and periodontal or gingival diseases, also tooth erosion. Hypo salivation, from the salivary gland hypofunction, might lead to a subjective sensation of xerostomia or dry mouth, which typically happens in the case when there is a decrease by half in the un-stimulated salivary flow rate compared to the normal in individuals. Yet, the rate could still be in the normal range, indicating that the saliva quality might

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be vital to determine the dryness' perceived degree. Xerostomia might be due not to a total lack of oral fluid, yet to localized areas related to mucosal dryness[6]. Commonly, xerostomia happens in the ones experiencing Sjögren disease or people subjected to radiation therapies for neck and head cancers[7]. Salivary gland tumors are neoplasia of the salivary glands, which are of two major categories, either benign or malignant (cancerous), benign tumors; the most common types are monomorphic adenoma, pleomorphic adenoma, canalacular adenoma, myoepithelioma, ductal papilloma, and basal cell adenoma[8]. The decreased salivary flow might result in problems in speaking, chewing, tasting, and swallowing; it might also be increasing the chances to develop dental decay, teeth demineralization, oral infections, and/or teeth sensitivity[9]. In xerostomia treatment, the major aims are to identify the possible cause(s), to prevent complications (periodontal infections and dental caries), also to relieve discomforts[10]. A patient with xerostomia is the first candidate in due course to experience gingival diseases. With no treatments, plaque and bacteria are allowed by xerostomia to stay on the teeth, which is going to results in gingivitis, after that periodontal diseases. As soon as the gingival disease is developed via the patients, they are going to be experiencing symptoms such as red or swollen gums might bleed, persistent bad breaths, sensitive or loose tooth, gum recession, and pain during e chewing [11].

Materials and Methods

Ninety patients who are suffering from gingival problems are involved in this retrospective cross-sectional study. Those patients are seeking out private clinics or Azadi Hospital in Duhok and Zakho city for periodontal treatment, and they are suffering from xerostomia and having a history of salivary tumors of the parotid, submandibular or sublingual salivary glands that we confirm by collecting their pathological reports from Duhok Central Laboratory in 10-year intervals. First, we collect our data from dental clinics; we look for those patients who are suffering from periodontitis, and then we looked for their history and probable causative factors for this periodontitis; then selecting those patients that suffer from xerostomia and then looked further for the causative factor for this xerostomia. And we select those patients that suffered from xerostomia due to the previous tumor in their oral region; then, matching their name in the central lab in Duhok city with those who had salivary gland tumors.

Inclusion criteria: those patients with periodontitis and history of salivary gland tumors.

Exclusion criteria: patients with periodontitis for another causative factor's examples; due to bad oral hygiene, due to bacterial, viral, autoimmune, or hormonal conditions also we exclude all patient suffering from xerostomia but didn't have the periodontal disease yet.

Results

Ninety patients involved in this study 47(%52.22) of theme with history of parotid gland tumor, 24(%26.66) with submandibular salivary gland tumor, and 19(%21.11 with sublingual salivary gland tumor. 68 (75.55%) are benign category while 22(24.44%) were the malignant type. Regarding the age group, 49 (54.44%) were under 50 years old while 41(45.55) were above 50 years old. Regarding xerostomia, patients having severe xerostomia are 28(31.11%) The moderate type of xerostomia is 14(%15.55) patients, while the mild type was 22(24.44%). And those with no xerostomia are 26(28.88%) patients. All patients included in this study were having periodontitis of different degrees. 53(58.88%) having severe periodontitis with partial bone loss, bleeding on probing attachment loss,18(20%) suffering from moderate type periodontitis with bone loss, bleeding on probing but mild attachment loss, while 19(21.11%) were having mild periodontitis with no attachment loss and mild bleeding on probing.

Table 1: incidence of salivary gland tumor by type

Tumor type	No. Of patients	Persentage%
Parotid S.G	47	52.22%
SubmandibularS.G	24	26.66%
Sublingual S.G	19	21.11%
Total	90	%100

Table 2: Distribution of study subjects based on age.

Age group (years)	NO. of patients	%
0—10	0	0
11—20	2	2.22
21—30	6	6.66
31—40	10	11.11
41—50	31	34.44
51—60	23	25.55
61—70	18	20

Table 3: Distribution of study subject based on the severity of xerostomia

Type of Xerostomia	No. of Patients	Percentage
Mild	22	(24.44%).
Moderate	14	(%15.55)
Severe	28	(31.11%)
No xerostomia	26	(28.88%)

Table 4: distribution of study subject based on the severity of periodontitis

Degree of periodontitis	No. of patients	percentage
Mild	19	21.11%
Moderate	18	20%
Severe	53	58.88%
Total	90	100%

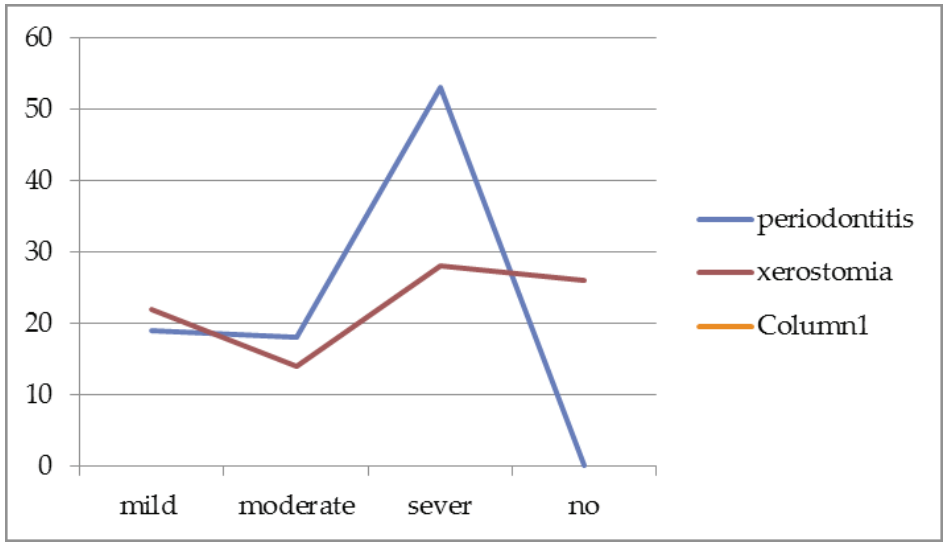

Fig 1: Relation graph between periodontitis and xerostomia

Fig2: Periodontitis due to dry mouth (xerostomia).

Discussion

Half of the people with a minimum age of 30 years are suffering from periodontal disease. This disease might lead to the loss of teeth, a need for dentures, along with series of more health problems. Due to the potential severity of the disease, there is a high importance in treating it adequately, in spite of how frequent the disease was indicated to be. The disease might be affecting people of all ages and all genders[3]. Periodontitis causes: The mouth bacteria are multiplying and forming a substance referred to as dental plaque. The mineral deposit is referred to as tartar, which is going to be encouraging more growth of bacteria towards the tooth's root[2]. Gum's inflammation results in due to the immune response of the body to such growth of bacteria. The gum's attachment to the tooth root was disrupted with time; also, a periodontal pocket (gap) might be formed between the root and the gum. In addition, the harmful anaerobic bacteria are colonized in the packet and after that multiplied, causing a release of toxins damaging the teeth, gums, and supporting bone structure. And the dryness of the mouth will aggravate the situation and make a change in oral flora balance and lead to gingivitis and periodontitis. Salivary gland tumors are neoplasia affecting the three major salivary glands, which are responsible for the secretion of more than 80% of saliva in the oral cavity. So salivary gland tumor will cause an obstruction in the ducts and decrease amount of saliva secretion during the course of the disease, and after excision of the affected gland, especially the parotid gland, which is responsible for the secretion of 750 -1000ml of saliva per day there will be a huge deficiency in saliva and dry mouth so there will be xerostomia and as we mentioned xerostomia would lead to several mouth problems.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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Effectiveness of Planned Teaching Programme on Knowledge Regarding Management of Common Behavioral Problems of School Children among Primary School Teachers

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Abstract

Infancy and childhood are of supreme importance in determining the future behavior. Behavioral problems always require individual attention. Teachers spend most of the school hours with children so that they can detect the signs and symptoms of these problems at the earliest.

The Title of the Study: Effectiveness of planned teaching programme on knowledge regarding management of common behavioral problems of school children among primary school teachers in selected school in Ernakulum

Objectives;1.Assess the knowledge of primary school teachers regarding the management of common behavioral problems of school children.2. Evaluate the effectiveness of planned teaching program on knowledge of primary school teachers regarding the management of behavioral problems of school children.3.Associate the socio demographic variables with the pretest & post test knowledge scores

Methodology: The research approach used in the present study was quantitative and research design was one group pre test post test design. The study was conducted among 30 teachers with the eligibility criteria following purposive sampling technique. The tools used for the study were structured questionnaire.

Results : The mean post- test knowledge score (16.18) is significantly ($p = 0.001$) higher than the mean pre-test score(13.7).This shows that structured teaching programme was effective in improving the knowledge of teachers regarding behavioral problems. There is no association between pretest & post test knowledge scores with demographic variables.

Conclusion: The study finding point out the responsibilities of a health professional to enlighten and educate the teachers regarding management of behavioral problems of school children.

Keywords: *Knowledge, Behavioral problems, Primary school teachers,*

Introduction

Introduction Infancy and childhood are of paramount importance in determining the future behavior. Gradually, children learn to adjust in the environment. But when, there is any complexity around them, they cannot adjust with that circumstances. Behavioral problems always require special attention. Teachers spend most of the school hours with children so that they can detect the signs and symptoms of these problems at the earliest. Children's mental health problems if neglected, can continue into adolescence and adulthood, adding further costs such as poor academic performance, school dropout, substance abuse, poor vocational outcomes, family and school violence, along with increased sick

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leave, unemployment, crime and risk of suicide[1].

According to the UNESCO report 2008 Ten percent of 5-15 year old children have a diagnosable mental health disorder and around 90% of these children are not currently receiving any specialist service.[2] MalhotraS et. al.(2009) in their study on incidence of psychiatric disorders among school children in India, have found that the annual incidence rate of psychiatric disorders in school children accounts for 18/1000/yr.[3]

According to WHO and UNICEF health reports as many a 20 percent of children worldwide are suffering from behavioural problems which could lead to serious public health problems[2]

Lian W B conducted a study on Pre-school teachers' knowledge, attitudes and practices on childhood developmental and behavioral disorders in Singapore. The results showed that there is knowledge deficit on normal development process (66%), autistic spectrum disorder (68%), and ADHD (32%) This study demonstrated education deficit among our pre-school teachers[4]. K M Jeenu and M G Sreejamol conducted a study on knowledge of mothers regarding behavioral problems of children in a selected community, Kerala. Study findings shows that Majority of the mothers had average level of knowledge(81.7%%), only 1.6% had good level of knowledge 16.7% and had poor level of knowledge on behavioral problems[5]·Family is the first source of support for a child's mental health. Children spent a significant amount of time at school and schools are excellent places to promote good mental health. So as trained caring adults , teachers require developing capacity both to reinforce children's mental health strengths and to intervene to children suffering from mental health problems. [6]

Materials and Methods

The research approach used in the present study was quantitative and research design was one group pre test post test design. The study was conducted among 30 teachers who met with the eligibility criteria following purposive sampling technique. The tools used for the study were structured questionnaire developed after reviewing related literature. The tool has two parts. Part – A: socio demographic profile. It includes age of the participant, Education, previous knowledge, year of experience, and marital status. Part- B: self developed structured questionnaire on knowledge of teachers regarding behavior problems among children. It includes 30 questions which consist of general aspects of behavior problems, risk factors of behavior problems, clinical manifestation of behavior problems, identification of behavior problems and management of behavior problems. The developed tool was sent to experts and their valuable suggestions were incorporated. The data collection was done, after obtaining ethical clearance from the thesis review committee of AIMS. Formal permission was obtained from the principal of the selected school, Kochi. The participants were explained about the study and confidentiality and anonymity was ensured. Then the questionnaire for data collection was administered to teachers those who present at the time of data collection. Data was analysed using descriptive and inferential statistics[9]

Results

Section 1 Demographic Characteristics of the subjects

Table 1: Distribution of subjects based on socio demographic characteristics

n=30

Serial Number	Demographic va riables	Frequency(F)	Percentage(%)
1	**Age in years**		
	25-30	5	16.6
	30-35	25	83.3

Cont... **Table 1: Distribution of subjects based on socio demographic characteristics**

n=30

2	Sex		
	Male	2	6.6
	Female	28	93.3
3	**Educational qualification**		
	Graduate	29	96.6
	Post graduate	1	3.3
4	**Year of experience**		
	Below one year	1	3.3
	2-5 years	8	26.6
	6-10 years	8	26.6
	More than 10 years	13	43.3
5	**Marital status**		
	Single	5	16.6
	married	25	83.3

Majority of the subjects belongs to the age group of 30-35years (83.3%). Majority of the teachers, 28 (93.3%) are females. Most of them are graduate 29(96.6%)only 1(3.3%) had post graduation . Nearly half of them had year of experience more than 10 years 13(43.3%). Most of the subjects were (83.3%) married.

Section II : pre test knowledge of teachers regarding behavior problems of school children

n=30

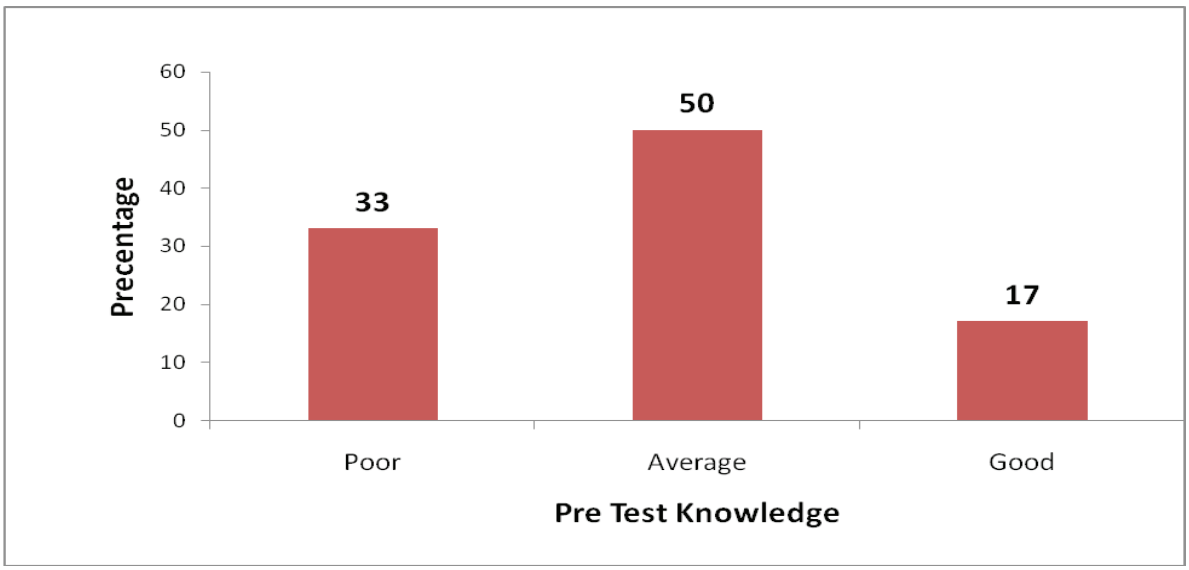

Figure 1: Distribution of primary school teachers based on Pre-test level of knowledge

The figure 1 shows that 33% of the subjects were having poor knowledge, and 50% were having average knowledge and only 17% were having good knowledge regarding management of common behavioural problems

Section III : post test knowledge of teachers regarding behaviour problems of school children n=30]

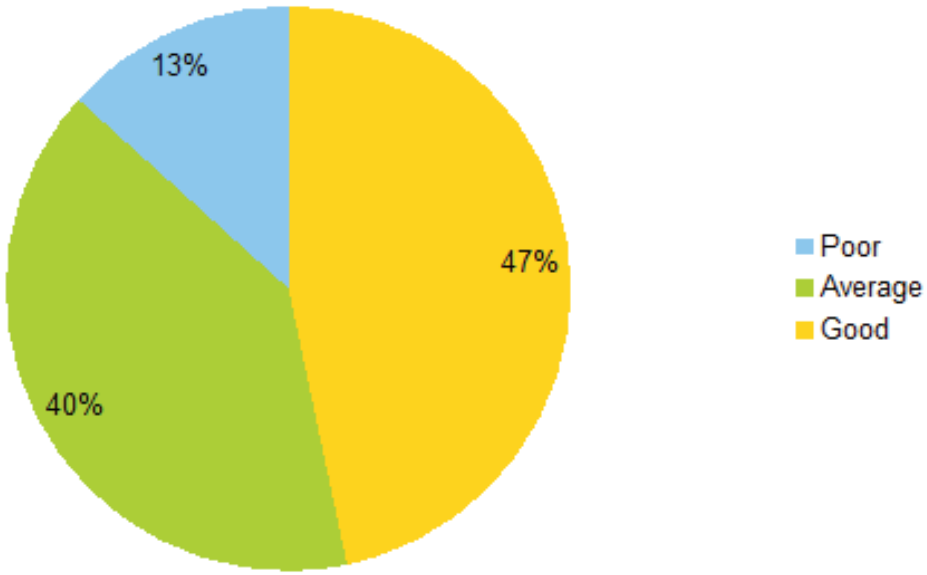

Figure 2: Distribution of primary school teachers based on Post-test level of knowledge

The figure:2 shows that 13% of the subjects were having poor knowledge, and 40% were having average knowledge and only 47% were having good knowledge regarding management of common behavioural problems

Section IV Comparison of the Pre test and Post test knowledge score of Primary school teachers regarding common behavioural problems n=30

Table 2: Comparison of the Pre test and Post test knowledge score of Primary school teachers regarding common behavioural problems

Groups	Scores of Knowledge		df	Paired t test value	P Value
	Mean	SD			
Pre test	13.7	3.79	29	3.569	0.001
Post test	16.18	2.74			

Table 2 depicts the mean pre-test score is 13.7 with the standard deviation 3.79 and the mean post-test knowledge score is 16.18 with the standard deviation 2.74.The mean post- test knowledge score (16.18) is significantly (t= 3.569,p = 0.001) higher than the mean pre-test score(13.7).This shows that structured teaching programme was effective in improving the knowledge of teachers regarding behavioral problems. There is no association between Pretest & Post test knowledge scores with demographic variables.

Discussion

There was statistically significant increase in the knowledge levels in each component after the

implementation of the education programme.Mean post test score(16.18) is higher than the pre test mean score(13.1 The mean post- test knowledge score (16.18) is significantly (t=3.569, p = 0.001) higher than the mean pre-test score(13.7)).Findings pointing out the need for training programmes for teachers. Sandeep Garg et.al conducted a study among primary school teachers on knowledge regarding common behavioural problems shows that in pre test, were having an average knowledge(49.40%) with mean score of 14.82+_ 3.372 and in post test the score was 75.83%with mean score of 22.75 +_ 2.802and the t value is 3.23 at .05 level of significance[7]

Abraham et.al.(1992) found that comprehensive orientation programs for teachers in various aspects of child mental health will enable them to function effectively in the promotion of child mental health.[8] Lian W B et.al. in their study identified that the pre-school teachers have an educational deficit in childhood developmental and behavioral disorders and they need to improve their skills in identifying and managing the mental health problems of children . In the Western literature there are numerous studies on training of school teachers on child mental health problems, enabling them to identify and manage the behavioral problems of school children[5] . As there is rise in the behavioral problems among school children and a scarcity in Indian literature on training programs for teachers on behavioral problems, there is a paramount need to conduct such programs for the school teachers

Conclusion

Based on the findings of the study it was concluded that the education program regarding management of common behavior problems in school children was effective in improving the knowledge of teachers regarding the same.

Conflict of Interest: Nil

Ethical Clearance: The researchers got the permission from the ethical committee of Amrita College of Nursing and Thesis review committee of Amrita Institute of medical sciences, Kochi before the study .

Source of Funding: From all authors contribution

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A Study of Knowledge, Attitude and Practice (KAP) Regarding Voluntary Blood Donation among Health Care Professionals in Dhiraj Hospital- Vadodara

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Abstract

Background: Blood components like PCV, Whole Blood, Fresh Frozen Plasma(FFP), Platelet Rich Plasma(PRP), Cryoprecipitate demands are increasing day by day in developing countries like India. So in our study to assess the overall knowledge, attitude and practice regarding the extent of blood donation practice among health care workers have keen importance.

Methods and Material: Our study which is a cross sectional study includes total of around 80 health care workers of Dhiraj Hospital - Vadodara. On the basis of socio-demographic characteristics and according to the levels of Knowledge, Attitude and Practice among participants data were collected using a self – administered questionnaire. A structured questionnaire was given to all the health care workers working in the Dhiraj hospital were taken into consideration.

Results: Eighty health care workers were involved in our study which was conducted at Dhiraj hospital Vadodara among which it was found that about 55% donated to serve for humanity. A large group of around 15% were donating as a replacement for their kith and kin. Around 10% were because of self motivated decisions. And on the other side the reasons for non-donation by non-donors was that nobody approached to them and it constituted to about 6%. Around 57% non donated due to some health related reasons while rest of them non donated due to fear of disease transmission and fear of needle which constituted to about 17%.

Conclusions: The health care workers of Dhiraj hospital were having overall level of knowledge, attitude and practice was satisfactory. Health Professionals, various hospitals and blood banks services are demanded to develop new ways so to make blood donation services more reliable and accessible

Key-words: *Knowledge, Attitude, Practice, Health Care Professionals.*

Introduction

"Blood donation and universal access to safe blood transfusion" is the WHO theme for blood donor day 2020. Globally, the demand for blood products in India is projected to increase substantially in coming future and many people die every year due to insufficient supply of blood products. Population growth and population ageing are the challenges which can endanger the supply of adequate blood products in the coming future. Millions of lives are saved every year through blood transfusion, yet the quality and safety of blood transfusion are still the matter of concern especially in the developing countries like India[1]. Health care workers if they are motivated and are willing to be voluntary blood donors can be a very good source of quickly accessible and quality blood[2].

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So, a cross sectional study was conducted to identify and assess the problems that prevent participants from becoming voluntary blood donors.

For blood transfusion a healthy blood supply is important as it would otherwise affect the health of targeted population. For overall blood transfusion inviting and maintaining low risk population groups for voluntary blood donation is the important management strategy. Constant knowledge, awareness and attitudes of each of the health care workers from the primary level is must. Therefore, combine effort by blood transfusion organization system to increase the number of participants who donate blood regularly is required and needs to be planned accordingly[2,3,4].The information so obtained could be helpful in developing appropriate messages to be used during recruitment campaigns, creating and strengthening positive attitudes towards blood donation and developing and implementing focused awareness programs for target populations[5]. In other developed countries with advanced health systems, the demand for blood products continues to rise sophisticated medical and surgical procedures, trauma care and the management of blood disorders[6].

Materials and Methods

Study population:

1. Study type and area: This was a cross-sectional study that was conducted from January- December 2019 among health care workers of Dhiraj hospital Vadodara.

2. Study population, sample size and study procedure: The study population included health care workers of Dhiraj hospital. The hospital has over 1500 health care workers in various departments among which a total of sample size of 80 health care workers were selected. Self- administrative paper pencil questionnaires were used as a tool of data collection. It includes six parts.

Part 1- Socio-demographic characteristics of participants.

Part 2- Awareness and knowledge of blood donation.

Part 3- Practice of blood donation.

Part 4 & 5- Attitude towards donating blood.

Part 6- Source of information.

3. Data Processing and Analysis: Questionnaires were collected and checked for any errors and completeness. Data were calculated and statistically analyzed. In the questionnaires, any information which was unclear was rechecked.

4. Ethical Clearance: Ethical clearance was sought from the Sumandeep Vidyapeeth ethical committee(SVEC) before starting the research. Written and informed consent was obtained from all the participants in the study after explaining the purpose of the study before distributing the questionnaires.

Study selection Criteria:

Inclusion criteria:

1. All the health care workers upto 60 years of age.

2. All the health care workers those working in Dhiraj Hospital of Vadodara.

Exclusion criteria:

1. Those health care workers who are absent in the time of data collection.

2. Health care workers of more than 60 years of age.

3. Not willing to participate in the study

Results

The results of the overall data collected from the health professionals are divided in three division:

1. Knowledge score in health workers:

Regular voluntary donor are the safest donor as their blood is checked at frequent intervals of time for example health care workers as they are having knowledge regarding all transfusion transmitted infections, their safety and risk factors associated with donation as shown in Table 1.

Table 1. OVERALL KNOWLEDGE REGARDING BLOOD TRANSFUSION TRANSMITTED INFECTIONS(TTI), SAFE PRACTICE AND RISK FACTORS IN HEALTH CARE WORKERS.

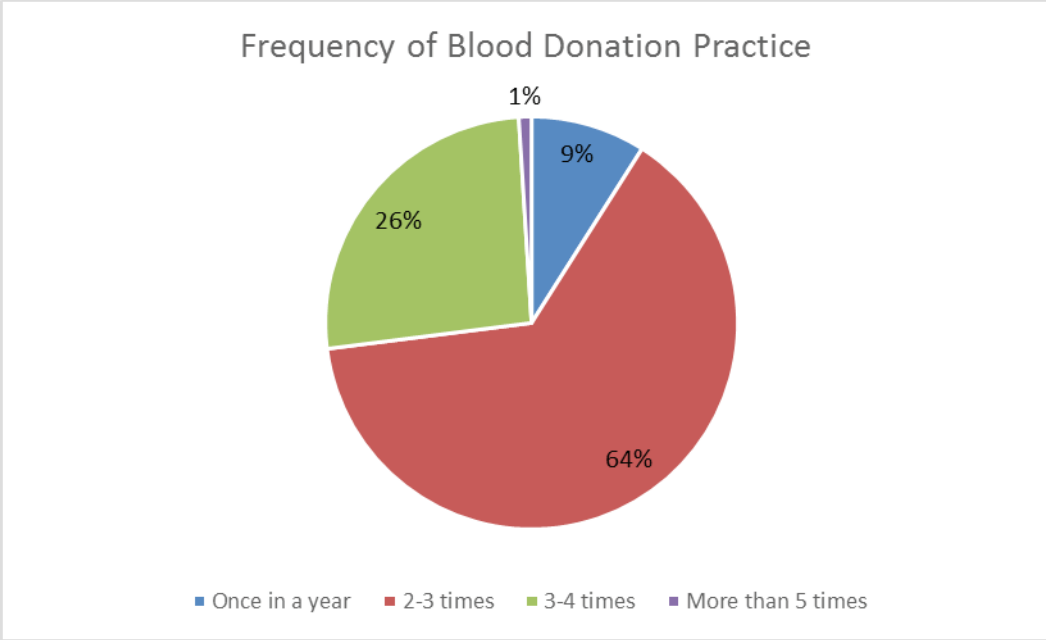
- KNOWLEDGE OF BLOOD TRANSFUSION TRANSMITTED INFECTIONS (TTI) AMONG WORKERS IN HEALTHE CARE INDUSTRY	**TTI IN HEALTH PROFESSIONALS**	**(A) TUBERCULOSIS**	**(B) HEPATITIS B AND C VIRUS**	**(C) HUMAN IMMUNO DEFICIENCY VIRUS**	**(D)HUMAN T –CELL LEUKEMIA VIRUS**	**TOTAL**
	TOTAL NUMBER	45	20	10	05	**80**
KNOWLEDGE OF HEALTH CARE WORKERS WITH RESPECT TO SAFE BLOOD DONATION PRACTICE	**KIND/TYPE OF DONOR**	**REGULAR VOLUNTARY DONOR**	**FAMILY REPLACEMENT DONOR**	**COMMERCIAL DONOR**	**PROFESSIONAL DONOR**	**TOTAL**
	TOTAL NUMBER	55	15	05	05	**80**
KNOWLEDGE OF RISKS ASSOCIATED WITH HEALTH CARE WORKERS REGARDING BLOOD DONATION	**RISK FACTORS**	SEXUAL ACTIVENESS	TATTOOING	UNSAFE SEX	I.V.DRUG ABUSERS	TOTAL
	TOTAL NUMBER	69	06	03	02	**80**

Table 2 reveals the knowledge about signs & symptoms and knowledge pertaining to various tests in health professional.

KNOWLEDGE ABOUT SIGNS AND SYMPTOMS	**(A)) CHRONIC DIARRHEA**	**(B) HUMAN IMMUNO DEFICIENCY VIRUS (HIV)**	**(C) SKIN REACTIONS AND SWOLLEN GLAND**	**(D) BRUCELLOSIS AND HERPES ZOSTER**	**TOTAL**
TOTAL NO OF HEALTH WORKERS	35	25	15	25	**80**
KNOWLEDGE ABOUT TESTS	**ABO AND RH INCOMPATIBILITY**	**HBSAG AND ANTI-HCV**	**ANTI HIV-1 AND ANTI HIV-2**	**ANTI TRYPANOSOMA CRUZI AND SYPHILIS**	**TOTAL**
TOTAL NO OF HEALTH WORKERS	44	16	15	05	**80**

2. Attitude of blood donation in health workers:

While conducting the study the overall attitude of the health workers regarding donation of blood by donor on one side was a main cause to serve humanity which constituted to about 55%. A large group of around 15% were donating as a replacement for their kith and kin. Around 10% were because of self motivated decisions. And on the other side the reasons for non-donation by non-donors was that nobody approached to them and it constituted to about 6%. Around 57% non donated due to some health related reasons while rest of them non donated due to fear of disease transmission and fear of needle which constituted to about 17%.



The above pie - chart shows the percentage of frequency of blood donation practice done by the health professionals are 64% of them donated 2-3 times in a year, 26% donated about 3-4 times in a year, 9% donated once in a year and 1% donated more than 5 times in a year.

3. Practice of blood donation in health workers:

Health care worker who were blood donors showed their active and voluntary involvement for donating blood when they were being studied for various practices involving in blood donation. The health care professionals provide and conduct predonation information, orientation, general health check ups and appropriate selection of donors. They are also involved with post donation information, orientation and supervision through blood tests. All this measures are aimed at safe blood donation practices involving appropriate donor selection, Transmission transmitted infection (TTI) reduction and quality transfusion to the people needing blood transfusion**.**

Discussion

To meet the increasing demand of blood products, there is overdependence on family replacement and remunerated donors which can poses serious danger to potential recipient. In our study serological screening tests play a vital role for detection of transfusion transmissible infections in potential donors; failure to take care of pathogen macromolecule detection technique, pathogen inactivation and irradiate blood products are more liable to infection. There are many similar studies conducted however only few of them can assess safety of blood through quality donors in blood transfusion services. There are two perspectives, on one hand health workers have knowledge regarding blood groups and possible TTIs and on other hand they are less involved in practice of blood donation. So overall knowledge, attitude and

practice is not up to the point which is expected in health workers. The major reason given by the health workers for not donating was that nobody approached to them. This shows that there is serious need to educate them for a safe donation practice. During our study on various health workers of Dhiraj hospital we also found that there is strong association between blood donor involved in donation and sex. In our region male health workers are more likely to donate blood than female health workers as the women which are in the donor age group usually have their monthly menstrual cycles, lactational period and pregnancy which prevent them from donating. So active education program must be conducted to educate all health workers for safe blood transfusion.

Conclusion

The study revealed that almost two-third of the health care workers in Dhiraj hospital in Sumandeep Vidyapeeth had ever donated blood with the majority of them willing to donate in the future. Awareness, motivation and positive attitude towards blood donation should be given to these potential blood donors. Proper information about the process of blood donation, TTI and the knowledge of blood in saving lives increase the frequency of blood donation. Along with it, education messages should be delivered to females to encourage more female health care workers to donate blood.

Ethical Clearance- Taken from institutional ethical committee.

Source of Funding- Self funded.

Conflict of Interest – Nil.

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Possible Pain Caused in Judicial Modes of Execution: A Physiological and Pathological Analysis

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Abstract

Undoubtedly, the application of Capital Punishment has attracted a torrent of worldwide criticism. Primarily the cause behind this criticism is the excruciating pain caused by ancient outdated and uncivilised methods like crucifixion, hanging, beheading, etc., unlike the cotemporary scientific processes and international instruments. In opposition to the backdrop of enumerated counterattacks, most of the developed countries devised/formulated and implemented some contemporary procedures like shooting, lethal injection, gas chamber, etc. These contemporary procedures were alleged by the formulating nations to be swift and causing minimum pain. In this research paper, each of these modern modes have been critically analysed after reviewing the physiological and pathological aspect of pain associated with each of these methods. The author finds out that these so called modern method do inflict agonizing pain on the convicted offenders. In conclusion the author does suggest that medicalized process of execution may, to some extent, lead to painless killings.

Keywords-*Capital Punishment, medicalized, contemporary, execution.*

Introduction

"The rights of every man are diminished when the rights of one man are threatened."

John F. Kennedy

Modes of judicial execution of death sentence have to be determined in light of various objective factors like prevailing atmosphere of the international opinion, international norms and standards, contemporary penological theories and ever progressing standards of human dignity. Diverse and numerous aspects varying from society to society, influenced by the sacred tradition and culture of that society, its philosophy and history and its sense of moral and ethical values, are the key determining factors which effect the evolving standards of human dignity.[1] With the gradual passage of time the standards and norms of human decency and dignity do vary within the same society as well. In a progressive society, these standards keep on elevating to higher altitudes.

Pain and suffering are not merely one but an important aspect of cruelty; lack of pain and suffering do not make the punishments free from being termed as "cruel and unusual". As broadly understood, any act that diminishes human dignity is termed as cruelty. The thought that separates the existence of human beings from other inanimate objects and even other living organisms and makes them unique creatures in the entire universe, is nothing but simple expression of dignity. The holy book of Quran has defined human being as "ashrafulmakluqat", which means supreme of all beings on the planet Earth. All inanimate objects and living creatures are only property existing at the disposal of man, as the only creation of Almighty capable of holding rights and performing duties are human beings.[2]

In this research paper, researcher will be analyzing the functioning and pathology of different methods of Death Sentence. All the Information for this analysis has been derived from observations on the condemned convicts, based on postmortem reports, physiological studies on animals where a similar procedure was applied for execution, and emergency medicine literature. It is extremely difficult to mark the intensity and duration of pain a person goes through when executed, because all the signs of pain are dependent on the procedure or on

the physical restraints,[3] but one can certainly identify those methods which are likely more painful.

Physiology and pathology in different methods of execution

Shooting

This method was carried out by either a single soldier or a policeman at short range who fired from behind the condemned person's occipital (back of the head) towards the forehead, or by a firing squad comprising of up to thirty soldiers who stood or knelt opposite to the blindfolded prisoners.[4] The soldiers aimed at the chest usually, since it was easier to target, than the head. Shooting from a short range literally destroyed the vital centers of the medulla, just like when a captive bolt is used for slaughtering cattle. When firing squad aimed at the head it produced the same type of lesions as that produced by a single soldier, while bullets fired at the chest ruptured the heart, great vessels, and lungs so that the condemned person dies of hemorrhage. A high velocity bullet produces a cavity which has a volume several hundred times higher than that of it.[5] Cavitation happens probably due to the heat dissipated by the bullet on body, an impact that boils the water and volatile fats in the tissue when bullet strikes. Convicts when hit by bullets feel as if they have been punched, pain comes later only if the person survives long enough to feel it. Shooting as a mode of death penalty and a potential substitute to hanging was discussed by the Royal Commission on Capital Punishment, however, it was discarded on grounds like; it did not possess the first and the most important requisite of an effective method that has the certainty of causing immediate death.[4] The authorized team furnishing evidence to the Commission often stressed on the point that any process of judicial execution recommended by them must not only be clean and swift but dignified as well.[6]

Beheading

Beheading as a mode of Capital Punishment was practiced in Congo, Qatar, Mauritania, the United Arab Emirates, Saudi Arabia, and Yemen.[7] In this method, the axe was wielded by a strong man who made repeated targeted sword cuts to the neck with immense force, or by guillotines a device consisting of a tall, upright frame in which a weighted and angled blade is raised to the top and suspended for beheading. Beheading does not always result from a single blow as the skin, muscles, and vertebrae of the neck are tough. The convict becomes unconscious within a few seconds, but not immediately after the spinal cord is severed. When biochemists had guillotined small rodents their eyes moved only for a few seconds. Anaesthetized sheep lose the flash-evoked responses of their electrocardiographs about 14 seconds after both the carotid arteries were severed, and it took 70 seconds after one carotid artery and one jugular vein were cut. It took merely 12 seconds for the dogs to be unconscious after the blood supply to their brains was obstructed. While the human brain stores enough oxygen for metabolism to carry on for 7 seconds after the supply is cut off. Nevertheless, the brain could also derive some of its energy from facial neck muscles and substrate in the scalp.[8] It may be concluded that a beheaded person dies from anoxia conceded by hemorrhage.

Hanging

Prior to the abolition of capital punishment in 1973, hanging as a judicial method of execution was lastly used in 1964 in Great Britain.[9] It would probably be used again if Parliament were in favor to reintroduce it as a mode of death penalty. In death penalty by hanging, the convict was weighed the day before the execution; also a try out was done using a sandbag of the same weight as that of the condemned. This was done to fix the length of the 'drop' that would ensure a rapid fracture-dislocation of the neck. Just before the execution, the convict's hands and legs were restrained, he was blindfolded and the noose was positioned around his neck.[10] The execution happened when the trapdoor was opened and the convict fell through. The weight of the convict caused a rapid fracture-dislocation of the neck, unless the condemned person had really sturdy neck muscles or the 'drop' was too short, or the noose had been wrongly positioned.[11] Whatever the case, the face became inflated and then cyanosed. The tongue protruded out and rapid violent body movements occurred which was usually due to the spinal reflexes. Usually the convict used to micturate or defecate or both. The heart continued to beat up till 20 minutes after the drop.

This procedure of capital punishment was most used by the British, but in most other countries hanging was a much less sophisticated practice, executions were

often carried out in public, and the bodies were often left dangling to frighten others intending a similar crime.

It was discovered through the post-mortem that the noose under the chin caused rotation and hyper flexion of the neck, and between the body and the pedicle of the axis, fracture of the junction was found, anterolateral on one side of the noose, and posterolateral on the other. The spinal cord got transected, with avulsed medulla, and there were extensive lacerations and bruising of the spinal cord. Such similar lesions were also seen in rats killed for biochemical experiments, who had cervical dislocation and their hearts continued to beat for 7 min approximately after dislocation.

It's usually implicit that instantaneous loss of sensation is caused by fracture-dislocation. Rupture of sensory pathways below the neck must take place without doubt. However, the sensory signals from the trigeminal nerve and skin above noose keep on reaching the brain till locked by hypoxia.[12]

In case there is no swift facture-dislocation, it's the asphyxiation which causes the death. In comparison to fracture-dislocation, asphyxia results in much slower death, because the vertebrae continues to supply blood to the brain as it protects the spinal and vertebral arteries and it's only the carotid arteries and jugular veins which are occluded by the noose.[13]

Gassing

Nevada was the first country to use Gassing in 1921. In this method of capital punishment, the condemned person is strapped into a chair in front of a sulphuric acid container, in an airtight chamber. The warders after strapping the convict withdraw from the chamber and it gets closed.[14] A lever is operated from outside the chamber which drops crystals of sodium cyanide into the pail. The condemned is instructed to take a whiff and breathe. Most convicts try to hold on their breath, and struggle as much as they can. It is still not known what sensation is felt when a person inhales cyanide, but in the post mortem reports of the signs and symptoms of accidental cyanide poisoning are vomiting, giddiness, ataxia, hyperventilation headache, dyspnea, and finally collapse to death.[15] Death is due to anoxia, because the cyanide is an inhibitor of the enzyme cytochrome oxidase, which is a key respiratory enzyme. Cyanide disrupts the electron transport chain by binding to the enzyme cytochrome oxidase. This prevents the transport of electrons from cytochrome oxidase to oxygen, thus resulting in anoxia.

Intravenous injection

United States was the first country to introduce execution by intravenous injection in 1977. In this method the convict is bound supine to a trolley and the vein in the angle of the elbow is annulated by a trained nurse or technician. There are times when the convict's veins are difficult to penetrate, for instance, if he or she does not cooperate, in case of phlebitis due to injection abuse of addictive drugs, or if there is scarring due to an attempt to slash the arm, due to all this, the procedure is fraught with complications. After the cannula is lodged successfully into the vein, three substances are injected one after the other: sodium thiopentone (a rapidly acting anesthetic), pancuronium bromide (a muscle relaxant to paralyzerespiration), and potassium chloride (stops the heart).[16] The condemned becomes unconscious within 10- 15 seconds after injection where death results due to anesthetic overdose and respiratory and cardiac arrest. In this method the doctor does not have any part in the execution, but he/she will certify after examination that the convict is dead.

The assessment of pain during execution

We will only consider the physical aspects of pain during execution. The ability to assess the intensity of pain, considering these signs in different modes of execution in death penalty, is indicated in the table:

TABLE 1.[17]

Signs of severe pain or distress in persons executed

(Sh) shooting, (H) hanging, (B) beheading, (G) gassing and (IV) intravenous injection.

+ indicates that the sign is often seen,

- that it is not seen,

*** that the sign can either not be made or not be seen, and**

? that there is no information.

Signs	Method of execution				
	Sh	H	B	G	IV
Shouting or screams?	?	*	+	+	-
Perspiration	?	?	?	?	?
Dilated pupils	*	?	?	?	+
Withdrawal from stimulus	*	*	*	*	*
Violent movements?	?	+	*	*	-
Contraction of facial muscles	*	+	?	+/-	-
Micturition	?	+	?	?	?
Defecation	?	+	?	?	?

TABLE 2.[18]: Factors occurring in execution which are likely to cause pain Intensity of likely pain is graded as little (+), moderate (+ +), severe (+ + + j, or not known (?), The likely duration of the sensations is not known.

Method of exxecution	Pain		
	Cause	Sensation	Intensity
Shooting	rupture of skin fracture of bone	sting or punch cracking	+or+ + ?
Beheading	stretch of skin prior to cut lacerations of skin	Burning sharp pain	+++ +++
Hanging	stretch of skin fracture - dislocation of vertebrae asphyxia	burning, stretching dislocation, fracture suffocation, distress	+++ +++ +++
Gassing	tracheal irritation asphyxia	burning suffocation	+++ +++
Intravenous Injection	missing or going through the vein	intramuscular injection	+

Following conclusions are drawn after examination of these tables:

(a) It is doubtful to state whether most of these signs happen or not even if some occur, they could not be seen;

(b) Some of them do not occur due to physical restraint;

(c) Various signs, like; micturition, contraction of the facial muscles and dilation of the pupils appear because of extreme pain, electrical stimulation, fear or even dying. Moreover, the intense stressful environment and circumstances surrounding the entire execution process may also either contribute to or suppress the pain.

The condemned when being stoned screams and shouts, but one cannot identify whether any of the other signs occur as well. Nor it is easy to assess the intensity and duration of pain when the head is decapitated. There are important areas of ignorance that cannot be measured, and certainly one cannot conclude for certain the extent of pain with respect to any particular method of execution. However, one can conclude the behavior which is likely to occur in each method and through them (table 2.), and hence the evidence for intensity of pain is derived (table 3).

Different stages at which pain occurs are shown in table 2. Based on the data, it is undeniable that with the exception of intravenous injection, all the methods of execution produce a similar sort of lesions as conditions. Also, it is highly likely that with the certain exception of intravenous injection and possibly the exception of shooting, all the modes of execution are likely to produce severe intensity of pain. Examples to justify this are given in table 3. The states practicing the modes of judicial execution like, hanging, electrocution, and gassing believe that these executions are painless and that the pain lasts only for a short time where the sentenced person does not suffer for long, or that they believe that the pain that a convict goes through is justified as based on the heinous crime for which the condemned has been found guilty.

TABLE 3.[19]: Evidence for Pain Being Likely During Execution

Method of execution	Evidence
Shooting	Evidence of victims of gunshot wounds (Beecher 1949; Owen-Smith 1981; Melzack et a1 1982)
Hanging	Dislocations are painful (Watson-Jones 1976), fractures are painful (Wu 1987)
Beheading	Skin receptors are active until sensory pathways are hypoxic (Donald Hatcher 1965; Safar et a1 19821
Gassing	Symptoms of cyanide poisoning include headache (Arena 1988; National Poisons Treatment Service 1991)
Intravenous Injection	No pain reported with successful cannulation

Conclusion

Subsequent to a thorough analysis as to the likelihood of suffering and pain that is caused to the condemned person in several modes of judicial execution of capital punishment like intravenous lethal injection, hanging, shooting, beheading, gassing, etc., the scholar reasonably reaches to the conclusion that all the judicial modes discussed above have the potential to cause severe pain and suffering in one form or the other, with only intravenous lethal injection as a possible exception. Apparently, the usual signs of intense pain seem absent, generally because these are being masked by the kind of procedures involved or because the physical restraint of the convict does not let him/her to demonstrate the same. Hence, lack of adequate evidence due to absence of signs of intense suffering and pain, leads to indecisiveness as to it's occurrence. Nevertheless, evidence offered by the science of physiology and derived from comparisons with

emergency and accidental medicine, makes it clear that almost all the procedures involved in judicial executions have the potential to cause severe pain and suffering to the condemned. However, in spite of the evidence shown and discussed above, it's extensively emphasized that processes involved in judicial executions are painless and humane, even though no evidence proving this has been published till date.

1. **Ethical Clearance -** Not Required

2. **Source of Funding-** Self

3. **Conflict of Interest-** Nil

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The Effect of Family-Centered Empowerment Model (FCEM) on Improving Family Functioning of Children Diagnosed with Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

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Abstract

Introduction and Aim: Caring for child with glucose-6-phosphate dehydrogenase (G6PD) deficiency causes a lot of stress for parents. Family-centered empowerment model is one of the educational programs.

Materials and Methods: A controlled clinical trial was conducted on parents of children with G6PD deficiency that was referred to Iran Hospital of Iranshahr. The participants were randomly divided into an experimental group (n = 30) and a control group (n = 30). To collect the data, the Family Assessment Device (FAD) was completed by the children's parents before and after the intervention. The FAD scales demonstrated fairly good internal consistency (the Cronbach's alpha of the questionnaire ranged from 0.72 to 0.92 and its validity and reliability were 0.76 to 0.66 indicating the acceptable values. The family-centered empowerment model was implemented during four sessions. One month after the intervention, the subjects completed the questionnaire again and finally the data were analyzed by the statistical tests.

Results: Both the paired and independent sample t-tests were used for all statistical analyses at a significance level of $P>0.05$. Also, both the chi-square test and the Fisher's exact test were used to compare qualitative variables in two groups. The mean FAD scores of the intervention group were significantly decreased from 152/4 to 1.120 ($P<0.001$), while no statistically significant difference was observed in the mean FAD scores for controls before and after the intervention. Furthermore, the mean FAD scores in the intervention group were decreased to 32.3 units and up to 0.6 unit in the controls, and the difference between two groups was statistically significant ($P<0.001$).

Conclusion: According to the results obtained, the implementation of family-centered empowerment model has a significant effect on improving the family functioning of children diagnosed with G6PD deficiency. Therefore, it is recommended to implement in health centers in order to prevent recurrent attacks of this disease.

***Keywords**: Glucose-6-phosphate dehydrogenase deficiency (G6PD) or favism; Family self-efficacy; Family-centered empowerment model*

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Introduction

The family-centered empowerment model (FCEM) is one of the effective ways to empower the chronic patients and their families too [1]. If there is a genetic defect in the production of the G6PD enzyme which

affect the cell membranes, especially red blood cells, and destroy their walls, leading to clinical complications known as the acute attacks of Favism [2].The prevalence rate of G6PD deficiency varies from 1% up to 50% worldwide. The prevalence rate of G6PD deficiency is 10-15% in Iran [3]. Also, the results of the study of Miri-Moghadamet al. (2002) showed that G6PD deficiency prevalence rate was 24.5% in Iranshahr city [4.] In their study, Mortazavi et al. (2010) demonstrated that the prevalence rate of G6PD deficiency in Iranshahr is one of the highest frequency in Iran cities [5].Given high prevalence of children with G6PD deficiency in Iranshahr and its frequent acute attacks of this condition due to cultural poverty, especially in the rural areas and the lack of their caregiver knowledge and awareness of the disease which can lead anxiety, suffering, economic burden and reduced family functioning for the patient's family [6]. With recent advancements of the removal of health problems, family caregivers have replaced health care institutions [7]. Nurses or health care providers are in a unique position to interact with individuals and family members [8]. Nurses have many duties, including caring for patients and helping their families to increase their hope and trust, contributing to the promotion of the health and well-being of the families [9].From the perspective of many experts, empowerment is a dynamic, positive, interactive, and social process which occurs in the interaction with others and helps patients feel greater responsibility to their health, interact with healthcare authorities more effectively, feel greater satisfaction, demonstrate better responses to treatments, prevent complications of disease , decrease their costs in healthcare, adopt a more positive attitude toward their problems, and have a higher quality of life (QoL)[10]. The FCEM is one of the Iranian health models published by a study conducted on the theory based on Bandura's theory in order to improve chronic diseases [11]. The FCEM is designed with emphasis on the effectiveness of an individual and role of other family members in three dimensions: motivational, psychological (self-esteem and self-control) and self-problematic features (knowledge, attitudes and perceived threat) [11]. Family empowerment has three main characteristics: (2) the ability to review and control the required educational resources (3), the ability to solve problems and make decisions, and (4) the ability to communicate to meet these needs [12].The main difference between the FCEM and traditional programs is that the FCEM can help the patients and health care providers [13] .The FCEM includes four major steps: (1) perception of threat (2), self-efficacy (3), self-esteem, and (4) evaluation [13]. Therefore, in order to achieve the best result, it is possible that the FCEM can simultaneously increase knowledge, skills, values, and beliefs of self-confidence and self-control in the patients and their families [14]. In Iran, many studies have been conducted on empowering the patients and their families, for example, a study conducted by Salehi et al (2011) on hemodialysis patients [15], a study conducted by Teymouri (2011) on children with asthma [16] the study performed by Vahedian-Azimi et al (2008) on patients with myocardial infarction (MI)[11] a study performed by Heidari et al. (2007) on adolescents with diabetes mellitus [12] and a study carried out by Allahyari et al(2006) on children with thalassemia. The results of many studies suggest that outcomes of the empowerment includes having positive self-esteem, having and achieving goals, gaining control over life and having a sense of hope for the future [17], and improving self-care , self-efficacy, and QoL of the patients and their families. Given that most research studies have recently been conducted on patient education, and less attention has been paid to the importance of the family as an important source for therapeutic goal setting, it therefore seems that the FCEM can be effective in empowering the patients, identifying their health care needs and helping them to follow a diet. To the best of my knowledge, a few studies have been conducted to investigate the effect of the family-centered empowerment model on improving the parental self-efficacy and family functioning of children diagnosed with G6PD deficiency. Due to the quasi-chronic nature of Favism and its recurrent attacks, it is therefore necessary to investigate the effect of the family-centered empowerment model on improving the parental self-efficacy and family functioning among children with G6PD deficiency, paying attention to the dimensions of knowledge, skills, values and beliefs of self-confidence and self-control of the patients and their families. Therefore, this study was conducted to investigate the effect of the family-centered empowerment model on improving the parental self-efficacy and family functioning of children diagnosed with G6PD deficiency.

Materials and Methods

A randomized controlled trial was conducted on parents of 6–14-year-old children with G6PD deficiencies that were referred to Iran Hospital of Iranshahr in 2019, used conveniently health records in health center were allocated to the study groups by table of random numbers. First 60 available children, who were referred to the Iran Hospital and previously diagnosed with G6PD deficiency, were selected according to the inclusion criteria. Then they were divided into intervention and control groups.

The parents filled out the informed constant, demographic information questionnaire and the FAD.

Based on the sample size formula, comparing the two means and based on the purpose of comparing the care load score before and after the intervention in the intervention group and the results of the study conducted by Jalilian et al.

$X1 = 5.25\ S1 = 1.66\ X2 = 6.9\ S2 = 1.37\ \alpha = 0.05\ \beta = 0.1$

The sample size in each group was estimated to be 18 people per group which was increased by 30 people to increase the strength and accuracy of the study and the probability of sample loss in each group of 30 people. First, 60 children with a history of hospitalization with a diagnosis of favism in Iranshahr hospital were selected according to the inclusion criteria, then the patients were divided into two groups based on a table of random numbers.

The inclusion criteria for parents were as follows: their willingness to participate in the study, giving informed consent to participate in the study, having the skills of reading and writing and the ability to complete the questionnaire, not being a members of group of health care workers or nursing group, having no family history of other chronic diseases, having no history of previous education regarding Favism prior to the start of the study, having no hearing and speech impairments and being responsible for the child's care daily. The inclusion criteria for children with G6PD deficiency were specified as follows: those between 6 and 14 years, having no hearing and speech impairments and having no history of other underlying diseases.

In this study, the data were collected using both the demographic information questionnaire (e.g., age, sex, marital status, occupation status, education level, type of relationship between the patient and his/her caregiver and economic status) and family assessment device (FAD). The FAD is a 60-item questionnaire designed to assess family functioning based on the McMaster Model of Family Functioning (MMFF) for family members above the age of 12 or older. The FAD was developed by Epstein, Baldwin, and Bishop in 1950 to assess structural and organizational properties of families and evaluate the family's ability to resolve family tasks using a self-reported scale. The model identifies seven dimensions of family functioning: problem-solving, communication, roles, emotional involvement, emotional response, behavioral control, and general function.

To score the FAD, all responses were coded as follows: 1 = strongly agree, 2 = agree, 3 = disagree and 4 = strongly disagree. Items describing unhealthy functioning are reverse scored, so lower scores represent healthier functioning. The scores range from 1 (healthy functioning) to 4 (unhealthy functioning). In the study of Yousefi (2012), the reliability of FAD was higher than 0.80 for all domains and the total instrument via calculating Cranach's alpha. After receiving permission from Iran Hospital of Iranshahr, the researcher examined the information related to patients admitted to the hospital. Considering inclusion criteria, 60 subjects and one of their parents who were eligible for the study were selected. A written invitation letter was sent to their parents requesting them to participate in the study. They were asked to participate in the initial session which was an introduction to participatory research in the hospital's conference hall at a specific time. After the participation of their parents in the initial session, the researcher introduced himself and explained the study goals, the significance of the problem and how to conduct the study and all participants had the right to leave a study at any time if they wanted to and their information would be kept confidential. The written informed consent was first obtained from parents then the demographic information questionnaire and the FAD were completed by their parents in the first session. A need assessment was conducted by discussion and then they were randomly assigned to an intervention group (n=30) and a control group (n=30), and the dates of the next sessions and the exact schedule of the sessions were announced to them. According to a previous schedule, the FCEM was implemented in four stages:

(1) discussion with the parents is implemented, which led to the identification of the parents' limitations, needs, and weaknesses in a variety of domains, (2) the content of the empowerment program was designed and (3) the intervention was performed. The study included two groups (experimental and control). The participants in the intervention group (three groups, n= 10 per group) received the intervention for four two-hour sessions. By viewing distance to participate in the study and the willingness of the participants to reduce the number of sessions, the duration of the sessions was increased. The intervention lasted one month and the control group received routine care. In this study, to achieve such goals, the FCEM was used in the experimental group. In the intervention group, the FCEM consisted of four steps and the first step was to enhance knowledge. To increase the level of knowledge, lectures, group discussions, PowerPoint presentations and educational books were used in the sessions. Parents were divided into three groups (n=30 per group) and received education on topics such as physiology, nature, symptoms, complications, prognosis of key factors in the exacerbation of G6PD deficiency or Favism, nutrition, physical activity, medication, and the ways to prevent the attacks related to Favism. The majority of parents talked about their own experiences and the researcher played a pivotal role. At the beginning of each session, 1 to 2 items were asked about the previous session, whether they had learned what was being discussed or not (process evaluation). The second step was to improve the self-efficacy. To increase the self-efficacy level, they received a practical demonstration session on training the self-efficacy skills such as attack symptoms (hematuria) and examination and convincing their child to avoid prohibited substances and how to inform a doctor that their child had Favism and a list of medications that should not be taken. Parents received each of the skills required through practical demonstration after the explanation. The second step was to increase self-esteem.

At this stage, parents were asked to participate in training their children so that they could transfer the content of the training sessions and what they learned through observation to their children, and the children could discuss what they learned from their parents. The fourth step was to evaluate the process (i.e., final evaluation). In process evaluation, knowledge and self-efficacy levels were evaluated during empowerment sessions. In order to evaluate the knowledge level, two oral questions were asked from the parents at the beginning of each session. Self-efficacy assessment was performed by demonetarizing or performing two skills. In the third stage (after the intervention), the final evaluation was performed for the experimental group and the post-test control FAD is conducted one month after the last empowerment session. In order to observe the ethical issues of the study, the participants in control group received all the educational issues related to Favism during a general session and an educational booklet was given to them. The content of empowerment sessions was presented for two hours each session. The first session included a process of randomly assigning subjects to experimental or control groups, completion of questionnaires before the intervention, a needs assessment, planning the sessions and the explanation of study objectives. The second session included the perceived threat in the form of one session through lectures, PowerPoint presentations, group discussion, providing an educational booklet on Favism to obtain familiarity with its causes, symptoms and complications, diet and ways to prevent its acute attacks and forbidden foods and chemicals in patients with Favism. The third session included the self-efficacy improvement through group problem solving and practical training related to self-efficacy skills such as: attack symptoms (hematuria), examination, convincing the child to avoid prohibited substances, informing physicians when prescribing drugs that their patient had G6PD deficiency and showing the film. The fourth session included self-esteem through the method of teaching methods on participation. In this session, caregivers were asked to train their child what they had learned (to implement the fourth step of process evaluation in each session, two questions related to the previous session were asked to them). The data were analyzed using the SPSS version 19. The normality of data was assessed by the Kolmogorov–Smirnov test. Due to the normality of the data, both the paired and independent sample t-tests were used for all statistical analyses at a significance level of $P>0.05$.Also, both the chi-square test and the Fisher's exact test were used to compare qualitative variables in two groups. The significance level considered less than 0.05.

Results

The mean age of the subjects showed that there

were no statistically significant differences between intervention and control groups in terms of demographic characteristics (e.g., age, sex, marital status, occupation status, education level, type of relationship between the patient and his/her caregiver and economic status) (Table 1). The mean problem-solving scores of the intervention group significantly decreased from 14.9 to 11.4 (P <0.001), while no statistically significant difference was observed in the controls with respect to the mean problem-solving scores before and after the intervention. Also, the mean problem-solving scores were decreased by 3.5 units in the intervention group and up to 0.1 units in the control group, and this difference between the two groups was statistically significant (P<0.001) (Table 2). The mean communication scores of the intervention group significantly decreased from 22.5 to 6.17 (P <0.001), while no statistically significant difference was observed in the controls with respect to the mean communication scores before and after the intervention. Also, the mean communication scores were decreased by 9.4 units in the intervention group and up to 0.1 units in the control group, and this difference between the two groups was statistically significant (P<0.001) (Table 2).

The mean role scores of the intervention group significantly decreased from 8.28 to 1.23 (P <0.001), while no statistically significant difference was observed in the controls with respect to the mean role scores before and after the intervention. Furthermore, the mean role scores of the intervention group were reduced by 7.5 units and up to 0.5 unit in the control group, and this difference between the two groups was statistically significant (P<0.001) (Table 2). The mean emotional response scores of the intervention group significantly decreased from 15.4 to 12 (P <0.001), while no statistically significant difference was found in the controls with respect to the mean emotional response scores before and after the intervention. Moreover, the mean emotional response scores of the intervention group were reduced by 3.3 units and the mean emotional response scores of the controls were increased by 0.1 unit , and this difference between the two groups was statistically significant (P<0.001) (Table 2). Also, the mean emotional involvement scores of the intervention group significantly decreased from 17.7 to 14.6 (P <0.001), while no statistically significant difference was observed in the controls in terms of the mean emotional involvement scores before and after the intervention. Additionally, the mean emotional involvement scores were increased by 3.1 units in the intervention group and up to 0.07 unit in the control group, and this difference between the two groups was statistically significant (P<0.001) (Table 2). Also, the mean behavioral control scores of the intervention group significantly decreased from 22.6 to 18.2 (P >0.001), while no statistically significant difference was observed in the controls in terms of the mean behavioral control scores before and after the intervention. In addition, the mean behavioral control scores were reduced by 4.4 units in the intervention group and up to 0.3 unit in the controls and this difference between the two groups was statistically significant (p>0.0001) (Table 2). The mean general function scores of the intervention group significantly decreased from 30.5 to 23.2 (P >0.001), while no statistically significant difference was observed in the controls with respect the mean general function scores before and after the intervention. Also, the mean general function scores of the intervention group were reduced by 7.3 units and the mean general function scores of the control group were increased 0.4 unit, and this difference between the two groups was statistically significant (P>0.001) (Table 3). The mean FAD scores of the intervention group were significantly decreased from 152/4 to 120.1 (P<0.001), while no statistically significant difference was observed in the mean FAD scores for controls before and after the intervention Furthermore, the mean FAD scores in the intervention group were decreased to 32.3 units and up to 0.6 unit in the controls, and the difference between two groups was statistically significant (P<0.001) (Table 3).

Discussion and Conclusion

FCEM can improve family functioning. Therefore, the use of given method for parents can emphasis the family and care given function for children with G-6PD deficiency. In this respect several research studies have been conducted in Iran and other countries. In a study on evaluating the effect of FCEM on the care burden of the parents of children diagnosed with cancer in Tehran Shahbazi et al. (2016) concluded that the implementation of the FCEM could reduce the care burden of the parents of children diagnosed with cancer[19]. In the above study both the patients and their caregivers are participated in the study, leading to increasing their awareness and knowledge, and the empowerment of parents of children

with cancer has an impact on reducing their care burden.

In this regard, Ross believes that the new care models have a comprehensive approach that pays special attention to the QoL and family functioning, so that the dynamics and complexity of various aspects of the patient and family are considered. It seems that what Ross has mentioned, we have achieved the same results in this study. If the care programs are implemented based on a family-centered approach, it can improve the effectiveness of the programs [20]. In their study, Masoudi et al. (2012) found that the FCEM influenced by the culture and beliefs of the community lead to results that may not be possible to control, it is therefore recommended that the FCEM should be implemented and compared in different cultures [21].Due to Baloch ethnicity, culture and beliefs of people in the city of Iranshahr are different from other cities of Iran. In line with previous studies, the result of the present study showed that there was a statistically significant reduction in self-efficacy and family functioning.

In this regard, Vahedian-Azimi et al. (2019) in their study showed that the FCEM is an indigenous model and compatible with culture and it as a simple, efficient and reliable model which can improve and improve various dimensions of quality of life and family functioning in adults with chronic disease. [22] .Wakarsin et al. (2016) conducted a study on the effect of FCEM on family functioning of children with thalassemia. The results showed that family functioning was significantly increased after the intervention [23].The results of this study also confirmed the findings of the present study. Roghanchi et al. (2017) conducted a study aimed at investigating the effectiveness of group counseling based on resiliency on family functioning of caregivers in Kermanshah [24] evaluated effectiveness of group counseling based on resiliency (GCR) on quality of parent-child relationship and family functioning in mother headed families. and the results showed that there was a statistically significant difference in the mean family functioning scores for intervention group before and after the intervention, leading a significant increase in family functioning. Although their intervention method was different from our study, their results were consistent with those of the present study. In addition, Liddle et al. (2001) found that the implementation of family group therapy and family□focused interventions can also improve family functioning [25]. Chen &Li used the principle of health empowerment theory for the cirrhosis patients to evaluate the health promoting lifestyle their findings showed that empowerment interventions are effective in increasing the quality of life and family functioning of children with chronic illnesses [26]. In their study, Masoudi et al. (2019) also demonstrated that implementing FCEM for caregivers of patients with multiple sclerosis could increase their knowledge, attitude and functioning, which were consistent with our results, suggesting that FCEM can increase the quality of life of patients with chronic diseases. The difference between their study and the present study is that only caregivers of patients received the training, while in the present study both the patients and their primary caregivers are participated in the intervention, which has a greater impact. Also, Ashoori et al. (2015) conducted a study with the aim of investigating effect of life skills training on self-efficacy and family functioning in women headed households in Varamin [27], their results showed that life skills training was effective in improving family functioning with the difference that a 60-item FAD was used in the above-mentioned study which only assessed the general functioning, but a 60-item FAD was used in the present study that assessed seven domains of family functioning. Also, the type of intervention and their methods were different from our study. In the present study, the FCEM was implemented during four stages by involving both the patients and their families, leading to empowering their families. Although the above studies have improved family functioning. Rahnama et al and Ebadi et al found spirituality to be an important factor for the acceptance and adoption of chronic diseases among the participants. They also expressed that spiritual adaptive strategies could assist people get meaning and goals during their illness [28, 29]. None of the previous studies have been conducted on caregivers of children with Favism. The intervention used in the present study is based on family-centered empowerment model, it has a significant impact on improving the family functioning of children diagnosed with G6PD deficiency, Therefore, it can be said that the number of training sessions and the time allotted to training both patients and their families, enough opportunity to learn the materials, discussion and active participation of the patients and their caregivers are among positive results obtained from the

present study. Findings from the present study suggest that family-centered empowerment model can contribute to adaptation to the patients' problems, their health and the prevention of adverse effects of care burdens of their caregivers. In this method, basic training is provided and the caregiver receives the necessary skills to care for the patient to help him/her to obtain the necessary skills and information for a healthy and successful life in his/her family. Ultimately, these factors improve family functioning. Moreover, group training has a positive effect on life style and health of an individuals and he/she feels that other individuals have similar problems and helps him/her to accept reality and cope better with them , which in turn increases self-esteem, self-efficacy and family functioning.

FCEM could significantly improve the family functioning of children diagnosed with G6PD deficiency. Therefore, due to its effects, the proposed model developed in this study should be considered by nurses and other healthcare workers in order to achieve more therapeutic achievements. The patients' support system should be determined so that their families of children diagnosed with G6PD deficiency can benefit from educational interventions. They families needed to improve health promotion. The goal of health promotion behaviors is to maintain performance, independence and QoL[30].

This study had several limitations. The family-centered empowerment model on Favism patients was implemented for the first time in the Iranshahr Hospital. It was a small study conducted at a single centre. The impact of empowerment on Favism attack was not assessed. Additional studies are required to develop these results.

Table 1. Frequency distribution based on demographic characteristics of primary caregivers in the intervention and control groups

Variable	Intervention group (Frequency Percentage)	Control group (Frequency Percentage)	The results of chi-square test and the Fisher's exact test	
Sex	Female Male	24(80) 20(6)	25(3.83) 5(7.16)	$\dot{X}$= 0.11 df=1 p=0.74
Marital Status	Married Single	27(90) 3(10)	23(7.76) 7(3.23)	$\dot{X}$=1.92 df=1 p=0.17
Education Level	Under high school diploma High school diploma Bachelor	12(40) 11(7.36) 7(3.23)	13(3.43) 14(7.46) 3(10)	$\dot{X}$=2 df=2 p=0.37
Economic Status/ Income	Low Mildel	10(3.33) 20(7.66)	14(7.46) 16(3.53)	$\dot{X}$=1.11 df=1 p=0.29
Occupation Status	Housewife Self-employed Employee	17(7.56) 8(7.26) 5(6.16)	20(7.66) 6(20) 4(3.13)	Fisher=0.7 P=0.76
Type of relationship between the patient and his/her caregiver	Mother Father Sister	6(20) 24(80) 0(0)	6(20) 21(70) 3(10)	Fisher=2.85 P=0.32

Table 2. Comparison of different scores in the experimental and control groups before and after the intervention

Type of Score	Group	before the intervention (n=30) $\dot{X}$±SD	after the intervention (n=30) $\dot{X}$±SD	The results of the paired t-test	Changes $\dot{X}$±SD
The mean problem-solving scores	Experimental group	14.9±1.8	11.4±2	t=7.27 p<0.001	-3.5±2.6
	Control group	14.1±1.8	14±1.7	t=0.29 p=0.77	-0.1±2.5
	The results of independent sample t-tests	t=1.66 df=58 p=0.1	t=5.3 df=58 p <0.001		t=5.1 df=58 p <0.001
The mean communication scores	Experimental group	22.5±2.2	17.6±2.1	t=8.6 p<0.001	-4.9±3.1
	Control group	22.9±2.2	22.8±2.8	t=0.14 p=0.88	-0.1±3.8
	The results of independent sample t-tests	t=0.77 df=58 p = 0.44	t=8.1 df=58 p <0.001		t=5.33 df=58 p <0.001
The mean role scores	Experimental group	28.8±2.3	23.1±2.4	t=10.06 P<0.001	-5.7±3.1
	Control group	28.9±2.5	28.4±2.9	t=0.79 P=0.43	-0.5±3.5
	The results of independent sample t-tests	t=0.16 df=58 p < 0.87	t=2.42 df=58 p =0.018		t=6.17 df=58 p < 0.001
The mean emotional response scores	Experimental group	15.4±2.2	12±2.4	t=5.9 P<0.001	-3.3±3.1
	Control group	15.5±1.7	15.6±1.9	t=0.2 p=0.84	-0.2±1.7
	The results of independent sample t-tests	t=0.26 df=58 p = 0.79	t=6.40 df=58 p <0.001		t=4.58 df=58 p<0.001

Cont... **Table 2. Comparison of different scores in the experimental and control groups before and after the**

The mean emotional involvement scores	Experimental group	17.7±1.9	14.6±2.1	t=6.46 p<0.001	-3.1± 1.6
	Control group	7.1±5.15	9.1±6.15	t=0.13 p=0.90	9.2±0.07
	The results of independent sample t-tests	t=0.07 df=58 p=0.95	t=5.68 df=58 p <0.001		t=4.41 df=58 p < 0.001
The mean behavioral control scores	Experimental group	22.6±2.5	18.2±2.4	t=5.89 p<0.001	4.4±-1.4
	Control group	23.6±2.04	23.3±1.8	t=0.79 p=0.44	-0.3±2.3
	The results of independent sample t-tests	t=1.68 df=58 p =0.098	t=9.22 df=58 p <0.001		t=4.74 df=58 p < 0.001

Table 3. Comparison of the mean FAD scores and the mean general functioning l scores in the experimental and control groups before and after the intervention

Type of Score	**Group**	**before the intervention (n=30) Ẋ±SD**	**after the intervention (n=30) Ẋ±SD**	**The results of the paired t-test**	**Changes Ẋ±SD**
the mean FAD scores	Experimental group	30.5±2.3	23.2±2.4	t=13.3 P<0.001	-7.3±2.9
	Control group	28.9±2.47	29.3±2.5	T=0.50 p=0.62	0.4±3.7
	The results of independent sample t-tests	t=2.51 df=58 P= 0.015	t=9.58 df=58 P <0.001		t=8.78 df=58 P < 0.001
the mean general functioning l	Experimental group	152.4±7.9	120.1±7.9	t=15.1 P<0.001	-32.3±11.7
	Control group	6.7±7.151	1.151±5.7	t=0.33 p=0.74	-0.6±9.3
	The results of independent sample t-tests	t=0.35 df=58 P= 0.73	t=15.58 df=58 P <0.001		t=11.58 df=58 P < 0.001

This study was conducted after obtaining the permission of the ethics committee of Birjand University of Medical Sciences (ethical code:IR.BUMS.REC.1398.107), registering at Iranian Registry of Clinical Trials (IRCT20190618043936N1) and receiving a written permission from Iranshahr University of Medical Sciences and obtaining written informed consent of all participants.

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Writ of Habeas Corpus Vis a Vis Mental Health Patients : An Analysis

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Abstract

Habeas corpus, as a writ has generally been associated with illegal incarceration of individuals. It is a writ, which is considered as an epitome of all remedies as far as implementation of human rights is concerned. Recently, the usage of the writ has surpassed the dimensions of technicalities of procedure of illegal detention and entered into more intricate aspects of an individual's life. Consequently, with the passage of time, the writ assumed varied dimensions, one among them being the usage of the writ in relation to individuals suffering from mental health issues, for instance, where the individual is being denied the freedom of movement, occupation and exercising the freedom of choice .

Apart from the political domain, the use of the writ has crept into facets of family laws, public laws. The researcher shall deal with the outreach of the writ in matters of health law. There has been very scant research in the outreach of the reach in aspects transcending political scenarios, owing to the fact that there exist explicit legislations on such issues and exploring the possibility of the use of the same in the above mentioned arena has been rarely considered. The researcher has recommended an invigorated use of the writ apart from illegal incarceration of prisoners through an analysis of several cases on this aspect.

Keywords: *Habeas Corpus, Health Laws, Judiciary, writ.*

Introduction

With the passage of time and with the change in the dynamics of society, the writ has assumed various dimensions not only in England but also in jurisdictions where it was brought in by the Britishers [(1)] . Despite its increased use, the prime focus of the writ has been safeguarding the liberty and life of the common man. According to Jenks, it's prime use has been to shield oneself against illegal detentions imposed by the Crown and being a dictum issued solely by the crown, the writ became a harbinger of justice against the excesses of the Crown itself[(1)].

In later years, in order to facilitate a wider outreach of the writ, its use was expanded in various statutes as well as rules that came up in England in due course of time[(2)]. This expanded use of the writ in various statutes, facilitated the transplantation of the writ into the private life of citizens', a stark departure from the usage of the remedy in political conditions. Emphasizing on the importance of the writ against arbitrary detentions, Justice Miller, in the case of *Philip S. Wales v. William G. Whitney*[(3)] observed that

There can no straitjacketed formula applicable to the nature of detentions that warrant the attention of the writ of Habeas Corpus. The spectrum of detentions can range from wives restrained by their husbands or children withheld in dire conditions by their parents to military abusing their powers on garb of suspicions. So the ambit of the reach can have far reaching dimensions.

Consequently, with the passage of time, the writ assumed varied dimensions, one among them being the usage of the writ in relation to individuals suffering from mental health issues, for instance, where the individual is being denied the freedom of movement, occupation and exercising the freedom of choice. Apart from the political domain, the use of the writ has crept into facets of health laws. The researcher shall deal with the use of the writ in foreign jurisdiction in arena of mental health

and the lessons that India can learn in this regard. There has been very scant research in the outreach of the reach in aspects transcending political scenarios, owing to the fact that there exist explicit legislations on such issues and exploring the possibility of the use of the same in the above mentioned arena has been rarely considered.

Findings

Owing to colonial domination, many facets of English law were imported into the Indian legal system. Now as far as India is concerned, there is a lot of incoherency in the development of this writ. The writ has witnessed several changes in the course of its history in India. The writ was not as potent a weapon as in England for safeguard of individual liberty[4].

To be more precise the Clause 4 of the Charter of 1774 gave the power to the Supreme Court of Calcutta to issue the writ of habeas corpus. There as we proceed into the legal history, the Supreme Court was abolished and High Courts established by the High Courts Act of 1861. Thus, the High Courts inherited the power to issue the writ of habeas corpus from the Supreme Court. Then comes the Criminal Procedure Code as Act X of 1872, wherein Sec. 81 explicitly conferred the liberty of European British subjects. Then a string of acts such as the Criminal Procedure Code Act of 1875 (Sec. 148), Act of 1882 which repealed 1872 and 1875 acts gave section 491 the power to issue the writ to the High Courts. Many amendments and repeals were introduced. But the peculiar feature that the benefit of Habeas Corpus was not available to Indians, it was restricted only to English. Change came via the Criminal Law Amendment Act XI of 1923 where it amended Section 491 of Criminal Procedure Code of 1898 and opened the gates of liberty to whole of India.

Thus, the story of habeas corpus shows its development from a luxury available only to Europeans to its recognition as a right available to all, specially after 1923[5].

Discussion

As far as the foreign jurisdictions are concerned, the instances of petitions praying for the writ of habeas corpus in relation to persons suffering from a mental illness, and their liberty being denied to them on the pretext of their illness, limiting their movement are not new. Indian courts needs to take lessons in this novel dimension of the writ. The foreign courts have validated the use of the writ for those detained in mental institutions only as permissible by the law in force and not surpassing the transcension of law [6] The pretext of care and concern towards mentally ill is not seen as a valid cause of curbing the freedom of movement of such individuals[7]. only to the extent where the concerned mentally ill person does not pose a threat to another individual.

A crucial decision on the use of writ of Habeas Corpus in context of mentally ill is the decision of Justice Eastham in Re C (Mental Patient :Contact)[8] where there occurred an altercation between a couple in relation to the custody of their adult daughter. The couple had judicially separated and the girl was under the care of her father. The mother had a grievance that the father restrained her from seeing their daughter. The mother applied for proper visitation rights so that she could meet her daughter through proper legal means as well as a written order by the court restraining the father from stopping her.

The issue which was raised was whether the court could entertain such a plea. The court through Justice Eastham answered in the affirmative [8]and observed that:

The unlawful obstruction by the parent in custody of a child to the visitation rights of the other parent can be rightfully remedied through the writ of Habeas Corpus. Furthermore, he added that if the young girl was not able to meet her mother and given access to her, this is a clear case of the writ of habeas corpus.

He further observed: [8]

The contentions of the mother, if true, must be supported through a writ of Habeas Corpus.

In A Local Authority v MA[9], a case concerned with the assessment of the decision making capacity of a deaf and dumb 18 year old girl specifically to marriage, Justice Mumby observed,

The writ is not limited to the testing of the legality of detention; as it requires the detainee to produce the body of the person before the court, it is also a method of analyzing whether the detention is illegal or not. And

if the court through a committee of medical experts can ascertain the mental health of the individual and establish the factum of coercion.

In another case[10], a woman named Zelika Antunovic, a woman aged 33, was forced into institutional mental care under the Victoria Mental Health Act 1986 (hereinafter referred to as the Act) in 2008. She was ordered to be under community treatment (hereinafter referred to as order) by Dr. Louise Dawson, a certified psychiatrist. The order underwent extensions in 2009 as well as 2010. The 2010 order was due to culminate on 2 May 2011. Now, the act provides specifically under section 14(3)(b) that a community treatment order issued by a certified psychiatrist may also mention the residential requirements of a person and ask the patient to comply to the same. These are the residential requirements under the act. But the same provision was never used by Ms. Antunovic's doctor neither were there any variations to her order. A proper treatment plan, under section 19A of the Act, was prepared by her doctor. At the same time, only on pretext of insufficiency, there was no bar on her to reside at her own home along with her mother. The Mental Health Review Board of Victoria analyzed the order on 18 June 2010. It gave the right of oral hearing to Ms. Antunovic as well as analyzed the findings of her doctor who did not personally appear before the Board. The order was prolonged and again reconsidered for a reanalysis after six weeks. In the reevaluation on the expiration of six weeks, the Board came to the conclusion that it was not satisfied with the treatment being administered to Ms. Antunovic by her doctor. It pinpointed the absence of any objection from Ms. Antunovic's end to continue her stay at the community care unit , where her treatment was underway and reside with her mother[11].

Ms. Antunovic approached the Supreme Court of Victoria stating that the specific requirement of her residence was nowhere mentioned in her treatment plan or there was no specific reference to a mandatory residence at the community care unit, she desired to reside along with her mother . Under Order 57.03 of Victoria's Supreme Court (General Civil Procedure) Rules, 2005, the court issued the writ of Habeas Corpus and ordered the release of Ms. Antunovic.

Justice Bell made an in-depth analysis of the impugned legislation which was the Victoria's Mental Health Act of 1986 and more specifically of the residential requirements of the patients in community treatment homes under it. He observed that such orders are tantamount to obstruction of fundamental rights and freedoms of individuals. The imposition of such orders can be mandated only in instances of pure medical requirements. He stressed that the requirement of a mandatory residential stay through a treatment plan which explicitly mentions it is what is mandated under the act and the plan of the petitioner has no mention of any mandatory residential requirements. Further, he also noted that the treatment plan of the petitioner also had no mention of her desire to cease to live at the commnity care unit and reside with her mother. He further observed that the intention of the legislature through the impugned legislation is to meet the requirements of the 1991 United Nations Principles for the Protection of Persons with Mental Illness and the Improvement of Mental Health Care which mandates for a treatment that respects the personal liberty and privacy of an individual, even though he is undergoing mental health treatment.[10]

He further noted that even through the petitioner was not under incarceration akin to those of prisoners, she had the liberty on her movements throughout the day but at night she was confined to four walls of the community care unit. This was not akin to complete control and domination but led to a degree of substantial control and domination by her authorize doctor in the sense that her freedom of movement as well as her wishes were being constrained owing to the treatment plan and the arbitrary review exercised by the Victoria Mental Health Board giving her doctor, a great degree of control which fell within the limits of the legal tests of incarceration.

He further admitted the case of the petitioner, making it a fit example of being subject to the jurisdiction of the writ of Habeas Corpus. He further ordered the release of the petitioner by her doctor from the confines of the Community Care Unit

Conclusion

The above-mentioned decisions clearly bring out the invigorated usage of the writ and the lessons that the Indian judiciary needs to learn. The right to personal liberty is also a divine right under Article 21 of the Indian Constitution. The above mentioned decisions

contain a treasure of analysis of on this cherished and most celebrated writ and its applicability to persons suffering from mental illness from which the Indian medical community can learn to a large extent.

The most unique aspect found by the author through the above mentioned cases is the use of the writ despite the cases not falling within the strict ambit of imprisonment and the individuals enjoying a considerable degree of freedom of movement and exercise of freedom of choice. It brings out an important facet that if there is an unlawful bar on the exercise of liberty and freedom of choice of a person , the writ is called for. Furthermore, the decisions occupies significance in light of its prime concern being people suffering from mental health issues and given equal rights and primacy akin to normal members of the public[11].

Thus, the decisions serve as a positive guiding light for the medical as well as legal community. The Indian medical community can also learn from such progressive decisions and incorporate healthy practices in course of their medical practice.

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Nanoparticles-Based Face Masks and Respirators for Preventing COVID-19 Transmission: Breathability Versus Biocidal Activities

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Abstract

Use of face masks and respirators are significant in preventing the transmission of coronavirus disease 2019 (COVID-19) via respiratory droplets or aerosols. The development of face masks and respirators have been focused on the modification using nanoparticles (NPs) to obtain biocidal activities. The incorporation of NPs can also increase the hydrophobicity of the material that assists the repelling of virus carrying droplets or aerosols. Nevertheless, the common cost of gaining those benefits is breathability. Previous studies have reported on the discomforts of wearing a face mask or respirator, one of which is stems from breathing difficulty. At the time of pandemic, maintaining the comfort wearing of face masks or respirators is even more crucial. Thus, this review article is important to keep the breathability aspect gaining a spotlight in the development of NPs-modified face masks or respirators. Herein, we discuss the relationship between the addition of NPs with breathability of the material. In the beginning of discussion, types of protective respiratory equipments, and biocidal activities of the modified fabrics are discussed. Strategies in maintaining the air permeability for long duration use and self-cleansing feature are also discussed.

Keywords: *Nanoparticle, Face mask, Respirator, Breathability, Antibacterial, Antiviral*

Introduction

The on-going coronavirus disease 2019 (COVID-19) pandemic has caused the cumulative death of more than 2.3 million worldwide, with more than 100 million confirmed cases as of February 14th, 2021.[1] Currently, COVID-19 has been recognized to be transmitted via viral-containing respiratory droplets or aerosols, close contact, and fomite routes.[2, 3] Responding the increase of COVID-19 cases, several restrictions have been in place differently in each country. US Centers for Disease Control and Prevention (CDC) released a recommendation for general public to wear face masks or mouth covering fabrics as a preventive measure against COVID-19 in public settings, even when six feet-distancing has been done.[4] Several other countries even legally mandate their citizens to wear face mask

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in public.[5-7] For healthcare workers, the use of surgical mask and respirators are a must to provide protection against transmission in a high-risk environment.[8]

Advancement in improving the performance of face masks or respirators has been significant in the field of nanotechnology during the pandemic. Nanoparticles (NPs) have received significant attention from scientific communities due to their biocidal activities, which can provide extra protection against COVID-19. Furthermore, microbial coinfection in patients with COVID-19 has been observed to add more complexity in the diagnosis and treatment.[9] To prevent the microbial coinfection, some research groups have showcased that fabric modification utilizing NPs could provide antibacterial properties.[10-31] Recently, the antiviral activities of NPs-modified fabrics have also been investigated.[32, 33] Hence, this future technology may contribute to more effective prevention of COVID-19 transmission or other respiratory viruses.

However, breathing comfortability of the face masks or respirators affected by the NPs-based modification has been scarcely discussed. Discomforts of using face masks and respirators have been underlined in several works.[34-36] It was reported that healthcare workers suffer headaches ascribed to the use of respirators.[37] Not to mention, various adverse skin reactions can be caused by wearing face masks or respirators.[38-40] Indeed, those reports[34-37] suggested that the burden is rather psychological than physiological. However, it is important to avoid a modification that can cause extra discomforts, especially in regard of breathing process. Hence, we will discuss how the beneficial biocidal properties of the modified respiratory equipment are intertwined with its breathability.

Transmission of COVID-19

COVID-19 is a respiratory disease caused by SARS-CoV-2, one of the five human coronaviruses, genus betacoronavirus. This spherical lipid-based enveloped virus is surrounded by spike proteins and has a diameter of around 100 nm.[41] While the etiology and pathogenesis of SARS-CoV-2 are not fully known, it has been reported that host cell entry is mediated by angiotensin-converting enzyme 2 (ACE-2),[42] a protein expressed in the epithelium of the human airway[43] as well as the lung parenchyma.[44] Comparatively with SARS-CoV, SARS-CoV-2 has higher affinity to ACE-2[45] and has faster infection rate.[46] Therefore, it is biologically plausible that the transmission of COVID-19 is mainly facilitated by respiratory droplets or aerosols.[47, 48]

There have been extensive works on how COVID-19 is transmitted via respiratory droplets which cover the investigation on the droplet sizes and environment settings. A study on the mechanistic movement of the droplets in a calm indoor setting revealed that aerosols are maintained longer in the air than droplets.[49] Droplets and aerosols can have longer transport in the air when they are generated by sneezing and coughing.[50, 51] As a physiological defence mechanism, coughing releases around 3,000 droplet particles, and more droplets are released during sneezing (40,000).[52] The diameter of droplets is varied (1 – 2,000 μm), but 95% of them are within the range of 2 to 100 μm. Droplet size of less than 100 μm in diameter can be transported up to 4 – 7 meter away by sneezing or coughing suggesting the physical distancing may not be effective to avoid the respiratory transmission.[53] Hence, the role of using face masks and respirators is one of the most significant preventive efforts for COVID-19 transmission.

Protective Respiratory Gears

Medical face masks

Originally, a simple single layer of gauze was used as a medical face mask, aiming to protect the patients from the contamination from the surgeons during operative procedures.[54] Later, it was developed with the overall design comprising three layers; hydrophilic layer (inner part), filter (middle part), hydrophobic layer (outer part). Hydrophilic layer has a function to govern the humidity by absorbing the aerosol from the wearer. Filter acts to selectively regulate the exchange of particles from both sides of the face masks through size exclusion. Hydrophobic layer provides protective repelling mechanism against the aerosols and droplets entering the side of the user. Medical face masks are regulated under Food and Drug Administration (FDA) jurisdiction as medical gears with loosely fitted and disposable characteristics. Though the masks may not tightly-fitted to the face, a study has reported its effective filtration against respiratory droplets (> 5 μm) and aerosols (≤ 5 μm) containing coronaviruses.[55]

Respirators

On contrast to loosely-fitting medical face mask, respirators have much tighter facepiece void with the ability of repelling particles whose size is less than 5 μm. These devices have different nomenclatures depending on the country. The National Institute for Occupational Safety and Health (NIOSH) of the USA classifies the respirators based on their filtration efficiency with N-, R-, and P- groups for non-oil resistant, moderate oil resistant, and strong oil resistant, respectively. The initials are followed by the indication of their filtration efficiencies; for instance, N95 for N-respirator with 95% filtration efficiency, R95 for R-respirator with 95% filtration efficiency, and soon.[56, 57] Nonetheless, the efficiency may fall lower than that stated at high flow rate of inhalation.[58] In particular, N95 has four fundamental layers from the outer layer, filter layer, support layer, and up to the inner layer. Its outer layer can be decorated with ventilator fan for easier respiration.[59] NIOSH has released the test standards for respirators, namely inhalation/exhalation tests, NaCl aerosol challenge, valve leak, and dioctyl phthalate (DOP) test for verification (NIOSH Procedure No. RC-APR-STP-0057, 0058 and 0059).

Homemade face masks

During the pandemic, scarcity in personal protective equipment, including face mask is expected. In such condition, non-medical homemade face masks can be the alternative. A previous study found a significant decrease in the amount of excreted droplet when a simple cloth face mask was in use.[60] Regulation on the mandatory use of face mask has been proven to yield significant reduction in daily COVID-19 cases in the USA.[61] As recommended by WHO, the homemade cloth face masks should be used with at least three layers of fabric.[56] Homemade face masks have been manufactured using various materials such as cotton, air-jet down-proof fabric, satin, polyurethane, polyester, linen, silk, nylon, and cellulose.[62-65] The filtration efficiency of these fabrics depends on their threads per inch (TPI), which is determined by the total number of threads in an inch counted on the length of a fastener. The filtration efficiency of 80% can be achieved by fabrics with >300 TPI.[66] However, for common polyester-, cotton-, silk, and nylon-based fabrics, their efficiencies only reach 5-25%.[63] Note that the use of these homemade cloth face masks is not recommended for high-risk environment.[67]

Nano Particles Embedment

NPs can be incorporated onto the surface of fabric materials through a variety of methods that affect the characteristics (including air permeability) as well as the stability of the immobilized-NPs. NPs embedment via electrospinning is conducted by simply homogenizing fiber materials and priorly prepared NPs.[15, 17, 33, 68] It is worth to mention that the NPs may have high conductivity which results smaller fiber diameter concomitant to the generation of high charge density on the surface of the ejected jet.[17] *In situ* NPs impregnations using green route with plant broths as reducing agent have been reported.[16, 21, 22, 24, 28] Simple adsorption technique, followed by metal ion reduction using UV irradiation, has also been applied.[27] However, coating or nanocoating via immersion and spraying is the most common method for NPs embedment onto fabrics.

Advantages and Disadventages of Nano Particles Embedment

Biocidal activities of nano particles

Incorporation of metal NPs onto fabric materials have shown the biocidal activities against various microorganism. Gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* are among the most widely used bacteria in the reported studies, to test the antibacterial activities of NPs-based fabrics.[11, 13, 17, 19, 20, 22-29, 32] Others have tested the biocidal activities against *Candida albicans, C. parapsilosis, Xanthomonas aconopodis, Enterococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. epidermidis, Bacillus subtilis,* and *Bacillus cereus*.[15, 19, 23, 24, 27, 28, 69] Based on those studies, the employment of various NPs could effectively kill the bacteria, including multidrug-resistant *S. epidermidis* and *S. aureus.*[70, 71]

Inhibition mechanism of the bacteria depends on the type of NPs. For instance, Ag NPs could cause bacterial cell death by interfering the cell respiratory system, interfering ATP production and DNA replication, increasing cell membrane permeability, and generating bacterial cell-derived reactive oxygen species (ROS).[72, 73] Metal oxide NPs (such as ZnO, MgO, CaO, CuO,

TiO_2 and so on) have biocidal activities attributed to the production of ROS from electrons and holes reaction under UV/Vis radiation.[31, 32] In particular, TiO_2 NPs show promising self-cleansing properties which can photodegrade the remnant of microorganism from the fabric surface.[19, 20, 31, 32]

A challenging factor for the metal oxide NPs is the need of light exposure to induce the photocatalytic activities. To overcome, some researches incorporate Ag NPs to provide biocidal effects even without the presence of light.[20, 23, 33] Despite the fact that Ag could have antioxidant activities,[16] the synergism between Ag and photocatalyst or active compound in promoting ROS-induced cell death has been observed.[17, 20] As another alternative, the fabric can be modified with 3,3′,4,4′-benzophenone tetracarboxylic acid to provide photo-induced active metastable structure which can still produce ROS during dark condition.[14, 17, 74]

Recently, NPs-based face masks have been investigated for its ability to inactivate viruses. ZnO nanorods and Ag NPs, incorporated in electrospun nanofibers-based face mask, has inhibitory activities against coronaviruses and influenza viruses.[33] Another study reported Cu NPs-decorated pristine face mask was effective in inactivating virus-like particle (VLP) of SARS-CoV-2.[32] The mechanism of the antiviral activities was attributed to the degradation of viral structure due to photocatalytic and photothermal effects. Another study reported that viral genetic encoding protein could be degraded after exposed with ROS promoted in photocatalytic activities of TiO_2.[75] In addition, it has been reported that NPs have antiviral properties against a wide variety of viruses.[76]

Droplets and aerosols rejection

Physical mechanism of antiviral protection on respiratory equipment relies on the filtration and repelling of virus containing droplets and aerosols. To repel the virus carriers, NPs can be added to enhance the surface hydrophobicity of the face masks or respirator's filter membrane.[32, 77] Hydrophobicity of the face masks can be measured by contact and roll-off angles.[30, 32] In particular, a study has achieved super hydrophobicity for various fabric material coated with SiO_2 NPs mixture.[30] Furthermore, a hydrophobic layer may repel humidity increasing the comfortability.

Moreover, the rejection of droplets, aerosols, and viral particles can be facilitated by size exclusion that depends on the pore size of the material. Filtration efficiency of Cu NPs-coated pristine mask was pretty similar to that of untreated face mask.[32] Filtration of NaCl particles by Ag NPs-based polyvinyl alcohol membrane revealed to be more efficient than that of N95, at a cost of increased pressure drop.[17] The study did not perform an investigation on the effect of Ag NPs addition, but the higher pressure drop means lower breathability and, as a consequence, lower comfortability.

Breathability

Prolonged use of surgical masks and N95 respirator has been recognized to lead the users to feel headaches, light-headedness, and suffocation.[35, 78] During COVID-19 pandemic, significant number of healthcare workers experienced headaches caused by wearing masks.[79] Factors responsible for the headache cases among healthcare workers include the design and psychological stress.[79] Difficulties in breathing and speaking as well as increases in temperature and humidity are responsible for such discomfort of wearing protective respiratory gear.[80]

Physiologically, wearing face masks or N95 respirators have been proven to yield no clinical relevance on the respiration and O_2 delivery.[35, 81, 82] However, there has been an observation on the significantly higher CO_2 level in N95 wearers in comparison with surgical face mask wearers.[35] Moreover, significant decrease in O_2 and increase in CO_2 was observed after the N95 wearers finished 1 h treadmill walking session.[81] Although that study concluded the effects are minor, the changes of O_2 and CO_2 levels can potentially lead to hypoxemia[82] and hypercarbia.[83] A previous study revealed increased nasal airflow resistance of more than 100% as a result of wearing N95 respirator.[84] The same study also revealed that N95 respirator may reduce air exchange volume as much as 37%. It is ascribed to only partial amount of air volume can pass through the pores of the respirator's layers (**Figure 1a**). Modification with nanoparticles may further decrease this air exchange volume due to smaller size of pore diameter as a consequence of fiber diameter expansion by binding agents, active agents, or NPs agglomeration (**Figure 1b**). Therefore, it is important to investigate the effect of NPs embedment with the

breathability of the material.

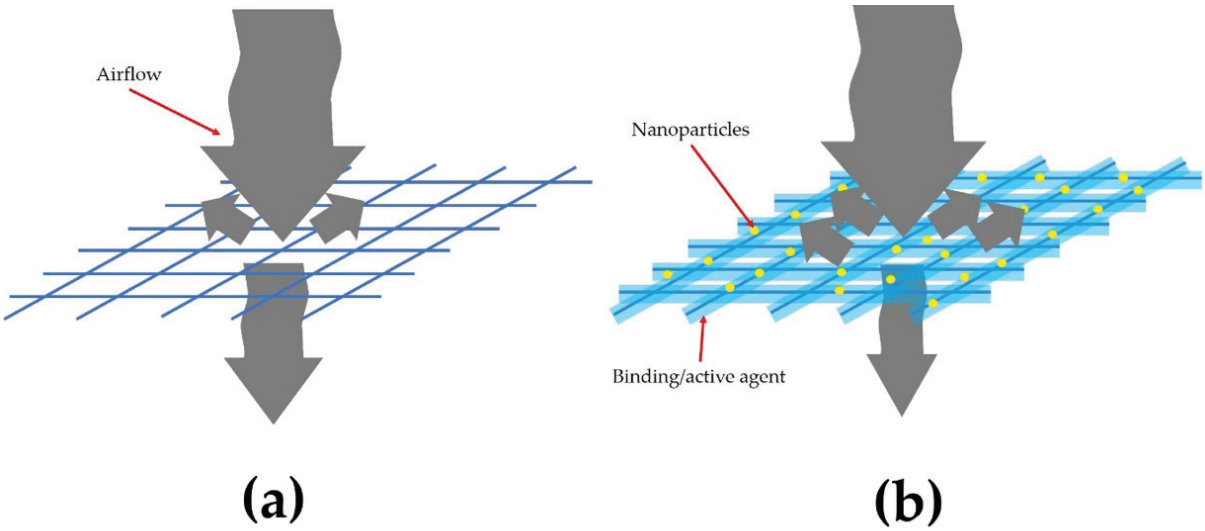

Figure 1. Representation of airflow passing through (a) unmodified fabric and (b) NPs modified fabric of face masks or respirators

Breathability is a parameter determining the comfortability of a face mask, since it has a correlation with moisture movement and thermo-regulation of the fabric.[85] NPs incorporation to the face masks have effects on the breathability. In one study, the coating of nanofibers onto commercial surgical face mask yielded lower air permeability leading to poorer breathability of the fabric.[68] The employment of mesoporous nano silica NPs with polydimethylsiloxane binder, however, did not change the original breathability of the spray-coated textile.[30] On another report, Cu NPs nanocoating results in similar pressure drop with uncoated mask at low air velocity.[32] Yet, increase in pressure drop is experienced by the Cu NPs-coated mask as the air velocity increased, indicating poorer breathability.[32] Lower breathability of the Ag NPs-based respirator membrane filtration, in comparison with that of N95, is associated with the dense properties of the matrix (polyvinyl alcohol).[17]

Observation of porous structure using scanning electron microscope (SEM) images can also suggest the breathability of the NPs-modified fabric. Insignificant changes in the SEM images have been observed in samples with spray-coatings of SiO_2,[77] TiO_2,[31, 86] and Cu NPs.[32] On contrary, significant changes in the pore structure and fiber diameter have been found in SEM images from SiO_2-TiO_2-Ag-embedded cellulosic membrane[20] and Ag NPs-based electrospun fiber,[17] respectively. Based on the explanation above, to optimize the breathability of the face mask, the selection of NPs attachment methods and textile material is important.

Self-cleansing

One of the recent advancements in the development of NPs-embedded protective respiratory equipment is photo-induced self-cleansing properties. Excellent self-cleansing was performed by bacterial cellulose (BC)/ SiO_2/TiO_2 composite against deposited crystal violet dye under UV irradiation for 50 minutes.[20] Similarly, effective self-cleaning was observed in the photobleaching of crystal violet dye (reaching 97% in 10h irradiation) by Mn:TiO_2-embedded textile.[31] Removal of microbial cells has been achieved by the NP-s modified materials, which can prevent bio-fouling and, further, maintain the breathability.[20, 32] Additionally, self-cleansing or self-sterilization can ease the disinfection process by simply light-irradiating the fabric and remove the risk of contamination or transmission during discarding process. The role of NPs technology prevention of COVID-19 transmission and its advantages and disadvantages are summarized in **Figure 2**.

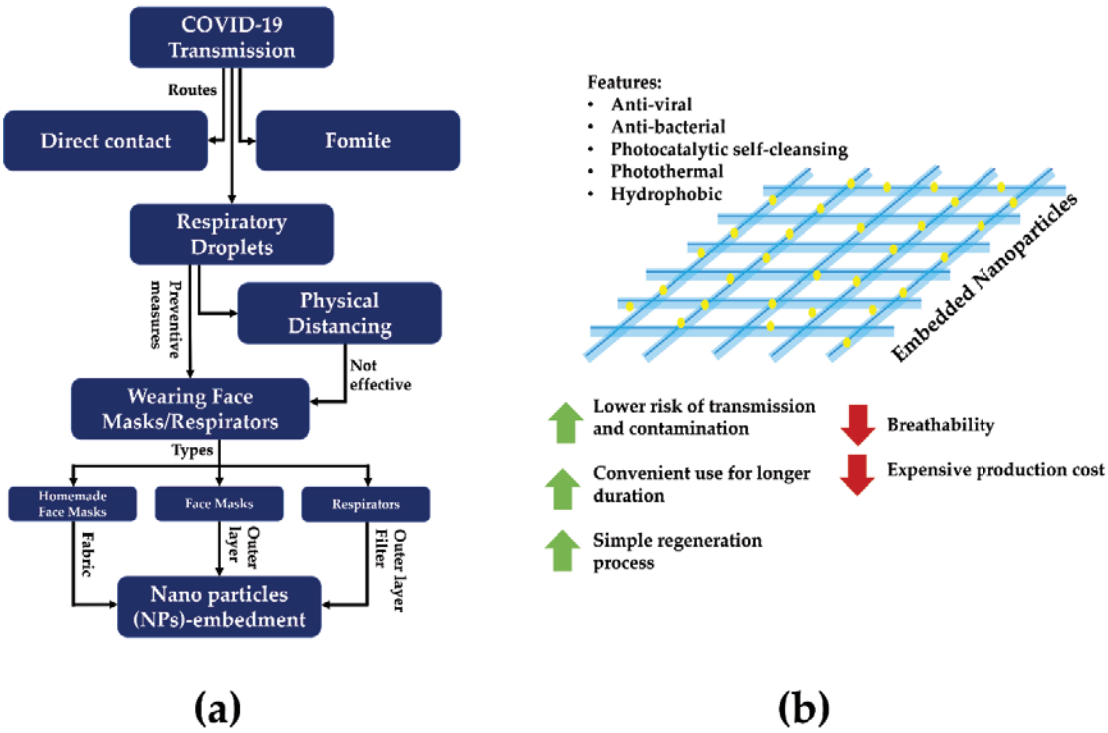

Figure 2. (a) Schematic diagram of the prevention of COVID-19 transmission through nanoparticle technology. (b) Features, strengths (green arrow), and weaknesses (red arrow) of NPs-based face masks or respirators.

Conclusion

Modification of face masks and respirator by employing NPs are found to have effective biocidal activities, where current researches have focused on developing antiviral fabrics. The antimicrobial activities can be attributed to the chemical and physical mechanisms. Despite their excellence in term of biocidal activities, the modification effects on breathing comfortability are rather problematic since several works reported poorer breathability. Spray-coated fabric, so far, has the least changed breathability. Additionally, self-cleansing feature from TiO_2 embedment may contribute in sustaining the breathability during long hour use by preventing microbial cells-induced biofouling. Studies regarding the effect of NPs incorporation on the breathability of the materials are still scarce and rather non-uniform. Thus, we recommend further researches to include the investigation of breathability of the material after the NPs embedment.

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Literature Review: Problems of Dental and Oral Health Primary School Children

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Abstract

Elementary school children or the age group 6-12 years who are in mixed teeth, it is important in maintaining the health of primary teeth which at this time has an important role in the development and maintenance of permanent teeth later. The results of basic health research by the Indonesian Ministry of Health in 2018 showed that 93% of children in Indonesia have oral health problems, which means only 7% do not have dental and oral health problems**.** Children this age really like sweet and sticky foods, and do not understand how to maintain good oral health. This study aims to collect dental and oral health problems in elementary school children and how to deal with them. The design used is a literature review with criteria published from 2007-2020. Based on the articles collected, there are several dental and oral health problems for elementary school children, both those affecting the hard tissues of the teeth and soft tissues or supporting teeth. Parents and schools play an important role in educating children of this age to become accustomed to maintaining oral health.

Keywords: *Dental health problems for children, Age group 6-12 years, Elementary school children.*

Introduction

The Global Burden of Disease Study 2017estimated that oral and dental diseases affect nearly 3.5 billion people worldwide, with permanent dental caries being the most common condition. Globally, it is estimated that 2.3 billion people suffer from permanent dental caries and more than 530 million children suffer from caries in their primary teeth.[1] The results of basic health research by the Indonesian Ministry of Health in 2018 showed that 93% of children in Indonesia experience dental and oral health problems, which means only 7% do not experience dental and oral health problems.[2]

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In elementary school children or the age group 6-12 years who are in mixed teeth, it is important to maintain the health of primary teeth which at this time has an important role in the development and maintenance of permanent teeth later. Dental and oral health problems can be broadly divided into two, namely those that attack hard tissue and soft tissue. Teeth and mouth problems in children can have a negative effect on their well-being, quality of life and health. In addition, it is known that the relationship between dental and oral health problems can develop into systemic conditions such as cardiovascular disease and diabetes later in life. Therefore, special attention needs to be given to handling these cases such as increasing prevention and handling measures from an early age.[3]

Methods

The method used in writing this article is a *literature review,* which is a literature search, both international and national, which is conducted using the ELSEVIER, PubMed database. A search for journal articles obtained 10 articles from 2007 to 2020 using the keywords "dental health problems in children", "dental health and elementary school children", "caries and periodontitis for children aged 6-12 years."

Results

Tooth eruption period Dental

development is divided into several stage: Van der Linden (1985) [4]

1. Period of primary teeth (primary dentition) Primary

Tooth eruption generally occurs between the ages of six to thirty months after birth, and lasts until the age of six years. In general, the sequence of eruption of primary teeth is the first molar, first incisor , second incisors, canines, and second molars

2. Mixed dentition

period There are several transitional periods, the first transition period occurring between the ages of six to eight years, marked by the deciduous incisors being replaced by the permanent incisors and the first permanent molars starting to erupt; then the inner transition period, starting at the age of nine years and the fixed incisors had all erupted; the second transition period y it is characterized by the canine and primary molars being replaced by the canines and permanent premolars and the second molars having erupted.

3. Permanent dentition period

This period is a period of complete tooth development, when the roots of the permanent third molars are fully formed and reach the occlusal peak

Dental and oral health problems in primary school children

Children aged around 6 to 12 years or elementary school children still do not know how to maintain oral hygiene. Therefore, efforts to maintain oral health must be done from an early age. Elementary school age is the ideal time to practice motor skills of children, including brushing their teeth. [4,5]

Caries in children

Caries is a dental and oral health problem that most often occurs in children in the world. *The World Health Organization* (WHO) states that the incidence of caries in children is 60-90%. The high prevalence of caries in school children is influenced by various factors (multifactorial) which include the host (teeth and saliva), agent (cariogenic bacteria), environment and time. Dental health efforts need to be viewed from several aspects, namely environmental aspects, and knowledge, education, public awareness and handling of dental health including prevention and care. Dental caries is a disease that attacks the hard tissues of the teeth by demineralizing the enamel. This demineralization process can worsen tooth enamel and dentin to the point of causing damage. This is more common in individuals who frequently eat foods that contain sugar. Caries can affect the child's growth and cause discomfort.[6,7,8,9,10,1,,12,13] Caries can cause toothache, discomfort, eating disorders, tooth loss, and stunted child development. In addition, caries can also affect the concentration of children in schools.[14,15,16]

First Permanent Molar Caries

Play a role in the development and growth of the dental arch, have the main task of mastication, affect the vertical distance of the maxilla and mandible, the height of the occlusion, and the proportion of esterica. Mandibular first permanent molars are generally the first permanent teeth to erupt at the age of around 6-7 years, so they are the teeth most at risk of caries, also because at that age children have the behavior to eat sweet and sticky foods. If the tooth is exposed to caries, it can result in extraction, which creates new risks such as changes in tooth position, affecting occlusion, TMJ disorders, and the mastication process that affects the absorption of food nutrients.[14, 34,35]

Periodontal disease of children

Periodontal disease is estimated to affect 90% of the world's population. Periodontal disease or so-called periodontitis, usually begins with gingivitis. This can occur due to poor oral and dental hygiene so that

plaque forms on the teeth. Periodontitis causes loss of connective tissue which can cause teeth to fall out of their socket. This of course can cause several negative impacts, such as disruption of chewing, speech, and quality of life of children. [3,6.36,,38]

Gingivitis induced plaque and gingival disease modified by systemic factors related to the endocrine system. Gingivitis, which is characterized by inflammation of the gingiva without loss of attachment to bone, often occurs in children. Although the microbiology of this disease has not been fully characterized, the increased levels of Actinomyces sp., Capnocytophaga sp., Leptotrichia sp., And Selenomonas sp. sub gingivitis has been found in experimental gingivitis in children when compared with gingivitis in adults. Therefore, this species is likely to play a role in its etiology and pathogenesis. Normal and abnormal fluctuations in hormone levels, including changes in gonadotrophic hormone levels during puberty, can alter the gingival inflammatory response to dental plaque. Likewise, changes in insulin levels in diabetic patients can affect gingival health. In both situations, there is an increased inflammatory response to plaque. However, the gingival condition usually responds to a thorough cleaning of bacterial deposits and improves daily oral hygiene.[15]

Periodontal tissue has an important role. Periodontal disease is a disease that often occurs in soft tissues and takes root in early childhood. Periodontal disease can include gingivitis and periodontitis. Untreated periodontal disease can damage the soft tissue and then damage the hard tissue of the tooth, which can lead to tooth loss. This of course can have a negative impact, such as disruption of chewing, speech, and quality of life, it is also known that there is a relationship between oral health problems that can develop into systemic conditions such as cardiovascular disease in the future[3]

Premature loss[7]

Premature loss of primary teeth because caries has an effect on the development of permanent teeth and not only reflects the unfortunate lack of knowledge about the course of the disease but also forms a negative attitude about the prevention of dental caries in adult teeth. Losing primary teeth can cause less space for permanent teeth. It is sometimes considered by ordinary people that the loss of primary teeth, which are sometimes referred to as baby teeth, has little effect because it is only temporary. However, primary teeth can be used from 2 to 7 years of age or older, or about 5 years or more in total. Multiple teeth were used from 6 months to 12 years of age, or 11.5 years in all. Thus these primary teeth are used and contribute to the health and well-being of the individual during the first years of his greatest development, physically and mentally.

Premature loss of primary teeth, retention of primary teeth, absence of congenital teeth, tooth anomalies, and insufficient space are considered important factors in the initiation and development of malocclusion. Premature loss of primary teeth due to tooth neglect is likely to result in loss of arch length and consequently a tendency to crowding in the permanent teeth.

Traumatic dental injury

can be an important public health problem, not only because of its relatively high prevalence but also because of its profound impact on children's daily life. This is due to physical and psychological discomfort, pain, and other implications such as a tendency to avoid laughing or smiling, which can affect social relationships. The main causes of traumatic dental injuries are falls and collisions, sports activities, traffic accidents, and violence. It is also known that increased dental overjet and inadequate lip coverage significantly increase the likelihood of dental injury when people have an accident.

Persistence of primary teeth

persistence of teeth is that the primary teeth have not fallen out properly, but the permanent replacement teeth have grown. The causes of tooth persistence include ankylosis, slow root resorption of primary teeth, hypothyroidism, nutritional deficiencies, genetic disorders such as cleidocranial dysplasia, or due to the abnormal position of permanent tooth seeds. This tooth persistence only occurs during the change of teeth which can result in disruption of the eruption of the permanent teeth. If there is disruption in the eruption of permanent teeth, it can cause malocclusion, esthetic disturbances, and disturbance of the masticatory muscles. Primary school age children are 6-12 years old on average. This age range is a mixed dentition period which is known as a critical period of growth and development, therefore

regular monitoring is very important to do so that there is no disturbance in this period. One of the disorders that can occur is the persistence of primary teeth. Lack of knowledge about the tooth replacement process can lead to tooth persistence. If the tooth persistence is not treated immediately, it will result in malocclusion. [17,18,19,20,21,37]

Malocclusion

Malocclusion is a deviation in the location of the teeth and / or a malrelation of the dental arch (jaw) outside the acceptable range and is considered aesthetically or functionally unsatisfactory. Malocclusion is a health problem that has received full attention. Malocclusion has been the third most common oral disease, after dental caries and periodontal disease. Malocclusion can cause dissatisfaction with aesthetics because it can interfere with smiling, besides that malocclusion can change the way of speaking, breathing, change facial posture, interfere with chewing, swallowing, cause *temporomandibular joint disorders,* and pain is found in many children with malocclusion can cause dissatisfaction with aesthetics because it can interfere with smiling, besides that malocclusion can change the way they speak, breathe, change facial posture, interfere with chewing, swallowing, cause *temporomandibular joint disorders,* and pain is commonly found in malocclusion children.[22,23]

Grabe divides the etiological factors of malocclusion into 2, namely extrinsic and intrinsic:[24]

1. Extrinsic factors: Hereditary, trauma, bad habits, malnutrition, malfunctions

2. Intrinsic factors: abnormalities in the number, shape and size of teeth, *premature loss,* persistence of primary teeth, and deciduous dental caries.

Treatment and prevention

Some preventive treatments that can be carried out to prevent dental and oral health problems in primary school children from an early age, namely:[10,11,12,13]

1. Prevention of plaque and bacteria by brushing and dental floss use

2. Systemic and local intake.

fluoride water as a systemic fluoride intake is said to be effective in reducing caries rates by 20-40%. Fluoridation of drinking water can provide topical and systemic effects, but is more effective when given in the pre-eruption age of permanent teeth. The recommended dosage limit for drinking water fluoridation is 0.7 ppm, to balance the benefits of preventing dental caries and reducing the likelihood of fluorosis. Another systemic fluoride preparation, namely fluorine tablets, is not recommended for children living in areas where fluorine content in water is 0.3 mg F / L. Indicated for children with high caries risk. Research by Ekstr and and Olive (1999) states that after 40 minutes of consuming fluoride tablets, the fluorine concentration in saliva increases and after 120 minutes the fluorine concentration decreases

. Topical application of fluorine is a technique of applying fluoride directly to the tooth surface with the aim of providing opportunities for fluoride to penetrate. into tooth enamel and then fluoride ion will replace the hydroxyl ion in the enamel so as to increase the resistance of the enamel to acid attack. The combined application of topical fluorine will increase the remineralization effect and increase the hardness of the enamel. Both individually and professionally, fluorine topically has 3 mechanisms of action, namely through increased remineralization, prevention of demineralization and inhibition of bacterial glycolysis.

3. Pit and fissure sealants

Materials that are placed in tooth pits and fissures that aim to prevent dental caries. Pits and fissures on the occlusal surface of the posterior teeth are one of the factors contributing to caries. This morphology facilitates the retention of debris and barriers that cause caries. Compared to the smooth surface of the teeth because the inside of the pits and fissures is difficult to reach by the bristles of the toothbrush. This results in a higher frequency of caries in the pits and fissures than in smooth surface caries. Fissure sealants are given at the beginning of the tooth eruption in order to prevent food residue bacteria in the pits and fissures. Materials that can be used for the application of fissure sealants include composite resin and glass ionomer cement. Composite resins and glass ionomer cements have different compositions and application techniques. The composite resin binds tightly to the enamel surface by means of micromechanical bonds created by the acid

in the etching technique. Meanwhile, glass ionomer cement bonds chemically in the presence of ion exchange and can release fluorine, thus making it anti-caries properties.[17,18]

4. Resin restorative preventive

Treatment which is the development of the use of sealants on occlusal surfaces, namely the integration of caries prevention with sealants and caries fillings with composite resin materials on the same surface. The goal is to stop the initial process of caries in pits and fissures, especially in teeth with deep pits and fissures, and to take caries prevention measures in pits and fissures in the same tooth.

5. Restoration

Restoration is a filling that is applied to the tooth by removing the carious tissue first without involving the pulp tissue. Indicated for carious lesions that do not involve the pulp. The use of restoration materials can be glass ionomer cement or composite resin

a. Glass Ionomer Cement

Pros: releases *fluorine* so it is indicated for patients with high caries risk

Disadvantages: low modulus of elasticity, making it difficult to withstand large chewing loads. GIC is also very soluble in saliva

b. Composite Resin

Advantages: not easily dissolved in saliva and good aesthetics

Disadvantages: polymerization continues and can endanger the vitality of the pulp [25]

6. Pulpectomy

Pulpectomy is the removal of all pulp tissue from the pulp chamber and root canals. In primary molars, mechanical retrieval of the entire tissue is not possible due to the complex morphology of the root canals. Pulpectomy can be done in 3 ways, vital pulpectomy, de-vital pulpectomy, and non-vital pulpectomy. The indication for pulpectomy treatment is primary teeth with infection beyond the pulp chamber of vital or non-vital teeth. Root absorption is less than apical 1/3. Internal absorption but not root perforation. Continuation of treatment if the pulpotomy fails. Contra indication is if the periapical abnormality is already involved. Widespread absorption of tooth roots. Public health is not good. The patient is not cooperative. Loose teeth due to pathological conditions The

The choice of pulpectomy cases for primary teeth is teeth whose pulp has been infected and the pulp tissue in the root canals is still vital. If left in this state the pulp degenerates / necrosis which will cause negative signs and symptoms, the situation will continue. Pulpectomy can still be performed but the success will decrease due to extensive pulp degeneration[26]

7. Root canal treatment

Canal treatment is a treatment that aims to relieve pain and control sepsis from the pulp and surrounding periapical tissues and restore the diseased tooth so that it can be biologically accepted by the tissue the surroundings. This means that there are no more symptoms, can function properly and there are no other pathological signs. Root canal treatment for permanent teeth in children is performed just like root canal treatment for adult teeth. However, what distinguishes the management is the need for a psychological approach and good communication to the patient.

In general root canal treatment is indicated for: 1) enamel that is not supported by dentin; 2) teeth with infections that pass through the pulp chamber, both vital teeth, partial necrosis and non-vital teeth; 3) periapex tissue abnormality on the radiographic image of less than one third of the apex; 4) dental crowns can still be restored and are useful for prosthetic purposes (for bridge restoration pillars); 5) the teeth are not loose and the periodontium is normal; 6) X-ray showed root resorption not more than one-third apical, no granuloma; 7) the patient is in good condition; 8) the patient wants his teeth to be preserved and is willing to maintain the health of his teeth and mouth; 9) the patient's economic situation allows.

In general, contraindications to root canal treatment include: 1) vertical root fracture; 2) restoration can no longer be carried out; 3) periapical tissue damage involving more than one third of the tooth root length; 4) alveolar bone resorption involves half of the root surface

of the tooth; 5) the patient's systemic condition, such as uncontrolled diabetes mellitus.[27]

8. Correction of malocclusion

Treatment for correcting malocclusion in children is adjusted according to cases of malocclusion in children, correction of malocclusion using orthodontic treatment. The goal of orthodontic treatment is to improve the appearance and profile of a person's face which affects the improvement of social life and quality of life, has good occlusion function so that masticatory function is normal, and tooth stability after treatment. For the correction of malocclusion in children, fixed devices or removable devices such as removable appliances, twin blocks, bionators, facemasks, chin cups, headgear, frankel, and others can be used for the correction of malocclusion. [4]

9. Extraction

Tooth Is a process of removing the tooth from the alveolus, where the tooth can no longer be treated. This procedure is usually done with routine procedures on patients, because tooth extraction is the easiest and best way to get rid of toothache if the tooth cannot be maintained anymore.[24,25,26,27] It is indicated in teeth that have extensive carious lesions that can no longer be treated, remaining roots, supernumerary teeth, persistence of primary teeth, and teeth that are due to be extracted. Contraindicated if the patient has uncontrolled systemic disease, the presence of a malignant tumor, and an acute infection.[28]

Discussion

Caries can cause toothache, discomfort, eating disorders, tooth loss, and stunted development of children. Apart from that, caries can also affect the concentration of children in school. Research by Martins L, et al. (2016) examined the impact of caries on the quality of children's daily schooling, that dental caries was found to be significantly associated with children's quality of life related to oral and dental health in activities such as eating, sleeping, and carrying out daily activities. Rebelo M, et al. (2018) examined the effect of dental and oral health in children on achievement and school attendance, that children with caries experience poor achievement and school attendance.[19,20]

Caries in primary school children

Several studies assessing the prevalence of caries in vulnerable primary school children aged 6-12 years in various parts of the world, the results of a study by Markovic et al., 2013 (Europe) stated that of 1240 children aged 6-12 years there were 88.8% of children experiencing caries, Jokic et al., 2013 (Europe) out of 1825 children aged 6 years, 57.86% of children had caries. Bhayat & Ahmad 2014 (Saudi Arabia, Asia) studied 360 children aged 12 years, 57.2% had caries, Iwasaki et al., 2014 (Japan, Asia) out of 1893 children aged 12 years, 97.3% had caries, Lingerew et al. al., 2014 (Ethiopia, Africa) there were 147 children aged 6-12, 17.6% had caries.

Akyuz et al., 2015 (Turkey, Europe-Asia) studied 325 children aged 6-12 years, 91% of children had caries. Arora & Bhateja, 2015 (India, Asia) out of 100 children aged 12 years, 57% of them have caries. Arangannal et al., 2016 (India, Asia) of 2976 children aged 6-12, 68.8% had caries, Hiremath et al., 2016 (India, Asia) of 13,200 children aged 6-11 years, 78.9% of children had caries , Hover et al., 2017 (Canada, America) of 133 children aged 6-12 years, 15.1% had caries, Doumit & Doughan, 2018 (Lebanon, Asia) of 1433 children aged 6-8, 88.05% had caries and age 12 years, 80.38% had caries.[3]

In Indonesia, the prevalence of tooth decay in children continues to increase from year to year, the results of the 2007 the prevalence of tooth decay in children aged 5-9 years is 21.6%, while DMF-T aged 12 years is 0.91. in 2013 the prevalence of tooth decay aged 5-9 years was 28.9%, aged 12 years who experienced tooth decay was 24.8% with an average DMF-T of 1.4, results in 2018 the prevalence of tooth decay in children aged 5-6 years, namely 93%, aged 12 years who experienced tooth decay by 65.5% with an average DMF-T of 1.89.[28,29,30,31,32,33]

Periodontal disease in primary school children

Periodontal disease is estimated to affect 90% of the world's population. Bhayat and Ahmad, 2014 (Saudi Arabia, Asia) out of 360 children aged 12 years, 70.8% have gingivitis, Rodan et al., 2015 (Jordan, Asia) of 40 children aged 6-11 years there are 70.2% have periodontitis, Markovic et al., 2013 (Bosnia and

Herzegovina, Europa) of 1240 children aged 6-12 years 43% have periodontitis, Arora & Bhateja, 2015 (India, Asia) out of 100 children aged 12 years, 20% have periodontitis.[3,20]

Conclusion

Teeth and mouth problems in children can have a negative effect on their well-being, quality of life and health. Caries and periodontal disease are the most common dental and oral health problems in children, the main cause of which is plaque buildup. Children aged 6 to 12 years or school age children still do not know how to maintain oral hygiene. Therefore, efforts to maintain oral health must be done from an early age.

Suggestion

Schools are the ideal social environment in which dental health promotion strategies can be applied to improve children's oral health and develop good and lasting oral health behaviors. School-age children can experience behavioral changes through interventions at school and improvement.

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Functional Generating Bite Therapy in Children During Growth and Development Period

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Abstract

Introduction: Function generating bite is an individualized functional appliance made from acrylic resin comprised of several components namely: SS bite blocks, buccal shields, expansion coil and palatal button. This appliance enables teeth reposition entirely based on the temoporomandibular joint physiological condition and avoiding cups-to-cusp misalignment. This is due to the fact that the SS bite block allows open contact in the posterior region to open the mandible and regulate/reposition the mandible in three different planes during orthodontic movement. One of the vital mechanisms of bite block is occlusal plane leveling and dental arch parallelism to prevent the occurrence of dental trauma. **Method:** In this systematic review, article search was performed using Google Search and Pubmed. Articles published from 2000 and 2020 were included. Seven articles were found which include: 7 articles from electronic search and 0 articles from manual search, 7 articles were screened, 1 article was excluded and 6 articles were included for feasibility test and 6 complete articles were included. **Results**: There were 6 articles on functional generating bite that can correct malocclusion whilst increasing the masticatory function. **Conclusion:** Function generating bite is not only able to correct malocclusion but can also have an effect on mastication, hence this appliance can be used as a therapeutic tool for masticatory function in children during growth and development.

Keywords: *Function generating bite, Mastication, Growth and development, Orthodontic therapy*

Introduction

Children are the future generation, who deserve attention and every child has the right to achieve optimal cognitive, social and emotional behavior development. In order to achieve better future of one nation, the nation itself requires children with good qualities.[1,2] The quality of a child can be assessed from the process of their growth and development.[2,3] Indonesian's children population is quite large, around 33% of the total population, which is around 83 million and each year the number of children population will increase.[1]

The term growth and development consists of two events that are different in nature but interrelated and difficult to separate. Growth is related to the changes in size, number or dimensions at cellular, organ and individual level.[3,4] Development is the improvement of a more complex bodily structure or function. Development involves the differentiation process of cells, tissues, organs and organ systems that develop in such a way that each can fulfill its own function.[3]

The development of children in Indonesia still needs serious attention. The rate of delayed growth and development is still concerning, namely around 5-10% of children experience general developmental

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delays.[1] Indonesia Pediatric Society in 2011 stated that the incidence of growth and development disorders in the world, in children aged 0-5 years reached 3 million children.[5] Two out of 1,000 babies have impaired motor development.[1]

Motor development in Indonesian children is classified as low, results of the research conducted by Research and Development Center for Nutrition of the Ministry of Health of the Republic of Indonesia in 2012, showed that Denver Development Screening Test (DDST) II survey found that the prevalence of fine and gross motor disorders in toddlers is 25%, or every 2 out of 1,000 children under five experiences impaired motor development. Based on the results of the 2013 Basic Health Research (Riskesdas), it shows that the percentage of children who experience gross and fine motor development disorders in Indonesia is 12.4% and 9.8%, respectively. Although this figure showed a decrease compared to the results of the same research conducted in 2010 where the data displayed 8.8% and 6.2%, respectively, but the data shows that children who experience motor development disorders are still a major public health problem (Riskesdas, 2013).[6]

The stomatognathic system is unison of organ, which functions relates to one another. These organs include the mandible, maxilla, temporomandibular joint (TMJ), dental structures and other supporting structures such as masticatory muscles, facial muscles and muscles of the head and neck. One of the functions of the stomatognathic system is the masticatory system which is the initial process of the digestive system.[7,8,9]

The masticatory system is a functional unit consisting of the teeth, temporomandibular joint (TMJ), muscles that support direct and indirect mastication and blood vessels also nerves that support all the supporting tissues of the masticatory system.[7] Muscles are moved by nerve impulses because there is pressure arising from the lower teeth in contact with the upper teeth so that the mandible can carry out the functional activities of the mastication system. The harmony between these components is very important to maintain its health and functional capacity. Functional disorders occur due to irregularities in the activity of one of the components involved in the mastication system function, such as abnormalities in the position and or function of the teeth or mastication muscles.[10]

Any disturbance in one component of the masticatory system will have an impact on other components; hence it is necessary to know the functional aspects and the movement of these components during mastication.[7] Such disorders can be classified as intrinsic disorders if they present pathological changes, or extrinsic disorders, if they indicate disorders of the neuromuscular system. The etiology of intrinsic disorders is internal derangements, rheumatoid arthritis, growth disorders, ankylosis of the jaw joint, etc; whilst extrinsic disorders are usually caused by excessive muscle usage.[10]

Oral motor problems in children will be easily detected when the child has a cough and/or chokes while eating. However, most of the problems associated with the chewing process (mastication) initially appear in a simple pattern, such as: difficulty getting the spoon into the mouth or the limited variety of foods the child can eat. In certain cases, some children sometimes experience oral motor problems in a more specialized pattern such as: uncoordinated tongue movement during lateralization or dysfunction of the movement of bullous food into the mouth for initiation of the ingestion reflex.[11]

It can be said that the experience and practice of mastication in children directly affects the oral motor pattern, which directly affects the response to mastication function. If the taste and texture of food do not change, the child cannot learn to accept food and cannot practice the new patterns needed for the manipulation of new types of food and the safe and efficient movement of food through the pharynx. Lack of proper practice can lead to loss of oral motor function or chewing function in children so that the child will fail to further learn the skill.[11]

The oral function therapy can be performed during the child's development and is performed through orthodontic action, namely the Function Generating Bite (FGB) appliance. This FGB appliance is a removable orthodontic appliance, which do not only provides action and effects on malocclusion but, is also able to improve masticatory function.[12,37,38,39]

Based on the background presented, the aim of this systematic review is to review the Function Generating Bite (FGB) appliance as an oral functional therapy

during childhood.

Method

Search Strategy

Search was carried out on Google Search and Pubmed. Articles were published from 2000 to 2020. The keywords used for search include: "function generating bite". For each search, the abstract and title will be screened and the full article that meets the criteria will be downloaded.

The flow chart in Figure 1 defines the articles that are excluded and involved at each stage. Screening was carried out on 7 articles obtained through electronic searches, 0 articles from manual searches, 1 article was excluded, and 6 articles were tested for their eligibility and 6 articles were included in the review.

Results

The following are the inclusion criteria for this systematic review: 1) Articles published in 2000-2020 2) In English or Indonesian 3) All types of publication and research designs were considered 4) A search was carried out on published and unpublished data. The exclusion criteria for this systematic review are articles that do not discuss the Function Generating Bite appliance.

Table 1. Articles covering function generating bite

No	Author	Year	Title	Conclusion
1	Maria Grazia Piancino, Francesca Talpone, Paola Dalmasso, Cesare Debernardi , Arthur Lewin dan Pietro Bracco	2006	Reverse-sequencing chewing patterns before and after treatment of children with a unilateral posterior crossbite	Before therapy, the percentage of reverse-sequencing chewing cycles, on the crossbite side, was significantly higher than that on the unaffected side with both a soft and hard bolus. After therapy, the percentage of reverse-sequencing chewing cycles on the crossbite side was significantly decreased with both a soft and a hard bolus. No significant differences were found in the percentage of reverse-sequencing chewing cycles on the non-crossbite side, before or after treatment, either with a soft or hard bolus.
2	M. G. Piancino, T. Vallelonga, C. Debernardi, P. Bracco	2013	Deep bite: a case report with chewing pattern and electromyographic activity before and after therapy with function generating bite	The improvement of both the chewing pattern and the muscular activation, after therapy with FGB-D, showed that in this case the functional appliance was able to correct the dental malocclusion improving the masticatory function

Cont... **Table 1. Articles covering function generating bite**

3.	M. G. Piancino, L. Roberi, G. Frongia, M, Reverdito, R. Slavicek, P. Bracco	2008	Computerized axiography in TMD patients before and after therapy with 'function generating bites	the study shows that the TMJ tracings of TMD patients before and after therapy with FGB significantly improve especially in young patients. FGB may be a useful appliance to improve TMJ function in the study shows that the TMJ tracings of TMD patients before and after therapy with FGB significantly improve especially in young patients. FGB may be a useful appliance to improve TMJ function in The study shows that the TMJ tracings of TMD patients before and after therapy with FGB significantly improve especially in young patients. FGB may be a useful appliance to improve TMJ function in young and adult TMD patients requiring orthodontic treatment.
4	Matteo Reverdito, Maria Grazia Piancino, Gianluigi Frongia, Andrea Adriano Bracco, Maurizio Gribaudo Fresi, Cesare Lorenzo Debernardi, Pietro Bracco	2011	Functional cephalometry analysis and computerized axiography before and after therapy with "Function Generating Bite" in a deep bite patient	There was an improvement in symmetry, movement of the condyles, Bennett's angle, morphology and symmetry of the tracing of the two condyles, as well as improvements between RFF and RKN relative to the sagittal plane.
5.	Maria Grazia Piancino, Stephanos Kyrkanides	2016	Therapy with Function Generating Bite Appliance: Actions and Effects on Malocclusion and Masticatory Function	*Function generating Bite* is a relatively simple appliance
6	T. Castroflorio, F. Talpone, A. Deregibus, M.G. Piancino, P. Bracco	2004	Effects of a functional appliance on masticatory muscles of young adults suffering from muscle-related temporomandibular disorders	FGB reduce torque index

Discussion

Children Growth and Development

Growth is an increase in the number and size of cells in all parts of the body. Growth is a physiological change as a result of the maturation process of physical functions that takes place normally in healthy children at normal times. Growth can also be interpreted as a process of transmission of a hereditary physical constitution (state of the body or physical state) in the form of a continuous active process. Meanwhile, development is the gradual change and the perfection of the function of the organs, the increase and expansion of one's capacity through growth, maturity, and learning.[13-17]

In general, the terms growth and development have the same meaning, namely that they both undergo change, but in particular the term growth is different from development. The term growth refers to changes in quantity, while development is more towards quality. The concept of growth is more towards physical definitions such as from small to large, from short or low to high and others. The nature of growth cannot return to its original form, for example from short to tall but it is impossible from tall to be short again. In addition, the most important thing about growth is the physical maturation process that is marked by the increasingly complex system of muscle tissue, nervous system and organ functions, this maturity causes the physical organs to feel ready to be able to carry out tasks and activities according to the stage of individual development. It is at this time that children begin to be able to develop and carry out activities to develop all their cognitive and affection potential properly. Development can be interpreted as a result of changes in physical maturity and readiness that have the potential to carry out an activity, so that individuals already have an experience.[13-17]

The period of child development is divided into:[18]

a. The pre-natal period (pregnancy to birth)

b. Infants and toddlers (born up to 2 years)

c. Early childhood (3 to 5 years)

d. Middle childhood (6 to 11 years)

e. Teenagers (12 to adults)

Masticatory System

The masticatory system is a functional unit of the stomatognathic system. Mastication is done to prepare food into smaller particles so that it is easier to swallow. Mastication occurs due to the complex interactions between the masticatory muscles and their supporting muscles, the teeth, and the TMJ. The presence of food that enters the oral cavity stimulates the muscles to open the mandible. Then the food enters the oral cavity and is moved by the tongue and cheek muscles so that it is on the tooth contact surface. This process occurs simultaneously with the closure of the mandible.[4,19-23]

During the mastication process, the tongue and cheeks also play an important role. The tongue will crush food. Assisted by the hard palate and the dorsal surface of the tongue (papilla) as well as mixing the food with saliva and transferring the food from one side of the oral cavity to the other and ensuring that all parts of the food are chewed. The lips and cheeks also play a role so that fluid does not come out of the oral cavity.[4,19-23]

Perfect chewing will produce food particles that are ready to be digested and absorbed in the digestive tract. The optimal absorption of food will have an impact on the absorption of nutrients that are important for oral health and systemic health.[4,19-23]

Function Generating Bite Appliance (FGB)

Oral motor is a coordination and movement of the hard tissue, soft tissue and the vascular system as well as control of nerves in the face and oral areas that form oral motor function. The coordination of these structures is very important for the functions of speech, mastication, and swallowing for a variety of food textures.

During the toddler years, the child will experience the formation stage of total motor function. This stage must be completed when the baby starts breastfeeding, which will then be switched to a form of food with various textures for the purpose of developing perfect oral motor function. The prevalence of oral motor disorders in children is quite high. Previous research shows that 39.8% of children suffer from disorders related to oral motor skills. Disorders of food intake and

mastication are reported in 10.25% of children, 40-70% of infants and 70-80% of children with special needs. Oral motor disorders sometimes form and are initially found in children from an early age. Parents sometimes do not realize the existence of this disorder in children. Healthy children are able to master all oral motor skills to get maximum nutritional intake at the age of five. Children who do not have this ability are feared that they will experience malnutrition and this will affect the child's growth.[24]

Mastication function therapy uses a variety of exercises to develop awareness, strength, coordination and movement and resistance of the lips, cheeks, tongue, and jaw. These actions include active muscle training, muscle stretching, passive exercises, and sensory stimulation aimed at influencing the physiology of the oropharyngeal mechanism.[25]

Knowledge regarding the physiology and biology of the masticatory function is based on scientific evidence through therapy during development. In fact, today, in the clinic, the success of orthognatodontic therapy is not only to reposition the teeth in the arch but also to have a therapeutic effect on function. This is mainly obtained for early therapy in developing children. Therefore, the ultimate goal of early orthognatodontic treatment is to obtain (through the teeth) a balance of functions, especially mastication, which refers to the principles of gnatology. This can be obtained by using functional tools. Function Generating Bite (FGB) "is not only for correction of dental malocclusion but especially for correcting abnormal masticatory patterns with significant results.[26]

The function generating bite (FGB) tool is a functional tool, tailor-made and made of acrylic resin with the characteristic of having a posterior bite enhancer made of metal. However, this tool is also often regarded as a gnatological tool.[12]

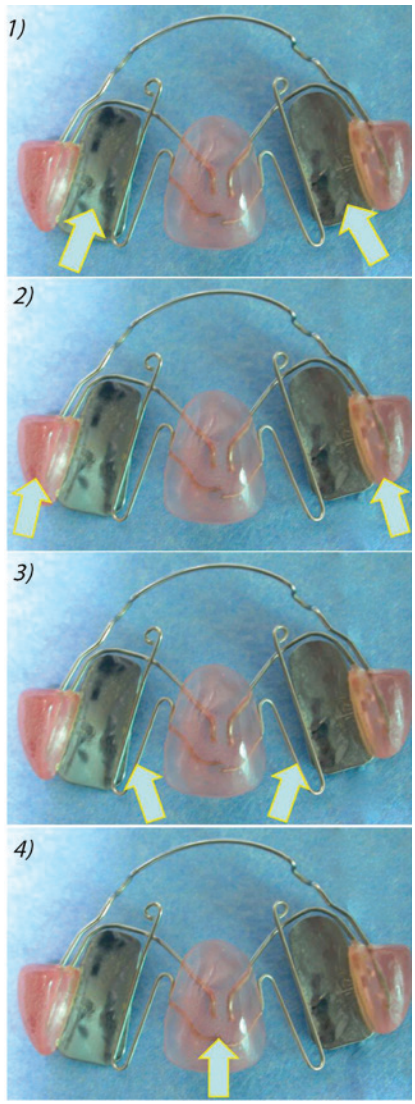

Figure 1. FGB components 1. Bite block SS, 2. Buccal Shields, 3. Expansion coil, 4. Palatal button

FGB main components are:[12]

1. Bite block made from stainless steel
2. Buccal shield
3. Expansion coil
4. Palatal button

FGB is considered a functional appliance because it meets the following criteria:[12]

1. Apparatus with two posterior bite enhancers
2. Apparatus with anterior bite elevators and two posterior bite elevators
3. Apparatus with multiple anterior bite elevators

and two posterior bite elevators

The actions and effects of FGB are:

1. Repositioning the mandible

2. Align the occlusal plane, avoid trauma and protect the dental cups

3. Anchoring muscles

4. Allows symmetrical and asymmetric activation

5. Reposition teeth by self-regulating, intermittent pressure (intermittent)

6. Directing the tongue, stabilizing the tool

7. Directing motor nerve control via self-regulation

The FGB is an individual functional appliance made of acrylic resin and a stainless steel bite block and wire. This appliance allows repositioning of the teeth according to the physiological condition of the TMJ and avoids misalignment of the tooth cup-to-cup contacts. This is because the bite block SS located in the posterior occlusion region plays a role in opening the mandible and regulating / repositioning the mandible on three planes during orthodontic movement. One of the important mechanisms of bite blovk is the leveling of the occlusal plane and alignment of the dental arch so as to avoid dental trauma.[26]

From an orthodontic point of view, the posterior bite block is activated simultaneously when the coil is enlarged which results in a series of forces / stresses and bodily movement of the teeth. It is important to stimulate bone growth so as to avoid the occurrence of tilting movements, to obtain stable orthodontic correction.[26]

FGB has muscular anchorage and is active during the ingestion process, so that orthodontic pressure moves the tooth intermittently when swallowing and regulates through the patient's muscles. FGB easily restores masticatory function because it prevents cups-to-cups contact during orthodontic movements, so it is considered an orthognatodontic tool.[26]

The action of the FGB appliance on the TMJ is due to the metal biteplane separating the mandible allowing the condyle to return to its physiological position compressing the joint through the application of traction to the ligament, thereby reducing load, reshaping function in the lubrication system and normalizing the meniscus tropism. It has been demonstrated that the FGB appliance for deepbite correction not only corrects dental malocclusion but also affects the joint function and neuromuscular structure of the patient.[27]

The correction of asymmetries in young patients using FGB appliances is due to the fact that the condyles have an adaptive growth type with an accelerated puberty. The therapy gives better results if applied during the maximum adaptation period to allow for condyles growth and capability. Thus, early therapy is preferable to functional rehabilitation.[28]

Improvements in both the masticatory pattern and muscle activity after FGB therapy showed that this appliance was able to correct dental malocclusions as well as improve masticatory function.[29]

The results from a study conducted by Castroflorio *et al*. Showed that the FGB appliance was able to correct an abnormal torque index after 12 months of therapy. As reported by Blanskma and Van Eijden, the anterior temporalis muscle is more associated with mandibular laterodevization than closure action. The data show that FGB has more effect on the anterior temporalis muscle. This is due to the stimulation of the periodontal mechanoreceptors, however when considering the position of the jaws, this is likely the result of different outputs from other peripheral sensory receptors such as joint receptors and muscle spindles.[30]

It should be noted that FGB is able to prevent maxillary and mandibular teeth from establishing occlusal contact through the use of a metal bite plane and static and dynamic control of mandibular position.[31,32,33,34,35,36]

Conclusion

Function Generating Bite (FGB) is a functional appliance made of acrylic resin with a posterior bite block made of stainless steel. This appliance is not only able to correct malocclusion but also can have an effect on mastication so that it can be used as a masticatory function therapy tool in children during growth and development.

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Vaccine Financing for the Treatment of Covid-19 of Banking Agencies' in Empowering Small and Medium Health Institution at Aceh

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Abstract

The purpose of this article is to explain the vaccine financing for the treatment of covid-19 of Banking Agencies in an effort to empower Small and Medium Institution in Aceh. This study applies qualitative legal methods with a descriptive-analytical approach. The results of the study indicate that the vaccine financing for the treatment of covid-19 and the existence of the Banking Agencies is instrumental in efforts to empowerment SMHi and covid-19 treatment, but the quantity of SMHi that can be financed very limited. Only a small proportion of SMHi can obtain loan facilities from bank agencies. In conclusion, bank agencies still not yet play the important role in empowerment the micro, small and medium Health Institution in Aceh.

Key Words: *vaksin, covid 19, Banking; Small and Medium Institution; Aceh Province.*

Introduction

The new corona virus vaccine made by *Sinovac* from China has arrived in Indonesia. A total of 1.2 million doses of vaccine. Some people are still unfamiliar with vaccines and why their administration is so important. Vaccines, quoting from Web MD, are biological products of viruses that are weakened or dead. Small and Medium Health Institution hereinafter abbreviated as (SMHi) plays an important role for national Health especially during the current covid-19 treatment. In addition, SMHi aims to cultivate business in order to develop a national health institution based on a just democracy, as suggested by Wilantara and Susilawati[1].

Vaccine and the important role of SMHi for the economy that affects current Covid-19 treatment not only prevails in the country of Indonesia, but also in every other developing country. Tambunan[2] states that in Asia, Africa and Latin America financed SMHi also plays a very important role especially from the perspective of labor and income sources for the poor and able to provide medical expenses during the current Covid-19 period, income distribution, poverty reduction and rural health development. From Tambunan's[2] statement, shows that the role of SMHi is so great, because SMHi can give access to employment opportunities from various business sectors. The same thing also stated by Muetia[3], "The development and SMHi" s role is not minor, since they can provide a large contribution in for a nation", as stated by Fitriati[4].

Komaruddin[5] views that the sector of Cooperatives and SMHi remains the largest contributor in the absorption of business and health sector opportunities. If the problem of SMHi can be solved well, judging from the position in supporting the economy then small health institution occupies a very strategic position because it accounts for more 17% employment and provide financing solutions for Covid-19 treatment, according to Fajar[6]. So that efforts to give vaccines to the community must be implemented immediately. Vaccines are very important during the current Covid-19 period. Vaccines are substances or substances that function to help the

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body fight certain diseases. The body that has been vaccinated will form antibodies against certain viruses.

Whereas the contribution of this group at the time is very large, said Wilantara and Susilawati[1]. The percentage of SMHi involvement in the national health contribution according to Fajar[6] is enough high during the current covid-19 treatment period, the legal subjects of consisting of small- in various sectors, especially health tools and trade of medicine and drug as well as services and processing others item. SMHi are also considered flexible enough and can easily adapt to the ebb and flow of the demand, said Djamil[7]. SMHi business sectors vary widely, ranging from industry, services, informal and medicine and related sectors during the current covid-19 treatment period.

In addition to creating employment opportunities and equity of health sector opportunities during the current covid-19 treatment period, SMHi also play a strategic role in overcoming the problem of unemployment and poverty. Through SMHi a lot of human resources can be absorbed. Similarly, SMHi act as contributors to the national economy through tax revenue, user charges and other forms of acceptance. SMHi are also considered as the spearhead of the health industry during the current covid-19 treatment period. From the reality above proves that SMHi have an important role in the life of the Indonesian as nation, according to Nadratuzzaman[8].

Providing the covid-19 treatment vaccine, In running its services, SMHi in Aceh faces various obstacles during the current covid-19 treatment period, both juridical, economic and financial constraints, as well as political constraints. From an economic and financial perspective, the most difficult constraints faced by SMHi are related to capital, corporate financial management, access, and a multi-related focus. This vaccine is expected to be able to provide health solutions for the community. According to Komaruddin[5] considers that capital is still a classic problem faced by some SMHi in Indonesia to increase the health coverage during the current covid-19 treatment period. Not to mention the problem of technology and information. So that the vaccine must be implemented immediately.

One of the efforts to suppress covid-19 is the provision of a covid-19 vaccine, Although the government has tried to overcome it by issuing various policies during the current covid-19 treatment period. through financing programs to strengthen SMHi. However, obstacles in the field show that access to capital is still a major obstacle for SMHi in managing their health services, in addition to other problems. The amount of capital has a significant relationship to the development of SMHi.

Vaccine and the development of Small and Medium Health Institution (SMHi) cannot be separated from the role and support of banking institutions in the channeling of credit/financing for SMHi Covid-19 treatment period. Therefore, the government should facilitate SMHi to gain access to the capital. The effort will not be realized if there is no government interference. According Kadir[9] "the government should intervene on the development and survival of the life of small and medium enterprise (including health sectors) during the current covid-19 treatment period, by giving capital cash loans with low interest besides public fund allocations.

Most in Indonesia Izziyana10 health belongs to the SMHi group. Based on statistical data the percentage of SMHi in Indonesia as much as 97%, the rest including large businesses. However, access to bank lending 84% is dominated by large health institution such as hospital and others groups. Based on the large amount of credit disbursement, Bank Indonesia made a policy through BI Regulation during the current covid-19 treatment period. The regulation requires banks to provide financing Small and Medium Health Institution under 20% at the end of 2018. Based on the above description it is interested to be studied in depth how vaccine financing for covid-19 of Banking Agencies in an effort to empower SMHi through capital strengthening national health program during the current covid-19 treatment period.

Methods

The study on vaccine financing for covid-19 of banks agencies in the empowerment of SMHi during the current covid-19 treatment period is juridical normative, using secondary data derived from primary and secondary legal materials. Primary sources were collected by analysing current literature. Secondary sources were obtained from previous research, journal, government reports and other institution documents.

Discussion

Vaccination during the Covid-19 treatment period

and in order to increase the role of SMHi for the national economy, the Government prepares the RPJPN for 2005-2025. This RPJPN contains the framework for Empowerment of Cooperatives and SMHi. To empower SMHi during the current covid-19 treatment period required a number of planned, systematic and comprehensive prerequisites, said Fitriati[4].

The Government of Aceh, through the RPJMA (Aceh Mid-Term Development Plan) 2012-2017, emphasizes the importance of developing SMHi during the current covid-19 treatment period. In the RPJMA 2012-2017, it is stated that the Government of Aceh's third mission is "Strengthening the health society and quality of human resources" which is manifested in, among other things, "Expanding health development opportunities through the development of infrastructure and support for SMHi and cooperatives." As well as supporting the provision of the Covid-19 treatment vaccine. Until now, the provision of the COVID-19 vaccine is the most effective solution to reduce the number of cases of the SARS-CoV-2 virus infection that causes COVID-19.

As a result of the monetary crisis, Indonesia's development has declined and the increasing number of of health facilities deteriorated. According to Sila[11] it requires the government to undertake various programs of health alleviation and eradication; one of them by providing access to the health and medical institution in order to increase additional income (income generating) through public fund allocation or other development programs. This program creates development in health sectors Covid-19 treatment and equity, trains self-reliance and enhances the creativity of the of the health development. One of the efforts to overcome this problem is the effectiveness and safety of the Covid-19 vaccine is still being studied in the clinical trial stage by the government and various related institutions. This is the government's step in ensuring that the Covid-19 vaccine that will be provided is suitable for use to prevent Covid-19.

Giving the Covid-19 vaccine is the solution that is considered the most effective way to reduce the number of cases of SARS-CoV-2 virus infection that causes Covid-19 disease. However, the distribution of vaccines is not evenly distributed among the people. The above conditions show that the banking institution has not performed its function and purpose as an agent in the development of the national health development as mandated in the Banking Act during the current covid-19 treatment period. Article 4 of the Law Number 10, 1998 concerning Banking which affirms that "Indonesian banking aims to support the implementation of national development in order to improve equity, growth and national stability towards the improvement of the welfare of the people". Based on the principle of equity, the banking function as an intermediary institution or agent of development can also be realized to SMHi through credit or financing assistance for the development.

Pursuant to 1945 Constitution, the health sector during the current covid-19 treatment period in Indonesia is one of the sectors that have a very important role in encouraging the improvement of the people. The activities of the sector are almost entirely services, of the citizen the development and advancement of the health sector, demands continuous improvement, both from the organizational, regulatory (policy), and human resources (HR) aspects.

Before there was a vaccine handling for the treatment of Covid-19, there is the fact that there are still very few commercial banks that provide credit to micro and small Health Institution during the current covid-19 treatment period saat ini. Many factors have caused Commercial Banks to be limited in serving SMHi, said Yuliana.[12] Generally the SMHi Customer come from the strata of low educated society and has capital in a very limited amount. This condition often leads to the administrative and technical requirements of small and medium Health Institution (SMHi) and those requested by the banking sector do not meet the feasibility during the current covid-19 treatment period.

The importance of vaccines to suppress the spread of covid-19 in society is because the main part of the profitability of banks is credit operations that is given to the community. Hence, the effectiveness of the system of credit risk management is important during the covid-19 treatment period, said Panfilova, et al. So, the procedure to distribute credit must be based prudential principle. SMHi face difficulties in obtaining credit from commercial banks due to the requirements requested by the bank, especially concerning the high interest rates charged to every debtor including SMHi due to the

impact of the virus and covid-19 treatment. The data of research conducted by Yuliana shows the interest rate of loan for financing the activities of MSMHi in Banda City an average of 9.5% per year. Viewed from the percentage is quite high. This of course will be very difficult for SMHi to pay off credit. In addition, banks require SMHi applying for loans to provide sufficient guarantees in anticipation of possible non-performing loans.

Although the government has channeled funds through government-owned commercial banks, such as BRI, BNI, Bank Mandiri with KUR programs to SMHi, the number is still very limited for current covid 19 treatment, so not all SMHi can enjoy the facilities of the Bank. Due to the difficulty of obtaining access to financing through the Banking Institution, many Micro and Small Health Institution are looking for other alternative ways, namely by increasing health service payment, or they decreased quality of citizen health service in line with the needs of SMHi for capital in the short term for current covid 19 treatment.

Deputy for Financing of the Ministry of Cooperatives and SME Braman Setyo from Tribunnews said Bank Rakyat Indonesia (BRI) is still the dominant bank in KUR -in the current covid 19 treatment period distribution, which is about 67 percent of the total budget allocated by the Bank credit channeled to SMHi is dominated in the type of working capital credit and expansion for strong health institutions group. Head of Finance of the Office of Cooperatives of Aceh, Muhammad Dahlan, said the target of the program for additional working capital, adding production equipment, and expansion in the current covid-19 treatment period. For example, micro, small and medium Health Institution, prospective migrant workers, prospective overseas apprentices, employee / worker or migrant workers, and workers affected by termination. The government's effort to overcome the Covid problem is to provide vaccines, but efforts to prevent COVID-19 even though there are vaccines, of course, must still be accompanied by health protocols.

The delivery of the Covid-19 treatment vaccine in Indonesia has not been evenly distributed, in terms of credit distribution to SMHi, both in the form of credit programs and commercial credit is still very limited. Based on Bank Indonesia, only 12% of SMHi enjoy access to finance in the current covid-19 treatment period. Whereas in the Medium-Term Development Plan (RPJM) until 2019 financing for SMHi from banks, must already be at the level of 16%. The Ministry of Healy and SMHi is currently working on a financing alternative for SMHi, which is a special financing scheme since the target of credit disbursement for SMHi faces many challenges as SMHi is not business institution in the current covid-19 treatment period.

Vaccines for the treatment of Covid-19 are very important**.** In an effort to accelerate the process funding distribution, the Government should involve the Regional Government, considering that MSMHi who will get financing facilities sourced from public fund or special loan (non or low interest) spread in the regions the current covid-19 pandemic treatment period. Before the distribution of financial program needs to be socialized to prospective recipients of on the mechanism of obtaining funds, so that from the beginning MSMHi who will get essential funds can prepare the requirements required at the time of submission of credit applications Covid-19 treatment period. Local Government also need to take steps to assist MSMHi will access the funding to upgrade or maintenance of the health or medical facility for people health protections

Conclusion

The provision of the covid-19 treatment vaccine is also supported by Banking Financial Agencies that serve as the importan of the funding SMHi besides of public fund through various sceme are formed to perform their duties and functions properly and optimally during the Covid-19 treatment period. Banking institutions that disburse loans to SMHi amounted to only 15.2% of existing credit sources. The rest of the credits of banking institutions are mostly distributed to business activity due to the impact of covid-19. From the data of Bank Indonesia's report on SMHi credit to total banking credit is minimum. As a solution during the Covid-19 treatment period, is to provide a vaccine to suppress transmission of Covid-19. From the amount of credit to SMHi Distribution of credit to Large Health Institution reaches 80% of total existing credit the current covid-19 treatment period. This shows that dominates access to financing capital of business is quite high compared

to SMHi. The above phenomenon shows that there is the only no interest can empower SMHi to strengthen their duty to protect citizen health the current covid-19 treatment period. Government need action to improve the SMHi Needs to as well as providing vaccine action as a treatment for Covid-19.

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Maternal and Perinatal Outcomes in Uncorrected Tetralogy of Fallot

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Abstract

Background: Tetralogy of Fallot (TOF) is the most common congenital heart disease in the population. Correction of TOF during infancy or childhood may increase the quality of patient's life. Uncorrected TOF may cause further complications later on. Pregnancy with uncorrected TOF may cause complications on both maternal and fetal sides.

Objective: to analyze maternal and perinatal outcomes in patients with uncorrected TOF during pregnancy.

Methods: This is a retrospective study. A total of 8 pregnant women diagnosed with TOF registered in the Obstetric-Cardiac unit in 3 years (2016-2018). Medical records were reviewed for clinical assessment, echocardiography results, and therapy during hospitalization. Patients were contacted by telephone to provide an obstetric history, including details of pregnancy and delivery. Five of 7 patients able to be contacted, and home visits were done to evaluate their conditions.

Results: There were 8 pregnant women with uncorrected TOF. Most cases (87,5%) were first diagnosed during pregnancy and delivery. Intra-Uterine Growth Restriction (IUGR) and abortion (62.5% and 25%, respectively) were the most obstetric complications. Cardiac complications were cardiogenic shock (12.5%), thromboembolic (12.5%), and various degree of heart failure (62.5%). The number of Small for Gestational Age (SGA) was 66.7%. Incidence of congenital heart disease (CHD) in the fetus was 20%. One death case and 2 abortions from a total of 8 cases were found.

Conclusion: TOF may cause many maternal and fetal complications. The most common maternal complications are heart failure, cardiogenic shock and thromboembolic, while perinatal complications include IUGR, SGA and abortion.

Keywords*: Uncorrected Tetralogy of Fallot, pregnancy, outcome, complication*

Introduction

Tetralogy of Fallot (TOF) is the most common form of congenital heart disease (CHD), representing 7-10% of all CHDs. It is characterized by a ventricular septal defect (VSD), overriding aorta, pulmonary stenosis, and right ventricular hypertrophy[(1)]. The incidence of CHD in Indonesia was relatively high, with 9 neonates per 1.000 live births and an estimated 40.500 babies born with CHD in Indonesia per year [(2)]. The discrepancy between the augmentation of cases per year and the number of surgeries contributed to the increase of uncorrected TOF.

If uncorrected TOF women reach adulthood and plan to conceive, a multidisciplinary team's supervision is needed during pregnancy and delivery. This study

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aims to analyze the maternal and perinatal outcomes in patients with uncorrected TOF during pregnancy. It consists of cases with uncorrected TOF in pregnancy that experienced delays in the diagnosis, and some were mismanaged. We hope this study could strengthen team works between obstetrician, cardiologist, a congenital cardiac surgeon, and anaesthetist in managing congenital heart disease in pregnancy.

Materials and Methods

This is a retrospective study. A total of 8 pregnant women diagnosed with TOF registered in the Obstetric-Cardiac unit in 3 years (2016-2018). Medical records were reviewed for clinical assessment, echocardiography results, and therapy during hospitalization. Patients were contacted by telephone to provide an obstetric history, including details of pregnancy and delivery. Five of 7 patients able to be contacted, and home visits were done to evaluate their conditions. Ethical approval obtained from Universitas Airlangga Academic Hospital, Surabaya Indonesia

Results

A total of 8 patients with TOF were identified. The characteristic of these patients were summarized in table 1. The mean age of the patients were 22.1 years old, all of them had never undergone surgery before. Diagnosis of TOF was late to be diagnosed, 87.5% cases were found during pregnancy and post-partum. Elective caesarean section (CS) was performed in 5 patients (71.4%), whereas forceps extraction and curettage were performed in 1 patient (14.3%).

Table 1. Maternal Characteristic of Pregnancy with Uncorrected TOF

Characteristic	Frequency (%)
Maternal Age 20 – 30 years old Mean age : 22,1 years old	8 (100)
Parity Primigravida	8 (100)
Time of Diagnosis Pre conception Gestational age < 20 w Gestational age > 20 w Post-partum	 1 (12,5) 3 (37,5) 3 (37,5) 1 (12,5)
Mode of Delivery (n=7) Cesarean Section Forceps Curettage	 5 (71,4) 1 (14,3) 1 (14,3)
Methods of contraception (n=7) IUD Sterilization	 1 (14,3) 6 (85,7)
Laboratory (n=8) Mean Hb : 15,35 g/dL Mean Hematocrit: 47,45%	
Oxygen Saturation (n=8) < 85% 85-90% >90%	 1 (12,5) 2 (25) 5 (62,5)

Obstetric and cardiac complications during pregnancy until post-partum periods were summarized in table 2. Since all women had never undergone cardiac surgery, heart failure was the most common complication, with varying degrees of DCFC. Most of them presented in DCFC II (62.5%). Cardiogenic shock was found in 1 patient (12.5%), occurring immediately post-delivery. Thromboembolic was also found in 1 patient (12.5%). On the other hand, intra uterine growth restriction (IUGR) was recorded in 62.5% cases, and became the highest obstetric complication in this group of study. Abortion was the second most common complication after IUGR. One case of abortion occurred spontaneously, and the remaining was elective termination during the first trimester.

Table 2. Cardiac and Obstetric Complications in maternal side

Complications	Frequency (n,%)
Cardiac	
Heart failure NYHA II	5 (62,5)
Heart failure NYHA III	2 (50)
Heart failure NYHA IV	1 (12,5)
Thromboembolic	1 (12,5)
Cardiogenic shock	1 (12,5)
Obstetric	
PROM	1 (12,5)
Preeclampsia	1 (12,5)
Abortion	2 (25)
IUGR	5 (62,5)
Oligohydramnion	1 (12,5)

Seven of 8 patients presented to the hospital during pregnancy, in a varying gestational age. Profile of pregnant women was summarized in table 3. There were 2 patients whom was diagnosed with TOF in the first trimester (A-1 & F-6), and before pregnancy (G-7). Even the pregnancy could endanger them, they were determined to continue the pregnancy. We did multidiscipline conference and did closely monitor the patients, and the pregnancy terminated in appropriate time.

Table 3. Maternal and perinatal outcome

Initial	Time of diagnosis	Hb (g/dl) SO2 (%)	HF NYHA	Indication of Termination	Time of termination (weeks)	Birth weight (g) BS (weeks) LS	MOD	Perinatal Complication	Last Condition
A-1	Pregnant (5/6w)	19,3 78	IV	Spontaneous Abortion					Death
B-2	Pregnant (26-27 w)	15,6 93	II	IUGR	29/30 w	1000 32; p<10	CS	ASD, RDS, Gastric outlet obstruction	NYHA II, SO2 71%

Cont... **Table 3. Maternal and perinatal outcome**

C-3	Pregnant (10/11 w)	14,3 93	II	PROM + IUGR	36 w	2100 36; p10-25	CS	(-)	NYHA II, SO2 94%
D-4	Pregnant (36/37 w)	11,9 98	II	IUGR + Oligohidramnion	36/37 w	2650 38; p10-25	CS	(-)	NYHA II, SO2 98%
E-5	Pregnant (33/34 w)	16,4 86	III	IUGR	33/34 w	1100 33; p<10	CS	RDS, EOS	NYHA II, SO2 80%
F-6	Pregnant (6/7 w)	17,4 86	III	DCFC III	7/8 w		Curettage		Loss of Follow Up
G-7	Before pregnant	16,2 92	II	Preeclampsia + IUGR	31/32 w	1000 32; p<10	CS	NEC, RDS	NYHA II, SO2 95%
H-8	Post partum	11,7 98	II	Suspect CHD & In-labor	38 w	2000 38; p<10	Forceps	(-)	Loss of Follow Up

BS: Ballard Score; LS: Lubchenco score; MOD: Mode of Delivery; CS: Cesarean Section; ASD: Atrium septal defect; RDS: Respiratory Distress Syndrome; EOS: Early Onset Sepsis; NEC: Necrotizing Entero Colitis; NYHA: New York Heart Association

One pink Fallot patient (D-4) adapted well during pregnancy. There was no dyspnea, orthopnea, or limitation of activity recorded. Diagnosis of TOF was accidentally found due to her confession in the late pregnancy, and then she was referred to the tertiary hospital for further management. The other two patients complicated with heart failure since the first trimester (F-6 & A-1) and ended up with pregnancy termination and spontaneous abortion. Two cases (B-2 & E-5) were referred to tertiary hospitals due to fetal compromise.

A total of 6 deliveries occurred within 30 months. All babies were life births, and 5 of 6 babies (83.3%) were delivered prematurely. One neonatal death (16.7%) occurred during the 7th day of hospitalization. Multiple congenital anomalies were noted in this baby, including atrial septal defect (ASD) and gastric outlet obstruction.

The correlation between maternal presentation and perinatal outcomes was summarized in table 4. Low levels of oxygen, high concentrations of haemoglobin (Hb), and degree of heart failure (NYHA) impacted pregnancy outcomes. We noted 2 abortions happened in the worst condition among the group. During hospitalization, some neonatal complications due to prematurity occurred, such as respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), and early onset of sepsis (EOS). The incidence of RDS was 40%, whereas NEC and EOS occurred in 20% of infants.

Table 4. Perinatal Outcomes Characteristic

	LB, n(%)	**Premature, n(%)**	**SGA, n(%)**	**NND, n(%)**	**Mean birth weight, kg**
Frequency (n=6)	6 (100)	5 (83,3)	4 (66,7)	1 (16,7)	1,641

LB: life birth, SB: still birth, SGA: small for gestational age, NND: neonatal death

Among 8 patients, there was 1 maternal death; hence the case fatality rate was 12.5%. The mean length of stay (LOS) was 8.25 days. All women needed intensive care, but none of them needed a ventilator. Pregnancy did interfere cardiac condition of uncorrected TOF women. 87.5% of patients were discharged well, but there was a significant decrease in oxygen saturation in 2 patients after 4 months and 2 years of follow up.

Discussion

TOF has various cyanosis degrees that require urgent surgical intervention in early life to cyanotic form that often undiagnosed or late to be diagnosed [4]. All patients in this study were diagnosed in adulthood or during the pregnancy and delivery process, whereas in other studies, 30-100% of cases of TOF had been diagnosed and repaired before pregnancy[3], [5], [6]. Hemodynamic changes in pregnancy and delivery may cause clinical deterioration due to an increase in right to left shunt and worsening of cyanosis. Moreover, the autotransfusion process, sympathetic activation due to pain, or obstetrical haemorrhage will put more strain on an already compromised heart, leading to increase right-to-left shunt, and worsening of cyanosis.

During pregnancy, the most common cardiac complication was arrhythmias (4,7%) and heart failure (1,6%) in pregnancy with congenital heart disease. Nonetheless, cyanotic heart disease (corrected or uncorrected) had a higher risk of cardiac complications, with OR 3,0 [7]. Many studies indicate that better maternal outcomes can be achieved in patients with uncorrected or palliated TOF or incomplete repair and residual cyanosis. The heart failure rate was higher in an uncorrected group compared to the repaired group (40% vs 2% respectively). Moreover, the uncorrected group's maternal mortality rate was 4-12 times higher (4-12% vs 0%) compared to corrected TOF [7]. On the other hand, all women in our study were at least in DCFC II; hence the heart failure rate was 100%. Other cardiac complications reported were thromboembolic in 12,5% and cardiogenic shock in 12,5%.

Cardiac complications in our study were relatively high. This could be caused by inadequate preconception counselling. A Scoring system to calculate the risk of cardiac complications during pregnancy in CHD women have been developed. If we calculated the risk of these women in pregnancy, we would find at least 7,5% risk of cardiac complication (ZAHARA score) could rise in such patients during pregnancy[7]. Assessment of the risk ideally conducted preconception period, and medication needs can be determined before pregnancy to minimize complications. Preconception counselling included clinical assessment, echocardiography, blood gas analysis, and electrocardiography in determining whether the pregnancy is contraindicated or not and to determine the need for surgery before pregnancy. The decisions should be made by a multidisciplinary team in a tertiary hospital[8].

Our country's problem was a late diagnosis of TOF seemed to cause less optimal management and monitoring of the experts. We missed preconception counselling and regular cardiologist visits as WHO recommends every 1 or 2 months to visit a cardiologist in WHO maternal class III group (uncorrected TOF)[8]. Also, fetal echocardiography should be conducted in the second trimester to screen congenital heart anomaly in the fetus. We had one case where the diagnosis of fetal ASD had not been made during pregnancy. Monitoring of fetal during the third trimester was aimed to screen whether the growth was restricted or not.

There was no consensus on the exact time of delivery for CHD women. Clinical presentation of the patient and fetal well-being were determinant factors to do the termination. Five pregnant women in our study were decided to terminate by CS due to obstetric indication (IUGR). The preferred mode of delivery is vaginal delivery that should take place in a tertiary centre with specialist multidisciplinary team care. Vaginal delivery is associated with lower blood loss and infection rate compared to caesarean delivery. On the other hand, caesarean delivery increases risk of venous thrombosis and thromboembolic, so that in general, CS is performed due to obstetrical indication [8]. It is recommended to use lumbar epidural analgesia during the delivery process. It reduces pain-related elevations of sympathetic activity, reduces the urge to push, and reduces cardiac output 37% during delivery [8], [9].

Previous research concludes that maternal hematocrit is more significant than 60%, oxygen saturation is less than 80%, and haemoglobin level > 18 g/dL were poor prognostic factors. These pregnant women were

associated with adverse fetal outcome [3], [9]. Another study explained that the chance of a pregnancy resulting in a live birth decreases to 12% if oxygen saturation was less than 85% or 8% if haemoglobin level > 20 g/dL [6]. Our study was consistent with the previous study. We had one case of spontaneous abortion whose maternal oxygen saturation was 78%, and haemoglobin level was 19,3 g/dL. The rate of abortions in our study (25%) was similar to the study in India (28,5%) [5]. All abortion cases occurred in uncorrected TOF women.

Some predictors of adverse neonatal outcomes were NYHA class greater than II, cyanosis, maternal left-ventricular obstruction, maternal smoking, maternal age under 20 years or over 35 years, multiple gestations and anticoagulation during pregnancy [10]. Siu *et al.* identified that the rate of neonatal complications has significantly increased in women with heart disease. Neonatal complications occurred in 18% of pregnancies. Preterm delivery occurred in 15%; fetal growth restriction occurred in 4%, respiratory distress syndrome (RDS) or intraventricular haemorrhage in 2% and neonatal death in 3% of pregnancies [11].

The rate of prematurity and small for gestational age (SGA) babies in our study were higher (66,7%), compared to the previous study in India and America, 30% and 40% respectively [3], [5]. The mean birth weight in our study was 1,64 kg. This number was similar to a study in India in an uncorrected TOF group of 1,62 kg. Preterm delivery was higher in our study (83,3%) than in other studies in India (40%). Termination in premature babies was common in our study due to fetal growth restriction antenatal, whereas the causes of prematurity in India had not been explained. A higher number of prematurity may result in a greater incidence of neonatal complication in 5 babies, such as RDS in 60%, necrotizing enterocolitis (NEC) in 20%, and early onset of sepsis (EOS) in 20%.

The risk of congenital heart disease (CHD) in the offspring of women with heart disease is increasing compared to the general population (1%). A previous study suggested various risks in lesion-specific and parents inherited. If the mother had TOF, the risk of CHD increased to 2,6%. Among 5 babies delivered in our hospital, we reported 1 case of ASD in the baby; hence, CHD incidence in our study was 20%. Moreover, previous medication before pregnancy could adversely affect the fetus (e.g., ACE inhibitors, warfarin, and statins). There was no medication before pregnancy in the woman whose baby had congenital heart disease. During hospitalization, she diagnosed with gastric outlet obstruction, and she died on the 7th day of care. Thus, neonatal death was 16,7%.

There is limited data on the effects of pregnancy on long-term outcomes in women with heart disease. One recent study showed a significant increase in sub pulmonary ventricular size persisting after pregnancy in patients with TOF [12]. Unfortunately, TOF patients involved were corrected TOF. There was no further study in the uncorrected group since the TOF patients' surgical intervention had been improved. All the women in our study survived during hospitalization and were discharged in a stable condition. Follow up and a home visit was done for 5 patients, but 2 of them were lost for follow up. The clinical condition of these 5 patients was DCFC II in 4 patients, DCFC I in 1 patient (pink Fallot). We did evaluate the oxygen saturation in every patient, and we found 2 patients had a significant decrease (from 86% to 80% within 4 months, and from 93% to 71% within 2 years). Nonetheless, they all refused to do the further evaluation in our hospital.

Conclusion

Uncorrected TOF leads to many complications that may arise in pregnancy and the outcomes. The most frequent maternal complications are heart failure, cardiogenic shock and thromboembolic, while perinatal complications involve IUGR, SGA and abortion.

What is already known

Proper multidisciplinary management is required for a better pregnancy outcome of the mother and the fetus.

What this paper adds

Knowing that many uncorrected TOF cases were to be found in developing countries, in which may cause many complications to the mother and the fetus, proper diagnosis in early pregnancy during antenatal care and multidisciplinary management are recommended.

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Hypertension Response after Stenting adult with Coarctaion of Aorta One Year Follow Up

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Abstract

Coarctaion of aorta occur in 6 % of patient with congenital heart disease, balloon dilation ,stenting and surgery have been proved as modality of treatment coarctaion of aorta.Usually untreated coarctation of aorta may lead to renal, vascular and cardiac complication that start to appear at binging of fourth decade of life.To knew hypertension response after stenting adult with coarctation of aorta. 75 patients with coarctaion of aorta 30 male (40%) and 45 female (60 %) was involve in this prospective longitudinal study, there age range from 16 to 41 year, for all echocardiography, CT chest done to confirm diagnosis then all undergo catheterization of aorta with 2 sheath one femoral and other radial to measure pressure gradient across stenosis and stent localization follow by stenting with pre and post dilation if needed then measure pressure gradient across stent. **A**ll patients stent by uncover stent including for 2 case with interrupted coarctaion, immediately after stent pressure gradient fall almost all our patient then follow up 6 month, 1 year by angiography assessment with CT chest show no stent fracture or aneurysm in aorta at stent site. The study conclude uncovered stent appear to be safe in treating coarctaion of aorta with less morbidity and mortality. stent adult with coarctaion of aorta have advantage of lowering blood pressure in those suffer from hypertension.

Keywords: *Coarctation of aorta, adult congenital heart disease, hypertension, interventional cardiology*

Introduction

Coarctation of the aorta (CoA), is a congenital disease in which the aorta is tightening, occurs most commonly following to the ductus arteriosus, also, coarctation can be defined as constriction of aorta of different degree that may occur at any part from transverse arch of aorta to iliac bifurcation but most commonly appear just below the beginning of subclavian artery. [1,2] This constriction of aorta may lead to total cut of aorta with fibrous remnant which is called interrupted coarctation of aorta or may lead to just narrowing of aorta which is called un interrupted coarctation of aorta, with variable degree of construction from mild without hemodynamic consequence to severe with hemodynamic consequence and this this usually assessed by invasive hemodynamic measurement.[3] On embryonic base CoA can be classified into post ductal and preductal according to presence of constriction pre or post to subclavian artery.[4-6] Usually untreated coarctation of aorta may lead to renal, vascular and cardiac complication that start to appear at beging of fourth decade of life.[7,8] Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. Blood is carried from the heart to all parts of the body in the vessels. Each time the heart beats[9-13], it pumps blood into the vessels. Hypertension classify according to level of blood pressure measure by sphygmomanometer to:

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Normal: systolic less than 120 mm Hg and diastolic less than 80 mm Hg.

Elevated: systolic between 120-129 mm Hg and diastolic less than 80 mm Hg.

Stage 1: systolic between 130-139 mm Hg or diastolic between 80-89 mm Hg.

Stage 2: systolic at least 140 mm Hg or diastolic at least 90 mm Hg.

Lifestyle changes and medications can lower blood pressure and decrease the risk of health complications. Lifestyle changes include weight loss, physical exercise, decreased salt intake, reducing alcohol intake, and a healthy diet[5] If lifestyle changes are not sufficient then blood pressure medications are used. Up to three medications taken concurrently can control blood pressure in 90% of people. Refractory hypertension is characterized by uncontrolled elevated blood pressure unmitigated by five or more antihypertensive agents of different classes, including a long-acting thiazide-like diuretic, a calcium channel blocker, and a blocker of the renin-angiotensin system. People with refractory hypertension typically have increased sympathetic nervous system activity, and are at high risk for more severe cardiovascular diseases and all-cause mortality. The current study aim to knew hypertension response after stenting adult with coarctation of aorta

Patient and Method

75 patients with coarctation of aorta, all these persons had been selected from those patients visit the cardiology consult department in Al-najaf cardiac center or from private clinic from the period January 2018 to January 2019. For all patient's data collected about age, sex, blood pressure, laboratory finding of glucose, renal function, lipid profile. For all echocardiography study done , and after diagnosed to have coarctaion of aorta, CT chest done to confirm the diagnosis with stenosis of aorta more than 50% , then all undergo catheterization of aorta with 2 sheath one femoral and other radial to measure invasive pressure gradient across the stenosis and if gradient more than 20 mmHg then intervention indicated or according to other indication list mention below. In Cath lab the femoral access done by modify seldenger approach

RADIAL ACCESS DONE BY SELDENGER APPROACH

We use then multipurpose guide catheter with guide wire through femoral sheath to reach coarctation and use pigtail catheter with guide wire through radial sheath to reach coarctation after pass from brachiocephalic artery to descending aorta, at that time pressure gradient through coarctation can measure.If coarctation indicated for intervention then we try to pass guide wire with multipurpose guide catheter through coarctation until reach aortic sinus then exchange with stiff guide wire then replace sheathe with bigger one as balloon and stent need bigger one usually we use 9 Fr size sheath. Localization of stent position by radial contrast injection with fluoroscopy follow by stenting with palmaz uncover stent, pre and post dilation if needed then measure pressure gradient across stent, success of procedure is defined as pressure gradient across coarctation zero mmHg. For all patients invasive blood pressure measurment before and after stenting during catheterization pre and distal to stenosis with follow-up by upper limb blood pressure measurement by sphygmomanometer on both arm in day 1, first month, 6 months and 1 yr. For all patients follow up with CT of chest done after 6 month and 1 yr. for any aortic wall injury or aneurysm develop.

INDICATION FOR INTERVENTION IN COARCTATION OF AORTA PATIENT:

Angiographic indication if invasive blood pressure across the stenosis more than 20 mmHg or stenosis of aorta more than 75%

Inclusion Criteria:

1. Native or recurrent aortic coarctaion

2. pressure gradient across the stenosis invasively more than of 20 mm Hg

3. CT scan of aorta show interrupted or un interrupted aorta

4. Symptomatic patient with invasive pressure gradient > 20 mmHg across the stenosis.

Exclusion Criteria:

1. Control blood pressure in pregnancy

2. Aneurysm of aorta fit for surgery.

3. Stent coarctation previously

4. Invasive pressure gradient < 20 mmHg across the stenosis.

5. Patient refusal

Statistical Analysis

For continuous variables we use mean, standard deviation as descriptive statistics, for categorical variables we use number, percentage, as descriptive statistics, for analysis we use paired t test for continuous variables and chi square for categorical variables. All calculations done by EXCEL Microsoft 2016 software.

Result

Over 12 month's period of the study, 75 patients with coarctation of aorta were assessed including 45(60%) women and 30 (40%) men. The age range was 16 years to 41 years with the mean age 25.7 years (SD ±6.6) as seen in (table 1), after diagnosis of coarctation all those patients enrolled in this research then invasive blood pressure measured in descending and ascending aorta before and after intervention done with pressure gradient across the stenosis to determine indication and success of intervention , no death or complication happened during intervention, after stent implantation sudden elevation of blood pressure seen in 5 patients (15%) , almost all patients experienced an immediate reduction in mean systolic blood pressure pre coarctation (from 159.80±20.5 to 120.87±31 mm Hg), (95% confidence interval of this difference From 30.5 to 47.4) (P value is less than 0.0001) as seen in (table 2).

At the 6-month follow up we found that 88.4% get better blood pressure control (no need for antihypertensive medication with normal blood pressure reading on measurement by sphygmomanometer on both arm) also became at same improvement at the 1-year checkup visit at which 84.9% control blood pressure (no need for antihypertensive medication with normal blood pressure reading on measurement by sphygmomanometer on both arm) as seen in table 3. 69 patients (92% of those who had a stent implanted) returned for the 6-month follow-up evaluation, 53 patients (70% of those who had a stent implanted) returned for the 1-year follow-up evaluation , at 6 month and 1 year for patient return for follow up CT angiography done in 39/69 patients (52% of those who had a stent implanted) at 6 month and in 18/45 patients (24% of those who had a stent implanted) at 1 year show no aortic wall injury or aneurysm development , and at 1 yr. follow-up no mortality occur as seen in Algorithm 1.

Table 1 demographic data

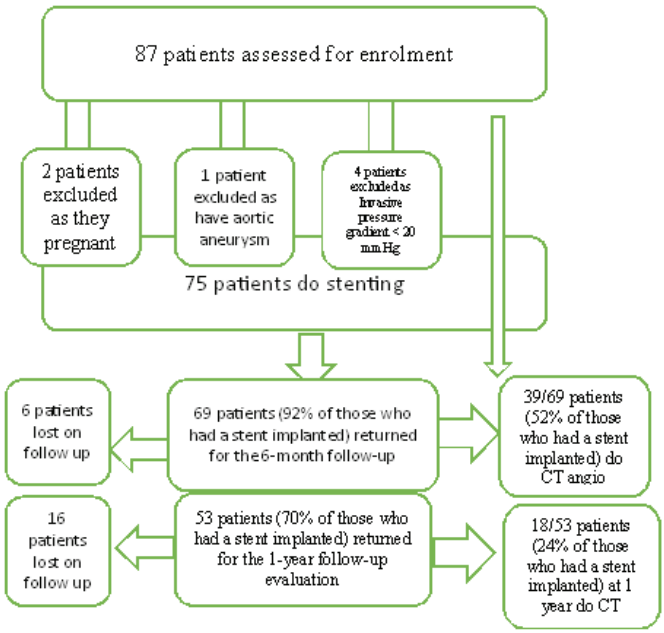
All patients	**75 patients**	
sex	30 Male (40%)	45 Female (60%)
Age(year)	16-41	25.7 years (SD ±6.6)
hypertension	75 patients	
diabetic	3 patients	
smoker	5 patients	
Previous surgical correction	0	
Interrupted CoA	2	
Previous stenting	0	
Associated bicuspid aortic valve	51 patients	
Renal impairment	0	
stroke	1	
Left ventricular hypertrophy	59 patients	

Table 2 comparison in invasive systolic blood pressure measurement by catheterization before and immediately after stenting in patient's underwent intervention for CoA

Blood pressure	Before stenting(n=75)	After stenting (n=75)	
Invasive systolic blood pressure (mmHg) proximal to coarctation area	159.80± (20.5)	120.87± (31)	P value < 0.0001
Invasive systolic blood pressure (mmHg) distal to coarctation area	97 ± (14.6)	121.5 ± (24.7)	P value < 0.0001
Average difference in invasive systolic blood pressure proximal and distal to coarctation area (pressure gradient)	38 ± (12)	2 ± (3)	P value < 0.0001

Table 3 comparison between blood pressure readings immediately, 6 month and 1 yr. in patient's underwent intervention for CoA

No. of patients On follow up Blood Pressure Measurement (mmHg)	Immediately Post intervention	6 months follow up	1 year follow up	Sig. Value
Normotensive	74(98.7%)	61 (88.4%)	45 (84.9%)	0.0001
Hypertensive	1(1.3%)	8 (11.6%)	8 (15.1%)	
Total	75(100%)	69(92%)	53(70%)	



Algorithm 1 patients flow diagram in patient's underwent intervention for CoA

Discussion

As hypertension has genetic and environmental risk factor, patient with coarctation carry both these factors. [14-18]. Untreated hypertension can lead to significant mortality and morbidity so management is vital for survival. [19]. Our research focuses on the benefit of reduced blood pressure by treating coarctation of aorta by stenting, only 15 % of patients enrolled in research persist to have hypertension after 1 year follow up while 85 % of patients get rid of hypertension and this in concordance with COSTA 2 trail [20-25]. One observation in this research is that most patients immediately after stenting of COA get improvement in blood pressure then after few week start to elevated but generally after 1 year follow up more than 2/3 of patients have controlled blood pressure reading with or without medication .

Conclusion

Uncovered stent appears to be safe in treating coarctaion of aorta with less morbidity and mortality. stent adult with coarctaion of aorta have advantage of lowering blood pressure in those suffer from hypertension.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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Survey Study for Causative Agents of Meningitis Patient in Babylon Province

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Abstract

One of the most dangerous infections that cause elevated morbidity and mortality rates is meningitis. Meningitis in children is one of the life threatening condition in pediatric age group, which needs proper and early diagnosis in the emergency department. According to findings and clinical diagnosis (35.3%cases) considered bacterial while (64.7% cases) considered non bacterial meningitis. Most rate of infection of meningitis occurred in 2017 (38.3%),Male sex, Age< 1 year with a higher prevalence of meningitis. We concluded that Bacterial meningitis are still occupying large percentage of cases admitted to hospital, and if un-diagnosed promptly will have a high mortality.

Key words : *meningitis, survey,epidemiology, Babylon*

Introduction

Meningitis is a subarachnoid space infection It appears to be a significant source of morbidity and mortality caused by a variety of pathogenic organisms[1]. It may have infectious causes, such as, or may be infected with, bacteria, mycobacteria, viruses, fungi, or parasites. drug reactions, cancer, or autoimmunity[2]. Bacterial meningitis are considered as one of the most serious infection in infant and children, Which, if not diagnosed and treated early, causes multiple complications and high mortality.. Early diagnosis, therefore,, early implementation Appropriate and important antimicrobial Therapy is important to prevent further treatment. complication untreated bacterial meningitis is highly lethal, leaving patients with serious neurological sequelae and impairing the lives of children[3,4]. Analysis of the CSF is critical to the diagnosis of meningitis and the Bacterial distinction from other etiologies., Other indications for CSF research include Guillain Barré syndrome diagnosis, other demyelinating mechanisms, and, diagnosing Care, and pseudo tumor cerebral[5]. Acute bacterial meningitis is serious with a high rate of fatality[6]. Viral and bacterial meningitis can put a tremendous strain on families, cultures, and communities[7]. While recurrent intellectual disability Often has been linked with bacterial meningitis, Some viral pathogens have also been identified, such as parechovirus, linked to impaired developmental achievement[8]. For this reason bacterial meningitis needs empiric treatment with appropriate potent antibiotics to avoid fatality. The empirical antibiotic regimens should be updated periodically to overcome the development of antimicrobial resistance. The combination of vancomycin with either ceftriaxone for adults or cefotaxime for pediatric ages is given empirically for those with suspected bacterial meningitis, based upon susceptibilities of isolated pathogens[9,10].

This study was carried out to describe the epidemiology of different types of meningitis and variables affecting the outcome.

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Methodology

A retrospective study carried out in Babylon, Iraq Babylon hospital for Maternity and Children from January 2016 to December 2018. Total sample 461pateint , The short-form was designed to record the personal information of the study sample as age(below>1 year,1-2,2-3,3-4,4-5,5-6,>6 yr) gender (male or female), The distribution of patients according year of infection , distribution of patients according cause of meningitis including viral or bacterial.

Results and Discussion

The results show that the incidence of **meningitis** in relation population was found to be different between reported age groups. Maximum group noted in age (low one year) at (49.5%) and minimum group note in age of (3-4 years)at(4.8%) just mention in figure one

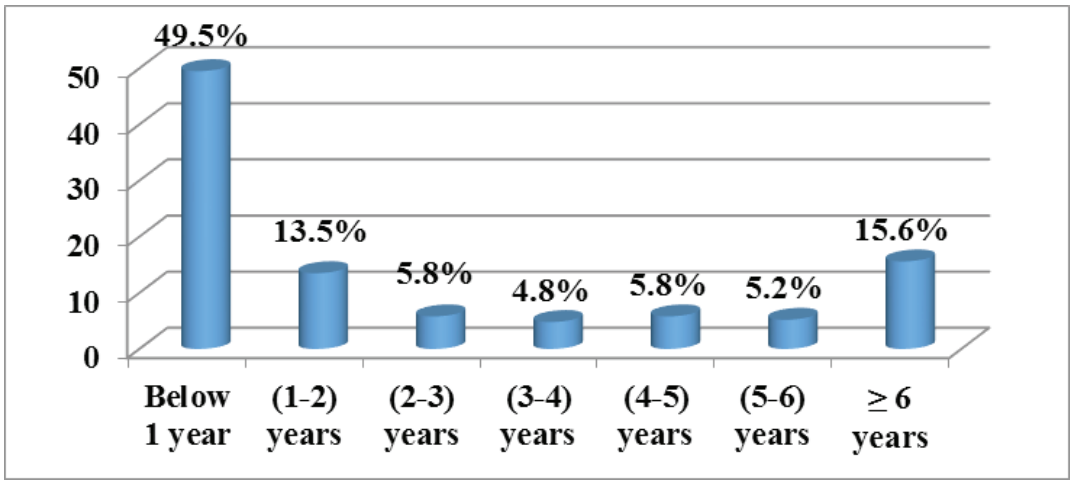

Figure 1: The distribution of patients according age

The incidence of neonatal meningitis is approximately (n=462). in age(low one year) at (49.5%) Compared to the global incidence of meningococcal in Iraqi children and increased to (15.6 percent) in the age group > 6 years , in addition to the possible emergence of new serogroups and the potential increase in the incidence of this bacterium, which needs more responsive techniques[11] .our results showed Age <1 year was found high infection with meningitis Some viral pathogens have also been identified, such as parechovirus[12]. prevalence of meningities was more (59.6%) in the age group of 2 months to 12 months of age in accordance with study by [13] Prevalence rate amongst below one-year age group has been reported as 77%, 65% and 75% and 61% by various authors.

Meningitis is caused by a large variety of bacteria[14]. The results exhibited distribution of patients according gender. Majority (64.5%) of patients was male. (n=462),while 36.5%) was female.

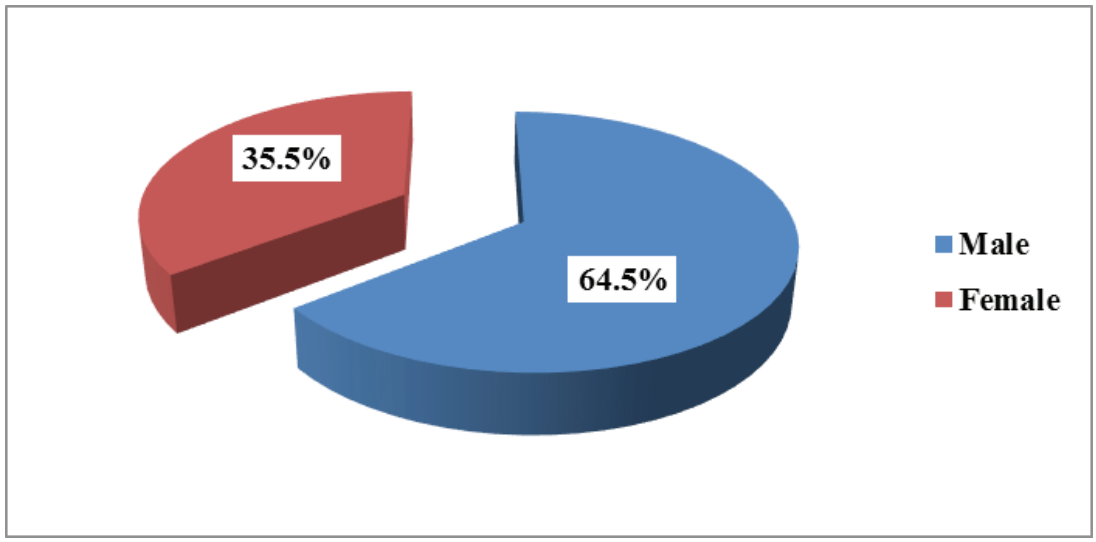

Figure 2: The distribution of patients according gender

The current results semi agreed with study of [15] who found that that 65 (68%) of cases were male, while 31 (32%) of cases were female in the ratio of (2:1). A male preponderance of 1.27:1,1.2:1 and 1.07 was also seen in other studies conducted in Iran/Tehran metropolitan area with a 2:1 ratio,(Mosavi-Jarrahietal,2009)[16] and other research respectively, There are poorly understood reasons underlying these sex differences, but a hypothetical model It has been designed to increase vulnerability to infectious diseases. Diseases such as relative immune deficiency[17].

The research results agreed also with (Mahmood, 2019)[18] who find that 73 patients (62.4 percent) were male and 44 patients (37.6 percent) were female with a male to female ratio of 1.65:1. No important prognostic factor P value 0.233 was found with regard to the prognostic factors male gender. Kooman *et al* discovered an important prognostic factor P value of 0.018[12].

The results of present study explained the distribution of patients according year of infection. Majority (38.3%) of patients infected during 2017. (n=462).

The rate of infection It is estimated that meningitis has fallen globally by 21.0 percent from 1990 to 2016[19]. This indicates that the development of meningitis will be much faster[20]. The present study exhibited also the distribution of patients according month of infection. Majority (13.9% and 13.4%) of patients infected during august and October respectively. (n=462).

Interestingly, throughout the seasonal period, an overall latitudinal trend could be seen, but latitudinal differences preclude a full quantification of this pattern. Second, the seasonal timing of bacterial meningitis was between February and March, during the dry season, in all African meningitis belt countries (between-5 ° N and 20 ° N).. Third, the incidence of disease caused by any of the pathogens peaked in countries below-20 ° N (South America, Oceania, and South Africa) from June to July (winter season)[21].

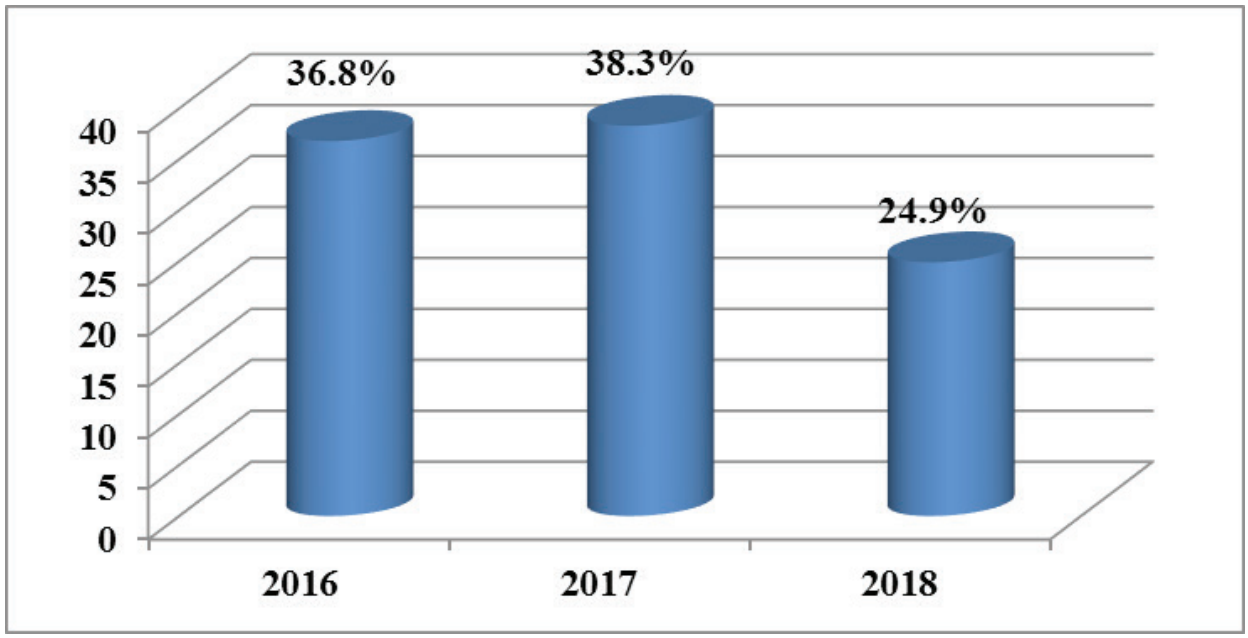

Figure 3: The distribution of patients according year of infection

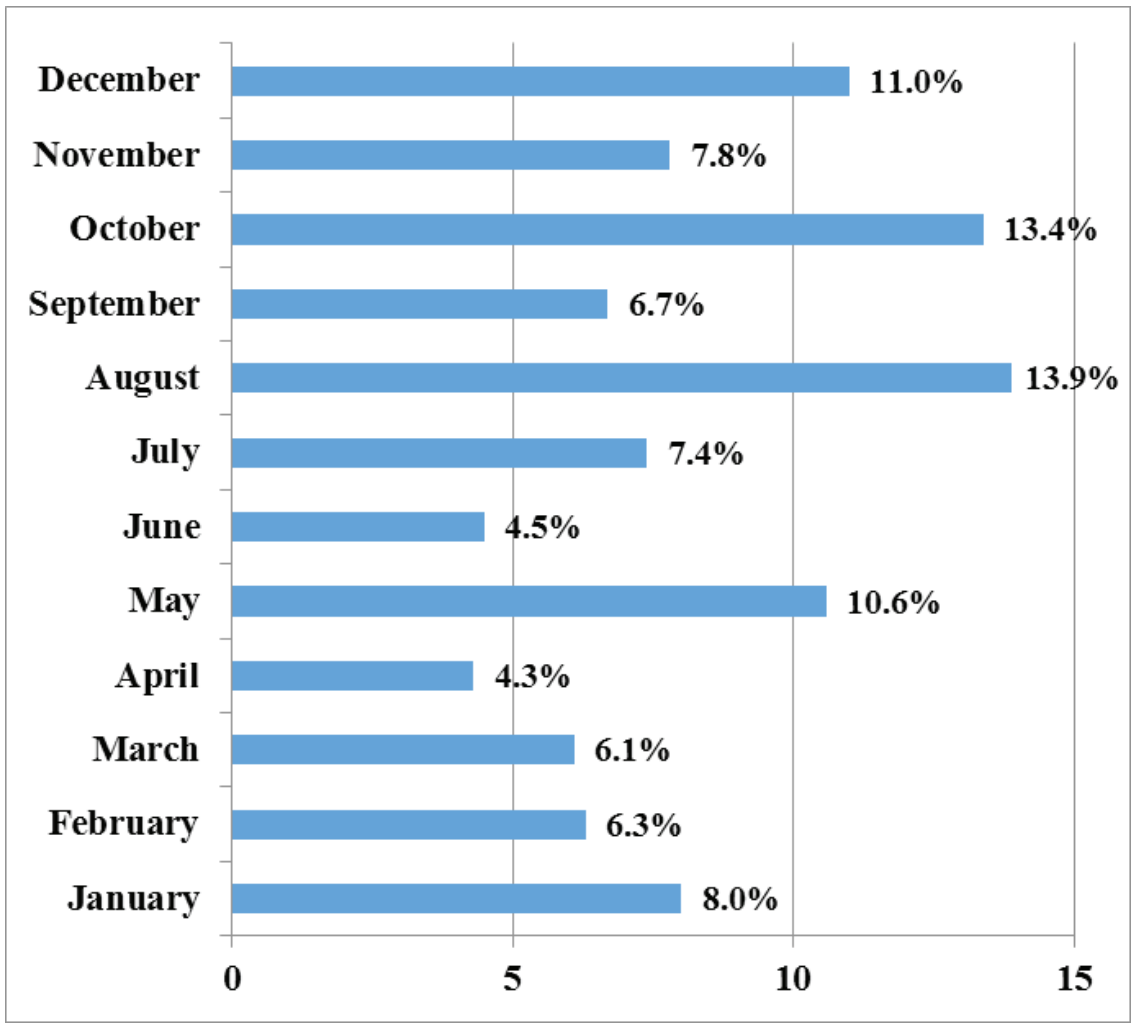

Figure 4: The distribution of patients according month of infection

To encourage the awareness of global seasonal trends in bacterial meningitis, we performed an observational, time-series study[22]. When considering the paradigm of the host-pathogen-environment and recognizing the multipart and nuanced collaboration Among these variables, we may be able to hypothesize A possible association between climate and the seasonality of bacterial meningitis over a wide geographical range is indicated by the latitudinal pattern[23].

The results of present study show the distribution of patients according cause of meningitis including viral or bacterial. Viral meningitis represent (64.7%) of patients and link between cause of meningitis and study variables including (age, gender, and year of infection and months of infection).just mentions in figure 5 and table 1 below

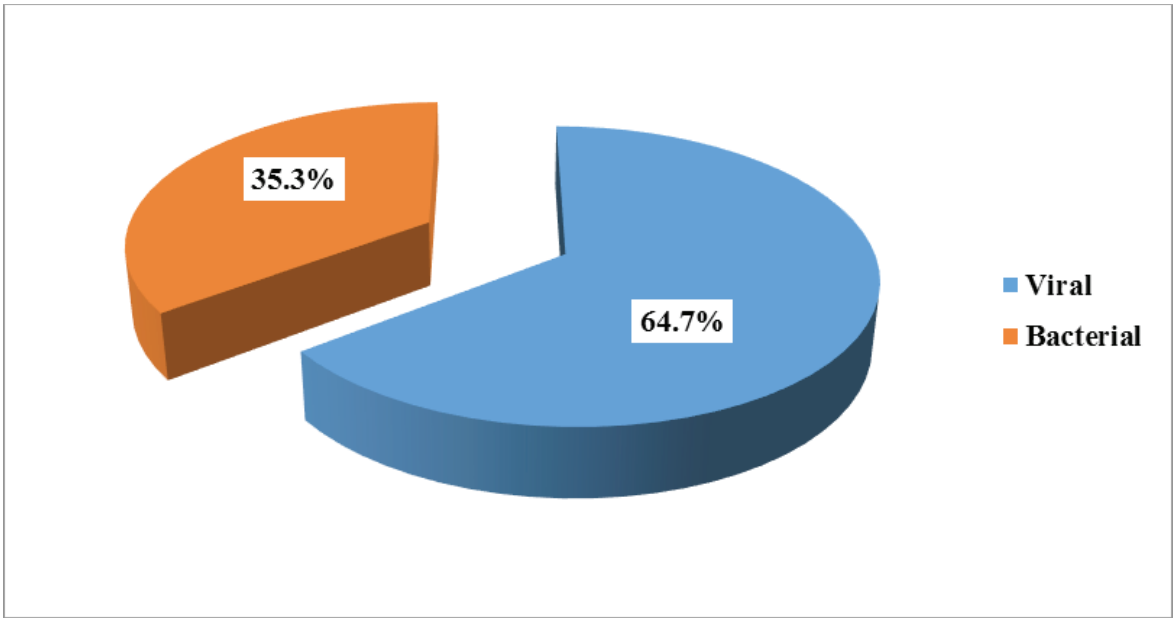

Figure 5: The distribution of patients according cause of meningitis

Table 1: The association between cause of meningitis and study variables

Study variables	Cause of meningitis		Total	X2	P-value
	Viral	Bacterial			
Age					
Below 1 year	148 (49.6)	80 (49.1)	288 (49.4)	2.819	0.42
(1-3) years	62 (20.7)	27 (16.6)	89 (19.3)		
(3-5) years	33 (11.0)	16 (9.8)	49 (10.6)		
≥ 5 years	56 (18.7)	40 (24.5)	96 (20.7)		
Total	299 (100.0)	163 (100.0)	462 (100.0)		
Gender					
Male	187 (62.5)	111 (68.1)	298 (64.5)	1.422	0.233
Female	112 (37.5)	52 (31.9)	164 (35.5)		
Total	299 (100.0)	163 (100.0)	462 (100.0)		

Cont... **Table 1: The association between cause of meningitis and study variables**

Year of infection					
2016	102 (34.1)	68 (41.7)	170 (36.8)		
2017	123 (41.1)	54(33.1)	177(38.3)	3.431	0.18
2018	74 (24.8)	41 (25.2)	115 (24.9)		
Total	299 (100.0)	163 (100.0)	462 (100.0)		
Month of infection					
1-3	57 (19.1)	37 (22.7)	94 (20.3)		
4-6	52 (17.3)	38 (23.3)	90 (19.5)	5.876	0.118
7-9	83 (27.8)	46(28.2)	129(27.9)		
10-12	107 (35.8)	42 (25.8)	149 (32.3)		
Total	299 (100.0)	163 (100.0)	462 (100.0)		

*p value ≤ 0.05 was significant.

In this study, (64.7%) Compared to (35.3 %) of cases diagnosed as bacterial meningitis, this slight rise in the number of incidences of bacterial meningitis agrees with the diagnosis of non-bacterial meningitis. Several reports written on childhood meningitis from almost every region of Saudi Arabia.(Abomelha *etal*,1988) [24] Another study done in Egypt. Bacterial meningitis accounted for 73.3 % of the 623 cases of meningitis, compared with 26.7 % of non-bacterial meningitis in the studied population[25].

While a higher ratio of non-bacterial meningitis (87.34%) seen in another study done in Pittsburgh by Negrini et al who studied one hundred fifty-eight cases of meningitis[26]. Compared to viral meningitis in other countries, a high percentage of bacterial meningitis in some countries is multifactorial, but a vaccination program is the most likely cause. There are successful vaccinations against the three main bacterial pathogens responsible for bacterial meningitis, *Streptococcus pneumonia, Haemophilus influenza,* and *Neisseria meningitis*[27]. Other study revealed that non bacterial meningitis (74.5%) While bacterial meningitis (36.7 %). In our country, service of meningitis vaccine is often not available, and also the low socioeconomic state is regarded as a risk factor for increasing bacterial infection.

Conclusion

Bacterial meningitis are still occupying large percentage of cases admitted to hospital, and if un-diagnosed promptly will have a high mortality.

Study limitation

The limitation of this study includes size of samples and other limitation not using advance technique such as PCR or For detecting N, RT-PCR.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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The Effect of Enhanced External Counter Pulsation on Improving Quality of Life in Patient with 3 Vessels Disease Treated Medically Versus Percutaneous Coronary Intervention, 1 Year Follow Up

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Abstract

Background : Refractory angina usually refers to as (equal or more than 3 months in duration) stable ischemic heart disease which cannot be regulated by a therapeutic treatment, surgical cardiac intervention, and where reversible cardiac ischemia has been stated to be the reason for the manifestation clinically.Aim: The efficacy of use enhanced external counter pulsation in treating refractory angina pectoris in those do PCI vs. medical alone and short-term outcome. PMethodology: 91 patients with refractory angina pectoris un respond to treatment and/or intervention or unfit for intervention or surgery have been enrolled in this study, for all ECG, echocardiography study done to assess LV and valvular function, Doppler study for lower limb artery to assess if there is peripheral vascular disease, abdominal ultrasound done to exclude the presence of aortic abdominal aneurysm, then if patient eligible for EECP inclusion criteria refer for EECP unit .After end of all session all patients assess improvement according to change in Canadian cardiovascular society grading CCS pre and post EECP and on follow up 6 month and 1 year. Result: Over 12 month's period of the study, 91 persons were assessed including 32(35%) women and 59 (65%) men. The age range was 45 years to 80 years with the mean age range 61 years (SD ± 8.2). Most patients treated with 30 session with 1 hour per session on average of 26 (SD ± 7) session with response rate at end of sessions 88.7% where response mean improvement of symptom and according to (CCS) change per and post EECP.

Conclusion: No befit from doing single vessel intervention in patient with 3 vessels disease in improving response to EECP or improving echocardiographic parameter. EECP appear as safe effective therapy in selective patient with refractory angina not respond to medical and unfit for intervention or surgery. Response to EECP in well selected patient persist for 1 year.

Keywords: *enhanced external counter pulsation, adult coronary heart disease, interventional cardiology.*

Introduction

Refractory angina usually refers to as (equal or more than 3 months in duration) stable ischemic heart disease which cannot be regulated by a therapeutic treatment, surgical cardiac intervention, and where reversible cardiac ischemia has been stated to be the reason for the manifestation clinically.[1] External Enhanced Counter pulsation is non-invasive therapy include putting external compressive sleeves for the calves, upper and lower thighs followed by consecutively blowing up them with beginning them from distally to proximally with time of heart cycle. Transforming an impact resemble to that

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produced from an intra-aortic balloon pump, the sleeves are blow nearby beginning of diastole to increase venous return furthermore coronary perfusion, and discharged in systole to diminish fundamental vascular opposition, improving cardiac workload and systemic perfusion. Therapy is done 1 to 2-hour sessions over a several weeks which approximate to 35 hours totally. The action mechanism by which counter pulsation has been exhibits to enhance the function of endothelium,[2-6] to diminish vascular stiffness [7-9] and to flow of friction reserve and collateral blood flow,[10,11] as well as promotion of peripheral vascular extension caused by blood flow, and additionally influencing endothelial determined vasoactive factors by decreasing proinflammatory cytokines and induction turnover of nitric oxide.[2,3,9]

The Worldwide EECP tolerant Registry explored cardiac failure patients (who with ejection fraction equal or less than 35 percent) accompanied with refractory angina furthermore detected upgrades for angina population, decreasing nitroglycerin utilization and life quality later termination of therapy. These impacts were preserved in a considerable ratio of patients after following up for three years.[10,12,13]. However, there is a bulk of studies of EECP have examined its using of EECP in cases of stable angina instead of refractory angina and, due to the intervention nature, EECP is restricted by a sum of contraindications which may include coagulation disorders with an international normal ratio of more than 2.5, severe chronic obstructive lung disease, cardiac arrhythmias, venous disease, severe peripheral arterial disease.[11,6]. However, EECP has been revealed reduction of hospitalization fees that will serve to encourage EECP adoption going forward. [14]

Equivalent to the progresses in cardiovascular carefulness, there is a growing of patients amount, mainly individuals with progressive coronary artery sickness, carry extreme manifestations caused by angina pectoris that not respond for ideal therapeutic treatment. [15]. The modern treatments for refractory angina include medications (such as Ivabradine and Ranolazine), noninvasive treatment compromise enhanced external counter pulsation (EECP) and extracorporeal shockwave cure, invasive therapy, neuromodulation, and others. [16,17] As indicated nowadays scientific revisions, noninvasive EECP has been confirmed to be a hopeful therapy for angina relieve caused by firm coronary artery illnesses.[18]. EECP method, a noninvasive and nonpharmacological outpatient management, has been accepted by the Food and Drug Administration (FDA) in united states in patients with unstable or stable angina pectoris, congestive cardiac failure, cardiogenic trauma and intense myocardial infraction.[19]. Nowadays the EECP technique is utilized for angina patients unqualified for routine medical treatment who are not hopeful for further revascularization methods and those desired to suspend invasive therapy.

A lot of trials have confirmed that EECP lessened symptoms of angina by protracted total occlusions,[20,21] advanced satisfaction of life quality,[21,22] improved exercise tolerance 21-24 and also increased the time to training provoked depression of ST-segment[23-25] and to enhance perfusion of myocardium.[23,24]. The instrument underlying the profit of EECP-derived is under assessment, which may be included the possible mechanisms include endothelial function enhancement, improvement of collateralization, atherosclerosis declining, and peripheral ‘‘training impacts ’’ resemble for exercising.[19] The intense hemodynamic impact resulted shear pressure that may cause enhancement of production of powerful elements of vasodilatation which include nitric oxide and prostacyclin from endothelial cells.[26]

Several studies approved that there was increased quantity of nitric oxide and reduction the of brain natriuretic peptide and serum enthelin-1 concentration following EECP treatment.[24,26]. Sessa et al.[27] established that prolonged practicing of exercise stimulates synthesis of nitric oxide gene expression and production of coronary nitric oxide in model of dogs. This procedure may have been started following EECP with enriching their training tolerance. Masuda et al. exhibited an increment in both resting furthermore post practice perfusion which may be enhance indirectly through creation preexist collaterals or opening new ones by expanded trans myocardial stress gradients throughout EECP.[28,29]. EECP-enhanced promotions in periphery of endothelial function furthermore rigidity of arteries should lead to an important decrease in left ventricular afterload and thusly, a decrease in oxygen gas needing of myocardial tissue. We have achieved 2 revisions exploring whether these EECP-induced

decreases in afterload of left ventricle are definitely connected with perfection in keys of myocardial oxygen request in coronary artery disorders patients with manifestations of chronic cardiac ischemia.[30]. the current study aim to study the efficacy of use enhanced external counter pulsation in treating refractory angina pectoris in those do PCI vs. medical alone and short-term outcome

Patient and Method

518 patients with refractory angina pectoris un respond to treatment and/or intervention or unfit for intervention or surgery have been selected to enter in this study, all these persons had been selected from those patients visit the cardiology consult department in Al-najaf cardiac center or from private clinic from the period January 2018 to December 2019, all patients how been selected to enter in this study undergo coronary angiography through femoral access and those with 3 vessels diseased patients where significant coronary stenosis (> 70 %) in each vessel (by opinion of two specialist) have been enrolled in this study and the patients divided in two group , first group those with 3 vessels disease and unfit for intervention or surgery ,second group those with 3 vessels and can do percutaneous coronary intervention for one or two vessels then those patients do PCI reassess for ischemia symptom after 6 weeks and only patients with persistent ischemic symptomatic post PCI enrolled in this study where those improve symptom after PCI excluded from study ,at the end only 91 patients where enrolled in this study including 48 patients in first group and 43 patients in second group , both group refer to EECP after 8 week from coronary angiography.

All patients treated according to guidelines and refer to EECP according to it, from all patients agree to enter in this research counsel have been taken. For all patient's data collected about age, sex, blood pressure, laboratory finding of glucose, renal function, complete blood count have been done.This patients includes 59 male and 32 female was involve in this prospective, randomized nonblind control trail , there age range from 45 to 80 year, symptomatic angina despite medication and/or intervention, for all ECG, echocardiography study done to assess LV and valvular function, Doppler study for lower limb artery to assess if there is peripheral vascular disease, abdominal ultrasound done to exclude the presence of aortic abdominal aneurysm, then if patient eligible for EECP inclusion criteria refer for EECP unit.

After end of all sessions all patients assess improvement according to change in Canadian cardiovascular society grading CCS pre and post EECP and do echocardiographic examination on follow up 6 month and 1 year, at follow up by clinical visit or telephone attachment , 31 patients miss contact and 3 patients die at period 2-8 months after end of session and 4 patients no tolerated session so stop prematurely and excluded from data analysis and only 53 patient complete 1 year follow up as seen in algorithm 1. In EECP unit we have 20 EECP device (vasomedical, Inc., Westbury, New York) each one compose of an air compressor that attach to pneumatic cuffs and control by computer system, also each device have figure plethysmography for monitor systolic and diastolic pulse wave and transmitted to computer, the pneumatic cuff applied to lower limb and inflated sequentially during diastole with pressure 250-300 mmHg for few second by computer control which detect diastole by ECG or figure plethysmography monitor, each session duration 1 hour and most patient's should complete 30 session if tolerated , before assessment for response and the need for extension of session.

Inclusion Criteria:

Any patient with refractory angina pectoris unrespond to medication and / or intervention or unfit for intervention / surgery.

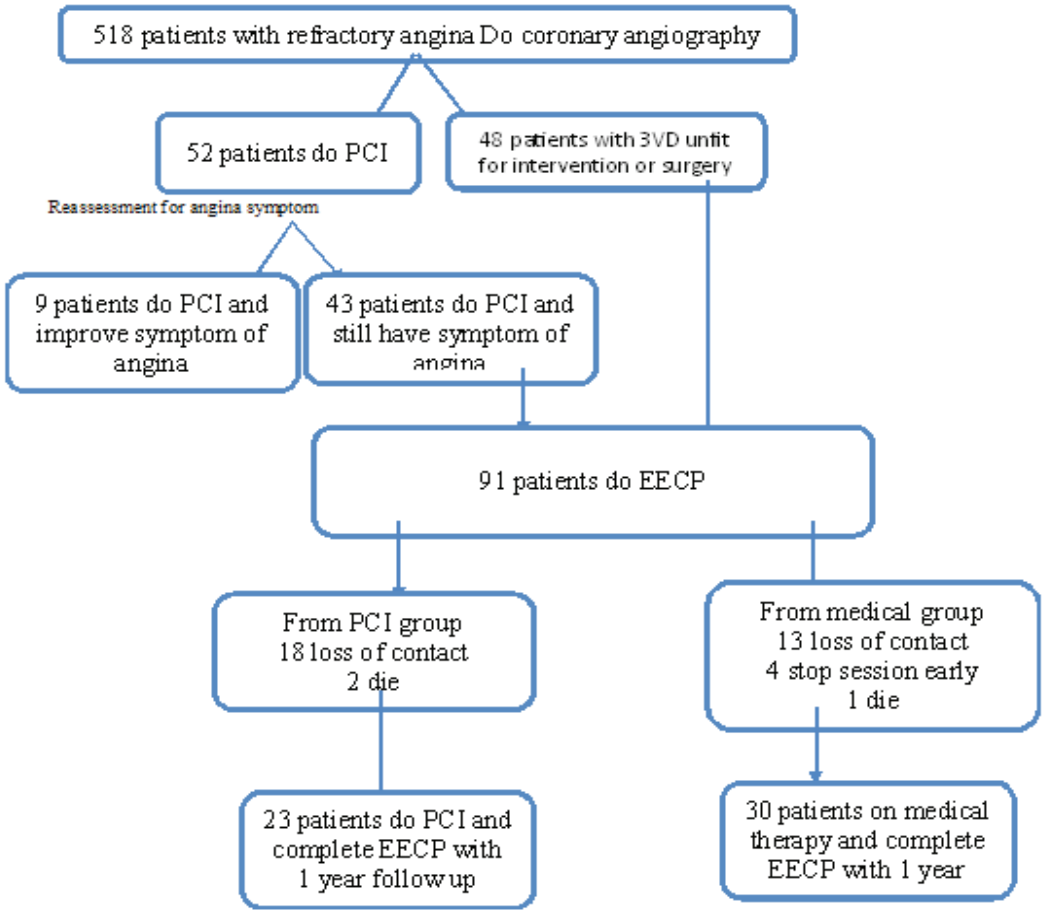
Exclusion criteria:

1. Severe peripheral vascular diseased
2. Abdominal aortic aneurysm
3. Severe aortic regurgitation
4. Uncontrolled hypertension
5. Uncontrolled congestive heart failure
6. Within 2 week from angiography from femoral access
7. Thrombocytopenia and bleeding tendency
8. Deep vein thrombosis

9. Significant varicose vein

Statistical Analysis

We use paired student t test and chi square test for both continuous variables and categorical variables, p value < 0.05 was regards as statistical significance.We use mean, with stander deviation to describe categorical variables and t test for continuous variables as blood pressure before and after intervention. Calculations done by EXCEL Microsoft 2016 software.



Algorithm 1 patient selection for study and 1 year follow up

Results

Over 12 month's period of the study, 91 persons were assessed including 32(35%) women and 59 (65%) men. The age range was 45 years to 80 years with the mean age range 61 years (SD ± 8.2), demographic data seen in (table 1). Most patients treated with 30 session with 1 hour per session on average of 26 (SD ± 7) session with response rate at end of sessions 88.7% where response mean improvement of symptom and according to (CCS) change per and post EECP. There is no satatistacaly diferance . We found no deference between PCI group and angiography only group in responding to EECP or improvement on echocardiographic parameter as it is not statistically significant (p-value = 0. 87 , p-value = 0.47) as seen in (figure 1 A and B). The response to EECP was not statistically (p-value = 0.185) affected by sex as seen in (figure 2A). The smoking not statistically (p-value = 0.67) affect the response of patients to EECP as seen in (figure 2B). The age appear as not statistically significant (p-value < 0.26) in response rate of patients

to EECP as seen in (figure 3). Hypertension appear to be non-effluence in response patients to EECP as it not statistically significant (p-value = 0.4) as seen in (figure 4A). Surprisingly that diabetes observe as no influence in response to EECP as it not statistically significant (p-value = 0.12) as seen in (figure 4B).

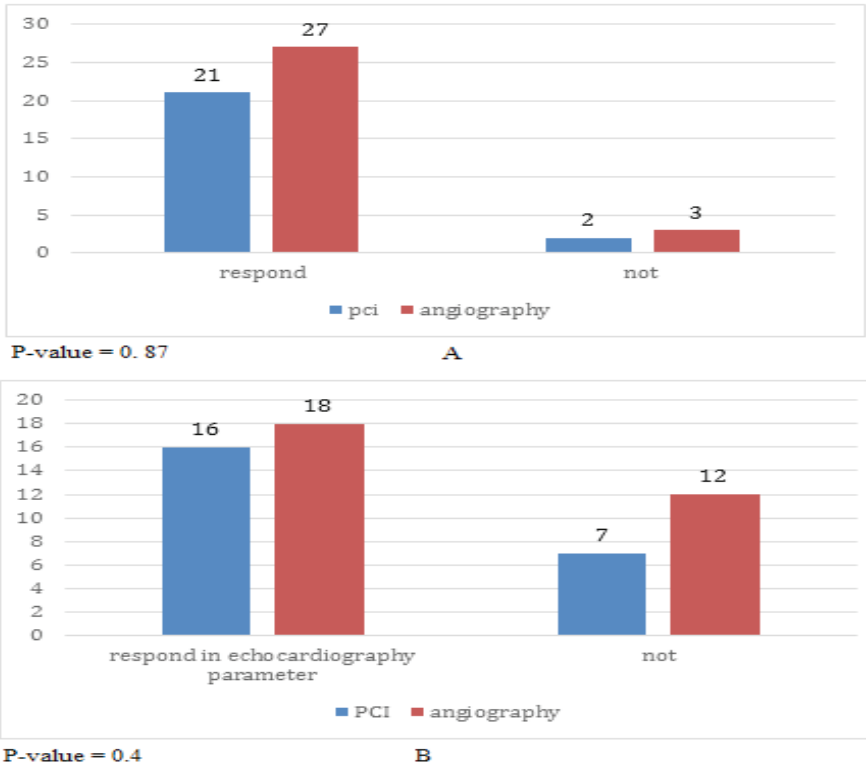


Figure (1): A):relation of PCI group and angiography only group in response to EECP. B):relation of PCI group and angiography only group in response to echocardiography parameter

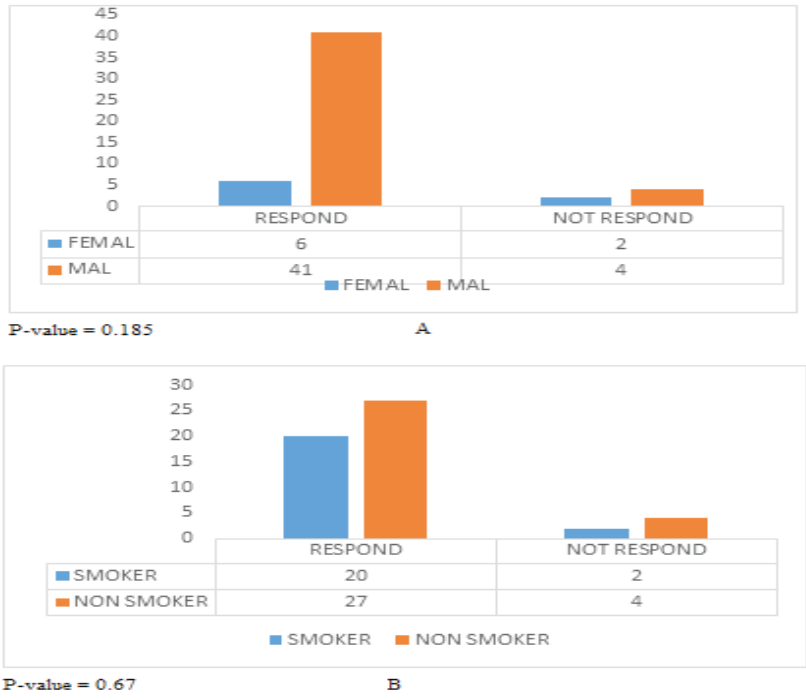
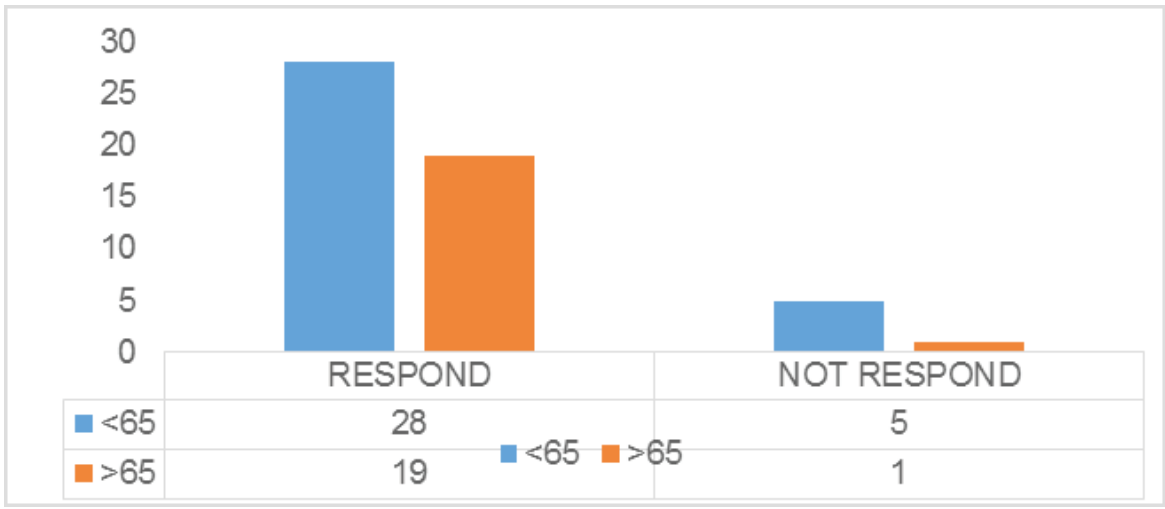
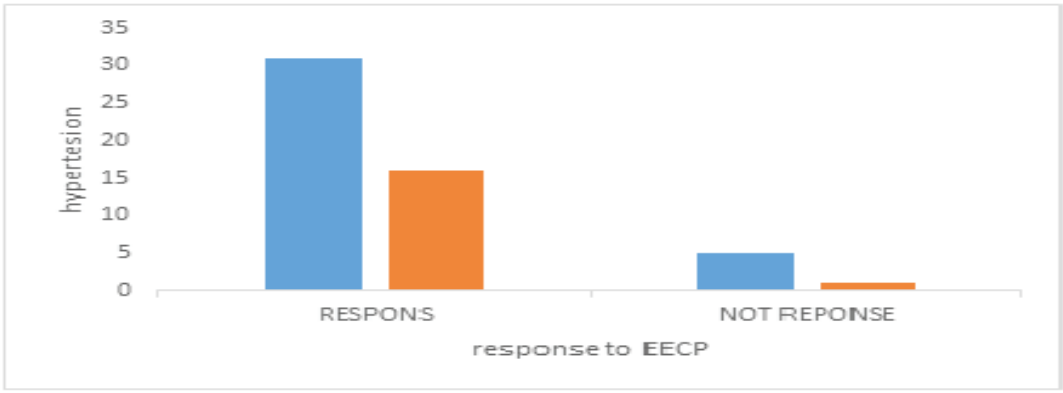


Figure (2): A):sex and response to EECP. B): smoker response to EECP

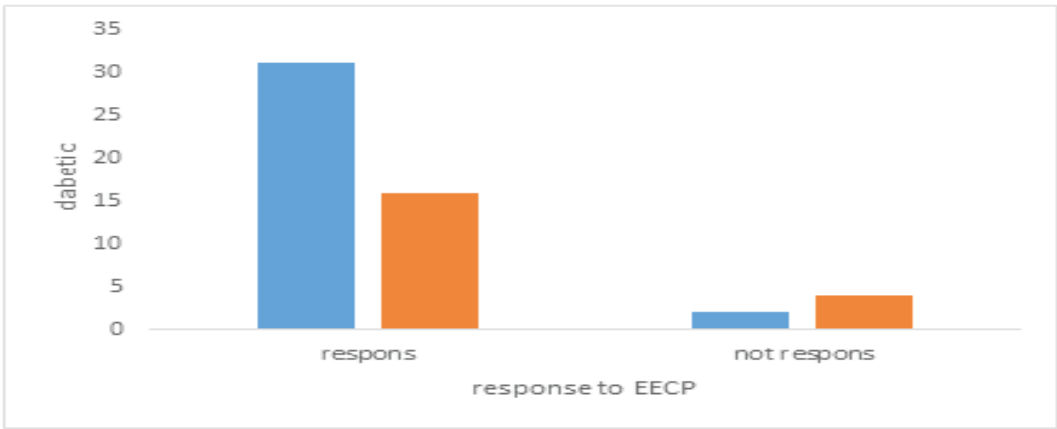


P-value < 0.26

Figure(3): age and response to EECP



P-value < 0.4 **A**



P-value < 0.12 **B**

Figure(4): A):hypertension and response to EECP. B): diabetic and response to EECP

Table 1 demographic data

sex	Male (59)	Female(32)
age	45-80	
No of session	26.1(SD ± 8.2)	
hypertension	36	
diabetic	33	
smoker	22	
Do angiography	50	
Do PCI	24	
Respond to EECP	47	
death	3	
complicated session	4	

Discussion

Most patients in this research have diffuse coronary disease confirmed by angiography some do intervention and other was beyond or unfit for it, so as they with refractory angina and unreasoned to medication they referred to EECP and in this research we found dramatic symptomatic response to this type of therapy specially that most patient was in CCS3 and CCS4 .After the end of EECP sessions most patients improve in their symptom from CCS3 and CCS4 to CCS1 and CCS2 and this in concomitant with MUST-EECP[31].Also we observe that this improvement in symptom persist in follow up till the end of year of follow up in majority of patients and this in concomitant with other research [32-34] and most patients with CCS4 get response that no patient in 1 year follow up enter this stage as most patients remain in CCS2 and to lesser extend in CCS3 [35], and that why we found dramatic improve in quality of life in our patients.Decrease in symptom (CCS) may appear without increment in myocardial perfusion, and the initial advantage of this therapy can related to secondary action comparable to those of physical rehabltaion.[36] The mode of action of persist long-term benefits are multi-factorial and include refinement in the endothelial function, angiogenesis, exercise training effect, and neurohormonal modulation.[37,38].The International EECP Patient Registry (IEPR)[39] reported sustained improvement in angina and quality of life in the majority of patients over a 2-year period, an observational study has reported that the benefits may be sustained for 5 years.[40]. In this research we found no deference in response for EECP and in echocardiographic parameter in those how do PCI or not after angiographic confirm 3 vessel disease. In our research we observe that diabetic and nondiabetic patients show significant refinement in symptom and quality of life with after EECP therapy as observe by other research [41,42].

Conclusion

No befit from doing single vessel intervention in patient with 3 vessels disease in improving response to EECP or improving echocardiographic parameter.

EECP appear as safe effective therapy in selective patient with refractory angina not respond to medical and unfit for intervention or surgery. Response to EECP in well selected patient persist for 1 year

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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Reducing of Hydrogen Peroxide Effects by Pomegranate Peel Extract in Vivo, in Female Wistar Rats

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Abstract

In this study, the effect of pomegranate peel aqueous extract (PPEA) in reducing toxicities associated with hydrogen peroxide consumption was investigated using rats. This was achieved by assessing the serum biochemical parameters as well as histopathological analysis of the liver and stomach sections. Forty, 6-week-old female Wistar albino rats weighing 11.6± 4.4 g were divided equally into six groups. The rats were administered the pomegranate extract and challenged with H_2O_2 via the oral route for 28 days. To achieve this, positive control rats ingested H_2O_2 at 5 mg/ 100g body weight (b.w), while the negative control groups were given saline solution. Rats in the other three experimental groups were given H_2O_2 and treated with PPEA at 20, 40 mg and Vitamin at 25mg per 100g body weight respectively. H_2O_2 at 5 mg/100 g b.w induced injury to the liver and stomach tissues, while increasing levels of malondialdehyde (MDA), the Aspartate Transaminase (AST) besides Alanine Transaminase (ALT) and, reduced of Glutathione GSH) were observed in comparison with the control groups. Treatment with PPEA demonstrated protective effects by reducing the MDA, ALT and AST values and increase of GSH level as well as, improving the liver and stomach tissues to recover. Our results indicate that the administration of treatments against H_2O_2 using PPEA decrease the harmful effect orally administered H_2O_2 at 5 mg/100g b.w.

Keywords*: Hydrogen peroxide, pomegranate, pomegranate peel aqueous extract, antioxidants biomarkers, liver enzymes, rats*.

Introduction

Hydrogen peroxide (H_2O_2) is an oxidizing agent that is utilized in the production of various household items, including some broadly useful disinfectants and bleach, element of multiple teeth brightening products, treatment of wounds as well as for the purpose of sterilizing instrumentations[(1)]. H_2O_2 induces toxicities by destructive or corrosive harm, and the direct action of their oxidizing features by the formation and development of oxygen gas alongside lipid peroxidation through the reactive oxygen species ROS (Watt et al., 2004). On the other hand, ROS increases oxidative damage and causes adverse adjustments to cell components, such as proteins, lipids as well as DNAs [(2)].

Organisms have devised strategies to manage H_2O_2, which involvee endogenous protection accomplished by enzymes like superoxide dismutase, catalase and glutathione peroxidase. Also, the occurrence of antioxidants of low-molecular weight nature such as glutathione and α-tocopherol helps remove the ROS and reactive nitrogen species RNS [(3)]. The consumption of normal dietary antioxidants such as vitamins, protein, phenolic mixes, terpene, coenzyme Q, and nitrogen mixes behave as a free radical scavenger molecules by authorizing redox metals to thwart free radical leading to the generation of anti-inflammatory action [(4,5)].

Pomegranate Tunica granatum L. (Lythraceae), belongs to the Punicaceae family which have been

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portrayed as an antediluvian extraordinary plants that were utilized in a few frameworks of medication for relieving an assortment of sicknesses [6,3,7]. Pomegranate producing juices and an enormous measure of peels as bye-products and accounts for about half of the entire organic fruit mass [8]. The pomegranate peels (exocarp and mesocarp) are the non-edible part and have largely been used as a source of bioactive composites, for instance, hydrolysable tannins pedunculagin, punicalin, punicalagin, ellagic and gallic acids [9]. Punicalagin, a unique ellagitannin of pomegranate, is the main polyphenolic component of pomegranate peels and has a strong antioxidant activity [10].

The probable therapeutic assets of pomegranate peel are enormous including its application in the treatment and counteraction for the progression of cancers [11], cardiovascular illness [3,12], diabetes [13] and antimicrobial activity [14], in addition to its antioxidant and anti-inflammatory properties [15]. However, there is limited information on ways to reduce the harmful effects of H_2O_2 above 3 mg/100g by natural bye-products. Therefore, this research is aimed at examining the potentials of aqueous extracts of pomegranate peel in reducing the effects of H_2O_2 following oral administration with dose of 5 mg/100 body weight. The study also seeks to evaluate the antioxidants properties of the pomegranate by analyzing liver functions biomarkers and as well as histopathological studies of liver and stomach organs.

Materials and Methods

Plant material collection and extraction

The pomegranate fruits were purchased from local markets at Salah Al-Din province, Iraq in the months of September–November which coincide with the peak of their season. The pomegranate peels (mesocarp and exocarp) PP was physically detached from washed fruits, slashed, sun dried and powered with a grinder. The pomegranate aqueous extract (PPAE) was prepared according to the method described by Qnais *et al.* (2007) [16] which used the fractional modification approach. One hundred and fifty grams (150 g) of dried PP powder was poured into 3000 mL of distilled water and boiled for duration of one minute while being agitated on a magnetic stirrer after which the blend was kept in a shaker at room temperature for 24h. The resultant solutions were sifted through the Whatman no.1 filter paper and the filtrate was dried at room temperature and stored in the refrigerator until required. About 2 g powder was obtained from a quantity of 150 g dried peels. The solution of the pomegranate peel aqueous extract (PPAE) was then prepared by thawing the resultant powder using physiologic salt solutions.

Animal Model

Forty female albino rats of Wistar strain, weighing 116.3 ± 4.4 gram were obtained from the central animal house of the Faculty of Veterinary Medicine, Tikrit University, Iraq. They were kept in the departmental animal house and maintained under standard environmental conditions with bedding of wood shaving, fed with standard commercially accessible pellets with potable water. The rats were assigned to groups after seven days of acclimatization.

The research study framework was based on the recommendation and approval of animal control and supervision committee of the Scientific Affairs at the Tikrit University in Iraq, in accordance with the OECD guidelines on laboratory animal usage in research.

Treatment

The under-test rats were placed into six groups (G1-G6) containing five rats each per group. All the groups were administered 2 ml of the pomegranate extract orally daily for 28 days. The LD50 dose of PPAE was determined by using the method described by Qnais et al. (2007) and Patel et al. (2008) [16,17]. While the route and dose of H_2O_2 was chosen depending on the toxic effects of hydrogen peroxide (H_2O_2) as earlier described by Kawasaki (1969) [18]. However, the group 1 G1 (negative control) was treated by administration of saline solution only. G2 was treated with 5 mg/100 g body weight (b.w) of hydrogen peroxide (H_2O_2), G3 were given 25 mg/100 g body weight (b.w) of vitamin (vit) C, G4 were treated with a combination of H_2O_2 and Vitamin C, while rats in G5 and G6 were treated with H_2O_2 and 20 and 40 mg/100g body weight (b.w) of pomegranate peels aqueous extract (PPAE) respectively. The rats were refused feed for 3–4 h before administration. General observations were made for any moribund animals twice each day. On the 29th day after the last treatment, the rats were anaesthetized using 200 mg/kg (b.w), i.p

of pentobarbital sodium in order to obtain samples of blood and relevant organs. Blood samples were taken by cardiac puncture following sacrifice. Serum samples were obtained after blood centrifugation and stored at -20°C until required for analysis.

Liver enzyme functions tests

The serum concentration of aspartate transaminase (AST) and alanine transaminase (ALT) were assessed by kits obtained from RANDOX Laboratories Ltd., United Kingdom, according to the manufacturers' instructions.

Biomarkers of oxidative stress estimation

Malondialdehyde (MDA) as a biomarker of oxidative stress

Spectrophotometric assay was applied to detect the level of oxidase stress marker, Malondialdehyde (MDA) in serum samples by quantifying thiobarbituric acid (TBA) reactivity as MDA. The TBA test is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) leading to the formation of MDA-TBA2 that produce a deeply colored chromogen fluorescent red adducts following absorption at 532 nm.

Glutathione (GSH)

Reduced glutathione (GSH) in serum was evaluated with reference to the Burtis method and Ashwood (2007) utilizing Ellman's reagent [19]. The assay is based on the decrease of the Ellman's reagent by sulfhydryl groups into 5, 5′-dithiobis (2-nitrobenzoic acid), which has a strong yellow color. The solution was weighted at 412 nm utilizing the Japanese spectrophotometers brand Shimadzu.

Histopathological assessment

Fresh liver and stomach organs from sacrificed animals were collected in sample bottles containing 10% formalin. The tissues from the harvested organs were processed into 4 to 5 µm thickness. The gut tissue segments were adhered on glass slides, de-parafinized, and embedded in a solution of Hematoxylin and Eosin stains. The stained sections were then viewed under a microscope at 40 X magnifications.

Statistical analysis

The recorded data from all the six groups were analyzed using one way analysis of variance (ANOVA) and statistical significance was deduced with the aid of Duncan's numerous range test (DMRT) on SPSS (version 25). Statistical significance was determined at $p < 0.05$. All the values were expressed as mean ± standard deviations (S.D).

Results

Effects on liver enzymes

The serum levels of AST and ALT were different among animals in the treatment and non-treatment groups (Fig.1). In group G2, rats were found to exhibit substantial ($p < 0.05$) levels of serum AST (by 104.5%) and ALT (by 58.8%) in comparison with control group G1. Similarly, rats treated with vitamin C at 25 mg/100 g b.w (G4) and PPAE at 40 mg/100g b.w (G6) showed significant ($p < 0.05$) reduction in both enzymes AST (by 28.8%; 20% respectively) and ALT (by 49.4%; 41.1% respectively) in comparison with G2 group, indicating the anti-inflammatory effect of PPAE at 40/100g b.w against H_2O_2 effects (Fig1, A & B) .

Oxidative stress parameters

Serum GSH activity level of rats in group (G2) showed a significant ($p < 0.05$) decrease (by 65.5%) and increase in MDA level (by 73.6%) in comparison with control group G1. Furthermore, treatment with vitaminC in G4 and PPAE in G6 revealed statistically signicant increase ($p < 0.05$) in the activity of GSH action (by 84%; 89% respectively) while MDA level decreased by by 52% and 68.8% respectively in comparison with group (G2) as shown in Figure 1 C & D, which shows the antioxidant activity of PPAE in reducing the harmful effect of H_2O_2 at 5mg/100g b.w.

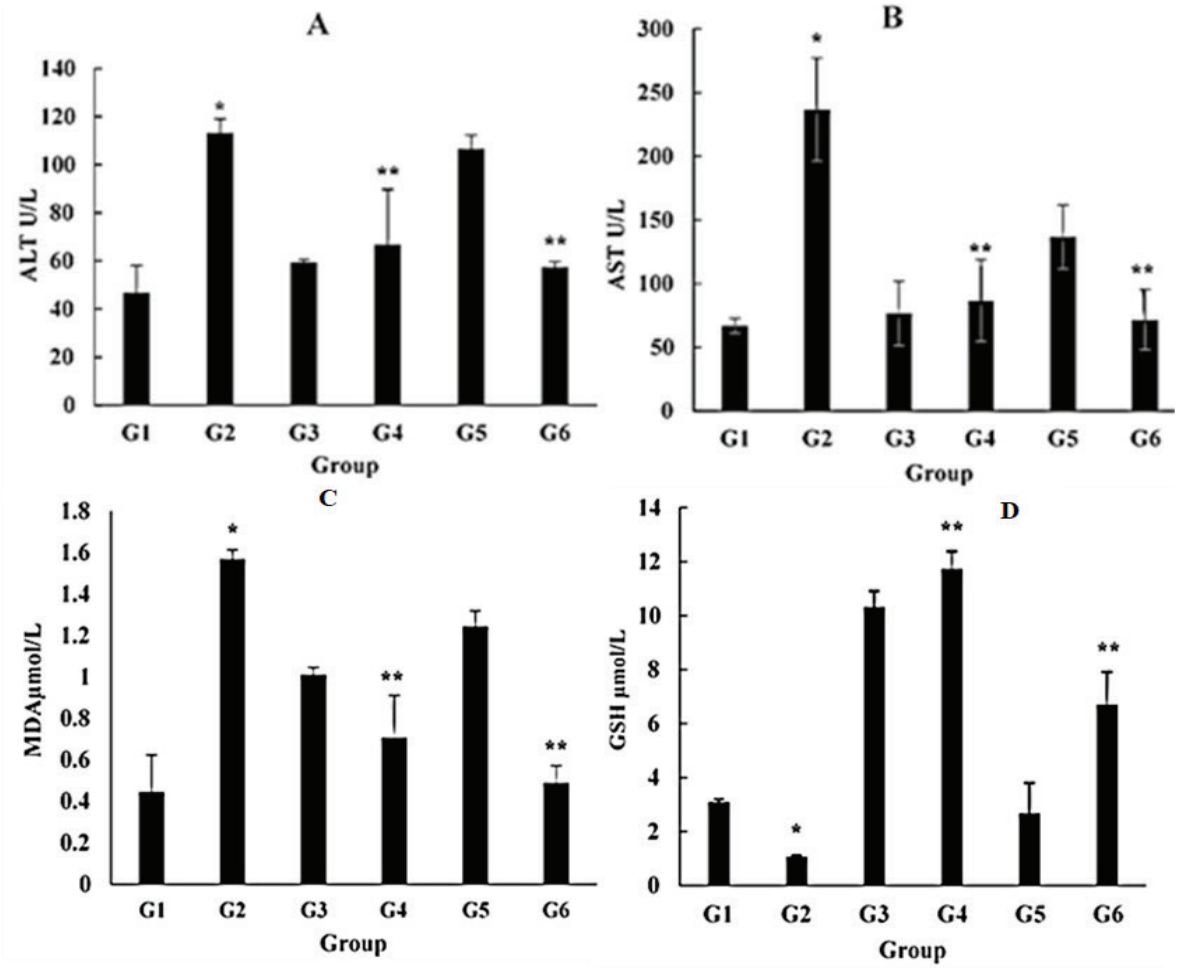

Figure 1. 2A, 2B, 2C and 2D indicates effects of PPAE and vitamin C on the AST and ALT activities as well as the oxidative stress parameters MDA and GSH in the serum of rats exposed to H_2O_2. The data was presented as means ±SD, (n = 5) animals. With asterisk* indicating statistical significance ($p < 0.05$) in comparison with the control group G1; while double asterisk indicate substantial increase ($p < 0.05$) in relation to the oxidative stress measure in group 2 G2.**

Histopathological examination

Microscopic examination of histological liver sections in the control group G1 (Fig. 2. A) showed a normal histological architecture. Normal hepatocytes (arrow) were organised in a number of several types of plates within the areas of the central vein (CV) and sinusoidal (S) plates. However, liver sections of rats in group G2 showed a marked damage (Fig 2B). The liver showed loss of the normal architecture, an invasion of inflammatory cells, with cellular infiltrations like neutrophils and Kupffer cells in the portal area, and hepatocytes indicating necrosis. Oral administration of the vitamin C 25 mg/100 g b.w and PPEA at 40 mg/100g b.w showed protection activity from the harmful effects of H_2O_2 (Fig 2 D, F). Remarkable change and reduction of inflammation and congestion in H_2O_2 intoxicated liver following treatment with PPEA at 40mg/100g b.w in comparison to group G2 was also observed. The observed regeneration in the hepatocytes (arrow) and binucleated liver cells was also shown.

The inner gastric surface of the stomach of the rats in the control group G1 (Fig 2 A), showed a normal histological structure of the gastric mucosa, where normal surface columnar epithelium (SCE), parietal and chief cells (PCs) as well as the gastric pits (GP) were observed. On the other hand, ingestion of 5 mg/100 g (b.w) dose of H_2O_2 for 28 days has shown destruction of the mucosal architecture, dissociation and erosion of

the mucosa and infiltration of inflammatory cell (arrow) (Fig 2B). Oral treatment with vitamin C at 25 mg/100 g b.w and PPEA at 40 mg/100g b.w reveal slight reduction of ulceration in the gastric mucosa of the stomach (Fig 2 D, F). Additionally, the gastric mucosa of the rats treated with PPEA at 40 mg/100g (b.w) show observable reappearance of the epithelial layers compared to group G2.

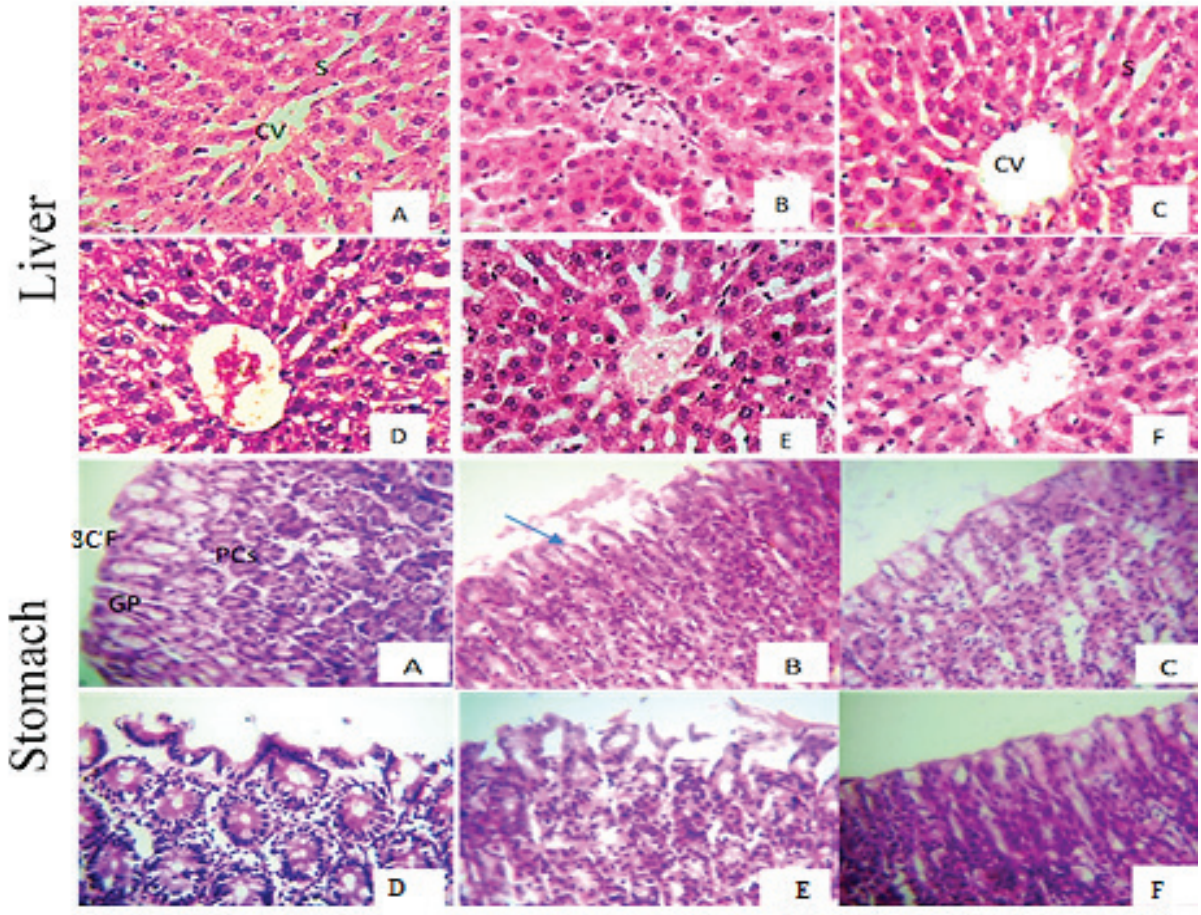

Figure 2. Microphotographs of the liver and stomach sections from the control and treatment groups stained by H & E and magnification, x400. G1 (A) control; G2 (B) 3 mg/100g H_2O_2; G3 (C) 25 mg/100g (b.w) vitamin C; G4 (D) vitamin C+H_2O_2; G5 (E) 20 mg/100 g (b.w) PPAE +H_2O_2 and G6 (F) 40 mg/100g (b.w) PPAE +H_2O.

Discussion

The marked increase in H_2O_2 within cells can bring about oxidative stresses as well as induce cellular destruction [21]. H_2O_2 in the presence of transition metals generates the often highly toxic reactive oxygen species (ROS) that provoke damage to multiple cellular organelles and process, leading to lipids peroxidation by influencing the irons redox cycling (30). Glutathione, is one of the major thiol-containing compound with low molecular weight within animals cells that are essential in many biochemical and pharmacological reactions due to the ease with which they are oxidized and the speed with which they can be regenerated, and they also successfully rummages ROS as antioxidants agent [22]. Because malondialdehyde (MDA) is one of the significantly formed aldehydes after lipids hydroperoxides failure, it is viewed as a decent biomarker contributing to free radical damages in pathologies associated with oxidative stress [23].

In this study, we demonstrated that oral administration of hydrogen peroxide (H_2O_2) with a dose of 5 mg/100 g body weight (b.w) result in increase of MDA and depletion of GSH levels in the serum of rats (group 2). As reported by Kawasaki et al. 1969 [18], when

rats are exposed daily, by oral gastric tube, to above 3 mg/100 g of H_2O_2 solutions, it causes significant blood chemistry changes. Findings of this study were similar to previous reports by Ganie *et al*. [24], and Rahim *et al*. [25] who both reported a considerable increase of MDA level in H_2O_2 diagnosed rats, indicating the likelihood of decreased fundamental creation, and bigger volume of lipoid peroxidation and induction of oxidative stress. However, treatment with PPEA with dose 400 mg/kg probably reduced the oxidative stress as indicated by the inhibition of lipid hydroperoxides due to depletion of MDA and increase GSH levels. Besides, the parameters were seen as near their normal levels in the serum of vitamin C treated group (G5). Both Osman *et al.* [26], and Cichoz-Lach *et al*. [27] demonstrated that the lipids peroxidation levels decrease in the pomegranate extract handled animals groups as compared to the control groups. Pomegranate peels are rich in antioxidants polyphenolic mixtures which has a protective effect from the harmful reactive oxygen species (ROS), through destruction of free radicals or by preventing the generation of these species.

Similarly, the antioxidant property of vitamin C is dependent on the decrease of lipid peroxidation through the removal of free radicals action [28]. Increase of MDA and depletion of GSH in the serum of rats of group G2 as evidenced by the significant increase observed in the levels of liver (AST and ALT) enzymes activities resulted in chronic hepatic injury following oral administration of H_2O_2. Rahim *et al.* [25], and Abozid *et al.* [29] indicated that oxidative stress induced by H_2O_2 bring about hepatic injury as depicted by the raise in the liver enzymes activities in the blood. Moreover, microscopic examine of the liver section of rats in group 2 showed damage in liver cells as result of H_2O_2 toxicity. Free radicals that invade the liver cell compartments cause changes in its composition and functions as result of oxidative stress [30,31]. The present research work realized a manifest impact of PPEA (400 mg/kg) diminished ALT and AST enzymes and produced a remarkable reduction of damage in liver cells. Toklu *et al*. [32] examined the impact of chronic organization of PPEA initiated by bile conduit legation within rats and got that serum ALT and AST were tremendously diminished. Which, indicate activity of pomegranate peel to preserve the auxiliary uprightness of the hepatocellular layer and liver cell design by particular phenolic mixes that may potentiate the defensive impacts against ROS [33]. Also, vitamin C protects the lipids and lipoproteins in the hepatocellular membranes versus oxidative harm brought about by dangerously toxic free radical scavenging activity [31].

After 28 days, ulceration of the gastric mucosa was observed in rats at group 2. Once ingested, H_2O_2 promptly interacts with tissue catalase such as mucous layers. This exothermic response quickly decays H_2O_2 to water, warmth, and oxygenated and is effortlessly changed over to hydroxyl radical which cause harm to numerous cell parts or even cell death [34]. On other hand, following treatment with PPEA, a slight reduction of stomach mucosal damage was noted. Inflammatory bowel disease is favorably influenced by consumption of several dietary natural plant products including polyphenolic compounds. Metabolism of polyphenol compounds such as ellagic acid in pomegranate peel by gut microbiota result in the formation of bioactive urolithins A, B, C, and D. Urolithin A (UA) is the most dynamic and compelling gut metabolite and goes about as a powerful calming element to oxidizing agents [10].

Conclusion

In conclusion, prolonged consumption of food contaminated with chemicals such as hydrogen peroxide can cause toxicity at a certain site of the body. Therefore, reducing this effect will require the intake of natural bio products such pomegranate peel extract which has enhanced antioxidative potentials, and can act as a free radical scavenger in addition to its anti-inflammatory action.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication of this article.

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Author contributions

Dhuha Salah Noori, Agharid A. Alrasheed: Conceptualization; **Dhuha Salah Noori; Agharid A. Alrasheed, Muna Salah Rasheed:**

Methodology; **Dhuha Salah Noori, Agharid A. Alrasheed, Muna Salah Rasheed, Bashiru Garba:** Writing- Original draft preparation. **Agharid A. Alrasheed, Bashiru Garba**: **Dhuha Salah Noori, Muna Salah Rasheed**: Writing- Reviewing and Editing.

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Which one affect the other, Recurrent Pregnancy Loss or Serum Level of E-cadherin and c-MycBP

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Abstract

Recurrent pregnancy loss (RPL) is a foremost life event and a common pregnancy complication, which defined as losses of two or more pregnancies before 20 weeks of gestation. It affects approximately 1% - 2% of couples trying to have children. To evaluate the potential role of cellular myelocytomatosis binding protein (c-MycBP) and epithelial cadherin (E-cad) serum level as biomarkers in maternal blood and focus on the similarity between tumour invasion and embryogenesis as phenomena by c-MycBP and E-cadherin. This case control study included 50 women with RPL (25 primary RPL and 25 secondary RPL) from three infertility clinics in Iraq. In addition to 50 control women with no history of RPL and at least one child birth. Both groups are matched in age and BMI. Blood sampling was done for all and protein level of c-Myc BP and E-cadherin were measured by ELISA Kit and analysis done according to the manufacturer recommendations. The result presented in this study showed a significant difference in c-MycBP level ($p<0.001$) and E-cadherin ($p<0.001$) between patients and control. A significant positive correlation has been found between these two biochemical markers ($p <0.001$, $r=-0.39$). The present study showed that decreasing both c-MycBP and E-cad serum level signpost possible hypothesis that these proteins participate in one way or another in RPL pathogenesis which affect cell proliferation and invasion. However, this binding protein transcription factor is a part of a coordinated network of various set of partners which need further studies in future. Taken together, additional studies are required to understand and clarify the importance of these biomarkers on this multifactorial condition.

Keywords: *Recurrent pregnancy loss (RPL), E-cad, c-MycBP*

Introduction

Recurrent pregnancy loss (RPL) recognised as two or more failed pregnancies according to the American Society for Reproductive Medicine (ASRM), not necessarily consecutive [1]. The two pregnancy losses or more with in less than 20 weeks of gestation. RPL occurs in 1–3% of women trying to conceive [2]. According to the evidence available, women should undergo comprehensive evaluations only after the loss of two or more pregnancies [3-5]. Even after extensive investigations, less than 50% of couples identify a cause for RPL [6]. Subsequently, most cases remain free from a modifiable risk factor [7]. Also associated with RPL are maternal lifestyle factors like smoking, consumption of alcohol and caffeine, and obesity [8]. Maternal age is a significant risk factor for loss of pregnancy, primarily based on the increased incidence with increasing age of foetal aneuploid abnormality [9]. There for, RPL is a great multifactorial condition; acquired and inherited thrombophilia, structural uterine defects, endocrine disorders, and parental balanced chromosomal translocations are among the multifaceted risk factors of RPL [10, 11]. Cellular myelocytomatosis is a family member of the myelocytomatosis (Myc) proteins (c-Myc, MYCN, and MYCL). These proteins are basic helix-loop-helix leucine zipper transcription factors with a vital role in proliferation, differentiation,

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cell cycle progression, metabolism, and cell survival/ apoptosis [12]. This protein regarded as an important cell cycle modulation cytokine. Cell proliferation and transition of resting phase to mitosis phase in cell cycle could be an expected outcome to c-Myc over-expression [13, 14]. Furthermore, phosphorylation of the mitogen-activated protein kinase (MAPK) is important pathway in c-Myc activation[15,16]. The expression and functional regulation of the Myc gene includes a range of mechanisms. One of these mechanisms is based on the Myc binding protein (MycBP) signaling pathway [17]. The MycBP gene encodes a ~11 kDa protein that can bind the Myc N-terminal region via its C-terminal structure, causing Myc to induce tumorigenesis [18]. Earlier studies were conducted to inhibit the expression of MycBP, and thereby Myc pathway, and observe the outcome suppression of the migration and invasion of cancer cells [19, 20].The functional mechanism of c-Myc, mainly, depends on the induction of mitosis and cell cycle growth response. Activation of c-Myc can regulate cell cycle, induce endometrium hyperplasia, upraise endometrium oestrogen receptor level, as well as the c-Myc transcription, eventually leading to gene amplification and over-proliferation of endometrial cell leading to carcinoma of endometrium [21].

Epithelial cadherin (E-cadherin or cadherin1 or E-cad) is transcribed from CDH1 gene into a precursor polypeptide of 135 kDa which is transmembrane glycoprotein that function as cell adhesion molecule (CAM) which bind to various cell types, and are vital in normal tissue morphogenesis and development [22, 23]. This transmembrane protein is regarded as a member of the classical cadherins including the neural cadherin (N-Cad), placental cadherin (P-Cad), and the vascular endothelial cadherin (VE-Cad). E-Cad is an evolutionarily preserved large single-pass transmembrane glycoprotein involved in Ca^{+2}-dependent cell–cell adhesion [24]. The complex of E-cad and β-catenin is closely associated with alteration in cell size, shape, and relative movements that are the foundation of the comprehensive morphogenetic processes producing stereotypical tissues and organs fulfilling their functions during embryonic development and adaptive processes of adult tissues [25-27]. Embryo implantation is a highly controlled event that is necessary for pregnancy establishment. Three successive stages are what implantation goes through which are implantation, adhesion and invasion [28]. The synchronous development of both the embryo and endometrium relies on positive embryo implantation. Often known as essential in implantation are numerous regulatory proteins recognized to play a key role in cancer cell invasion[29].E-cad is found in humans in trophoblasts, and homophilic interaction between cytotrophoblast and endometrium are thought to be mediated through it. This may mean that E-cad involves in the embryo implantation initial stage [30], and it is down-regulated in placental villi during early pregnancy in patients with spontaneous miscarriage may be regarded as one of risk factors of this condition [31].However, no evidence exists to indicate whether or not the down-regulation of E-cad expression in villi or the up-regulation of E-cad expression in extravillous trophoblasts marks the beginning or the terminal stages of trophoblast differentiation in patients with missed and threatened miscarriage[32]. In our study, we evaluated the serum level of MycBP and E-cad and explored the correlation between these two biomarkers with this clinical condition.

Material and Methods

Subjects

in this case–control study a total of 50 RPL subjects (25 primary RPL and 25 secondary RPL) with at least two or more recurrent miscarriage before 20 weeks of gestation were recruited from three different infertility clinics: the Obstetrics and Gynaecology department at Imam Al-Hujjah Hospital, Karbala province, Iraq; Infertility Centre, Babylon Teaching Hospital, Babylon province, Iraq; and Teba Centre for infertility, Babylon province, Iraq. As well as 50 women apparently healthy control group with no history of pregnancy loss and at least one child birth. Furthermore, we excluded individuals with history of smoking, alcohol abuse, infections, endocrine or metabolic disorders, anatomic abnormalities and autoimmune diseases based on history and medical and biochemical examination. All patients with RPL groups experienced recurrent abortions with same partner. The sample has been collected for the period from October 2018 to August 2019. All subjects matched in age, 19-35 years, and body mass index (BMI). PRL patients vs Control Mean ±SD for age (30 ± 4.2 vs 30 ± 4.1), BMI (25.72 ± 2.05 vs 25.25 ± 1.75), gravidity (3.5 ± 1.27 vs 2.42 ± 0.91), parity (1.00 ± 1.23 vs 2.42 ± 0.91) and abortion (2.5 ± 0.81 vs NA)

Ethical Issues

All participants gave written informed consent to participate in this study with objectives and methodology explained to all of them individually. The official approval for this study obtained by The Scientific Committee of Babylon Medical College, University of Babylon, and Research and Development Department of Babylon Health Directorate, Babylon province, Iraq.

Materials

Commercial ELISA sandwich kit for human c-Myc binding protein and E-cad were used as per manufacturer, Bioassay Technology Laboratory, instructions with Cat.No E2901Hu (sensitivity: 0.04ng/ml) and Cat.No E0209Hu (sensitivity: 0.52ng/ml) respectively.

Methods

Blood sample collection, venous blood sample drawn from all subject by using disposable syringe (5 mL) in the sitting position. The needle was disconnected and the blood is evacuated slowly into plain disposable test tubes without anticoagulant. The blood was allowed to clot at 37˚C for 10-15 minutes, and then centrifuged at 3500xg for five minutes. Separated serum was used for protein level measurement (Human c-MycBP, human E-cadherin).

Statistical Analysis

The collected data were tabulated and analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows version 20th version. Data were expressed as (mean ± SD). Independent sample T-test was used to compare means between two groups. ANOVA test was used to compare means of more than 2 groups. Correlation coefficient (r) was used to find the relationship between two continuous variables. P values < 0.05 were considered statistically significant.

Results

Based on our results of the present study, multiple comparisons of c-MycBP serum concentrations (ng/ml) were shown among control and RPL groups, and the significant differences were expressed as ($p < 0.05$) (Table 1, 2). The c-Myc is a transcription factor which play as an essential regulator for cell proliferation, differentiation and embryogenesis [33]. This result indicates that c-MycBP protein may be involved in the pathogenesis of RPL agreeing with Wu *et al* findings that c-Myc regulation of peroxiredoxin2 might have an important role in trophoblast proliferation and apoptosis [34], these results support out this study work outcome.

Table 1. Descriptive of c-MycBP in Control and RPL Groups

Groups	Mean ± SD	95% Confidence Interval for Mean	
		Lower Bound	Upper Bound
Primary RPL	2.18 ± 0.35	2.01	2.30
Secondary RPL	2.00 ± 0.42	1.84	2.18
Control	2.64 ± 0.45	2.51	2.77

Table 2. Multiple comparison dependent variable of c-MycBP

Groups	Comparative Group	95% Confidence Interval		p. value	Mean Difference
		Lower Bound	Upper Bound		
Primary RPL (n=25)	Control	-0.68	-0.27	<0.001	-0.48*
	Secondary RPL	-0.09	0.39	0.21	0.15
Secondary RPL (n=25)	Control	-0.83	-0.42	<0.001	-0.63*
	Primary RPL	-0.39	0.09	0.21	-0.15
Control (n=50)	Primary RPL	0.27	0.68	<0.001	0.48*
	Secondary RPL	0.42	0.83	<0.001	0.63*
*The mean difference is significant at the 0.05 level.					

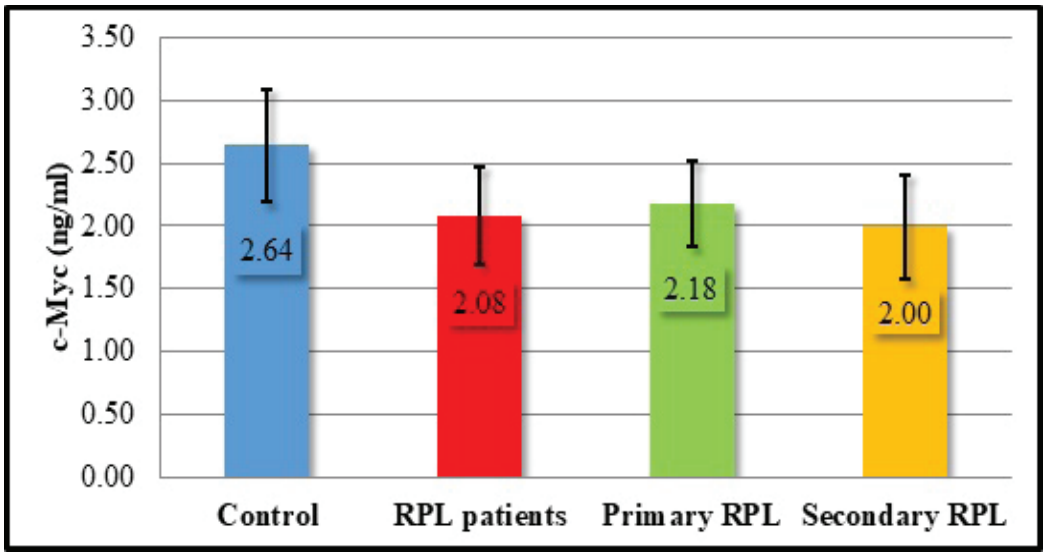

Figure 1. c-MycBP mean ± SD (ng/ml) in control and RPL groups

RPL may be due to infection or attributed to physiological aspects or heavy physical activity during pregnancy [35-37]. The result of the present study showed a very high significant difference in E-cad between RPL groups and control as shown in (Figure 1 and 2). This is consistent with results from Yang *et al*, which indicated a possible role of E-cad in implantation processes and altered expression in women with reproductive failure and E-cad low level linked to RPL [37]. Furthermore, Wu *et al* suggested that placental syncytiotrophoblast is accountable for the transport of oxygen, nutrients and wastes; it also produces hormones for foetal development and maintains immune tolerance. E-cad dynamically changes during the process of cytotrophoblast fusion and that its downregulation is coincident with cell fusion [34]. Likewise, Verma *et al* have shown that the expression of E-cadherin is essential for embryonic development. Mice with E-cad knockout are unable to form functional trophectoderm and therefore could not survive during implantation. Moreover, a decrease in the expression of E-cad has been reported in trophoblast cells during EMT when extravillous trophoblasts (EVTs) migrate or invade into the cell column [38].

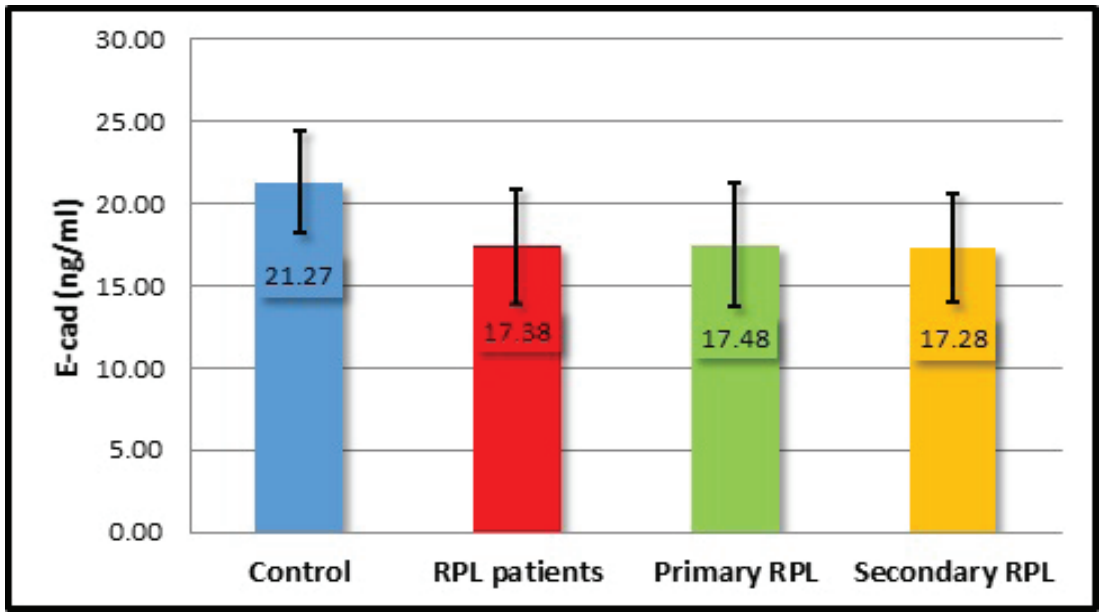

Figure 2. E-cad mean ± SD (ng/ml) in control and RPL groups

ANOVA COMPARISON OF E-CAD BETWEEN CONTROL AND RPL GROUPS

Current study results shown multiple comparisons of E-cad serum concentrations (ng/ml) among control and RPL groups (Mean±SD=17.48 ± 3.72 for Primary PRL, 17.28 ± 3.32 for secondary PRL and 21.27 ± 3.10 for control), and the significant differences were expressed as ($p<0.05$)

Table 3. Multiple comparison dependent variable of E-cad

Groups	Comparative Group	95% Confidence Interval		p. value	Mean Difference
		Lower Bound	Upper Bound		
Primary RPL (n=25)	Control	-5.41	-2.18	<0.001	-3.79*
	Secondary RPL	-1.66	2.07	0.82	0.21
Secondary RPL (n=25)	Control	-5.66	-2.39	<0.001	-4.00*
	Primary RPL	-2.07	1.66	0.82	-0.21
Control (n=50)	Primary RPL	2.18	5.41	<0.001	3.79*
	Secondary RPL	2.39	5.62	<0.001	4.00*
*The mean difference is significant at the 0.05 level.					

CORRELATION OF C-MYC AND E-CADHERIN

There is a very high significant positive correlation ($p\leq0.001$), ($R^2 = 0.1429$) between c-MycBP and E-cad in patients and control groups as shown in (Table 4). This result represented for the first time in our study which may reviled the hidden factor affecting both c-MycBP and E-cad causing their down regulation in RPL and this will need more investigations and researches. This demonstrated result conflicting what Liu *et al* has found. They stated that c-Myc oncoprotein is overexpressed in most human cancers and regulates different genes and pathways in different cell types. E-cad expression is supressed by Myc through a post-transcriptional mechanism, but the exact mechanism remains elusive [39]. Likewise, Fonseca-Alves *et al* determined that carcinogenesis of the canine prostatic epithelial cells produced a strong and elevated cytoplasmic c-Myc protein expression associated with a related loss of the tumour suppressor proteins NKX3.1 and E-cad [40]. Another recent paper verified that upregulation of E-cad in PC4-knockdown cells were reversed by overexpression of c-Myc [41]. In an interesting coincidence finding, Miller *et al* has found that Myc activates the expression of miR-9 which inhibits E-cad expression [42]. Present results, in contrary, supported by Liu et al outcome. He and his colleagues concluded that data presented indicated decreasing cellular polyamines represses E-cad transcription, which is prevented by ectopic c-Myc overexpression [43].

Table 4. Correlation between c-Myc and E-cad

Parameter		E-cad	c-Myc
E-cad (ng/ml)	r	1	0.39***
	p		<0.001
c-Myc (ng/ml)	r	0.39***	1
	p	<0.001	
***Correlation is very highly significant at the 0.001 level (2-tailed)			

Conclusion

This study concluded that there is a significant positive correlation between c-Myc and E-cad in control and patients' groups demonstrating for the first time, to our knowledge, that decreasing both c-Myc and E-cad serum level signpost possible hypothesis of these two proteins participate in one way or another in RPL.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Transferring of Heavy Metal Co-Resistance and Antibiotics Resistance of Bacteria Isolated from Sewage Water

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Summary

Fifty-five samples of water have been collected from Baghdad sewage stations in the period between 9th till 18th of Jul in 2018. Forty-two *Klebsiella pneumonia* isolates have been identified from water samples according to its morphological, cultural, and chemical characteristics. The susceptibility of the isolated bacteria to antibiotic were performed against ten types of antibiotics representing most common antibiotic categories, the results showed differences in the bacterial susceptibilities against the currently antibiotics ranged according to the antibiotic itself. The results were ranging from medium to low of bacterial resistance. In addition four of the bacterial isolates were multidrug resistant bacteria (MDR). The results of bacterial abilities to tolerate the toxicities of nickel and zinc showed high levels of the metal tolerate. Plasmid profiling of bacterial isolates were performed, and then plasmids curing processes were done to delete the bacterial plasmid and then both antibiotic resistance and heavy metal tolerance of cured isolates were performed to determine the origin of resistance and tolerance encoded gene. Nor resistance or tolerance patterns of cured bacteria showed difference when compared with non-cured (original) isolates such as have been showed from results. The conjugation results of four *Klebsiella pneumonia* isolates didn't transfer metal tolerance genes between two successfully conjugated bacteria, while some of resistance genes transfer conjugationally from donor to recipient cells.

Key words: *Conjugation, K. pneumonia, antibiotic, nickel, zinc, and sewage water.*

Introduction

Water pollution refers to any parameters changes in water characteristics and may create a new case differ from the normal one on all or some living thing that drinks or uses or lives in it. Because of their harmful effects on approximately all living organisms, the increased presence of heavy metals in water areas in recent decades has piqued public and scientific interest.[1] This has prompted researchers across the world to investigate heavy metal emissions in order to prevent their adverse effects and decide if they are safe to consume by living organisms. As a result, numerous experiments were conducted to assess the concentration of heavy metals in spices as well as to investigate their potentially harmful effects. WHO and other healthy organisms consider heavy metals, which may found as dissolve or suspend chemical forms according to their particle diameters, can be consider as serious pollutants of the aquatic environment.[2] They cause serious im pairment in all biological systems as a result of their occurrence in more than their normal levels in the environment. [3] The concentration of heavy metals within living organisms (which is called bioaccumulation) is the most significant factor in their harmful or lethal influence on bacteria and other organisms. 4,5 To stay alive with heavy metal concentrations, To avoid the accumulation of heavy metal ions, bacteria have acquired a number of mechanisms; some of these mechanisms are typical in the antibiotic resistance state, such as cell membrane permeability and heavy metal ion efflux outside the cell, as well as other unique mechanisms. [6] This study aims to determine and screaming the antibiotic and heavy metals resistance of waste water Klebsiella pneumonia isolates, and the permeability of these isolates to transfer the resistance genes to another bacteria isolates by conjugation.

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Materials and Methods

Samples collection.

Twenty-eight water samples were collected in the period between 9th till 18th of Jul in 2018 from four stations in army canal in Baghdad as shown in figure (1); samples were collected in 500 ml sterilizing containers from 30 cm depth away from surface area, the GPS parameters of the isolation areas were illustrated in table (1).

Bacterial identification.

Klebsiella pneumonia isolates were identified according to their cultural, morphological, and biochemical characteristics which include gram stain, catalase, oxidase, indol, methyl red, citrate, urease, H_2S, motility and VP tests.[7]

Table (1): GPS parameters of the studied area.

Station no.	Longitude			Latitude		
Station One	33°	32’	76”	44°	47’	31”
Station Two	33°	34’	03”	44°	45’	66”
Station Three	33°	34’	98”	44°	44’	48”
Station Four	33°	36’	82”	44°	42’	82”

Figure (1): Satellite image of the study area.

Antibiotic susceptibility test.

Suspensions of refresh growth of all bacterial isolates were prepared separately with turbidity equal to McFarland standard solution, and then 100 µl of these suspensions were seeded on Muller-Hinton agar plates. Ten antibiotics were used in this study and then antibiotic discs (ampicillin, amoxicillin, cefoxitin, cephalexine, meropenem, erythromycin, tetracycline, chloramphenicol, rifampicin, and nalidixic acid) were placed on the surface of media, the plates were left to dry for 10 minutes at room temperature; and incubated at 37°C for 18 hrs.[8] Bacterial susceptibility to antibiotics was recorded by comparing the diameters of inhibition zone around the antibiotics discs with reference of national committee for clinical laboratory standard.[7]

Heavy metals tolerance test

100 µl of serial dilutions of both nickel and zinc were added separately to plates containing Muller Hinton medium after cooling to 45-50°C, and then fresh growth of bacterial isolates were spread on plates and then incubated at 37º for 24 hrs. The sub-MICs values were recorded as the highest tolerance concentration of heavy metals that bacteria can tolerate. [9]

Plasmid extractions.

DNA of the bacterial plasmids isolates were extracted according to QIAamp® DNA Mini Kit (QIAgen, 2010). [10]

Profiling of bacterial plasmid.

The plasmid profiling were done by preparation of agarose by boiling 400 µg agarose and 50 ml TBE buffer, and then 10 µg/ml of ethidium bromide was added when the mixture reached to 45º. 25 µl of the last preparation of bacterial plasmid was transferred to new eppendorf tube containing loading buffer (5 µl). After mixing, five micron of it was transferred into the comb wells in the agarose. Then the tray was moved and putting into the tank which filled with TBE buffer, then the gel was examined by 336 nm ultra-violets light as well as photographed after two hours of passing the electric current. [11]

Curing of bacterial plasmids

Two different curing agents were selected and used to determine the plasmid curing due to their high efficiency; Sodium dodecyl sulfate (SDS) and acridin orange. [11] Two-fold serial dilutions of SDS and acridin orange were separately performed starting from 16 to reach 2048 µg/ml, then 100 µl of the isolated bacteria were transferred to these tubes containing 100 µl of these curing agents' concentrations, MIC and sub-MIC of each curing agents was determined by using ELISA reader spectrophotometer (580 nm) after incubation of these tubes in at 37 C for 18 hrs. MIC and sub-MIC of each curing agents was determined by using ELISA reader spectrophotometer (580 nm), where Sub-MIC refers to a maximum concentration of curing agent that allows bacteria to live in it, while MIC stands for lowest inhibitory concentration, which has the potential to inhibit bacterial growth. Starting from 10 -1 till 10 -8 , decimal serial dilutions of sub-MIC tubes were done, and then 100 µl of latest four tubes which contains the highest concentration of the agent (which were starting from 10 -5 – 10 -8) were inoculated on the prepared media plate and incubated at 37 C for 18 hrs. [12] Curing isolates were electrophoretically run in agarose to confirm that their plasmids were lost, and then resistance and tolerance abilities of these bacteria were tested against antibiotics and heavy metals, respectively.

Bacterial Conjugation.

Four distinct bacterial isolates were selected and conjugated according to the conjugation protocol. 13 one single colony of Escherichia coli standard (MM 294) was transferred to sterilized tubes containing Luria-Bertoni broth. Secondly, single colonies of four isolates of Klebsiella pneumonia were separately added to the tubes, where E. coli MM 294 was considered as a recipient cell and K. pneumonia isolates were considered as donor cells, the tubes were incubated in 160 rpm shaker incubator at 37C for 18 hrs. 1000 µl of both donor and recipient cells were transferred and mixed in new test tubes. After 1-2 hours of incubation at 37C, the bacterial combination was strongly shaken for few minutes to differentiate between donor and recipient cells by breaking conjugation Bridge. Following that, a series of tenfold dilution (starting from 10 -1 up to 10 -6) were prepared and 0.1 ml of the each diluted concentration were spread on the surface of the prepared media which containing both antibiotic markers (Rifampicin 100 mg/ml and antibiotic don't effect on K. pneumonia isolates). After incubation at 37º for 18-24hrs, 100 µl of the last three dilutions was spread on plates contain

Muller Hinton media with rifampicin (100 mg/ml) to measure the number of recipient cells. On the other hand, To detect and measure the spontaneous mutated cells, 0.1 mi of the broth culture of both bacterial cells (recipient and donor cells) were transmitted on the selective media and incubated at 37C for 18 hours.

The frequency rate of conjugation was determined using the equation below:

$$\text{The conjugation frequency} = \frac{\text{Trans-conjugation cells}}{\text{Recipient cells}}$$

For the sake of maintaining the acquired characteristics of the new bacterial strain, the conjugational bacterial colonies were moved and inoculated to new selective media.

Results

Eleven isolates of *Klebsiella pneumonia* were identified from fifty-five water samples were collected from the five stations (eleven samples from each station). *Klebsiella pneumonia* colonies appeared as smooth, mucoid, and pink colonies on Mackonky media as a result of lactose fermentation, and according to microscopic examination it was gram negative rod non-spore forming bacteria. According to biochemical tests, it was positive in catalase, urease, citrate, and vp tests, while it showed negative results in oxidase, indol tests, methyl red, H_2S, and motility tests.[7]

Antibiotic susceptibility tests of *Klebsiella pneumonia* isolates showed that amoxicillin and cephalexine had the greatest effects on bacterial isolates, *K. pneumonia* isolates resistance to them were (18.1%), while the highest resistance percentages were recorded against erythromycin (54.6%), in addition to moderate resistance against to ampicillin (36.4), cefoxitin (27.3%), cephalexine (18.1%), meropenem (27.3%), Chloramphenicol (27.3%), rifampicin (27.3%), and nalidixic acid (45.5%), as shown in table (2). Multi drug resistance of bacterial isolates may be because some antibiotic resistance genes express themselves and encode enzymes which have the ability to antibiotic degradation, or may be resisting by using one or more processes to avoid the antibiotic actions.[14,15]

Table (2): Antibiotic diagram of *K. pneumonia* isolates.

Antibiotics	Symbol	µg/disc	Resistance %
Ampicillin	AM	10	36.4
Amoxicillin	AX	25	18.1
Cefoxitin	FOX	30	27.3
Cephalexine	CL	30	18.1
meropenem	MEM	10	27.3
Erythromycin	E	15	54.6
Chloramphenicol	C	30	27.3
Rifampicin	PRL	5	27.3
Tetracycline	TE	30	36.4
Nalidixic acid	NA	30	45.5

The bacterial ability to tolerate zinc and nickel concentrations was performed, as well as the limits of these tolerances. Figures (2) show the bacterial tolerance percentages against both nickel and zinc. Based on the collected results, the toxicity effects of nickel was more than zinc toxicity,

The bacterial heavy metals resistance may be considered as an indicator for the metal contamination in the waste water, many evidences referred to consider microbial tolerance value as a pollution indicator. [16] This point agreed with what Díaz-Raviña *et al*. recognized, the magnitude of the increase in heavy metal tolerance was found to be linearly related to the logarithm of the amount of metal concentrations.[17] The results showed that *K. pneumonia* isolates could tolerate 6.1 mM of nickel, and 4.2 mM of zinc as shown in figure (2), this may happen due to the high levels of these metals in bacterial environments, which may turn a response of their presence and concentration in media.[18]

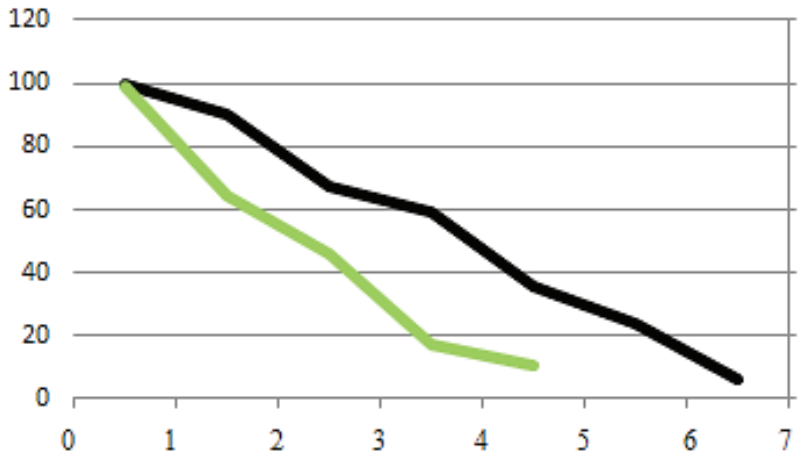

Figure (2): Heavy metals resistance of *K. pneumonia* isolates (mM).

Zinc concentrations

Nickel concentrations

Bacterial resistance to antibiotics and heavy metals showed to be gathering in bacterial isolates, one of the suggestions assumed that bacterial isolates used the same mechanisms in resistance of both antibiotics and heavy metals, such as efflux system, changing the permeability of bacterial membrane, as well as other resistance mechanisms.[19] Or it may be occurred due to some specific genes in bacterial isolates which can provoke both resistance and tolerance processes.[19,20]

To determine if the resistance genes are encoded by a plasmid or not, curing agents were used to eliminate the plasmid from the strain to determine location of the resistance and tolerance bacterial genes. The electrophoretic of the plasmids profile of non-cured isolates were compared with the plasmid extract of the same isolates after curing to confirm if the resistance and/or tolerance genes are encoded by plasmid or chromosomal genes. Plasmid curing process of the isolated strain was performed by using two curing agents; SDS and acridin orange. The present study revealed the high efficiency of acridin orange in plasmid curing as compared with SDS efficiency, it could cure all the bacterial isolates, this result showed similarity to other results which carried out the same result of the acridin activity comparing with SDS,[21] while it differs from Raja and Salvan (2009) results which showed batter activity of SDS comparing with other curing agents.[21,22]

Nickel resistance gene was shown to be present on the chromosomal DNA rather than the plasmid DNA as the cured and uncured cultures remained similar in lead resistance. Similar to nickel, zinc results didn't record any difference in metal tolerance values between both cured and non-cured isolates, although the comparison clearly showed existence of slightly decrease in cadmium tolerance in one isolate from 0.9 to 0.6, as well as the other two isolates from 0.8 to 0.7 and from 1.2 to 0.9. It was

reported that resistance to some heavy metals has been found in bacteria and genetics determinant of resistance are frequently located on plasmids or transposons.[22] In addition, many other results agreed with the results of this study and showed the chromosomal origin of some heavy metals tolerance gene, such as nickel and zinc, without any effect of plasmid presence on the tolerance state.[23] Berg *et al.,* (2010) showed there was no linkage between plasmid presence and tolerance to heavy metals; he didn't found any difference in bacterial tolerance to cadmium, zinc and other metals although some of them were free plasmid.[24] The results of chromosomal origin of bacterial tolerance to nickel and zinc where confirmed by the conjugation data which reviewed in table (3), these results showed that no one of all these metal tolerance success to transfer conjugationally from donor to recipient bacteria.

Table (3): The conjugation results of *Klebsiella pneumonia* isolates

Isolate	Donor cells	Recipient	frequencies
1	FOX, MEM, E, NA	-	-
2	AM, AX, FOX, CL, NA	AM, AX, FOX, CL	2*10-4
3	AM, COL, AX, CL	AM, AX, CL,	2.2*10-3
4	COL, E, PRL	-	-

Conjugation results showed that four bacterial isolates of *Klebsiella pneumonia* isolates, only two isolates could successfully conjugate with the frequencies equal to $2*10^{-4}$ and $2.2*10^{-3}$ respectively, while other two isolates couldn't perform conjugation. These results represent another evidence of the chromosomal origin of the heavy metals tolerance genes, while many antibiotics cloud transferred by the conjugation process. Although they had two or more plasmids as well as some resistance pattern, two isolates fail to make the conjugation, that may happened because the resistance genes were located on non-conjugational genes, or a mutation was happened in the extraction enzymes which may prevent the conjugation of these genes.[25] Many previous studies referred to failure of metal tolerance to success in transfer conjugationally from donor to recipient cells. Kamala-Kannan and Lee (2018) studied the tolerance origin of zinc, nickel, chromium, manganese, magnesium, mercury, and cobalt, they mentioned the chromosomal origin of all these metal except two isolates showed susceptibility to mercury after curing indicating a plasmid boron mercury resistance.[26] another study also referred to chromosomal origin of metal tolerance.[27] It suggested that the resistance mechanisms are chromosomally encoded or, more often, plasmids of different size and showing conjugative capabilities are carriers of metal-resistance genes.[27] Many other studies viewed the chromosomal origin of lead tolerance, Roan and Roan and Kellog did not find plasmids in lead resistance isolates collected from contaminated soils.[27] Similar report was presented for loss of plasmid linked drug resistance after treatment curing agent.[23]

Conclusion

The high percentages of MDR (30.76%) may indicate a serious case in both healthy and environmental states, in addition to high bacterial resistance in general against the selected antibiotics. While many other studies illustrate the bacterial tolerance against many heavy metals. The results of antibiotic susceptibility and heavy metal tolerance after bacterial curing showed that all the thirteen *Klebsiella pneumonia* isolates which isolated in this study were recorded the same result comparing with non-cured isolates, and that illustrate the fact that

all antibiotic susceptibility as well as metal tolerance of the selected isolates were coded by chromosomal gene, or the bacteria could resist antibiotics and heavy metals by using another strategy such as cell membrane permeability and/or pumping technique.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Toxicity of Cadmium Chloride on White Rats Liver and the Protective Role of *Brassica Nigra* Seed Extract

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Abstract

The study designed for the toxic role of cadmium chloride on the liver of mice from both a physiological and histological aspects. The animals were treated with a dose of 5 mg/kg of body weight for 30 days. And the protective role of *Brassica nigra* seed extract against cadmium chloride toxicity. This pilot study was conducted on 20 adult white rats divided into 4 equivalent groups including the control group: Animals in this group received a dosed of distilled water for 30 days. The placebo group was treated with cadmium chloride at a dose of 5 mg/kg of body weight for 30 days and returned as an infected control group. While in the third group, animals treated with cadmium chloride were dosed with *Brassica nigra* seed extract at a dose of 200 mg/kg of body weight for 30 days. The fourth group was dosed with *Brassica nigra* seed extract at a dose of 200 mg/kg of body weight for 30 days. After 30 days, liver enzymes including aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured by spectrophotometric method. In addition to making tissue sections of the liver. The treatment of rats led to a significant increase ($P \leq 0.05$) in liver enzymes compared with the control group. It also led to histopathology in the liver tissue, while the *Brassica nigra* seed extract acted as a protective role against the toxicity of cadmium chloride.

Keyword*: cadmium chloride, Brassica nigra, aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP).*

Introduction

The increased emissions of both minerals into the environment and their lack of ability to rapidly biodegrade have increased the risk of human exposure to them. The main methods of exposure to cadmium and lead are ingestion and inhalation due to their presence in food and air, as well as in tobacco smoke[1]. The American Agency for Toxic Substances and Disease Registry (ATSDR) has ranked cadmium seventh and lead second on its priority list of hazardous substances[2]. Cadmium poisoning is a global health problem that leads to infection in many organs, and in some cases it may cause death. Cadmium production, consumption and release into the environment without proper treatment resulted in air, water and soil pollution. And that long-term exposure to cadmium through water, air and diet leads to the problem of different organs of cancer problem for these organs[3]. Cadmium is a heavy metal, which is very toxic and its use in various forms in the chemical industry (dye, paint) as well as the metallurgical industries (alloys) [4]. Numerous studies have demonstrated its toxic nature to the liver by administering cadmium chloride to mice that produce cell necrosis and lipid changes [5].

The chemical is highly toxic the liver generally metabolizes it into a non-toxic chemical, but sometimes the non-toxic or less toxic substance also activates to the highly toxic metabolite. Therefore, the chemical burden increases stress on hepatocytes and leads to hepatic disturbance due to degenerative cells, necrosis, tumors, etc. [6] . Cadmium has the ability to spread through various organs of the body quickly, and most of the cadmium ingested enters the liver and kidneys [7] . The liver is the organ most targeted by cadmium toxicity

and hepatotoxicity is the main cause of death in the organism [8] .

Medicinal plants and processed natural products have an important role in eliminating many of the harmful liver effects caused by exposure to environmental pollutants such as cadmium [9,10] . A medicinal plant can be defined as if it has the use of any part of the plant or its seeds as a treatment [11] . People all over the world use the mustard plant. And it has been mentioned as the greatest herb ever. It is used in the treatment and prevention of many ailments such as cancer, as well as has antidiabetic , antioxidant and antimicrobial properties [12,13]. The therapeutic efficacy of the mustard plant is due to the fact that it contains selenium as well as magnesium and B-complex vitamins are also among its main ingredients [14] . *Brassica nigra* seeds also have important medicinal uses in treating rheumatism, joint pain, liver and spleen diseases, oral tumors, and a laxative. Recent studies reveal that the mustard plant has antioxidant and antimicrobial properties [15,16] .

MATERIALANDMETHODS :

A- **Prepare the animals :**

Use 20 white rats. Their ages ranged between (2.5-3) months, and their weights ranged between (190-220 grams). The animal was bred in the College of Veterinary Medicine, University of Tikrit. All animals were exposed to uniform environmental conditions in terms of ventilation and temperature, where the temperature ranged between (20-25) C , and the lighting period was 12 hours of light and 12 hours of darkness (natural lighting). Animals were fed with standard feed.

B- Prepare mustard seed extract :

After obtaining the mustard seeds, they were left to dry, after which they were ground by an electric grinder, and the extraction was carried out according to the method Parthiban *et al*. [17] .

C- Experience design :

The animals were divided randomly into four groups (5 animals in each group). After the preparation period ended, the animals were dosed daily for 30 days through tube feeding, as follows :

1- Group I : This group was injected with distilled water for 30 days.

2- Group II : This group was treated with cadmium chloride at a dose of 5 mg / kg of body weight for 30 days [18] .

3- Group III : This group was dosed with mustard extract at a dose of 200 mg / kg of body weight for 30 days [17] .

4- Group IV : This group was dosed with cadmium chloride with mustard seed extract at a dose of 200 mg / kg of body weight for 30 days.

D- Collecting blood samples :

At the end of the experiment, the animals were silent for 12 hours, after which they were anesthetized with chloroform. Blood samples were taken directly by heart stab, and (3-5) ml of blood were collected using a medical syringe and placed in plastic tubes with dry caps. Using centrifugation, serum samples were obtained. Samples were collected to determine the enzymes of aspartate and alanine transporter (AST and ALT).) And alkaline phosphatase (ALP).

E- Prepare histological sections:

After dissecting the animals, liver tissue was obtained, and then cleaned with water were fixed in 10% formalin for 24 hours, dehydration in increasing concentrations (70%, 80%, 95%, 100% and 100%), purified with xylene and then mixed with paraffin. . Upon analysis, all paraffin-embedded tissues were divided at 5 µM , and stained with eosin and hematoxylin. Samples were examined under an optical microscope with a magnification of 400 X [19].

Results

It is noted from Table No. (1) that the treatment of rats with cadmium chloride led to a significant increase ($P \leq 0.05$) in the level of ALT, AST and ALK compared with the control group. Whereas, dosing of rats treated with CdCl2 with Brassica nigra seed extract resulted in a significant decrease in the level of ALT and AST ALK compared with the group treated with cadmium chloride, However, no statistically significant differences were observed upon dosing of Brassica nigra seed extract on healthy animals.

Table (1) concentration of liver enzymes in blood serum.

Parameters / Groups	AST (IU/ml)	ALT (IU/ml)	ALP (IU/ml)
Control group	42.2±4 b	62.3 ± 5 b	29.4±2 c
cadmium chloride	86.3±6 a	97.6 ± 5 a	57.2±4 a
CdCl2 and Brassica nigra seed extract	33.4±7 b	66.4 ± 2 b	46.2±8 b
Brassica nigra seed extract	30.6±4 b	58.2 ± 4 b	44.1±3 b

-Values represent arithmetic mean ± standard error.

-Vertically different letters mean that there is a significant difference at a significant

level ($P \leq 0.05$) .

- The number of animals is 5 in each group.

Histological:

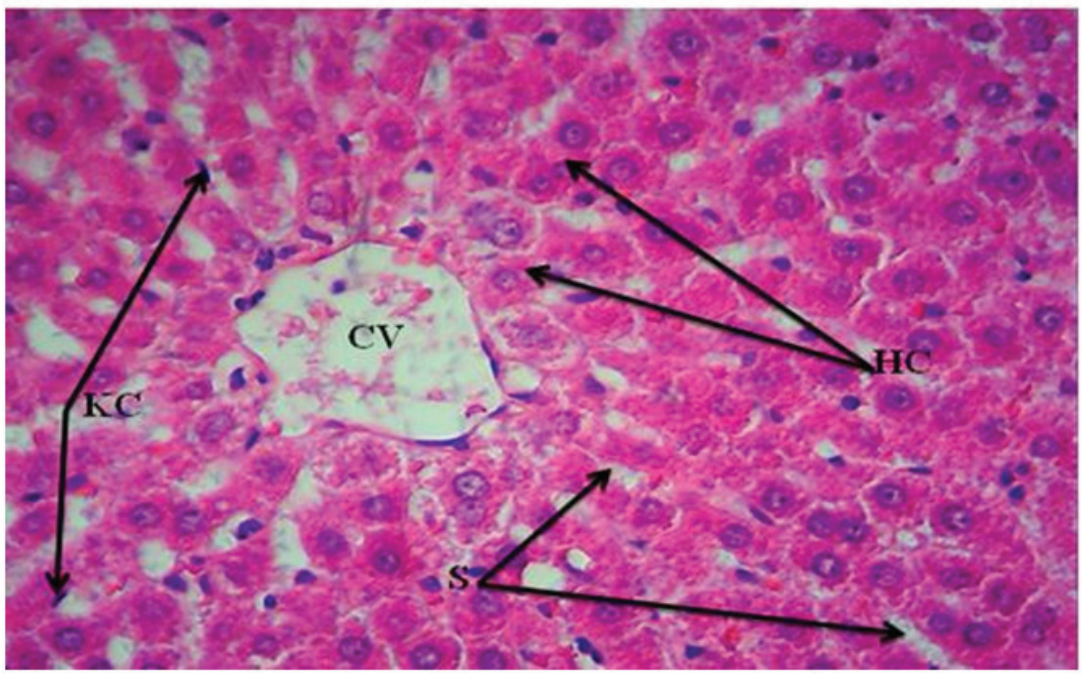

Image (1) of a rat liver section from the control group showing the central vein (CV), hepatocytes (HC) arranged in the blood vessels as ropes, as well as blood sinusoids cells (S) and Koffer cells (KC). H & E 400X.

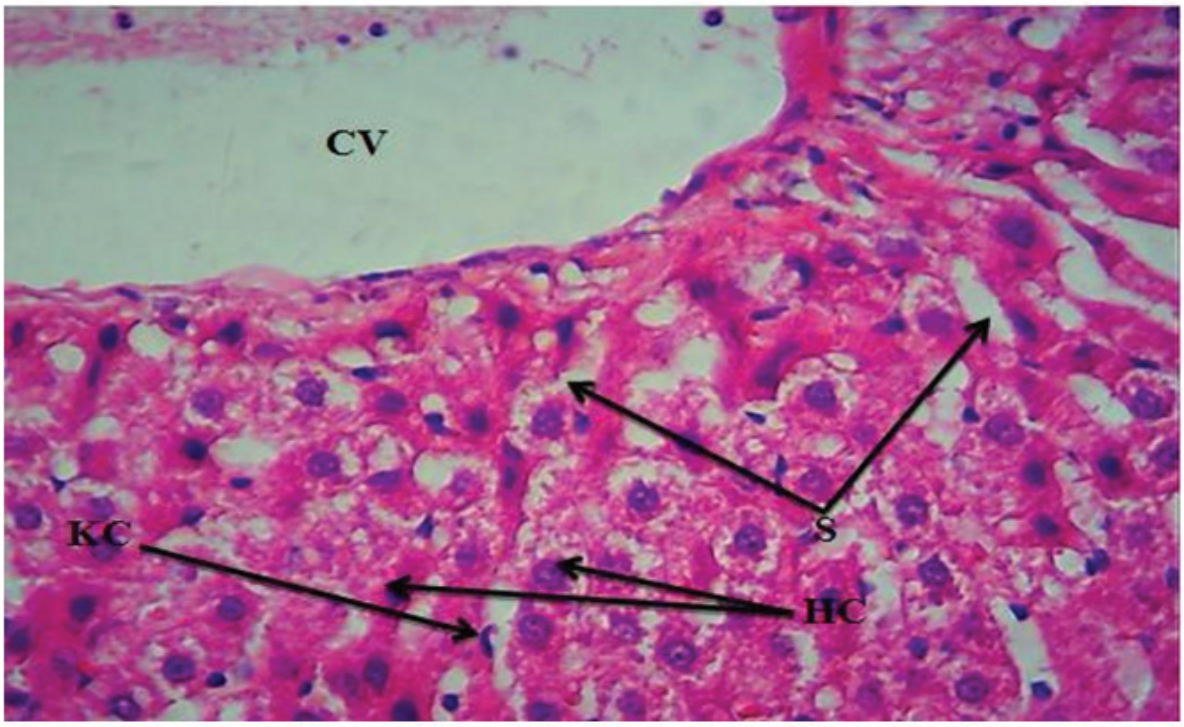

Image (2) section of rat liver of the group treated with brassica nigra seed extract shows the central vein (CV) and hepatocytes (HC) arranged vascularized in the form of cellular cords as well as blood sinusoids (S) and Koffer cells (KC) in their normal form.

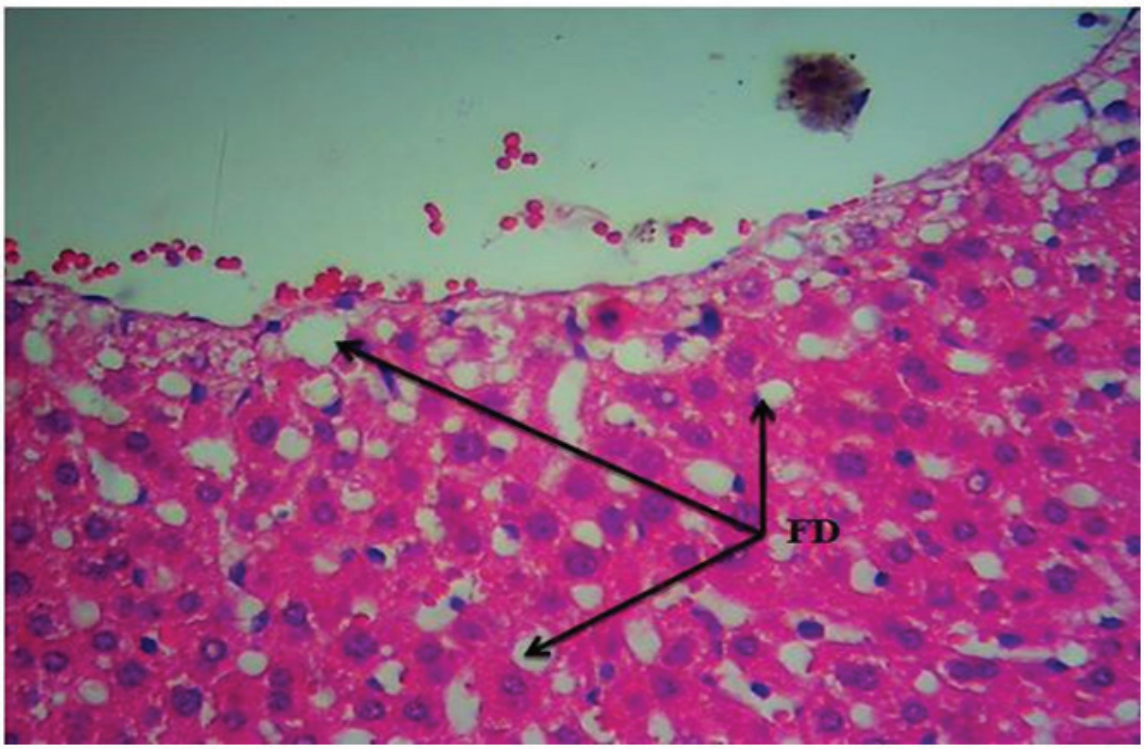

Image (3) of a group of rat liver section treated with cadmium chloride shows fatty degeneration in hepatocytes (FD). H & E 400X .

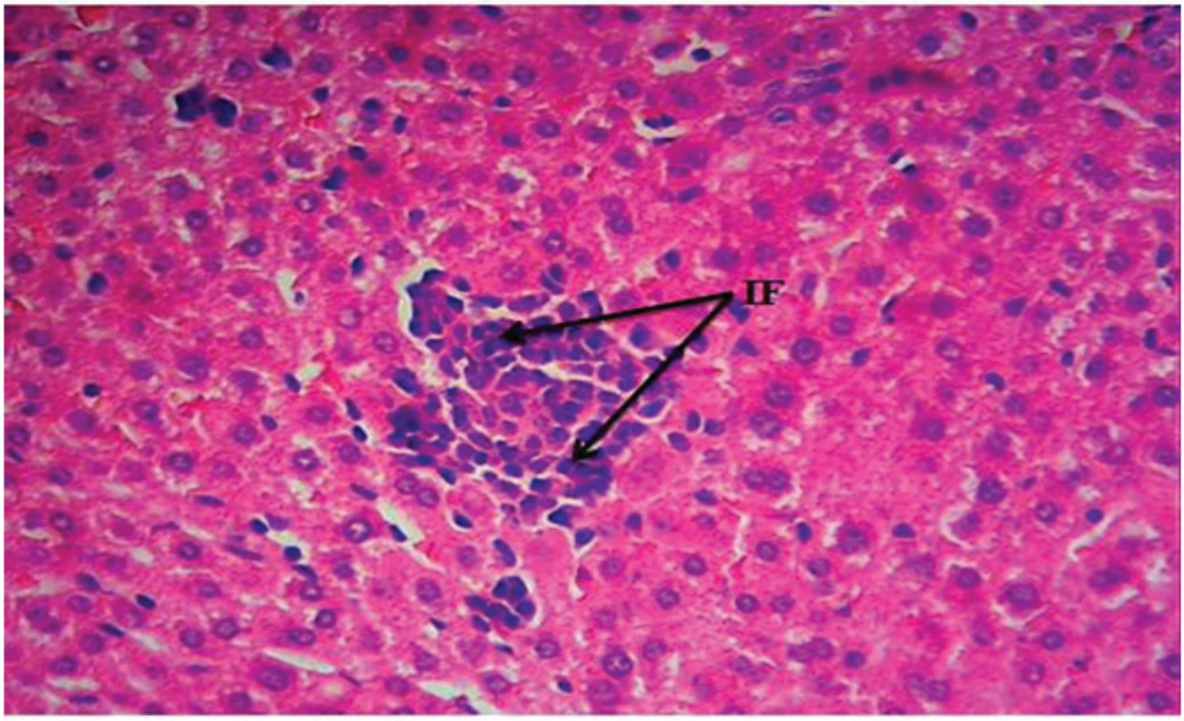

Image (4) of a group of rat liver section treated with cadmium chloride showing a focal infiltration of inflammatory cells (IF) H&E 400X .

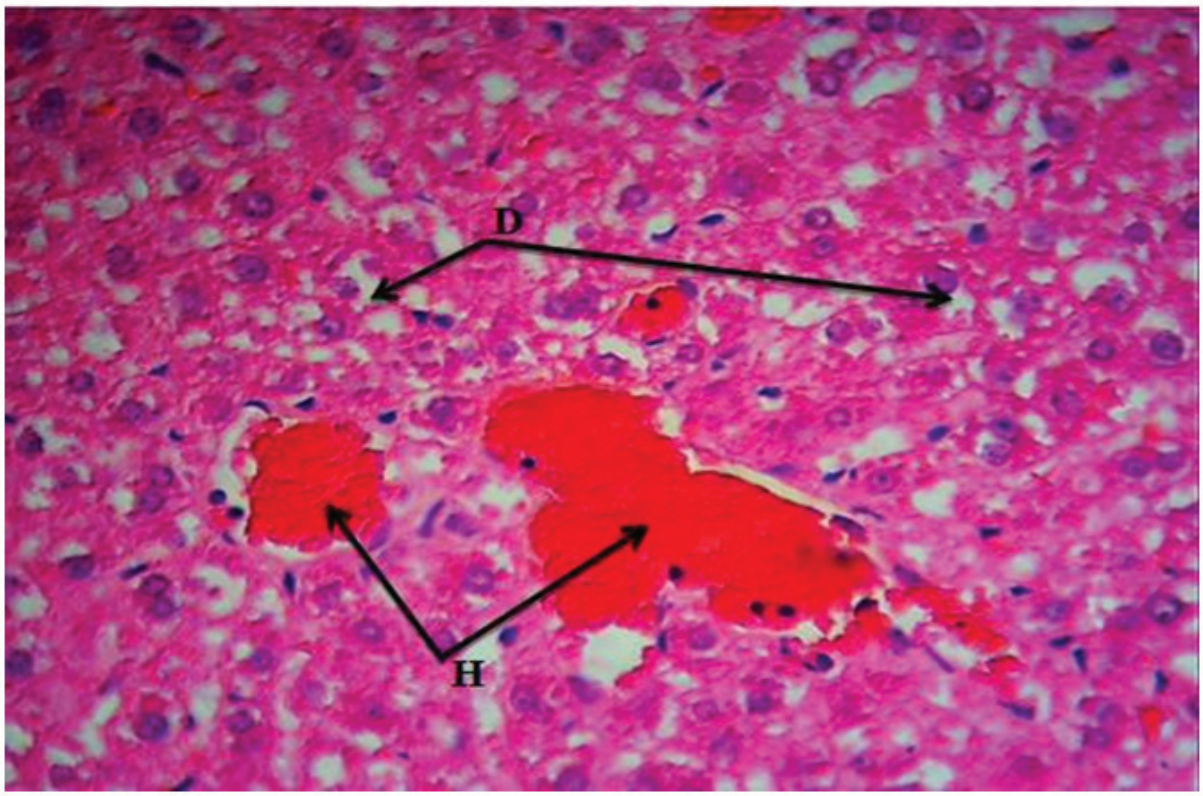

Image (5) of a group of rat liver section treated with cadmium chloride showing the degeneration of some hepatocytes (D) with hemorrhage (H) within the liver tissue H&E 400X.

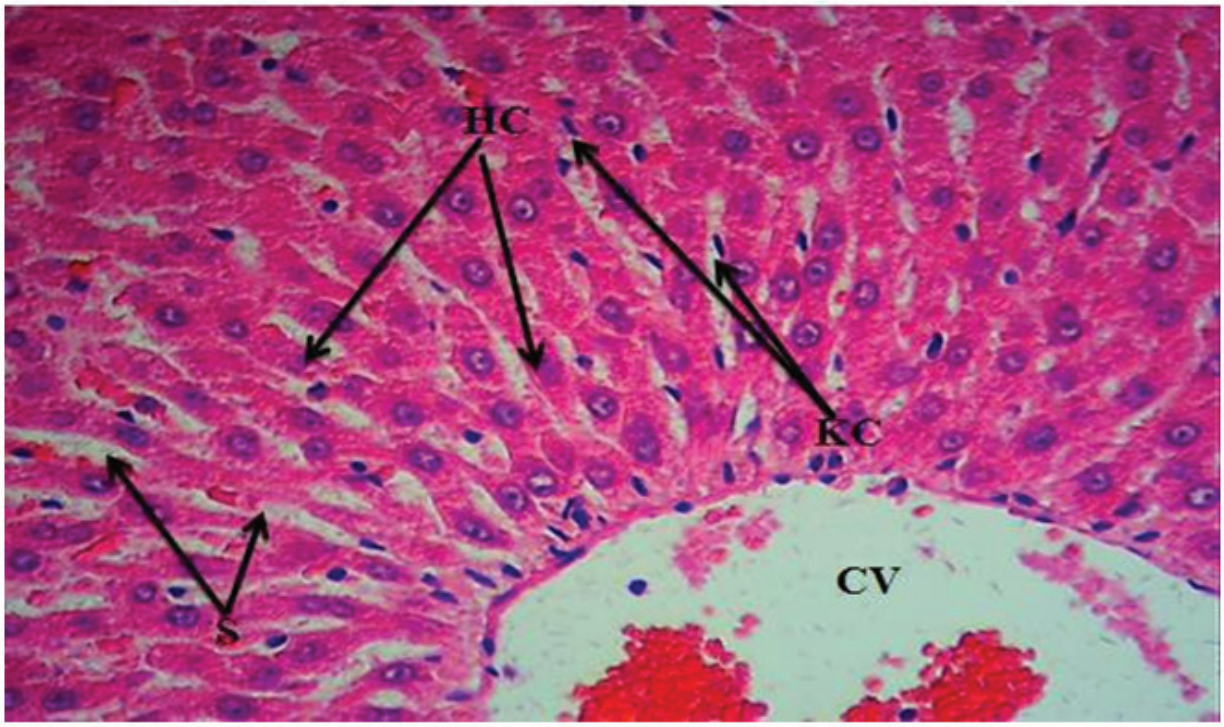

Image (6) section of the rat liver of the group treated with cadmium chloride and mustard seed extract showing the central vein (CV) and hepatocytes (HC) arranged vesicles in the form of cellular cords as well as blood sinusoids (S) and Koffer cells (KC) naturally. H & E

400X,

Discussion

Through Table No. (1) it is noticed that the treatment of rats with cadmium chloride led to a significant increase ($P \leq 0.05$) in the level of ALT, AST and ALP compared to the control group. These results agreed with the results of Mohamed *et al* [20] , And Sajjad *et al.* [21] . who found that levels of liver enzymes increased significantly in the group of animals treated with cadmium chloride due to the occurrence of hepatocyte toxicity.

As they observed that cadmium chloride caused a significant increase in liver enzymes . Cadmium causes toxicity to hepatocytes and its enzymes AST, ALP and ALT that are released into the bloodstream, and thus the level of these enzymes in the blood rises above the normal range [22].

As the evaluation of the activity of the enzymes AST and ALT is important for evaluating the function of cells of some tissues, especially the liver, it is known that both are present in hepatocytes, renal, muscle, and others. Therefore, their high activity outside the cell indicates the presence of functional impairment or the presence of damage to the cells of these tissues [23] , So the treatment with cadmium chloride to the use of radicals and active oxygen species (ROS) , which worked on loading neurons, [24] , And the increase in the level of these two enzymes explained the effect of cadmium in changing the permeability of the membranes of the liver cells, which leads to the leakage of these enzymes into the blood, or due to the occurrence of Hepatocellular necrosis that occurs as a result of toxic substances or the occurrence of liver cirrhosis and also leads to leakage of these Enzymes from broken cells and their arrival into the blood [25,26] .

However, when rats treated with cadmium chloride with mustard extract resulted in a significant decrease ($P \leq 0.05$) in the level of liver enzymes (ALT, AST and ALP) compared to group of rats treated with cadmium chloride . These results are in agreement with those of Rajamurugan *et al.* [27] . Who used *Brassica nigra* against liver and kidney damage . Rajamurugan *et al.* [28] . found that *Brassica nigra* extract has an antioxidant

effect in vivo. Also, *Brassica nigra* extract has been shown to have antioxidant activity in vitro . Several studies by previous researchers have confirmed that cruciferous vegetables can work as a good source of natural antioxidants because they contain high levels of carotenoids and phenolic compounds. Most of the antioxidant effects have been associated with the phenolic compounds that reduce levels of oxidative stress [29] . Kuyutork *et al*. [30] found that the hepatotoxicity of cadmium was affected in two ways, first by the onset of the inflammatory condition, and second by the toxic and direct effect of cadmium on liver cells. The present study showed that administration of *Brassica nigra* seed extract after cadmium chloride restored enzyme levels in animal serum as an indicator of the productive effect of mustard seed extract against cadmium-induced liver damage due to the presence of high levels of carotenoids and phenolic compounds.

Tissue sections of rat liver treated with cadmium chloride show clear fatty degeneration in hepatocyte (FD) . and Degeneration of some hepatocytes (D) with hemorrhage (H) . These results are consistent with several studies, including the study of Zhai *et al*. [31] and Albasha and Azab [24] . Who found that cadmium toxicity causes vacuoles in liver cells, increased nuclear chromatin density with compression of the nuclei in cells, in addition to degeneration of the fat mass of cells with large vacuoles occurring in the cytoplasm of nuclei of hepatocytes, In addition to other studies, it was found that cadmium causes the generation of active oxygen species (ROS) that lead to damage to cellular membranes due to oxidative damage to their lipid contents, and this leads to cell toxicity in the liver [32,33] .

While histopathological sections of the liver in rats treated with cadmium chloride and dosed with aqueous extract of *Brassica nigra* seeds showed that it has a protective role against toxic damage to cadmium chloride as it contains effective compounds against oxidative stress [34,29].

Conclusions

Through the research, we noticed that cadmium chloride has a toxic effect on hepatocytes histologically and physiologically, while *Brassica nigra* seed extract proved a good protective role against cadmium chloride toxicity.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

Funding: The work was supported by the researchers themselves.

Conflict of Interest: The author declare no conflict of interest regarding this research.

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Molecular Investigation of Quinolone Resistant Genes among Clinical *Psedomonas aeruginosa* Isolates

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Abstract

The current research was performed in Al-imam AL- Sadiq Hospital, Hilla Teaching Hospital, Babylon city, Iraq to investigate the resistance gene of fluoroquinolones (FQs) in *P. aeruginosa* clinical isolates of taken from different origins. Mutations arising at the bacterial gyrA, gyrB, parC, par E gene belong to DNA gyrase and topoisomerase IV are the fluoroquinolone tolerance essential mechanism in *P. aeruginosa*.. and over expression of efflux pumps . (80) clinical isolates collected by Transport swabs from various source [(burn 45(56.25 %), wound 15 (18.75 %), ear 3 (3.75 %), blood 2 (2.5 %), urine 4(5 %),sputum 11(13.75)] that initially identified by culturing on MacConkey agar, blood agar and cetrimide agar selective media for *Pseudomonas aeruginosa, P. aeruginosa* chromogenic agar then diagnosed by performing some morphological and biochemical tests. The second diagnosis to (10) isolates was done by VITEK 2 Compact system using VITEK 2GN,and by using PCR technique for genotyping detection by the 16s rRNA gene. A susceptibility test was applied to all (80) isolates using 9 antibiotics through resorting to Kirby-Pour method. The results of this test showed that isolates which represented the highest resistance to ciprofloxacin (71%),levofloxacin(75%) , aztreonam(87.5%), but isolates showed low resistance to impenem 18.75% and 0%resistant to meropenem. (gyrA, gyrB) subunit of DNA gyrase is target of flouroquinolon antibiotic has been screened by PCR reaction. The results showed that these genes present in *P. aeruginosa* at (100 percent, 100 percent,), respectively..

Keywords: *Fluoroquinolone, gyrA,gyrB. aeruginosa, 16s rRNA, antibiotic resistance.*

Introduction

P. aeruginosa is an ubiquitous bacterium known as a pathogenic microbe responsible for serious opportunistic infections, especially in the immunocompromised individuals. The spread of this organism in health institutions is very dangerous, with infiltrates, via any defect at the basic defense line of the human host, Through the skin, especially in the hospital intensive care units (ICUs) that lead to nosocomial infections,. The pathogenesis of *p.aeruginosa* is multifactorial., due to the existence of multiple mechanisms of veritable resistance to most antibiotics, resulting in the production of a variable set of cellular structures and extracellular molecules which play a significant role in increasing pathogenicity[1].Practicing hand hygiene and knowledge regarding the same is essential for reducing Health Care-Associated Infections (HCAI) and drug resistance. [2]Antibiotics are either cytotoxic or cytostatic to microorganisms, allowing the body's natural defenses such as the immune system, to eliminate them. [3]. A typical explanation for hospital-acquired infections is a rod-shaped Gram-negative bacteria *Pseudomonas aeruginosa*. While it normally does not affect healthy persons, it may effectively colonize any portion in a human body that retains enough moisture to create a Oh. Niche. *P. aeruginosa* is implicated in about 8-10 percent (51,000 Cases registered in the US in 2013) to all health-related infections in the healthcare environment. Alarmingly, multidrug-resistant strains were found in around 13% of these cases and a growing number of pan-drug-resistant specimens were identified that could

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not be treatment by any of the anti-pseudomonal agent that present in the clinic[4] . Drugs are been administered from various routes for delivery to the systemic circulation. Numerous routes such as oral, nasal, rectal, topical, parenteral, etc. [5]. Fluoroquinolones are categorized as being vigorous versus both the Gram-negative and Gram-positive bacteria as the broad-spectrum antibacterial products. (formally called simple quinolones). The effectiveness of fluoroquinolones can be due, in addition to broad-spectrum efficacy, so their features, like strong during oral administration bioavailability, beneficial pharmacokinetics and very low toxicity. While unfavorable reactions do occur when using fluoroquinolones. There are also some of the major antimicrobial agents that are, and in the sense to fluoroquinolones, where structural evolution has been resorted to leading to new analogues of enhanced potency. The main use of fluoroquinolones was to treat urinary tract infections (UTIs), Respiratory-tract diseases, diseases that are sexually transmitted, infections of gastrointestinal and stomach, infections of skin and soft tissues, and the infections of bones and joints[6].Quinolone consist of bicyclic ring structureand different functional groups are substituted at positions which are shown in structure**.** The structure, activity and relationship of fluoroquinolones have been investigated, the C-7 position of fluoroquinolone posseses the great impact on potency[7]. DNA gyrase and topoisomerases are the major targets of quinolones. For DNA transcription, recombination, replication and simplified remodeling of DNA, these enzymes are important and act by performing transient single- and double-strand breaks. DNA supercoiling is modulated by topoisomerases to allow proper operation and interaction with proteins[6]. One of the largest problems facing western medicine (AMR) is Antimicrobial resistance. It is predicted that when no action is taken, AMR will cause 10 million deaths. 2050, mainly due to the infections resulted from Gram-negative bacteria (GNB). GNB can be graded according to the degree of resistance as either to the pan-drug resistant (PDR) or the extensively drug resistant (XDR) the multi-drug resistant (MDR). The former exhibiting the resistance to all forms of antibiotics[8]. FQ resistance is produced by mutation in the bacterial chromosomal gene encoding DNA gyrase or topoisomerase IV or by successful drug transfer out of the cell [9]. *Pseudomonas aeruginosa* is very common in nosocomial infections. It is the causative agent of about 10-15% of these infections. It exhibits high resistance to different groups of antibiotics by intrinsic or acquired mechanisms. This remarkable resistance makes the treatment of *P. aeruginosa* infections very difficult. It is of great value to investigate new drugs to overcome such resistance either by use of these agents individually or in combination with antibiotics. [10] Patients with wound infections have longer hospital stays, more expensive hospital admissions, and increased mortality. The development of wound injuries depends on the safety of protective skin functions. [11]. Urinary Tract Infection (UTI) remains one of the most common bacterial infections and second most common infectious disease . [12]. Hospital-acquired infection or nosocomial infection applied to any clinical infection that was neither present nor was in its incubation period when the patient entered the hospital. Nosocomial infections may also make their emergence after discharge from the hospital, if the patient was in incubation period at the time of discharge. [13]. Antibiotics which are used for animal agriculture will be ingested by human beings, when they consume food. Antibiotics used for agricultural use will affect the environmental microbes. About 90% of the antibiotics given to live stock are excreted in urine and stool, and then widely dispersed through fertilizer, ground water and surface run off . [14]. Multidrug Resistant (MDR) organisms thus transmitted through the hands of health care workers are a major cause of HCAIs. MDR organisms are microbes (predominately bacteria) resistant to more than one class of microbial agents. [15]. In recent years, In pharmacy, overuse of FQs has facilitated bacterial resistance to FQs, posing a significant anti-infective treatment challenge of *P. aeruginosa*. Important evidence has reaveld the resistance for FQs is largely attributed to: (i) The point mutations in the genes of DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE), (ii) the presence of transmissible plasmid-mediated quinolone resistance (PMQR) determinants, and (iii) gene mutations controlling efflux pump expression and reduced porin outer membrane expression. This possessions benefits from the interplay between the drug efflux mechanisms and the microorganism's low external membrane permeability [16]. *P. aeruginosa* has at least 12 structural genes for multidrug efflux pumps belonging to the MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM efflux

pumps family of resistance-nodulation-cell division (RND) transporters. It has been found to be of therapeutic significance. The efflux pumps MexAB-OprM leads to this organism's inherent tolerance to quinolones, tetracycline, chloramphenicol, novobiocin, macrolides and β-lactams, and its over-expression confers cross-resistance or diminished susceptibility to several antibiotics[17]. Furthermore, *P. aeruginosa* also exhibits insubordination toward conventional antimicrobials due to its capacity to create protective communities known as biofilms. Before *P. aeruginosa* becomes more capable of environmental stress, host immune variables, It is used in the biofilm matrix and antimicrobial therapy [18]. MDR *P. aeruginosa* has been a worthy anxiety in the treatment of nosocomial infections, largely because its has inherent and acquired immunity to a wide range of antimicrobial agents[19].

Materials and Methods

Collection of clinical samples

overall of 80 clinical samples that collected from varied clinical sources of patients and teaching laboratories of (AL-Imam Al-Sadiq Hospital, Hilla Teaching Hospital), in Al-Babylon city, Iraq during the period from 15 Septemper to 15 November ,2020. Samples which were collected by sterile swabs and containers had been cultured on macConkey agar and blood agar, to get pure colonies subculture done on macConkey agar, incubated for overnight at 37C°. The most common origin of isolates were burn (56.25%), followed by wound (18.75%), sputum (13.75 %), and urine (5%). Isolate confirmations were conducted using conventional biochemical tests and confirmed by the Vitek 2 using VITEK 2GN system, followed by the genotyping detection using PCR technique.

Antibiotic Susceptibility Assays

The *P. aeruginosa* isolates were seeded with Muller-Hinton agar plates (by spreading) and the antibiotic discs were then added according to CLSI (2019). The inoculum that be used in this test were prepared by adding growth from 2-5 separated colonies grown on cetrimide agarplates(Himedia/India) to 2.5ml of nutrient broth. It was conducted using a pure culture of previously identified bacterial isolates. This culture was then incubated for (3-4 h) to create typical mild turbidity bacterial suspension equivalent to the standard McFarland drain (0.5). The growth was then roughly standardized to 0.5 McFarland and the standardized inoculums were derived from the sterile swab. On a Muller-Hinton spread, this inoculum was propagated. On the surface of the medium atevenly spaced intervals, the antibiotic disks were mounted with flamed forceps. Incubation was normally at 37oC overnight. Inhibition zones for antibiotics is assessed. In order to assess the sensitivity or tolerance of the organism to each antibiotic, zone sizes were relative to average (CLSI, 2019). 9 antibiotic discs were used as follows: amikacin (30 µg), gentamicin (10 µg), tobramycin, , ceftazidime (30 µg), , imipenem (10 µg), meropenem (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg),.

DNA extraction Genomic DNA

This process was done according to the genomic DNA purification Kit complemented by (G-spin^{TM}). When isolate that inoculated in Luria broth (LB) media (incubated at 37C ° for 24 hours) .

PCR reaction

PCR technique was used to amplify 16s rRNA and gyrA ,gyr Bgenes, using specific primers (table 1). A volume of 25µL was used for amplification in accordance with the user manual of the manufacturer company, which included 12.5µL of master mix, 1.5µL of each primer, 3 µL of the DNA template, and 6.5 µL of nuclease free water. The proper program for this experiment is presented in (table 1). Several trails were conducted to determine the optimum conditions to amplify 16s rRNA and gyrA,gyr B genes in a thermal-cycler device depending on different primers and the DNA template. Afterward, 5 µL was analyzed to detect 16s rRNA PCR product through electrophoresis on 1% agarose gel containing 3 µL of Red safe, and then viewed on UV transilluminator.

DNA sequencing

In the current study, PCR products of the 3isolates (for 16s rRNA) were sent to the MacroGenecompany, South Korea, to define the basic nucleotides sequences for the genes. The results of sequencing were then analyzed by BioEdit and MEGA7 software with the NCBI engine.

Table (1): Nucleotides sequences for primers

Gene	primer	sequences	Expected size (bp)	Annealing
16SRNA	Forward	5-TGCCTGGTAGTGGGGGATAA-3	505bp	55 Cº for 30 sec.
	Reverse	5-GGATGCAGTTCCCAGGTTGA-3		
gyrA	Forward	TGACGGCCTGAAGCCGGTGCAC	418	58 Cº for 30 sec.
	Reverse	GCCCACGGCGATACCGCTGGA		
gyrB	Forward	GCGGTGGAACAGGAGATGGGCAAGTAC	510	58 Cº for 30 sec.
	Reverse	CTGGCGGAAGAAGAAGGTCAACA		

Results

Amongst the 80 clinical samples, from different sources were obtained. The isolates of P.aeruginosa distributed among clinical samples based on their source are listed in (Table 2). All clinical isolates were identified as *P.aeruginosa* by a set of a variety of techniques (biochemical tests, cultural, pigment production and then (10isolates) by VITEK2 Compact.

Antibiotic sensitivity test

Eighty isolates (PA1 to PA80) of *P. aeruginosa* were tested against 9 common antibiotics. According to the results in Fig (1), the antibiotics susceptibility test to (80 isolates) showed that the percentages of the resistant toward these antibiotics were as follow: levofloxacin 60 (75%),ciprofloxacin 57 (71%), gentamycin 62(77.5%), Amikacin 59(73.5%) ,Tobramycin 66(82.5%), Impenem 15(18.75%), Meropenem 0(0%),Aztreonam 70(87.5%) , ceftazidim 55 (68.75%)

Table(2): Distribution of P.aeruginosa isolates.

Clinical samples	No. of isolate	Percentage %
Burn	45	56.25
Wound	15	18.75
Ear swap	3	3.75
UTI	4	5
Blood	2	2.5
Sputum	11	13.75

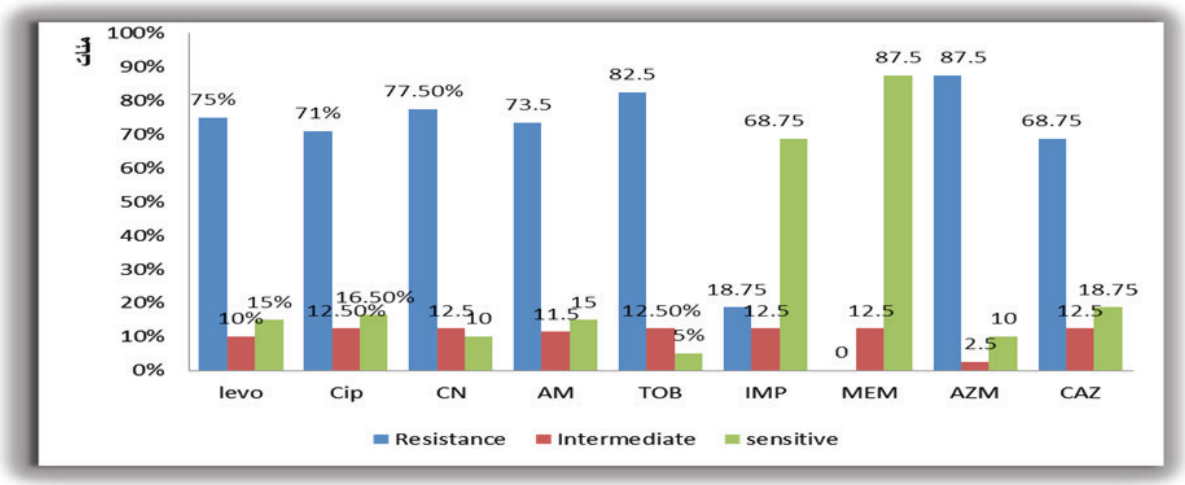

Figure (1): Susceptibility patterns of *P. aeruginosa* via using different antibiotics in this study.

In the present study, the highest resistant rate across the antibiotics were found with flouroquinolon (Levofloxacin, Ciprofloxacin) (75%), (71%) respectively aminoglycoside(Gentamycin,Amikacin,Tobramycin), (77.5%), (73.5), (82.5) respectively while the lowest resistant rate were found with carbpenem(Impenem(18.75%),Meropenem(0%)When the, aminoglycoside, or quinolone is ineffective, This result coincided with a previous study [20] in Babel city, which found that the resistance of *P. aeruginosa* isolated from burns and wounds to gentamicin and amikacin, was 70%, 93.4%,Tobramycin 83.4% respectively. Also, Compatible with the results of [21] in Baghdad, that *P. aeruginosa* were resistant to ceftazidime,(77.7 %) and disagree with [22] who found the resistance range was 44.4 %, ,38.8 % for ciprofloxacin, levofloxacin, respectively. This study showed that high number of *P. aeruginosa* isolates (38/80) (47.5 %) were multi-drug resistant (isolate resistance at least to 3 different categories of antibiotic) .This result agree with study in Iran [23] which found (44.4 %) of the isolates were MDR, and with [24] in Tehran, Iran which found 46.5% of the isolates were MDR and lower the finding of study in Western Yemen [25] was found 65.2% of the isolates were MDR. In the present study the MDR isolate are taken in high rate from burns and wound and lower than from sputum

confirmation detection of *p.aeuroginosa* by 16SRNA

Methods of genotype-based detection circumvent the variable phenotype issue and provide more precise identification of organisms. However, there is a barrier to genotypic recognition assays due to the taxonomic uncertainty, unclear phylogeny, and lack of genomic sequence evidence of the thousands of species within the large genus Pseudomonas. The 16S rRNA gene is the component of the DNA widely used in bacteria for taxonomic purposes[26] Because of the outcomes of rotten studies such as biochemical tests that have always failed to accept the identity of this isolate, the PCR commodity 506bp Statistic shows this testing accreditation on the universal primer pair used (Fig.2A). The amplification findings were compatible with the online amplification of the same primer pair with the figure of *P. aeruginosa*.

Molecular detection gyrA, gyrB

PCR study for GyrA subunits of topoisomerase II genes revealed the presence of genes is 100 % among (10) *P. aeruginosa* isolates. as shown in Fig. (2B). The results of primer **Gyr B** where it showed That all (10) *P. aeruginosa* isolates possitive as shown in (Fig. 2C).

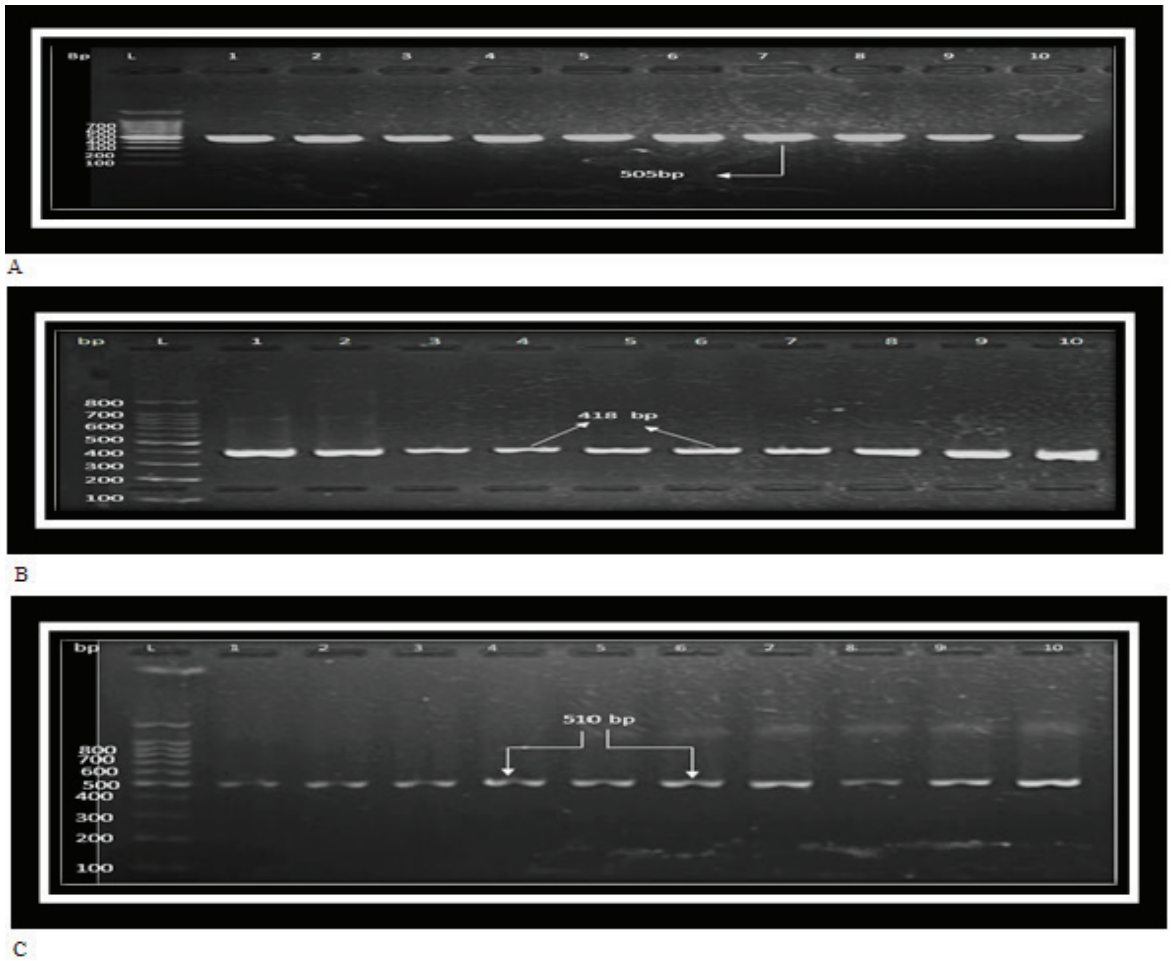

Figure (2): A)1.5% Agarose gel electrophoresis at 70 volt for 90 min of PCR for 16srna genes (505bp) ; lane M represent DNA marker size(100bp). Lanes (1,2,3,4,5,6,7,8,9,10,) were positive.

B): 1.5% Agarose gel electrophoresis at 70 volt for 90 min of PCR for Gyr A genes (418bp) ; lane M represent DNA marker size(100bp). Lanes (1,2,3,4,5,6,7,8,9,10,) were positive

.

C) 1.5% Agarose gel electrophoresis at 70 volt for 90 min of PCR for Gyr B genes (510bp) ; lane M represent DNA marker size(100bp). Lanes (1,2,3,4,5,6,7,8,9,10,) were positive

fluoroquinolones such as ciprofloxacin and levofloxacin, which act by inhibiting the activity of target enzymes, DNA gyrase, and topoisomerase IV, with both enzymes playing a major role in DNA replication. Heterotetrameric enzymes consist of two subunits encoded by gyrA, gyrBand parC, parE, respectively, DNA gyrase and topoisomerase IV. The genes gyrA and gyrB are respectively homologs of parC and parE. Fluoroquinolone tolerance pathways in *P. aeruginosa* include DNA gyrase and topoisomerase IV mutations in efflux pump system overexpression and intrinsic membrane impermeability [27] .

Phylogeny Tree based on study of pairwise sequences:

The three sequence of(AL-Imam Al-Sadiq Hospital) isolates of *P. aeruginosa* with close related bacterial sequences were by MEGA 7 software multiple alignment constructing the phylogeny tree. Thus, two aims are provided: firstly the specify of primer pair designed for P. aeruginosa to be confirmed nor closed related Enterobacteraceae bacteria such as E.coli neither other Citrobacter spp.; secondly, as a result the P. aeruginosa stains 1,2 and 3 came with reference strains of *P. aeruginosa* is shown in one cluster, with accession numbers CP033744.1,CP033742.1 and CP024677.1, although became so with the E.coli. in separate clusters

other *P. aeruginosa* , Figure(3)

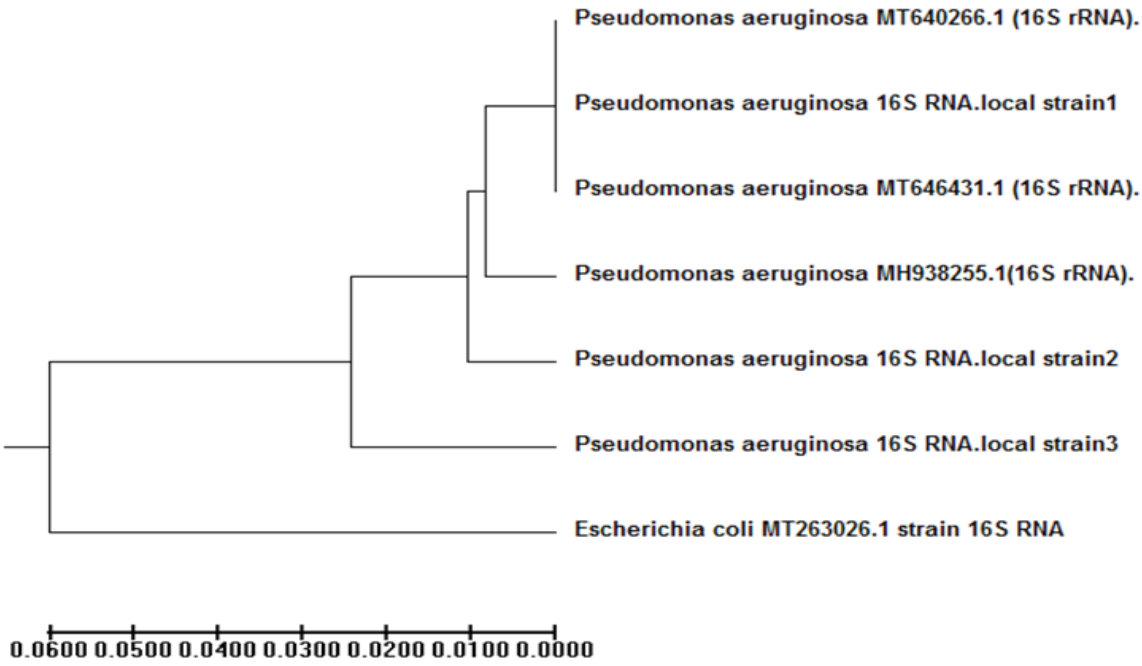

Figure (3): The phylogeny tree of three isolates of P. aeruginosa 1,2 and 3 was obtained from the NCBI website with other reference strain sequences deposited in genbank and it is performed in MEGA7 program. Using the method called UMPG, the evolutionary distances were determined

Multiple alignment of *P. aeruginosa*:

For more conformation of accuracy for amplification of targeted partial sequence of 16s rDNA gene, the three selected of *P. aeruginosa* strains 1,2 and 3 the sequence variance was spontaneously generated and the two isolates demonstrated a strong identity with reference strains of P. aeruginosa with accession numbers MT640266.1 and MH938255. The multiple alignment was done on the basis of BioEid 2.7. fig(4). The three local strains under interest shown polymorphism in their sequence compare with leading sequence of reference strain MT640266.1.

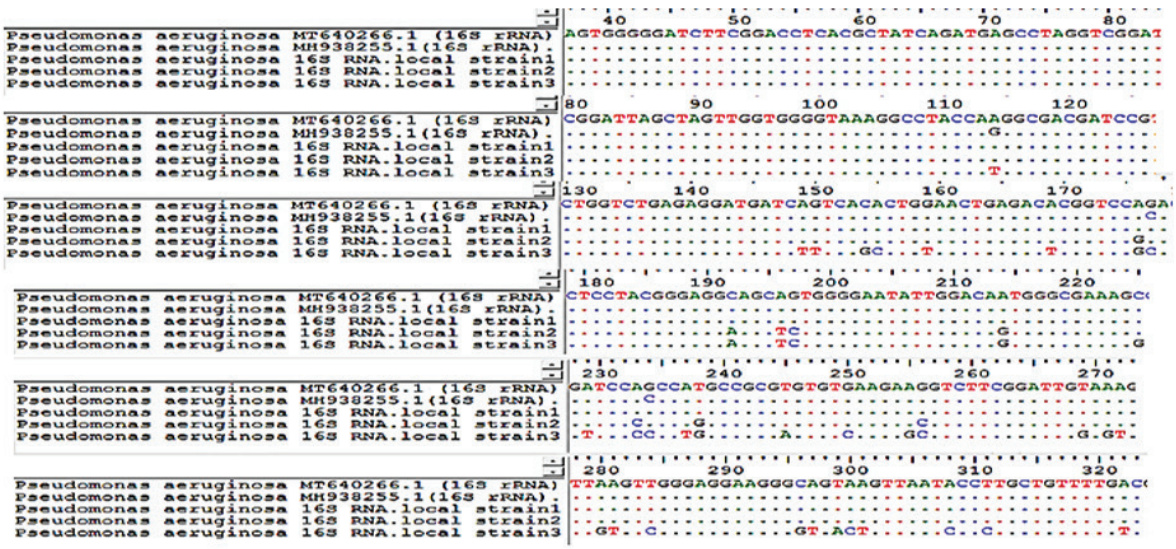

Figure (4):Multiple alignment sequences of *P. aeruginosa* strains 1,2 & 3 with two reference strains of *P. aeruginosa* with accession numbers MT640266.1 & MH938255. was carried out on the basis of BioEid 2.7 program.

Conclusion

The current study conclude presence of genes responsible for quinolone resistance among all studies *P. aeruginosa* isolates

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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Clinical Diagnostics in Patients with Cerebral Infarction Due To Covid-19

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Abstract

The Corona Virus Disease 19 (COVID-19) outbreak is a deadly disease of concern to the world, including Indonesia. Reported cases of death show a high number with more than 3800 deaths reported. This disease can induce Cytokine Storm Syndrome (CSS) and interfere with the Blood-Brain Barrier (BBB), causing coagulopathy and hypoxia, which in turn lead to cerebral edema in the brain. This research uses a case study approach, with a detailed examination of one event. In this case, cerebral infarction was caused by COVID-19 as evidenced by laboratory results in the presence of symptoms of inflammation, CSF, sepsis, and coagulopathy. These symptoms can result in cerebral infarction and hypercoagulability. therapeutic interventions are carried out in reducing inflammation, reducing cerebral edema, and preventing secondary infection. COVID-19 in this case can have a deleterious effect on patients with cerebral infarction. Proper treatment is needed to give better results.

Keywords: *Diagnostics, Cerebral infarction, Corona Virus Disease 19 (COVID-19)*

Introduction

The Corona 19 Virus Outbreak (COVID-19) is of global concern and has had a deadly impact on society (Ahmad & Rathore, 2020; Ellul, et al., 2020), including Indonesia. More than 3800 cases of death were reported in Indonesia (Covid Task Force, 2020)[1]. This epidemic certainly has an impact on every aspect of people's life, including the economy, health, and psychology (WHO, 2020). Covid-19 can be transmitted through direct contact or by human-to-human saliva droplets (Ahmad & Rathore, 2020; Wang, Wang, Ye, & Liu, 2020). In his research revealed that this plague can spread to the brain through the olfactory epithelium. In the brain, this outbreak can induce Cytokine Storm Syndrome (CSS) and severely disrupt the Blood-Brain Barrier (BBB), resulting in hypoxia and coagulopathy. The result of this process causes cerebral edema (Ahmad & Rathore, 2020; Chen, et al., 2020; Nordvig, et al., 2020). Symptoms that arise from this outbreak are dizziness, headache, decreased level of consciousness, and seizures (Chen, et al., 2020; Whittaker, Anson, & Harky, 2020)[2]. Cerebral infarction is reported as a result of cerebrovascular complications of COVID-19 and tends to occur in individual sufferers with comorbidities such as diabetes, hypertension, dyslipidemia, and vascular disease. Also, coagulopathy is associated with inflammation, with endothelial and platelet activation, dehydration, and cardiac embolism which is the cause of stroke[3].

Coronavirus Case

The 68-year-old man came to the hospital with a weak body condition 1 day before admission. Three days before admission, the client experienced fever, headache, decreased appetite, and shortness of breath. Six days before entering, the patient attended the funeral procession of his relative, who was suspected of being infected with the COVID-19 virus. The client denied not consuming coffee and smoking. His vital signs are 210/107 (blood pressure), 100 (pulse), 10 (respiration rate), and 37 (temperature). Physical examination results reported a decrease in Glasgow Coma Scale (GCS) with global aphasia (E4M5Vx). The client also did not show neck stiffness, middle left facial palsy, and a positive Babinsky sign. Head CT scan showed brain infarction in the right hemisphere. The laboratory test results reported leukocytosis (13400 / µL), positive SARS-CoV-2 IgM antibody, and positive SARS-CoV-2 IgG antibody. Chest CT scan also revealed atypical bilateral

pneumonia with bilateral pleural effusions. The client was initially treated with citicoline, clopidogrel, aspilet, azithromycin, acetaminophen, ceftriaxone, zinc, vitamin B, dan vitamin C[4].

The client on the first day his condition worsened. The client has a decreased level of consciousness, accompanied by fever and seizures. Blood pressure 196/137 with temperature 38.5[5]. The GCS decreases to E1M2V1 and neck stiffness occurs. Hypertonicity was observed in all extremities. Laboratory studies showed leukocytosis (18700 / µL), increased D-dimer (11.8 µg / mL), increased lactate (4.0 mmol / L), increased ferritin (<1200 ng / mL), increased quantitative C-Reactive Protein (308.2 mg / L)), increased procalcitonin (4.82 ng / mL), and respiratory alkalosis (pH 7.542)[6]. Polymerase Chain Reaction (PCR) from nasopharyngeal swab was positive (Cycle threshold (Ct) 38.62)[7]. Aspilet and clopidogrel were discontinued, and treatment for meningitis was started. Ampicillin, dexamethasone, phenytoin, and levofloxacin were added to the regimen. On the fifth day was given amlodipine and telmisartan, while phenytoin was replaced with levetiracetam.

After entering the sixth day, the client's condition improved. The client can open his eyes spontaneously and follow instructions, although it is still difficult to speak. The client does not feel headaches, fever, and tightness. Her blood pressure dropped to 152/102 with GCS E4M6V4. You can still feel the stiff neck. On day 10, the client was fully conscious (GCS E4M6V5) and transferred from the COVID-19 special intensive care unit to the neurology high care unit, then transferred to the general neurology ward, and finally discharged[8].

Method

This research uses a case study approach. According to Bogdan and Biklen (1982), a case study is a detailed examination of one setting or one subject, or one particular event. Placing the research subject as a case or a way of looking at the research subject as a 'case'.

A case is something that is seen as different from what generally happens, both positively and negatively. Performed in real-life conditions, examining the subject in conditions related to the context. Involves multiple data sources. In conducting case studies, researchers can stand on a certain theoretical point of view.

Result and Discussion

The brain infarction discussed in this study can be linked to the COVID-19 outbreak, which is transmitted through direct contact with other individuals through saliva droplets from patients who have contracted the outbreak. At first, the client contracted the COVID-19 outbreak as evidenced by the results of serological tests (positive SARS-CoV-2 IgM and IgG antibodies). Clients who are diagnosed with COVID-19 are known through their PCR test results (positive from a nasopharyngeal swab).

Gullian et al., (2020) reported that cases of simultaneous cerebral infarction were found in several arterial areas in COVID-19 clients, with symptoms of an increase in the number of white blood cells, fibrinogen, D-dimers, lactate dehydrogenase, CRP, and ferritin. Brain Magnetic Resonance Imaging (MRI) shows ischemic lesions involving the posterior cerebral artery (PCA), and superior cerebellar artery (SCA), middle cerebral artery (MCA). Clients included in this study reported similar laboratory findings (leukocytosis, elevated levels of ferritin, lactate), and elevated levels of CRP, which indicate inflammation, particularly CSF. Manolis et al., (2020) suggested that D-dimer was proposed as an important key in clinical practice. They also describe viral coagulopathy involving macrovascular and microvascular structures, hypercoagulable states, thrombosis/thromboembolism, and fibrinolytic disorders. The client's D-dimer in this study has increased.

Procalcitonin (an indicator of sepsis) also shows an increase and is suspected to be the cause of worsening the client's condition. All of the factors described above can lead to hypercoagulability and cerebral infarction. The therapeutic regimen was immediately changed to a meningitis regimen to prevent secondary infection, reduce cerebral edema, and reduce inflammation. MRI and spinal tap were not performed in this study due to the limitations of the procedure and the client's health condition. On the first day, the client's condition worsened, then started to improve after 7 days, and was finally allowed to go home on the 20th day with a minimal deficit. This condition gets better because of the reduction in the inflammatory process.

Conclusion

COVID-19 in this case can have a devastating effect on patients with cerebral infarction. Proper treatment is needed to give better results.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Community Empowerment Policy Toward Graha Mina Tourism Model in Natural Resource Management at Kepulauan Seribu

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Abstract

This study aims to analyze community empowerment policy through Graha Mina tourism in natural resource management at the Kepulauan Seribu group. This study was conducted at Pulau Kelapa and Pulau Harapan, Kepulauan Seribu, by using a study case method with a qualitative approach. The result shows :(1). The policy of community empowerment has been conducted as follows: Intensification program of fish breeding, community empowerment with captured fishery based, seaweed farming fishery based, cage cultivation fisheries based, tourism and protection based, counseling and training based, and strengthening community institution based; (2) fisheries based of cage cultivation become a priority program that need to be applied at Pulau Kelapa and Pulau Harapan. Based on the finding, it was concluded that the community's understanding of natural resource management in the island cluster is still low. Besides, the model and construction of Graha Mina marine tourism is a novelty in this research.

Key words: *Policy, Graha Mina Tourism Empowerment Model, Management, Natural Resource*

Introduction

Kepulauan Seribu consists of a group of small islands and has highly productive natural resources and biodiversities, such as mangrove, coral reefs, seaweed, and other creatures. The most productive utilization of kepulauan Seribu areas is fishery capture and cultivation, along with tourism[1]. Mainland natural resource is limited, on the contrary, coastal and ocean natural resource, and environmental services are highly potential[2]. The human resources of kepulauan Seribu are generally as fishermen, and as seaweed farmers, fish farmers, merchants, and civil servants. Poverty, low education and knowledge and lack of information as a result of isolation of small Islands are characteristic of small island communities[3].

The results of development have not been enjoyed by people living in remote island areas. The community was laid as a development object and not as the subject of development[4]. Thus, it takes high attention and desire to advance the condition of coastal community as a maintainer of small islands resources in order to take place sustainably or in other words it is necessary to increase community participation. Community participation is a dual interactive communication between the government and society (Li & Liu, 2019)[5]. Additionally, active community participation is an important process to increase long-term sustainable tourism heritage (Zhao, Hu, Liang, & Qian, 2019)[6].

Community participation is more focused on how the quality of the resources owned. Human resource plays an essential role in organizational change. Specifically, human resources can conduct specific practices in commitment development for a change (Baran et al., 2018)[7]. Society in Kepulaun Seribu generally works as fishers, and some of them work as seaweed cultivators, grouper cultivators, merchants, and civil servants. Several problems that occurred in the island groups are coral reef damaged, water pollution, mangrove logging, fish bombing, and people who live in the area are littering and voiding directly to the sea. All those problems need to be solved by involving society in the processes and practices[8]. Community need to have

abilities and creativities to manage natural resources becoming a potential for coastal economy[9].

Community empowerment policy concept is required as the direction of empowerment program objectives so that people do not throw garbage and bridge to the sea so that the water condition is not polluted because of household waste. Because if the sea is polluted then many dead fish that result in society will become increasingly poor. Thus it takes some related policies[10].

State of the art in this research is that there is a graha mina model tourism that manages natural resources so that the coastal economy can improve through tourism and fish cage cultivation. The use of Graha Mina Wisata Bahari is the structure of the building does not make the fish easily attacked, not easy to stress and the water quality can be stable, have a good water exchange (current), not easily polluted from the fuel of the passing vessel. Graha mina model is the researcher's finding as well as a novelty in this study. This study aims to analyze community empowerment policy through Graha Mina tourism in natural resource management at the Kepulauan Seribu group[6].

Literature Review

Natural Resource Management

Natural resource management is a model created by participation that involved stakeholders so that the activities are considered as the participative model (Bonté et al., 2019)[11]. Natural resource management in community-based is a governance mechanism that directly involves the societies to have control toward the use of natural resources (Addison et al., 2019)[12].

Other than that, sustainable governance mechanisms about natural resource soil, water, and ecosystem need to be conducted for life sustainability and a climate change adaptation (Getnet, Pfeifer, & MacAlister, 2014)[13]. Also, economic development is hugely dependent on natural resources, and the increase in demand creates permanent pressure on the primary resources (Bonnin, Azzaro-Pantel, & Domenech, 2019)[14].

Community Empowerment

Empowerment can be interpreted as that building individual strength and competence, natural presentation structure, and practice behavior for social policy and social change (Bonté et al., 2019)[15]. Community empowerment is a social action which promote communities' participation and organization to the societies' (Ni, Xiao, Liu, & Wang, 2019)[9]. Community participation is an active role and societies involvement in a process of decision making, also the ability of the society to implement those decision (Shafique & Warren, 2018)[13].

From those several concepts of community involvement, there is a crucial fundamental concept, namely community independence, which focuses on the ability to conduct and to make decisions in many aspects based on ownership of local resources (Santosa, 2017). Additionally, referring to the process that allows society to increase control over their lives, it needs to be collective efforts and integrations from various groups and social institutions in order to increase their income (Iffah et al., 2018)[7].

Method

This study addressed the case study method with a qualitative approach. The case study method is used to gather accurate information to answer the research problems, namely condition, and potential natural resources ansd human, especially the level of participation in the preservation of the natural resources and factors that influenced natural resources condition in a group of islands, in this case the Kepulauan Seribu (Pulau Kelapa and Pulau Harapan). The primary data sources (key informant) in this study were the society, oceanology, Bapedal, Bakosurtanal, Inkopal of Ministry of maritime and fisheries, and the local governments of the Island groups. From the sources of the information was conducted several data collection methods such as interviews, documentation, and observation, to get as much information about empowerment community policy and empowerment programs.

Result and Discussions

A. Community Empowerment Policy in the Utilization and Management of Coastal Natural Resources for Sustainability**.**

Several community empowerment policies which are implemented in Pulau Kelapa and Pulau Harapan

include:

1. Intensification program of fish breeding

Intensification program of fish breeding (INBUDKAN) is a one of the aquaculture development programs, focusing on the movement of various parties to develop a fish cultivation business, which is carried out on the basis of cooperation between members of the fish farmers group (Poktakan) as program participants in the area, which implements recommended technology to improve the quality of production and productivity of fish cultivators in an efficient and sustainable manner. Business patterns consist of: (a) the company pattern guidance, (b) the development service unit (UPP), and (c) the private pattern. Determination of business and coaching patterns in the framework of program implementation is done by taking into account (a) the level of business development, (b) the existence of private companies, (c) the availability of service facilities in the area concerned.

2. Capture Fisheries Based Community Empowerment

For the capture fisheries - based community empowerment in Pulau Kelapa and Pulau Harapan which has fisheries and marine resources are low with the level of utilization experienced *overfisihing,* empowerment program focused on:(a) increasing quality result and utilizing cool box; (b) adding value through processing fishery products;(c) improving supervision of marine and fisheries resources through the society; (d) increasing understanding of the natural resource marketing network through restocking.

3. Community Empowerment of Seawed Farming Fishery Based.

For the cultivation-based community empowerment, the empowerment program used for seaweed cultivation as follows: (a) attempt to the increasing of seaweed quality result, such as increasing the quality of drying seaweed, and seaweed processing;(b) training program; (c) increasing the quality and handling production facilities along with marketing networks; (d) natural resource development and enrichment program through restocking.

4. Community Empowerment Cage Aquaculture Based.

For the community empowerment of aquaculture cultivation based, the programs were conducted as follows: (a) prioritozing quality of fishery products; (b) adding cultivations facilities; (c) training programs; (d) increasing the quality and handling the quality of marine products; (e) Improving marine cultivations; (f) improving and handling the quality of production facilities; (g) natural resource development and enrichment programs.

5. Tourism and Protection Empowerment.

Community empowerment in the islands, which are converted dan tourism areas, aims to give important additional knowledge of the environment around the group of islands. Therefore, societies do not break biodiversity but protect the conservations and tourism areas. The empowerment is conducted by looking for alternative jobs through empowering the national economy. For instance, by preparing tourist accommodation, becoming souvenir handmade, selling souvenirs, renting snorkeling tools, or renting fishing tools. Also, increasing human resources such as conducting training for tourist guides, sellers and handcrafter, and marine sports.

6. Community Empowerment in The Field of Counseling and Trainings.

For the community empowerment on understanding the importance of the environmental value of natural resources and environmental pollution, several programswere conducted as follows: (a) training of coastal communities through a clean sea program; (b) environmental protection programs and natural resources on mangroves, coral reefs, and degraded pastures; (c) pollution control programs; (d) maritime and fisheries resources supervision and control training program through monitoring, controlling and surveillance systems.

7. Strengtening Community Institution.

Strengtening Community Institution is (a) institutional and organizational performance programs and (b) fisherman's joint program.

The programs of policies explained above are having strengths and opportunities in the implementations. The explanations can be seen as follows:

Tabel 1. Empowerment programs opportunities and challenges

Strengths	Weaknesses
a. Natural resource of ocean (cultivations and aquaculture) considered moderate in terms of quality. b. Motivation and working culture of fishermen are high. c. Local wisdom needs to be increased to support cultivation.	a. The numbers of poor fishermen are extremely high. b. Low quality of human resources around coastal areas. c. Cultivation facilities are limited. d. Lacking capital of fishery business. e. Institution capacities are low. f. Lack of technology to handle the quality of fish. g. Lack of government attentions. h. Lack of information regarding fish market. i. The area of the sea in Kepulauan Seribu is too large so that, it is hard for a protection.
Opportunities	Threats
a. Higly demand of fishery product both domestics and foreigns. b. Higly opportunities for community empowerment funding. c. Marine tourisms are highly in demand.	a. Pressure of traditional monetary institutions. b. High exploration of fish and destruction of coral reefs. c. Domestic household waste pollution d. Population density and growth rate e. Small collective awareness of the importance of the environment.

The impact of community empowerment policy can look like the increase of community participation in preserving nature, the mangrove forests, the people do not dispose of household waste to the sea, not the bridge to the sea, the community understands the various potential natural resources that should be maintained and developed.

For all these reasons that the researchers chose to conduct research in the area of cluster islands. Some studies related to the research conducted as research conducted by Santosa which states that of the many communities the concept of community empowerment, there is one key concept that is fundamental and important that their independence in the community focused on the ability to perform and make decisions in the interests based on ownership of local resources(Santosa, 2017). Likewise, the results of research conducted by Erum and Hussain which says that the natural resources leads to economic growth in local communities (Erum & Hussain, 2019).

B. Fishing Community Empowerment Program Priorities in Accordance with Potential Natural and Human Resources.

1. Program priorities

In the implementation of empowerment programs, cage cultivation based, and the second priority is the development of marine tourism and implementation. Both programs can work together and benefit each other through the graha mina marine tourism model, which is a combination of floating net cage fish culture with marine tourism. Above the fish cage in the phase of grouper or white snapper growth is built houses where the tourist can enjoy the sea panorama while fishing and having a big meal. The management is fully controlled by the society who live around the coastal under supervising by facilitators. In order to achieve the goals of the program, institutional strengthening is needed in the community.

Tabel 2. Community Empowerment Program

No	Community Empowerment Programs	Weight	Priorities
1	Strengthening the institution in order to increase community economic status and natual resources preservation.	0,0042	3
2	Capture fisheries empowerment based	0,0033	5
3	Empowerment on cage cultivation based	0,0044	1
4	Seaweed empowerment based	0,0041	4
5	Empowerment of tourism around the conservation	0,0043	2
6	Counseling and training in the field of environmental sanitation	0,0031	6

Based on the priorities table 2, it can be concluded that Empowerment on cage cultivation based becomes a main priority that need to be implemented in Pulau Kelapa and Pulau Harapan.

Based on table 2, the priorities of the community empowerment program can be explained that empowerment on cage cultivation based weights 0.0044 so as a top priority, empowerment of tourism around the conservation with a weight of 0.0043 so that the second priority, strengthening the institution in order to increase community economic status and natual resources preservation with a weight of 0.0042 so that the third priority, seaweed empowerment based with a 0.0041 weight, capture Fisheries empowerment based at a weight of 0.0033 so it becomes the fifth priority, and counseling and training in the field of environmental sanitation 0.0031 become the sixth priority.

2. Model and Construction of Graha Mina Tourism.

Floating net cage will be suggested in the empowerment policy program in Pulau Harapan dan Pulau Kelapa with 3m x 3m x 3m size. At the Graha Mina marine tourism area, the number of cages that can be used is 12 with 154m. The floating net cage is connected to the housing adjusted the size of the cage with 6m x 6m large.

Floating net cage should be placed at the quite water location (protected from direct waves or between islands) with adequate water flow. The depth of the water when it is tide minimum five meters (ideally 15-30 meters) and having water exchange (flow) well, no stirring of the water, and a flow speed of at least 0.1 meters. Besides, site selection should be avoided from ship traffic, so that fish in the cage is not stressed, and water quality is not directly contaminated with fuel from passing ships. The depth factor influences the type of cage depth above 10 meters should use the floating type, while less than 10 meters use a stake. Using types of building materials are recommended lightweight building types that fit cage construction.

The impact of Graha Mina Wisata Bahari can look like the condition and potential of increased natural resources, natural resources in the form of coral reefs, for the better, the increase in the society Perekonomiam, the quality condition of the sea water is always awake, sea water is not polluted, income from the cultivating grouper fish, seaweed is increasing. Based on the results of the above research supported by the results of research conducted by Dalu, and Shackleton that there is a fairly basic understanding of the contribution of natural resources to the resilience of poor urban communities living in coastal settlements implicates the economic level of the surrounding community(Dalu & Shackleton, 2018).

Based on the results of the study of Lessmann, and Steinkraus explained that natural resources are a blessing to the region, but the natural resources will be catastrophic if not keep it well(Lessmann & Steinkraus, 2019). So also according to Zalle (2018) that natural resources can lead to a good increase in economic performance, as well as vice versa can be a disaster for an area(Zallé, 2018).

Conclusion

Based on the all explanation above, it can be concluded that that various community empowerment policies have been carried out such as Intensification program of fish breeding (INBUDKAN), capture fisheries community empowerment-based, seaweed farming community empowerment-based, community empowerment on cage aquaculture based, tourism community empowerment and protection based, community empowerment in the field of counseling and training based, and community empowerment on community institutional strengthening based. Furthermore, cage-based empowerment is a priority program that needs to be implemented at Pulau Kelapa and Pulau Harapan. Finally, the model and construction of the marine tourism mina graha is a novelty in this study.

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The Government's Responsibility for the Fulfillment of the Right to Education for Child Labour in Working Relationships

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Abstract

The purpose of this study is to analyze the setting of the education right for child labour in working relationships. The method used in this study is empirical normative. The results showed that the regulation of the fulfillment of the education right for child labour in the working relationship have not been maximal, because there is still a legal void in the Employment Law and the absence of synchronization of Law No. 20 of 2003 on the National Education System, Government Regulation No. 48/2008 on Education Funding. The implementation of the 20% education budget has not been achieved so that uneven education is enjoyed. Therefore, a revision of the rules on the fulfillment of educational rights for child labour in an integrated and harmonious working relationship is required.

Keywords*: Responsibility, Education rights, Child Labour, Employment Relations*

Introduction

Human beings and human rights are two things that are difficult to separate because man since his birth carries integral natural rights in his life conferred by the almighty God (Fattah, 2017)[1]. Its existence is so fundamental to human in protecting themselves and the human dignity. It is also used as a moral foundation in associating or connecting with fellow human beings, so that no one can take it away (Endrawati, 2011)[2].

In human rights law, countries in this case represented by the government are obliged to respect, protect and fulfill human rights in the constitution of their respective countries specific to Indonesia (Sakharina, 2016)[3]. This obligation is set out in Article 28I of the NRI Constitution of 1945, which determines that the protection, promonation, enforcement, fulfillment of human rights is the responsibility of the state, especially the government[4]. One of the human rights is the right to education as affirmed in Article 28 C paragraph (1) of the 1945 Constitution that everyone has the right to develop themselves through the fulfillment of their basic needs, are entitled to an education and benefit from science and technology, arts and culture in order to improve their quality of life and for the welfare of mankind. Education is the right of all persons as stated in Article 31 paragraph (1) and (2) of the 1945 Constitution that every citizen is entitled to education and must attend basic education and the government must finance it[5]. The embodiment of educational rights in the Constitution of the NRI year 1945 is spelled out through legislation and various government policies including Law No. 39 of 1999 on Human Rights Article 12 that everyone is entitled to his personal development, obtaining an education and smartening himself[6]. Furthermore, Law No. 20 of 2003 on the National Education System Article 5 that is every citizen is entitled to a quality education. Article 6 paragraph (1) that every citizen aged 7-15 years old shall obtain a primary education[7].

In order to achieve the objectives of the state of education becomes the main target of the government[8], therefore through education every citizen is given the rights to be enjoyed fairly without discrimination, specifically for children implicitly affirmed in Article 31 paragraph (2) of the NRI Constitution of 1945 and Article 6 paragraph (1) of Law No. 20 of 2003 on the National Education System, that primary education becomes the main perioritas of the government and is responsible for funding it especially for citizens who are 7-15 years old connotations in the child[9], because the child is every person who is less than 0-18 years old[10]. Therefore, in terms of national and state life, the child is the future of the nation and the next generation of

national ideals so that every child is entitled to survival[11], growth and development, participate and be entitled to protection from violence and discrimination as well as civil rights and freedoms as stipulated in Article 28B paragraph (2) of the NRI Constitution of 1945. One of the human rights of children is the right to growth, especially intellectual development through education that must be fulfilled by the state (Sirait, 2017)[12]. Therefore, the state, government and local governments are obliged to conduct education democratically and not discriminatoryly through the formulation of policies in the field of child protection and local governments support national policies in the implementation of child protection by building kabupeten / city worthy of children[13]. A form of national commitment is to ratify the Convention on the Rights of the Child which was ratified by the United Nations in 1989 through Kepress No. 36 of 1990 on the Ratification of the Convention on the Rights of the Child Article 28 paragraph (1) KHA 1989, that every child deserves a quality education[14]. Law No. 39/1999 on Human Rights Article 60 that every child is entitled to an education and teaching in order to develop their interests, talents and level of intelligence[15]. The affirmation in Law No. 20/2003 on the National Education System Article 6 paragraph (1) that as well as every citizen aged 7-15 years shall attend primary education, strengthened in Law No. 35/2014 on Child Protection Article 9 paragraph (1) that every child is entitled to education and teaching. Based on this, each child has the right to get an education and teaching in order to develop his/her personal and intelligence level according to his talents and interests[16]. But in reality not all Indonesian children have the opportunity to obtain their rights in the field of education in full, especially children born to poor families or very poor households[17]. According to the Education Monitoring Network together with Result International, there are three main issues of education in Indonesia today, namely the quality of education, educational discrimination and inspitable schools (Sudjatmoko, 2010)[18]. The quality of education is mainly concerned with the lack of facilities and infrastructure such as the lack of availability of books and decent school buildings, the most affected groups are children living in rural areas with limited facilities and educational opportunities causing the quality of education to be very low[19]. The issue of education discrimination, especially concerning the cost of education, causes uneven education. Education is still dominated by the upper middle class, so that the lower middle class does not feel the education[20]. Therefore there is a gap between education for the rich and the poor and lastly is the problem of schools that are not child friendly, nowadays the education system is more oriented towards memorization with such a schedule that causes the child not to have time to play so happy to learn to be anxious in some cases there are children trying to kill themselves because they are tired of learning[21]. Even the results of research by educational institutions in Yogyakarta reveal that some children stop attending school and choose to work because the education system is boring[22].

The implication according to RTEI is the emergence of marginalization of education and marginalized groups are children of poor families, so the implementation of basic and free education as guaranteed in the constitution in fact cannot be enjoyed evenly especially children from economically incapable families so that early on their children are involved in the world of work (Kemenakertrans, 2020)[23]. The issue of poverty, lack of opportunities for education, lack of legal protection and lack of effective implementation of laws are the causes of the difficulty of child labour issues. From 2008-2019, the government has tackled child labour by 1346 out of 1,709 child workers, and the government has targeted attracting 9000 Child Labour workers by 2000 (Children's Profile Book 2019)[13]. As a result of the covid-19 pandemic that hit 216 countries including Indonesia became the biggest challenge to realize the program. Covid-19 has an impact on the economic, health and education sectors. In the economic sector, according to ILO data by 2020, there are 25 million workers potentially losing their jobs, 3 million labours in the informal and vulnerable sectors in layoffs and 5.6 million homeless (World Day commemorations, 2020a)[3]. The condition affects declining household income and has the potential to affect child development, health and education. Education is one of the social problems caused by covid 19 with online learning policy. One of the groups affected by online learning is poor children because of the limited facilities and infrastructure they have[17].

According to UNICEF, 120 million children worldwide are affected by pandemics and 30 percent of

poor families have difficulty learning distances, resulting in 1.6 million dropping out of school (World Day commemorations, 2020b). LPPPA data reveals that as a result of online education policy causing marginalization of education at all levels of education, piaud level there are about 120 million unaffordable children, elementary school level as many as 271 million children and junior high school level of 79 million children (Bapenas, 2020)[14]. Poor families are the most vulnerable group, especially the limited online learning packages they have. As a result families become permissive to involve children in the world of work in place of loss of family income. The condition is particularly concern to all parties including the ILO for Indonesia and the East East predicting an increase in the number of child labours who over the past 20 years have managed to reduce child labor by about 94 million. It is necessary to protect the children of low-income families, especially the emergence of family access to credit, debt and urbanization from city to village, because the main epicenter of covid 19 is the vulnerable rural economy, especially farmers and fishermen at risk of children getting stuck in child work (LPPPA, 2020). Whatever the context, the government should not eliminate the obligation of the government to continue to grant the rights of children who are forced to work they are still entitled to protection for their rights as stipulated in several regulations including Article 32 of the Convention on the Rights of the Child that the child has the right to be protected from economic exploitation and from doing any work that may interfere with the child's education, endangering the child's physical, mental, spiritual, moral and social health/development. Article 64 of Law No. 39 of 1999 on Human Rights that every child must obtain protection from economic exploitation activities and any work that endangers him, so as to interfere with education, physical health, morals, social life and spiritual mental life, Law No. 20 of 1999 on the Ratification of ILO Convention No. 138 on Minimum Age for children Allowed to Work in Article 3 paragraphs (1) and (3), the minimum age allowed for occupations that endanger the health, safety, or morale of the child must not be under 18 years of age and the age to perform light work is 16 years. The legal protection of child labour is also stipulated in Law No. 1 of 2000 concerning the ratification of ILO Convention No. 182 of 1999, concerning the Prohibition and Immediate Action for the Elimination of The Worst Forms of Employment for Children (BPTA) which has been ratified in Law No. 1 of 2000 specifically governing restrictions and prohibitions to involve children in the worst or harmful occupations. The convention also contains norms directly related to the concept of child protection as a worker, determining the steps that must be taken, namely determining the minimum age, proper regulatory work permit regarding working hours. Law No. 13/2003 concerning Employment Article 68, Article 69 paragraphs (1) and (2), Article 70 paragraph (1), which has subtancially governed concerning child labor, ranging from the determination of the age limit can work as well as protection of the rights of child labours.

Implicitly the existence of child labour has been guaranteed from some of the most specific regulations in Law No. 13 of 2003 on Employment. But in the implementation is still not maximal, there are still various parties that utilize the weakness of the child to make it a labour with the minimum wage, and give birth to a work system that only prioritizes the advantages of safety, health and educational rights, inevitably still found child workers with long working hours and resulted in neglected right to attend school. A survey by the Indonesian Child Welfare Association in 2018 found that 1.92 million children aged 10-14 worked at least 4-5 hours per day. The association believes this figure is already higher due to higher poverty (Child Labor, 2018). This condition is due to the provisions of Article 69 paragraph (1) and (2) Law No. 13/2003 concerning employment not clearly governing the type of light work, the reference is the Decree of the Minister of Manpower and Transmigration Number Kep-235/MEN/2003 concerning Types of Work that Harm the Health, Safety or Morale of the Child, which in principle prohibits children from working in certain types of work. While the right to attend school for child workers, the reference is not yet clear because the Regulation of the Minister of Women Empowerment and Child Protection No. 5 of 2011 on the Fulfillment of Child Education Rights does not specifically regulate child labor. Then Law No. 20 of 2003 on the National Education System as the basis for the implementation of education has not accommodated the policy of special education services for child labours both regarding the schedule and curriculum. Full day implementation in schools has implications for not maximal child labor adjusting school schedules and working hours. Therefore, the phenomenon of child labor

is still an issue and an agenda that continues to be sought solutions. Based on data from lpppa in cooperation with BPS RI in indonesia's children's profile book in 2019 records that out of 79 million indonesian children today, there are about 5.6 million children working especially the age of 10-14 years, out of 5.6 million children who work there are about 52 percent in school, half the rest drop out of school or have never even been to school. The highest child workers are in the countryside with the majority working in the informal sector, particularly in plantations, agriculture and as domestic assistants, presentation of the highest child labour in Papua, NTT, South Sumatra and Sulawesi (Children's Profile Book 2019). Nevertheless, child labour remains and still exists scattered throughout the province the lowest percentage is in DKI. Sakernas 2019 data reports there are about 1.33 percent of 15-17 year olds working in the trade sector and more in urban areas. Child labour is believed to continue to grow as long as poverty has not been alleviated, especially the current state of poverty of the nation tends to remain high, BPS data in 2019 there are 4.7 million poor people in Indonesia and potentially increasing due to covid that is shaking the global economy, especially Indonesia (LPPPA, 2020). The impact of covid 19 on Indonesia based on reports from KPAI in the field of exploitation and traffic revealed that during the pandemic there is a tendency for children to work, among others in East Jakarta there are children working in stalls and restaurants, while in Depok it is found that children work as scavengers in TPS.

Based on the composition of child labour it is concluded that the trend of increasing child labour each year is rationalized to meet the economic needs of poverty-stricken families. Therefore, efforts are needed to increase the role of government, local government and community in the fulfillment of educational rights for children such as the establishment and implementation of more appropriate and consistent regulations as well as the implementation of activity programs aimed at supporting the fulfillment of educational rights for child labour in working relationships.

Method

The type of research used is empirical normative legal research. This means normative research is supported by empirical data. This type of research is adapted to the formulation of the problems studied, namely the study in the field of law. Legal research with normative legal research types is a legal study that examines written law from various aspects of theory, history, philosophy, comparative law, structure and structure, scope and material, consistency, general explanation article by article, formality and binding power of a law and the language of law it uses, but reviews its application or implementation.

Result and Discussion

The arrangement of fulfillment of the right to education for child labour in the relationship of discussion work begins with several basic concepts, namely:

1. Child Labour and Government Responsibility

In general, the child according to The Minimum Age Convention No. 138 of 1973 is a person who is 15 years of age or under. Meanwhile, in the 1989 Convention on the Rights of the Child, which was ratified by the government through Kepress No. 39 of 1990, it was revealed that the children were those aged 18 and under. Meanwhile, UNICEF defines children as residents between the age of 0 and 18. In Article 1 number 2 of Act No. 4 of 1979 on Child Welfare states that the child is a person who is not yet 21 years old and has never been married. Furthermore Article 1 number 1 of Law No. 35 of 2014 on Child Protection defines that a child who is not yet 18 years old including a child who is still in the womb. Then in Article 1 number 5 of Law No. 39 of 1999 on Human Rights states that the child is every human being aged 18 years and under and unmarried including the child who is still in the womb, the last definition of a child under Law No. 13 of 2003 on Employment that the child is any person under the age of 18.

From some sense of the child, it can be concluded that, the child is those aged 0-18 years. As for this time the child will be in the study period and need more time to socialize with his peers. This will allow the child to develop as they should. But there are also children who decide to work to help their family's economy.

Child labour is children who do work regularly for their parents or for others who need a large amount

of time, by receiving rewards or not. According to Manurung, child labour is those aged 10-14 and they are working at least one hour continuously a week. Referring to the Employment Act child labour is children under the age of 18, children can be employed on the condition of parental consent and work a maximum of 3 hours per day. The Decree of the Minister of Home Affairs and Regional Autonomy No. 5 of 2001 on Child Labour Management in Article 1 defines that child labor is a child who performs every type of work that endangers health and hinders the learning process and growth.

Child labour referred to in this paper is limited to children who do work regularly requiring a certain amount of time by receiving wages or benefits and fulfillment of other rights as set out in the employment relationship. Child labour in this category is mainly minors who work in this type of light work and are not hampered by the right to attend school. Be aware that children who become child laborers have a susceptibitas to disturbing situations that must be protected and full of rights, because child labour is a human being who deserves to be respected, upheld by the state, law and government and parents as affirmed in Article 1 number 12 of Law No. 35 of 2014 on Child Protection that the rights of the child are part of human rights that must be guaranteed, protected, and fulfilled by parents, families, communities, governments and countries (Aswanto, 2014).

UNICEF states that the true measure of a nation's achievements is the safety of the child, its well-being, its education and socialization, feelings of pity, being valued and included in the families and communities in which they were born (Sudjatmoko, 2010). The attention to the child world is a measure of the extent to which a society places a child's position in its national development. Therefore, it is not permissible for actions that lead to the loss of children's rights let alone hinder future hopes, including economically disadvantaged children who cannot get an education because they have to work to meet their family's needs.

In the history of the nation, education was important even before the independence of education was used as a means of transformation of the nation even education was used as a means of fighting for the nation. In the new order, education was once the nation's priority by getting priority in the budgeting of the country as reflected by the construction of presidential instruction elementary schools in various regions including remote areas. In the reform era, education priorities are affirmed in the constitution that education funding is allocated at 20 percent of the state budget and state budget as well as the responsibility of the state to finance 9 years of primary education with the aim of equalization of educational fulfillment for all citizens. However, one of the problems that arises is that the implementation of basic human rights still exists that is not in line with the concept of human rights i.e. the right to access free or free education still contains discrimination. Education today is still dominated by the upper middle class so there are some lower middle class who can not feel education, because this is the gap of education in the city and in the village especially in the border area, so the issue of the quality of education is still dominated in the city and for the upper middle class. Quality education still belongs to the rich, while the poor still have difficulty obtaining quality education due to the high cost of education. As a result, the responsibility of the state in the fulfillment of education for all citizens, especially for children, is still not maximal because the impelementation of the education budget by 20 percent for education has not been achieved, as a result of a levy in education that implications the emergence of the phenomenon of dropping out of school and choosing to work. In fact, children should have time to learn, play and interact in err with the conditions they have to be on the street or elsewhere that demand it to work with various risks that must be faced and harm the child's development process.

On that basis, the Indonesian government has committed to addressing dropouts since 1970 by making education a priority with compulsory learning programs for 7-12 year olds nationally. Success achieved with the mandatory 6-year study program motivated the government to improve the compulsory 9-year study program since May 9, 1994. The compulsory 9-year study program is based on the concept of basic education for all, which in essence means the provision of equal access for all children. The goal that this program wants to achieve is to stimulate the educational aspirations of parents/foster carers and children who in turn are expected to increase the work productivity of the population nationally.

This 9-year compulsory learning program is contained in Article 48 of Law No. 35 of 2014 on Child Protection states that the government must hold a primary education of at least 9 years for all children. The reason the government implements a compulsory 9-year study program is that there are more than 805 workforce only educated elementary or less, or junior high school does not end, in addition this mandatory 9-year study program will improve the quality of human resources and can add value to economic growth and mandatory study 9 years will increase the minimum working life from 10-15 years. However, the program did not run optimally, because the dropout rate remained high at 3.01%. Since 2018, Indonesia's Human Development Index has been at 71.39 and one of the basic dimensions of IPM that is still difficult to improve is access to education/knowledge. Related to the IPM component of the educational element used is the average length of school, the expectation of old school. It was found that the average low school length for national IPM is 8.17 years. The level of South Sulawesi Province is 7.95 years. It turns out that the average school length in Indonesia in South Sulawesi province is still less than 12 years old (UNICEF, 2018). The long duration of education coupled with the high cost of education causes some parents to prefer not to send their children. One of the challenges in child care is the high cost of education (Sugianto, 2017). Some studies report that children drop out of school because of low family income to fund education. The phenomenon of children breaking up in Makassar is due to poverty factors, according to Makassar social service data mentioning the number of poor households in Makassar city in 2018 is about 87.90 percent. Economic necessity forces parents to engage their children to meet household needs by ignoring educational rights (Nursita, 2018). So it is natural that in South Sulawesi province the children drop out of school still expected 28 thousand, especially in the midst of the covid-19 outbreak.

According to the Ministry of Education because the cost of education and poverty is the main factor so that children drop out of school should be, then the responsibility of the government is to have to finance as affirmed in Article 53 of Law No. 35 of 2014 on Child Protection that the government is responsible for providing free education and/or assistance or special services for children from disadvantaged families, displaced children, and children living in remote areas. In addition, it is persuaded to participate in package A and Package B programs, coupled with programs to expand open schools so that the illiteracy eradication movement can be eradicated (Saraswati, 2015). This is in accordance with the mandate in Article 12 of Law No. 39 of 1999 on Human Rights that everyone is entitled to protection for his personal development, to obtain an education, educate himself, and improve his quality of life. However, the implications arising from the progra of package A, B and Package C indirectly perpetuate the culture of dropouts and child labor on the grounds that ahead of exams they are just taking the equality exam to obtain a diploma. So out put more education on a mere formal orientation and ignore the quality of graduates.

Children of package graduates tend to have low skills and competitiveness so that children from families who are not able still find it difficult to obtain an education becomes a powerful way to prosper them-selves in the future. This condition is really concerning, child labour in its position as a child which is the basic capital for national development and the successor of the ideals of the struggle of the nation that will be expected to be able to carry out its duties and responsibilities for the sustainability of the nation and the state, in other words the child is a long-term investment for the planning of the nation going forward should take into account the child as a very important variable. Moreover, Indonesia's child population occupies a third of Indonesia's population is certainly a nation asset that must be prepared from now on through education so that it creates a quality generation (LPPPA, 2019). Therefore, the responsibility of the state in this case is an inevitable in a country that adheres to the concept of the welfare law state, one of the characteristics of the welfare law state is the recognition of human rights, among which is the right of education. So that the improvement and fulfillment of quality education for all citizens without exception especially for children in working conditions is non-bargainable because education is a systematic process of improving human dignity and human welfare, especially creating a better life for child labour in the future.

The responsibility of the government is essentially the obligation as a stakeholder to be responsible in protecting, enforcing and advancing the human rights of citizens. The government must be accountable to its

people because the government in carrying out various legal actions that are set out in legal instruments such as laws, policies and decisions and certainly cause legal consequences both general and special (Kattharilda, 2013). The existence of government or state accountability is an inevitable in a country that adheres to the concept of a legal state, one of the characteristics of the state of law is the recognition of human rights. In the concept of international human rights law, the responsibility of the state is closely related to the rights that have an international nature, actions and actions that give rise to the responsibility of the state including violations of international treaties relating to contractual obligations and liability due to losses to citizens of other countries. Breach of duty can be acts or omissions (Ardhiwisastra, 2003).

Referring to the theory of human rights law and the doctrine of public law firmly shows that the enforcement and fulfillment of human rights is the responsibility of the government. Therefore, the enforcement and protection of the human rights of normative citizens is imposed on the state that is fully implemented by the government as the trustees of the constitution of the country. One of the mandates of the constitution as stated in the Opening of the Constitution of the Republic of Indonesia is the mandate to educate the nation through the educational process. Education as part of economic, social and cultural rights has been guaranteed recognition of the rules of international law. This rule is legally binding on the country that has ratified it (Mudyahardjo, 2015). Education as a constitutional right in Indonesia is not separated from the adoption process arising through international treaties both the Convention on the Rights of the Child, The International Covenant on Economic, Social, and Cultural Rights was subsequently abbreviated as ICESCR which was accepted through UN General Assembly Resolution No. 2200 A (XXI), 16 December 1966, the Covenant was declared effective on 3 January 1976 and signed by 153 countries. The ratification of some international instirumen has the consequence that such international treaties have normative powers to apply as a rule of law, therefore, requiring further implementation in national law. One of the basic rights that has been recognized and guaranteed in the international instrument is the right to education as affirmed in Article 13 of ICESCR that each country of participants of this covenant recognizes everyone's right to education should be directed at the full development of human personality and awareness of self-respect, and strengthen respect for human rights and basic freedoms. Furthermore, the state of the covenant recognizes that to exercise that right fully where primary education should be compulsory and freely available to everyone.

Further affirmed in Article 2 paragraph (1) of the Covenant of Civil and Political Rights ratified by Indonesia in Law No. 10 of 2005 reads that every country must respect and guarantee the rights of individuals, in the event of the non-existence of the relevant rules, the state shall make local rules, even if necessary to perform bilateral or mulitilateral treaties, and each country is obliged to demonstrate good faith to comply with the provisions of international law both material law and procedural law.

Indonesia is one of the countries that has ratified the ICCPR Covenant and the ICESCR Covenant respectively in Law No. 11 of 2005 for ICESCR and Law No. 10 of 2005 for the ICCPR, bringing the consequence that the Indonesian government must carry out its obligations in a positive way based on the principle of achieving results and the principle of the implementation of covenant instruments that have been ratified in the form of requiring the country to protect, fulfill , advancing and respecting human rights both civil and economic, social and cultural rights. The obligation to fulfill the state's actions in the form of facilitating the fulfillment of such rights by properly incorporating in the legal system, administration, finance, legal process, promotion and other actions that can support the fulfillment of such rights, among others the act of provision in this case the state is asked directly to provide assistance or services for the realization of such rights. The obligation of the state to protect is the most basic obligation and not only to protect the human rights from violations committed by the state, but also against violations or actions committed by other entities or parties that would interfere with the protection of such human rights, so that the state is required to take appropriate action so as to prevent outside parties from intervening in terms of enjoying those rights.

The consequence of the above three obligations is that the state in this case the government is required

to take certain actions to carry out the fulfillment of a right and obligation to achieve certain goals specific to educational rights. Thus, the country is obliged to carry out its international obligations in fulfilling its educational rights to the maximum based on existing resources. The absence or lack of resources is not an excuse not to carry it out. The realization of the implementation of the commitment of the ecoosob covenant is to require the state to create a specific action plan or program for the fulfillment of human rights based on the application of effectiveness principles in its territory and/or within its jurisdiction. One of the Indonesian government's programs is to commit to achieving the Sustainable Development Goald (SDG's) program by 2030. One of its main targets is Indonesia's decent children in 2030 (LPPPA, 2019). The government's commitment proves that the fulfillment of the right to education is a fundamental effort of the state that places education as one of the functions of the state including advancing public services in this case education services as part of the constitutional right of citizens as a manifestation of the purpose of the state in the Opening of the NRI Constitution of 1945 the fourth paragraph that is to educate the life of the nation, which is then spelled out in various regulations, specific regulations regarding the fulfillment of the right to education for children who are forced to work.

2. Analysis of Education Rights Fulfillment Arrangements for Child Labour in Working Relationships

Education under Law No. 20 of 2003 on the Education System is a conscious effort to prepare students through guidance, teaching and training activities for their future roles. The goal is to educate the nation and develop Indonesian people as a whole, virtuous worker that have knowledge and skills. On that basis, education is an important component in life so that the fulfillment of education can be realized evenly then the foundation of education arrangements is needed for the sustainability of education in Indonesia.

The philosophical foundation of education in Indonesia is pancasila, as affirmed in Article 2 of Law No. 20 of 2003 on the National Education System that the national education based on pancasila and the NRI Constitution 1945, this confirms that national development seeks among others the establishment of pancasila people as human development of high quality and able to be independent. While the cornerstone of the education juridical point of rejection is the NRI Constitution of 1945, clearly contains about the main purpose of the implementation of education in Indonesia is to educate the life of the nation, this means that education can free people from all shackles. As a manifestation of the country's purpose in smartening the nation, departing from the philosophy of education arrangements in Indonesia namely pancasila and NRI Constitution in 1945, the implementation of education becomes the responsibility of the state and should be enjoyed by all citizens without exception specific to children. Children are the buds, potential and the next generation of the struggle ideals of a nation that has a role and has special characteristics and traits that ensure the continuity of the existence of the country to a better future.

Thus, education as a child's human right, the right of children to obtain an education has been guaranteed and recognized from various countries both on a global scale and on a national scale. On a global scale, the right to education is guaranteed in the Universal Declaration of Human Rights of 1948 in Article 26 paragraph (2) affirming that education aims to strengthen the universal humanitarian relationship, as well as education directed at the development of personality. The next development is the 1989 United Nations Convention on the Rights of the Child affirming that the rights of the child are inherent in the child and are human rights, so that the state cannot escape responsibility for the provision of rights to the child. Children's rights under the Convention on the Rights of the Child are grouped into 4 (four) categories including the right to survival, the right to protection, the right to growth and the right to participate (Djamil, 2013). The Convention was reviewed by Indonesia in Presidential Decree No. 36 of 1990. In KHA 1989 it is stated that the child has the right to obtain an education as stipulated in Article 28 paragraph (2) that there must be a guarantee that the school discipline is carried out in a manner that is in accordance with the human dignity of the child and in accordance with this convention without violence. Furthermore, the International Covenant on Economics, Social and Culture in 1966 known as the ekosob covenant of the Ekosoc Covenant contains 5 categories of rights: the right to employment, the right to social security, the right to a decent life, the right to

education and the right to cultural participation. Education as part of the ecosoc right is stipulated in Article 13 and 14 of the covenant which is essentially that the state of the covenant participant recognizes everyone's right to education. The Government of Indonesia ratified the Ekosob Convention in Law No. 11 of 2005. The next concrete efforts of countries in fulfilling children's right to education are held in an International Agreement on May 26, 2004 in Bangkok that agreed on the need to improve access and quality of education through a child-friendly learning environment, promote systematic programs and capacity building and school-based management and build more effective partnerships and collaborate with other countries in an effort to achieve quality education from a comprehensive perspective. UNESCO emphasizes that all children should have access to quality primary education and recommends the need to focus on improving the quality of education through social, emotional and physical development as well as children's academic attainment, the issue of educational discmination should be limited, governments and non-governmental organizations should collaborate in efforts to achieve friendly and equitable learning for all children.

Further development of the idea of promonding and fulfillment of the right to education for children can be found in the Millennium development Goals established in September 2000 as a form of commitment from UN member states to create an environment conducive to the development and alleviation of poverty. Hasyim (2000) stated this millennium declaration contains 8 points, namely: Eradicating poverty and poverty, realize basic education for all, Promote gender and women's equality, lower maternal mortality rate, improve maternal health, ighting HIV and AIDS, malaria and other diseases, and ensuring environmental sustainability

The arrangement and guarantee of the right to education in a global level carries consequences for the countries of the participants to conduct the adaptation process in their respective national laws, including the Government of Indonesia. The arrangement of the fulfillment of the right to education for children still refers to the principles of international and national legal instumen among others must be based on Pancasila especially social justice syllaby for all Indonesians, as well as based on the principles of the Convention on the Rights of the Child i.e. non-dskriminasi, best interests for the child, the right to life, the right to survival and development and the appreciation of the child's opinion.

In the national level, the arrangement of the fulfillment of the right to education for children is concretely set out in the constitution article 31 of the NRI Constitution of 1945 which is the right of every citizen, and citizens are entitled to basic education and the government must finance it. Law No. 39/1999 on Human Rights Article 60 that every child is entitled to an education and teaching in the framework of personal development in accordance with his interests, talents and level of intelligence. Furthermore, Law No. 35 of 2014 on Child Protection mentions the rights of children including the right of every child to obtain education and teaching in the framework of his personal development and his level of intelligence according to his interests and talents.

The policy of fulfillment of the basic rights of children is further affirmed in Law No. 20 of 2003 on the National Education System Article 6 paragraph (1) that every citizen aged 7-15 years old must attend primary education, Article 11 paragraph (2) that the government and local government shall guarantee the availability of funds for the implementation of education for every citizen aged 7-15 years. Then it published Government Regulation No. 59 of 2019 on the Coordination of Child Protection in Article 6 that monitors the implementation of the fulfillment of children's rights to fulfillment of civil rights and freedoms, family environment and alternative parenting, fulfillment of health rights and fulfillment of educational rights. Furthermore, the Regulation of the Minister of Women and Child Protection No. 5 of 2011 on the Policy of Fulfillment of Children's Education Rights, in particular implements Article 11 paragraph (1) of Law No. 20 of 2003 on the National Education System which requires governments and local governments to provide services and facilities and ensure the implementation of quality education for every citizen without discrimination (See Regulation of the Minister of Women Empowerment and Child Protection, 2011). The core of the Government Regulation and Regulation of the Minister of LpPPA does not require children aged 18 years and under not to attend primary and secondary education. The policy philosophy is explicitly related to child labor to get an

education like other children. Child labour is a child aged 18 and under who is forced to work due to poverty and economic inequality. The expected condition is the implementation of articles in national and international legal instruments along with the principles contained therein, so that it can be realized district / city worthy of children with assessment indicators is the accrebility of basic education for all citizens without exception, high educational participation at every level of education or low dropout rate and can create child-friendly schools.

However, due to the limited cost of education funding and poverty of the nation that is still difficult to alleviate, the implementation of the regulation is not optimal in the reality that there are still many citizens who can not have primary education, especially children of poor families have to quit or drop out of school because of the cost of expensive education. As a result, the phenomenon of dropping out of school is inevitable, of course, a violation of the basic rights of children who have been guaranteed both in the constitution and in the instruments of international law that have been ratified by the government (Waluyadi, 2009).

In reality the issue of child labour is not just an issue of children doing work with wages, but vulnerable to exploitation, dangerous work, inhibited access to education and inhibits the physical, psychic and social development of the child (Joni, 1999), of course contrary to the provision stipulated in Article 64 of Law No. 39 of 1999 on human rights that every child is entitled to protection from economic exploitation activities and any work that endangers him, so as to interfere with his education, physical, moral, social and mental life. If the child is forced to work then a work agreement is required in the form of a working relationship. Working relationships are very helpful for child labour especially ensuring clarity of the type of work charged to the child so that the child can be protected from acts of economic exploitation that can hinder the fulfillment of the rights of child labours especially concerning the right to attend school. On that basis, The Employment Act Articles 69 paragraphs (1) and (2) affirm that the provisions referred to in Article 68 may be excluded for children aged 13 to 15 to do light work as long as it does not interfere with development and physical, mental and social health. Article 69 paragraph (2) that employers who employ children in light work as referred to in paragraph (1) shall be qualified including written permission from a parent or guardian, employment agreement between the employer and the parent/guardian, maximum working time of 3 hours, carried out during the day and does not interfere with school time, occupational health and safety, the absence of a clear working relationship and receiving wages in accordance with the applicable provisions. Article 70 paragraph (1) that a child may perform work in a workplace that is part of an educational or training curriculum authorized by an authorized official. Article 70 paragraph (2) of the child in question is at least 14 years old. Article 71 paragraph (1) the child can do work to develop his talents and interests. Paaal 74 paragraph (1) that the government can make efforts to combat children working outside the working relationship. As well as the Decree of the Minister of Manpower No. 235/2003 in article that a 15-year-old can do a job unless it is harmful to the child. On that basis, the existence of child labour and its rights has been guaranteed from regulation and the government is legally responsible for fulfilling it. But in its implementation is still less maximal, still found child labours with minimum wage, a work system that only prioritizes profit over safety, health and educational rights. A survey by the Indonesian Children's Welfare Association in 2018 found that 1.92 million children aged 10-14 working at least 4-5 hours per day could potentially be hampered by schooling rights. This association believes this figure is already higher due to higher poverty especially in the midst of covid-19 (Child Labor, 2018). According to the authors' observations, this is due to several factors, namely the absence of costs, mostly child labor due to poverty factors, so the cost of education that should be provided by the government and the state does not meet it. Then the time constraints caused child labour to work to meet the needs of daily life, most of child labours work with long working time causing education rights to be neglected. The last is the low willingness to study. This condition is caused by a school environment that is not conducive, an education system that is less attractive to children so prefers to work than school. The results of the Institute of Education survey in Jogja against tobacco companies reported that the reason respondents worked was largely because the education system was boring and considered un attractive.

Various child labour issues as outlined earlier, realized that the phenomenon of child labour is still

difficult to resolve because from the point of view of subtansi law realized that Law No. 13 of 2003 on Employment is not yet clearly regulated regarding the type of light work and provisions regarding the right to attend school, so there is still a legal void. Then a provision requiring employers to report employees above 5 is not appropriate because it has the potential to be a loophole for employers to hire minors without a working relationship on the grounds that the number of workers under 5 is of course contrary to Article 74 of the Employment Act which prohibits the hiring of children without a clear employment relationship. Then the ambivalent attitude of the government that shows an unequivocal attitude in dealing with child labor, the government prohibits hiring children and expects all children to go to school on the other hand the government can not shy away from the fact that there are still many poor families. So the Employment Law looks not so firm especially in Article 68 which prohibits the hiring of children whereas in Article 69 paragraphs (1) and (2) allow with some conditions. The condition caused the optimal fulfillment of children's educational rights even at the age of 18 still not fulfilled. Menakertrans has conducted a company investigation to provide protection assistance for child labor in order to learn, protecting children to be returned to their habitat as children to attend formal education in order to speed up the withdrawal of child labor. However, these efforts are still less maximal due to the provisions in Article 176 of the Employment Law concerning the employment watchdog, as well as Presidential Regulation No. 21 of 2010 concerning the Establishment of Employment Supervision Unit unit is still not effective in carrying out supervisory duties to the company due to the spread and the number is not comparable to the number of companies. In South Sulawesi Province the real condition of the supervisor's spread has not been evenly distributed, currently only reaching about 12 districts / cities by dividing the surveillance area into 5 regions. Each region has 8 functional supervisors supervising 4-5 districts with the number of companies above 100 of course ineffective supervision.

Then there are still regulations that are harmonious, especially Law No. 20 of 2003 on the National Education System as the basis for the implementation of education has not accommodated the policy of special education services for child labours. Even the policy of full day enforcement has implications on the fulfillment of the right to education for child labour is not maximal because child labour is increasingly difficult to divide time between work and school. Technical provisions of Law No. 20/2003 on the National Education System include Government Regulation No. 19/2005 on National Standards of Education, Government Regulation No. 47/2008 on Compulsory Learning, Government Regulation No. 48/2008 on Education Funding and Government Regulation No. 17/2010 on The Management and Implementation of Education of Basic Education Programs 9 Years. In fact, 20% of the allocation of education budgets in the state budget and state budget in the implementation of 85% of the provincial/regency/city government is unable to allocate 20% of its BUDGET for education, thus affecting the less optimal education units, lack of facilities and infrastructure of learning resources as well as increasing dropout rates due to the cost of education that is difficult to reach especially in the midst of the covid-19 outbreak potentially dissingena and hiring children due to the economic difficulties of parents (Limpo, 2018). The government's role in improving education during the pandemic includes legal protection of poor families in the form of social assistance, suppressing poverty, preventing children from entering the workforce, maintaining the quality of education, affirmative efforts to catch up with learning and improving children's access to health and nutrition services. Strategic programs are human resources development formed through good education, regulatory simplification, economic transformation and infrastructure development.

The realization of a 12-year compulsory learning program is not relevant to the provision stipulated in the National Education System Act which still adheres to the mandatory 9-year study program as affirmed in Article 6 paragraph (1) of the National Education System Act that every citizen aged 7-15 years old must attend a basic education. There needs to be a revision in Article 6 paragraph (1) of Law No. 20 of 2003 on the National Education System Jo Article 2 decree of the Minister of Education and Culture of the Republic of Indonesia No. 0306/U/1995 on the Implementation of Compulsory Learning of Primary Education 9 years to 12 years, then it is necessary to increase the mandatory age of study to 7-18 years. In order to make the compulsory 12-year study program necessary clear coordination between the

government and the local government so that there is no reason to correct the uneven access and quality of education. In addition, menakertrans's efforts in order to attract school-age child workers are facilitating the implementation of training for child workers in BLK, PPA-PKH by referring to Permenakertrans No. 36 of 2016 on the Implementation of Internment in the Country especially mentioned that the internment participants are job seekers of the minimum age of 17 years. However, government policy clearly does not involve National Tripartite institutions. As a result it can be read that the rules of this apprenticeship program are very dry protection against interns from misguided practices that make apprentices become pure workers without being changed and without obtaining their rights as labours as stipulated in the legislation. Article 22 of the Employment Law expressly states that apprenticeships carried out without going through an apprenticeship agreement between participants and employers made in writing are deemed invalid the status of apprentices to be pure workers and employers should be punished. However, the weak regulation of ministers in protecting apprentices due to provisions regarding the rights and obligations of participants and employers within a certain period of time is not regulated more firmly the provision stipulated in the law causes the company to still mushroom by practicing interns instead of apprentices, which of course is very beneficial to the company because participants are not paid or paid. This condition is especially vulnerable, especially in times of covid 19 children labour has the potential to work longer working hours.

The most appropriate step taken by the government is to immediately fill the legal void in Law No. 13 of 2003 on Employment, especially arrangements related to the type of light work and the policy of fulfillment of school rights for child labours. Currently the reference refers to the Decree of the Minister of Manpower and Transmigration on The Type of Light Work as well as the Decision of the Minister of Child Support and Protection on the Fulfillment of Child Education Rights. The right approach according to the author's frugality that can be used in looking at the problem of child labor is the approach of protection of the rights of the child, especially the child as an individual and citizen is entitled to work because work is part of the human rights by not ignoring the other basic rights that are the right of education It is the responsibility of the government to strive for the fulfillment of the right to work for the child in addition to the right to attend school through the issuance of separate laws related to the fulfillment of the rights of child labours to obtain an integrated and harmonious education with the regulations that Related.

Conclusion

The regulation stipulated in Article 69 paragraph (1) and (2) of Law No. 13 of 2003 on Employment that basically allows underage labours as long as labours' rights are fulfilled, including the right to attend school, is completely ineffective because the Employment Law does not specifically regulate the right to attend school, resulting in a blurring of work norms. In addition, there is no synchronization between the relevant regulations, especially Law No. 20 of 2003 on the National Education System on education service-specific policies for child workers in working relationships, so that the rights of children in obtaining education are far from expected. Therefore, special regulations are required that regulate child labour in the form of separate laws regarding the fulfillment of the educational rights of child workers that are inocentic and harmonious with the relevant regulations.

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The Role of HMGB1and KIM-1 Repair Genes in the Progression of Kidney Failure Disease

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Abstract

Renal Failure(RF) is a leading clinical characteristic of patients in nephrology , it represents significant concern and the main challenge for healthcare systems. The incidence rate of RF is rising globally , mainly as a result of increased aging population. The current study , was performed to identify the effect of HMGB1and KIM-1gene expression in renal failure disease focusing on it is role on the repair processes. This study comprises 100 patients diagnosed clinicaly and seriologicaly with renal failure)52 males and 48 females) their age vary from (17 to 80 years) and 30 healthy individuals as a control group(18 males and 12 females). RNA extracted from blood samples and converted to cDNA which subjected to real-time PCR for detection gene expression .HMGB1 and KIM-1 have significantly overexpression (3.51 folds and 5.37 folds) respectively in RF patients compared with healthy control subjects ($p < 0.05$). However , HMGB1 and KIM-1 gene folding elevated gradually with advance the severity of the RF disease.

Keywords: *Renal Failure , repair , HMGB1, KIM-1, gene expression*

Introduction

Renal Failure disease is defined as renal damage or a glomerular filtration rate <60 mL/(min1.73 m2) for > 3 months, with or without kidney damage. The pathological events involve renal structural or functional abnormalities[1,2] . However, acutely the kidney damage mounts a molecular response to repair itself[3]. Both HMGB1 and KIM-1 gene expression have been increasingly linked to the renal repair . Therfore, when gene expression repair is impaired and dysregulated leading to renal failure and progression of disease. High mobility group box 1 (HMGB1) is a DNA-binding protein that belongs to HMG superfamily, it nuclear non-histone chromosomal protein that bends DNA. Inside the nucleus of cell, HMGB1 has a structural role such as DNA transcription[4], replication and repair[5,6]. Under normal circumstance, HMGB1has been considered as a ubiquitous nuclear protein with an architectural function . HMGB1 effectively contributes to the performance modulation of all 4 main repair processes of DNA, i.e., nucleotide excision repair (NER)[7] , base excision repair (BER)[8] , mismatch repair [9] and repair of double-strand break and nonhomologous end-joining[10] . HMGB1 has been found to bind to DNA lesions based on the identification of a particular structure and bend DNA. HMGB1 can modulate the interaction between protein repair and DNA containing lesions[11] thereby affecting damage repair efficiency [12]

Kidney injury molecule-1(KIM-1) is a type I membrane protein that is expressed at very low levels in the normal kidney, consisting of two portions a cytoplasmic and an extracellular. KIM-1 is primarily expressed in a proximal tubular cell's apical membrane, but also in a glomerular epithelial cell[13,14] . After kidney injury, the KIM-1 ectodomain undergoes membrane-proximal cleavage and entered tubule lumens rapidly,which lead to soluble KIM-1 to be released into the urine. After kidney injury, a dramatic up-regulation of KIM-1 expression correlated with a transition from normal epithelial cells to dedifferentiated cells. This differentiation and proliferation includes the reconstitution of a functional epithelial layer of viable cells that border the damaged areas[15]. Consequently ,

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The purpose of this study was to examine the role of HMGB1 and KIM-1 gene expression in renal failure patients focusing on the repair processes.

Materials and Methods

Clinical Baseline of The Study Population

The study was involved in 100 random patients (52 males and 48 females) . These cases were diagnosed as a renal failure by specialized physicians and pathologists as well as their serum levels for urea and creatinine was checked. Also, the control group comprised 30 healthy volunteers (18 males and 12 females), who had no pathological state at the time of the study . All individual in the control group were matched to patients in age groups and gender . The study protocol in agreement with ethics of Al- Zahraa Teaching Hospital,and College of Science , and all subjects gave written informed consent.

Classification of Renal Failure : The National Kidney Foundation's as part of its Kidney Disease Outcomes Quality Initiative (NKF KDOQI) clinical practice guidelines, proposed classification system table (1). This system is calculated from the estimated glomerular filtration rate(eGFR) based on severity determined by renal function levels , irrespective of the renal disease etiology.This classification includes five stages initiate from mild, moderately to severely decreased eGFR .Finally, the stage 5 (< 15 GFR) also called renal failure or end-stage renal disease (ESRD), is considered a serious disease with short lifespan if untreated .The patients should be undergoing renal replacement therapy if uremia present . The MDRD equation was used to calculate eGFR and that proposed by [16]as following :

MDRD eGFR = 186 × [serum creatinine (mg/dL)]$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female)

Table (1): Distribution of patients according to different stages of RF disease classified by eGFR

Stages of renal failure disease	Glomerular filtration rate (mL/min/1.73 m3)	Patients No(%)
Stage 1	≥90	5(5%)
Stage 2	60–89	7(7%)
Stage 3	30–59	41(41%)
Stage 4	15–29	19(19%)
Stage 5	<15 (or dialysis)	28(28%)

Molecular study : HMGB1 and KIM-1 genes expression was carried out using reverse transcription RT- qPCR . The entire RNA of all samples was collected using the AccuZol reagent in accordance with the manufacturer 's protocol. The extracted RNA was reversely transcribed to complementary DNA (cDNA) using (СИНТОЛ Company / Russia). The procedure was carried out in a reaction volume of 25 µl following the instructions of manufacturer . Real-time PCR quantitative was applied in this study by using EVA green. The fluorescent dye recognized any double-stranded DNA including cDNA and the amplification was recorded as a cycle threshold (Ct) value. The mRNA level of endogenous control gene *B*- actin was amplified and used to normalize the mRNA levels of HMGB1 and KIM-1 genes . Table (2)displays the primers used in RT-qPCR

Table (2): The primers sequences used in RT-qPCR

Genes	Primer	Sequence (5' to 3') direction	References
HMGB1	Forward	5' TCATCTGCTGCAGTGTTGTT '3	(17)
	Reverse	5' CTCAGAGAGGTGGAAGA 3'	
KIM-1	Forward	5'- AACCAGTAGCCACTTCAC-'3	(18)
	Reverse	5-'CTGTCACGGTGTCATTCC-'3	
B- actin Reference gene	Forward	5' CTGGAACGGTGA AGGTGACA-'3	(19)
	Reverse	5' CGGCCACATTGTGAACTTTG '3	

Statistical Analysis

The data have been translated into a computerized structure . SPSS version 25 with Microsoft Excel 2010 were used to perform statistical analysis. Prevalence of variable of variable as gender, age and RF stages are detected by approximate percent .Data were normally distributed ,an unpaired (t-test) was used to compare the difference between patients with RF and control group. An estimation was statistically considered if its P-value was below 0.05. Gene expression was calculated by $2^{-\Delta\Delta Ct}$ equation [20]

Results

The present study reveals there are non- significant difference between the mean age of patients and control group (49.19 ± 15.52 and 42.92± 13.88) respectively (P>0.05) table (3).Although the sample is randomly selected, the number of male patients (52) very close to the number of female patients (48).The decline in eGFR value less than (>60 ml/min/1.73m^2) indicates renal failure.The median eGFR for all patients was (27.66±20.82 mL/min/1.73 m^2), which significantly reduced compared with healthy control (124.57±18.20 ml/min/1.73 m^2) with (p<0.001). The prevalence of RF patients stratified the patients into three age groups, youth(25<) ,middle-aged(26-55), and elderly(56>) for statistical analysis to see the effect of age on kidney damage ,figure(1). Data in table (4)showed RT-qPCR analysis for relative gene expression by using the formula 2^{-DDC} . HMGB1 and KIM-1 have overexpression in renal failure patients (3.51 and 5.37)compared with the control group (1.00), that reflect the significant difference between both groups (p<0.05).

Table (3) Clinical baseline characteristics of patients and the control group

Subjects	Patients		Control	
Gender	No	%	No	%
Male	52	52%	18	60%
Female	48	48%	12	40%
Age range				
Age(year)	17-85 year		17-66 year	
Mean±SD	49.19±15.52		42.92±13.88	
SE	1.77		2.71	

Cont... **Table (3) Clinical baseline characteristics of patients and the control group**

P-value	0.17**	
eGFR		
Total Mean (ml/min/1.73m2) ± SD	27.66±20.82	124.57± 18.20
p- value	0.001*	

*= significant ; **= non-significant

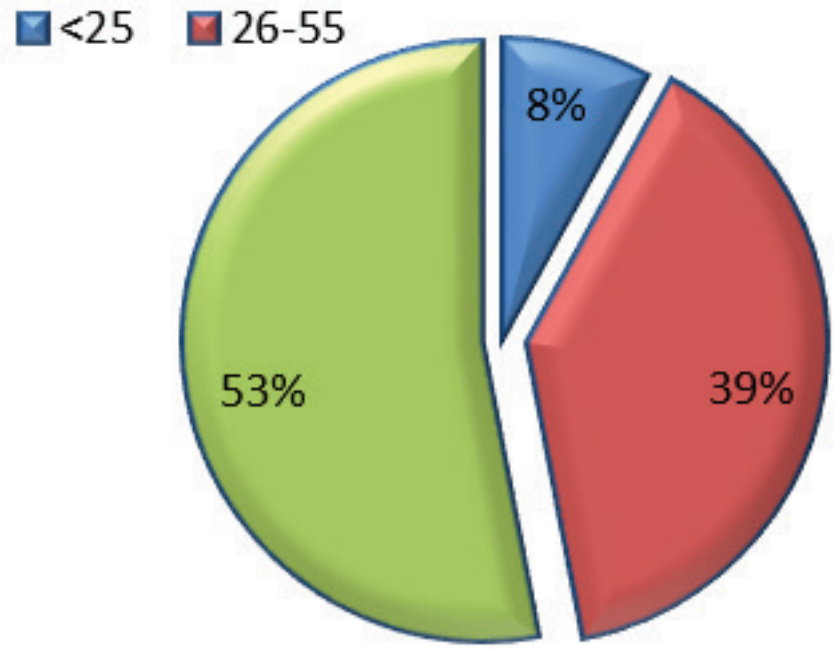

Figure (1) : The prevalence of patients according to age groups

Table(4): mRNA expression of HMGB1 and KIM-1 in RF patients and control group

Subjects	Mean Ct gene	Mean Ct actin	Mean Ct test Δ	Mean Ct ΔΔ	Folding
HMGB1					
Patients	16.28	13.33	2.89	-1.81	3.51*
Control	18.01	13.30	4.71	0.00	1.00
KIM-1					
Patients	18.42	17.77	0.64	-2.42	5.37*
Control	20.65	17.58	3.07	0.00	1.00

*= significant

According to five stages of RF disease ,our results detected that both HMGB1 and KIM-1 gene folding elevated gradually with advance the stages from to five stages of the RF patients when compared with control group (1.23 ,2.22, 3.02, 3.69 ,3.36 ,3.52) respectively .Regarding KIM-1 gene expression , also it was gradualy overexpressed in RF patients according to five stages of disease (1.53 ,2.45 ,4.08 ,4.06 , 4.87 , 5.49) respectively, comparing with control group ,table (5). However, both genes were showed the highest folding value in stage five the end-stage of renal disease.

Table(5) : HMGB1 and KIM-1 gene expression according to RF stages

Subjects	Mean Ct gene	Mean Ct actin	Mean Ct test Δ	Mean Ct ΔΔ	Folding
HMGB1					
Stage 1	17.80	13.39	4.41	-0.30	1.23*
Stage 2	16.95	13.39	3.56	-1.15	2.22*
Stage 3 A	16.51	13.39	3.12	-1.60	3.02*
Stage 3B	16.22	13.39	2.83	-1.88	3.69*
Stage 4	16.35	13.39	2.96	-1.75	3.36*
Stage 5	16.29	13.39	2.90	-1.82	3.52*
Control	18.01	13.30	4.71	0.00	1.00
KIM-1					
Stage 1	20.23	17.77	2.46	-0.61	1.53*
Stage 2	19.55	17.77	1.78	-1.30	2.45*
Stage 3 A	18.82	17.77	1.04	-2.03	4.08*
Stage 3B	18.82	17.77	1.05	-2.02	4.06*
Stage 4	18.56	17.77	0.79	-2.28	4.87*
Stage 5	18.39	17.77	0.62	-2.46	5.49*
Control	20.65	17.58	3.07	0.00	1.00

*= significant

Discussion

The result in the table (2) showed the patients' number was nearly closed in both sexes even though the sample was random . The number of male patients (52) and female patients (48),this indicated that the RF disease can affect both genders equally, and not related to the type of gender**.** The study reveals the majority of patients were older than 56 years , figure (1) at the time of diagnosis with an estimated percentage of (53%) from the total of patients. We believe that RF disease elevated progressively with age of patients, and that may be to decline in GFR and increase creatinine level . In addition, the elderly patients have associated diseases such as Hypertension and Diabetic Mellitus that affect the glomerular .Our findings were compatible with the results of studies in the world , [21] showed the prevalence of RF in the US adult population was much higher of persons aged 50+ years versus of persons aged 40–50 years and 20–39 years. Recently, with rapidly developing economy of China ,the 2015 Annual Data Report of the China Kidney Disease Network found that approximately half of the patients with RF were aged 60 years or older [22].The present study, showed significant decrease in eGFR for RF patients compared with control ,this result in line with [23,24]. eGFR is a parameter which defines a clinician's

kidney function .Regardless of its origin, a decline in eGFR is linked to renal disease, and the severity of renal damage is closely yet inverse to eGFR. A typical eGFR (~125 mL / min) is presumptuous evidence of functioning healthy kidneys. When the activity of eGFR the kidneys decrease, urinary excretion of creatinine and urea also declines and both increase concentration in blood flow [25, 26].On the other hand, our result focus on repair genes HMGB1 and KIM-1in RF disease ,the results in table (4) showed that both genes have significant overexpression than control group .This findings in line with the result reported by [27, 28]. In a pathological condition such as renal injury, the HMGB1 exhibits pro-inflammatory activities during the course of apoptosis and autophagy as well as necrosis . The expression HMGB1 released outside the nucleus, and rapidly translocates from the nucleus which is a normal residence position into the cytoplasm , and then extracellular upon stimulation by molecules signaling damage or injury. After cellular injury, HMGB1 releases rapidly which can indicate HMGB1 plays a critical role in the early stages of injury signal propagation following damage [29,30]. Ichimura, and his colleagues [31] detected that KIM-1 overexpression is indicator for the long-term prognosis of chronic renal diseases . For acute renal injury ,the KIM-1 expression is actual promising sensitive and specific biomarker. After ischemic or toxic insults, KIM-1 is markedly upregulated in damaged and regenerating renal proximal tubular epithelial cells, suggesting a potential role for this molecule in kidney repair process [32] . The proximal tubule epithelium would regenerate and repair following renal injury, and some cellular events are involved respectively, including cell migration, proliferation, and dedifferentiation [33] . One of first mechanisms of epithelial repair is the movement of the regenerating cells to cover the denuded basement membranes. In fact, the overexpression of KIM-1 significantly increases the migration of renal epithelial cells [34] .

The findings in table (5) detected both HMGB1 and KIM-1 genes folding elevated progressively with 5 RF stages .This results coming in line with results of Jin,X., *et al*.,[35] they noticed that HMGB1 was elevated in RF patients in relation with stages of disease and upregulation of markers of renal dysfunction. Furthermore, this results compatible with results of Rabadi,M., *et al*.,[36] who reported that HMGB1 was elevated parallel with increasing of renal stages disease . Rabadi,M., *et al*.,[36] also reported that HMGB1 is a damage-associated molecular pattern molecule that binds to chromatin, but undergoes nuclear-cytoplasmic translocation and renal cell release when it is signaled (particularly vascular and tubular cells) into the venous circulation after injury.

On other side, the results of KIM-1 gene expression agree with results of Sabbisetti ,V.,*et al*., [37]and Panduru,N.,*et al*., [38]who showed that increased KIM-1 levels were inversely correlated with lower eGFR. The gradual decline in eGFR results in advance to end-stage renal disease (ESRD). In addition to the urine, KIM-1 is released into circulation following renal proximal tubule injury. A high level of KIM-1 is a major indicator for the risk of ESRD in RF patients [39,40]. Although the level of KIM-1 elevated with development stages of RF disease , no other published data has explored the role of KIM-1 circulation in advancing early progressive renal decline[41,42] .

Conclusion

The incidence of renal failure can be distributed equally between males and females. Elderly people over 50 years old are more likely to be affected than youth. HMGB1 and KIM-1 have significantly overexpression in renal failure disease . likewise , HMGB1 and KIM-1 increase progressively with severity of renal failure disease.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Identification of Novel Bacteria by Using 16S rRNA from Clinical and Soil Samples

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Abstract

The 16S rRNA gene is a useful tool and molecular indication for identifying novel bacterial species from different sources. It is widespread for members of this field and thanks to the continuous expansion of information sequence databases. We isolated Gram-negative and Gram-positive bacilli from the respiratory infections and soil respectively, and phenotypical and molecular identifications were performed. Genetic analysis indicated that the isolated bacteria were a new species belonging to *Bacterium* strain *and Proteus sp. Bacterium* strain JND-RSIb-21A with Accession number MW013547.1 which closely related (99.8%) with *Bacillus subtilis strain JND-RSIb-21A* for its Transversion point mutation (G instead of C) at the position 445 bp. *Proteus sp.* strain AMJ131 with Accession number MW015095.1 the isolate related closely (99.8%) to *Proteus mirabilis* strain AMJ131for two-point mutations Transition of (G instead of T) and Transversion of (T instead of A) at position 9bp and 10bp respectively.

Keyword: *Bacterium* strain, *Proteus sp.,* 16S rRNA gene, soil isolate, Gram-positive bacilli

Introduction

The genus *Proteus,* apart of the *Enterobacteriaceae* family of rod-shaped bacilli, Gram-negative bacteria [1], facultative anaerobic, swarming motility by flagella, ability to self-elongate and secrete a polysaccharide when in contact with solid surfaces; this allows for attachment and easy motility along surfaces. Its ability to form biofilms and is suggested to contribute to resistance to host defenses and certain antibiotics [2]. Widely spread in the environment mainly in water, soil, humans, and animals[3]. It is an opportunistic pathogen that is implicated in various human diseases of the respiratory tract, eye, ear, skin[4], and urinary tract [5]. Its hardy, adaptable, and potentially pathogenic residents of the human gastrointestinal tract and have been underappreciated as a cause of the gastrointestinal disease [6]. Soil microbiology emerged as a distinct branch of soil science [7]. Soil contains a wide range of microorganisms described as a 'black box' [8]. Soils are one of the world's hotspots for biodiversity [9] and it is a natural habitat in which microbes live, multiply and die. Interest in microbial diversity has grown rapidly in the scientific community. Increasing attention is being drawn to microorganisms because the fertility of soil depends not only on its chemical composition but also on the qualitative and quantitative nature of microorganisms inhabiting it [10]. The activity of microorganisms in soil is important for the robust functioning of soil and related ecosystem services.[11]. Most of the antibiotic producers used today are soil microbes [12]. One of the strategies to reduce the time for microbial identification is the use of molecular biology techniques which may also be supplemented with numerous molecular fingerprinting techniques[13]. Fast detection and identification of microorganisms is a challenging and significant feature from industry to medicine. Standard approaches are known to be very time-consuming and labor-intensive (e.g., culture media and biochemical tests). Conversely, screening techniques demand a quick and low-cost grouping of bacterial isolates, and current analysis call for broad reports of microorganisms, involving the application of molecular techniques (e.g., 16S ribosomal RNA gene sequencing-based on polymerase chain reaction) [14].

Materials and Methods

Sample collection

Sputum samples were collected from patients with respiratory infections in the respiratory and chest disease center in Basra city. Soil samples were collected from the pharmacy college garden/Basrah university and prepared by paraffin baiting technique [15].

Isolation and purification of bacterial isolates

Sterile dry swabs were used to streak sputum samples and soil samples onto sterile Petri dishes containing Sabouraud dextrose agar for 3 weeks at 37°C [16]. The antifungal agent cycloheximide (actidione) at 50 µg /ml was added to the sterilized media at 46°C [17]. Single colonies were obtained using sterile inoculation needles. Colonies were then stained with Gram staining [18]. Conventional and specific biochemical tests were used for identification [19].

DNA Extraction from Bacteria

The bacterial isolates were transported to an eppendroff tube (1.5) ml. The extraction of genomic DNA was achieved by Geneaid bacterial DNA extraction kit(GEE150), and the extraction steps were performed according to the instructions of the kits supplied company. then DNA was detected by gel of 1% agarose containing 0.5 µl ethidium bromide and electrophoresed at 60 volts for 1.5 hours.

PCR for 16S Ribosomal DNA

Primers 27-forward 5'-AGAGTTTGA TCCTGGC-3' and 1492-reverse 5'-GGTTACCTTGT TACGACTT-3'[20]. were applied to amplify 16SrDNA genes in eppendroff tube (20 µl) mixture (Intron, Korea) consisting of 5 µl Mastermix, 10 pmol primers (1µl) for each bacterium sample, 1µl DNA template, and 34.5 µl nuclease-free water. PCR program was 92 ºC for 2 min, 35 cycles of 94 ºC denaturation for 30 sec., 51.8 ºC annealing for 45 sec. and 72 ºC extension for 1.5 min., finally, 72 ºC for 5 min. The bands of 1500 bp were observed by adding 5 µl of PCR product in 2% agarose gel with 0.5 µl ethidium bromide and electrophoresed with 5 µl of 1 kbp DNA ladder (Bioneer) [20].

Sequencing and Identification of 16S rDNA gene

The PCR 16SrDNA gene was purified from gel using MEGAquick-spin™ Total Fragment DNA Purification Kit (Intron) Korea and then sequenced according to Macrogen Company conditions using an automated DNA sequencer. And by "BLAST" The alignment was identified for each bacteria, from the website http://blast.ncbi.nlm.nih.gov, and "CLUSTAL Omega" http://www.ebi.ac.uk /Tools /msa /clustalo/. were used for comparing all sequences [20].

Phylogenetic Tree

The 16SrDNA sequences data for each identified bacterial isolates were aligned for the concatenated of different lengths for isolates ranging from (537-706) bp and phylogenetic trees were inferred by using the Molecular Evolutionary Genetics Analysis" MEGA7" software.

Results

Identification by 16Sr DNA Gene

Sequencing 16SrDNA gene from 5 bacterial isolates was observed on agarose gel at a suitable size (1500 bp) in comparison with the DNA ladder as in Figure (1). Only 4 were identified by 16S rDNA gene sequencing and compared with their type strains. The bacterial isolates belong to, *Bacillus sonorensis*, *Bacillus subtilis*, *Bacterium* strain, *and Proteus sp.*

Phylogenetic Tree of Bacterial Species

The phylogenetic tree (Figure 2) shows the distribution and phylogenetic relationships among the studied bacterial species, the bacterial strains showed closely related isolates (0.1549, 0.0140) for branch value. While for others identical were 0 and scale value=0.050). While the Phylogenetic tree for newly recorded isolate *Bacterium* strain JND-RSlb-21A illustrates closely related isolates (0.0027, 0.0018 and for others identical were 0 and scale 0.0005), and for *Proteus sp* strain AMJ131 the phylogenetic tree shows closely related isolates with (0) for identical isolates, and scale= 0.001. (Figure 3and 4)

Identification of New Global Bacterial Strains

Two bacterial isolates were identified as new strains

showing differences with their type strains in some numbers and placements of the bases isolates. *Bacterium* strain JND-RSIb-21A with Accession number MW013547.1 which closely related (99.8%) with *Bacillus subtilis strain JND-RSIb-21A* for its Transversion point mutation (G instead of C) at the position 445 bp (Figure 5). *Proteus sp.* strain AMJ131 with Accession number MW015095.1 the isolate related closely (99.8%) to *Proteus mirabilis* strain AMJ131for two-point mutations Transition of (G instead of T) and Transversion of (T instead of A) at position 9bp and 10bp respectively as shown in (Figure 6).

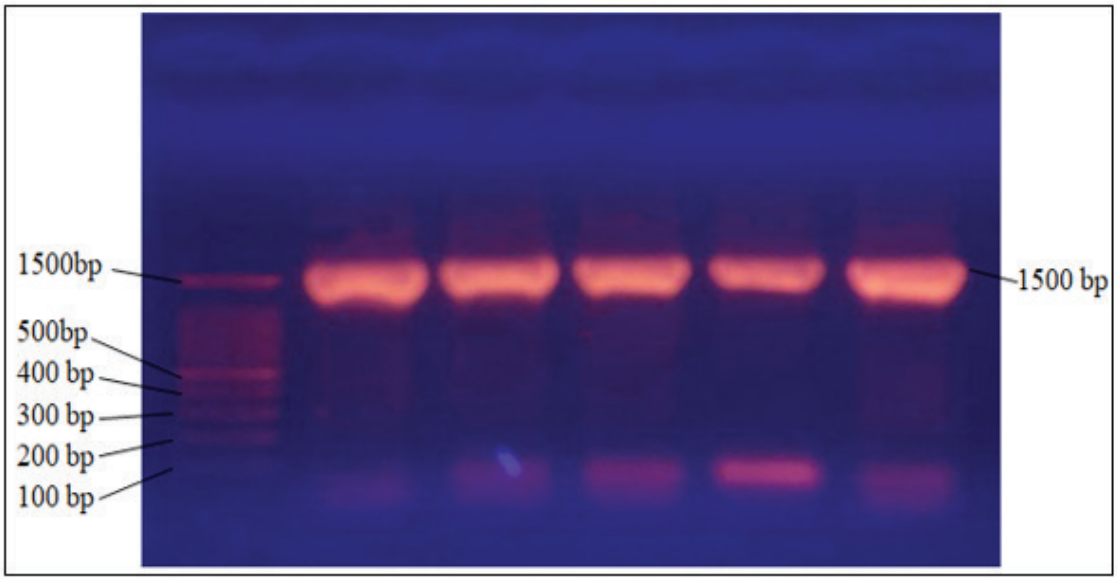

Figure 1: the Agarose gel electrophoresis of the 16SrDNA gene for each bacterial isolate.

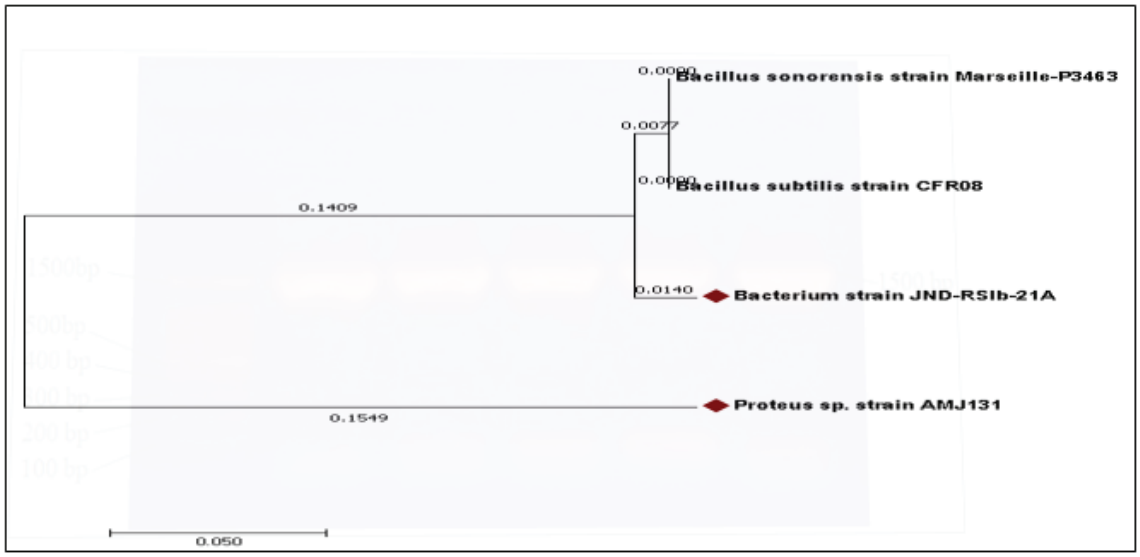

Figure 2. The phylogenetic tree for the bacterial isolates, newly recorded isolates

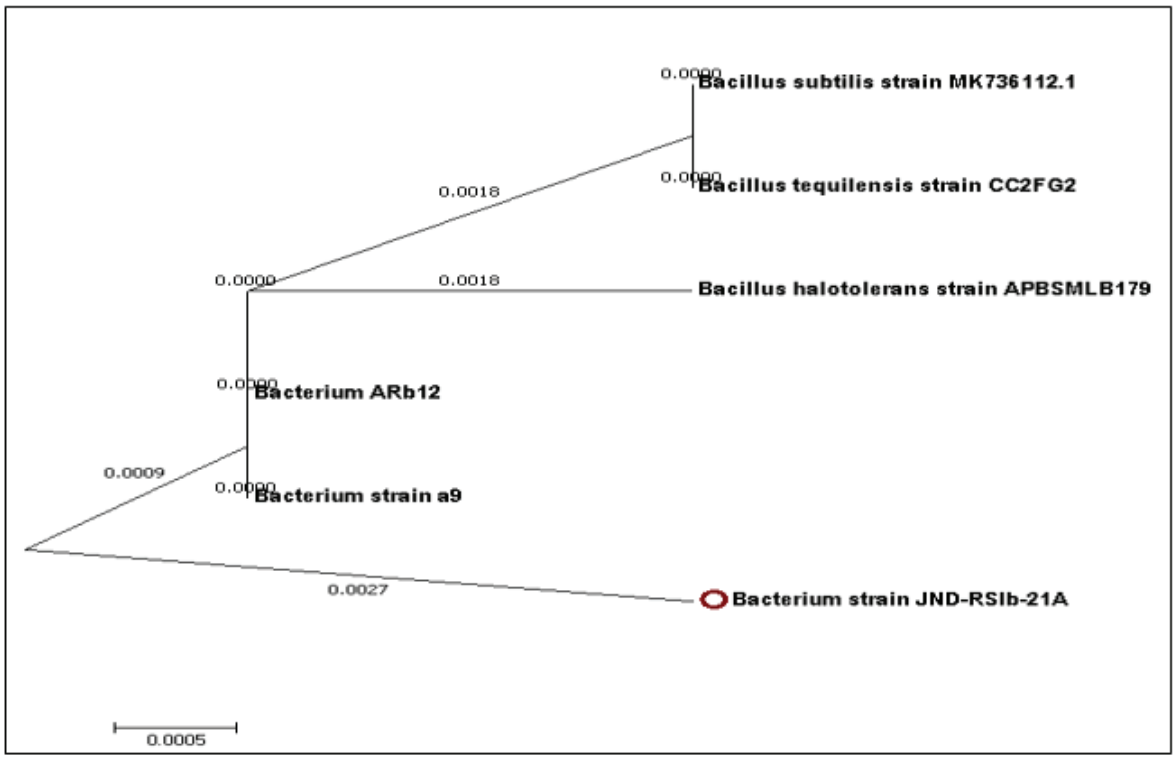

Figure 3: Phylogenetic tree for newly recorded isolate Bacterium strain JND-RSlb-21A

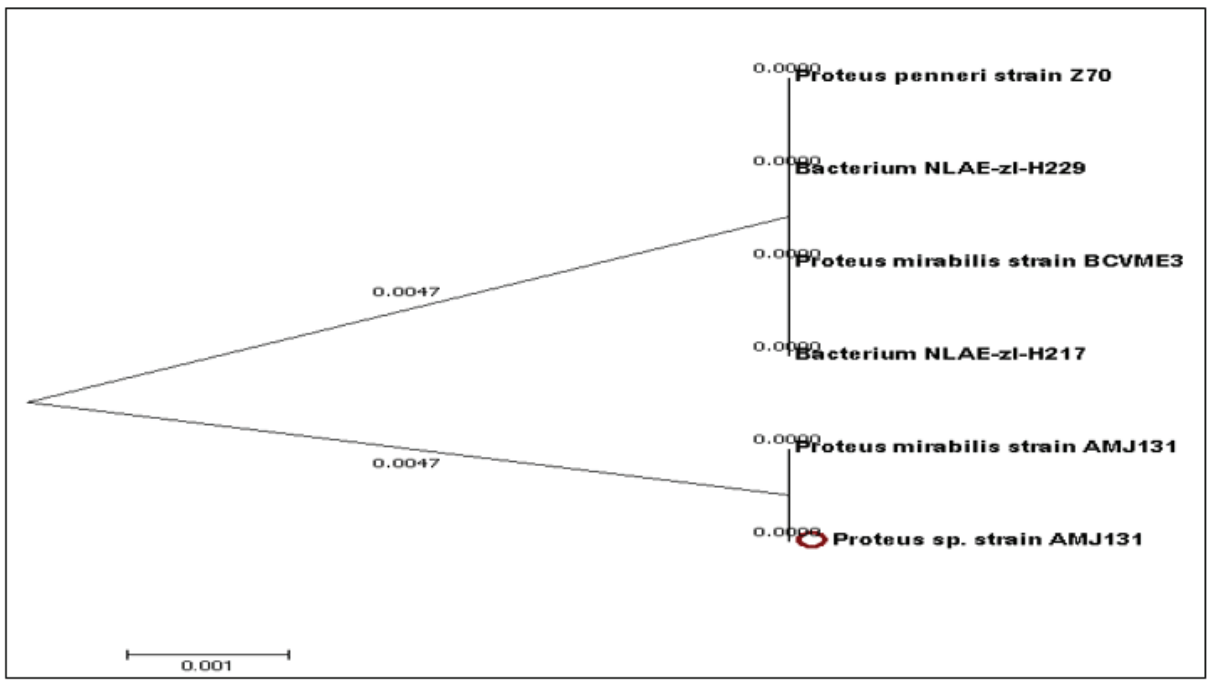

Figure 4: Phylogenetic tree for newly recorded isolate Proteus sp. strain AMJ131

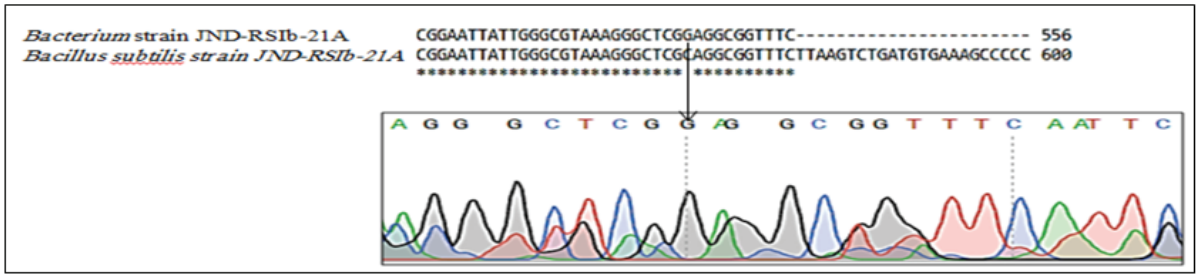

Figure 5:. Comparison of 16S rDNA nucleotide sequences (556 bp) for the isolate *Bacterium strain* JND-RSIb-21A from present study and *Bacillus subtilis* strain JND-RSIb-21A. A gene or point mutation type Transversion (G instead of C) at the position 445 bp.

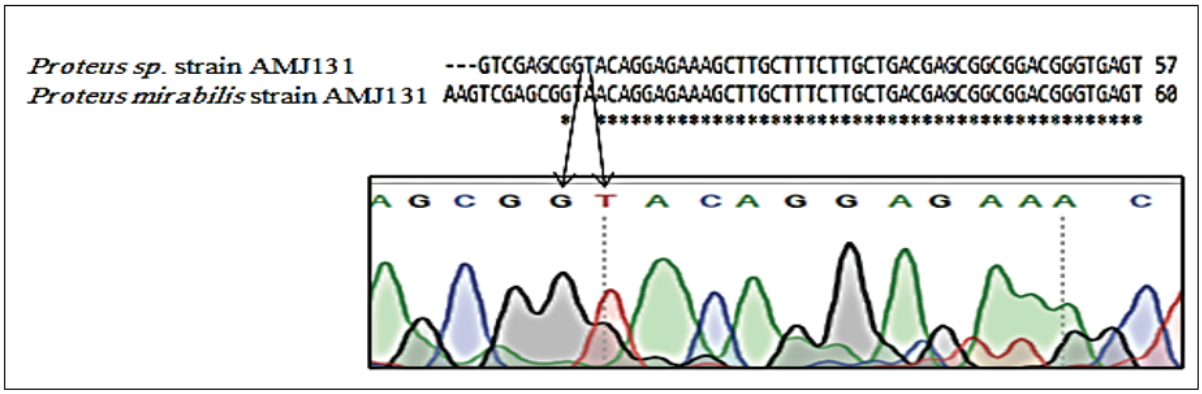

Figure 6: Comparison of 16S rDNA nucleotide sequences (539 bp) for the isolate *Proteus sp.* strain AMJ131 from the present study and *Proteus mirabilis strain AMJ131* gene or two-point mutation type Transition (G instead of T) and Transversion (T instead of A) at the position 9 bp and 10 bp respectively.

Discussion

Proteus belongs to the family Enterobacteriaceae and the tribe of Proteeae [21]. In the late 19th century, Hauser was reported characterized the first isolates of Proteus [22]. Proteus was isolated from sputum cultures according to the morphological and microscopical features, biochemical and API 20 E tests confirm [23]. The microorganisms in soil are very important to the planet in our lives, according to their role in the nutrient cycles [24]. Therefore, soils rich in nutrients are considered a fertile environment for a wide range of microorganisms, but the number of bacteria in the soil exceeds the total of other populations of microorganisms in number and type. [25]. *Enterobacter* spp., *Pseudomonas* spp., *Ralstonia* spp., *Proteus* spp., *Aeromonas* spp., *Burkholderia* spp., *Pantoea* spp., *Raoultella* spp., *Achromobacter* spp., *Escherichia coli,* and *Leclercia* spp. respectively isolated from the soils of Iraq [26]. Rapid replication of DNA from less starting material by PCR makes it a more sensitive technique for the detection of bacterial species. PCR-based identification of bacterial DNA and sequencing of the 16S rRNA gene has become a standard molecular method and highly specific to each bacterial species. PCR-based methods are used for the identification of bacteria that are difficult to grow in laboratory conditions [27]. The 16S rRNA gene is commonly used for the identification and classification of microbes from environmental samples. In this study, we use this gene for the identification of different isolates from soil and the human respiratory system. The 16S rRNA gene could be used as a phylogenetic marker because of its functional constancy and the presence of conserved and variable sequence regions evolving at very different rates [28]. A phylogenetic tree is a diagram that refers to evolutionary relationships among different species based on similarities and differences in their genetic characteristics. The phylogenetic tree was designed according to the bacterial isolates which showed 99%

or 100% similarity with the type strains, giving two bacterial isolates as new strains, in this study (Figure 2) for the phylogenetic tree of the isolates illustrate. The *Bacillus sonorensis* strain Marseille-P3463 is closely related to *Bacillus subtilis* strain CFR08 but differs from the newly recorded strain *Bacterium* strain JND-RSlb-21A and *Proteus sp.* strain AMJ131 because of the changing in 16s rRNA gene as a result of mutation [20]. To explain the relationship between newly recoded isolates with other strain we draw two phylogenetic trees (Figure 3) shows the newly recorded strain *Bacterium* strain JND-RSlb-21A differ from other strain selected from GenBank (*Bacillus subtilis* strain MK736112.1, *Bacillus tequilensis* strain CC2FG2, *Bacillus halotolerans* strain APBSMLB179, *Bacterium* ARb12, *Bacterium* strain a9) which were isolated from the various sources, and that may be for transversion point mutation (G instead of C) at the position 445 bp as mention above in (Figure 5). The newly recorded strain *Proteus sp.* strain AMJ131 is closely related to *Proteus mirabilis* strain AMJ131 as illustrate in the phylogenetic tree (Figure 4) but it differs from other strain from different sources selected from GenBank (*Proteus penneri* strain Z70, Bacterium *NLAE-zl-H229*, *Proteus mirabilis* strain BCVME3, *Bacterium* NLAE-zl-H217) and that difference explain according to two-point mutations Transition of (G instead of T) and Transversion of (T instead of A) at position 9bp and 10bp as in (Figure 6) [20,29]. A mutation is a permanent alteration in the sequence of the nitrogen base of the DNA that is generally may change the end product of the specific gene, mutations lead to change in the genes that are very important in bacterial evolution and that make a difference in the distribution of isolates in the phylogenetic tree. Mutations may occur by exposure of the bacteria to certain environmental factors such as radiation and chemical mutagens. Also, overuse of broad-spectrum antibiotics leads to a mutation and emergence of a new strain such as in bacteria isolated from humans such as the urinary tract [20].

Conclusion

Respiratory infections are the most prevalent and a chronic health problem in humans all over the world. Different bacterial species have the important role in the environment especially in soil. In this study, new bacterial strains were isolated from the soil samples of pharmacy college garden and patients with respiratory infections belonging to *Bacterium* strain JND-RSIb-21A *and Proteus sp.* strain AMJ131 respectively. Result of 16S rRNA analysis showed that *Bacterium* strain JND-RSIb-21A with Accession number MW013547.1 which closely related (99.8%) with *Bacillus subtilis strain JND-RSIb-21A and Proteus sp.* strain AMJ131 with Accession number MW015095.1 which closely related (99.8%) to *Proteus mirabilis* strain AMJ131.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Antibacterial Activity of Eucalyptus Camaldulensis Extracts on *Staphylococcus aureus* Isolated from Nasal Cavity

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Abstract

To study the effect of prepared *Eucalyptus camaldulensis* in aqueous, ethanol and flavonoid extracts by conducting susceptibility tests against *Staphylococcus aureus* isolated from nasal carrier of healthy subjects, in addition to detection some of virulence factors by conducting β-lactamase enzyme test, and biofilm formation test. This study included 250 healthy subjects, the males were 125(50%) and 125(50%) females. Two swab samples were collected from each subject, the studied samples collected during one year period from (April / 2018- April 2019). Different culture media and biochemical tests were used for isolation and identification the isolates as *Staphyllococcus aureus*, in addition to prepared three types of *Eucalyptus camaldulensis* extracts in form of aqueous, ethanol and flavonoid. Susceptibility test was conduct by disc diffusion method, then study some of virulence factors of *Staphyllococcus aureus* by conducting β-lactamase enzyme test and biofilm formation test. Among 250 healthy subjects involved in this, study *Staphyllococcus aureus* 68 (27%) isolates were detected, isolates from male was 40 (59%), while from female was 28 (41%), only 39 (57 %) isolates were sensitive to different concentrations of *Eucalyptus camaldulensis*, of both aqueous and ethanol extracts, ethanol extract had a higher effect on *Staphyllococcus aureus* than aqueous extract, while 45 (66%) isolates were sensitive to flavonoid extract. Twelve (18%) isolates form a biofilm in tube method, and 48 (71%) isolates showed *β*-lactamase test positive. A higher nasal carriage were in males in this study than females. The anti *Staphyllococcus aureus* effect of *Eucalyptus camaldulensis* extracts were flavonoid followed by ethanol then aqueous extracts, some of *Staphyllococcus aureus* isolates were highly virulence by showing a positive result for β- lactamase enzyme and biofilm formation test. this study shown that the flavonoid extract of *E. camaldulensis* have a great potential as antimicrobial agents on S. aureus than crude aqueous and ethanol extracts, many nasal pharmaceutical preparation can prepare from *E. camaldulensis* in crude and flavonoid form for treatment infections and reduce nasal carrier of *S. aureus*.

Keywords*: Eucalyptus camaldulensis, Staphylococcus aureus, Virulence factors.*

Introduction

Eucalyptus camaldulensis has the widest geographical range of any eucalypt in Australia; It is one of the most widely cultivated eucalypts across a range of tropical ,arid and temperate countries, with southern provenances occasionally better suited to temperate, Mediterranean-type climates and more northern provenances to tropical and subtropical climates. It has smooth white or cream-coloured bark, lance-shaped or curved adult leaves, flower buds in groups of seven or nine, white flowers and hemispherical fruit with the valves extending beyond the rim. Leaves extracts of *E. camaldulensis* have been used for treatment diseases all over the world before advent the modern drugs, its use in folk medicine as a treatment remedies of various medical conditions, these extracts contain an important source of bioactive molecules for drug discovery[1]. Isolated bioactive molecules serve as starting materials for laboratory synthesis of drugs as well as a model for the production of biologically active compounds that associated with various phytochemical compounds of

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tannin, flavonoids and sterols, which poses medicinal properties such as anesthetic, antiseptic, astringent, in addition to its medicinal effect on colds, colic, sinuses congestion, cough, laryngitis, pharyngitis, sore throat, spasm, and used in many topical preparation for wounds infections, in addition to antibacterial, antifungal, anti-inflammatory, antiviral, and antoxidative activities[1,2].

Staphylococcus aureus is a Gram-positive, spherical bacterium, arranged and appeared in grape like clusters, non-spore forming, they reside mainly on the skin, particularly in moist areas such as throat, axilla and groin, although multiple sites of body can be colonized in spite of this the main ecological niche where *S. aureus* resides was the anterior nares of the nose, nasal carriage often lead to infections[3]. One third of individuals carry these organisms at any time, staphylococcal infections occur worldwide, and newly emerging hyper virulent or multi-resistant strains of *S. aureus* spread rapidly over wide geographical areas[4]. Many virulence factors that associated with *S. aureus* infections which include hemolysins, leukocidins, proteases, production of toxins, and adhesion properties[5]. This work has several aims: first, to know the nasal carrier of *S. aureus* among healthy subjects, second to evaluate the anti *S. aureus* of prepared *E. camaldulensis* in flavonoid, aqueous and ethanol extracts by conducting susceptibility tests, third conducting β-lactamase enzyme test, and biofilm formation test as a study some of virulence factors associated with *S. aureus*.

Materials and Methods

Studied samples: two hundred and twenty five healthy subjects were involved in this study of both sexes the males were 125 (50%), while the females were 125 (50%) and their age ranged from (18-22 years) from them two swabs were obtained by swabbing the nasal vestibule of each right and left nares with a sterile swab. The studied samples were collected during 1 year period from (April / 2018- April 2019).

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS AUREUS:

Each specimen was inoculated on non-selective media as nutrient agar, and blood agar, the plates were incubated at 37 °C in the presence of oxygen for 24 hours[6]. When growth was appeared and identified macroscopically as Staphylococcus species, then the diagnosis was confirmed by catalase and coagulase enzymes test, in addition to culture on Mannitol salt agar when the cultural growth that had appositive results of both culture and biochemical test tests, then the diagnosis was confirmed by slide smear, using Gram stain and tested microscopically for appearance of bacteria as clustered Gram positive cocci to confirm the isolation and identification of *S. aureus*, in addition to susceptibility test was applied to each isolated *S. aureus* by using chloramphenicol disc[6].

PREPARATION OF EUCALYPTUS CAMALDULENSIS EXTRACTS:

Eucalyptus camaldulensis leaves were collected from trees of *E. camaldulensis* in Mosul city, the leaves were washed with distilled water, then dried in shed and grounded into fine powder to pass 100 mm sieve[7]. Fifty gram of *E. camaldulensis* fine powders was weighted and soaked in 250 ml of sterile distilled water (D.W) for aqueous extraction, however ethanol extraction was prepared by soaking 50 gram of *E. camaldulensis* in 250 ml of ethanol, both extracts were prepared by maceration at room temperature with stirring for 48 hours. The aqueous and ethanol extracts were separately filtered with filter paper (Whatman No. 1), then solvents were evaporate. Finally the extracts of *E. camaldulensis* leaves were kept freeze dried in refrigerator until use[7]. Every 50 gram of *E. camaldulensis* leaves extract yielded 7.3 (14.6 %) gram for ethanol and 4.6 (9.2 %) gram for aqueous extract as showed in (Table 1). The extract had deep yellow - brown colour with gummy-like appearance.

Table 1. Weight and percentage of aqueous and ethanol of Eucalyptus camaldulensis leaves extracts

Aqueous extract		Ethanol extract	
Wt.(gram)	%	Wt.(gram)	%
4.6	9. 2	7.3	14.6

PREPARATION OF FLAVONOID EXTRACT

From Eucalyptus Camaldulensis Leaves:

Initially 50 gram of *E. camaldulensis* leaves powder was soaked in 250 ml ethanol for 24 hours at room temperature with stirring. Then the mixture was filtered by filter paper (Whatman No. 1). The filtration were repeated several times, then 50 ml 1% lead acetate solution was added to extract, with continues stirring for 4 hours for complete precipitation. A mixture of 125 ml acetone and 15 ml hydrochloric acid was taken and mixed with the extract. Deep brown colored precipitate after evaporation was obtained by using a rotary evaporator under reduced pressure[8]. From 50 gram of *E. camaldulensis* leaves extract yielded 0.01 (0.02 %) gram of flavonoid. The extract has a deep green- brown colour with crystal-like appearance.

Susceptibility Test:

Susceptibility test was applied to each *S. aureus* isolates, which processed by transferring 2-3 of pure culture colonies of identified *S. aureus* and mixed with 3-5 ml Muller- Hinton broth to prepare a bacterial suspension that equal to 0.5 MacFarland, then cotton swab was soaked in bacterial suspension and streaked over a whole dried surface of Muller–Hinton agar[9].

Preparation the Discs

A stock concentration of 2000 µg/ ml was prepared form each flavonoid, aqueous and ethanol extracts. The aqueous extract dissolved in distilled water, while ethanol extract dissolved in dimethyl sulfoxide (DMSO), however flavonoid extract dissolved in ethanol. Paper discs (radius 6mm) prepared from sterile filter paper (Whatman No.1) were saturated with different concentrations (50µg/ disc, 100µg/ disc, 150 µg/ disc, 200 µg/ disc, 250 µg/ disc, 300 µg/ disc) of each *E. camaldulensis* extracts overnight . With a sterile forceps the discs were applied on the inoculated Muller–Hinton agar with *S. aureus*, and the plates were aerobically incubated for 24 hours at 37°C. The diameter of inhibition zone were measured in millimeter with a ruler[10].

BIOFILM FORMATION TEST

This test was applied to each *S. aureus* isolates to test if they have an ability to form a biofilm or not by tube method. 10 ml of fresh trypticase soy broth with 1% glucose inoculated with a tested 3-5 colonies of *S. aureus* in a test tube. The tubes were incubated at 37 °C overnight. After incubation, tubes were poured and then washed with a PBS and left to dry, then stained the tubes with crystal violet. The tubes were left to dry upside down, positive results were concedered when a visible film lined up on the bottom and wall of the biofilm tube. The amount of biofilm formed was scored as none, weak, moderate, and high according in comparison with a results of the control isolates[11].

B-Lactamase Enzyme Test

vvvFor detection the ability of 68 *S. aureus* isolates for production β-lactamase enzyme by using rapid standard iodine method. This test was done by transferring 3-5 young colonies to plates that contain wells in each contain 100 µl of penicillin G and mix well by wood stick then incubated at 37 °C for 30 minutes. After incubation, then added 50 µl of starch solution and 20 µl of iodine solution to each well and mix well by a wood stick if the color remain blue that negative result, but when the color change from blue to white - yellow in 5 minutes that mean its positive results for β-lactamase enzyme production[12].

Results and Discussion

This study included 250 subjects, the males were 125 (50%) while females were 125 (50%), from them 68 (27 %) *S. aureus* isolates were revealed from their clinical specimens. From 68 (100%) *S. aureus* isolates, the isolates from males were 40 (59 %), while from female was 28 (41 %) as shown in (Figure 1). The rhinal carriers of *S. aureus* have an increasing risk of acquiring an infection with this pathogen, the region in the nares act as the main inhabitant sites of *S. aureus* colonization based on longwise studies with repeated samples of many population that has *S. aureus* nasal carriage patterns, although *S. aureus* often causes life threatening deep seated infections as bacteremia, endocarditis and pneumonia[13]. Among 250 healthy subjects involved in this study from them 68 (27%) subjects showed positive results for the presence of *S. aureus* in their nasal cavity, the ratio of male to females involved in this study were the same 50% for males vs. 50% for females. From 68 (100%) healthy subjects with nasal carrier from male

was 40 (59%), while from female was 28 (41%). Wertheim and Coworkers 2012[14] showed that (20-30%) of healthy individuals were carrier of *S. aureus*, and the rate of nasal carriage vary with age and gender which is higher in children and white males than females.

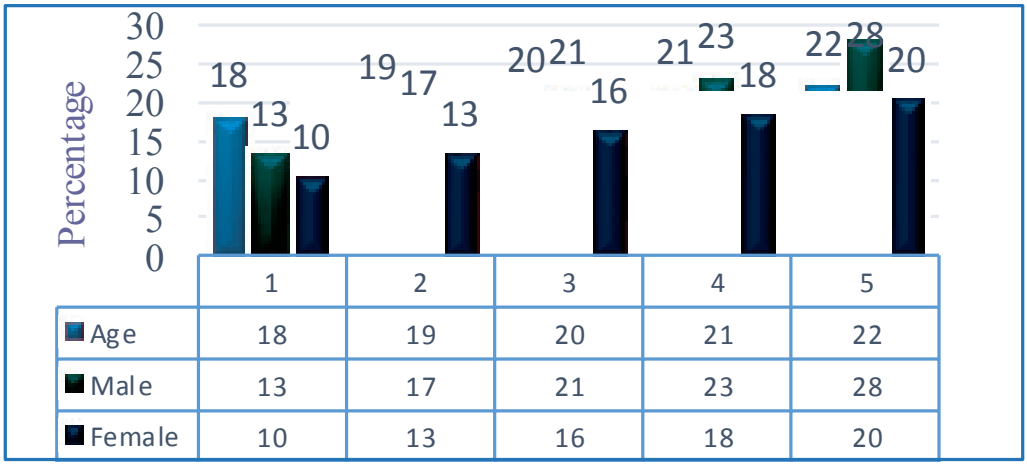

Figure 1. Number of Staphylococcus aureus isolates in studied groups by age, and gender.

Susceptibility Test:

From 68 isolated *S. aureus* there were 39 (57 %) isolates were sensitive to different concentration of *E. camaldulensis* aqueous and ethanol extracts, while 29 (43 %) isolates were unsusceptible to all concentrations of both extracts. The sensitive isolates to *E. camaldulensis* both extracts there zone of inhibition increase with increase the concentrations, however inhibition zone of ethanol extract was higher than inhibition zone of aqueous extract as shown in (Table 2,3 and Figure 2). Based on the results, of susceptibility test of ethanol and aqueous extracts of *E. camaldulensis* have a good antimicrobial activity on the studied *S. aureus*. From 68 isolated *S. aureus* there were 39 (57%) isolates were sensitive to different concentrations of *E. camaldulensis* both extracts, the sensitive isolates to *E. camaldulensis* both extracts there zone of inhibition increase with increase the extract concentrations. The results indicate that the extract of ethanol of *E. camaldulensis* has a greater effect by their zone of inhibition which ranged from (15-30) mm in compare to aqueous extract by their zone of inhibition which ranged from (11-25 mm) as shown in (Table 2,3 and Figure 2), this might be due to the extraction procedure by ethanol used as a dissolvent were more efficient than aqueous extraction procedure used distilled water as a solvent for extraction the active phytochemical compounds[15]. Behbahani and Coworker, 2013[16] showed that in his study the zone of inhibition by disc diffusion method of ethanol of *E. camaldulensis* extracts ranged from (20-27 mm), which higher than the zone of inhibition of aqueous extracts of *E. camaldulensis* was ranged from (15-26 mm).

Table 2. The mean diameter of inhibition zone in millimeter against different concentrations of both Eucalyptus camaldulensis extracts

Type of extracts	Concentration in µg/disc of extract					
	50	100	150	200	250	300
	Mean zone diameter in (mm)					
Ethanol extract	15	18	20	25	30	30
Aqueous extract	11	13	15	18	25	25

Table 3. The mean diameter of inhibition zone in millimeter against different concentrations of Eucalyptus camaldulensis flavonoid on Staphylococcus aureus

Type of extracts	Concentration in µg/disc of extract					
	50	100	150	200	250	300
	Mean zone diameter in (mm)					
Flavonoid	18	18	20	25	30	30

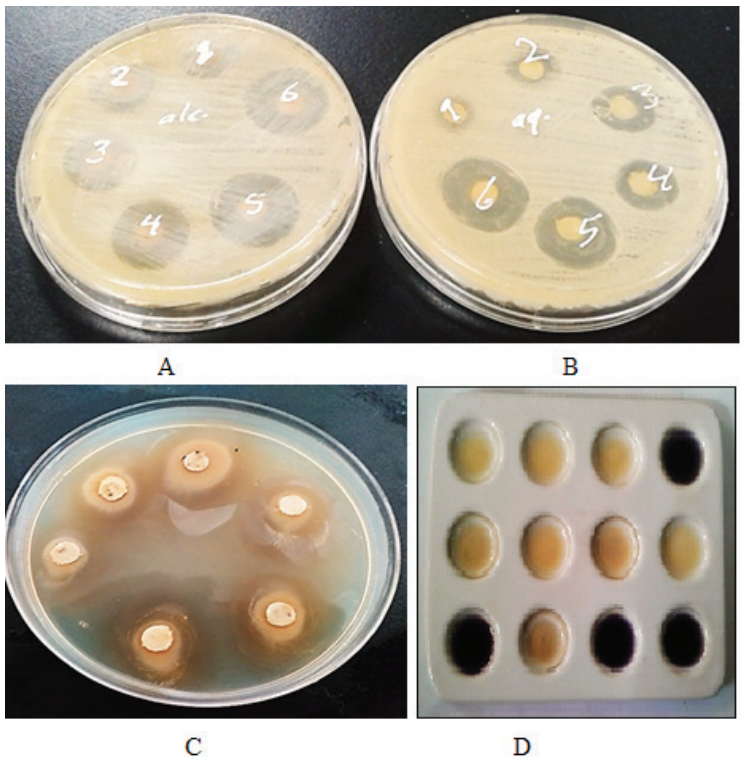

Figure (2): A and B) Susceptibility test of Eucalyptus camaldulensis aqueous and ethanol extracts.

C):The effect Eucalyptus camaldulensis flavonoid on Staphylococcus aureus

D):The presence and absence of β - lactamase enzyme of Staphylococcus aureus.

B-LACTAMASE:

This test were done to each *S. aureus* isolates in this study. From 68 isolates there were 48 (71%) isolates showed β-lactamase test positive (Figure 3-A-B). In this study there were 48 (71%) isolates showed β-lactamase test positive. From this result obtained in this study, which showed that increase in the number of *S. aureus* that produce β-lactamase enzyme this could be due to increase intake of anti β–lactam antibiotic as a reason that stimulate the production of β–lactamase enzyme[17]. Researcher showed that approximately 90% of *S. aureus* produce β-lactamase[6].

BIOFILM FORMATION TEST:

Among 68 (100 %) isolates only 12 (18%) form a biofilm from them there were 8 (12%) form weak biofilm, in addition to 2 (3%) were moderate biofilm former, while 2 (3%) isolates were strong biofilm former, however 56 (82%) isolates not showed a biofilm in tube method as shown in (Table 4). Biofilm consist of densely

packed communities of microorganisms adhered to each other and to the surfaces, which represent the major virulence factors attributed to *S. aureus*, these biofilm is resistant to conventional antimicrobial therapy[4]. The adherence property of biofilm was scored as non, weak, moderate, and strong, the present study showed that only 12 (18%) isolates have an ability to form a biofilm by a tube method, from them 2 (3%) isolate form strong biofilm, followed by 2 (3%) form moderate biofilm, and 8 (12%) form weak biofilm, however 56 (82%) isolates were negative results for biofilm formation test (Table 4). The strong adherence of the isolates were the highly virulence and resistance to conventional antibiotics[4] .

Table 4. Number and percentage of the isolates that form a biofilm by tube method

	Biofilm formation	Tube method	
		No.	%
Number of isolates (12)	High	2	3
	Moderate	2	3
	Weak	8	12
	None	56	82

Conclusions

The results of this study shown that the flavonoid extract of *E. camaldulensis* have a great potential as antimicrobial agents on *S. aureus* than crude aqueous and ethanol extracts, many nasal pharmaceutical preparation can prepare from *E. camaldulensis* in crude and flavonoid form for treatment infections and reduce nasal carrier of *S. aureus*.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

Source of Funding: the research funded by the authors only

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Emotional Intelligence and its Relationship to Psychological Cohesion and Social Communication Skills among Students of the College of Physical Education and Sport Sciences at the University of Samarra

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Abstract

The aim of this study is to identify the relationship between emotional intelligence, psychological cohesion and social communication skills among students of the College of Physical Education and Sport Sciences at the University of Samarra, that is to identify a group of students from this college whose number was about (140) students through the use of the following: emotional intelligence scale prepared by psychological cohesion scale, social communication scale designed by and Statistical Package for Social Sciences (henceforth SPSS) was also used. Accordingly, the study deduced that students of the college of Physical Education and Sport Sciences at Samarra University have a considerable level of emotional intelligence which was positively reflected in their social solidarity and social communication skills. For this reason, the researcher recommends more expansion to clarifying the concept of emotional intelligence, psychological cohesion and social communication skills, as they are among the novel concepts in sports field.

Keywords*: emotional intelligence, psychological cohesion, social communication skills.*

Introduction

The capture of material life over many of humans' concerns led to disregarding the emotional aspect, which is one of the basic components of the human psyche, while some came to a lack of awareness of their emotions and sentiments, and their inability to manage themselves as they are caught in their psychological pressures, so, consequently, they succumbed to sadness, depression and anxiety. All of the aforementioned reasons had a demonstrable impact on a lack of empathy with others, understanding their feelings and then poor communication with them, to reach to what is called emotional illiteracy. Many studies and researches have proved that mental intelligence alone is not sufficient for future successes, but emotional intelligence must be present alongside it, as it represents a person's abilities and skills that might be present or not, but it can be acquired, developed and psychologically trained.

The significance of emotional intelligence lies in the link between feelings, personality, and theoretical moral preparations that are the basic ethical positions in life stemming from the basic emotional capabilities of the human being. So, emotion, for a person, is the (medium) of passion, that explodes inside a person to express him/herself in any action [(1,2)].

In this regard, many studies dealt with emotional intelligence and its relationship to stress confronting skills[(3)]; among these studies is a study done by which examined emotional intelligence with methods of confronting stress among university students, whereas others are concerned with emotional intelligence and its relationship to some other variables as in a study by [(4)], which examined the relationship between emotional intelligence and some emotional variables among university students.

Moreover, regarding psychological cohesion, since 2007, Antonovsky's theory has become a central issue

for health promotion in the world, and it has received great attention and wide support in terms of application and field research by the International Union for Health Promotion and Education in various fields [5].

The concept of psychological cohesion has been attractive to many psychologists and health professionals because it assumes that health results from the tendency of the individual's personality towards life, in addition to the situational factors, such as personal perception of stress, perception of the level of social support in stressful situations, as well as the extent of its impact on various body systems such as, the central nervous system, the endocrine system, and the biological immune system, as it represents a tool for successful reconciliation, and a source of proper supply in times of crisis, as well as a means of refining and filtering in assimilating and processing the information [6,7].

In this regard, the study conducted by found that feelings of social solidarity correlate positively with feelings of hope, while, on the other side, found a significant correlation between feelings of psychological cohesion and each of the encountering strategies as well as a quality of life of chronic patients. This means that individuals with a high level of feelings of psychological cohesion use effective resistance strategies to cope with the disease and to maintain their mental health[8,9].

Bowman (1996), Flannery (1994) and Matsuura, et al., (2003) also found that individuals who are marked as having feelings of cohesion have in turn a lower level of depression and anxiety [6]. Social skills are also very important in the domain in which students live, as they are considered as indicators of mental health [10]. Consequently, it is regarded as one of the determinants of academic consistency, distinction in academic achievement and decision-making ability [11].

Social solidarity is regarded as a basic factor in the processes of socialization from an early age, and its impact remains for all life stages[12] So, the significance of the study lies in its approach to variables that are still novel, especially Arab studies, so the current study aims to identify emotional intelligence and its correlation with psychological cohesion and social communication skills among students of the college of Physical Education and Sport Sciences at the University of Samarra.

Procedures and Measurments

Participants

The study was conducted on all students of the College of Physical Education and Sport Sciences at the University of Samarra, whose number is (140) ones representing four stages.

Procedures

The researcher applied the emotional intelligence scale of [13], which is based on [14] model of emotional intelligence consisting of 83 items distributed into five areas: social skills 19 items, self-awareness 15 items, empathy 15 items, motivation 17 items, and self-organization 17 items. In the scale itself there are five repeated items to ensure the validity of the answer, thus the scale consists of 88 items.

The psychological cohesion scale was used to assess the overall life orientation of the individual to cope with pressures and adapt to them. Also, the answer sheet consists of 13 items that were formulated towards the negative direction, in which each item of the scale corresponds to seven answer alternatives due to Likert's method [3,15].

Also, the researcher applied the Social communication Scale [16], the scale consists of 45 items that measure three skills:

- The skill of social expression: It consists of 15 items
- The skill of social sensitivity: It consists of 15 items
- The skill of social control: It consists of 15 items

The answer to these items is done through a pentagonal graduation.

Results

Table (1) The arithmetic mean, standard deviation, value of the simple correlation coefficient, and the level of statistical significance and significance level between emotional intelligence and psychological cohesion

No.	Variables (scale)	Unit of measurement	The arithmetic mean	standard deviation	correlation coefficient	Statistical Sig.	sig
1	Emotional intelligence	score	348.5214	76.8852	0.861***	0.000	sig.
2	Psychological cohesion	score	53.6143	22.68			

*sig if the level is >(0.05).

Table (2) The arithmetic mean, standard deviation, value of the simple correlation coefficient, and the level of statistical significance and significance level between emotional intelligence and social communication

No.	Variables (scale)	Unit of measurement	The arithmetic mean	standard deviation	correlation coefficient	Statistical Sig.	sig
1	Emotional intelligence	score	348.5214	76.8852	0.645***	0.000	Sig.
2	Social communication	score	99.5071	50.1646			

*sig if the level is >(0.05)

Discussion

Through results achieved with regard to emotional intelligence, they can be explained according to the nature of the emotional atmosphere that prevails in the Iraqi environment, where the regulation of their emotions, their sympathy, their wills, their social communication and their ability to know their emotional knowledge, which, in turn, helps them to successfully practice their daily life despite the difficult conditions that the country is going through, which are of a strong influence on how we perceive these emotions with our feelings and better deal with them. In this way, emotional awareness is the essential building block in emotional intelligence, which is able to get rid of the disordered mood [14].

It is evident from table (1) that the current research sample is characterized by feelings of psychological cohesion which means that individuals possess in-depth resistance resources that contribute to understanding events, predicting them, controlling different situations and their related internal and external requirements, and that they greatly contribute to the cognitive representation process in dealing with the external environment. Our neurological data enter into the formation of basic skills to practice life known as emotional intelligence in being able, for example, to control our tendencies and impulses and to look at the subtle feelings of others and deal with flexibility in our relationships with others. This makes the person smart and emotional where his/her emotions exist in the personal abilities revolution in dealing with others [17].

Conclusions

The study deduced that students of the college of Physical Education and Sport Sciences at the University of Samarra have a good level of emotional intelligence, which was reflected positively in psychological

cohesion and social communication skills. Thus, the researcher recommends conducting more expanding studies to clarify the concept of emotional intelligence, psychological cohesion and social communication skills, as they are among the novel concepts in the field of sports.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

Funding: The work was supported by the researchers themselves.

Conflict of Interest: The author declare no conflict of interest regarding this research.

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Diagnosis of Bernard Soulier Syndrome and Glanzmann's Thrombasthenia in Iraqi Patients

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Abstract

Background: Bernard–Soulier syndrome (BSS) and Glanzmann Thrombasthenia (GT) are rare inherited platelet function disorders, defined by a permanent history of mucocutaneous bleeding. The objective of this project was to identify biological and clinical characteristics of BSS and GT patients. **Methodology:** This study included 52 patients with bleeding disorders, the clinical, haematological and demographic features of patients were determined and the level of GpIIb/IIIa and GPIb/IX was assessed. **Results:** From a total of 52 patient, 20 were diagnosis with BSS (9 females and 11 males), their age range from 6 to 42 year and 23 diagnoses with GT (10 females and 13 males), their age range from 4 to 50 year. Epistaxis, easy bruising, menorrhagia and ecchymosis were the most frequent symptoms. Prolonged bleeding time (BT), normal PT and PTT were seen in all cases. Variable thrombocytopenia, large platelets with decreased GPIb/IX level were seen in BSS patients, while the level of GPIIb/IIIa was decreased in GT cases. **Conclusion:** Since BSS and GT are infrequent disorders diagnosis may be postponed, both diseases are combined with crucial bleeding tendency, therefore earlier detection would have been important for patients management.

Keywords: *Inherited platelet function disorders, Bernard–Soulier syndrome, Glanzmann Thrombasthenia.*

Introduction

Inherited platelet function disorders (IPFDs) are a heterogeneous group of bleeding disorders caused by defects of soluble agonist receptors, adhesive protein receptors, platelet granules and membrane phospholipids[1]. Inherited platelet disorders may change the number of circulating platelets as well as their function, cause mucocutaneous bleeding signs with varying intensity, generally bruise easily, epistaxis, ecchymosis and gingival bleeding[2]. Among IPFDs Glanzmann thrombasthenia (GT) and Bernard Soulier syndrome (BSS) are exceedingly rare, but their intensity and biological features make them especially important[3]. Bernard-Soulier syndrome is autosomal recessive bleeding disease resulting from a qualitative and/or quantitative defect in the GPIb/IX/V platelet receptor, it is prevalent in male and female with 1:1 ratio[4,5]. It is estimated to occur in 1 per million individuals however, due to under recognition and misdiagnosis the prevalence may be much higher than the estimated[6,7]. The typical BSS manifestation begins at birth and continues throughout life. Thrombocytopenia, giant platelets, prolonged BT and low/ absence of GP1b/V/IX expression are main indicators in BSS cases [8]. GPIb/IX/V is platelets restricted receptor bind with von Willebrand factor (vWF) to initiate platelet adhesion at site of damage, this interaction will transmit signals to the cytoplasm of platelet to trigger a series of events which lead to creation of clot[9]. GPIb/IX/V formed by the association of four proteins related to the leucine rich motif (LRM) family[10]. The GPIb/IX/V subunits are encoded by GPIBA, GPIBB, GP5 and GP9 genes which located at chromosomes 17p12, 22q11.2, 3q29 and 3q21 respectively[11]. Diverse mutations in genes encodes for GPIb/IX/V subunits impaired synthesis and expression of the receptor on the platelet surface and consequently cause BSS[12].

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Glanzmann's thrombasthenia (GT) is a rare autosomal recessive bleeding disease, in which the platelets have defective or low levels of GPIIb/IIIa, the diagnostic features of GT include normal count and shape of platelets, prolonged bleeding time, irregular clot retraction, lack of platelet aggregation in response to arachidonic acid, collagen and ADP, as well as GPIIb/IIIa defective or deficiency [13].

GPIIb/IIIa is a large heterodimeric cell transmembrane complex, express in large amounts in the platelet plasma membrane consist of a larger αIIb subunit linked non-covalently to a smaller β3 subunit [14]. Activation of αIIbβ3 and binding of soluble ligands (primarily fibrinogen) are critical for platelet aggregation. αIIb and β3 subunits are encoded by ITGA2B and ITGB3 genes which are closely situated at chromosome 17q21.31-32[15]. GT mutation screening reveals a wide diversity of nonsense and missense mutations involving small insertion, deletion, frameshifts and splice site defect that occur through the 45 exons of ITGB3 and ITGA2B genes results in a qualitative or quantitative defect in the GPIIb/IIIa complex [16,17].

Patients and Methods

Patients

This study included 52 patients suffering from bleeding complication and 36 apparently healthy individuals without bleeding symptoms (control group).

Hematological examination

Five ml of blood were collected from each patient and control, the collected blood samples were divided in two tubes, one of which containing sodium citrate for PT and PTT test and another containing EDTA for CBC and ELISA. Duke's method was used for detection the BT and blood smears were prepared and stained with Leishman's stain to examine platelets morphology.

Evaluation of platelet glycoproteins

The level of GPIb-IX and GPIIb/IIIa glycoproteins was assessed in cases and control group with commercial ELISA kits (Bioassay Technology/ China) by Human Reader Systems, (Germany). The microtiter plate has been pre-coated with antibody (capture antibody). The platelet surface antigens in the sample are added and bind to the immobilized capture antibodies, then the antigens bound to the immobilized antibodies are sandwiched with an enzyme-labeled antibodies for color development. The color develops in proportion to the glycoprotein amount, absorbance was measured at 450 nm.

Statistical Analysis

The statistical analysis was carried out using SPSS version 26, parametric variables were presented as mean ± standard deviation (SD). For all tests P-value less than 0.05 was considered as statistically significant.

Results

The study included 52 patients (26 females and 26 males) have normal PT and PTT, but a history of hemorrhage and prolonged bleeding time that shows evidence of a primary hemostasis defect.

The patients' mean age was (17.56±10.9, mean ±SD) year ranged from (1-61)year. The main clinical manifestations in patients were epistaxis, easy bruising, menorrhagia and ecchymosis. The consanguineous marriages in parents were reported in 84% (n= 44) of patients and a majority of patients have positive family history of bleeding. Statistical analyses have shown that there was a considerable differences in BT ,Hb, platelet count and MPV comparing between patients and the control group and the results of the PT, PTT and WBC were within the normal range, and non-significant difference from control (P higher than 0.05), table(1).

Depending on the hematological examination and level of platelets surface glycoproteins, 20 patients were diagnosed with BSS and 23 with GT. Variable thrombocytopenia, large platelets and decreased GPIb/IX level were seen in BSS patients, while the level of GPIIb/IIIa was decreased in GT cases, the basic characteristics of patients are shown in tables (2).

Table No.(1): Correlation between patients and control regarding laboratory data

Item	Patients (Mean±SD)	Control (Mean±SD)	P value
Age/ years	17.56±10.9	18.5±9.2	0.8
Hb g/dl	9.5±1.6	12.9±1.7	0.001*
WBC	8.8±2.7	8.6±2.3	0.69
Platelet	166±73.6	260±59.8	0.001*
MPV fL	12.4±2.2	9.5±1.8	0.001*
PT sec	11.9±1.3	12±1.4	0.6
PTT sec	32.7±3.1	32.6±3	0.9
Bleeding time /min	12±2.3	5±1.5	0.001*

*= Significant difference (P value ≤ 0.05

Table No.(2): Demographic data in patients groups and control

Diagnosis	N. of p.	Age in years (Mean±SD)	Sex		Consequent state%	
			Male	Female	Positive	Negative
Glanzmanns	23	18±13.2	13	10	58.5	41.4
Bernard soulier	20	18.4±9.4	11	9	60%	40%
Thromasthenia	9	12.5±4.5	2	7	55.5%	44.4%
control	36	18.5±9.2	19	17	80.5%	19.4%

Bernard Soulier syndrome group

BSS was diagnosed in twenty patients (11 males and 9 females), the mean of age was (18.4±9.4) ranged from 6 to 42 year. All hematological parameters showed non-significant differences (P higher than 0.05) when compared between patients groups except platelet count and MPV, in BSS group platelet count was always low with median(111±40) and the MPV was elevated (14.1±1.4) range from 10.5 to 16.8 fL.

The bleeding time was prolonged (12.7±2.3), median hemoglobin level was (9.5±1.6) g/ dl, PT, PPT and WBC within normal range, table (3). Peripheral

blood smear analyzed showed the presence of larger platelets and the GPIb/IX expression was reduced, while the GPIIb-IIIa level found to be no statistically significant difference from control group as indicated in table(4).

Table No.(3): Correlation between patients groups regarding laboratory data

Parameter	BSS (Mean±SD)	GT (Mean±SD)	Unclassified thrombasthenia Mean±SD	P value
Hb g/dl	9.5±1.6	9.5±1.7	9.3±1.5	0.9
WBC	8.2±2.9	9.2±2.2	10±3.5	0.2
Platelet 10 e3/µL	111±40	225±62	148±33	0.001*
MPV fL	14.1±1.4	11±1.9	11.1±1.4	0.001*
PT sec	11.7±1.3	12.2±1.4	11.6±1.3	0.4
PTT sec	33±2.9	32.2±3	32±3	0.9
Bleeding time /min	12.7±2.3	11.3±2.2	12.1±2.2	0.1

*= Significant difference (P value ≤ 0.05)

Table No.(4): Correlation between BSS group and control regarding Elisa test results

Glycoprotein	BSS (Mean±SD)	Control (Mean±SD)	P value
Alpha	.993±1.7040	2.77±3.122	0.006*
Beta	2219.1±1476.2	3867.6±1891.9	0.001*
GP9	191.50±238.8	401.55±447.4	0.055
GPII/IIIa	22.3±13.7	19.8±5.6	0.44

*= Significant difference (P value ≤ 0.05)

Glanzmann thrombasthenia

Twenty-three patients were diagnosed with GT(10 females and13 males) the mean of age was (18±13.2) ranged from 4 to 50 year. GT patients had a normal platelet morphology and count (225±62), prolonged bleeding time (11.3±2.2 min), PT and APTT within normal range, hemoglobin level was reduced (9.5±1.7 g/ dl),table (3). The expression of integrin GPII/IIIa was reduced whereas the level of GPIbα, GPIbβ and GPIX found to be no statistically significant difference from control as shown in table(5).

Table No.(5): Correlation between GT group and control regarding Elisa test results

Glycoprotein	Glanzmann (Mean±SD)	Control (Mean±SD)	P value
Alpha	2.2 ±2.5	2.77 ± 3.12	0.52
Beta	3478±1965	3867.6±1891.9	0.44
GP9	354.65 ± 347.8	401.55 ± 447.4	0.6
GPII/IIIa	12.6 ± 8.2	19.8 ± 5.6	0.001*

*= Significant difference (P value ≤ 0.05)

The remaining nine patients with unclassified thrombasthenia had a normal platelet morphology, prolonged bleeding time (12.1±2.2), hemoglobin level was reduced (9.3±1.5 g/ dl),table (3) and the expression of GPIb/IX and GPII/IIIa glycoproteins found to be non-considerable difference compared with control(P value higher than 0.05).

Discussion

The demographic and clinical characteristics of 52 patients with bleeding manifestation were examined. The consanguineous marriages in parents were reported in 84% of patients and a majority of patients have positive family history of bleeding. Toogeh *et al*., (2004) demonstrated that the family history with consanguinity marriage would definitely increase the probability of having children with IPDs. So the occurrence was found to be high in populations where marriage between the close relative is common, such as Jordan, Iran, Pakistan, India and Saudi Arabia [18].

Twenty patients diagnosis with BSS(11 males and 9 females) with mean age (18.4±9.4) ranged from 6 to 42 year. The appearance of markedly larger platelets on peripheral blood smear, low platelet count and reduce the expression of GPIb-IX expression support the diagnosis of BSS in these group. Similar to previous studies in the literature, we found a relatively equal gender distribution and bleeding symptoms are usually evident from early childhood. The GPIb-IX-V receptor defect is combined with abnormal platelet structure and function. The prolonged bleeding time in BSS is most likely due to GPIb-IX-V defect, thrombocytopenia and reduced thrombin creation[19]. The GPIb–IX–V receptor is important for development of proplatelet due to its participate in the dynamic reorientation of the underlying microtubular cytoskeleton which is needed to enable MKs to expand the proplatelet[20]. The interaction between GPIbα subunit and filamin A adjusts platelet size, morphology and ligand adhesion, so diminished this association can lead to the development of giant platelets[12].

GT was diagnosed in twenty-three patients(10 females and 13 males) the mean of age was (18±13.2) ranged from 4 to 50 year, this study has shown slight male predominance (56%). Some previous reports show slight female predominance in which revealed 53% in 113 patients and 66% in 64 patients[21]. This is not significant as GT is an autosomal recessive disease. The results of laboratory investigations showed normal platelet count and morphology, PTT and PT are within normal limit, BT was prolonged with an average of 11.3 min. The analysis of platelet glycoproteins revealed reduce in the level of GPIIb/IIIa. In the 1970s, Nurden and Caen established that GT patients had specific defects in the composition of platelet membrane glycoproteins, the platelets have defective or reduced levels of GPIIb/IIIa[22]. Physiologically this platelet complex binds numerous adhesive plasma proteins and this enables attachment and aggregation of platelets to ensure thrombus development at vascular injury sites[23]. Impaired binding of GpIIb/IIIa with fibrinogen or other adhesive proteins that bind platelets result in no cross-linked bridges between platelets which lead to defect in platelet aggregation and reduced retraction of clot and BT is considerably prolonged in GT cases [24].

Conclusion

The diagnosis of BSS and GT may be postponed due to their rarity, many cases with inherited thrombocytopenias misdiagnosed with immune thrombocytopenia or less frequently myelodysplastic syndrome, exposing patients to unsuccessful and harmful treatments, so earlier diagnosis might be beneficial for their medical management and must be specifically sought in the consanguineous family offspring that have a history of bleeding.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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The Concentration Factor Effect Study of Etoposide Drug and *Annona squamosa* L. Seeds Extracts on Topoisomerase II Inhibition Model: Catalytic Inhibitory and Interfacial Poisons

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Abstracts

Background: Topoisomerase II plays an important role in cell proliferation and DNA regulation. It is responsible for loosening the supercoiled form of DNA and for condensing chromosomes in eukaryotic cells. Custard apple (*Annona squamosa* L.) (Annonaceae) has been one of the main ingredients of ethnomedical preparation for the treatment of tumors in some countries, and has cytotoxic effects on cancerous cell lines. Aim: assessment of Qualitative Inhibition (Catalytic Inhibitor or Interfacial Poisons) and Quantitative (IC50%) of Custard apple Plant Seeds; Crude Extract (CCE) and Alkaloid Extract (CAE) of Human Toposomerase IIA (TOP2A) compared to etoposide (VP-16) as a controlled drug. Methods: TOP2A (0.5 unit) and pHOT1 DNA plasmid (62.5ng), DNA plasmid relaxation assay required for inhibition testing. Inhibitors, the seeds plant was extracted by 100% methanol for the preparation of crude with concentrations (240, 160, 100, 80, 40, 20, and 10 µg/ml) and alkaloid (100, 80, 60, 40, 20, 10 and 5 µg/ml), and the VP-16 as a positive control drug (150, 100, 50, 25, 12.5, and 3.125 µg/ml). **Results and Discussion:** The CAE showed a higher DNA plasmid relaxation inhibition, IC50% (27.46). The higher concentrations of CCE and CAE play as a Catalytic Inhibitory Compounds CICs (as suppressor compound) rather than Interfacial Poisons IFPs (as poisons compounds), quite the opposite at low concentrations. Higher concentrations may inhibit the formation of the enzyme-DNA complex, resulting in the accumulation of the supercoiled form of the DNA plasmid due to inactivation of the enzyme function**.** In comparison, at low concentrations of inhibiting factors the linear structure is accumulated, which means that both strands cannot be rejoined. Conclusions: The CAE had the highest inhibition activity on the TOPO2A compared to the other agents. In contrast to the VP-16 drug, the CAE and CCE concentrations of this plant affected inhibition models.

Keywords: *Etoposide, TOP2B, Custard apple, Poison, Suppressor.*

Introduction

Cancer is one of the health issues the planet suffers from and is continually rising. In Iraq, the recently announced data from the National Cancer Registry in Iraq (NCRI) suggest that there are more than 31,500 cancer cases and tumours linked to cancer in Iraq as of 2017-2018 because of the weapons wars and the worsening of the health situation [1]. Cancer is one of the country's leading causes of death, contributing to an estimated 11% of all deaths. This has drawn the attention of the World Health Organization (WHO) to create a program for the prevention and treatment of the disease[1]. Topoisomerase is an enzyme that has two forms, type I and type II, type I, which breaks one strand and passes through the other, while type II breaks two strands and passes through the other double strands [3-4]. Topoisomerase II (TOPO II) is a remarkable enzyme that is widespread in eukaryotic organisms, responsible for

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the decoupling of double-stranded DNA segments during the translation and replication process. This enzyme is considered useful to separate the newly-duplicate sister chromosomes. To understand how this can be used to promote the development of chemotherapy, it is important to know how to control and inhibit the activity of this enzyme and its effect on the progression of the cell cycle [5-9]. There are two isoforms of Topoisomerase II: Topoisomerase (TOPO2A) and Topoisomerase (TOP2B). Both involved in a large part of the genome and all cell cycles[10]. The inhibitors of toposomerase are divorced into two categories: (a) topoisomerase poisons that inhibit relegation or rotation of strand, and (b) catalytic topoisomerase inhibitors (CICs) which inhibit the DNA binding or DNA cleavage [11-13]. Natural and synthetic drugs in different collaborations have been able to influence the normal topoisomerase feature in recent years. Synthetic drugs have been developed and used in clinical trials as topoisomerase inhibitors, but they have significant side effects[14]. Etoposide is a highly active anticancer agent that has been used for almost 30 years to treat a number of blood-borne and solid human malignancies[15], TOP2 poisons are effective anticancer agents, but their use is limited because of associated side effects, e.g. etoposide therapy also causes secondary malignancies and anthracyclines associated with therapy[16-18].

As a result, several researchers have been compelled to study the potential to mitigate the hazards of these drugs and decrease their toxicity, such as minimizing the cytotoxicity of doxorubicin and methotrexate to normal cells via synergistic activity with natural products[19-20]. Most anti-cancer medications are not cure for cancer and, in addition to their high costs, have adverse side effects. A significant proportion of cancer patients (80 per cent) accept complementary and alternative drugs as primary or adjunctive treatment[21-22]. Plant products are one of the healthy therapeutic alternatives available, cheap and available[23-24]. It is cultivated in Thailand and usually found in India, and its origin is from the West Indies and South America [25]. Some of the newly isolated chemical components have demonstrated anti-cancer, anti-HIV, and anti-diabetic properties [26-27]. It can treat pernicious sores as is common in southern China [28]. AIM: The research aims to study the possibility of using the *Annona squamosa* (Custard apple) seed extract to inhibit the topoisomerase II enzyme and compare it with VP-16 (Etoposide) as a controlled drug. Determination of the inhibition mechanism of this enzyme (Catalytic Inhibitory and Interfacial Poisons).

Materials and Methods

2.1 CHEMICALS

TopoGEN Kit (TG2000H-2) bought from TopoGEN Company, Contains pHOT1 DNA supercoiled (25 ug), relaxed pHOT1 DNA marker in gel loading buffer (25ul, load 2ul as a marker). Linear pHOT1 DNA marker (25ul, load 2ul as a marker) in a gel buffer. Topoisomerase II enzyme 2 units/ul Buffer (A) contains 0.5 M Tris-HCl (pH 8), 1.50 M NaCl, 100 mM MgCl2, 5 mM Dithiothreitol, 300 ug/ml bovine serum albumin (BSA), Buffer (B) contains 20mM ATP in sterile distilled water. The stop Buffer contains 10% sodium dodecyl sulfate (SDS) (300 ul) to terminate reactions used 1/10 volume, loading. 10x loading buffer (300 ul) contains a 5% Sarkosyl, 0.125% bromophenol blue, 25% glycerol. Proteinase K (500 ul) at 0.5 mg/ml. methanol solvents for plant extraction and HCL purchased from sigma-Aldrich.

2.2 PLANT MATERIAL

The Custard apple seeds It was extracted from imported fruits. The plant was classified in the herbarium of the Department of biology, Faculty of Science, University of Kufa. The research was conducted in the Biotechnology Laboratory in cooperation with the Faculty of Pharmacy/Department of Pharmaceutical Science/ University of Kentucky/USA.

2.3 PREPARATION OF CUSTARD APPLE PLANT EXTRACTS

A half kilogram of seeds powder of Custard apple plant was extracted with 100% methanol solvent, 10 cycles in a hot continuous extraction by 3000 ml soxhlet apparatus, the extract then filtered, the volume decreased by rotary evaporator (40-50C^{o}) until getting 1-litre volume. Then, the solution was separated into two parts; part one 250ml, remains crude, evaporated to get dry powder, while the part two (750ml) was used to purify the alkaloid, the crude extract was transferred to a separating funnel (1 litre) volume, 2N HCL was added to obtain pH2, then. The solution fractioned by

500 ml chloroform three times to remove the phenolic compounds. The pH of remain solution within the separating funnel adjusted to 10 with 2M NH_4OH and again partitioned with 500 ml chloroform three times. The chloroform will draw free alkaloids from the upper aqueous layer. The chloroform layer was collected and evaporated well to get alkaloids powder. The dry alkaloid extract placed in the refrigerator in an opaque glass container[29].

2.4 PREPARATION OF INHIBITOR CONCENTRATIONS

The various concentrations of negative inhibitors control (CCE and CAE) and drug a positive inhibitors control (VP0-16) were dissolved by

Dimethyl sulfoxide solvent (24% DMSO) and Tris buffer (10 Mm Tris HCL, pH7.5). The mother concentrations of CCE, alkaloids CAE and VP-16 were (2400, 1600, 1000, 800, 400, 200, and 100 µg/ml), (1000, 800, 600, 400, 200, 100 and 50 µg/ml), and (1500, 1000, 500, 250, 125, and 31.25 µg/ml) respectively.

2.5 PROTOCOL FOR TOPOISOMERASE II UNIT ASSAY (FINAL VOLUME OF 10 UL)

The procedure depended on TopoGEN methods with slight modulation; the final reaction solution was 10 ul. Firstly, to prepare a fresh stock buffer, add equal volumes of buffer A and B together to make a 5x working buffer assay. The reaction solution comes from mixing two prepared solutions parts (5µl for each). **Part 1**: PCR tubes (0.25 ml) contain 5µl of a stock buffer (5x) with 0.5 µl (0.62ng/ml) of pHOT1 DNA plasmid, the PCR tubes inserted into an ice container during work. **Part 2**: PCR tubes (0.25 ml) contain a 5µl stock buffer (5x) with (2.5, 2, 1.75, 1.5, 0.75, 0.5 and 0.4 enzyme unit per test). Then these solution parts mixed carefully and incubated by PCR (type bioneer) for 30 min at 37º, lid temperature 105º, and stopped by adding 1ul 10% Sodium Dodecyl Sulfate (SDS). The PCR tube returned to the ice container again for 30min, then added 1µl of proteinase k enzyme, and The PCR tubes of the reaction solution incubated again for 15min at 37C˚ by PCR to break down the topoisomerase II enzyme. Added 0.1 volume loading buffer (blue juice), 7ul of the samples loaded directly onto 1% Non-Ethidium Bromide gel. Fresh electrophoresis buffer (1x of TAE Gel buffer; 242g Tris base, 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA), 1.5V/Cm (measured between electrodes). Run the agarose until the dye front (bromophenol blue) is about 4-6 cm down the gel. After running, stained with EB (0.5µg/ml) for 20min and then washed with distal water for 15 min before photo documentation (Bioneer) [29]. The activity of enzyme % calculate according to the following equation (Eq. 1):

Activity of enzyme% (AE %) = Sc. no-enzyme – Sc. enzyme/ Sc.no-enzyme x 100 (Eq.1)

-Where Sc.no-enzyme means the amount of the supercoiled DNA band without enzyme.

-Sc. enzyme means the amount of the supercoiled DNA band with the enzyme.

2.6. The Effect of the Ethidium Bromide Stain on the Plasmid Band Sites Assay.

A 0.5 µl (0.62ng / ml) pHOT1 DNA plasmid with half an enzyme unit was used to study the change of plasmid sites according to the effect of the ethidium bromide dye, and the above-described method was used. The electrophoresis (Bioneer type) was conducted in two ways according to the staining stage with Ethidium Bromide (EB).

Non-Ethidium Bromide (EB)-gel; Run 1% agarose gel (1x TAE), adding 7µl of the reaction solution, Fresh electrophoresis buffer (1x of TAE Gel buffer; 242g Tris base, 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA), 1.5V/Cm (measured between electrodes). Run the agarose until the dye front (bromophenol blue) is about 4-6 cm down the gel. After running, stained with EB (0.5µg/ml) for 20min, then washed with distal water for 15 min before photo documentation (Bioneer type) **EB-gel (0.5µg/ml, EB)**; was prepared to illustrate the locations changing of bands. The gel washed by distal water (15 min) before photo documentation.

2.7 PROTOCOL FOR TOPOISOMERASE II INHIBITION ASSAY (FINAL VOLUME OF 10 UL)

The reaction solution (10µl) consists of mixing two parts previously prepared separately in a PCR tube (0.25 ml). **Part 1:** PCR tubes (0.25 ml) contain 7µl of a stock buffer (5x) with 0.5 µl (0.62ng/ml) of pHOT1 DNA plasmid, the PCR tubes inserted into an ice container

during work. The contents mixed very carefully before adding the inhibitor agents. One µl of inhibitors agents prepared above to get the final concentrations of CCE (240, 160, 100, 80, 40, 20, and 10 µg/ml), CAE (100, 80, 60, 40, 20, 10 and 5 µg/ml), and the VP-16 (150, 100, 50, 25, 12.5, and 3.125 µg/ml) in final reaction solution volume (10 ul). **Part 2:** 3µl of a stock buffer contains 0.5 unit of Topoisomerase II enzyme. Then these solution parts mixed carefully and incubated by PCR (type bioneer) for 30 min at 37º, lid temperature 105º, and stopped by adding 1ul 10% Sodium Dodecyl Sulfate (SDS). Before gel documentation, remove the extracts from the reaction solution to prevent them from interfering with plasmid bands, this occurred by adding a 13µl of Chloroform: Isoamyl Alcohol (CIA) (24:1), vortex briefly, and then microfuge for 5 sec. The blue top layer pulled and loaded onto an agarose gel. The PCR tube returned to the ice container again for 30min, then added 1µl of proteinase k enzyme, and The PCR tubes of the reaction solution incubated for 15min at 37C° by PCR to break down the topoisomerase II enzyme. Added 0.1 volume loading buffer (blue juice), 7ul of the samples loaded directly onto 1% Non-Ethidium Bromide gel. Fresh electrophoresis buffer (1x of TAE Gel buffer; 242g Tris base, 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA), 1.5V/Cm (measured between electrodes). Run the agarose until the dye front (bromophenol blue) is about 4-6 cm down the gel. After running.

The concentration of agents that inhibit 50% of topoisomerase II relaxation activity (IC50%) depends on the amount of the remaining supercoiled form, The amount of plasmid forms is measured as optical density using a program Image 1.4 (version 2). The inhibition of the relaxation activity of topoisomerase II calculated according to the following formula[30].

(Inhibitory action of compounds % (IAC %) = (S - S0) / (S control – S0) X 100) (Eq. 2)

- S control: pHOT 1 DNA supercoiled %, in the control (no enzyme and test compounds).

-S0: pHOT 1 DNA supercoiled %, with enzyme and DMSO only.

-S: pHOT 1 DNA supercoiled %, with enzyme and test compound dissolved in 2.4%.DMSO.

The inhibitor concentration that reducing 50% of relaxation activity of the enzyme (IC50%)

Results and Discussion

Topoisomerase II Unit Assay

The enzyme units (0.065, 0.125, 0.25, 0.5, 0.75,1, 1.5, 2, and 2.5) showed relatively convergent efficacy in relaxing the supercoiled plasmid DNA. Where is the enzyme unit (0.5) was ideal for use in subsequent examinations with an appropriate potency value (75.7%), Figure (1).

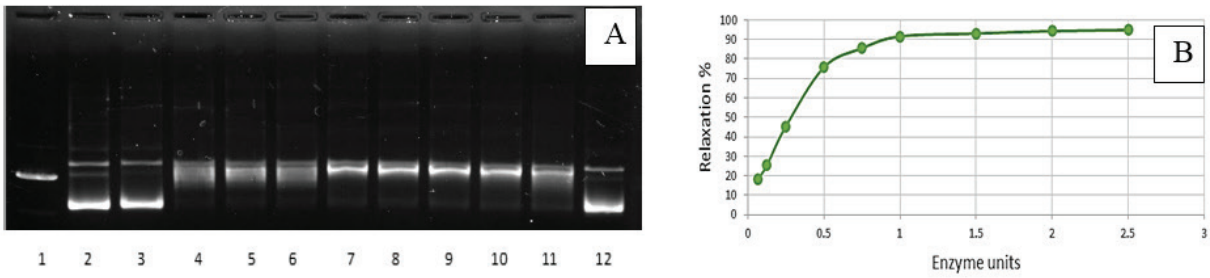

Figure1.: The Topo II enzyme activities on (62.5ng/ml) of pHOT 1 plasmid, non- EB agarose gel 1%. Lane 1, relaxed pHOT1 plasmid marker, lane 2; supercoiled pHOT 1 plasmid without enzyme, lane 3; plasmid with DMSO, Lane 4-12; plasmid with deferent units of Topo II enzyme. Lane 4; 2.5 units, lane 5; 2 units, lane 6; 1.5 units. lane 7; 1 unit, lane 8; 0.75 units, lane 9; 0.5 units, lane 10; 0.25 units, lane 11; 0.125 units, and lane 12; 0.065 units. Deference unites activities of Topo II enzyme (0, 0.5, 1, 1.5, 2, 2.5, and 3 unite) on (62.5ng/ml) of pHOT 1 plasmid

3.1 THE EFFECT OF THE ETHIDIUM BROMIDE STAIN ON THE PLASMID BAND SITES.

Two models of electrophoresis were used for agarose, one Non-Ethidium Bromide-Gels and Ethidium Bromide-Gels to study the effect of the dye on the band sites.

the Ethidium bromide is an intercalating dye agent that interacts with supercoiled DNA and causes the plasmid to unwind, in the opposite side, if excess Ethidium bromide is added, the plasmid will rewind in the opposite direction[31].

-Supercoiled DNA plasmid + EB = unwind DNA plasmid

-Relaxed DNA plasmid+ excess EB = rewind in the opposite direction1

Therefore, those two forms of plasmid exchanged their position according to present or absent EB-dye in a gel. On the other side, VP-16 drug used to get clear nicked (OC) by blocking relegate of the nicked one strand of plasmid and by this way p HOT1 DNA plasmid containing at least one single-stranded nick, the position of this form didn't change its position and affected by EB dye Figure (2).

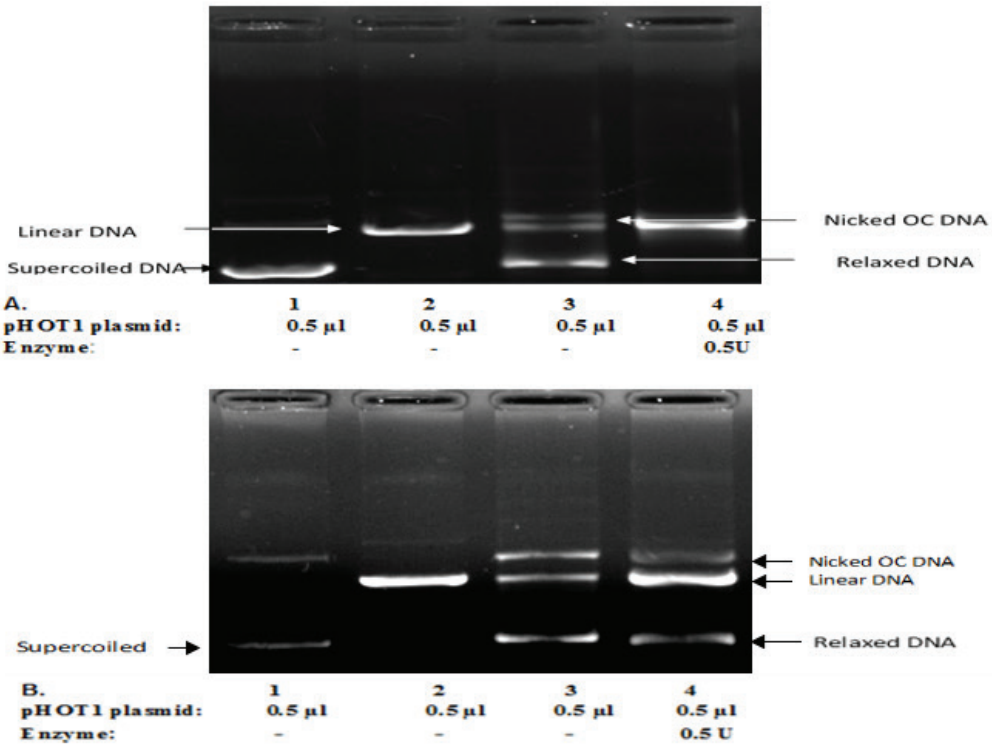

Figure 2. These figures show the difference between Non-EB Gels A, and EB Gels B by utilizing the same Deferent state for each lane to get the deferent shape. 0.5µl of supercoiled pHOT 1. The lanes of the tow gels above are the same contains; lane 1, Sc marker. lane 2, Linear marker. Lane 3, pHOT 1 without enzyme. Lane 4, pHOT 1 with 0.5 units of the enzyme

3.2 TOPOISOMERASE IIA INHIBITION ASSAY

Cancer cells need increased activity in topoisomerase to promote fast cell division and indeed, many kinds of cancers have overexpressed these enzymes[32-35]. The targeting of topoisomerases by small molecule inhibitors in different cancers is therefore an interesting area of investigation[13,36]. Ethoposide drug is used widely to treat a number of cancers including cancer of the lung, testicular cancer, lymphoma, some forms of leukemia, multiforme glioblastoma, Kaposi and Ewing[13]. Commonly, the etoposide compound is

toxic to topoisomerase II leading to the development of secondary malignancies, in particular acute myeloid leukaemia related to therapy[37-38]. Acute promyelocytic leukaemia and therapy-related[39].

The current study showed that CAE had the best TOPO 2A relaxation inhibition compared to CCE and VP-16, with IC50% being 27.46, 216.63 and 73.41 µg/ml respectively, as shown in Figure (3, and 4). The Custard apple isolated alkaloids have anti-cancer activity on different cell lines (HepG-2, MCF-7, and HCT-116).[40].

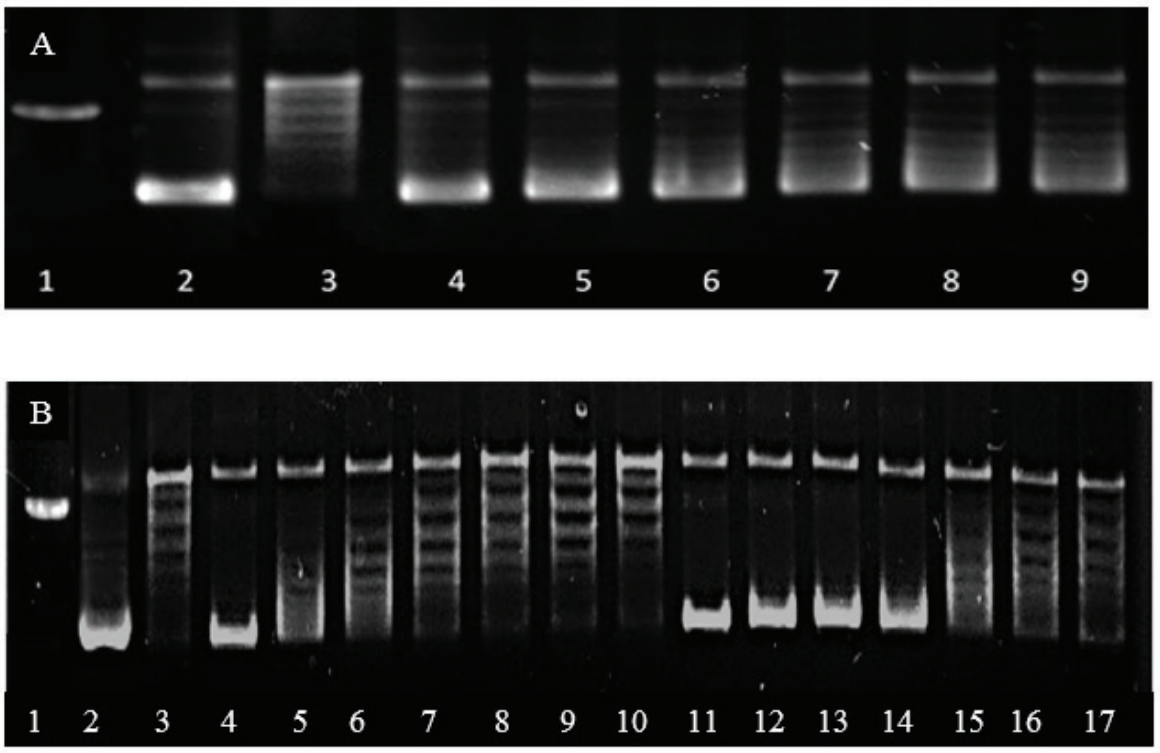

Figure 3. Etoposide activity on 0.5 units of Topo II enzyme, pHOT1 plasmid 62.5ng, 1% of non- EB agarose gel. Lane 1, linear marker. Lane 2, supercoiled marker. Lane 3, p HOT1 plasmid plus enzyme. Lane 4-9, etoposide (150, 100, 50, 25, 12.5, 3.125 µg/ml). The CCE and CAE inhibitors, 0.5 units of TOPO 2A, 62.5ng pHOT1 DNA plasmid, non- EB agarose gel1%. Lane 1-3, marker. Lane 1, linear DNA plasmid. Lane 2, supercoiled DNA plasmid. Lane 3-17, Plasmid with enzyme 0.5 units. Lane 3, with one µl DMSO (2.4%). Lane 4-10, CCE (240, 100, 160, 80, 40, 20, and 1 µg/ml). Lane 11-17, CAE (100, 80, 60, 40, 20, 10, and 5 µg/ml).

Annona squamosa contains many natural products that contain 1 atom of nitrogen and have anti-cancer activity [40]. It contains almost all natural products as compounds flavonoid, steroid, , glycoside, saponin, alkaloid, terpenoids and phenolic [41]. Ari pointed to Anona's richness in structural diversity and pharmacological activities that call for further pharmacological exploration and the ability to develop new anti-cancer drugs[42]. Compound AA annonacin (3) promotes apoptosis by activating the Bax pathway, while the squamocin compound has the potential for apoptosis through the expression of proapoptotic genes Bax and Bad, inducing PARP cleavage and enhancing caspase 3 activity in T24 bladder cancer cells [44-45].

3.3 CATALYTIC INHIBITORY COMPOUNDS (CICS) AND INTERFACIAL POISONS (IFPS) ANALYSIS

The inhibitors of toposomerase are divorced into two categories: (a) topoisomerase poisons that inhibit relegation or rotation of strand, and (b) catalytic topoisomerase inhibitors (CICs) which inhibit the DNA binding or DNA cleavage[11-13] . In this study, in addition

to high concentrations, etoposide had a toxic effect at low concentrations, which means that the compound was allowed to form a plasmid-enzyme complex of DNA and to cut the two strands, but the re-ligation step is not allowed. This means it is poison at all concentrations in this study. On the other hand, the increase of this agent concentration directly affects the accumulation of the supercoiled forms because of enzyme prevention from forming the DNA plasmid complex, thus increasing the proportion of the supercoiled forms with increasing etoposide concentrations. (Figure 3, 5). Severity of etoposide toxicity (IFPs), Kumar Jain and colleagues reported stabilization of covalent enzyme-DNA complexes with topoisomerase poisons that generates DNA lesions and ultimately induces apoptosis[46]. The risk of etoposide as a poison [36] increases at low concentrations[47]. The production of secondary malignancies, on the other hand, is associated with DNA damage that may be permanent; exposure to low concentrations of leukaemia cells may induce resistance to subsequently elevated concentrations[48]. On the contrary, the high concentration of both the CCE and CAE showed a decrease in their toxicity, with a decrease for linear DNA as shown in (Figure 3, 5). Due to the effect of increasing concentrations of these extracts, this shows an improvement in suppression as a (CICs) and decreased toxicity (IFPs). The *Annona squamosa* plant's CIC activity may be indirectly inhibited by inhibiting the ATPase and this agrees with Dinesh and colleagues, which suggested the *annona squamosa* contains compounds capable of inhibited the gastric H+ K+-ATPase activity, [49]. Cells with defective decatenation checkpoint pathway fail to stop the cell cycle during the G2 phase and enter the M phase with catenated and undercondensed chromosomes resulting in impaired mitosis and eventually cell death[50]. For this reason, TOP2A catalytic inhibitors are also promising agents for targeting decatenation checkpoint defective cancer cells during cancer chemotherapy[51].

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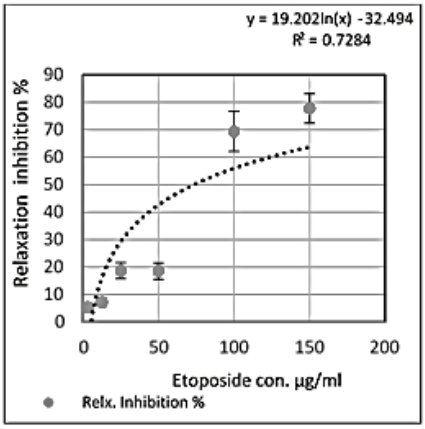

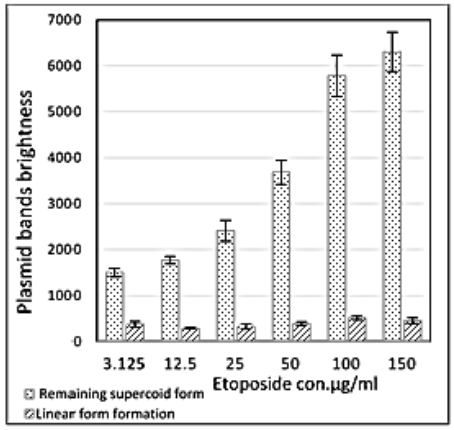

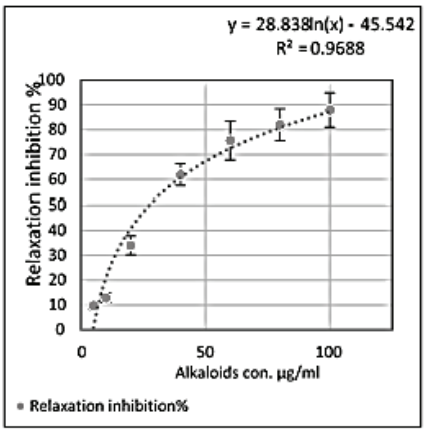

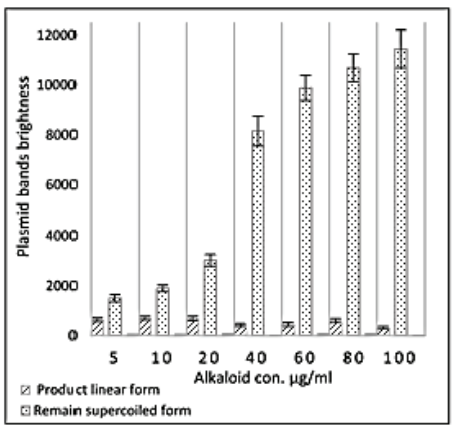

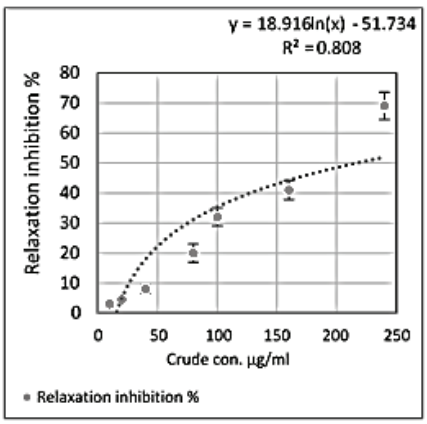

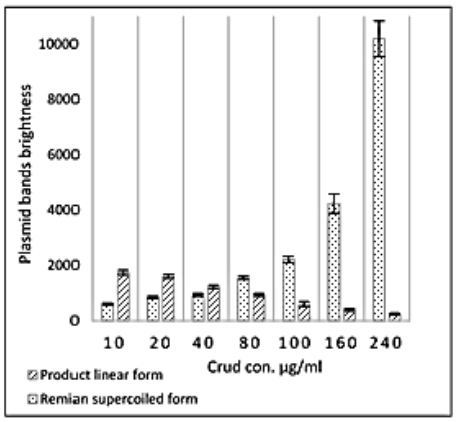

Figure (4) DNA pHOT1 plasmid relaxation inhibition activity%±SD, IC50% of etoposide, alkaloids and crude extract were 73.41, 27.46, and 216.63µg/ml respectively.

Figure (5): The Catalytic Inhibitory Compounds CICs (suppressor compound activity depend on the brightness value of supercoil form ±SD, and the interfacial poisons IFPs (depend on the brightness value of linear form ±SD) of standard Etoposide compounds, alkaloids and, crude extract.

Conclusions

1. Compared with its crude extract (CCE) and etoposide (VP-16), Custard apple seeds alkaloid extract (CAE) was the strongest as a TOPO 2A inhibition .

2. Interfacial poisons (IFPs) are a means of eliminating the drug's side effects. Relative to the suppressor (CICs), it does not need to have a high concentration and relies on the speed of cell division, so we can rely on the faster speed of cancer cell division compared to the normal cell, which increases the killing of cancer cells higher than the normal cell.

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Conflict of Interest: We declare that there is conflict of interest

Ethical Approval: The research approved by scientific and ethical committee at our department

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Study the Negative Effects of Pseudoephedrine on Brain Tissues in Adult Albino Male Rats

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Abstract

In this study, (24) male animals were used, which were divided randomly into four groups. Each group included (6) animals, which are: the control group that was given drinking water and food daily, and the group of rats treated with pseudoephedrine at a dose of (40) mg / Kg of body weight for a period of (30) days, the group of rats treated with pseudoephedrine at a dose of (40) mg / kg of body weight for a period of (40) days, the group of rats treated with pseudoephedrine at a dose of (40) mg / kg of body weight for a period of (50) days, the animals dosed the drug by the oropharyngeal tube at a dose of (1) ml, and after the end of the experiment, the animals were scarified , brain samples were taken and preserved with formalin at a concentration of 10% until the tissue sections were made, when the meningeal membrane appeared the upper layers of the cerebral cortex in the group treated with pseudoephedrine for a period of 30 days were close to normal, while the group treated with pseudoephedrine for a period of (40) days showed shrinkage in the deep layers of the cerebral cortex and hemolysis in many blood vessels, and the meningeal membrane appeared.

Key words: *nervous system, pseudoephedrine, brain tissue, male rats.*

Introduction

The nervous system in the human body consists of the central nervous system and the peripheral nervous system, and the nervous system is responsible for controlling the functions of the human body, analyzing incoming stimuli and integrating internal and external responses, and the central nervous system (CNS) includes the brain and spinal cord, and the peripheral nervous system (PNS) consists of sensory receptors that bring information to the central nervous system and motor nerves that transmit information away from the central nervous system to facilitate response to stimuli, and the autonomic nervous system uses components of the central nervous system and the peripheral nervous system to regulate automatic or involuntary responses to stimuli [(1)] People use many types of drugs in the treatment of various diseases, including pseudoephedrine, which is a drug that mimics the sympathetic nervous system of the chemical class of amphetamines, It is used as a sinus decongestant and as a tonic or vigilance enhancing agent that is found singly or in combination with antihistamines or with paracetamol [(2)].

The mechanism of action of pseudoephedrine depends directly on the adrenergic receptor system, as it is believed that the narrowing of the blood vessels produced by pseudoephedrine is primarily a response to the α-adrenergic receptors [(4)], alpha-adrenergic receptors are found on the muscles lining the walls of blood vessels, and when these receptors are activated, the muscles contract, which leads to constriction of the blood vessels that allow less fluid to enter the lining of the nose, throat and sinuses and thus Reduced inflammation of the nasal membranes as well as decreased production of mucus, relaxation of smooth muscles in the bronchi and expansion of the airways, which reduces congestion [(4.14)].

Materials and Methods

Experimental animals used in the study

In this study, adult white male rats, whose weight ranged between (230-270) gm, and their ages (2.5-3) months, were used in this study. They were prepared from the animal house of the College of Veterinary Medicine / University of Tikrit, and placed in cages for creditors with metal covers. With dimensions of (12 x 24 x 40) cm, water and food were given in sufficient quantities throughout the period of the experiment, which lasted 30 days, until the animals were dissected and brain samples were taken for the purpose of making the histological sections, examining and imaging them.

The drug used in the study

Pseudoephedrine drug was used in the form of a powder, it was dissolved in distilled water and the animals were dosed at (1) ml at a concentration of (40) mg / kg of body weight [5], the solution was prepared daily.

Experience design

In this study, (24) animals were used from the adult white male rats, which were randomly divided into four groups. Each group included (6) animals, which are as follows: -

1- The first group (G1): the control group. This group was given regular drinking water and food (standard diet) daily for (50) days.

2- The second group: the group of rats treated with pseudoephedrine at a concentration of (40) mg / kg of body weight, and the drug was dosed by the oropharyngeal tube at a dose of (1) ml and given water and normal food for a period of (30) days.

3- The third group: a group of rats treated with pseudoephedrine at a concentration of (40) mg / kg of body weight, and the drug was dosed by the oropharyngeal tube at a dose of (1) ml and given water and regular food for a period of (40) days.

4- **The fourth group:** the group of rats treated with pseudoephedrine at a concentration of (40) mg / kg of body weight, and the drug was dosed by the oropharyngeal tube at a dose of (1) ml and given water and normal food for a period of (50) days.

Histological Study

Tissue sections were prepared and stained with hematoxylin and eosin stain according to the method by [6].

Results and Discussion

Control group

In the control group rats, the cerebral cortex appeared surrounded by the inner meningeal membrane (pia mater), below it the external molecular layer containing a few small neurons, and the number of neurons and glial cells increased at the external granular layer. Then, the cells increased more in the external pyramidal layer, while the inner granular and inner pyramid layer, most of the neurons were surrounded by a transparent band devoid of tissue features, as shown in Figure (1).

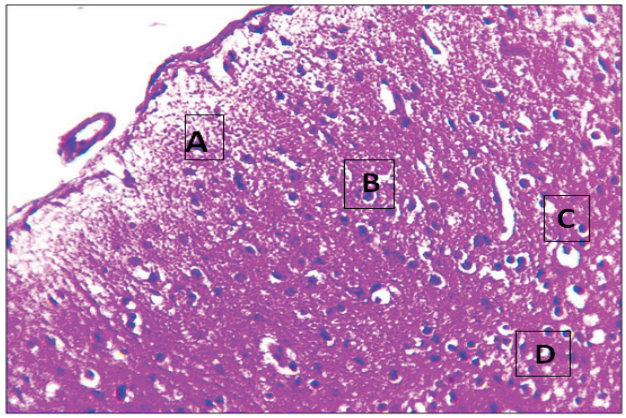

Figure (1) cerebral cortex in the control group: shows the inner meningeal membrane (A), the molecular layer that contains neurons and glia (B) the outer granular layer (C) the inner pyramidal layer (D), magnification force X40, stained with H&E stain.

2. The group treated with pseudoephedrine for 30 days.

The meningeal membrane and the upper layers of the cerebral cortex appeared in the group treated with pseudoephedrine drug for a period of 30 days, close to the normal state, as in figure (2), while the deep layers of the cerebral cortex showed thickening in the nuclei of nerve cells, breaking down and degenerating some other neurons and vacuolation around each other as in Figure (3).

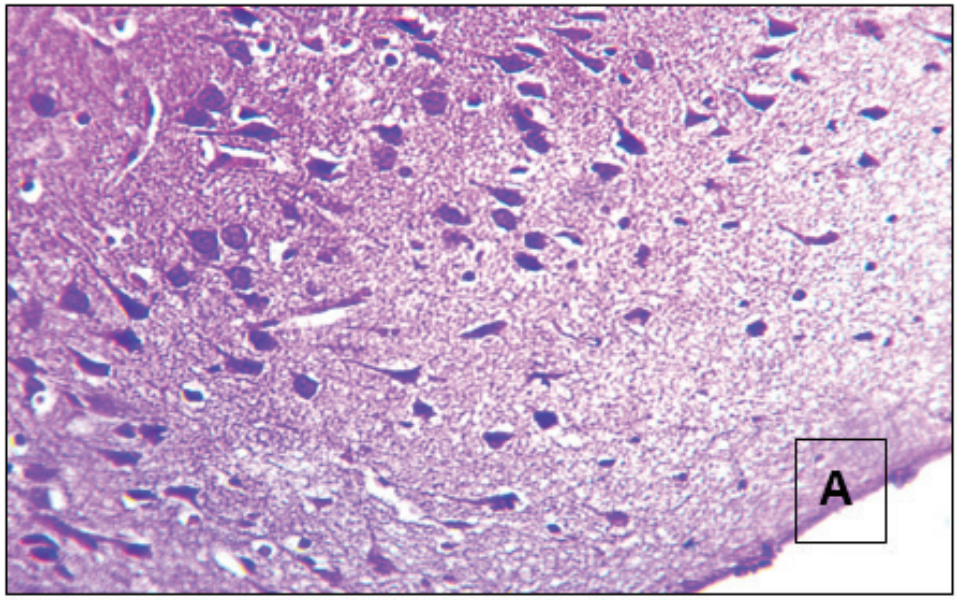

Figure (2) the cerebral cortex in the treatment group for 30 days: the meningeal membrane appeared (A), the layers of the cerebral cortex close to normal, magnification force X40, stained with H&E stain.

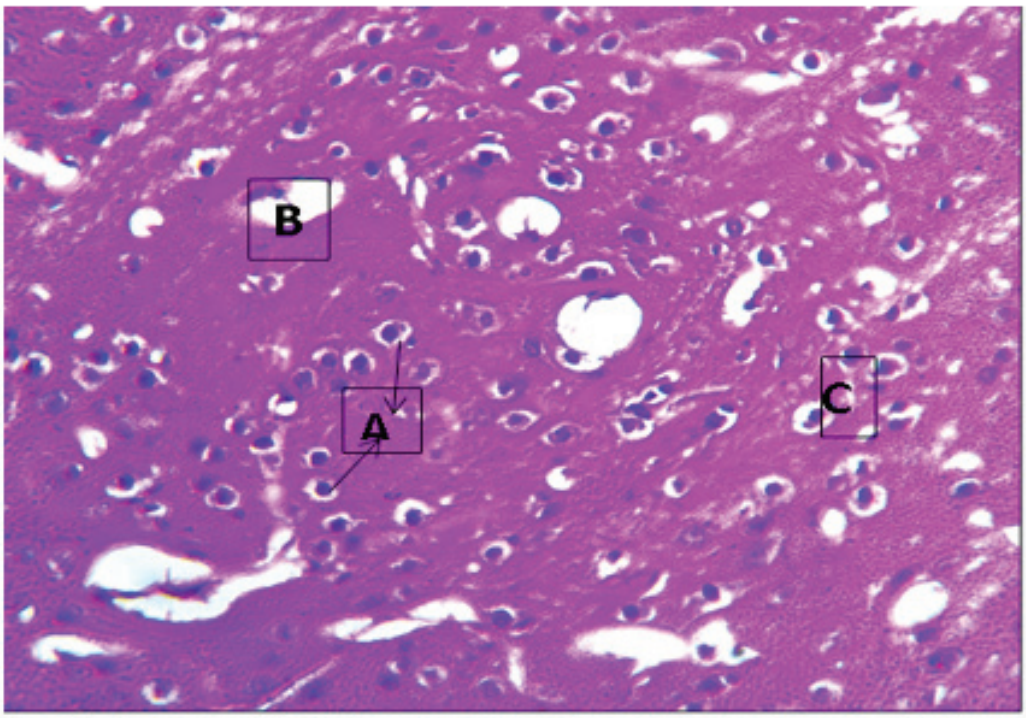

Figure (3) the cerebral cortex in the pseudoephedrine group for 30 days: the nuclei of the neurons thicken (A), the nerve cells are broken and ruptured (B), the cells around the nerve cells are shattered (C), Magnification force X40, stained with H&E .

3. The group treated with the Pseudoephedrine drug for (40) days.

The meningeal membrane of the cerebral cortex appeared in the group treated with pseudoephedrine for a period of (40) thickened days, and many vacuole were found around many pyramidal cells as in figure (4), while the deep layers of the cerebral cortex showed shrinkage of neurons and hemolysis in the blood vessels as in the figure (5).

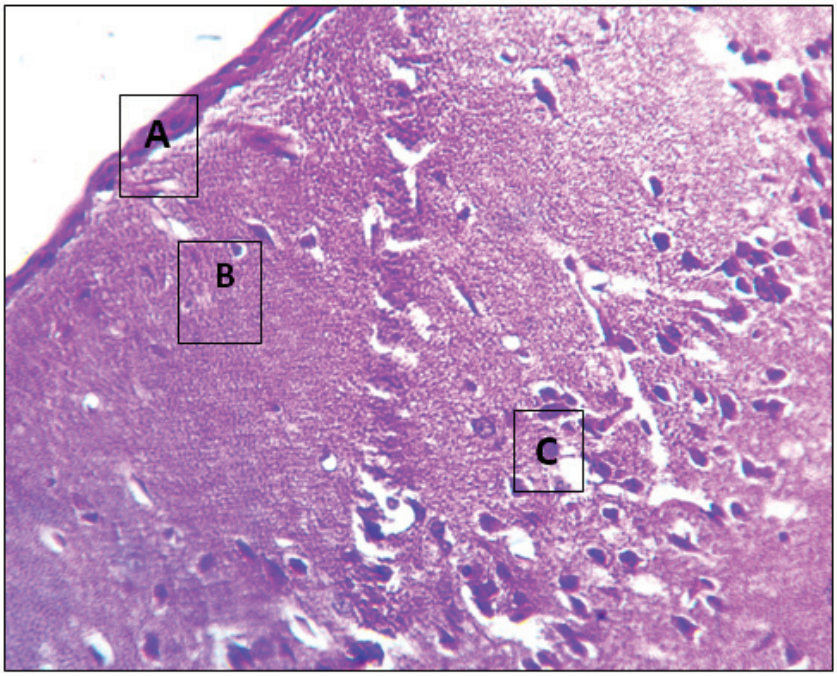

Figure (4) Cerebral cortex in the pseudoephedrine group for 40 days: the meningeal membrane of the cerebral cortex thickens (A), the molecular layer with specific neurons (B) vacuolation around the outer pyramidal cells (C), magnification force X40, stained with H&E stain.

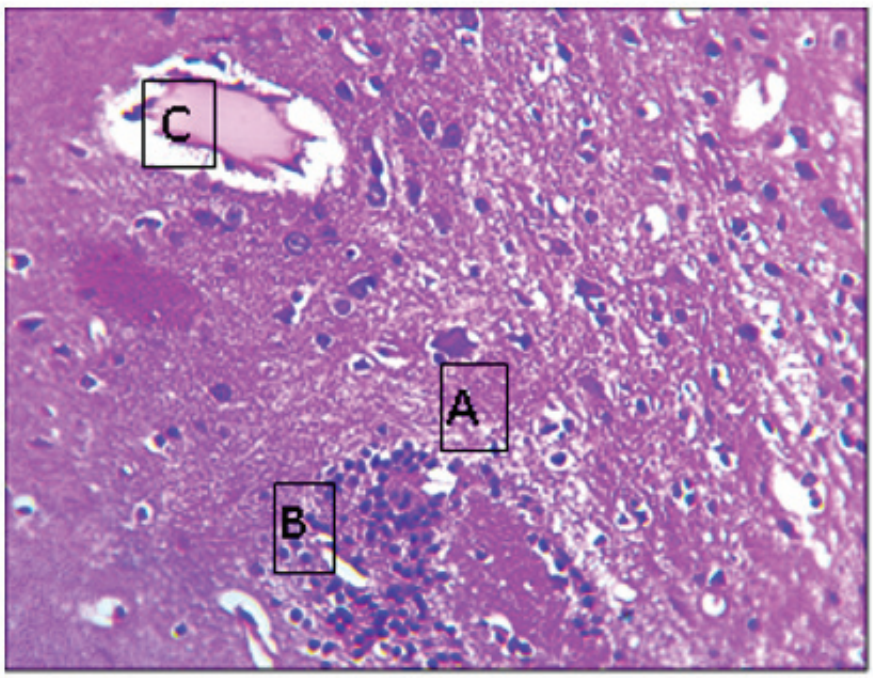

Fig. (5) The cerebral cortex in the pseudoephedrine group for 40 days: Neuronal shrinkage (A) vacuolation (B) hemolysis in a blood vessel (C),.magnification force X40, stained with H&E stain.

4. The group treated with pseudoephedrine for (50) days.

The meningeal membrane (arachnoid pain) appeared in the group treated with the drug ephedrine for a period of (50 days) thickened, highly tortuous and semi-separated from (pia mater). The meningeal artery was subarachnoid with large blood congestion, and a pool of blood appeared under the pia mater and surrounded by numbers of Glial cells, as the molecular layer appeared in the form of multiple foamy vacuoles and similar to a sponge pattern as in Figure (6).

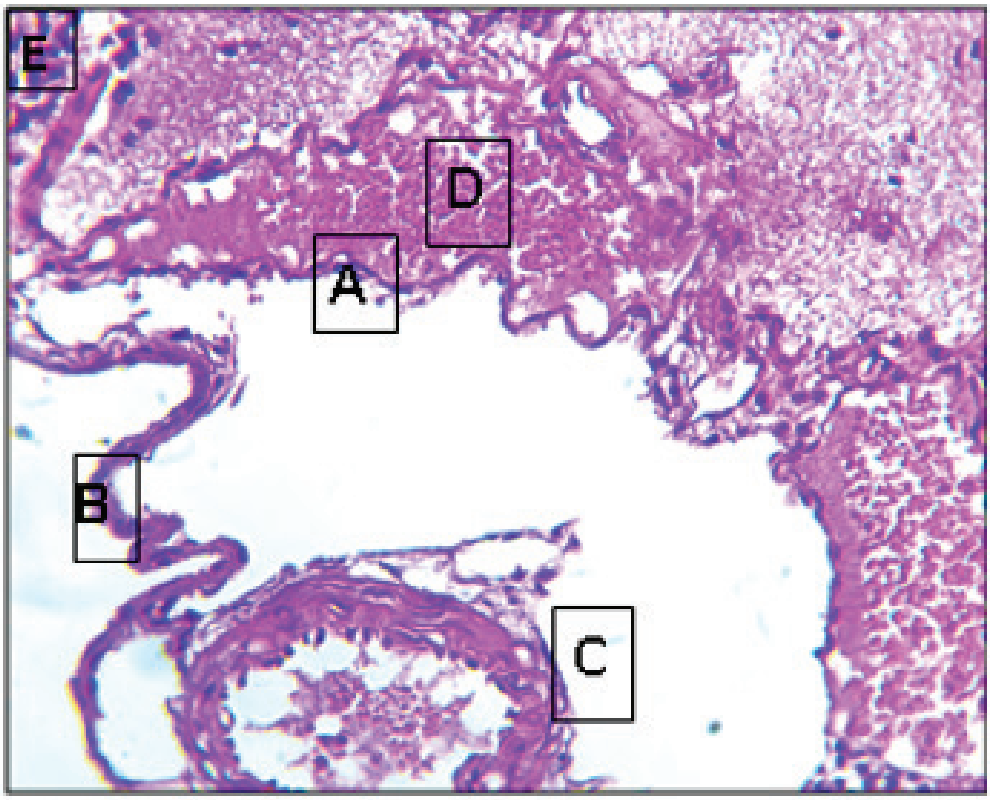

Fig (6) cerebral cortex in the pseudoephedrine group for 50 days: the inner meningeal membrane of the cerebral cortex (A), arachnoid membrane (B), almost complete detachment, congestion (C), pool of blood under the inner meningeal (D), glial cells (E) ,agnification force X40, stained with H&E stain.

The results of the histological examination of the brains of rats treated with the drug Pseudoephedrine for (30) days showed that the nuclei of some nerve cells thicken, degenerated in other nerve cells and vaculation around many cells of the cortex and medulla, as well as the degeneration of Purkinje cells. These effects may be due to the occurrence of neuritis resulting from take pseudoephedrine drug, which leads to the overproduction of dopamine and thus worsens the neurodegeneration, and thus this study agrees with a study [7,8] which showed the occurrence of neurodegeneration by increasing the intake of the drug Pseudoephedrine.

The tissue injury appeared more severe in the brains of rats treated with the drug pseudoephedrine for a period of (40) days, as the meningeal membrane was thicker than in the previous group and degeneration and shrinkage occurred in many nerve cells, and in many layers of the brain, as well as hemolysis and glial cell enlargement, which it may indicate that pseudoephedrine has triggered an immune response in the brain and activated a special type of immune cells called c-fos immunohistochemistry that is an indicator of neuronal damage [9], which is expressed under the influence of some addictions inducing drugs such as opiates, amphetamines and ephedrine [10,11] the effect of pseudoephedrine reached

its maximum in the brains of the group treated with this drug for a period of (50) days, as the arachnoid membrane appeared almost separate from the pia mater. Arterial congestion and widespread vacuolization and thickening of the nuclei of nerve cells and the gathering and infiltration of a number of glial cells, which in most cases leads to the occurrence of gliosis which is a non-specific, reactive change of glial cells that occurs in response to the central nervous system(CNS) injury and in which the enlargement of different types of glial cells such as astrocytes, microglia and oligodendrocytes, leads in chronic cases to the formation of glial scars[12], or perhaps chronic use of pseudoephedrine leads to the activation of a special type of neuron that is considered a protein called Regenerating islet-derived protein 3 alpha that is encoded by the REG3A genes and these cells have an effect on the functions of the meninges and thus increase the degeneration of neurons, especially in the cerebral cortex. Thus, this study is in agreement with the study [13.15], which showed the occurrence of degeneration in nerve cells after chronic use of the pseudoephedrine drug.

Conclusion

The results of the histological examination of the brains of rats treated with the drug Pseudoephedrine for 30 days showed that the nuclei of some nerve cells thicken, degenerated in other nerve cells and vaculation around many cells of the cortex and medulla, as well as the degeneration of Purkinje cells. These effects may be due to the occurrence of neuritis resulting from take pseudoephedrine drug, which leads to the overproduction of dopamine and thus worsens the neurodegeneration, and thus this study agrees with a study, which showed the occurrence of neurodegeneration by increasing the intake of the drug Pseudoephedrine.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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Conflict of Interest: The author declare no conflict of interest regarding this research.

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Genotype Frequency of Cytochrome P450 2C9 (CYP2C9) Mutations in Iraqi Ethnic Groups

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Abstract

In Middle East, There is a little knowledge about the cytochrome P450s genotyping and there has not found any investigation about the CYP2C9 allelic diversity in Iraqi population nowadays .CYP2C9 genotypes were established in a total of 400 subjects of healthy Iraqis volunteers from different areas in Iraq (Arab ,Kurd and Turkmen). After extraction of DNA from blood cells and polymerase chain reaction, SNP genotyping for CYP2C9 variants (2,3,4 and 5) was performed by using the primer extension KASP® genotyping assay. The agreement with "Hardy-Weinberg equilibrium" for detected the distribution of CYP2C9 genotype was proved by (X^2 – test) .The CYP2C9*2 , CYP2C9*3 and CYP2C9*5 alleles variant were identified in Iraqi population , whereas the CYP2C9*4 was absent , The results revealed that there was non- significant association observed in all CYP2C9 genotypes among Iraqi ethnic groups .then The CYP2C9 genotypes results in our study were compared to other populations and The results exhibited that the frequency of Iraqi population CYP2C9 variants were comparable with other countries and ethnic groups. This is the first report of CYP2C9 variants from Iraq; the results are assist as a database on CYP2C9 gene polymorphisms and clinical data baseline for dosing and avoiding adverse reactions of drugs metabolized by CYP2C9 in Iraqis patients.

Key Words: *Cytochrome P450 2C9 ; SNP , polymorphism ; population*

Introduction

The CYP2Cs form are an important subfamily of CYP enzymes that are accountable for metabolizing approximately 20–30% of most therapeutic drugs [1,2]. P450 2C9 is a member of four P4502C monooxygenases (P450s, 2C19, 2C9, and 2C8) that are expressed In human liver. They significantly contribute to hepatic capacity to metabolize drugs [3] and consider the most essential enzymes for metabolizing of drug in humans. It occupied about 20% of the total P-450 cytochrome protein in microsomes of liver [4] and hydroxylates around 16% of current clinical usage drugs [5]. P450 2C9 Cytochrome (CYP2C9) catalyzes the metabolism of several essential drugs include: the anticoagulant, the anti-diabetic agents,tolbutamide and glipizide, ,warfarin , the anticonvulsant phenytoin, the antidepressant fluoxetine , the antihypertensive losartan[6] "and a number of nonsteroidal antiinflammatory drugs (NSAIDs) include : ibuprofen[7]celecoxib[8] meloxicam [9] and lornoxicam" [10] . This enzyme is coded by a gene mapped on 10q24 chromosome which displays genetic polymorphism [1]. Beside to wild-type protein(CYP2C9*1), the CYP2C9 has at least five single nucleotide polymorphisms (SNPs) described within CYP2C9 gene coding region resulted in different allozymes are: CYP2C9*2 (Arg-144-Cys), CYP2C9*3 (Ile-359- Leu), CYP2C9*4 (Ile-359-Thr), CYP2C9*5 (Asp-360-Glu) and CYP2C9*6 (null allele) [5] . This polymorphism may alter a catalytic activity and specificity of encoded enzyme to drug response [6 ,11] There are 3 alleles in most different ethnic groups are CYP2C9*1 (wild-type allele), CYP2C9*2 and CYP2C9 *3 . The individuals with the *2 and *3 alleles display lower in enzymatic activity, and these subjects have been designated as **"poor metabolizers**" that they may

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tolerate drug toxicity[12 , 5] . Therefore, determination in CYP2C9 genotypes in different ethnic groups is necessary to prevent a toxicity of drugs. The objective of the present study was to detecting and comparing the frequency of the cytochrome P450 CYP2C9 genotype polymorphisms in the Iraqis population with the frequency of other ethnic populations.

Materials and Methods

Subject:

The genotypes of CYP2C9 were recognized in a total of 400 subject of healthy Iraqis individuals distributed in different areas in Iraq (200 Arab ,100 Kurd and 100 Turkmen) . Following DNA extraction from blood cells.

***CYP2C9* GENOTYPING :**

The analysis of genotype variation for CYP2C9*2,*3,*4, and *5 alleles was accomplished by using a competitive allele-specific PCR (KASP) SNP genotyping system using FRET quencher cassette "oligonucleotides. For this purpose, the gDNA was sent to LGC genomics (LGC Hoddesdon, UK) and was genotyped using the SNP line system. In brief, two allele-specific forward primers and one common reverse primer were designed for each supposed varietal SNP, according to SNP location (Table 1) (LGCgenomics, UK). Gene Pro Thermal cycler (Bioer Technology, Hangzhou, China) was used for genotyping "in a total volume of 5 lL containing 1X KASP Reaction Mix (KBioscience), 0.07 lL Assay mix (comprising 12 lM from each allele-specific forward primer and reverse primer 30 lM) and 10–20 ng genomic DNA. The subsequent cycling conditions were used: 15 min at 94 ˚C; 10 touchdown cycles of 20 s at 94 ˚C, 60 s at 65–57 ˚C (decreasing 0.8 ˚C per cycle); and 26–35 cycles of 20 s at 94 ˚C, 60 s at 57 ˚C. Fluorescence recognition of the reactions was made using an Omega Fluorostar scanner (BMG LABTECH GmbH, Offenburg, Germany), and the data were analyzed using the Kluster Caller 1.1 software (KBioscience)".

Table(1): SNP polymorphism for CYP2C9*2, *3, *4, and *5 alleles.

CYP2C9 Allele	Exon	Position	SNP	Reference
*2	3	430C > T	AGGAGCATTGAGGAC[C/T]GTGTTCAAGAGGAAG	[13]
Primers			"F:5'-GTATTTTGGCCTGAAACCCATA-3' R:5'-CACCCTTGGTTTTTCTCAACTC-3' Sequencing primer 5'-GGGAAGAGGAGCATTGAGGC-3'"	
*3	7	1075A > C	GAGGTCCAGAGATAC[A/C]TTGACCTTCTCCCCA	
*4	7	1076T > C	AGGTCCAGAGATACA[C/T]TGACCTTCTCCCCAC	
*5	7	1080C > G	CCAGAGATACATTGA[C/G]CTTCTCCCCACCAGC	
Primers			PCR Primers for *3,*4, and*5 "F:5'-TGCACGAGGTCCAGAGAT-3' R:5'-GATACTATGAATTTGGGACTTC-3' Sequencing primer for *3,*4, and*5" "5'-GCTGGTGGGGAGAAG-3'"	

Statistical Analysis

The sample size in our study was detected by statistical power analysis with SAS software "SAS Institute, Cary, NC". Statistical analyses were achieved using the program SPSS version 23. The frequent of each genotype was calculated by gene counting method (14) . The Hardy-Weinberg equilibrium agreement of CYP2C9 genotypic distribution was established by chi-square(X^2) tests. P value was statistically significant if $P \geq 0.05$. The comparisons among population were also accomplished by Chi-square(X^2) test for population diversity [14] .

Results and Discussion

The distribution of CYP2C9 genotype frequencies between the Iraqi ethnic group are revealed in table (2) . the results exposed three allelic variant among Iraqi population : two common alleles (Cyp2C9*2 and Cyp2C9*3) and one appear in low frequent (Cyp2C9*5), while the (CYP2C9 *4) was not detected , the Hardy-Weinberg equilibrium of the detected genotypic distribution was demonstrated , using chi-square test ,the predominant genotype variant of Cyp2C9*2 is T/C with highest freq. in Turkmen (TR) with (0.24) then in Arab (AR) about (0.24), the low freq. in Kurd (KR) with (0.12). Moreover, the predominant Cyp2C9*3 genotype variant was C/A with highest frequency in TR (0.25) followed by AR (0.17) ,then KR (0.13) .

Table (2): CYP2C9 genotypes frequency in Iraqi ethnic groups (Arab (AR), Kurd (KR) and Turkmen (TR).

CYP2C9 variants	Genotype	Iraqi Ethnic Groups CYP2C9 variants Frequency			Sig.
		AR	TR	KR	
Cyp2C9*2	C:C	0.76	0.76	0.84	0.8
	C:T	0.22	0.24	0.12	0.12
	T:T	0.02	0	0.04	0.4
Cyp2C9*3	A:A	0.82	0.71	0.84	0.54
	C:A	0.17	0.25	0.13	0.13
	C:C	0.01	0.04	0.03	0.42
Cyp2C9*5	C:C	0.99	1	0.97	0.000*
	C:G	0.01	0	0.03	0.32
	G:G	0	0	0	/

Hardy-Weinberg Equilibrium was performed to calculate the genotype frequencies

*p<0.05

Tables (3) exhibit the variances in frequency of CYP2C9 genotypes among the Iraqi ethnic group and other populations . The results showed that 0.19 ; 0.14 and 0.01 of the Iraqi subjects had the heterozygote CYP2C9*2 (C/T) , CYP2C9* 3 and CYP2C9* 5 genotypes respectively . In the meanwhile the frequency of CYP2C9*2 (C/T) in Iraqi population was higher than the Asian 0% and African American (0.002) with p<0.05 . on the other hand , our results were comparable in comparison with other countries and ethnic groups e.g. Egypt (0.117) ; Caucasian (0.143) and Turkish(0.106) . The frequency of CYP2C9*3 (A/C) genotype freq. (

0.14 %) was higher in the Iraqi ethnic group compared to Asian 0% and African American (0.002) . While the frequency of CYP2C9*5 genotype was detected with 0.01 in Iraqi population according to Hardy-Weinberg Equilibrium and there are no further studies detected the distribution of CYP2C9*5 allele and genotype among populations . CYP2C9*5 variant (C1080G transversion on exon 7) was demonstrated from a study among African American and European American genomic samples . The variant, resulted in Asp-360-Glu substitution in the encoded protein which cause increasing the Km value by 12-time for (S)-warfarin, 5-time for diclofenac and 3-time for lauric acid, when it was compared to CYP2C9*1[15] .

Table (3): Comparison of CYP2C9 genotypes frequency in Iraqi ethnic groups with other different populations.

Gene	Samples No	Genotype	Allele Frequency					
			Iraq	Egypt	Asian	African American	Caucasian	Turkish
Cyp2C92	220	C:C	0.79					
		C:T	0.19	0.117	0 *	0.02 *	0.143	0.106
		T:T	0.02					
Cyp2C93	253	A:A	0.85					
		A:C	0.14	0.092	0 *	0.02*	0.109	0.100
		C:C	0.01					
Cyp2C95	261	C:C	0.99					
		C:G	0.01	0.01	0	0	0	0
		G:G	0					

Hardy-Weinberg Equilibrium was performed to calculate the genotype frequencies

*p<0.05

Similar results were obtained by Yoon *et al*.,(2001) [16] . on Caucasians populations, they exposed that the CYP2C9*2 and CYP2C9*3 were more allele frequent comparing to African American and Asian populations while Chinese and Japanese have not showed the CYP2C9*2 allele . The allele frequencies of CYP2C9 differ among different populations and many investigates on CYP2C9 variation in different populations have revealed a significant difference in alleles and genotypes frequency[17,18] . This may be the reason behind the probable ethnic differences to drug response. It is necessary to study the pharmacokinetic effects of CYP2C9 genotypes in different populations because a variety ethnic groups from all over the world custom medications. Many researches have indicated the frequent of CYP2C9 alleles is diverse between African , Caucasian and Asian populations, the most prevalence allele in Chinese populations is CYP2C9*3 with a frequency about 3.3%; whereas CYP2C9*2,CYP2C9*4 and CYP2C9*5 alleles are rare or absent[19 ,20] .A number of investigators show that the Arg-144-Cys allelic variants of (CYP2C9*2) and Ile-358-Leu of (CYP2C9*3) were encoded for enzymes with decrease substrate turnover [21,22] and this amino acid substitutions

may decrease the rate of phenytoin hydroxylation by impairing the 6- & 7-hydroxylation of "S-warfarin" and the latter reduce the rate of "tolbutamide" metabolism[23-25]. Apparently, these variants in amino acid may not effect in some NSAIDs metabolism like diclofenac to the same extent[6]. The differences in genotype of enzyme have displayed the association of certain genotypes with a great risk of s-warfarin over bleeding events and anticoagulation in comparing to a wild type . Clinical studies have also suggested that subject with the mutated genotypes require lower doses of S-warfarin (10- 20% and/or 20-50%) when compared to a wild type subject[17]

Conclusion

In term of genotyping, the most frequent allele in a sample of Iraqi population were CYP2C9*2 and CYP2C9*3 while the CYP2C9*5 was detected with low frequent in Iraqi population .Furthermore, the difference in CYP2C9 genotypes may effect on pharmacokinetics of various drugs. Therefore , determination the CYP2C9 genotypes may assist physicians to give optimal dosage of a drugs for treatment and prevention of their side effects.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Physiological and Histological Study of Doxorubicin Effect on Kidney Tissue in Cancer Induced Rats

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Abstract

Doxorubicin (Adriamycin) is one of the chemical drugs commonly used worldwide for the treatment of various types of cancer , and high doses of it cause unwanted toxic side effects on the kidney. This study aims to demonstrate the physiological and histopathological effect of the doxorubicin drug on kidney of rats. For this purpose , twenty adults male were used in this study and were divided equally into four groups (A: control group, B: a group treated with the carcinogen only , C: a group treated with the carcinogen and doxorubicin and D: a group treated with doxorubicin only). Five rats were included in each group and the cancer was developed by injection with azoxymethane once a week for the B and C groups. Compared to the other groups, there was a significant decrease in the weights of animals for the carcinogen-treated group. The concentrations of the hematological parameters of the groups, such as Hb, PCV as well as some tests related to the kidney functions like the concentration of urea , uric acid ,albumin ,globulin, creatinine and total protein were measured and it was found differences between these three groups in term of these testes compared with control group. Regarding to the histological examination of the kidney present study showed specific tissue changes in the treated-carcinogen groups , such as lymphocytes infiltration , glomeruli condense , destroyed of urinary tubules in addition to the presence of bleeding. As for the group treated with doxorubicin , pathological changes were observed include, destroyed of glomerular basement membrane , glomeruli and urinary tubules.

Keywords: *Cancer, Chemotherapy, kidney, Doxorubicin, histopathology*

Introduction

Cancer a major public health problem and the second leading cause of death worldwide. Many chemotherapy drugs are used to many various types of cancer, doxorubicin drug is one of the most highly effective agents against a wide variety of cancer because Quinine-containing anthracycline antibiotic [1]. In spite of its high antitumor efficacy but used in chemotherapy has limited due to side effects and toxicities on kidney, testis, cardiac, lung, liver and blood [2, 3,4]**.** In addition, this chemotherapy causes disorder antioxidant systems [5]. Although, the mechanism of nephrotoxicity that occurs by this drug is unknown , it is believed that this toxicity is through the formation of free radicals and the oxidative damage caused by these free radicals to the tissues [6]. Doxorubicin also causes direct toxic glomerular damage because of its impact on the glomerular filtration barrier composed of glomerular endothelial cells containing glycocalyx, glomerular basement membrane and podocytes, thus affecting membrane permeability, as doxorubicin leads to a decrease in glycocalyx thickness, increasing the size of the holes in the glomerular endothelial cells and decreasing the selectivity of the glomerular charge. All these changes end with passage of large molecules as protein lead to proteinuria and decrease glomerular filtration rate (GFR) [7]. The presence of this protein in the lumen of tubular stimulate to reabsorbed more protein by the proximal tubular cells lead to protein accumulation

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causing tubular obstruction and increased pressure inside the renal tubular and tubular expansion , as well as rupture of the basement membrane and leaking of this protein to the intestitium which trigger the inflammation [8,9]. Furthermore, administration of doxorubicin causes vasoconstriction in vessels and the concentration of the drug in the blood leads to glomerular tuft atrophy and urinary space expansion **[10]**.

Material and Methods

1- Animals experimental design : In this study , twenty adult male Sprague Dawley rats, aged (3-4 months) and (225-240 gm) body weight . The animals were divided into four experimental groups. Five rats for each group. Group A was considered a control group as it was only injected with normal saline , while B and C group were injected with the carcinogen (azoxymethane) (15 mg/kg body weight) once a week for two weeks intraperitoneally for cancer induction [11], in addition to carcinogen, group C were also injected intraperitoneally with doxorubicin drug (25 mg/kg body weight) once a week for two months, while group D was only recived doxorubicin drug (25 mg/kg body weight) once in a week interperitoneally for two months [12].

2- Sample collection and haematological , biochemical tests: The body weight of the animals were recorded before and after the injection. (6 ml) of rat blood were drawn, (1 ml) of it was placed in plastic tubes with anti-coagulant EDTA for some blood analyses such as calculating the haemoglobin concentration (Hb) [13] and packed cell volume (PCV) [14]. The rest of the blood was placed in tubes without anticoagulant and centrifuged to obtain blood serum for biochemical tests of kidney function such as urea [15], uric acid [16], albumin [17], globulin [18], total protein [19] and creatinine [16].

3- The histological study: two weeks after, the carcinogen-injected rats were investigated to detect the occurrence of cancer, while the other groups were exaimend a week after the last dose, the rats kidney were then sectioned and fixed in 10% formalin for the purpose of the histological study [20] .

4- Statistical analysis: Using the 2008 statistical package (Social Science, version 20(SPSS)) program, the statistical analysis of the current study results was carried out to study the effect of the materials used in the experiment and to determine the significant differences between the average probability level ($p \leq 0.05$) [21].

Results and Discussion

The current study showed a significant reduction in the weight of the rats group treated with the carcinogen compared with the other groups and the control group ($p \leq 0.05$) as shown in figure (1-A). The reason is that during the treatment period, the negative effect of the carcinogen on the animals causes a decrease in their nutrition, an increase in the production of free radicals, and therefore an increase in lipid peroxidation, as the decrease is due to the loss of fatty tissue in the muscle [22, 23]. Doxorubicin drug also has an effect on the DNA of the cancer cell, which leads to apoptosis of the cancer cell and its non-proliferation [24). It was also observed that there was a significant decrease below the level $p \leq 0.05$ in some haematological parameters such as Hb concentration and PCV in cancer-induced group (B group) compared with control group and as shown in figure (1-B), this is due to the effect of tumour cells on intestinal cells and microvilli enzymes in the intestine impact their effect on absorption and metabolism of iron [22]. Tumor cells also cause side effects on the kidneys, such as cases of bleeding and a decrease or lack of erythropoietin hormone secretion that is important for blood cell production. In addition , the tumor cells collecting in the organs may be cause ulcers and scarring , especially in the gastrointestinal tract , leading to malabsorption of iron and other minerals , as well as a decrease in body proteins as a result of lack appetite and difficulty in feeding and the occurrence of cachexia condition in carcinogenic rats , an example of which is the transferrin protein, which has the ability to bind to iron and transporting it to the inside of the cells and since iron is important in the formation of blood cells, so the lack of this protein causes anaemia. In addition to a decrease erythropoietin hormone, which is secreted by the adrenal gland and responsible for the process of formation of blood cells (erythropoiesis) due to the effect of the carcinogen on the kidney tissue [25]. A significant decrease was also observed in group C and D compared with the control group due to the effect of this drug on the kidneys and induced alterations in kidney functioning, as well as their effects on erythropoietin hormone secretion [26]. Doxorubicin disrupts the production of blood cells and cause blood clotting disorders and anaemia [27].

Consistent with our results, [28] observed that rats treated with this drug exhibited a sharp decrease in red blood cell number and haemoglobin level.

Current results also observed a significant decrease ($p \leq 0.05$) in the concentration of Urea, uric acid , albumin, globulin , total protein and creatinine as shown in figure (1-C,D,E) for the group treated with the carcinogen compared to control group, and this may due to the effect of the carcinogen on the kidney tissue [22], while the group that subjected to the carcinogen and drug, it was proven that there was a significant increase in the previous concentration of above tests compared to the carcinogen group only. As for the drug treated group only, due to the increased production of free radicals in the body, there was an insignificant decrease in the concentration of above parameters compared to the control group, causing tissue damage, especially the kidney tissue, causing a nephrotoxicity condition [29]. Proteinuria causes mesangial cell injury, which in turn causes mesangial proliferation and increased mesangial matrix production by doxorubicinin causing glomerular expansion [30] .

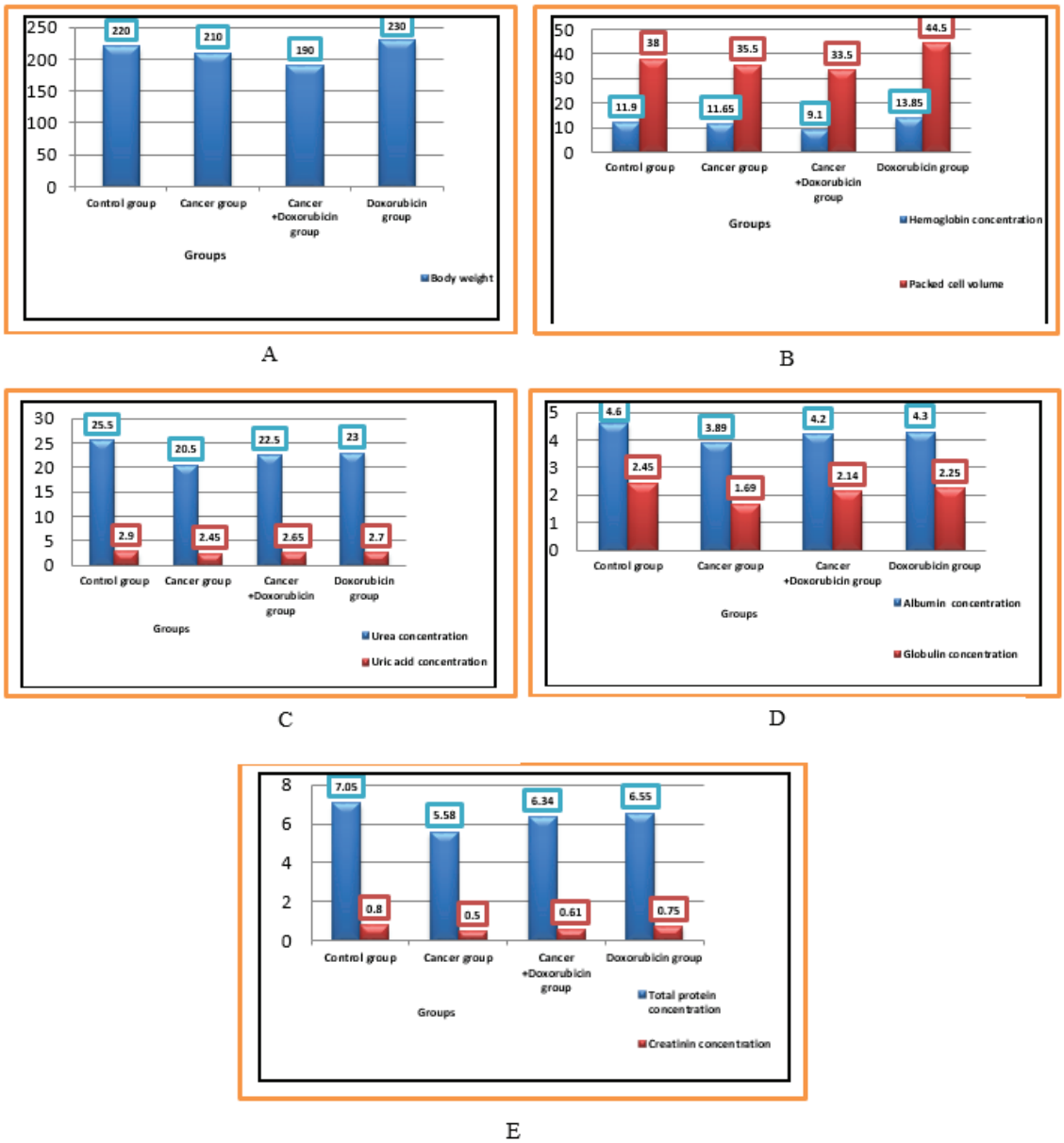

Figure (1): A): Body weight for experimental groups.

B) Hemoglobin concentration (Hb): Packed cell volume (PCV)

C) Urea , Uric acid concentration for experimental groups.

D) Albumin, Globulin concentration for experimental groups.

E) Total protein , Creatinine concentration for experimental groups

Histological study :

The microscopic examination of the kidney tissue of the control group reveal normal shape and structure , as shown in figure (2-A), while the histological examination of the group treated with carcinogen (figure2-B,C,D), showed the presence of bleeding between the renal tubules , lymphocytes infiltration, glomeruli condense, destroyed of urinary tubules, degeneration of cells lining the renal tubules, dilation of the tubules, presence of non- living materials in tubules lumen, expansion of bowman space, destroyed basement membrane, destroyed urinary tubules, and epithelial cells degeneration due to the effect of the carcinogen on the renal cells causing tissue damage [22]. Regarding to the group treated with the carcinogen and doxorubicin drug, the following effect were observed, presence case degeneration in renal tubules, cell necrosis, lysis of cortical tubules, lymphocyte infiltration, glomerulus destroy ,tubules destroy, glomerular thickening. Basement membrane breakdown, epithelial cell degeneration tubules expansion and bleeding between the cells (figure2-E,F,G), these disorders may due to the effect the carcinogen and drug on the kidney tissue [22, 26,31, 32, 33].

Different pathological changes have been observed for the group treated with doxorubicin drug, including thickening of the basement membrane, metaplasia of the epithelial lining of the tubules, bowman space expansion, complete dissolution in the lining of tubules and necrosis of medulla tubules, expansion of their lumen (figure2-H,I,J). High dose of doxorubicin causes acute toxic side effects in kidney tissue which in turn induced nephrotoxicity [29,33,34, 35, 36]. In addition , doxorubicin drug causes cell damage due to oxidative stress and this is consistent with [32]. The presence of protein in the kidney causes mesangial cell injury, which in turn causes mesangial proliferation and increased mesangial matrix production, causing doxorubicin glomular expansion. [35].

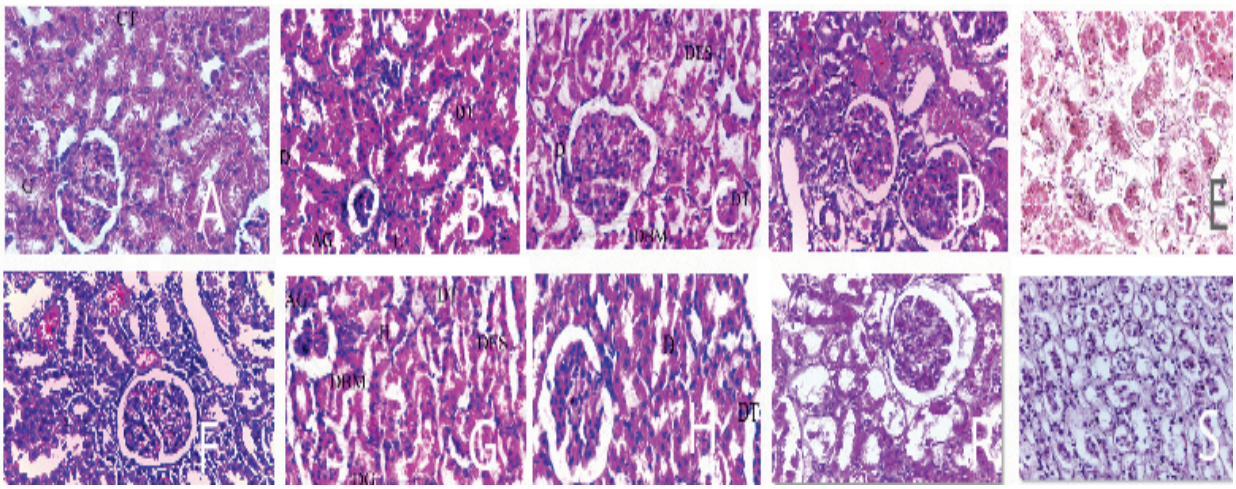

Figure (2): A):kidney rat control group illustrates the normal shape of the kidney, glomular(G),urinary tubules(CT) (H&E) (400X).

B):kidney rat carcinogen group illustrate lymphocyte infiltration(IL), glomeruli condense and destroy(AG,DG) , destroyed urinary tubules(DT) , endothelial cell degeneration(D) (H&E) (400X).

C):kidney rat carcinogen group illustrate destroyed basement membrane(DBM) , destroyed urinary tubules(DT) , epithelial cells degeneration (D) (H&E) (400X).

D):kidney rat carcinogen group illustrate dilation of the tubules(TD) , presence of non- living materials in tubules lumen(N), expansion of bowmans space(EB), hemorrhage(H),Congestion(CON) (H&E) (400X).

E):kidney rat carcinogen and doxorubicin drug group illustrate cell necrosis(N), lysis of corticol tubules (L) (H&E) (400X).

F):kidney rat carcinogen and doxorubicin drug group illustrate epithelial cells degeneration ,tubules degeneration and expansion ,hemorrhage (H&E) ,(400X) . G) : kidney rat carcinogen and doxorubicin drug group illustrate lymphocyte infiltration(IL), glomerulus destroy(DG) ,tubules destroy(DT) , glomerular thickening(AG), Basment membrane breakdown(DBM), epithelial degeneration(DES) (H&E) (400X).

H):kidney rat doxorubicin drug group illustrate tubules destroy(DT), epithelial cells degeneration(D) (H&E) (400X).

I):kidney rat doxorubicin drug group illustrate bowman space expands(BE) , completely dissolves in tubules lining(DT) (H&E) (400X).

J):kidney rat doxorubicin drug group illustrate necrosis of medulla tubules(N) , expansion of their lumen€ (H&E) (400X).

Conclusion

The study showed the effect of carcinogen (azoxymethane (AOM)) on the hematological parameters and biochemical tests specially kidney function. Effects carcinogen upon the kidney tissues. Side effects for doxorubicin on kidney tissue .

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Ramadan Fasting and Risk of Coronavirus Disease 2019 (Covid-19) in Healthy People: A Review

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Abstract

Fasting the month of Ramadan is one of five obligatory pillars of the Islamic creed, observed strictly by hundreds of millions of Muslims every year. The fasting process includes absolute abstinence from eating and drinking for an average of around 15 hours daily for one month. As the coronavirus disease 2019 (COVID-19) pandemic has been ongoing during the month of fasting, there are public concerns that fasting may increase the susceptibility for infection of COVID-19. According to Islamic jurisdiction, if adequate scientific evidence of harm on health is available, fasting is discouraged and should not be practiced. Such concern is not only relevant to the Muslim population but is becoming increasingly relevant to other populations due to the practice of intermittent fasting which is a popular current health trend worldwide. There is no direct evidence of any disadvantage effect of Ramadan fasting during the COVID-19 pandemic on healthy people, for this purpose, we performed a systematic review of 37 articles published between 2005 and April 2020 which investigated the effect of fasting on markers of the respiratory system function and immune response. Our main findings from this analysis show that there is no evidence that fasting the month of Ramadan have a negative impact on the respiratory function and the immune system of healthy individuals.

Key words: *Ramadan fasting; COVID-19; Immunity, Respiratory system function*

Introduction

Ramadan requires healthy Muslim adults to strictly abstain from eating and drinking from sunrise to sunset [1, 2, 3]. The duration of fasting can range from 12 to 22 hours/day depending on the geographical area and season [4,5]. Fasting in Ramadan is obligatory with some valid exemptions for children, pregnant, breastfeeding, and menstruating women, elderly, and sick individuals [6,7,8].

Ramadan is the 9th lunar month migrates throughout the seasons. During Ramadan 2020 as the COVID-19 has been spreading globally, people were more concerned about their immunity and the possibility that fasting may increase their vulnerability to acquiring this infection.

COVID-19 belongs to a large family of viruses, the coronaviruses when transmitted to humans, they can result in a variable clinical course ranging from asymptomatic carrier status, mild flu-like symptoms, pneumonia, and up to an aggressive systemic infection that require hospitalization and intensive care. Infection is transmitted by droplets through direct contact or at the community level by aerosol transmission based on different reports [9, 10, 11,12].

With the rabidly pandemic of COVID-19, the high growing interest of individuals in potential preventive, dietary, change lifestyle, and remedies that may enhance the immunity against covid-19. a growing debate rose toward the expected impact of Ramadan fasting on the body's immunity and respiratory system functions.

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As a first step to investigate this hypothesis, we performed a systematic review of studies that evaluated the effect of Ramadan fasting on intermediate markers that can indicate susceptibility to such infection. The main intermediate markers searched included the respiratory function parameters and markers of the immune system. Other relevant markers such as dehydration and inflammation were also included. Our aimed to collect the current evidence regarding the effect of Ramadan fasting on the immune system, respiratory function and vulnerability to COVID-19 infection in healthy subjects.

Methods

In this article, we reviewed recently conducted studies on the impact of Ramadan fasting on the immune system, dehydration, infection, and respiratory system function. MEDLINE (http://www.pubmed.com) was searched by using "Ramadan" as keyword and the most recent articles in mentioned topics since 2005 until April 2020 were selected.

A systematic review of the following databases was used: Institute for Scientific Information ISI/ Web of Science (WoS), Scopus, PubMed/MEDLINE, Google Scholar, Directory of Open Access Journals (DOAJ), EbscoHOST, Scirus, Science Direct, the Cochrane Library, and ProQuest.

The search was carried out using a string made up of a combination of keywords including "Islam," "Ramadan," "Fasting," "Immunity", "Immune system", "Respiratory system"," Pulmonary function" and "Inflammatory".

Review articles relevant to the aim of this systematic review were scanned in order to improve the chances of finding potentially relevant studies, the aim of this review was excluded, while all other research articles and each reference of the potentially relevant articles were manually consulted and screened in an iterative way until no new references were found.

Results

After removing duplicates and not pertinent Articles, A total of 37 studies were included in the current systematic review, Twenty-three studies focused on the impact of fasting on the immune system and inflammatory in healthy individuals in Healthy individuals while fourteen focused on the impact of fasting on respiratory system structure and function.

The impact of Ramadan fasting on the immune system and inflammatory in healthy people

Maliji et al., 2006 [15] investigated the effects of Ramadan fasting on key parameters of the immune system including complement components of C3 and C4. Researchers applied SRID and CH50 test on two different occasions during the fourth week of Ramadan and one month later. Fifty healthy male and female volunteer students, aged 20-25, were included in that study. The study concluded that Ramadan fasting had no negative effect on markers of innate immunity including complement components. In the study of Latifynia et al., 2007 [13], the effect of fasting on circulating immune complex (CIC) level and immune system was investigated. The serum levels of CIC of 28 healthy adult male students were compared before and after Ramadan by using the polyethylene glycol method. The blood samples were examined using quantitation chemiluminescence and circulating immune techniques, based on the results, no significant difference between the CIC level before and after Ramadan and fasting in Ramadan does not have a bad effect on the immune system in healthy people. In another research by Latifynia et al., 2007 [16] the Neutrophil Respiratory Burst (Innate Immunity) in 24 healthy male adults from Tehran aged 18-35 years were measured and compared before and after Ramadan, using the chemiluminescence method, this study has shown the fasting in Ramadan does not effect on blood neutrophil respiratory burst function and has no critical effect on the immunity of the healthy individuals. The same group, Latifynia et al., 2008 [14] has studied the effects of Ramadan fasting on neutrophil's respiratory burst (as markers of innate immunity) and circulating immune complex (CIC), in 21 young fasting individuals using standardized chemiluminescence and polyethylene glycol methods respectively. Their findings showed that 11 (52%) of the study participants had normal chemiluminescence (CL) activity, and CIC levels before and after Ramadan fasting, despite an insignificant decrease or increase in CL activity or CIC level. Therefore, the changes in the immunological parameters were not significant, and the levels remained in the range of normal.

In study of Latifynia et al., 2009 [18], the Blood samples were tested, neutrophil phagocytosis, serum opsonization power, and NBT reduction for thirteen healthy males, aged between 28-54 years, to investigate the effect of Ramadan fasting on opsonization, phagocytosis, and nitroblue tetrazolium (NBT) reduction by leucocytes, they found a decrease in the neutrophil phagocytic index and serum opsonization index, while the neutrophils participating were increased in phagocytosis Ramadan. Although there was a decrease in the opsonization of the serum, the increased percentage of opsonization compensated for this defect. The result demonstrates the beneficial effect of fasting during Ramadan on neutrophil phagocytic function in healthy people.

The study of Chennaoui 2009 [35] was included, 40 healthy fasting males and females volunteers (20 females aged 20–38 years and 20 males aged between 23–39 years) with normal weight, they were compared with 28 healthy non-fasting volunteers (14 males, and 14 females). The biochemical parameters were investigated including C-reactive protein (CRP), IL-6, and homocysteine or hcy levels. The levels of the inflammatory markers (IL-6, CRP, and hcy) were shown to be significantly decreased as a result of Ramadan fasting in both genders compared to their primary basal values.

Mohammed & Mahmood, 2010 [21] performed a study on the effect of Ramadan fasting on immune cytokines, by evaluating the level of IL-1α, IL-2, IL-6, and IL-8 in 30 healthy fasting individuals compared with 30 healthy non-fasting individuals. Interleukin 1α, interleukin 2, IL-6, and interleukin were shown to be decreased although not reaching a significant level, which seems to suggest that fasting in Ramadan is safe and causes immunomodulation for healthy people. A study by Trepanowski & Bloomer, 2010 [24], on fifty healthy fasters, were reported measurements of pro-inflammatory cytokines IL-1 β, IL-6, and TNF-α, was conducted in three phases (7 days before the fasting, 21 days after fasting, and 1 month after Ramadan fasting). Significant reductions in cytokines IL-1 β, IL-6, and TNF-a levels compared to baseline (78%, 57%, and 71%, respectively) were noted. IL-6 levels remained significantly low one month after the end of Ramadan fasting, implying a potential long-term beneficial role of Ramadan fasting on inflammatory profile and fasting can be a time of a great improvement of the health. Faris et al., 2012 [17], designed A cross-sectional study to investigate the advantage of Ramadan intermittent prolonged fasting RIF on selected inflammatory cytokines and immune biomarkers in Fifty (21 men and 29 women) healthy volunteers who practiced Ramadan fasting for 8-24 hours during the hours between dusk and dawn, they were recruited for the investigation of circulating pro-inflammatory cytokines "interleukin [IL]-1β, IL-6, and tumor necrosis factor α" immune cells " total leukocytes, granulocytes, monocytes, lymphocyte", and anthropometric with dietary assessments. The researchers found that the Immune cells significantly decreased during the month of Ramadan, and still within the ranges. The results showed that RIF attenuates the inflammatory status of the body by suppressing pro-inflammatory cytokine expression and decreasing body fat and circulating levels of white blood cells (WBC).

In a study by Lahdimawan et al., 2013 [30] Blood sampling was drawn for the examination of IFN-gamma, TNF-alpha, iNOS and SOD level from Twenty-seven healthy volunteers males aged 18–22 years who were fasting in Ramadan, Blood sampling was conducted before 7 days of Ramadan, 7th day of Ramadan, and 21st day of Ramadan. The authors suggest that Ramadan fasting induce classically activated, inflammation, decrease the oxidative stress on macrophage, altered activated macrophage regulation-signaling and induces macrophage function.

In Develioglu et al., 2013 [31] study, blood, and saliva samples were collected from 35 healthy men one week before Ramadan and during the last week of Ramadan to determine the Albumin, total lymphocyte count, electrolytes, IgG and IgM concentrations, and IgA concentrations. The results showed that although IgG concentration was decreased after-Ramadan in comparison to before-Ramadan, it was not out of the normal range. The reduction in the concentration of salivary IgA was also observed, while the lymphocyte number was elevated. The results show that Ramadan fasting has no severe effect on the immune system. Mohajeri et al.2013 [32] determined the levels of biochemical parameters for 58 healthy fasting subjects, aged 20–40 years, chemokine (C–X–C motif) ligand 1 (CXCL1), chemokine (C–X–C motif) ligand 10 (CXCL10) and chemokine (C–X–C motif) ligand 12

(CXCL12) chemokines levels using enzyme-linked immunosorbent assay (ELISA). The results showed a significant decrease in the pro-inflammatory chemokines (CXCL1, CXCL10) and the constitutive chemokine (CXCL12).

Lahdimawan et al., 2014[19] took blood samples from thirty healthy volunteers males seven days before Ramadan and on the seventh and 21st day of Ramadan to determine whether fasting in Ramadan change the ability of serum, peripheral blood mononuclear cells (PBMC), and macrophages kill Mycobacterium tuberculosis. Blood sample were measured by ELISA: Complement C3, inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD) levels in serum, PBMC, and the killing ability of immune components determined by measuring colony-forming units (CFUs). They found that CFU in PBMC infected with M. tb (PIM), and macrophages infected with M. tb (MIM) decreased, the CFUs in serum infected with M. tb (SIM) increased, and CFUs in MIM decreased compared with that before Ramadan fasting. The levels of iNOS in serum and the CFUs in MIM decreased and the CFUs in SIM and PIM increased and the levels of iNOS in PBMC significantly increased on the 7th and 21st days of Ramadan compared with before Ramadan fasting. The study suggested that Ramadan fasting has a beneficial effect on host defense against M. tb and decreases the risk of tuberculosis (TB) infection in healthy people.

In another study of Siadat et al., 2014 [26]. The pre-Lymphocytic subpopulations analysis was conducted using flow cytometry for 38 healthy peoples (9 females and 29 males), age range 17-51 years, were assessed before Ramadan and one day before the end of Ramadan. The results showed that the percentage of the total lymphocytes was 25.82% and 26.23% in the pre and late-Ramadan, respectively, therefore, the results seem Ramadan fasting does not affect the lymphocyte count, percentage ratio, and the main lymphocyte subpopulations of healthy individuals.

Feizollahzadeh et al., 2014 [33] studied the effect of the Ramadan fasting on serum levels of adiponectin and tumor necrosis factor-α (TNF-α) as two inter-related peptides involved in cells sensitivity to insulin and glucose metabolism for seventy healthy men, with age range equal or greater than 30. The results showed that the Ramadan fasting resulted in augmented adiponectin levels which may help in improving metabolic stress induced by insulin resistance in people with predisposing factors of type2 DM. In Gorjipour et al., 2015 [20] Study, the Plasma granulysin levels as a biomarker of the immune system were investigated in 44 healthy male volunteers with a mean age of 41.15±13.6 years. Blood samples were obtained on the 29th day of Ramadan and four months after Ramadan. They investigated Plasma Granulysin, triglyceride (TG), Cholesterol (Chol), low-density lipoprotein (LDL), and High-density lipoprotein (HDL), FBS, Uric acid, and CRP. They found that the mean concentrations of CRP serum on the 29th day of Ramadan were significantly lower than after Ramadan, which shows Fasting caused modulation in the CRP level but did not affect the immune system. A study by Bahijri et al., 2015 [25] investigated the effect of Ramadan fasting on secretory patterns of PTH, markers of bone metabolism, and serum immunoglobulins for healthy young volunteers before (Shaban) and 2 weeks into Ramadan. Calcium, phosphorus, magnesium, albumin, alkaline phosphatase, 25-OH vitamin D, intact PTH (iPTH), and immunoglobulin (Ig) A, M, and G were measured, the levels of immunoglobulin M remained stable, with no significant increase or decrease. and Ramadan fasting was not shown to result in drastic disturbances.

Mohammadzade et al., 2017 [27] performed a study on the effect of fasting during Ramadan on biochemical parameters and inflammatory markers. The studies were done on 30 healthy fasting men, blood samples were taken one week before Ramadan, during the last week of Ramadan, and four weeks after Ramadan. Serum interleukin-6, high sensitivity C-reactive protein, fasting blood sugar (FBS), insulin, total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein levels were measured. No significant change was observed in the results and the showed that the inflammatory factors do not change significantly during and after fasting.

Orucunun et al., 2019 [34] conducted a study on 30 fasted female subjects during Ramadan month. They investigated the red blood cells (RBC) and total White blood cells (WBC) before the 3rd and 28th day of the Ramadan month using a fully automatic hematological

analyzer. The results of Lymphocytes percent and Neutrophils percent were significantly increased during the Ramadan fasting period as compared to the pre-Ramadan fasting period. WBC count decreased significantly at the end of the Ramadan month as compared with pre-Ramadan fasting.

In addition, A cross-sectional study for Mushtaq et al., 2019 [22] at Federal Urdu University of Arts, Science & Technology investigated the effect of Ramadan fasting on plasma adiponectin and TNF-α levels for 55 females and 55 males, age 20 and 40 years fasting in Ramadan, the Plasma adiponectin and TNF-alpha levels were assayed with ELISA kits. Fasting in Ramadan significantly decreased TNF-α (pg/mL) levels of post-obese males and females than Pre-Ramadan-groups. fasting showed a beneficial effect on health. A cohort study was done by Alam et al., 2019 [29] on 78 adult men who were fasted for 29 consecutive days from sunrise to sunset in Pakistan, aged 20 to 85 years, All CRP level < 2 mg/l during the fasting period and a similar trend were observed for TNFα. Most SASP molecules were decreased after the fasting period, heightened levels of IL-8 and IL-6 suggest that some inflammatory markers may be elevated by recurrent circadian fasting. The results highlighted that circadian fasting is beneficial at the cardiometabolic and inflammatory levels. According to the review results of Massoud et al., 2020[23]. Ramadan fasting has a positive influence on the body weight, lipid and glucose levels, while exerting antioxidative effects, increasing longevity, and improving the renal and immune function.

In an additional study of Mindikoglu et al., 2020 [28] An untargeted serum proteomic profiling was performed using ultra-high-performance liquid chromatography/ tandem mass spectrometry for 14 healthy fasted people from dawn to sunset for over 14 h daily for 30 consecutive days. Samples of Seram were collected before 30-day of fasting, at the end of 30-day of Ramadan, and one week after 30-day intermittent fasting. the results highlighted that 30-day fasting can be a preventive and adjunct therapy in cancer, DNA repair, cytoskeleton remodeling, immune system, and cognitive function.

The impact of Ramadan fasting on Respiratory structure and function in healthy people

Siddiqui et al., 2005 [37] studied the impact of Ramadan fasting on pulmonary function values in Healthy people. They found no significant change in the pulmonary function parameters during fasting in Ramadan compared to before the Ramadan period. However, FVC was decreased significantly after the Ramadan period compared to Ramadan. Relative Topre-Ramadan Baseline Values, there was no change in spirometric variables during Ramadan fasting.

In addition, Subhan et al.,2006 [38] determined the effect of Ramadan fasting on the expiratory flow rates in healthy subjects. There was a significant reduction in body mass in Ramadan compared to pre- and post-Ramadan. They found no significant changes in expiratory flows during Ramadan as compared to the pre-Ramadan period. forced expiratory flow rates at 75%-85% of the vital capacity showed a significant increase in the post-Ramadan period compared to Ramadan. Their results show that Ramadan fasting did not affect expiratory flow rates in healthy subjects.

Moosavi et al.,2007 [39] assessed changes in lung volume during and after Ramadan fasting. They found that the mean of FEV1% increased both during fasting and after Ramadan. Moreover, the mean vital capacity and peak expiratory flow rate were significantly increased during Ramadan. Although the mean maximum mid-expiratory flow decreased at the beginning of Ramadan, however, it was subsequently increased significantly. The researchers concluded that fasting increased lung volume and improved lung function. Moosavi et al.,2007 [44] designed a cohort study that included 117 healthy male subjects' professors and student volunteers, to evaluate the changes in pulmonary volume during and after Ramadan fasting. All participants underwent spirometry 10 days prior to Ramadan, two times during Ramadan, and one time 10 days post-Ramadan. Although the mean maximum mid-expiratory flow decreased at the beginning of Ramadan and significantly increased subsequently, MEF50%, and MEF 75% increase at the beginning of Ramadan and decreased subsequently the result clarifies that fasting increases lung volumes and might improve pulmonary function.

The study of Askari et al.,2015 [47] evaluated the total and differential white blood cells, inflammatory biomarkers, and respiratory symptoms in fifteen

asthmatic patients compared with a matched control group of fourteen healthy volunteers before and after the one-month fasting. There was no negative impact found on the pulmonary function, total white blood cells, and inflammatory biomarkers on healthy nor on asthma subjects.

In a study by Soori et al., 2016 [36], the effects of Ramadan fasting on respiratory muscle strength were investigated in a group of 35 fasting healthy males, maximal inspiratory muscle pressure (MIP) and peak inspiratory flow (PIF) were measured in the last week of Ramadan month in summer, after 3 months of Ramadan the measurements were repeated in 12 individuals. the results showed Ramadan fasting may cause reduction of respiratory muscle strength through reduction of body weight but no other effects.

Latiri et al., 2015 [45] Evaluated the effect of Ramadan Fasting on Spirometric values in 29 non-smoking healthy males (mean age of 27±6 yrs) fasting during Ramadan for at least the 5 past years for three periods, before-Ramadan, mid-Ramadan and, after – Ramadan, the results showed Ramadan fasting did not have any significant changes in the spirometric only affected FEV1 and FVC of healthy male adults.

Askari et al., 2016 [42] investigated the pulmonary function tests (PFT) and respiratory symptoms, in 15 asthmatic patients compared with 14 healthy volunteers who had no history of respiratory complaints. They measured PFT before and after the one-month fasting duration. The results showed that the values of MCH, MCHC in both groups, and monocyte counts in asthmatic patients. Respiratory symptoms in asthmatic patients were non-significantly increased and wheezes were significantly reduced following Ramadan fasting in the asthmatic group of patients.

Latiri et al.,2017 [40] conducted a study of whether Ramadan Fasting affects Spirometric values in Twenty-nine nonsmoking healthy males during fasting month for three periods before-Ramadan, mid-Ramadan, and after-Ramadan, to conclude, RF did not have any significant changes in the spirometric values of nonsmoking healthy adult males.

The study of Roy A. & Bandyopadhyay A.2018 [41] aimed to investigate the effects of Ramadan fasting on lungs function tests (PFTs) in Fifty sedentary nonsmoking healthy young Muslim male individuals aged 20 to 25 years without any history of pulmonary or other diseases were recruited by simple random sampling from Kolkata, India. Participants completed the American Thoracic Society questionnaire for their personal demographic data, health status, PFTs were within the normal range and did not show any significant variation during the RIF and RIF did not affect the normal range of PFTs in young Muslim males.

The Study was done by Sayeed et al.,2018 [43] to determine the effect of Ramadan fasting on spirometric parameters for 50 apparently healthy young adults aged between 17-27 years. It was done at three different time points: First: 5–10 days before the start of Ramadan (pre-Ramadan); second: Within 10 days of the beginning of RF (Ramadan); third: Within 7 days of the end of Ramadan (post-Ramadan). There were no statistically significant differences between the three phases, RF does not have any significant effect on PFTs as assessed by spirometry. Hence, the diagnosis and prognosis of a respiratory disorder made on spirometry findings are reliable and need no error correction with an individual is fasting.

In another study of Dodderi & Philip, 2018 [48] aimed to measure the pulmonary function of thirty-seven healthy adults between 18-23 years volunteers, Twenty non-fasting and seventeen fasting healthy adults, Individuals under fasting performed spirometry tracings across the 1st week of fasting, last week of fasting and one week after breaking of fasting, the results showed reduced pulmonary function in fasting volunteers during 1st week and last week of fasting, with the increase in pulmonary function 1st-week post breaking of fasting and the study concludes that fasting in Ramadan improves the pulmonary function. Fraj et al.,2019 [46] Evaluated the weather of Ramadan Fasting affects Spirometric values in a group of 36 healthy males adolescents aged 12 to 15 years for three periods Before-Ramadan, Mid-Ramadan and Post-Ramadan, to conclude, Ramadan Fasting did not have interaction effect in the spirometric values of healthy male adolescents.

Conclusion

In Ramadan, as the COVID-19 continues to spread

globally, and people go on to self-isolate in their homes and most of them afraid of getting sick, moreover, they work to strengthen their immunity. healthy people wanted to consider the religious license to break their fast in consultation because fasting during Ramadan may have a negative effect on the immune system, pulmonary function, and increase the risk of infection. however, guidelines statements that help to guide healthy people in addressing these issues are not clear, moreover, There has been no research on the impact of fasting on an increased risk of COVID-19 disease, for this purpose, we performed this review, in this review, our aimed to collect the current evidence regarding the effect of Ramadan fasting on the immune system and respiratory structure and function in healthy subjects. We can conclude that:

1. Ramadan fasting has been shown no negative impact on the immune system in healthy people.

2. Fasting in Ramadan may improve the immune system function.

3. Ramadan fasting has been shown no negative impact on the respiratory system in healthy individuals.

4. fasting in Ramadan may improve pulmonary function.

5. Ramadan fasting doesn't increase the chances of getting sick with the COVID-19.

6. Studies of healthy people have shown that Ramadan fasting is safe

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Author's contributions

BQ, participated in the design of the study, data collection, and drafted the manuscript. BQ, KSF and AOA contributed to the e writing of the manuscript. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

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Argyrophilic Nucleolar Organizer Regions in Premalignant and Malignant Oral Lesion: A Quantitative and Qualitative Assessment

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Abstract

Background: Argyrophilic nucleolar organizer regions (AgNORs) has been considered to be useful in diagnostic pathology, mainly to distinguish benign lesions from their malignant counterparts.

Aim: This study was done to evaluate the significance of various AgNOR parameters in differentiating premalignant and malignant oral lesions.

Materials and Methods: The study was performed on thirty five archival tissues consisting of sample comprised of five normal mucosa, fifteen premalignant lesions and fifteen malignant oral lesions. AgNORs were assessed both quantitatively and qualitatively. The data were analyzed using ANOVA and Chi square test.

Results: Quantitatively and qualitatively significant difference was noticed. Quantitatively significant difference in the mean value of AgNORs noticed between the premalignant and malignant lesions. In terms of qualitatively, the premalignant and malignant oral lesions differed significantly from normal mucosa.

Conclusion: To conclude, the AgNOR count is the most appropriate marker to differentiate between premalignant and malignant oral lesions.

Keywords: *Argyrophilic nucleolar organizer regions, epithelial dysplasia, oral squamous cell carcinoma.*

Introduction

Leukoplakia is one of the most common premalignant lesion affecting the oral mucosa. It's potentially malignant transformation rate ranges from 3 - 33% over 10 years.[1] Leukoplakia as a clinical entity may have varied histopathological presentation ranging from mildly hyperkeratotic lesions to those exhibiting severe dysplastic features. Oral epithelial dysplasia (OED) is the diagnostic term used to describe the histopathologic changes seen in a chronic, progressive and premalignant disorder of the oral mucosa. Epithelial dysplasia is the histologic marker of premalignancy and as such it is predictive of an increased rate of development of oral squamous cell carcinoma (OSCC).[2] OSCC is the third most common form of malignancy in the developing countries whereas in the developed countries, it is the eighth most common malignancy.[3]

Histopathological typing, grading and staging of a tumor is of proven value in the clinical management as well as prognostic outcome of the cancer patients. The development of staining technique like

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"Argyrophilic Nucleolar Organizer Regions (AgNOR) for histopathological diagnosis as well as prognosis of the diseases.4 The silver staining is a useful tool for the study of the structure of the nucleolus and the variation of the nucleolar activity.5 Nucleolar organizer regions (NORs) are loops of DNA that transcribe for ribosomal RNA (rRNA). They are located on the short arm of chromosomes 13, 14, 15, 21, and 22.Associated with these regions are certain acidic and argyrophilic , non-histonic proteins called NOR- associated proteins. NORs can be demonstrated in tissue sections by staining their associated proteins with colloidal silver and these silver- stained reaction products represent the AgNORs.[5] Transformation of a normal cells to a neoplastic cell is characterized by an increase in protein synthesis and strong basophilia of cytoplasms. Additionally, an increase in size and number of the nucleoli, which implies an amplified synthesis of the ribosomal RNA in order to meet the increased demand for proteins in tumor.[7]

The AgNOR is demonstrated to be useful in diagnostic pathology, mainly to distinguish benign lesions from their malignant counterparts.[6] AgNORs reflect the state of activation and the proliferation activity of the cell and the degree of malignant transformation of certain tissues.[8] The aim of this study was to evaluate the proliferative activity, through AgNOR count in oral premalignant and malignant oral lesions and to assess the malignant transformation and clinical behavior of epithelial dysplasia and OSSC by qualitative and qualitative assessment.

Materials and Methods

The study sample consisted of thirty-five archival biopsy specimens which included Group I (control group- 5 cases of normal oral mucosa), Group II (15 cases of epithelial dysplasia) and Group III (15 cases of OSCC) retrieved from the Department of Oral & Maxillofacial Pathology, Santosh Dental College and Hospital, Ghaziabad. The histopathological diagnoses were reviewed using routine hematoxylin and eosin stained sections.

The tissue sections were stained by using AgNOR staining procedure described by Ploton et al (1986).[8] Two solutions were prepared: Solution- A which consists of 2% gelatin solution dissolved in ultrapure water, to which formic acid is then added to make a final 1% solutions; Solution- B consists of 50% silver nitrate solution in ultrapure water. Then the staining solution was obtained instantly by rapidly mixing one part of solution- A with two parts of solution- B into a glass cylinder. 3-4 µm thick sections were cut from the paraffin block, dewaxed in several baths of xylene and hydrate through graded alcohols to ultrapure water. Sections were deparaffinized in xylene and hydrated groups decreasing grades of alcohols to deionized water. Ensuring a dark environment was maintained throughout the reaction time. The sections were reacted with freshly prepared silver colloidal solution in a closed coplin jar for thirty-five minutes at room temperature. The silver colloidal solution was washed with distilled ionized water. The sections were washed in double distilled deionized water. Then sections were dehydrated through increasing grades of alcohol. The sections were cleared in Xylene-I and then Xylene II. And then sections were mounted by using mounting media DPX.All sections were examined for analysis.

For quantitative assessment: by careful focusing, AgNOR dots were counted manually.

Intranucleolar and extranucleolar dots were included in the counting regime. Dots that were overlapped or those with indiscernible AgNORs were excluded.

For qualitative assessment: Morphological variations of AgNORs were assessed in terms of size and shape of individual AgNOR dots and their pattern of distribution of AgNOR, as defined by Wanakulasuriya and Johnson.

Results and Discussion

AgNORs appeared as dark brown or black dots or blobs against yellowish background.

AgNORs were counted in each nucleus and categorized as large NORs which appeared as blebs and small NORs which appeared as distinct small dots.

The morphological assessment of AgNORs was done on the basis of their size, shape and the pattern of distribution shows certain differences among the various study groups. In case of

normal oral mucosa (control), the individual

AgNOR dots were large sized, uniformly round or oval (Figure-1). In case of leukoplakia (premalignant lesion), the AgNORs were not so uniformly round or oval ,irregular in shape(Figure-2). In case of OSCC (malignant lesion), the

AgNOR were distributed throughout the nucleus (Figure-3).They were appear in cluster and are

abnormal in shape. Sometimes they appear very fine, minute and numerous and distributed thoughout the nucleoplasm, giving rise to granular appearance.

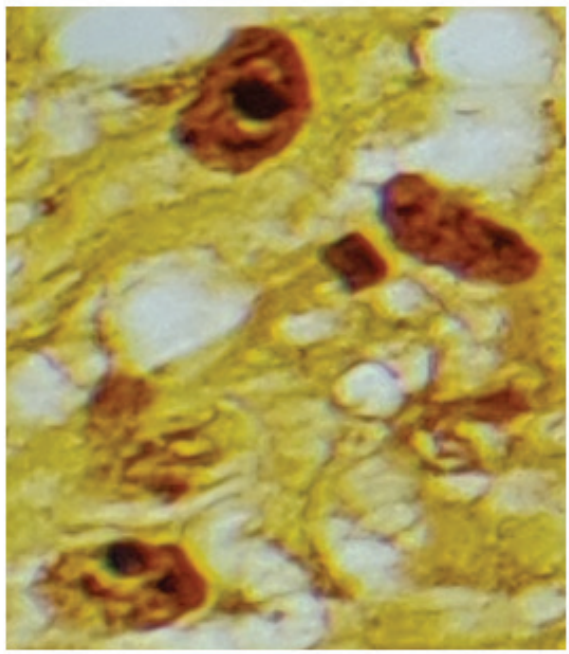


Figure 1: Silver stained section of normal oral mucosa

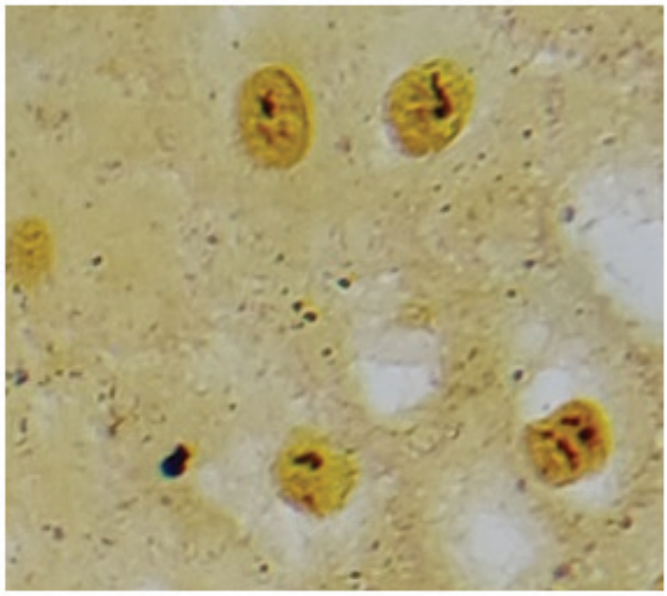


Figure 2: Silver stained section of leukoplakia

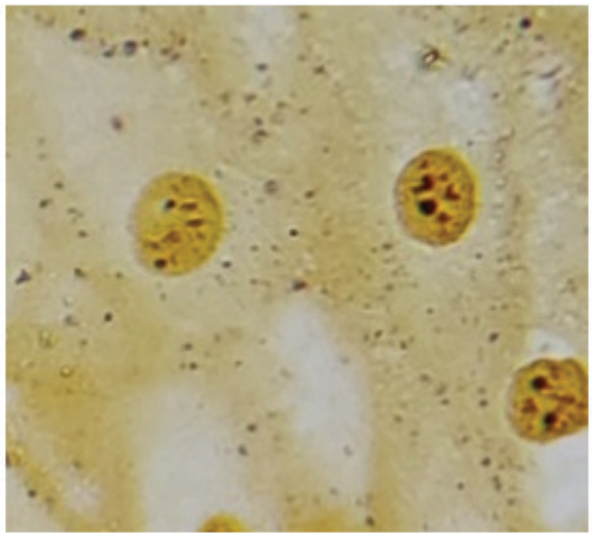
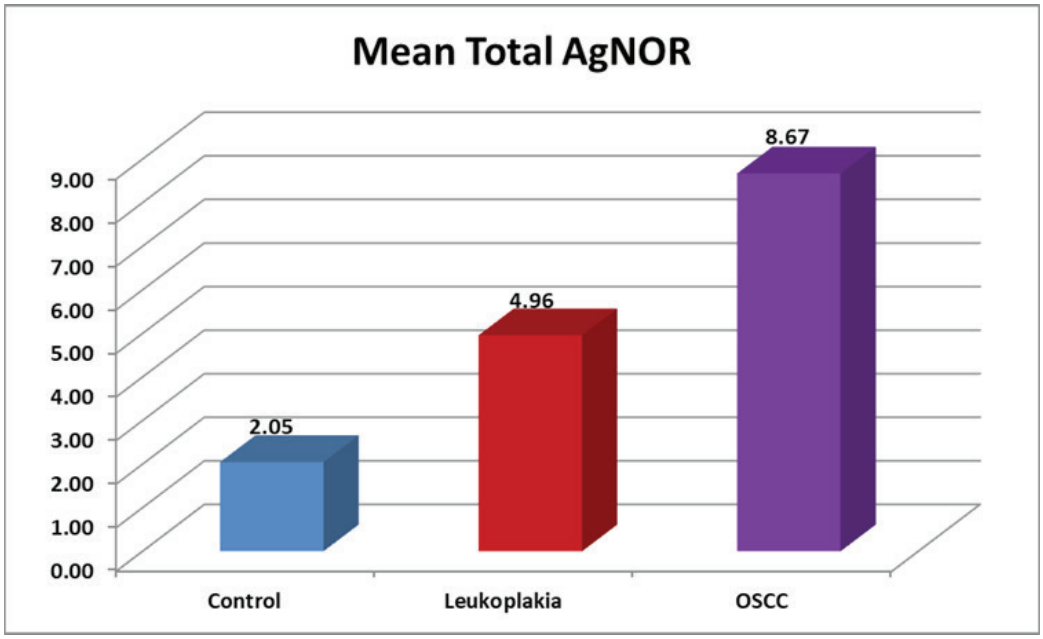


Figure 3: Silver stained section of Squamous cell carcinoma

The mean AgNOR count was 2.05 of normal mucosa. The mean value of premalignant lesion was 4.96 lower than malignant lesions (8.67). One-way ANOVA test shows a significant difference in mean of total AgNOR count between study groups (p value <0.001), as shown in graph.



NORs are loops of DNA that transcribe for ribosomal RNA. They are present on the short arms of acrocentric chromosomes and are associated with ribosomal RNA activity, protein synthesis, and cell proliferation.[9] AgNORs are considered to reflect the biosynthetic and nucleolar\ functional activity of a cell and serve as an indicator of the rapidity of cell cycle. The AgNOR count increases with increased cell ploidy and with increased transcriptional activity during active cell proliferation.[10] Variations in the size and /or number of the AgNOR dots may depend on the stage of the cell cycle, the transcriptional and metabolic activity of the cell or the number of NOR-bearing chromosomes in the karyotype. AgNOR count is used as a marker of cellular proliferation and for this reason it has been shown as an important tool to characterize the epithelial dysplasia

in leukoplakia and OSCC. Thus, AgNORs have found widespread application in tumor histopathology, in assessing growth potential and malignant potential of tumors, in distinguishing between benign and malignant neoplasm, to assess the prognosis and to evaluate the risk of occurrence.[11]

Various newer techniques, which are used for assessing the tumor tissue are based on nuclear studies. The staining of AgNORs by a silver compound has become popular for its simplicity, ease of use, low cost and good correlation with other proliferative markers.[11]

The present study was conducted to evaluate the importance of AgNORs in differentiating pre-malignant and malignant lesions of the oral cavity. The results of this study clearly indicate significant differences in qualitative assessment of the mean AgNOR count of the different study groups and also a steady increase in the count from the normal mucosa (2.05) to leukoplakia (4.96) to oral squamous cell carcinoma (8.67).

In our study, the frequency of total AgNORs increased with malignant progression from normal mucosa to squamous cell carcinomas of the oral cavity ($P < 0.001$). This clearly indicates that the AgNOR counts increase with the changes occurring in the normal epithelium towards a dysplastic change and its malignant transformation. Qualitative assessment of AgNORs based on their size, shape and pattern of distribution showed significant difference among the different study groups. The appearance of the individual AgNOR dots in control was uniformly round or oval shape in size (80%) and irregular shape (20%). Gulia S P et al (2011) observed that the AgNOR dots tended to be large, homogenously stained and regular in the nucleus of the normal epithelial tissues, whereas significant differences (irregular, giant and bizarre clusters) were seen in oral squamous cell carcinoma.[11] Warnakylsuriya K & Johnson NW (1993) observed that single, double or few AgNORs per nucleus (Type – I product) were seen in most of the benign lesions. The epithelial dysplasia predominantly demonstrated multiple small AgNOR dots within the nucleolus (Type II) whereas in OSCC, multiple small AgNOR dots were seen scattered throughout the nucleoplasm (Type III). As per them, on the other hand dysplasia group also showed an admixture of all the three types of silver stained products. [12] While Chowdhary A. et al (2013) found significantly different mean value of normal mucosa (1.95) to oral squamous cell carcinoma (5.19).[13]The reason for the greater number of AgNORs in the nuclei of malignant lesions is undetermined it may represent an escalation in ploidy, increased gene amplification or a rise in chromosomal segregation with more cells being in the Sphase of their cycle.[13] In the present study, the mean value of smaller AgNOR size in the control group was 1.03. In this group, type I distribution pattern (60%) and type II pattern of distribution (40%) were observed. While on comparing the normal mucosa with the premalignant group, there were noticeable differences in the appearance of the individual dots. Leukoplakia showed larger and more scattered AgNOR dots with a mean value 2.36. AgNOR dots in the premalignant group were not uniformly round or oval as in normal mucosa. AgNOR dots in the premalignant group comprised of irregular (80%) and oval (20%) in shape. Pattern of distribution showed a significant difference, type II pattern of distribution more among leukoplakia. In OSCC, the dots were predominantly small and were irregular in shape. The size of silver stained NORs is also related to the degree of transcriptional activity of the rDNA concerned. In OSCC, AgNORs were of both large and small size and their abnormal aggregation resulted in significant AgNOR pleomorphism. Several studies have shown variations in the number and shape of the AgNORs in normal mucosal cells and in the malignant cells.

Conclusion

AgNOR count appears to be a useful tool in distinguishing between normal epithelium, dysplasia and squamous cell carcinoma of the oral cavity. The present study finding shows the significance of qualitative and quantitative changes as a diagnostic indicator for premalignant/ malignant lesions. AgNOR pleomorphism was a reliable criterion in defining and differentiating between malignant and premalignant lesions. A correlation between the AgNOR count and diagnosis was found in premalignant and malignant lesions of the oral cavity.

Ethical Clearance: The ethical clearance was obtained from the Institutional Ethical Committee.

Source of Funding: Self

Conflict of Interest: Nil

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Role of Serum Malondialdehyde (MDA) and Vitmin- C Level in Non-Smokers and Chronic Smokers with Acute Myocardial Infarction (AMI) in Male

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Abstract

Introduction: Cigarette smoking is recognized as a serious health hazard as each cigarette tears away 7-11 minutes of human life. The present study aimed to investigate the serum MDA and vitamin c levels in non-smoker and chronic smoker with AMI patient and its association with cigarette consumption. Cigarette smoke contains many oxidants capable of generating reactive oxygen species and reactive nitrogen species. These species play a key role in oxidative stress, leading to the development and progression of many disorders, including hypertension, cancer, diabetes mellitus and cardiovascular diseases.

Methods: This study was conducted in the Medicine Department at DMMC & SMHRC, Nagpur in collaboration with ABVRH, Sawangi (Meghe) during September 2020 to January 2021. Informed consent was obtained from all the subjects. 40 controls who were apparently healthy non-smokers were included after appropriate matching (for age and sex) and 40 apparently healthy chronic smokers were selected 40 chronic smokers with diagnosed acute myocardial infarction were selected from SMHRC. All of them were male subjects

Result: The MDA and vitamin C were compared between Group A and Group B and also between Group B and Group C. There was a significant rise in MDA ($p<0.0001$) and significant decrease in vitamin C ($p<0.01$) in Group B compared to Group A. There was a significant rise in MDA ($p<0.0001$) and significant decrease in vitamin C ($p<0.001$) in Group C compared to Group B.

Conclusions: The increase in serum MDA level and decrease in vitamin C was found in chronic smokers compared to non-smokers. It was also found that there is increase in serum MDA and decrease in vitamin C in smokers with AMI compared with smokers without AMI, and the reason for this inter-subject variability of MDA and vitamin C levels may be due to gene-environmental factors.

Key Words: *Chronic smokers, Myocardial infarction, MDA, Vitamin C*

Introduction

Cigarette smoking is a major health issue and causes large number of deaths worldwide. Smoking has been reported to be a risk factor for oral cancer, oesophageal cancer, lung cancer and even liver cirrhosis.[1]

Cigarette smoke is a complex mixture of chemicals containing more than 4000 different constituents. Some of the compounds identified include pyridine alkaloids such as nicotine, ammonia, acrolein, phenols, acetaldehyde N-nitrosamine, polycyclic aromatic hydrocarbons such as benzopyrine, combustion gases such as carbon monoxide, nitrogen oxides, hydrogen cyanide, and trace metals, α-emitter radioactive elements such as polonium, radium and thorium etc.[2]

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Smoking is a potent but modifiable risk factor of cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular diseases. It acts substantially via reactive species to initiate and promote pathology and is consistently associated with elevated oxidative damage to DNA, lipids and proteins.[2,3] It has been suggested that cigarette smoking done regularly more than 10 per day, constitute a major risk for coronary heart disease (CHD).[4]

Cigarette smokers have an increased risk of cardiovascular diseases (CVD), possibly mediated by elevated levels of oxidized macromolecules owing to heightened ROS production. Smokers are exposed to significant quantities of ROS in both gas and tar phase. Further ROS production mediated through inflammatory processes may exacerbate those produced through direct exposure. Blood of cigarette smokers routinely displays decreased antioxidant capacity and increased oxidized lipids compared to non-smokers. [5]

Malondialdehyde is a organic compound with the formula CH2 (CHO) 2. This reactive species occurs naturally and is a marker for oxidative stress. Reactive oxygen species degrade polyunsaturated lipids present on cell membrane forming malondialdehyde. This aldehyde product is used as a biomarker to measure the level of oxidative stress in an organism.[6]

MDA is a sensitive marker of lipid peroxidation. Free radicals released from smoking degrade polyunsaturated lipids present mainly in cell membranes forming malondialdehyde (MDA). This compound is a reactive aldehyde and is one among the many reactive electrophilic species that cause toxic stress in cells and form covalent protein adducts which are referred as advanced lipoxidation end products.[6]

Antioxidants depletion or deficiency may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of oxidative stress related diseases.[7]

Vitamin C is a potent water-soluble antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. The species formed after the loss of one electron is a free radical, semidehydroascorbic acid or ascorbonyl radical, a reactive and possibly harmful free radical. Many studies have demonstrated low plasma concentration of vitamin C in smokers and acute myocardial infarction (AMI) patients.[2,8]

Vitamin C functions as antioxidant by preventing others substances from being oxidized by donating its electrons. However in this process vitamin C oxidized itself. The compound formed after loss of electron is ascorbyl radical which is relatively stable with half life of 10─5 seconds and is fairly unreactive which explains the antioxidant function of ascorbic acid.[9]

The mechanism involved in the reduction of vitamin C level in smokers may be due rapid oxidation of ascorbic acid by free radicals. The negative relationship between vitamin C and MDA may be due to the depletion of vitamin C when the oxidant burden is increased.

MDA is a sensitive marker of lipid peroxidation. Free radicals released from smoking degrade polyunsaturated lipids present mainly in cell membranes forming malondialdehyde (MDA). This compound is a reactive aldehyde and is one among the many reactive electrophilic species that cause toxic stress in cells and form covalent protein adducts which are referred as advanced lipoxidation end products.[10]

Present study is undertaken to evaluate serum malondialdehyde as indicator of oxidative stress and serum vitamin C as indicator of antioxidant level in non -smokers and chronic smokers with acute myocardial infraction AMI in male.

Materials and Methods

This study was conducted in the Medicine Department at DMMC & SMHRC, Nagpur in collaboration with ABVRH, Sawangi (Meghe) during September 2020 to January 2021. Informed consent was obtained from all the subjects. 40 controls who were apparently healthy non-smokers were included after appropriate matching (for age and sex) and 40 apparently healthy chronic smokers were selected 40 chronic smokers with diagnosed acute myocardial infarction were selected from SMHRC. All of them were male subjects.

Inclusion Criteria: Both chronic smokers and chronic smokers with myocardial infarction, who smoked more than 10 cigarettes per day for more than 09 years, were selected.

Exclusion Criteria: Those excluded from the study were persons abusing alcohol, ex-smokers, patients with diabetes mellitus, hypertension, renal diseases, hepatic impairment, endocrine disorders, obese individuals and

patients on drugs like multivitamins etc.

Sample Collection: 5 ml blood sample was collected venipuncture method in all subjects and care was taken to ensure that in AMI patients, blood sample was collected before thrombolysis of patient. Serum was separated in 3000 rpm for 1 min and analyzed for the MDA and vitamin C level.

Biochemical Analysis:[11,12]

· MDA was measured by thiobarbituric acid reactive substances assay (TBRAS) method.

· Ascorbic acid (Vitamin C) measured by dinitrophenyl hydrazine (DNPH) method.

Statistical Analysis

The data were analyzed using SPSS software program, version 20.0. The mean and standard deviation were measured. Analyzed and interpreted using descriptive and inferential statistics. The probability value is less than 0.05 ($p < 0.05$) and it was considered as statistically significant.

Result

Table no 1 shows the serum levels of MDA and vitamin C in non-smokers (Group A) and chronic smokers (Group B). The MDA of Group A was 238±3.54nmol/l and in Group B was 432±9.0nmol/l. The mean vitamin C in Group A was 1.20±0.10mg/dl and that of Group B was 0.98±0.18mg/dl. MDA was significantly higher ($p<0.0001$) in Group B compared to Group A. Vitamin C level was significantly lower ($p<0.01$) in Group B than in Group A.

TABLE NO: 01: serum levels of MDA and vitamin C in non-smokers (Group A) and chronic smokers (Group B).

Parameter	Non-Smokers (Group A)	Chronic Smokers (Group B)	P- Value
MDA nmol/l	238±3.54	432±9.0	P<0.0001
Vit C mg/l	1.20±0.10	0.98±0.18	P<0.01

Table no 02 shows the serum levels of MDA and vitamin C in chronic smokers (Group B) and chronic smokers with MI (Group C). The MDA of Group B was 432±9.0nmol/l and in Group C was 530±15.90 nmol/l. The mean vitamin C in Group B was 0.98±0.18mg/dl and that of Group C was 0.73±0.20 MDA was significantly higher ($p<0.0001$) in Group C compared to Group B. Vitamin C level was significantly lower ($p<0.001$) in Group C than in Group B.

TABLE NO: 02: serum levels of MDA and vitamin C in chronic smokers (Group B) and chronic smokers with MI (Group C).

PARAMETER	CHRONIC SMOKERS (GROUP B)	CHRONIC SMOKERS WITH AMI (GROUP C)	P- VALUE
MDA nmol/l	432±9.0	530±15.90	P<0.0001
Vit C mg/l	0.98±0.18	0.73±0.20	P<0.01

Discussion

The present study showed that there was a significant elevation of serum MDA ($p<0.0001$) and significant decrease in serum ascorbic acid (vit c) levels ($p<0.01$) in chronic smokers compared to non-smokers.

Cigarette smoking is probably the most addictive and dependence producing form of object-specific, self-administered gratification known to man. According to present estimates, tobacco is responsible for causing more than 5 million deaths every year (World Health Organization, 2008). About 19% of tobacco consumption in India is in the form of cigarettes, while 53% is smoked as bidis. Regular smoking doubles the chances of stroke in men.[13]

It has been estimated that 1016 radicals are present in one puff of cigarette smoke. Free radicals can oxidize lipid, protein and carbohydrate molecules, damaging cell membranes and DNA, thereby altering cellular structure and function. Cell membranes are rich sources of polyunsaturated fatty acids, which are more prone to be attacked by oxidizing radicals causing lipid peroxidation.[14]

Chole et al. reported association of lipid peroxidation with the habit of either chewing betel nut or betel leaf or tobacco or smoking in the control subjects.[15]

Solak et al. also reported that MDA levels in plasma also correlated with the daily exposure to cigarette smoke.[16]

In this study, we have also compared chronic smokers and chronic smokers with acute myocardial infarction. The serum MDA was significantly higher ($p<0.0001$) and serum ascorbic acid was significantly lower ($p<0.001$) in chronic smokers with AMI than in chronic smokers, even when both groups were matched for age, body weight, BMI and smoking habits. Although cigarette smoking is a well-established risk factor for vascular diseases, the genetic mechanisms that link cigarette smoking to an increased incidence of cardiovascular diseases are not well understood.

Several environmental factors are documented to influence redox metabolism, but relatively little is known about genetic effects. TAS, an indicator of redox homeostasis, is under strong genetic control, especially in smokers and this could lead to the difference in MDA and vitamin C levels in chronic smokers with MI than in chronic smokers without MI.[17]

Conclusion

In conclusion, the present findings indicated that cigarette smokers had higher lipid peroxidation levels and lower antioxidant capacity compared to non-smokers. Inter-subject variability of both elevated serum MDA and reduced serum vitamin C levels in chronic smokers with AMI may be influenced by gene-environmental factors.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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Quadrifurcation of Coeliac Trunk- A Case Report

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Abstract

During routine dissection of a cadaver in the department of anatomy at Seth GS Medical College and KEM Hospital, we discovered a very rare type of branching pattern of the Coeliac Trunk (CT). The CT measured 3cm in length and was quadrifurcating into replaced right hepatic artery, common hepatic artery, left gastric artery and splenic artery. The replaced right hepatic artery had unusual course where it ran behind the portal vein and bile duct to reach the right lobe of liver. Common hepatic artery after giving gastroduodenal and right gastric artery continued as middle hepatic artery while left hepatic artery originated from left gastric artery. A complete knowledge of these variations are helpful for hepatobiliary surgeons and also for radiologists.

Keywords: *Coeliac trunk, Quadrifurcation, Replaced right hepatic artery, Liver transplantation, cholecystectomy*

Introduction

Coeliac trunk is a short stump measuring about 1.25cm in length arising from abdominal aorta just below the aortic hiatus of diaphragm opposite the disc between T12 and L1 vertebra. It mainly supplies the foregut part of gastrointestinal tract and associated viscera like liver, spleen, gall bladder and pancreas. Coeliac trunk usually trifurcates into the left gastric, splenic and common hepatic arteries. The common hepatic artery after giving right gastric artery, divides into gastro duodenal artery and hepatic artery proper. The gastro duodenal artery ends by dividing into superior pancreatico duodenal artery and right gastroepiploic arteries.[1]The classical type of branching of coeliac trunk is known as trifurcation and was first described by Haller in 1756. This "TripusHalleri" is considered to be the normal branching pattern of coeliac trunk and the coeliac trunk other than its usual branches are referred to as collaterals.[2]

It is said that anatomical variations in the hepatic arterial supply are due to variant embryological development of the ventral arteries of the foregut and failure of these vessels to regress.[15]

A complete knowledge of these variations in branching pattern of CT is clinically important for in liver transplantation, cholecystectomy, hepatobiliary surgeons and also for radiologists. Variations in the celiac trunk may predispose to iatrogenic injury during surgical procedures of pancreaticoduodenectomy and hepatic artery infusion chemotherapy. Knowledge of these variations is also important in planning whole and split liver transplantation.

Also it may be useful in planning and executing radiological interventions such as celiacography and chemoembolisation of hepatic tumors.[16]

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Case Report

During dissection of embalmedadult male cadaver at Seth G.S. Medical College and K.E.M. Hospital, Mumbai, The CT measured was 3 cm in length and was quadrifucating into replaced right hepatic artery, common hepatic artery, left gastric artery and splenic

artery. The replaced right hepatic artery had unusual course where it ran behind the portal vein and bile duct to reach the right lobe of liver. Common hepatic artery after giving gastroduodenal and right gastric artery continued as middle hepatic artery while left hepatic artery originated from left gastric artery. While cystic artery originated from replaced right hepatic artery in the Calot's triangle.

This a very rare case where the length of coeliac trunk is longer than usual i.e. about 3 cm in length and it was quadrifurcating into replaced right hepatic artery, common hepatic artery, left gastric artery and splenic artery [figure]

The left gastric and splenic arteries were normal in course and distribution.

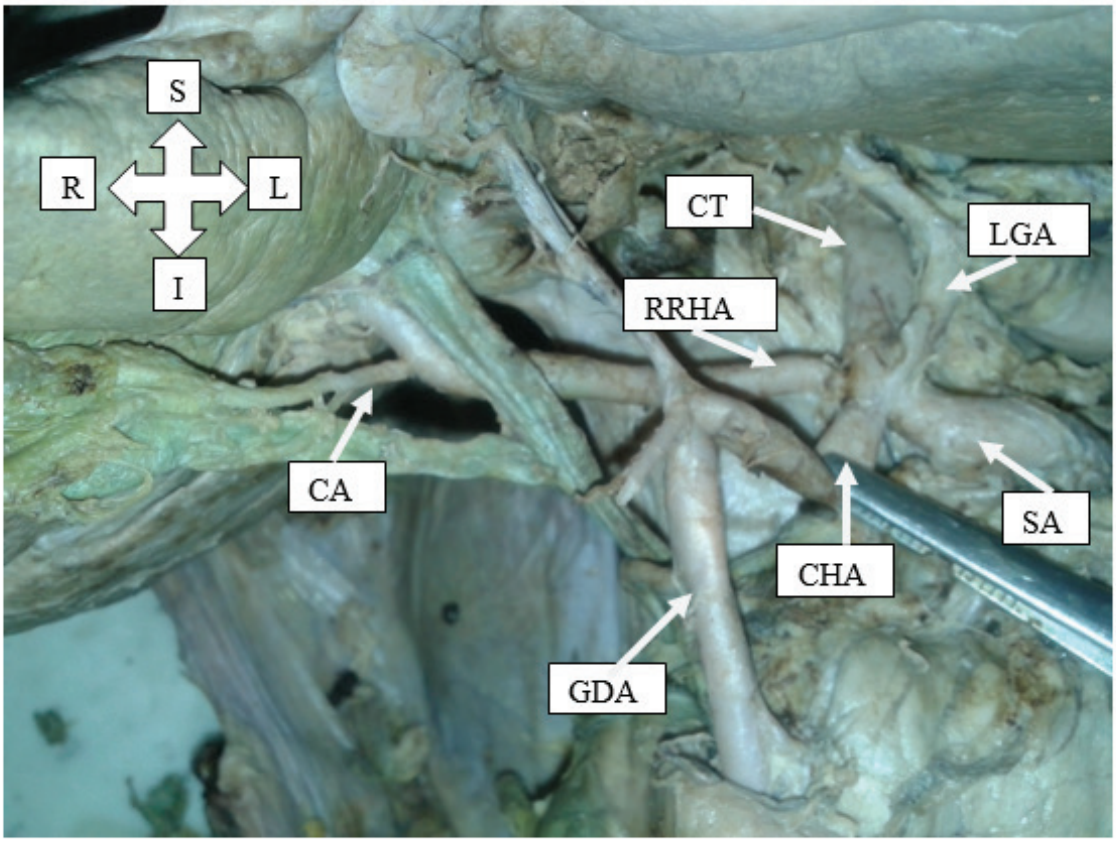

Figure : Quadrifurcation of CT (CT=Coeliac Trunk, RRHA=Replaced Right Hepatic Artery, CHA=CommonHepatic Artery, LGA=Left Gastric Artery, SA=Splenic Artery, CA= Cystic artery, GDA=Gastroduodenal artery)

Discussion

Anatomical variations in the branching pattern of the celiac trunk are of considerable importance. Previous studies have described many variations in the branching pattern of CT. Michels classified the branching of CT into 6 different types. Type-1: Usual branching (Trifurcation) Type-2: Hepatosplenic trunk, left gastric from aorta Type-3: Hepatoslpeenomesenteric trunk, left gastric from aorta Type-4: Hepatogastric trunk, splenic artery from superior mesenteric artery Type-5: Spleenogastric, common hepatic from superior mesenteric artery Type-6: coeliacomesenteric trunk,common hepatic, splenic, left gastric and superior mesenteric artery form a common trunk.The variation of celiac trunk found in this study is not classifiable in either system.[4]

Depending on the number of divisions, the branching pattern of coeliac trunk can be classified as bifurcation, classical and nonclassical trifurcation, quadrifurcation,

pentafurcation, hexafurcation.[2,5,6,8,9]

SateeshaNayak in 2012 reported quadrifurcation of the celiac trunk into common hepatic, left gastric, left hepatic and splenic arteries.[5]

Sathidevi reported quadrifurcation of CT into gastroduodenal artery, common hepatic artery, left gastic artery and splenic arteries.[6]

The present case confirms with the findings of SatishNayak and Sathidevi with reference to quadrifurcation, but the pattern of quadrifurcation differs as replaced right hepatic artery is present in place of left hepatic artery and gastroduodenal artery.

According to the study by SumanTiwari, the length of celiac trunk ranges from 1.3-1.8cm with a maximum length about 2.5 cm. According to the author the celiac trunk with longer length showed trifurcation – in 84%, bifurcation- in 2%, quadrifurcation- in 8% and pentafurcation- in 6%.[7]

In the present case CT measured 3 cm from its origin from aorta till its quadrifurcation.

Ronald Bergmann reported variation out of 165 specimens, about 0.8% the right hepatic artery arose directly from coeliac trunk where it coursed anterior to the bile duct in 64%, anterior to portal vein in 0.1% and posterior to portal vein in 9%.[10]

In the present study the course of right proper hepatic artery was observed behind the portal vein and bile duct.

Variation in origin and importance of accessory and replaced hepatic arteries are well documented by Michels NA, Niederhuberet al., Kemeny et al., Chain H et al., Hiatt et al., and Braun et al.[4,11,12,14,15,16]

Preoperative knowledge of interposition of hepatic artery variations can be important in guiding the surgical approach to the gallbladder, liver, pancreas, stomach, and portal system. A retro portal course of right hepatic proper is surgically significant during portocaval shunt. Vascular variations are usually asymptomatic. They may become important in patients undergoing coeliacography for gastrointestinal bleeding, celiac axis compression syndrome, prior to an operative procedure or transcatheter therapy; chemoembolization of pancreatic and liver tumors.[16] Careful identification and dissection of celiac trunk branches is therefore important to avoid iatrogenic injury.

Conclusion

Although various types of quadrifurcations are reported, this rare variation of quadrifurcation of CT into replaced right hepatic, common hepatic, left gastric and splenic arteries becomes utmost importance.

It is important for the surgeons to appreciate variant origin of the right hepatic artery during laparoscopic cholecystectomies, vascular radiological procedures, while carrying out a pancreaticoduodenectomy procedure or dissecting out the portahepatis during resection of the liver and during both right lobe liver living donor and split graft transplantation. Since it is an end artery, its presence must be recognized so as to prevent damage to the artery. This is essential to avoid excessive bleeding from the injured right hepatic artery during surgery and prevent postoperative complications.

Presence of arterial variations may result in erroneous interpretation of angiograms. Hence the knowledge of variations of CT is very useful and should be kept in mind to avoid intra operative and post operative complications.

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Physical and Psychological Health of Nursing Staff During the Pandemic: Effect of Stress

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Abstract

Stress is part of life and it affects the people in different ways. If stress is not managed properly it can lead to anger, frustration or depression. High stress levels can cause physical or psychological issues. It might cause headache, weight loss or weight gain, dizziness, teeth grinding, lack of sleep, laziness, body pain, anger, irritation, anxiety or depression. The nursing staff being part of a sector which falls under essential services was at work in hospitals during the pandemic COVID 19. They had to report to services in spite of the fact that they were equally exposed to the risk of getting the infection. In such situation the nursing staff was working under stress. This study aims to investigate the relationship between the stress and health of nursing staff employed in the COVID 19 ward.

Key Words: *Stress, Nursing, Physical issues, psychological issues*

Introduction

Every individual faces some situations in life which exposes them to stress. Stress can affect the people in different ways.[1,2] However not all the individuals can deal with stress easily and have to face the consequences. The people who are not able to manage their stress levels can have negative impact on their health.[3] Stress does not cause illness but it makes the body vulnerable to various diseases. The higher stress levels cause various health issues which are the symptoms of negative impact on health.[4] High stress levels can cause physical or psychological issues. It might cause headache, weight loss or weight gain, dizziness, teeth grinding, lack of sleep, laziness, body pain, anger, irritation, anxiety or depression.[5,6]

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The sudden outbreak of pandemic had led to increase in stress level of all the individuals and especially of the hear care professionals.[7] As the health care professionals doctors, nurses etc had to deal with the corona infected patients directly. The situation was very turbulent the infection rates were increasing erratically and the even the death rate was very high.[8,9] This situation exposed to the risk of getting infected by the novel Corona virus. At such a point the Nursing staffs employed in the COVID 19 ward were suspended to the risk of infection.[10] This increased the stress level among the nursing staff which started having a negative impact on their physical and psychological wellbeing. The fear of infection led to increased absenteeism also higher dissatisfaction, increased organization turnover.[11,12]

This Research focuses on the impact of higher stress levels on the physical and mental health of the nursing staff employed in the COVID ward. For the purpose of the study primary data was collected from 50 nurses employed in the COVID ward through a questionnaire with a variety of questions to examine their stress levels and health issues caused. The data so collected was further analysed and interpreted to reach the conclusion.

Objective of Study

1. To investigate the relationship between the stress and health of nursing staff employed in the COVID 19 ward.

2. To evaluate the impact of Stress on the health of nursing staff employed in the COVID 19 ward.

Data Collection:

Primary data was collected for the purpose of the study. The data was collected through a questionnaire form 50 nurses employed in the COVID 19 ward.

Technique of Data Analysis:

The primary data collected is analyzed using statistical tools and techniques. Analysis was made using MS-Excel and SPSS software.

Results

Table 1: Data Analysis and Interpretation:

	Mean	Standard Deviation
Health issues physical and psychological increase of the staff employed in the COVID 19 ward due because of the stress due to increased risk of infection	4.08	0.941
Health issues of the nursing staff increased whenever there is increase in stress levels	3.58	1.244
Health issues physical and psychological does not increase if the stress levels increased	2.29	1.425
More health issues both physical and psychological will be caused even if the nursing staff id exposed to higher level of stress	2.77	1.713
Average	3.18	1.33075

The analysis represents that the respondents agreed the objective with a mean range of 3.18. It shows that the Heath issues physical and psychological have increased due to the increased stress level of the nursing staff in the hospitals. The increased stress is because the nursing staff employed in the COVID ward is exposed to the risk of getting infected. It is also found that the stress levels increases it leads to increase in both physical and psychological health problems.

Table 2: Pearson Correlation

		Stress	Health Issues
Stress	Pearson Correlation	1	0.454**
	Sig. (tailed)		0.004
	N	35	35
Health issues	Pearson Correlation	0.454**	
	Sig. (tailed)	0.004	
	N	35	35

**Correlation is significant at the 0.01 level (2-tailed)

The above analysis shows the correlation between the stress levels and health of the nursing staff employed in the COVID 19 ward based on the primary data collected from the respondents, the correlation between the two variables is positive. However the significance of relationship between the two variables is lower than the significant level of 0.01.

Conclusion and Recommendation

The research concluded that the nurses employed in the COVID 19 ward were working under high stress levels. The stress was caused mainly due to the risk of exposure to the corona infection. The other factors were fear of infection to the family, strict hospital norms, household responsibilities during the lockdown, etc. Increased stress levels had led to a negative impact on the health of the nursing staff. Common health issues include frequent headaches, dizziness, sleeplessness, anxiety, anger, frustration, lack of appetite, body pain and weight loss. Thus the increased stress levels have impacted the health of the nursing staff. In order to avoid the health hazards the nursing staff must ensure a balance diet, adequate sleep, practicing regular exercise, meditation. The staff must ensure having positive attitude toward their health. The hospitals must provide utmost safety top the nursing staff in form of all the facilities to protect them from the risk of infection. The staff must be employed in the COVID ward on rotation so that the staff gets relaxation time. Hospitals must provide social security, Insurance, training, best quality PPE kits, masks and sanitizers and also incentives must be provided to keep the nursing staff motivated and positive during such difficult times.

Scope & Limitations:

The study suggests the impact of stress due to the pandemic on the physical and psychological health of the nursing staff. The study concentrates on the impact of only stress on the physical and psychological health of the nursing staff owing to the pandemic. The data is time bounded. The sample is restricted to the Nagpur city

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Vitamin D Levels and Its Correlation with Severity of Breast Cancer

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Abstract

Introduction: Vitamin D has wide range of actions in the body which include immunogenic actions, differentiation of immune cells and keratinocytes, expression of growth factors etc. Genes encoding for proteins that regulate cellular differentiation, cell proliferation, apoptosis and angiogenesis contain vitamin D response element.

Materials and Methods: A total of 135 newly diagnosed cases of breast cancer and equal number of controls were recruited in the study. The histo-pathological grading of cancer, Hormone-receptor status (estrogen receptor-ER, progesterone receptor PR and HER-2 receptor) was done by immunohistochemistry, stage of tumor (based on TNM Classification), details of metastasis were noted. Vitamin D levels were done by HPLC method.

Results: The mean age, age at menarche, BMI, calcium intake, sun exposure, Ser PTH levels, Ser Calcium levels were insignificantly different in both the groups. Ser Vitamin D levels were 30.95±17.65 ng/Ml in cases and 27.21±7.97 ng/Ml in controls, with a p value of 0.198 which is not significant. The levels of Vitamin D in ng/Ml in Stage I, Stage II, Stage III and Stage IV were as follows: 26.06±11.45, 29.61±17.23, 47.33±21.13, 42.67±28.92 and a p value of 0.190(NS). Thus there was no significant difference in Vitamin D levels of cases and controls. The association between the biomarker status and stages showed significant results as indicated by p-value of 0.043.

Conclusion: This study fails to find any inverse association of vitamin D with severity of breast cancer and receptor status.

Key Words*: Vitamin D, Breast Cancer, hypovitaminosis D, Receptor Status*

Introduction

Vitamin D receptors (VDRs) are found in many tissues other than kidneys, bones, and intestines like, skin, lymphocytes, cardiac muscle, breast tissue, and anterior pituitary gland.[1] In addition to its role in calcium metabolism, Vitamin D has wide range of actions in the body which include immunogenic actions, differentiation of immune cells and keratinocytes, expression of growth factors etc. Genes encoding for proteins that regulate cellular differentiation, cell proliferation, apoptosis and angiogenesis contain vitamin D response elements.[2,3] The factors associated with breast cancer are genetic mutation, reproductive factors, family history, breast

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density, increasing age and nutritional risk factors. Retrospective and prospective epidemiologic studies have revealed that vitamin D deficiency is associated with an increased risk of initiation, development, prognosis, progression and dying of breast cancer. Several recent reports have found vitamin D intake is beneficial not only for cancer prevention but also for women recently diagnosed with breast cancer. In contrast many studies including randomized controlled trials have failed to establish beneficial effects of Vitamin D in breast cancer.[4] Laboratory studies have demonstrated that vitamin D3 and its analogs inhibit cell proliferation and promote apoptosis in cancer cells in culture.[5,6] The major circulating form of vitamin D is 25-hydroxyvitamin D [25(OH) D] formed after the first hydroxylation of vitamin D3 or vitamin D2 in the liver. In the kidneys, 25(OH) D is converted to the active metabolite 1, 25(OH) D after second hydroxylation. 1, 25(OH) D has a short half life and tight homeostatic control. Once 1, 25(OH) 2 D completes the task of maintaining normal cellular proliferation, inhibition of angiogenesis, differentiation and apoptosis, it induces expression of the enzyme 24-hydroxylase (24-OHase) which enhances the catabolism of 1, 25(OH) 2D. This action of Vitamin D3 is also seen in breast tissue in vitro, where Vitamin D3 is shown to reduce cell proliferation, induce apoptosis and also promotes cell differentiation.[7-9]

Methods

This is a case-control study which was approved by the Institutional Ethics Committee. A total of 135 cases and an equal number of healthy age and sex matched controls were included in the study.

Statistical Analysis

All the analysis were performed using SPSS version 20.0 (IBM Corp.) and significance was evaluated at 5% level.

Results

Table 1 provides the descriptive statistics for various characteristics in cases and controls. The difference in mean age in two groups was statistically insignificant, as indicated by p-value of 0.15. Further, other characteristics like BMI were comparable between case and control groups as revealed by p-values > 0.05. As regards vitamin D, although the mean in cases (30.95 ± 17.65) was higher than that of control group (27.21 ± 7.97), the difference was statistically insignificant (p=0.198). The number of pre-menopausal cases is 90 and post-menopausal cases is 45. In control group premenopausal women are 99 and postmenopausal 36 in number. Similarly Ser PTH and Calcium levels were also comparable in both cases and controls.

Table 1: Descriptive statistics for cases and control group patients

Characteristics	Cases (N=135)	Controls (N=135)	P Value
Age in completed years	49.16±11.37	46.31±6.54	0.150(NS)
BMI in Kg/m2	21.79±3.56	28.21±30.08	0.162(NS)
Age at Menarche in years	12.8± 2.3	12.5±1.8	0.174(NS)
Calcium Intake in mg/day	760±265	780±236	0.231(NS)
Vitamin D Intake in mcg	2.3±1.1	2.2±1.3	0.182(NS)
H/O Smoking	Nil	Nil	-
Family h/o Breast Cancer	13%	0%	0.000
Use of Oral Contraceptives	21%	20%	0.44(NS)
Use of HRT post-menopausal state	0	0	-
Number of Children	2.1±0.9	2.0±1.3	0.53(NS)
Ser 25(OH)D3 ng/Ml	30.95±17.65	27.21±7.97	0.198(NS)
Ser Calcium mmol/L	2.24±0.58	2.36±0.76	0.137(NS)
Ser PTH pg/ml	28.5±11.6	27.96±12.54	0.179 (NS)

*Obtained using *independent samples t-test*; NS: Not significant

Table 2 shows the distribution of Vitamin D levels in both cases and controls.

Table 2: Distribution of Vitamin D levels across cases and Controls

	Vitamin D Levels, Deficiency, Insufficiency, Sufficiency and Toxicity			
	Deficiency Vitamin D3<10	**Insufficiency Vitamin D3 11-29**	**Sufficiency Vitamin D3 between 30-150**	**Toxicity Vitamin D3 > 150**
Cases	21(15.55%)	45(33.33%)	69(51.11%)	0
Controls	18(13.33%)	75(55.55%)	42(31.11%)	0

Table 3 shows comparison of biochemical parameters across patients with different stages of cancer. In the case group, the patients were sub-grouped based on the stages of cancer and the biochemical parameters were compared statistically across these stages. Vitamin D showed statistically insignificant difference across the stages as indicated by p-value > 0.05. The reason for higher vitamin D mean in case group was mainly due to stage 3 and 4 with levels higher than that of stage 1 and 2. However, the sample number of cases in 3 and 4 groups was much smaller, and hence insignificance was observed across comparison.

Table 3: Comparison of biochemical parameters across patients with different stages of cancer

Parameters	**Stage I N=27**	**Stage II N=90**	**Stage III N=9**	**Stage IV N=9**	**P Value***
Vitamin D3 in ng/Ml	26.06±11.45	29.61±17.23	47.33±21.13	42.67±28.92	0.190(NS)

*Obtained using *ANOVA*; NS: Not significant

In conclusion, no relationship was observed between vitamin D levels and stage of cancer based on this sample study. The association of receptor status and stage of cancer was also observed on the studied sample. The results are shown in Table No 4. For HER2, out of 135 evaluations, 61.48% showed negative while 38.51% had positive result. The association between test result and stages of cancer was statistically insignificant with p-value of 0.585. For PR, in stage II, the proportion of positives was 85.5%,which mainly contributed to the significant association of PR outcome with stage of cancer(p=0.035). Further, for ER, 72.22%were positive. The association between the biomarker status and stages showed significant results as indicated by p-value of 0.043.

Table 4: Association of biomarker outcomes with stages of cancer

Biomarker	Stage1(n=27)	Stage2(n=90)	Stage 3 (n=9)	Stage4(n=9)	P-value*
HER2					
Negative (17)	4(14.81)	69 (76.66)	3 (33.33)	7(77.77)	0.585(NS)
Positive(8)	23(85.18)	21 (23.33)	6 (66.66)	2(22.22)	
PR					
Negative (5)	9(33.33)	13 (14.44)	8(88.88)	8(88.88)	0.035(S)
Positive (20)	18 (66.66)	77 (85.55)	1(11.11)	1(11.11)	
ER					
ER Negative (n=7)	0	25(27.77)	8 (88.88)	9(100)	0.043 (S)
Positive (n=18)	27 (100)	65 (72.22)	1(11.11)	0	

*Obtained using Chi-Square test; S: Significant; NS: Not Significant

*Figures in bracket indicate percentage

Discussion

The main aim of our study was to evaluate the co-relationship of breast cancer, its severity and biomarker status with the levels of vitamin D. The study fails to find any correlation between vitamin D deficiency, sufficiency and breast cancer severity and marker status. The difference in Vitamin D levels in cases and controls is insignificant. Also the levels were insignificantly different in stage I, stage II, stage III and stage IV. The main reason for this insignificance could be the small number of cases in Stage III and Stage IV. The receptor status of HER-2, Estrogen receptor ER and progesterone receptor PR were also assessed in this study and there was no difference in the levels of Vitamin D based on positive or negative status of either of the receptors. In this study we have taken into account the calcium intake, exposure to sun and PTH levels which are known confounders for the vitamin D levels and we have adjusted for these known confounders in our analysis. We have also excluded patients and controls who were or had been on calcium and Vitamin D supplements.

Two clinical trials of vitamin D and breast cancer incidence have been completed. In the Women's Health Initiative, a total of 36,282 women were randomly assigned to receive either 1000 mg calcium plus 400 IU vitamin D or placebo and were followed for an average of 7y. No effect of the intervention was observed for breast cancer incidence.[11] Similar inconsistent results were reported by Lowe et al.[3] Our study resembles these findings, though VDR and 1 alpha hydroxylase were not done in our study. Since the above mentioned follow up studies and interventional studies don't prove any significant difference in vitamin D levels and any beneficial effects of calcium and vitamin D supplementation, the role of vitamin D deficiency in cancer needs to be re-explored. Similar findings echoed by another study which aimed at studying the levels of vitamin D in breast cancer survivors post treatment and breast cancer specific mortality in multivariate analysis during a follow up period of 9 years.[12] This study design and end points were different from our study as we had enrolled newly diagnosed cases and ours was a cross-sectional study. Though the message conveyed by both studies is coherent. Many studies have reported Vitamin D deficiency as one of the risk factors for breast cancer and also inverse relationship of Vitamin D levels with the severity of breast cancer. These findings contradict our observations. A few of these studies have a very large sample size,5in which higher levels of Vitamin D3 were associated with lower risk of breast cancer specially in younger age group. In another trial by Lappe et al[13] women who were supplemented with1400–1500

mg calcium plus 1100 IU vitamin D had a significantly reduced risk of overall cancer incidence, with lower rates of breast cancer in the calcium- and vitamin D–supplemented group than in the placebo group. A few Asian studies from Pakistan and Korea have similar observations with low levels of Vitamin D in breast cancer patients, inverse relationship with severity of cancer and poor outcome with low vitamin D levels.[9,14,15] All these findings are contradictory to ours. It becomes all the more important to undertake multicentric project to define the role of Vitamin D in breast cancer specially when the trials from other Asian countries are reporting increased cancer risk with hypovitaminosis D. Some data available from India indicated that VDR poly-A polymorphism is significantly associated with BC risk in north Indians especially with early onset disease.[16] Analysis on VDR gene polymorphisms in breast cancer in Indian population has revealed that some VDR gene polymorphisms (Taq 1 genotypes) are associated with BC risk.[17] Asians have low exposure to sunlight, there is high prevalence of various malabsorption syndromes, hyper pigmentation and rare vitamin D supplementation. The different studies from India have reported a very high prevalence rate of Vitamin D deficiency and insufficiency ranging between 70-90%.[18]

Conclusion

This study has a main constraint of small sample size and thus fails to establish any cause effect relationship between Vitamin D levels and Breast Cancer though it may form a basis for designing a larger study to see the association of Vitamin D levels with severity of breast cancer and also with the marker status (ER, PR and HER-2) of breast cancer. Thus the role of vitamin D and its levels in severity, recurrence and prognosis is far from being definitive. In conclusion this study fails to find any inverse association of vitamin D with severity of breast cancer and receptor status.

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A Review Article on Ahar Vihar According to Ritus in Ayurveda

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Abstract

In general there are mainly three seasons: winter, summer, rainy season. According to Ayurveda, six seasons are considered: Vasant, Grishma, Varsha, Sharad, Hemant and Shishir. Acharya Sushrut has divided the 12 months of the year into these seasons. There are two parts of the year in which the sun moves towards north in the first part and in the second part, the sun moves towards the south in Visarga. These are termed as adanakala and visargakala. There are winter, spring and summer seasons in the input period and varsha and Hemant seasons in the Visarga period. At the time of exchange, the Sun remains strong and the moon weak. The peak season is strong, spring is medium and summer is beautiful. During the Visarga period, the Moon is strong and the Sun is weak. Lunar is nutritious. The rainy season is of great strength, the autumn season is of medium strength and the spring season is of great strength.

Keywords: *Ritu, Ahar, Vihar, Pathya, Apathya*

Introduction

Ayurveda is the ancient life science in this period of life on earth. In Ayurveda there are many ways to cure disorders by following certain regimen. As Ayurveda has mentioned in the text that it has two main aims that are "Swasthya Swathya Rakshana and Aaturasya Vikara Prashman" that means Ayurveda has said that maintain the longevity of a person and provide shelter from the disease[1,2]. Thus by following these proper regimen, it is possible to avoid disorders which further leads to a healthy lifestyle as well. So according to ayurveda, the proper diet and regimen according to various ritus can be elaborated here[3].

Vasant Ritu

Winter and summer season is spring season. At this time there is neither too much winter nor too much heat. In this season, the air filled with the aroma of mango mangoes blossoms everywhere. Spring season is also called Rituraj. On the auspicious festival of Vasant Panchami, nature starts to dress by wearing mustard yellow flowers. In Vasant Ritu, blood circulation becomes intense due to which the body is energized[4,5].

Vasant Ritu has neither the heat of the heat nor the flood of rain, nor the cold wind of the winter, snow and fog. For these reasons Vasant Ritu has been called 'Rituraja'. According to Charaka Samhita, Kapha, which is collected in Hemant Ritu, is fueled by the rays of the Sun in Vasant Ritu (fluidized) due to which the spring is in the morning, cold, swelling in tonsils, sore throat, lethargy and heaviness in the body etc. persists[6,7].

Diet in the Varsha Ritu:

In the last days of the rainy season and before the onset of Sharadrutu, it starts to get strong sunshine and the accumulated bile starts to fade. Therefore, one should not consume bile acids in these days.

In these days water causes many diseases due to getting dirty and containing bacteria. Therefore, in this season one should boil and drink water or rotate alum piece in water which will reduce the dirt.

Vihara: In these days to avoid malaria etc. diseases put a net and sleep. Take care of the cleanliness of the body to avoid skin diseases. Avoid Consumption of impure and contaminated water which causes skin diseases like jaundice, cholera, diarrhea etc.

Sleeping during the day, bathing in rivers and getting more wet in rain is harmful.

In the rainy season, a small amount of churna and a pinch of rock salt should be consumed with fresh water[8,9].

Sharad ritu:

From September to November in the view of the whole of India, sharad or autumn can be considered. Autumn comes after the rainy season. Naturally accumulated pitta in the rainy season - the outbreak of dosha increases in the autumn. Due to this, the digestive nature of bile gets removed during this season and it becomes ingenious. This may results in fever, dysentery, vomiting, diarrhea, malaria etc. In all the seasons in Ayurveda, autumn is called 'Mother of diseases'. In this season, Pitta-dosha and grow naturally[10,11]. The heat of the sun also seems particularly strong. Therefore, Pitta - Dosha, and heat should be used to mitigate these three. Such as, Tikta and Kashay rasas should be used specially. Increase of Pitta dosha, such sour and pungent items should be discarded. For the relief of the outbreak of Pitta, take special items of sweet, cold, heavy, bitter and astringent. Vegetables are plenty in this season, but due to the new water in the rainy season, they are defective.They contain a lot of salt (saline) juice. So, as far as possible, take less vegetables in the autumn and consider them in the month of Bhad (Bhadrapada)[12]. Ghee - Milk is the antidote to Pitta-dosha, so our ancestors must have organized Shraddha Paksha in the Bhads. Wheat, barley, jowar, paddy, sama (a type of grain) should be taken in grains during this season! Take gram, tur, mung, mutt, lentils, peas in pulses. Vegetables can include cabbage, kakoda, (khekhasa), carrot, maize corn, turai, chaulai, gourd, spinach, pumpkin, spring beans, potatoes, etc. In fruits, ripe bananas, jamfal (Bihi), berries, watermelon, pomegranate, grapes, coconut, ripe papaya, moussmi, lemon, sugarcane etc. can be taken. Walnuts, potato bukhara, cashew nuts, dates, charoli, almonds, water chestnuts, pistachios etc. can be taken in dry fruits! Cumin, amla, coriander, turmeric, poppy seeds, cinnamon, black pepper, fennel etc. can be taken in spices! Apart from this, coconut oil, castor oil, ghee,milk, butter, sugar candy, rice etc. are good to take. Eating kheer, rabri etc. in the autumn is beneficial for health. Eating ghee and cardamom in ripe banana is beneficial Sugarcane juice and coconut water are very beneficial[13].

In the Autumn, curd, sour buttermilk, salt-sour rasas are notable. Especially do not take sour buttermilk, ladyfinger and cucumber. Use of ghee in place of oil is best in this season. Those who have bile disorder should use bitter things like Mahasudarshan churna, neem etc. By taking laxatives in this season, the bile-defect is removed from the body and thus protects from biliary disorders Applying camphor and sandal on the body during this season, sitting in the moonlight in the open, wandering, is beneficial. The sleep of the day, the intake of sunny snow, the extreme obstruction, and the wind coming from the east are harmful in this season In the autumn, it is beneficial to play such a game of sweating at night by performing Havan, performing Deepmala purifies the atmosphere.

Season of Hemant and Shishir

Their properties can be taken advantage of due to the coming of both the winter season and the immersion period, because the nutritive power of the Visargakaal period supports us in the hemant season. At the same time the exchange starts in the peak season, but the rays of the sun are not so intense that we can exploit us by drying the ras, but the beginning of the interchange makes the sun's light and primal rays look pleasant.

Man gets natural strength naturally during winters. Due to the coldness of the body of naturally formed humans due to cold, the pores of the body get protected in the stomach, and due to this, it becomes more powerful. This strong gastrointestinal cold causes more air to flare up. If this jatharagni suffers less dietary fuel then it burns the dhatus of the body. Therefore, one should drink pure, sour and sweet foods including season. To make the body strong in this season, nutritious, powerful and beneficial dishes should be consumed[14].

In this season, delicious and nutritious dishes made from wheat, oil, wheat, urad, milk, dry ginger, peppers, gooseberry, etc. should be eaten. If the diet is not taken according to the Jatharangni in this season, there is a possibility of outbreak of diseases. Those who are not financially economical, should eat soaked desi gram in the morning as a snack and chew it. Those who do more physical work, it is highly beneficial to eat banana, til,

jaggery, coconut, dates etc.

One thing is like keeping in mind that nights are long and cold in this season. Therefore, only in this ritual, Ayurveda texts have asked to have early breakfast in the morning such as guru ahar etc.

For the people who have become weak due to consumption of more laghu ahar, various drug such as Vardhman Pippali, Bhallatak , Shilajit , Triphala, Chitra can be used by consulting vaidyas. Those who are suffering from constipation should eat empty stomach Hara and jaggery or Yashtimadhu and Triphala in the morning etc[15].

Vihara: It is necessary to be careful with the diet as well as in the Vihara and the living! In this season, one should massage oil to make the body stronger. Use of gram flour, Lodhra or Amla boiled is beneficial. Exercising means punishing- sitting, kusti, running, swimming, etc. and should practice pranayam and yogasanas, suryanamaskar, sun bath and sun exposure are beneficial in this season. Apply agar on the body. Due to normal hot water bath but do not put hot water on the head. No matter how cold it is, you should wake up early in the morning and take a bath. The excessive heat generated in our body by sleeping at night goes out by taking a bath, which gives the body an enthusiasm. Sleeping till late in the morning causes the loss of increased body heat, which affects the head, eyes, stomach, gall bladder, bladder, rectum, etc. organs, causing different types of diseases. In this way, by taking a bath early in the morning, these can be kept healthy by protecting them from diseases. Wearing warm clothes in sufficient quantity, wearing warm blankets at night to avoid excessive cold, using quilts, etc., sleeping in hot rooms and warming bonfire is beneficial Weakness: Extreme cold in this season, cold water, cold air, earthquake, fasting, dryness, bitter, astringent, cold and stale substances, daytime sleepiness, keeping the mind from work, anger etc occur.

Shishira Ritu: Indications: Massage with oil. Ubvartan with fine paste/powder of kumkum (kesar). Exercise (vyama). Clothing-leather, silk and wool. Exposure to sunlight and fire to keep yourself warm.

Grishma ritu: In this season, Sunrays become powerful. Kapha decreases vata increases day by day

Indications: Anoint body with chandan paste and take bath with cold water. Stay in cool places. Wear light dresses.

Varsha ritu: The agni (digestive activity) weakens further and gets vitiated by doshas.Hence all methods to mitigate doshas and measures to enhance digestive activity can be adopted.

Indications: Panchkarma can be done. Perfumes can be used. Avoid sleeping at daytime, exposure to sunlight.

Sharad ritu: Sudden exposed to sunlight after cold season aggravates pita.

Indications: Udvartan with chandan. Bath with warm water. Pearls give soothing effect from aggravated pita.

Conclusion

Today`s society is so alienated from Nature. Who knows what day of the cycle the nature is in. To really heal one must return to balance and know our place in the Universe. One way to describe the daily cycle is in terms of the doshas, Vata, Pitta, and Kapha. Vata is dominant from 2 to 6 in the morning and afternoon. Kapha is dominant from 6 to 10 in the morning and evening, and Pitta is dominant during mid day and midnight. Keep in touch with these energies during the day and move with them, not against them. In ayurveda hygiene, ahar and vihar are crucial to good health. To attain good health, Ayurveda prescribes the specific daily routine dinacharya and the seasonal regime ritucharya. Before going any further, it is important to make clear what considers to be health. For healthy life, the lifestyle should also be considered along with the diet. We can control the diet to a considerable extent. But we cannot control much of the lifestyle. It does depend on others, but whatever lifestyle is under our control, if we try to control it properly, we can become more and healthier. Ayurveda has given a deep thought to the daily routine also. Maintaining good health and treatment of disease are two sides of the same coin. The food, drugs and regimen that prescribe for both are similar. The components are the same, they are all found in nature. Equal importance is given to the kind of food to be taken as to the drugs prescribed. Each reinforces the other and both act in similar ways to maintain the equilibrium of the doshas in the body which is the ultimate key to a sound health.

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A Critical Review on Area of Research in Standardization of Panchakarma Procedures

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Abstract

Background: *Panchakarma* procedures belonging to a class of cleansing procedures called *shodana* therapy (purification) in *Ayurveda*. The unusual fact *Panchakarma* now convening is, the same *Panchakarma* procedure is giving different percentage of efficacy rate in different regions, in patients with the same disease and base-line parameters. To ensure uniformity of the procedures and achieving maximum efficacy, standardization of these *Panchakarma* procedures became an emergency necessity.

Aim: The present article focus at exploring the area of research in standardization of *Panchakarma* procedures and to enhance and make sure standardized *Panchakarma* procedures should be followed everywhere thereby certifying maximum possible safety and uniform efficacy.

Review Results: The methodology for Standardization of *Panchakarma* procedures has to be implemented in an emergency basis to ensure complete safety and maximum efficacy.

Discussion: The methodology for Standardization should be prepared by absorbing the concept of *Panchakarma* on scientific lines and should be well ordered, practical and cost-effective.

Conclusion: The present article outlines the concept behind *Panchakarma* procedures, the methodology for Standardization and area of research in it.

Keywords: *Panchakarma, Standardization, Research*

Introduction

The nature has taught the man how to be healthy before science has discovered the law of health[1]. In Ayurveda *Panchakarma* procedure is practised for both healthy and diseased person. *Panchakarma* therapy is called as purificatory therapy in *Ayurveda* because of its detoxification action. *Panchakarma*, a comprehensive, and an essential part of *Ayurvedic* treatment and having its role in each therapeutic condition. Due to its long lasting and absolute relief of chronic diseases, it is now evolving globally. In other way we can sense, *Panchakarma* is nothing other than the peculiar trans-cellular bio-purificatory mechanism ever evolved[2].

Concept regarding evolution of *Panchakarma* therapy:

In *Ayurveda* textbook it is explained that our body is made up of *sthula* and *sukshma srothas*[2] (macro and micro cellular channels) through which body elements are as well as ejection of metabolic waste materials are going on. Due to various reasons including improper food habits, sedentary life- style, attack of germs etc, these cellular channels get slowly and slowly assemble with "*aama"* [3] or accumulated cellular toxins. When blockages of these srothas are occurring, *Ayurveda* calls it as a beginning of pathological procedure called as "*aavarana*" or blockage. This *aavarana*, according to *Ayurveda*, is causing not only hiddenness to free

movement of *vaata dosha* or free transport of body elements and metabolic wastes, but also, make the toxic elements or vitiated *dosha* which will get deposited in prone areas in body. As per the site of its *sthanasamsraya* (deposition), the disease appears as *amavata, vatarakta*, (rheumatoid problems), *kushta* (skin problems) etc[4]. So, the very first step required while preparing a *panchakarma* protocol is eradicating these cellular toxins by pooling them into digestive tract.

1. Standardization of Idea behind formulating of a Panchakarma protocol:

The occulted cellular toxins (*aama*) have the peculiar property of *pichilatwa* (stickiness). So, the initial step needed is to bring it into the digestive tract is nothing other than causing its liquefaction and therefore increasing its volume (*abhishyandana*)[5] which is achieved by a *poorvakarma* procedure called "*snehapana*" in which medicated/non-medicated, ghee/oil/ fat is given orally in a properly increasing dose under controlled conditions for maximum up to 7-9 days or up to the development of *samyak snigdha lakshana* (symptoms of proper oleation)[6]. After causing liquefaction of cellular toxins through *snehapana* procedure, next step is *swedana* (sudation)[7] through which these liquifacted toxins will be assisted towards alimentary canal. When these cellular toxins reach digestive tract, it can be removed out as per the adjacency by *Vamana* (vomiting) therapy or *Virechana* (purgation) therapy. If the patient's age or any other parameters is contraindicated for *snehapaana* or *swedana* procedures, alternative procedures like *abhyanga* (external oleation), *pinda sweda* (pottali sudation) etc can be selected. Once *poorvakarma* procedures are done, then according to the adjacency and site of *dosha, vamana* procedure (emesis) or *virechana* procedure (purgation) can be opted. Usually, enema (*Basti)* procedure is doing after the initial level of detoxification or can be done previously for some specific medical indications and even during emergency conditions. When *doshas* are localized on head and neck area, *nasya* therapy (nasal administration of medicine) is recommended[8]. This Idea behind the developing of a protocol for *Panchakarma* procedure should be standardized.

2. Standardization of *poorva karma* procedures:

(I) Standardization of medicine required for *poorva karma* (oil/ghee/*pottali*):

The principle methodology behind the selection of which type of *snehana* and *swedana* procedure needed to be administered for a patient can be developed by following the steps of *dasavidha pareeksha* (ten levels of examination)[9].

(ii) Standardization of methodology of preparation:

When prepration of *pinda sweda* is concerned, its ingredients, quantity of each ingredient required for *pottali sweda* and preparation method should be standardized. As per patient's health factor, quantity of each ingredient can be scientifically altered. For example, when the patient has more *kapha dosha dushti* symptoms like Swelling and feeling of heaviness, quantity of coconut scrapings in *Patra Pinda Pottali Sweda* can be reduced and quantity of lemon which have anti-inflammatory property can be increased. The standardization should also be done for order of steps for the preparation of *potali sweda*.

(iii) Standardization of administration of procedure:

The methodology in which a procedure is carry out should be standardized and authenticate or in other words, authentification of Standard Operative Procedure (SOP) should be finished for *snehapaana, abhyanga, pinda sweda, pottali sweda* or any *poorvakarma* procedure.

(iv)Standardization of *samyak lakshana* (symptomatologies) and authentification of tool to assess the procedure:

Development of pro forma can be done under the following steps:

a. Collection of symptomatologies:

It should be selected particularly from all the available *Ayurveda* classical literature.

b. Item Scrutiny:

This include cutting of *lakshanas* (symptoms) which have similar meanings. Each and every collected *lakshanas* (symptoms) should be examined by its root word, meaning, implication etc by thorough analysis and which symptoms having same meaning should be cut out and only one *lakshanas* (symptoms) should be selected.

c. Review of Literature:

Recent available research, review works and monograph should be systematically analyzed to search for any other additional *lakshanas* (symptoms) observed during the performance of the procedure.

d. Clinical observation:

With this developed pro forma, the researcher has to go to the *panchakarma* theatre for inspection of these *lakshanas* (symptoms) in patients.

e. Conclusion:

After clinical inspection of *lakshanas*, concluded pro forma should be made. This concluded pro forma should be tested to various stages of validation like face validity, content validity by subject experts, construct validity and criterion validity which involve the comparison of developed pro forma with existing gold standard.

Standardization of *Pradhaana karma*:

***Vamana* (Emesis) therapy**:

Vamana therapy is exactly medically promoted vomiting by intake of prescribed drug which causes the activation of Chemoreceptor Trigger Zone (CTZ), solitary tract nucleus and finally the vomiting centre medulla oblongata leads to activate phrenic nerve, vagus nerve and intercostals nerves to activate vomiting[10]. The medicine which are used, collected and processed for *vamana*, its dosage, *anupana* should be standardized along with the standardized achievement of *vamana* protocol and patient regimen.

***Virechana* (Purgation) therapy:**

Regarding *virechana* therapy is concerned, prior diet, level of *virechana* needed in the peculiar condition of disease, procedure of *virechana* as a whole, tool to asses *samyak virechana lakshana* in a particular disease, time of intake of *virechana* medicine etc need standardization. Level of *virechana karmukta* should be selected, whether it is having *anulomana* or *sramsana* or a *bhedana karma*, it depends on various factors including strength of *dosha* vitiation of *dosha, rogi bala* etc. For example, in *pakshaghatha* (hemiplegia) disease, only *anulomana*[11] is advised whereas in *kushta,* due to bahudoshavastha(huge vitiation of *dosha*), *bhedana virechana* is indicated.

***Vasti* (Enema) therapy:**

Vasti by its action on *moolasthana* get control on *vata* all over body[12]. *Vasti* therapy consists of both *niruha vasti* (enema with medicated *kwatha*) and *anuvasana* (enema with medicated oil). It can be performed as specific designed packages *like kala vasti, yoga vasti, karma vasti* or can be done alone in specific conditions like *amavata (Vaitarna Vasti), anaha (Vaiswanara churna vasti)*. The selection for designing to perform into packages or doing it alone needs standardization. Standardization of *vasti* therapy also includes standardization of *peedana kala* (pressing time of vasti bag), standardization for *mixing* of *vasti dravya,* positioning of patient, diet taken during *vasti,* time of administration of *vasti* etc.

***Nasya* (Errhinne) Therapy:**

Nasya (Errhine therapy) is said to be effective in curing diseases of *Urdhvajatru* (supraclavicular region)[13]. Many Modern research works enlightens the existence of naso-brain pathway which is the fastest drug delivery route. To ensure rapid drug absorption directly to the brain, drug has been considered to be absorbed through arachnoid matter sleeve which extends along olfactory nerve[14]. For that standardization of *nasya* therapy has to be observed including for factors like position of patient head while performing *nasya karma*, standardization of *hastaswedana* procedure which may cause facial efferent stimulation, standardization of doze and also *bindu pramana*[15] as mentioned in *ayuvedic classics* w.s.r.to each *nasya dravya*, standardization of time, tool implemented for *samyak nasya lakshana* etc.

Standardization of diet taking during *Panchakarma* treatment protocol:

Habit of food taking during *panchakarma* therapy course cannot be authetified fully because of the cultural sensitivity of the patients of various regions where *Panchakarma* is practised. Even though we cannot standardize food habits, the idea behind diet used during various stages should be standardized. Scientifically arranged diet protocol implemented after *panchakarma* procedure aiming at the rejuvenation of elements in body which were medically-altered during procedure is mentioned as *samsarjana karma*[16]. The principle idea of *samsarjana karma* for *panchakarma procedure*, which starts with carbohydrates followed by proteins and ends with fats should be standardized and implemented. The dietary food items prescribed while

doing *snehapana, swedana, vasti* etc should also be standardized on scientific basis. Efficacy of *vamana* and *virechana* procedure mainly depends on the dietary food items consumed on previous day which is mentioned as *utklesana* diet planned for liquefaction of *kapha* and *pitta dosha* respectively also need standardization[17].

Standardization of Panchakarma theatre Room:

Panchakarma procedure room should be set and standardized as per NABH criteria which involves proper electricity and proper ventilation, hygienic, with separate hand washing area, separate washrooms in every procedure room, storage area, recovery room, hot water supply etc with accurate instruments. The room should be also free from vectors, bacteria and infections.

Standardization of investigations required during panchakarma therapy:

Investigations advised during *pancakarma* therapy are to make sure the safety of the therapy and to make scientific observation on parameters. Specific Vitals should be examined before and after every therapy. The particular investigations vary according to the procedure, for example, *rakta moksha* or bloodletting procedure should not be advised in case of anaemia, abnormal bleeding and clotting times. Portal hypertension and oesophageal varix should be examined before *vamana* procedure. In bleeding haemorrhoids, *vasti* cannot be done. Hypotensive patients cannot be advised to do purgation procedure. The investigation list needed to be examined prior and after to each *panchakarma* therapy should be analyzed scientifically and validated.

Discussion:

Standardization methodology should be planned only on scientific factors without abstracting essence of *panchakarma* therapy. Standardization methodology should include the idea behind formulating of a *panchakarma* protocol, standardization of dietary habits, investigations, examination, standardization of *panchakarma* theatre room and formulating of standard operative procedure protocol for every procedure which will cover standardization of medicine used standardized involvement of the procedure and standardized patient regimen protocols.

Conclusion

It is right now very high time to carry out urgent researches on standardization of *panchakarma* therapy to make sure its absolute safety with uniform and maximum viable efficacy and thereby expurgate *Ayurvedic* medical sciences.

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A Therapeutic Action of Vatari Guggulu in Amavata, Sandhigata Vata & Grudhrasi

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Abstract

Drug (Ausadha) is a part of quadruped of the treatment, which has been placed next to the physician. The comprehensive knowledge of the drug is very important to physician because without knowledge of drug patient cannot be treated properly. Vatari guggulu is such herbal remedy mentioned in the ancient ayurvedic texts of Bhaishajya Ratnavali Amavata chikitsa prakarana for the treatment of various diseases like Samavata, Katishool (Low back ache), Grudhrasi (Sciatica), Khanja, Pangu, Vatarakta (Gout) ,Shotha, Daha (Relives burning sensation) and Koshtukshirsha. It having Vatashamaka, Kaphashamaka, Aamapachana, dipana, Vedanasthapana and Rasayana properties. Various study is carried out to evaluate the efficacy of drug Vatari Guggulu in diseases like Amavata,Sandhigata Vata and Grudhrasi and significant results were obtained.The present review article aim to evaluate the Theurapeutic action of Vatari Guggulu in aamavata,Sandhigata Vata,Grudhrasi.

Key Words: *Vatari Guggulu, Aamavata, Grudhrasi, Sandhigata Vata, Ayurveda, Theurapeutic action*

Introduction

Ayurveda is ancient system of life. It is an eternal source of knowledge has a multi-angled textual material including pharmaceutical knowledge. Drug (Ausadha) is a part of quadruped of the treatment, which has been placed next to the physician.[1] The comprehensive knowledge of the drug is very important to physician because without knowledge of drug patient cannot be treated properly. In this way, all Ayurvedic classics advocate specific formulations for particular disease.

The Ayurveda believes in maintaining the balance of Tridosha in the body to keep person healthy. The effects of certain Ayurvedic preparation with the concerned Vyadhihara Dravyas have been explained with their therapeutic actions in the ancient Ayurvedic literature. The Pharmacodynamics of these drugs and their compound preparations have been explained on the basis of Rasa, Guna, Virya, Vipaka, and Prabhava. According to Ayurveda, the drug or diet articles that reverses or breaks the Samprapti is ideal for the particular disease. The drug can be used singly or in combination to achieve the prescribed objective. It is often the total effect of all the ingredients in case of a formulation rather than the action of individual drugs that plays a vital role in therapeutics. In Ayurvedic Samhita, Ample of herbal remedy are mentioned.one formulation can be used in various diseases according to involved Dosha, Dushya, Strotasa and Vyadhi avastha .Vatari guggulu is one among them .Its Therapeutic uses mentioned in Bhaishajya Ratnavali Amavata chikitsa prakarana are Samavata**,** Katishool (Low back ache)**,** Grudhrasi (Sciatica)**,** Khanja**,** Pangu**,** Vatarakta (Gout)**,** Shotha**,** Daha (Relives burning sensation)**,** Koshtukshirsha.[2]

Various studies were carried out to evaluate efficacy of Vatari guggulu in aamavata sandhigata vata and Grudhrasi and significant results were obtained. It having Vatashamaka,, Kaphashamaka, Aamapachana, Dipana, Vedanasthapana and Rasayana properties. These properties may easily invade in the pathogenesis of disease and ultimate cure. There for to evaluate the Theurapeutic action of of Vaatari Guggulu in disease Amavata ,Sandhigata Vata, and Grudharasi, these review was planned with following aims and objectives.

Aims and Objectives

To evaluate Theurapeutic action of Vatari guggulu in Amavata Sandhigata vata, and Grudharasi..

Material and Method

Available Ayurvedic literature, article, journal, research work, monograph related to disease and drug Vatari Guggulu.

Ingredients of Vatari Guggulu: [3]

Sr.No	Drug Name	Latin Name	Part Used	Quantity
1.	Eranda	Ricinuscommunis.Linn.	Seed	1 Part
2.	Guggulu	Commiphoramukul hook Ex.stocks.	Exudate	1 Part
3.	Gandhaka	Sulphar (purified)	-	1 Part
4.	Haritaki	TerminaliachebulaRetz.	Pericarp	1Part
5.	Bibhitaki	Terminalia belerica Roxb.	Pericarp	1 Part
6.	Amalaki	Emblicaofficinalis Gaertn.	Pericarp	1Part

Method of preparation:

Guggulu was made soft by adding Erand Taila. The drugs enlisted from 3 – 6 were made into powder form in equal quantity. All above drugs were added and mix well. The final product was prepared in the form of Vati.

Therapeutic Benefits of the Ingredients:

1. ERAND[4]

RASA - Madhura (API)

Madhura Tikta, Katu, Kashaya Anurasa (B.P)

GUNA - Pichhila, Tikshna, Guru, Snigdha, Sukshma

VIRYA- Ushna

VIPAKA- Madhura

DOSHGHNATA - Kaphavatahara

KARMA– Deepana,Vrishya,Twachaya,Bayastahpana, Medhakantibalaprada, Yonisukravishodhana

ROGAGHNATA– Visamajwara, Hridya, Pristha-guhyatashula, Vatodara, Anaha,

Gulma, Asthila, Katigraha, Vatashonita, Vidavibandha,

Braghna, Shotha, Àma, Vidradhi, Àmavata.

Pharmacological activity: Anti-inflammatory, Spasmogenic, Hepatoprotective, Anti-fertility, Purgative, Immunizing, CNS depressantant.

2. GUGGULU[5]

RASA - Tikta, Katu, Kashaya

GUNA - Vishada, Ruksha, Laghu, Picchila, Sukshma, Tikshna

VIRYA - Ushna

VIPAKA- Katu

PRABHAVA - Tridoshahara, Rasayana

DOSHGHNATA- Tridoshahara

KARMA- Lekhana, Ropana, Vedana, Sthapana, Nadibalya, Dipana,

Saraka, Mutrala, Raktavardhaka, Rasayana, Bhagna

Sandhankar, Jantughna,

ROGAGHNATA- Aamavata, Kushta, Prameha, Vatavyadhi, Granthi, Sopha,

Gandamala, Medoroga Oleo-gum-resin used for reducing

Obesity and in rheumatoid Arthritis, osteoarthritis, sciatica in

the treatment of hyperlipidemia, hypercholesterolaemia and

Obesity.[6]

Pharmacological Activity:

Hypolipidemic,Antibacterial, Anti-atherosclerotic, Anthelmintic, Antiarthritic, Antiviral, ca2+Antagonist activity, Anti-inflammatory, Anti-rheumatic, Hypocholestremic, Increase serum cholesterol fibrinolytic activity. Guggulu has traditionally been used in the treatment of various conditions and disorders including rheumatism, neurological disorders, obesity and related disorders, syphilis, bronchitis, catarrh, gingivitis, inflammation, pyorrhea, sores, tonsillitis, hysteria, mania, skin and urinary disorders.[7]

3. GANDHAKA[8]:

ENGLISH NAME- Sulphur

RASA - Tikta, Katu, Kasaya, Madhura

GUNA – Usna, Snigdha, Sara, Laghu

VIRYA - Ushna

VIPAKA- Katu

PRABHAVA - Twakavikar Nasaka, Dadru Nasaka, Kusthghna

DOSHGHNATA - Vatakapha Shamaka, Rakta Shodhana

KARMA - Garavisahara, Dipana, Amapacana, Kandughna, Krimighna

ROGAGHNATA: Visha, Kushta, Kandu (itching), Mutrakrucchata, Twakvikara,

Krimi (worms), Visarpa (Erysipelas) and Plihavruddhi. It has Rasaayana properties. Gandhaka (Sulphur) is pungent, bitter and penetrating. It has a strong smell.

ACTION: - Externally parasiticide and internally mild laxative and blood-purifier.

USES: - it is given in combination with Thriphala and pepper as Gandhaka rasaayana. In combination with mercury, it is very much used in Ayurvedic Pharmacopoeia as black-sulphide and red sulphide. It is mentioned as virya-vriddhikara and rasaayana.Gandhaka is used for therapeutic purpose and for drug formulation in various forms, viz. as a Sneha, Rasayana, Lepa, Pralepa, Druti and Pishti etc.for preparing Kajjali, Parpati, Pottali, Kharaliya Rasayana, and Kupipakwa Rasayana etc.

4. HARITAKI: [9]

RASA - Kashaya Pradhana, Tikta, Madhura, Katu, Amla

GUNA - Laghu, Ruksha

VIRYA- Ushna

VIPAKA - Madhura

PRABHAVA - Tridoshashamaka

DOSHGHNATA- Tridoshashamaka

KARMA- Deepana ,Pachana ,Anulomana ,Shothahara , Hridya, Vednasthapana , Vrishya , Kusthaghna

ROGAGHNATA- Nadidaurbalya, Grahani, Vatarakta, Pratishyaya, Kasa, Swarabheda, Hikka, Mutrakrichhra, Prameha, Kustha, Visarpa, Vishama Jwara.

PHARMACOLOGICAL ACTIVITIES:

Antimicrobial, antifungal, antibacterial, antistress, antispasmodic, hypotensive, indurance promoting activity, anti hepatitis B virus activity, hypolipidaemic, inhibitory activity, against HIV-1 protease, anthelmintic,

purgative.

5. BIBHITAK[10]

RASA - Kashaya

GUNA- Laghu, Ruksha

VIRYA- Ushna

VIPAKA- Madhura

DOSHGHNATA - Tridoshahara

KARMA- Kasaghna, Keshya, Shothahar, Krishnkarana, Anulomana,

Raktavikarahara, Chakshushya, Bhedaka

ROGAGHNATA- Chardi, Kasa, Krimimiroga, Vibandha, Svarabheda,

Netraroga, Swarbheda, Raktavikara, Keshavikara

PHARMACOLOGICAL ACTIVITIES:

Fruit- purgative when half ripe, astringent when ripe, antipyretic, diarrhoea, dyspepsia, biliousness, cough, bronchitis and upper respiratory tract infections, tropical pulmonary eosinophilia and allergic eruptions, vitiligo, anti-spasmodic, antibacterial, CNS stimulant, amoebicidal, antistress. The fruits produce hepato-protective effect in CCl4-induced liver injury in mice and hypotensive effect of varying magnitude in a dose dependent fashion on isolated rat and frog.

6. AMALAKI [11]

RASA - Pancha Rasa Lavan varjita Amla Rasa Pradhana

GUNA - Laghu Ruksha

VIRYA- Sheeta

VIPAKA- Madhura

DOSHGHNATA - Tridoshahara (Especially Pitta Shamaka)

KARMA - Dahaprashmana, Kesya, Rochana, Deepana, Anulomana, Hridya, Yakriduttejaka, Kusthaghna, Rasayana and Vrishya

ROGAGHNATA- Aruchi, Hridroga, Kasa, Swasa, Raktapitta, Jwara, Prameha,

Kustha.

PHARMACOLOGICAL ACTIVITIES:

Spasmolytic, mild CNS depressant, hypolipidaemic, antiatherosclerotic, antimutagenic, antimicrobial, antioxidant, immunomodulatory, antifungal, antitumour, hypoglycaemic, antiinflammatory, antibacterial, antiulcer, adrenergic potentiating, HIV-1 reverse transcriptase inhibitory action. Aqueous extract of the fruit increases cardiac glycogen level and decreases serum GOT, GPT and LDHin rats having induced myocardial necrosis.Preliminary evidence suggest that the fruit and its juice may lower serum cholesterol, LDL, triglycerides and phospholipids without affecting HDL levels and may have positive effect on atherosclerosis. (Eur J clin Nutr, 42, 1988, 939-944; Phytother Res, 14, 2000, 592-592.)

Therapeutic Dosage:

According to Bhaishajya Ratnavali dosage of Vatari Gugguluis 1Masha (1g)[12] Mardita homogenous mixture of 1g was taken and rolled between thumb and index finger. The minimum effective dosage of Vatari Guggulu is roughly 500 mg, taken once or thrice a day, according to severity before or after food or as advised by your doctor.

Disease Review

Amavata: The term Aamvata is derived from two terms Ama and Vata. Ama means formation of toxin that is produced by imbalanced body fire. The toxin ama is carried by imbalanced Vata (one of the three energetic forces) and reaches the kapha (one of the three energetic forces) dominated places like joints etc. This toxin becomes sticky due to imbalanced doshas and blocks the vital channels which nourish the body. The ama which gets harbored in joints acts like a foreign substance and triggers the immune system. This leads to inflammation of linings of joints.[13] In Ayurveda RA is described as 'Amavata'and its cause are very well explained. Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. About 1% of the world's population is affected by rheumatoid arthritis, women three times more often than men. The disease generally sets in the people between the ages of 40 and 50, but people of any age can be affected. The disease manifests in swelling, pain, redness, stiffness and warmth in the affected region and may lead to deformity

of joint and restriction to mobility.

Grudhrasi: disease Gridhrasi mentioned in Ayurveda under the umbrella of Vatavyadhi, and here piercing type of pain which restricts the movement of the affected leg, make his walking pattern-like bird vulture and put him in disgraceful condition. The disease is listed under the heading where disease develops due to vitiation of Vata only, i.e. Nanatmaja Vatavyadhi.[14] Still, while enumerating the diseases, the disease Gridhrasi has been mentioned of two types, (1) Vata dominant and (2) Vata-Kapha dominant. The common symptoms of Gridhrasi are–pain starts from Sphik (buttock) and then radiates to Kati, Prushta (back), Uru (thigh), Janu (knee), Jangha (calf), and Pada (foot) along with Stambha (stiffness), Toda (pricking pain), Spandana (twitching)[15] and causes the Sakthiutkshepa Nigraha (restricted movement of lifting of the leg). Whereas in Vata Kaphaja type of Gridhrasi, Arochaka (aversion to food), Tandra (feeling of drowsiness), and Gaurava (feeling of heaviness) are found additionally.

Disccusion

Effect of Vatari Guggulu in Sandhigatavata : Composition of Vatari guggulu is collectively having Vatashamaka, Kaphashamaka, Aamapachana, Dipana, Vedanasthapana and Rasayana properties. Due to Ushna Virya and Vatanulomana properties, it normalizes the movement of Apana Vaayu and Vyana Vaayu which in turn helps to relieve pain. Furthermore, the Kaphashamaka properties of Eranda (Ricinus communis Linn) and Guggulu (Commiphora wightii (Arn.) Bhandari) by its laghu (lightness), Ushna (hot), Sukshma, Strotoshudhikara properties; it checks blockage of path occurred due to kapha dosha and so helps to relieve stambha (stiffness) and shotha (inflammation)[16-18]

Effect of Vatari Guggulu in Grudhrasi : In Grudhrasi mainly Apana and Vyana dushti occur .Due to Ushana Virya and Vatanulomana property it normalize the movement of Apana Vaayu, and Vyaana Vaayu which in turn helps to relive pain (Ruka). Also the Kaphashamaka properties of Eranda and Guggulu by its Laghu, Ushna, Sukshma, Strotoshudhikar properties, it checks blockage of path occur due to Kaphaand so helps to relive Stambha and shotha. Again by Aampachan and Dipana properties it corrects Agni and relieves generalized symptoms like Aruchi, Tandra, and Gaurava too. Generally the ingredients are more useful in Upastambhita Vaayu, and this can be the reason that the patients of Vaatakapaja type of Grudhrasi have shown better symptomatic improvement than Vataja type of Grudhrasi patients. Vatari Guggulu has Anti-inflammatory and Peripheral analgesic activity[19] which reduces the pressure on Sciatic nerve and improves the degree of movement i.e. Sakthiutkshepanigraha. In this way VatariGuggulu works very well in Vatakaphaja type of Grudhrasi than Vataja type.and shows highly significant relief in symptoms like Ruka, Toda, Spandana Gaurava Tandra and Aruchi. [20]

Conclusion

Vatari guggulu having Vatakaphhara, Vatanulomaka, Dipana, Pachana and Shulaprashamana properties.Ingredients having Anti-inflammatory and Peripheral analgesic activity.These properties helps to relieve pain , inflammation and stiffness. Vatari guggulu is effective where Vata dosha is vitiated along with Kapha dosha .(i.e vatakaphaja condition). In Sandhigata vata Aamvata and Grudhrasi for complete cure along with Vatari guggulu , Basti karma ,some vatahar taila for local application is effective. All these show synergistic and cumulative effect responsible for improvement.

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Clinical Assessment of a Local Application of Kasisadi Ghrita in Parikartika (Fissure-in-ano)

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Abstract

Background: Parikartika is charecterised by sharp cutting pain in anal region. In Parikartika Teevrashoola, bleeding is observed, equally extreme pain& slimy blood discharge are seen in Fissure-in-ano

Objective: To test the efficacy of thelocal application of Kasisadi Ghruta in managing

Methods: Classical signs and symptoms ofParikartika (fissure inAno) Age groups of 20 to 60 years have been recruited for the researchThe trial group obtained treatment with Kasisadi Ghruta local application for 28 days, twice a day.

Results: Average relief observed was observed 62.5% for most symptoms Shothahara, Vedanasthapana and Ropana are in Kasisadi Ghruta. Properties because of which it helps in healing the ano fissure

Conclusion: Local Kasisadi Ghruta The programme has better results in the Fissure in ano.

Key words: *Parikartika, Vedanasthapana, Shothahara, Ropana, Kasisadi Ghruta*

Introduction

Fissureinano is frequently found in current circumstances. Train from day to day. Parikartika defined byKartanavatvedana in Guda. Fissureinanosimilarly is Sharp cutting pain in anal pain is often characterised by Area.[1] Bleeding in Parikartika,Teevrashoolaextreme pain and slimy discharge of blood They can be seen at Fissureinano.Thevariables responsible for Parikartika is known as VyapadaThese areBasti,Virechana,Vamana, Arsha (piles), Atisara, etc. Indicated in different texts.[2,3] The symptomsdescribed in Sushruta Samhita are described as Pain in the anus, penis, umbilical area, cutting/burningpain,Urinary bladder and stomachpain, cessation of flatus. The features were identified by Acharya Charaka, such as In the groyne and sacral zone, pricking pain, scanty pain Per rectum, constipated stools and frothy Hemorrhages.[4,5]

The popular fissureinano site is 6 o'clock, which is, Posterior midline, the bottom half of the anal canal, which is In young adults, usually found. In fissure in males Typically 90% and a lot of them occur in the midline posterior. Less generally, the previous 10% In the fissures of females on In retrospect the midline is significantly more prevalent than Backwards (60:40)[6]

Objective Assessing the productivity of the local Kasisadi Ghruta Application in fissure-in-ano management.

Material and Methods

Kasisadi Ghruta

Ingredients of Kasisadi Ghruta

1. Shudha Kasisa 2.Katuki Mula 3Jati Mula 4,Haridra 5.Goghruta

Method of preparation Each one was taken by Shudha Kasisa, Katuki, Jati, Haridra 1part Where 4 sections were added as Goghruta. Jala has been 16 **part**s

were poured, then Ghruta, preparedby Snehapakak Vidhi.

Drug administration details

Drug : Kasisadi Ghruta

Rout of Administration : Local Application/Tropical

Duration : 28 days

Follow up : 7th , 14th , 28th days.

Diet Regimen : All patients were advised Pathya-Apathya related to Guda Vikara, light fiber diet was advised as per the status of Agni.

Criteria of selection-

Inclusion Criteria

The patient has classic symptoms and signs of Ano fissure with an age group of 20 to 60 years was Invited to participate in this Shalyatantra OPD/Clinic

Exclusive Criteria

Patients suffering from fissure-in-ano due to any secondary cause, Perianal Abscess, Chronic Fissure.

Results

Treatment was initiated after complete examination Although in groups, patients were allocated randomly. Trial Kasisadi Ghruta was treated by the community twice a day, 28day local application.Subjectiveness The test was conducted before and after the procedure. Scores of Parikartika signs and symptoms For the interpretation of subjective parameters. The Wilcoxon Signed Rank Test was applied in the Trial Group For Fissure-in-ano Symptom's Ranking.

Table 1: Wilcoxon Signed Rank Test of symptoms in Fissure-in-ano

Sr.No.	Symptoms	Mean ± SD BT	Mean ± SD AT	Median BT	Median AT	p value
1	Vedana	1.70±1.2	0.56±0.6	2.0	0.5	<0.001
2	Bleeding P/R	1.26±0.8	0.40±0.4	1.0	0.0	<0.001
3	Itching at Anus	1.4±1.22	0.43±0.6	1.5	0.0	<0.001
4	Burning	1.46±0.8	0.53±0.6	1.5	0.0	<0.001
5	Discharge	0.66±0.4	0.13±0.3	1.0	0.0	<0.005
6	Sentinel tags	0.60±0.4	0.40±0.4	1.0	1.0	0.12
7	Inflamation	0.60±0.6	0.20±0.4	0.5	0.0	<0.005
8	Tenderness	1.0±0.98	0.40±0.6	1.0	0.0	<0.001
9	Sphincter spasm	1.03±0.7	0.46±0.5	1.0	0.0	<0.001

Percentage of Relief in Symptoms Score: (SubjectiveCriteria)

The percentage of relief noted in the patients, Vedana/shool was 66.42 percentage, symptoms such as bleeding per rectum relief was noted as 68.42 percent, in scratching in the anus 69.04 percent, in sentinel tags 33.33 percent,in burning sensation 63.63 percent, 80 percent in discharge, 66.67percent in inflammation,60percent in tenderness & in sphincter spasm 54.83percent respectively. In all the median percentage was 62.50 percent relief with symptoms.

Table 2: Percentage of Relief (Subjective Criteria) ineach Symptom of Fissure-in-ano.

Sr.No.	Symptoms	Before Treatment	After Treatment	Percentage of Relief
1	Vedana	51	17	66.66
2	Bleeding P/R	38	12	68.42
3	Itching at Anus	42	13	69.04
4	Burning	44	16	63.63
5	Discharge	20	04	80.00
6	Sentinel tags	18	12	33.33
7	Inflamation	18	06	66.67
8	Tenderness	30	12	60.00
9	Sphincter spasm	31	14	54.83

In the this sample size, 13 patients [43.3%] were significantly improved. 08 patients [26.7%] moderately, while only 1 patient improve slightly.

Discussion

Shift or irregularity occurs in the age of fast food Timings for food and diet and type of sedentary life. In In addition to diet and lifestyle changes, most People are under immense mental stress at all times. Both of these stimuli disrupt the digestive system, which It results in many constipations and contributes to many constipations. Parikartika, because of hard stoolleads in anal tears Domain. Area.One of the main causes of Anal Fissure is Analarea pain. It is very popular to have fissureinano Patients have a debilitating and painful disorder that hampers Standard of living. Acharya Dalhana has characterised the Parikartika as a Guda state in which there is a word. The pain of cutting and tearing is .7 The trial group was treated with Kasisadi in the study. Ghruta local application for 28 days, twice a day. Acharya Sushruta identified Kasisadi Ghruta as having Healing Vrana, which includes Shudha Kasisa, Mula Katuki, Jati Mula, Haridra and Goghruta Properties: Pittnashak, Vranaghana, Kandughna. Vata Kasisadi Ghruta's Pittahara property may be attributable to To the base of Ghrita and it probably takes away the Accumulated secretions in the bed of the fissure, enabling Healing, and also decreases secondary infection. Getting it Attributes such as Vrana Sodhana, Vrana Ropana,Properties of Sothahara andVedana Sthapana. In Parikartika, the superiority of Vata and Pitta is there, Dosha is responsible for the clinical features and for the care of The VataPitta Shamak drugs that will be used for this disease.[8-11]

Conclusion

In Kasisadi Ghruta, the ingredients used are Shothahara, Vedanasthapana and Ropana, which is

why it assists in Fissure Healing in Ano. Along with diet and oral care Kasisadi Ghruta medication may be the best choice for Healing wound without any hand of Parikartika Repercussions.

Conflict of Interest: Nil

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Ethical Clearance: taken from institutional ethics committee

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Genetic Polymorphisms of the Efflux Transporter Gene ABCB1 and Their Effects on the Anastrozole Response in Iraqi Breast Cancer Patients

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Abstract

Background: The ABCB1 gene (ATP-binding cassette) encodes the ABCB1 transporter. Anastrozole is a substrate for the ABCB1 efflux transporter. The objective of the study was to detect the genetic polymorphisms of this efflux transporter in Iraqi breast cancer patients and their effects on the anastrozole response.

Method: Blood samples were taken from breast cancer patients, and a portion of each sample was used for biochemical analysis (measuring estradiol and cancer antigen 15.3 levels), while the remaining portion was used for genetic analysis. Genotyping demonstrates that the mutant genotype being the most frequent type for both C1236T and C3435T SNPs.

Results: The result indicated nonsignificant differences in the serum levels of estradiol or cancer antigen 15.3 among different genotype groups of the ABCB1 gene.

Conclusion: It is concluded that the ABCB1 gene is highly polymorphic in Iraqi women with breast cancer. This study indicated that ABCB1 gene may not affect the response to anastrozole.

Keywords *Anastrozole, ABCB1 gene, estradiol, cancer antigen CA15.3, arthralgia.*

Introduction

Breast cancer (BC) is one of the most frequent cancers worldwide, with an elevated incidence rate in all countries [(1)]. In Iraq, BC is the first among the top ten malignancies affecting society. In 2016, hundreds of females died from this disease, which is recorded as the leading cause of cancer-related mortality among Iraqi women [(2)]. Of the risk factors that have been associated with BC, such as family history, genetic mutations, lifestyle and others, estrogen is a major factor in breast cancer pathogenesis. Factors such as obesity, age at menarche and menopause, and hormone replacement therapy have been demonstrated to increase the risk of BC [(3)].

Tumor markers are biomarkers that are produced at higher levels by cancer cells or by the host in response to cancer. One of these markers is cancer antigen (CA) 15-3, which is used to determine breast cancer prognosis and to monitor the efficacy of therapy [(4)].

Because of the role of estrogen in carcinogenesis, hormonal therapy targeted at decreasing estrogen production or its action has been applied in various ways to manage BC [(3)]. Decreasing the quantity of estrogen by inhibiting its production can be achieved with aromatase inhibitors (AIs), such as anastrozole [(5)]. Anastrozole is a nonsteroidal inhibitor that inhibits aromatase enzyme competitively by binding to the heme group of the enzyme, thus decreasing estrogen biosynthesis in the breast and periphery [(6)].

One study showed that anastrozole is a substrate for ATP-binding cassette B1 (ABCB1) transporter [(7)], an energy-dependent xenobiotic efflux pump that is expressed at the apical membrane in tissues that are involved in excretion or form blood-tissue barriers.

Several studies have confirmed the notable impact of drugs transport via ABCB1 on the pharmacokinetics of these drugs in humans [8]. Additionally, the ABCB1 gene has been identified to have multiple single nucleotide polymorphisms (SNPs) that effect on drug disposition and clinical outcomes [9]. Of these mutations, the most well studied are two synonymous transitions (C1236T and C3435T) located in exons 12 and 26, respectively [10].

Patients and Methods

Study samples

This cross-sectional observational study included Iraqi females with breast cancer taking 1 mg of anastrozole by tablet daily. This study was carried out at the Oncology Center in Kerbala at Imam AL-Hussein Medical City.

The protocol of the study was approved by the Ethical Committee of Pharmacy College at Kerbala University, and each participant was given a written informed consent form for their participation.

The study was conducted on 100 females aged 34-76 years, who had estrogen receptor and/or progesterone receptor (ER, PR)-positive breast cancer.

The exclusion criteria for this study involved: taking other adjuvant endocrine therapies, taking anastrozole therapy concomitantly with either adjuvant chemotherapy or adjuvant radiotherapy (or both), females with a history of gastrointestinal surgery or disorders, women who took CYP3A4/5 or UGT1A4 inducers or inhibitors or any drugs affecting ABCB1 transporter, and females who were pregnant or lactating.

Clinical data collection

The clinical data were taken from the medical records of the patients and from the women themselves and included weight, height, age, workplace, academic achievement, marital status, breast feeding, pre- or postmenopause, family history of breast cancer, date of breast cancer and duration, site, grade and stage of breast cancer, presence of liver or any other diseases, and time and duration on anastrozole.

Sample collection and analysis

A 5 ml peripheral blood sample was taken from each female, 3ml of the blood was placed in a gel tube and used to measure the biochemical parameters, and 2 ml was placed in an EDTA tube for the genetic assay.

Biochemical parameters

Estradiol level (E2)

E2 levels in the serum of breast cancer patients were determined by using a CL-series chemiluminescence immunoassay (CLIA) analyzer (Mindray/China). This assay is a competitive binding immunoenzymatic assay [11]. The method was performed according to a kit.

Normal values:

Postmenopausal (<25-84 pg/ml)

Follicular phase (20-138 pg/ml)

Ovulation phase (100-440 pg/ml)

Luteal phase (31-317 pg/ml)

Tumor marker CA15.3

Levels of the serum tumor marker CA15.3 in breast cancer patients were determined by using a Minividas (Biomerieux/France) instrument, which utilizes the enzyme linked fluorescent assay (ELFA) technique [12, 13]. The method was performed according to a kit.

Normal value: 0-30 U/ml.

Genotyping

Genomic DNA was extracted from blood samples using the G-DEX™IIb kit (iNtRON/Korea).

ABCB1 gene polymorphism genotyping

C1236T and C3435T were detected using amplification refractory mutation system PCR (ARMS PCR). Primers (synthesized by Macrogen/Korea) were used for C1236T and C3435T identification, and the detection of alleles is shown in Table (1).

Table (1) Primer sequences for determining the genotypes of C1236T and C3435T based on product size (14).

SNPs	Primer sequences	Product size
1236P1	5' AAT GTT CAC TTC AGT TAC CCA TCT CG 3'	508
1236P2	5' AAT GAT TTC CCG TAG AAA CCT TAC 3'	
1236C	5' TGG TAG ATC TTG AAG CGC 3'	305
1236T	5' TGC ACC TTC AGG TTC TGA 3'	238
3435P1	5' TGC TGG TCC TGA AGT TGA TCT GTG AAC 3'	300
3435P2	5' GGC CAG AGA GGC TGC CAC AT 3'	
3435C	5' GTG TCA CAG GAA GAG TTC 3'	126
3435T	5' TCC TTT GCT GCC CTC TCA 3'	209

The PCR mixture was prepared in an AccuPower® PCR PreMix (Bioneer/Korea) tube by adding 1 µl of each primer at 10 pmol/µl, and 5 µl of DNA, and the volume was brought to 20 µl with distilled water. PCR was carried out with the following program: initial denaturation for 3 minutes (min) at 94ºC, followed by 35 cycles of denaturation at 94ºC for 30 seconds (s), annealing at 60ºC and 58 ºC for C1236T and C3435T respectively for 30 s, and extension at 72ºC for 55 s. Final extension was performed at 72ºC for 5 min. Amplified segments were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and observed under ultraviolet (UV) light.

Statistical Analysis

For statistical analysis, SPSS software for Windows (version 15.0 USA) was used. Single-factor ANOVA was performed to examine the differences in the mean of parameters tested within genotype groups. Odds ratios and the corresponding 95% CIs were used to examine associations. For all tests, p-values of <0.05 were considered statistically significant.

Results

Study Population

The general characteristics of the participants: the mean age of women who participated in this study was 57.51 (range: 34-76); the percentages of women who were married and unmarried were 94% and 6%, respectively; the menopausal status of the patients was 95% postmenopausal and 5% premenopausal; the proportion of women who depended on lactation to feed their babies was 64%, while 19% did not lactate and 17% undertook mixed feeding; the percentages of females who had and did not have a family history of breast cancer were 12% and 88%, respectively; some patients presented with breast cancer on the right side (48%), others on the left side (49%) and only 3% presented with breast cancer on both sides; the percentage of patients who were both ER- and PR-positive was 94%, while only 6% of patients were ER- or PR-positive; and the proportion of women who suffered from arthralgia (as a side effect of anastrozole) was 89%, while 11% did not have arthralgia.

Frequency and distribution of ABCB1 gene polymorphisms

The percentage of ABCB1 genotypes detected in 100 Iraqi breast cancer patients are shown in Table (2). For C1236T, the frequencies of patients who carried CC, CT, and TT were 28%, 18%, and 54%, respectively. For C3435T, 9% patients had the wild genotype (CC), 76% had the mutant genotype (TT), and only 15% patients carried the heterozygote genotype (CT). The most frequent genotype for both SNPs was TT.

Table (2): The percentage of C1236T and C3435T detected in Iraqi breast cancer patients.

SNPs	Genotypes	Percentage
C1236T	CC (wild type)	28%
	CT (heterozygote)	18%
	TT (mutant type)	54%
C3435T	CC (wild type)	9%
	CT (heterozygote)	15%
	TT (mutant type)	76%

SNPs: single nucleotide polymorphisms, C: cytosine, T: thiamine.

Effect of ABCB1 gene polymorphisms on estradiol levels (E2)

Table (3) shows the mean and standard deviation (SD) of estradiol levels in the detected genotypes for C1236T and C3435T SNPs. For C1236T, patients with CC had a lower mean level of E2 (19.482 ± 15.06 pg/ml), while those with CT had the highest mean level of E2 (22.656 ± 8.02 pg/ml), and the mean level in those carrying the TT genotype was 19.716 ± 9.717 pg/ml.

In C3435T, the mean and SD values of E2 in patients who had the CC, CT, and TT genotypes were 15.275 ± 6.817 pg/ml, 23.023 ± 18.803 pg/ml, and 20.2 ± 9.45 pg/ml, respectively. There were nonsignificant differences in the mean levels of E2 and genotype groups for both SNPs.

Table (3) Mean and standard deviation of E2 levels in the detected ABCB1 (C1236T and C3435T) genotypes.

SNPs	Genotypes	E2 level Mean ± SD Pg/ml	P value
C1236T	CC	19.482 ± 15.06 a	0.585
	CT	22.656 ± 8.02 a	
	TT	19.716 ± 9.717 a	
C3435T	CC	15.275 ± 6.817 a	0.26
	CT	23.023 ± 18.803 a	
	TT	20.2 ± 9.45 a	

P value derived from ANOVA test, $p < 0.05$ is significant, $p > 0.05$ is nonsignificant. Same letters indicate nonsignificant differences. SD: standard deviation.

Effect of ABCB1 gene polymorphisms on cancer antigen CA15.3 levels

Table (4) shows the mean and standard deviation values of CA15.3 levels in different genotype groups for C1236T and C3435T. For C1236T, the mean and SD of CA15.3 in patients with the wild genotype was 15.616 ± 6.26 pg/ml which is the lowest mean among the different genotypes. The highest mean of CA15.3 appeared in the heterozygous genotype (23.235 ± 14.73 pg/ml). The mean and SD of CA15.3 in patients with

the mutant genotype was 20.507 ± 16.446 pg/ml. There were significant differences in the mean levels of CA15.3 between the wild type and heterozygote genotypes, while there were nonsignificant differences between the wild type and mutant or between the heterozygote and mutant genotypes.

For C3435T, there were nonsignificant differences between the mean CA15.3 and genotype groups. The mean and SD values of CA15.3 for CC, CT, and TT were 14.438 ± 5.43 pg/ml, 14.882 ± 6.42 pg/ml, and 21.417 ± 15.93 pg/ml, respectively.

Table (4) Mean and standard deviation of CA15.3 levels for the detected genotypes of ABCB1 gene polymorphisms.

SNPs	Genotypes	CA15.3 level Mean ± SD	P value
C1236T	CC	15.616 ± 6.26a	0.163
	CT	23.235 ± 14.73b	
	TT	20.507 ± 16.446ab	
C3435T	CC	14.438 ± 5.43a	0.09
	CT	14.882 ± 6.42a	
	TT	21.417 ± 15.93a	

SNPs: single nucleotide polymorphisms, CA15.3: Cancer Antigen 15.3, SD: standard deviation. P value derived from ANOVA test, $p< 0.05$ is significant, $p> 0.05$ is nonsignificant. The same letters indicate nonsignificant differences, and different letters indicate significant differences.

Association of ABCB1 gene polymorphisms and the elevation of CA15.3 levels

The association between the genetic polymorphisms in ABCB1 and the elevation in serum levels of CA15.3 was determined by using odds ratios (Table (5)). For both SNPs, there was a nonsignificant association ($p> 0.05$) between ABCB1 gene polymorphisms and the elevation in CA15.3.

Table (5) Association of ABCB1 gene polymorphisms (C1236T and C3435T) with the elevation of CA15.3 levels.

SNPs	Odds ratio (CI-95)	P value
C1236T	1.56 (0.427-5.721)	0.499
C3435T	1.477 (0.296-7.364)	0.633

CI-95: confidence interval 95%, $p< 0.05$ is significant.

Association of ABCB1 gene polymorphisms with the occurrence of arthralgia

Table (6) shows the association between ABCB1 gene polymorphisms and the onset of arthralgia. For C1236T and C3435T, there were nonsignificant associations with the development of arthralgia ($p> 0.05$).

Table (6) Association of ABCB1 gene

polymorphisms (C1236T and C3435T) with the occurrence of arthralgia.

SNPs	Odds ratio (CI-95)	P value
C1234T	0.401 (0.099-1.611)	0.1979
C3435T	1.214 (0.295-4.994)	0.787

CI-95: confidence interval. $p< 0.05$ is significant.

Discussion

An in vitro study introduced by Miyajima et al. indicated that anastrozole is a substrate for ABCB1 transporters [7]**.** Because ABCB1 is a highly polymorphic transporter, variability in this gene has been associated with changes in drug response, disposition, and toxicity [9]. Therefore, to obtain an effective therapeutic response and lower the side effects of anastrozole, it is important to study the effects of genetic polymorphisms of ABCB1 on the therapeutic response (by measuring serum E2 and CA15.3 levels) in Iraqi women with breast cancer treated with anastrozole.

The association between the development of breast cancer and the continued increase in serum estrogen levels has been studied by several researchers. These studies suggest that estrogen is a mammary gland carcinogen [15]. Therefore, the use of aromatase inhibitors is a common strategy to treat women with hormone receptor-positive breast cancer [16]. Although anastrozole was used as an aromatase inhibitor, this study found a detectable quantity of E2 in the serum of breast cancer patients that was still within normal limits (Table 3), which suggests that there may be other pathways for estrogen synthesis. These results were in agreement with a study introduced by Abd-Allateef et al. (2016), who found a detectable concentration of estrogen in females with breast cancer treated with anastrozole despite complete inhibition of the aromatase enzyme [17]. In this study, both SNPs (C1236T and C3435T) exhibited nonsignificant differences in the mean E2 among genotype groups, with those who carried the heterozygote genotype CT (in both SNPs) having the highest mean E2 level, although it was still within normal limits. A study conducted on females with breast cancer found that patients who carried TT genotypes had significantly lower ABCB1 expression [18]. Accordingly, the capacity of ABCB1 transporters may be lowered, so patients with TT may respond better to treatment than those with wild genotypes, although this was not indicated in our results[20].

Serum tumor markers are soluble molecules present in the blood that are discharged from tumor cells, and they are usually used to determine the response to anticancer drugs or determine prognosis because their concentrations may indicate the presence of hidden metastasis or reflect the extent of the tumor mass [19,21]. In the present study, there were nonsignificant associations between serum CA15.3 and the genotype groups for both SNPs, except for the CT genotype of C1236T, which showed a significant difference in the mean CA15.3 levels relative to the CC genotype (Table 4). [19]

This study revealed that there were nonsignificant associations between different genotype groups of the ABCB1 gene and the elevation of CA15.3 (Table 5), which may be because these SNPs (C1236T and C3435T) are synonymous and do not alter amino acids or affect activity [10].

Arthralgia, one of the common adverse effects of anastrozole, usually affects quality of life and may lead to the patient discontinuing treatment [22]. In the present study, most women suffered from arthralgia, but there was nonsignificant association between the occurrence of this side effect and ABCB1 gene polymorphisms (Table 6). This supports the findings of a study by Gervasini et al. (2017), who did not report a statistically significant association between C1236T and the occurrence of arthralgia, although the same study found that C3435T was inversely associated with arthralgia development [23].

Conclusions

It can be concluded that the ABCB1 gene is highly polymorphic, with mutant genotype TT being the most frequent for both SNPs in Iraqi breast cancer patients. This study revealed that ABCB1 gene polymorphisms may not impact the anastrozole response.

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Ethics approval: This study was approved by the ethical committee of the College of Pharmacy at the University of Kerbala.

Conflict of Interest - The authors declare no conflict of interest.

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Clinical important of E-Cadherin, CA15-3 and CEA in Diagnostic and Flow Up for Breast Cancer Patients

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Abstract

Background: The normal prevailing cancer in the women is Breast cancer, other than nonmelanoma skin cancer. E-cadherin performs an essential role in epithelial cell adhesion and the decline of its role is the main donor to cancer rise because the majority of solid tumors are carcinomas that occur from epithelial tissue. A segment of the mucin-1 (MUC-1) is the cancer antigen 15-3 (CA15-3) order of glycoproteins, is classified as a serviceable tumor marker inspired by revised glycosylation of itself. The carcinoembryonic antigen (CEA) levels is raise in the blood, as a sort of cell adhesion molecule are associated with tumor metastasis.

Methods A cross-sectional study held implemented on100 specimens, 50 patient with breast cancer ere and subsequent chemotherapy therapy with 50 healthy individuals (healthy patients).

Results The result reveals significant disparities patient group the same patient as a novel cause, following initial chemotherapy procedure and matched with the control group in some tumors markers such as (CA15-3, CEA) exact (E-cadherin) shows non-significant.

Conclusions Our cross-sectional study designates that the E-Cadherin was non-significant for breast cancer patients including Tumors markers (CA15-3, CEA) bestows significance with breast cancer patients.

Keywords**:** *CA15-3, CEA, E-cadherin, MUC-1, CEA*

Introduction

Mortality's important cause in females is Breast cancer; in 2018, about 2.1 million females to be supplementary breast cancer cases, rating 25% of female cancer. (1). the composite tumor of breast cancer with four administrator molecular subtypes and discrete in prediction. Now, the evaluation and strategy are determination of guess in the polyclinic based on distinguished molecular subtypes. The breast cancer patients are forecasts by primarily delimited by pathological components of tumors, for example grade, stage "human epidermal growth factor receptor2 (HER2)", and hormone-receptor status (1). CEA & CA15-3 are the usual castoff and supported markers. The predictive importance of CA15-3 had been established by certain researches (2) Research is ongoing for new predictors to alter the production of predictive durability, and more recently, the significance of tumor markers when assessing has led to more interest. a member of the mucin-1 (MUC-1) family of glycoproteins is The cancer antigen 15-3 (CA15-3), is known as a helpful tumor marker and is overexpressed in cancers is enforced by renovated glycosylation of itself (1). the carcinoembryonic antigen (CEA) levels is raised in the blood as a sort of cell a adhesion molecule are associated with tumor metastasis (3). The essential labor has proceeded into the relationship between tumor markers and the prediction of breast cancer. The European Collection on a Tumor Markers has advised appropriating CEA and CA15-3 to weigh a patient's appearance in the eternity a the arrangement of the disease, initial discovery of the development of the illness, and chosen curative approaches a in breast cancer(4). Because CA15-3 is a slowly glycosylated protein, glycosylation variations have the fabulous potential for indicating carcinogenesis and unfounded

reasoning have admittedly pointed to glycosylation shifts of CA15-3 in breast cancer[5]. Novel reflections confirmed that successive ascertainment of CEA and CA15-3 levels while the postoperative follow-up of breast cancer cases maybe serviceable for immediate exposure of preclinical recurrence or metastatic disorder. Besides, the serum levels of CA 15-3 moreover CEA may be done to prophesy rejoinder to chemotherapy in patients[6]. Any researchers have informed that CEA is not a predictor in initial and metastatic breast cancer[7][8][9]. though others have described those high concentrations of CEA were associated with a reduced diagnosis in breast cancer[10][11][12]. E-cadherin is a one of the well-studied establishing members of the superfamily and an influential tumor suppressor because down regulation of E-cadherin is frequently seen in cancerous epithelial cancers[13]. E-cadherin is tumor suppressor and frequently seen in cancerous epithelial cancers [13]. E-cadherin is essential in improving the homeostasis of tissues and sustaining the epithelial phenotype by tempering distinct signaling pathways [14]. Deviations, chromosomal deletions, protolytic cleavage, epigenetic regulation, and transcriptional silencing of CDH1 promoter were shown to prevent the functionality of E-cadherin in the extension of altered malignancies including pancreas, gastric, liver, breast, and skin[14]. E-cadherin is a certified to assist nucleotide extraction a improvement by improving the composition of xeroderma a pigmentosum complementation a group C (XPC) and DNA a damage-binding protein 1 (DDB1) in the a case of ultraviolet-induced a DNA impairment[15]. Experimental considerations determine that reexpression of E-cadherin in cancer cells wanting it can arrest tumor rise and invasion advancing E-cadherin a typical tumor suppressor[16]. Investigation of E-Cadherin, CEA and CA15-3 tumor markers in diagnosis and monitoring of breast cancer before and after chemotherapy treatment and compare with healthy patients.

Materials and Methods

Medical Ethics a Committee, Faculty of Medicine / University of Kerbala accepted this study scheme. The questioners are registered in Appendix-A. In this cross-sectional study, 50 cases were selected about 100 of the breast cancer patients as new patients and after the first chemotherapy regimen. The study was achieved throughout the term from February 2020 to September 2020. The samples were solicited from Al-Hussain Center for cancerous tumors in the Al-Hussein medical city; the parameters were entire worked in the laboratory of Imam Zain Alabiden hospital.

A blood specimen (5 ml) was earned from various groups, the serum appropriated for multiple tumors marker, including (E-Cadherin, CA15-3, CEA level). The E-Cadherin level was estimated by ELISA, the CA15-3 and CEA were mechanical calculations by using the ARCHITECT PLUS i 1000 SR. Equalized data are exhibited as the means ± SD. Pearson's equivalence test was done in order to test each association between the values of the above patients with breast cancer parameters. Statistically significant was P-Value < 0.05.

Results

The current study included 100 subjects (50 patients with breast cancer as new patients, after first chemotherapy treatment and 50 with healthy patients. The tumors marker characteristics of the recruited individuals are presented in table (1.1). When shows significant differences patient group the same patient as new case and after first chemotherapy treatment and compared with control group in some parameter such as (CA15-3 and CEA) expect (E-Cadherin) shows non-significant.

Table 1.1: Tumors markers characteristics of study subjects.

Tumors Parameter	Pre	Post	Control	P value
E-cadherin ng/ml	59.27±18.22	56.4±19.94	65.99±10.33	0.353
Ca15-3 ng/ml	57.88±40.11	43.91±36.6	13.56±2.38	0.004*
CEA ng/ml	4.04±3.19	2.67±2.49	1.43±1.13	0.036*

Student t test was used, *significant P< 0.05,

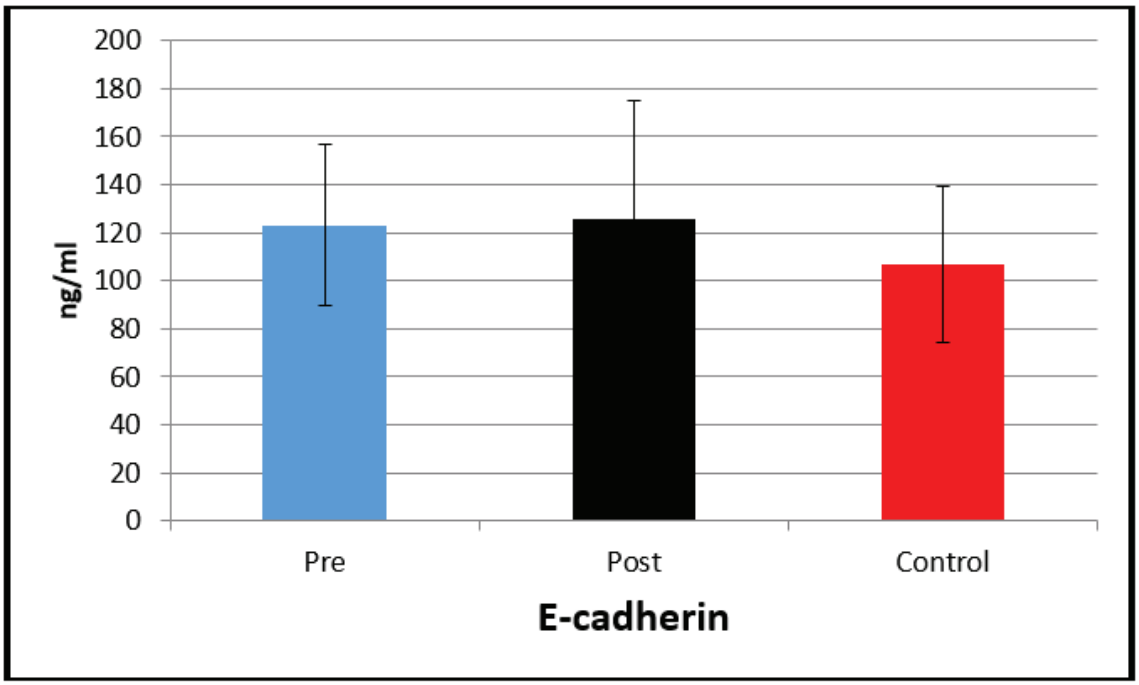

Figure 1.1: Tumor marker E-cadherin

There was non-significant diversity in E-cadherin between the patient (as a new patient and after the first chemotherapy medication and control groups table (1.1).

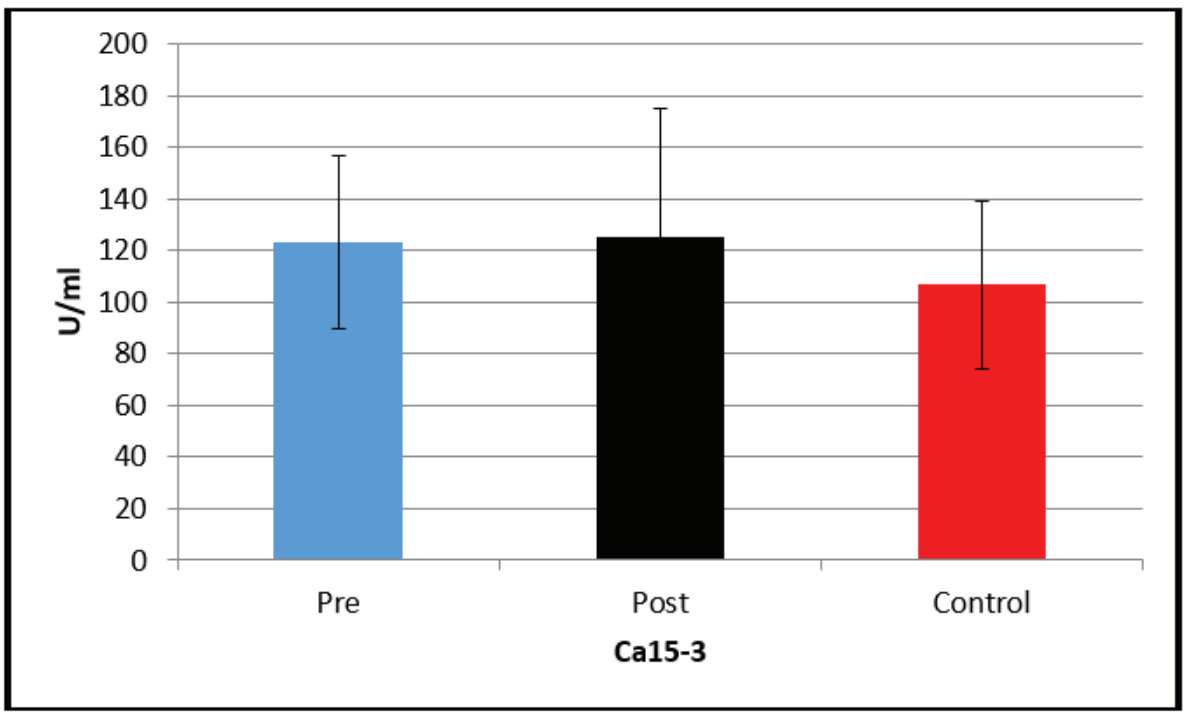

Figure 1.2: Tumor marker CA15-3

There were significant variances in CA15-3 linking the patient (as a new patient, after the first chemotherapy medicine and control groups figure (1.2).

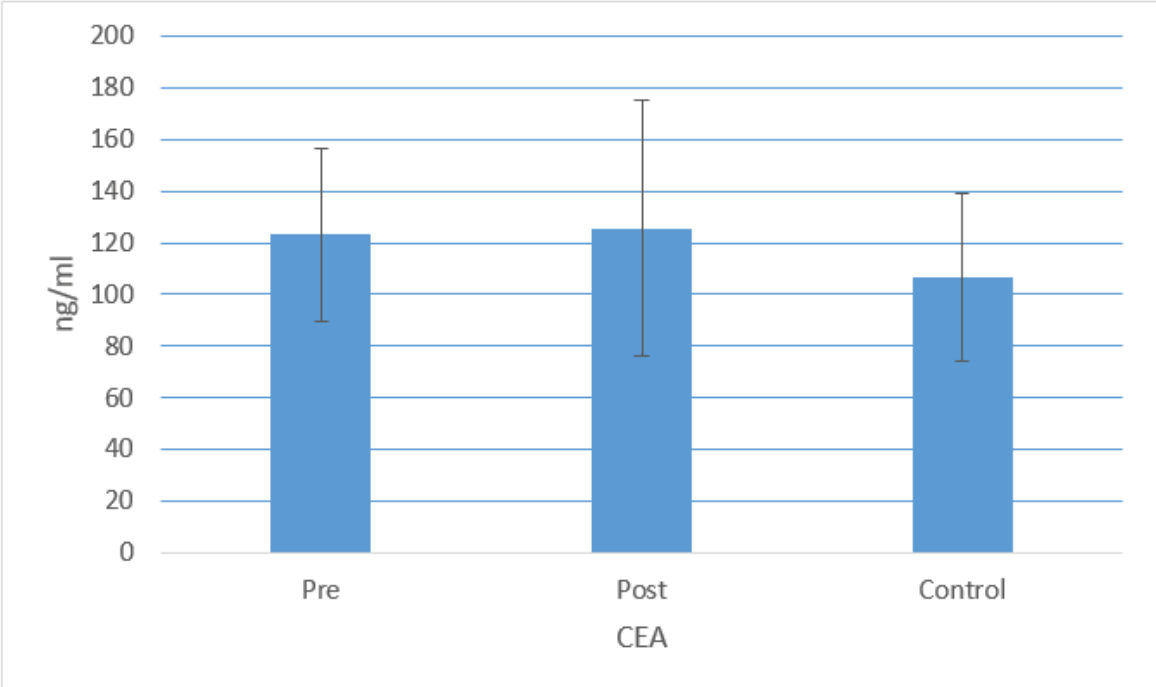

Figure 1.3: Tumor marker CEA

There were significant differences in CEA between patient (as new patient and after first chemotherapy treatment and control groups figure (1.3).

Discussion

E-cadherin, a fragment of the cadherin superfamily of transmembrane glycoproteins, is a linker protein of cell-cell connections [17]. The practical loss of E-cadherin is known to have been viewed as several essential hallmarks of epithelial and mesenchyme translocation (EMT), which causes tumor cell distribution and finally increases cell migration and invasion[18]. Additionally, the absenteeism of E-cadherin appearance has an intimate relation with the resistance of tumor cells to chemotherapy and radiotherapy[19]. Countless researches have appraised the cooperation amid decreased E-cadherin diagnosis and the prognosis of breast cancer patients. However, the results are variable[17]. E-cadherin immunostaining can be used in determining patients with an agreement with outcomes among node-positive patients. Loss of the appearance of E-cadherin is a very urgent change in lobular breast carcinogenesis, and the rutile protein acts to suppress the tumor and suppress the invasion. E-cadherin staining can improve discrimination within lobular carcinoma in situ (LCIS)/ lobular carcinoma and ductal in-situ (DCIS)/infiltrating duct carcinoma denoting the anchorage of DCIS or infiltrating duct carcinoma[20]. The contrasting reports of E-cadherin damage as a predictor of heightened invasiveness, metastatic potential, and lower endurance [21]. Two markers of tumor are used widely to screen and monitor cancers, CA15-3 and CEA, and the FDA has been called upon to monitor breast cancer [22][23]. Unfortunately, they are not usually used for screening due to their flatulence and breast cancer specificity [24].Tumor markers are more prominent in targeted or metastatic breast cancers than in early stage breast cancer [10]. Tumor markers are regularly elevated linked with frequency ora medication position, which can be beneficial in the outcome of the observing strategy [25]. The significance of preoperative tumor marker rise was many kinds of research have reviewed, but a unison has not yet reached. However, many early outlines show compatible results. In breast cancer cases, CEA and CA15-3 levels of preoperative serum are more prominent in progression with massive tumor mass, LN metastasis,

or higher histological grades. This hints that raised tumor markers are correlated to an improved tumor burden[23][12][26][27]. Some previous studies revealed that aerial CEA and CA15-3 are predictors of all breast cancer subtypes, while others have noted differences in predictive power according to the subtype. It was announced that elevated CEA and CA15-3 are more regularly recognized in the luminal subtypes than in the non-luminal subtypes [28]. The CA15-3 elevation is uniform in the Luminal A subtype, and that patients have lower expectations [29]. The more sensitive predictive marker for breast cancer is CA15-3 than CEA [30].

Conclusion

The important tumor marker E-Cadherin was shown the followed that non-significant for breast cancer patients furthermore Tumors markers (CA15-3.CEA) shows significance with breast cancer patients.

Declaration of contending class: The scholars state no dispute of credit work.

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Microsafe (Oral Care) to Control COVID-19 Infection

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Abstract

Dental practice poses a possible risk of cross-infection with COVID-19 among patients, dentists, and their assistants by direct saliva exposure and indirect contact. At Al-Kafeel Hospital in Karbala, Iraq, This study has focused on using Microsafe (Oral car) as a mouthwash product to COVID-19 control infection.

Materials and Methods: One hundred people used the Microsafe (oral care) as a mouthwash product to prevent COVID-19 for two months. Fifty persons from Al-Kafeel Hospital staff (dentists) are fully committed to prevention measures, and fifty persons from the Al-Kafeel Hospital patients regularly attend hospital treatment. Not all patients can be guaranteed and apply these preventive measures.

Results: After two months of using Microsafe (oral care) as a mouthwash, only eleven of the total patients were affected with COVID-19. Only three of the hospital staff and eight patients were affected.

***Keywords**: - Microsafe, oral care, COVID-19, dentists,*

Introduction

Overview of COVID-19: The Virus Taxonomy has established the etiologic agent responsible for this disease as Extreme acute coronavirus-2 respiratory syndrome (SARS-CoV-2). It has similar genomic characteristics to SARS-CoV, which triggered a pandemic in 2002. COVID-19 has been the primary pathogens of emerging respiratory illnesses or respiratory diseases. These are a big family of single-stranded RNA (+ssRNA) viruses distinguished from various species of animals (1) these viruses can cross species boundaries for reasons yet to be clarified and may cause human diseases differing from common colds to more severe conditions such as MERS and SARS.

The majority of infected people with the COVID-19 virus, and without requiring any medical treatment, will undergo mild to moderate respiratory disease and recover. Like those underlying medical problems such as cardiovascular disease, diabetes, chronic respiratory disease, cancer, and older individuals are more likely to experience severe illnesses. The COVID-19 virus propagates primarily by saliva particles or nasal discharge when infected individual coughs or sneezes.

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COVID-19 Transmission

When primary cases of COVID 19 were directly linked to a market, "Huanan Seafood Wholesale Market of Wuhan." The primary cause was believed to be animal-to-human transmission. Still, the following reports of infection were not related to this kind of transmission, so it is believed that the infections were transmitted from one person to another. Individuals who display symptoms are most of the group have the only way to control this outbreak is by isolation.

It is suspected that an infection is spread by droplets, coughs, or sneezes, as is the case for other respiratory pathogens such as flu and rhinovirus.

The incubation period can generally be within 3 to 7 days (mean 5.1 days, SARS equivalent)(2) and up to 2 weeks as the maximum period from infection to symptoms was based on information from the first infections in Wuhan and on research conducted by the CDC and local CDCs in China. 12.5 days Yeah, 12.5 days (95 percent CI, 9.2 to 18) (3). This data shows that this latest outbreak doubled every seven days, whereas the simple reproduction number (R0 - R naught) is 2.2. This means, on average, each patient transmits the infection to an additional 2.2 human beings. Of note,

in 2002-2003, the R0 estimate for the pandemic was approximately (3, 4).

The main type of human coronavirus transmission

There is an interaction between one person to another by respiratory particles caused by respiration, sneezing, coughing, etc., as also by direct or indirect contact with the infected person, virus transmission from infected fomites to the mouth, nose, and eyes via a hand-mediated transmission (5, 6). This virus mainly and directly targets the respiratory system, and the virus is transmitted to the respiratory system through the nose and mouth.

Preventing infection and slowing COVID-19 transmission:

The vaccine of COVID-19 is predicted to be ready at the beginning of 2021(7). The "US National Institutes of Health" recommendations do not prescribe any drug for the avoidance of COVID-19, prior-to or following SARS-CoV-2 virus exposure, outside the framework of the clinical trial (8, 9). A crucial aspect of handling COVID-19 is attempting to minimize and prolong the outbreak peak, called "flattening the curve" without a vaccine, other prophylactic steps, or successful therapies (10). This is achieved by slowing the infection rate to minimize the risk of overwhelming health care facilitating better care of existing cases, and avoiding new cases until appropriate treatment or vaccine is available (10, 11).

Infection control Personal protective equipment:

1. Face masks

WHO recommends that human being wear non-medical clothing and masks when in public places to decrease infection with COVID 19 (12, 13, 14, 15); this recommendation aims to restrict the spread of the virus by people without symptoms, as these masks limit the transmission of exhaled droplets scattered when talking, breathing, and coughing (7).

2. Social distancing

Through the closure of schools, workplaces, travel restrictions, the elimination of large crowded gatherings, social distance strategies aim to restrict contact among large groups of infected individuals (17). Distancing recommendations also involve people staying at least 2 meters apart.

3. **Hand-washing and hygiene**

WHO and the Centers for Disease Control (CDC) recommend a tissue over the mouth and nose during coughing or sneezing, and if no tissue is available, WHO suggests using the inside of the elbow (13, 14*, 18)*. Good hand hygiene is recommended after any cough or sneeze (14, 19). The WHO also advises people to clean their hands daily with soap and water for no less than 20 seconds, especially after using the toilet or dirty hands before meals. After blowing the nose (20). The Centers for Disease Control suggests using an alcohol-based hand sanitizer of at least 60 percent alcohol, but only when soap and water are not readily accessible (5). For areas where hand sanitizers are not available, WHO offers two local production formulations. In these mixtures, the antimicrobial activity comes from ethanol or isopropanol. The hydrogen peroxide is used to kill bacterial spores in alcohol "it is not an active substance for hand antisepsis." Glycerol is used as a moisturizer (1).

Background about Dermacyn

Dermacyn is a stable free radical solution, was initially applied to the surface and equipment as an antibacterial. Nowadays, Dermacyn was proven effective in controlling and treating infections of diabetic foot ulcers.

By cleaning the wound mechanically prevents side effects as a topical agent and develops resistance through antibiotics. This product has been proven to eliminate many bacteria after 15 minutes of topical application on a wound ، and, most importantly, bacteria MRSA.

Ingredients of Dermacyn

"Oxidized Water 99.97%, sodium chloride 0.023%, hypochlorous acid 0.003%, sodium hypochlorite 0.004%."

Properties

1. Neutral ph solution.
2. Super oxidized.
3. Non-toxic.
4. Alcohol-Free.
5. Non-Corrosive.
6. Non-Flammable.

7. Non- irritant.

8. Indicated to have vast and powerful bactericidal, fungicidal and virucidal action in less than 30 seconds.

9. Zero side effects.

"Dermacyn effectively kills gram-positive and gram-negative bacteria (including MRSA) viruses, fungi, and bacterial spores."

STUDY

Name of Organism	Log10 Reduction	Time to Kill	Percent Reduction
Acinetobacter baumannii	6.3692	30 seconds	99.9999%
Bacteroides fragilis	7.6435	30 seconds	99.9999%
Candida albicans	6.3345	30 seconds	99.9999%
Clostridium difficile (spores)	5.8779	30 seconds	99.9977%
Enterobacter aerogenes	6.0881	30 seconds	99.9999%
Enterococcus faecalis-VRE	6.3646	30 seconds	99.9999%
Enterococcus faecium-VRE MDR	6.5119	30 seconds	99.9999%
Escherichia coli	5.6990	30 seconds	99.9998%
Escherichia coli ESBL (Carbapenemase +)	5.9865	30 seconds	99.9999%
Haemophilus in uenzae	5.1775	30 seconds	99.9993%
Klebsiella oxytoca-MDR	6.0492	30 seconds	99.9999%
Klebsiella pneumoniae	6.1430	30 seconds	99.9999%
Klebsilla pneumonia KPC3 (Carbanpenemase +)	6.0952	30 seconds	99.9999%
Micrococcus luteus	5.8420	30 seconds	99.9999%
Proteus mirabilis	6.2028	30 seconds	99.9999%
Pseudomonas aeruginosa	5.8096	30 seconds	99.9998%
Serratia marcescens	5.9978	30 seconds	99.9999%
Staphylococcus aureus-MRSA	6.3454	30 seconds	99.9999%
Staphylococcus aureus	6.2266	30 seconds	99.9999%
Staphylococcus epidermidis	6.0233	30 seconds	99.9999%
Staphylococcus haemolyticus	5.9112	30 seconds	99.9999%
Staphylococcus homins	5.4456	30 seconds	99.9996%
Staphylococcus saprophyticus	5.9590	30 seconds	99.9999%
Streptococcus pyogenes	6.7160	30 seconds	99.9999%

Figure 1 Dermacyn working mechanism:

Foreign matter is not eliminated but destroyed by a purely natural physical process. The critical factor in the body's innate immune system is hypochlorous acid (HOCL).

Disruption of the cell wall

The hypochlorous acid that surrounds single-celled microorganisms attacks the cell walls of the microorganisms and increases their permeability.

Cytolysis, or osmotic lysis -the bursting of the cells

The hypertonic Dermacyn Wound Care solution ensures that the solution increasingly flows into the cells to equalize the osmotic. Gradient. The result is osmosis increasing internal pressure causes the cells to burst.

This effect causes a decrease in the effective elimination of pathogens.

Microsafe (oral care) (Mouth & Throat Antiseptic)

An oral sterile solution of stable, super oxidized water with neutral ph (6.2–7.8) Proven efficacy.

The respiratory system is the primary focus of the COVID-19 virus. Since it can reach the respiratory system through the nose and mouth, it is essential to wear a mask to minimize the infection risk and avoid touching the face and eyes because the virus can also be transmitted through the eyes. Therefore, maintaining not to touch the face, as well as avoiding touching the eyes.

Benefits Microsafe (oral care) in 30 second

1. Non-toxic.
2. Non- irritant to the oral mucosa.
3. Zero potential for microbial resistance.
4. Zero side effects.
5. Compatible with any additional topical or systemic

treatment.

6. Enhance wound healing in the oral cavity.
7. Direct application, without diluting—Ready to use (21, 22). Some studies showing the Microsafe (oral care)

1. Superior efficacy and tolerability over Povidone-iodine 1% w/v (23).
2. Important and potent antibacterial impact towards E. Biofilms of faecalis in root canals (25).
3. Significantly favorable safety profile compared to NaOCI (24)
4. Significantly broader & faster bacterial kill rate compared to Povidone-iodine 1% w/v (23).
5. Reducing inflammation through inhibits 80% of histamine release caused by mast cell degranulation (24).
6. Tissue oxygenation levels doubled and sustained for up to 36 hours from baseline after 30sec exposure to Microcyn Technology.
7. Clinically proven to promote fibroblast migration (25).

Materials and Methods

In 2020 from April to September, 100 People used 15ml twice daily of Microsafe (oral care) as a mouthwash product to prevent COVID-19. 50 persons from Al-Kafeel Hospital staff (dentists) are fully committed to prevention measures, and 50 persons from the Al-Kafeel Hospital patients regularly attend hospital treatment. All persons who suffered from respiratory symptoms were excluded from this study.

The 50 dentists were in direct contact with COVID 19 patients. All study populations were followed clinically, and there are no disorders recorded.

Conclusion

This research provides direct evidence of Microsafe (oral care) viral activity against SARS-COV-2 in just 30 seconds. Microsafe (oral care) mouthwash can aid personal protection from the risk of infection and transmission of COVID 19, especially when there are oral interventions, as is the case in dental practice.

Ethical Clearance- Taken from the Department of dentistry scientific committee in Al Kunooz University

Source of Funding- This study is self-funded

Conflict of Interest - The authors declare no conflict of interest.

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The Impact of Antioxidants in Blood Pressure and Free Radicals of Athletes

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Abstract

Many reports have defined oxidative stress as an imbalance between an enhanced reactive oxygen/nitrogen species and the lack of protective ability in antioxidants. From that point of view, free radical-induced damage caused by oxidative stress seems to be a probable contributing factor to the pathogenesis of many newborn diseases, such as respiratory distress syndrome, bronchopulmonary dysplasia, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, and retinopathy of prematurity. This study was conducted on a sample of (30) players. researchers used the experimental approach (equivalent groups for its suitability and method of study. the sample was divided into two groups, (15) players representing the control group and (15) players representing the experimental group. the study aims to identify the effect of antioxidants on oxygenated blood pressure and free radicals for athletes. the study showed that there is an effect of antioxidants (vitamins on increasing the activity of the enzyme (SGOT) involved in ATP rebuilding processes, and an effect of antioxidants on reducing oxygen blood pressure while not the effect of antioxidants on SGPT enzyme activity is clear, which shows that it is not involved in reducing free radical, muscle fatigue and blood pressure. Hence, the researchers came out with a set of recommendations, including the need to pay attention to taking vitamins because of their role in reducing the effects of free radicals and emphasizing the gradual increase in training loads.

Keywords: *free radicals, oxidants, antioxidants, Blood Pressure, Proteus*

Introduction

The rapid development of the science of sports physiology and its comprehensiveness for much athletic performance and interpreting physiological responses and adaptations provided by this science is the cornerstone of the development of motor learning methods and sports performance, and understanding and interpreting the results and its evaluation is the way to an increase in the level of physical performance. [1,2]

Sports games have witnessed a remarkable and rapid development through studies and research conducted by researchers and experts at various levels, fields, and training programs developed by coaches and experts the level of players' performance and make it advanced, thus this is reflected in the functional and physiological systems of athletes, which may lead to negative changes in these devices. Especially if the diet is weak. Therefore, the concepts of free radical have appeared recently, associated with harm and damage to athletes, especially in cases of high or medium-intensity

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training, and for a long period. Because sports training has become a scientific study based on facts, theories, and foundations, the training processes, must on the continuous physiological evaluation of performance under safe limits and not expose the athlete to any damage for his living cells as a result of their oxidation. Which causes damage and deficiency in immunity, and diseases that may shorten the athlete's life, including the rise in oxygen blood pressure, which is one of the dangerous diseases to human life. They cause damage to some tissues of the body and reduce the supply of oxygen-rich blood to the tissues that will lead to its death. [3,4]

This increase in blood pressure will lead to chemical changes occurring inside the athlete's body, including changes that occur in the enzymes. These changes interpret the presence of damage or ulceration in the muscle that makes the athlete unable to continue with athletic performance or the damage may appear two days after the stressful random exercise. The trainer and athlete must be familiar with the correct training methods and according to the athlete's abilities. He must also be familiar with the nutritional programs that help the athlete get rid of free radicals, and these programs are called antioxidants. [5,6]

As antioxidants are an important prerequisite for athletic excellence as they provide the body with fluids and energy, but the choice of antioxidants (food) must be appropriate to the nature of the practitioner's activity and the goal of this food is to resist oxidation and work to increase the body's ability to possess antioxidant elements to get rid of free radical (Muscle ulcers). [7–9]

Through the foregoing, the importance of the research lies in clarifying the importance of antioxidants (nutrition) on oxygenated blood pressure and muscle pain of late-onset resulting from the free radical. [10–12]

The Research Problem:

Through the experience and the follow-up of researchers for many sports team players. They noticed that, for most coaches, when the trainer gives training units of high intensity to the players, this affects the functional tissues and systems of the body and thus the feeling of fatigue and acute pain in the body's muscles after the end of the training unit.

Hence, the research problem lies in identifying the physiological causes that lead to the occurrence of pain and fatigue, as well as ways to eliminate or reduce these pains by administering antioxidants before the severe and moderate training units.

The Research Objectives:

1- Identification of the effect of antioxidants on the oxygen blood pressure of athletes for the two research groups.

2- Identification of the effect of antioxidants on the free radicals of athletes for the two research groups.

The Research Hypotheses:

1- There are statistically significant differences for the antioxidants on the oxygenated blood pressure between the pre-and post-tests.

2- There are statistically significant differences for the antioxidants on the loose fissures between the pre-and post-tests.

Theoretical Studies:

Decentralizing Exercise (contraction by extension):

Muscle strength is one of the basic components of athletic training, so it is necessary to analyze the components of strength in addition to muscle inflation and nervous adaptation. It is the muscle contraction by lengthening (decentralizing), which accompanies strength training. The summary of muscle contraction is the regulation of the oppositional action resulting from the spindle sensory particles that inform the central nervous system about the length and rate of muscle length change, which increases muscle contraction. Sensory particles present in muscle tendons, where these particles inform the central nervous system about the tension on the tendon, which reduces muscle contraction. Muscle contraction can be defined as lengthening. It is a muscle contraction in which the length of the muscle changes without a change in the amount of tension. The movements produced by them remain stable, such as the movements of lifting, pushing, walking, running, and jumping. An athlete can continue to produce muscle strength for a short period before the energy stored in the muscle begins to deplete. [13,14]

Researches have shown that endurance and strength training places a burden on the antioxidant mechanisms in the blood and cells that work to protect the body from free radical and associated damage, as well as violent physical training accompanied by muscle damage even among athletes with high levels. Antioxidants are compounds that can get rid of free radicals. It has been found that people who practice these exercises randomly are more vulnerable to damage compared to athletes who exercise regularly. (15,16)

Antioxidants:

From a chemical point of view, any compound of free nature is placed on top of its symbol, and on the right is a point indicating the extra electron acquired, such as O2, which is the free form of oxygen, in addition to the electron negative signal due to this electron, and it is noticed that oxygen exists in the form of a molecule of two atoms because both of them are free-form. Thus, they interact with each other and form an oxygen molecule. (17,18)

Oxidation is known as removing electrons or hydrogen or adding oxygen and vice versa. While Reduction is: adding electrons or hydrogen or removing oxygen, and because of the danger of the oxidation process by free radicals, the cells of the body are armed with impenetrable defenses for that, which are types of them. (19,20)

1- Chelating Metal Traps.

2- Breaking Free Radical into Less Dangerous Forms.

3- Suicidal Antioxidants.

Protein blockers of transition minerals, such as iron and copper that stimulate the production of free radicals, as well as suicide antioxidants that interact with free radical without turning into a free radical, as well as regenerative antagonists such as vitamins (E, C) as well as enzymes that break free radical into Less dangerous forms. (21)

All major cell molecules in living organisms are potential targets for oxidative damage. Therefore, cells are prepared with specialized fibers to prevent this damage, which are antioxidants, by preventing or terminating oxidative chain reactions, removing free radicals, or repairing their damage. (22)

Therefore, antioxidants can be defined as any substance or mechanism that prevents the formation of free radicals or removes them after their formation, or fixes the resulting damage. These antioxidants consist of integrated systems in the human body that include: vitamins, enzymes, and other non-specialized minerals. (23,24)

Some sports disciplines have shown that supplementing with vitamin E and C or other antioxidants leads to a decrease in evidence of oxidative stress induced by physical performance, as well as that continuous physical training leads to a decrease in oxidative stress resulting from physical training as well as a more efficient level of antioxidant mechanisms compared to non-athletic individuals. (25,26)

At the end of this topic, we recommend that individuals with physical activity have a diet rich in antioxidants.

Oxygen Blood Pressure:

Blood pressure is defined as the pressure caused by a rush of blood from the heart to the arteries, causing it to expand, and its amount ranges between systolic pressure and diastolic pressure. The similarity in weight, height, and age. To understand what is happening in the blood circulation inside the body, we must know that the heart presses the blood and pushes it to reach the various parts of the body. When the heart contracts, the blood pressure reaches its peak and is called systolic pressure. Blood pressure in the form of diastole of the heart by one-third. So, if it turns out that the blood pressure measured for an athlete is 80/120, these two numbers express the clear difference between the systolic and diastolic pressures and that the athlete is in good health. (27)

Many causes lead to high blood pressure, including unregulated random physical exertion, which leads to a temporary increase in systolic blood pressure, which may reach from 30-50 mm. However, it quickly returns to normal after the end of the physical exertion. (28)

The researcher explains this increase in blood pressure after physical exertion as the result of the decrease in the amount of oxygen in the muscles of the

athlete, which leads to an increase in the need to fill this deficiency by increasing the work of the heart to deliver sufficient amounts of blood loaded with oxygen and nutrients to the athlete's muscles to return to a normal state, especially after exercise ends, so we find the athlete breathing very quickly and through this increase in the heartbeat, the pressure will be generated on the walls of blood vessels, which is called oxygen blood pressure, and thus this increase will lead to collateral damage in the case of high pressure, including tissue damage as a result of the arrival of large amounts of oxygen to muscles irregularly. (29)

Kiyici confirmed this, "There are many external influences on the formation of free radical, including inhalation of air containing oxygen at a concentration greater than normal." (30)

Delayed-onset muscle pain:

Those physiological states that are ongoing in the body as a result of tense activity are understood under the term muscle pain. Represented by the incompatibility of its functions and the temporary decline of its ability to perform. The phenomenon of pain occurs in humans in a more complex manner. This is related to the biological role in protecting the body at a time from exhaustion in the event of a prolonged period of muscular effort or its tension in tolerating physiological changes in the case of severe fatigue and clear features, where the reaction to it is pain in the working muscles. (31)

As for Abu Al-Ela, he says that the general outcomes of performance that have not been trained or violent training is the occurrence of muscle pain delayed onset, as this pain appears after a day or two after training, while muscle damage appears after three days of physical performance. (3,6,12,31)

Although training with lengthening muscles is the cause of both delayed muscle pain and muscle damage, the damage to the connective tissue around these muscles, and this damage is the cause of a sense of muscle pain source. (24,25)

It has been found that the entry of fluids into the fibers after damage to them leads to an increase in the pressure inside them, as well as the sensitivity of these receptors may increase due to the chemicals released from the inflammatory reaction of the damaged muscles or as a result of the activity of free radical. (14,15)

Free Radical:

First of all, we must know what the free radicals are. To be confident that the main enemy of the athlete is the formation of free radicals if this component is not alerted and also not to work to resist this enemy. (5,18)

We must also be sure that as long as we use oxygen and the body processes it to produce the energy needed for it, about 2: 5% of it leaks out of the cellular control range and this leads to the formation of free radicals. (2,3)

As it was in the recent past that the concept of free radical is: the presence of an atom or atom in a molecule that has one or more non-double electrons, this lack of pairing makes it more voracious to interact with other materials to complete the pairing process and during that process damage to tissues and cells occurs. But as a result, scientific development and accurate research found that free radicals consist of four types, which are: 1- Nitrogen radical 2- Oxygen radical 3- Fat radical 4- Free toxin radical. (22,24)

Oxygen-free radicals are formed during the physical performance from oxidation reactions in the respiratory chain, as these clefts represent 4: 5% of oxygen during breathing, in addition to that, physical training results in free radical by other means, including: (11)

The endogenous oxidation of adrenaline and its derivatives, the accumulation of lactic acid that converts the relatively weak radicals, such as superoxide, into other radicals such as the hydroxide radical, and also produces an inflammatory reaction associated with muscle damage. (24)

When performing physical training, the muscle's need to consume oxygen increases, as we showed it previously and this tremendous increase leads to an increase in free oxygen radicals as a waste of oxygen leaked from this process, and the changes that occur in the dynamics of the blood after the end of physical activity and the rapid rush of blood to the organs from which it came (re) Perfusion) This process creates the most dangerous free radicals. (26)

Research Methodology and Field Procedures:

1-Research Methodology:

The researchers used the experimental method (equivalent groups) due to its suitability to the nature of the study.

2-The Research Sample:

After the researchers identified the research community represented by some (40 advanced) volunteer athletes, then the research sample was randomly selected.

After that, the researcher distributed the sample, randomly, into two groups, in the manner of even and odd numbers, and they were distributed as follows:

o (15) players representing the experimental group.

o (15) players representing the control group.

To ensure the homogeneity and parity of the two groups, the researchers applied the T-test of independent and independent samples on the results of the pre-and post-tests of the two groups, and after detection (1) it appeared that there were no significant differences between the two groups, if the value was smaller than the tabular one, this indicates the homogeneity of the two samples and their equivalence as in Table (1).

Table (1): The homogeneity and equivalence of the two Groups' samples on the research variables

#	Variables	Measurement units	Control (Before)		Experiment (Before)		T Value	Significant level
			Mean	SD	Mean	SD		
1	The enzyme (SGOT)	U/L	11.54	0.72	11.87	0.69	0.8	Not Significant
2	The enzyme (SGPT)	U/L	7.8	0.55	7.77	0.84	1.02	Not Significant
3	Blood Pressure	Mm Hg	11.7	0.48	11.24	0.38	0.55	Not Significant

The homogeneity and equivalence of the two research samples with the research variables show the significance of the tabular T value 2.10 at the level of significance 0.05 and freedom 9.

Tests and measurements used in the research:

The researchers used blood pressure measurements and the enzyme SGPT, SGOT, by analyzing blood in the laboratory.

How tests were taken:

The pre-tests on the sample 6/15/2020 by drawing a blood sample for each player before the start of the exercise and placing it in ampoules titled with the names of the players in order not to be confused. They were taken to the laboratory for analysis and discovery of the research variables (SGPT, SGOT) and measuring blood pressure. The purpose is to achieve equivalence between the control and experimental groups, and Table (1) shows that.

Training Unit:

After the experimental and control sample, the doses of vitamins C and E as tablets, to the experimental group and for a period of (10) ten days before the pre-test, while the control group was not given vitamins, and after that, the research sample (experimental and control) was given a high-intensity training unit.

Post-Tests:

The post-tests were conducted on a sample 6/25/2020

and under the same conditions and variables that were in the pre-tests except for the experimental group variable which is vitamins E, C, A. The test was conducted at the end of the training unit after performing a jogging exercise for (18) minutes continuously and with high intensity. After completing the training unit and after (24 hours), a blood sample is taken from the experimental and the control research samples for analysis in the laboratory to find out the amount of SGPT, SGOT, which reflects the amount of free radical in the body.

Statistical Methods

1- The arithmetic mean

2- Standard deviation

3- T-test for independent samples

4- T-test for non-independent samples

5- Presentation, analysis, and discussion of the results.

The researchers presented the results got through implementing the nutritional program and after giving a physical effort; the data were taken and treated statistically and the results were arranged as tables for all research variables and both the control and experimental groups in the pre and post-tests. To find out the truth of the differences, the researchers used the T-test for asymmetric samples between the two groups. T-test for the corresponding samples to find out the difference between the pre and post-tests for one group

Presentation, analysis, and discussion of the results of the pre and post-tests for the control group:

Table (2): The values of the arithmetic mean and standard deviations of the control group in the pre and post-tests, the calculated and tabular T value, and the level of significance on the research variables

#	Variables	Measurement units	Control				T Value	Significant level
			Before effort		After effort			
			Mean	SD	Mean	SD		
1	The enzyme (SGOT)	U/L	11.54	0.72	10.2	0.22	2.63	Significant
2	The enzyme (SGPT)	U/L	7.8	0.55	7	0.75	1.75	Not Significant
3	Blood Pressure	Mm Hg	11.7	0.48	13.6	0.35	3.27	Significant

It shows the values of the arithmetic mean and the standard deviations of the control group in the pre-and post-tests, the calculated and tabular T value, and the level of significance.

Tabular T value 2. 26 at a significance level of 0.05 and freedom 9

By displaying the results of the tests shown in Table No. (2) before the effort and after the effort for the control group, it emerged that the arithmetic means of SGOT enzyme activity is (11.54) and the standard deviation is (0.72) in the pre-test, while the arithmetic mean was reached in the post-test of activity The enzyme is (10.2) and a standard deviation (0.22), while the calculated T value was (2.63), which is greater than the tabular value, which is 2.26, at a level of significance (0.05) and freedom (9). This shows the significant difference between the pre-and post-tests. And this drop in the enzyme activity that the researchers attribute to its use during rebuilding ATP energy and its depletion and the lack of compensation, which leads to the occurrence of free radicals and therefore the player feels tired, and this was confirmed by Dekkers, "that the supply of vitamin E besides its ability to increase the (Oxidative) In the muscles, it improves the body's reserve of enzymes, inhibitors of oxidation [26]

As for the activity of the SGPT enzyme, the test showed that the arithmetic mean is (7.8) and the standard deviation is (0.55) in the pre-test, while the arithmetic means in the post-test for the enzyme activity is (7.0) with a standard deviation (0.75), and the calculated T value is (1.75) and it is smaller than the tabular value of (2.26) at the level of significance (0.05) and freedom (9). This shows the non-significant difference between the pre and post-tests of the control group. This confirms that physical exertion does not affect the activity of this enzyme. [17]

As for the blood pressure measurement test, the arithmetic mean is (11.7) and a standard deviation (0.48) in the pre-test and the arithmetic mean in the post-test reached (13.60) and with a standard deviation (0.35), while the calculated value of T was (3.27), which is greater than the tabular value of (2.26) at the level of significance (0.05) and freedom (9). This shows the significant difference between the pre and post-tests of the control group. Whereas, this increase is caused by high physical exertion, which Ahmad confirmed, "that high physical exertion leads to a temporary increase in systolic pressure that may reach (30 - 50 mm Hg) and quickly returns to normal after rest.". The researchers also attribute this. The increase in blood pressure means that players do not take vitamins that help reduce blood pressure and maintain the amount of energy that the player needs during the performance and thus reduce the work of the heart to deliver the largest amount of blood loaded with oxygen, which causes high blood pressure inside the blood vessels, and during this process free radicals took place. [29]

Presentation, analysis, and discussion of the results of the pre-and post-tests of the experimental group:

Table (3): The values of the arithmetic mean and standard deviations of the control group in the pre and post-tests, the calculated and tabular T value, and the level of significance on the research variables

#	Variables	Measurement units	Experiment				T Value	Significant level
			Before		After			
			Mean	SD	Mean	SD		
1	The enzyme (SGOT)	U/L	11.87	0.69	12.6	0.36	3.15	Significant
2	The enzyme (SGPT)	U/L	7.77	0.84	8.1	0.11	1.54	Not Significant
3	Blood Pressure	Mm Hg	11.24	0.38	12.53	0.59	2.02	Not Significant

It shows the values of the arithmetic mean and the standard deviations of the control group in the pre and dimensional tests, the calculated and tabular T value, and the level of significance

The tabular T value is 2.26 at a level of significance 0.05 and degree of freedom 9

By displaying the results of the tests shown in Table No. (3) before the effort and after the effort of the experimental group, it emerged that the arithmetic mean of the activity of the SGOT enzyme is (11.87) and the standard deviation is (0.72) in the pre-test, while the arithmetic mean was reached in the post-test of the activity of the enzyme is (12.6) with a standard deviation

(0.32), while the calculated T value was (3.15), which is greater than the tabular value, which is 2.26, at a level of significance (0.05) and a degree of freedom (9). This indicates the significant difference between the pre-and post-tests, which indicates To an increase in the activity of the SGOT enzyme in favor of the post, but within the normal limits that range between (9-13 U / L) (3), and this increase is attributed by the researchers to the number of antioxidants that the players took before the physical effort, which contributes to the increase in the activity of enzymes The mitochondria where mixture supply of (A, C, E) To maintain or increase muscle enzymes (4), and that this increase will help the player not to feel tired after physical exertion and not have free radicals. [5]

As for the activity of the SGPT enzyme, the arithmetic means reached (7.77) in the pre-test with a standard deviation (0.84), while the arithmetic means in the post-test for the enzyme activity was (8.1) with a standard deviation (0.11), and the calculated T value was (1.54), which is smaller than the tabular value of (2.26) at the level of significance (0.05) and degree of freedom (9), which indicates that the difference is not significant between the pre-and post-tests of the experimental group. This indicates that the vitamins do not affect the activity of this enzyme. [15,18]

As for the blood pressure measurement test, it appeared that the arithmetic mean is (11.24) and a standard deviation (0.38) in the pre-test and the arithmetic mean in the post-test reached (12.53) and with a standard deviation (0.59), while the calculated value of T reached (2.02) and it is smaller than the tabular value of (2.26) at the level of significance (0.05) and the degree of freedom (9), and this indicates the non-significant difference between the pre-and post-tests of the experimental group. The researchers attribute this to the effect of antioxidant (vitamins) in not raising blood pressure except to a small extent, and thus this helped prevent fatigue and free radicals [7,12,16]

To find out the difference between the control and experimental groups in the dimensional tests of the research variables, the researchers resorted to the T-test for non-correlated samples, as shown in the following table.

Table (4): The values of the arithmetic mean and standard deviations of the control and experimental group in the dimensional tests and the calculated and tabular T-value and the level of significance

#	Variables	Measurement units	Control (After)		Experiment (After)		T Value	Significant level
			Mean	SD	Mean	SD		
1	The enzyme (SGOT)	U/L	10.2	0.22	12.6	0.36	4.12	Significant.
2	The enzyme (SGPT)	U/L	7	0.75	8.1	0.11	1.26	Not Significant
3	Blood Pressure	Mm Hg	13.6	0.35	12.53	0.59	3.14	Significant.

It shows the values of the arithmetic mean and the standard deviations of the control and experimental group in the post-tests and the calculated and tabular T value and the level of significance

The tabular T value is 2.10 at a significance level of 0.05 and freedom 9

Through Table No. (4) the calculated T value (4.12) is greater than the tabular value of (2.10) at the level of significance (0.05) and freedom (18), showing that the difference is significant between the post-tests of both the control and experimental groups. The researchers attribute the difference to the nutritional program

comprising vitamins A, C, and E, which had a clear effect in raising the activity of enzymes, including SGOT, and thus avoiding the risk of free radicals (tissue damage) and the feeling of muscle fatigue after exertion, and this is what Dekkers pointed out in One of the "Downhill Running Studies" found that late-onset muscle pain and oxidative stress resulting from physical training were significantly reduced when taking a dose of antioxidants that included vitamin E [26]

As for the activity of the SGPT enzyme, the difference between the arithmetic media appeared in the post-tests of the two research groups, as the calculated value of T reached (1.26), which is smaller than the tabular value of (2.10) at a level of significance (0.05) and freedom (18), which shows that the difference is not Significant among the post-tests. This shows that the vitamins and physical effort do not affect the activity of this enzyme, which shows that the enzyme does not take part in energy processes and therefore does not directly contribute to the reduction of free radicals. [13,26]

Whereas a difference appeared in the arithmetic means for the post-tests of the two research groups in measuring blood pressure, where the calculated value of T reached (3.14), which is greater than the tabular value of (2.10) at the level of significance (0.05) and freedom (18), which shows that the difference is significant for the favor of the experimental group. Which the researchers attribute to the nutritional program comprising vitamins A, C, and E, which had a clear effect in maintaining high blood pressure only slightly, and this is what El Abed showed: "The researchers discovered that a diet rich in vitamins and low in fat helps Reducing blood pressure enough to reduce or stop the need for medication. [1]

Conclusions

- The presence of an antioxidant effect (vitamins) on increasing the activity of the enzyme SGOT inside ATP reconstructions.

- There is no effect of antioxidants on the activity of the SGPT enzyme, which shows that they do not involve it in reducing free radicals, physical fatigue, and blood pressure.

- The presence of an antioxidant effect on blood pressure.

- the results obtained allow us to conclude that the modality of running exercise determines the antioxidant response. From a practical view, during endurance training sessions, it is suggested for coaches and players to adopt an intermittent running training session to avoid free radical damage occurring from continuous exercise.

Recommendations

Through the conclusions reached by the researchers, we can offer some recommendations to coaches and those in charge of college teams, clubs, and national teams.

- Take care of taking vitamins because of their importance in reducing loose fissures before performing the effort

- Increasing physical loads on players gradually, not suddenly

- Future research linking antioxidants to physical exertion and rest

- Establishing laboratories in clubs and universities specializing in blood analysis and providing everything new in raising the level of athletes.

Contributions:

The authors of this original article. Collected, organized, and analyzed all of the data utilized in this article and concluded from the results of the data analysis.

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A Study to Assess Awareness Levels and Status of Lipid Parameters in the Subjects with Coronary Artery Disease in Tertiary Care Setup in a Rural Area

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Abstract

Background:-. Among the various cardiovascular diseases, Coronary artery disease is one of the major etiology of mortality and morbidity in the world wide population. It is a prevalence in mainly older adults and exists in the entire health context. The CAD prevalence was strongly associated with various lipid parameters such as cholesterol and different lipoprotein fraction, which contributed as a major factor for the progression of CAD. In spite of various therapeutic advances, so for safe and effective management of CAD, there are requirements for consideration of risk/benefits and goals of care.

Objective: To determine the current level of knowledge of the symptoms and duration of diseases and also comparison of lipid parameters in cases and control.

Material and Methods:-*The study was a case control observational study conducted in the department of biochemistry, SBKSMI& RC. A total of 200 subjects of 100 Coronary artery disease (CAD) and 100 healthy control were enrolled to assess lipid parameter and duration of diseases.*

Result: - There were 82% male and 18% female in case group and CAD caused majorly by the angina which was 65% and in the symptom of duration, majority of people aware with their symptoms in less than 1 months. In the measurement of dyslipidemia there was significantly higher in atherogenic component i.e. LDL-C and anti atherogenic component i.e. HDL-C was also higher and found significant.

Conclusion: - *The majority of subjects who were suffering from CAD were males compared to females. Most of the patients from a rural areas so they had a chest pain comparison to heart attack and found highly significant with lipid parameters.*

Keywords- *Non-communicable diseases, cardiovascular diseases, coronary artery disease, dyslipidemia*

Introduction

There has been upward graph in the pattern of non-communicable diseases all over the world. The primary reason which can be attributed is to the imbalanced nutrition which is leading to many metabolic abnormalities including diabetes mellitus, cardiovascular diseases.(1) Cardiovascular disease is one of the major contributor of both mortality and morbidity in both developed and developing countries especially like highly populated countries like India.(2)

Development of atherosclerotic plaque within the coronary artery ultimately leading into stenosis in the

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arteries [3]. This is mainly due to an imbalance between the myocardial oxygen demand and supply ratio. The most common causes of CAD is restriction of blood flow to the heart due to narrowing lamina of the artery. [4] This is not a onetime event ant this process takes place over the years as lipids go on accumulating and finally leading to plaque formation. The process of atherosclerosis affects large and medium sized arteries and causes coronary artery disease such as angina pectoris and myocardial infarction , cerebrovascular disease (ischemic stroke and vascular dementia and peripheral vascular disease such intermittent claudication and gangrene. [5]

Coronary artery disease (CAD) is caused by the deposition of atherosclerotic plaque to the coronary arteries results restraint in the continuous blood flow in the heart. Atherosclerosis is a process that develops slowly over time. [6] CAD has become the largest cause of death worldwide and the leading cause of death globally, accounting for 21.9 per cent of total deaths, and are projected to increase to 26.3 per cent by 2030. [7]

One of the established risk factor for coronary artery disease which occurs before the onset of other is risk factors is dyslipidemia. The impact of dyslipidemia on the burden of CAD has large contribution due to its atherogenic property and therapeutic implication. [8] The parameters which are used in the characterization of extent of dyslipidemia are of total cholesterol, low density lipoproteins, triacylglycerols and decreased levels of high density lipoproteins. These parameters are conventionally used in the assessment of the risk for coronary artery disease. Components of circulating lipids include reduced plasma LDL-C and triglyceride concentration have associated with atherogenesis. A single serum HDL-C levels have a role as cardio protective lipid. [9]

In view of the increasing prevalence of this disease one important aspect is if population and those with this disease and without disease have the awareness regarding its symptoms and various therapeutic and dietary management issues that would ultimately improve the disease outcome and help a great way in the prevalence. [10, 11]

Inability to recognize signs and symptoms or duration of causes of diseases has become important reason for delayed presentation. So looking into above aspect we hypothesized this study to determine the current level of knowledge of the symptoms and duration of diseases and also comparison of lipid parameter in cases and control.

Methods and Methodology

The present study was a type of case control study on total 100 outdoor coronary artery diseases (CAD) patients from Dhiraj general Hospital. This study was conducted in collaboration with Biochemistry and cardiology Department, SBKSMI & RC, and Sumandeep Vidyapeeth after getting ethical approval from institutional ethical approval **(Approval No. SVIEC/ON/Medi/BNPG19/D20066).**

Ø A Total 200 subjects of either sex having age group of 40-70 years were enrolled for this study and were categorized into the following two groups.

Ø Case:-100 patients with Coronary Artery Disease (CAD)

Ø Control:-100 Healthy individuals termed as Control Group.

Ø Subjects regarding general information, clinical assessment including symptoms, etiology of diseases and duration of diseases were recorded for each and every subjects with their consent.

Ø Subjects of known case of diabetes mellitus, Liver diseases, kidney diseases, any Nutritional disorder and other than CAD were excluded from the study.

Sample collection: - Blood sample was collected after an overnight fasting. Approximately 3ml of blood samples were collected from an each and every individual and wait for 10 minute and then centrifuge centrifuged at 3000 rpm for 10 minutes in order to get serum.

All the Biochemical parameter were analyzed by enzymatic method on EM-200 fully automated Biochemistry analyzer at central Clinical Laboratory, Dhiraj General Hospital, Sumandeep Vidyapeeth, piparia.

Lipid parameter including total cholesterol (TC), triglyceride (TG) and high density lipoprotein were estimated by standards enzymatic methods. The low

density lipoprotein cholesterol (LDL-c) was calculated by using Friedewald formula (FF) is: LDL-c (mg/dL) = TC (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)/5 [12]

Statistical Analysis: Data were analyzed with the help of software program Statistical Package for Social Sciences (SPSS version 21, Chicago). The data of this study results were presented in the form of mean ± SD values and categorical data were presented in frequency. . Kolmogorov-Smirnov and Shapiro-Wilk tests were used to see that data is following normal distribution. Test of significance (P value) was less than 0.05 was considered as statistically significant.

Results

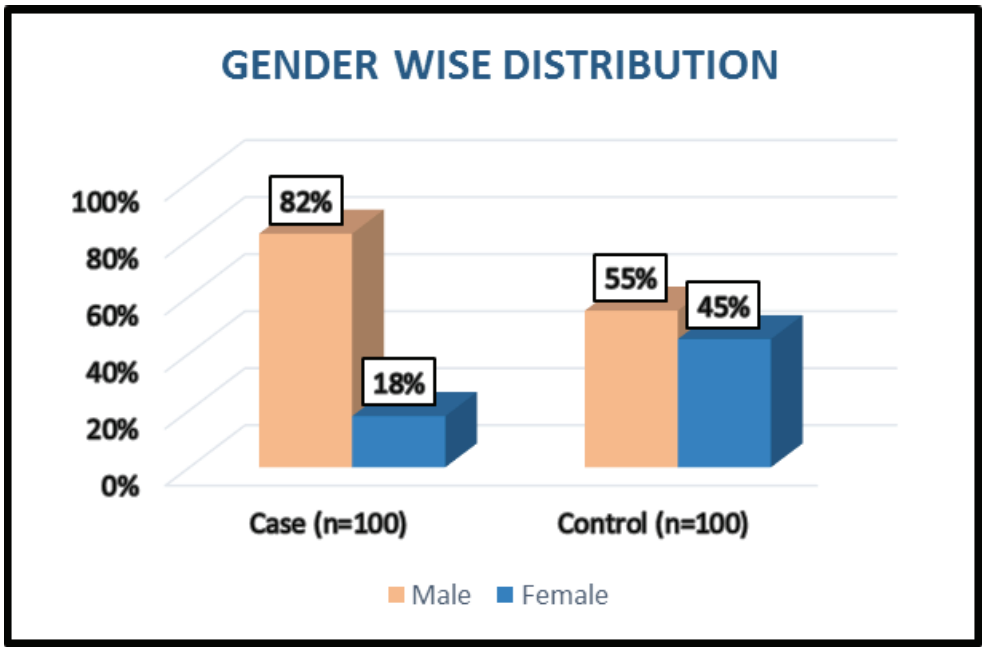

Figure-1: Age & gender wise distribution in Case and Control Groups

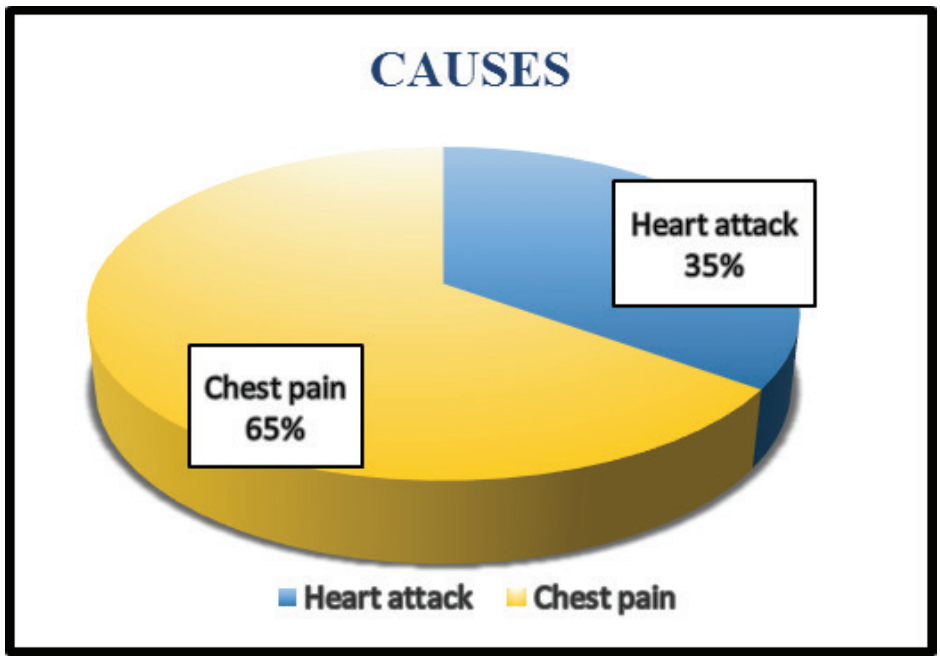

Figure-2: Etiology of CAD in study subjects

Table-1: Duration of disease in Case Groups

Duration Of disease	Frequency
Less than 1 month	67%
1-6 month	15%
1 year	2%
2-3 years	6%
3-6 years	6%
6-8 years	4%

Table-2: - Comparison of age and Lipid parameters between Case and Control Groups.

Variables		Case (Mean ±SD)	Control (Mean ±SD)	Test of significance (P value)
Age		54.38±7.92	45.99±8.67	<0.001*
Lipid parameter	Cholesterol	248.94 ± 55.4	170.7±31.9	<0.0001**
	Triglyceride	226.27 ± 60.8	124.92±38.3	<0.0001**
	HDL	37.25 ± 8.3	48.31±9.0	<0.0001**
	LDL	164.34 ±58.0	99.93±32.8	<0.0001**
	VLDL	36.57±12.1	22.927±41	<0.0016*

P < 0.0001** highly significant, P< 0.05* significant & P> 0.05 # not significant

Discussion

Among the various non-communicable diseases, cardiovascular diseases contributed a major factors which are responsible for the deaths in global population. CVD involves the abnormality in heart and blood vessels (arteries and vein). Various diseases which come under the category of CVD include, CAD. Which is one of the major health problems in developing countries like India. [13] So a total of 200 subjects were enrolled as per inclusion criteria. Out of which 100 were CAD cases and 100 were healthy controls. In this study we have

done the gender wise distribution and found males are more prone to CAD as compared to females **(Figure-1)**. Among the subjects female were 18% compared to the controls, which was 45%. As we all know that gender is non-modifiable risk factor. The incidence of CAD was much more in males compared to females, this may be due to late onset of disease in females after menopause. However, in the present study we did not find much difference between males and females as per the age is concerned. The number of female subjects included in the study was very low so making it difficult to come up any type of conclusion.

Approximately 2% of chest pain patients with acute coronary syndrome (ACS) which is associated with a twofold increase in 30-day morbidity and mortality. Indeed, the absence of typical chest pain does not exclude an ACS [13], to find out the etiology of CAD in rural area and in this study, majority of patients having CAD is due to chest pain (65%) and others due to heart attack (35%) (Figure-2) and in the duration of disease we categorize the duration into less than one month, 1-6 month , 1 year and 2-3 years , 3-6 years and 6-8 years in which the percentile of patients lies under each category was 67% , 15% , 2% , 6%, 6% and 4% (table-1)

Age is also an unmodifiable risk factor for CAD and the mean levels of age in case 54.38±7.92 and control 45.99±8.67 and also found statistically significant (P-0.001). Age advances females at the proportional of death among females also increases. **[14]** A major non modifiable risk factor is an age. A study reported from southern Iranian population **[15]** shows that the age factor of CAD subjects and controls were matched.

There were a close association between the levels of serum lipids in the development of CAD. Among the lipids, serum cholesterol, triglycerides, LDL are the major parameters. When they are increased, lead to the increase in the risk. Whereas, HDL-C known to be protective. These are some important aspects with respects to the various lipids in the subjects with CAD **[16]**

Many epidemiological studies confirm that there is strong relationship between serum cholesterol and Cardio vascular risk**. [17]** In the present study, (**Table-2)** shows the details of age and Lipid parameter of both Case and controls groups. In the case group the Mean ± SD of age was 54.38±7.92 and cholesterol was 248.94 ± 55.4, Triglyceride is 226.27 ± 60.8, HDL is 37.25 ± 8.3, LDL is 164.34 ±58.0 and VLDL is 36.57±12.1. In the control group the age group was is 45.99±8.67 and Lipid parameter includes Cholesterol, Triglyceride, HDL, LDL, VLDL is 170.7±31.9, 124.92±38.3, 48.31±9.0, 99.93±32.8 and 22.927±41 respectively.

The serum cholesterol level in control was less than that of serum cholesterol in the study subjects and Serum triglycerides level in the study subjects and corresponding controls. Derivation of LDL-C varies depending upon serum Triglyceride for deriving in our set up. We have used friedewald's formula. After deducting VLDL + HDL from total cholesterol, the values were taken as the values of LDL cholesterol. Over or under estimation of TAG will affect the net result of LDL. Increase in HDL-C is associated with decrease risk of CHD. **Das and Kumar et.al (2018)** [18] reported a significant decrease in HDL-C levels in the patients with CAD risks. All parameters and age was highly significant p value (<0.05).

Conclusion

The majority of subjects who were suffering from CAD were male compared to corresponding age group females. In this study we have observed that, while eliciting information from them almost 2/3 patients had complaints of chest pain and only 1/3 patients had complaints of heart attack. Out of this majority of those subjects with chest pain found to have CAD. It can be inferred that in this study majority of the subjects had chest pain subsequent to CAD. Like in any other population in this population also the component of dyslipidemia is a major predictor for CAD.

Limitation of the study: The sample size of the present study was small hence a definitive conclusion cannot done. To draw a conclusive trend in this aspect there is a need for the study with large sample size. Furthermore study is needed to establish the concept as we have limitation in sample size.

Issue of Conflict: None

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A Study to Assess the Knowledge on Food Practices among Postnatal Mothers

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Abstract

Introduction: Women need to receive special health and social support during postnatal time to prevent problems. Inadequate care might result in complications for the mother and her child. Nutrition plays an important role in the course of the postpartum period. It is well documented that dietary modification during this period has a profound effect on the nutritional status of both the mother and the newborn. According to recent studies, Sueel Raj and Mishra (2008) examined the food consumption pattern and associated habits of the Bhil tribe of Dhar district of Madhya Pradesh. They found that no special foods are being consumed during pregnancy. Sahoo and Panda (2005) while studying in the Balasore District of Odisha found that some sort of food restrictions during pregnancy. The general concept was that the restricted food items would cause cold and cough to the mother or would lead to abortion. **Aim**: To assess the knowledge on food practice among postnatal mothers. **Materials and Methods**: A descriptive survey research design was done from March 2019 - July 2019 in 30 postnatal mothers. Data was collected with structured questionnaire on knowledge on food practices among postnatal mothers. Questions are How much kcal required for PNC mother. Green vegetables helps in, Dairy product helps in, Protein helps in..etc. Descriptive statistics were represented in Frequency and percentage. Statistical tests used were One way ANOVA test and independent T –test and Chi square test. The level of significance was considered to be statistically significant when the p-value was ≤0.05. **Result**: Distribution of postnatal mothers according to their age in years shows the majority (76%) of them belongs to the age of 20-30 years, Distribution of postnatal mothers according to types of diet that majority of (73%) have vegetarian, 20%non vegetarian, 6% have taking mix diet. The majority 16 (53%) women were having average level of knowledge , 14(46%) women were having good level of knowledge .The minimum score was 4 and maximum score was 11, , the mean score was 7.80 and standard deviation is 2.18 respectively **Conclusion:** This reveals that age group of 20-30years have more knowledge, 16 (53%) women were having average level of knowledge , 14(46%) women were having good level of knowledge

Key Word: *Cultural beliefs, customs, taboos, food practices*

Introduction

The Postpartum period is the state of physiological stress characterized by profound metabolic and hormonal changes. This period is the time of transition for women and their families. Mothers during this time undergo a lot of pressure and concern about how they can get their health and shape back after childbirth, which in some situations may cause psychological problems or disorders. Therefore, women need to receive special health and social support during this time to prevent such problems. Inadequate care might result in complications for the mother and her child. Nutrition plays an important role in the course of the postpartum period[1]. It is well documented that dietary modification during this period has a profound effect on the nutritional status of both the mother and the newborn (Geneva, 1984). During this period, culture plays a major role in the way a woman perceives and prepares for her birthing experience (Al-Kanhal & Bani, 1995). In fact, the notions of birth and postnatal care vary considerably with cultural beliefs and traditional practices[2] Each culture has its own values,

beliefs and practices related to pregnancy and birth. According to recent studies, eating habits after delivery among American women are not very complex. Women simply continue to eat a good quality diet similar to the diet that they used to consume during pregnancy. In contrast, in the Saudi culture, a postpartum female is treated as a sick patient for the first six weeks after her childbirth. A long list of food restrictions and work limitations is typically enforced on her[3]

Good nutrition is important, but it assumes greater importance for women during their pre and post pregnancy period. During pregnancy nutritional needs are higher and meeting those needs will have a positive effect on the health of both the mother and her unborn baby. A pregnancy without negative effects of poor nutrition on the health is the best possible nutritional state. Nutrition deserves special attention during pregnancy and breastfeeding because of the high nutrient needs and the critical role of appropriate nutrition for the fetus and infant's poor maternal diet could be a barrier to women's health so, it must be maintained properly[4]

Any country's social or cultural element highly bears an effect on its citizens, in context of food habits. In spite of the fact that the way of life of nourishment practices are infrequently considered as an imperative instance of health, numerous practices are particularly intended to secure and advance wellbeing. It is genuine; however some food practices and taboos in a few social orders may lead to healthful insufficiencies among specific group of people. Some dietary standards are connected with an exceptional period in women's' life, for example, pregnancy[5]. Despite the fact that this practices and taboos have been rehearsed following genealogical period, there is no single hypothesis as evidence. All individuals whether rural or urban have their own cultural practices. Pregnant women require the need of extensive additional calorie and supplement prerequisites. Taboos are in fact characterized as a practice which are approved or considered legitimate by a society[6]

Pregnancy, Birth, Lactation, Periods of illness or sickness. The temporary food avoidances are more concerned of vulnerable groups, including the pre and post pregnancy. Some of these restrictions may appear to be odd from an experimental perspective, however, there is regularly an unnoticed logic behind it[7] Women must be aware of their nutritional intake during pregnancy period and must understand what should be done to ensure the successful delivery of a healthy child. The temporary food avoidance also includes the principles of fasting during the pre-natal period.

Prohibited acts are present in every society. Every religion represents its own perspectives on specific foods that are viewed as taboos. Every religion characterizes the avoidance of particular food for particular reason relating to their judgment and accordingly people follow such taboos according to their religion[8]

Objective

1. To assess the knowledge on food practice among postnatal mothers.

2. To associate the knowledge score with selected demographic variables.

Hypothesis

H1: There is a significant association between knowledge on food practices among postnatal mother with their selected demographic variables.

Material and Methods

A descriptive survey research design was done from March 2019 - July 2019 in 30 postnatal mothers at AVBR Hospital of Wardha. The approval for the study was taken from the Institutional review board and the Ethical Committee of DMIMS (Deemed to be University). Informed consent was taken from all the patients before they underwent the procedure. Inclusion criteria- Postnatal mothers whose gestation age was 36-42 week. Both vaginal delivery and caesarian section delivery mother can participate. Exclusion criteria is Postnatal mothers who have systemic disease like diabetes, hypertension, cardiac disease etc.

The content validity of the tool was done by 13 experts. The reliability of the tool was 0.87 which is done by Guttmann Split Half Coefficient. After receiving opinions from the experts and consultation from the guide required modifications were done in framing the item and same were incorporated into the tool. The tools were divided in to two sections. The first section deals with demographic information sheet. The

second section deals with structured questionnaire on knowledge on food practices among postnatal mothers. he data analysis was planned to include descriptive and inferential statistics. Descriptive statistics: Frequency and percentage distribution will be used to describe the socio-demographic variables and Mean; mean percentage and standard deviation will be used for knowledge on food practices. Inferential statistics: One way ANOVA test and independent T –test: Assessment of knowledge and Chi square test will be used to determine the association between knowledge with selected socio demographic variables.

Result

Section I: Distribution of women in reproductive age according to their demographic characteristics

This section deals with the distribution of women in reproductive in AVBR Hospital Wardha with regards to their demographic variables A sample of 30 subjects was drawn from the study population who were selected from postnatal mothers admitted in AVBR hospital Wardha. The data obtained to describe the sample characteristics included age, religion, occupation, parity, type of family and type of diet.

Table no I : Distribution of postnatal mothers according to their demographic variables n=30

Demographic variables	Postnatal mothers	Percentage
Age		
20-30	23	76%
30-40	3	12%
40-50	2	6%
50 above	2	6%
Religion		
Hindu	20	66%
Muslim	7	23%
Christian	3	11%
Occupation		
Housewife	25	84%
Working women	4	13%
Own business	1	3%
Parity		
P1	25	83%
P2	3	11%
P3	1	3%
Above P3	1	3%
Types of family		
Nuclear	25	83%
Joint	5	17%
Type of diet		
Vegetarian	22	73%
Non vegetarian	6	20%
Mix diet	2	7%

Section Ii

Assessment of the knowledge on food practice among post natal mothers

This section deals with the assessment of existing knowledge on food practice among post natal mother .The level of knowledge is divided under following headings poor, average ,Good, Excellent. There were total 14 questions , this divided into four i.e.0-3 is poor,4-7 is average , 8-11 is good and 12-14 is excellent.

Table no II : Distribution of women according to level of knowledge

Level of knowledge score	Percentage score	Score range	Knowledge score	
			Frequency	Percentage
Poor	0-25	0-3	0	0%
Average	26- 50	4-7	16	54%
Good	51 – 75	8-11	14	46%
Excellent	76-100	12-14	0	0%
Minimum score	4			
Maximum score	11			
Mean score	7.80			
SD	2.18			

The above table shows that majority 16 (54%) women were having average level of knowledge , 14(46%) women were having good level of knowledge ,11 (18%) women were having good knowledge .The minimum score was 4 and maximum score was 11, the mean score was 7.80 and standard deviation is 2.18 respectively.

SECTION- III

Association of knowledge score in relation to demographic variables

There was significant association of knowledge score in relation to types of diet..The tabulated 'F' value was which is lower than the calculated 'F' value .i.e 3.655 at 5% level of significance .Also the calculated 'P' = 0.039 which is similar to the acceptable level of significance .i.e 'P' 0.05.Hence it is interpreted that the type of diet knowledge is associated with their knowledge score.

There was no significant association of knowledge score in relation to age, occupation , religion, parity and family.

Discussion

In present study, the majority (76%) of postnatal mothers belongs to the age of 20-30 years, majority of women 66% were Hindu, their occupation shows majority of 83% were house wife, majority of 83% were belongs to nuclear family, types of diet that majority of (73%) have vegetarian. Majority 16 (54%) women were having average level of knowledge , 14(46%) women were having good level of knowledge ,11 (18%) women were having good knowledge. The findings of the study have been discussed with reference to the objectives and with findings of other studies. A descriptive study on food practice among postnatal mothers in a hilly township in north eastern Nepal was carried out with 50

mothers (aged 20-39 years) of different ethnic groups (Adibasi-Janajati: 46.0%, Bahun/ Chhetri: 34.0% and Dalit 20.0%)[9]. Findings show that this township was representative of a rural area in northeastern Nepal in terms of demographic characteristics like mixed ethnic composition, high illiteracy rate (especially among women), joint family structure in majority of the households (80% of the total participants) and agriculture as the more common occupation (44% of the total participants). It was seen that various factors are responsible for affecting food practices of mothers during the postnatal period in this region. Socio-cultural beliefs are contributory to the food practices followed, like the frequency of meals/day eaten by the mothers. Other factors that affected were: Lower economic status of the family (33% of Dalit women and 50% of women who work as labourers were only eating meals two times a day); Support from the family (75% of women living in joint families were eating meals four times a day while 80% of the women living in nuclear families were eating meals three times a day); It also showed more attention and care was given to younger, first time mothers (younger mothers who were mostly first-time mothers were eating meals four times a day while experienced and older mothers were eating meals three times a day). The study also showed that there are food taboos surrounding specific food items, which were not consumed from 11 days to six months after delivery based on various socio-cultural beliefs and practices[10]

Findings of the study can be used by the nursing administration in creating policies and plans for providing education to the postnatal mother about food practice. Findings will help the nursing students to understand about need to be equipped with adequate knowledge. Findings will help nursing faculty to give more importance, for planning and organizing health education programme on food practice in nursing curriculum to improve knowledge and clinical practicing skill of students. Findings of the study will motivate nursing personnel to improve the knowledge regarding postnatal food practice.

Conclusion

The above table shows that majority 16 (53%) women were having average level of knowledge , 14(46%) women were having good level of knowledge ,11 (18%) women were having good .The minimum score was 4 and miximum score was 11, the mean score was respectively

Recommendations

On the basis of the findings of the study, it is recommended that the following studies can be conducted

ü A similar study on a large scale including rural areas across the country can be carried in order to estimate the level of knowledge regarding food habits of postnatal mothers.

ü A similar study on a large scale including urban areas across the country can be carried out in order to estimate the level of knowledge food

ü A study can be conducted to evaluate the effectiveness of planned teaching programme on food practice.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: The study was approved by the Ethics committee of the institute.

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Study of Acute Renal Failure in Anemic Patients at Vidharbha Region

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Abstract

Introduction: Anemia is commonly characterised as haemoglobin in men of less than 13.0 g/dL and in premenopausal women of less than 12.0 g/dL. Chronic kidney disease (CKD) anaemia is a type of normocytic normochromic hypo-proliferative anaemia. It is also associated with poor results in CKD and raises mortality among other CKD complications. After a greater than 50 percent loss of kidney function, the disease normally begins to progress, generally when the glomerular filtration rate (GFR) falls to less than 60 mL/min. When chronic kidney disease (CKD) progresses, the seriousness of anaemia continues to worsen. Erythropoietin deficiency in renal development and the incidence of anaemia do not always appear to correlate with renal impairment severity.

Material and Methods: Total 60 patients of iron deficiency anemia were taken for the study which contains 55% (33) male patients and 45% (27) of female patients. This is a cross-sectional investigation wherein cases were out-patients. Patients went to the Directly Observed Treatment Short-course focus in the Dept. of General Medicine. The samples were used to analyze serum urea, Creatinine and Hematocrit value.

Result: The level of urea in male (260.69±38.25) is non-significantly high than in female (241.0±34.16) patients.The hematocrit value was significantly low in both men and women (p=0.0006) indicating no difference irrespective of gender. However, it was discovered that the mean value of females (20.14±2.63) was higher than that of males (17.90 ±2.16).

Conclusion: Our study concluded that hematocrit value had a strong association with acute renal failure irrespective of the gender and the severity of the anemia is independent of the maximum or minimum value of the creatinine or urea.

***Key Words**: Anemia, Hematocrit, Acute Renal Failure, CKD and GFR.*

Introduction

Anemia is commonly characterised as haemoglobin in men of less than 13.0 g/dL and in premenopausal women of less than 12.0 g/dL.[1]Chronic kidney disease (CKD) anaemia is a type of normocytic normochromic hypoproliferative anaemia. It is also associated with poor CKD outcomes and increased mortality, among other complications of CKD.[2]

After a greater than 50 percent loss of kidney function, the disease normally begins to progress, generally when the glomerular filtration rate (GFR) falls to less than 60 mL/min. When chronic kidney disease (CKD) progresses, the seriousness of anaemia

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continues to worsen. Erythropoietin deficiency in renal development and the severity of anaemia do not always appear to correlate with the severity of the disease. At least 90% of patients who end up on dialysis will inevitably experience chronic disease anaemia.[3]

Renal failure or illness is called the inability of the kidney to expel the toxic material and metabolic waste from the blood. There are two forms of renal failure: acute and chronic renal failure. Acute renal failure is a condition with a high level of urea and creatinine due to an unexpected decline in kidney function resulting in a substantial decrease in filtration of glomerulus.[4]

Laboratory analysis showing elevated levels of serum creatinine or Blood Urea Nitrogen (BUN) is the diagnostic normal.[5]

Acute renal failure (ARF) is divided into causes of kidney damage, including pre-renal, intrinsic, and post-renal, for further understanding. With repeated signs of vomiting, diarrhoea, inadequate fluid intake, fever, use of diuretics and heart failure, pre-renal ARF is the reduction of blood supply to the kidney. By causing damage to kidney tubules, interstitium and glomeruli, intrinsic ARF occurs. Post renal ARF is due to one or both of the urinary tracts being blocked. ARF is a threatening health issue for surgical patients, with a high mortality rate depending on disease status.[6]

Normocytic, normochromic, and hypo proliferative are usually anaemia in CKD. The discovery of a factor developed in the renal cortex and responsible for stimulating erythropoiesis, later known as erythropoietin (EPO), led to the belief that the key cause of anaemia in patients with CKD may be its deficiency.[7]

Chronic kidney disease (CKD): It is regarded as a major health concern that affects about 13% of the population of the United States. There will continue to be an increase in the number of prevalent CKD patients, reflecting the growing elderly population and the increasing number of patients with diabetes and hypertension. If the number of CKD cases grows, the treatment of complex medical conditions specific to patients with permanent renal disease would be handled by primary care practitioners. The nephrologist seldom handles the medical needs of CKD patients before renal replacement therapy is needed, as is well established in the literature. CKD staging will be described in this chapter and five risks associated with CKD will be discussed: anaemia, hyperlipidemia, diet, osteodystrophy, and cardiovascular risk.[8]

CKD is characterised as the presence of kidney injury, manifested by excessive excretion of albumin or decreased renal function, quantified by glomerular filtration rate (GFR) measured or calculated, which persists for more than three months.[9]

Although creatinine clearances can be calculated from urine creatinine concentration measured A more realistic solution in the office is to measure GFR (estimated GFR or eGFR) from the serum creatinine concentration in a 24 hour urine sample and a concomitant serum creatinine concentration, using either the Cockcroft-Gault or the Alteration of Diet in Renal Disease (MDRD) Research estimation equations.[10]

Materials and Methods

Study area:

Total 60 patients of iron deficiency anemia were taken for the study which contains 55% (33) male patients and 45% (27) of female patients. This is a cross-sectional investigation wherein cases were out-patients. The present study was conducted in the Dept. of General Medicine in collaboration with Dept. of Biochemistry, Datta Meghe Medical College and Shalinitai Meghe Hospital and Research Center, Nagpur and collaboration with Jawaharlal Nehru Medical College (Datta Meghe Institute of Medical Sciences Deemed to be University), Sawangi, Wardha, Maharashtra.

Sample Collection:

5ml of blood sample were taken from each patient and divided into EDTA and Plain Vial. EDTA sample were used for the estimation of Hematocrit and the plain sample were used to estimate the level of serum creatinine and serum urea

Inclusion Criteria:

patients with proven acute renal insufficiency.
endocrinopathy,
anemic patients
hepatosplenomegaly

Exclusion Criteria: No patient had a blood transfusion or dialysis.

Biochemical Analysis:

Estimation of urea and creatinine was done by using by Beckman Coulter AU480.

Results

Table 1: Gender wise distribution of patient with acute renal failure

Gender	Number of patients	Percentage
Male	33	55
Female	27	45

Table 1 shows gender wise group distribution of patients with acute renal disease, 55% male and 45% female patients were included in this study.

Table 2: Relation of creatinine and urea among male and female with ARD

Parameters	Male(Mean±SD)	Female (Mean±SD)	P-value
Creatinine (mg/dl)	6.10±1.56	5.89 ±1.54	P=0.6038
Urea (mg/dl)	260.69±38.25	241.0±34.16	P=0.0419

Table 2 shows the average levels of creatinine and urea in patients with acute renal failure. The level of creatinine was 6.10±1.56 in male patients as compare to female patients i.e. 5.89±1.54. Level of creatinine is non-significantly increased in male than in female. The level of urea in male (260.69±38.25) is non-significantly high than in female (241.0±34.16) patients.

Table 3: Relationship between hematocrit value among men and women with acute renal disease

Sex	Mean±SD	P-Value
Male	17.90 ±2.16	P = 0.0006
Female	20.14±2.63	P = 0.0006

Table 3 shows The correlation between the importance of hematocrit in males and females with acute renal disease. In acute renal failure (p=0.0006), the hematocrit value was considerably low, suggesting a close interaction between them. In both men and women, the hematocrit value was slightly low (p=0.0006), suggesting little distinction, regardless of sex. Nevertheless, the mean value of females was found to be (20.14±2.63) It was better than the men's (17.90 ±2.16)

Discussion

Table 1 shows gender wise group distribution of patients with acute renal disease, 55% male and 45% female patients were included in this study.

Table 2 shows the average levels of creatinine and urea in patients with acute renal failure. The level of creatinine was 6.10±1.56 in male patients as compare to female patients i.e. 5.89±1.54. Level of creatinine is non-significantly increased in male than in female. The level of urea in male (260.69±38.25) is non-significantly high than in female (241.0±34.16) patients.

Table 3 shows The correlation between the importance of hematocrit in males and females with acute renal disease. In acute renal failure (p=0.0006), the hematocrit value was considerably low, suggesting a close interaction between them. In both men and women, the hematocrit value was slightly low (p=0.0006), suggesting little distinction, regardless of sex. Nevertheless, the mean value of females was found to be (20.14±2.63) was higher than that of males (17.90 ±2.16)

Acute renal failure or disease (ARF) is a complex condition that induces a sudden decline in kidney function due to the rapid increase in serum creatinine and urea from multiple sources[11]. The prevalence of incidence of acute renal failure rose more than 10 years ago, accounting for 20% of hospital admissions, most of which are seriously ill.[12.] The risks of acute renal failure are as follows: progression to progressive renal dysfunction, coronary disease, long patient life and elevated hospital mortality.[13] Recognition of clinical investigation of acute renal disease, however, has decreased the mortality rate and mortality will occur only when the condition is severe for dialysis-requiring acute renal failure.[14]

91 % of anaemic patients with acute renal failure due to elevated urea levels and the presence of oliguria were observed in several previous studies. 53 of the 56 patients with moderate anaemia (hematocrit<35 percent) were hospitalised and 433 of those with hematocrit below 30 percent were hospitalised.[15] The same result was found in another study in which the majority of patients (87.5 percent) with acute renal failure had less than 35 percent hematocrit and less than 30 percent hematocrit in just 12.5 percent of patients. These results are not consistent with existing findings in which all our patients (100%) with acute renal failure have extreme hematocrit anaemia below 30%, with just 1.8% of patients having hematocrit hematocrit. Extreme anaemia can be caused primarily by a complication of renal insufficiency due to reduced development of erythropoietin in the kidney.[16] The anaemic disease leaves patients vulnerable to oxidative stress and kidney hypoxia. Numerous anaemic patients also have undetectable kidney failure that is predisposed to renal insults.[17]

Conclusion

Our research concluded that, regardless of gender, hematocrit value was closely correlated with acute renal failure and the severity of the anaemia was independently of the maximum or minimum value of creatinine or urea. Women with acute renal failure were also shown to have a higher hematocrit value than men as a result of a decreased male testosterone level. This research has taught us that acute renal failure should be examined for any given serious anaemia.

Extreme anaemia can be linked with other disorders of acute renal failure, such as male infertility. Another insight is that the cause of a high degree of hematocrit value in males is testosterone than females. More precise information is that the low value of female hematocrit is not correlated with their menstrual menstruation.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: Taken From institutional ethics committee

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Effect of Prolonged Use of Facemask on Ambulance Driver During Covid 19: A Questionnaires Study

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Abstract

Background: The nose is a complex organ that shapes and has many roles as an integral part of the face. The nose's primary purpose is to humidify, warm the inspired air, and also helps to remove the destructive particles from entering the lower respiratory tract. In general, an average adult inspires about 10,000 L of air each day. Nasal mucosa is a highly vascular structure and has a large surface area of 150 cm square. Physiologically, the nose has 50% resistance in the entire airway, which when affected plays a significant role in total respiratory function. that prolonged use of facemasks induces difficulty in breathing on exertion and excessive sweating around the mouth to the Ambulance driver which results in poorer adherence and increased risk of susceptibility to infection

Objectives: The aim of this study is to determine the effects of prolonged usage of N95 respirators and surgical facemasks on Ambulance driver

Material and Methods: A self constructed questionnaire containing 10 questions regarding the prolonged use of N95 respirators and surgical mask on Ambulance driver between the age group of 35 – 50 years were handed to them

Results: This study suggests that prolonged use of facemasks induces difficulty in breathing on exertion and excessive sweating around the mouth to the Ambulance driver which results in poorer adherence and increased risk of susceptibility to infection.

Keywords: *N95 respirators, Surgical masks, COVID-19, Ambulance driver*

Introduction

The nose is a complex organ that shapes and has many roles as an integral part of the face. The nose's primary purpose is to humidify, warm the inspired air, and also helps to remove the destructive particles from entering the lower respiratory tract. It is the respiratory system's frontline defender.

Usually, an average adult inhales about10,000 L of air per day. The nasal mucosa is a highly vascular system with a wide 150 cm square surface area. Physiologically, the nose has 50 percent resistance in the entire airway, which when compromised plays a major role in overall respiratory function. All of these parts contribute to normal body homeostasis. Individual respiratory safety devices and face masks are vital instruments to protect health care staff in hospitals and clinics and play a key

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role in reducing the spread of 2019's high-risk pandemic infection [1,2,3]

Coronavirus disease (COVID-19) is caused by a newly identified coronavirus and is an infectious mild to moderate respiratory disease. In patients with other pathologies, this infection is a serious illness, especially in the elderly with underlying medical conditions such as chronic respiratory disease, cancer, cardiovascular disease, and diabetes. With a high mortality rate, these patients develop extreme acute pneumonia .The primary way in which the virus spreads is through saliva droplets, formed by an infected individual during coughs or sneezes or through discharge from the nose. Infectious diseases that can become major public health issues can be transmitted by airborne bacteria or viruses. If masks are not used properly, the use of protective face masks (PFMs) will not be successful. Because of airflow resistance and discomfort associated with facial heat build-up, particularly in hot and humid weather, many individuals use a PFM without complying with safety regulations. [4,5]

The direct surgical mask has a low/moderate filter output with lower airflow resistance levels, while the high heat and humidity under a PFM may cause the PFM's outer surface to condense moisture, which therefore impairs the loss of respiratory heat and imposes an increased heat burden. It has been suggested that a rise in facial temperature may induce a panic disorder with hot flashes and sweating caused by elevated CO2 levels under the PFM. In reality, a significant increase in skin temperature, especially under the mask, is created by wearing a surgical mask or respirator[6,7,8]. A PFM causes a large increase in the effects of facial skin on thermoregulation. For this purpose, PFM is misused by several individuals without covering the nose or, after a few minutes, can lead to partial detection of the nasal region. Impatience with the thermal effects of PFM leads to discomfort and can simultaneously cause decreased usage and decreased consumer protection[9,10]. Impatience with PFM's thermal effects leads to irritation and can cause decreased usage and reduced safety for the user at the same time. The aim of the present study was to evaluate facial skin temperature, discomfort and hands moving the mask with thermal Infrared Imaging while wearing surgical masks or N95 respirators. [11,12]

Methodology

1) **Study design-** self constructed questionnaire

2) Study set up-Hospital Ambulance driver

3) Selection criteria-Ambulance driver who are willing to participate in the study

4)Sample size:30

Inclusion criteria-

Ambulance driver between the age group of 35-50 years

Exclusion criteria-

co-morbidities like diabetes, hypertension, epilepsy,cardiac illness,asthma & other respiratory illness

Materials used-structured questionnaire

Procedure-

Permission to carry out the research were taken from the ethical committee of the Datta Meghe college of Physiotherapy, Nagpur.

According to the inclusion criteria the Ambulance driver who were volountarily willing to participate in the study were selected from various multispeciality hospital .

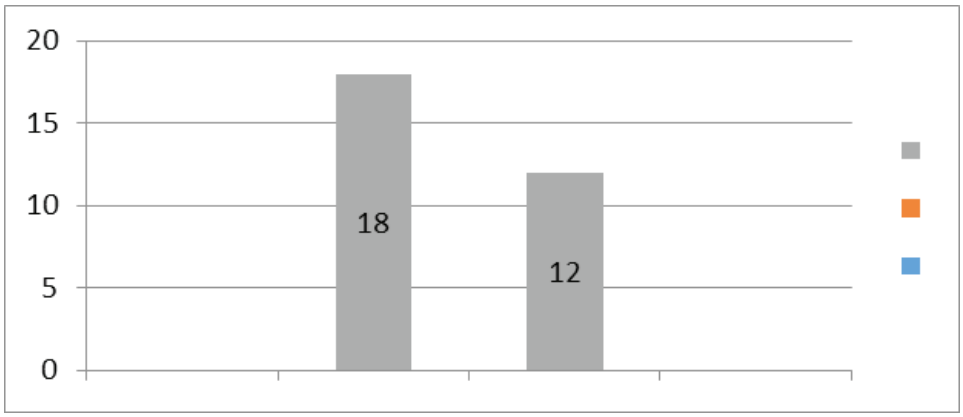
The aim and protocol of the study were described to the driver and their written consent were obtained .On the same day , the questionnaire were handed to the Ambulance driver by the therapist, and requested then to tick in the appropriate boxes of the questionnaire according to their most suitable answers.

A stipulated time of 10-15 minutes were given to them to fill the questionnaire and were asked to hand over the questionnaire to the therapist within the time limit.

The questionnaire was simple but even if the driver face any problem while filling the questionnaire, the therapist was explain it and solve the problem. The questions which were unticked and which contained any other marking other than tick, those questionnaire were not included for data analysis

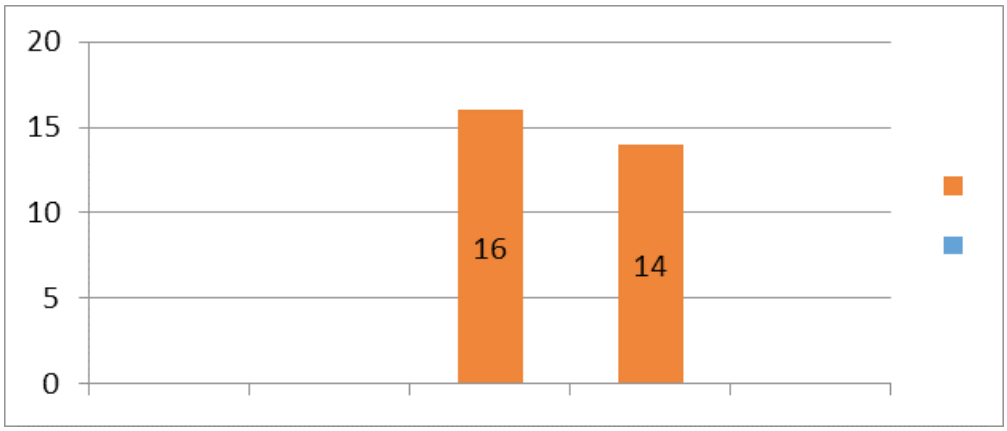
Results

QUE1:FEELING OF GENERALIZED NASAL DISCOMFORT



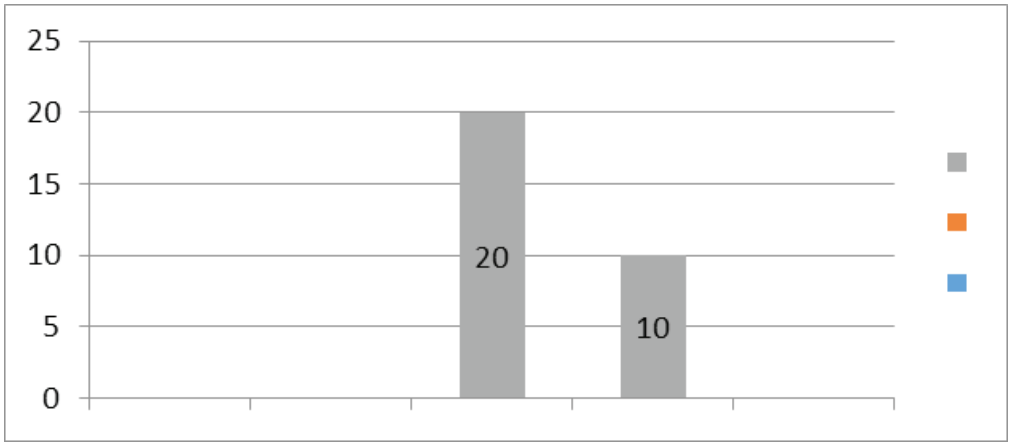
Graph 1 shows that 60% of the participants experienced of generalized nasal discomfort and 40% of the participants had not experienced

QUE2:FEELING OF HOT BURNING NOSE



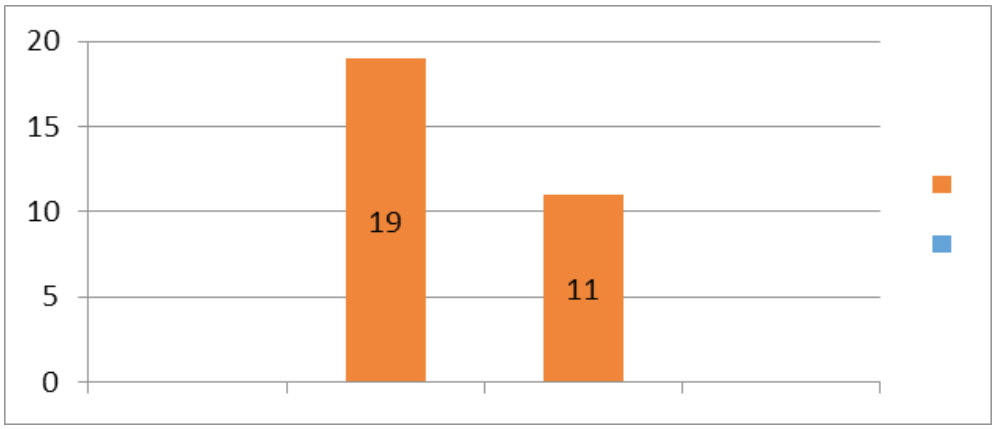
Graph 2 shows that 53.5% of the participants experienced hot burning nose whereas 47% of the participants had not experienced

QUE3:EXPERIENCE EXCESSIVE SWEATING AOUND MOUTH



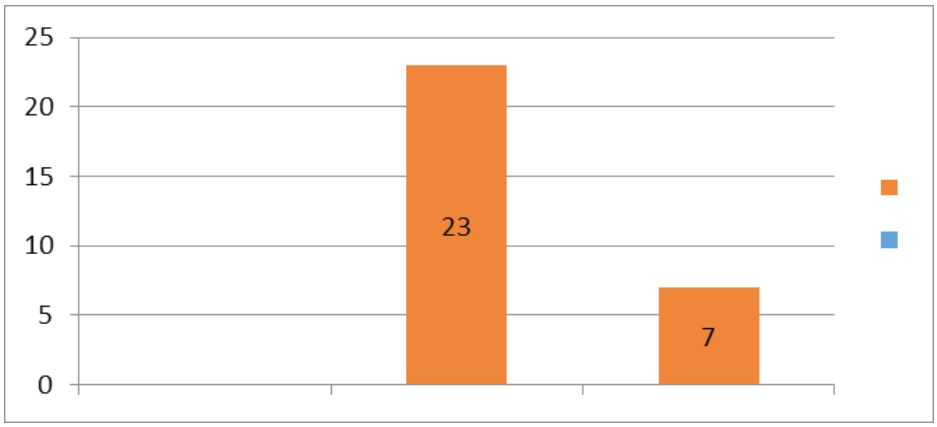
Graph 3 shows that 67% of the participants experienced excessive sweating around the mouth and 33.3% of the participants had not experienced

QUE4: EXPERIENCE PAIN BEHIND EAR



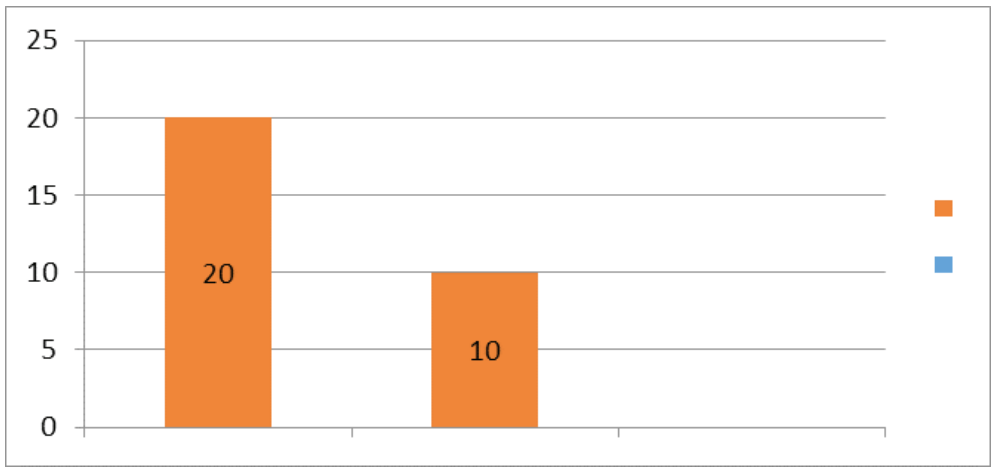
Graph 4 shows that 67% of the participantsexperienced pain behind the ear and 37% of the participants had not experienced

QUE5: EXPERIENCED TROUBLE BREATHING ON EXERTION



Graph 5 shows that 77% of the participants experienced trouble breathingon exertion whereas 23.3%of the participants had not experienced

QUE6: EXPERIENCED BAD BREATH



Graph 6 shows that 67% of the participants experienced bad breath whereas 33.3% of the participants had not experienced

DISCUSSION

The result of the present study shows that facemasks can lead to a wide spectrum of nasal discomfort and complaints pertaining to the facial skin and oral cavity due to its prolonged usage. There is a decrease in humidification of air beneath the facemask and decrease in transpiration of the skin around the nasal and perioral region[13,14]. 60% of the participants had generalised nasal discomfort on wearing the facemask for a prolonged period of time. Wearing the facemask for a prolonged period causes reduced heat loss from the body by various mechanisms such as conduction, convection, evaporation and radiation[15].

This is supported by **Antonio Scarano, Frances co Inchingolo** who compare the facial skin temperature & heat flow when wearing medical surgical masks to same factor when wearing N95 respirators ,each subjects were invited to wear surgical mask on respirator for the infrared thermal evaluation was performed to measure the facial temperature of perioral region & perception ratings related to humidity, heat breathing difficulty & discomfort were recorded, he concluded that N95 respirators are able to induce an increased skin temperature, greater discomfort & lower wearing adherence when compared to medical surgical masks.[15]

67% of the participants experienced excessive sweating around the mouth and pain behind the ear. This is supported by **Y.Li,H.Tokura et al,** investigated the effect of wearing N95 & surgical facemask with and without nano-functional treatments on thermo physiological responses and subjective perception of discomfort , he concluded that 1) the subjects had significantly lower average heart rate when wearing nano- treated & untreated surgical facemasks than when wearing nano-treated and untreated N95 facemasks .2) The outer surface temp of both surgical facemask was significantly higher than that of N95 facemasks.3) But surgical facemask had significantly higher absolute humidity outside the surface than both N95 facemasks.4) Both surgical facemasks were rated significantly lower for perception of humidity , heat,breath resistance & overall discomfort than both N95 facemask, there was significant differences in preferences between the nano-treated & untreated surgical facemask and between the surgical & N95 facemasks.[16]

83.3% of the participants experienced that the nose is irritated and itchy ,The most common adverse reaction reported to the N95 mask was acne , a hot and humid microclimate is created in regions of the face covered by the mask, which predisposes to a flare-up of acne. Secondly, occlusion of pilo sebaceous ducts due to local pressure on the skin from the close-fitting mask could result in a flare-up of acne. This study is supported by **Chris C,Anthony.T.J Goon et al,** presented the prevalence of adverse skin reactions to personal protective equipment against severe acute respiratory syndrome in Singapore, he concluded that those who use mask regularly reported acne 59.5% ,facial itch (51.4%) and rash 35.8% from N95 mask , the usof PPE is associated with high rates of adverse skin reactions.[16]

Conclusion

This study concluded that, the use of facemask plays an important in causing significant discomfort in all the Ambulance drive during its prolonged usage which can limit the efficient usage of facemask, leading to decreased protection. Therefore facemasks are essential to protect us from COVID-19.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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A Comparative Study on Assessment of Pain as an Outcome by Vas Score in Patients of Adhesive Capsulitis Treated by Hydrodilatation with and without Corticosteroids

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Abstract

Background: Adhesive Capsulitis is a painful condition commonly occurring in middle age group population and injectable corticosteroids have shown significant improvement in the pain as an outcome variable. **Methodology:** A prospective study was conducted from June 2010 to June 2012 for a period of 2 year, at a tertiary care hospital in central India. **Results:** The marked difference in pain relief was seen on VAS scale on pre intervention to follow up of 3rd week. The mean on pre intervention of steroid group was (8.10) while that post intervention 3rd week was (4.90). On the same basis, the pre injection mean was (7.55) in saline group while post injection 3rd week mean came out to be (5.75). Thus, pain was relieved on injecting both solutions. But, more improvement was observed in steroid group than saline group. **Conclusion:** Intra saline group and steroid group analysis revealed a marked improvement in shoulder range of motion and pain relief on immediate post intervention and follow up in hydro dilatation of steroid group of patients.

Keywords: *Adhesive capsulitis, hydrodilatation, corticosteroids, Frozen shoulder, acromioclavicular joint.*

Introduction

The condition 'adhesive capsulitis' or 'Frozen shoulder' is a clinical diagnosis; Physical examination is crucial and history also. It is essential to confirm the characteristic features of the condition on grading, its severity and exclude other contributory systemic or local causes which may require laboratory investigations, radiographs and imaging. The majority of patients with adhesive capsulitis do not seek medical attention until weeks to months after the onset of stiffness and pain.

The pain is characteristically severe, felt diffusely around the shoulder girdle, with a deep burning quality. Except other intermittent causes of shoulder pain, would have been around for more than one month.

Neviaser RJ [1] ***(1987)*** and ***Nicholson GP***[2] ***(2003)*** stated that key diagnostic feature is intense night pain and disturbing sleep, and difficulty in doing most normal daily activities. Pain from the acromioclavicular joint is common, because the restricted glenohumeral movement increases the stress on this joint.

Hannafin JA[3] ***(2000) Reeves B***[4] ***(1975)***, divided into three consecutive stages:

'freezing' (10 – 36 weeks) with acute pain and stiffness,

'frozen' (4- 12 mths) with established stiffness and reduced pain and

'thawing' (5-26 mths) with the return of movement.

However, clear stages of development are often difficult to define, or may be absent.

The history and physical examination are

essential to differentiating between painful shoulder and stiffness with identifying true adhesive capsulitis. Patients will often describe an insidious onset of vague, dull pain at the deltoid insertion, a pain pattern that may be due to innervations of the joint capsule by the axillary nerve. Night pain is a very common feature, and sleeping is not possible on affected shoulder is one of the symptom. Pain, restricted elevation and external rotation are common. As the patient progresses from the freezing to frozen stage, the pain increases more, and the restriction in elevation and rotation increases[4-6].

Methodology

Type of study: It was a hospital based prospective study carried for 2 years (June 2010 to June 2012) in a single centre in a tertiary care hospital in Central India. Patients were randomized in two groups of 20 patients each

1. Group A-Injection of corticosteroid, local anaesthetic and saline,

2. Group B-Injection of normal saline and local anaesthetic.

Inclusion Criteria:

1. Limitation of passive movement of the glenohumeral joint compared with the unaffected side, more than 30 degrees for at least two of three movements: forward flexion, abduction or external rotation.

2. Patients with previous adhesive capsulitis in the opposite shoulder were accepted even if the differences between sides were smaller than 30 degrees.

3. Patients with history of diabetes on medication (controlled blood sugar levels) and limited range of motion.

All included patients were clinically assessed for restriction of active and passive range of motion. Plain radiographs of shoulder joint to rule out other pathologies were done and ultrasonography of shoulder joint for confirming the diagnosis of adhesive capsulitis was carried out.

Exclusion Criteria:

1. Patients not willing to give consent for study.

2. Patients contraindicated for steroid injection-bleeding disorders, known drug allergy.

3. Patients whit history of trauma to shoulder needing immobilization.

4. Patients with serious mental illness.

5. Patients with age under 18 or over 70.

6. Patients currently taking oral corticosteroid therapy.

7. Patients with less glenohumeral range of motion for reasons other than adhesive capsulitis with X-ray signs of glenohumeral arthritis, dislocation or full-thickness rotator cuff tears with dislocations of the humeral head.

Technique: The proper consent of the procedure was taken from the patient and Xylocaine sensitivity test was performed 45 minutes before the procedure. The procedure was performed according to the ***Kaye-Schneider technique***. The patients were placed supine on a table with an overhead X-ray tube and a supporting pillow under the opposite shoulder. Under image-intensified fluoroscopy or ultrasonographically guided a marker was placed over the glenohumeral joint space at about the junction of its middle and lower third or just lateral to the coracoid process in collaboration with internventional radiology department for guidance.

This point was then marked on the skin with a pen. The skin area was cleaned with an antiseptic. The joint was punctured by a needle (18 or 22 Gauge intramuscular spinal needle) and its position was checked frequently by fluoroscopy during the procedure. The needle was connected to a 20 ml syringe. Upto 16 ml of sterile normal saline , 2 ml of local anesthetic (Bupivacaine hydrochloride, 5 mg/ml), and an injection of 2 ml Depomedrol (80 mg Methyl Prednisolone) as total of 20 ml solution was injected slowly in group “A” patients.

Similar procedure was carried out for hydrodilatation in group “B” patients with 18ml of sterile normal saline and 2 ml of local anesthetic (Bupivacaine hydrochloride, 5 mg/ml),

In both the groups, following hydrodilatation, manipulation of affected shoulder joint was done.

Results: A total of 40 patients were selected based on the inclusion and exclusion criteria for the study, which were then divided into 2 groups of 20 patients each.

The present study is to compare the pain on VAS pre intervention and post injection (Hydrodilatation) after 3 weeks. The intra group analysis of steroid group, pre intervention to that on post injection resulted in a significant p value on student's paired t test. The mean pre intervention value was 8.10 and post injection on the 3rd week follow up was 4.90. This shows pain reduces post injection on 3rd week follow up in steroid group. Similarly the intra saline group analysis pre intervention to that on 3rd week post injection gave a mean value of 7.55 and 5.75 respectively, Hence the pain was reduced post injection 3rd week comparatively.

The comparison between saline and steroid groups using student's unpaired t test with respect to VAS on 3rd week follow up resulted in a significant p value. The mean value pre intervention was 8.05 and 7.55 in steroid and saline groups respectively. There was no significant difference on comparing the two groups on pre intervention analysis.

The post injection 3rd week follow up had a mean of 4.8 and 5.7 in steroid and saline groups respectively. The resultant p value was 0.033 giving a significant result.

The inference derived was that an improvement was observed using both steroid and saline groups. But the use of steroid was more effective in pain relief on 3rd week post intervention.

Table 1 showing Comparison of pain on VAS at pre intervention and post injection 3 weeks in both the groups.

Group		Mean	N	Std. Deviation	Std. Error Mean
Steroid	Pre Intervention	8.10	20	1.05	0.23
	Post Injection 3 wks	4.90	20	1.07	0.23
Saline	Pre Intervention	7.55	20	1.14	0.25
	Post Injection 3 wks	5.75	20	1.44	0.32

Table 2: Student's paired t test

Group	Paired Differences					t	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Steroid	3.15	1.26	0.28	2.55	3.74	11.11	19	0.000 p<0.05
Saline	1.80	1.28	0.28	1.20	2.39	6.28	19	0.000 p<0.05

Table 3 showing Comparison of VAS in both groups at pre intervention, And post injection 3 weeks in both the groups

	Group	N	Mean	Std. Deviation	Std. Error Mean
Pre Intervention	Steroid	20	8.10	1.05	0.23
	Saline	20	7.55	1.14	0.25
3 wks	Steroid	20	4.90	1.07	0.23
	Saline	20	5.75	1.44	0.32

Table 4: Student's unpaired t test

	t	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Pre Intervention	1.43	38	0.158 NS,p>0.05	0.50	0.34	-0.20	1.20
3 wks	2.11	38	0.041 S,p<0.05	0.85	0.40	0.07	1.66

Discussion

The main aim of this study was to assess and compare hydrodilatation with corticosteroids and without corticosteroids (with normal saline) along with a comprehensive physiotherapy programme in the outcome of patients with adhesive capsulitis[6-10].

On comparison of pain on VAS index in pre intervention and post injection after 3 weeks the intra group analysis of steroid group pre intervention to that on post injection resulted in a significant p value, suggestive of significant pain reduction post injection on 3rd week follow-up in steroid group[11-13].

Similar results were obtained in saline hydrodilatation group 3rd week post intervention.

But, the comparison between saline and steroid groups with respect to VAS on 3rd week follows up resulted in a significant p value. The mean value pre intervention was 8.05 and 7.55 in steroid and saline groups respectively. There was no significant difference on comparing the two groups on pre intervention analysis.

The post injection 3rd week follow up had a mean of 4.8 and 5.7 in steroid and saline groups respectively. The resultant p value was 0.033 giving a significant result.

The inference derived was that an improvement was observed using both steroid and saline groups. But the use of steroid was more effective in pain relief on 3rd week post intervention[14,15].

In our study volume used for hydrodilatation was 20 ml, in both the groups

Bal A[15] ***(2008)*** said that intra-articular steroid injection was significantly associated with an improvement in range of motion and pain for 2 weeks (but not for 12 weeks) after conclusion of their study.

Ryans I[16] ***(2005)*** found that patients having intra articular corticosteroid therapy had better outcome in disability scores but not in pain and range of motion in the 6th week.

Van der Windt DA[17] ***(1983)*** in his trial of fluoroscopically guided injection with and without physiotherapy found corticosteroid-injected patients had less disability and good range of motion outcome at six weeks compared with physiotherapy alone or placebo injection.

In our study both the groups received single shot hydrodilatation.

Each patient in the study was subjected to proper physiotherapy programme, as per scheduled appointments and home exercises and was evaluated accordingly.

Comprehensive exercise therapy under close follow-up is a fundamental choice.

Hannafin JA[3] ***(2000)*** stated that physiotherapy is critically important in adhesive capsulitis. It is important to educate the patient regarding the improvement in range of motion. Stretching should be the basic treatment. It can be taken beyond the limits of the available range of motion.

Griggs SM[18] ***(2000)*** quoted that 90% improvement can be achieved using only multi directional stretching exercises.

On the contrary ***Carette***[19] ***(2003)*** stated that it is important to weigh up the potential benefits and risks of the use of steroid treatment, especially in self-limited disorders such as adhesive capsulitis.

No randomized trials have controlled distension alone with placebo. The combined intervention of steroid injection and distension has been compared with steroid injection by ***Gam AN*** [20] ***(1998)***.

Conclusion

Intra saline group and steroid group analysis revealed a marked improvement in shoulder range of motion and pain relief on post intervention follow up with a maximum improvement observed on 3rd, 6th and 12th week but more in steroid group comparative to saline group.

Limitations: The limitations were Small study sample, Single dosage of hydrodilatation. and Multiple operators, Lack of timely follow up and Lack of patient compliance for Physiotherapy.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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Study of LDH Isoenzymes in Myocardial Infraction at Vidharbha Region (Central India)

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Abstract

Introduction: A significant cause of morbidity and mortality worldwide is myocardial infarction (MI). Within the first few hours of the beginning of MI, the greatest risk of death occurs. Thus, for the effective treatment of patients with MI, early detection of cardiac ischemia is essential. Inappropriate diagnosis of patients with chest pain sometimes leads to improper admission, and vice versa, of patients without MI. Physical examination, precise ECG results and evaluation of cardiac biomarkers play an important role in the early diagnosis of acute ischemia, in addition to clinical history. In the present analysis, the cardiac biomarker lactate dehydrogenase released during a MI event is discussed in depth.

Aim: We conducted a cross sectional study to examine the different levels of LDH isoenzymes in myocardial infarction patients those admitted in cardiac care unit in SMHRC Nagpur.

Material and Methods: The present study included 100 subjects of age group 30-80 years. Patients admitted to the coronary care unit at SMHRC & AVBRH between January 2020 and June 2020 was considered eligible for the study. These guidelines ensured that sufficient blood samples were available and the criteria for diagnosis were consistent. Lactate dehydrogenase isoenzyme was fractionated by agarose gel electrophoresis as described by Cawley and Eberhard. The stained slides were scanned on Chromoscan at 520 mix and the percentages of the fractions were quantitated.

Results: The results obtained in the present study indicate that serum lactate dehydrogenase enzyme activity increases 4-5 folds in the first 24-72 hours after the onset of clinical infarction with a peak on the second day. It then declines gradually towards the normal within two weeks. Thus, determination of total serum LDH is valuable if patients present themselves for clinical check up even after a week from the day of infarct. Although the contribution of myocardium to total serum lactate dehydrogenase is large, its interpretation becomes difficult if the diseases of other organs such as liver, kidney, etc.

Conclusion: In conclusion, the present data strongly support the use of Lactate dehydrogenase for the diagnosis of MI. Such a strategy should boost both the precision and sensitivity for myocardial infarction diagnosis.

Keywords*: Lactate Dehydrogenase, Myocardial Infarction, Coronary Artery Disease, Isoenzymes, ECG.*

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Introduction

Because of prolonged ischemia, myocardial infarction is known as myocardial cell death.[1] Myocardial cell death does not occur straight away after the beginning of myocardial ischemia, but occurs at 6 hour. The most frequent cause of myocardial infarction is, by far, atherosclerosis.

Myocardial infarction is typically associated with chest pain or discomfort, fatigue, sweating, nausea, vomiting, and arrhythmias. Common risk factors include old age, obesity, smoking, high blood pressure, hypercholesterolemia, and DM. Atherosclerosis, a gradual accumulation of cholesterol and fibrous tissue in plaques inside the arterial wall over decades, is by far the leading cause of MI.[2-5] Atherosclerotic plaques may, however, become unstable, burst, and produce a thrombus that occludes the artery. It contributes to thrombosis and complete vascular occlusion when a major plaque rupture occurs in the coronary arteries, ending with the incidence of MI. [6,7]

The leading cause of death worldwide is cardiovascular disease, responsible for an estimated 16.7 million deaths per year[8.] Coronary artery disease (CAD) incidence varies across various geographical areas across the globe. Pakistani people have the highest known rate of CAD among the South Asian populations, for example. According to careful figures, in 2002, almost 100,000 individuals in Pakistan suffered from acute myocardial infarction (AMI)[9.] Chest pain or discomfort, fatigue, sweating, nausea, vomiting, and arrhythmias are commonly presented with acute MI, often loss of consciousness and syncope. This happens with the abrupt disruption of the flow of coronary blood and is a life-threatening medical emergency requiring immediate management.[10, 11]

For better prognosis, rapid recognition of MI is necessary to initiate successful therapy. The new definition of MI diagnosis emphasises the importance of the 12 lead ECG and the evaluation of early cardiac biomarkers, since MI is often insufficient to diagnose the ECG itself. Kidneys, and. Because of its lack of cardiac tissue specificity, it is no longer used for the diagnosis of MI[12, 13]. LDH was used for the diagnosis of MI[14] in the year 1960. Finally, the WHO recommended the LDH panel for the diagnosis of MI in the year 1979.[15]

Table 1: The five isoenzymes are found in different amounts in tissues throughout the body.[16]

Isoenzyme	Location
LDH I	Heart and red blood cells
LDH II	White blood cells. It is also found in heart and red blood cells, but in lesser amounts than LDH-1.
LDH III	lung tissue
LDH IV	white blood cells, kidney and pancreas cells, and lymph nodes
LDH V	liver and muscles of skeleton

Serum LDH activity exceeds the normal range within 24 to 48 hours in a typical patient with acute myocardial infarction, reaches a peak elevation of two to tenfold within 3 to 6 days, and decreases to the normal range within 8 to 14 days.[17] Differences in substrate specificity for LDH isoenzymes were then used to develop serum a-hydroxybutyrate dehydrogenase (HBD; LDH isoenzyme1) assays, which were then used to develop serum a-hydroxybutyrate dehydrogenase (HBD; LDH isoenzyme1) assays. [18]

Accordingly, we undertook the following study to determine the sensitivity of Lactate dehydrogenase cardiacisoenzymes, forthe diagnosis of myocardial infarction.

Material and Methods

Our study was carried out in the Biochemistry Department, DMMC&SMHRC Nagpur, from January 2020 to June 2020. The study was approved by Institutional Ethical Committee and informed consent was taken prior to the study. A total 100 subjects of age between 30-80 years were enrolled in this study. Out of 100 subjects, 50healthy normal as the same age group and 50were suffering myocardial infarction as a study group. hundred patients belonging to both the

sexes between the age group of 30 -80 years who had an attack of myocardial infarction diagnosed by W.H.O. criteria and admitted to the Intensive Cardiac Care Unit of Shalinitai Meghe Hospital Nagpur were selected for the present study.

Inclusion criteria

Inclusion criteria were age between 30-80 years both [Male and Female], diagnosed myocardial infarction and admitted to the Intensive Cardiac Care Unit of Shalinitai Meghe Hospital Nagpur.

Exclusion criteria

Patients were not included if they refused informedconsent, could not be sampled serially, or had bypass surgery,which precluded sampling throughout their hospital stay.

Blood sample collection and processing

Blood was collected in heparin bulbs by venepuncture soon after the acute episode of myocardial infarction. Haemolysis was avoided while separating the plasma which was immediately subjected to the enzyme assay. The blood samples were collected serially upto three days and a week from the day of infarction. Samples were processed in the clinical chemistry laboratory of Shalinitai Meghe Hospital Nagpur. LDH isoenzymes were measured within 24 h from serum samples stored at room temperature. Aliquots of thesamples were frozen at -70^{0}Cfor subsequent measurement of LDH.

Biochemical analysis

Lactate dehydrogenase isoenzymes were fractionated by agarose gel electrophoresis as described by Cawley and Eberhard.[19] The stained slides were scanned on Chromoscan at 520 mix and the percentages of the fractions were quantitated.The ratio of LDH1/LDH2 isoenzymes was determined in theclinical chemistry laboratory at Shalinitai Meghe Hospital by using agarose gel electrophoresis toseparate the LDH isoenzymes eletrophoretically and then quantifyingtheir activity by their NADPH fluorescence. Electrophoreticmethods have been reported to have variable accuracy[20].

Result

Table-2: Lactate dehydrogenase (LDH) isoenzymes levels in study and control group

Isoenzymes	Control Group (n=50) Mean ± SD	Study Group (n=50) Mean ± SD	P value
LDH 1 (U/L)	295.8±65.8	1157±843	P < 0.0001
LDH 2 (U/L)	22.5±5.82	92.64±83.12	P < 0.0001
LDH 3 (U/L)	0.45±0.36	0.58±0.49	P = 0.1338
LDH 4 (U/L)	0.52±0.39	0.78±0.62	P = 0.0137
LDH 5 (U/L)	0.86±0.63	0.97±0.64	P = 0.3385

LDH1 and LDH2 both are statistically significant as compared to other isoenzymes.

Table 2 shows the level of lactate dehydrogenase (LDH) isoenzymes in myocardial infraction patients. Hence in this study shows significantly increase the level of LDH 1 and LDH 2 in study group as compared to control group during the stroke and there is no significant difference in the level of LDH3, LDH4 and LDH5 in study group as compared to the control group.

Table 3: Atypical Symptoms in Myocardial Infraction in Elderly Patients.

PERCENTILE OF PATIENTS WITH SYMPTOM			
SYMPTOMS	**Age 31-60 yrs**	**Age 61-80 yrs**	**Age >80 yrs**
Chest Pain	80	77	40
Shortness of Breath	50	60	62
Sweating	50	25	20
Syncope	5	19	21
Acute Confusion	8	14	26
Stroke	6	18	22

Table 3 shows that the percentages of myocardial infraction symptoms in patients. The most common symptom is chest pain it's seen mostly in age criteria between 30-60 yrs, shortness of breath symptom seen in age group above 80 yrsother symptoms are also show in different percentile in different age criteria.

Discussion

The findings obtained in the current study show that the activity of the serum lactic dehydrogenase enzyme increases by 4-5 folds in the first 24-72 hours after the onset of clinical infarction, with a peak on the second day. It then progressively decreases to normal within two weeks. Therefore, the assessment of total serum LDH is useful even after a week from the day of infarction if patients submit themselves for clinical check-up.While the proportion of myocardium to total serum lactic dehydrogenase is high, if other organ diseases such as liver, kidney, etc. coexist, its perception becomes difficult.

For laboratory diagnosis, the electrophoretic isolation of serum enzymes identified by tissue-based isoenzyme patterns is more specific. Agarose gel was found to have a distinct advantage over the others from the various supporting media such as paper, agar, starch gel, polyacrylamide used for gel electrophoresis, Both staining and separation are stronger and it is easier to scan on the densitometer.

In 1973, Cohen et al.[21] found that a 1:2 increase in LDH above 2.0 was observed in all patients with myocardial infarction. LDH 1:2 improvement in angina pectoris patients with evidence of myocardial infarction.

In the diagnosis of acute infarction, **Roe et al.**[22] researched the use of LDH isoenzymes in combination with CPK isoenzymes. They showed that the diagnosis was supported by an increase in LDH1:2 to more than 1.0. The increase in LDH isoenzymes occurred about 12 to 24 hours after an acute infarction in their study.

When they used LDH 1:2 in combination with CPK isoenzymes, **Wagner et al**[23] noted 90 percent sensitivity and 95 percent specificity. Although the LDH 1:2 was relatively specific and fragile, they concluded. They found that LDH1:2 typically increased within 24 to 48 hours of symptom onset. LDH 1:2 was particularly helpful in their study in patients whose infarction occurred several days prior to admission.

Our study shows that the determination of LDH isoenzymes is a useful addition to the enzyme diagnosis of myocardial infarction when performed by a rapid and sensitive process. The LDH 1:2 would help to validate the findings of the CK-MB in most cases.

Conclusion:

The usefulness of complex enzyme reactions is becoming increasingly evident in clinical medicine. Patients suffering from the loss of different tissue cells

in their serum have increased enzymatic activity. When cell death happens, the enzymes usually present within the cells are released into the blood stream. As an aid in the diagnosis of myocardial infarction, the assessment of serum lactic dehydrogenase (LDH) has two advantages: first, there is an increased elevation for as long as six to eight days after the attack; second, there is a much less complex technical method than other enzyme tests. In particular, the determination of LDH, which is usually not important in the diagnosis of acute myocardial infarction, is useful when the electrocardiographic pattern masks recent changes in acute infarction.

Cardiac biomarker analysis has become the frontline diagnostic method for MI and has significantly allowed clinicians to quickly diagnose and prepare for timely treatment, thus greatly reducing the mortality rate. The study of a panel of markers for the diagnosis and prognosis of myocardial infarction would, however, track the future of cardiac biomarkers.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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Relation of CRP, Ferritin and Procalcitonin Level in People Infection with Covid- 19

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Abstract

Introduction: Since December 2019, the coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has rapidly developed into a global outbreak characterized by a human-to-human transmission. On March 11, 2020, WHO declared the COVID-19 a pandemic. It has caused a total of 30 675 675 confirmed cases, including 954 417 deaths as of September 20, 2020. Patients with comorbidities such as diabetes, cardiovascular disease, underlying respiratory diseases, and cancer are at high risk of severe complications and even death. This is a global crisis that requires the joint efforts of all mankind to fight it.

Method: This study was a descriptive study, conducted in the respiratory Department and molecular Research laboratory at Datta Meghe medical college (DMMC) Wanadongri, Nagpur and Shalinitai Meghe hospital and research center (SMHRC), Nagpur in collaboration with ABVRH, Sawangi (Meghe).

Result: The Present study showed the relationship between the age factor and the prevalence of infection with the COVID virus-19, as the virus can infect all age groups, but the age group (20-79) years were the most affected age (26/68) 38.23%. Also, the study shows that infection with the virus Covid-19 has a significant effect on the level of the serum Ferritin (p-value < 0.001) and in both sexes (520.25ng/ml in the Men, 460 ng /ml In the Female compared to the Control group (232.30 ng/ml,77.217 ng/ml), respectively. significant increase in the level of C- Reactive Protein (p – value< 0.001 as the protein level reached (46 .60 IU/ML) in infected people compared to the control group (2.41 IU / ML). The current study coronavirus infection caused a significant increase level of PCT (P- value < 0.001) as the PCT level (16.2 ng/ml) in infected people compared to the control group 0.16 ng /ml.

Conclusion: We conclude from the current study that the age and gender factor play an important role in the prevalence of Covid-19 infection and that Covid-19 infection causes an increase in both the effective protein level C, PCT the level of the ferritin of blood, so it can be relied upon in the early diagnosis of COVID-19 infection.

Key words: *CRP, COVID-19, Ferritin, PCT.*

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Introduction:

Coronavirus disease-2019 (COVID-19) is an emerging infectious disease deemed by the World Health Organization (WHO)to be a global public health emergency. Over 3,500,000 cases and 243,403 deaths have been reported worldwide since its founding in Wuhan, China.[1] A small proportion of patients experience

extreme pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure, and may even die, while most patients with COVID-19 have a mild influenza-like disease or may be asymptomatic.[2]

The cause why certain people, while others do not, become seriously ill remains an unanswered mystery. For risk stratification, co-morbidities and laboratory markers have been suggested.[3-6] There is growing evidence that hyperinflammation traits consisting of elevated serum C-reactive protein (CRP), procalcitonin (PCT), D-dimer, and hyperferritinemia occur in critically ill patients. In COVID-19 pathophysiology, these results indicate a probably crucial function of a cytokine storm.[7]

COVID -19 has strong infectivity and a rate of a high incidence example CRP. CRP is one of the proteins that are made in the liver and sent to the bloodstream in response to the occurrence of inflammation and its level in the normal state is low and its height is a sign of inflammation, disease or disorder because it is considered one of the interactive indicators in the body.[8]

The disease transmitted via close contact with infected person and from respiratory droplets when an infected person talks, sneezes, or coughs. also, the disease transmitted through direct contact with the contaminated surface a virus then touching eyes, nose, or mouth. Treatment and prevention options are limited, including the use of antibody therapy, that is, the use of convalescence plasma taken from infected people after recovery from the disease, where encouraging clinical results emerged after taking this plasma and improving the chance of survival.[9,10.] This is because neutralizing antibodies are of particular importance as they prevent the adhesion of the virus to the surface of the cell and inhibit the fusion of the host's membrane, Consequently, rapid viral removal, which indicates that it is an anti-viral agent. As for non-neutralizing antibodies, it has a role in removing the virus according to the need for the phagocytic process mediated by the antibody-dependent cell and the antibody-dependent cellular cytotoxicity, as well as the activation of the complement.[11,12]

While most patients have mild symptoms and a strong prognosis, acute respiratory distress syndrome (ARDS) and systemic inflammation can be present in extreme COVID-19 cases. Therefore, determining the seriousness of the disease and examining potential biomarkers is urgent in order to make quick and accurate clinical decisions. One recent research has shown that serum C-reactive protein (CRP) (58.3 percent), lactate dehydrogenase (LDH) (57.0 percent) and erythrocyte sedimentation rate (ESR) are typically increased in patients with COVID-19 (41.8 percent). [13]

In order to predict the severity of COVID-19, laboratory biomarkers are important in a pandemic, as the allocation of resources, particularly in the context of respiratory support readiness, must be carefully planned. A systematic review and meta-analysis was performed in the present study to examine the relationship between several biomarkers, including serum CRP, PCT, D-dimer, and serum ferritin, as well as the magnitude of COVID-19.

Materials and Methods

This study was a descriptive study, conducted in the respiratory Department and molecular Research laboratory at Datta Meghe medical college (DMMC) Wanadongri, Nagpur and Shalinitai Meghe hospital and research center (SMHRC), Nagpur in collaboration with ABVRH, Sawangi (Meghe).

Sample size: 60 COVID-19 patients and 50 control subjects.

Study steps: -clinical examination and laboratory diagnosis.

Inclusion criteria: people infected with COVID-19.

Exclusion criteria: people infected with other respiratory system infection.

Sample Collection: Sample collection a vein blood specimen for 5ml was collected in the plain test tube for a measure of CRP, PCT and Ferritin levels. serum was isolated after centrifugation at 3500rpm for 10 min.

Biochemical Analysis:

· **C- Reactive protein (CRP**) level was estimated by turbidimetric method.

· **Ferritin level** was estimated by two – site immunoenzymatic (Sandwich) assay**.**

· **Procalcitonin (PCT)** level was immunoturbidimetric method.

Statistical Analysis

The data were analyzed using SPSS software program, version 20.0. The mean and standard deviation were measured. Analyzed and interpreted using descriptive and inferential statistics. The correlations of C- Reactive Protein (CRP) with Ferritin, and serum Procalcitonin (PCT) were calculated by Pearson's correlation test and relevant 'p' value was calculated as level of significance. The probability value is less than 0.05 ($p < 0.05$) and it was considered as statistically significant.

Result

The Present study showed the relationship between the age factor and the prevalence of infection with the COVID virus-19, as the virus can infect all age groups, but the age group (20-79) years were the most affected age (23/60) 38.33%

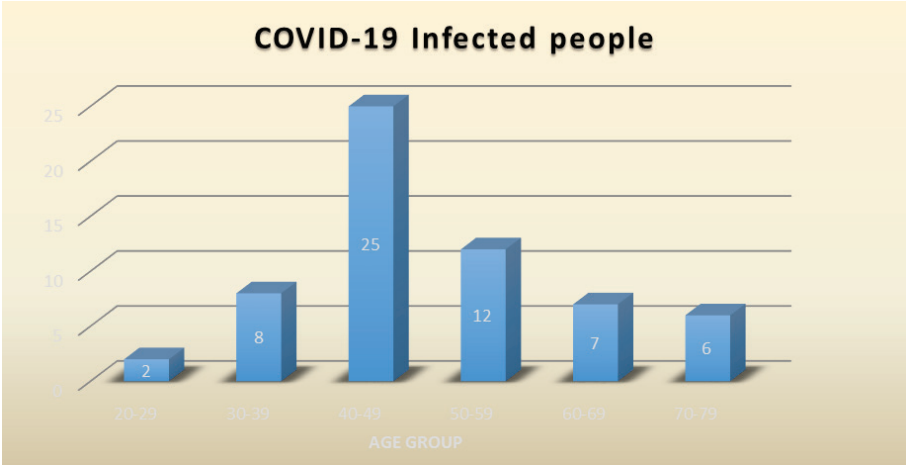

Figure No 1: show the relationship between age and infection by COVID-19. Age group of 40-49 more infected in COVID-19.

The study also showed that the prevalence of coronavirus infection is influenced by gender, as males are more susceptible to infection than females, where the percentage of males was 63.33% (38/60), while the female percentage is 36.66% (22/60).

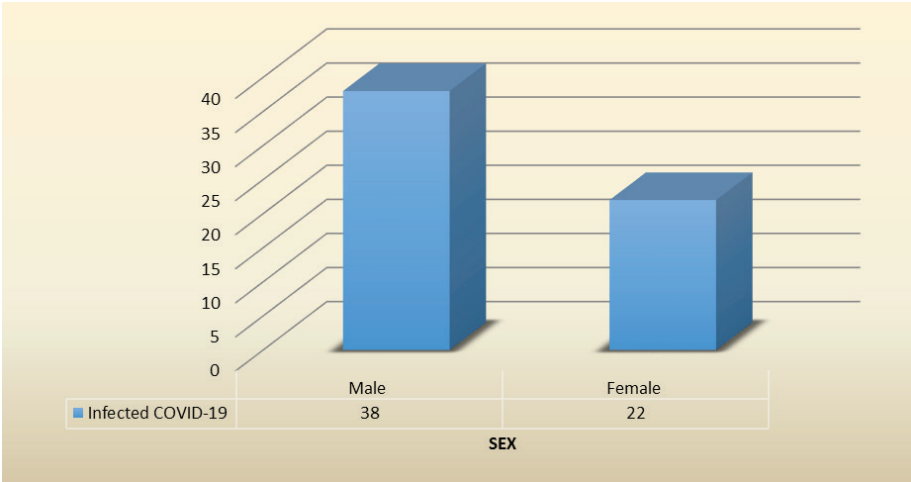

Figure No 2: show the relationship between sex and infection by COVID-19

Figure No 2: show the relationship between sex and infection by COVID-19. Also, the study shows that infection with the virus Covid-19 has a significant effect on the level of the serum Ferritin (p-value < 0.001) and in both sexes (520.25ng/ml in the Men, 460 ng /ml In the Female compared to the Control group (232.30 ng/ml,77.217 ng/ml), respectively.

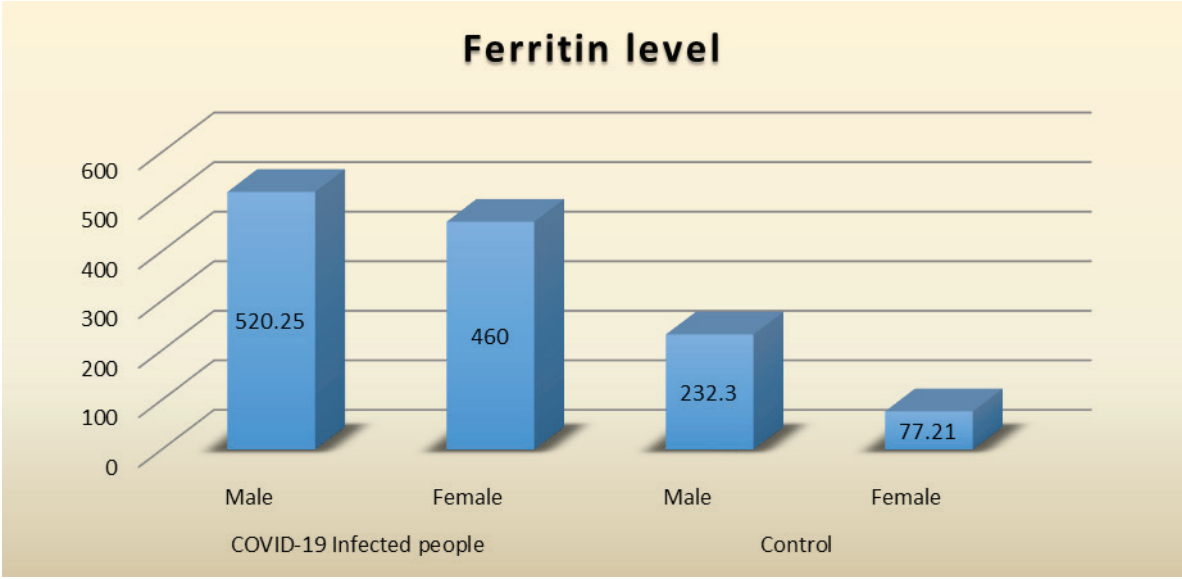

Figure No 3: show the relationship between Ferritin Level and infection by COVID-19.

The present results demonstrated that coronavirus infection caused a significant increase in the level of C-Reactive Protein (p – value< 0.001 as the protein level reached (46 .60 IU/ML) in infected people compared to the control group (2.41 IU / ML).

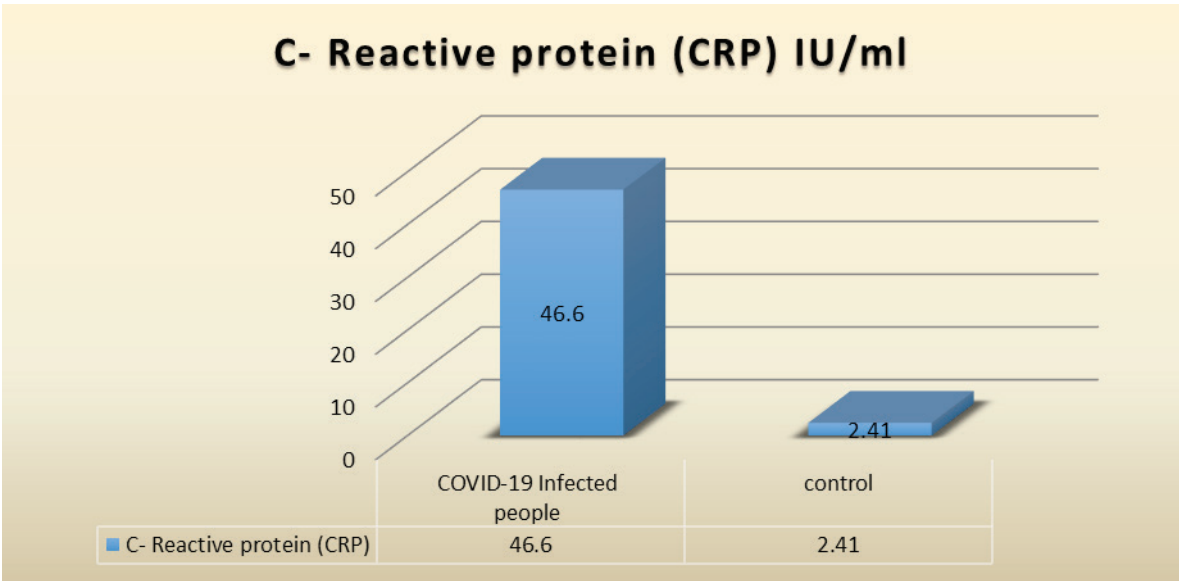

Figure No 4: show the relationship between CRP level and infection by COVID-19.

The current study coronavirus infection caused a significant increase level of PCT (P- value < 0.001) as the PCT level (16.2 ng/ml) in infected people compared to the control group 0.16 ng /ml.

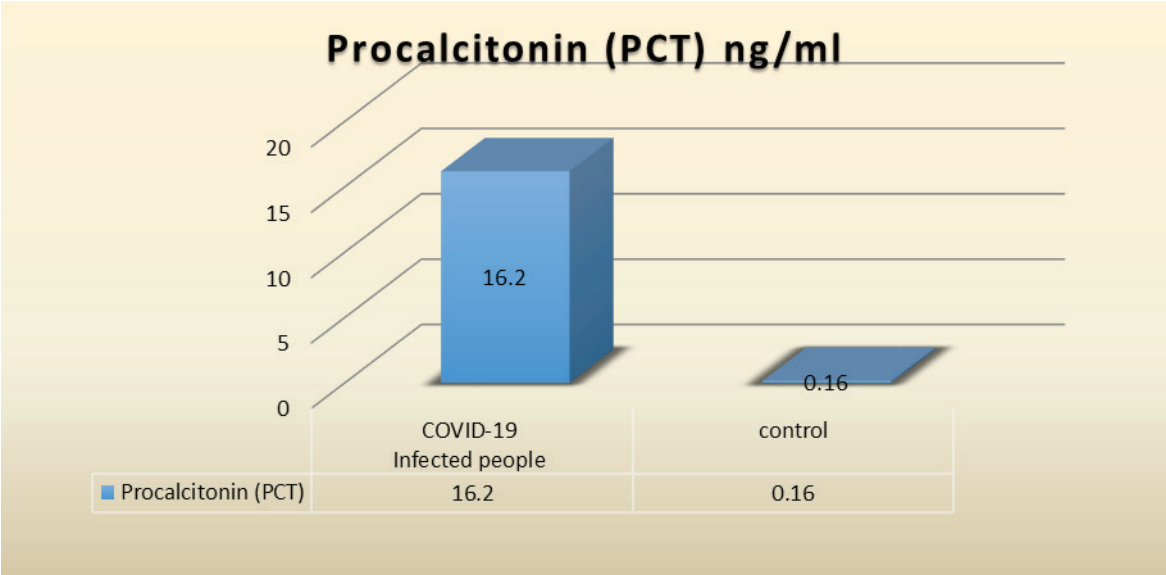

Figure No 5: showing the relationship between procalcitonin level and infection by COVID-19.

Discussion

COVID-19 pandemic is a significant general health danger that requires quick activity. Notwithstanding the extreme endeavours to discover novel medications for SARS-CoV2, this procedure is tedious with constrained advancement to date. Hence, medicate repurposing has been recognized as the quickest method of figuring it out restorative specialists for COVID-19 to meet the desperation of the situation.[14]

The present study showed that elevated serum CRP, PCT, and serum ferritin levels in COVID-19 patient compare to control group.

CRP(C-Reactive protein) is an inflammatory acute phase protein that can be elevated in many conditions, such as inflammation, cardiovascular disease, and infection, developed by the liver. [15]

infection by coronavirus effect on a biomarker of body, as the level of CRP and Ferritin.in this study, the CRP and Ferritin levels in the serum are increased in patients with COVID-19. This excess might cause secondary infection by bacteria and exacerbate of COVID -19 infection. In the current study evaluated levels of ferritin in the patient's serum COVID -19, which are significantly high.[16]

As we known, when inflammation or tissue damage happens, CRP can be significantly increased in serum, which is usually used as a unique inflammatory marker in the current clinical practice.[17]

On the other hand, PCT, as the precursor of calcitonin, is a kind of glycoprotein without hormone activity, which is significantly higher in bacterial infection, but remain normal or slightly increased in viral infection [17,18]

several studies supported our results about the level of CRP e.g. (**wel Chen et. al**) who demonstrated an increased level of CRP in COVID-19 infection, reaching 23.40 mg/l. so clinically depend on an increased level of CRP as an indicator of nosocomial infection COVID-19 patients who were slow to recover. [19,20]

Mr. Kais Khudair also find from the current study that the age and gender factor play an important role in the prevalence of Covid-19 infection and that Covid-19 infection causes an increase in both the effective protein level C and the level of the ferritin of blood, so it can be relied upon in the early diagnosis of COVID-19 infection. [21]

Conclusion

We conclude from the current study that the age and gender factor play an important role in the prevalence of Covid-19 infection and that Covid-19 infection causes an increase in both the effective protein level C, PCT the

level of the ferritin of blood, so it can be relied upon in the early diagnosis of COVID-19 infection.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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21. Mr. Kais Khudair study the relationship between CRP and Ferritin in people infection with COVID-19 Al-Kufa University Journal for Biology 12.1: 2020.

To Assess the Study of Pro- BNP, CPK -MB and Troponin Level in Acute Myocardial Infarction

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Abstract

Introduction: One of the greatest causes of death and morbidity in the world is acute myocardial infarction (AMI). Atherosclerotic coronary artery disease (CAD) with plaque erosion or breakup, causing transient, partial or total arterial occlusion, is the most common cause of AMI. Without appropriate blood flow, the heart cannot continue to function, and if it is severely compromised, death is inevitable. Simple and objective measures of cardiac function are brain natriuretic peptide (BNP) levels. These measurements can be used to diagnose heart failure, including diastolic dysfunction, and have been shown to save money by using them in the emergency room setting. For the diagnosis of acute myocardial infarction, the most sensitive and most precise measure available is creatine phosphokinase (CPK-MB). The main biomarker for AMI diagnosis is cardiac troponin I. The level of troponin may also be elevated with important prognostic value in many other disorders, including heart failure.

Methods: This study was a case-control study, conducted in the Medicine and cardiology Department at DMMC & SMHRC, Nagpur in collaboration with ABVRH, Sawangi (Meghe) during Nov 2020 to Jan 2020. Total 80 individuals were considered for the study, 40 each in case and 40 in control groups.

Results: As present study show the Pro-BNP Levels were increased significantly (P<0.001) in the AMI (2200±601.30) as compared in controls (90.20±3.20). The mean values of serum CK-MB and troponin I were significantly (P<0.001) higher in the AMI (165±47.21, 1.48±0.45) as compared to those in the healthy controls (11.50±0.80± 3.250, 0.03±0.01) respectively.

Conclusions: These findings therefore indicate that the combined detection of CK-MB, Troponin I and NT-Pro-BNP levels will contribute significantly to the early diagnosis of AMI. It can also provide the clinic with diagnostic evidence and thus reduce AMI mortality in the acute phase.

Key words: *AMI, N-terminal Pro-BNP, CPK-MB, Troponin I.*

Introduction

In the acute phase of myocardial infarction, post-infarction heart failure is a growing medical and epidemiological issue, especially in the community of patients over 65 years of age, despite the increasingly successful treatment available.[1]

Easy and objective measurements of cardiac activity are brain natriuretic peptide (BNP) levels. These measurements, including diastolic dysfunction, can be used to detect heart failure and have been shown to save money by using them in the emergency room setting. To rule out heart failure, the high negative predictive value of BNP tests is especially helpful. Treatment with angiotensin-converting enzyme inhibitors, angiotensin-

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II receptor blockers, spironolactone and diuretics decreases the level of BNP, which means that BNP testing can play a role in tracking heart failure patients. Patients with chronic, stable heart failure, however, can have levels above the normal range (i.e. less than 100 pg/mL of BNP and less than 125 pg/mL of Pro-BNP N-terminal for patients younger than 75 years). Increases in BNP levels may be due to intrinsic heart disease or may be caused by secondary causes, such as pulmonary or renal disorders (e.g., chronic hypoxia). Other tests of cardiac status, such as the New York Heart Association ranking, are associated with BNP examinations. In patients previously diagnosed with heart failure or heart dysfunction, the BNP level is a good predictor of the risk of death and cardiovascular events.[2]

In order to maintain stable blood pressure and plasma volume, and to prevent excess salt and water retention, the heart secretes natriuretic peptides as a homeostatic signal. In the atrial myocardium of rats, an auric natriuretic peptide (ANP) was initially identified.[3]

Due to their stretching, volume overload and elevated fi ling pressure, BNP is released from cardiac myocites Both of these activities result in high wall stress that initiates the release of the BNP precursor Pre-Pro-BNP- it first cleaves to pro-BNP, then to the biologically active BNP and the inactive amino terminal fragment, BNP-NT-Pro-BNP N-terminal prohormone. BNP release is part of a compensatory operation in the failed heart, such as renin-angiotensin-aldosterone system (RAAS) activation and sympathetic nervous system activation. In addition to its function as an intense mechanical pump, the heart has now become a new endocrine organ—the heart communicates its pain by releasing BNP. In various trials in clinical and epidemiology. The direct association between the reduction of the left ventricle's systolic function and the elevation of natriuretic peptides has been shown, allowing for a potential biochemical diagnosis of heart failure. BNP levels are up to 100 pg/ml in the heart with good systolic activity. If the level of BNP is 100-500 pg/ml, further diagnostic evaluation is required.[4] For the diagnosis of acute myocardial infarction, the most sensitive and most precise measure available is creatine phosphokinase-MBB (CPK-MB). With the exception of post-cardiac surgery, the degree and duration of serum CPK-MB elevation approximates the level of acute myocardial infarction, although a number of factors can undermine the reliability of such an index. Conflicting documentation concerning the presence of CPK-MB in tissues other than myocardium and the release of CPK-MB under conditions other than acute myocardial infarction has been provided by differences in fractionation and assay methods for creatine phosphokinase isoenzymes. Also worthy of consideration is the embryological growth of the CPK-MB isoenzymes, as well as the different conditions involving increased serum CPK-BB activity.[5]

While CPK-MB is of great benefit in the environment of a coronary care unit (ccu), following cardiac surgical procedures, it is less useful. CPK-MB is normally elevated postoperatively for procedures including coronary bypass grafting, valve replacement or reconstruction of congenital defects. The release of CPK-MB occurs intraoperatively, and in 74 percent of cases, CPK-MB has been reported back to normal levels 18 hours after surgery.[6,7]

The creation of delicate and precise biochemical markers of myocardial injury (cardiac troponin) has helped to diagnose and treat acute myocardial infarction in recent years (MI). The diagnostic criteria for acute MI were redefined by the European Society of Cardiology (ESC) and the American College of Cardiology (ACC) to include the measurement of cardiac troponin as the gold standard.[8] As a result of these recommendations and the increased availability of troponin assays, the diagnosis rate of MI may increase,[9] Which is likely to influence the treatment and epidemiology of patients.[10]

In addition to its diagnostic application, the ACS setting is prognostic, clinically actionable, and can be tracked as a proxy for progress during therapy. Troponin is a valuable biomarker of prognosis in acute decompensated heart failure (AHF), but it does not help in the diagnosis of AHF, and its function as an actionable biomarker is uncertain. Guidelines suggest that troponin levels be assessed in patients presenting with AHF for evaluation of the event of ACS as well as for assessment of prognosis and seriousness of disease.[11]

Materials and Methods

This study was a case-control study conducted between Nov 2020 and Jan 2020 in the Department of Medicine and Cardiology at DMMC & SMHRC, Nagpur

in collaboration with ABVRH, Sawangi (Meghe). For the analysis, a total of 80 participants were considered, 40 in each case and 40 in the control groups.

Inclusion criteria for myocardial infraction:

Patient suffering for Myocardial infraction (AMI).

Exclusion criteria for myocardial infraction:

Patients with chronic diseases such as tuberculosis, HIV, cirrhosis of the liver, acute & chronic kidney failure, etc.

Sample Collection:

5ml Blood sample was collected from each subject by venipuncture with standard blood collection technique in a plain vial for serum separation.

Biochemical Analysis

Pro-BNP, CK-MB, and Troponin I were detected by chemiluminescence immunoassay (CLIA)in 40 AMI patient (AMI group) and 40 Non-AMI Subject (control group).

Result

Table 1: Percentage wise distribution of subjects in Control Group & Study Group.

Sr. No	Group	No of patient	Percentage (%)
1	Control group (Healthy Individuals)	40	50%
2	Study group (AMI Patients)	40	50%
	Total	80	100%

Table 1: Shows percentage wise distribution of control group and study group. The present study includes total 80 subjects. Total 80 subjects were divided into two groups: Control Group consists of 40 (50%)) Healthy Individuals and study group consists of 40 (50%) (AMI Patients)

Table No 02: The BNP, CPK-MB, Trop I Levels

Parameter	Study group (MI patient)	Control group (Healthy subject)
Pro-BNP (pg/ml)	2200±601.30	90.20±3.20
CPK-MB(IU/L)	165±47.21	11.50±0.80
Trop I(ng/ml)	1.48±0.45	0.03±0.01

As shown in table no. 2 The BNP Levels were increased significantly ($P<0.001$) in the AMI (2200±601.30) as compared in controls (90.20±3.20). The mean values of serum CK-MB and troponin I were significantly increase ($P<0.001$) higher in the AMI (165±47.21, 1.48±0.45) as compared to those in the healthy controls (11.50±0.80± 3.250, 0.03±0.01) respectively.

Discussion

Natriuretic hormones are a family of vasoactive peptides that, as their name implies, promote natriuresis and diuresis, which serve as healthy arterial and venous vasodilators. The ability to measure the circulating concentration of these hormones has led to an interest in enhancing the diagnostic and prognostic evaluation of cardiovascular disease patients through the use of these

levels.[12,13]

Sabatine et al. It has been shown to be closely associated with the severity of an acute ischemic insult and the amount of Pro-BNP circulating.[14]

In this study, we observed increased levels of Pro-BNP relative to healthy populations in patients with AMI. Among the patient classes, Pro-BNP levels were found to be the highest in the cardiovascular patient population,

In interpreting myocardial infarction, CK and, more specifically, its isoenzyme CK-MB also have a formal function. The new definition, however, is not very beneficial because studies have shown that patients with myocardial infarction and unstable angina have similar results, as currently described.[15,16]

As expected, in patients with AMI, CK-MB levels were higher than in the healthy population in the current study. According to this study, CK-MB therefore appears to be a better predictor of MI compared to Troponin, especially within the first few hours of MI. There were significantly higher CK-MB levels comparison with the control group ($p<0.05$). Troponin I is a complex troponin-regulatory protein involved in contractility of the heart. Troponin I have a very high myocardial tissue specificity, is not detectable in the blood of healthy people, and, compared to a mixture of ECG and traditional biochemical markers, provides improved sensitivity and specificity for AMI. Cardiac-specific troponin are highly sensitive and reliable markers of myocardial injury.[17] and therefore cardiac troponins are the preferred markers for the diagnosis of myocardial infarction.[18] In this study, AMI patients were found to have increased levels of troponin I as compared to healthy controls, as predicted.

Conclusion

These findings have therefore indicated that the combined detection of CPK MB, Troponin I and NT-Pro-BNP levels will contribute significantly to the early diagnosis of AMI. It can also provide clinical diagnostic evidence and thus lower AMI mortality in the acute stage.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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Perception of Final Year Physiotherapy Students about Effectiveness of Virtual Skills Lab

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Abstract

Background: "The art of physical therapy is elaborately tied to the art of communication." In ancient medical course of study, communication isn't schooled formally and this results in a spot in dependability and consistency of the teaching. **Aim**: To study the concept of Final year physiotherapy students about Effectiveness of virtual skill lab. **Materials and Methods:** This observational study was carried out at Virtual skill Lab of Datta Meghe College of Physiotherapy, Wardha. Feedback was obtained with the help of a pre-validated questionnaire from 40 final year students of physiotherapy about their concept about utility of the model taught in the Virtual Skill Lab including factors which helped them and which affected there learning. **Results:** A total of 78.46% students were of the idea that Virtual Skill Lab posting is must for all medical undergraduates. A 93.83% perceive that the module taught was very relevant and useful and were satisfied with the duration of posting (81.47%). A 78.46% students experienced improvement in their virtual skill lab. They opined that more emphasis should be given on communication between doctor and patient (61.53%). **Conclusion:** The students found virtual skill Lab very useful. They desired more emphasis on communication between doctor and patient and sought more interactivity, video demonstrations to be part of the module.

***Key Words**: Virtual, Medicine,Physiotherapy, Medical Education Unit*

Introduction

"The artwork of drugs is intricately tied to the artwork of verbal exchange"[1]. "Medicine is an art whose magic and creative potential have long been identified as dwelling within the interpersonal factors of patient-doctor relationship"[2,3]. Clinical digital skill lab embody a sequence of abilities that facilitate the verbal exchange between health practitioner and affected person[4].

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However, for the duration of interviews of sufferers, maximum docs tend to pay attention on medical factors associated with ''disease'' (Signs and symptoms) as opposed to on the patient's specific revel in of his or her ''infection'', consisting of the affected person's ideas concerning the cause and results of the sickness[5,6,7]. This changed into illustrated by means of a take a look at showing that docs interrupted their patients' commencing statements after a median length of 18 seconds[8]. This behaviour might also lead to the loss of valuable statistics this is crucial to arriving at a accurate prognosis[8]. Placing the sufferers' issues at back foot creates a terrible health practitioner and

patient relationship[9]. Ultimately the goal of any shape of physician-affected person conversation is to discover the purpose of patients problems and improve the affected person's fitness and hospital treatment[3,10]. Even although many docs considered their communique good enough or maybe great, studies on doctor affected person verbal exchange have confirmed discontent from sufferers' view point[10-12].

There is also an increased interest in research in this field of doctor-patient communication, recognizing the need to experiment with the teaching methods and to measure the clinical skills [1]. The attitude of medical students towards learning virtual skill lab have long been a matter of concerns for medical teachers, curriculum planners and policy makers[13–15]. Like any other skill, good communication skill is an art which can be acquired and improved by putting conscious efforts in day to day practice. Such skills should also be incorporated as part of medical teaching curriculum [5].

Objectives

To study the perception of medical students about usefulness of virtual skill lab b to study factors facilitating and hindering learning in the lab and to study their preference regarding particular components of virtual skill lab.

Materials and Methods

This cross-sectional observational study was carried out over a period of 6 months, from 1st January 2018 to 30th June 2019 in Virtual skill Lab at Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, (DU), Sawangi (M), Wardha after approval from the Institutional Ethical Committee.

Inclusion criteria

- Students of final physiotherapy who were posted in virtual skill lab during the study period.

- Students who attended the full five days posting without absentee.

- Students who consented to be a part of the study.

Exclusion criteria

- Students who did not wish to participate in the study.

Materials and Methods

The III-I physiotherapy students who were posted in virtual skill lab were taught with a virtual skill lab module during their posting of 5 days for 2 hours each day. Each day a different faculty member, trained in virtual skill lab, engages the students. These faculties are members of Medical Education Unit (MEU) and were trained in communications skills in a workshop conducted by School for Health Professions Education (SHPER) for this purpose.

Module of Communication Skill Lab for III- I physiotherapy: The module deals with various aspects of communication and professionalism including rapport building with the patient, history taking, consent, examination of patient and grief announcement. The teaching methods used are debates, role plays, group activities and videos. The students are also sensitized about time management, stress management and conflict management skills. On the last day of posting the students are taught about common vernacular terminologies regarding the symptoms **[Tabl- 1].**

[Table-1]: Details of the module are as follows:

Day	Topic
1	Get acquainted Power point presentations on virtual skill lab Discussion on the power points Planning /practice for role play

***Cont...* [Table-1]: Details of the module are as follows:**

2	Role plays Importance of History talking – Deviant, Ideal Rapport Building – Deviant, Ideal Examination of patient - Deviant, Ideal Ward & Bedside manners - Deviant, Ideal Behaviour in the clinical postings – Deviant, Ideal
3	Debate:Topics: Good communicators are born as good communicators Language is an important barrier for communication Communication gap and generation gap are inter-related Health professional students should be posted in the communication skill lab. The generation of family physicians was the generation of excellent communicators.
4	Powerpoint presentations on: Conflict Management Time Management Stress Management Team Performance Sympathy and Empathy Leadership skills
5	English to Marathi Dictionary

Statistical Analysis

For close ended questions, various percentages were calculated and also average score on a scale of five was estimated. Open ended responses were qualitatively analysed using coding and categorization.

Results

During the study period 85 students of final MBBS were posted in virtual skill lab lab. Out of these 65 met the inclusion criteria and feedback forms were obtained from them. Before posting in the virtual skill lab 12 out of 65(18.46%), students were unaware about virtual skill lab . A total of 30 (46.15%) students were slightly aware and remaining 23 (35.38%) were well aware of Virtual Skill Lab as they are posted during the 1st and 2nd MBBS as well (But attendance is not compulsory) [Table-2].

[Table-2]: Student's awareness about virtual skill lab lab before posting

Awareness	Unaware (%)	Slightly aware (%)	Well aware (%)
No of students (N)- 65	12 (18.46)	30 (46.15)	23(35.38)

In the feedback questionnaire, among the closed ended questions, 78.46% students opined that Virtual Skill Lab posting should be compulsory to the medical students. Out of these 40% students strongly agreed and 38.46% agreed. Only two students (3.07%) strongly disagreed and five (7.69%) disagreed and 10.76% were neutral on this question. Average score on 5 point scale was 4.04. The students who strongly disagreed were probably concerned with the timing of the posting i.e. last semester. They thought that they should get more time to study.

Four students said that the posting duration is inadequate. Whereas 53 (81.53%) students felt that the posting duration was adequate. Out of this 43.07% (28) strongly agreed to adequacy of duration of posting. Only four (6.14%) students felt that, there was no improvement in their virtual skill lab after the posting in Virtual Skill Lab (Two strongly disagreed and two disagreed). These were the students who also thought that the duration of posting was inadequate and the timing of posting was not correct. On the other hand 51 (78.46%) students felt that, their virtual skill lab improved after the posting in Virtual Skill Lab. This includes 38.4% students who agreed strongly and 40% who agreed. Average score was 4.07 [Table-3].

[Table-3]: Student's reaction to virtual skill lab posting using a 5 point Likert scale

Parameter	SDNo (%)	DNo (%)	SD+D(%)	NNo (%)	ANo (%)	SANo (%)	A+SA(%)	Average score
Virtual Skill Lab posting should be compulsory	2(3.07)	5(7.69)	7(10.76)	7(10.76)	25(38.46)	26(40)	51(78.46)	4.04
VIRTUAL SKILL LAB is well equipped	0	4(6.15)	4(6.15)	5(7.69)	26(40)	30(46.15)	56(86.15)	4.26
Modules taught are helpful	0	0	0	4(6.15)	40(61.53)	21(32.30)	61(93.83)	4.26
Faculties are competent	0	3(4.61)	3(4.61)	11(16.92)	25(38.46)	26(40)	51(78.46)	4.13
Faculties are approachable	1(1.53)	3(4.61)	4(6.14)	8(12.30)	26(40)	27(41.53)	53(81.53)	4.15
Duration of posting is adequate	4(6.15)	0	4(6.15)	8(12.30)	25(38.4)	28(43.07)	53(81.47)	4.12
Posting has improved your virtual skill lab	2(3.07)	2(3.07)	4(6.14)	10(15.38)	26(40)	25(38.4)	51(78.46)	4.07

Five point Likert scale – SD (Strongly disagree) – 1, D (Disagree) - 2, N (Neutral) -3, A (Agree) – 4, SA (Strongly agree) – 5

Maximum students (61.53%) wanted to learn about communication between doctor and patient. Thirty five percent (35.38%) i.e. 23 students opined that communication between student and teacher should be taught in the module of Virtual Skill Lab. Surprisingly almost 18.46% students preferred modules on student-student communication, indicating the communication gap between peers. Only one student (1.53%) wanted to learn about communication between student and non teaching staff. This aspect of communication is important in the clinical settings but mostly overlooked [Table-4].

[Table-4]: Student's preference about communication skill module

Module for communication between	No of students (%)
a. Student – teacher	23 (35.38)
b. Student- student	12 (18.46)
c. Student- non teaching staff	01 (1.53)
d. Doctor- patient	40 (61.53)
e. Any other	Nil

Analysis of the open ended questions suggested that, the students found group discussions (33%) and interactive sessions (20%) very helpful for learning in Virtual Skill Lab, followed by role play (18.46%), games (16.92%). The students were of the opinion that, power point presentations (7.69%) were not very helpful in learning. In fact it was stated as the top most factor (9.23%) causing hindrance to learning and 4.61% students found the infrastructure and ambience of Virtual Skill Lab as one of the factors facilitating learning [Table-5].

[Table-5]: Factors facilitating learning in virtual skill lab*

Factor	No of students (%)
1. Group discussion	22(33)
2. Interactive sessions	13 (20)
3. Role play	12 (18.46)
4. Games	11 (16.92)
5. Power point presentations	5 (7.69)
6. Infrastructure and ambience	3 (4.61)
*Open ended question. All students did not respond to these questions.	

Top five factors causing hindrance to learning, as stated by students were power point presentations (9.23%), less interactivity, small duration of posting, communication gap between teacher and students (6.15% each) and interruption in power supply (4.61%). The reason for felt or perceived communication gap between teacher and student could be due to more use of power point presentations by the teachers, which sometimes become monotonous [Table-6].

[Table-6]: Factors causing hindrance to learning*

Factors	No of students (%)
1. Power point presentations	6 (9.23)
2. Less time	4 (6.15)
3. Less interactivity	4 (6.15)
4. Communication gap between teacher and student	4 (6.15)
5. Interruption in power supply	3 (4.61)

*Open ended question. All students did not respond to these questions

Discussion

Our results are comparable to other studies done on virtual skill lab in medical students. Neupane MS et al., in their study of, "Attitude towards learning virtual skill lab in medical students of Chitwan Medical College, Nepal" also found similar results, where in the students in lower terms had a stronger tendency to learning virtual skill lab than the ones in higher terms[15]. Not only is it recommended to place more importance on virtual skill lab but also to provide greater opportunities for students to learn the virtual skill lab[15-18]. Overall 51 students (78.46%) agreed to the point that Virtual Skill Lab posting had helped them in improving their virtual skill lab. Similar were the findings of Towle A et al., and also Wagner PJ et al.,[16,19]. There is some evidence that taking courses that emphasized virtual skill lab training also influenced the medical students' attitudes towards them[20,21]. In the study by Rees C et al., which examined the attitudes before and after the course for virtual skill lab, the students rated their virtual skill lab significantly lower at the end than before the start of the course. By the end of the course even positive attitudes towards learning virtual skill lab become significantly lower as compared with the start. In this study as per the researchers, the reason might have been the overconfidence of students about their abilities to communicate with patients, which was brought down to more realistic levels after learning the communication issues during their training [20,21]. patient is responsible for the development of attitude towards virtual skill lab training [21]

Conclusion

The students found virtual skill lab lab very useful in improving their virtual skill lab. They desired more emphasis on communication between doctor and patient. They sought more interactivity and video demonstration to be part of the modules. The methodology used to teach the modules should be more interactive. Apart from doctor patient communication, communication between students and teacher and between students themselves also needs to be taught. Video demonstrations should be added in teaching methodology. Power back up should be provided to the virtual skill lab lab.

Five point Likert scale – SD (Strongly disagree) – 1, D (Disagree) - 2, N (Neutral) -3, A (Agree) – 4, SA (Strongly agree) – 5*Open ended question. All students did not respond to these questionsNote - *Open ended question. All students did not respond to these questions

Conflict of Interest: Nil

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Ethical Clearance: taken from institutional ethics committee

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Quality Assurance in Physiotherapy Education in India

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Abstract

Objectives: To investigate the quality assurance system in Indian physiotherapy education. **Design:** A cross-sectional survey with mixed method was employed in this research. **Participants:** Forty two participants were involved in the study and responded to a questionnaire. Seven participants out of the 42 were selected for video interview. **Materials and Methods:** A questionnaire and an audio or video interview technique were used for the data collection. Descriptive statistics was used to analyze the questionnaire. A qualitative method was analyzed with content analysis. **Results:** The questionnaire result showed that hardly one-third of participants were aware about quality in general in the physiotherapy education. In the analysis of the interviews, four categories emerged: They indicated that awareness, skills, rating, and requirements related to quality seem to be important in developing quality assurance in physiotherapy. **Conclusion:** As a small scale study of quality assurance system, this study can contribute to develop an overall awareness of quality system in the Indian physiotherapy education. It can also contribute to the educational and professional life ahead by developing experience and updating the upcoming new physiotherapy researches. However, affirmative effects can also extend physiotherapists in advance.

Keywords: *Awareness, accreditation, credentials, professional recognition, students' educational quality, skills*

Introduction

People and Countries are relying upon schools and universities to make a successful future; to catch the advantage of this more focal fixation and advanced training Foundations (HEIs) need to change their crenellation, task, cycles, and undertakings to be both more versatile and more open to changing social necessities[1]. After the achievement of self-sufficiency in 1947, India has achieved broad progression in tutoring. We have gained gigantic ground with respect to growing the horizons of fundamental, helper, and high level training[1,2]. Instruction has covered about two-third of the populace. The improved guidance system has been much of the time alluded to as one of the standard allies of the headway of India's economy[3]. The headway of guidance in India has been credited to the development in the Establishments of higher learning close by specific progression. There are central, state, thought of, saw self-financed universities and private schools. The state-upheld schools are on square honors or upkeep grants. The private tutoring market in India is surveyed to be esteemed at $40 billion of each 2008 and we foresee that it should augmentation to $68 billion by[4]. Regardless; we see it that while broadening horizons in consonance with growing solicitations HEIs are standing up to critical challenges to ensure quality in guidance which

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is apparently a concern of huge clinical Organizations the country over. These challenges were perceived by coming in the strategy for esteem improvement estimates endeavored by the College which anyway were unequivocal to us yet can be summarized for the HEIs in the country[5,6,7].

Materials and Methods

Design

Cross-sectional survey designs with mixed (quantitative, qualitative) methods were employed. A questionnaire with audio or video interview techniques was used for the data collection. Mixed methods were also used to analyze the data. Permission to perform the research was obtained from the head of the Institution.

Participants

A purposeful selection of 42 participants willing to participate was considered for this study. They were faculty members, post-graduate students, and interns at the College of Physiotherapy [Figure 1], Pravara Institute of Medical Sciences, Loni, Maharashtra State, India – 413 736. Out of the 42 participants who were recruited for this study, 32 (76%) were females and 10 (24%) were males. They had variable professional experience from none to 10 years. The average age of participants was 25.83 ± 7.564 years with a range from 21 to 53 years.

Figure 1: Demographic presentation of participants

Figure 2: Barriers of quality assurance in physiotherapy education

Data collection

A questionnaire was developed specifically for this study. The questionnaire had close-ended questions with yes and no answers and was constructed to give an idea of quality assurance. A number of background questions and six questions related to quality assurance were included like, have you heard about quality assurance? Do you think quality assurance exists in India? Is quality assurance system necessary? Do you think it can maintain academic standards? And, What are the barriers [Figure 2] for quality assurance, will quality assurance be of any help? A restricted number of participants were selected for audio or video interview. As per the convenience and willingness of the participants, they could choose interview from either audio or video. The interviews were performed on the same day in a small office at the physiotherapy. The duration was about 5-10 min. The interview was conducted by the investigator and one supervisor. It was formal interviews with five structured questions like what is quality in general? Will quality assurance be of any help in physiotherapy? How could a quality system in Indian physiotherapy develop? Will quality assurance improve our professional skills? Will it improve academic standards and requirements needed to create a quality assurance system? The mixed type of questions in the questionnaire and the interviews were used to receive a broad picture and to know the participants' different opinions and ideas about quality.

Data Analysis

The analysis of the questionnaire responses used descriptive statistics. The interview text was analyzed with content analysis. The process of analysis was constructed in four steps: Meaning unit, condensed meaning unit, coding, and category. Later, the codes with the same ideaswere grouped into the different categories. This content is abstracted by only audio\ video interview.

Results

Out of a total 50 participants, 13 interns, 11 Ist Yr MPTh students, 10 IInd Yr MPTh, and 8 Faculty Members available at COPT on the day of the data collection, 42 participated in this research yielding a response rate of 84% and rest 8 participants were not interested to respond for the study. It showed that the results of completed Questionnaire revealed that 29.5% of participants heard about the quality assurance in physiotherapy, 11.9% felt that quality assurance exists in physiotherapy, 95.2% felt that quality assurance will be of any help, 97.6% felt that quality assurance is necessary for Indian physiotherapy education, and 92.8% felt that quality assurance will help in maintaining the academic standards. The last question consisted of sub-questions as barriers of quality assurance and response.

Structured questionnaire result: Out of 42 participants, only 7 participants agreed for audio\ video interview, of which 5 (12%) agreed for audio interview and 2 (5%) agreed for video interview. The interpretation of the video interview was done as it can be used for

other educational purpose. Their responses formed four categories such as Awareness, Skills, Rating, and Requirements

Awareness: Responses showed that there was unclear idea about the quality assurance and no concentration on quality. General idea suggested that quality was given but not working on quality assurance. The majority response was that participants were unaware about quality assurance. Even barriers of quality were included, by which conductance of quality assurance is not possible.

Skills: Responses ensured of basic professional skills competence. Some didn't find any relation between professional skills and quality assurance. It showed that 57% felt that quality assurance can improve our professional skills.

Ratings: Participants felt that academic standards can be improved by quality assurance to certain extent. The responses indicated that 29% perceived that quality assurance will help in rating academic standards.

Requirements: Regarding the requirements, some have no idea of requirements; some suggested of clinical practice license, credentials (a documentation and certification), accreditation (grading of students academics), awareness of quality and practice guidelines, regulatory bodies for physiotherapy education are needed, so they can be confident about their knowledge and some proof that no one can replace them. Seventy two percent felt that these are the requirements so as to ensure the quality assurance in physiotherapy education.

Discussion

The findings of this qualitative pilot study suggested that the current status pertaining to quality assurance system in physiotherapy is probably missing. The questionnaire had in the beginning a filter question asking if the participant heard of quality assurance system in physiotherapy. One-third of the participants reported an affirmative response to this question, suggesting the greater extent of unawareness about quality assurance system in physiotherapy education[8,9]. This response is because there is lack of knowledge, time, and lack of awareness. Since there has been hardly any similar study in India, this topic needs to be highlighted. The results of the study could not be interpreted in the light of previous knowledge Secondly, a restricted number of participants reported positive but lower response rate for existence of quality assurance in physiotherapy education, but suggested that they had a vague idea of quality system and its existence[10,11,12]. Thirdly, almost all the participants reported an encouraging response to help in quality assurance in physiotherapy and suggested that development of quality assurance is low and the awareness with its existence is near to the ground. Fourth, majority of participants reported that there is necessity of quality assurance in physiotherapy and suggested that it should be made necessary for progress of physiotherapy education and increase confidence in their ability in the near future. Almost all participants reported confirmatory responses for maintaining academic standards and recommended that by introducing new techniques and some uniform rules could help in maintaining academic standards[13,14]. The quality assurance can improve theoretical and practical knowledge. It can give greater confidence to students and also some responsibility to their own learning and later can act as facilitators. The old techniques will not be further developed if the importance of quality has not been discussed and would follow the same path. Even higher bodies are almost non-existing to regulate physiotherapy. The findings also suggest time constraints related to perceptions of burden, so lack of time is also more in common. The former questionnaire consists of structured questionnaire done by interviewing participants[15,16]. In the interviewing process, many described their clarification about the study: The comments made more commonly on awareness, skills, rating and important aspects illustrates that participants have a vague idea about quality in general as well as quality assurance as it is self-contradictory word, so participants think that quality assurance is lacking. It describes that there should be improvement in educational point of view and once developed in proper manner, it can provide apropriate learning opportunities and improve their knowledge as well[17,18]. Here also, we got affirmative response by participants that introducing new techniques in our theoretical knowledge will improve professional skills and can keep updating our knowledge and implementation of that knowledge or skills and keep the records, which can show quality indeed and quality assurance can help in improvement

of skills. It can be maintained by approaching to students' education, structuring supervisions, structured orientation programs, and increase confidence in their ability of academic knowledge. For requirement, even regulatory bodies are needed, so that people can get good quality service and also be confident about the quality they are giving to the patients. Teaching and facilitation of learning are likely to become even more important as physiotherapist adapt to policy changes and adopt a more preventative and educative role in practice. The questionnaires used in this study were close-ended and structured questionnaire. It is the investigator's design and study[19]. The questionnaire was made simple to comprehend for the participants. In addition, interview was conducted of the participants since it is believed that using more than one method of data collection may be helpful in obtaining a more complete picture of human behavior and experience. By this help of the questionnaire, even participants got aware of quality assurance and its existence. So, there should be need to study the quality assurance of physiotherapy education in India.

Conclusion

Public interest in accountability is rooted in the growing importance of physiotherapy education and uncertainty concerning its adequacy and affordability. Quality assurance of Indian physiotherapy academic programs is highly perceived as non-existing or missing, and ineffective physiotherapy education effectively serves public and individual priorities.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from institutional ethics committee

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Effect of Cigarette Smoking on Vitamin C in Non Pregnant and Pregnant Women

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Abstract

Introduction: It is perceived that smoking cigarettes during pregnancy is unfortunate and can prompt expanded unconstrained early abortion in the first trimester, premature abruption of the placenta, preterm delivery, decreased birth weight and SIDS. All things considered, children destined to mothers who smoke during pregnancy weigh 150-300 gram not exactly those destined to mothers who don't smoke and the danger of little for-gestational age among ladies who smoke is at any rate twice as high as among ladies who don't smoke. For more seasoned ladies, the effect of smoking during pregnancy on fetal development and preterm conveyance are more noteworthy. The danger of placental issues can be expanded by long haul smoking.

Aim: Effect of cigarette smoking in non-pregnant and pregnant women's on the blood serum level of vitamin C

Material and Methods: The present study included 150 subjects of age group 25-35 years. Out which 75 werenon-smokers pregnant women as a control group and 75 were smoker pregnant women as a study group. During this stage of pregnancy, the vitamin C levels in the maternal serum were colorimetrically determined. The respondents also answered a questionnaire about their smoking habits during pregnancy. Vitamin C intake was measured during the third trimester by monitoring food consumption over a 5-day period (including Sunday) and vitamin C registration with dietary supplements.

Results: In the present study, we found significant decreased levels of serum vitamin C and in pregnant cigarette smokers as compared to pregnant non-smokers.Vitamin C was protective for placental abruption in nonsmokers but not in smokers (P=0.01).

Conclusion: If the production of antioxidants (vitamin C) in smokers is lower compared to pregnant women who are not smokers, this could aggravate their newborn's peroxidation problems. Supplementation of vitamin C tends to be connected with a decrease in placental abruption and preterm birth in pregnant smokers.

Keywords: *Lung development, pulmonaryfunction test, Vitamin C, Preterm birth, Smoking and Pregnancy*

Introduction

Smoking during pregnancy is the key preventable cause of childhood respiratory disease[1-3] and is a significant public health issue because, despite the alert of the Surgeon General of the related health issues, more than 50 percent of smokers who become pregnant continue to smoke.[4,5]This is equal to at least 12 percent of American women who choose to smoke while pregnant, resulting in more than 450,000 babies born annually exposed to smoke.[6] Maternal smoking adversely affects lung development during pregnancy, as shown by lifelong declines in pulmonary function and increased risk of wheezing, infections of the respiratory tract and asthma.[1,2]

In spite of the well-known adverse health effects of smoking cigarettes, consumption rates remain high. In addition, smoking rates are highest among women of childbearing age (20-24 years), affecting one in six females. Increased rates of abortion, prematurity and low birth weight are among the dangers of smoking during pregnancy. In addition, maternal smoking risks to the child extend far into the neonatal period and are known to include an elevated risk of sudden infant death syndrome, low lung function, and inflammation of the lower respiratory tract[7]. Despite these known risks, only one-fourth of women will quit before pregnancy and another 20% will quit during pregnancy[8], meaning that many women are either unaware of the risks or unable to quit smoking.

The smoking is allied with a lesserconsumption of antioxidants,specifically vitamin C[9, 10]. It has also been reported that vitamin C in smokers' tissues and plasma may be at suboptimal concentrations[11]. In particular, deficiency of vitamin C during pregnancy hasbeen linked with an increased risk of contracting infections,premature rupture of the membranes[12,13], prematurity[12] andeclampsia[14]. In pregnant women, cigarette smoking is a source of oxidant tension, indicating that in infants exposed to utero[10], it may be a source of the same. At birth, compared to in utero conditions, the newborn is brought into an atmosphere that is hyper toxic and vitamin C deficiency can lead to inadequate antioxidant defences[15]

The aim of the present investigation was to determine the effect of cigarette smoking in non pregnant and pregnant women's on the blood serum level of vitamin C.

Material and Methods

Our study was carried out in the Biochemistry Department, DMMC&SMHRC, Nagpur, from April 2020 to November 2020. The study was approved by Institutional Ethical Committee and informed consent was taken prior to the study. A total 150 subjects of age between 25-35 years were enrolled in this study. Out of 150 subjects, 75 were non smoker pregnant women as a control group and 75 were smoker pregnant women as a study group. The pregnancy periods of 150 women was followed. During their third trimester (between weeks 32 to 36) dietary, anthropometric and biochemical studies were made.

Inclusion Criteria

Inclusion criteria were age between 25-35 years , mother contain either Single and multiple gestation, gestational age between 130/7 and 226/7 weeks based on clinical information and ultrasound and pregnant women's smoking history (Current cigarette smoking of at least 1 cigarette in one day).

Exclusion Criteria

- Age less than 25 and above 35 years
- Diabetics or using insulin or oral hypoglycemic agents
- Hypertensive or using antihypertensive medication
- Hyperlipidemia or using lipid lowering drugs,
- Obese
- Abnormal renal function

Blood sample collection and processing

A 2 ml venous blood sample was collected from each participant, into a plain vial. After centrifugation at 1500 rpm for 3 minutes, the serum was assayed.

Biochemical Analysis

Blood samples were taken first thing in the morning from subjects fasted overnight. Since vitamin C is unstable in storage[16], the serum component was separated and vitamin C levels determined colorimetrically[17] (BoehringerMannheimGMbH, Mannheim, Germany) immediately after extraction.

Result

Out of 150 non- gestational diabetic, non preeclampsia subjects, 75 werenon-smoker pregnant women as a control group and 75 were smoker pregnant women as a study group. The age in smoker group and non smokergroup ranged from 25 to 35 years.

Table-1: Personal and Anthropometric Data of Subject and Vitamin C Intake during the Third Trimester of Pregnancy Differences between Smokers and Nonsmokers pregnant women

Variables	Non-Smokers	Smokers
Number of subjects	75	75
Weight (kg)	68.2±8.8	62.4±4.2*
Height (cm)	180.3±9.1	178.8±3.5
BMI (kg/m2)	42.5±4.4	41.2±4.4
Anthropometric data in 3rd trimester		
Weight (kg)	71.3±9.1	68.3±7.5
Height (cm)	180.3±9.2	178.8±3.4
Body mass index (kg/m2)	45.5±4.3	45.1±3.3
No. of children previously born	0.66±0.80	0.83±0.93*
Cigarettes smoked/day (n)	0	6.9±2.8*
Vitamin C supplied by supplements (mg/day)	0	35.7±85.5
Vitamin C supplied by supplements +diet		
Total intake (mg/day)	159.1±83.4	162.3±109.6
Coverage of RI (%)	190.6±98.8	199.9±135.3
Intake, RI (%)	27.4	41.8

Mean±SD. *p<0.05 between smokers and nonsmokers

RI= Recommended intakes. No significant differences between smokers and nonsmokers.

For the purpose of the present study, the total 150 subjects divide into smokers and non-smokers pregnant women were group of non smokers (N=75) and smokers (N=75). **Table 1** shows some of the mothers''personal and anthropometric data. Smoker pregnant women's showed lower bodyweights and had previously born more children, though noother significant differences were seen between the two groups

Smoker pregnant ladies have indicated a marginally lower intake of foods grown from the ground than did Non-smoker pregnant ladies, however these distinctions were not sufficient. These outcomes legitimize the absence of finding any significant contrast between the vitamin C intake of Non-smoker and Smoker pregnant women. During the third trimester, just three Smoker subject took a vitamin C containing supplement, in amounts of 700 to 3500 mg/day. The dietetic outcomes are, accordingly, intensely modified when supplements are considered.(**Table1**).

Table No 2: Levels of Vitamin C in Maternal Serum during the Third Trimester

Variables	Non-Smokers	Smokers
Serum data, third trimester of pregnancy		
Vitamin C (µmol/L)	541.7±369.5*	312.4±108.1*
% low serum levels		
<10. 2µmol/L	31.5	60.8
<15.0 µmol/L	35.6	61.0
<24.5 µmol/L L	39.9	55.1

*P<0.05 between smokers and nonsmokers.

However, Non smoker pregnant women's showed significantly greater vitamin C levels as compared to smokers pregnant women's. (**Table 2**)

Discussion

Our analytical data indicated that a decline in serum vitamin C levels is associated with cigarette smoking. Despite correction of variables that independently affected serum vitamin C levels, such as age, gender, ethnicity, and BMI, this association persisted. In smokers, dietary vitamin C intake was lower than in non-smokers, a result that may theoretically explain this inverse association. Further adjustment for consumption of vitamin C, however, showed that the correlation between smoking and serum levels was independent of dietary intake.Smoking status actually clarified more of the overall difference in serum vitamin C levels than the self-reported vitamin C dietary intake. In addition, there was no major reduction in the increased risk of hypovitaminosis C in smokers while controlling for reduced dietary vitamin C intake associated with smoking. These results indicate that cigarette smoking has a substantial effect on the levels of serum vitamin C, which occurs mainly through a mechanism independent of reduced dietary vitamin C intake.

With regard to the consumption of supplements, **West et al.**[18] indicate that maternal use during pregnancy of a vitamin supplement supplement containing ascorbic acid can provide foetal protection against lead toxicity and/or free radical damage. Despite this, the use of supplements was rare in the current research, and was only found in subjects who smoked.

Many studies have shown a lower intake of vitamin C among smokers[19]. This was not seen in the present research, and because they were pregnant, these participants could have taken better care of their diet (pregnant women typically increase their fruit and vegetable intake[20]. Instead, it may be due to the low cigarette consumption (Table 1). Only ten women smoked 10/day, and only twenty women smoked 15/day.

Decreased preterm births among women receiving regular vitamin C supplements prior to 32 weeks of gestation have already been recorded by **Hauth et al. 2010**, which was due to a decrease in preterm PROM.20 While smoking was regulated in this research, the possible association with smoking was not discussed. Smoking, however, is associated with an increased preterm birth rate prior to 32 weeks of gestation.[20]

We found that self-reported smoking was associated withincreased incidences of the maternal compositeoutcome, neonatal composite outcome, preterm birth, low birthweight and small for gestational age.

The high percentage of subjects with serum vitamin deficiency levels is consistent with that recorded by **Pfeffer et al 1996**[21], who, despite sufficient vitamin C intake, found low levels of leukocyte vitamin C in 60 to 80 percent of a different group of pregnant women, irrespective of their weight gain.This may indicate a high prevalence of subclinical infections, according to **Pfefferet al**[21], which may be the cause of increased peripheral tissue absorption of vitamin C. This result supports the idea of using biochemical estimates for the detection of women with "low" levels of vitamin C.

It is probable that attempts to enhance the nutritional status of pregnant women will be better served by encouraging all women of childbearing age to optimise the nutritional content of their diets, **Borrud et al 1993**[22]. Special care is needed for pregnant smokers, and an increase in their fruit and vegetable intake is recommended.Considering the **West et al**.[18] criterion, it may be appropriate to recommend such nutrient supplements (e.g. vitamin C) for pregnant smokers.

Conclusion

Our findings indicate that the risk of placental abruption and preterm birth at<37 weeks of gestation may be decreased by maternal supplementation with vitamin C among smokers. We do not agree that a supplementation trial limited only to pregnant women who are smoking is recommended at this time because multiple vitamin C supplementation trials have been performed to avoid preeclampsia.Additional systematic analyses and even individual meta-analysis of patient data combining the data and women from these randomised trials with smoking status consideration will further determine the robustness of our findings and confirm them. Such research should discuss abruption and preterm birth explicitly. Therefore, the possible role of vitamin C supplementation as an adjunctive intervention in this at-risk population to prevent adverse outcomes needs further study, while smoking cessation remains the most significant intervention to prevent these outcomes in smokers.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: Taken from institutional ethics committee

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The Effects of Traditional Strengthening Exercises Versus Functional Task Training on Pain, Strength and Functional Mobility in Knee Osteoarthritis

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Abstract

Osteoarthritis (OA) is the most common in India. The incidence of osteoarthritis in adults over the age of 45. Although there is no evidence to suggest that genetics, heredity, histology and biochemistry play a strong role in its development. To date no cure for the disease exists. However, it has been proven that risk factors for the onset and progression of disease can be decreased or avoided through lifestyle modifications such as weight loss, increased physical activity and dietary changes. Twenty subjects were divided into two Groups. Training exercise group 7 subjects and functional task training 13 subjects. Baseline measures for performance outcome measures were insignificant between groups for all of the following: Berg Balance Scale (BBS), (p=0.304), stair climb test (SCT), (p=0.567), timed up and go test (TUG), p=0.970. This study support that functional task training is a better option in improving walking speed in this 45-65 year old population having osteoarthritis knee.

Key Words: *WOMAC, knee OA, Stair Climb Test, Berg Balance Test*

Introduction

Osteoarthritis (OA) is the most widely recognized type of joint inflammation in the US (Lawrence et al., 2008) in grown-ups beyond 45 2009 years old, (McDougall and Keefe, 2009). Albeit the etiology has not been completely depicted, there is proof to recommend that hereditary qualities, heredity, histology and natural chemistry assume a solid part in its turn of events (Hinton, Davis, and Thomas, 2002)[1,2]. Until now, no solution for the illness exists. Notwithstanding, proof proposes that danger factors for the beginning and movement of the illness are reducible or avoidable through way of life adjustments, for example, weight reduction, expanded active work and dietary changes[3,4]. Epidemiologic examinations affirm that these alterations may help control the beginning and movement of knee OA (Zhang, 2010). Restorative boundaries demonstrated to be effective in treatment incorporate patient instruction, exercise based recuperation, pharmacological specialists, social help, assistive gadgets, and support in joint inflammation programs[5]. The impacts of the sickness go with optional weaknesses that remember changes for step, varus/valgus arrangement distortions, muscle irregular characteristics and different illnesses related with maturing (Altman, Hochberg, Moskowitz and Schnitzer, 2000). As indicated by the World Wellbeing

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Association, knee OA positions the fourth most normal reason for incapacity in ladies and the eighth in men (Simmons, Mathers, and Pfleger, 2000). Knee OA addresses a significant reason for torment and brokenness and addresses a financial weight to society[6,7]. The US spends more than $56 billion every year on treatment and pay for people with knee OA (Joint inflammation Establishment, 2009). Radiographs affirm the analysis of knee OA[8].

Methodology

Materials and Methods

Source of data

Data was collected from patients, who are referred to the outpatient physiotherapy department of data Meghe College of physiotherapy Wanadongri, Nagpur with diagnosis of OA Knee after obtaining informed consent.

Research Design: Experimental design of study.

Methods of Sampling: A sample of convenience of twenty patients Aged between 45 to 65 Years diagnosed with Knee Osteoarthritis was selected and equally divided in to two groups randomly, the Traditional exercise group (TE) or the Functional task training group (FTT).

Instruments Used

- Western Ontario **and** MacMaster Universities **OA** lndex **(WOMAC)**
- Timed Up and Go Test
- The Stair **Climb** Test
- Berg Balance Scale (88s)

Results

Subject Characteristics and Baseline Outcome Measure Scores Twenty subjects were divided into the TE (n=7) or FTT (n=13) groups. Results of an independent t-test show that there were no significant differences between groups regarding subject demographics, age (p=0.353), height (p=0.355), weight (p=0.721), BMI (p=0.871), right leg length (p=0.833) and left leg length (p=0.793). In addition, other insignificant differences between groups at baseline were the measures of self-reported WOMAC subsets: pain (p=0.807), stiffness (p=0.996), and function (p=0.531). Baseline measures for performance outcome measures were insignificant between groups for all of the following: Berg Balance Scale (BBS), (p=0.304), stair climb test (SCT), (p=0.567), timed up and go test (TUG), p=0.970.

Table 1 Subject Characteristics and measurement for Groups Traditional Exercise (TE) Versus Functional Task Training (FTT)

Characteristics	Traditional Exercise (TE) (n=7)	Functional Task Training (FTT)	Significance
Age	57.4(± 5.6)	55(±5.3)	0.353
Male	2	5	
female	5	8	
height	65.7(±2.9)	67.1(±3.1)	0.355
weight	214.1(±38.5)	219.8(±30.7)	0.721
BMI	35.2(±8.1)	34.7(±4.9)	0.871
Leg length Right	81.0(±6.5)	81.7(±7.6)	0.833
Left	81.0(±6.5)	81.9(±7.3)	0.793

Table 2: Means and ± Standard Deviation for stair climbing test scores for both traditional exercise and functional task training group at baseline week 6 and week 12

Group	Time	Mean	S.D
Traditional Exercise	1	13.8229	3.39176
	2	13.2000	3.22016
	3	12.7443	3.10723
Functional Task Training	1	15.0354	5.26861
	2	12.7469	3.20549
	3	11.5654	2.93465

Outcome Measures for Stair Climb Test (SCT)

Table 2 addresses the means and standard deviations of SCT scores for the two groups. Mauchly's test found that an assumption of sphericity is highly significant, **W=.206, x2(2) =26.862,** p=0.000. A **two-way** repeated measures **ANOVA** demonstrates there was no interaction effect for Group and SCT scores F (2, 36) =7.46, p=0.079. However, there was a main effect for time for both groups, F (2.36) = 1 1.436, p=0.002, indicating that **both** groups improved after exercise. Pain rise comparisons using Bonferroni's correction indicated that differences occurred between baseline and week 6 as well as baseline and **week12 (peO.01).** Therefore, both groups improved in their ability to Ascend/descend stairs after 6 and 12 weeks of exercise as compared to baseline; however, scores were lower in the **FTT** group.

Outcome Measures for WOMAC Total Score and Subset Scores: Total WOMAC Scores (VVT). WOMAC Pain WP), WOMAC Stiffness (WS), and WOMAC Function (WF):

The means and standard deviations of VVT, WP, WS, and WF scores. Mauchly's test of sphericity was highly significant for one subset, that of WS (stiffness), W=.353, x2(2)=17.723,. p=.000. Results of a two-way repeated measures ANOVA demonstrated there was no interaction effect for group total scores. However, there was a main effect of time for WP scores, F(2, 36)=44.226, p=O.OO, WF scores, F(2,36)= 14.918, p=O.OO and WT scores, F(2,36)= 37.131, p=O.OO, indicating a significant improvement after exercise. Pairwise comparisons show that there was a significant difference for time points for W, WP, and WF at all time levels, p~O.01, indicating that there was a decrease in pain and an improvement in function in both groups from baseline to 12 weeks. WS pairwise comparisons show that there was a significant difference and improvement at 6 and 12 weeks compared to baseline, p< 0.01.

Discussion

In this study, two rehabilitation approaches were utilized to examine if any significant differences existed in measures of strength, pain (as measured by the WOMAC self-assessment scores), gait velocity and functional outcomes on the Timed Up and Go Test, stair climb test and Berg Balance Scale (BBS)[9,10]. Results indicate that both groups improved in all measures of strength, pain and functional outcomes. However, an interaction effect was found for velocity, which was significant across all three testing periods for the functional task training group[11,12,13]. The demographic characteristics of the sample recruited for this study are consistent with those of individuals with knee OA observed in the current literature regarding age, weight and gender[14].

Pain, Stiffness and Function

Regardless of group assignment, there were no significant group differences in pain, stiffness and

function, and both groups displayed significantly lower scores on the WOMAC. Both the TE and FlT group demonstrated significant decreases over time for pain from baseline to the completion of the study with a larger, yet non-significantly different decline in the FlT group[15,16]. Overall, the results from this study are consistent with those found in the literature and suggest that regardless of group assignment, strengthening exercises decrease pain, stiffness, and improve self-reported function in individuals with knee OA (Deyle et al., 2005; Fransen & McConnell, 2001; Hinman, Heywood, & Day, 2007; Jamtvedt et al., 2008; van Baar et al., 1998).

Outcome Measures

Timed Up and Go (TUG)

The TUG scores were significantly different over time for both groups in the study; however, a greater change was observed for those subjects in the FTT group. This change, while not significant for groups, warrants further investigation. The TE group improved their time by ***8.7%*** **(-82** seconds), while the FTT group improved by 19.7% (2.076 seconds). Mean TUG scores have

been reported between six and 1 1.2 seconds in community dwelling women without any **known** pathology. Published studies report that times of 8.5 seconds to 14 seconds indicate pre-clinical functional ability limitations (Fried, Young, Rubin, & Bandeen, 2001) and that a decrease of 1.5 seconds reflects a minimally clinically significant change (Piva et al., 2002)[17,18].

Berg Balance Scale

In this study, both groups were not at risk for falls at the beginning of the study, and both groups consisted of high functioning individuals. The BBS has been noted to be less sensitive to change in individuals with high levels of balance abilities (Bogle & Newton, 1996). Despite this fact, there was an observed significant improvement in the FTT group. Balance is a complex, multifactoral phenomenon involving the vestibular, visual and somatosensory systems, so manipulating the task and environment, as was done with the functional task training group, may help to cope with the constraints of knee OA. Effective and timely muscle control is necessary to maintain balance, and static as well as dynamic control is required for both simple and challenging tasks (Hinman et al., 2002). Several studies demonstrate the relationship between knee pain and balance (Hinman et al., 2002; Jadelis et al., 2001) while others indicate that poor muscle strength and decreased proprioception are greater contributors to balance deficits (Bennell & Hillman, 2005). Jadelis et al. (2001) found that when subjects had greater muscle strength, pain did not influence balance performance. Postural control is a functiop of the aforementioned.

Stair Climb Test

Interestingly, both the TE (increase of 7.8%) and the **FTT** group (increase of 23%) improved in stair climb times with greater improvements noted in the functional task training group. Again, if the study was adequately powered, this noted trend between groups may have been significant[18,19]. As the **FTT** group practiced stair climbing as part of their exercise regimen, it was expected that they would perform significantly better. Although this was not the case, the **p** value was approaching clinical significance. Researchers report that the stair climb test performance is related to quadriceps strength (Asay et al., 2008; Mitzer et al.), pain and function (Stratford et al., 2006; Sowers, et al., 2006) and walking speed (Perera, Mody & Woodman, & Studenski, 2006) in individuals with knee OA. In this study, the larger observed improvements in the functional task training group for all of these variables reflect upon these findings[20,21].

Conclusion

In this study, the FlT group demonstrated a slight improvement in strength (35%) when compared to the TE group **(33%),** which thus could not account for the functional ability differences seen between groups. In addition, although both groups demonstrated significant changes in time to rise from a chair and ascend/descend stairs, the observed change was greater in the functional task training group. Taken together, the data may suggest that increases in strength alone at the impairment level may not result in improved task performance. The FlT group performed exercises which required the subject to change directions, stand on one foot, and negotiate around other individuals and objects and may have resulted in greater task performance and muscular

strength as completion of these activities required strength, coordination, balance, postural control, stability, mobility as well **as** the environmental demands associated with those tasks.

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Determination of Age from Shoulder Joint by Radiological Examination of Epiphyseal Union of Proximal End of Humerus in Boys from Ahmedabad Region

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Abstract

In law, crime and punishment is entirely based on criminal responsibility and this in turn depends on the age of a person. It is well known that appearance and fusion of epiphysis of long bone occurs at particular period of time and this in turns help in age estimation. As there is variation in appearance and fusion time of epiphysis, aim of the present study is to determine Epiphyseal union of proximal end of humerus in boys in Ahmedabad region. Present study was conducted by department of Forensic Medicine and Toxicology, B.J. Medical College Ahmedabad. Total 100 boys of age group between 14-20 years were included in the study and were subjected for radiological examination. To study the appearance and fusion of ossification centre of upper end of humerus digital X-ray with AP view was taken. Result was tabulated and statistical analysis of result was done comparing with previous studies.

Keywords: *Age estimation. Epiphyseal Union, boys, proximal end of humerus, shoulder joint.*

Introduction

In both criminal and civil cases estimation of age of an individual is one of the difficult problems faced by forensic experts. Whether it is a case of living, dead or human remains it always creates trouble in court of law. In cases where authorities are in doubt about the age of a suspected criminal, forensic age estimation is often requested in order to determine if the suspect is of an age in which he may be charged with a crime.[1] In law, crime and punishment is entirely based on criminal responsibility and this in turn depends on the age of a person. Determination of age of an individual from Epiphyseal union is a well accepted fact in the field of medical and legal professions. Epiphysis of the bone unites during age periods which are remarkably constant for a particular epiphysis.[2]

India is a developing country and because of illiteracy and ignorance they do not have proper documentation of births and death registration. Despite the fact that there are a number of laws requiring registration of births and death (e.g, Registration of births and deaths Act 1969), most births are not properly recorded.[3] The age of an individual can be determined from teeth, ossification of bones, and secondary sex characteristics and general development in case of children.[4] It is an established fact that the sequence and ultimate ossification of epiphyses varies in both sexes in different parts of world

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to the extent that there are wide variation even in the population of the different states of the same country.[5] So taking an account to this variation, the present study is to determine the ages of ossification of shoulder joint radiologically in boys in Ahmedabad region.

Materials and Methods

The present study was conducted in the department of Forensic Medicine and Toxicology at B.J. Medical College and Civil Hospital, Ahmedabad. A total of 100 boys were included in our study of age group 14-20 years of Ahmedabad region. Those subject having valid authentic documentation of their date of birth like Birth certificate, adhar card, School leaving certificate, Hospital records etc where included in our study. Those subjects not having valid Date of Birth certificates, bony deformity or pathology, congenital malformation, nutritional deficiency, endocrinal disorders, history of chronic drug intake (e.g. anti-epileptic drugs, steroids), chronic illness thus affecting the skeletal growth and development of the individual etc where not included in our study.

Procedure

Written informed consent was taken for the X-ray of shoulder joint. The X-ray of the shoulder joint was taken in antero-posterior (AP) view using a factor 55 KVP and 9 MAS. To minimize the exposure, minimum shots were taken to expose the joint involved in study and appropriate precautions were taken to avoid needless x-ray exposure to the subjects, by giving them lead gown. Skeletal maturity was evaluated according to the Jits and Kulkarni's classification of four stages i.e. appearance, non fusion, partial fusion and complete fusion. X-Rays showing clear gap between the epiphysis and diaphysis, showing saw tooth like appearance end were designated as "Non-fusion" (NF) X-rays. The X-rays showing a line replacing the hiatus between the epiphyseal and diaphysial ends and not showing saw tooth like appearance were designated as "Partial Fusion" (PF) X-rays. X-Rays showing the same bony architecture in the diaphysis and epiphysis and showing scar of the previous stage were designated as "Complete Fusion" (CF)[6]. On the basis of code given above master chart was prepared and tabulated.

Results

A total of 100 boys where included to study fusion of proximal end of humerus in age group 14-20 years of Ahmedabad region. In which age group between 14-15 years and 15-16 years show 100% and 78% cases of non fusion of proximal end of humerus, whereas proximal end of humerus shows partial fusion in 7%, 100%, 87% and 7% cases in 15-16 years, 16-17 years, 17-18 years and 18-19 years respectively. Complete fusion was seen in 13% and 93% cases in age group 17-18 years and 18-19 years, whereas in age group 19-20 years and 20-21 years, 100% cases shows complete fusion of proximal end of humerus as shown in table no. 1.

Table no.1 Percentage of fusion of Proximal end of humerus

Age (Years)	Non-Fusion (NF)	Partial-Fusion (PF)	Complete Fusion (CF)	Total
14-15	14(100%)	0(0%)	0(0%)	14(100%)
15-16	14(93%)	1(7%)	0(0%)	15(100%)
16-17	0(0%)	11(100%)	0(0%)	11(100%)
17-18	0(0%)	14(87%)	2(13%)	16(100%)
18-19	0(0%)	1(7%)	13(93%)	14(100%)
19-20	0(0%)	0(0%)	14(100%)	14(100%)
20-21	0(0%)	0(0%)	16(100%)	16(100%)
Total	28(28%)	27(27%)	45(45%)	100(100%)

Discussion

Analysis of the above data revealed that, the process of fusion of proximal end of humerus does not start before 16 years of age and as age increases stages of fusion also increases in number of candidates. Complete fusion of head of humerus was earliest seen in 17-18 years of age group in only 13% of cases and 93% cases in age group 18-19 years show complete fusion. Whereas age group 19-20 years and 20-21 years shows 100% fusion of proximal end of humerus.

In the comparative analysis done from the previous studies from India and other countries, we have found that in significant number of cases the age of fusion of proximal end of humerus is 18-19 years in male, which is similar to study done by Reddy (Andhra Pradesh 1973)[4], Saini (Jaipur-2005)[7], Pimple (Mumbai 2013)[8] and Flecker (Melbourne-1932)[9]. It is in contrast to the study done by Galstaun (Bengal-1937)[10] found that fusion of proximal end of humerus occurs at 15-17 years of age. However, in the study done by Davies and Parson (England-1927)[11] and Cardoso Hugo (Spain-2008) shows that there is delayed fusion by one to two years. Observation of our study was also found similar with the study done by Hepworth's (1929)[13] in the Punjabi population and Das Gupta et al (1974)[14] in the population of Uttar Pradesh. Human identification is of paramount importance[15,16]. Apart from shoulder joint, rest of the joints should also be assessed to get the more precise range for age estimation[17-20]. Comparative study of previous researchers in relation to fusion of proximal end of humerus are shown in Table no.2

Table No. 2. Comparison study of average age of fusion of proximal end of humerus in boys in India and world

Hepworth (Punjabi-1929)	17-18
Pillai(Madras-1936)	14-17
Galstaun (Bengal -1937)	14-18
Reddy KSN (Andhra Pradesh-1973)	18-19
Saini O P(Jaipur -2005)	18-19
Pimple D H(Mumbai-2013)	18-19
Paterson(Manchestor-1926)	20
Davies and Parson(England-1927)	19-21
Flecker (Melbourne-1932)	19
Knight B(UK-1961)	16-23
Present study	18-19

Conclusion

From the present study we can conclude that the complete fusion (100%) of head of humerus occurs at the age of 18-20 years in the boys of Ahmedabad region. Our study was found to be in accordance of most of the Indian studies; however there were few variations in some of the Indian and other countries study. This variation can be due to change in region, dietary pattern, behaviour, life style changes etc. This study also emphasize that standard method should be used for age estimation of the living as most of the cases are of medico-legal importance. Owing to the variation, a periodic study on age estimation is recommended in every region.

Conflict of Interest: Nil

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Ethical Clearance: taken from institutional ethics committee

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Evaluation of Nurses' Knowledge and Attitudes Concerning Fever Management for Children at Babylon Teaching Hospital for Maternity and Pediatric at Al-Hilla City

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Abstract

Background: Fever is part of the body's normal reaction to inflammatory or immunological disorders and infections. Since health care providers (HCPs) and parents are still a source of great concern, attempts have been made to include guidance for their management worldwide. Objectives of the study: To evaluate of nurses' knowledge and attitudes concerning fever management for children. Methods of the study: a descriptive design study was carried out (November/2018-April/2019) at Babylon maternal and child teaching hospital on (100) nurses, a convenience non probability study sample, the validity of the questionnaire was accomplished through (8) experts. The researchers were collected the data by self-administration report. Data analyzed by using statistical package for the social science (SPSS). Results: the result show that the (49%) of the nurses are college and above graduate. Regarding marital status, (64%) were married. In addition, the years of experience for nurses were (47%) less than 5 years. Concerning Participating in any training course, the most of nurses (57%) were participating in the training courses, and most of sample (90%) are lived in urban area, overall all assessment of nurses knowledge are moderate. Conclusions: the study conclude that Early adulthood nurses who were graduate with a college degree and above, married, who live in urban areas, and had experience from 1-5 years .as well as, who participated in training courses regarding fever management.

Key words: *Nurses, Knowledge, Attitudes, Fever management, Children*

Introduction

In pediatric patients, fever is a common complaint and a common concern in both the intensive care unit and the pediatric ward. Increased hospital stays lengths, increased morbidity, and greater impairment have been associated with elevated temperatures in infants. Fever is not a disease in itself; a virus or bacterial infection typically triggers it. It's believed to be part of the natural reaction of the human body to an infection. Childhood fever management is a dynamic behavior, with several research describing nurses' similar myths and misconceptions globally. To develop a deeper understanding of this difficult issue [(1)].

In addition, until it exceeds at least 41.7ºC, fever alone causes no damage. Fortunately, nearly all untreated fevers due to infection < 41.7ºC are maintained by the brain thermostat. Very high temperatures, however, are dangerous when the rectal temperature is 41 ºC for long periods, resulting in some permanent brain damage. Heat stroke occurs when it's over 43ºC and death is prevalent. Fever is also a physiological reaction to infection, marked by an increase in body temperature above normal everyday changes. The increase in body temperature is related to a decline in microbial replication, which tends to minimize the spread of the infection. [(2)].

For as many as one third of all pediatric consultation in general practice, fever is the most common signal of childhood diseases serving as the main complaint. In several countries, the use of antipyretic pharmaceutical drugs (acetaminophen and ibuprofen) is frequent and appears to be increasing. Many health professionals

view fever as dangerous and assess the severity of the disease by the height of the fever. The fever phobia of nurses is still recorded in the literature and negative fever attitudes remain unchanged. [3]

In addition, symptoms and indications indicating a diagnosis or at least referring to an organ system, such as headache, cough, diarrhea, dysuria, etc., are frequently accompanied. Furthermore, this is not always the case. For acute fever episodes, between 20 and 30% of all children's visits to the emergency room are for acute fever episodes. Most illnesses cause a fever ranging from 38 ° C to 40.5 ° C with an average fever of 39.5 ° C [4] . To determine fever, it is also important to consider the temperature measurement location. Internationally, childhood fever management is a complex behavior in which physicians, nurses and parents have some myths and misconceptions (this behavior is defined in more detail in the literature review. Therefore, this study aimed to evaluate the attitude of nurses in the field of childhood fever and awareness of childhood fever management [5]

Objectives of the Study

the study aim to:

1- To evaluation of Nurses' knowledge and attitudes concerning Fever Management for Children.

2- To find out the relationship between Nurses' knowledge and attitudes concerning Fever Management for Children and certain sociodemographic characteristics.

Methods and Materials: The study design: Descriptive design study was conducted from November/2018 to April/2019.

The study sample: by using a non-probability convenience sample of (100) nurses.

The study setting: The study was carried out at maternal Babylon and child teaching hospital

Statistical Analysis: using statistical package for social sciences (SPSS), version 24. Appropriate statistical tests were used accordingly, P. value ≤ 0.05 considered significant difference or relationship by using (Mean, ANOVA).

Instrument: Through an extensive review of related review, the instrument of the study was developed and constructed by the researcher. the Instrument comprise the following of three parts: A three-part questionnaire was used, the first part included an inquiry into participants' socio-demographic characteristics, and the second part included questions about fever management skills (questions), while the last part included questions about the attitude of nurses towards fever management (questions). A level Likert scale was used for knowledge items to measure the variables (know, I not sure, and don't know), 3= know, 2=I not sure and 1= don't know.. The following statistical analysis approach is used in order to analyze and evaluate the results of the study (frequencies, percentages, mean of score, Poor= (1.00 – 1.66) ; Moderate = (1.67 – 2.33), Good= (2.34 – 3.00).

Results

Table (1): Shows the distribution of the demographical characteristics of the sample.

Demographic Data	Groups	Frequency	Percentage
Age / Years	20 to 29	72	72.0
	30 to 39	18	18.0
	40 to 49	6	6.0
	50 to 59	4	4.0
	Total	100	100.0
Resident	Urban	90	90.0
	Rural	10	10.0
	Total	100	100.0

***Cont...*Table (1): Shows the distribution of the demographical characteristics of the sample.**

Marital Status	Single	36	36.0
	Married	64	64.0
	Total	100	100.0
Level of Qualification	Secondary Nursing school	17	17.0
	Health Institute	34	34.0
	College and above	49	49.0
	Total	100	100.0
Years of Experience	Less than 5 Year	47	47.0
	6-10 Year	29	29.0
	More than 10 Year	24	24.0
	Total	100	100.0
Participating any training course	Yes	57	57.0
	No	43	43.0
	Total	100	100.0

This table reveals that the majority of the sample (72%) of them their age between 20-29. concerning the level of qualification, results indicate that (49%) of the nurses are college and above graduate. Regarding marital status, (64%) were married. In addition, the years of experience for nurses were (47%) less than 5 years. Concerning Participating in any training course, the most of nurses (57%) were participating in the training courses. Lastly, in this table, the study results show that most of the nurses (90%) living in urban areas.

Table (2): Show the overall of assessment knowledge about fever management

Total	Classification	Frequency	Percent	Mean of scale	Std. Deviation	Assessment
Overall knowledge	Not Sure	52	52.0	2.299	.26125	Moderate
	Know	48	48.0			
	Total	100	100.0			

This table revealed the overall responses of nurses regarding fever management knowledge, which was moderate with total mean score of (2.299).

Table (3): Show the overall assessment of attitude about fever management

Total	Classification	F	%	Mean	S.D	Assessment
Over all Altitude	Not sure	54	54.0	2.1455	.16408	Moderate
	Agree	46	46.0			
	Total	100	100.0			

This table revealed the overall responses of nurses regarding fever management Attitudes, which was moderate with total mean score of (2.1455).

Table (4): Analysis of variance of nurse's knowledge and their demographical characteristics

Demographic Data		d.f	Mean Square	F	Sig.
Age	Between Groups	21	65.114	1.171	.299
	Within Groups	78	55.588		
	Total	99			
Marital status	Between Groups	21	.295	1.363	.164
	Within Groups	78	.216		
	Total	99			
Environment	Between Groups	21	.096	1.073	.394
	Within Groups	78	.090		
	Total	99			
Years of Experiences	Between Groups	21	.972	1.674	.054
	Within Groups	78	.581		
	Total	99			
Level of Education	Between Groups	21	.706	1.344	.175
	Within Groups	78	.525		
	Total	99			
Participating any training course	Between Groups	21	.396	1.905	.022
	Within Groups	78	.208		
	Total	99			

This table indications there was no statistical significant relationship between nurses' knowledge regarding fever management and their sociodemographic characteristics except Participating in any training course whereas there was statistical significant relationship between nurses' knowledge and participation in any training course at p-value 0.022.

Table (5): Analysis of variance of nurse's attitudes and their demographical characteristics

		D.F	Mean Square	F	Sig.
Age	Between Groups	20	80.503	1.554	.087
	Within Groups	79	51.813		
	Total	99			
Marital status	Between Groups	20	.303	1.411	.142
	Within Groups	79	.215		
	Total	99			
Environment	Between Groups	20	.086	.932	.550
	Within Groups	79	.092		
	Total	99			
Years of Experiences	Between Groups	20	.955	1.620	.068
	Within Groups	79	.590		
	Total	99			
Level of Education	Between Groups	20	.577	1.031	.438
	Within Groups	79	.560		
	Total	99			
Participating any training course	Between Groups	20	.335	1.486	.110
	Within Groups	79	.225		
	Total	99			

This table shows there was no significant relationship between nurses' attitudes regarding fever management and their sociodemographic characteristics

Discussion

1- Sociodemographic characteristics of the sample:

The study result reveals that the vast majority of the sample (72%) of them their age between 20-29. concerning the level of qualification, results indicate that (49%) of the nurses are college and above graduate. Regarding marital status, (64%) were married. In addition, the years of experience for nurses were (47%) less than 5 years. Concerning Participating in any training course, the most of nurses (57%) were participating in the training courses. Lastly, the study results indicate that most of the nurses (90%) living in urban areas.

This result consistent with the study that carried out by descriptive research design who found that the majority of sample their age between 20-30 years [(1)]. Concerning years of experience, the results of study under hand incongruent with study that carried out by quantitative cross-sectional survey to investigate fever management practices among physicians and nurses in Hebron district, who found that majority of participant has experience above 15 Years with 28% [(5)]. The findings of the study not in the same line with study that

conducted in Egypt [6] aimed to assess nurses' knowledge and practice regarding to fever management of neonates. Who found that majority of sample not participation in training course. Finally, regarding years of experience, the findings of present study incongruent with study that carried out by [5] who found the most of sample with years' experience (33.9%) more than 10 years.

2- Show the overall assessment of attitude about fever management

The study revealed the overall responses of nurses regarding fever management knowledge, which was moderate with total mean score of (2.299). the results of current study not in the same line with study that carried out by a cross-sectional descriptive design to test the knowledge of nurses about fever found that about half of the sample had low knowledge of fever and fever control. [7].

3- Show the overall assessment of attitude about fever management

The study revealed the overall responses of nurses regarding fever management Attitudes, which was moderate with total mean score of (2.1455). The findings of study under hand not harmony with the results of the study that conducted in Egypt [1] who reported that the most of nurses had positive response about attitudes toward fever management.

4- Analysis of variance of nurse's knowledge and their demographical characteristics

The study result shows there was no statistical significant relationship between nurses' knowledge regarding fever management and their sociodemographic characteristics except Participating in any training course whereas there was statistical significant relationship between nurses' knowledge and participation in any training course at p-value 0.022. these results go along with study that carried out in Ireland, who found that there no significant difference in the total knowledge scores of nurses and level of education at p-value= 0.836 [8]. While, the results of the present study congruent with the prospective one-group pretest-posttest design to investigates the effect of the training on fever and febrile convulsion management given to pediatric nurses on their knowledge level, who found that there was statistical relationship between training about fever management and pediatric nurses knowledge [9] .

5- Analysis of variance of nurse's attitudes and their demographical characteristics

The study shows there was no significant relationship between nurses' attitudes regarding fever management and their sociodemographic characteristics. These findings consistent with the study that carried out by [8] who found non statistical significant between demographical characteristics of the sample such as level of education and nurse's attitudes.

Conclusion: Early adulthood nurses who were graduate with a college degree and above, married, who live in urban areas, and had experience from 1-5 years .as well as, who participated in training courses regarding fever management. The overall knowledge of participant nurses was moderate. The vast majority of the participants' nurses had a moderate attitude toward fever management. The socio-demographic characteristics of nurses had not been the effect on their knowledge, except participation in the training course; where shows a significant association between nurse's participation in any training course and their Knowledge about fever management had been reported. No significant association was found between nurses' attitude toward fever management and their socio-demographic characteristics.

Recommendations: The results indicate that changes in pediatric nurses' management practices are needed. Educational programs are advised to improve pediatric nurses' awareness of fever control and to reinforce positive attitudes towards childhood fever. The study indicated that certain training and seminars on fever control are required to enhance the awareness of nurses. Providing up-to-date training services to educate nurses can be a successful way of changing the management of fever by nurses, reinforcing positive attitudes, challenging negative attitudes, and enhancing the quality of knowledge provided to parents about fever when their child is discharged home. Continuous monitoring and examination of nurses is needed to assess any knowledge-related defects. The current process should be more studies on the nursing management of fever and the physiological responses associated with fever and all nurses should be involved in this process.

Conducting health education activities to include a general reduction in fever phobia for both nurses and parents and to facilitate safer drug use.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Therapeutic Effects of Fenugreek Seeds on Diabetic Rabbits

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Abstract

The aim of this study was to investigate the therapeutic effects of Fenugreek on rabbit with diabetes induced experimentally. A total of thirty rabbits were divided into 3 groups, each group contain 10 animals, G1 (control negative), G2 (Control positive), G3 induced diabetes by giving alloxan 135mg/kg IP then after 2 weeks of diabetes induction, animals were drenched with Fenugreek seeds 100mg/kg orally. The results showed that rabbits in G2 suffered from hypoglycemia due to Fenugreek effects as compared with G1 group, also, G3 showed a significant gradual decrease in glucose levels especially after 96hrs of giving the Fenugreek.

In conclusion, Fenugreek showed a therapeutic effect as anti-diabetic in rabbits.

Key words: *Fenugreek, rabbits, diabetes, alloxan.*

Introduction

A large number of plants and their isolated constituents have shown physiological effects [(1)]. Some medicinal plants have shown immunological, anti-inflammatory anticancer effects [(2)]. Fenugreek seed is one of the oldest cultivated plants and it has been applied widely as food additive [(3)]. The plant part used as a medicine has generally been the seed. The Ebers Papyres of 1500 B.C in Egypt listed a preparation of Fenugreek for skin [(4)]. The Greek physician Hippocrates had considered it a valuable soothing herb while Dioscorides recommended Fenugreek for all gyneocological problems. Fenugreek has been used since Biblical times for stimulation of milk production. Also it was used adding to food of nursing mothers to increase milk production. [(5)]. Fenugreek seed extract have a very large scale of application, they are used as hypo-glycemic and hypocholestrmic effects [(6,7)]. It has particular Importance effects on the physiology of different body organs and systems. It also has the ability to kill germs [(8)]. In both animal models and humans, fenugreek seeds have been reported to have antidiabetic and hypocholesterolaemic properties. The activity was primarily due to the saponin and high fibre content of fenugreek and is possibly not related to its significant trigonelline alkaloid. Antihyperglycaemic effects have been associated with delayed gastric emptying caused by fiber content and with components that inhibit digestive enzymes from carbohydrates (unidentified). Plasma insulin levels in vivo may be increased by fenugreek administration [(9,10)].

The aim of this study was to investigate the therapeutic effects of Fenugreek on rabbit with diabetes induced experimentally.

Materials and Methods

Plant:

The seeds of Fenugreek were bought at the local market, Seeds were cleaned, dried for 4 hours and then grounded to fine powder.

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Animals

A total of 30 adult rabbits were divided into three groups:

G1 contain 10 rabbits serve as control negative

G2 contain 10 rabbits serve as control positive

G3 contain 10 rabbits, these rabbits were given alloxan at a dose of 145 mg/kg IP according to [11], after 2 weeks of diabetes induction, 100 mg/kg of Fenugreek was given orally.

Blood collection:

The blood samples were taken at zero, 24hrs, 48hrs, 72hrs and 96hrs for glucose estimation.

Statistical Analysis

The statistical analysis was done by using SPSS software.

Results and Discussion

The results showed that rabbits in G2 suffered from hypoglycemia due to Fenugreek effects as compared with G1 group, also, G3 showed a significant gradual decrease in glucose levels especially after 96hrs of giving the Fenugreek (Table and figure).

Table: blood glucose levels in experimental groups

Groups	Blood glucose levels				
	Zero	**24hrs**	**48hrs**	**72hrs**	**96hrs**
G1	102.1 ± 1.6 Ab	105.3 ± 0.8 Ab	99.7 ± 1.3 Ab	104.9 ± 0.5 Ab	104.3 ± 2.9 Ab
G2	105.6 ± 2.6 Ab	100.1 ± 0.7 Ab	93.3 ± 1.8 Ab	88.4 ± 1.0 Bb	85.4 ± 2.5 Bb
G3	380.1 ± 3.4 Aa	320.8 ± 4.1 Aa	301.6 ± 4.5 Ba	288.3 ± 1.4 Ba	220.2 ± 1.7 Ca

Capital letters denote significant ($p<0.05$) differences between groups horizontally

Small Letters denote significant ($p<0.05$) differences between groups vertically

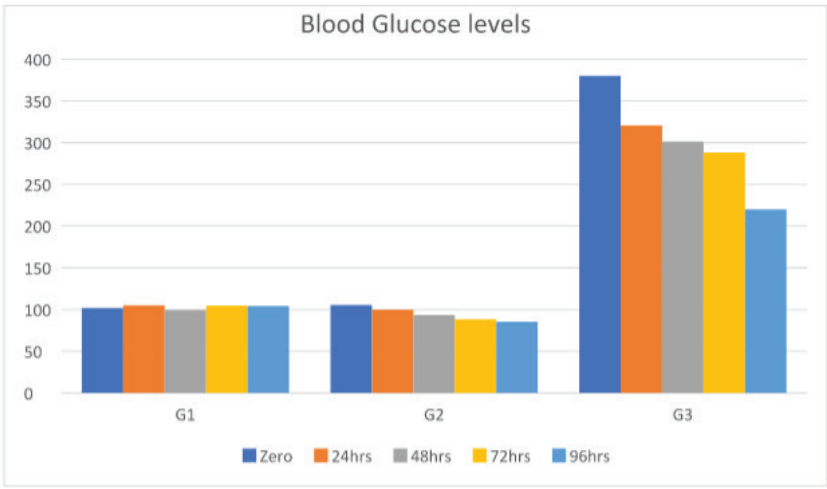

Figure: blood glucose levels in experimental groups

Many traditional treatments have been recommended in the alternative system of medicine for treatment of diabetes mellitus among them herbal plant like (*Trigonella Foenum graecum*) which has a hypoglycemic effect in patient with diabetus mellitus [12]. When 20-25 adult male albino rats to whom was in duced by using 185 mg/kg B. W. alloxan. Then these rats were fed fenugreek seed (12.5% of the diet/ 15 days). The effect of the plant on Fasting-blood-glucose (FBG) was estimated. The FBG of the untreated group (positive control) were (268 + 7.7) while that of the healthy group was (67+ 4.1). In the fenugreek treated group FBG (189+ 4.6). There was significant difference of FBG between groups, received the plant and positive control [13].

Sharma et al. [6] observed that adding fenugreek seed to the diet of diabetic patients lead to clear decrease in sugar and cholesterol level. Adding the defatted part of fenugreek seed to the diet of the induced diabetic dogs lead to a significant decrease in blood sugar and glycogen as compared with alcoholic extract of the fenugreek seed [14].

Geberemeskel et al. [15] concluded that in type II diabetic patients with no adverse effects, the administration of fenugreek seed powder solution had a marked effect on improving lipid metabolism. Therefore, new alternatives for the clinical management of type II diabetes can be provided by Trigonella foenum-graecum seed. Also, clearly indicated a significant anti-diabetic activity with the seed powder of T. foenum-graecum and supported the traditional usage of the seed powder by Ayurvedic physicians for the control of diabetes [16].

Conclusion

The usage of fenugreek has been showed a therapeutic effect as antidiabetic, so, the authors recommended to use fenugreek in treatment of diabetes.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Nurses' Perception about Medication Errors Types, Causes and Barriers to Report it

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Abstract

Background: Since there is a continuous rise in the number of patients requiring quantity of prescribed medications and medical care, there is always an increasing requirement for a complete system that can carefully evaluate all the medication errors that may occur.

Objective: Determine types, causes, and barriers of medication errors report

Setting: The current study was conducted in Imam Sadiq Hospital - Babel City during 2019 year

Subjects: The study comprised of 100 nurses with at least two years of clinical experience

Tools: The **Medication Administration Errors (MAEs)** method was used to gather the data by reporting ruestionnaire that was formulated by Wakefield et al.(1996) and revised by the researchers. The questionnaire has 65 questions; 15 items are related to the reasons of medication errors occurrence, 7 items concerning the reasons of not reporting medication errors and 5 items related to the percentage of each medication error type reported on units which classified into 9 items for non- intravenous and 11 items for intravenous medication administration errors.

Results: most of nurses reported no mistakes related to wrong route, wrong dose, wrong method; Whilewrong time, allergic reaction and infusion rate mistakes were more repetitive errors according to their perception. Fears about punishmentand from patient relatives was most common barriers to report medicationerrors.

Conclusion: Medication errors is highly priority health system problem; more than half of nurses reported no mistakes related to wrong dose, wrong method, and wrong patient, most of them indicated that no clearly definition of medication error was identified, unclear reporting system wasexisted.

Recommendations Assessment and surveillance system should be followed to minimize medication errors potentiality. Nurses have to always remember that they are responsible and accountable to the results of their decisions andactions whatever they are.

Keywords: *Medication, errors, causes, types.*

Introduction

A medication error is known as any preventable incidence that could lead or result in an inappropriate usage of drugs or patient hurt; as long as the drug is under the control of the patient or the healthcare professionals. These incidences might be linked to procedures, professional practice and systems involving communication, ordering,compounding, prescribing, packaging, product labeling, distribution, dispensing, nomenclature, monitoring, education, and administration.[1,3]

Over the world the medical error is considered as one of the main causes leading to harm and death of patients and the most challenge threatening the whole of the healthcare system [2]

Prescribing medication is complex, widespread, and increasingly hazardous.In the United States, about one- third of the adults take 5 ormore drugs. Obviously, the development in clinical therapeutics produced great improvements in health for patients with different diseases, but these advantages may also have been accompanied by increased risk.[2,4]

Administering Medication is a high- risk essential function in healthcare settings involving various disciplines. The techniques associated with the provision of drug to a patient need communication, accuracy, and thorough attention to the details by hospital workers. The medicationadministration has huge consequences if conducted wrongly.[3,5]

Additionally, these errors have negative implications like increased the hospitalization and medical costs and higher rates of patientsmortality. [1,5]

Such errors can make patients become doubtful of the services provided by the healthcare system and can lead to dissatisfaction with that system. Moreover, it can lead to ethical conflicts and stress among nurses[6]

Errors in drug administration negatively have an effect on the quality of care provided to patients. Nonetheless, it is very important to early diagnose the errors and take the right actions to decease their occurrences. On the same hand, many studies have been carried out aiming to examine obstacles avoiding nurses to report such errors. Two important factors were identified as barriers among nurses are management and fear of the negative consequences of reporting errors.[5]

Prescription errors has been classified by Neville RG into 4 categories on the basis of their potential clinical outcomes: type A (potentially serious to patient); type B (major nuisance-pharmacist/doctor/contact required) type C (minor nuisance-pharmacist must use professional judgment); and type D (trivial).[6]

Unfortunately, medication errors still occur even in the best healthcare systems designed. It is wrongly thought that incompetent healthcare providers make many medication errors who have made such errors several times during their career. Generally, healthcare professionals rarely make such errors many times. Usually, there is amedication use process and a medication error occurs due to a breakdown of that process. This breakdown usually arises at more than one step within this process.[3,4]

There are five major categories in the medication use process, each one has several steps including: "prescribing, documenting, dispensing, administering, and monitoring". The average number of steps in the process of using medications is estimated by the Institute for Safe Medication Practices (ISMP) is 40. Any mistake in each of these steps can cause a medication error. The majority of healthcare professionals believe that most of the errors happen in the process of medication use occur in the dispensing category. Though, this believe can be inappropriate. [1,7]

Medication errors, in both community settings and hospitals, remain to be a worry to patients and healthcare practitioners. The Institute of Medicine's (IOM) report was the first to bring the gigantic scope of the problem to the public's attention. It was estimated by this report that American dead cases reached to 44,000-98,000 case yearly due to medical errors in hospitals and about 40% of these could have been avoided. This caused an estimated lost wages of$17 to $29 billion, additional medical care, disability payments, and other costs.[6]

After the occurrence of a medical error, formulating a secure system for the nurses may reduce the level of errors to the lowest level as possible and raise the reporting inevitable errors without the fear of the negative consequences. Thus, we have carried out this study to examine the main factors and reasons leading nurses to keep away from reporting medication errors[3,8]

One more study, in Florida, in a tertiary hospital, about 6,000 patients were involved. The administration error rate per 1,000 admissions was found of 5.8, whereas per 1,000 dispensing and preparation errors happened at a rate of 2.6. [8]

Two more studies were exclusively about intravenous (IV) medications. One of them, at one British and two German hospitals, revealed a preparation errors ratio of 26% per observed preparation (88 preparation errors out

of 337 observations). The second one, at acommunity and tertiary hospital in the U. K., discovered a preparation errors ratio of 49% per observed preparation "(212 preparation and administration errors out of 430 doses)".[9]

Other studies evaluated errors of transcription/ documentation for medications in pediatric carehospital. In a study of two academic pediatric units, in 10,778 orders (0.8 %), 85 documentation errors were found. In a second study, out of 540 (9 %), 49 pediatric medication cardexes were found to differ with a main method (wrong frequency or duration, different dose, missing route, wrong medication) from the physician's original medication order. In another study, at the first transcription, 11.8 % (16 out of 135) of chemotherapy prescriptions and 20.7% (41 out of 198) of non-chemotherapy prescriptions were transcribed wrongly in a pediatric on hematology unit.[10-12]

In order to administer medicationscorrectly,extensive knowledge and skill are needed (7,29). Despite the crucial role of nurses identifying and reporting such errors when they take place, there has been a few researches trying to assess their perception. Indeed, each stage of the patient care process for the nursing staff has a potential for making errors and some level of danger to the safety of patients, particularly in regards to drug errors. In fact, the first step toward preventing such errors is the appropriate comprehension of the contributing factors for why these errors occur, why they are not reported and to what extent these errors are actually reported. Moreover, it is very important to understand their perception about it asit helps health organizations to highlight the factors threatening safety of the patient (29). Thus, this piece of work shows the responses of nursing staff in a survey designed with the aim of measuring their perception and solicit information regarding their personal experiences with drug errors.[13-15]

Because of medication errors common occurrence and potential risk to patient, they are often used as indicators of patient safety inhospitals.

Significant efforts have been directed to understand this problem in recent years. Therefore, this study was conducted to addressing drug errors and related areas for improvement, as perceived bynurses.

Aim of the Study

The aim of this study is to determine types, causes, and barriers to report of medication errors.

Research Questions

The researcher of the current study answered the following research questions:

§ What is the type of medication errors as perceived bynurses?

§ What are the causes of medication errors, as perceived bynurses?

§ What are nurses' views about reporting medicationerrors?

Materials and Method Materials

Design:

A descriptive cross-sectional survey used to conduct this study by using self-report questionnaire.

Setting: The study carried out at Imam Sadiq Hospital - Babel City – Iraq during 2019 year **Subjects:** 100 nurses who had to fulfill the criteria of having less than two-years of experience were included in the study.

The exclusion criterion was those who refuse to participate in thestudy.

Tools:

The revised Gladstone's scale was used to collect data on rate, causes and reporting of medication errors (Osborne et al. 1999, Mayo & Duncan 2004). It consisted two parts: first part for gathering nurses' socio-demographic characteristics such as nurse age, level of education, years of experience, and employmentstatus.

Second part included four group of questions about medication error: (I) type of medicationerrors(15items); (II)nurses'perceived causes of medication errors (11 items); and (III) Sources of medication errors (5 items), and (IV) nurses' points of view about reporting medication errors (7 items).

A pilot study was carried out with the purpose of assessing the comprehensiveness and appropriateness of the research instrument to the Syrian health care setting.

Revisions were completed before collecting the data, especially excluding the inappropriate items.

For reliability measurement, a test-retest was used. The questionnaire was distributed twice among twenty subjects of the population with two weeksinterval. In both times, Cronbach's alpha the reliability coefficient was 0.76, which showed that the questionnaire has an acceptable level of utility.

Ethical Consideration

Numerically coded made to all questionnaires before being given to the nurses.

Nurses' anonymity and confidentiality of information were assured.

All questionnaires were handled by the research team only.

Methods

- The official approvals from the competent authority wereobtained.
- All studied nurses surveyed to identify those fulfilling the studycriteria.
- Informed consent of the nurses obtained after explanation of the purpose of the study.
- Nurses were asked to answer the questionnaire through 45 minutes after receivingthem.
- Data collection conducted during January - Mars2019.
- Statistical analysis performed using Stata (version23).

Results

Table (1) shows the socio-demographic characteristics of the nurses; their age ranged from 21 to 59 years, nurses aged 20-25 years constituted 69% of them, and the rest 22% and 9% were either 26-40 years or more than 40 years respectively.

In relation to educational level 35% of studied sample had diplomaornursing school certificates while 30% had BSC nursingdegree.

Also the table shows that most of nurses had less than 5 years of experience constituted 79% while 8% of them had more than ten years of experience.

Concerning the nurses' job position; the table shows that ordinal nurses constituted 88% of the sample and nurse supervisor amounted 8%, the head nurse were the lowest percentage 4% of studied sample.

Table (1) Distribution of the nurses according to their socio-demographiccharacteristics

Socio-demographic Characteristics	Frequency N=100	% 100
Age in years:		
Less than 26 years	69	69%
26- 40 years	22	22%
More than 40	9	9%
Mean – SD	38±14	
Level of education:		
Diploma	35	35%
Nursing School	35	35%

Table (2) Medication error type as perceived by nurses:

BSC	30	30%
Years of experience		
Less than 5 years	79	79%
5- 10 years	13	13%
More than 10 years	8	8%
Employment status		
Nurse	88	88%
Nurse supervisor	8	8%
Head nurse	4	4%

Concerning types of medication errors in clinical settings the most of nurses indicatedto low percentage of medication errors during their work as the table 2 show that nurses reported 51%, 69%, 65, 73% no mistakes related to wrong dose, wrong route, wrong method, and wrong patient respectively. While, in relation to wrong time, allergic reaction and infusionrate mistakes 35%, 29%, 32% of them respectively said as never incidence in theirshifts.

In addition; the table shows just 12% of studied sample reported that almost mistakes related to time of medication administration in relation to drug- drug interaction the table shows that 58%, 26%, 11%, 5% of nurses respectively reported never, rare, sometimes, almost incidence percentage related medicationerrors.

Table (2) Medication error type and incidence percentage

Medication error type & incidence percentage	Never	Rarely	Sometimes	Almost	Always
	N/%	N/%	N/%	N/%	N/%
- Wrongdose	51%	39%	9%	1%	0%
- Wrongmethod	69%	21%	6%	3%	1%
- Wrongtime	35%	32%	19%	12%	2%
- Wrongdrug	65%	18%	10%	7%	0%
- Wrongpatient	73%	22%	3%	2%	0%
- Give med for wrong diagnosis	62%	26%	6%	7%	0%
- Twice doses	75%	15%	5%	3%	0%
- Give un-prescribed drug	86%	9%	4%	1%	0%
- Misseddose	57%	28%	9%	6%	0%
- Wrongstorage	55%	28%	12%	5%	0%
- Allergicreaction	29%	37%	27%	6%	1%
- Wrong preparation	59%	28%	12%	1%	0%
- Drug-Druginteraction	58%	26%	11%	5%	0%
- Wrong infusionrate	32%	45%	17%	4%	2%
- Expiredmedication	89%	11%	0%	0%	0%

Table (3) Causes of medication errors as perceived by nurses:

Table (3) show the causes of medication errors; most of nurses reported that physician order, work-overload and confusion by visitor co-worker 82% 72% respectively as common causes; while 40% of themreported that physician font and patient name confusion as common causes of medication errors; 43% of studied sampleconsidered verbal order as common causes.

Table (3): Causes of medication errors as perceived by nurses.

Medication error type	Yes	No
	N%	N %
Unclear medication name	45%	55%
Different medications look alike	55%	45%
Resemblances of patient names	40%	60%
Physician 'medication orders are not legible	40%	60%
Wrong orders(dose or name) by physician	82%	12%
Verbal order of medication	43%	57%
Wrong transcription, documentation by other nurse	51%	49%
Work overload and exhaustion	72%	28%
Low experience level	46%	54%
Confusion by co-worker or visitors or accidents	72%	28%
"Pharmacists are not available 24 hours a day". Pharmacy delivers or prepares incorrect doses	50%	50%

Table (4) shows medication errors reporting barriers**;** 76 percent of nurses indicated that drug error is not defined clearly as main reasons of not errors reporting occurrence; nurses fear of patient's relatives/parents and nurses fear of job penalty constituted 62%, 61% respectively, while 47 percent of nurses stated that negative response of administrators as major causes of not reporting drug errors

Table (4) Medication errors reporting barriers as perceived bynurses

Medication error reporting barriers	Yes	No
	N%	N %
Medication error is not clearly defined	76%	24%
Nurses afraid of head nurse or physician or manager	48%	52%
Nurses afraid from patient's parents or relatives	62%	38%
Nurses afraid from job punishment	61%	39%
Negative response by administrator	47%	53%
"Nurses may not think the error is important enough to be reported"	53%	47%
Medication error accident report form not present	50%	50%

Table (5) shows nurses opinion about error phase; the outcomes shows that documentation phase constituted about 34% as most repetitive errors phase followed by prescription phase21%.

Table (5) Nurses opinion about error phase

Nurses opinion about error phase	
Medication prescription	21%
Medication preparation	19%
Medication administration	10%
Documentation	34%
Monitoring	16%

Discussion

Clinical nurses have complex and multifaceted roles in medication management. These roles include administering medication efficiently and safely, monitoring and assessing desirable and unwanted effects, giving patient education, and planning for discharge (27). For nurses to be effective carrying out these roles; thus our study conducted to identify all information related medication errors from nurses prospective.

The outcomes of this experiment revealed that most of nurses were aged less than 26 years and had less than five years of experience; this make their opinions come near together and in congruence with

In relation to the types of medication errors the results showed that more than half of nurses reported no mistakes related to wrong dose, wrong method, and wrong patient, While, they said the wrong time, allergic reaction and infusion rate were more prevalence in their clinical settings; that can be interpreted by their fear and anxiety about punishment or fear from patient relatives because most of them were with medium experience.

Regarding to the most common causes of medication errors; this study revealed that most of nurses reported that physician order, work-overload and confusion by visitor co-worker were more common in clinical settings; while less than half of studied sample considered verbal order and low experience level as common causes That is in concurrence with Mrayyan MT 2007 who reports medication errors occur when nurseswere distracted by other patients, coworkers or events on the unit; and Abusaad F 2015 indicated that highest cause of medication errors was due to heavy workload, lowest cause of medication errors was due to unfamiliarity with medication. That mean it is very important to computerize and ensure correct medication order by two references in addition to optimize nurses shift schedule and emphasize on importance of rest periods during working day, and ensure clear administrative disciplines about visitors and patient relativesnumber.

Moreover; the results revealed that most of nurses indicated that not clearly defined medication error as main barrier of errors reporting; and the nurses expressed their fears from patient relatives and punishment as causes of not reported errors. That was in congruence with Mrayyan MT 2007 who said in their study conducted in Jordan (the most frequent causes related to nurses' failure to report medication errors were because nurses were fear that they might be subjected to disciplinary actions or even lose their jobs). While Aboshaiqah A 2013 said that administrative response was collectively perceived by the participants as a factor why MAEs were not reported. All of that inforce the importance of find clear definition of medication error in addition of establishing rules and standard to protect nurse's rights.

A study was conducted by Bates et al. revealed that 56% of errors happen during prescribing, 34% at administration, 6% during transcription and just 4% during dispensing; that was in congruence with ourstudy

Conclusion

It can be concluded from the study that the medication errors are a multifactorial problem should be studied deeply and periodically to be resolved; more than half of nurses reported no mistakes related to wrong dose, wrong method, and wrong patient, most of them indicated that no clearly definition of medication error was identified. So an integrated safety health care system should be established; strategies and Solutions are required to be formulated andimplemented.

Recommendations:

1. Assessment and surveillance system should be followed to minimize medication errors potentiality
2. Nurses always need to remember that they are responsible and accountable personally to the result of their decisions andactions whatever it is.
3. A designed in-service training program about medicationadministration.
4. Errors reporting methods should be clear identified

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Mothers' Self-Efficacy for Preventing Diarrhea in Children Under 5 Years

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Abstract

Diarrheal disease is the second leading cause of under-five mortality, about two billion cases of diarrheal disease are registered annually worldwide, and 1.9 million children under the age of five die each year from diarrhea.

A descriptive study was conducted to assessing the self-efficacy among mother′s for preventing diarrhea in children under 5 years and find out the association between self-efficacy of mother′s for preventing diarrhea with her selected demographic variables. The study started from the period of 19th September 2020 to 15th March the 2021.

Convenience sample of 200 mothers of children under five years was selected. The data were described statistically and analyzed through the use of descriptive and inferential statistical analysis procedures.

Results revealed that mothers self-efficacy evaluated moderate level in the prevention of diarrhea in their children represented (64.3%), with respect to the relation of there is non significant differentiation at P>0.05 among mother's responding regarding studied domains and their (SDCv.) except between occupation variable level of education variable and socio-economic status since significant relationships are accounted at P<0.01.

The study concludes there is non significant differentiation among mother's responding regarding studied domains and child (SDCv.) except between method of breastfeeding in the first six months**,** at any age the mother began to give her child extra food has the child previously suffered diarrhea since significant relationships and recommended that community based diarrhea prevention programs" need to adopt the mothers self-efficacy scale to assess mothers 'perceived risks in order to implement specific interventions that will boost and sustain Diarrhea prevention strategies at the health household levels.

Keywords: *Mothers' self-efficacy, Diarrhea, Children under five years*

Introduction

Diarrhea is a symptom of a number disorders and is one of the major causes of infant and child morbidity and mortality worldwide [1].

A higher prevalence of diarrheal diseases in young children is associated with young age, low socio-economic status (SES), poor maternal literacy, the involvement of under-five siblings in the household, low birth weight, insufficient breastfeeding, malnutrition, poor sanitation and poor maternal hygiene practices [2].

Diarrheal disease prevention and control interventions not only include improved water quality, but also measures to improve sanitation, improve quality and access to water supply, and promote hand washing and other hygiene practices in domestic and community environments [3].

Diarrhea is a global health concern for children under 5 years of age. This causes nearly 1.9 million deaths annually every year [4]. Most of these occur globally in rural and suburban areas of developing countries and are the second leading cause of under-5 mortality,

accounting for 700,000 to 800,000 preventable deaths. In arresting and reversing its prevalence, correct awareness of the dynamics of the disease is important [5].

That adequate self-efficacy in mothers plays a role in the management of the diarrhea process, self-efficacy has been shown to be a good predictor of child health and protective behavior habits in mothers, so enhancing mothers' self-efficacy will affect the prevention of diarrhea [6].

Mothers' self-efficacy depends upon their knowledge of child care strategies that are developmentally appropriate to the child, confidence in their own abilities to execute those strategies, and a belief that their children will respond to this strategy in the manner desired by the mother [7].

Prevention of diarrhea and other interventions to reduce morbidity and mortality due to diarrhea will be effective if it is adjusted to the level of maternal self-efficacy in preventing diarrhea. Prevention of diarrhea is the key to reducing morbidity and mortality due to diarrhea. Prevention of diarrhea carried out by mothers includes washing fruits and vegetables before cooking and eating, washing hands using soap and water, giving breast milk, immunizing children, and consuming healthy drinking water [8].

Methodology

Design of the Study:

The study was descriptive using quantitative data collection method. In which assessment approach is applied to achieve the objectives of the study and was conducted for mother's self-sfficacy for preventing diarrhea in children under 5 years at primary health care centers in AL-Diwaniyah city during the period of the study from 19th September 2020 to the 15th of March 2021.

Ethical Consideration:

The participants were fully acquainted with the current study and its aims and then a voluntary verbal consent was obtained in order to participate in the study. Besides, the confidentiality of information obtained from mother's children under five years has been taken into account. Also, ethical approval was obtained from the ethical committee of research in the Faculty of Nursing/ University of Baghdad regarding confidentiality and anonymity of participants.

Setting of the Study:

The study is conducted in AL- Diwaniyah health directorate, central sector. (9) primary health care centers from total (18) primary health care by random selection These setting are the designated site for data collection, because all them are representing health care facilities for children.

Sample of the Study:

A convenience sample of (200) mothers children under five years were selected from the primary health care centers when they companied their children at the time of attend is the health centers. The mothers accepted to participate in the study after explaining the researcher's need for their contribution and filling of the questioner form.

Instrument of the Study:

A questionnaire is constructed for the purpose of the study throughout a review of relevant literature and consultation from panel of experts and related studies. It consists of four parts; demographic characteristics of the mother children, demographic characteristics of the child, factors related to managing the home to stop diarrhea and scales which supposed to measure levels of mother's self-efficacy for preventing diarrhea in children under 5 years.

Statistical Analysis

Researcher using the statistical package of social science (SPSS) version (24) to analyze the data and assess the result of present study. The researchers used descriptive and inferential data analysis to obtain results.

Results and Discussion

Discussion of Socio-Demographic Characteristics for Mothers (in the table 1):

Findings reveal that the majority highest percentage of the age mothers is located in age group of (25-30) in the table (1). This finding similar to a study done by (Artifasari & Irawati, 2020). In their study (self

efficacy relationship towards improvement of mother's knowledge in childhood management with diarrhea), the mention that majority of the study sample (73.3%) were within age group (20-29) years. From researcher view point mothers age played a significant role in the occurrence of childhood diarrhea. Teenage mothers had the highest risk of experiencing childhood diarrheal diseases. The occurrence of childhood diarrhea decreased with mother's age.

In addition, the present results agree with a study in Sub-Saharan Africa [9]. In titled (prevalence of diarrhoea and associated risk factors among children under-five years of age in Efoulan health District-Cameroon, Sub-Saharan Africa), they reported that the majority of mothers age 63.0% were within age group (26-38).

The current study concerning the educational level of the study sample that found that higher proportion that participants were educational level (represented less than quarter of participants were primary and college 68(34%). These finding agree with (Artifasari & Irawati, 2020)[8]. Who mention that high school as many as 24 respondents with a percentage of 40%.

Furthermore, the current study found Occupation, which illustrated that most of studied mothers don't work, and they are accounted 149(74.5%). These finding agree with study in Bangalore in titled (a study to assess the effectiveness of self instructional module on home management of diarrhea among mothers of under five children in selected hospital, Bangalore). who mention that the maximum number of mothers 55% were noticed as house wives [1].

Also, Solomon et al., (2020). Were consistent with the findings of the current study in titled (diarrheal morbidity and predisposing factors among children under 5 years of age in rural East Ethiopia). They mentioned that the majority of mother housewife and accounted for (73.6) [10].

Based on the marital status of respondents, the study found out that majority of the respondents, 194 representing (97%). These findings agree with Lopes et al., (2014) [11]. In their study (assessment of the maternal self-efficacy for child diarrhea prevention), they reported that the majority of the sample were married 76 (50.7) and agree with (Faith, 2016). In titled (caretakers' home management and health seeking behaviour for diarrhoeal diseases among children under five years in Eldoret Municipality, Kenya) were married 155 (43.1) [12].

Furthermore, the current study found that the number of the mothers living in nuclear 91(45.5) and mothers living in extended family accounted 109(54.5). These result agree with the study in (Bangalore) who that the majority number of the mothers (70%) belongs to joint family and 30% belong to nuclear family [1].

In additional, urban residency formed the most of studied mothers, and they are accounted 154(77%); from researcher view point, the vast majority of people in Iraq live in urban areas, perhaps because there more service for citizen than in rural areas. Therefore, there is an increasing population density towards the city center.

In relation to the family socioeconomic status (SES), the present study findings revealed that most of the sample came from the moderate level of the SES. The results of this study is opposite to study in (Brazilian). In titled (self-effectiveness in preventing diarrhea and child care: A transversal study) who mentioned most of the sample living under the poverty line, that is having a per capita income under a quarter of the minimum wage (n=188; 43.8%) [13].

Also the result of present study agree with study done in by (Turkish). In titled (reliability and validity of a Turkish version of the maternal self-efficacy scale for preventing early childhood diarrhea) who stated that most of his study sample accounted 58.8% of the families had their incomes equal to their expenditures [6].

Discussion of the Factors Related to Home Management to Stop Diarrhea (in the table 2):

In the table (2), regarding item of "soap near the tap", most of studied sample were assigned a positive answered, and are accounted 189(94.5%), as well as asking about water treatment (filtration, boiling, chlorine), most of studied sample were assigned a positive answered, and are accounted 196(98.0%).

With respect to "presence of flies at home" item, 120(60%) of studied sample were supported positively. Not a few of the respondents responded by throwing waste into the river, and accounted 56(28%), and most of them are answered for covered of water reservoir,

and they are 197(98.5%), as well as most of them were used flushing for toilet latrine treatment, and they are 192(96.0%). Finally exclusive breastfeeding until 6 month are formed 112(56.0%).

The results of the present study support with (Joventino et al., 2013) they mention that The socio-demographic characteristics of study children with previous diarrhea show significantly precarious water management 86 (67.2), and toilet latrine with flushing 19 (55.6)[13], soap near the tap 230 (58.5), covered of water reservoir 182 (61.9) and not a few of the respondents responded by throwing waste into the river, and accounted 14 (73.7) and agree with study conducted by (Oliveira et al., 2017). In their titled (influence of socio-economic conditions and maternal knowledge in self-effectiveness for prevention of childhood diarrhea) who reported that the most of them used flushing for toilet accounted 87 (56.1) and exclusive breastfeeding formed 119 (50.4) [14].

Main Domains and Overall Evaluation maternal Self-Efficacy Scale for Preventing Diarrhea Concerning Studied Main Domains:

Table (2) show that mother self-efficacy scale for preventing diarrhea concerning studied main domains since grand mean of score is evaluated moderate level.

The results of current study agree with (Githae, 2018). In their titled (psychometric scale for measure mothers' self-efficacy in diarrhea prevention) the mentioned that the study established that there was a correlation between the perceived risk variables of child feeding, feeding practices, maternal hygiene, household environment and the proximate variable of health service awareness and the mother self-efficacy scale with a p-value < .05. [14],

Also,the current study agree with (Joventino et al., 2013). The majority of mothers reached the moderate level numbered the least in the survey group (n=105; 23.5%).

Relationships among Mother's Responding and Their (SDCv.) in the table 3:

Table (3) shows relationships for mother's responding regarding studied main domains: [(feeding and general practices), and (family hygiene)], as well as mothers self-efficacy scale for preventing diarrhea generally, and mother's (SDCv.), results observed weak relationships with non significant levels such as (age of mother , marital status , family type , residency) except between occupation variable, as well as level of education variable and finally between "socio-economic status" since significant relationships. From view point researcher in general, when individual gets older, the self-efficacy increases, they can effectively respond with child rearing and caring tasks. And mothers who were not limited by working time and could spend more time for child care task.

The result of the current study agree with (Oliveira et al., 2017) they reported that the some variables of the present study related to maternal age, schooling, marital status, and occupation had no significant association with maternal self-efficacy to prevent diarrhea in the sample investigated and association between socioeconomic and maternal self-efficacy variables [14].

On the other hand, a study carried out in Fortaleza, Brazil. In their titled (socio-demographic factors relating to mothers' self-efficacy in preventing childhood diarrhea) with 90 mothers of children under 5 years of age, found a significant level of self-efficacy with these same variables. However, such study resembles this one insofar as family income was determinant for self-efficacy[15].

Conclusion and Recommendations

The study concludes there is non significant differentiation among mother's responding regarding studied domains and child (SDCv.) except between method of breastfeeding in the first six months**,** at any age the mother began to give her child extra food, has the child previously suffered diarrhea since significant relationships and recommended that community based diarrhea prevention programs" need to adopt the mothers self-efficacy scale to assess mothers 'perceived risks in order to implement specific interventions that will boost and sustain diarrhea prevention strategies at the health household levels.

Table (1): Distribution of the studied group according to (SDCv.)

Factors related to Home Management To Stop Diarrhea	Groups	No.	%
Soap near the tap	No	11	5.5
	Yes	189	94.5
Water treatment (filtration, boiling, chlorine)	No	4	2
	Yes	196	98
Presence of flies at home	No	80	40
	Yes	120	60
Household garbage disposal	Driven to public collection	144	72
	Drop in river or open area	56	28
Water reservoir	Covered	197	98.5
	Uncovered	3	1.5
Toilet latrine	With flushing	192	96
	Without flushing	8	4
Exclusive breastfeeding until 6 month	No	88	44
	Yes	112	56

Table (2): Distribution of the studied group according to (SDCv. of Infected Child

Factors related to Home Management To Stop Diarrhea	Groups	No.	%
Soap near the tap	No	11	5.5
	Yes	189	94.5
Water treatment (filtration, boiling, chlorine)	No	4	2
	Yes	196	98
Presence of flies at home	No	80	40
	Yes	120	60
Household garbage disposal	Driven to public collection	144	72
	Drop in river or open area	56	28
Water reservoir	Covered	197	98.5
	Uncovered	3	1.5
Toilet latrine	With flushing	192	96
	Without flushing	8	4
Exclusive breastfeeding until 6 month	No	88	44
	Yes	112	56

Table (3): Summary Statistics of main domains of mother's responding and overall maternal self-efficacy scale for preventing diarrhea

Main Domains	No.	Min.	Max.	GMS	PSD	PRS%	Ev.
Family Hygiene	200	1.696	3.000	2.590	0.219	68.3	M
Feeding and General Practices	200	1.400	3.000	2.466	0.335	62.2	M
Maternal Self-Efficacy Scale for Preventing Diarrhea	200	1.959	2.957	2.528	0.241	64.3	M

Ev. : Evaluated (20 – 46.67) Low (L) ; (46.68 – 73.3) Moderate (M) ; (73.34– 100) High (H).

Table (4): Relationships among mother's responding regarding studied domains and their (SDCv.)

Mothers Demographical Characteristics variables	Family Hygiene			Feeding and General Practices			((Overall)) Maternal Self-Efficacy Scale for Preventing Diarrhea		
	C.C.	Sig.	C.S.	C.C.	Sig.	C.S.	C.C.	Sig.	C.S.
Age of mother (Yrs.)	0.119	0.719	NS	0.188	0.199	NS	0.211	0.096	NS
Level of Education	0.225	0.153	NS	0.298	0.007	HS	0.339	0.001	HS
Occupation	0.202	0.003	HS	0.239	0.001	HS	0.286	0.000	HS
Marital Status	0.004	0.960	NS	0.031	0.662	NS	0.004	0.960	NS
Family Type	0.008	0.907	NS	0.056	0.424	NS	0.008	0.907	NS
Residency	0.037	0.605	NS	0.080	0.258	NS	0.060	0.393	NS
Socio-Economic Status	0.158	0.077	NS	0.137	0.148	NS	0.286	0.000	HS

(*) HS : Highly Sig. at P<0.01; NS : Non Sig. at P>0.05 ; S : Sig. at P<0.05 ; Testing are based on a Contingency Coefficient test.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Effectiveness of Educational Program on Nurses' Knowledge toward Malignant Solid Tumors of Children at the Oncology Center

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Abstract

Background: Solid tumor is one of the problem facing the early childhood and the common types of solid tumors are (Lymphomas, Nephroblastoma and Wilm's Tumors)

Methodology: The study was designed as quiz-experiment design (non -probability sample) using (pre and post-test). Participants were 50 nurses working in the oncology center , and being, tested in three periods pre-test and post-test-1, and post-test-2 after applying an education program regarding improving their knowledge toward malignant solid tumor.

Results: The study outcomes low (33.3%**)** in assessment of nurses' knowledge before applying the educational program and became high in test -1 (81.24%) and in test-2 (87.93%) assessment of nurses' knowledge after applying the educational program.

Conclusions: The level of knowledge regarding the solid tumor malignancy among nurses' enrolled in the pre-test assessment of the study and control groups was low level. The educational program is an effective in improvement of nurses' knowledge regarding solid tumor malignancy.

Recommendations: The need for educational activities to improve and update the more efforts should be done to improve and update nursing staff knowledge.

Keywords: *Effectiveness, Educational Program, Nurses, Malignant Solid Tumors*

Introduction

Pediatric solid tumors are a gathering of non-hematologic, extracranial malignancies that happen during adolescence and childhood. This heterogeneous gathering of tumors speaks to roughly 40% of every pediatric disease. Numerous pediatric strong tumors are alluded to as embryonal or formative diseases since they emerge in small kids or youths because of adjustments in the cycles of organogenesis or typical development [1].

Many types of cancer can develop during childhood, such as carcinoma, thyroid cancer due to Hashimotos thyroiditis, Hodgkin and non-Hodgkin lymphomas, leukemia, neuroblastoma, Wilms tumor, rhabdomyosarcoma, retinoblastoma, bone cancer (including osteosarcoma and Ewing sarcoma), tumors of the brain and spinal cord [2] [3].

While cancer mortality rates have decreased during this period, there are still more than (100,000) individuals lost among U.S. children each year [4]. The number of pediatric cancers has risen all over the world. Malignancy, data processing for 10 percent-12, is the 2nd most typical reason for child death in the developed world. 3% of all deaths in children's years [5].

The oncology nurse should have instructions for determining cultural values that, following a cancer diagnosis, could affect the coping style of the family. By

reviewing the family condition at home, the nurse could offer assistance; it could be helpful if parents contact a teacher or counsellor in school about the changes taking place at home [6].

Self-management education services enable patients with serious cancer to learn or retain the skills needed to handle their lives with a chronic illness. This entails medical disease management, changing responsibilities and relationships, controlling the emotional and psychosocial effects of disease and care, and maintaining a balanced lifestyle to improve health through the solid tumors pathway[7], [8].

Objectives of the study:

1-Assessing the nurses' knowledge regarding children with malignant solid tumors.

2-constructing an education program for nurses' knowledge of children with malignant solid tumors.

3-Evaluating the effectiveness of an educational program on nurses' knowledge of children with malignant solid tumors.

Methodology

Design of the study

The study was designed as quiz-experiment design using test retest approach for study group participants employed in the Children oncology center in Al-Hussein medical city in holy Karbala governorate the period of the study from 19th September 2020 to 15th March 2021.

Ethical consideration

In order to participate in the study, the participants were fully informed of the current study and its goals and then received voluntary verbal consent. In addition, the confidentiality of information gained it has been taken into account by nurses. Ethical approval was also received from the ethical research committee at the faculty of nursing at the University of Baghdad regarding confidentiality and anonymity of participants.

Settings and sample of the study

A non - probability sample selected from nurses who working at oncology center in Al-Hussein medical city in holy Karbala governorate. The sample (25) nurses enrolled as a control group and (25) nurses enrolled as a study group. The study group participants were exposed to an educational program.

Questionnaire parts

To achieve the goals of the study, a questionnaire format is designed and constructed after reviewing related literature and previous studies by the researcher. The questionnaire consists of four parts including 38 items.

Part 1: Include socio-demographic data of nurses includes gender, age, level of education, social status, years of experience in general nurses and years of experience inside oncological unit, did you participate in oncology-related training courses, do you have a training courses related to oncology nursing.

Part 2: Nurses' knowledge toward children with solid tumors malignancy this part includes three sections:

Section I: Nature of solid tumors malignancy: This section includes (10 items).

Section II: The signs and symptoms of solid tumors malignancy this section include (6 items).

Section III: Diagnoses and treatment of solid tumors: This section includes (2 items).

Part 3:- Knowledge of nurses regarding nursing care for children with malignant solid tumors (20 items).

Study instrument validity

The educational program and the study instrument underwent series of revisions and modifications according to panel experts views, those experts had more than seven years of experience in their field of specialty. The researcher proposed each expert member to review the study questionnaire and program for content, simplicity, relevance, style, and suitability.

To make the instrument more valid, it was presented to a panel of (16) experts in the different fields

Statistical Analysis

Researchers using the statistical package of social science (SPSS) version (24) to analyze the data and

assess the result of present study .the researchers used descriptive and inferential data analysis to obtain results.

Discussion of the Results

5.1. Discussion of socio-demographical characteristics variables in the table (1) :

The results of the present study reveal that the gender highest percentage (52 %) is female, the lowers percentage (48 %) is male in both groups (study and control). This result agrees with study in (Iraq) was conducted on nurses who worked in hemodialysis ward in , Baghdad city reported that more than three quarters of the study sample were female and remaining were male[9].

The researcher believes this result give as the nurses job are more likely for the female than male and this job it is established on female gender also support this finding

The result of the present study concerning the age that revealed that the majority of participants is located in the age group (20-29years) in both of study and control groups, these finding agreed with study in Baghdad city was conducted on nurses who worked in oncology ward in Baghdad, reported that more than half of sample were in age (20-30 years) [10].

Furthermore, the current study found that the highest percentage nurses were from the intermediate level of education (institute) (32%) in both of study and control groups. This results are agree with (Hassan& Hassan, 2013) was conducted on nurses who worked in cardio ward in Kirkuk's, that reported educational status most of the nurses (37.5%) were institute [11].

Based on the years' experience the study found of the half participates was within of (1-5 years) experience in nursing field in both of study and control groups . Also the results of current study similar with study in Iraq in which that the majority of the half participates was within of (1-5 years) experience in nursing field[9].

5. 2. Discussion of experience variables:

In relation to the years of experience in the oncology ward in both of study and control groups , the present study findings revealed that the most of the sample (less than 5 years) 56% and most them participated training courses in the field of oncology. These results agree with study conducted on nurses who worked in oncology ward in Baghdad done by (Zaid, 2010). reported that more than half of sample were in (1-5years) of experience in the oncology ward[12].

5.3. Discussion of the effectiveness educational programs according main domains and overall assessment:

Table (2) Shows the levels of nurses' knowledge regarding malignant solid tumors pre, post1, and post2-test; the findings indicate that nurses have moderate level of knowledge prior educational program (Pre-test: 47.45%) while the level of nurses' knowledge is increased to good after the educational program during post-test I (81.24) and post-test2 (87.93).

From the researcher's point of view when looking at the results in posttest 1 and posttest 2, the results were higher than those of the pretest. This is evidence that the participants have benefited from the educational program. Which was applied in an integrated manner through the diversity of the lectures and the methods of presenting the program, and this leads to encouraging the participants to attend and obtain new information. Also, use means of encouragement, including an official session, for the purpose of annual promotion.

5.4. Discussion relationships among overall assessment nurses' knowledge and their socio demographic characteristics variables :

Table (3) Indicates that there is no significant relationship between nurses' knowledge and socio demographic characteristics variables were accounted at P>0.05. These findings ,these results of current study agree with (Zaid, 2010) was conducted on nurses who worked in oncology ward in Iraq . they stated there is no significant association between nurses' knowledge and their years of experience in hospital. that there is no significant between nurses' knowledge toward solid tumors' and their years of employee in oncologic ward [12].

These result of present study disagree with (Mohammed & Aburaghif, 2018). That indicates that there is no significant association between demographic characteristics of nurses and their knowledge regarding

the side effects of chemotherapy among children with leukemia domains, except that their educational level was significant in both pre-test and post-test . Also the years of experience in hospitals and years of experience at Oncology wards in post -test there were significant association [13].

Conclusions

The level of knowledge regarding the solid tumor malignancy among nurses' enrolled in the pre-test assessment of the study and control groups was low level. The educational program is an effective in improvement of nurses' knowledge regarding solid tumor malignancy.

Recommendations: The need for educational activities to improve and update the more efforts should be done to improve and update nursing staff knowledge. Periodic monitoring of the nurses to determine the level of knowledge and performance about the management of children with solid tumors and apply their appraisal score to their annual evaluation. The nurses need to applicate of global educational standards to promote their knowledge and practices by sharing in training courses regarding solid tumor malignancy.

Table(1): Distribution the general data of the study

Socio–demographic variable	Classes	Study		Control		P-value
		F	%	F	%	
Gender	Male	12	48	12	48	P=1.000 (NS)
	Female	13	52	13	52	
Age Groups Yrs.	20 – 29	17	68	12	48	P=0.233 (NS)
	30 – 39	7	28	9	36	
	40 – 49	1	4	4	16	
Educational level	Secondary School	5	20	7	28	P=0.886 (NS)
	Institute Graduate	8	32	8	32	
	College Graduate	8	32	6	24	
	H. Dip./or M.Sc.	2	8	1	4	
	Ph. D.	2	8	3	12	
Number of years of experience in the nursing field	< 5	13	52	11	44	P=0.364 (NS)
	5 – 9	7	28	4	16	
	10- 14	4	16	6	24	
	15 – 20	1	4	4	16	

Table (2): Distribution of the studied groups according to main domains and overall assessment of knowledge with comparisons significant.

Domains and Overall Assessment	Period	F	Study			Control		
			PGLMS	SD	Ass.	PGLMS	SD	Ass.
First Domain : the nature of malignant solid tumors	Pre	50	48.40	17.00	M	40.40	17.91	L
	Post 1	50	80.40	10.60	H	56.80	16.51	M
	Post 2	50	88.40	8.00	H	64.00	12.91	M
Second Domain : Signs and symptoms related to malignant solid tumors	Pre	50	38.00	20.71	M	32.00	22.01	L
	Post 1	50	80.67	14.97	H	41.33	19.32	M
	Post 2	50	87.33	12.98	H	47.33	20.23	M
Third Domain : Diagnosis and fundamentals treatment related to malignant solid tumors	Pre	50	48.00	39.48	M	48.00	33.79	M
	Post 1	50	74.00	32.66	H	60.00	35.36	M
	Post 2	50	82.00	28.43	H	62.00	33.17	M
Fourth Domain : Nurses 'knowledge regarding care of children with malignant solid tumors	Pre	50	55.40	11.08	M	59.20	9.99	M
	Post 1	50	89.90	4.11	H	66.00	10.97	M
	Post 2	50	94.00	4.02	H	70.10	11.35	H
Nurses' knowledge about solid tumors malignancy	Pre	50	47.45	11.45	M	33.3	13.31	L
	Post 1	50	81.24	9.57	H	56.03	12.95	M
	Post 2	50	87.93	8.05	H	60.86	11.90	M

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing based on repeated Measurement test; Assessments Intervals Scoring Scales of Percentile Global Mean of Score (PGLMS): [L: Low (0.00 – 33.3)]; [M: Moderate (33.3 – 66.7)]; [H: High (66.7 – 100)]. Testing are based on Wilcoxon Signed Ranks Test.

Table(3): Relationships between overall assessment nurses knowledge and their soci-demographic data.

Group	Source of Variations	Type III Sum of Squares	d.f.	Mean Square	F Statistic	Sig. Levels	C.S. (*)
Study	Corrected Model	773.152	11	70.287	0.294	0.908	NS
	Intercept	6231.868	1	6231.868	26.087	0.123	NS
	Gender	240.886	1	240.886	1.008	0.499	NS
	Age Groups	159.111	1	159.111	0.666	0.564	NS
	Educational level	275.433	3	91.811	0.384	0.795	NS
	No. of yrs. Service	122.013	2	61.006	0.255	0.814	NS
	Service in the O.P.	0.489	1	0.489	0.002	0.971	NS
	Have you courses ?	71.191	1	71.191	0.298	0.682	NS
	Error	238.887	1	238.887	R-Squared = 0.764		
	Total	99126.32	13				

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Adolescents Health and Using Information Communication Technologies (ICT)

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Abstract

The social support networks become important to guide the feelings and conduct adopted by relatives. Social media provide an additional informal source of data that can be used to identify health information not reported to medical office or health departments and to reveal viewpoints on health-related topics, especially of a sensitive nature. Babylon secondary schools were hunted to be the setting of the study in order to achieve the objectives of the study. This was a correlation study of Babylon adolescents aged 12–15 years. A total sample of 125 participants was achieved via convenience and snowball sampling. Instruments were developed and used in order to collect data. Data were collected through self-administration of questionnaire regarding the study. The Statistical Package for Social Sciences (SPSS) version 23 was used for data analysis. Conclusion: Internet health resources have the potential to be extremely valuable in reducing peer isolation for adolescents with chronic conditions and facilitating their transition to adult health care. A variety of Internet resources are available. The study recommended providing guidelines for engagement in daily moderate to vigorous physical activity which may have had a protective effect on the intensity of IT-related discomfort reported.

Key words: *Adolescents Health and Information Technologies*

Introduction

Social media communication is an increasingly utilized out let for people to freely create and post information that is disseminated and consumed worldwide through the Internet. News media, traditional scientific outlets, and social media create a plat form for minority viewpoints and personal information, which is not being captured by other sources. Social media can create a sense of anonymity, allowing for un adult rated personal expression when compared to traditional face-to-face meetings, especially among young people and about intimate matters. (1).

In the is respect, social media provide an additional informal source of data that can be used to identify health information not reported to medical office a ls or health departments and to reveal viewpoints on health-related topics, especially of a sensitive nature. In the past 10years, research articles connecting disease surveillance with Internet use have increased in number, most likely due to the increase in availability of health-related information from various I internet (2)

Network causes a lot of disease including, Cerebral palsy (CP) consists of a set of motors and postural disorders that cause functional impairment to the child, Is considered a non-progressive condition, resulting from injury or abnormalities that occur in the brain in fetal or child development, until the second year of postnatal life. Disabling character, CP cause great impact on family, and may destabilize it, requiring sudden changes in family dynamics, causing conflicts and changes of routines and functions (3)

In the process of living, the family builds a world of symbols, practices and knowledge that are built, shared and redefined in social interactions intra and extra family (4)

In addition, it is well known that the availability of certain features social and psychological influences on care to the chronic sick and contributes to improving the

quality of life of families. [5]

In this way, the social support networks become important to guide the feelings and conduct adopted by relatives [3]

The first objective of this study was to quantify adolescents' self-reported exposure to ICT, the amount of daily physical activity in which they participate, and their musculoskeletal discomfort. The second objective was to determine any associations between the reported discomfort and exposure to ICT tasks

Methodology

Design: This was a correlation study of Babylon adolescents aged 12–15 years. A total sample of 125 participants was achieved via convenience and snowball sampling,

Settings: Babylon secondary schools were hunted to be the setting of the study in order to achieve the objectives of the study.

Participants: Stratified and randomly selected students between Jan 2016 and April 2017. Students were handed covering letters explaining the purpose of the study, and assuring the confidentiality of information.

Instruments: Instruments were developed and used in order to collect data. A questionnaire including demographic information and tow validated instruments assessing ICT,

Data collection: Data were collected through self-administration of questionnaire regarding the study. Participants provide self-report information across their participation in the study.

Data analysis: The Statistical Package for Social Sciences (SPSS) version 23 was used for data analysis. All data were double checked for accuracy. Frequency distributions were examined to check for outliers and normality of distributions. Means and standard deviations for continuous variables and numbers and percentages for categorical variables were computed.

Ethical considerations:

Ethical approval was obtained from a scientific research commute at the nursing college and governmental health department (2016/11).

Results

Table (1) shows the items of using information communication technologies (ICT)

On a typical school day, how much of your leisure time do you spend		
	Frequency	**Percent**
Watching television	63	50.4
Reading (not for school)	5	4.0
Playing computer/electronic games	5	4.0
Doing homework/projects	16	12.8
Using social networking sites (including instant messaging, chat rooms, face-book etc)	10	8.0
On the phone/text messaging	20	16.0
Playing sports or other physical activity	6	4.8
Total	125	100.0

Cont... **Table (1) shows the items of using information communication technologies (ICT)**

On the weekend, how much of your leisure time do you spend		
	Frequency	**Percent**
Watching television	33	26.4
Reading (not for school)	29	23.2
Doing homework/projects	9	7.2
Using social networking sites (including instant messaging, chat rooms, Facebook, etc.)	10	8.0
Talking/spending time with your family	20	16.0
On the phone/text messaging	1	.8
Playing sports or other physical activity	23	18.4
Total	125	100.0
Where exactly in your home are the computer/laptop(s) that you regularly access the internet from?		
	Frequency	**Percent**
Bedroom	82	65.6
Brother/sister's bedroom	13	10.4
Parents' bedroom	12	9.6
Lounge/family/dining room	8	6.4
Study/office	5	4.0
Hallway	2	1.6
Move around (laptop)	3	2.4
Total	125	100.0
Where else do you go to access the internet?		
	Frequency	**Percent**
Friend's house	43	34.4
Relative's house	22	17.6
School library	18	14.4
Community library	5	4.0
Youth centre	11	8.8

Cont... **Table (1) shows the items of using information communication technologies (ICT)**

Internet café	13	10.4
Shopping centre	13	10.4
Total	125	100.0
How often do you use your own mobile phone (or someone else's mobile phone) to access the internet or visit social network sites?		
	Frequency	**Percent**
Several times a day	74	59.2
About once a day	8	6.4
A few times a week	6	4.8
About once a week	16	12.8
Less than once a week	9	7.2
Hardly ever	2	1.6
Never, I don't have access to a device with this service	10	7.2
Total	125	100.0

Table (2) shows the adolescents using ICT

	Frequency	**Percent**
Low	6	4.8
Moderate	73	58.4
High	46	36.8
Total	125	100.0

This table showed the 58.4% of adolescents using ICT in moderate matter and 36.8 % with high used which impact upon the adolescent health and life style

Discussion

Many studies have shown that the using of communication technology among adolescents has become addictive, which leads to poor physical, psychological and social health and affects the educational level. Participants in our study reported using ICT frequently and for long durations of time before taking a break; both of which are considered risk factors for increased musculoskeletal discomfort. Discomfort prevalence was high among the sample; with nearly all participants reporting discomfort at least once during the study. The most commonly reported areas of the body being the head/neck, back and shoulders, similar to prior reports from child and adolescent ICT, [6]

Pfeiffer et al. [6] investigated the prevalence of problematic Internet use among Chinese adolescents. They found that those who used the Internet more were more likely to suffer from psychosomatic symptoms, emotional and behavioral symptoms as well as physiological dysfunction. Furthermore, their results showed that problematic Internet use was more severe in high school students where they experience more serious dramatic problems than other ages.

Internet use and playing games on the Internet had significant effects on how many hours adolescents slept per night. On average adolescents in the study slept seven hours per night, previous studies have shown that SNS use induces difficulties in sleep and lead to less overall sleep compared to non-users [7]

Other study showed opposite results like Sawyer et al. [8] study results which showed the benefits of using online technologies were reported as increased self-esteem, perceived social support, increased social capital, safe identity experimentation and increased opportunity for self-disclosure. Harmful effects were reported as increased exposure to harm, social isolation, depression and cyber-bullying. The majority of studies reported either mixed or no effect(s) of online social technologies on adolescent wellbeing.

Conclusion

Internet health resources have the potential to be extremely valuable in reducing peer isolation for adolescents with chronic conditions and facilitating their transition to adult health care. A variety of Internet resources are available, and the ease of access allows adolescents to use them as often as mutually desired by parent and child. Pediatric providers can play a role in introducing families to relevant and reliable sources, and teaching them how to evaluate sources to maximize child safety and learning

Recommendation:

The study recommended providing guidelines for engagement in daily moderate to vigorous physical activity which may have had a protective effect on the intensity of IT-related discomfort reported. There is a need for future studies to involve a larger representative sample population in order to gain a better understanding of the association between IT exposure and health promotion. An educational program for family caregivers is important to enhance the ability of caring outside hospital

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Knowledge of Early Childhood‘s Mothers Regarding Toilet Training at Primary Health Care Centers in Alrusafa District in Baghdad City

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Abstract

Toilet training refers to the acquisition of skills necessary for controlling of both the bowel and bladder for the right use of toilet by child at appropriate age and time. A descriptive study (cross-sectional design)was used; To aseess the Knowledge of early childhood‘s mothers Regarding Toilet Training and to find out the relationship between between the knowledge of early childhood‘s mothers’ demographic variables such as (age, educational level, occupational status et al ….) and their child’s demographic variables (age, gender, number of children et al….).

Non probability (convenience sample) of (225) early childhood’s mothers (have children’s age between 1-5 years).

The study concludes that the early childhood’s mothers with moderate border to low level of knowledge regarding toilet training. No significant association among parent’s socio-demographical characteristics andchild’ssocio-demographical characteristics with the knowledge, except between “Starting toilet training or not” and knowledge, also significantassociation found between mother’s source of information with the knowledge. The study recommends that extensive health educational programme should be applied to increase the early childhood’s mother knowledge regarding toilet training.

Keywords: *Early Childhood‘s Mothers,¶ Knowledge,Toilet training.*

Introduction

Toilet training is a training process of the child to control defecation and urination properly and regularly. Its one of the most difficult facets of early childhood. So, failure to train can result in serious physical and psychological effects, such as loss of child’s autonomy. It is not advised that plans concerning toilet training begin when the family experiences major life stressful events [1], [2], [3].

There are many signs of readiness which indicate that the child is ready for toilet training such as physical, psychological, and intellectual.Toilet training in general should be carried out in a child who has started to reach the stages of independence, usually between the ages of 18 and 24 months. Completion’s age of toilet training was described as the age at which the child was fully trained in terms of urine and stool [4[, [5], [6].

There are different types of toilet training methods available, such as the child’s oriented method of Brazelton and the Azrin and Foxx method, other methods include variations of operant conditioning, assisted infant toilet training, and the Spock method [7].

The number of toddlers who were difficult to control urination at preschool age reached 75 million children, this phenomenon is triggered by many things, one of them is the inadequate mother’s knowledge about how to train children to go to the toilet. So,lack of understanding about toilet training among mothers must be determine [8] , [9].

Fathers and mothers are the primary care givers of the children, because they have an important role in toileting process, but it has been revealing that they have only a little knowledge about the toilet training aspects of their children [10].

Toilet training should be an optimistic, non-threatening and normal process, using reinforcement after progress or rewards.Its beneficial in many ways, it reduces necessary care time for the parents, reduces costs, providing children with a sense of independence, and leads to an increase in sanitation [11] &[12].

later age of training was more associated with showing disruptive behavior. And if it is conducted to children older than 3 years, the child may experience developmental decline. Incorrect toilet training may lead to chronic urinary tract infections, enuresis, constipation, and encopresis [1], [6], [13].

Method of the Study

A descriptive (cross-sectional design) study was conducted from the period of 19th September 2020 to the 15th March 2021. The study was carried out in the primary health care centers (PHCCs) in Alrusafa district in Baghdad city to assess the knowledge of early childhood's mothers regarding toilet training.

Non probability (convenience sample) of (225) early childhood's mothers who attend the (PHCCs), where selected as (10%) from the average of a (3) previous monthly visits of mothers to the immunization units. The study including two primary health care sectors (PHCSs) which was selected as (20%) from all (PHCSs), which account (10) sectors in Alrusafa district. A total of (6 PHCCs) was selected as (20%) from each sectors "Al-Sadr Sector" (Third, Al-Shahid Kadeem Abdalnabi, Al-Shahid Ibrahim Al-Ashiqi, and Al-Shahid Jamal Al-Mousawi) PHCCs, and "Al-Baldiat second Sector" (Al-Jawadeen, and Al_Imam Mohammed Al-jawad)PHCCs and randomly for the purpose of the study.

The Study instrument

The tool for the study, including two parts, first concerned with the demographic characteristic of early childhood's mothers such as (age, marital status et al…), and the socioeconomic status scale which have items related to the child's Parent (occupational status, educational level, family type, crowding index, house ownership, house size, house expenses and possession of a car),and also include the child's demographic characteristic which include (age, gender, number of children, child's birth order, starting toilet training or not, age of starting, and information source about the toilet training). The second part is comprised of structured questions to assess the knowledge of the early childhood's mothers regarding toilet training. It includes (20) questions, all were multiple choices question (MCQ) that having four options with one appropriate option, and it was categorized under the following sub domain (General information, Influencing factors, Initiation of toilet training andImportant consideration).

To make the instrument more valid, it was presented to a panel of (15) expertswhich have more than (10) years' experience in their field. Those experts were asked to review the instruments of the study. Acceptable reliability of structured questionnaire format depending on the value of the Cronbach's Alpha which was (0.7333).

Rating and Scoring:

Multiple choices question (MCQ) scored for the "Knowledge item's", using the nominal binary dichotomous categories responding (True, and False) which are scored by (1, 0) scales respectively. Evaluation intervals of relative sufficiency: [L: Low (0.00 – 33.3)]; [M: Moderate (33.3 – 66.7)]; [H: High (66.7 – 100)] after transforming both scored to (true, and false) by the values of (1, and 0) respectively.

The Statistical Data Analysis

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS) ver. (22.0). Descriptive data analysis (Frequencies, Percentages, Mean, Standard deviation, Relative Sufficiency (RS %), Percentile Grand Mean of Score, Percentile Global Mean of Score (PGMS), Pooled Standard Deviation (PSD %), Reliability Coefficient ans Alpha Cronbach). and the Inferential data analysis (One sample Chi-Square test, Binomial test and Contingency Coefficients (C.C.) test).

Results and Discussions

Table (1): Distribution of the parent's socio-demographical characteristics variables (SDCv.), and the Socio-Economic Status.

Parent's (SDCv.)	Groups	No.	%
Age of mother in years	< 20	29	12.9
	20 _29	109	48.4
	30 _39	75	33.3
	40 _ above	12	5.3
	Mean ± SD	27.43 ± 6.79	
Marital status	Married	216	96
	Divorce	3	1.3
	Separate	5	2.2
	widow	1	0.4
Type of family	Extended	107	47.6
	Nuclear	118	52.4
Educational level of (child' Father)	Illiterate	29	12.9
	Read & write	37	16.4
	Primary graduate	72	32
	Secondary graduate	47	20.9
	College graduate	32	14.2
	High Education	8	3.6
Educational level of (Mother)	Illiterate	31	13.8
	Read & write	36	16
	Primary graduate	68	30.2
	Secondary graduate	65	28.9
	College graduate	25	11.1
Occupational status (child' Father)	Employed	105	46.7
	Retired	5	2.2
	Free jobs	113	50.2
	Unemployed\ House wife	2	0.9
Occupational status (Mother)	Employed	23	10.2
	Without Work	3	1.3
	Unemployed\ House wife	199	88.4
Socio-Economic Status	(80 - 100) High	4	1.8
	(50 - 79) Middle	135	60.0
	(0 - 49) Low	86	38.2

No.=Number, % = percentages

The table (1) Reveal that the vast majority of mothers were at the second and third age groups (20 – 29), (30 – 39) yrs., respectively. Most of the mothers in these two age groups are usually healthy young's, newlyweds and aware of the process of visiting PHCCs for the vaccination of their children . The study findings agree with a study counducted at "Jaipur, Rajasthan" reveals that 50(100%) of samples were in the age group of (21-35) years [9].

In related to "Marital Status" most mothers 216(96%) were married, this is due to the cultural nature of the sample area that encourages early marriage. This findings agree a study conducted in sudia Arabia in which (91%) of the mothers were married [14].

The child's family "Nuclear Family" type has recorded vast majority 118 (52.4%). This finding agree with a study conducted in Bangalore in which most of mothers 32(53.33%) belong to nuclear family [15].

The present study reveals thatmajority of child's parent "Father and mother" 72(32%), 68(30.2%) respectively were primary graduates. This finding agree with a study conducted with "Kalika Municipality" in which the level of education for the husbands (father) 81(56.6%) focused on the primary and secondary graduate,also agree with the study conducted in Erbil reported that 102(34%) of mothers have completed primary school [16] & [17].

The present study shows more than half of child's fathers 113 (50.2%) were free jobs, and most 199(88.4%) of mothers were house wife. The study findings agrees with a study conducted in "Egypt" which reported that (72%)fathers were free jobs, and consistent with a study counducted at "Jaipur, Rajasthan" reported that 45(90%) of mothers were housewife [18] & [9].

The present study indicate that majority of the studied sample at middle level "Socio-Economic Status" 135 (60%).This finding agree with a study conducted in "Kalika Municipality" in which in "Not enough for a year class" account 76(49%) of the respondent family [16].

Table (2): Distribution of child's socio-demographical characteristics variables (SDCv.) and the Source of Information about toilet training.

Child's SDCv.	Groups	No.	%
Age of the child in years	1	42	18.7
	2	39	17.3
	3	53	23.6
	4	51	22.7
	5	40	17.8
	Mean ± SD	3.04 ± 1.37	
Gender of child	Male	143	63.6
	Female	82	36.4
Number of children (brothers and sisters)	None	32	14.2
	1 _ 2	101	44.9
	3 _ 4	65	28.9
	5 _ 6	21	9.3
	≥ 7	6	2.7

Cont... **Table (2): Distribution of child's socio-demographical characteristics variables (SDCv.) and the Source of Information about toilet training.**

Child's birth order	The First	77	34.2
	The Second	58	25.8
	The Third	40	17.8
	The Fourth	23	10.2
	The Fifth and above	27	12.0
Starting toilet training or not	No	87	38.7
	Yes	138	61.3
Age of starting toilet training	None	87	38.7
	After age of one year	13	9.42
	After age of two years	87	63.04
	After age of three years	30	21.74
	After age of four years and above	8.0	5.80
Source of Information	Social media (TV, Net, Magazine)	47	20.9
	Household and relatives	113	50.2
	Primary health care centers	9	4.0
	Previous experience	56	24.9

No.=Number, % = percentages

Table (2) Reveal that highest percentages refer to three years old of child's age 53(23.6%). The convergence of age groups may be attributed to the closeness of the periods of pregnancy and childbearing in Iraqi women. The present study findings agree with the study conducted in "Erbil" in which most 180(60%) age group were (2 and 3) years old [17].

The present results reveal that male child's account 143(63.6%). The present study finding agrees with Saudi study reported that (54%) of the toddler is males [14]. The present study results focused at (one to two) number of children 101(44.9%). This is due to the trend twoards preparing and creating a promising generation, educating and rearing them well. The study findings agree with astudy to "Assess the Knowledge Regarding Toilet Training among Mothers of Toddlers" in which the number of children ranging between (1 to 2) child 28 (92.9%) [19].

The results show that the first and the second child's birth order were vast majority 77(34.2%), 58(25.8%) respectively. This findings agree with a study conducted in "Kalika Municipality" reveals that first and second child were vast majority 108(69.7%) [16].

The results show that most of children 138(61.3%) start toilet training and 87(63.4%) of them started training after the age of two years.This findings agree with a study conducted in "Bangalore" in which 55(91.67%) of children started toilet training. Also agrees with a study in Erbil in which 165(55%) of children starting their toilet training at equal or more than twenty four months old. [15] & [17]

The results reveal that "Household and relatives" group has formed the most 113(50.2%) sources of

information for mothers. The present study findings agree with a study conducted in "manamadurai", shows that (64%) of the mothers were got information on toilet training from elders, relatives of the family and friends [20].

Table (3): Distribution of sub and main domains of knowledge concerning early childhood's mothers regarding toilet training

Sub and Main Domains	No.	Min.	Max.	PGMS	PSD	Ev.
General Information	225	0.00	100	47.11	33.11	M
Influencing Factor	225	0.00	100	36.44	33.82	M
Initiation	225	0.00	100	46.07	30.47	M
Important Consideration	225	0.00	90.91	43.03	18.32	M
Main Domain: Knowledge	225	2.27	97.73	43.17	18.00	M

Ev. : Evaluated (0.00 – 33.33) Low (L) ; (33.34 – 66.66) Moderate (M) ; (66.67– 100) High (H).

Table (3) shows that the responses of mothers accounted a moderate level border to low for knowledge sub and main domain. Most of mother get information from "elders & relatives" so it normally that they have moderate level of knowledge.

The present study result agree withstudy conducted in "mysuru" reported that majority of mothers 34(56.6%) are having average knowledge regarding toilet training. And agree with study conducted in "moradabad district" reported reported that majority of mothers (57%) were having moderate knowledge [10] ,[21].

Table (4): Relationships among mothers responding regarding studied sub and main domains of knowledge with parent's and child's Socio-Demographical Characteristics variables (SDCv.).

Parent's (SDCv.)	Mothers' Knowledge			Child's (SDCv.)	Mothers' Knowledge		
	C.C.	Sig.	C.S. (*)		C.C.	Sig.	C.S. (*)
Age of mother in years	0.076	0.724	NS	Age of the child in years	0.122	0.495	NS
Marital status	0.055	0.875	NS	Gender of child	0.112	0.091	NS
Type of family	0.056	0.403	NS	Number of children (brothers and sisters)	0.179	0.113	NS
Educational level of parent (Father)	0.200	0.094	NS	Child's birth order	0.166	0.173	NS
Educational level of parent (Mother)	0.064	0.922	NS	Starting toilet training or not	0.137	0.039	S
Occupational status (Father)	0.121	0.344	NS	Age of starting toilet training	0.164	0.285	NS
Occupational status (Mother)	0.038	0.847	NS	Sources of Information	0.217	0.011	S
Socio-Economic Status	0.145	0.088	NS				

S : Sig. at P<0.05 ; Testing are based on a Contingency Coefficient test

Table (4) Reveal that no significant levels has recorded at P>0.05 among parent's (SDCv.) and child's (SDCv.) with knowledge, except between "Starting toilet training or not" and mothers knowledge, at P<0.05, result also show significant relationships are accounted at P<0.05 among mother's source of information and knowledge level.

The present study findings agree with study conducted in "mysuru" reveal that there is no significant association at (0.05) between knowledge level of mothers of toddlers regarding toilet training and personal variable (age, educational status, number of children, family income, types of family).This present findings agree with a study conducted in "Bangalore" showed that there is no significance association between mothers level of knowledge and study variables such as (child'age, child's gender). The present study findings agree with the study conducted in "manamadurai" in which there is a significant association between knowledge level and source of information on toilet training. [10], [15], [20].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Impact of Chemotherapy upon Psychological status of School Age Children with Acute Lymphoblastic Leukemia at Pediatric Teaching Hospitals in Baghdad City

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Abstract

A descriptive design study was conducted to assess the psychological status of the impact of chemotherapy upon school age children with acute lymphoblastic leukemia in Baghdad city and find out the relationship between Impact of Chemotherapy upon psychological status of school age children with acute lymphoblastic leukaemia and their demographic characteristics like children's(age ,gender, level of education et a)

A non-probability (Purposive) sample of 114 children with ALL under chemotherapy was selected in attending hospital wart out pation and counseling clinic.The results of the present study indicated that impact of chemotherapy upon psychological status of acute lymphoblastic leukemia children were effected at moderate level, with regard to relationships among impact of chemotherapy upon psychological status and child's Socio-demographic characteristics,and no significant association was apper

The study concludes that most of the children with acute lymphoblastic leukemia had moderate level, and recommended that Psychosocial support should be provided to school age children and their parent's to facilitate their coping with disease and its treatment. Establishment of counseling clinic especially for treatment of children with acute lymphoblastic leukemia, to maintain care, reduce the anxiety, and their consequently.

Keywords: *Impact of Chemotherapy, Psychological status, Acute Lymphoblastic Leukemia.*

Introduction

Leukemia is a common malignancy in children that causes changes in the normal regulatory processes of cells and alterations hematopoietic stem cells to proliferate uncontrollably in the bone marrow. The prevalence rate of age-adjusted leukemia in the United States is 12.8 per 100,000 individuals each year[1].

Acute Lymphoblastic leukaemia (ALL) is the most common type of cancer in children, and is a leading cause of death by disease in developed countries [2]. It is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extramedullary sites [3]. Leukaemia accounted for the largest number of childhood cancer and is the primary cause of cancer concerning mortality for children in the United States(US) at an average rate of approximately 41 cases per 1 million people between the ages of (0 – 14) years and approximately 17 cases per 1 million people between the ages of (15-19) years old. ALL leukaemia is the most common cancer diagnosed in children, accounting for around 25% of diagnoses of cancer in children younger than 15 years of age. Each year, approximately 3,100 children and adolescents younger than 20 years are diagnosed with ALL in the United States Since 1975, and the incidence of ALLL has risen steadily since 1975[4].

In the last 20 years, cancer survival has increased, affecting the long-term risk of mood disorders [5]. Neurocognitive and psychological issues as a result of chemotherapy have been identified in adult cancer survivors across a variety of cancer types. However, most paediatric have focused on the treatment of children

with brain tumours or central nervous system (CNS)-directed radiation, In ALL children, the deleterious neurocognitive effects of cranial radiation are well known. While the initial treatment of both, cranial radiation is now rarely used and usually performs CNS prophylaxis with systemic and intrathecal chemotherapy [6]. Psychological morbidity associated with CNS treatment has been observed among survivors of childhood ALL and brain tumors. Although indicate rates of psychological distress that are only marginally elevated relative to that seen in the general population, others show increased mood disturbance (especially depression), Symptoms of posttraumatic stress, poor body image, and insufficient coping strategies may be difficult to diagnose a patient because adjustment difficulties and mood problems can manifest as diminished focus or decreased academic achievement [7].

Chemotherapy alone or in conjunction with radiotherapy plays a significant role in cancer care. The psychological state of these children can be impaired by long recovery times, frequent hospitalizations, failure to adapt to social and occupational life and side effects of chemotherapy. All of these can increase anxiety and depression, including awareness of having cancer, as well as lower self-esteem for children with cancer. Due to change in the physical appearance, the challenge of handling the therapy and the side effects, and re-adaptation after treatment, cancer children undergoing chemotherapy frequently suffer from loss of self-esteem [8].

The important to recognize psychosocial risks before and after ALL care for children and their families, as well as ways to minimize anxiety and help patients and families. However, in various paediatric oncology contexts, there is a wide variability in psychosocial services provided to patients. In addition, there are no written, systematic, evidence-based paediatric psycho-oncology treatment guidelines [9].

Methodology

Design of the study:

A descriptive study design (cross-sectional study) was conducted on school age children both gender having acute lymphoblastic leukemia under chemotherapy treated and their age between 6 years to 12 years. The study started from the period of September, 19th 2020 to March,1st 2021 and aimed to assess Psychological status of School Age Children with Acute Lymphoblastic Leukemia at Pediatric Teaching Hospitals in Baghdad City.

Ethical Consideration:

The participants were fully acquainted with the current study and its aims and then a voluntary verbal consent was obtained from in order to participate in the study. The confidentiality of information will keep silent and has been taken into account. ethical approval was also obtained from the ethical committee of research in the Faculty of Nursing/University of Baghdad regarding confidentiality and anonymity of the participants

Settings of the Study:

The present study was conducted in Baghdad City; Oncology Centre at the Children Welfare Teaching Hospital and at Oncology Child's Central Teaching Hospital At AL-iskan sector of Baghdad city.

Sample of the study:

A non-probability (Purposive) sample of 114acute lymphoblastic leukemia under chemotherapy children with their parents either (father or mother) were selected from the oncology center at the children welfare teaching hospital and child's central teaching hospital, At AL-iskan sector of Baghdad city The parents accepted to participate in the study after explaining the purpose need for their contribution and filling of the checked form

Instrument of the study:

A questionnaire is constructed for the purpose of the study throughout a review of relevant literature and consultation from panel of experts and related studies. It composed of four parts (Demographic Characteristics, Disease Related Information, Pediatric Psychosocial symptoms inventory scale PPSIS and Hospital Anxiety and Depression Scale HADS)

Statistical Analysis

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS) ver. (22.0): Descriptive data analysis(Frequencies, Mean

and Standard deviation and Percentages) and Inferential data analysis(One sample Chi-Square test, Binomial test for testing the different of distribution of the observed frequencies of two categories nominal /or ordinal scale and there is none restricted of an expected outcomes at 50%.

Results and Discussion

Table (1): Distribution of the sample by their Socio-demographic characteristics (SDCv.)

Discussion of the school age children with Acute lymphoblastic leukaemia according to their Demographic.

Child's (SDCv.)	Groups	No.	%
Gender	Male	63	55.3
	Female	51	44.7
Age Groups Yrs.	6 _ 7	24	21.1
	8 _ 9	28	24.6
	10 _ 11	42	36.8
	12 above	20	17.5
	Mean ± SD	9.53 ± 1.87	
The age of the mother when she gives a birth to a child (Yrs.)	< 20	7	6.1
	20 _24	44	38.6
	25 _29	32	28.1
	30 _34	23	20.2
	35 -39	8	7
	Mean ± SD	26.19 ± 5.22	
Brothers and sisters numbers	1 _ 2	44	38.6
	3 _ 4	54	47.4
	5 above	16	14
His / Her lineage between sons	1 _ 2	64	56.1
	3 _ 4	39	34.2
	5 above	11	9.6
The education level for a child	Non Applicable	19	16.7
	First primary school	9	7.89
	Second primary school	11	9.64
	Third primary school	17	14.9
	Fourth primary school	17	14.9
	Fifth primary school	16	14.03
	Sixth primary school	25	21.92
Doesn't enrolled in school	Non Applicable	95	83.3
	Was rejected by school	10	8.76
	Incapable of learning	9	7.89

Table (1), indicates that more than half of the study sample were males,it has been mentioned that males are more affected than females in percentage 63(56.3). male was the dominant gender and represented approximately more than half sample of acute lymphoblastic leukemia children this might be related to prevalence rates for ALL are two times higher in males than females in ratio 3:1 .This result agree with a study conducted by (Lau, 2014) he said that parent of 159 children with ALL participant in the study (83 (52%) males children and 76 (48%) female children[10], and this result agree with a study conducted by (de Vries, et al., 2010) concerning his study who indicated that the majority of study sample 23 (56%) were males[11].

The result of the present study revealed that the dominant age group of the study sample is (10-11) years old of age group and accounted for 42 (36.8),because in this stage characterized by activity , effectiveness and emptying of energies, and the child remains lying on the bed for a long time as a result of the effect of disease leads to disturbance of his/her psychological status. This findings agree with study in Egyptthat was reported that the average age of the children with ALL in the (9-11) age group was (45.6%)[12].

Throughout the course of the present study in table (4-1). It is indicated that most of the acute lymphoblastic leukaemia children were the first- two child's birth order in the family 64 (56.1%). This be related to several factors such as the child family size and the risk of acute lymphoblastic leukaemia increases for the first-two birth children. This result agrees with study in Egypt was mentioned that most of the Acute Lymphoblastic leukaemia children were for the first- two birth order in the family and accounted for 116 (65.4)[12].

In regarding to the his /her lineage between sons , the findings of the present study revealed that the dominant of the study sample their order in the number of their siblings is with (3-4) 39 and reported that (34.2%). This phenomena perhaps related to several factors such as parent's early marriage and not having the domestic planning to the number of children . This finding agree with (Sherief et al., 2015) they reported that 61% had 3 to 5 siblings[13].

The present study mention also that the education level for children with acute lymphoblastic leukemia treatment under chemotherapy more than in Sixth primary school and account for 25(21.92 %) This result might be development of the cultural awareness of the family and the children's love to completing his-her education. This result agree with Alias, Mohd Naziand Chong (2019), In them study they mention that 29(61.7%)was primary school[14].

The age of the mother when she gives a birth to a child that the dominant of study sample at age group with (20-24) accounted for 44 (38.6%). theis result agree with (Bangun, et.al, 2014) entitled (Risk factors of childhood leukemia) who mention that the dominant age group of study sample is with in age group (20-34) years old and accounted for (75.7%)[15].

Table (2): Distribution of main & Sub domains concerning impact of chemotherapy upon psychological status of school age children with acute lymphoblastic leukemia at pediatric teaching hospitals

Main and Sub Domains	No	Min.	Max	GMS	PSD	GRS%	Ev.
Negative Feelings	114	1.333	3.000	2.227	0.408	74.219	M
Self-Esteem	114	1.200	2.800	1.798	0.345	59.942	M
Physical Satisfaction	114	1.500	2.750	2.132	0.351	71.053	M
Personal Signs	114	1.400	3.000	2.421	0.379	80.702	H

Cont... **Table (2): Distribution of main & Sub domains concerning impact of chemotherapy upon psychological status of school age children with acute lymphoblastic leukemia at pediatric teaching hospitals**

Signs of Learning Problems	114	1.000	3.000	1.900	0.405	63.333	M
Psychosocial of child Score	114	1.520	2.587	2.095	0.257	69.850	M
Signs of Depressions	114	1.000	2.714	1.825	0.419	60.821	M
Signs of Anxiety	114	1.200	3.000	2.279	0.493	75.965	M
Hospital Anxiety and Depression Score	114	1.272	2.857	2.052	0.423	68.393	M
Overall Evaluation	114	1.446	2.702	2.074	0.318	69.121	M

Ev. : Ev. : Evaluated (33.33 – 55.55) Low (L) ; (55.56 – 77.77) Moderate (M) ; (77.78– 100) High (H). GMS: Global Mean of Score, PSD%:Pooled Standard Deviation, GRS%: Grand/or Global Relative Sufficiency

Discussion of the overall of the impact chemotherapy upon psychological status of school age children with acute lymphoblastic leukemia

The present study finding revealed that the overall main domane assessment of psychosocial child's Score of acute lymphoblastic leukaemia children were at moderate level in percentage (68.39) table (2). Their level of psychosocial may related to diagnosis and treatment of acute lymphoblastic leukaemia in early stage during childhood has the potential effects on the social development, emotional health , and academic progress of survivors.

The result also revealed that the overall main domane hospital anxiety and depression score of acute lymphoblastic leukemia children were at moderate level and indicated(68.39) , which may refer to the presence of anxiety problems and unsatisfactory level of depression of acute lymphoblastic leukemia child's stay in the bed and limited to activity ,social isolation in addition to that side effected of chemotherapy such as bone pain nausea vomiting and hair loss. The results of this study are supported by (Myerset al., 2014) who reported that acute lymphoblastic leukemia child's show moderate level of anxiety and accounted Mean anxiety scores were 53.2, 52.3, and 50.0 and depression scores were 52.1, 53.1, and 52.9 at one, six, and twelve months after diagnosis, respectively from 159 sample and reported that overall hospital anxiety and depression of children with ALL had a high level of anxiety (15%) and depression (28%) . the result of present study may be related to the healthy family functioning and more reliance on social support coping strategy people around the child and their parent's [15].

Table (3): Relationships among studied main domains and child's Socio-demographic characteristics (SDCv.)

Child's (SDCv.)	Socio-Psycho of child Score			Hospital Anxiety and Depression Score			((Overall))		
	C.C.	Sig.	C.S.	C.C.	Sig.	C.S.	C.C.	Sig.	C.S.
Gender	0.169	0.068	NS	0.100	0.284	NS	0.150	0.105	NS
Age of child (Yrs.)	0.148	0.468	NS	0.179	0.285	NS	0.165	0.364	NS
Brothers and sisters numbers	0.126	0.398	NS	0.051	0.861	NS	0.073	0.738	NS
Brothers and sisters numbers	0.081	0.689	NS	0.198	0.099	NS	0.189	0.122	NS
The education level for a child	0.146	0.871	NS	0.174	0.734	NS	0.163	0.795	NS
He wasn't enrolled in school	0.188	0.405	NS	0.188	0.405	NS	0.188	0.405	NS
The age of the mother when she gives a birth to a child	0.254	0.097	NS	0.168	0.505	NS	0.192	0.358	NS

NS : Non Sig. at P>0.05; Testing are based on a Contingency Coefficient test.

Discussion of the association between psychological impact of chemotherapy upon school age children and their socio-demographic characteristics

The result of the present study showed that socio-demographic characteristic that includes (child age, gender, child's age at diagnosis,Brothers and sisters numbers, the education level for a child and the age of the mother when she gives a birth to a child) did not affect in relation to the psychological impact of chemotherapy upon school age children table (3).

Some side effect of chemotherapy, such as pain, vomiting, nausea, hair loss and feels weak in his body. These symptoms can lead to limit their ability to engage in normal activities such as attending school. The results of the current study agrees with new study that was stated that no significant association was found between sociodemographic characterize of acute lymphoblastic leukemia children and their psychological impact [16].

So, Indication the results also supported withstudy in Iraq was reported that there was no significant association between ALL child and their gender[17].

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Parents' Knowledge toward Pediatric Immunization and its Relation with their Compliance at Primary Health Care Centers in Karbala City

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Abstract

Parents are their children's primary health decision makers, their knowledge towards immunization in general have a significant effect on the immunization status of their children. A descriptive (cross-sectional study) was conducted to assess parents' knowledge toward immunization and its relation with pediatric immunization compliance at primary health care centers in Karbala city, Iraq

A non-probability (convenience) sample of 292 parents selected from (5) primary health care centers. These centers distributed throughout (2) primary health care sectors selected randomly as (20%) from each sectors. The results of the present study indicated that the majority of studied parents had moderate level of knowledge, which was a significant associated with their age of fathers, level of education of father and mothers, occupational status of mothers, family type, , more than sixty present of studied parents have children partially immunized. In addition, the present study revealed that parents' knowledge not had a significant influence on child's immunization status. So, this study recommendedit is necessary to raise parents awareness of the importance of immunization by implementing educational programs and by traditional and social media with emphasis on time, side effects, contraindication, and disease preventable by immunization and promote periodically assessment for immunization coverage.

Keywords: *Parents, Knowledge, Immunization, Compliance.*

Introduction

Immunization against diseases is one of the most important intervention in public health, with cost-effective ways of reducing childhood morbidity, mortality and disability. However, a proportion of children are not completely immunized with the recommended vaccines[1]. Immunization is important to reduce disease burden in young children and it is estimated that it prevent 2 to 3 million deaths every year. There are currently an estimated 19.5 million children in the world who have not received basic vaccine. Global vaccination coverage has stalled at 86%, with no significant improvements during the past year [2].

In the literature on public health, the contributors to lower immunization rates are well established. Increases in the number of vaccines required and changes to the immunization schedule create difficulties for parents and care providers in tracking immunization status[3].

More than 330,000 children die from VPDs per year. Perinatal disorders (20%), respiratory infection (18%), diarrheal diseases (17%), VPDs (15%), and malaria (7%) are among the top killers in children under five years old[4]. Approximately 1 of 5 children worldwide have no received routine life-saving immunization every year, according to the (WHO), and 1.5 million children have died of diseases that could have been prevented by vaccines [5].

Parents are their children's primary health decision makers, their knowledge towards immunization in general have a significant effect on the immunization status of their children, the tremendous amount of conflicting vaccine safety information and misinformation on the

internet may have a negative impact on the decisions of parents. Thus, there is a critical need to evaluate knowledge and practice of parents about their children's immunization in order to enhance and improve the coverage and completeness of vaccination[6].

Parental behaviours such as unawareness of adverse effects follow vaccination and vaccine contraindications, negative views regarding vaccination in mild illness, negative attitudes, for example, the fear of vaccination by parents was considered as one of the major barrier to childhood vaccination[7].

Methodology

Design of the Study:

A descriptive design (cross-sectional study) was approved throughout the present study aimed to assess parents' knowledge toward immunization and its relation with pediatric immunization compliance at primary health care centres in Karbala city through the period of 19th September 2020 to the 6th March 2021.

Ethical Consideration:

The participants were fully acquainted with the current study and its aims and then a voluntary verbal agreement was obtained in order to participate in the study. Besides, the confidentiality of information obtained from parents of children has been taken into account. Also, ethical approval was obtained from the ethical committee of research in the College of Nursing / University of Baghdad regarding confidentiality and anonymity of participants

Setting of the Study:

The accessible population included parents of children who attend to the immunization unit in the PHCCs in Karbala city. The study conducted in Karbala city including two primary health care sectors (Central Sector and Al-Hur Sector**).**

Sample of the Study:

Non probability "convenience" sample of (292) parents (father and/or mother) who attend the PHCCs where selected in and out of the unit of immunization at the (5) PHCCs which involved in the study in Karbala city. These centres distributed thought (2) primary health care sectors.

Instrument of the study:

A questionnaire is constructed and developed as a tool for data collection by the researchers, after an extensive review of relevant literatures and consultation from panel of experts and related studies. A questionnaire format was contained three parts were constructed to include all aspect associated to the study **(Socio-demographic characteristics, Parents' knowledge toward immunization and Parental compliance with the routine immunization schedule)**

Methods of data collection:

Data were obtained through face to face interview techniques as a method of data collection. The period from (8th December 2020 to the 1stFebrile 2021), interview was conducted with parents who were attended to PHCCs in Karbala city to fill in the questionnaire after a permission was arranged from primary health care sector and an agreement of participation was obtained from the parents to the interview.

Statistical Analysis:

The researcher using the Statistical Package of Social Science (SPSS) version (22.0) to analyze the data and relation the results of study .The researchers used descriptive and inferential data analysis to obtain results.

Results and Discussion

Table (1): Distribution of parents by their socio-demographic characteristics variables.

The findings of the present study showed in the table (1) that more than 80% of the studied sample were mothers This finding agree with a study in Hail City that was carried out upon (420) parents found that the majority of the study sample were mothers and accounted 277 (66%)[8].

Furthermore, most of fathers and mothers of the present study sample are within (30-34) and (25-29) years respectively. This result consistent with a descriptive study in Nigeria was reported that the majority of fathers within age group (30-34) and accounted 86 (19.46%), and most of mothers 116 (26.24%) falls within age group (25-29) years old [9].

The current study found that higher proportion of participants were married (represented more than three-quarters of the participants), These findings agree with study conducted in Saudi Arabia was reported that the majority of the study sample (mothers and fathers) were married and accounted 458 (91.6%)[10].

This results is consistent with a study in Saudi Arabia was revealed that the majority of the study sample were living in urban environment. Furthermore, the current study indicate that more than half of the study sample was from a nuclear family [11].

The result of the current study that concerning with the educational level of the study sample mentioned that the majority of fathers and mothers obtained institute / university degree and accounted 28 (49.1%), 79 (33.6%) respectively. they reported that most of the participants (fathers and mothers) graduated from college 366 (61%) . Furthermore, the result of the current study in table (1) founded that the majority of the participants got information about immunization (vaccination) by health care workers which accounted 241(82.5%). We thought that the health care workers can be a trusted source to promote parent's knowledge and attitudes toward immunization. This result agree with a descriptive study done by (Verulava et al., 2019) Their study result mention that the majority of participants 93 (49.5%) got information about immunization by health care workers [12].

Table (2): Distribution of sub main domains of parents' knowledge towards immunization and overall assessment of parents' knowledge.

No.	Sub Domains	GMS	PSD%	Ass.
1	Parents' Knowledge about concept of immunization	0.754	0.216	H
2	Parents' Knowledge about the important of immunization	0.853	0.177	H
3	Parents' Knowledge about the expected side effect vaccination	0.787	0.175	H
4	Parents' Knowledge about routine immunization	0.490	0.237	M
5	Parents' Knowledge about contraindication of immunization	0.329	0.210	L
A	Parents' Knowledge about contraindication of immunization-False considered	0.354	0.267	M
B	Parents' Knowledge about contraindication of immunization-True considered	0.304	0.235	L
6	Parents' Knowledge about BCG vaccine	0.396	0.260	M
7	Parents' Knowledge about oral polio vaccine	0.534	0.239	M
8	Parents' Knowledge about hepatitis B vaccine	0.361	0.421	M
9	Parents' Knowledge about (diphtheria, tetanus, pertussis) vaccine	0.170	0.261	L
10	Parents' Knowledge about measles	0.425	0.336	M
11	Parents' Knowledge about measles, mumps, rubella	0.091	0.241	L
12	Parents' Knowledge about Haemophilus influenza type b vaccine	0.019	0.119	L
13	Parents' Knowledge about Rotavirus vaccine	0.026	0.125	L
Overall assessment of parents' knowledge		GMS	PSD	Ass.
	Parents' Knowledge towards immunization (vaccination)	0.447	0.123	M

***GMS: Global Mean of Score, PSD: Pooled Standard Deviation, ,* Ass. : Assessment(0.00 – 33.33) Low (L) ; (33.34 – 66.66) Moderate (M) ; (66.67– 100) High (H).**

Overall assessment of parents' knowledge towards immunization:

The results of the study on hand showed that the majority of the studied parents had a moderate level of knowledge towards children immunization as shown in table (2). This findings was agree with the results in Yemen that was carried out a cross-sectional survey study on (400) parents, the study revealed that the (63.3%) of parents had a moderate level of knowledge[13].

Furthermore, a descriptive prospective study conducted on (140) nursing mothers at a child hospital in south-west Nigeria was reported that the majority of study sample (71.4%) has adequate knowledge. On the other hand, another study in Jeddah City was indicated that most of the participants (87.2%) had a high level of knowledge about the preventive measures and the benefit of vaccination for children [14], [15].

Table(3): Association between overall assessment of parents' knowledge and their socio-demographic characteristics:

Demographical Characteristics variables	Parents' Knowledge towards immunization (vaccination)		
	C.C.	Sig.	C.S.
Gender	0.044	0.451	NS
Age of mother (Yrs.)	0.184	0.070	NS
Age of Father (Yrs.)	0.198	0.035	S
Marital Status	0.122	0.218	NS
Residency	0.009	0.881	NS
Level of Education of Father	0.306	0.000	HS
Level of Education of Mother	0.423	0.000	HS
Family Type	0.171	0.033	HS
Health care workers	0.130	0.025	S
Social media	0.121	0.035	S
Television	0.004	0.946	NS
Family, neighbor, relatives	0.016	0.787	NS

HS : Highly Sig. at P<0.01; NS : Non Sig. at P>0.05 ; S : Sig. at P<0.05 ; Testing are based on a Contingency Coefficient test.

The findings of this study indicated that no significant association was found between parents' knowledge and their some socio-demographical characteristics.

From the researcher's point of view the educational level is the most important factors that helps the parents to understand the importance of immunization for the children. Otherwise, occupation although could be supposed that the employed mothers are better educated and have more access to information through a broader social environment. The results of the present study similar to results of study in Lagos State was reported that there was a statistically significant relationship between level of knowledge and some socio-demographic variables like (mother's occupation, level of education, and type of the family) [16].

Theses result supported by a study in Turkish was mention that there was a significant relationship between parents' knowledge and the economic status of the family at P < 0.001. In other hand a descriptive study done in Iraq indicated that that there was no significant association between the level of participant's knowledge and their age, marital status, and residency [17],[18].

The findings of the study on hand stated that no significant association was existent between parents' knowledge, attitudes toward immunization and source of information, except the social media that has a significant correlations with parents' knowledge toward immunization, table (4-12).

The result of the present study go in line with a descriptive survey study done by Vinish (2016), who reported that no significant correlation between knowledge of participants and source of information. While study Saudi Arabia was found that there were a significant relationship between parents' attitudes and the source of information about immunization at P-value <0.001 [19][20].

Table(4): Parents' compliance and Association between (child's immunization status) and Parents' Knowledge

Immunization Status	Groups	F.	%	C.S. (*) P-value
Child's immunization status	Fully immunized	115	39.4	P=0.000 (HS)
	Partially immunized	177	60.6	
Child's immunization status and Parents' Knowledge				
Relationships	C.C.	Sig.	C.S.	
	0.044	0.409	NS	

NS : Non Sig. at P>0.05 ; Testing based on a Contingency Coefficient test (C.C).

Regarding the immunization status of children, the present study findings as in table (4), indicated that more than half of the studied children 177 (60.6%) were partially immunized. These results consistent with a cross-sectional study in the Urban Slums of Bijapur city, Karnataka, India was revealed that the most of studied children were partially immunized and accounted 97 (62.58%) [21]. From the researcher's point of view child's illness lead to partial immunization of studied sample and not related to parents' knowledge and attitudes toward immunization.

The results of the this study showed that no significant association was found between knowledge of parents towards immunization and their compliance (child immunization status) in a table (4-15). These results agree with Trushitkumar et al. (2017), they reported that no significant association was found between the parents' knowledge and the immunization status of their children.

From the researcher's view pointa major reason for this can be the fact that most of the parents with moderate level of knowledge score, 82 (46.3%) of participants mention that the reason for non-compliance with paediatric immunization was sickness or illness of child this mean it's not related to parents lack of information about the important of immunization for their children[21].

From the researcher's point of view, might be related to the reason that mentioned by the researcher previously.

Conclusion and Recommendation

The results of the present study indicated that the majority of studied parents had moderate level of knowledge, which was a significant associated with their age of fathers, level of education of father and mothers, occupational status of mothers, family type, , more than sixty present of studied parents have children partially immunized. In addition, the present study revealed that parents' knowledge not had a significant influence on child's immunization status. So, this study recommendedit is necessary to raise parents awareness of the importance of immunization by implementing educational programs and by traditional and social media with emphasis on time, side effects, contraindication, and disease preventable by immunization and promote periodically assessment for immunization coverage.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Interventional Nursing Program for Nurses Practices about Enteral Feeding Guidelines in Critical Units

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Abstract

Background: Malnutrition among critical ill patients is a common and serious problem followed by sever clinical results, nutrition is one of the most important principles in treating many diseases and directly intervenes in pathophysiological changes and critical results of the disease. Regular enteral feeding is an effective intervention for critically ill patients to meet their nutritional and physiological needs. Nursing care for enteral feeding patients plays a vital role in ensuring enteral feeding efficiency and adopting evidence-based methods to improve patients' feeding and clinical outcomes.

Objectives of the study: this study aims to find out the effectiveness of interventional program concerning enteral feeding guideline on nurses' practices in critical care units.

Methodology: A pre-experimental study design was carried out in the critical care units of Al Ramadi Teaching Hospital and Al Fallujah Teaching Hospital at Al-Anbar Governorate between the period 3rd of November 2020 to 25th of February 2021. A non-probability sampling method consists of 32 nurses was selected purposively based on the study criteria.

Results: The participants in present study was 87.5% were males and 12.5% were females and age between 20-30 years was 59.4% of sample. In addition to that, 40.6% of the patients were morning shift and 59.4% were night Shift and there were significant statistical differences between the pre and posttest for intervention program at $p \leq 0.05$ value, and there were a highly significant correlation between the effectiveness of interventional program and educational level of nurses, and time working at $p \leq 0.05$.

Conclusion: The study concluded that the interventional nursing program had a positive impact on nurses' practices regarding enteral feeding.

Recommendations: The study recommends to apply the guideline for Parenteral and Enteral Nutrition in order to avoid the complication of enteral feeding for critical patients and conducting continuous training programs about safe administration of enteral nutrition and medications as well as skills related to patients nutritional assessment to develop and improve nurses' knowledge and practices regarding the management of patients who are receiving enteral feeding.

Key Words: *Interventional, Nurses Practice, Evidence-Based Guidelines, Enteral Feeding, Critical Care Units.*

Introduction

Patients with critical illness are patients with severe life-threatening conditions with significant consequences, including malnutrition. Typically associated with increased hypermetabolic and lean body weight reduction leading to malnutrition [1].

Most patients including critical care patients fail to intake the required nutrition like normal people [2]. Regular enteral feeding is an important intervention for critically ill patients to meet their nutritional and physiological needs [3]. Enteral feeding is one of the most efficient nutritional methods in intensive care. In addition to its cost effectiveness, it has become more

popular with other methods of nutrition in the promotion of patient immunity and survival [4]. Nutrition support is now seen as an important part of the strategy for managing critically ill patients in the ICU [5]. Enteral feeding has a particular benefit in helping reduce some of the inflammatory responses to lack of nutrition. It can increase intestinal contractility, which helps control bacterial overgrowth [6]

Nutritional support is currently designed to limit catabolic response and systemic inflammation as well encouraging return to physiological baseline. Appropriate energy and protein consumption is related to better health outcomes in critical illness [7]. Evidences-based practices (EBPs), particularly in critically ill patients have substantial impact on reducing variations in clinical practice and ensuring standardized nutritional care. EBP allows healthcare professionals to make the best medical judgments, resulting in less harm being caused [8]. In recent studies, nutrition support guidelines have been shown to be effective in maintaining nutritional outcomes in a wide range of patients in intensive care. It is important to find methods to improve early enteral feeding delivery that meets requirements and encourages positive performance [7]. The American Society for Enteral and Parenteral Nutrition (ASPEN) is a major source of nutritional recommendations, and the nurse-centered guidelines included in this study are based on the most recent ASPEN guideline update[9].

Methodology

Design and setting of the study: A pre-experimental study design was carried out in the critical care units at Al Ramadi Teaching Hospital and Al Fallujah Teaching Hospital of Al-Anbar Governorate from the period 3rd of November 2020 to 25th of February 2021, in order to find out the effectiveness of the intervention program concerning enteral feeding on nurses' practices.

Sample of the study: a purposive sample was consists of (32) nurses was selected according to study criteria and after obtains verbal and written consent permission from them.

The study instrument: Part one, included the study sample's socio-demographic characteristics, and Part two focused on the nurses' practices regarding enteral feeding, the instruments consist of 30 items of practical knowledge and 46 items of observational checklist based on American Society for Parenteral and Enteral Nutrition guideline (ASPEN 2017), includes two domains: First domain concerning nurses' practices regarding enteral feeding administration which contain (26) items, Second domain: concerning nurses' practices regarding medications Administration via enteral feeding tube which of (20) items.

Statistical Analysis: The IBM Statistical Package of Social Sciences (SPSS) Version 22 was used to analyze the results. Both descriptive statistical analysis and inferential statistical analysis approaches were used in order to investigate or predicts the relationships between variables.

Results

Table (1): Distribution of the Study Sample by their Socio-demographic Characteristics (n=32).

Variables	Classification	Frequency (F)	Percentage (%)
Age/ years	20-30 years	19	59.4
	31-40 years	10	31.3
	41-50 years	2	6.3
	51-60 years	1	3.1

Cont... **Table (1): Distribution of the Study Sample by their Socio-demographic Characteristics (n=32).**

Gender	Male	28	87.5
	Female	4	12.5
	Total	32	100
Level of Education	Nursing School	5	15.6
	Nursing Institute	26	81.3
	College of Nursing	1	3.1
Years of Experience in nursing	1-5 years	14	43.8
	6-10 years	11	34.4
	11-15 years	5	15.6
	16-20 years	1	3.1
	31-35 years	1	3.1
Years of Experience in Critical Care Unit	1-5 years	28	87.5
	6-10 years	3	9.4
	11-15 years	1	3.1
Time Working	Morning Shift	13	40.6
	Night Shift	19	59.4
Training course about Enteral Feeding	Yes	-	-
	No	32	100
Self-Learning	Yes	32	100
	No	-	-
Type of Self-Learning	Internet: Social Media	17	53.1
	Internet: Scientific Websites	9	28.1
	Personal reading (library)	6	18.8

F=Frequency; %=Percentage.

Table (1) shows that the high percent (87.5%) of nurses were males at age group (20-30) years old which of (59.4%), high percent of them (81.3%) was graduated from nursing institute, (43.8%) of them have (1-5) years of experiences, 87.5% of them have experiences in critical care units, and 100% of the nurses not participant in a training courses related to enteral feeding.

Table (2): Statistical Differences between Pre and Posttest for Interventional Program

Paired Samples Statistics							
Program Domains		**Mean**	**N**	**SD**	**T**	**Df**	**Sig. P≤ 0.05**
Pair 1	pre practical nurses knowledge	44.125	32	5.097	12.986	31	0.00 H.S
	post practical nurses knowledge	55.781	32	1.773			
Pair 2	Pre applying to administration of nutrition	43.968	32	2.822	19.602	31	0.00 H.S
	Post applying to administration of nutrition	63.281	32	4.017			
Pair 3	pre applying to administration of medication	34.468	32	2.199	22.220	31	0.00 H.S
	post applying to administration of medication	48.718	32	2.997			

T. = T.test, Df. = Degree of freedom, Sig. =Significance (≤0.05=significant, ≤0.001=high significant & ≥ 0.05=no significant).

Table (2) presented that there were significant differences between Pre and Posttest practical nurses knowledge, practices applying to administration of nutrition, and their practices applying to administration of medication according to guideline at **P≤ 0.05.**

Table (3): Statistical Differences between Pre and Posttest for Interventional Program

Paired Samples Test								
Sig. P≤ 0.05	**Df.**	**T**	**Paired Differences**					**Periods of program**
			95%Confidence Interval of the Difference		**Std. Error Mean**	**Std. Deviation**	**Mean**	
			Upper	**Lower**				
.000 H.S.	31	29.806	73.287	63.900	2.301	13.018	68.593	Pre- post Program

T. = T.test, Df. = Degree of freedom, Sig. =Significance.

Table (4): Relationship between Effectiveness of Interventional Program and Nurses Years experiences in nursing, Experience in critical care unit, Time working, and Level of Education

program	Level of education	Time working	Experience in critical care	Years experiences	setting	Variables	
.189	-.139	-.250	.250	1	-.092	Pearson Correlation	Years experiences in nursing
.300	.449	.167	.168		.615	Sig. (2-tailed)	
32	32	32	32	32	32	N	
.044	-.235	-.284	1	.250	-.092	Pearson Correlation	Experience in critical care unit
.810	.195	.115		.168	.618	Sig. (2-tailed)	
32	32	32	32	32	32	N	
-.347	.365	1	-.284	-.250	.214	Pearson Correlation	Time working
.051 (S.)	.040		.115	.167	.241	Sig. (2-tailed)	
32	32	32	32	32	32	N	
-.389	1	.365	-.235	-.139	.078	Pearson Correlation	Level of education
.028 (HS)		.040	.195	.449	.672	Sig. (2-tailed)	
32	32	32	32	32	32	N	

According to table (3), there were high significant statistical differences between the pre and posttest for intervention program at $p \leq 0.05$ value.

Sig. =Significance (≤ 0.05=significant, ≤ 0.001=high significant & ≥ 0.05=no significant)

The findings of table (4) revealed that there were significant statistical correlation between the effectiveness of interventional program and education level and time working of the study sample at $p<0.001$. While there was no significant statistical correlation between the effectiveness of interventional program andtheir setting, years of experience in nursing field and experience in critical care unit.

Discussion

Applying the standard of enteral feeding for critical patients is very important procedure must be used by nurses to avoid complication and other feeding disorder. For these reason the researchers conduct the present study to apply the standard guideline of (ASPEN) version 2017 for nurses, which includedthirty two nurse working in critical care units in order to find out the effectiveness of the intervention program concerning enteral feeding on their practices. There were 87.5% of them was males at age group (20-30) years old, graduated from nursing institute which of 81.3%, 59.4% of them working at night shift, 43.8% of them have (1-5) years of experiences in nursing and 87.5% of them had 1 to 5 years of experience in critical care units especially in ICU and 100% of the nurses had no participant in a training courses related to enteral feeding.

This socio-demographic data of the study participants consistent with the study in Uganda which conducted at International Hospital Kampala/Uganda, the participants in the study was 62.5% at age group (20-30) years old [10]. Another study that was done inYemen to assess the level of ICU nurses' knowledge regarding the management of enteral nutrition conducted on 174 nurses in Yemen's capital city showed that more than half of ICU staff have a nursing diploma degree and work experience of five years or less in nursing[3], as well as study in Saudi Arab which assess the effect of educational nursing guidelineson nurses' knowledge and practices regarding enteral feeding in critical care units among 55 nurses at Benha University Hospital, reported that more than two thirds of nurses had 1 to less than 5 years of experience in ICU [11].

According to findings of present study, the practical knowledge of nurses related to mechanism of enteral feeding was 52.91% incorrect answer at pretest, while the correct answer of total practical knowledge of them was improved after applying the nursing interventional program at posttest to 85.73% . Regarding the nurse's practice about enteral feeding, the nurses practice not applying the correct guideline for enteral feeding administration at pretest was 22.22%, while the correct applying of enteral feeding administration was improved at 66.82%. On the other hand, nurses Practice regarding the correct applying to administration of medication according to guideline at pretest was 23.125%, and their practices was improved in posttest at 67.03%. The present study findings revealed that there were highly significant statistical differences between the pre and posttest intervention program at ($p<0.05$). Bedier, et al., (2016) conducted a study on 60 nurses working in intensive care units to assess their performance in providing nutrition support, they reported that the total mean practice score regarding caring of patients receiving nasogastric tube feeding were improved as good score was 40.18±3.30 pre-program, while it reached 87.09±14.55 after implementation of their educational program[12]. A study which was done in South Korea that was documented the designed education program was having an effective impact on improving nurses' knowledge and practice about enteral feeding in the critical care unit[13], Khalil, (2017) As well also reported that there was significant and positive correlation between subjects' knowledge and skills before providing them education program about enteral feeding and their knowledge and skills after health education program at ($p<0.001$)[14].

The results of present study revealed that there were highly significant relation between the effectiveness of interventional program and demographic characteristics regarding education level and time working of the study sample at $p<0.001$, Bedier, et al., (2016), reported that there were no significant association was found between nurses knowledge and practice regarding nutritional support and their socio-demographic characteristics. Similarly Mula, (2014) conducted a study on 51 nurses to assess competency and determine challenges experienced in enteral feeding practice in the intensive care unit in Malawi, reported that there was no significant association between nurses age and their knowledge level[12],[15].

Conclusions

The study concluded that the interventional nursing program had a positive impact on nurses' practices regarding enteral feeding.

Recommendations:

According to results of presents study the researcher's recommended

• Tray to apply the guideline for Parenteral and Enteral Nutrition in critical units to avoid the complication of enteral feeding .

• Establishing a written update protocol regarding enteral feeding to ensure sufficient and safe nurses knowledge and practice.

• Conducting Continuous training programs about safe administration of enteral feeding and medications as well as skills related to patients nutritional assessment to develop and improve nurses' knowledge and practices regarding the management of patients who are receiving enteral feeding.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Mothers' Knowledge Regarding Infant Physical Needs

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Abstract

Parents' most important need was for accurate and honest information. Needs focused around sensitive infant care and involvement in decision-making. The greatest stressor for parents was alteration to the parental role, followed by infant appearance.A descriptive / Cross-sectional study design is carried to assess the mothers' knowledge regarding infant physical needs in Babylon governorate.

The study conducted in Babylon Governorate in AL-Hashemia district, health centers. The sample size was (100) mother's .The sample of study was multistage sampling, The data were collected through the training of two groups of specialized community health nursing student, each groups consisting of 2 students. They were trained on how to collect the sample and data collection method. The study used a developed questionnaire (Arabic version(The data of the present study are analyzed through the use of Statistical Package of Social Sciences (SPSS) study concluded A third of the female participants in the study breastfeed their children through a bottle of milk and half of them feed their children 5 times a day, more than half of the participants had moderate information, while 40% of them had little information about caring for their children physically. study recommended new mothers depend on the care of their children on the family inheritance, especially the mother or older sister without adopting the scientific aspects and the development in the care of infants. Therefore, the study suggests developing an educational program that is part of the motherhood and childhood program.

Keywords: *Infant physical needs, Mothers'knowledge.*

Introduction

The connection between mother–infant physical contact and maternal responsiveness has not been investigated [(1)]

Infant feeding is a significant predictor of health outcomes for both mother and infant. Among mothers, never breastfeedingnonexclusive breastfeeding, or early cessation of breast feeding,are associated with increased risks of breast cancer, ovarian cancer, diabetes, hypertension, and myocardial infarction [(2)]

Parents' most important need was for accurate and honest information. Needs focused around sensitive infant care and involvement in decision-making. The greatest stressor for parents was alteration to the parental role, followed by infant appearance [(3)]

A conceptual model of breastfeeding decisions, this contextual approach to infant feeding focuses on multi-directional influences and the dynamic nature of balance between self and infant care that women experience over time [(4)]

Sleep develops rapidly during the first few years of life and is a highly dynamic process. At birth, infants lack an established circadian rhythm and hence sleep across multiple intervals throughout the day and night in short bouts, which may also be due to infants' feeding needs [(5)]

Sleep is a highly dynamic developmental process, particularly in the first 2 years of life, with a high inter- and intra-individual variability. While many studies have focused on children, the link between variations in sleep and developmental outcomes in healthy infants remains less explored. The objective of this narrative

review is therefore to summarize and evaluate available literature on the relation between infant sleep during the first 2 years of life and cognitive and physical growth in healthy typically developing infants and young children. (6)

Parents respond to their infants' emotions in ways they believe are most appropriate. These reciprocal interactions make up the infants' social-emotional environment and appear to guide future development and relationship formation,perceptions of infant emotions were associated with beliefs about basic needs for infants and some mothers expressed stress when their infants fussed or cried. Mothers who reported stress showed fewer mother-infant interactions. However, mothers who did not express stress had infants that fussed and cried more than infants of mothers who reported stress. (7)

The first 1000 days of a child's life are a delicate yet highly consequential period affecting future physical, cognitive, and socio-emotional growth. The bond between mother and infant within the first 1000 days is especially critical as it is within the bounds of this relationship that a child is fed, cared for, and kept safe. Furthermore, mother–infant bonding lays an essential foundation for future development (8).

This study aims to assess mothers' knowledge regarding infant physical needs in order to conform the educational program or instruction for new mothers how don't know how to caring their first infant.

Methodology

Study Design

A descriptive / Cross-sectional study design is carried to assess the mothers' knowledge regarding infant physical needs in Babylon governorate.

Setting of the Study

The study conducted in Babylon Governorate in AL-Hashemia district, health centers

Study Sample

The sample size was (100) mother's .The sample of study was multistage sampling:

Tools of the Study

During an extensive review of relevant literature the study instrument is constructed for the purpose of the study by the researcher. It is comprised of five questionnaires parts and the overall items included in these questionnaires are (24) items, including mothers' knowledge regarding infant physical needs

Method of data collecting

The data were collected through the training of two groups of specialized community health nursing student, each groups consisting of 2 students. They were trained on how to collect the sample and data collection method. The study used a developed questionnaire (Arabic version)

Statistical Data Analysis Approach

The data of the present study are analyzed through the use of Statistical Package of Social Sciences (SPSS-23). The following statistical data analysis approaches are used in order to analyze and evaluate the results of the study.

Results

Table (1) shows the demographic data of the mothers

Mother age		Frequency	Percent
	18-25 Years of age	18	18.0
	26-33 years of age	46	46.0
	34- 41 years of age	25	25.0
	42-49 years of age	11	11.0
	Total	100	100.0

Cont... **Table (1) shows the demographic data of the mothers**

Age of Child		Frequency	Percent
	1-4 Months aged	31	31.0
	5-8 Months aged	34	34.0
	9-12 Months aged	35	35.0
	Total	100	100.0
Educational Level of the mothers		Frequency	Percent
	Primary schools	43	43.0
	Secondary schools	21	21.0
	University graduated	30	30.0
	Postgraduate	6	6.0
	Total	100	100.0
Place of residence		Frequency	Percent
	Urban	69	69.0
	Rural	31	31.0
	Total	100	100.0

This table indicated that 46% of mother aged 26-33 years,35.5% their infant aged9-12 months with education level 43% of primary school and 69% of them life in urban area.

Table (2) identify the infant physical needs

How to feed a child		**Frequency**	**Percent**
	Breast feeding	34	34.0
	Bottle feeding	38	38.0
	Mixed method	28	28.0
	Total	100	100.0
Number of feeding times per day		Frequency	Percent
	Less than 5 times / day	48	48.0
	5-8 Times / Day	43	43.0
	More than 9 times / Day	9	9.0
	Total	100	100.0
Number of hours of sleep during the night		Frequency	Percent
	Less than 5 hours / day	45	45.0
	5-7 hours / Day	49	49.0
	More than 9 hours / Day	6	6.0
	Total	100	100.0

Cont... **Table (2) identify the infant physical needs**

Number of hours of sleep during the day		Frequency	Percent
	Less than 4 times / day	40	40.0
	4-6 Times / Day	54	54.0
	More than 6 times / Day	6	6.0
	Total	100	100.0
Baby sleep		Frequency	Percent
	Back sleep	31	31.0
	Abdominal sleep	69	69.0
	Total	100	100.0

The table showed that 38% of mothers feeding their infant by bottle method,48% of infant had less than 5 times / day,49% sleep s-7 hours during the night and 54% sleep 4-6 hours during day, th 69% of infant had sleep on abdomen.

Table (3) identify the infant cleanness care

How many diapers does he need during the day?		Frequency	Percent
	Less than 5 times / day	40	40.0
	5-7 times / Day	50	50.0
	More than 9 times / Day	10	10.0
	Total	100	100.0
How many diapers does he need during the night?		**Frequency**	**Percent**
	Less than 2 times per day	37	37.0
	3-5 Times per day	45	45.0
	more than 5 times per day	18	18.0
	Less than 5 times per day	30	30.0
	6-9 Times per day	50	50.0
	more than 10 times per day	20	20.0

The table showed that 50% of infant diapers changed 5-7 times per day,45% 3-5 times per night

Table (4) shows the mothers' knowledge regarding infant physical needs

Mothers' knowledge	frequency	%
Low knowledge	40	40.0
Moderate knowledge	54	54.0
High knowledge	6	6.0

The table indicated that half of the participants had moderate knowledge and 40.0% had low knowledge

Dissection

Study results indicated that 46% of mother aged 26-33 years,35.5% their infant aged 9-12 months with education level 43% of primary school and 69% of them life in urban area.

The results showed that mothers still young with infant child and low educational level

Mjwara, N., & Maharaj, P. [10] study showed that Young mothers wanted to assume financial responsibility for their children but they were finding it difficult to do so. Many faced numerous obstacles as they were no longer in a relationship with the father of their child and depended on their own parents for support. Participants described how the difficulties they faced in their daily lives and their new roles as mothers impacted negatively on their education and career aspirations.

In another hand 38% of mothers feeding their infant by bottle method,48% of infant had less than 5 times / day,49% sleep s-7 hours during the night and 54% sleep 4-6 hours during day, th 69% of infant had sleep on abdomen this result agree with such study

Infant feeding is essential for survival. The primary infant feeding modalities for infants under 6 months of age are breastfeeding and bottle-feeding. The ideal feeding modality for mothers and infants is breastfeeding. Global initiatives aim to enhance uptake and duration of breastfeeding (World Health Organization (WHO) & United Nations Children's Fund (UNICEF), 2003).

Bottle-feeding advice concentrates on more procedural recommendations: cleaning and sterilizing of feeding equipment, correct preparation of formula, and the storage and transport of formula (United Nations Children's Fund (UNICEF) UK, 2015).

Sleep problems, typically identified as difficulty settling to sleep and frequent night waking, are common in young children, with prevalence estimates of 25% children across the world

Past research indicates that sleeping arrangements are one of the strong predictive factors for young children's sleep problems. For example, co-sleeping was shown to be associated with persistent child night waking and bedtime struggles [7,9, 10,11]

Conclusion

A third of the female participants in the study breastfeed their children through a bottle of milk and half of them feed their children 5 times a day. Half of the participants let their children sleep more than seven hours a day and two-thirds of the participants let their babies sleep on their abdomen

The study concluded that half of the mothers participating in the research work on cleaning their babies well, about 5-7 times a day.

When analyzing the information regarding mothers' knowledge about the physical care of the infant, it was found that more than half of the participants had moderate information, while 40% of them had little information about caring for their children physically..

Recommendations:

New mothers depend on the care of their children on the family inheritance, especially the mother or older sister without adopting the scientific aspects and the development in the care of infants. Therefore, the study suggests developing an educational program that is part of the motherhood and childhood program and is the basis for continued care and follow-up by doctors and nurses in health centers or private clinics within the care program, from the beginning of registration in the center, the completion of pregnancy and childbirth.

Finding methods and supporting mothers 'participation in an educational program as part of community support or non-governmental community-based organizations

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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A Study of Self- Medication Uses without a Prescription among Iraqi Patients in Baghdad City

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Abstract

Background: Medicines are those chemical compounds which consist of a single substance or more and are obtained through natural (plant or animal), as well as industrial methods, by chemical reactions that utilized in the treatment of diseases that can infect human and animals or alleviate illness, prevention or diagnosing. Self-medication is the unwanted medical use of prescription and / or over-the-counter drugs. These practices have become an encouraging global form of self-care when it comes to minor ailments. Well it is not limited to over-the-counter medicines. This also includes the use of prescription medications such as antibiotics.

Objective: This study explores the extent of a widespread phenomenon of medicines usage without a doctor prescription, and studied the self-medication practices reasons among patients in the city of Baghdad.

Methods: A cross-sectional descriptive study was conducted by questionnaires were distributed randomly to 300 adults in different age group, sex and level of education at PHC in Baghdad city.

Result: In this study, findings depict that the (34.3%) of study male participants are aged (30-39) years old. In regarding with education, thirty-four percent are primary school graduated and work a housewife resident in rural areas. Also, the (53 %), (12.3%) respectively take drugs in the last months for flu. The (64.7%) of drugs was prescribed by physician and (35.3%) of drug take without prescription because (16.3%) of them not have access to physician care. The source of drugs supplies, the (31.7%) take it from others source and (38.3%) of information on the use of these drugs was obtained from physicians. The most common types of drugs used without prescription the result indicated that (39.0) was antibiotics and (53.3%) of sample not commitment by dose, Reading instructions & advertence with complication. Also the (73%) of sample don't uses or depending upon herbs.

Conclusion & Recommendations: The continued use of medications without a prescription is an alarming problem in the country of Iraq. The prevalence of self-medication management is high in Middle Eastern countries and is often associated with inappropriate drug use. Therefore, it is necessary to understand the relationship between the various factors that favor the management of self-medication and to evaluate the evolving trends to help us develop strategies to reduce the health risks associated with drugs among the people of the Middle East.

The recommendations included the imposition of regulatory measures that restrict access to prescription medications only in countries where antibiotics are available over the counter. Another recommendation, which has emerged in several studies, is to improve the use of media campaigns to effectively guide public education programs.

Keywords: *Self-medication, Prescription, Patient.*

Introduction

Medicines are those chemical compounds which consist of a single substance or more and is obtained through natural (plant or animal), as well as industrial methods, by chemical reactions that utilized in the treatment of diseases that can infect human and animals

or alleviate illness, prevention or diagnosing.

If every medication and each drug dose of its own quantity and time of use and any defect in it, will inevitably lead to complications, future very serious immune system natural and other services for people used the drug, the drug double-edged sword in I the one he is treating wholesome and effective as what the best use of hand-marking assassin on the other hand as misused [1].

In developing countries, self-medication is a global problem and a common practice [2]. Self-medication can be defined as the use of drugs to treat self-diagnosing symptoms or disorders, or the use of a drug prescribed for chronic or recurring intermittent or persistent diseases or for other symptoms without a prescription. In many studies, inappropriate self-medication has been shown to increase resistance to pathogens, waste resources, and generally lead to serious health problems such as harmful drug interactions, prolonged suffering, and drug addiction. [3].

In Iraq it can be considered a common complex problem due to the multiplicity of its causes as well as its repercussions on the patient's health and therefore on the country's economy. One of the previous studies carried out in Saudi Arabia showed that about thirty-five percent of patient's attendance primary health care centers (PHCC) have some experience of self-medication [4]. The most common over-the-counter medications are antibiotics and pain relievers / fever reducers [5]. The most common reasons for buying OTC drugs are that symptoms were too few to seek advice and save time, pursuant to modern study [6][7].

The ignorance in the use of drugs is extremely dangerous health, economic and social. These effects do not stop at this point and even beyond to the next generation, which requires the state to take measures to actors in the Medication Awareness and deliver medication sound ways for the citizen and the lowest possible cost does not make evades citizen of the proper use of drugs and resort to ways to hurt citizens in particular and society in general [1].

In Iraq, research conducted by the University of Baghdad in the capital Baghdad noted 195 samples that 46.5% of people use drugs without a prescription and 37.4% use medicinal herbs [8].The World Health Organization reports indicate that 50% of patients do not take medication the right way that has been described by the doctor, there are also a lot of patients who depend on their handling of the drug on the advice of their relatives or friends to save time and to reduce to spend money and this thing shows a lack of pharmaceutical and health education and non-compliance with the rules of medical and health systems [9].

Methods and Materials

The study design: Descriptive cross-sectional study was conducted at the Baghdad primary health care center. About 300 adults with different levels of education and socio-economic status are enrolled. A questionnaire involved open and closed questions is revalidated.

The questionnaire was randomly distributed to all patients who attended the primary care center in March 2018. The questionnaire has five parts. First part consisted questions related to demographic data such as (age, gender, education level, marital status, employment status, and health status). Second part covered the source of self-treatment and information from pharmacists, family / friends, remaining prescription drugs, etc. The third part covered the most common symptoms of self-medication and the most common therapeutic classes of drugs, and the end part of the questionnaire was used to assess the reason for people self-medicate.

Statistical Analysis: Through the used descriptive statistical (SPSS) version 20 analysis approach that includes, frequencies and percentages,

Study instrument: Questionnaire was used to find differences between the two groups who visited primary health care clinics in one month and based on some characteristics that included (age, gender, marital status, level of educational and occupational status). Aspects related to health involved the health status of the respondents themselves, if they had a chronic disease or perceptions of access to medical care and satisfaction with the quality of the medical care they receives.

Results

Table (1): Sample Characteristic

No (%)	Variables	Rating
103 (34.3)	30-39	Age (years)
100 (33.3)	40-49	
85 (28.3)	50-59	
12 (4.0)	60+	
171 (57.0)	Male	Gender
129 (43.0)	Female	
60 (20.0)	Unable to read &write	Level of education
102 (34.0)	Primary school graduate	
39 (13.0)	Intermediate school graduate	
62 (20.7)	Preparatory school graduate	
12 (4.0)	Institute	
25 (8.3)	College	
36 (12.0)	Employee	Occupation
85 (28.3)	Free work	
105 (35.0)	Retired	
43 (14.3)	Jobless	
31 (10.3)	Housewife	
160 (53.3)	Rural	Location
140 (46.7)	Urban	

In this table, findings depict that the (34.3%) of study male participants are aged (30-39) years old. In regarding with education, thirty-four percent are primary school graduated and work a housewife resident in rural areas.

Current Findings reveal that (53 %), (12.3%) respectively take drugs in the last months for flu. The (64.7%) of drugs was prescribed by physician and (35.3%) of drug take without prescription because (16.3%) of them not have access to physician care. Regarding the source of drugs supply the (31.7%) take it from others source and (38.3%) of information on the use of these drugs was obtained from physicians. Regarding the duration of drugs intake was (43.0%) take 1-3 times per day. The (53%) of the sample take medicines with prescription. Concerning with the most common types

of drugs used without prescription the result indicated that (39.0) was antibiotics and (53.3%) of sample not commitment by dose, Reading instructions & advertence with complication. Also the (73%) of sample don't uses or depending upon herbs. Regarding to themost common symptoms and reason to use self-medication the results show (40.3%), (40.3%) respectively of them used for fever with reason lack of time to visit health care facilities. The (74.0 %) of sample reported that the physician prescriptions are costly. The majority of the study sample (61.3%), don't look at the expiration date before using it, and (59.0%) are committed to the duration of usage of prescribed treatment.

Discussion

Self-medication uses are very common among the Baghdad people despite that the ministry of health in Iraq had strict legislation for these improper uses.

Table (1): This study showed that one of the characteristicsof the sample was the age, which recorded (34.3%) for the (30-39) group.

Male was recorded 171 people who they were often attending the PHC where the sample conducted from. In regarding with educational level, thirty-four percent are primary school graduated, they were retired and residents in rural areas.

These results do not come along with a study at Tabuk City in Saudi Arabia [2], owing to their results was (45.3%) of single Saudi man studying in good health in high school and college and (73.9%) of single Saudi women studying in high school and college in good health.

Whereas in another study which the population was 204 cases taken from Al-Najaf province in Iraq [1]. The highest number of cases in males and females occurred in age groups (20-24) year with percentage 34.3%, 22.5%.

Regarding the profession, the housewives recorded the highest number with the percentage 36.3% followed by students 26.9%, the officers were 21.1% and free jobs were 15.7%. The highest percentage 23.5% was recorded for the academic's levels of education. Also, their results pointed to the most cases was from the urban area with percentage of 87.3% followed by rural area by percentage 12.7% depending on the geographical locations [1].

These differences in ratios and results in these studies and other studies found in the various researches and articles in different cities are due to different size of the research sample and the different characteristics of it. Therefore, all researchers should always take larger samples to be the most comprehensive and comprehensive results.

Table (2): Findings reveal that (53 %), (12.3%) respectively take drugs in the last months for flu. The (64.7%) of drugs was prescribed by physician and (35.3%) of drug take without prescription because (16.3%) of them not have access to physician care. Regarding the source of drugs supply the (31.7%) take it from others source and (38.3%) of information on the use of these drugs was obtained from physicians. Regarding the duration of drugs intake was (43.0%) take 1-3 times per day. The (53%) of the sample take medicines with prescription. In another study conducted by (Skliros and others) [10], the result shows that 888 participants (77.9%) reported on antibiotic use in the past 12 months. 508 people (44.6%) reported receiving non-prescription antibiotics at least once. The main source of self-medication was the over-the-counter pharmacy (76.2%). Concerning with the most common types of drugs used without prescription the result indicated that (39.0) was antibiotics and (53.3%) of sample not commitment by dose, Reading instructions & advertence with complication. Also the (73%) of sample don't uses or depending upon herbs. Results in parallel with the study conducted by (Abasaeed and others) [11], The result shows that of the respondents, 393 (46%) reported having intentionally used antibiotics as self-medication without medical consultation, a behavior that is significantly influenced by the level of education ($p < 0.001$). Two hundred forty-five (28%) participants kept antibiotics at home. These antibiotics were mainly purchased from pharmacies without a prescription ($p < 0.001$). Even in studies with pharmacists in Saudi Arabia 34 and Qatar 35, the pharmacy owner's influence in maximizing revenue, customer pressure, and weak regulatory mechanisms explain the sale of antibiotics without a prescription. Therefore, strong community awareness and enforcement campaigns are needed to limit the sale of over-the-counter antibiotics. As for the most frequent symptoms and the reasons for self-medication, the results

show respectively (40.3%), (40.3%) those used for fever, due to lack of time to recover. (74.0%) of the sample indicated that medical prescriptions are expensive. In another study (Skliros et al.) [10]. The results indicate that a high percentage of the rural adult population uses non-prescription antibiotics, preferably for fevers and colds. Furthermore, in line with another study (Alhomoud et al.) [12], the results show that sociocultural, economic and regulatory factors were the most frequently cited reasons for self medication administration. Penicillins were the most widely used antibiotics; Antibiotics were mainly obtained through stored drug leftovers, over-the-counter pharmacies, and friends / family. The SMA was mainly for upper respiratory problems. The main sources of drug information were family / friends and previous successful experiences. Misuse of medications has been reported, including misdirections, short and long term treatment, shared use of antibiotics, and storage of antibiotics at home for later use. Both negative and positive self medication administration results were identified.

Conclusions

The continued use of medications without a prescription is an alarming problem in Iraq. The prevalence of self-medication management is high in Middle Eastern countries and is often associated with inappropriate medication use. Therefore, it is necessary to understand the relationship between the various factors that favor the management of self-medication and to evaluate the evolving trends to help us develop strategies to reduce the health risks associated with drugs among the people of the Middle East.

Educational interventions targeting public and public health professionals and regulations mandating the use of over-the-counter drugs can help reduce the challenge of using over-the-counter antibiotics

Recommendations:

The recommendations included the imposition of regulatory measures that restrict access to prescription medications only in countries where antibiotics are available over the counter. Control of the prescription by the doctor through suitable interventions, for example, advising to prescribe the exact number of capsules or tablets for a given duration of treatment.Raise awareness among healthcare professionals, particularly pharmacists, nurses to empower their role in advising patients on the proper use of prescription and to prohibit the dispensing of any medications without a prescription. Develop a continuous national system for antibiotic consumption and resistance monitoring. Update of antibiotic policy and antibiotic treatment guide. Another recommendation, which this study emphasized on is to improve the use of media campaigns to effectively guide public education programs.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Influence of Fluoxetine Therapy Combination of Rational Emotive Behaviour Therapy Againts the Improvement of Depression Symptoms, Cognition Function and Improvement of Brain-Derived Neurotrophic Factors Serum Levels in Patients with Depressive

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Abstract

Introduction: Depression is a health problem in the world that has affected more than 300 million people around the globe. This health problem is characterized by feeling sad, depressed, irritability as well as distortion of cognition such as self-criticism, feeling of guilt, feeling of worthlessness, lower self-confidence, pessimism and hopelessness where their manifestations may vary for each individual. **Method:** In this study, it compares the fluoxetine group combination of Rational Emotive Behavior Therapy (REBT) with the fluoxetine group in the treatment of reducing symptoms of depression and improving cognitive function. This research is an experimental research with pre-test and post-test control group design. The sampling technique was done by using consecutive sampling for each group. **Results:** From the results of the study it was found that the mean of Hamilton Depression Rating Scale (HDRS), the Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina) and Brain-Derived Neurotrophic Factor (BDNF) serum levels in the samples given by both groups experienced statistically improvements. **Conclusion:** there is an effect on the provision of fluoxetine therapy combination of REBT compared to fluoxetine therapy alone in improving depressive symptoms, cognitive function as well as increasing BDNF serum levels.

Key words: *Depression, Cognitive function, Fluoxetine, REBT, BDNF*

Introduction

Depression is a health problem in the world that has affected more than 300 million people around the world which is characterized by feelings of sadness[1] depressed, irritability and distortion of cognition such as self-criticism, guilt, feelings of worthlessness, lower self-confidence, pessimism and hopelessness manifestations may vary for each individual ([2],[13]).

In Canada, approximately 79% of depressed patients experience functional impairments in work function, both decreasing work productivity and absenteeism. Occupational disorders often have an impact on family and psychosocial relationships in depressed patients. Cognitive dysfunction can be a major mediator of functional disorders in depression. In the study of observation for 3 years against depressed patients, complain of cognition has been reported as much as 94% during the acute depression episode, and the remaining as much as 44%, with full or partial remission of symptoms during treatment. Meta-analysis showed that cognitive deficits in executive function are still found in patients after treatment for depression, this may explain persistent psychosocial disorders in remission ([4],[6],[9]).

Cassano *et al*. conducted a double blind study comparing paroxetine treatment (20-40 mg daily) and fluoxetine (20-60 mg daily) for 1 year, and as many as 242 patients were involved. Cassano *et al*. concluded that both of these antidepressants proved to be suitable for long-term treatment of depression, and that both could improve cognition [11].

The combination of psychotherapy and pharmacotherapy is increasingly common in psychiatry as the evidence accumulates that many conditions respond better to combined treatment than to either modality alone (Gabbard and Kay 2001). Because both treatments affect the brain, in a very real sense, they are both biological treatments. However, the mechanisms of action of the two treatments may occur in very different areas of the brain. Goldapple et al. (2004), using PET, scanned 17 unmedicated patients with unipolar depression before and after a 15- to 20-session course of cognitive-behavioral therapy. They compared the findings to a separate group of 13 depressed patients who responded to paroxetine. The psychotherapy appeared to alter brain regions that medications did not touch. The psychotherapy was associated with increases in metabolic activity in the anterior cingulate and the hippocampus. By contrast, paroxetine showed increases in metabolic activity in the prefrontal cortex and decreases in the brain stem and subgenual cingulate [20].

Analysis of research conducted in the TADS (Treatment for Adolescents with Depression Study Team) study has shown that a new CBT strategy that can potentially heal (i.e., Rational Emotive Behavior Therapy (REBT)), clinical improvement is mediated by changes in perfectionism and distorted thinking. Felicia *et al*'s results show that the REBT/CBT group, pharmacotherapy (i.e., sertraline), and a combination of both are equally effective in the management of young depression ([7],[18],[20]).

Based on previous studies where it compared levels of Brain-Derived Neurotrophic Factor (BDNF) fluoxetine and sertraline groups in week 6 showed significant differences. From week 6 BDNF comparison of the two groups, it showed that the fluoxetine group was superior in increasing BDNF compared to the Sertraline group. Ramanathan *et al*. claimed that fluoxetine can improve the cognitive condition of patients suffering from depression. The improvement will occur mainly in learning capacity and memory. However, this particular study was developed in the experimental animal model of Wistar rat[3],[9],[19].

Based on the facts above, the researchers were interested to find out whether REBT can increase the response of fluoxetine therapy can accelerate the improvement of clinical symptoms of depression, restore cognitive function as before the patient suffers from depression as well as increasing levels of BDNF serum as the main indicator of improvement in psychosocial function. In this case the researchers will use a research instrument namely Hamilton Depression Rating Scale (HDRS) and the Indonesian version of the Montreal Cognitive Assessment (MoCa-Ina). The HDRS is a questionnaire consisting of 21 items used to find out indications of depression and as a guide in evaluating the recovery process. Cognition function can be measured using the MoCa-Ina instrument, which includes visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation [17].

Method

This research is an experimental study with the design of pre-test and post-test control group design. This study was planned to be performed on November 2019 - finish.

This research was planned to be carried out in Psychiatry Polyclinic of UNHAS Makassar Hospital, and its hospital channels. The populations in this study were all depressed patients based on the fifth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and the WHO's tenth edition of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) who underwent outpatient treatment at the Psychiatry Polyclinic of UNHAS Hospital and its channel hospital.

The sample in this study were depressed patients based on DSM-5 and ICD-10 who underwent outpatient care at the Psychiatry Polyclinic of UNHAS Hospital and its channel hospital, which met the inclusion criteria: all male and female patients diagnosed with depression based on DSM-5 and ICD-10, ages 18-35 years old, minimum education was elementary school, depressed patients who sought treatment for the first time, recurrent depressed patients who dropped out of antidepressant drugs for a minimum of 3 months and patients willing to take part in the study. Sampling techniques for each group were carried out by means of consecutive sampling, i.e. all patients who meet the research criteria until the sample required is fulfilled. The type of data in this study was primary data obtained directly from research subjects. The research instrument used in this

study consisted of HDRS and MoCA-Ina questionnaire sheets. Measurement of BDNF serum levels in the blood of study samples.

Ethical statement

This study received ethics approval from the Universitas Hasanuddin hospital ethical committee.

Data Processing Techniques

Processing was carried out after recording the medical record data needed into the questionnaire by using a computer program SPSS 23.0 and Microsoft Excel to obtain the expected statistical results.

Paired T test and unpaired T test are used when the data are normally distributed, as well as Wilcoxon Test and Mann Whitney Test are used when the data are not normally distributed.

Results

Table 1. Sociodemographic Characteristics based on Frequency (N=37)

Variable	Variable Group	n	%
Sex	Male	18	45.9
	Female	19	51.3
Job	Civil Servant	2	5.4
	Employee	16	43.2
	Entrepreneur	5	13.5
	Housewife	12	32.4
	Unemployed	2	5.4
Status	Married	25	67.6
	Not Married	11	29.7
	Divorce	1	2.7
Education	Middle School	7	18.9
	High School	15	40.5
	Higher Education	15	40.5
Tribe	Bugis-Makassar	21	56.8
	Toraja	2	5.4
	Jawa	4	10.8
	Luwu	3	8.1
	Tionghoa	4	10.8
	Enrekang	1	2.7
	Mandar	2	5.4

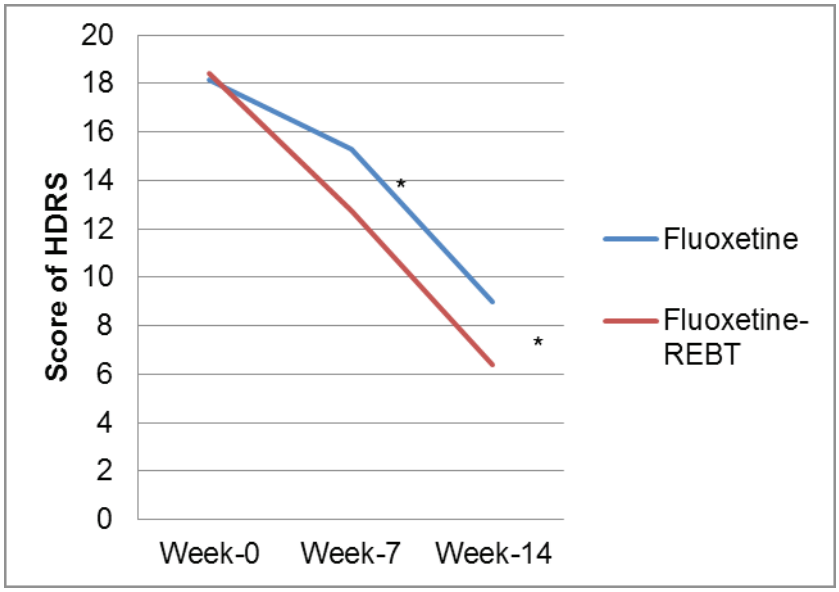

Figure 1. Average Comparison of HDRS Scores for Fluoxetine and Fluoxetine-REBT

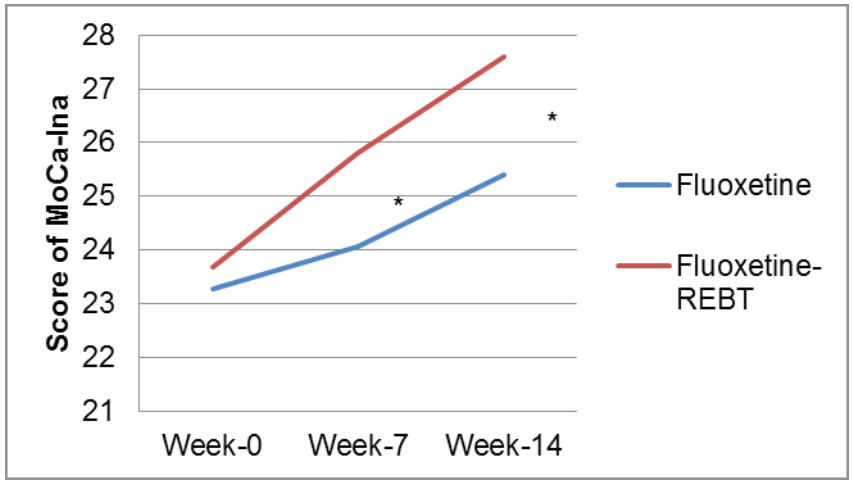

Figure 2. Average comparison of MoCa-Ina scores of the fluoxetine group and fluoxetine-REBT

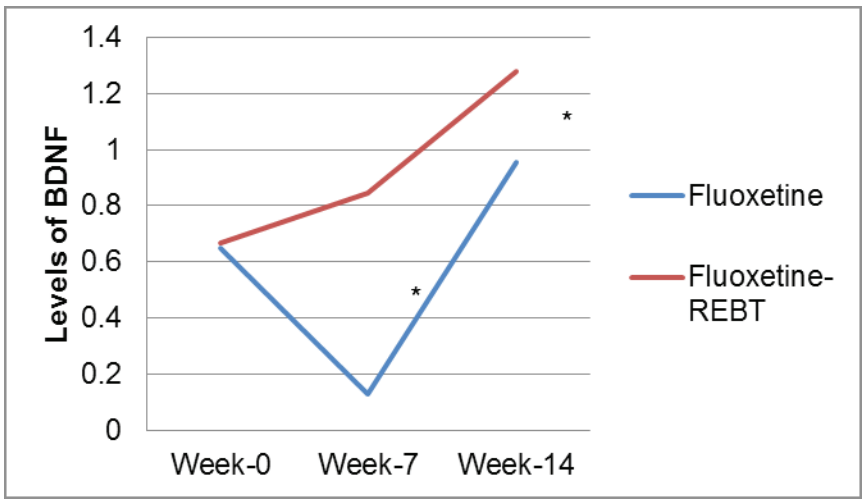

Figure 3. Average comparison of BDNF levels in the fluoxetine group and fluoxetine-REBT

Table 2. Comparison of BDNFS levels (ng/ ml)

Observation	Group		P
	Mean (+SD) Fluoxetine (N=15)	Mean (+SD) Fluoxetine – REBT (N=15)	
Week-0	0.651+0.009	0.668+0.006	0.563
Week-7	0.130+0.221	0.845+1.101	0.000
Week-14	0.954+0.171	1.276+0.326	0.000

Mean + SD

** Significant p<0.05*; Analysis of multivariate (Mann-Whitney U with t-test)

Table 3. Difference in HDRS score

Observation	Group		P
	Mean (+SD) Fluoxetine (N=15)	Mean (+SD) Fluoxetine – REBT (N=15)	
Difference week 0 – 7	2.86+0.639	5.67+0.617	0.000
Difference week 7 – 14	6.27+1.668	6.33+2.663	0.935
Difference week 0 – 14	9.13+1.506	12.00+2.449	0.001

Mean + SD

** Significant p<0.05*; Analysis of multivariate (Mann-Whitney U with t-test)

Table 4. Difference in MoCa-Ina scores

Observation	Group		P
	Mean (±SD) Fluoxetine (N=15)	Mean (±SD) Fluoxetine – REBT (N=15)	
Difference week 0 – 7	0.80±0.414	2.13±1.060	0.000
Difference week 7 – 14	1.33±0.723	1.80±0.861	0.204
Difference week 0 – 14	2.13±0.743	3.93±0.883	0.000

Mean + SD

* *Significant p<0.05*; Analysis of multivariate (Mann-Whitney U with t-test)

Table 5. Difference in BDNF Serum Levels (ng/ ml)

Observation	Group		P
	Mean (+SD) Fluoxetine (N=15)	Mean (+SD) Fluoxetine – REBT (N=15)	
Difference week 0 – 7	0.065+0.216	0.778+0.099	0.000
Difference week 7 – 14	0.824+0.223	0.431+0.343	0.001
Difference week 0 – 14	0.889+0.163	1.209+0.324	0.000

Mean + SD

* *Significant p<0.05*; Analysis of multivariate (Mann-Whitney U with t-test)

Discussion

2. 3. *Participants*

This study is an experimental study using a pre-test and post-test control group design, which compares the fluoxetine group with fluoxetine-REBT. This research was conducted at the Psychiatry Polyclinic Hospital of South Sulawesi Province, Ibnu Sina Hospital, Makassar Public Hospital with a sample of 43 people. After taking the identity and data of research subjects, HDRS checks were performed. After fulfilling the criteria for the value of HDRS that has been determined the sample was divided into the fluoxetine group and the fluoxetine-REBT group, then MoCa-Ina examination and blood sampling were performed to check BDNF levels. During the follow-up sample, 7 people dropped out.

From the analysis conducted on the demographic characteristic variables in the study subjects (N = 37) the mean age was 31.07 ± 3.25 years, with the majority of female (51.3%), employee as occupation (43.2%), marital status (67.6%), high school and college education (40.5%). A study reported that depression is more common at a young age. The average age was between 20-40 years. This study shows a greater susceptibility of depression to women than men according to the literature [18]. Age and education are also important contributors to heterogeneity. At the level of individual education, patients with low levels of education and older patients exhibit severe cognitive deficits in depressive disorders ([1],[3],[17]).

Statistical Analysis

From the research it was found that the mean HDRS score in the samples given the therapy were both statistically significant on depressive symptoms. Samples who received fluoxetine-REBT therapy seen from statistical data at week 7 (mean 12.73 ± 1.580) a significant decrease in HDRS scores p <0.05 (0.000) and week-14 (mean 12.73 ± 1.580) significant decrease in HDRS score p <0.05 (0.001). The control group seen from the statistical data at week-7 (mean 15.27 ± 1.792) a significant decrease in HDRS scores p <0.05 (0.000) and week-14 (mean 9.00 ± 3.286) had a significant decrease in HDRS scores p <0.05 (0.001). The mean MoCa-Ina score for the sample given by the therapy was both statistically significant for cognitive function. Samples who received fluoxetine-REBT therapy were seen from the statistical data at week-7 (mean 25.80 ± 1.146) a significant increase in MoCa-Ina score p <0.05 (0.001) and week-14 (mean 27.60 ±1.595) a significant increase in MoCa-Ina score p <0.05 (0.001). The control group seen from the statistical data at week-7 (mean 24.07 ± 1.100) a significant decrease in HDRS scores p <0.05 (0.001) and week-14 (mean 25.40 ± 1.595) a decrease of MoCa-Ina scores significantly p <0.05 (0.001). The mean BDNF levels in the sample given the therapy were both statistically significant in depressive disorders and cognitive function. Samples who received fluoxetine-

REBT therapy seen from statistical data at week-7 (mean 0.845 ± 1.101) a significant increases in BDNF levels p <0.05 (0.000) and week-14 (mean 1.277 ± 0.326) an increase of BDNF levels significantly p <0.05 (0.000). The control group seen from the statistical data at week-7 (mean 0.130 ± 0.221) a significant increase in DNF levels p <0.05 (0.000) and week-14 (mean 0.954 ± 0.171) a significant decrease in BDNF levels p = 0.05 (0.000*)*.

From the research it was found that the therapeutic effect based on HDRS scores on the samples given the therapy were both statistically significant on depressive symptoms. Samples who received fluoxetine-REBT therapy seen from statistical data at week 0-7 (mean 5.67 ± 0.617) a significant decrease in HDRS scores p <0.05 (0.000). Weeks 7-14 (mean 6.33 ± 2.663) had a decrease in score but not significant p <0.05 (0.935). Weeks 0-14 (mean 12.00 ± 2.449) a significant decrease in HDRS scores p <0.05 (0.001*)*. The control group seen from the statistical data at week 0-7 (mean 2.86 ± 0.639) a significant decrease in HDRS scores p <0.05 (0.000). Weeks 7-14 (mean 6.27 ± 1.668) a decrease HDRS scores but not significant p <0.05 (0.935). Weeks 0-14 (mean 9.13 ± 1.506) a significant decrease in HDRS scores p <0.05 (0.001). The therapeutic effect based on the MoCa-Ina score in the sample given by the therapy was both significant in cognitive function. Samples receiving fluoxetine-REBT therapy seen from statistical data at week 0-7 (mean 2.13 ± 1.060) a significant increase in MoCa-Ina score p <0.05 (0.000). Weeks 7-14 (mean 1.80 ± 0.861) a decrease in score but not significant p <0.05 (0.204). Weeks 0-14 (mean 3.93 ± 0.883) a significant increase in MoCa-Ina score p <0.05 (0.000). The control group seen from the statistical data at week 0-7 (mean 0.80 ± 0.414) experienced a significant increase in MoCa-Ina score p <0.05 (0,000). Weeks 7-14 (mean 1.33 ± 0.723) an increase in MoCa-Ina score but it was not significant p <0.05 (0.204). Weeks 0-14 (mean 2.13 ± 0.743) experienced a significant increase in MoCa-Ina score p <0.05 (0.000). The therapeutic effect was based on BDNF levels in the samples given both therapies were statistically significant in depressive symptoms and cognitive function. Samples who received fluoxetine-REBT therapy seen from statistical data at week 0-7 (mean 0.778 ± 0.099) a significant increase in BDNF levels p <0.05 (0.000). Weeks 7-14 (mean 0.431 ± 0.343) a significant increase in BDNF levels p <0.05 (0.001*)*.Weeks 0-14 (mean 1.209 ± 0.324) a significant increase in BDNF levels p <0.05 (0.000). The control group seen from statistical data at week 0-7 (mean 0.065 ± 0.216) a significant increase in BDNF levels p <0.05 (0.000). Weeks 7-14 (mean 0.824 ± 0.223) a significant increase in BDNF levels <0.05 (0.001). Weeks 0-14 (mean 0.889 ± 0.163) a significant increase in BDNF levels p <0.05 (0.000).

Discussion

In general, the results of the current research showed that fluoxetine group and combination of REBT both respectively was effective in the treatment of depression. However, there seems to be difference significantly, where the group of fluoxetine-REBT is better in treating the symptoms of depression, cognitive functions increase the levels of BDNF serum.

HDRS scale consists of 21 questionnaire items, each item range from 0 to 4 or 0 to 2, the researcher used the 14-20 point limit and we did not classify specifically according to mild, moderate and severe symptoms of depression. The seven core depressive symptom items consisted of: mood, guilt, suicidal thoughts, work and activities, psychomotor retardation, somatic symptoms, sexual symptoms [[17],[18]].

The clinical response rate after 14 weeks of therapy showed there was an improvement in HDRS scores, the fluoxetine-REBT group responded better than fluoxetine therapy. The level of decline in HDRS scores after 7 weeks of therapy was around 50% of the initial HDRS score, According to [17] that from 6 weeks of antidepressant treatment clinical trials there is a reduction in scores of 50% or more on the standard depression scale. However, depressed patients who responded with a 50% decrease in HDRS scores may still show significant symptoms, especially in mood items, feelings of guilt and somatic symptoms. Some depressive symptoms are mediators of cognitive dysfunction, including psychomotor retardation, lack of motivation, fatigue, insomnia, and mood. With the combination of REBT therapy it is expected to accelerate the improvement of symptoms remaining from antidepressant treatment because REBT can help patients to cope with emotional, behavioral and irrational thoughts through a good coping mechanism in dealing with every problem that the patients face. The formation of a good coping mechanism can help patients

manage stress, so as to reduce cortisol levels in the blood. Cortisol levels in the blood that return to normal conditions can reshape neuroplasticity in the anterior region of the anterior cingulate and hippocampus ([14],[18]).

Regarding the function of cognition, we use the MoCa-Ina instrument, which includes visuospatial/ executive, naming, memory, attention, language, abstraction, delayed recall and orientation. MoCa-Ina was chosen because its sensitivity is better for assessing cognitive function than other instruments. According to the literature, we used cut-off points 26 to illustrate between clinical and non-clinical symptom scores ([3],[18]).

The results of this study showed that cognitive impairment appeared early in the disease. The clinical response rate after 14 weeks of therapy showed there was an improvement in MoCa-Ina score, the incorporation of fluoxetine with REBT response rates was better than single therapy. The results confirm that this combination is more effective than the drug alone in reducing symptoms of cognitive impairment. We also found that cognitive impairment, especially executive functioning, memory, attention and delayed recall were most often affected by depressive disorders which became the major factor in the workforce performance decline ([4],[18],[19]).

After 7 weeks of fluoxetine-REBT combination therapy, BDNF levels significantly increased compared to the control group. This increase in BDNF is consistent with the literature, where SSRI antidepressants regulate cAMP Response Element-Binding Protein (CREB), transcription factors that depend on Cyclic adenosine monophosphate (cAMP), and BDNF in the course of time associated with therapeutic measures (10 to 20 days). CREB transcription factors are involved in the induction of BDNF gene expression in neurons. This effect on the cAMP pathway provides a link between monoamine antidepressants and neurotropin action [18]. Some research on depression also shows an increase in BDNF levels after undergoing psychotherapy for 4 weeks [11]. BDNF is mostly found in the hippocampus and cortex, the hippocampus is a center of learning and memory. The hippocampus is also connected to the cortex region, and is very important for the integration of cognitive and emotional processes. According to previous studies in the Psychiatry Section (research in the process of publication) showed the Fluoxetine group was superior in increasing BDNF serum levels compared to the Sertralin group. The study also assessed BDNF serum levels in normal people to determine the standard of whether depressed patients have BDNF serum levels below the BDNF serum levels in normal people ([11],[18]).

Based on pharmacotherapy, the administration of fluoxetine in the first week to the fourth week did not have a significant therapeutic effect, thus it did not experience a change on the BDNF serum levels because fluoxetine only works in the prefrontal cortex. Improvement of depressive symptoms, cognitive function and increased BDNF serum levels can be achieved earlier by adding REBT therapy, where REBT works in areas of the brain that are different from fluoxetine, namely in the anterior cingulate cortex and hippocampus. This explains why fluoxetine administration in the first week to the 7th week actually experienced a decrease on the BDNF serum levels. Although in the end after 14 weeks, both the fluoxetine-REBT group and the fluoxetine group will reach normal BDNF serum levels. As a clinician, it is very important to provide appropriate therapy through pharmacotherapy combined with REBT in order to accelerate the improvement of depressive symptoms through improvement in BDNF serum levels that do not respond to fluoxetine therapy in the first week. Thus the clinician helps patients not to fall into a severe major depressive disorder and prevents suicide ideas, which according to research that suicide attempts can occur early in the beginning of therapy for severe major depressive disorders ([15],[18]).

This research is not without limitations. First, the sample size is not large enough to determine the correlation between improvement in depressive symptoms and improvement in function with an increase in BDNF serum levels. Second, the researcher did not set standards and minimum intelligence scores on the participants [15].

Conclusion

The conclusions in this study found there is an effect on the provision of fluoxetine therapy combination of REBT compared to fluoxetine therapy alone in improving depressive symptoms, cognitive function as well as increasing BDNF serum levels.

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The Effect of Spiritual Qur'anic Emotional Freedom Technique (SQEFT) Therapy on Reducing Anxiety Value and Blood Cortisol Levels in NAPZA (Narcotics, Psychotropic, Other Addictive Substances) Residents

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Abstract

Introduction: The patients in general and, in particular, the patients of drug/NAPZA (Narcotics, Psychotropic, and Other Addictive Substances) residents seek spiritual support during illness and undergo rehabilitation. Both religion and spirituality are related to positive coping mechanisms (psychological) and exercise (physical). NAPZA (drug) residents undergoing rehabilitation experience anxiety, including moderate anxiety and severe anxiety, which can adversely affect physical and mental disorders. This research aims to determine the effect of Spiritual Qur'anic Emotional Freedom Technique (SQEFT) therapy on reducing anxiety values and blood cortisol levels in NAPZA residents. **Method:** The experiment was used with a pre-post control group design, and purposive sampling was as the sampling technique. This research was conducted at Mandatory Report Recipient Institution (IPWL) Pelita Jiwa Insani Foundation in Padang, consisting of 44 respondents who were divided into two groups, namely the experimental group (n=24) and the control group (n=20). Data were collected using the Hamilton Rating Scale questionnaire to determine the reduction in anxiety values and venous blood sampling for measuring blood cortisol levels. Data were analyzed using the Wilcoxon signed-rank test and the Mann-Whitney test. **Results and Analysis:** the results of the research on the anxiety variable obtained p-value = 0.004 ($p<0.05$), which means that there is a significant difference given the Spiritual Qur'anic Emotional Freedom Technique (SQEFT) therapy in reducing the value of anxiety in NAPZA residents currently undergoing rehabilitation. The cortisol variable results showed $p=0.00$ ($p<0.05$), so it can be interpreted that there is a significant difference in the difference value of cortisol in reducing blood cortisol levels in NAPZA residents who are undergoing rehabilitation. **Discussion and Conclusion:** There is a significant effect of giving Spiritual Qur'anic Emotional Freedom Technique (SQEFT) therapy on reducing anxiety values and blood cortisol levels in NAPZA residents.

Keywords: *NAPZA, SQEFT therapy, anxiety, blood cortisol levels.*

Background

NAPZA (Narcotics, Psychotropic, and Other Addictive Substances) Residents need family support and motivation because they experience stressful conditions and are not prepared to face reality. Confused thoughts will lead to mood swings, anxiety, depression, decreased immunity, and more severe signs and symptoms. At present, in addition to pharmacological treatment, non-pharmacological treatment is also carried out. Therapy is currently in demand by patients in general and in particular NAPZA residents. Besides having no side effects, the cost is relatively cheap, easy, affordable, and can be applied to everyday life without others' help. One of the efforts to prevent the problems experienced by NAPZA addicts is by conducting Spiritual Qur'anic Emotional Freedom Technique (SQEFT) therapy. SQEFT is a combination development of *ruqyah syar'i*

therapy and EFT (Emotional Freedom Technique). The SQEFT intervention principle stimulates the body's energy points by using the tapping method at specific points on the body while doing dhikr, praying according to the patient's main complaint, accompanied by an attitude of surrender to God, the Creator. SQEFT therapy can improve positive symptoms, negative symptoms, cognitive, and disease acceptance (Rosyanti, 2018)[1]. Many NAPZA residents seek spiritual support during illness. Religion and spirituality are related to positive coping mechanisms (psychological) and exercise (physical).

In general, people who are suffering from illness are overwhelmed by anxiety and an uneasy spirit. Apart from seeking treatment from the experts, praying and doing dhikr (remembering Allah) can calm their soul. God recommends that mental peace be maintained under any circumstances because God promises heaven's reward (Hawari, 2008)[2]. Al-Qur'an therapy is a healing physical, the spiritual, and social disease for Muslims (Muhammad, 2017)[3]. Reading and listening to the Qur'an scientifically will have a calming effect, promote relaxation, and eliminate negative physical and mental disorders, stimulate the release of endorphins in the brain, which have a positive impact on mood and memory. Focusing on positive thoughts and experiences, distracting negative thoughts, reducing stress, anxiety, and depression become non-pharmacological treatments for existing therapies (Koening, 2015, Rahim, 2014, Rosli, 2018)[4].

Anxiety is a normal thing as a sign of a threat, a reaction to a threat from pain or the outside world that is not ready to be overcome and serves to warn individuals of danger. The anxiety that cannot be overcome is referred to as traumatic (Safaria, 2012)[5]. Another source states that anxiety is an affective disorder characterized by deep and continuous feelings of fear or worry and does not experience disturbances in assessing reality. Moreover, Reality Testing Ability (RTA) is still good, personality is still intact (no splitting of personality), and behavior can be disturbed but even within normal limits (Hawari, 2013). Anxiety is common in everyday life, and if this anxiety continues and enters the anxiety disorder phase, it will impact the quality of life. Based on data from the United Nations Office on Drugs and Crime (UNODC, 2019)[6], it is estimated that 29.5 million people have suffered from drug use disorders, and 190,900 people have died due to drug abuse. Statistically, in Indonesia, it shows that the number of drug abuse cases is estimated to reach 4.1 million people or around 2.1 to 2.35% of the total population in 2019 (Ministry of Health, 2019). The projection results of the calculation of drug abuse cases show an increase in the number from 4.2 million in 2019 to 5.0 million in 2019 (BNN, 2019)[7]. In West Sumatera, the figure is 1.78 or around 63,523 people. Anxiety is an internal factor that causes drug users to experience a recurrence of 38.1%. Research conducted at Mandatory Report Recipient Institution (IPWL) of the Pelita Jiwa Insani Foundation in Padang from August to October 2020 totalled 51 people.

Research Methods

The purpose of this research is to determine the effect of Spiritual Qur'anic Emotional Freedom Technique (SQEFT) therapy on reducing anxiety values and blood cortisol levels in NAPZA residents. This research used purposive sampling and was conducted at Mandatory Report Recipient Institution (IPWL) of Pelita Jiwa Insani Foundation in Padang, consisting of 44 respondents who were divided into two groups, namely the experimental group (n=24) and the control group (n=20). Data were collected using the Hamilton Rating Scale questionnaire to determine the reduction in anxiety values and venous blood sampling to measure blood cortisol levels. Data were analyzed using the Wilcoxon signed-rank test and the Mann-Whitney test.

Research Result

This research indicates that both groups' respondent data showed a homogeneous data variant p=0.545 based on age. The data of both groups showed homogeneous data variants with p-value = 0.069 based on gender[8]. Meanwhile, based on rehabilitation length, both groups' data showed a homogeneous variant with p-value = 0.080. The two groups' data also showed a homogeneous data variant with p-value = 0.892 based on occupation. Last, the two groups' data showed a homogeneous data variant with p-value = 0.632 based on education. Based on the results of the Mann-Whitney test between the pre-experimental and pre-control groups, the p-value was 0.343 ($p<0.05$), meaning there was no difference/effect in the pre-experimental and pre-control groups. The Mann-Whitney test between the post-experimental and post-

control groups obtaining p-value was 0.004 ($p<0.05$). It shows that there was a significant difference in the post-experimental group and the post-control group that was given SQEFT therapy in reducing the value of anxiety in NAPZA residents currently undergoing rehabilitation. The results of the different tests between groups using the Mann-Whitney test showed that the value of $p=0.002$. It means that there was a significant difference between the pre-experimental cortisol and pre-control cortisol groups[9]. Thus, the delta test (difference) was carried out to determine the difference between the pre-test and post-test, where the data distribution was abnormal, and performed the Mann-Whitney test. The result showed that $p=0.00$ ($p<0.05$), meaning that there was a significant difference in the value of cortisol difference in reducing blood cortisol levels in NAPZA residents undergoing rehabilitation[10].

Discussion

SQEFT therapy that was carried out for seven weeks showed significant results, which means that there was an effect of SQEFT therapy in NAPZA residents who were undergoing rehabilitation[11]. This research is almost the same as the research conducted by Lilin Rosyanti, 2018, which states that SQEFT therapy can reduce the Brief Psychiatric Rating Scale (BPRS) levels in schizophrenia patients[12]. Another research was by Asmawati, 2018, who says that the Spiritual Emotional Freedom Technique (SEFT) therapy can reduce the value of anxiety in NAPZA residents[13]. Through this therapy, the physiological reaction of people who experience tension will subside, such as the heart rate begins to slow down, breathing is regular, the mind relaxes, and the blood flow returns to normal, causing the reduction in the value of anxiety[14]. There was a significant difference in the difference value of blood cortisol in NAPZA residents undergoing rehabilitation in cortisol measurement results. According to Kendall-Tackett et al. (2019), blood cortisol levels, besides a predictor of chronic stress, can also be used to predict a person's acute psychological stress[15].

Religious involvement provides cognitive stimulation involving social and recreational activities to lead to more efficient brain networks (Blazer, Corsentino, 2009)[16]. Hawari (2010)[17] also said that religion's role is essential in drug victims' therapy and rehabilitation. Medical therapy itself, which is not accompanied by prayer and dhikr, is incomplete because prayer and dhikr are medicine. Spirituality helps a person to overcome various diseases, even in various stressful situations. Positive emotions include well-being, happiness, hope, optimism, meaning and purpose, high self-esteem, and a sense of control over life. Positive energy is positive psychological energy such as being kind, loving or forgiving, and grateful (Van Ness & Kasi, 2003)[18]. Spirituality emphasizes love for others, compassion, and altruistic action while encouraging mutual encounters during social events. It can prevent stress and lead to needed social support in difficult times.

Spiritual helps encourage others to increase positive emotions. Spirituality encourages human virtue, honesty, forgiveness, gratitude, patience, increases positive emotions, and neutralizes negative emotions (King K & Carson, 2012). Spirit/religion (including spiritual awakening) and altruism are associated with the spiritual awakening of drug addicts attending religious activities (Marc Galanter, Helen D, Stephen, 2013)[17]. Spiritual Qur'anic therapy becomes a medium of therapy for someone. Itis what Allah *'Azza Wa Jalla* says in the Qur'an, Surah Yunus verse 57: *"O mankind, there has to come to you instruction from your Lord and healing for what is in the breasts and guidance and mercy for the believers"* (Rakhmat, 2008). Calm conditions obtained from the process carried out can suppress the parasympathetic nervous system (Sholeh, 2010). Passive attitudes, which are performed with submission and surrender during dhikr, increasingly give a multiple relaxation response, causing acceptance. The tension caused by life problems can be tolerated by the body (Purwanto, 2006)[3].

NAPZA residents who are undergoing rehabilitation feel anxious and afraid of their physical and psychological conditions. Thus, spiritual support is needed in undergoing rehabilitation and family support when NAPZA residents return home and prevent a recurrence. The intervention carried out in the experimental group, namely SQEFT therapy, affects reducing anxiety values and blood cortisol levels in NAPZA residents undergoing rehabilitation without ruling out pharmacological therapy and community therapeutic in IPWL.

Conclusion

1. There is a significant difference in cortisol's difference value in reducing blood cortisol levels in NAPZA residents undergoing rehabilitation. There is an effect ($p<0.005$) of SQEFT therapy with anxiety levels on NAPZA residents who are undergoing rehabilitation at Mandatory Report Recipient Institution (IPWL) Pelita Jiwa Insani Foundation in Padang.

2. SQEFT therapy is one of the most beneficial therapeutic methods for NAPZA residents, inexpensive, and easy to implement for health workers and the patient's family.

Recommendation

SQEFT therapy can be used as an alternative to religious psychotherapy to reduce anxiety levels and the value of cortisol levels in the blood. It is recommended to apply SQEFT therapy for NAPZA residents who are undergoing rehabilitation, especially for NAPZA residents who are Muslim.

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Conflict of Interest: Nil

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Analysis of Risk Factors affecting Stunting at Sub-District Health Center Galesong Takalar Regency

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Abstract

Stunting (short) is a linear growth disorder caused by malnutrition of chronic nutrient intake or chronic or recurrent infectious diseases indicated by a z-score of height by age (TB/U) of less than -2 elementary school. The purpose of this study is to find out the analysis of risk factors that affect stunting in Sub-District Health Center Galesong Takalar Regency. The types of research used in this study are stunting case studies in toddlers as dependent variables whereas the independent variables studied are birth weight, energy intake, protein intake, age and gender of toddlers, maternal education, number of household members, residential areas and family economic status. The sample is a baby that has complete data according to the research variables (nothing is missing). As recorded in Sub-District Health Center Galesong Takalar Regency 2019 using Lemesshow formula N = 298. The results showed that psychosocial stimulation affects stunting problems with a risk of 33.84 times. These results showed that the four free variables tested found no correlation with each other, so they were declared free of multicollinearity.

Keywords: *Risk Factors, Stunting, Nutrition, Mother, and Child*

Introduction

Stunting in Indonesia has been widely done to analyze the risk factors of stunting at the individual or household level. Based on the PSG Survey held as a monitoring and evaluation of activities and achievements of the program. Based on PSG's 2015 results, the prevalence of short toddlers in Indonesia is 29%. This decreased in 2016 to 27.5%. But the prevalence of short toddlers again increased to 29.6% in 2017. Based on the background above, researchers need to research to find out what risk factors affect stunting cases[1].

Stunting is a failure to achieve linear growth potential due to insufficient nutrition or low levels of health (World Health Organization., 2003)[2]. The usefulness of this indicator is to measure the nutritional imbalances that have occurred over a long period of time that lead to shortness. Growth retardation due to lack of incense and repeated infections tends to be more at risk of causing morbidity and mortality in children (Oktarina, Z., & Sudiarti, 2014)[3]. Malnutrition can be caused by imbalance/inadequacy, or due to medical conditions such as infections affecting digestion of food or absorption of nutrients from food (Wilson, PWF., D'Agostino, RB., Sullivan, L., Parise, H., Kannel, 2002), or socio-cultural factors (World Bank 2006)[4]. The term stunting is to indicate a low age-based height index (TB/U). This indicator describes malnutrition that occurs over a long period (Reinhard I et al., 2002)[5]. Children are considered stunting when they have a TB/U index value below -2 of 2005 WHO anthropometric standard SD. For children under the age of 12 months, the term used is body length, not height (World Health Organization., 2003)[6].

The assessment of nutritional status itself is directly and there are indirect. The assessment of nutritional status directly is by anthropometric method (Supariasa et al, 2001a)[5]. In society, the most commonly used method of measuring nutritional status is anthropometry. Anthropometric methods relate to a wide range of measurements of body dimensions and body composition of varying levels of age and nutritional levels. The use of anthropometry in general is to look at imbalances. The determining factors of stunted growth are family income, maternal nutrition knowledge,

parenting practices, history of infection, immunization, protein intake, and maternal education. Meanwhile, under-20s primary school children have low academic achievement. The higher the stunting rate, the lower the academic achievement of elementary school children (Picauly, I., & Toy, 2013)[6].

This imbalance is seen in physical growth patterns and the proportion of body tissues such as fat, muscle, and amount of water in the body. There are two types of nutritional anthropometric measures, linear and tissue mass. Linear growth describes the nutritional status associated at the time and the growth of tissue mass describes the nutritional status associated at present or at the time of measurement (Supariasa et al, 2001b)[5]. The shape of the linear size is the size associated with the length. Examples of linear size are body length, chest circumference, and head circumference. Low linear size usually indicates poor nutritional state due to lack of intake suffered in the past. The most commonly used linear size is height or length.

Anthropometry is the most commonly used method in community nutrition programs because it has advantages such as the tools are cheap, easy to bring, easy to obtain and use, durable, can be ordered and made in the local area; simple user procedure; measurements can be repeated easily and objectively; not only done with special professional personnel / relatively do not need experts; relatively cheap cost; can evaluate changes in nutritional status in a given period, or from one generation to the next; the result is easy to infer; scientifically recognized truth because the method is precise and accurate, standardized; the result can be used for the screening of nutrient-prone groups (Supariasa et al, 2001b)[5].

In addition to the above advantages, anthropometry also has drawbacks, among others: insensitive, factors beyond nutrition such as disease, genetics, and decreased energy use can decrease the specification and sensitivity of anthropometric measurements; errors that occur during measurement can affect the precision, accuracy, and validity of nutritional anthropometric measurement. Errors can be caused by erroneous measurements, analyses, and assumptions. The source of the error is usually due to insufficient training of officers, errors of tools or tools are not marked with a test, and difficulty measuring (Supariasa et al, 2001b)[5].

Anthropometry as an indicator of nutritional status can be done by measuring several parameters. Parameters are the single size of the human body, among others: lifespan, height, and length (Supariasa et al, 2001b)[5]. The age factor is very important in determining nutritional status. Age-defining errors can lead to incorrect interpretation of nutritional status. Accurate measurements of height and weight become meaningless when not accompanied by proper age determination. According to (World Health Organization., 2003)[7], the age limit used for children aged 0-2 is the month of full age (completed month)[8]. The example of the calculation of the full age month as follows: the age of 4 months 5 days is considered to be 4 months and the age of 3 months 27 days is considered to be 3 months. In rural areas, many families do not have a record of their child's date of birth or write down numbers easily for example 1 or 1.5 years. If after trying to dig up the date of birth information but still can not be known precisely, while the month and year are known, then the date of birth of the child can be determined the date of the 15 months in question (Supariasaetal. 2001)[5]. The recommended age grouping for data analysis and interpretation, especially in the under-two age group is 0-5 months, 6-11 months, 12-23 months (World Health Organization., 2003)[9].

Height is an important parameter to describe past circumstances and current circumstances if age is not known appropriately. Besides, height is an important second measure, because, by linking weight to height, age factors can be ruled out. Measurements for toddlers who are already able to stand are done by measuring the height of the child. Install the measuring instrument according to the instructions. Place the measuring instrument on a flat floor or surface. Remove the footwear, headgear/hat, or hat/cap from the respondent to be measured. Respondents to be measured were asked to go up to the base of the measuring instrument with the position of the measuring instrument. Respondents were asked to stand perpendicular, straight-forward views. Note, the back wall of the measuring instrument must be in the middle of the measured back body, do not go left or right. Five parts of the body namely the head, shoulders, back of the buttocks, and heels attached to the wall of the measuring instrument. When this is not possible at least 3 parts are attached to the wall of

the measuring instrument. The gauge position is in front of the measured one. When measured higher than the gauge use a tool such as a seat. The movement of the sliding tool until it touches the head, do not press too much.

Note that the back of the sliding tool should stick tightly to the back wall of the measuring instrument. When a high number is read from the front: see the scale number in the reading window located at the front of the slide right on the red line. When a high number is read from a scale located next to: read the number located at the bottom of the slide tool. As for babies or children who have not been able to stand, the way used is to measure the length of the baby. Combine the measuring parts (only 2 rods combined)[10]. Install the support iron so that the sliding tool can move freely. The installation of this support iron should be in the direction of the protruding part of the fixed elbow (the motionless elbow at the end of the measuring instrument)[11]. Select a flat floor or table to place the measuring instrument. The body length scale number should be in the top position for easy reading. The fixed elbow position should be to the left of the gauge, and the measuring helper position is behind the fixed elbow. The child is laid down with his head attached to the elbow fixed. The measuring helper holds the child's chin and cheeks from behind the fixed elbow. The imaginary line (from the point of the ear hole to the tip of the eye) must be perpendicular to the floor where the child is laid. The gauge holds the child's knee so that the child's feet are attached to the floor. While holding the child's knee, the gauge moves the sliding device towards the soles of Luanda's feet.2014[12].

The position of both soles of the child's feet should be tight and perpendicular when attached to the sliding tool. The gauge should quickly move the sliding device until it attaches to the sole of the child's foot, and immediately read the scale of the length of the body[13]. Read the body length scale located to the left of the slide tool. Moving the sliding and reading scale should be done quickly because the child is often fussy and moving. The combination of several parameters is called the anthropometric index (Supariasaetal. 2001)[5]. Nutritional status is a condition caused by the balance between the amount of nutrient intake and the amount needed by the body for various biological functions such as physical growth, development, activity, and health maintenance (Kartini, S., & Abdurrab, 2016)[14].

Nutritional status is one of the factors that determine human resources and quality of life. Therefore, the nutrition improvement program aims to improve the nutritional quality of food consumption, to improve the nutritional status of the community (Muchtadi, 2002)[15]. While according to (Almatsier, 2010)[16] nutritional status is the state of the body as a result of food consumption and nutritional use adriato and ningrum, 2019[17]. Toddlerhood is a rapid growth process that requires attention and compassion from parents and their environment. Besides, toddlers need balanced nutrition to have good nutritional status, as well as the growth process is not inhibited, because toddlers are the age group that suffers most from malnutrition (Wijayanti, 2008)[18].

Toddlers are declared a critical period to obtain quality human resources, especially in the first 2 years is the golden age for optimal brain growth and development, therefore at this time, it needs serious attention. Around the world, protein-energy shortages such as marasmus, kwashiorkor, and stunting remain one of the main nutritional problems in children. Lack of energy contributes to brain growth and long-term development. The impact of protein-energy deficiency (KEP) on each individual is strongly influenced by several factors such as age, length of malnutrition, the speed of recovery to normal nutrition, the home environment, and nutritional rehabilitation, and the absence of disease-related and micronutrition deficiencies[19].

Chronic energy deficiency in children can cause toddlers to be weak, physical growth is late, and subsequent development is disrupted. In adults it is characterized by decreased weight and decreased work productivity. Malnutrition at all ages can lead to the easily affected by infections and other diseases as well as the slow process of regeneration of the body's cells (Veriyal N, 2010)[20].

Hunger and inadequate food supplies still affect a portion of the world's population with serious impacts on health and well-being, especially in children. Malnutrition in children interferes with physical and mental development. Adequate nutrition from an early age is a prerequisite for the prosperity of a society. Diet plays a special role because of the importance of

micronutrition for growth and development. So far, efforts to combat malnutrition and malnutrition, and make progress towards the Millennium Development Goal (MDG), which aims to 'eradicate poverty and hunger'. Thus, the proportion of children under the age of 5 with malnutrition has been lowered from 33% in 1990 to 26% in 2006. Around the world, however, the number of malnutrition continues to increase (Team, 2010)[21].

Malnutrition in children is a result of a variety of factors, which are often associated with poor food quality, insufficient food intake, and severe and recurrent infectious diseases, or often some combination of all three. This condition, in turn, is very closely related to the overall standard of living and whether a population can meet its basic needs, such as access to food, housing, and health care (World Health Organization., 2003). The state of malnutrition in children has an impact on the slowness of growth and development that is difficult to heal. Therefore, children who are malnourished can learn and work and behave more limited than normal children (Santoso, S and Ranti, 2004)[8].

Stunting is a short and very short state of the body that exceeds the deficit of -2 elementary school below the median length or height (Manary, M. J., and Solomons, 2009)[13]. Stunting can be diagnosed through a high-age anthropometric index that reflects the linear growth achieved in pre and postpartum with indications of long-term malnutrition, as a result of inadequate nutrition and or health. Stunting is a linear growth that fails to reach genetic potential as a result of poor diet and disease (ACC/SCN, 2000)[15]. Stunting is defined as an indicator of TB/U nutritional status equal to or less than minus two standard deviations (-2 SD) below the average of standards (WHO, 2006a)[14]. It is an indicator of the health of chronically malnourished children who provide an overview of nutrition in the past and which is influenced by the environment and socioeconomic circumstances. Worldwide, 178 million children under the age of five (toddlers) suffer from stunting with the majority in South Central Asia and sub-Saharan Africa. Stunting is a major public health issue in low- and middle-income countries due to its association with an increased risk of death during childhood, (Irmawaty Bentian, N. Mayulu, 2015)[19]. In addition to causing death in childhood, stunting also affects the physical and functional of the body (Boerma, T., Mathers, C., AbouZahr, C., Chattergi, S., Hogan, D., & Stevens, 2015)[14]. Stunting is a form of the stunted child growth process. Until now stunting is one of the nutritional problems that need attention.

In 2003, 27.5% of toddlers in Indonesia suffered from moderate and severe skinnyness, or only 10 percentage points lower than in 1989, and nearly half were stunting. Children suffering from low birth weight and stunting in turn grow into adolescents and undernourished adults, thereby perpetuating a cycle of malnutrition (Nuraini B., 2015)[13]. In 2005, for all developing countries, an estimated 32% (178 million) children under the age of 5 had tb/U scores with a Z Score of less than -2. The highest prevalence in the UN sub-region is east and middle Africa at 50% and 42%, respectively, with the highest number of children affected by stunting, 74 million, living in South Central Asia. Stunting risk factors include birth weight length, intake, disease, and infection, genetic, and socioeconomic status of the family. Stunting especially in children over the age of 2 is difficult to overcome, so research on stunting risk factors in children over the age of 2 is required.

The prevalence of stunting in Asia in 2007 was 30.6 %. In developing countries, 11.6 million child deaths under the age of five, an estimated 6.3 million (54%) from the death of children is associated with malnutrition, which is largely caused by malnutrition (Dalimunthe, 2003)[11]. The prevalence of skinny and stunting is highly correlated, moving together through time, and telling the same story. However, for Central and South America, the prevalence of stunting remains substantial and is an issue that must be addressed. In Africa, the prevalence of malnutrition is 20% and the prevalence of stunting is 39%. In Asia, the prevalence of malnutrition is 22% and the prevalence of stunting is 31%. But in South and Central America & the Caribbean, the prevalence of malnutrition is only 4%, while the prevalence of stunting is 15%. Most countries start with a high prevalence of malnutrition and stunting from about 30-60%, which will probably continue to drop at up to 0.5 percentage points per year (Zahroh, 2016)[21].

There are several reasons why stunting happens in toddlers. In toddlerhood, the nutritional needs are greater, concerning weight gain, than in adolescence or adulthood. High nutritional needs for rapid growth,

including growth in adolescence. Thus, the chance of failed growth is greater in toddlers, as more growth occurs Martorell: 1994

Research Methods

The types of research used in this study are stunting case studies in toddlers as dependent variables whereas the independent variables studied are birth weight, energy intake, protein intake, age and gender of toddlers, maternal education, number of household members, residential areas and family economic status. The sample is a baby that has complete data according to the research variables (nothing is missing). As recorded in Sub-District Health Center Galesong Takalar Regency 2019 using limes show formula N = 298. Data processing will be done data editing, data coding, tabulation. Data analysis activities that include entering, processing, and analyzing data using computer software. Analysis of the data used in this study includes univariate and bivariate.

Results of Research and Discussion

The use of parametric statistical tests and nonparametric tests are based on the distribution of data used as one of the data assumptions. Population data will be distributed normally if the average value is the same as the mode and equal to the median and as the score collects in the middle position. According to the Kolmogorov-Smirnov test, the Asymp Sig (2-tailed) value of 0.001 > 0.05 is declared normal distributed. Multicollinearity testing aims to see no link between free variables that are biased to influence the prediction of regression results. A data is declared free of multicollinearity if, the tolerance value is greater than 0.01 and the VIF value is not greater than 10. Based on the results of multicollinearity testing stated this research data is free of multicollinearity with the following values:

Table 1: Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	1.527	.615		2.481	.015		
	feeding practices	.051	.036	.129	1.440	.152	.975	1.026
	Psychosocial disorder	.138	.053	.232	2.598	.011	.984	1.016
	hygiene practices	-.101	.059	-.160	-1.727	.087	.923	1.084
	breastfeeding	-.077	.087	-.080	-.881	.380	.959	1.043

a. DependentVariable: status stunting

Based on the table above for the variable feeding practice obtained a value of tolerance = 0.975 and a value of VIF = 1,026. The psychosocial stimulation variable obtained a tolerance value = 0.984 and a value of VIF = 1,016. Variable practice hygiene tolerance value = 0.923 and value VIF = 1,084. The breastfeeding variable values tolerance = 0.959 and the value VIF = 1,043. These results showed that the four free variables tested found no correlation with each other, so they were declared free of multicollinearity. Univariate analysis was conducted to get an overview of each variable in the form of frequency distribution i.e. parental work, parental education, parental income, and number of family members.

Table 2: Family Income

Variable	Case		Control	
	N	%	N	%
Mother's Education				
Not school	2	3,3	3	5
SD	31	51,7	33	55
SMP	15	25	13	21,7
SMA	9	15	7	11,7
DIPLOMA	3	5	2	3,3
SARJANA	0	0	2	3,3
Family Income				
500.000	40	66,7	39	65
1.000.000	13	21,7	15	25
2.000.000	6	10	1	1,7
>2.000.000	1	17	5	8,3
Number of Family Members				
3	23	38,3	20	33,3
4	17	28,3	17	28,3
>5	20	33,3	23	38,3
Parental Work				
URT	57	95	59	98,3
Honorer	3	5	1	1,7

Parental Education

Based on the above data obtained results that the education of parents of children with stunting 3.3% is not attended school; 51.7 % of primary school education; 25% are middle school educated; 15% are high school educated and only 5% are diploma educated and 0% are undergraduate educated. The education of parents of normal children 5 % do not attend school; 55% are elementary school educated; 21.7% are middle school educated; 11.7% are high school educated; 3.3% are diploma educated and 3.3% are undergraduate educated.

Family Income

Based on data on the income of parents of stunting children with a total of 500,000 as much as 66.7%; 1,000,000 by 21.7 %; 2,000,000 by 10%; and <2,000,000 by 17%. While the income of parents of normal children with the amount of 500,000 as much as 65%; 1,000,000 by 25%; 2,000,000 by 1.7 % and 2,000,000 by 8.3%.

Number of Family Members

From the above data obtained that the number of family members of children with stunting is 3 people as much as 38.3%; 4 with 28.3% and 5 33.3%. While the number of family members of normal children is 3 people as much as 33.3%; 4 as many as 28.3% and 5 people as many as 38.3%.

Parental Work

From the above data obtained results that the work of parents of children with stunting is URT as much as 95% and 5% as honorer. While the parental work of a normal child is 98, D3% is URT and 1.7% honorer.

Concurrent Test of Logistics Regression Parameters

Concurrent tests are tests that have a function to know the signification of parameters on constants narrowly. Below is a table describing the results of concurrent tests with the hypothesis:

Table 3: Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	13.082	5	.023
	Block	13.082	5	.023
	Model	13.082	5	.023

Based on the table above it is obtained a significant model value of 0.023 because this value is smaller than 5% so reject Ho so it is concluded that the free variables used together affect stunting. The formation of models on simultaneous bias tests is seen in the table above. In the test, it is expected that Ho is rejected so that the variables that are being tested go into the model. For a partial test, bias is seen in which significant value is greater than alpha. The corresponding significant value on the table is psychosocial variables (psychosocial stimuli).

Model Conformity Testing

This test was conducted to find out if there is a difference between the observation results and the possible results of the model prediction. Based on the logistic regression model above to further test the design of the model as follows:

Table 4: HosmerandLemeshowTest

Step	Chi-square	df	Sig.
1	13.288	8	.102

The Chi-Square value is close to 13,288 with a significant value of .102 then accept Ho, so it can be concluded that the resulting model on binary logistic regression that is stunting is influenced by the free variable factor. In other words, there is no significant difference between the observation results and the model prediction because it is seen from the table that the value of sig = 0.102 which means greater than 0.05 (accept Ho) with a confidence rate of 95% can be believed that the logistic regression model used has been sufficiently able to explain the data/ accordingly.

Table 5: Contingency Tablefor Hosmerand Lemeshow Test

		status = stunting		status = normal		Total
		Observed	Expected	Observed	Expected	
Step 1	1	12	10.089	1	2.911	13
	2	7	8.652	6	4.348	13
	3	6	7.045	6	4.955	12
	4	10	7.867	4	6.133	14
	5	8	6.085	4	5.915	12
	6	5	5.135	6	5.865	11
	7	2	4.334	8	5.666	10
	8	2	5.109	11	7.891	13
	9	4	3.662	8	8.338	12
	10	4	2.022	6	7.978	10

Based on the table above can be seen the frequency of observation and the expectation of the data that the observation 1 for stunting cases out of 12 observations diagnosed is 10 so it can be concluded that the difference between the two does not pass far so that the model can be said to be appropriate. While on observation 1 for case-control from 1 diagnosed observation there are 2 so it can be concluded that the model is not suitable.

Conclusion

The conclusion in this study was that psychosocial stimulation affected stunting problems with a risk of 33.84 times. These results showed that the four free variables tested found no correlation with each other, so they were declared free of multicollinearity. The univariate analysis was conducted to get an overview of each variable in the form of frequency distribution i.e. parental work, parental education, parental income, and the number of family members.

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Prevalence and Prevention of Diarrhoea among Infants in Rural Areas of Ekiti State, Nigeria

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Abstract

In Nigeria, diarrhoea has emerged as one of the common causes of gastroenteritis in children and adult population. Generally speaking, it is a condition in which three or more liquid or loose bowel movement per day happen. Basically, prevalence of diarrhoea as a disease is highly caused due to poor sewerage disposal, hygiene conditions and contaminated water at the household level. This study sought to investigate the prevalence and prevention of diarrhoea among infants in Ekiti State, Nigeria. The survey research design was used to accomplish this study. Among the rural population of the area, a sample of one hundred (N=100) mothers were selected randomly to gather information for the study. The primary data was collected by using the questionnaire and secondary data was collected from textbooks, research journals/articles published on the subject in the past. The findings shown, that the potential causes and prevention mechanisms of diarrhoea among children (less than 5 years) in the study area are not known to most of the nursing mothers. Therefore, it is imperative to engage community and women in particular aimed to ensure the success of educational interventions.

Keywords*: Diarrhoea, Prevalence, Infants, Prevention, Rural, Ekiti, Nigeria*

Background to the Study

In Africa countries, Nigeria specifically, diarrhoea still remains one of the common causes of gastroenteritis both in children and adult. It is a condition of having three or more loose or liquid bowel movement per day (WHO, 2018). In the main, it has remained in recent time the most common cause of infant mortality in developing countries, most especially, where safe drinking water and/or adequate sanitation hygiene are not available and it is the second most common causes of mortality among infant worldwide (WHO, 2018). Diarrhoea is preventable and treatable. Diarrhoea which is usually due to bacterial, viral, or parasitic infection is a common problem that usually lasts 1 or 2 days and resolves on its own without special treatment (Chen, Chin, Lai, Tung, Chiou, Hsu and Chang, 2010)[1].

The loss of fluids through diarrhoea can cause electrolyte imbalances and dehydration. Dehydration alters the child's natural balance of water and electrolytes (sodium, potassium, chloride) and can be serious if not treated promptly. Diarrhoea is not a disease, but is a symptom of a number of illnesses (Kandakai, Mawak and Ochai, 2009)[2]. Studies have shown that bacterial agents are important causes of infantile diarrhoea in many developing countries. Prolonged diarrhoea persisting for more than 2 days may be a sign of a more serious problem and poses the risk of dehydration (Katribe, Bogomolnaya, Wingert, Andrews-Polymenis, 2018)[3]. It has been demonstrated in previous studies that diarrhoea infection is more severe in younger children and incidence of occurrence is highest in the six month period following child birth.

Diarrhoea still remains one of the leading causes of morbidity and mortality among children and infants globally. In the main, the burden still remains disproportionately high among children in developing countries like Nigeria. Young children are specifically prone to diarrhoea as a disease with the larger proportion of the mortalities occur in the first 2 years of their lives (Black, Morris, and Bryce, 2013)[4]

Globally, the lager percent of the deaths related to diarrhoea happens among the children in Africa and South Asia. On the whole, virtually, fifty percent (50%) of the deaths from diarrhoea among infants and children occur in Africa, where diarrhoea has become the largest cause of mortality among infants under the age of 5 years old, which is the major cause of childhood morbidity (Black, Morris and Bryce, 2013)[5]

In African countries, with Nigeria inclusive, every child averagely suffers from five episodes of diarrhoea on yearly basis, while the two weeks prevalence usually ranges from 10 to 40% (Alambo, 2015)[6]. Diarrhoea as a disease has constantly been the first or the second cause of utilization of health facilities in the Nigeria. Basically, diarrhoea alone has contributed up to 19% of the experienced under-five mortality globally, while 22.5% had experienced hospitalization while up to 20% of the vulnerable children are outpatients (Alambo, 2015)[7]. Diarrhoea death ratio, for instance, the number of children mortality as a result of diarrhoea over the total number of children mortality as a result of any other cause is around 46%. The dangers associate with diarrhoea are not but related to malnutrition and dehydration. Furthermore, dysentery is another relative cause of deaths As a result of the fatal complications related to it.

On the whole, the prevalent diarrhoea cases globally require a lot of care that is virtually expensive and usually ineffective. This may definitely cause almost 25% of all diarrhoea associated mortalities. In addendum to causing high rate of morbidity and death, diarrhoea as diseases has been one of the major causes of childhood malnutrition. Moreso, over 30% of pediatric beds in most of the developing countries are always occupied with children suffering from diarrhoea diseases (Belachew, Jira, Faris, Mekete, Asres and Aragaw, 2011)[8]. Based on this, diarrhoea as a disease levies a very heavy and high burden on the available healthcare facilities and generally, on the national health budgets yearly. This research defined is aimed at determining the prevalence and prevention of diarrhoea among infants, using rural areas of Ekiti State as a case study.[9]

The Problem

Basically, many of the indicating factors associated with prevalence of diarrhoea among children in Nigeria such as maternal education, maternal history of recently experienced diarrhoea, availability of toilets (latrine) facilities, sources of drinking water, low level of exclusive breastfeeding by nursing mothers, duration of breast feeding, living in a house with few number of bedrooms and age of the child, have been identified, diarrhoea is still a major public health problem among children and infants.

The high level of prevalence of diarrhoea disease is largely attributable to contaminated water, poor sewerage disposal and poor hygiene at household level. In many villages in Ekiti State, many of the households have little or no access to effective sanitation infrastructure and clean water supply, which always leads to the health of children and infants negatively affected and the rate of diarrhoea among infants is very high.

The major contributors of diarrhoea among infants have been found to be contaminated drinking water and food, lack of proper toilet facilities, ineffective disposal of household wastes, lack of hand washing practice knowledge after they visit toilets and before they handling foods as well as low education levels especially among mothers of infants (0-12 months) children. This research therefore aims to investigate Prevalence and prevention of diarrhoea among infants in Ekiti State, Nigeria.

The Study Objectives

The main objective of this study sought to investigate the prevalence and prevention of diarrhoea among infants in Ekiti State, Nigeria.

Other objectives are to;

- To determine if mother's level of education have an effect on diarrhoea prevention in infants
- To determine if the source of water affect the

occurrence of diarrhoea in infants

· To find out the relationship between hygiene practices and occurrence of childhood diarrhoea in Ekiti State

Theoretically, this research was based on health action process for its framework. The health action process approach contends that; different factors are really at work, when a person is determining which of the health action(s) to be adopted. That is, a period of time at which he calls the motivation phase, which is also known as the decision-making phase. This decision is in an operation during the action, which is as well known as volition phase. This model in furtherance is of the view that the most imperative predictors of intentions are always the risk perception outcome of expectancies and self-efficacy.

The aim of this research was to determine the level of knowledge mothers about the childhood diarrhoea. Specifically, the nursing mothers may or may not perceive the real consequences of their behaviours in any case of emergency such as dehydration among others. Basically, delays in seeking management practices may likely emanate from the environment such as traditional birth attendants, friends and/or relations, hence the significance influence entrench on mothers by the immediate environment may definitely hamper health behaviour and/or adequate management of the phenomenon.

Research Methods

The survey research design was used in which the researcher used the sample drawn from the total population in order to have an insightful perception about the problem under examination and as well used the research findings in making comprehensive generalization. The study was carried out in the rural areas in Ekiti State, Nigeria. The inhabitants of the area are mainly farmers while some few hands are engaged in civil service and trading business. A sample of One hundred (100) mothers representing was utilized for the study.

The primary data was collected by the researcher, using the research instrument (questionnaires) while secondary data was collected from textbooks, journals and former research carried out on the study. The collected data was arranged, tabulated and analyzed with the use of descriptive statistics using percentage distribution. The ethical approval was sought and obtained from the Ekiti State Ministry of Health, Ado – Ekiti. Permission was sought and obtained from management of Hospitals used for this study. Only eligible and consenting respondents were used for the study after due explanation of the purpose, objectives, benefits and risks of the study. Confidentiality was ensured by avoiding writing names on the research tools; instead they were coded for the purpose of follow up.

Findings and Discussion

This session presented the data analysis, interpretation and discussion of findings based on the research questions. The researcher made use of simple percentages to illustrate the findings on Prevalence and prevention of diarrhoea among infants in rural Ekiti State, Nigeria.

Table 1: Socio-Demographic Characteristics of Respondents

Variables		Percentages (%)
Age	Below 20 years	15
	21-30 years	31
	31-40 years	29
	41-50 years	17
	51 years and above	8

Cont... **Table 1: Socio-Demographic Characteristics of Respondents**

Education of respondents	Tertiary	55
	Secondary	33
	Primary	10
	No formal Education	2
Religion of respondents	Christianity	63
	Islam	25
	Traditional	12
Marital Status of respondents	Unmarried but with a child	41
	Married	48
	Divorced	11
Occupation of respondents	Civil Servant	46
	Self-employed	34
	Farmer	20
Family size	Less than 5 members	52
	5 – 10 members	43
	More than 10	5
Has your child ever had diarrhoea?	Yes	80
	No	20

Source: Fieldwork, 2019

The total number of mothers interviewed was 100. They were interviewed on their age, education, marital status, occupation, and occurrence of diarrhoea in children. The majority of the respondents (29%) were women aged between 31 to 40 years, 15% were aged below 18 years, 31% were from 21 – 30, 17% were from 41-50 years while only 8 respondents were aged between 51 years and above.

On education of the respondents, 55% of the respondents had tertiary education, 33% had secondary education, 10% had primary education and 2% had no formal education. Analysis of the religion distribution of the respondents showed that 63% of the respondents were Christians, 25% were Muslims, while 12% were traditionalist. Forty eight percent of the respondents were married, 41% were single and 11% were divorced. Most of the women interviewed were civil servants 46%, 34% were self-employed, while 20% were farmers. On their family size, 52% of the respondents had less than 5 family members, 43% had from 5 – 10 family members and 5% of the respondents had more than 10 members in their family.

Table 2: Mother's Level of Education about Diarrhoea Prevention in Infants

Statement	YES	NO
Oral rehydration solution (ORS) helps to replace prevent dehydration	69%	31%
Boiling water before drinking it helps prevents diarrhoea	83%	17%
Breastfeeding protects the baby against diarrhoea	71%	29%
Hand washing is very effective in preventing diarrhoea	74%	26%

Source: Fieldwork, 2019

Table 2 shows that 69% of the respondents agreed that oral rehydration solution (ORS) helps to replace prevent dehydration while the remaining 31% disagreed. Also, Majority of the respondnets, (83%) agreed that boiling water before drinking it helps prevents diarrhoea and 17% wrongly disagreed. Furthermore, 71% of the respondnets correctly agreed that breastfeeding protects the bay against diarhoea but 29% disagreed. In addition, 74% of the respondnets agreed that hand washing is very effective in preventing diarhoea while the remaining 26% disagreed.

Table 3: Mother's Knowledge about Causes of Diarrhoea in Infants

Statement	YES	NO
Contamination of drinking water is the cause of diarrhoea	81%	19%
Eating food prepared with dirty hands cannot lead to diarrhoea	37%	63%
Eating too much rice is the cause of diarrhoea	58%	42%
Diarrhoea is caused by invisible microorganisms	80%	20%
Major cause of death in children with diarrhoea is loss of water and essential minerals	85%	15%

Source: Fieldwork, 2019

Table 3 shows that the 81% of the respondnets interviewed agreed that contamination of drinking water is the cause of diarrhoea while 19% disagreed. Also, 37% of the women interviewed wrongly agreed that eating food prepared with dirty hands cannot lead to diarrhoea while 63% disagreed. Also, 80% agreed that diarrhoea is caused by invisible microorganisms while 20% disagreed. The results further showed that 85% of the respondnets agreed that the salient cause of deaths in children suffering from diarrhoea is loss of water and nutrients. The results showed that the respondnets have good knowledge of about the causes of diarrhoea.

Table 4: Hygiene Practices and Incident of Childhood Diarrhoea

Statement	YES	NO
Defeacating in open places can cause diarrhoea	87%	13%
A child who has diarrhoea should not be allowed to defecate in bushes or open spaces	72%	28%
Using different utensils for raw and cooked foods can lead to occurrence of diarrhoea	63%	37%
Eating food on the floor instead of on tables is very hygienic	57%	43%
Diarrhoea can be transmitted from one person to another	62%	38%

Source: Fieldwork, 2019

On hygiene practices that lead to incidence of diarrhoea, 87% of the respondents agree that defecating in open places causes diarrhoea while 13% disagreed. Furthermore, 72% of the respondents agreed that a child who has diarrhoea should not be allowed to defecate in bushes or open spaces. The results further revealed that 63% of the respondents agreed that udifferent utensils for raw and cooked foods can lead to occurrence of diarrhoea while 37% disagreed. Also, 57% of th respondnets agreed that eating food on the floor instead of on tables is very hygienic while 43% disagreed. Furthermore, 63% of the respondents agreed that diarrhoea can be transmitted from one person to another 38% disagreed. The results are also presented in the chart below.

Table 5: Knowledge of Diarrhoea Management among Nursin•g Mothers

Statement	YES	NO
Lost fluids should be replaced with Oral Rehydration Therapy	56%	44%
Antibiotics like Ampiclox is best used for treatment of diarrhoea	58%	42%
Eating solid foods like eba will stop diarrhoea	59%	41%
A child with diarrhoea for more than 24 hours should be taken to the hospital	47%	53%
A babalawo or pastor must be consulted when a child has diarrhoea	55%	45%

Source: Fieldwork, 2019

Table 5 indicates that more than half (56%) of the respondents agreed that lost fluids should be replaced with Oral Rehydration Therapy while 44% disagreed. In addition, 58% of the respondents agreed that antibiotics like Ampiclox is best used for treatment of diarrhoea while 42% disagreed. Also, 59% of the respondents agreed that eating solid foods like eba will stop diarrhoea while 41% disagreed.

The table further revealed that 47% of the respondents agreed that a child with diarrhoea for more than 24 hours should be taken to the hospital while 53% disagreed. Also, 55% of the respondents agreed that a babalawo or pastor must be consulted when a child has diarrhoea while 45% disagreed.

Discussion of Findings

Childhood diarrhoea has emereged as important social, mental and health issues in most of the developing nations including Nigeria. In rural Ekiti State where this study was carried out, diarrhoea in children under-five is a common occurrence. The result shows that there was a gap in knowledge about the management of diarrhoea among the mothers where 31% disagreed that oral rehydration solution (ORS) helps to replace prevent dehydration.

Generally, sociodemographic backgrounds variables including age, professions, education of respondents, income of family including women and husband job status and family size and structure are all significant factors and above all nursing mothers' knowledge about diarrhoea and mothers' individual attitude, skills, managememt, treatment and prevention approach toward diarrhoea have been shown as important variables in the findings of the study.

These findings are predominantly aligned with the results of previous research conducted by Mwambete and Joseph (2010)[4] who reported that nursing mothers' primary, relevant information, knowledge and responsiveness about diarrhoea all depends on various important variables including prior knowledge and experience related to the beginning, spread and prevention techniques of this disease, ethnic background and the awareness/education of the nursing mothers.

The results showed that; though, nursing mothers had knowledge about diarrhoea and its management at homes but the level of their awareness about the management of the disease was poor and insufficient. Occurrence of the disease may be linked to various social factors ranging from unavailability of a good water source, education, unavailability of latrines in their homes, attitude, poverty, social status, ethnicity, monthly

income and locality (rural). Notwithstanding the fact that, nursing mothers were informed related to disease main signs and symptoms of dehydration but level of their knowledge about the real signs and symptoms of dehydration as a result of diarrhoea was very poor. In corroboration to the findings, similar studies conducted in Tanzania and Indonesia as well got similar results (MacDonald, Moralejo and Mathews, 2007).

In addition, some of the mothers (17%) do not believe that boiling water before drinking can prevent diarrhoea in under 5 years' children. There is plausible reasons of this fact as nursing mother might have no prior experience, or knowledge or education related to diarrhea among children under this age. Regarding mothers knowledge about the cause of diarrhoea, an encouraging result was that 81% had the knowledge that contamination of drinking water is the cause of the disease.

However, there are still some gaps in knowledge about the causes of diarrhoea which can be seen from some of the respondent who believed that eating too much rice is the cause of diarrhoea. This is a popular belief among residents of the study area and often times the mothers attributes the disease to rice consumption.

Although many of the women interviewed correctly affirm that the major cause of death in children with diarrhoea is loss of water and essential minerals. There are still some who do not agree. Also, 44% of the women do not agree that lost fluids should be replaced with Oral Rehydration Therapy.

Moreover, lack of proper knowledge about the successful rate of effectiveness of ORS in the treatment of diarrhoea by some nursing mothers is also related to nursing mother' poor information about the diction of dehydration and rehydration. In similar vein, strong beliefs among nursing mothers remain important factor regarding ORS role in increasing or decreasing the extent, level and prevention of diarrhoea among children. Their knowledge of the preparation of SSW as compared to ORS solutions was very poor. This finding shows that nursing mother might have no exposure to ORS or prior knowledge regarding the usage of ORS in treatment of diarrhoea.

The findings of this research show that there are predominantly misconceptions about diarrhoea its potential causes, onward transmission and spread, as well as lack of appropriate controlling strategies to prevent this disease. So it can be safely suggested that increased information on the spread and transmission of this disease along with effective awareness campaign on preventive and treatment mechanism are the factors which could contribute toward ensuring reduction in the destruction of damage of this disease. But this is only possible with help of mobilization of individual-nursing mothers and community at large.

Conclusion

From the findings, it could be concluded that some nursing mothers still have misconceptions regarding the causes and prevention of diarrhoea among under 5 children in the study area. The research also showcased various wrong assumptions about prevention of diarrhoea in children. Although diarrhoea occurrence is not limited to children alone the proof of this submission is beyond the scope of this study. The repercussions of inadequate management of diarrhoea are many and grim. These include dehydration, electrolyte loss, and in extreme cases death of the child. These repercussions have costly implications on the social, psychological, emotional and economic well-being of individual child and their families with associated negative impact on public health resources.

Around the globe, it is estimated that 1.7 billion cases of diarrhoea come to happen every year. It is Diarrhoea disease that badly affects almost all kinds of population with different age brackets. However, young people are mostly affected by this disease. Various researches shown, that it has emerged as the major cause of death among 5 years old children all over the world and third world in particular combined with other kinds of diseases like malnutrition and dehydration; therefore, all stakeholders should join hands to decrease the frequency of its spread and transmission onward.

Recommendations

From the findings of this study, the following are recommended:

i. There is a need for extensive educational

interventions especially rural community dwellers for the improvement in their knowledge and practices about childhood diarrhoea and its management at home.

ii. The indiscriminate use of antibiotics for diarrhoea treatment should be criticized on the grounds of drug toxicity and the risk of increased wide-spread antimicrobial resistance.

iii. Interventions aimed at improving sanitation, hygiene and child birth spacing should be put in place to decrease the occurrence of diarrhoea.

iv. Health education needs to be given priority and significance should be given to clean/treated water, may be through boiling and storage this water in covered place.

v. Water treatment should be ensured and approved by WHO in order to ensure effective control local ways and means of water storage, preservation and carriage from one plae to another.

vi. The advocacy and aggressive awareness campaign is required to educate children regarding the importance of hand hygiene for under age children and their care takers are to be advised and educated to spread information of treatment and prevention strategy against diarrhoea.

vii. Health education specifically targeted at the preparation and use of ORS should be made available to nursing mothers and the practice encouraged instead of indiscriminate use of antibiotics.

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The Family Hope Program (PKH) Collective Partnership among Beneficiary Families (KPM) For Healthy Living through the Clean Friday Campaign

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Abstract

Concerning the importance of **collective partnership,** to encourage a Safe and Balanced Way of Life, PKH Facilitators in Bone District, South Sulawesi, carry out the Clean Friday Campaign initiative as a **collective partnership** in enforcing a sanitation or cleaning program for the village by including all KPM PKH. This paper explains what the Clean Friday Campaign is, how PKH facilitators inspire KPM PKH The study site was situated in the Bone Regency, South Sulawesi Province of the Cina District. Interviews and findings, and documentation were carried out as data collection procedures, which were then analyzed by Miles and Huberman using research theory. The study's findings show that every Friday morning, the Clean Friday Campaign is carried out every week towards public facilities guided by the collaboration of PKH Facilitators with village and sub-district governments to increase the self-awareness of KPM in ensuring a safe atmosphere for a safe and stable existence.

Keywords*: Clean Friday Campaign; Health; Family Hope Program; Indonesia*

Introduction

Participants providing government social assistance through the Ministry of Social Affairs, both aids in the form of Non-Cash Food Assistance (BPNT) and assistance from the Family Hope Program (PKH) disbursed to KPM to alleviate poverty in Indonesia, are beneficiary families (KPM). Based on data from the Central Statistics Agency (BPS), PKH has been in operation for 17 years with a notable degree of success in lowering the poverty rate (Misnawati et al., 2019)[1].

KPM has responsibilities that must be met through its membership status as PKH to receive its benefits as beneficiaries of government aid, such as the responsibility to attend community meetings organized by PKH Social Assistants, and also the duty to monitor household members to satisfy their commitment to participation and education in health facilities. Early childhood and pregnant mothers are expected to receive medical exams, and children of school age are required to attend a minimum attendance of 85% per month or equal to a maximum absence of 3 face-to-face class sessions.

Furthermore, PKH Social Facilitators are expected to help KPM boost their financial quality of life and shape a mentality (Yusriadi, Tahir, et al., 2020). KPM is also armed with some knowledge of family management by optimizing family skills at monthly community meetings known as the Family Growth Session (FDS) in PKH (Yusriadi, Awaluddin, et al., 2020)[2]. The Family Development Session (FDS) is an education for KPM that seeks to structurally and consistently improve their potential to build a community of meaningful thought habits. The meeting topics explored in the FDS were family financial control, child care and schooling patterns, and a Clean and Balanced Way of Life (CHBS)[3].

In partnership with the local village government, PKH Facilitators in Bone District, South Sulawesi, are introducing the Clean Friday Campaign program

to encourage CHBS. This clean Friday campaign is an initiative of collective partnership in the execution of a village cleaning or cleaning program involving all KPM PKH in the cleaning up public amenities, such as mosques, village offices, road shafts, hallways of the village, and other public areas. To ensure cleanliness and environmental sustainability, Clean Friday events are held every Friday morning so that people can live in a safe, clean, and enjoyable atmosphere. Besides, this campaign should also include the friendship between all PKH beneficiary participants. In the Islamic world, the Clean Friday trend has also become a culture, as cleanliness is more than religion. This paper would explain how PKH facilitators are mobilizing KPM to engage in the Clean Friday Campaign activities, how this action is done, and what benefits the group, especially KPM PKH, feels.

Research Method

In addition to obtaining a complete description of the Clean Friday Campaign events according to the informants' opinions, this writing is a comprehensive method of writing using a qualitative approach to identify and illustrate the study outcomes in writing. As reported by Putu Laksman Pendit (2003)[4], qualitative analysis is normally designed and carried out to acquire real experience and meaning as what is generated in the field of research through direct contact between the researcher and the individual being studied (Pendit, 2003)[5].

The Bone District of the Cina District, South Sulawesi Province, is the place for data collection and analyses about the Clean Friday Campaign events. Where almost the entire PKH Facilitators population in Bone Regency applies this initiative against the following KPM, accidental sampling that is important to the research subject is selecting KPM in the District of Cina as the research goal. Interviews and findings and documentation were carried out as data collection methods that were then analyzed by Miles and Huberman using methodological philosophy, which included transcripts of outcomes of interviews, data elimination, examination, interpretation of data, and triangulation. The scientific results were derived from the consequences of data processing[6].

Discussion

What is the Clean Friday Campaign?

As far as the importance of cooperation is concerned, Indonesia's citizens have long been recognized as a country with a high value for cooperation. In this matter, collective partnership, please help or support, not just about working together. The collective partnership is essentially an operation carried out intentionally by the society to attain a shared interest without considering material benefits for those participating in it (Badruddin, 2011). Since Indonesian culture is in a time of social change, the different values that evolve in society are influenced directly or indirectly (Tamsah, Ansar, et al., 2020)[7]. The ideals reflected in collaboration as a societal principle have also shifted (Tamsah, Ilyas, et al., 2020). As part of shared cooperation in the neighborhood, inspire the community with the Clean Friday Campaign[8].

The Clean Friday Campaign in the Cina District of Bone Regency is one of the events encouraged by PKH Facilitators with the key goal of growing the self-awareness of KPM in ensuring a clean atmosphere for a good and happy existence. The key implementers who plan and carry out exercises and decide the venue to be held every Friday are PKH beneficiary partners. The clean-up movement is still similar on Fridays, according to the informant, since Friday is the major day for Muslims, where this day has its own Muslim rights. Friday is said to be the shortest day of the week, so it will restrict Friday activities, provided that Muslims will jointly conduct Friday prayer activities. For the word Clean Friday to appear, individuals must be clean before undertaking Friday prayer practices. One of the cultural-based community events is Clean Friday.

Furthermore, cleanliness is essential to the community and personal life. Since a clean atmosphere is a safe environment, it is necessary for people who care about environmental cleanliness to live a pleasant life and avoid the spread of diseases. This activity is intended to shape the community's mentality to maintain a safe atmosphere to produce a healthier life such that people live happily and enjoy their place of residence. Furthermore, with this shared assistance activity on Clean Friday, it can be an event to increase togetherness and, at the same time, reinforce the fraternity between the group, especially the recipients of PKH social

assistance.

How do PKH Facilitators get KPM to join in events with the Clean Friday Campaign?

In the sense of poverty alleviation in Indonesia, PKH companion is a government extension. According to Syahabbudin, Head of the Ministry of Social Affairs of the Republic of Indonesia's Department for Education, Study and Social Counselling, assistants act as soldiers for the Ministry of Social Affairs to combat hunger. Still, the arms of the PKH facilitators are information. It can use this knowledge to open up KPM's innovations and knowledge to change the quality of life for the better (Humas BBPPKS Yogyakarta, 2020).

Given that KPM is a society with low-class economic and educational circumstances, PKH facilitators' role for KPM is vital. The figure of PKH assistants is a requirement to help fulfill their responsibilities to gain the privileges they deserve. Informants agree that PKH facilitators are like angels to them who listen patiently to their grievances as KPM. PKH facilitators easily support, mediate, and overcome these barriers regardless of the difficulties they face.

By mobilizing all KPMs as executors and activists in it, the Clean Friday operation, a PKH companion initiative, only happens simultaneously without complicated preparation. According to informants, this operation resulted from a plan by the Cina district government to foster a clean atmosphere within the scope of villages and sub-districts. The village government also communicates with PKH facilitators to include KPM as assistance recipients, taking into account the huge number of KPM. If mobilized by them, it would be very successful. Besides, KPM can also contribute to the government as the target beneficiary of government assistance. One of the contributions alluded to is its role in preserving a safe and balanced climate, namely by partnership efforts to clean the environment by prioritizing public facilities. This suggestion was then forwarded to the KPM as a distribution for community meeting events for this purpose.

Positive motives for the public interest are obviously stuff that KPM embraces very well. Given that certain persons who have not earned government social assistance for KPM PKH have become socially jealous, this proposal was accepted by them as a means of compensation and appreciation to the government for being given a chance to boost their quality of life by PKH. They will make a positive contribution to the world where they work, at least through this Clean Friday Campaign activity.

What are the actions of the Clean Friday Campaign carried out?

Every Friday morning, Clean Friday Campaign operations are carried out. The tasks begin at about 07.00 - 10.00 am. In this operation, based on their groups allocated by PKH Facilitators, KPM PKH gathered where the group divisions were divided depending on the hamlet or climate. There are typically 1 to 4 groups in one village, depending on the number of KPM in the hamlet, with a typical maximum number of groups of 30-35 KPM individuals.

In public locations such as the Grand Mosque or the hamlet mosque, in the village office yard, in the schoolyard, on the side of the main lane, in the village and hamlet alleys, and public cemeteries, the site of the Clean Friday Campaign is. Cleaning exercises in the mosque area are a priority given that Friday prayer activities will be conducted in the congregation on that day so that the mosque must look clean to be used by Muslims for worship.

Some female KPMs do not refuse to do hard work as men do since some KPMs consist of most women and a few men. Women sweep around them leaf litter and plastic waste, digging out the growing vegetation, while some of the males' hoe boost the drainage. Any KPMs included in the elderly age group are typically portrayed to partake in this task by their children or grandchildren, so cleaning tasks usually include many viewpoints of young children of pre-school age.

In addition to washing the area, this is achieved such that the waste pile does not become a spawning ground for germs in the rainy season, as well as a resting spot for mosquitoes as a vector of dengue hemorrhagic fever (DHF), a mosquito-borne infectious disease that is one of the containers for replication by waste and stagnant water. This happens in areas of the tropics and subtropics. If not washed, garbage will go anywhere in the dry season, where it is still very vulnerable to fires.

In the dry season, the garbage is exposed to a flame, so it has the ability to ignite.

Supplemented by some family medicinal plants (TOGA) and poison-absorbing plants such as the tongue of the in-laws, the weeds that flourish near public buildings will be coordinated with the city authority and the society in the future. Clean Friday activities, including a group meal (breakfast) and group pictures, are always closed. To improve the emotional bond between neighbors, the advantages are in environmental cleanliness and friendship.

Every week on Friday, PKH Facilitators, the Village and District Governments of Cina, invite both parties to become more conscious and concerned about environmental cleanliness and the issue of waste through the Clean Friday Campaign. So, we're going to protect nature and the world by worrying about pollution, and everything depends on each of us.

What are the benefits felt by the community, KPM PKH in particular?

A very vital supporting factor in supporting a healthier life is the cleanliness of the living environment. The development of an ecosystem that is secure, safe, and attractive would positively affect society. The Clean Friday Campaign Initiative is a regular work program carried out by KPM PKH every week to encourage a feeling of belonging to society regarding the value of environmental cleanliness for well-being. The culture of the community feels secure. The climate is well preserved. In the world in which we live and adapt, clean living habits define a balanced lifestyle. A safe atmosphere will enhance a relaxed feeling and create a harmonious environment that can be taken into the family environment so that it is far from feeling depressed.

The advantage of preserving cleanliness is also Islamic advice for Muslims that cleanliness is part of religion. One of those hadiths must be used by Muslims as guidance, where they must likewise uphold cleanliness as a believing group. The hope of all people is a safe world. With a clean, they can carry out their daily activities safely and comfortably. Informants have revealed that if they look at the outside of us, including the inhabited region, other entities will give us an appraisal of ourselves. When our home environment is safe, individuals or our visitors will not hesitate to come. It's not hard to get started, first by tossing the garbage in its place. Our world would be tidy and comfortable to look at in that way.

KPM PKH experiences a considerable influence from the Clean Friday Campaign's events, such as the sensation of still feeling satisfied when the world is clean. Since this practice instills a pure character in the mentality of KPM so that seeing trash lying around, his heart is motivated to pick it up and throw it away. With clean environments around him, he discourages littering because of the cleanliness that exists in front of him. It will destroy their already beautiful eyesight. The home atmosphere of KPM also becomes a target for cleanliness to be cleaned. For their eyes, if they can clean and look stunning with their works, if they can clean it and look beautiful with their work, why not for the home environment that is their place of residence, a place to return from activities and built-in places to start operations.

Most notably, the group photo activity is one of the events that motivate you to attend these activities. Suppose you don't want to skip-proof since the Clean Friday Action activity becomes a fun activity within the community. In that case, they get closer to the normal activities carried out every week. The biggest attraction to meet and partake in this program is to eat together periodically to close these events.

Conclussion

A very vital supporting factor in supporting a healthier life is the cleanliness of the living environment. The development of an ecosystem that is secure, safe, and attractive would positively affect society. The action of the Clean Friday Campaign, which takes place every week on Friday morning, has made the atmosphere clean and safe so that it is comfortable for the community, particularly for public facilities such as mosques, village offices, classrooms, highways, village hallways, and even public cemeteries.

KPM PKH actively conducts this every week to encourage a feeling of belonging to the society in terms of the value of environmental cleanliness for wellbeing so that the community climate is secure. The ecosystem is well protected. In the world in which we live and

adapt, clean living habits define a balanced lifestyle. A safe atmosphere will enhance a relaxed feeling and create a harmonious environment that can be taken into the family environment so that it is far from feeling depressed.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Neonatal Mortality Rate and Its Causes at Maternity Wards in Baghdad City's Hospitals

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Abstract

Background: The first 28 days of life – the neonatal period – is the most vulnerable time for a child's survival. Children face the highest risk of dying in their first month of life at an average global rate of (17) deaths per (1,000) live births in 2019.

Objectives: To find out the neonatal mortality rate at maternity wards in Baghdad City's Hospital for the year 2019, and to assess the causes of neonatal mortality rate.

Methods The study included a non-probability (convenient) sample of (150) Neonate who have beendyingin ten hospitals in Baghdad City. The study subjects are recruited from 10 teaching hospitals. Data is collected from neonatal medical records in the Department of Statistics; data are analyzed *using* descriptive and inferential data analysis process.

The Results: The results of the current study indicate that the neonatal mortality ratefor the year of 2019 is (0.636).The highest number of causes of neonatal mortality is accounted for asphyxia (57.7%) and congenital anomalies (29.6%).Most of the neonates are (1-5) day of age (83.8%); Males (64.1%); (3000-3.999) gm of weight (42.9%); urban residents (60.6%); are born at early term (37 to 38+6 days) (35.9 %) and in hospital (95.8%) through cesarean section (64.8%). There is no significant relationship between neonates' age and the causes of neonatal mortality.

Conclusion: The neonatal mortality rate is (0.636) for the year of 2019 on the maternity wards in Baghdad City's hospitals per 1000 of the population at risk for the same year; The most accounted cause of neonate mortality is asphyxia and congenital anomalies.

Keywords: *Neonatal Mortality Rate,Causes.*

Introduction

The neonatal period, the first 28 days of life, is the most vulnerable time for the survival of a child. In their first month of life, children face the highest risk of dying at an average worldwide rate of (17) deaths per (1,000) live births in 2019, down by (52) percent from 38 deaths per (1,000) in 1990.

In comparison, the probability of dying after the first month and before reaching age (1) is estimated at (11) deaths per (1,000) and the probability of dying after reaching age 1 and before reaching age (5) is estimated at (10) deaths per (1,000) in 2019. Globally, (2.4) million children die in the first month of life in 2019 – approximately (6,700) neonatal deaths every day – with about a third of all neonatal deaths occurring within the first day after birth, and close to three-quarters occurring within the first week of life[1]. The neonatal death can be classified as an early age which occurs through the first seven days while the late stage who happens after seventh day but before 28 days of life, therefore they considered as the greatest risky period in reason of facing many problems and diseases which lead to rising the morbidity and mortality rates[2].

Wherever babies die as it is pointed the prematurity, severely malformed, obstetrical complications or poorly hygienic practices were issues lead to infections, which end to death of the neonates[3].

Methodology:

Descriptive correlational study is conducted at Maternity wards in Baghdad City to find out the Neonatal Mortality Rate during the year 2019. The study was carried out for the period from October 10th 2019 to June to 15th 2020, at the maternity wards in Baghdad's City Hospitals.A non-probability (convenient) sample of (150) newbornwho have been diedat ten hospitals in Baghdad City.

The study assessment tool is designed and constructed for the purpose of the study. It is consisted of three parts; Part I is about neonatal demographic characteristics, part II is concerned with neonatal death causes, and part III is include the formula for calculating the neonatal mortality rate as follow:

$$\text{Neonatal Mortality Rate} = \frac{\text{Number of Neonatal deaths under 28 day of age}}{\text{Number of live births in the same year}} \times 1000$$

Validity is determined through a panel of experts and reliability of assessment tool using Alpha Correlation Coefficient)is(0.85) which considered significant through of pilot study.

Results and Discussion

Table (1): Neonates Demographic Characteristics

Variables	Groups	F.	%
Age (Days)	1-5	119	83.8
	6-10	18	12.7
	11-15	2	1.4
	16-20	1	0.7
	> 20	2	1.4
Gender	Male	91	64.1
	Female	51	35.9
Weight (gm)	< 1000	12	8.5
	1000-1.999	30	21.1
	2000-2.999	39	27.5
	3000-3.999	61	42.9
Residence	Urban	86	60.6
	Rural	56	39.4
Gestational Age (Weeks)	Extremely preterm (less than 28 weeks)	6	4.2
	Very preterm (28 to 32 weeks)	30	21.1
	Moderate preterm to late (32 to 37 weeks)	46	32.4
	Early term (37 to 38+6 days)	51	35.9
	Full term (39 to 40+6 days)	9	6.3

Cont... **Table (1): Neonates Demographic Characteristics**

Place of Labor	Home	3	2.1
	Hospital	136	95.8
	Others	3	2.1
Type of Labor	Normal Vaginal	50	35.2
	Cesarean Section	92	64.8

The result of this table depict that most of the neonates are (1-5) day of age (83.8%); Males (64.1%); (3000-3.999) gm of weight (42.9%); urban residents (60.6%); are born at early term (37 to 38+6 days) (35.9 %) and in hospital (95.8%) through cesarean section (64.8%).Results out of

$$\text{Neonatal Mortality Rate} = \frac{\text{Neonate Death}}{\text{Lived Birth}} \times 1000$$

$$= \frac{142}{223{,}21} \times 1000$$

$$= 0.636$$

Figure (1): Neonatal Mortality Rate

Result, out of this table, indicates that the neonatal mortality rate is (0.636) for the year of 2019.

Table (2): Causes of Neonatal Mortality

List	Causes of Neonatal Mortality	Yes	Percent	NO	Percent
1	Tetanus	4	2.8	138	97.2
2	Congenital Anomalies	42	29.6	100	70.4
3	Sepsis	4	2.8	138	97.2
4	Asphyxia	82	57.7	60	42.3
5	Preterm Labor	6	4.2	136	95.8
6	Trauma	2	1.4	140	98.6
7	Others	13	9.2	129	90.8

Results out of this table indicate that the highest number of causes of neonatal mortality is accounted for asphyxia (57.7%) and congenital anomalies (29.6%).

Discussion of Neonate's Demographic Characteristics

Neonatal Age

In the study under hand, the group of the neonates is (1-5) day of age (83.8%) as being die during the first week after delivery. This result come in the same line with study conducted in Babylon Governorate deals with neonatal mortality. Findings depict a majority of neonate (85.5%) die during the first week after delivery[4]. Also, results isconsisting with retrospective study conducted AL-Gumhouri Teaching Hospital, Sana'a, Yemen to identify the chief reasons of newborn morbidity and death and to ascertain some of the potential danger

factors related to newborn mortality in the (NCU). Their results reveal the majority of deaths (80%) occurred during the first week of life [5].

In addition to, descriptive case series, hospital based prospective study entitled "Morbidity and Mortality Among Neonates Admitted to a Neonate Intensive Care Unit of a Tertiary Care Teaching Hospital of Jammu and Kashmir (India)". Results indicated that about four-fifth (79.4%)out of 1017 neonates were in the age group of 0-7 days [6]

This result may due to newborn during the first week of life more liable to infection and sepsis due to low immunity, in addition most cases of extreme prematurity and birth asphyxia die during the first week of life. These findings are logical and expected as neonates in the early neonatal period are at risk of contracting diseases and at-risk neonates are identified by healthcare workers immediately if they are born in hospitals. and referred to NCU and It signifies that main neonatal problems arise in early neonatal period.

Neonatal Gender

There is a male predominance in our study which deliberated as (64.1%) due to number of births of the male gender is more than the female gender, or it may be due to biological, cultural and social factors. This consistent with retrospective study has been assessed the "factors causing mortality rate of neonate in AL-Batool Teaching Hospital in Mosul City". Their finding indicated the predominance of males from study sample it constituted (53%) out total number[7].

A study of Welaga and others in (2013), have been conducted in Northern Ghana to determine the NMR in the Kassena-Nankana District, which resulted higher percentage (50.7%) of study sample were males[8]. Another descriptive study deals with predictors of neonatal mortality referred to a tertiary care teaching institute. Findings reveals (60.8%) were male[9].

Neonatal Weight

Our finding depicts that (3000-3.999) gm of weight (42.9%). This corresponds with the study determinates of neonatal mortality in Indonesia to identify the neonatal mortality, for a nationally representative sample of births for the period of 1997 to 2002. In which the highest percentage of neonate weight about (58.4%) out total study sample their weight was (2500-3500 kg) [10].

The findings of current study also, go along with the study conducted by Kotwal and others (2011) in India to determine the morbidity and mortality pattern of among the neonates admitted to a NICU. reported that Majority 58.3% out of 1017 neonates admitted to NICU were of normal weight 2500-3500 grams.

Residences

Urban residents were dominate in our study finding, it composed (60.6%) out total number. As being mostly those who inhabited at urban and these services are widely distributed primarily in urban areas and are easily accessible. This result come along with a case-control design investigated the risk factors of prenatal and neonatal mortality at Alexandria, in Egypt to identify the social and biomedical risk factors contributing toward PM and NM. Their result indicated that most of participants (58%) were urban they live with high level of neonate death[11]

While result disagrees with Debelew and others (2014), have been studied the "determinants and causes of neonatal mortality in Jimma Zone, Southwest Ethiopia: A multilevel analysis of prospective follow up". Their results indicated that most of participants (75.1) Out of 3463 neonates were from rural areas[12].

Neonatal Age of Birth

Neonate who borne at early term were mostly in our findings and (37 to 38+6 days) and composed (35.9 %) and in hospital (95.8%). It corresponds with the present study finding in relation to gestational age which ranged between (36 to 41) weeks and constituted (45.7%) out total number of the study sample. While, other a descriptive case series, hospital based prospective study also concerned with morbidity and mortality among neonates admitted to the neonatal intensive care unit of a tertiary care teaching hospital of Jammu and Kashmir India. Findings indicated that the majority of (60.6%) of the neonates were born at full term of gestation[13].

A descriptive quantitative study of secondary data, correlated with the causes of death and hospitalization according to classification by ICD-10 which entitled "Factors associated with mortality in a neonatal intensive

care unit" which targeted to recognize the "factors associated with mortality in a neonatal intensive care unit" for the period of 2012 to 2015. Which the findings indicated about (36.0%) of study sample the gestational age (37-41) weeks [13].

Also, study of Lahasehin (2014), has been conducted a case-control design study entitled "Determinant of Neonatal Mortality in Palestine - 2012 (Northern West Bank)" to determine the major risk factors contributing to neonatal mortality, and to describe the health reporting system regarding mortality of neonates and infants in the northern districts of the West Bank. Their findings indicated (84.7%) was government hospital delivered[14].

Type of Labor

Through cesarean section were delivered (64.8%). While study of Abed, (2018) the highest percentage of the study sample (71.8%) normally delivered[4], which consisting with a study conducted by Jehan and other in 2009as a prospective design aimed to identify the "neonatal mortality, risk factors and causes: A prospective population-based cohort study in urban Pakistan" to evaluate the prevalence, gender distribution and reasons of newborn deaths, as well as its danger factors. Findings indicated (63.8%) of study sample were N.V.D[15].While another retrospective design was inconsistent with results of present study which conducted by Azize and others (2017) entitled "the causes of early neonatal death in Sulaimani intensive care unit at Maternity Teaching Hospital for the period of (Jan 2016 to Feb 2017)" in which there were (67.3%) of mothers had C/S[16].

Discussion of Causes of Neonatal Mortality

Results out of this table indicate that the highest number of causes of neonatal mortality is accounted for asphyxia (57.7%) and congenital anomalies (29.6%). While the results of the study of Abed (2018), were (41.6%) of neonate die by the respiratory distress syndrome as considered the greatest causes of neonate mortality. While meningitis and encephalitis recorded the lowest causal mortality in neonates (2%) out total number of the study sample for the 2016 and 2017 due to that most of neonate (78.8) enter the premature unit (NICU) directly after delivery[4].

Recommendations:

Plans for strategies for reducing neonatal mortality to improve the care of babies at the time of birth by improving the knowledge and skills of health care providers.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Co Management Health and Economic Development Indexes: Grand Strategy for Health Development in the Madura Island-Indonesia Coast through Mangrove planting

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Abstract

Poverty in Indonesia's coastlines is currently an unresolved national issue. This study aims to analyze the mangrove co-management in the economic and health development in the coasts, as an effort to tackle poverty. This research uses the qualitative and quantitative methods, analyzed using the SEM WarpPLS. The research results show that in the coastlines of Madura Island, the Instructive Co-Management is most dominantly applied in managing mangroves. The mangrove co-management has positive and significant effects towards the economic and health development of the coastlines. The economic development of Madura Island's coasts is marked by the increase of the people's income, the creation of new job fields, and also the increase of health conditions, in line with the increase of mangrove preservation quality and quantity.

Keywords: *Co-management, Mangrove, Development, Economy, Health, Coastlines.*

Introduction

The development of the economy as well as the health quality in the coasts of Indonesia are still crucial national issues which require special attention. Some problems in the coastlines include the weak education, the low-quality human resources, the lack of access, the low rate of life expectancy and health, the lack of technological access and network, and also the deteriorating natural resources. Kunarjo[1] stated that the root cause for poverty is the low income, which affects the education and health conditions, causing low productivity. Muqtafa and Nadila[2] and Imron[3] stated that other factors causing the poverty of fishermen are more complex, such as the destruction of the coastal ecosystems and destructive exploitation of natural resources. Such environmental destruction has decreased the fishermen's income.[4]

The natural resources in the coasts of Madura Strait have experienced a worsening decrease of quality and quantity, due to the demographic pressures, industrial waste, and developmental demands, as stated by Kusnadi.[5] Many fishermen shift to seek jobs on land, as fishing no longer becomes a reliable source of income. Thus, to guarantee the welfare of the people as well as to preserve the natural resources of the coasts, there needs to be a comprehensive developmental policy combined with structured steps to achieve it.[5]

The terrible destruction of the coastal ecosystem further affects the quantity of the fish catch. The issue of the coastal people's poverty is basically multidimensional, thus there needs to be an integrated social engineering to resolve it, instead of partial solutions.

Because of that, there needs to be some efforts to save the fishermen from poverty. One of the ways is by developing the diversification of off-fishing economic ventures. There needs to be integrated economic and ecologic management of the coastal resources to achieve sustainable coastal development. To support it, there

needs to be the increase of health facilities which are subsidized by the government, the optimization of ecosystem conservation potentials, and the preservation of environmental functions.[6,7]

Mangroves are one of the natural resources with high potential which are not yet optimally explored. The mangrove forest ecosystem has highly crucial values for human life. The mangroves can provide various environmental products and services for economic gain. They even support the health of the people by fulfilling some of their needs. These potentials may give the hope of fulfilling the needs and the welfare of the society. Thus, the coastal areas become a crucial part of the economic and developmental activities.

Indonesia has a wide area of coastlines and mangroves. Indonesia has 293.464 hectares of seagrass and it boasts 3,5 million hectares of mangrove. But the mangrove management so far is still partial, with the orientation of "work project" and with "sectoral ego" of policies. These things inhibit the objective of development.

There needs to be a balance between the ecologic, social, cultural, governmental, and economic aspects in integratedly managing the coastlines. An important concept in this management is the *co-management*, where the local society has an active role in determining the policies and in utilizing the coastal areas, bringing positive impacts. Pomeroy and Williams[8] found that the co-management approach requires the division of responsibilities between the related parties, such as the government and the society, in managing the existing resources and the environment.

The co-management principle is manifested in the form of the fishery natural resource ownership right transfer to the society. This concept may accommodate the interests of the society and other users. Co-management is used to manage the mangrove ecosystem in an integrated manner in the economic and the health development of the coastal communities.[8]

The research results will then yield a good mangrove co-management model which may be applied in the society. Based on this framework, it is crucial to carry out an analysis on the mangrove co-management's effects towards the economic development of the coastlines in Madura Island.

Research Methods

The research was carried out along the shorelines of Madura Island, East Java Province, Indonesia. This is a quantitative research combined with a profound qualitative analysis. The sampling of location and respondents are carried out purposely. The data is collected through observation, profound direct interview, and giving out questionnaires to respondents. This study is carried out at four regencies, which are the Bangkalan, Sampang, Pamekasan, and Sumenep Regencies. There were 170 samples which consisted of communal groups, governmental institutions, academia, environmentalists, health facility workers, non-governmental organizations, and CSRs. This research analyzes the relations between co-management and the economic development of the coastal areas. The data is analyzed using the Structural Equation Model (SEM)-Partial Least Square method. Then, the quantitative results are interpreted and combined with the qualitative data.

Results and Discussion

The Importance of Mangrove Co-Management for the Health and Economy

The hypothesis test is a reflection of the relations between the examined variables in this research, as shown in Table 1. A model has a discriminant validity if the AVE root for each construct is greater than the correlation between one construct and another. The test results are shown as follows:

Table 1. The Validity and the Reliability Tests

No	Variable	A.K.AVE	Composite Reliability	Cronbach Alpha
1	Mangrove Co-Management(Y1)	0.516	0.020	0.885
2	Economic and Health Development (Y2)	0.615	0.777	0.713

Source: Data Processing Results using WarpPLS 5.0

Table 1 shows that the square root value of the average variances extracted (AVE) of every construct is greater than the correlation value between constructs. These values show that the research instrument in the form of questionnaires for all variables are valid. The coastal economic development variable has the highest AVE (0,615), which is then followed by the mangrove co-management (0,516). These results show that all variables measured by the chosen indicators are valid. It also shows that there is a link between the mangrove co-management and the economic and health developments.

The analysis of the mangrove co-management types shows that the *instructive co-management* is most dominating, as shown in the table below.

Table 2. Indicator Factor Loading of Every Variable

Indicator Factor Loading of Every Mangrove Co-Management Variable (Y1)				
Variable	**Indicator/Form**	**Item**	**Factor Loading**	**P value**
Mangrove Co-Management (Y1)	Instructive Co-Management	Y1.1	0.765	<0.001
	Consultative Co-Management	Y1.2	0.434	<0.001
	Cooperative Co-Management	Y1.3	0.722	<0.001
	Advocative Co-Management	Y1.4	0.701	<0.001
	Informative Co-Management	Y1.5	-0.433	<0.001
Indicator Factor Loading of Every Economic Development Variable (Y2)				
Variable	**Indicator**	**Item**	**Factor Loading**	**P value**
Economic Development (Y2)	Increase of Income	Y2.1	0.908	<0.001
	Increased Work Opprotunities	Y2.2	0.886	<0.001
	Mangrove Preservation	Y2.3	0.487	<0.001

Source: Data Processing Results Using WarpPLS 5. 0

Table 2 shows that the most dominant type formed or reflected in the co-management variable is the instructive co-management, with the factor loading of 0.765. Thus, this type of co-management is most dominantly applied in the coasts of Madura Island, where the government gives information on the determined decision, then the society applies it. Such type of program is an effort to revive the degrading mangrove condition in Madura.

The research results show that the mangrove co-management has significantly positive effects towards the economic development of the coastal areas. The table below presents one of the evidences of the mangrove co-management's effects towards economic development.

Table 3. Results of the Hypothesis Test

No	The Relationship Between Variables (Explanatory variable à Response Variable)		Path Coefficient	P value	Significance
1	Co-management (Y1)	Coastal Economic and Health Development (Y2)	0,212*	0,012	Significant

Note: * = significant at $\alpha = \leq 0.05$ (5%).

Source: Data Processing Results Using WarpPLS 5. 0

The table above shows that the hypothesis tested in this research is the effect of co-management towards the economic and health development of the coastal areas. The analysis results using the WarpPLS yields the path coefficient value of 0,212 and the p-value of 0,012, which is significant at $\alpha = \leq 0{,}05$ and with the error rate of merely hanya 5%. Thus, this hypothesis is valid. The path coefficient value is positive and this value shows the tendency that the better the co-management, the better economic development of the coastal areas.

During the New Order Era, the developmental policies rest on the paradigm of "growth first and distribution later" and "growth and redistribution", which are actually proven to fail in bringing this nation to achieve welfare. An approach which has prioritized the economic sector as the commander of development has resulted in poverty, unemployment, lack of access to health facilities, and various inequalities, such as that between groups of society, between sectors and between regions.

Influence of the Mangrove Co-Management towards the Coastal Economic and Health Development

There is a shift from the economic growth-oriented developmental approach towards income equality and social justice, or the developmental framework which emphasizes the society's capability in controlling the condition and the environment. The research results show that economic and health developments are necessities which are relevant to the developmental policies of coastal areas and villages in the regional autonomy era, which aims to tackle unemployment and mass poverty. The greater the roles of the government and the stakeholders, the better the economic developments of coastal communities. The results of this research's analyses show the mangrove co-management's influence towards the coastal economic developments in Madura are as follows:

Increase of Income and Health Access

Basically, integrated regional developmental strategies require a synergy of sustainable social and economic development. The objectives are not limited to increasing income and social welfare, but also to encourage the increase of community participation in economic activities to develop all of their potentials.

The research results and the empirical facts show that the people of Madura coastlines have succeeded in obtaining extra money from the result of managing mangroves in the area, including from the sales of mangrove seeds, crabs, coffee, tea, and mangrove honey. They also profit from mangrove ecotourism. This results in the increased welfare of the society; thus, they are able to access health services and facilities. This shows that the mangrove co-management is effective in increasing the society's income. It is an indication of a good coastal economic growth.

New Job Opportunities

The research results show that the mangrove co-management increases the job opportunity indicator, with the factor loading of 0,886. This value shows that the availability of job opportunities is an important factor and is an indicator of the coastal area's economic development. The Madura Island coastal communities which have managed the mangroves have new job

opportunities. This surely has positive impacts towards the people's income and economy.

Mangrove Preservation

The integrated coastal area development approach is a developmental policy which aims to achieve sustainable usage of coastal and marine resources and to protect them from destruction. This approach is also used to analyze developmental implications. Kusnadi[5] stated that a systematic developmental policy framework is required to resolve poverty of fishermen. It must also depend on the resources which are easily-accessible.

The mangrove preservation indicator has the factor loading value of 0,487. This shows that it is a crucial indicator of the coastal people's economic development. The condition of the mangrove forests in the coasts of Madura Island has improved in several areas, especially those which are managed by community groups. The increase of the environmental quality means that the people's health conditions will increase, as environmental health is highly beneficial to society.

The statistical analyses show that the mangrove co-management has positive and significant effects towards the coastal economic developments if carried out by integrating the ecology, the human resource quality, the institutional roles, and the technological accesses. Other researches which support the link between co-management and natural resource and environmental management include Jentoft[9], Pomeroy and Williams[8], Tulungen[10], Indrawasih.[11]

In this research, the co-management variable is measured using five indicators, which are the Instructive, Consultative, Cooperative, Advocative and Informative co-managements. The empirical facts from the field show that the mangrove management in the coasts of Madura Island has improved and the society's income has increased. This proves that the co-management has positive effects towards the economic developments of the coastal communities. The analysis results are presented in the Figure 1 below:

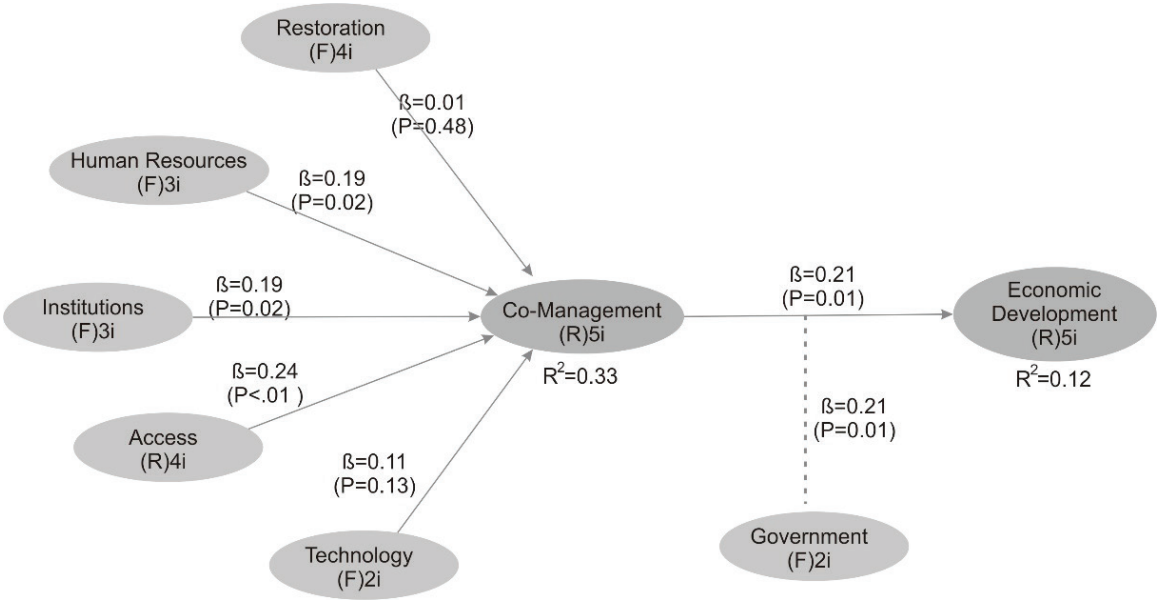

Figure 1. Model Testing Results using the WarpPLS 5.0

Source: The study's conceptual framework

In this research, the coastal economic development variable is tested with the affecting factor, which is the mangrove co-management. The analysis results show that the mangrove co-management has positively significant effects, with the value of 0,212. Thus, the co-management concept carried out in the coasts of Madura Island has strong effects towards the coastal communities' economic and health developments.

The co-management model's success is highly

influenced by the government's willingness to decentralize the responsibility and authority to the society and the stakeholders[12]. Thus, this co-management requires support from the government and the society.

The main elements which must be paid attention to in the mangrove co-management are: the delegation of responsibilities and authorities in mangrove management; social, cultural, economic, and health objectives; also, the sustainable mangrove management. The indicators of the coastal communities; economic development include the increase of the society's income; the availability of new job opportunities, and the increase of mangrove conservation. This condition is experienced by the coastal communities of Madura Island who have managed the mangrove ecosystem.

Conclusion

Based on the tests and the analyses on the hypothesized effect, it can be concluded that:

1. There are some forms of mangrove co-management in the Madura Island coasts, sequentially written as follows: instructive, cooperative, advocative, consultative, and informative co-managements.

2. Mangrove co-management has positively significant effects towards the development of health and economy in the coasts. If it is carried out in a good and an integrated manner, the condition of the coastal economic development will also improve.

3. The economic development of Madura Island's coasts through the mangrove co-management is marked by the increase of the people's income, the availability of new job opportunities, and also the improving mangrove condition, with better quality and quantity.

Ethical Clearence : No Need

Conflict of Interest : No

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A Better Choice for Primary Anterior Tooth Restoration Between Strip Crowns and Zirconia Crowns: A Narrative Review

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Abstract

Pediatric dentists very commonly encounter patients with carious primary maxillary anterior teeth demanding esthetic treatment. Meeting this need of the patient and their parents is the greatest challenge to the clinician, considering the tender age of the patient and limited cooperation available during the treatment. Strip crowns were popularly used conventional esthetic restorative option to treat such teeth with a good degree of acceptance from patients and their parents. However, they are technique sensitive and have other disadvantages. The process of overcoming these drawbacks introduced zirconia crowns to the primary anterior esthetic armamentarium. This review discusses the comparison between strip crowns and zirconia crowns for primary anterior tooth restoration. The PubMed database was accessed to search for relevant articles using key terms: (primary anterior teeth) AND (strip crown) and (primary anterior teeth) AND (zirconia crowns).

Key words: *Primary anterior teeth, Esthetics, Strip crowns, Zirconia crowns*

Introduction

Early childhood caries (ECC) is highly prevalent global disease that specifically affects children with deciduous dentition.[1] High sugar intake commonly associated with improper feeding habits and poor oral hygiene are the clearly stated causes for ECC, which typically results in smooth surface caries affecting the maxillary anterior.[2,3,4] Severe loss of coronal tooth structure of maxillary anterior of pre-school children affects the speech and esthetics demanding their immediate restoration. Restoration of these teeth to near normal form and function becomes a great challenge for a paediatric dentist, vowing to the reported high failure rates.[5]

One of the popular esthetic treatment options, that is widely practiced for more than 3 decades is strip crown composite resin restoration.[6,7] Strip crowns are the crown forms used for restoring primary anterior teeth having large multi surface caries, however with sufficient tooth structure available following caries removal.[6,8] While strip crowns were known to offer immediate supreme esthetics to carious primary anterior teeth, their questionable long-term esthetics, durability and technique sensitivity were the biggest concerns.[6,9]

To overcome the said disadvantages of the strip crowns, primary anterior teeth esthetic dentistry saw lot of advent in terms of various full coverage crowns.[5]

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Among them zirconia esthetic crowns are the latest ones marketed since 2008 and are greatly accepted.[8] Prefabricated zirconia crowns are said to offer superior long term esthetics to the patient.[8, 10] These crowns are fabricated using yttrium-stabilized zirconium by milling technique or injection molding and they are autoclavable.[11] While these crowns have mechanical properties akin to a metal crown, they can't be adjusted via contouring or crimping or trimming thus demanding greater tooth reduction for a passive fit.[10, 12] Another disadvantage of zirconia crowns is that, the crown color can't be adjusted to match the adjacent tooth color.[12] The awareness and the knowledge of pediatric dentists seems to be lacking to use zirconia crowns as compared to strip crowns as per a web based survey conducted in 2017.[13]

At present, both strip and zirconia crowns are popularly available in the market. When there is an indicated case in which either of the above-mentioned restoration options can be used, paediatric dentist must weigh the pros and cons of each to choose one. Through our search we could find only one randomized controlled trial published till date, which compares these two widely used crowns in paediatric dentistry as primary anterior teeth restoration.[8] This review aims at comparing different properties of strip and zirconia crowns as studied individually and quoted in the literature to help the clinician make a better decision for restoring anterior esthetics of the child.

Methodology and Analysis

The PubMed database was accessed to search for relevant articles using key terms. Articles published in English and full articles were included in the final analysis. The search strategy used the following keywords: (primary anterior teeth) AND (strip crown) and (primary anterior teeth) AND (zirconia crowns). Figure 1 describes the details about the articles found on search strategy and included during this review. Title, abstract and full text screening were done by three reviewers. Any sort of disagreement found was settled by discussing among three reviewers. The quality assessment of the included cross-sectional studies were based on Newcastle – Ottawa Scale with three components (selection, comparability and outcome measure) .The total quality score range from 0–10 (Table 1).

Table 1 depicting quality assessment of included cross-sectional studies based on Newcastle – Ottawa Scale

	Selection	Comparability	Outcome	Total score
Holsinger et al. (2016)	***	-	**	5
Halawany et al. (2017)	*****	*	**	8

Analysis of one randomised controlled trial (RCT)[8] included in the study carried low risk in terms of random sequence generation, allocation concealment and attrition rate. It carried high risk in terms of blinding, outcome assessment for blinding and selective reporting.

Results and Discussion

One of the greatest challenges encountered by the paediatric dentists is restoring the primary teeth to good esthetics.[14] It's directly related to the child/ parent's concern about the esthetic impact of restorative therapy that you suggest for an anterior tooth. While, the existing literature provides a broad idea on the various indications and choices available for primary esthetic zone restoration, the treatment decision is finally dependent on the clinician's opinion, clinical expertise and experience.[15, 16] Currently, strip crowns and zirconia crowns are trending esthetic restorative options for anterior teeth with few common overlapping indications between the two.

Strip crowns are the simple clear plastic crown forms, which will be trimmed and adjusted to fit the tooth to be restored. Adequate seating of the crown form requires minimal incisal and interproximal reduction of the tooth. Following filling the crown form with composite, it will be placed on the tooth. After curing, the crown form will be cut, stripped out and hence the name strip crown. The final restoration requires minimal finishing and polishing.[17, 18] While isolation is critical for the success of strip crowns, Psaltis et al[19] described an alternative simple and cost effective isolation technique for placing strip crowns sing orthodontic elastomers.

Kupietzky et al[20] reported excellent overall parent satisfaction with resin composite strip crown restorations of the multi surface carious lesions of primary maxillary anterior teeth. This study also found that the durability of the crown was the parental concern and it negatively affected overall parental satisfaction than the perfect colour match. Various restorative materials have been tried for filling the strip crown forms. Vignesh et al[21] suggested Protemp 4 as a possible alternative to packable pedo shade packable composite resin to be used inside strip crown forms. Kupietzky et al[6,7] have also studied elaborately the clinical performance of strip crowns. They suggested this crown form to be an esthetic and durable choice for restoring primary anterior teeth with sufficient tooth structure. In an interventional study followed until 9 months by Duhan et al.,[22] strip crowns showed loss of retention at 9 months with no changes in anatomic form and loss of surface texture in the retained samples.

One of the said limitations of the composite strip crown is that it can't be used when there is limited tooth structure available.[18] However, there are case reports available in the literature[5, 23, 24] which describe restoring even grossly decayed primary maxillary anterior teeth using strip crowns with good success using the support of various posts. A clinical study by Sharaf AA[25] reported significantly improved fracture resistance of strip crowns following placement of intraradicular composite cores. Versatile strip crowns have even been tried successfully for restoring permanent maxillary anterior teeth with developmental defects.[26]

Zirconia crowns are the latest addition to primary tooth esthetic dentistry, which has gained lot of popularity with it's promising properties.[18] They are referred to as "ceramic steel" as they combine the esthetics of ceramics and strength of steel.[27] They also offer excellent biocompatibility, colour stability and durability.[18, 27] Literature reports comfortable function and pleasing esthetics achieved with the use of zirconia crowns and suggests them as an alternative for the restoration of primary maxillary anterior teeth.[11, 27] The child patient's, as well as the parental satisfaction was high with the esthetic results obtained using zirconia crowns.[12, 28, 29] The highly polished surface of zirconia crowns, repels the plaque accumulation thereby offering excellent gingival health.[27, 30] Yanover et al[31] reported good marginal integrity with well adapted healthy gingiva following restoration of carious primary maxillary anterior teeth.

These are full coverage restorations, unlike strip crowns and inability to adjust them, demand greater tooth reduction and passive fit.[10, 18] Clark et al[10] reported in their in vitro study that zirconia crowns require more tooth preparation in comparison to stainless steel crown for both primary anterior and posterior tooth. However, zirconia crowns can even be used to restore severely mutilated teeth and while doing so, clinicians may take the help of various posts and core build ups.[30, 32]

Upon reviewing the clinical performance of strip and zirconia crowns, the former has been reported to have a success rate greater than 80% on 2 years follow-up, while the later had a success rate of 100% on 6 months follow-up as reported in a systematic review by Schmoeckel et al[4] on different interventions for early childhood caries. Alternatively, the failure rate for strip crowns ranged between 0- 51% while done chairside and under general anaesthesia, as reported by various authors.[9, 33, 34]

During our search, we could find only one RCT comparing the zirconia crowns to strip crowns.[8] This was 12 months follow up study, which showed no secondary caries and better gingival response in relation to zirconia crowns throughout the study period, however with greater wear of the opposing tooth. Alternatively, 6.7% of teeth restored using strip crowns had secondary

caries. Crown failure was seen only with 2 teeth restored with zirconia crowns and the cause for failure was trauma. With respect to the strip crowns 30% showed small but noticeable area of restoration loss and 8.3% showed larger restoration loss at the end of 12 months.

To summarize strip crown is a crown former which is indicated in restoring primary maxillary anterior teeth having 1) multisurface caries with or without endodontic treatment; 2) developmental defects or fractured due to trauma. It is contraindicated in cases where isolation can't be achieved and in teeth with inadequate tooth structure to support the restoration. The advantages include- it involves a simple procedure, good immediate post-op esthetics, shades can be matched with the composite, cost effectiveness and the repair of the restoration is possible. The disadvantages of the same are technique sensitivity owing to poor bond strength and discoloration following contamination with blood or saliva, no long-term durability, questionable esthetics over a period and questionable marginal integrity (Figure 1).

On the other hand zirconia crowns are full coverage crowns for primary anterior and posterior teeth with 1) Multisurface caries with or without endodontic treatment; and 2) Developmental defects or fractured/discolored due to trauma. The contraindications include- the crowded dental arch, uncooperative children, the teeth with less than 2mm of supra-gingival margin and the teeth, which are to be used as abutments for space maintainer. While they have excellent long-term esthetics, good marginal integrity, good gingival health (Figure 2), better strength and retention and are not technique sensitive, the disadvantages are the crown can't be manipulated in the form of crimping, contouring or trimming, precise and greater tooth reduction is required, color matching not possible and are expensive.

3M^{TM} is the popular commercially available strip crown form, where as Cheng Crown, EZ Pedo, Kinder Krowns , NuSmile and Kids-e-crowns are the different brands of zirconia crowns available in the market.

(Figure 1: A : Pre-op, B: Immediate post-op, C: 12 months follow up)

(Figure 2: A : Pre-op, B: Immediate post-op, C: 12 months follow up)

Conclusions and Recommendations

Clinicians have to wisely choose between these two for a given case. This article gives an overview of the discussed crowns and may easy down the decision-making task for a paediatric dentist. The limitation of this review is that we included only one database for our search. Based on our search clinical studies comparing various aspects of these two widely used crowns in paediatric esthetic dentistry are lacking and recommended in the future.

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Successful Cardiopulmonary Resuscitation Rate and Related Factors in Emergency Departments

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Abstract

Cardiopulmonary Resuscitation, also called CPR, is a set of actions taken by conscious and willing individuals to restore the functions of two vital heart and lung organs and deliver blood and oxygen to the brain to prevent brain damage. This is an immediate intervention to prevent or delay death in a person who has suddenly had a cardiopulmonary arrest. About 40 years have passed since the start of cardiopulmonary resuscitation, so the survival rate is low. Research has shown that about 10 to 15 percent of people survive after resuscitation. Cardiac resuscitation is on the rise among hospitalized patients, so these measures and the factors that contribute to its success are crucial.Our aim is to review the success rate of cardiopulmonary resuscitation and its associated factors in emergency departments and hospitals. In this review study, keywords such as cardiopulmonary resuscitation, cardiopulmonary arrest, emergency, underlying cause, success rate, resuscitation outcome and related factors and search on reputable Google scientific databases Scholar, Pubmed, Web of Science, WHO Web site, Articles and theses published between 1989 and 2019, and finally a number of sources were selected and critiqued, interpreted, and analyzed. We concluded at the time of cardiopulmonary arrest, the speed of technicians, equipment, and devices and medicines used will increase the chances of successful cardiopulmonary resuscitation.

Key words*: cardio-pulmonary resuscitation, cardiopulmonary arrest, emergency, underlying cause, rate of success, CPR outcome*

Introduction

Cardiopulmonary resuscitation is all that is done to return to life after death for two important organs of the body, such as heart and lung. It has been found to be more effective in patients who have died from sudden death than those who have died from underlying and dangerous diseases. Sudden death is a death that occurs naturally without any known or underlying disease and loses life symptoms (1, 2).

One of the greatest and most important scientific achievements in the world is immediate intervention to delay death or prevention of one's death, who has sudden stroke and has lost his life symptoms. Favorite in hospital resuscitation, is complete recovery of one's life. Many factors influence the success of resuscitation including types of underlying disease that a person has, the interval time between cardiac arrest and resuscitation, the availability of hospital and emergency services, equipment and supplies needed for resuscitation (3). For the first time, in 1960 Kowenhoven, performed cardiopulmonary resuscitation but still despite of developments in techniques and medical staff, mortality rates after cardiopulmonary arrest is high compared to other causes of death. Cardiopulmonary resuscitation is a series of organized actions performed on patients with cardiopulmonary arrest and is an attempt to keep the circulatory and respiratory systems active enough to provide enough oxygen to keep the body's vital organs alive. Until the spontaneous physiological activity of the circulatory system returns to normal (5). On average, the United States annually loses about 400,000-350000

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people because of sudden cardiac death that most of them had an unsuccessful Cardiopulmonary resuscitation (6, 7). In studies of American Heart Association has repeatedly emphasized the need for a more immediate Cardiopulmonary resuscitation in 3 to 5 minutes which increases person survival after a cardiac arrest. Also, public education to learn about resuscitation and its principles has been emphasized before EMS arrived (8). Cardiopulmonary arrest is responsible for half of all deaths (9). Overall, 50% of deaths occur suddenly, of which only 25% perform cardiopulmonary resuscitation (10). Most of the deaths that occur in different parts of a hospital are largely dependent on one's underlying disease and are not similar to those of cardiac arrest outside the hospital, so researchers are now studying Cardiac arrest mainly uses and emphasizes epidemiological records and knowledge. The epidemiology of cardiac arrest focuses on factors that are individually and increase the risk of cardiac arrest (11).

The number of successful cardiopulmonary resuscitation cases is one of the most important and most noteworthy features of the emergency, for example, the high rate of which confirms the success of the emergency (12). In previous studies, some factors such as location and time of cardiac arrest were considered. for example, Dehaven et. al. declared cardiac arrests which occurred out of emergency will have an unfavorable resuscitation (13). Nurses are among the first people to prepare for their responsibilities at the time of cardiopulmonary arrest. They must have had extensive experience in the field of cardiopulmonary resuscitation. Since the late 1980s, there have been numerous studies on CPR skills (14). Some of these studies have shown the impact of having educated people on the resuscitation process (15-19).

In many cases, at the moment of cardiopulmonary arrest, the individual may return to life if performed quickly and correctly (20). Nearly 40 years after the start of the procedure, the survival rate after resuscitation is not ideal, and mortality rates after cardiopulmonary arrest remain high compared to other causes of death. In developed countries, cardiac arrest survival rates in hospital and out of it are less than 30% and 10%, respectively (21). Out-of-hospital cardiac survival rates range from 2% to 26%. Reports of success rates of cardiopulmonary resuscitation in different countries vary widely (11, 22, 23) 2 to 27% at in-hospital cardiac arrest and 2 to 10% at out-of-hospital cardiac arrest. Reported (24-27)

Methods and Materials

In this Narrative Review study, we used key terms including cardiopulmonary resuscitation, cardiopulmonary arrest, emergency, underlying cause, success rate, resuscitation outcome by searching the databases. International sites including: pub med, Web of science, Google Scholar, Scopus, Elsevier, and internal science databases including: Barakatkns Knowledge System, Academic Jihad Scientific Database, Medlib Iranian Medical Library, Magrian journals database, Civilica knowledge reference and search on the WHO site.

A total of 70 scientific sources, including books, articles, theses, and reports published between 1989 and 2019 in Farsi and English on the success of cardiopulmonary resuscitation and related factors, were collected. Unrelated articles and sources were excluded and references related to our review were studied. Finally, 55 articles and scientific sources were selected and analyzed according to the purpose of the study.

Findings

Much has been done to increase the impact of cardiopulmonary resuscitation on patients who have died. Considering the factors that can contribute to cardiopulmonary resuscitation including the types of underlying disease that a person has, the time spent between cardiac arrest and cardiopulmonary resuscitation, the availability of experts, emergency services, Required Devices and Supplies (3). Factors that were found to be effective in resuscitation failure include age, time interval between cardiac arrest and resuscitation, cardiac arrest time, hospital staff skills, and some physiological variables such as systolic blood pressure, arterial acidity, serum potassium level and primary rhythm (28).

Patient's age

Different people of different ages have different reactions to disease, cardiac arrest and resuscitation. Generally young people have shown a better response to cardiac resuscitation.

Thus age has been identified as one of the most important factors in the ultimate success of cardiopulmonary resuscitation. Due to Jaberi's study, people under 50 years old had a higher successful resuscitation rate also based on study of Keivanpazhouh et al. approximately 42% of patients under 55 years old and 35% of patients over 55 had successful resuscitation (30).

Cardiopulmonary arrest location

Cardiac arrest can occur in different locations, including outside the hospital, as well as in the hospital and its various departments, where people's response to cardiopulmonary resuscitation varies both outside and inside the hospital.

According to the study of Khoshfetrat, 57.3% of people in different parts of the hospital except ICU had a cardiac arrest, 24.6% of patients in the emergency and 16.1% outside of hospital had cardiac arrest, 81 percent of whom Out of the hospital, they were resuscitated and 63% of those resuscitated in the hospital were unsuccessful (31).

Systolic pressure

When the heart contracts or is the pressure inside the arteries. In the elderly, because of the reduced elasticity of their arteries, they cannot be easily expanded, leading to elevated systolic pressure. In Borimnejad study of 63 patients who had cardiopulmonary resuscitation and whose systolic blood pressure was controlled several hours before cardiac arrest occurred, 89.7% of them had blood pressure above 130 mm Hg (28).

Potassium

Potassium is a chemical (electrolyte) in blood that is required for proper functioning of nerve and muscle cells, especially heart muscle cells. Sometimes the amount of potassium is higher or lower than normal. 43% of patients with cardiac arrest had high blood potassium and there was a significant relationship between arterial blood pH and primary cardiac rhythm (28).

Discussion and Conclusion

Cardiac arrest is known as stopping blood circulation. In this case, the heart muscle has no contraction and heart has no outflow or blood flow (32). Cardiac arrest is different from a heart attack or bloodflow disorder, although cardiac arrest may also be due to a heart attack (33). Heart disease is more common in old age, and is more common in men than in women (34). Before taking any cardiopulmonary resuscitation procedure, make sure that it does not threaten the health of you or the injured person with the spread of diseases such as AIDS and hepatitis. The most important change in 2010 resuscitation guidelines is the change from A-B-C (airway-breathing-circulation) to C-A-B (circulation-airway-breathing) (35). With the exception of newborn infants, out-of-hospital CPR stages are (left to right) respectively: cardiac massage, airway opening, and artificial respiration (36). Symptoms of cardiac arrest are sudden and severe and include:

Sudden fainting, lack of pulse, interruption of breathing and loss of consciousness. Sometimes other signs and symptoms are seen before cardiac arrest. For example, fatigue, faint, dizziness, chest pain, shortness of breath, weakness, palpitations, or vomiting are among the symptoms that occur before cardiac arrest. But cardiac arrest often occurs without any warning. The first cause of cardiac arrest is usually a disorder of the heart rhythm (arrhythmia), which is the result of a problem with the heart's electrical system. Unlike other muscles in the body that rely on neural connections to get the electrical stimuli they need, our heart has its own electrical stimulus, a special group of cells called the sinus node, located in the right upper chamber of the heart (right atrium). The sinus node generates electrical impulses that flow through the heart in an orderly manner to synchronize the heart rate and coordinate the pumping of blood from the heart to other organs.

If there is a problem with the sinus node or the flow of electrical pulses to the heart, it may lead to an arrhythmia that will cause the heart to become too fast, too slow or irregular. Often, these interruptions in the rhythm are transient and harmless, but some types of arrhythmias can be serious and lead to sudden stoppages in heart function (heart arrest). If the person has pulse but has stopped breathing, he should continue mouth-to-mouth breathing (37).

The most common cause of cardiac arrest is a type of arrhythmia called ventricular fibrillation. Ventricular fibrillation occurs when rapid electrical pulses cause

your ventricles to shake in vain rather than pumping blood.

Most often, cardiac arrest-induced arrhythmias do not happen by themselves. In a person with a healthy heart, a sustained irregular heart rhythm, without an external factor, such as electrical shock, drug use, or a severe trauma to the heart cycle (heartbeat) is unlikely to progress.

The most common cause of cardiac arrest out of hospital and its deaths in adults is cardiovascular disease. Coronary artery disease and cardiomyopathy causes 10-15% of sudden deaths (38). sudden death incidence increasing at age 30 with its peak at age 45 to 75. The incidence in adult population is approximately 1 to 2 cases per 1000 individuals per year (38,39).

As a sign of death in most heart disease is sudden death to further reduce related mortality from disease, according to CPR and basic determinants of its success that increase the efficiency of cardiopulmonary resuscitation outside hospital is in the center of researcher's attention. Outpatient cardiovascular outcomes are reported to range from 1.4% to 29%, and this success is more in towns and less in cities (40). Possibility of successful CPR Outside of hospital depends on several factors. The probability of success depends on the patient's first recorded rhythm, better prognosis in ventricular tachycardia (VT) and then ventricular fibrillation (VF) and resuscitation time, collapse interval to resuscitation, and collapse interval to first electric shock (40).

In Jaberi et al.'s study of age variables (final success only), collapse interval time to endotracheal intubation, non-monitoring of cardiac arrest, underlying disease (initial success only), first rhythm on monitor, and hospitalization (final success only) increased resuscitation success. However, only the variables of the collapse interval time to the resuscitation team presence, non-monitoring of the patient before cardiac arrest and the first rhythm on the monitor independently predicted resuscitation success. Gender, hospitalization to cardiac arrest interval time, collapse interval time to resuscitation, number of resuscitations were not correlated with resuscitation success (29).

The largest number of cardiac resuscitation trials in four hospitals by Branner and his colleagues accounted for 433,985 of patients over the age of 65 in the United States from 1992 to 2005 (41); The rapid review of these studies shows despite of outside-hospital resuscitation, hospital resuscitation speed related factors are not as important as resuscitation quality related factors(42). Alba et al. regarding to America heart association declared quality of resuscitation as an important factor in successful resuscitation. In Karnsted study resuscitation success increased from 8am to 4pm, another manifestation of the effect of resuscitation quality on its success (43, 44). Marik et al. believe the speed of resuscitation is important on its success (45). In the Boyd and Peters study in Australia, similar results have been observed and the emphasis on the speed of resuscitation has been successful in resuscitation (45). Based on Jaberi's study, time interval between hospitalization and cardiopulmonary arrest has no effect on resuscitation outcome (29).

According to Nazri et al.'s study, the highest number of cardiopulmonary resuscitation occurred in the morning shift with a frequency of 41.2%, followed by night shift with frequency of 31.2% and evening shift with frequency of 27.6%, but There were no significant correlations between shift and outcome of cardiopulmonary resuscitation as well as the gender of the patients on resuscitation rate (46). Also, the mean age of patients studied was 43 and this was similar to the mean age in the Jafarian study (47) in Nichols and selenium study, age has not had much effect on success rate. It seems additionally to age there are some other effective factors on success of CPR (48,49).

Cardiopulmonary arrest location was one of the factors influencing the outcome of resuscitation; more preparations and the continued presence of anesthesia technicians to perform resuscitation and airway resection was an important determinant of resuscitation success. As of most Cardiopulmonary arrests occur in ward, it is recommended that respected authorities provide the conditions in the ward and add to the success statistics.

The interval between cardiopulmonary arrest and the onset of cardiopulmonary resuscitation has been one of the most discussed variables during the study of Nazri et al. According to research one of the most important reasons for the success of the first minute resuscitation after 5 cardiopulmonary resuscitations

is timely and effective cardiopulmonary resuscitation, which significantly increases the chance of success; The most important objectives of this variable in this study were to obtain this time interval to improve the quality of cardiopulmonary resuscitation and hospital readiness in emergency departments and wards, but, contrary to expectations, in all patients with cardiac arrest, the time of onset of cardiac arrest and the onset of resuscitation in the files were quite similar, which, according to personnel's quotes , this value may be due to inaccurate recording of data because it may take a short time after being recalled from the patient's bedside. The purpose of this study was to obtain this interval and measure the speed of the resuscitation team, but this time Recorded in file was not valid (46).

One of the most important factors in a successful resuscitation is the opening of the airway and the patient's intubation in less than 5 minutes. According to the hospital staff, 115 technicians do not intubate the patient and act only in transferring the patient to hospital. This is due to insufficient education so that the necessary education and awareness can help patients successfully resuscitation (46).

Conclusion

Given above, at the time of cardiopulmonary arrest, the speed of technicians, equipment, and devices and medicines used will increase the chances of successful cardiopulmonary resuscitation.

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Covid-19: Information, Transference, Properties of Human Coronaviruses, and Enhancing Immunity by Dietary Recommendations

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Abstract

In Wuhan City, China was found a new type of viral infection could be a novel CoV strain (2019-nCoV) which as an acute respiratory syndrome CoV-2 (SARS-CoV-2) and formerly, severe acute respiratory syndrome (SARS). All these emerging infectious diseases are caused great damage to general health.New Coronavirus has diffused very quickly at the world and the World Health Organization has declared it a pandemic. It usually affects people who are immunocompromised. Immunodeficiency often comes from nutritional deficiency and this makes viruses stronger. Therefore, this review focused on the identification of the SARS-CoV-2 virus, its transmission and spread, and the history of Coronavirus. In addition, how to boost immunity by learn about nutrition recommendations and healthy diets during the COVID-19 pandemic.

Key words: *2019-nCoV; SARS-CoV-2; COVID-19; Nutrition; immunity; dietary recommendations.*

Introduction

Coronaviruses (CoVs) are widely present that symptoms began with the development of severe acute respiratory syndrome (SARS) and MERS-CoV in 2002 and 2012, respectively. Currently, Coronavirus Syndrome (Covid-19) appeared Coronavirus Syndrome (Covid-19) appeared in delayed 2019, which poses a health danger due to the continuing epidemic in the world [(1).]

Health workers are making efforts to control the new Coronavirus that was detected in Wuhan, China, in December 2019. Whilst, in 2020, the World Health Organization (WHO) started the disease is "COVID-19" may be due to SARS-CoV-2, and it is concerning to the southern Chinese human seafood market in Wuhan[(2).] Coronavirus (CoVs) belong to the family *Coronaviridae* which producing diseases like SARS, MERS, and also current, COVID-19 [(3),] however, this novel virus is genetically distinct. Until 2020, Coronavirus (CoVs) have caused in great mortality and also keep connected with upper-respiratory diseases [(4).]

Genomic examination showed that SARS-CoV-2 is relatively concerning SARS-like bat viruses and its transmission to humans is not known. However, few large-scale antiviral drugs have been evaluated against COVID-19 in clinical trials, which may lead to clinical recovery [(5)].

What is the virus (SARS-CoV-2)

Coronaviruses are RNA viruses had contained natural hosts and influence multiple systems [(6).] Coronaviruses can reason clinical illness to great severe respiratory diseases such as SARS and MERS [(7).] The SARS-CoV-2 has caused a comprehensive to the worldwide population; in addition, the WHO announced an official name for this disease as COVID-19 [(8).]

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The SARS-CoV-2 brings under the group2B coronavirus [2]. The genome sequences of SARS-CoV-2 shared by 79.5% sequence similarity to that of SARS-CoV [9].

Coronaviruses encode four main proteins, namely Spike glycoprotein (S), Membrane protein (M), Envelope glycoprotein (E), and Nucleocapsid protein (N), in Figure (1) [10].

Transmission, spread, and emergence of SARS-CoV-2

The new coronavirus was identified during four weeks (28 days) from beginning, compared to the time for SARS virus identification (125 days) in Foshan, China [11]. From analyzes, it was found that the primary infection among the infected was common in a seafood market in Wuhan, China (Fig. 2). These restaurants are serving various kinds of overland animals to human consumption, and this may be due to the transmission of infection from animal to human (zoonotic) [12]. Therefore, the SARS-CoV-2 is could be infected from an animal to human and also human-to-human (Fig. 2), and also the potential of food-carried transportation for happening a connected potential [1]. In addition, possible and predictable ways connected with the transmission, similarly respiratory viruses; by immediately relate polluted hands, and surfaces (Fig. 2). It could know if blood transfusion and organ transplantation [13], and also perinatal may be possible for SARS-CoV-2 transmission (Fig. 2).

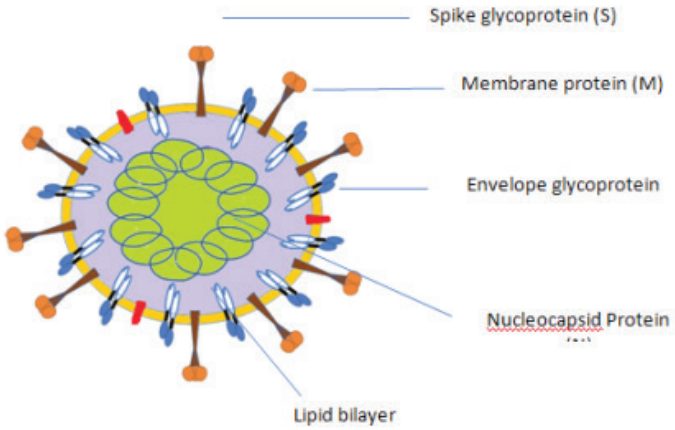

Figure (1): SARS-CoV-2 virus structure [10].

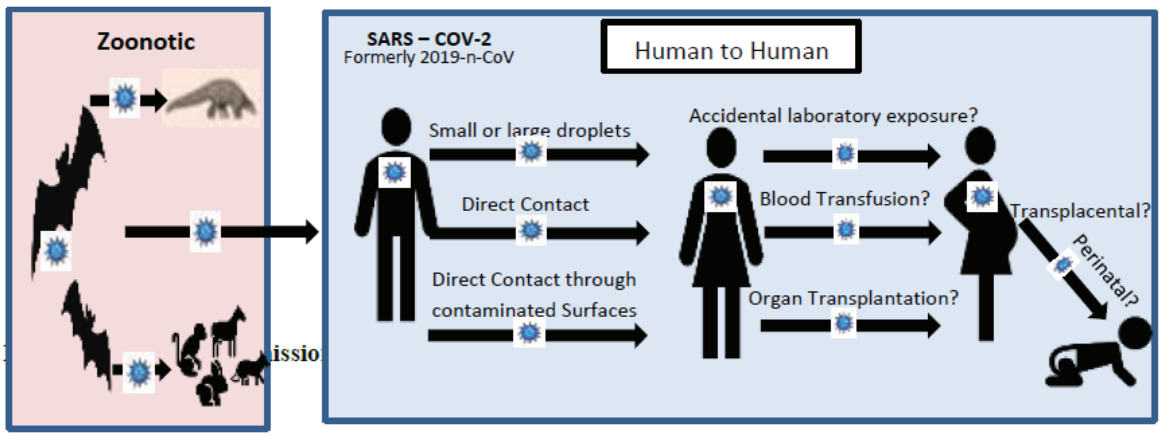

Fig (2): Potential transmission routes for SARS-CoV-2 [1]

Phylogenetic estimated for whole-genome sequences of SARS-CoV-2 found that CoVs of the bat origin were pointed to during 2018 in China [14]. It was pointed to that SARS-CoV-2 have to utilize ACE-2 as an entry receptor whilst showing the same receptor-binding domain as SARS-CoV [15]. Therefore more countries were recommendations for their people when traveling to China [16]. In the past, the efficiency in transmission of SARS-CoV-2 for human-to-human is less, which may be due to the health workers were less influenced by deadly coronaviruses [1]. It could be due to the super infecting for transmission of SARS and MERS [17]. About half of the MERS-CoV situations pointed out in Saudi Arabia had occurred during contact with infected-asymptomatic or with symptomatic [18]. The SARS and coronaviruses can affect the lower respiratory system with average symptoms [19].

Coronaviruses (CoV) in humans – SARS, MERS, AND COVID-19

WHO [8] suggested that the various SARS and MERS infection COVID-19 can be based on the human-to-human transmission that happens during immediate relate. Modern research showed that the aerosol transmission of SARS-CoV-2 is feasible may be due to the virus being able to stay viable in aerosols for more hours and on surface all-day [20].

Peiris *et al.* [21] definition SARS as a viral respiratory illness that may be due to the animal coronavirus produced from the seafood in China after becoming suitable to the human host, and it can be transmitted among humans. **WHO** [22] found that the SARS setting in the year 2002-03 has identified total deaths 9.6%, the coronaviruses have exhaustively influenced China [23]. Thereby the dead rate coronaviruses are less than SARS-CoV, there occurs a heavy worry related may be due to its epidemiological likeness to influenza viruses [24] therefore happen a pandemic [25].

Through the year 2012 is respiratory illness the MERS that was first pointed out in Saudi Arabia. The dead of this illness was rate about 35% [26]. The examination observed incubation period of SARS-CoV-2, SARSCoV, and MERS-CoV, was similar. Whereas, the incubation periods of SARS-CoV-2 (COVID-19) was the longest (14 day), therefore the individuals are isolated for 14 days to protect from the danger of infect [27]. The genome sequence was similar of novel coronavirus (SARS-CoV-2) and SARS-like CoVs, therefore different estimates in the splitting location in the SARS-CoV-2 S protein was lost in other SARS-like CoVs [28]. The furin-like division site is an act to treatment target for furin inhibitors. The infection of SARS-CoV-2 was higher than to it is previously SARS; these results may be due to a mutation that happened in the endosome-linked with protein-connected the domain of nsp2 protein.

There are two potentialities for the transport of COVID-19 it is transmitted from human to other human or directly from bats [10]. Moreover, **Graham *et al.*** [29] indicated that the SARS-CoV originated from the bats and jumped to as a host to become better bounding to intermediate host. This intermediate host-appropriate virus, through their following exposure to humans, elevated to the epidemic strain.

Enhancing immunity by dietary recommendations during the COVID-19 pandemic

Coronavirus disease (Covid-19), points out a significant general health danger to the world. Meanwhile, it would not be a nutrition inhibitor or therapy Covid-19 transmission and it could be a balanced food to give a strong immune system, activity for the body and healthy sleeping habits.

When healthy eating is estimated for coronavirus pandemic, recommendations for health are as follows;

Healthy eating is important in quarantine applications.

Covid-19 is social isolation, and all contacts are minimized, which could be two weeks period to therapy additionally nutrition as a portion of quarantine. The diet is rich in proteins, fibers, vitamins, minerals, and antioxidants, as well as balanced nutrition, is of great significance through these times with a better diet.

Vegetable and fruit consumption should be prioritized.

Vegetables and fruits for their daily and enough consumption should be preserved for every main meal. Therefore, a healthy meal plate had divided into three-quarter the first quarter consists of vegetables, the second quarter is grain products, and the residual quarter has contained fruits, protein foods, and dairy products (Figure 3). In addition, it is suggested to include enough water consumption and utilize of olive oil in daily meals. Thus, food bought had contained vegetables and fruits. Therefore, could preserve healthy food during the quarantine.

Figure (3): "Healthy Food Plate: Healthy Food Plate According to Food Groups" from Turkey Dietary Guidelines [26].

Importance of minerals and vitamins consumption

Micronutrients are minerals and vitamins which are required in very tiny amounts [30]. But together both are extremely important for the normal body functioning in the organism [31]. They are involved in triggering many important biochemical reactions [32]. However, micronutrients are getting attention all over the world during the COVID-19 pandemic for its ability to alter the susceptibility to infection [33, 34]. Cytokines (certain cells secreted substances that affect other cells) lead to inflammation through damaging the lining of the lungs, leading to pneumonia. Vitamins and cytokines modulate immunity and inflammation. Immunity involves vitamins renovate the capability of some cells to produce certain cytokines that affect the mechanism of immune cells [35]. Vitamin E is indispensable to get rid of chronic viral infections [36]. Different water-soluble vitamins like vitamin B complexes, vitamin C, and fat-soluble vitamins (Vitamin A, vitamin D, and vitamin E), different trace elements (Zinc, Magnesium, Iron, and Selenium) have been proved to show a satisfactory effect on enhancing human immune response. Adequacy of iron can protect from respiratory tract infections in severely critically infected coronavirus patients. Essential fatty acids such as omega-3 fatty acid that modulate immune function by its action on inflammatory response [37]. Magnesium is associated with the immune system in both innate and acquired responses. It acts as a cofactor for participation in immunoglobulin synthesis and antibody production. Magnesium is the most overlooked electrolyte, although it has an enormous role in immune function [38]. Hence, the good nutritional status of the host plays a crucial role to deal with different infectious diseases [39]. Therefore, proper nutrition must be ensured to deal with unexpected infections with people who are vulnerable or who are already attacked by a novel coronavirus. If through diet, the daily required amount of different nutrients are not met, different processed food, and fortified with different nutrients can be a solution. These Micronutrients were found the significant role of some food nutrients to tackle harmful RNA viruses including SARS virussee-through boosting up immunity [40].

Vitamin C is the production of interferon that can influence the immune system in addition is absolutely necessary to the white blood cells that help to fight

infections and overall immune system health. Vitamin C is of great significance for iron absorption, and iron deficiency can confirm vulnerability to infections in general [41]. Therefore, food fortification with vitamin C in places of high coronavirus recrudescence is recommended.

As COVID-19 happened the coagulopathy and thromboembolism are predominant and connect to reduce survival. Therefore, vitamin K management becomes better results in patients with COVID-19 [42]. Thus, it could be recommended that utilize vitamin K-rich food sources such as green leafy vegetables and vegetables in food formulations in places of high coronavirus recrudescence especially for COVID-19 patients

Zinc shortage is responsible for 16% of all respiratory infections worldwide and also it could be found the link of zinc shortage with the danger of infection and acute progression of COVID-19 [43]. Supplement of Zn has the possibility to promote antiviral immunity, both innate and humoral, and to become better normal immune cell function, in immune-compromised or elderly patients. Zn may also act synergistically when co-administered with the standard antiviral therapy, for patients with SARS-CoV-1. In addition, it was protecting the cell membrane which could contribute to blocking the virus entry into the cell. Therefore, it may be hypothesized that Zn supplementation may be of potential benefit for prophylaxis and therapy of COVID-19, especially for those who have a Zn shortage [44].

Legumes could be consumed every day.

Legumes are a protein source alternative with a high nutritional value. Therefore, different varieties of legumes could be consumed every day. In addition, these products can be soaking in water, Thus, that is cannot take time during cooking, and then stored in the freezer to consumed. Consequently, these different varieties of legumes were prepared at home as an alternative to buying them in quarantine times.

Importance of high-quality animal protein consumption

Eggs and cheese are contained a high-nutrition value of animal protein therefore it could be consumed enough every day due to include the achievement of antibodies, which great significant defense of the bodies against microorganisms [45]. Moreover, useful bacteria as probiotic was found in yogurts are support the immune system. Probiotics defined as "live microorganisms that, when administered inadequately amounts, confer a health benefit on the host" [46]. Probiotics can act improve immune function, by the production of antimicrobial and organic acids compounds additionally, being improved the gut [47]. **Azad *et al.*** [48] studied that the probiotic species as the *Lactobacillus* and *Bifidobacterium genera*. The results have shown that fermented dairy products could be a good choice to become better the gut microbiota.

Effect of natural antioxidants in coronavirus infections

Glutathione is a strong natural antioxidant in the body, it improves the immune system and it is also used as a nutritional supplement. More research on viral infections for rats showed that N-Acetylcysteine production from glutathione has decreased the period of symptoms by elevating cellular defense and repair. Therefore the glutathione can be taken orally at level 500 mg [49].

Quercetin is a natural antioxidant was found in fruits and vegetables. Quercetin can inhibit infections involved in coronavirus. Quercetin protects lung tissue and also supplement is interested in vitamin C. Therefore, it could be taken orally at level 500 and 1000 mg daily [49]. Quercetin is found in fruits and leafy green vegetables, and black tea [49].

Tannins were known for antiradical activities and anti-inflammatory influences, thus tannins as strong antioxidants can decrease disease dead may be due to redox balance conservation. Gallotannin from tannins was found as anti-inflammatory influences [50]. Tannins are found in constituencies' herbs, spices, nuts; legumes; orange juice; and tea, as well as could be utilized for food formulations in places of high coronavirus infection.

Nutrition recommendations for healthy humans

1. To the power immune system is supplying enough

diet daily for healthy humans.

2. Healthy humans should be to the consumption of fruits and vegetables through period coronavirus pandemic which presents of great significance public health threat in the world

3. Healthy humans could supplement their diet with nutritional supplements to include the constituent of their duties.

4. Healthy humans should be not be found in crowded places and also using digital platforms for meetings.

Potential therapeutic strategies against COVID-19

Uses interferons as an antibiotic, and antiviral to decrease the viral load [51, 52] however, the only remdesivir has shown promising impact against the virus [53]. Remdesivir is an interferon-beta blocker the SARS CoV- 2 replication and patients were declared as clinically recovered [54, 55]. **Richardson *et al.*** [56] estimated combining the antiviral with traditional Chinese medicines were estimated against SARS CoV-2 induced infection in humans and rats. The results reported that exhibit moderate when tested against infection inpatients and in-vitro clinical isolates [57]. Doctors isolated the blood plasma from patients which return to a normal state of health from COVID-19 and inject it into the infected patients the results were found positive recovery for patients [58]. New research specified that the monoclonal antibody (CR3022) binds with the SARS-CoV-2 may be caused to the divergent ACE2 receptor-binding. Moreover, it has been the possibility to be as a therapy, alone or in collection with other antibodies for the treatment of COVID-19 infection [59].

The balance of intestinal micro-ecology and nutritional support

COVID-19 patient is to the stomach and the intestines symptoms that may be caused to immediate viral infection of the intestinal mucosa. The intestinal micro-ecological balance is broken to COVID-19 patients, which is an important lowering of the intestinal probiotics. Therefore it was significant to preserve the micro-ecology using nutritional support.

Microecologics Intervention

(1) Microbiologics can increase the activity of gut bacteria, and decrease infection reduced by gut microflora dysbiosis.

(2) Microecologics can become better the stomach and the intestines symptoms of patients. It can decrease water in feces and lowering diarrhea by preventing intestinal mucosal atrophy.

(3) The hospital analyzed and discovered the disturbance of intestinal flora. Therefore, it could decrease the available as another possibility of intestinal bacterial translocation and gut-derived infection.

(4) Food assistance is significant to preserve intestinal micro-ecological equilibrium. Therefore, it could be utilized based on the influential estimations of food dangers, gastroenteric roles, and ambition hazards.

Nutrition support

Disease COVID-19 patients are at elevate nutritional risks. Therefore, happening estimations of nutrition dangers, stomach, and the intestines roles, and enteral food assistance is significant to the patient's prognosis [60].

(1) Feeding orally is preferred could be, early intestinal nutrition can supply nutritional support, nourish intestines, and preserve intestinal microecology.

(2) Almost all patients often harbor sharp stomachs, and intestines harm apparently as abdominal distension, and diarrhea thus could be recommended using post-pyloric feeding.

(3) It could be recommended that the chosen nutrient solution for patients with intestinal danger, had contained predigested small peptide which absorption in intestinal. For hyperglycemia patients, it could be recommended that nutrition is beneficial to the blood sugar controlling.

(4) Means of nutritional supply was taken by the pump of nutrients utilized at a uniform speed and gradually increasing. In addition, the nutrients can be heated before feeding to lower intolerance.

Conclusions

This review provides an insight into the COVID-19 current situation and represents a picture of the current state of the art in terms of public health impact, immunity, and the role of food in prevention, case management, emergency response, and preparedness. People with short immunity are the most affected by this global epidemic. To help or boost the immunity, the plant-based foods play vital role by promoting beneficial bacteria in the body.

Future aspects need more study to know the behavior of the Coronavirus and the role of food in preventing it. In nutshell, healthy vegetables, fruits, proteins, and drinks are essential against the new Coronavirus by improving the immunity of all age groups.

Consent of Ethics

No possibility of a conflict of benefit was reported by the authors.

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Original Research Article

Estimation of Cephalic index in 17-20 Years old population of Nimad region of Madhya Pradesh

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Abstract

Identification is determination of the individuality of a person based on certain physical characteristics and is of importance in solving criminal as well as civil cases on a regular basis. Examination of fragmentary remains by a doctor can aid the police in complete identification of an individual. Cephalic index is one such parameter that can help in identification of the individual. Cephalic index (CI) and head shape are greatly affected by geographical, sex, age and racial factors. Such data is of great medico legal and forensic importance which indicates that cephalic index can be used as marker of ethnicity. There have been various instances where the race of the individual bone has to be ascertained in solving issues related to forensic or archaeological importance where Cephalic index is an important tool to determining the race and sex of an individual whose identity is unknown. This study was carried out to determine Cephalic index of 400 healthy individual, including 200 Male and 200 Female individuals between 17 years to 20 years the of age, from the Nimad region of Madhya Pradesh.

Key Words: *Cephalic Index, Race, Identification, Craniofacial, Anthropometric assessment*

Introduction

The human body dimensions are affected by ecological, biological, geographical, racial, sex, and age factors[1]. All the factors are an adaptive change brought by natures to adapt to the living environment. The study of the craniofacial relations and variations in man has long been used to differentiate various racial groups in physical anthropology. Morphological features of different races and ethnic groups are not randomly distributed but appear in geographic clusters. [2] Anthropometric assessment aids in determination of geographic distribution of different races and helps in identifying the ethnicity of an individual by means of accurate measurements from the human remains. On the basis of cephalic index, head shapes are grouped into four international categories, which are dolichocephal, brachycephal, mesocephal and hyperbrachycephal.[3]

The study is carried out in Nimad region of Madhya Pradesh as there is no data available on the racial features of the people who belong to this region of the country. The data can be used for identification of race in solving issues of forensic and legal importance.

Materials and Methods

The present study conducted on 400 healthy subjects including 200 males and 200 females, age ranging from 17years to 20 years. All the measurements were taken with the subject sitting on chair, in relaxed condition and the head held in anatomical position.

The head measurements were recorded by using the Martin spreading calliper. The length of the head

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i.e. the maximum antero-posterior diameter was measured from Glabella to Inion, and the breadth of the head was measured as maximum transverse diameter i.e. the bi-parietal diameter between the two parietal eminences.

Cephalic index was calculated using the following equation[4].

$$\text{Cephalic Index} = \frac{\text{Head width}}{\text{Head length}} \text{X } 100$$

Depending upon this index, the types of head shapes are as in Table- 1.

The data were collected and analyzed using student t-test.

Observations and Results

Male Cephalic index is found to be 77.69 ± 4.40, while female Cephalic index is found to be 76.23 ± 4.13. The difference in Cephalic index between male and female was significant (p value < 0.05). **Table-2** shows incidence of cephalic index. **Table-3** shows relationship of sex with cephalic index.

In males length of head varies from 172 mm to 211 mm, the mean length of head is 191.25 mm and standard deviation is 6.88. In female the length of head varies from 166 mm to 197 mm, the mean length of head is 178.72 mm and standard deviation is 5.57. **Table- 4** shows incidence of Head lengths of males and females.

In male breadth of head varies from 129 mm to 160 mm, the mean breadth of head is 145.61 mm and standard deviation is 5.90. In female the breadth of head varies from 122 mm to 154 mm, the mean breadth of head is 138.74 mm and standard deviation is 6.12. **Table- 5** shows incidence of head breadth of males and females.

Table-1: Types of head shapes depending upon cephalic Index

Head shape	Range of Cephalic index (CI) %
Dolichocephalic	< 74.9
Mesochepalic	75 - 79.9
Brachycephalic	80 - 84.9
Hyperbrachycphalic	85 -89.9

Table – 2: Incidence of cephalic Index

Cephalic Index	No. Observed	Cephalic Index	No. Observed
66-67.99	4	80-81.99	46
68-69.99	6	82-83.99	27
70-71.99	38	84-85.99	12
72-73.99	50	86-87.99	9
74-75.99	68	88-89.99	-
76-77.99	81	90-91.99	-
78-79.99	58	92-93.99	1

Table – 3: Relationship of sex with Cephalic Index

Cephalic Index	Male	Female	Cephalic Index	Male	Female
66-67.99	3	1	80-81.99	18	28
68-69.99	5	1	82-83.99	8	19
70-71.99	22	16	84-85.99	4	8
72-73.99	31	19	86-87.99	4	5
74-75.99	35	33	88-89.99	-	-
76-77.99	42	39	90-91.99	-	-
78-79.99	27	31	92-93.99	1	-

Table – 4: Head length in males and females

Head length (mm)	No Observed	
	Male	Female
166-171	0	24
172-177	4	52
178-183	22	90
184-189	50	25
190-195	68	8
196-201	44	1
202-207	10	-
208-213	2	-

Table – 5: Head breadth in males and females

Head breadth (mm)	No Observed	
	Male	Female
122-126	0	2
127-131	4	24
132-136	9	51
137-141	29	57
142-146	78	46
147-151	49	15
152-156	22	5
157-161	9	-

Table 6: Mean cephalic Index in various studies

S. No.	Worker	Race	Mean Cephalic Index
1.	Bhargava et al, 196011	Bhils of central India	76.98
2.	Shah et al 196010	Gujrat, India	80.81
3.	Bhargava et al, 19618	Barelas of central India	79.80
4.	Basu et al. 196312	K. Vanjara, India	79.50
5.	Shah et al 200411	Gujrat, India	80.42
6.	Del Sol et al, 20058	IX Region of Chile	80.42
7.	Golalipur et al., 200710	Turkman, Iran	80.40
8.	Fawehinmi et al., 200813	Port Harcourt, Nigeria	79.80
9.	Eroje, M. A et al., 201014	Ogbia, Nigerua	72.96
10.	Present study	Nimad region of Madhya Pradesh, India	76.96

Discussion

In the present study, the mean cephalic index in both sexes is ranging from 72.83 – 81.01. The cephalic index in male and female is 77.69 ± 4.13 and 76.23 ± 4.13 respectively.

The cephalic index of male is significantly higher than female (p value < 0.05).

Present study finding are closely similar to Oladipo, G. S. & Olotu et al [5] in which it was found that the cephalic index for Ijaw male and female as 80.98 and 78.24 respectively.

In our study, dominant type of head shape was Dolichocephalic (65.66%). This finding is similar to Bhatia et al[6] in India which 58.5% are dolicocephalic.

Dominant type of head from this study is not similar to del Sole et al [7] in Chile, Bhargava et al [8] in central Indian.

Also, the dominant head type was reported as brachycephalic in IX Region of Chile, del Sol et al [7] Turkman of Iran, Golalipour *et al*[9], and in Gujarat, India, Shah et al[10].

It is worthy to note that the percentage of head types also varies in different populations. In a study on 50 individuals in the IX Region of Chile, del Sol reported that 66% of the individuals were mesocephalic, 28% brachycephalic, 4% hyperbrachycephalic and 2% dolichoephalic.

In present study cephalic index is 76.96, very similar to Cephalic index of Bhil community of central India which is 76.98 reported by Bhargava et al, 1960[11].

Conclusion

Present study points out that the mean cephalic index of people belonging to Nimad region of central India including both sexes is ranging from 72.83 – 81.01, cephalic index of male and female is found to be 77.69 ± 4.13 and 76.23 ± 4.13 respectively. The cephalic index of male is also found to be significantly higher than

female (p value < 0.05). Considering the limited racial features distributed over a wide geographic area there is an obvious overlap of features among the population distributed into geographic zones. It is also noted that people of particular origin residing in a particular area have a significant similarity like the Nimad population has a Mesochepalic skull features as revealed from this study. Hence, if the subject under study is of a particular geographic location and there is a data available to compare the skull bone with the data of a particular geographic location, the identification of race is possible. This study has made a significant effort to make such data available now for the population of Nimad region of Madhya Pradesh. Similar study involving larger study group in future will help to substantiate the results and enhance the authenticity of the data.

Funding: Self

Conflict of Interest: None

Ethical Clearance: All ethical parameters were taken care of in the study

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Assessing the Preparedness for Emergency Management of Status Asthmaticus in Pediatric Patients' among EMS Professionals, Pune, India

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Abstract

Background: Asthma is one of the most common diseases affecting children. Children affected by Asthma need recurrent hospitalization and show high degree of absenteeism in schools due to exacerbations. It is characterized as a state of hyper responsiveness triggered by allergens such as pollens, foods, dust mites and animal dander which irritate and cause bronchoconstriction. EMS professionals shoulder great responsibility in pediatric respiratory emergencies as their actions determine the long term quality of life of these children

Aim: The present study is aimed at assessing the preparedness with regards to management of Status Asthmaticus amongst EMS professionals in Pune, India.

Methodology: The study was conducted amongst 189 Emergency medical Services professionals residing in Pune India. A pretested and validated questionnaire was utilized for the study. The questionnaire consisted of 21 items meant to assess the knowledge and skills with regards to Emergency Management of Status Asthmaticus amongst EMS professionals. Each correct answer was given a score of one with maximum score of 21. The questionnaire was administered online.

Result: EMS professionals showed average level of preparedness with reference to management of Status Asthmaticus.

Conclusion: EMS professionals need periodic training to improve their level of knowledge and skills. Simulation play a pivotal role in augmenting the skills of EMS professionals in order to identify suspected cases and provide accurate emergency management.

Keywords: *Pediatric, Status Asthmatics, Hyper responsiveness, Simulation.*

Background

Asthma is a chronic, non-communicable disease ranked 16th amongst the leading cause of diseases around the world contributing to higher rates of mortality and morbidity among children and adults. It is estimated that by the year 2025, 100 million people will get affected by Asthma.[1]

Asthma is one of the most common diseases affecting children. The disease prevalence is nearly 6.8% for age group of 6-7 years and less than 5% for age group of 3-14 years. Asthma has been increasing steadily and prevalence affecting a significant number of children leading to detoriation in their quality of life [2, 3]

Children affected by Asthma need recurrent hospitalization and show high degree of absenteeism in schools due to exacerbations .[4]

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Asthma is a state of hyper responsiveness triggered by allergens such as pollens, foods, dust mites and animal dander which irritate and cause bronchoconstriction. Most frequently change of temperature, cold air, viral infections, exercise and exposure to cigarette smoke can trigger asthma attack .Pediatric patients present with breathlessness and non-productive cough. Significant wheezing may be audible on auscultation of the chest, the child can develop signs of increasing respiratory distress if early intervention is delayed. The child Cn develop severe respiratory distress if early timely intervention is not carried out. [5, 6]

Dharmage e.t al[1] in a study (2019) opined that childhood asthma can lead to long term impairment of lung function with gross anomaly in airway development and gradual progression to Chronic Obstructive Pulmonary Disease (COPD) along with associated co-morbidities like bacterial pneumonia.

A study by Yosef e.t al[7] (2015) found that factors like frequency of attack, night time wakefulness, effect on the daily activities and frequency of exacerbations helps in categorizing asthma as controlled, partially controlled or uncontrolled stage.

Emergency Medical Services need to encounter cases of severe Asthma presenting as Status Asthmaticus. A study by Amorha e.t al[8] (2018) recommended that health care professionals should have adequate knowledge on quality of management and lifestyle of the Asthmatic patient to restrict the Asthma to the controlled stage. Status Asthmaticus occurs due to repeated exposure to triggering factors in the uncontrolled stage. Although asthma can't be cured yet episodes of Status Asthmaticus can be prevented by implementation of proper management guidelines. The preventive drugs indicated in uncontrolled asthmatic are grossly underutilized.

A study by Almutawa e.t al[9] (2014) stated that Health care professionals show low adherence to guidelines of asthma management.

EMS professionals shoulder great responsibility in pediatric respiratory emergencies as their actions determine the long term quality of life of these children[10].

Aim

The present study is aimed at assessing the preparedness with regards to management of Status Asthmaticus amongst EMS professionals in Pune, India.

Methodology

The study was conducted amongst 189 Emergency medical Services professionals residing in Pune India. A pretested and validated questionnaire developed by Aziz e.t al[5] was utilized for the study.

The questionnaire consisted of 21 items meant to assess the knowledge and skills with regards to Emergency Management of Status Asthmaticus amongst EMS professionals. 6 items tested the knowledge on pathophysiology, 4 items on symptomatology and 11 items on the management. Of the 21 items, 14 required binary responses while 7 items were multiple choice questions with one or more correct option. Each correct answer was given a score of one with maximum score of 21. The questionnaire was administered online.

Written and informed consent was taken by the EMS professionals. The participants were asked to submit the completely filled questionnaire in one day by mail. Responding to all the items in the questionnaire was mandatory. All the 189 EMS professionals submitted the duly filled questionnaire.

The data collected was tabulated and statistically analyzed using SPSS version 23.0.

Table 1. Demographic Data Distribution:

Sex	Number (%)
Male	138 (73%)
Female	51 (27%)
Age	Number (%)
< 30 years	174 (92.1%)
>30 years	15 (7.9%)
Qualification	Number (%)
BAMS	99 (52.4%)
BHMS	82 (43.4%)
Others	8 (4.2%)
Experience	Number (%)
< 1 year	94 (49.7%)
> 1 year	95 (50.3%)

Result

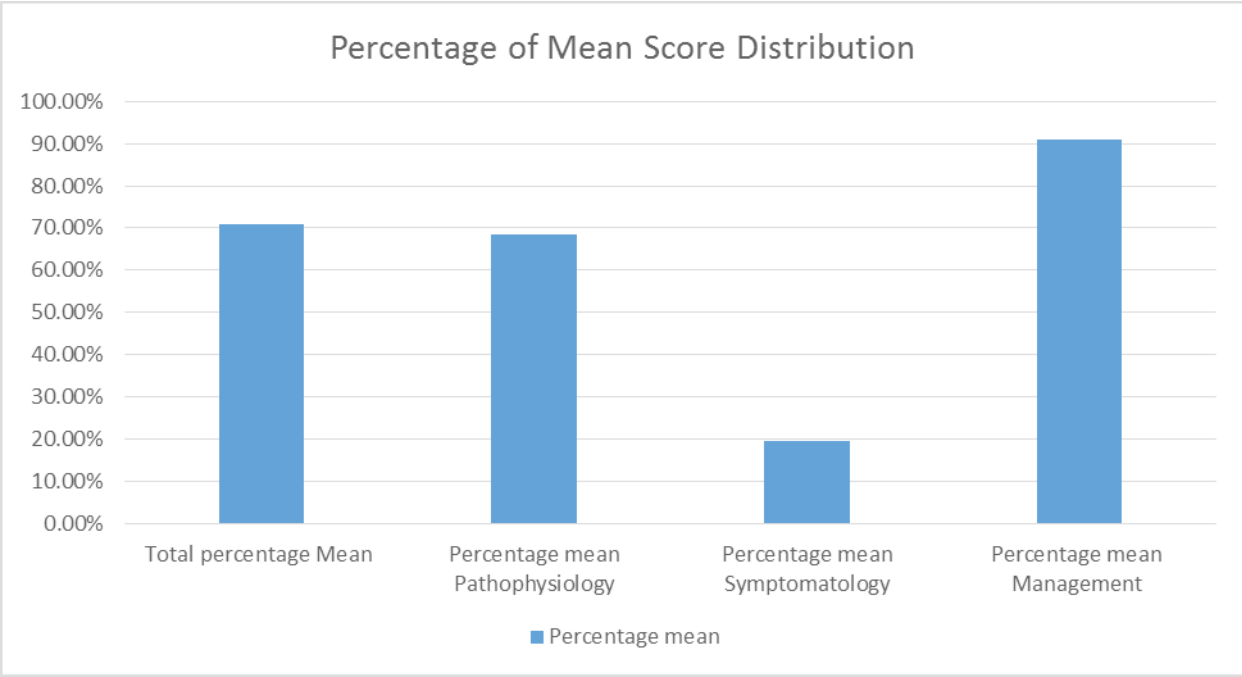

Fig 1. Percentage of mean score distribution

Table.2 Preparedness assessment score

Total Mean Score	14.9
Standard Deviation	3.05

As depicted in fig 1 & table 2, EMS professionals showed average level of preparedness with reference to management of Status Asthmaticus. The poorest score were obtained on the items testing symptomatology knowledge. Fairly high scores were obtained on the items testing the skills and knowledge regarding management aspects.

Discussion

Pediatric uncontrolled Asthma presenting as Status Asthmaticus is a life threatening disease with significant impact on the long term quality of life of the child. EMS professionals are generally more focused on cardiac and trauma emergencies among adults, hence various studies testing their knowledge and skills on the management of cardiac and trauma emergencies yield fairly high scores. The scores obtained in the present study are as best average and reflect the lack of prioritization on pediatric respiratory emergencies in the EMS curricula and on-the-job training. The abysmally low scores obtained on the items pertaining to knowledge of symptomatology are alarming. They point towards the fact that EMS professionals are not equipped to identify suspected cases of Status Asthmaticus, cases of controlled Asthma and the possible complications. Failure to do so can adversely affect the clinical condition of a child suffering from the disease.

The score obtained on items pertaining to emergency management and rehabilitation are encouraging. This indicates that EMS professionals can manage diagnosed cases of Status Asthmaticus astutely.

The importance of continued medical education cannot be over emphasized. Practical sessions using case based vignettes and high fidelity simulation mannikins can positively reinforce the knowledge and skill sets required in effectively handling cases of Status Asthmaticus in pediatric patients.

Simulation offers a safe environment to learn with a team based approach. The short comings found during the simulation sessions can be dealt with during debriefing sessions.

Conclusion

Pediatric Status Asthmaticus requires early diagnosis and prompt management. EMS professionals need periodic training to improve their level of knowledge and skills. Simulation play a pivotal role in augmenting their skills to identify suspected cases and provide accurate emergency management. Further studies can be conducted to study the impact of simulation in this regard.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Obtained from IEC, SIU

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Assessment of Interpersonal Communication Skills Among Emergency Medical Professionals in Pune, India

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Abstract

Introduction: Effective interpersonal communication is one of the most important skills for healthcare providers to master, in order to deliver high quality patient care. Doctors need to communicate with a wide range of people including peers, administration staff, patients, their family members and caregivers. Of course, the most significant is doctor-patient communication as it forms the foundation of the healthcare delivery system.

Poor inter-personal communication skills can jeopardize the patient care process and hence it is expected that healthcare professionals undergo formal training both as part of their medical curriculum and on the job Continued Medical Education. Studies have shown that effective interpersonal communication skills help improve overall healthcare outcomes especially while managing difficult encounters and breaking bad news.

Designing training programs with contemporary relevance requires regular needs assessment. It is essential to evaluate the baseline levels of inter-personal communication skills amongst practicing professionals to devise appropriate training strategies.

Objective: To assess inter-personal communication skills among Emergency Medical Professionals in Pune.

Methodology: The study was conducted among 140 Emergency Medical Professionals (EMPs) employed in various hospitals in the city of Pune, India. A questionnaire tool designed in 2019 by Piyush Ranjan *et al* was utilized to assess inter-personal communication skills among Emergency Medical Professionals.

The tool was administered through online mode. The respondents were given one-day deadline to revert with responses. Any queries pertaining to the questionnaire were clarified during data collection. 131 respondents reverted with completely filled questionnaire.

Conclusion: It relies on self-assessment to assess interpersonal communication skills and has a limited sample size. Yet, the study is an eye-opener to the incidents of conflicts in emergency healthcare settings and the barriers that prevent a healthy doctor patient relationship.

Keywords: *Inter-personal communication skills, emergency medical professionals, conflicts, doctor-patient relationship*

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Introduction

Effective interpersonal communication is one of the most important skills for healthcare providers to master, in order to deliver high quality patient care.[1] Doctors need to communicate with a wide range of

people including peers, administration staff, patients, their family members and caregivers. Of course, the most significant is doctor-patient communication as it forms the foundation of the healthcare delivery system. It includes the process of history taking, explaining a diagnosis or prognosis, giving instruction for treatments, and counseling.[2]

Poor inter-personal communication skills can jeopardize the patient care process and hence it is expected that healthcare professionals undergo formal training both as part of their medical curriculum and on the job Continued Medical Education. Studies have shown that effective interpersonal communication skills help improve overall healthcare outcomes especially while managing difficult encounters and breaking bad news.[3,4,5]

In the everchanging healthcare environment, interprofessional and doctor patient relationships are rapidly evolving. This apart Emergency Medicine is a challenging branch of Medicine where healthcare professionals are highly stressed and their burnout rates are high.[6,7]

In such scenarios, effective inter-personal communication skills are an important tool to ensure the delivery of quality healthcare. Lack of empathy in communication is the key reason behind increasing incidence of conflict and violence in healthcare settings.[8]

Designing training programs with contemporary relevance requires regular needs assessment. It is essential to evaluate the baseline levels of inter-personal communication skills amongst practicing professionals to devise appropriate training strategies.[9] Self-assessment of communication skills by healthcare professionals yields valuable information which can be utilized to improve the skillset during on the job training.

Objective

To assess inter-personal communication skills among Emergency Medical Professionals in Pune

Methodology

The study was conducted among 140 Emergency Medical Professionals (EMPs) employed in various hospitals in the city of Pune, India. A questionnaire tool designed in 2019 by Piyush Ranjan *et al* was utilized to assess inter-personal communication skills among Emergency Medical Professionals.[10]

The questionnaire consisted of forty-one items divided into three sections. Section A devoted (four items) to doctor-patient conflicts. Section B focused on (twenty-nine items) self-assessment of communication skills in different scenarios like dealing patient in IPD, OPD and breaking bad news. This section is further divided into four domains, covering important aspects such as components of communication, dealing with patients in outpatient and intensive-care settings, breaking bad news, and communication with colleagues. Section C (eight items) pertained to barriers in practicing good inter-personal communication skills. The reliability of the questionnaire is found to be high (Cronbach α: 0.497, 0.885 and 0.771 for sections A, B and C, respectively).

Section A of the questionnaire required a numerical response whereas items in Section B and C require response on a five-point Likert scale. Informed consent was taken during administration of the questionnaire.

All forty-one items were required to be mandatorily filled. The tool was administered through online mode. The respondents were given one-day deadline to revert with responses. Any queries pertaining to the questionnaire were clarified during data collection. 131 respondents reverted with completely filled questionnaire. The data was tabulated and statistically analyzed with the help of SPSS version 23.

Result

Table 1: Demographic Data

Participants	131
Age	
21 to 25 years	55 (42 %)
25 to 30 years	66 (50.44%)
Above 30 years	10 (7.6 %)
Sex	
Female	101 (77.1%)
Male	30 (22.9%)
Qualification	
BHMS	57 (43.5%)
BAMS	54 (41.2%)
BUMS	14 (10.7%)
BNYS	3 (2.3%)
Others	3 (2.4%)
Work Experience	
Below 1 year	64 (48.9%)
1 to 3 years	50 (38.2%)
3 to 6 years	7 (5.3%)
More than 6 years	10 (7.6 %)

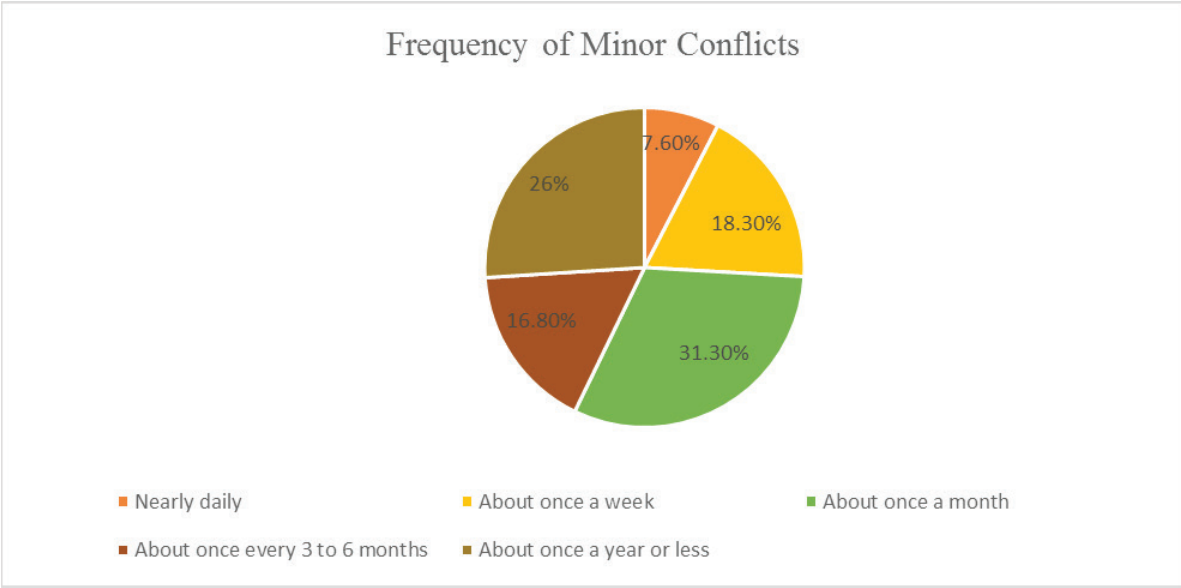

Figure 1: Frequency of Minor Conflicts

As seen in Fig.1 majority EMS professionals encountered minor conflicts very frequently.

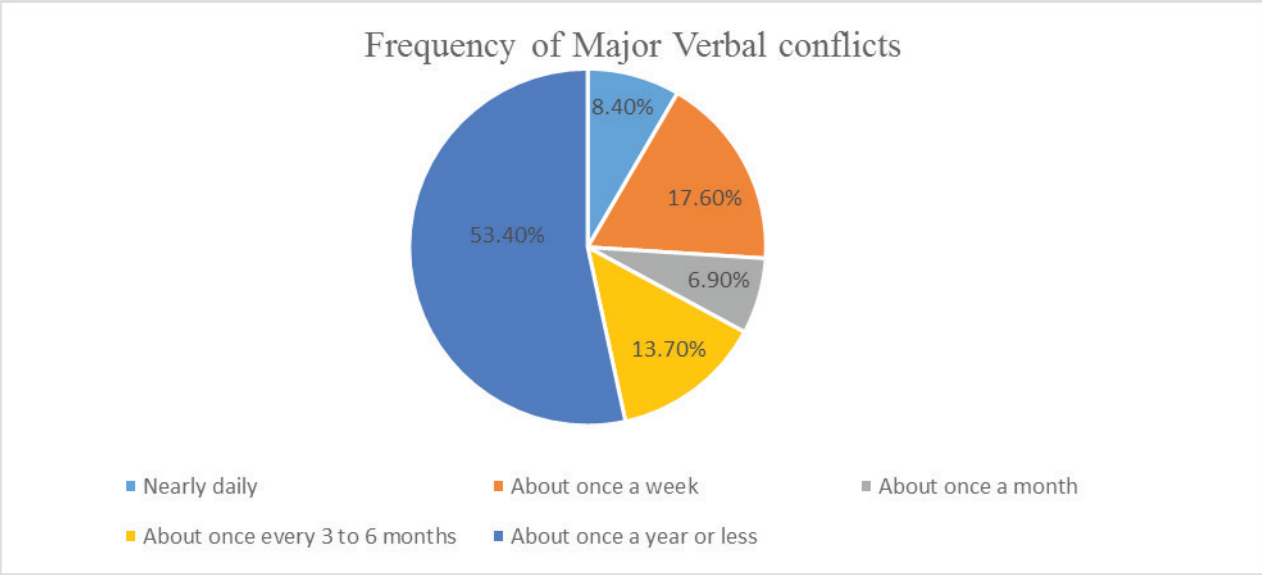

Figure 2: Frequency of Major Verbal Conflicts

As seen in Fig. 2, nearly 8 % of EMS professionals face major verbal conflicts nearly daily. Another 17 % of them faced it once a week. as many as 13.7 %of EMS professionals were victims of major conflicts once in 3 to 6 months while another 53.4 % of EMS professionals had experienced it atleast once during their career.

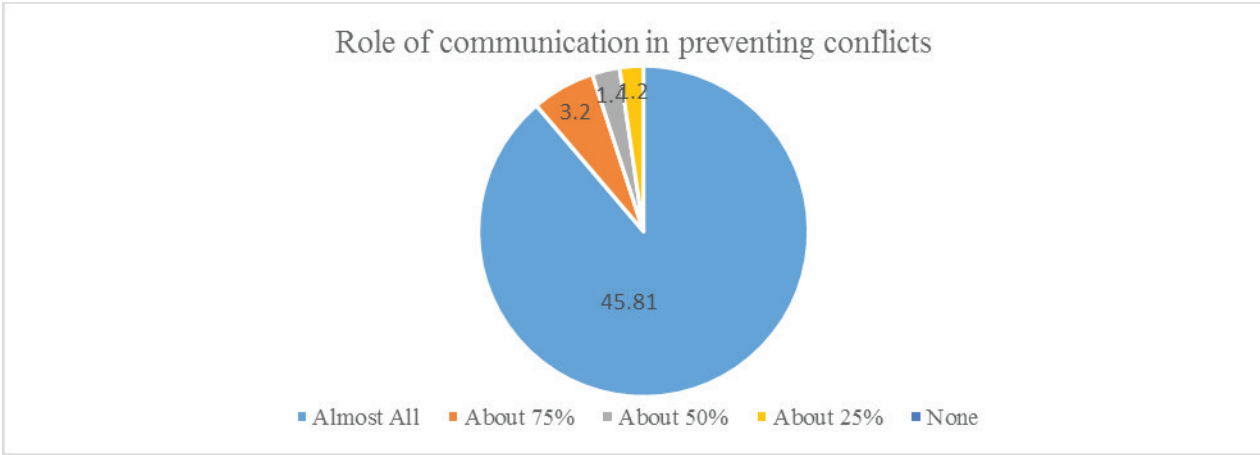

Figure 3: Role of communication in preventing conflicts

As depicted in Fig 3. most EMP's felt that effective communication can be extremely helpful in preventing conflicts.

Table 2

	Range	Total Mean Score	Standard Deviation
Section B	(90, 145)	28.21	12.49
Section C	(11, 40)	26.92	5.82

As seen in Table 2 the respondents scored themselves high on various aspects of doctor patient communication. But they also identified vital barriers that hamper effective communication.

Discussion

Conflict occurs as a result of poor communication. The increasing incidences of minor and major conflicts including physical violence in healthcare settings is extremely disturbing and demoralizes the healthcare professionals. Emergency Medical professionals work in high stress environment and in many cases the patients are already critical and might deteriorate further. This exposes them to greater frequency of conflict with the patient caregivers. Hence, effective communication among this cadre is extremely significant to ensure smooth workflow and healthy doctor patient relationship.

The results demonstrate the high frequency of conflicts encountered by EMS professionals as part of their job. This is inspite of the fact that most EMS professionals scored high on the self-assessment scale of doctor patient communication. This points to the fact that most EMS professionals tend to suffer from a relative bias in terms of their communication skills. This is further reiterated by the high score obtained in the barriers scale of the questionnaire. The disparity in the scores in the two scales could also be because although EMS professionals follow all communication etiquettes yet there are many environmental barriers that are not always under their control. This could include language barriers, paucity of time, infrastructure deficits like lack of proper place or overcrowding, long working hours and lack of subject knowledge required for comprehensive communication.

The results justify the need for improving the insight of EMS professionals with regard to their communication skills as well as searching for possible solutions to overcome the barriers. All aspects of inter-personal communication should be covered thoroughly during the course of medical education. This should be reinforced during the professional career by way of interactive workshops utilizing the tools like case studies, roleplay and standardized patient encounter. Self-assessment should be supplemented with peer assessment and debriefing sessions to ensure more accurate evaluation of the skillset.

Conclusion

The study has a few limitations. It relies on self-assessment to assess interpersonal communication skills and has a limited sample size. Yet, the study is an eyeopener to the incidents of conflicts in emergency healthcare settings and the barriers that prevent a healthy doctor patient relationship.

Source of Funding: Self

Conflict of Interest: Nil

Ethical Clearance: Obtained from IEC, SIU

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 DOI Number: 10.37506/ijfmt.v15i3.15440

Assessment of the Level of Clinical Preparedness amongst EMS Professionals in Managing Life Threatening Emergencies in Suspected Covid 19 Patients

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Abstract

Introduction: SARS-CoV-2 virus has infected millions of people worldwide and has claimed lakhs of lives in its wake. The sudden onset of the pandemic has led to increased levels of anxiety and depression amongst healthcare workers. EMS professionals are the first responders and need to provide emergency Medical Care to their patients. They are required to perform lifesaving interventions in a short period of time. As it is not possible to ascertain the Covid status of the patient, hence EMS professionals are at the greatest risk of getting infected. The study aims to assess the level of clinical preparedness of EMS professionals and suggests recommendations to improve upon it.

Objective: To assess the level of clinical preparedness amongst EMS professionals in tackling life threatening emergencies in suspected Covid 19 patients.

Methodology: The study was conducted amongst 150 Emergency Medical Professionals in Pune, India.

Respondents were asked to respond to each item using 5 point Likert scale. The Questionnaire was administered to the respondents through online mode after obtaining informed consent. Responding to all questions was mandatory. The respondents were given a period of two days to fill the questionnaire.

Discussion: The present study was aimed at examining the current level of preparedness with regards to the disease after nearly a year of the pandemic. The result obtained in terms of knowledge were encouraging and pointed to the fact that the EMS professionals had received adequate training in identification of suspected cases and their initial management. Majority of the respondents suffered from high grade anxiety of contracting the infection. The study highlights the urgent need to conduct counselling and stress management sessions for better mental health of EMS professionals and showed that hospitals are slowly but steadily devising appropriate protocols and safety measures to protect their employees.

Conclusion: The contribution of EMS professionals in providing emergency lifesaving care to suspected Covid 19 patients cannot be over emphasized. As the pandemic continues it is essential to ensure the mental and physical of all healthcare professionals. The study is a small attempt to portray the level of knowledge and mental attitude amongst EMS professionals. The findings need to be corroborated with a larger multicentric study.

Keywords: *Emergency Medical Services (EMS) professionals, Covid 19, Pandemic, Anxiety, Clinical Preparedness.*

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Introduction

Since the first case of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus has infected millions of people worldwide and has claimed

lakhs of lives in its wake. The high degree of infectivity of the virus has led to rapid spread of infections among health care professionals. This has led to a plethora of psychological issues amongst healthcare and frontline workers who have been forced to deal with the pandemic. [1,2]

It has been determined that the main mode of transmission is through direct human contact, via respiratory droplets and fomites with the clinical manifestations in the form of fever, dry cough, breathlessness and patches in lung[3]. The disease is associated with higher rate of mortality in people with underlying comorbidities and healthcare professionals. A study by Mohd. Hanawi K etal[4] in 2020 laid emphasis on the significance of targeted health education interventions for vulnerable population, to reduce the risk of contracting COVID-19.

The sudden onset of the pandemic has led to increased levels of anxiety and depression amongst healthcare workers as they are concerned about the wellbeing of their families. The pandemic has led to a condition called state anxiety described first by Konter [5] in 1996, wherein, distress, worry and tension occurs before or during alarming and dangerous situations due to logical reasons. The concerns of healthcare professionals with regards to Covid 19 are logical as they have to come in close contact with the infected patient and the transmission is airborne.

It is expected that healthcare professionals have to work longer hours during this pandemic. Hence their mental and physical preparedness as well as positive attitude are essential to their optimum performance. A similar situation had occurred in 2003 during the SARS pandemic wherein the mental health of HCP was adversely affected with fear of contracting the virus and infecting family, friends and colleagues.[6]A recently published study by Jianyu Que etal [7] laid emphasis on the higher risk of anxiety, insomnia and psychological issue amongst front-line healthcare workers compared to their counterparts who were not involved in care of Covid 19.

EMS professionals are the first responders and need to provide emergency Medical Care to the patients. Timely provision of medical aid even before the patient can reach the hospital can play an important role in saving the life of the patient.[8]

EMS professionals are required to perform lifesaving interventions in a short period of time. In such cases it is not possible to ascertain the Covid status of the patient. Hence EMS professionals are at the greatest risk of getting infected [9]. The nature of tasks performed by EMS professionals include coming in close contact with the patient, travelling in enclosed environment during patient transport with extremely limited medical information[10,11].

The Centre for Disease control, Atlanta has formulated detailed guidelines to be followed by EMS professionals so as to ensure minimum risk of contracting the infection.[11] But it remains to be seen whether EMS professionals are aware of the guidelines and follow.

Managing emergencies even under Covid 19 era is inevitable. The quality of care provided to the patient cannot be compromised due to lack of knowledge or anxiety amongst EMS professionals.

The study aims to assess the level of clinical preparedness of EMS professionals and suggests recommendations to improve upon it.

Objective:

To assess the level of clinical preparedness amongst EMS professionals in managing life threatening emergencies in suspected Covid 19 patients.

Methodolgy

The study was conducted amongst 150 Emergency Medical Professionals in Pune, India. A

previously tested and validated questionnaire designed by Reem Al-Dossary *etal*[9] in 2020 was utilized for the study.

The sixty-two item questionnaire meant for Healthcare Professionals consisted of 38 items assessing the Knowledge and Awareness levels, 14 items pertaining to Prevention of Covid 19, 6 items assessing the mental attitude of professionals and 4 items regarding their perception of Covid 19 infection.

Respondents were asked to respond to each item using 5 point Likert scale: (1 = Strongly Disagree 2

= Disagree, 3 = Neutral, 4 =Agree, and 5 = Strongly Agree). The Questionnaire was administered to the respondents through online mode after obtaining informed consent. Responding to all questions was mandatory. The respondents were given a period of two days to fill the questionnaire. Any queries pertaining to the questionnaire was clarified during data collection.

All 150 respondents reverted with completely filled questionnaire. The data was tabulated and statistically analyzed with the help of SPSS version 23.

Results

As per the demographic data, 63% of the respondents were between 20 to 25 years of age with 69% of female respondents. Majority of them had less than 5 years of clinical experience. When the duty hours before and after covid outbreak was compared it was observed that 50% of them had to work for more than 8 hours/day after covid outbreak whereas only 37% of them had to work for 8 hours/day before covid outbreak.

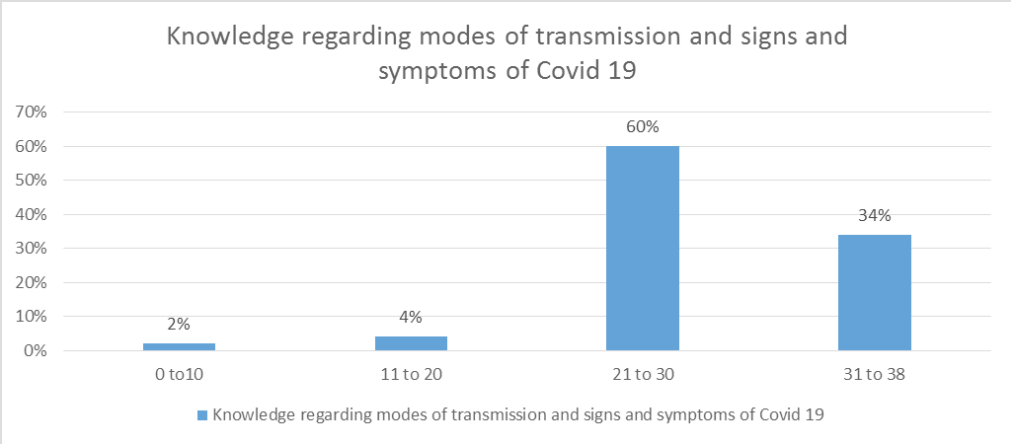

Figure 1: As seen in figure 1. Out of the 38 parameters pertaining to the knowledge regarding transmission, Signs and Symptoms and initial management: Majority of the respondents scored well with 34% of them scoring above 30 out of 38.

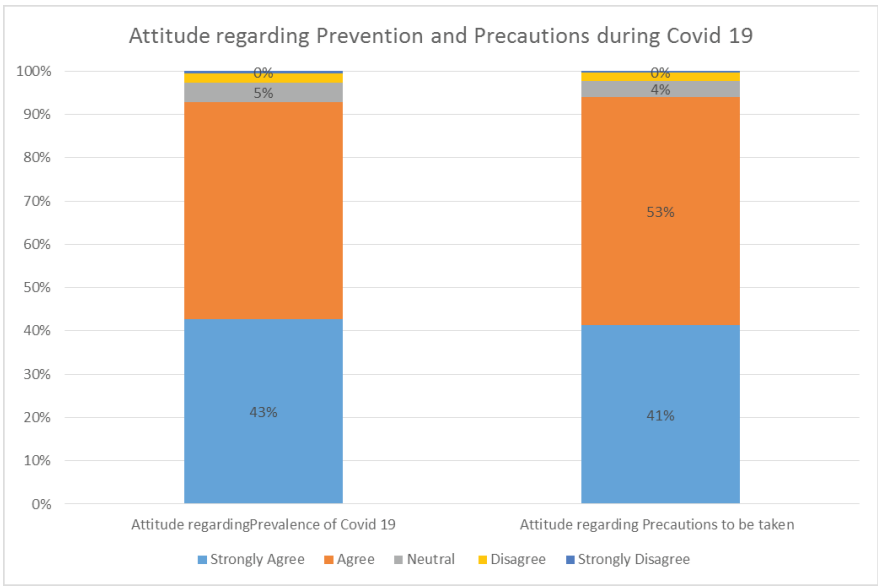

Figure 2: As shown in figure 2, majority of the respondents showed a positive attitude regarding the steps to be taken for prevention of Covid 19 and precautions required to minimize its spread.

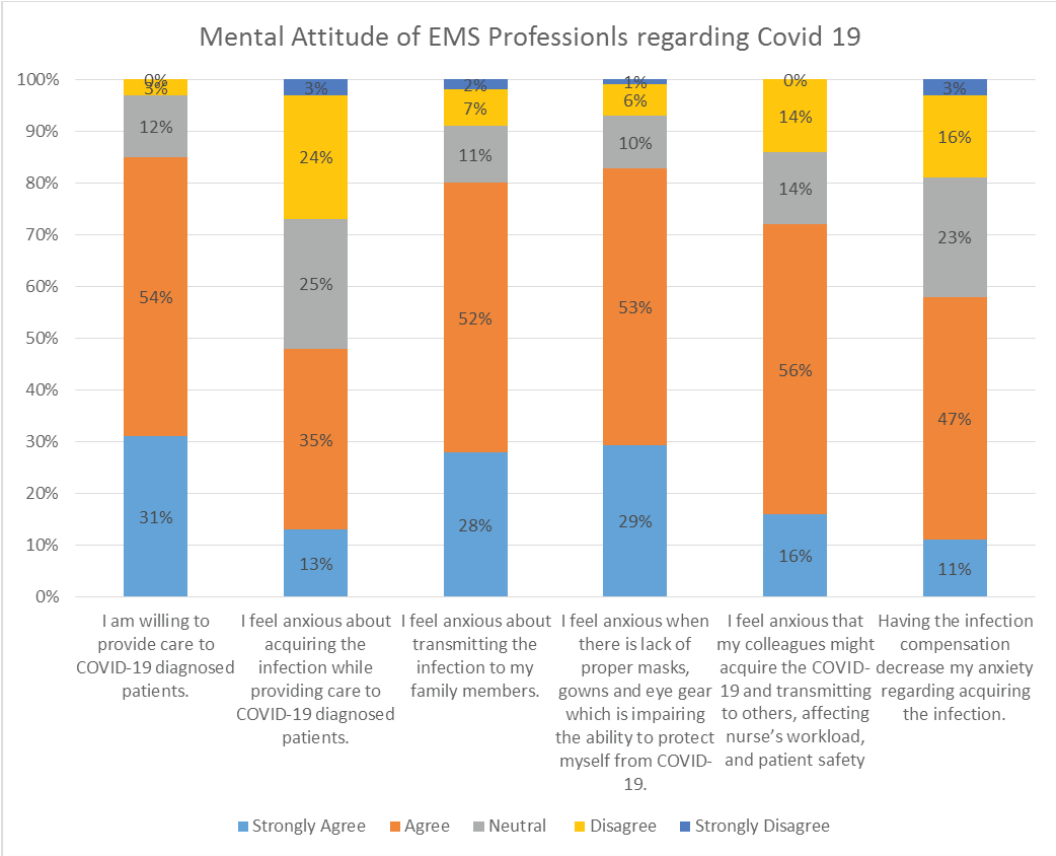

Figure 3: As seen in figure 3, majority of the respondents were willing to provide care to Covid 19 patients. Although most of them were anxious about acquiring infection and transmitting it to their family members. Most of the respondents felt anxious regarding lack of availability of personal protective equipment and spread of infection at workplace.

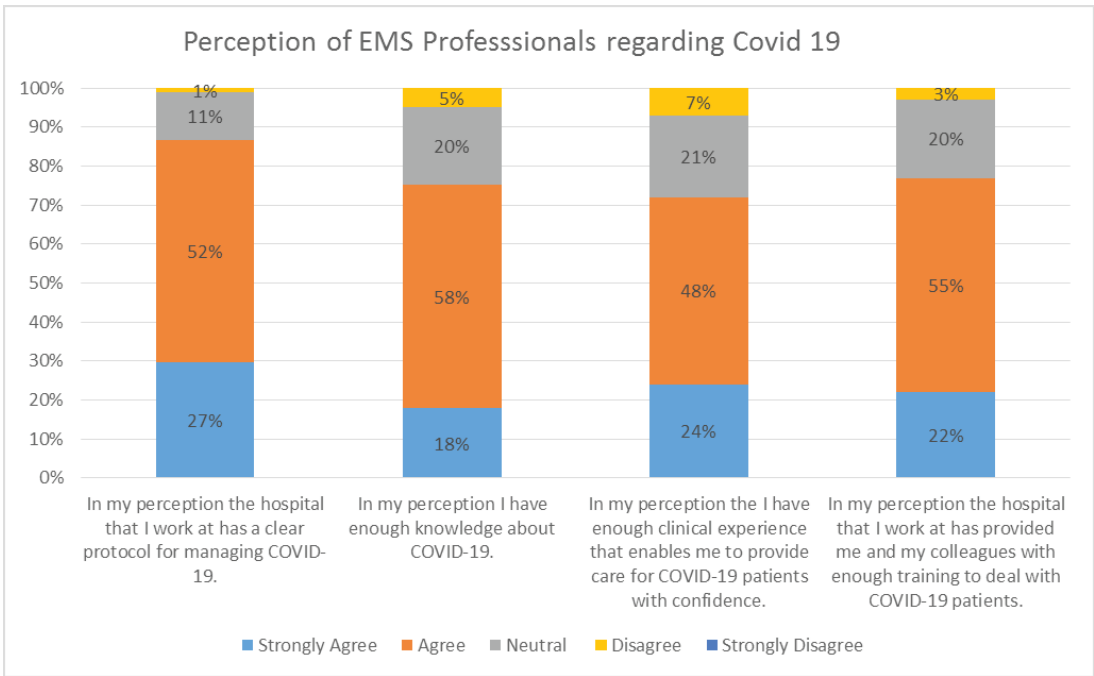

Figure 4: As seen in figure 4, a vast majority of the respondents displayed positive perception towards their hospital efforts in following Covid 19 protocols. Most EMS professionals felt that they possessed sufficient knowledge and clinical experience to provide emergency care to suspected Covid 19 patients.

Discussion:

The Covid 19 pandemic spread in a very short span of time and Healthcare professionals were caught unaware. Among them the EMS professionals had to continue dealing with various kinds of emergencies as the first line of care. This predisposed them to acquire the infection from the patient as the Covid 19 status of the patient could not be ascertained at the time of emergency. Even though EMS professionals were advised to take all precautions to avoid transmission of infection, this led to a high degree of anxiety amongst them.

Knowledge of the clinical signs and symptoms and transmission pattern of the disease is essential for all healthcare professionals. Suspecting Covid 19 in a respiratory emergency patient is essential for both, providing optimum first line of care and avoiding infection transmission. The present study was aimed at examining the current level of knowledge with regards to the disease after nearly a year of the pandemic. It is imperative that EMS professionals are well versed with the clinical features, basic emergency treatment and prevention of transmission in all suspected emergency patients. A vast majority of the respondents scored well on the items pertaining to the knowledge of the disease. Nearly all the EMS professionals were aware about the importance of PPE, handwashing at frequent intervals and following social distancing norms wherever possible. The result obtained in terms of knowledge were encouraging and pointed to the fact that the EMS professionals had received adequate training in identification of suspected cases and their initial management.

Majority of the respondents were willing to handle suspected Covid 19 patients. They suffered from high grade anxiety of contracting the infection. The respondents also communicated anxiety with regards to acquiring the disease at the workplace and professional colleagues. They were also worried about transmitting the infection to their loved ones. Serving the population irrespective of their Covid status is the duty of all healthcare professionals and EMS professionals are fulfilling their duties. But it is essential to allay their fears and anxiety for their own wellbeing and to ensure quality care to the patient. The study highlights the urgent need to conduct counselling and stress management sessions for better mental health of EMS professionals.

Inspite of the anxiety EMS professionals showed positive attitude towards contributing to their emergent cate of suspected patients. They also showed a high degree of trust on their employers i.e. hospitals in making relevant arrangements for personal protective equipment. Provision of compensation in case they develop infection helped reduce their level of anxiety. The findings show that hospitals are slowly but steadily devising appropriate protocols and safety measures to protect their employees.

Conclusion

The contribution of EMS professionals in providing emergency lifesaving care to suspected Covid 19 patients cannot be over emphasized. As the pandemic continues, it is essential to ensure the mental and physical of all healthcare professionals. The study is a small attempt to portray the level of knowledge and mental attitude amongst EMS professionals. The findings need to be corroborated with a larger multicentric study.

Acknowledgement: We thank Reem Al-Dossary *etal* for granting permission to use the questionnaire for the research study.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Obtained from IEC, SIU

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Type of Article- Original Article

Efficacy of *Dhataki ghrit* Tulle in Comparison with Framycetine Sulphate Tulle in Management of Dusta Vrana

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Abstract

Background: Since life, humans have been susceptible to injury caused by wound healing deficiency. Modalities are needed to treat wounds from very early developmental stages. Ayurveda has various treatment modalities mentioned in texts for treatment of vrana. Modalities have been described in the form of local application, oral medication as per the stages and *sadhyasadhyata* of disease. Local use of ghrita with harbal formation is best practice for acharyas from ancient times until now. **Aims and Objectives**: Efficacy of *Dhataki ghrit* tulles in the management of *Dusta vrana* in comparison with Framycetine sulphate tulles. And to study the effect of dhataki ghrit , plain ghrit and framycitene sulphate on dusta vrana. **Methodology**: in this study total 210 patient will be divided randomly into 3 groups (70 each).

In group A (Experimental) Dhataki Grita tulle will be applied on wound locally in group B(Control) Plain Go ghrita will be applied and in group C(control) Framycetine sulplate tulle will be applied . Assessment will be recorded as per criteria. **Results**: Changes will be observed in objective outcomes. **Conclusion:** Dhataki ghrit tulle may be the best and cheap modality used as dressing material. Will be drown as per sutaible data.

Keywards: *Dhataki, Wound, Vrana, Wound healing , Framycitene sulphate , Sofratulles*

Background

Wound healing plays an significant role in Ayurvedic literature. The earliest medical literature mention wound care thoroughly, wound treatment is undoubtedly the first medical issue faced by humans. In reality, the nonhealing existence of ulcer often times poses a challenge. Vrana healing is a natural process but it becomes dusta and normal healing process delayed due to interference of vitiated doshas . Chronic wound is long standing ailment with profuse discharge and slough where more importance should be given in removing the debris and enabling the proliferation of healthy tissue. The prevalence of wounds in the surveyed population (n=6917) was 15.03 for every 1000. The prevalence of acute and chronic wounds in the population was 10.55 and 4.48 per 1000, respectively. The lower extremity was the most common site for both acute and chronic wounds. Unlike Western studies, an untreated, acute, traumatic wound was the most common etiology for a chronic lower extremity wound. This in effect underlines the need for community-based wound-care teams in India [1]

Acharya sushruta mentioned Dusta vrana in its definition of shalya tantra with other significant factors [2]. He further defines 60 therapeutic steps known as shasti-upakrama for the management of vrana [3].

For the *Shodhan* and *Ropana* seven *kriyakalpas* are mentioned that are *kashaya, varti, avachurnana, taila and sarpi.* Mode of *kriyakalpas* is according to the combination of drugs used. Among these Sarpi is the one in which ghrit is used along with different kalpas

and different ways [4]. *Dhataki* (***Woodfordia fruticosa)*** plays important role in *vranaropana*[5].

Debridment consists of three surgical, chemical , and mechanical forms. Removing dosha from dead and vitiatead by surgical means damages healthy tissue. For chemical debridement various chemicals like povidone ointment, eosol, hydrogen peroxide,Framycetine sulphate etc in the form of ointment, liquids and tulles are being used. Povidone ointment should not be use in case of sensitive participants. Flushing or hydrotherapy is used in mechanical debridement since chemical matrials used for dressing have their own setbacks in this study. *Dhataki ghrit* will be used in the form of tulles.

Rationale of the study- Sofra tulle (Framycetine sulphate) has been used from several years which is tropical antibiotic. Framycetine sulphate is very useful for wound healing but cost of one tulle is very high as for dressing of any type of wound there is need of may tulles for daily dressing, hence it cost high to patient. Similarly overuse of any antibiotic can cause resistant to specific bacteria or specific component. The main purpose of this study is to improve the care of the participants and to reduce the costs associated with Dusta vrana (chronic non-healing ulcers) also by using this study we can reduce the use of antibiotics.

Dhataki due to its *vranaropak* activity heals *vrana* by reducing pain,itching and discharge[5]. *Dhataki* is available freely hence it is economically viable and it become easy to prepare with *Goghrit* which is also easily available. This research is therefore being suggested to evavulate the efficacy of Dhatakighrit tulles as comparison to Framycetine sulphate.

Aim and Objectives

Aim: Efficacy of *Dhataki ghrit* tulles in the management of *Dusta vrana* in comparison with Framycetine sulphate tulles.

Objectives:

· To study the effect of *Dhataki ghrit* tulles on healing of *Dusta vrana*

· To study the effect of Framycetine sulphate tulles on healing of *Dusta vrana*

· To study the effect of Plain *Goghrit* tulles on healing of *Dusta vrana*

· To compare effect of *Dhataki ghrit* tulles, Framycetine sulphate tulles and plain *Goghrit* tulles on healing of *Dusta vrana*.

Case definition

Dusta Vrana-

Participants of *vrana* on extremities with following Clinical features:-

Samruta (Narrow maunted), *Kathina* (Hard),

Avasanna- depressed, *Vedonarvn*- (severepain),

Vivrutta(Wide mounted), *Ushna*(Hot) ,

Daha(burning sensation at the site), *Paka*(suppuration),

Raga (Redness), *Puyastrava*(Discharging pus), *Manojnadarshana*(with ugly site),

Kandu(Itching), *shopha*(swelling) , *Pidaka* (with boils), *Mrudu* (soft),

Bhairava(Frightful), *Putimamsasirasnayu*(full of pus muscles , vessels, ligaments)[6]

Dusta Vrana (Chronic non healing wound) which is difficult to heal. *Dusta* is one in which there is localization of *doshas vata pitta* and *kapha* . *Vrana* which has a bad smell, has abnormal color with profuse discharge , intense pain and it takes long period to heal. [7]

Research Question:

Is Dhatakighrit tulle effective as that of Framycetine sulfate tulles in management of Dusta vrana. **Hypothesis:**

Dhataki ghrit tulle may be effective in management of *dustavrana* than Framycetine sulphate tulle due to its *vranaropak* activity.

Null Hypothesis:

Dhataki ghrit tulles may not be effective than Framycetine sulphate tulles in management of *Dustavrana.*

Trial design: Randomized Standard controlled Study called Double Arm Open. Interventional study with ratio of 1:1:1 on 3 parallel group. This is an experimental analysis.

Methodology

Study setting : The study will be conducted in academic hospital MGACH & RC, Salod (H), Wardha.

Inclusion criteria

· Participants will be selected irrespective of sex, age, religion, occupation, economic and educational status.

· Participants between the age group of 18-55 years will be selected.

· Participants of *vrana* presenting with minimum 4 signs and symptoms of *Dusta vrana* which are mentioned in case definition and willing to participate in study will be recruited.

Exclusion criteria

1. Participant with

· Known case of Malignancy,

· Known case of Tuberculosis ,

· Known case of Leprosy and

· HIV and HbSAg positive will be excluded from this study

Criteria for discontinuing or modifying allocated interventions:

The subject will be withdrawn from the study if any incidence, drug sensitivity characteristics or any other disease or problem arises the subject will be offered free treatment until the problem subsides.

Follow up: 0 and 30th day

Primary outcomes – we will see the effect of interventional drug on the basis of assessment criteria on pain, itching, discharge. The data will be expressed as a standard error of 5 % significance, it is hypothesized that Dhataki pushpa tulle in the form of dressing material would be more effective in wound healing.

Secondary Outcome – we will se the changes of wound within its bacteriological phenomenon . It is hypothetized that dhataki pushpa tulle will be also effective in controlling bacteriological growth.

Discussion

Many research studies regarding *Dusta vrana* (Chronic wound healing) are carried out in modern medicine as well as in *Ayurveda*.

Modern drugs and dressing materials used in management of chronic wound are very limited and preparations like sofratulles are much costly for general population also there are many setbacks with the overuse of tropical antibiotics and antimicrobial drugs[8,9], hence there is need of study to prove efficacy and safety in management of *Dusta vrana*(Chronic Non healing Ulcer). So, this study is being undertaken to prove the effectiveness of Dhataki ghrita tulles in Dusta vrana management compared to Framycetine Slphate tulles.

Dhataki cures vrana because of its vranaropak activity by reducing pain, itching and discharge. Dhataki is freely available, which makes it economically viable and easy to prepare with easily available Goghrit.

Analysis plan (Statistical test):

The statistical analysis will be carried out using descriptive and inferential statistics using Chi square test, Dunnett D test, One way ANOVA and the software used in the study will be SPSS 22.0 and Graphpad prism 7.0 and EPI-INFO 7.0 edition.

Intervention period: 30 days

Follow up period on– 60 day

Time schedule of enrolment, Intervention period: 30 days

Recruitment: 210 Participants (70 in each group) will be selected using simple random sampling Lottery process, and PI will assign and enroll the patient.

Methods: Data collection, enrolment then management, and analysis

Data collection methods:

Assesement criteria

Objective parameters-

Size (Blotting paper Technique) **Bacteriological test** (Conventional Method)

Data management: The data entry coding will be done by PI

Statistical methods: Paired and Unpaired 't' Check objective criteria.

Ethics and dissemination:

Research ethics approval; approval from research ethics committee has taken. No-Ref.No.DMIMS (DU)/ IEC/2018-19/7598

Consent or assent: The written consent will be taken from the participant prior to start the study.

The confidentiality of the individual participants will be protected throughout the study.

Dissemination policy: The data will be disseminated on paper.

Eligibility requirements for authorship and any planned use by qualified authors

Informed consent materials: With all the information model, the participants will be given consent form and other relevant documentation.

Strengths: If Dhataki Improves wound healing it will be a very effective and versatile dressing device. Unless the proposed study results in a positive outcome then the best parallel modality for wound management will be given.

Limitations: Single drug therapy is used to determine the efficacy of dhataki ghrit tulle in Dustavrana (Chronic Non Healing Wounds) treatment. Additional drugs may be added for better outcomes and combined formulation may be prepared.

Gantt chart (in Quarterly based)

Item	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Enrollment of Volunteer								
Data collection								
Writing the thesis parts up to methods								
Data analysis								
Writing the thesis parts up to results and Conclusions								
Submission								

Conclusion

Conclusion is drawn by careful examination of the results.

Conflict of Interest: None

Funding: None

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